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Seasonal variation in effects of urea and phosphorus on phytoplankton abundance and community composition in a hypereutrophic hardwater lake

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Abstract

1. Urea accounts for half of global agricultural fertiliser applications, yet little is known of its role in eutrophication of freshwater ecosystems, nor how it interacts with phosphorus (P) in regulating phytoplankton composition, especially during spring and autumn.
2. To identify when and how urea and P inputs interact across the ice-free period, we conducted seven monthly fertilisation experiments in 3,240-L mesocosms from ice-off to ice-formation in a hypereutrophic lake. In addition, we ran bioassays with ammonium (NH_4^+) to compare the effects of urea with those of NH_4^+ , the immediate product of chemical decomposition of urea.
3. Analysis of water-column chlorophyll *a* and biomarker pigments by high-performance liquid chromatography revealed that addition of inorganic P alone ($100 \mu\text{g P L}^{-1} \text{ week}^{-1}$) had no significant impact on either algal abundance or community composition in hypereutrophic Wascana Lake. Instead, fertilisation with urea ($4 \text{ mg N L}^{-1} \text{ week}^{-1}$) alone, or in concert with P, significantly ($p < 0.05$) increased algal abundance in spring and much of summer, but not prior to ice formation in October. In particular, urea amendment enhanced abundance of cryptophytes, chlorophytes, and non-diazotrophic cyanobacteria during April and May, while fertilisation in summer and early autumn (September) increased only chlorophytes and non-diazotrophic cyanobacteria.
4. Comparison of urea mesocosms with NH_4^+ bioassays demonstrated that urea lacked the inherent toxicity of NH_4^+ in cool waters, but that both compounds stimulated production during summer experiments.
5. This study showed that urea pollution can degrade water quality in P-rich lakes across a variety of seasonal conditions, including spring, and underscores the importance of quantifying the timing and form of N inputs when managing P-rich freshwaters.

KEYWORDS

cyanobacteria, eutrophication, management, nitrogen, water quality

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1 | INTRODUCTION

Enrichment of freshwater and marine habitats with nitrogen (N) and phosphorus (P) fuels proliferation of harmful algal blooms (Elser et al., 2007; Glibert, Maranger, Sobota, & Bouwman, 2014; Schindler, 1977). Although nutrient management strategies for freshwater ecosystems have traditionally focused on the effects of P (Bormans, Maršálek, & Jančula, 2016; Dillon & Rigler, 1974; Litke, 1999; Schindler, Carpenter, Chapra, Hecky, & Orihel, 2016), research shows that addition of N to P-rich lakes can additionally degrade water quality, stimulate phytoplankton abundance, and promote growth of toxic cyanobacteria at the expense of other phytoplankton taxa (Andersen, Williamson, Gonzalez, & Vanni, 2019; Bunting, Leavitt, Gibson, McGee, & Hall, 2007; Conley et al., 2009; Glibert, Maranger, et al., 2014; Harris, Wilhelm, Graham, & Loftkin, 2014; Van de Waal, Smith, Declerk, Stam, & Elser, 2014). Recent studies also suggest that hypereutrophic lakes may exhibit seasonal variation in nutrient limitation, with P regulation of phytoplankton production during winter and spring, while N limitation is paramount during summer and autumn (Andersen et al., 2019; Chaffin, Bridgeman, & Bade, 2013; Hayes, Vanni, Horgan, & Renwick, 2015; Paerl et al., 2011, 2015). However, predictions of the seasonality of N effects are complicated by temporal variation in the predominant chemical form of N added to surface waters (Cade-Menun et al., 2013; Lomas, Trice, Glibert, Bronk, & McCarthy, 2002; Newell et al., 2019; Stepanauskas, Laudon, & Jørgensen, 2000), as well as inherent differences in the unique effects (i.e. stimulation or suppression) of these forms on specific phytoplankton taxa (Glibert et al., 2016; Swarbrick, Simpson, Glibert, & Leavitt, 2019). Thus, improved management of P-rich freshwaters requires information on how the effects of individual N compounds on the abundance and community composition of phototrophs may vary among seasons.

Global influx of N to freshwater systems has increased ~ five-fold since the pre-industrial period (Green et al., 2004) due to a combination of elevated atmospheric deposition (Holtgrieve et al., 2011), increases in discharge of storm- and wastewater effluents from cities (Bernhardt, Band, Walsh, & Berke, 2008), and application of N-based fertilisers (Glibert, Harrison, Heil, & Seitzinger, 2006). Presently, over 50% of global N fertiliser is applied as urea due to its low intrinsic toxicity, chemical stability (i.e. non-explosive), and ease of manufacture (Belisle et al., 2016; Glibert et al., 2006). These agricultural applications are forecast to nearly double again by 2050 to meet global demands of an additional 2.5 billion people (Glibert et al., 2006; Millennium Ecosystem Assessment, 2005). Use of urea is most prevalent in regions where long-term agricultural activity has often saturated soils with P, resulting in surface waters enriched with soluble reactive phosphorus (SRP) from diffuse sources (Bennett, Carpenter, & Caraco, 2001; Bogard, Donald, Finlay, & Leavitt, 2012; Bunting et al., 2007). Export studies estimate that up to 40% of urea applied to farmlands may be lost to downstream surface waters, particularly during pluvial periods (Davis, Tink, Rohde, & Brodie, 2016; Kibet et al., 2016; Siuda & Chróst, 2006).

In general, research on the effects of urea on water quality in agricultural regions has been conducted during late summer (e.g. July–September) when influx of urea from diffuse sources is less likely to occur (Bogard et al., 2017; Donald, Bogard, Finlay, & Leavitt, 2011; Finlay, Patoine, Donald, Bogard, & Leavitt, 2010). These studies show that fertilisation of warm, P-rich waters with urea stimulates growth of chlorophytes and non-diazotrophic cyanobacteria at the expense of N₂-fixing cyanobacteria (Bogard et al., 2017; Donald et al., 2011; Finlay et al., 2010), with more pronounced N effects if SRP exceeds c. 50 µg P/L and ratios of total dissolved N (TDN) to SRP are less than 20:1 by mass (Donald et al., 2011). Further, these experiments indicate that effects of urea and ammonium (NH₄⁺) on phytoplankton biomass and community composition are comparable (Donald, Bogard, Finlay, Bunting, & Leavitt, 2013; Donald et al., 2011), suggesting that chemically reduced forms of N, rather than the specific compound (e.g. urea, NH₄⁺), favours cyanobacterial growth (Blomqvist, Pettersson, & Hyendstrand, 1994; Donald et al., 2011; Finlay et al., 2010; Hyenstrand, Nyvall, Pettersson, & Blomqvist, 1998; Newell et al., 2019). However, while NH₄⁺ is known to selectively stimulate bloom-forming taxa in summer, this compound can also suppress growth of some spring phytoplankton (diatoms, cryptophytes, unicellular cyanobacteria) (Glibert et al., 2016; Swarbrick et al., 2019), whereas little is known about whether urea exhibits similar differential effects among seasons, either directly or via its principal decomposition product, NH₄⁺ (Lee, Cherry, & Edmonds, 2016; Siuda, Chróst, Kalinowska, Ejsmont-Karabin, & Kiersztyn, 2016; Solomon, Collier, Berg, & Glibert, 2010).

Preliminary research suggests that effects of urea on water quality in P-rich lakes may vary among seasons (Belisle et al., 2016; Mitamura, Tachibana, Konda, Ueda, & Seike, 2010; Siuda & Kiersztyn, 2015). First, export of urea is expected to be highly seasonal (Glibert et al., 2006; King et al., 2017), particularly in northern regions where agricultural fertiliser application and release of wastewater lagoon effluents tend to occur in spring and autumn, when low temperatures coincide with reduced microbial and enzymatic activity (Siuda & Chróst, 2006) and elevated runoff (Pham, Leavitt, McGowan, Wissel, & Wassenaar, 2009; Pomeroy et al., 2007). Together, these factors reduce the extent of urea hydrolysis and favour export to lakes (Di & Cameron, 2008; Silva, Cameron, Di, & Jørgensen, 2005; Swensen & Singh, 1997). Second, hydrolysis of urea may suppress diatom and cryptophyte growth in cold, illuminated waters of spring and autumn, as NH₄⁺ prevents nitrate (NO₃⁻) uptake and constrains the dissimilatory nitrate reduction (DNR) needed to quench photo-oxidative cell damage (reviewed in Glibert et al., 2016). Third, seasonal variation in the timing of sedimentary organic matter mineralisation and water-column circulation may affect the magnitude and chemical composition of internally loaded nutrients (e.g. urea, NH₄⁺, SRP) (Bogard et al., 2012; McCarthy, Gardner, Lehmann, Guindon, & Bird, 2016; Patoine, Graham, & Leavitt, 2006; Søndegaard, Jensen, & Jeppesen, 2003). Fourth, the annual phenology of phytoplankton is expected to lead to seasonal variation in plankton composition (Dickman, Vanni, & Horgan, 2006; Donald et al., 2013; Klausmeier, Litchman, Daufresne, & Levin, 2004;

McGowan, Leavitt, & Hall, 2005). Thus, while urea is ubiquitous in lakes and may exceed 0.5 mg N/L in some freshwater systems (Bogard et al., 2012; Glibert et al., 2006; Swarbrick, 2017), relatively little is known of how these annual patterns lead to seasonal variation in urea effects on P-rich lakes.

This research seeks to quantify how urea effects on phytoplankton abundance and community composition may vary among seasons in eutrophic lakes of the northern Great Plains within Canada. To this end, effects of urea, alone and in combination with P, were assessed using large-scale (3,240-L) mesocosm experiments, repeated monthly from April to November. We also compared mesocosm results with those from concurrent bioassays fertilised with NH_4^+ to determine whether urea acted directly or through its main decomposition product. We hypothesised that urea would not stimulate phytoplankton growth during spring and autumn when ambient P concentrations were low and water was cool (Paerl et al., 2015; Swarbrick et al., 2019), but that it would selectively stimulate toxic, non-diazotrophic cyanobacteria and chlorophytes during warm, P-rich conditions of late summer (Donald et al., 2011; Finlay et al., 2010; Harris et al., 2014). Instead, we hypothesised that urea amendment in spring and autumn may suppress growth of diatoms and cryptophytes due to microbially or chemically mediated hydrolysis of urea to NH_4^+ (Alexandrova & Jorgensen, 2007; Solomon et al., 2010; Swarbrick et al., 2019). Finally, we expected that fertilisation with P would favour phytoplankton growth only in spring, when ambient SRP concentrations and internal release from sediments are often low (Nürnberg, 2009).

2 | METHODS

2.1 | Study area

Experiments were conducted in Wascana Lake, a small (0.5 km²), polymictic, hardwater lake within the City of Regina, Saskatchewan, Canada (50°26.17'N, 104°36.91'W). The study lake is part of the Qu'Appelle Valley Long-Term Ecological Research programme, and was sampled bi-weekly between May and September following methods in Vogt, Sharma, and Leavitt (2018). Regional climate is characterised 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 (~ 1°C) with high seasonal variability (Leavitt, Brock, Ebel, & Patoine, 2006). Spring snowmelt accounts for 80% of annual surface runoff (Pham et al., 2009; Pomeroy et al., 2007), leading a rapid flushing rate (mean water residence time = 0.15 year) that varies substantially among years (Table 1). Wascana Lake receives nutrients from agricultural runoff (wheat, canola, livestock) from its 1,400 km² catchment via Wascana Creek, as well as inputs from urban diffuse and storm-water sources, migratory water fowl (Fremaux, Boa, & Yost, 2010), and internal sedimentary sources (Patoine et al., 2006). As a result, Wascana Lake is hypereutrophic with elevated mean (\pm SE) concentrations of chlorophyll *a* (Chl *a*; 42.8 \pm 2.9 μ g/L) and soluble reactive P

TABLE 1 Morphometric, chemical, and biological characteristics of Wascana Lake

Area (km ²)	Volume (m ³ 10 ⁶)	Water residence (yr)	Z _{max} (m)	TDP (μ g P/L)	SRP (μ g P/L)	TDN (μ g N/L)	Urea (μ g N/L)	TDN: SRP	DOC (mg/L)	DIC (mg/L)	Conductivity (μ S/cm)	pH	Secchi depth (m)	Chl <i>a</i> (μ g/L)
0.5	0.7	0.05	3.4 (0.06)	357.8 (26.5)	261.5 (26.5)	1,441.2 (60.7)	111.6 (19.7)	13.5 (2.6)	17.7 (0.6)	43.2 (1.2)	918.5 (12.2)	9.0 (0.1)	0.8 (0.1)	42.8 (2.9)

Note: Data, except urea, are mean values (\pm SE) of measurements taken between May - Aug of 1996-2012. Urea was measured between May-August of 2008-2012. Abbreviations represent maximum depth (Z_{max}), total dissolved- and soluble reactive phosphorus (TDP, SRP), total dissolved nitrogen (TDN), dissolved organic and inorganic carbon (DOC, DIC), and chlorophyll *a* (Chl *a*)

(SRP; $261.5 \pm 26.5 \mu\text{g P/L}$), low transparency (Secchi depth $< 0.8 \text{ m}$), and low mass ratios of dissolved N:P (13.5 ± 2.6 ; Table 1). The lake also exhibits elevated pH (8–10.5) and concentrations of dissolved organic (DOC) and inorganic carbon (DIC) characteristic of regional prairie lakes (Finlay et al., 2015; Finlay, Wissel, & Leavitt, 2009).

Wascana Lake exhibits pronounced seasonal patterns of community succession typical of shallow, productive lakes, with spring phytoplankton communities of diatoms and cryptophytes giving way to mid- to late-summer blooms of chlorophytes, diazotrophic cyanobacteria, and non- N_2 -fixing cyanobacteria (Donald et al., 2013; McGowan, Leavitt, et al., 2005). Although chrysophytes are present during spring, they generally contribute $<20\%$ of the phytoplankton abundance during the open-water period (Donald et al., 2013). Summer taxa include N_2 -fixing *Aphanizomenon flos-aquae* in June–July, non-heterocytous *Microcystis* spp. in late August–September, and *Planktothrix agardhii* throughout June–September, while *Phormidium* spp. and diatoms (e.g. *Cyclotella* spp.) increase during September (Donald et al., 2013). Heterocytes are not routinely enumerated in Wascana Lake, although they have been recorded for a decade in both Katepwa and Crooked lakes, downstream of Wascana Lake (Hayes et al., 2019). The pelagic invertebrate community is composed mainly of large-bodied *Daphnia* spp. (e.g. *Daphnia magna*, *Daphnia pulicaria*, *Daphnia galeata mendotae*) during April–June, but is replaced with abundant copepods (*Diaptomus thomasi*, *Leptodiaptomus siciloides*) in July–September (McGowan, Leavitt, et al., 2005; Patoine et al., 2006) due to seasonal predation by zooplanktivorous fish, including emerald shiner (*Notropis atherinoides*), five-spined stickleback (*Culaea inconstans*), and yellow perch (*Perca flavescens*).

2.2 | Mesocosm experiments

Mesocosm experiments were based on designs of Finlay et al. (2010) to facilitate comparison among studies evaluating effects of different chemical forms (Donald et al., 2011, 2013) and concentrations of N (Bogard et al., 2017; Bogard, Vogt, Hayes, & Leavitt, 2020) on planktonic communities in late summer (July–September). Briefly, 12 cylindrical mesocosms (2m diameter \times 1m deep, $\sim 3140 \text{ L}$), made of poly-weave plastic, closed at the bottom and open to the atmosphere, were secured to floating frames, and deployed in a sheltered bay of Wascana Lake. Each frame was anchored independently to the sediments and held six enclosures, which received randomly assigned treatments. Prior to each use, mesocosms were cleaned, rinsed, and then filled passively with lake water by drawing the submerged poly-weave material up from depth. Filling was then completed using a portable water pump. Minnow traps were deployed in each mesocosm and checked routinely to remove fish. Triplicate mesocosms were deployed for each treatment including amendments of urea alone (+U), phosphorus alone (+P), urea and phosphorus (+UP), and no fertilisation (–UP controls). Amended mesocosms received either $4 \text{ mg N L}^{-1} \text{ week}^{-1}$ as urea or $100 \mu\text{g L}^{-1} \text{ week}^{-1}$ of P as orthophosphate (K_2HPO_4), concentrations known to saturate

autotrophic demand in previous experiments (Bogard et al., 2017, 2020; Donald et al., 2011; Finlay et al., 2010). Mesocosm amendments of 4 mg N/week were selected because they are characteristic of N-polluted waters, and tend to elicit clear responses without inducing the profound anoxia and strongly heterotrophic conditions found in wastewater lagoons and intensive livestock effluent (Bogard et al., 2020). Enclosures were mixed completely using a paddle after fertilisation. Mesocosms were removed, pressure-washed without detergents, and dried at the end of each experiment.

Experiments were conducted each month from April to October 2012, a period which covered the entire ice-free season. Experiments were run for 2-week durations during April, May, June, and October, with sampling between 10:00 and 14:00 h on days 0 (immediately after nutrient amendments), 7, and 14 (prior to nutrient amendments). Shorter experiments were selected because most phytoplankton responses to fertilisation were evident by 14 days in past trials. Experiments in July, August, and September were conducted for a 3-week duration (including day 21 samples) to facilitate direct comparison with previous summers' experiments (Bogard et al., 2017, 2020; Donald et al., 2011; Finlay et al., 2010). Onset (Bourne, MA, USA) HOBO® Pendant Data Loggers were installed at 0.1 and 0.5m depths in each mesocosm, and recorded temperature and light intensity (lx) at 10min intervals over the duration of each experiment. On each sampling date, water temperature ($^{\circ}\text{C}$), specific conductivity ($\mu\text{S/cm}$), salinity (g/L) and dissolved oxygen (DO; $\text{mg O}_2/\text{L}$) were recorded at 0.5m depth using a YSI Inc. (Yellow Springs, OH, USA) model 85 meter, pH was measured at 0.1m depth using a handheld Oakton Instruments (Vernon Hills, IL, USA) pHTestr 10 meter, and water transparency was estimated using a 20cm diameter Secchi disk.

Whole-water samples were collected from 0.5m below the surface of each mesocosm, and returned to the laboratory within 1 hr for subsequent analysis and storage. Whole water was transferred using sterile technique into 10mL pre-sterilised evacuated vials and preserved with 1% glutaraldehyde for microbial enumeration at University of Quebec at Montreal. Particulate organic matter for analysis of pigments was collected on GF/C filters (nominal pore size $1.2\mu\text{m}$) and frozen at -10°C until analysis for biomarker pigments (see below). The remaining whole water was filtered through a GF/F $0.45\mu\text{m}$ pore membrane filter, sealed, and either frozen (nutrients) or refrigerated (DIC, DOC) until chemical analysis. Although periphyton was monitored following standard procedures (Donald et al., 2011; Finlay et al., 2010), wall growth typically accounts for $<5\%$ of total mesocosm Chl *a* and was not considered further.

2.3 | Bioassay experiments

On the first day of each experiment, a nutrient-limitation bioassay was conducted on $243\mu\text{m}$ screened, depth-integrated water from Wascana Lake, and used to indicate the instantaneous nutrient status of the phytoplankton community (Finlay et al., 2010). Triplicate acid-washed and rinsed borosilicate 250 ml bottles received one of

four treatments; 1 ml of 17.12 g/L NH_4Cl (+N) for a final concentration of 68.48 mg/L, 1 ml of 4.352 g/L K_2HPO_4 (+P) for a final concentration of 17.41 mg/L, both nutrients (+NP), or no addition (-NP controls). Bottles were incubated for 72 hr in a growth chamber at ambient seasonal lake temperatures, a fixed light: dark regime (12 hr: 12 hr), and an irradiance equivalent to that observed at Secchi depth (Finlay et al., 2010). After the incubation period, particulate organic matter was filtered onto GF/C filters and processed for Chl *a* concentration using spectrophotometric methods. Additional microcosm experiments were conducted in 2017 using these protocols to compare the effects of urea with NH_4^+ at equivalent concentrations of N (Supporting Information).

2.4 | Laboratory analyses

All water chemistry analyses were conducted on depth-integrated water that had been filtered through a 0.45 μm pore membrane filter. DIC and DOC were analysed using a Shimadzu Scientific Instruments Inc. (Kyoto, Japan) 5000A total carbon analyser following Finlay et al. (2009). Nitrate and nitrite (hereafter as NO_3^-), NH_4^+ , TDN, SRP, and TDP were analysed using a QuikChem 8500 FIA automated ion analyser (Lachat Instruments, Milwaukee, WI, USA) following standards methods (APHA-AWWA/WEF 1998). Dissolved urea concentrations were measured by the diacetyl monoxime method of Revilla, Alexander, and Glibert (2005), as modified by Bogard et al. (2012).

Phytoplankton abundance as Chl *a* ($\mu\text{g/L}$) was determined using standard trichromatic spectrophotometric methods (Jeffrey & Humphrey, 1975), while phytoplankton community composition (as relative % pigment abundance) was quantified using high-performance liquid chromatography (HPLC) on an Agilent Technologies Inc. (Santa Clara, CA, USA) model 1100 HPLC system (Leavitt & Hodgson, 2001). Briefly, pigments were tentatively identified on the basis of chromatographic position and spectral characteristics in comparison to authentic standards from DHI LAB Products (Hørsholm, Denmark), and an internal reference standard of 3.2 mg/L of Sudan II (Sigma Chemical Corp, St. Louis, MO, USA). HPLC analyses were restricted to the most abundant biomarker pigments, including fucoxanthin (mainly siliceous algae), alloxanthin (cryptophytes), Chl *b* (chlorophytes), lutein-zeaxanthin (chlorophytes and cyanobacteria), myxoxanthophyll (colonial cyanobacteria), and aphanizophyll (potentially N_2 -fixing cyanobacteria). On this HPLC system, carotenoids from *Aphanizomenon* (aphanizophyll), *Anabaena* (4-keto-myxoxanthophyll) and the Oscillatoriaceae (oscillaxanthin) were incompletely resolved and were presented together as aphanizophyll (Leavitt & Hodgson, 2001). Similarly, structural isomers lutein (chlorophytes) and zeaxanthin (cyanobacteria) were not separable on the HPLC system and were used as an index of bloom-forming taxa (Leavitt et al., 2006; Leavitt & Hodgson, 2001).

Bacterial abundance was measured according to del Giorgio, Bird, Prairie, and Planas (1996). Briefly, bacterioplankton were

stained with the nucleic-acid dye STYO 13 and enumerated using fluorescence at 488 nm on a FACScan (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) flow cytometer equipped with an argon-ion laser.

2.5 | Numerical analyses

Response variables, including total phytoplankton abundance (as trichromatic Chl *a*) and community composition (as HPLC carotenoids) were inspected for normal distribution of residuals using a Shapiro-Wilks test and were $\log_{10}(x + 1)$ transformed as required. Repeated-measures analysis of variance (ANOVA) was used to test for significant differences due to treatment, time, and the interaction of time and treatment on response variables in each experiment (Donald et al., 2011; Finlay et al., 2010). Tests were run on actual length of each experiment (2 or 3 weeks) and on the first 2-weeks only; however, as there were few differences, in results, we present statistics from actual experimental duration. Mauchly's test was used to determine if response variables met the assumption of sphericity. Violations from sphericity were addressed by applying the Greenhouse-Geisser correction when Mauchly's test correction estimate ϵ values were <0.75 , or the Huynh-Fledt correction when test correction estimate ϵ values were above >0.75 (Girden, 1992). Pair-wise comparisons were conducted using Tukey's honestly significant difference post-hoc test. ANOVAs with Tukey's honestly significant difference tests were used to evaluate the differences between treatment responses in each of the short-term bioassay experiments.

3 | RESULTS

3.1 | Lake conditions

Seasonal changes in lake conditions resulted in a wide range of initial conditions for each experiment (Figure S2). For example, surface water temperatures varied in a unimodal fashion (2.6–29.8°C) during the ice-free period (day of year 95–303), while O_2 content varied inversely with temperature, with elevated values in spring (20.0 mg O_2/L) and autumn (11.6 mg O_2/L) and a minimum during June (5.2 mg O_2/L). Overall, mean initial pH generally declined from 9.2 in April to 8.1 in October, whereas DIC concentrations increased from spring (49.1 mg C/L) to a peak in June (63.6 mg C/L) before declining to intermediate values thereafter. Water transparency (as Secchi depth) displayed patterns typical of eutrophic lakes (Lathrop, 2007), with a minimum of 0.25 m in April and May followed by a maximum of 1.50 m during the June clearwater phase, and slowly declining transparency during summer (0.55 m in September) before increasing to 0.83 m in October. Overall, specific conductivity increased from $\sim 1,200 \mu\text{S/cm}$ in spring to a plateau of $\sim 1,600 \mu\text{S/cm}$ from July onwards. Although DOC concentrations also increased during summer, the most rapid changes occurred following the clearwater

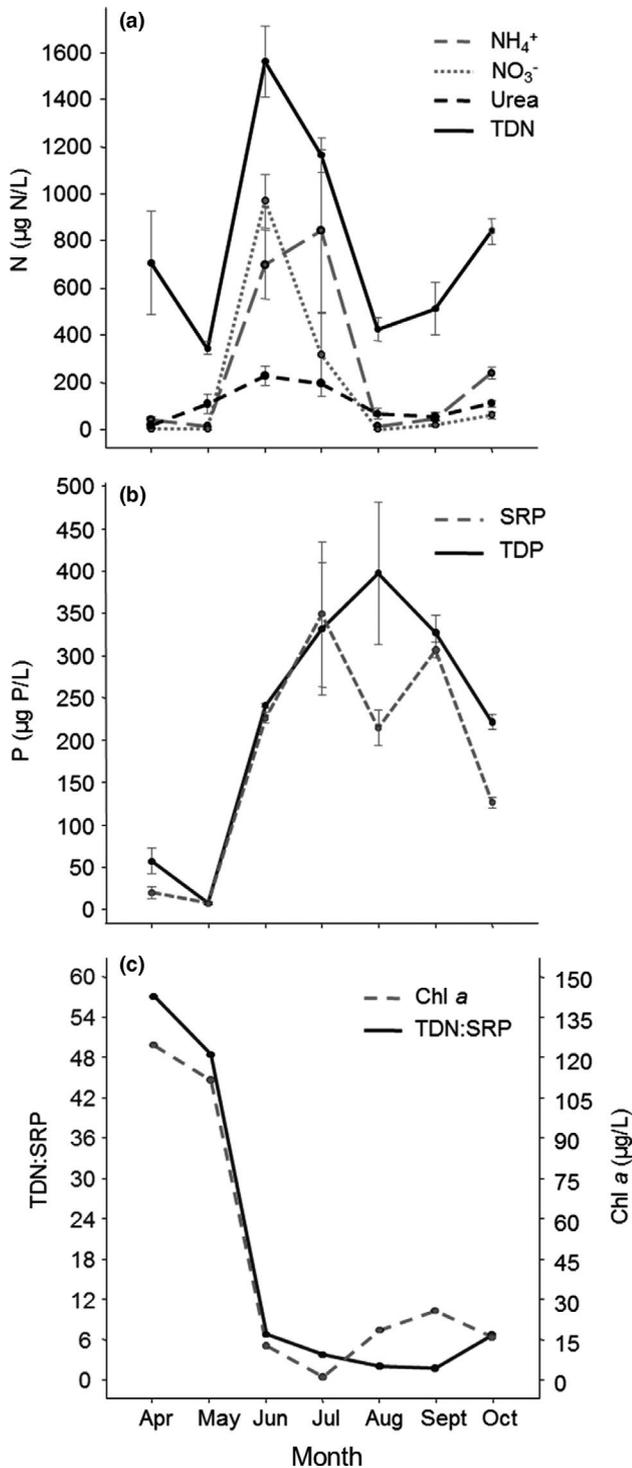


FIGURE 1 Seasonal limnological conditions in Wascana Lake during the open-water period of 2012. Panels show (a) dissolved nitrogen species, including total dissolved nitrogen (TDN), ammonium (NH_4^+), nitrate (NO_3^-) and urea (all $\mu\text{g N/L}$), (b) total dissolved (TDP) and soluble reactive phosphorus (SRP) (both $\mu\text{g P/L}$), and (c) TDN:SRP and Chlorophyll *a* (Chl *a*; $\mu\text{g/L}$). Triplicate values represent mean \pm standard error.

phase, when values increased from ~ 23 mg C/L in early summer to ~ 35 mg C/L thereafter.

Nutrient concentrations varied more than 10-fold during the open-water season (Figure 1). Generally, concentrations of all

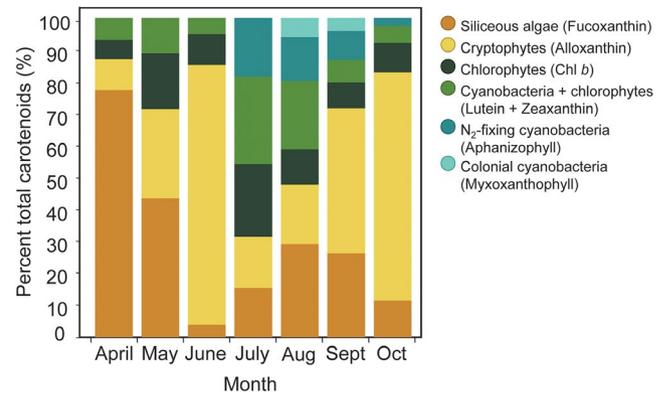


FIGURE 2 Seasonal community composition in Wascana Lake during 2012, expressed as relative (%) abundance of pigment biomarkers measured using high-performance liquid chromatography. [Colour figure can be viewed at wileyonlinelibrary.com]

dissolved forms of N were lower in spring and late summer and exhibited peaks during June and July (Figure 1a). Concentrations of biologically available N, including urea ($19\text{--}107$ $\mu\text{g N/L}$), NH_4^+ ($13\text{--}46$ $\mu\text{g N/L}$), and NO_3^- ($1\text{--}61$ $\mu\text{g N/L}$), were lowest in spring and autumn when together they made up $<30\%$ of the TDN pool, but accounted for most of the TDN during June and July (urea = 227 ± 41 $\mu\text{g N/L}$, NH_4^+ = 841 ± 345 $\mu\text{g N/L}$, NO_3^- = 969 ± 113 $\mu\text{g N/L}$). In contrast, average concentrations of TDP and SRP were lowest in spring (TDP: 32 ± 13 $\mu\text{g P/L}$; SRP: 13 ± 4 $\mu\text{g P/L}$), and rose to maxima during July–September (TDP: 397 ± 67 ; SRP: 349 ± 85) $\mu\text{g P/L}$ (Figure 1b). SRP comprised only 35% of TDP in spring, but rose to nearly 100% of dissolved P during most of the summer, despite concentrations of Chl *a* characteristic of eutrophic waters (Figure 1c). Seasonal changes in N and P concentrations resulted in a sharp decline in mass ratios of TDN:SRP (Figure 1c) from elevated values characteristic of P-limited systems in spring (57.1 ± 21.9) to low ratios representative of N-limited conditions during summer and autumn (1.7 ± 0.7).

In general, seasonal variation in total phytoplankton abundance as Chl *a* followed the annual trajectory known from other eutrophic lakes (Dröscher, Patoine, Finlay, & Leavitt, 2009; Lathrop, 2007), with a spring maximum (125 $\mu\text{g/L}$) followed by a minimum (~ 1.2 $\mu\text{g/L}$) during the clearwater phase in June, before rising to late-summer values of ~ 30 $\mu\text{g/L}$ (Figure 1c). Changes in Chl *a* were correlated positively to TDN:SRP ($r^2 = 0.87$, $p < 0.001$), negatively with concentrations of SRP ($r^2 = 0.62$, $p < 0.01$) and TDP ($r^2 = 0.62$, $p < 0.01$), and weakly with alterations in individual N compounds (i.e. NH_4^+ , urea; $r^2 = 0.24\text{--}0.25$, $p < 0.01$; NO_3^- : $r^2 = 0.14$, $p = 0.10$).

High-performance liquid chromatography analysis of biomarker pigments demonstrated that the phytoplankton community was composed mainly of siliceous algae (as fucoxanthin) and cryptophytes (as alloxanthin) in April, with increasing relative abundance of cryptophytes during May and June as that of diatoms declined (Figure 2). Community composition shifted again after the June

clearwater phase, with increased abundance of N_2 -fixing cyanobacteria (as aphanizopyll) and chlorophytes (as Chl *b*) in July giving way to non-diazotrophic cyanobacteria (as myxoxanthophyll) in August and September. Autumn communities were characterised by increasing relative abundances of cryptophytes. In contrast, dinoflagellates were rarely observed during 2012.

3.2 | Bioassay experiments

Short-term bioassay experiments revealed that phytoplankton growth was strongly and significantly suppressed ($p < 0.05$) by fertilisation with NH_4^+ relative to unamended control treatments during April to July (Figure 3). In contrast, the P treatment had no significant effect on phytoplankton growth during the open-water period. Increases in Chl *a* concentration in response to added N were only modest in August (+NP) and October (+ NH_4^+) but were not statistically significant at $p < 0.05$. In contrast, no toxic effect of urea was recorded in microcosm experiments during spring 2017, in contrast to phytoplankton suppression by NH_4^+ (Supporting Information Figure S1).

3.3 | Effects of nutrient amendments on mesocosm environments

Effects of fertilisation on the chemical environment of mesocosms were similar to those seen in prior experiments (Bogard et al., 2017, 2020; Donald et al., 2011; Finlay et al., 2010) and are presented in detail in

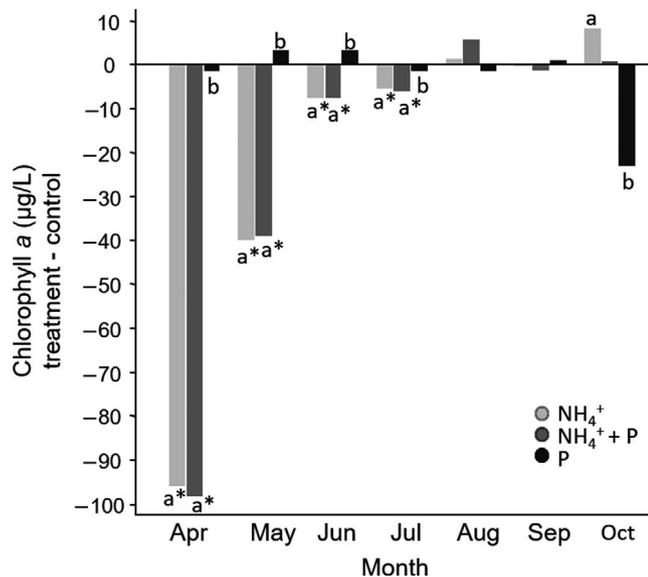


FIGURE 3 Phytoplankton response (as $Chl\ a_{treatment} - Chl\ a_{control}$) of 243 μm screened water and natural assemblages from Wascana Lake fertilised with NH_4^+ . Bioassays were conducted on the initial date of each mesocosm experiment. Letters indicate treatments that were significantly different from each other ($p < 0.05$), and asterisks mark treatments that were significantly different from control treatments ($p < 0.05$), as determined by ANOVA and Tukey's honestly significant difference post-hoc test.

Supporting Information (Figures S2 and S3). Briefly, urea was elevated in amended trials but did not accumulate in mesocosms. Instead, urea was partly converted to NH_4^+ , especially by the end of trials conducted during warm summer months. There were few changes in nutrient contents of reference mesocosms, nor those fertilised with P alone, aside from modest changes in SRP content in amended trials later in summer (Figure S3). In general, DIC concentrations declined, whereas those of DOC increased, in urea-fertilised mesocosms alone (Figure S2).

3.4 | Effects of nutrients on phytoplankton abundance and community composition

Urea amendments did not suppress phytoplankton growth in mesocosm experiments during cool-water intervals (Figure 4, Figure S4). Instead, fertilisation with urea stimulated phytoplankton abundance up to 400% relative to that in control mesocosms in all months between April and September when either added alone or in concert with P. Repeated-measures ANOVAs (Table 2) revealed that Chl *a* abundance was significantly higher ($p < 0.05$) in urea-amended treatments than -UP control trials during April, May, June, and September experiments. Although not statistically significant, Chl *a* concentration was also elevated in +UP and +U treatments relative to either the control or +P treatments during July and August experiments (Figures 4 and 5). In contrast, there were no significant effects ($p > 0.05$) on Chl *a* due to fertilisation with P alone in any of the seven experiments (Figure 4). Neither added N nor P had a significant effect on total phytoplankton abundance during October (Table 2).

Analysis of taxonomically diagnostic pigments using HPLC revealed that the effect of nutrient amendment varied across season and phytoplankton group. For example, +UP treatments resulted in significantly higher abundances of cryptophytes ($p < 0.01$) and chlorophytes ($p < 0.01$) during April experiments (Table 2, Figure 4), mainly at the expense of diatoms and siliceous algae (Figure 6). During May, chlorophyte abundance ($p < 0.01$) was elevated by fertilisation with +U or +UP, siliceous algae ($p = 0.04$) increased in the +U treatments (but not +P or +UP), and chlorophytes and cyanobacteria together were significantly higher in +UP treatments than in +P treatments ($p < 0.05$). In contrast, although communities in urea-amended treatments comprised higher relative abundances of chlorophytes, colonial cyanobacteria, and cryptophytes, and reduced relative abundances of N_2 -fixing cyanobacteria than controls (Figure 6), the concentrations of individual pigment biomarkers were not significantly different between treatments in June, July, or August ($p > 0.05$, Table 2). During September, fertilisation with +U and +UP resulted in significant ($p < 0.05$) increases in abundances of all major taxonomic groups relative to +P and control treatments (Table 2), with chlorophytes and colonial cyanobacteria again increasing at the expense of N_2 -fixing cyanobacteria (Figure 6; $p < 0.01$; Table 2). Finally, experiments in October revealed no notable differences ($p > 0.05$) in phytoplankton abundance (Figure 4) or gross community composition (Figure 6) among nutrient treatments (Table 2) when irradiance was low and waters were cool (Figure S4).

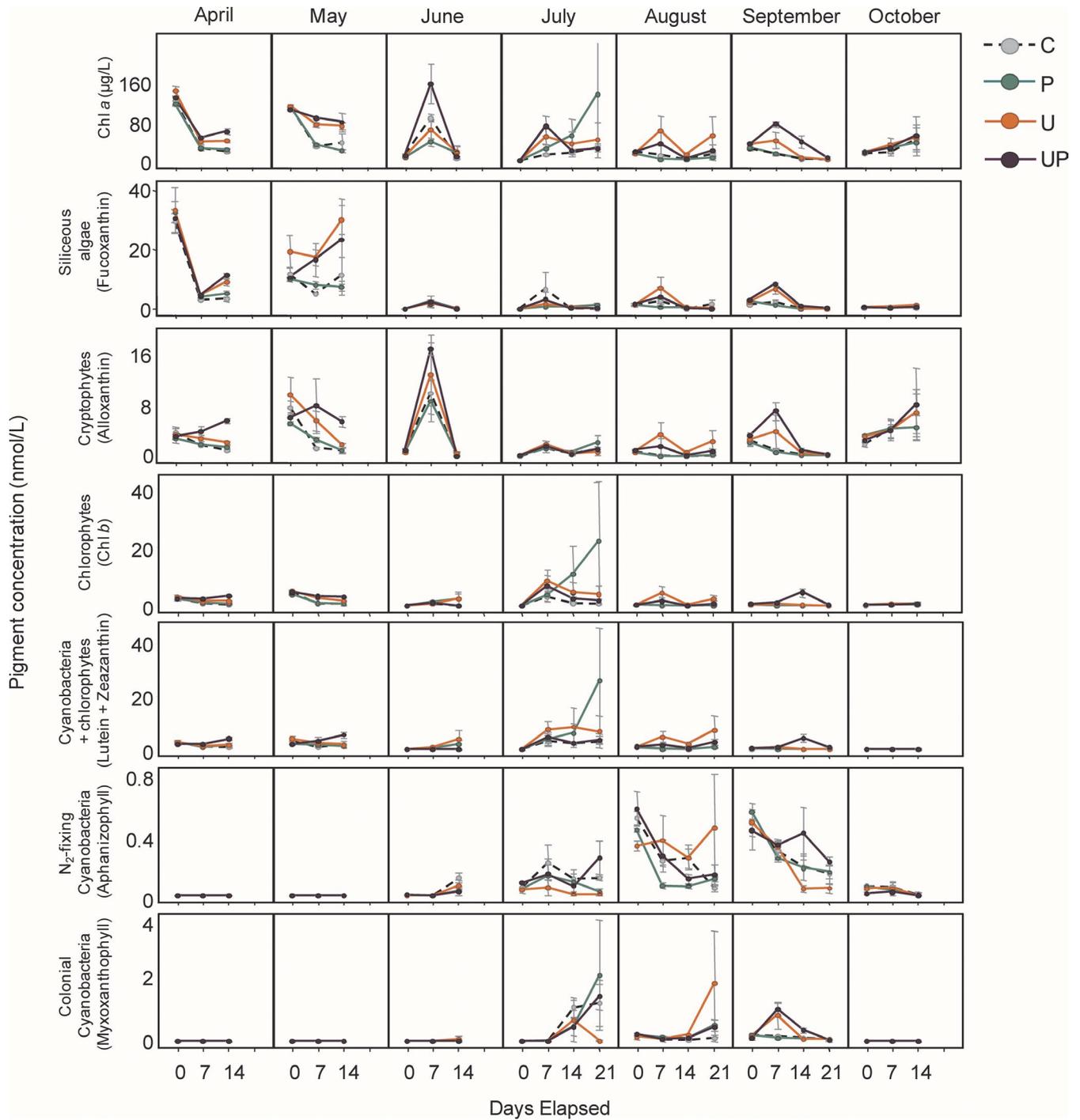


FIGURE 4 Time series of biomarker pigment (chlorophyll and carotenoids) concentrations in each experiment between April and October measured by high-performance liquid chromatography (nmoles pigment/L) or trichromatic methods (Chl *a*; $\mu\text{g/L}$). From top to bottom, panels represent total algal abundance (Chl *a*), siliceous algae (fucoxanthin), cryptophytes (alloxanthin), chlorophytes (Chl *b*), chlorophytes and cyanobacteria (lutein + zeaxanthin), potentially N_2 -fixing cyanobacteria (aphanizophyll), and colonial cyanobacteria (myxoxanthophyll). Points represent means of triplicate mesocosms, with error bars denoting ± 1 SE, for the control (grey line), phosphorus only (+P; green line), urea only (+U; orange line), and urea plus P (+UP; purple line) amended treatments.

4 | DISCUSSION

Analysis of seasonal mesocosms demonstrated that fertilisation of P-rich lakes with moderate concentrations of urea, either alone or with P, increased total phytoplankton abundance in most

open-water months. Specifically, addition of environmentally relevant concentrations of urea to mesocosms in hypereutrophic Wascana Lake increased total phytoplankton abundance by up to 400% relative to values in -UP controls, both alone and in combination with added P. However, unlike in previous years (Bogard et al., 2017, 2020; Donald et al., 2011, 2013; Finlay et al., 2010), the

TABLE 2 Repeated-measures ANOVA statistics for treatment effects and their interaction with time on Chl *a* and phytoplankton community composition.

	April		May		June		July		August		September		October	
	<i>p</i>	Post hoc	<i>p</i>	Post hoc	<i>p</i>	Post hoc	<i>p</i>	Post hoc	<i>p</i>	Post hoc	<i>p</i>	Post hoc	<i>p</i>	Post hoc
Chl <i>a</i>														
Treatment	0.01	c, p < u, up	< 0.01	p, c < c, u < u, up	0.02	p, u, c < c, up	0.74	-	0.13	-	< 0.01	c, p, u < u, up	0.97	-
Interaction	0.15	-	0.01	-	0.04	-	0.07	-	0.52	-	0.07	-	0.97	-
Siliceous algae														
Treatment	0.51	-	0.04	c, p, up < p, up, u	0.99	-	0.98	-	0.23	-	< 0.01	p, c < u, up	0.69	-
Interaction	0.66	-	0.54	-	0.94	-	0.60	-	0.41	-	< 0.01	-	0.74	-
Cryptophytes														
Treatment	< 0.01	c, p, u < u, up	0.12	-	0.24	-	0.94	-	0.09	-	< 0.01	c, p, u < u, up	0.98	-
Interaction	< 0.01	-	0.17	-	0.55	-	0.38	-	0.37	-	0.01	-	0.87	-
Chlorophytes														
Treatment	< 0.01	c, p, u < up	< 0.00	c, p < u, up	0.64	-	0.65	-	0.11	-	< 0.00	c, p, u < up	0.82	-
Interaction	< 0.01	-	< 0.00	-	0.52	-	0.35	-	0.26	-	< 0.00	-	0.98	-
Chlorophytes + cyanobacteria														
Treatment	0.09	-	< 0.05	p, c, u < c, u, up	0.52	-	0.83	-	0.08	-	< 0.01	c, p, u < up	0.64	-
Interaction	< 0.01	-	0.05	-	0.06	-	0.44	-	0.44	-	< 0.01	-	0.58	-
N₂ fixing cyanobacteria														
Treatment	-	-	-	-	0.25	-	0.38	-	0.37	-	< 0.01	u, c, p < c, p, up	0.26	-
Interaction	-	-	-	-	0.23	-	0.79	-	0.16	-	0.36	-	0.89	-
Colonial cyanobacteria														
Treatment	-	-	-	-	0.43	-	0.60	-	0.40	-	0.01	c, p, u < up	-	-
Interaction	-	-	-	-	0.43	-	0.34	-	0.65	-	< 0.02	-	-	-

Note: Probability (*p*) values were calculated for treatment and treatment-time effect, and are indicated in bold if significant. Results of post-hoc Tukey's honestly significant difference tests are shown with mean treatment values ordered from smallest to largest, and significant differences (<) at = 0.05 for controls (c), phosphorus (p), urea (u), and urea + phosphorus (up) treatments. Treatments that appear on both sides of the (<) were not significantly different from the treatments on either side.

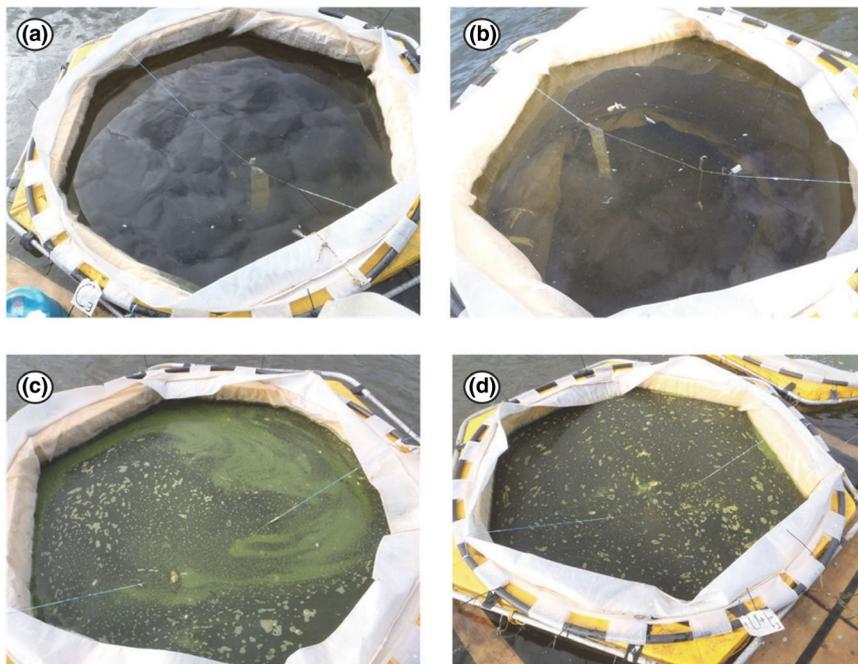


FIGURE 5 Mesocosm enclosures on day 21 of the August experiment, with unfertilised controls (a), phosphorus-amended (b), urea-fertilised (c), and dual-fertilised (d) treatment. Note obvious effect of urea despite lack of statistical significance on this date ($p \sim 0.1$). [Colour figure can be viewed at wileyonlinelibrary.com]

most substantial effects were recorded in April and May rather than during late summer, despite only modest excesses of SRP and elevated TDN:SRP mass ratios characteristic of P-limited ecosystems (Hecky & Kilham, 1988). Conversely, substantial amendment with P alone ($100 \mu\text{g P L}^{-1} \text{ week}^{-1}$) had no significant effect on phytoplankton abundance or composition at any time during the open-water season, consistent with ambient SRP $> 200 \mu\text{g P/L}$ after June and in other years (Donald et al., 2011; McGowan, Leavitt, et al., 2005; Vogt, Rusak, Patoine, & Leavitt, 2011). Comparison of mesocosm and bioassay findings suggested that urea lacked the toxic effects of NH_4^+ observed in cool waters (Glibert et al., 2016; Swarbrick et al., 2019), in part because of limited hydrolysis of urea during spring and late autumn. Overall, growth stimulation by urea was consistent with previous short-term laboratory (Belisle et al., 2016; Berman & Chava, 1999; Yuan et al., 2012) and mesocosm experiments (Bogard et al., 2020; Donald et al., 2011; Finlay et al., 2010), but showed that phytoplankton growth was strongly enhanced in spring, when urea influx is expected to be elevated (Bogard et al., 2012; Glibert et al., 2006) and temperature-dependent enzymatic hydrolysis to NH_4^+ is low (King et al., 2017; Siuda & Chróst, 2006).

4.1 | Seasonal effects of urea: Spring

Contrary to our initial hypothesis, amendment of spring waters (April, May) with urea alone, or in combination with P, stimulated phytoplankton biomass by up to 4-fold relative to the controls, despite ambient SRP concentrations as low $<15 \mu\text{g P/L}$ and TDN:SRP mass ratios in excess of 45. These findings contrast sharply with empirical and theoretical expectations of nutrient limitation based on research during summer, which predict that effects of N fertilisation should be limited to conditions in which ambient mass ratios of N:P are below

~ 20 (Donald et al., 2011; Downing & McCauley, 1992; Smith, 1983). Instead, our findings suggest that threshold for N effects on phytoplankton growth may be mediated by differences in nutrient acquisition strategies of spring taxa (Hecky & Kilham, 1988; Sterner & Elser, 2002), as well as by vernal limnological conditions (high terrestrial subsidies of dissolved organic matter, low temperature, no thermal stratification), which together may modify nutrient requirements of spring phytoplankton relative to mid-summer taxa (Hayes et al., 2015; Kolzau et al., 2014; Paelr et al., 2015). In particular, phytoplankton growth rates may be lower in cool, spring waters, leading to lower P demands relative to those of N, and potentially higher requirements for N (Elrifi & Turpin, 1985; Sterner & Elser, 2002).

Increased abundance of total phytoplankton in response to urea amendment in spring may also reflect the physiological characteristics of the predominant cryptophyte, diatom and chlorophyte algae. For example, these taxa exhibit mixotrophy under P-limited conditions (Burkholder, Glibert, & Skelton, 2008; Sanders, Porter, & Caron, 1990; Sparber et al., 2015). In addition, cryptophytes grow preferentially on chemically reduced N (Antia & Chorney, 1968; Watson & McCauley, 2005), have particularly high rates of urea uptake (Berg et al., 2003; Solomon et al., 2010), and are more competitive under high NH_4^+ : low SRP conditions (Gervais, 1998; Glibert, Wilkerson, et al., 2014). Similarly, the moderate positive response of sub-dominant chlorophytes to added urea may reflect their diverse N uptake strategies (Fernandez & Galvan, 2007; Swarbrick et al., 2019) and rapid growth rates, which enable them to outcompete other taxa for nutrients when light is sufficient (Jensen, Jeppesen, Olrik, & Kristensen, 1994). Although further research is needed to identify the precise mechanisms underlying the pronounced stimulation of diatoms, cryptophytes, and chlorophytes by fertilisation with urea during spring, these data suggest that phytoplankton communities can be susceptible to urea pollution even when biogeochemical parameters

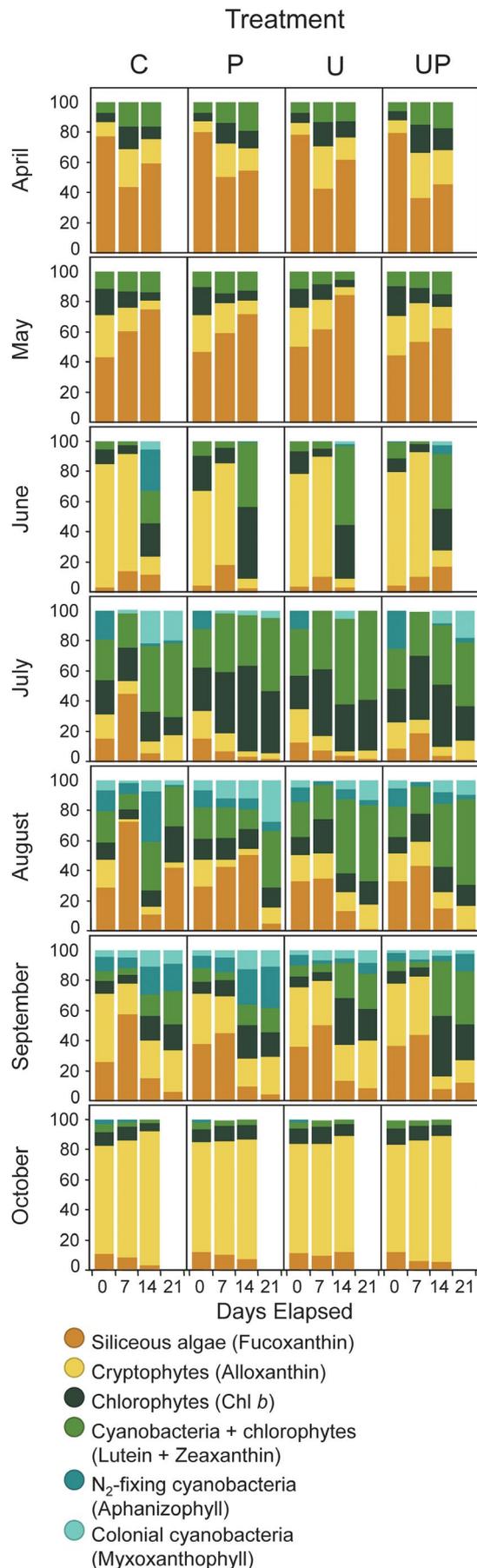


FIGURE 6 Relative abundance of major phytoplankton groups within each mesocosm experiment as determined by high-performance liquid chromatographic analyses of biomarker pigments (all nmol pigment/L). Bars within each plot represent the average relative pigment abundances from three replicate mesocosm treatments, with unfertilised controls (C), phosphorus-amended (+P), urea-fertilised (+U), and dual-amended (+UP) treatments. [Colour figure can be viewed at wileyonlinelibrary.com]

(i.e. low SRP content, elevated N:P ratios) would predict P-limited growth.

4.2 | Seasonal effects of urea: Summer

As originally hypothesised, and seen elsewhere (Barica, Kling, & Gibson, 1980; Donald et al., 2011; Harris et al., 2016; Hayes et al., 2015), fertilisation of warm, P-rich waters with N as urea increased phytoplankton abundance 3.5- to 5.0-fold, particularly in early September. In addition, urea fertilisation promoted replacement of N₂-fixing cyanobacteria by chlorophytes and non-heterocysteous cyanobacteria, consistent with physiological and genetic mechanisms in which N assimilation suppresses heterocyst formation (Flores & Herrero, 2005; Solomon et al., 2010). Such low-light adapted non-diazotrophic cyanobacteria (e.g. *Planktothrix* spp., *Limnothrix* spp.) are common in these and other warm, hypereutrophic systems (Huisman et al., 2004; McGowan, Patoine, Graham, & Leavitt, 2005; Patoine et al., 2006) and are enhanced by influx of chemically reduced N under conditions of low N:P and elevated SRP in this (Donald et al., 2013; Finlay et al., 2010) and other aquatic systems (Berman & Chava, 1999; Scheffer, Rinaldi, & Mur, 1997; de Tezanos Pinto & Litchman, 2010). As these Oscillatoriaceae often produce the potent toxin microcystin in response to N fertilisation (Donald et al., 2011; Finlay et al., 2010), selective stimulation of these taxa during summer poses additional challenges for water-quality management (Paerl & Otten, 2013).

4.3 | Seasonal effects of urea: Autumn

Addition of urea had no effect on phytoplankton abundance or community composition during October, despite low mass ratios of dissolved N:P. This finding is consistent with expectations derived from the Plankton Ecology Group model paradigm in which nutrient limitation during summer gives way to physical control of primary production during autumn (Sommer et al., 2012; Sommer, Gliwicz, Lampert, & Duncan, 1986). For example, while ambient nutrient concentrations remained characteristic of an N-limited, hypereutrophic lake, mean temperatures declined from $13.1 \pm 0.07^\circ\text{C}$ in September to $3.6 \pm 0.1^\circ\text{C}$ in October, well below optimal conditions for most phytoplankton (Carey, Ibelings, Hoffman, Hamilton, & Brookes, 2012; Litchman, de Tezanos Pinto, Klausmeier, Thomas, & Yoshiyama, 2010; Lürling, Eshetu, Faassen, Kosten, & Hurszar, 2013). Irradiance also declined substantially in October due to

a combination of elevated concentrations of DOC (~ 40 mg C/L), greatly reduced illuminance, a 3-hr reduction in day length relative to that in September, and the formation of ice on mesocosms (~1 cm by end of experiment). As noted by Sommer et al. (2012), declines in autumnal irradiance to values below those required for most phototrophic taxa (Deblois, Marchand, & Juneau, 2013; Litchman & Klausmeier, 2001; Schwaderer et al., 2011) signals the end of the growing season.

4.4 | Differential effects of urea and ammonium

Comparison of mesocosm and bioassay experiments suggests that urea and NH_4^+ can have divergent seasonal effects on hyper-trophic systems and that urea may lack the inherent toxicity of NH_4^+ under cold, spring conditions (Collos & Harrison, 2014; Glibert et al., 2016; Swarbrick et al., 2019). Specifically, fertilisation with urea stimulated phytoplankton growth during April and May and in spring microcosms in 2017, whereas NH_4^+ substantially suppressed rates of Chl *a* accumulation over the same intervals in which cryptophytes and diatoms predominate. Originally, we expected urea supplements to suppress phytoplankton growth during spring because extracellular ureases are common in lake water and hydrolyse urea to NH_4^+ (Dyrman & Anderson, 2003; Mobley & Hausinger, 1989; Solomon et al., 2010) and the assemblage is composed mainly of NH_4^+ -sensitive taxa. Recent research shows that NH_4^+ suppresses growth of diatoms (and possibly cryptophytes) at elevated concentrations by inhibiting DNR and favouring accumulation of photo-oxidative damage during cool, illuminated conditions (Glibert et al., 2016; Swarbrick et al., 2019). Instead, we found little evidence of urea hydrolysis during spring and note instead that diatoms grown on urea can up-regulate nitrate reductase activity, potentially maintaining the photo-quenching capabilities of DNR pathways (Hildebrand & Dahlin, 2000). Although further study is required to validate this hypothesis, our results reinforce recommendations that lake managers should consider not only the timing and magnitude of N pollution, but also the chemical form of N (Newell et al., 2019) when setting policy to protect water quality in P-rich lakes.

4.5 | Absence of P effects

P-amendment failed to significantly stimulate phytoplankton abundance relative to the controls at any time between April and October, indicating that ambient concentrations (~15–350 $\mu\text{g P/L}$) within the lake were sufficient to meet growth demands of phytoplankton. We infer that the accumulation of SRP in the water column is derived from internal sedimentary sources (Allan, Williams, Josh, & Warwick, 1980; Nürnberg & LaZerte, 2016) because >75% of total annual runoff and associated influx of allochthonous P (Cade-Menun et al., 2013) occurs during a 3-week snowmelt period in the spring (Pham et al., 2009; Pomeroy et al., 2007), while dieback of macrophyte populations and associated P release occurs mainly

in August after SRP levels have peaked (McGowan, Leavitt, et al., 2005). Overall, the limited response of phytoplankton to added P is consistent with persistently high SRP concentrations after May and the lack of positive correlation between SRP and Chl *a* in much of the Northern Great Plains (Allan, 1980). More surprising is the observation that addition of 100 $\mu\text{g P L}^{-1} \text{ week}^{-1}$ had little effect during spring, when SRP content was relatively low (~ 15 $\mu\text{g P/L}$), mass ratios of dissolved N:P were high (57.1 ± 21.9), and vernal phytoplankton were abundant. As noted above, we speculate that response of diatoms and cryptophytes to added P may have been limited by a combination of high physical turbidity (lake Secchi < 20 cm), sub-optimal temperatures (<10°C), and mixotrophic growth of algae on added urea.

4.6 | Limitations of the study design

Despite the widespread use of mesocosm experiments to identify nutrient limitation (Abell, Oezkundakei, & Hamilton, 2010; Elser et al., 2007; Spivak, Vanni, & Mette, 2011), debate remains over the utility of mesocosm and bioassay experiments to generalise findings to larger temporal and spatial scales (Carpenter, 1996; Paerl et al., 2016; Schindler, 1998; Scott & McCarthy, 2010). Previous studies within this system (Bogard et al., 2017, 2020; Donald et al., 2013; Finlay et al., 2010) have provided detailed rationale for the use of a constant experimental design (3,140-L closed-bottom mesocosms) and its value for preliminary investigations of the effects of urea on pelagic freshwater ecosystems. In principle, the shortcomings of smaller-scale experiments could be addressed by a whole-lake nutrient amendment; however, urea is included on the Canadian Domestic Substance list (Government of Canada, 2020) and is prohibited from being purposefully dispersed into surface waters. Instead, we note the high degree of consistency between our findings and those of similar N amendment experiments conducted in different years, as well as other large enclosure experiments (Barica et al., 1980; Levine & Schindler, 1999), mass-balance studies (Bunting et al., 2007; Leavitt et al., 2006), and whole-ecosystem studies (Barica et al., 1980; Lathrop, 1988). Together, these diverse experiments provide strong evidence of pronounced degradation of seasonal water quality by urea pollution of P-rich prairie lakes.

4.7 | Implications for water-quality management

This study shows that urea can alter water quality and phytoplankton production in freshwater ecosystems even during cool, spring conditions. Currently, North American regulations and guidelines on point source pollution with N are generally restricted to NH_4^+ , NO_3^- , and TN (e.g. CCME, 2010; United States Environmental Protection Agency, 2013; Canada Wastewater Systems Effluent Regulations SOR/2012-139, 2015). Based on the results herein, we suggest that government agencies also establish seasonal effluent limits for urea, and that consideration of previously identified

thresholds for N effects (e.g. TDN:SRP < 20 and SRP > 50 µg P/L) (Donald et al., 2013) will be most effective in preventing additional water-quality degradation during late summer effluent releases. However, given the results of our spring experiments, we also recommend that additional research, including whole-embayment or whole-lake experiments, be conducted to better establish the thresholds for N effects in spring and autumn periods, which coincide with agricultural fertiliser applications and seasonal wastewater lagoon releases (CCME, 2006). In addition to limiting point source urea pollution, we recommend that resource managers also work towards improved control of non-point source urea pollution, such as through outreach and education (Newell et al., 2019), incentivised catchment management programmes for land owners, and development of policy and regulatory tools to minimise export of urea from agricultural activities. This is particularly relevant to the Northern Great Plains, a region where urea makes up more than 50% of all nitrogenous fertiliser on farms (Glibert et al., 2006), the majority of small communities use wastewater lagoons to treat domestic wastewater (CCME, 2006; Holeton, Chambers, & Grace, 2011), and lakes and wetlands often exhibit elevated concentrations of SRP (Allan, 1980). Finally, we suggest that future research is needed on the sources, transport, and transformation of urea in freshwater ecosystems (Belisle et al., 2016; King et al., 2017; Lee et al., 2016) to better assess the integrated risk of urea pollution arising from agriculture intensification and urban expansion.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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