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Eutrophication mediates rapid clonal evolution in *Daphnia pulicaria*

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Abstract

1. Laboratory studies have revealed that *Daphnia* species can evolve to tolerate toxic cyanobacteria in the diet. Specifically, *Daphnia* from eutrophic lakes where cyanobacteria are common tend to have higher growth rates and survival when fed toxic cyanobacteria than populations from oligotrophic environments with low abundance of cyanobacteria.
2. We conducted an in-lake mesocosm (i.e. limnocorral) experiment during the autumn of 2009 to assess the effects of nutrient enrichment on clonal evolution in *Daphnia pulicaria*. As nutrient enrichment often favours grazing-resistant cyanobacteria, we hypothesised that fertilisation would influence the genotypic composition of *D. pulicaria* that vary in tolerance to cyanobacteria. Mesocosms were fertilised to manipulate phytoplankton and cyanobacterial abundance and concentrations of a cyanobacterial toxin (microcystin). Thus, half of the mesocosms were high-nutrient and half were low-nutrient. We then stocked half of the mesocosms with a mixture of six genetically-distinct *D. pulicaria* genotypes (three genotypes from oligotrophic lakes and three from eutrophic lakes) leaving half of the mesocosms *Daphnia*-free to assess grazing effects, using a fully factorial design.
3. When compared to the low nutrient treatment, high nutrient mesocosms had nearly five-fold higher chlorophyll *a* concentrations, eight-fold higher cyanobacterial dry biomass, and three-fold higher microcystin levels at the start of the experiment. In contrast, low nutrient mesocosms had phytoplankton concentrations typical of mesotrophic lakes.
4. Fertilisation strongly affected *Daphnia* genetic diversity in the mesocosms. Final *Daphnia* genotype diversity in the mesocosms with low-cyanobacteria (richness = 5.83, Shannon–Weiner index = 1.55, evenness = 0.88) was similar to the initial stocked diversity (richness = 5.50, Shannon–Weiner index = 1.48, evenness = 0.87). In contrast, final diversity in fertilised mesocosms with high cyanobacteria was greatly reduced (richness = 2, Shannon–Weiner index = 0.17), with one *Daphnia* genotype that originated from the most-eutrophic lake being highly dominant (evenness = 0.25). Thus, eutrophication mediated strong clonal selection of a cyanobacteria-tolerant *Daphnia* genotype over just 10 weeks.

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5. By the end of the experiment, *Daphnia* significantly reduced phytoplankton biomass in the high-nutrient, but not in the low-nutrient treatment. This difference in effect size was largely driven by the five-fold higher initial phytoplankton biomass in the high-nutrient treatment. Thus, the ability of *Daphnia* to reduce phytoplankton biomass in eutrophic lakes may be driven more so by the abundance of planktivorous fishes, as opposed to the prevalence of cyanobacteria and their associated toxins.

KEYWORDS
cyanobacteria, ecological stoichiometry, *Microcystis*, nitrogen, phosphorus, phytoplankton

1 | INTRODUCTION

A growing body of evidence indicates that trait variation can have important consequences for ecological interactions (Chislock, Sarnelle, Olsen, Doster, & Wilson, 2013; Post, Palkovacs, Schielke, & Dodson, 2008; Thompson et al., 2001; Whitham et al., 2006). Phenotypic expression, plasticity, and inducible defences can all be important in mediating species interactions (Akbar et al., 2017; Des Roches et al., 2018; Jeyasingh, Weider, & Sterner, 2009; Tollrian & Harvell, 1999; Turner & Mittelbach, 1990; Werner & Peacor, 2003). Although trait variation within keystone and foundation species is expected to have large effects on ecological interactions (Hughes, Inouye, Johnson, Underwood, & Vellend, 2008; Hughes & Stachowicz, 2004; Miner, De Meester, Pfrender, Lampert, & Hairston, 2012; Post et al., 2008; Whitham et al., 2006), there are relatively few field experiments that examine the role of intraspecific trait variation in freshwater environments (Bassar et al., 2010; Palkovacs & Post, 2009). Environmental factors can strongly influence the presence and importance of species, including the evolution of trait frequencies (Darwin, 1859), yet our knowledge is limited regarding the consequences of ecological–evolutionary interactions on ecosystem function (Alexander, Vonlanthen, & Seehausen, 2016; Rudman, Kreitzman, Chan, & Schluter, 2017).

Nutrient enrichment of freshwater ecosystems leads to a simultaneous increase in total phytoplankton biomass, the relative abundance of cyanobacteria, and cell toxin quota (Horst et al., 2014; Lürling, Van Oosterhout, & Faassen, 2017; Paerl & Huisman, 2008; Smith, 1983; Watson, McCauley, & Downing, 1997). In freshwater lakes and ponds, cladocerans within the genus *Daphnia* can have large effects on phytoplankton abundance, transparency, and water quality (Chislock, Sarnelle, Jernigan, & Wilson, 2013; Chislock, Sarnelle, Olsen, et al., 2013; Ger, Hansson, & Lurling, 2014; Ger et al., 2016; Leibold, 1989; Sulcius et al., 2017). Strong trophic cascades are well documented in lakes with *Daphnia* (Leibold, 1989). Furthermore, the magnitude of top-down control is thought to be affected by ecosystem productivity (Carpenter et al., 1995; Sarnelle, 1992). A large body of literature has demonstrated that cyanobacteria and associated cyanotoxins, which are often concomitant with nutrient enrichment of lakes, can have strong negative effects on the survival and reproduction of several zooplankton species (DeMott, Zhang, & Carmichael, 1991; Wilson & Hay, 2007). Furthermore, it is well established that predation rates on zooplankton by fishes can be greatly enhanced in eutrophic lakes, and that the success of biomanipulation in eutrophic lakes depends on the effectiveness of strategies aimed at reducing zooplanktivory (Carpenter et al., 1987; Sarnelle, 1992).

Recent research has shown that populations of *Daphnia* in eutrophic lakes may evolve to tolerate toxic and grazing-resistant cyanobacteria, with tolerant *Daphnia* genotypes showing higher survival and growth rates when fed diets of toxic cyanobacteria (Frisch et al., 2017; Hairston et al., 1999, 2001; Orsini et al., 2013; Sarnelle & Wilson, 2005). It is also well known that many herbivores (including *Daphnia*) often trade-off the ability to exploit high resource levels with the ability to depress resources to low levels (Jeyasingh et al., 2009; Tessier, Leibold, & Tsao, 2000; Tessier & Woodruff, 2002). Numerous recent studies have documented substantial variation within *Daphnia* populations for traits associated with resource use, particularly with respect to nutrient enrichment and surface water management (Chislock, Sarnelle, Jernigan, & Wilson, 2013; Chislock, Sarnelle, Olsen, et al., 2013; Duffy, 2010; Hairston et al., 1999; Sarnelle & Wilson, 2005). Ecological trade-offs associated with food quantity and quality are hypothesised to cause clonal replacement in *Daphnia* populations that favour cyanobacteria-tolerant genotypes in eutrophic systems (Gustafsson & Hansson, 2004; Hairston et al., 1999, 2001; Sarnelle & Wilson, 2005), but experiments to test this hypothesis are lacking and the time scale of clonal replacement is little documented.

In this study, we used a mesocosm (i.e. limnocorral) experiment to explore: (1) the role that fertilisation-mediated shifts in phytoplankton communities have on clonal selection among six *Daphnia* genotypes that vary in their tolerance to cyanobacteria and their associated toxins (Sarnelle & Wilson, 2005; Supporting Information Figure S1); and (2) the ecosystem-level consequences of changes in genotypic and trait variation of a generalist aquatic consumer. We hypothesised that fertilisation-mediated increases in grazing-resistant cyanobacteria would subsequently increase the prevalence of *D. pulicaria* genotypes that are tolerant of cyanobacteria.

2 | METHODS

2.1 | *Daphnia* genotypes

The six *Daphnia* genotypes used in this experiment were descendants of single females isolated in 2004 from each of six small lakes (<0.3 km^2) in southern Michigan (Table 1). Three of the lakes (Lawrence, Sixteen, and Warner) are oligotrophic with few cyanobacteria, whereas the other three lakes (Baker, Wintergreen, MSU Lake 1) are eutrophic and have high cyanobacterial abundance during the summer months (Sarnelle & Wilson, 2005). Notably, there was much greater variation in phosphorus level among the eutrophic lakes than the oligotrophic lakes (Table 1), which is typical (Downing, Watson, & McCauley, 2001; Watson et al., 1997).

All six *Daphnia* genotypes were grown under common-garden conditions to provide animals for stocking the enclosures. Prior to the field experiment, each *Daphnia* genotype was first maintained in the laboratory (25°C, 12 h light: 12 h dark) in 1-L glass beakers filled with autoclaved lake water and fed a nutritious green alga (*Chlorella vulgaris*) grown in a nutrient-rich medium (modified BG-11 medium; Wilson et al., 2005). Each *Daphnia* genotype was then transferred to separate outdoor 160-L tanks filled with 35-µm sieved lake water and supplemented with *Chlorella* as a food source. Clonal populations were grown in the tanks for several weeks before the start of the experiment.

2.2 | Study site

The experiment was conducted at the E.W. Shell Fisheries Research Station at Auburn University, Alabama, in a small, eutrophic reservoir pond (S1). This is a shallow, polymictic pond with a surface area of approximately 8 ha, maximum depth of 3.5 m, and total nitrogen (TN) and total phosphorus (TP) concentrations in the mixed layer averaging about 1,000 µg/L and 150 µg/L, respectively, in the autumn (Boyd & Shelton, 1984; A. E. Wilson and M. F. Chislock unpublished data). Consequently, S1 tends to be nitrogen limited (Chislock, Sharp, & Wilson, 2014). Cyanobacteria begin to dominate phytoplankton communities in S1 during late spring (April), and cyanobacterial blooms typically persist into September (A. E. Wilson and M. F. Chislock unpublished data). Immediately prior to the initiation of the experiment (5 October 2009), chlorophyll *a* in S1 was approximately 30 µg/L, and cyanobacteria comprised <10% of total phytoplankton biomass.

2.3 | Mesocosm experiment

We manipulated nutrient concentrations in 2,500-L, clear polyethylene enclosures (i.e. mesocosms) that were sealed at the bottom, open to the atmosphere at the top, and suspended from a floating platform (EZ-Dock) anchored in the middle of the pond. Twenty-four enclosures were filled on 5 October 2009 by pumping pond water through a 75-µm mesh net to exclude resident *Daphnia*. All enclosures were enriched at 400 µg/L phosphorus added as K_2HPO_4 at the beginning of the experiment. Low nitrogen enclosures received no addition of nitrogen (ambient), while high N enclosures were also enriched with 7,000 µg/L nitrogen added as NH_4Cl (n = 12 enclosures per high N treatment) at the beginning of the experiment. Therefore, the ratio of TN to TP in low N:P enclosures was *c.* 2:1, by mass, and high N:P enclosures had a TN:TP ratio of *c.* 16:1, by mass.

We stocked approximately equal densities of six genetically-distinct (based on microsatellite analysis: see *Daphnia genotyping* section below) *Daphnia* genotypes from oligotrophic and eutrophic lakes (n = 3 genotypes per lake type) into half (n = 12) of the enclosures at a total density of 0.1 per L on 17 October 2009, and the remaining 12 enclosures served as no-*Daphnia* controls. Thus, the experimental design was completely factorial across both N and *Daphnia* treatments (six replicates per treatment). During *Daphnia* addition, we preserved two subsamples of the *Daphnia* inoculum in 95% ethanol for genetic analysis to assess the initial genetic composition of *Daphnia* stocked into each treatment. We sampled all enclosures biweekly-to-monthly beginning on 5 October 2009, and the experiment was concluded on 10 December 2009 (10 weeks).

2.4 | Sample collection

Depth-integrated water samples for chlorophyll *a*, phytoplankton biomass and species composition, and microcystin were collected

TABLE 1 Source lakes and genetic characterisations of the six *Daphnia* genotypes. Three *Daphnia* genotypes were collected from oligotrophic lakes where cyanobacteria were absent (Lawrence, Sixteen, and Warner), and three genotypes were collected from eutrophic lakes with abundant cyanobacteria (Baker, MSU Lake 1, and Wintergreen). Each *Daphnia* genotype was characterised using two microsatellite markers (Dp3 and Dp339; Colbourne et al., 2004), and microsatellite nucleotide lengths were determined for both loci

Lake	Location Lat. N, Long. W	TP (µg/L)	Chl (µg/L)	Dp3 length (bp)	Dp339 length (bp)
Lawrence	42°26′27″, 85°21′03″	8–10	4	282, 291	175, 180
Sixteen	42°33′90″, 85°36′80″	9–12	5	282, 286	175, 180
Warner	42°28′16″, 85°31′30″	12–14	3	282, 282	175, 180
Baker	42°26′27″, 85°21′03″	21–40	9–74	291, 291	175, 180
Wintergreen	42°23′50″, 85°23′07″	50–70	13–25	282, 282	175, 175
MSU Lake 1	42°40′53″, 84°28′57″	170–300	60–250	282, 286	175, 190

TP, total phosphorous; Chl, chlorophyll

from each enclosure with a tube sampler (inside diameter = 51 mm) on each sampling date. We collected depth-integrated samples for *Daphnia* density, biomass, and genotypic composition at the end of the experiment only (10 December 2009) to minimise opportunities for contamination. Zooplankton samples were preserved in 95% aqueous ethanol. Chlorophyll *a* concentrations were measured by extracting phytoplankton collected on Pall A/E filters in 90% ethanol for 24 h in the dark at 4°C followed by measurement with a fluorometer (Sartory & Grobbelaar, 1984). Particulate microcystin concentrations were quantified using enzyme-linked immunosorbent assay (An & Carmichael, 1994) after extraction from 75% aqueous methanol. Phytoplankton species abundance and composition were determined via the inverted microscope technique (Utermöhl, 1958) using water samples preserved in 1% Lugol's solution. Biovolumes for each species were calculated using cell counts and estimates of cell volume based on measurements of cell dimensions. We then converted biovolumes (mm^3/L) to dry biomass (μg/L) assuming a specific gravity of 1 g/cm^3 and a dry biomass: wet biomass ratio of 0.40 (Knoll et al., 2008; Riemann, Simonsen, & Stensgaard, 1989; Sarnelle & Wilson, 2005).

2.5 | *Daphnia* genotyping

We genetically characterised 20–25 randomly selected *Daphnia* individuals from ethanol-preserved macrozooplankton samples for each inoculum subsample and for all *Daphnia* enclosures at the conclusion of the experiment. Prior to genotyping, *Daphnia* were measured and counted at 40× in a Sedgwick-Rafter cell, and *Daphnia* lengths were converted to biomass using a length-weight regression (O. Sarnelle unpublished data). *Daphnia* genotypes for each of the six clones used in the experiment were genetically discriminated using variation in two microsatellite loci (Dp3, Dp339; Colbourne, Robison, Bogart, & Lynch, 2004) that have proven to be highly polymorphic for *Daphnia pulicaria* collected from several of our study lakes in Michigan (A. E. Wilson unpublished data). *Daphnia* from ethanol-preserved samples were rinsed thoroughly with distilled water to remove attached bacteria and phytoplankton. Genomic DNA was extracted by heating individual *Daphnia* to 95°C in 10 μL Lyse-N-Go polymerase chain reaction (PCR) reagent (Pierce Chemical Co., Rockford, IL, U.S.A.). Forward primers were modified with (-29)/IRDye-labelled 19-mer M13 primer sequence in order to visualise PCR products on a Li-Cor 4300 DNA Analyzer (Li-Cor Biosciences, Lincoln, NE, U.S.A.). Amplification of microsatellite alleles was performed using PCR in 12.5 μL volumes (*c.* 40 ng of DNA, 1× buffer [Promega Go Green Colorless Buffer], 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1 pmol M13-labelled forward microsatellite primer, 2 pmol reverse microsatellite primer, 0.5 pmol IRDye-labelled M13 primer, and 0.5 units of *Taq* DNA polymerase). Each locus was separately analysed for each *Daphnia* individual. Polymerase chain reaction used a touchdown protocol under the following conditions: 95°C for 3 min followed by 10 cycles of 94°C for 35 s, 65°C (−1°C/cycle) for 35 s, 72°C for 45 s, followed by an additional 30 cycles with a constant annealing temperature of 55°C with a final extension at 72°C for 10 min. Reactions were stopped with 6 μL stop buffer, diluted as necessary with deionised water, denatured for 3 min at 90°C, and snapped cold before loading on a 6.5% polyacrylamide gel.

2.6 | Statistical analyses

We used analysis of variance (ANOVA) to compare chlorophyll *a*, cyanobacterial dry biomass, relative abundance of dominant phytoplankton taxa, and microcystin concentrations between low- and high-nitrogen treatments immediately prior to the addition of *Daphnia* (16 October 2009). The effects of fertilisation (primarily nitrogen) and *Daphnia* presence on chlorophyll *a* and cyanobacterial dry biomass over time were tested using repeated measures ANOVA (sampling date = repeated measures), and pairwise differences among treatments were assessed with Tukey's test. The effect of fertilisation on *Daphnia* density and biomass at the conclusion of the experiment was tested using ANOVA. Standard diversity metrics, including richness, Shannon–Weaver, and evenness indices, were calculated for the *Daphnia* inoculum (two subsamples) and each fertilisation treatment (six replicates per treatment) at the end of the experiment to determine the effect of fertilisation on *Daphnia* composition via ANOVA followed by Tukey's multiple comparison tests. We also compared the relative abundance of *Daphnia* genotypes using grouping based on their source lakes (i.e. *eutrophic* versus *oligotrophic*) using ANOVA. To compare *Daphnia* genotypic composition at the beginning and conclusion of the experiment for low- and high-nitrogen treatments, we used principle components analysis (PCA). We then used ANOVA of factor score #1 to compare *Daphnia* genotypic composition at the beginning and conclusion of the experiment for low- and high-nitrogen treatments. Relative abundance data for *Daphnia* genotypes were logit-transformed prior to all statistical analyses. In some cases, specific genotypes were absent (e.g. 0%) or only one genotype was present in an enclosure (e.g. 100%). To allow for logit-transformation in these cases, we added or subtracted a small value (1%) where a genotype was absent or the only genotype present, respectively. All other data were checked for normality and homogeneity prior to being transformed, when necessary. All non-proportional data (e.g. chlorophyll *a*, microcystin, cyanobacterial biomass, *Daphnia* biomass) were log_{10}-transformed while proportional data were logit-transformed. Statistical analyses were conducted using SPSS and R.

3 | RESULTS

Elevated nitrogen concentration had a large and significant positive effect on the concentrations of chlorophyll *a*, cyanobacterial dry biomass, and the toxin, microcystin (Figure 1), prior to adding *Daphnia* (i.e. the first 11 days of the experiment) and over the duration of the experiment in the no-*Daphnia* control enclosures. Immediately prior to the stocking of *Daphnia* genotypes (day 11: 16 October 2009), mean chlorophyll *a* and cyanobacterial biomass for high-nitrogen enclosures were nearly five- and eight-times higher,

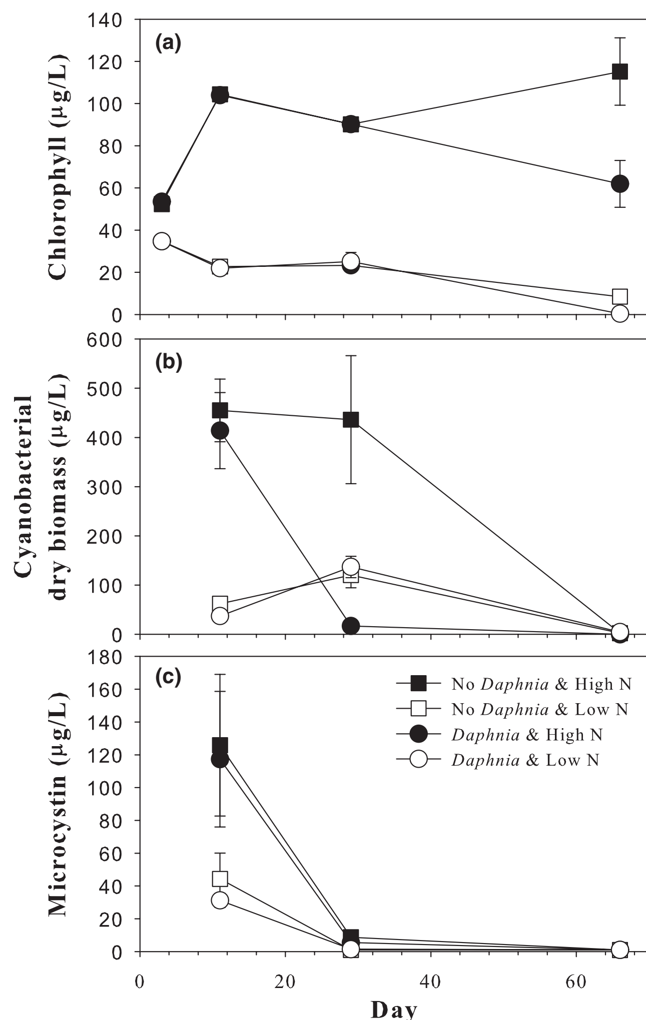

FIGURE 1 Dynamics of (a) chlorophyll *a* (µg/L), (b) cyanobacterial dry biomass (µg/L), and (c) microcystin (ng/L) over the 10-week enclosure experiment across four treatments, including no *Daphnia* under high nitrogen (black square), no *Daphnia* under low nitrogen (white square), *Daphnia* under high nitrogen (black circle), and *Daphnia* under low nitrogen (white circle). The experiment was initiated on 5 October 2009 (day 0), *Daphnia* were added on 17 October 2009 (day 12) after sampling, and the experiment ended on 10 December 2009 (day 80). Data represent means ± one standard error for each treatment. Note: the figure legend for all three panels is provided in (c)

respectively, than for low-nitrogen enclosures (Figure 1a, chlorophyll *a*: $F_{1,22}$ = 19201.816, p < 0.0001; Figure 1b, cyanobacterial biomass: $F_{1,22}$ = 62.285, p < 0.0001). Furthermore, mean concentrations of microcystin were over three-times higher under high nitrogen than low nitrogen on day 11 (Figure 1c, $F_{1,22}$ = 8.002, p = 0.010). By the 4th week of the experiment (*c.* 2 weeks after *Daphnia* addition: 3 November 2009), *Daphnia* had suppressed cyanobacterial biomass by *c.* 96% relative to no-*Daphnia* controls at high nitrogen (Tukey's test: p = 0.009) but had no effect on chlorophyll *a* (Figure 1a, Tukey's test: p = 1.000). At low nitrogen, *Daphnia* had no effect on either chlorophyll *a* (Figure 1a, Tukey's test: p = 0.972) or cyanobacterial biomass (Figure 1b, Tukey's test: p = 1.000) by day 29 (3 November

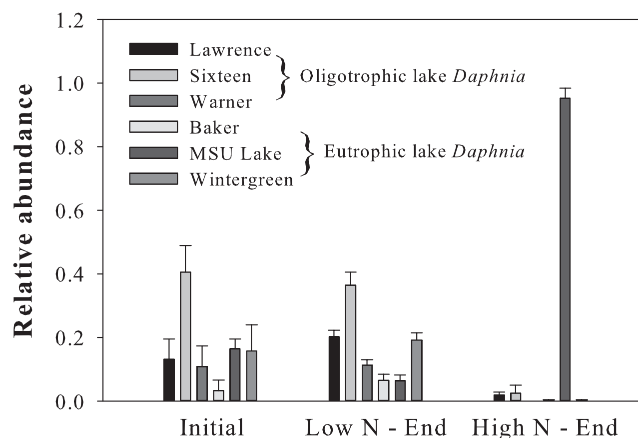

FIGURE 2 Relative abundance of each of the six *Daphnia* genotypes at stocking (Initial = 16 October 2009) and the conclusion of the experiment (10 December 2009). The first set of three bars represent *Daphnia* from oligotrophic sites (Lawrence, Sixteen, Warner) and the second set of three bars represent *Daphnia* from eutrophic sites (Baker, MSU Lake 1, Wintergreen). Data represent means + one standard error for each treatment

2009). Repeated measures ANOVA over the 10-week experiment revealed significant effects of nitrogen addition ($F_{1,20}$ = 519.876, p < 0.0001), *Daphnia* presence ($F_{1,20}$ = 9.931, p = 0.005), and the interaction of nitrogen and *Daphnia* presence ($F_{1,20}$ = 6.221, p = 0.022) on chlorophyll *a*. Furthermore, nitrogen ($F_{1,20}$ = 31.837, p < 0.0001), *Daphnia* presence ($F_{1,20}$ = 8.635, p = 0.008), and the interaction of nitrogen addition and *Daphnia* presence ($F_{1,20}$ = 8.241, p = 0.0009) all had significant effects on cyanobacterial biomass over time. At the conclusion of the experiment (day 66), *Daphnia* had reduced chlorophyll *a* by approximately 46%, relative to the no-*Daphnia* control at high nitrogen (Tukey's test: p = 0.005). In contrast, *Daphnia* had no effect on chlorophyll *a* at low nitrogen (Tukey's test: p = 0.963).

Although *Daphnia* biomass was similar for low- and high-nitrogen enclosures (p = 0.78) at the conclusion of the experiment, *Daphnia* genetic diversity was significantly different between nitrogen treatments (Figure 2; Table 1). The relative abundance of eutrophic-lake *Daphnia* was nearly 100% for high-nitrogen enclosures where cyanobacteria dominated, while *Daphnia* genotypes from oligotrophic and eutrophic lakes were equally represented at low nitrogen (Figure 2). In fact, relative abundances of the six *Daphnia* genotypes were similar (all Tukey pairwise comparisons p ≥ 0.83) at the start (richness = 5.5, Shannon–Weiner index = 1.48, evenness = 0.87; Table 2) and end (richness = 5.83, Shannon–Weiner index = 1.55, evenness = 0.88) of the experiment in the low-nitrogen treatment (Figure 2) where phytoplankton abundance or species composition changed little over time (Figure 1). In contrast, the high-nitrogen treatment differed significantly from the inoculum (i.e. initial) and low-nitrogen treatment (all Tukey pairwise comparisons p ≤ 0.001; Table 1). For example, in the high-nitrogen treatment, three genotypes were undetectable or near the detection limits, and a single genotype (MSU Lake 1) dominated at the end of the experiment (Table 2; richness = 2,

TABLE 2 Diversity estimates of *Daphnia* populations at the start (inoculum; 2 subsample replicates) and end the mesocosm experiment for the two treatments (Low N and High N; 6 replicates each). Twenty to 25 individuals were sampled from each inoculum subsample and for all enclosures at the end. Note that two High N enclosures only had the MSU Lake 1 *Daphnia pulicaria* genotype so evenness could not be calculated for these enclosures. Means (one *SE*)

Diversity metric	Inoculum	Low N end	High N end
Genotypic richness	5.50 (0.5)[a]	5.83 (0.17)[a]	2.00 (0.37)[b]
Shannon–Weiner index	1.48 (0.16)[a]	1.55 (0.04)[a]	0.17 (0.10)[b]
Evenness	0.87 (0.05)[a]	0.88 (0.02)[a]	0.25 (0.11)[b]

Note. Letters denote statistically similar treatment means ($\alpha < 0.05$; ANOVA followed by Tukey's multiple comparison tests).

Shannon–Weiner index = 0.17, evenness = 0.25). PCA of relative abundances of *Daphnia* genotypes revealed that 87% of the total variance could be explained by a single factor (factor 1) that could be attributed to the relative abundance of the MSU Lake 1 genotype. ANOVA using PCA factor 1 scores indicated that the relative abundance (based on PCA factor 1 scores) of the MSU Lake 1 genotype at the end of the experiment was significantly higher at high nitrogen versus low nitrogen ($p < 0.0001$; Tukey's test: $p < 0.0001$) or when compared to the beginning of the experiment ($p < 0.0001$). In contrast, the relative abundance (based on PCA factor 1 scores) of the MSU Lake 1 genotype was similar at the beginning versus end of the experiment for low-nitrogen enclosures (Figure 2; $p = 0.216$). Similar analyses using only the MSU Lake 1 genotype logit-transformed relative abundance data showed the same patterns (initial and low-nitrogen treatment were statistically similar (Tukey's test: $p = 0.362$); however, both relative abundances of initial and for the low-nitrogen treatment were different from the high-nitrogen treatment at the end of the experiment (both Tukey's tests: $p < 0.001$).

4 | DISCUSSION

Nutrient enrichment of freshwater ecosystems frequently leads to increased phytoplankton biomass, especially cyanobacteria (Paerl & Huisman, 2008). As grazing-resistant cyanobacteria can inhibit *Daphnia* feeding, growth, survival, and reproduction (DeMott et al., 1991; Gliwicz & Lampert, 1990; Lürling & van Der Grinten, 2003; Tillmanns, Wilson, Pick, & Sarnelle, 2008; Wilson, Sarnelle, & Tillmanns, 2006), eutrophication could influence the genotypic composition of *Daphnia* populations in which tolerance to cyanobacteria varies (Hairston et al., 1999; Sarnelle & Wilson, 2005). We created environments with high and low concentrations of cyanobacteria via two fertilisation regimes and exposed a mixture of *Daphnia* genotypes to these two environments. The *Daphnia* genotype from the most eutrophic lake (MSU Lake 1) dominated the *Daphnia* population at high nitrogen, accounting for 95% of the *Daphnia* population at the conclusion of the experiment. Despite the increased abundance of cyanobacteria at high nitrogen by week 4, *Daphnia* addition resulted in a large reduction in cyanobacterial biomass after only 2 weeks and the effect was maintained until the end of the experiment. In contrast, *Daphnia* had no effect on cyanobacterial biomass at low nitrogen. Limited sampling precluded our ability to better assess *Daphnia* grazing effects and potential grazer selectivity over time. However, initial grazing by *Daphnia* on cyanobacteria may have indirectly facilitated small, rapidly growing chlorophytes (e.g. *Scenedesmus* species) by reducing competition with cyanobacteria (e.g. increased light availability) in the high-nutrient treatment.

A single *Daphnia* genotype (MSU Lake 1) dominated the *Daphnia* population in the high-nitrogen treatment, which was characterised by higher phytoplankton biomass, cyanobacterial abundance, and concentration of the cyanotoxin, microcystin. The MSU Lake 1 genotype was collected from a eutrophic lake and identified to be tolerant to toxic *Microcystis* in a laboratory experiment. Interestingly, the other two genotypes from eutrophic lakes nearly disappeared in these fertilised enclosures. These data suggest that being from a eutrophic lake may not fully explain tolerance to cyanobacteria or their toxins or alternatively that tolerance is a continuous, rather than dichotomous, phenomenon (Sarnelle & Wilson, 2005). In the case of this study, it appears that clonal replacement may have been driven by the rapid growth rate of the MSU Lake 1 genotype on nutritious green algae (i.e. *Ankistrodesmus*), in addition to surviving on the toxic diet (i.e. *Microcystis*). While we estimated tolerance by comparing somatic growth rates of *Daphnia* genotypes on toxic versus nutritious phytoplankton, shifts in *Daphnia* populations will also be affected by differences among genotypes in genotype-specific birth and death rates. Alternatively, different *Daphnia* genotypes from eutrophic lakes may also differ in diel vertical migration behaviour, allowing access to additional food sources (e.g. bacteria) and affecting vulnerability to zooplanktivorous fishes. All three genotypes from oligotrophic lakes that were identified to be sensitive to toxic *Microcystis* were quickly driven to (Warner) or near (Lawrence and Sixteen) extinction in the high-nitrogen environment. These patterns follow observed phenotypic bottlenecks for a *Daphnia* population in Lake Constance as it underwent eutrophication over a decade (Hairston et al., 1999). In our study, near exclusion of half of the stocked genotypes occurred in just 2 months.

In the low-nitrogen treatment, we found no significant changes in genotypic composition in the *Daphnia* populations suggesting that the mesocosm environment (that lacked planktivorous fish) was suitable habitat for all six *Daphnia* genotypes. Moreover, considering that the low-nitrogen treatments maintained chlorophyll *a* at elevated levels (>20 μg/L), competition for resources may have been low, thus minimising the potential for one or a few genotypes to out-compete the others. The low abundance of cyanobacteria and microcystin in the low-nitrogen treatment probably also limited the potential for eutrophic lake *Daphnia* (especially MSU Lake 1) to dominate; however, as we only sampled *Daphnia* populations at the end of the experiment to minimise contamination of *Daphnia* genotypes,

the dynamics of relative abundances for *Daphnia* genotypes over time are unknown.

Ecological genetics is a rapidly growing field that merges aspects of population genetics and community ecology to understand the development and consequences of intraspecific variation for communities and ecosystems (Wade, 2007; Whitham et al., 2006). In freshwater ecosystems, rapid adaptive evolution by major consumers may play an important role in the response of lake ecosystems to cultural eutrophication and food web manipulations (Frisch et al., 2017; Hairston et al., 2001; Jiang et al., 2013; Lyu et al., 2015; Orsini et al., 2013; Sarnelle & Wilson, 2005). Hairston et al. (1999) made the seminal observation that a generalist herbivore in freshwater lakes (*Daphnia*) can evolve to tolerate toxic cyanobacteria in the diet in response to nutrient enrichment. *Daphnia* tolerance to toxic cyanobacteria has now been observed for a *D. galeata* population following eutrophication of Lake Constance in Europe (Hairston et al., 2001) and for several *D. pulicaria* populations in North America (Frisch et al., 2017; Sarnelle & Wilson, 2005). Despite several recent studies documenting tolerance of zooplankton to toxic cyanobacteria, understanding the specific mechanisms allowing for grazer tolerance has remained elusive (but see Macke, Callens, De Meester, & Decaestecker, 2017). In this study, we highlight a significant, unexplored threat of eutrophication, namely that it mediated, through the promotion of toxic cyanobacteria, the significant loss of *Daphnia* genetic diversity within a single growing season.

Understanding the causes and consequences of intraspecific variation within *Daphnia* may aid in the future management of eutrophic lakes (Ger et al., 2016; Lyu et al., 2016, 2017). Given the considerable variation in ecologically important traits observed for *Daphnia* populations (Duffy, 2010; Tessier et al., 2000), an emphasis on intraspecific trait variation provides an interesting conceptual framework for linking diversity to ecosystem function, and the results of our study and several previous studies suggest that this approach may be profitable, particularly for plankton food webs (Duffy, 2010; Hairston et al., 1999; Orsini et al., 2013; Post et al., 2008; Sarnelle & Wilson, 2005; Tessier et al., 2000).

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CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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