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Biological control of toxic cyanobacteria by mixotrophic predators: an experimental test of intraguild predation theory

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Abstract. Intraguild predators both feed on and compete with their intraguild prey. In theory, intraguild predators can therefore be very effective as biological control agents of intraguild prey species, especially in productive environments. We investigated this hypothesis using the mixotrophic chrysophyte *Ochromonas* as intraguild predator and the harmful cyanobacterium *Microcystis aeruginos*a as its prey. *Ochromonas* can grow photoautotrophically, but can also graze efficiently on *Microcystis*. Hence, it competes with its prey for inorganic resources. We developed a mathematical model and parameterized it for our experimental food web. The model predicts dominance of *Microcystis* at low nutrient loads, coexistence of both species at intermediate nutrient loads, and dominance of *Ochromonas* but a strong decrease of *Microcystis* at high nutrient loads. We tested these theoretical predictions in chemostat experiments supplied with three different nitrogen concentrations. *Ochromonas* initially suppressed the *Microcystis* abundance by >97% compared to the *Microcystis* monocultures. Thereafter, however, *Microcystis* gradually recovered to ~20% of its monoculture abundance at low nitrogen loads, but to 50–60% at high nitrogen loads. Hence, *Ochromonas* largely lost control over the *Microcystis* population at high nitrogen loads. We explored several mechanisms that might explain this deviation from theoretical predictions, and found that intraspecific interference at high *Ochromonas* densities reduced their grazing rates on *Microcystis*. These results illustrate the potential of intraguild predation to control pest species, but also show that the effectiveness of their biological control can be reduced in productive environments.

Key words: eutrophication; lake restoration; mixotrophy; omnivory; predator–prey interactions; resource competition; toxic cyanobacteria.

Introduction

Studies of competition and predation, the two major structuring forces of ecological food webs, have played a key role in the development of community ecology. Intraguild predation encompasses both types of species interaction (Polis et al. 1989, Polis and Holt 1992). Intraguild predation is defined as "the killing and eating of species that use similar resources and are thus potential competitors" (Polis and Holt 1992). In other words, intraguild predators eat their competitors. Attacking prey from two sides, through both competition and predation, can be a very efficient strategy to suppress the population abundances of intraguild prey (Polis and Holt 1992, Thingstad et al. 1996). Therefore, intraguild predators that utilize pest species as their intraguild prey might be very effective as biological control agents (Bampfylde and Lewis 2007).

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Most models predict coexistence of intraguild predators and their prey in habitats of intermediate productivity, provided that the intraguild prey is a better competitor for the common resource (Polis and Holt 1992, Holt and Polis 1997, Diehl and Feissel 2000). However, intraguild prey populations are predicted to decrease with increasing productivity due to an enhanced predation pressure, and intraguild predators will tend to exclude their intraguild prey from highly productive environments (Holt and Polis 1997, Diehl and Feissel 2000, Mylius et al. 2001, Van de Wolfshaar et al. 2006). These theoretical predictions are supported to various degrees by microcosm experiments with aquatic protists (Morin 1999, Diehl and Feissel 2000, 2001) and by field experiments with an insect and its parasitoids (Borer et al. 2003). Yet, other experiments observed persistence of intraguild prey in productive environments (Liess and Diehl 2006, Montserrat et al. 2008). Moreover, coexistence of intraguild predators and their prey appears widespread in natural communities, including productive environments (Polis and Strong 1996, Arim and Marquet 2004). During recent years, theoretical studies have therefore proposed a plethora of possible mechanisms that promote the

persistence of intraguild prey in productive environments (e.g., Křivan and Diehl 2005, Finke and Denno 2006, Janssen et al. 2007, Amarasekare 2008, Rudolf and Armstrong 2008, Abrams and Fung 2010, Hin et al. 2011).

Intraguild predation has received considerable interest in agricultural studies (Rosenheim et al. 1995, Straub et al. 2008, Van Maanen et al. 2012), but may also find application in other fields including water management. In particular, cyanobacterial blooms are favored by high nutrient loads, and have increasingly become a major nuisance in many lakes and reservoirs (Chorus and Bartram 1999, Huisman et al. 2005, Paerl and Huisman 2008). Dense cyanobacterial blooms may cause nighttime oxygen depletion, sometimes leading to large fish kills. In addition, the high turbidity of cyanobacterial blooms reduces the growth of aquatic macrophytes, suppressing important underwater habitat for other aquatic organisms (Scheffer 1998). Furthermore, several cyanobacterial species can produce toxins causing serious and sometimes fatal liver, digestive and neurological diseases in birds, mammals (e.g., pets, cattle) and humans (Codd 1995, Carmichael et al. 2001). Cyanobacterial blooms are therefore a major concern in water quality management (Codd et al. 2005, Verspagen et al. 2006, Qin et al. 2010).

Mixotrophic organisms combine autotrophic and heterotrophic nutrition. For instance, many photosynthetic microalgae can also ingest bacteria and phytoplankton species by phagocytosis (Stoecker 1998, Jones 2000). Hence, mixotrophs often act as intraguild predators that compete with phytoplankton species for nutrients and light, but also graze on them (Thingstad et al. 1996). Mixotrophs are widespread in plankton communities of both freshwater and marine ecosystems (Isaksson 1998, Zubkov and Tarran 2008, Hartmann et al. 2012, Flynn et al. 2013), and several species are capable to form dense blooms in eutrophic environments (Burkholder et al. 2008). In particular, some mixotrophic chrysophytes of the *Ochromonas* genus can graze efficiently on toxic cyanobacteria, and are even capable of degrading cyanobacterial toxins (Van Donk et al. 2009, Wilken et al. 2010). Their function as intraguild predators might make these mixotrophs ideal candidates as biological control agents against harmful cyanobacteria in eutrophic lakes.

In this paper, we test the predictions of intraguild predation theory with the toxic cyanobacterium *Microcystis aeruginosa* as intraguild prey and the mixotroph *Ochromonas* sp. as intraguild predator. We first develop a simple model to describe the population dynamics generated by intraguild predation along a productivity gradient. The model predictions are tested in a series of chemostat experiments at three different nitrogen levels to create an experimental productivity gradient. The experimental results are used to evaluate the potential for mixotrophic predators to control the development of harmful cyanobacterial blooms in productive environments.

Theory

Model structure

Our model is a variant of the earlier intraguild predation models developed by Holt and Polis (1997) and Diehl and Feissel (2000). These authors assumed that the basal resource was a living organism (e.g., a bacterium) obeying logistic growth, the intraguild prey was a heterotroph feeding on the basal prey, and the intraguild predator consumed both the basal and intraguild prey. In our application, the basal resource is an inorganic nutrient, the intraguild prey is an autotrophic organism that takes up inorganic nutrients from the environment, and the intraguild predator is a mixotroph that obtains nutrients through both uptake of inorganic nutrients and ingestion of the autotrophic prey (Wilken et al. 2014).

We consider a variable-internal-stores model, where nutrients are first taken up by the organisms, and subsequently the organisms can utilize the stored nutrients for population growth (Droop 1973, Grover 1991). Let N denote the concentration of dissolved inorganic nutrient, let Q_A and Q_M denote the cellular nutrient contents of the autotroph and mixotroph, respectively, and let A and M denote the population densities of the autotroph and mixotroph. Our intraguild predation model then reads

$$\frac{dN}{dt} = D(N_{\text{in}} - N) - u_A(N, Q_A)A - u_M(N, Q_M)M \quad (1)$$

$$\frac{dQ_A}{dt} = u_A(N, Q_A) - \mu_A(Q_A)Q_A \quad (2)$$

$$\frac{dQ_M}{dt} = u_M(N, Q_M) + f_M(A)Q_A - \mu_M(Q_M)Q_M \quad (3)$$

$$\frac{dA}{dt} = \mu_A(Q_A)A - f_M(A)M - m_A A \quad (4)$$

$$\frac{dM}{dt} = \mu_M(Q_M)M - m_M M. \quad (5)$$

Here, D is the nutrient turnover rate, N_{in} is the nutrient load in the incoming media entering the chemostat, $u_A(N,Q_A)$ and $u_M(N,Q_M)$ are the nutrient uptake rates of the autotroph and mixotroph, $\mu_A(Q_A)$ and $\mu_M(Q_M)$ are the specific growth rates of the autotroph and mixotroph as functions of their cellular nutrient status, $f_M(A)$ is the functional response of the mixotroph grazing upon its autotrophic prey, and m_A and m_M are the mortality rates of the autotroph and mixotroph, respectively.

We assume that the nutrient uptake rates of the autotroph and mixotroph increase with the ambient nutrient concentration according to Michaelis-Menten kinetics, and are suppressed when cells become satiated

with nutrient (Morel 1987, Ducobu et al. 1998)

$$u_i(N, Q_i) = \frac{u_{\max,i} N}{K_i + N}\left(\frac{Q_{\max,i} - Q_i}{Q_{\max,i} - Q_{\min,i}}\right), \qquad i = A, M \tag{6}$$

where $u_{\max,i}$ is the maximum nutrient uptake rate of species i, K_i is its half-saturation constant for nutrient uptake, and $Q_{\max,i}$ and $Q_{\min,i}$ are its maximum and minimum cellular nutrient contents, respectively.

The model further assumes that the specific growth rates of the autotroph and mixotroph are increasing saturating functions of their cellular nutrient status

$$\mu_i(Q_i) = \mu_{\max,i}\left(1 - \frac{Q_{\max,i} - Q_i}{Q_{\max,i} - Q_{\min,i}}\right), \qquad i = A, M \tag{7}$$

where $\mu_{\max,i}$ is the maximum specific growth rate of species i. This formulation resembles Droop's (1973) classic growth equation, but in addition to a minimum cellular nutrient content it also incorporates a maximum cellular nutrient content.

The mixotroph feeds upon the autotroph with a Holling type III functional response, in accordance with the experimental data of Wilken et al. (2010)

$$f_M(A) = \frac{f_{\max}(A/k_M)^b}{1 + (A/k_M)^b} \tag{8}$$

where $f_{\max}$ is the maximum ingestion rate, k_M is the half-saturation constant for prey ingestion, and b describes the curvature of the type III functional response.

Our model assumes that the predation rate depends on prey abundance only, while it is independent of the inorganic nitrogen source and does not cease at high cellular nitrogen contents of the mixotroph. Conversely, however, the uptake rate of inorganic nutrient can be suppressed by the ingestion of prey via an increase of the cellular nutrient content of the mixotroph (see Eqs. 3 and 6). Hence, our model assumes that the mixotroph prefers heterotrophic over autotrophic growth, in agreement with the predominantly heterotrophic nutrition of the mixotroph *Ochromonas* (Andersson et al. 1989, Sanders et al. 2001, Wilken et al. 2013). Yet, at low prey abundances its heterotrophic lifestyle is less profitable, and the mixotroph shifts to autotrophic growth through its type III functional response.

Parameter estimates

The model was parameterized based on the settings of our chemostat experiments and a series of independent experiments with the autotroph *Microcystis aeruginosa*, the mixotroph *Ochromonas* sp., and ammonium as the limiting nutrient. In chemostats, the nutrient turnover rate D and the nutrient load N_{in} (see Eq. 1) are set by the dilution rate of the chemostat and the nutrient concentration in the mineral medium, respectively. We assume that the specific loss rates of both species were governed by the dilution rate of the chemostat (i.e., $m_A = m_M = D$). The maximum specific growth rate ($\mu_{\max}$) and maximum cellular nitrogen content ($Q_{\max}$) of *Microcystis* were measured during exponential growth in an independent chemostat experiment provided with a saturating nitrogen concentration of 500 μmol/L in the mineral medium. The maximum specific growth rate of *Ochromonas* was measured during exponential growth under mixotrophic conditions, by inoculation of a small amount of *Ochromonas* into the *Microcystis* chemostat experiment after *Microcystis* had reached high population densities. We were not able to measure the maximum cellular nitrogen content of *Ochromonas* during this experiment, because the *Ochromonas* cells could not be separated from the *Microcystis* cells. The minimum cellular nitrogen contents ($Q_{\min}$) of *Microcystis* and *Ochromonas* were measured under autotrophic conditions, using nitrogen-limited batch cultures in which the cells became nitrogen starved at the stationary phase. The functional response of *Ochromonas* grazing on *Microcystis* was obtained from the grazing experiments of Wilken et al. (2010), adopting their values for the maximum ingestion rate ($f_{\max}$) and the curvature of the functional response (b), but using the half-saturation constant (k_M) as a tuning parameter to account for differences in predator-prey encounter rates between the batch experiments of Wilken et al. (2010) and the much more turbulent conditions in our chemostat experiments. The remaining model parameters ($u_{\max}$ and K of both species; $Q_{\max}$ of *Ochromonas*) were obtained from earlier monoculture chemostat experiments with *Microcystis aeruginosa* and *Ochromonas* sp. using ammonium as the limiting nutrient (Wilken et al. 2014). The parameter values are summarized in Table 1.

Model predictions

The model predictions are illustrated in Fig. 1. From these results, which are in good agreement with other intraguild predation models (Holt and Polis 1997, Diehl and Feissel 2000), we deduce the following theoretical predictions: (1) at low nutrient loads, the autotroph dominates; (2) at intermediate nutrient loads, the autotroph and mixotroph coexist; (3) with a further increase in nutrient loads, the mixotroph becomes dominant, while the population density of the autotroph decreases; (4) the inorganic nutrient concentration shows a slight increase after the mixotroph has entered the system, but remains rather low; (5) the community does not show alternative stable states or non-equilibrium dynamics such as predator–prey oscillations (Fig. 1).

With respect to prediction 3, we note that the type III functional response of the mixotroph implies that its grazing pressure on the autotroph becomes negligible when the autotroph becomes rare. Hence, the model predicts that the mixotroph will suppress the autotroph at high nutrient loads, but will not drive it to extinction (Wilken et al. 2014). Furthermore, we note that prediction 5 is specific for our parameter estimates,

TABLE 1. Definition and values of model parameters

Symbol	Definition	Value	Unit	Source
State variables				
N	dissolved inorganic nitrogen concentration		mol/L	
Q_A	cellular nitrogen content of the autotroph		mol/cell	
Q_M	cellular nitrogen content of the mixotroph		mol/cell	
A	population density of the autotroph		cells/L	
M	population density of the mixotroph		cells/L	
System parameters				
D	dilution rate	0.12	d^{-1}	a
N_{in}	nitrogen load in the incoming media entering the chemostat	10^{-8} to 10^{-2}	mol/L	a
Species parameters				
$u_{max,i}$	maximum N-uptake rate of species i	12.0×10^{-14}†, 24.0×10^{-14}‡	mol·cell^{-1}·d^{-1}	e
K_i	half-saturation constant for species i	9.0×10^{-7}†, 6.5×10^{-7}‡	mol/L	e
$\mu_{max,i}$	maximum specific growth rate for species i	0.70†, 2.2‡	d^{-1}	b
$Q_{min,i}$	minimum cellular nitrogen content for species i	2.6×10^{-14}†, 1.0×10^{-13}‡	mol/cell	c
$Q_{max,i}$	maximum cellular nitrogen content for species i	9.5×10^{-14}†, 32×10^{-13}‡	mol/cell	b, e
f_{max}	maximum prey ingestion rate	na†, 53.0‡	cells·cell^{-1}·d^{-1}	d
k_M	half-saturation constant for prey ingestion	na†, 4.0×10^8‡	cells/L	f
b	curvature of type III functional response	na†, 2.37‡		d

Note: Sources are: a, measured system parameter; b, measured in an independent chemostat experiment with saturating nitrogen concentrations; c, measured after nitrogen starvation in batch culture; d, measured in the grazing experiment of Wilken et al. (2010); e, estimated from earlier monoculture chemostats (Wilken et al. 2014); f, tuning parameter estimated from model fits to the species mixtures.

† *Microcystis*; na, not applicable.

‡ *Ochromonas*.

while intraguild predation models can display alternative stable states and predator–prey oscillations for other parameter combinations (cf. Holt and Polis 1997, Verdy and Amarasekare 2010, Hiltunen et al. 2013).

MATERIALS AND METHODS

Species

Our experiments were performed with the mixotrophic chrysophyte *Ochromonas* sp. as intraguild predator, and the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806 as intraguild prey. *Microcystis* PCC 7806 is a single-celled strain producing the hepatotoxin microcystin-LR. It has a cellular biovolume of 29.6 ± 1.0 μm^3 (mean ± SD), and did not show any colony formation during the experiments. Our *Ochromonas* strain is probably *Ochromonas globosa* Skuja (determined by R. Bijkerk). It was originally detected as infection in a large-scale mesocosm experiment with *Microcystis*, from which it was isolated with micro-needle techniques (Van Donk et al. 2009). Its cellular biovolume is 211.9 ± 21.9 μm^3. We showed in earlier experiments that this *Ochromonas* strain can feed effectively on *Microcystis*, and is not affected by its toxin microcystin (Wilken et al. 2010).

Chemostat experiments

The theoretical predictions were tested in chemostat experiments, using three different nitrogen concentrations in the mineral medium (N_{in} = 20, 100, and 500 μmol/L of NH_4Cl) to generate an experimental productivity gradient. These concentrations reflect the range of total nitrogen concentrations typically found in eutrophic lakes with dense *Microcystis* blooms (Verspagen et al. 2006, Jöhnk et al. 2008, Xu et al. 2010). All other nutrients were provided in excess using a nutrient-rich mineral medium (Wilken et al. 2013). First, monoculture chemostats of *Microcystis* and *Ochromonas* were grown at each nitrogen level. After the monocultures had been maintained at steady state for at least two weeks, mixed cultures were produced by cross-inoculating each monoculture with 5% from the monoculture of the other species at the same nitrogen level. This resulted in two mixed cultures at each nitrogen level, one in which a small amount of *Ochromonas* was added to a steady-state monoculture of *Microcystis* and the other in which a small amount of *Microcystis* was added to a steady-state monoculture of *Ochromonas*. Hence, this procedure provided a straightforward method to test for alternative stable states.

The chemostat experiments were performed in 1.6-L flat culture vessels as described in Huisman et al. (1999, 2002), with a dilution rate of $D = 0.12$ d^{-1}. The temperature was kept constant at 23 ± 1°C by a metal cooling finger connected to a water bath. Light was supplied by white fluorescent tubes (PL-L 24W/840/4P; Philips, Eindhoven, The Netherlands) at a constant incoming irradiance (I_{in}) of 50 ± 2 μmol photons·m^{-2}·s^{-1}. The chemostats were aerated with filter-sterilized air at a flow rate of 20 L/h. To prevent introduction of gaseous ammonium, the air flow was cleaned by leading it through a 0.166 mol/L phosphoric acid solution before entering the chemostat cultures. The photosynthetic activity of dense cultures may deplete the CO_2 concentration and raise the pH. Therefore, the pH was continuously monitored, and carbon limitation was avoided by enriching the air flow with adjustable concentrations of CO_2 using Brooks Mass Flow Controllers (Brooks Instruments, Hatfield, Pennsylvania, USA), targeted to stabilize the pH at 7.8. Wall growth was removed daily by scraping the walls with a magnetic stirrer.

The chemostats were sampled approximately every other day. Samples for cell counts were fixed with a mixture of glutaraldehyde and formaldehyde (final concentration of 0.1% and 0.01% by mass, respectively) and stored at 4°C until counting at a flow cytometer (MoFlo XDP cell sorter; Beckman Coulter, Miami, Florida, USA). The flow cytometer distinguished between *Ochromonas* and *Microcystis* based on their cell size and pigmentation. Samples for determination of the dissolved ammonium concentration were centrifuged for 10 min at relative centrifugal force of 3200 g or 31.38×10^3 m/s^2 and the supernatant was stored at −20°C until further analysis. Ammonium concentrations were measured using the fluorometric method described in Holmes et al. (1999). To determine particulate organic carbon and nitrogen concentrations, cultures were filtered onto pre-combusted glass fiber filters (Whatman GF/F), dried at 60°C overnight, and measured using an Elemental Analyzer (Flash 2000; Thermo Fisher Scientific, Waltham, Massachusetts, USA). We also calculated the cellular nitrogen content from the difference between the dissolved inorganic nitrogen concentration in the mineral medium and in the chemostat vessel using mass balance considerations. The incident light intensity (I_{in}) and the light intensity transmitted through the chemostat vessel (I_{out}) were measured for at least six time points during steady state of both monocultures and mixed cultures. Measurements were performed with a LI-COR LI-250 quantum photometer (LI-COR Biosciences, Lincoln, Nebraska, USA) at 10 randomly chosen positions on the front and back surface of the chemostat vessel, respectively. The depth-averaged light intensity in the cultures (I_{avg}) can then be calculated as $I_{avg} = (I_{in} - I_{out})/(\ln[I_{in}] - \ln[I_{out}])$ (Huisman et al. 2002).

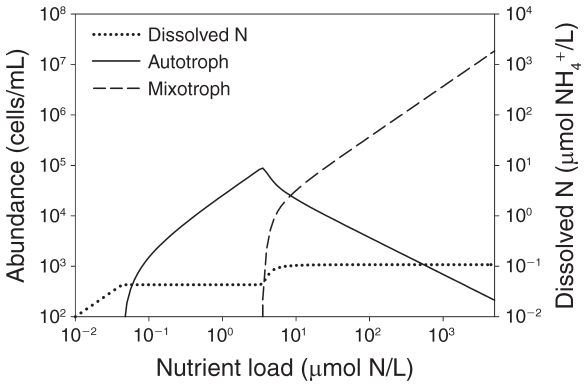

FIG. 1. Model predictions of the steady-state abundances of the autotroph and mixotroph and the dissolved inorganic nitrogen concentration as function of the nutrient load. Parameter values are provided in Table 1.

RESULTS

Chemostat experiments

The autotroph *Microcystis* and the mixotroph *Ochromonas* both grew well in monoculture, and increased in abundance with increasing nitrogen load (Fig. 2). *Ochromonas* had a lower growth rate than *Microcystis*, as can be seen from the longer time span needed until steady state was reached (compare Fig. 2E and F). Due to its smaller cell size, the cellular nitrogen content of *Microcystis* was lower than that of *Ochromonas*, reaching steady-state values of 0.034–0.038 pmol/cell in *Microcystis* as compared to 0.26–0.32 pmol/cell in *Ochromonas*. Consequently, *Microcystis* reached about 10-fold higher steady-state abundances than *Ochromonas* (Fig. 2; Fig. 3A). The measured cellular nitrogen contents were in good agreement with the steady-state values of 0.038 pmol/cell for *Microcystis* and 0.27 pmol/cell for *Ochromonas* predicted by the model.

The linear increase in steady-state abundance of both species with increasing nitrogen load confirmed that nitrogen was indeed the limiting factor, even at the highest nitrogen level (Fig. 3A). Furthermore, *Microcystis* cells showed high C:N ratios of ~17 at all nitrogen loads (Fig. 3B). C:N ratios of *Ochromonas* were lower, especially at 100 and 500 μmol/L N load, but also remained well above the Redfield ratio of 6.6. The depth-averaged light intensity was slightly reduced to $I_{avg} \approx 33$ μmol photons·m^{-2}·s^{-1} in the cultures provided with high nitrogen loads (Fig. 3B), indicative of mild self shading by the increasing population abundances. At steady state, *Microcystis* reduced the dissolved ammonium concentration to lower levels than *Ochromonas* (paired t test, $t_2 = 5.05$, $P < 0.05$; Fig. 3C). Hence, *Microcystis* has a lower critical ammonium concentration (i.e., a lower R^* sensu Tilman [1982]), and resource competition theory therefore predicts that *Microcystis* will be the better competitor for ammonium (Grover 1991, Smith and Waltman 1994).

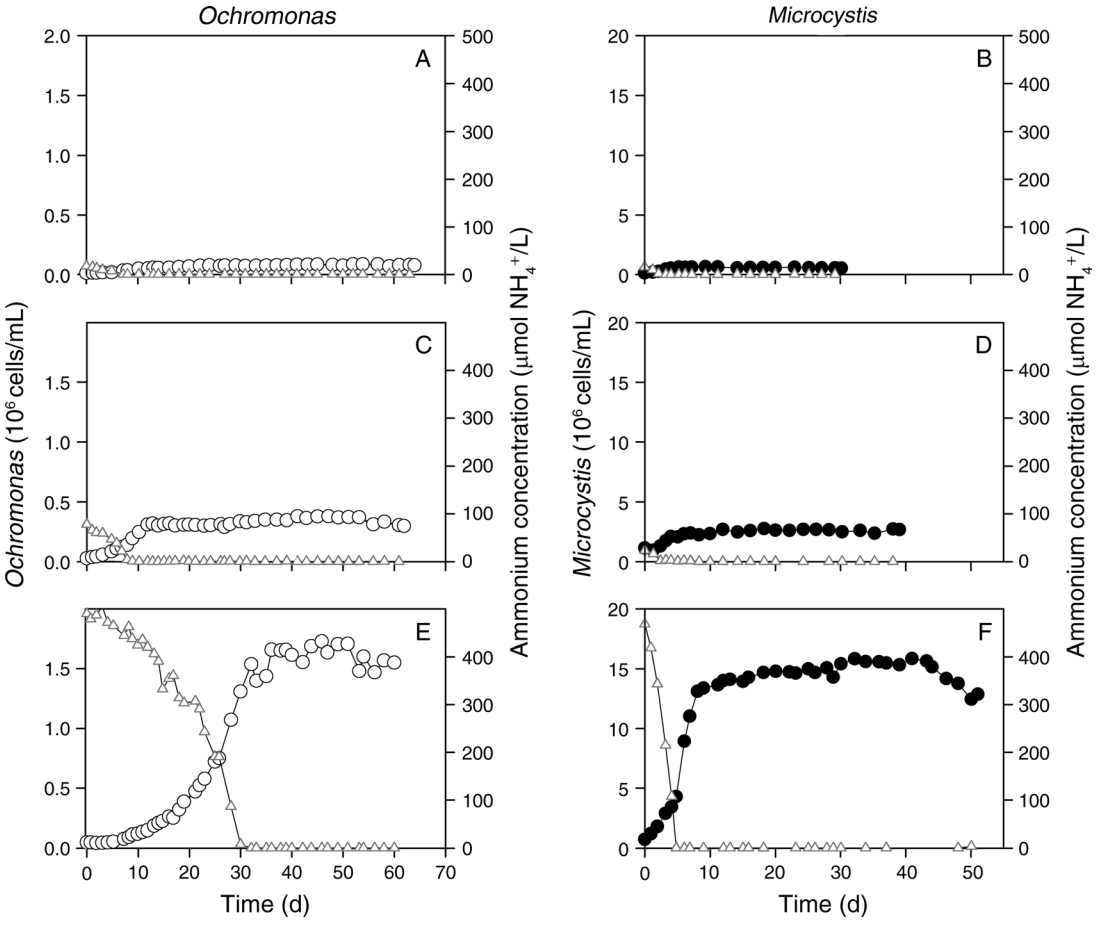

FIG. 2. Monoculture experiments of (A, C, E) *Ochromonas* and (B, D, F) *Microcystis*. The experiments were performed at (A, B) low nitrogen load of 20 μmol NH_4^+/L, (C, D) intermediate nitrogen load of 100 μmol NH_4^+/L, and (E, F) high nitrogen load of 500 μmol NH_4^+/L. Open circles are *Ochromonas*, solid circles are *Microcystis*, open triangles show the ammonium concentration.

After inoculation of *Microcystis* into the monocultures of *Ochromonas*, the *Microcystis* abundance remained low for several weeks and then slowly increased (Fig. 4A, C, E). At low nitrogen load, the *Ochromonas* population was not much affected by the invasion of *Microcystis* (Fig. 4A). However, at intermediate and high nitrogen load, the increase of *Microcystis* came along with a clear decline in *Ochromonas* abundance (Fig. 4C, E). Conversely, after inoculation of *Ochromonas* into the *Microcystis* monocultures, *Ochromonas* showed high initial grazing rates on the *Microcystis* populations, resulting in a rapid increase of the *Ochromonas* population. *Microcystis* quickly declined within the first one to two weeks, from 450 000 to less than 1000 cells/mL at the low nitrogen load (Fig. 4B), from 2.6 million to 50 000 cells/mL at the intermediate nitrogen load (Fig. 4D), and from 12 million to 300 000 cells/mL at the high nitrogen load (Fig. 4F). Hence, within the first few weeks *Microcystis* was reduced by >97% at all three nutrient treatments. After some time, however, the growth rates of *Ochromonas* dwindled, and concomitantly *Microcystis* was able to slowly grow up again (Fig. 4B, D, F). Steady-state abundances of *Ochromonas* and *Microcystis* converged to similar levels, irrespective of whether the species mixtures were started from the *Ochromonas* or *Microcystis* monocultures (compare left column vs. right column in Fig. 4). This indicates that the outcome of the species interactions was independent of the initial conditions. The species abundances at high nitrogen load seemed to fluctuate mildly, suggestive of small-amplitude predator–prey oscillations (Fig. 4E, F). The dissolved ammonium concentration remained low in all treatments.

In the species mixtures, both *Ochromonas* and *Microcystis* increased in steady-state abundance with increasing nitrogen load (Fig. 5A). At the lowest nitrogen load, *Ochromonas* reached slightly higher steady-state abundances than in monoculture and suppressed *Microcystis* by 80% relative to its monoculture abundance (Fig. 5B). Hence, the mixotroph was facilitated by the presence of its prey at low nitrogen loads. In contrast, at the intermediate and high nitrogen loads *Ochromonas*

reached lower steady-state abundances than in monoculture, while *Microcystis* was suppressed by only 40–50%. Thus, although both species increased with the nitrogen load, the intraguild prey *Microcystis* benefitted relatively more from the increasing nitrogen load than its intraguild predator *Ochromonas*. As in the monocultures, the depth-averaged light intensity was slightly reduced at high nitrogen loads, indicative of mild shading (Fig. 5C). Due to their similarity in size the two species could not be separated from the mixture, and hence, the C:N ratio could not be determined for each species individually. The C:N ratio of the species mixtures was intermediate between the C:N ratios of the monocultures of *Microcystis* and *Ochromonas* (compare Figs. 3B and 5C). Likewise, the dissolved ammonium concentration in the species mixture was slightly higher than in the *Microcystis* monocultures, while it was slightly lower than in the *Ochromonas* monocultures (Fig. 5D; Friedmann ANOVA $X^2 = 2.67$, df = 2, $P = 0.26$).

Evaluation of theoretical predictions

We can now evaluate to what extent the theoretical predictions are supported by the experiments:

Prediction 1: at low nutrient load, the autotroph dominates.—The autotroph *Microcystis* was not the dominant species at the lowest nutrient load of 20 μM. In fact, the mixotroph *Ochromonas* strongly suppressed its autotrophic prey (Fig. 4A, B). However, the parameterized model predicts dominance of the autotroph only at very low nitrogen loads of $N_{in} < 2$ μmol/L, but coexistence of the autotroph and mixotroph at higher nitrogen loads (Fig. 1). Hence, the applied nutrient load of 20 μmol/L was apparently not low enough to enable dominance of the autotroph.

Prediction 2: at intermediate nutrient load, the autotroph and mixotroph coexist.—This prediction is supported by the experiments.

Prediction 3: with a further increase in nutrient load, the mixotroph becomes dominant while the autotroph decreases in abundance.—This prediction is not supported. The autotroph and mixotroph both increased in abundance with the nutrient load (Fig. 5A). In relative terms, the dominance of the mixotroph *Ochromonas* even decreased and the autotroph *Microcystis* was suppressed less strongly at high nutrient loads (Fig. 5B).

Prediction 4: the dissolved inorganic nutrient concentration slightly increases after the mixotroph has entered the system, but remains rather low.—As predicted, there was a slight increase in the dissolved ammonium concentration in the species mixtures compared to the *Microcystis* monocultures, but the ammonium concentrations clearly remained low in all experiments.

Prediction 5: the community does not show alternative stable states or predator–prey oscillations.—Despite completely different initial conditions, the two experiments at each nutrient level rapidly converged to the same population dynamics. Hence, we did not find evidence for alternative stable states. The population dynamics suggest convergence to equilibrium at low nutrient load, but mild predator–prey oscillations at high nutrient load.

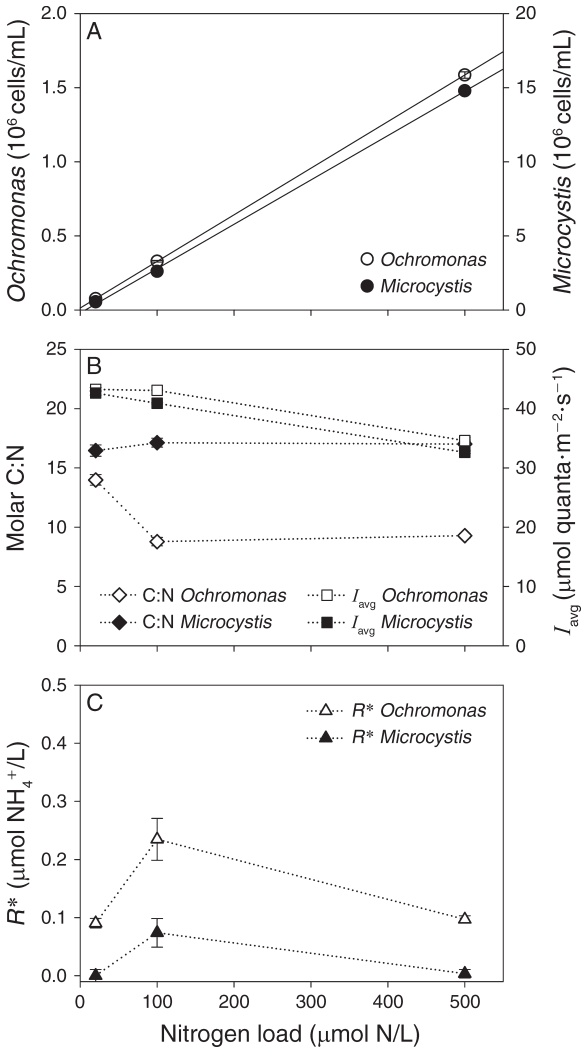

FIG. 3. Steady-state characteristics of the monocultures plotted as function of the nitrogen load. (A) Steady-state abundances of *Ochromonas* (open circles) and *Microcystis* (solid circles) in the monoculture experiments. (B) Molar C:N ratios of the cells (diamonds) and depth-averaged light intensity (I_{avg}; squares) in the monocultures. (C) Concentration of dissolved ammonium (R^*) in the monocultures. Open triangles indicate *Ochromonas*, while solid triangles indicate *Microcystis*. Error bars indicate standard errors of repeated measurements during steady state ($n = 12$ measurements).

Summarizing, several of the model predictions are supported by the experiments. However, the experimental results clearly contradict the expectation based on prediction 3, that mixotrophs (intraguild predators) will dominate at high nutrient loads, while autotrophs (intraguild prey) will decrease with increasing nutrient loads.

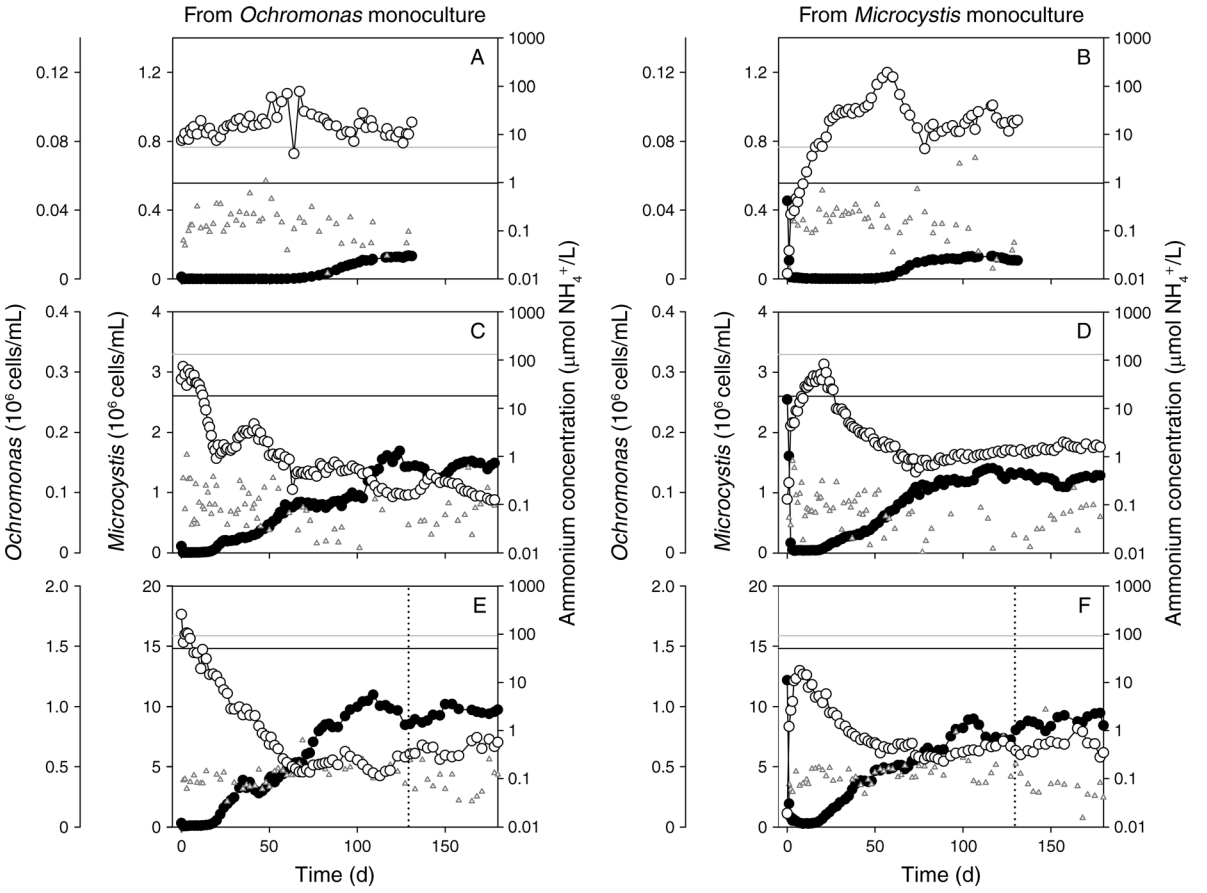

FIG. 4. Intraguild predation experiments. In panels (A, C, and E), a small amount of *Microcystis* was inoculated in monocultures of *Ochromonas*. In panels (B, D, and F), a small amount of *Ochromonas* was inoculated in monocultures of *Microcystis*. The experiments were performed at (A, B) low nitrogen load of 20 μmol NH_4^+/L, (C, D) intermediate nitrogen load of 100 μmol NH_4^+/L, and (E, F) high nitrogen load of 500 μmol NH_4^+/L. Open circles are *Ochromonas*, solid circles are *Microcystis*, open triangles show ammonium concentration. For comparison, horizontal lines give the steady-state abundances of *Ochromonas* (gray lines) and *Microcystis* (black lines) in monoculture. The vertical dotted lines in panels (E and F) indicate the time point at which the incident light intensity was increased.

Explanations for the Discrepancy Between Theory and Experiments

Similar to our findings, earlier studies have been puzzled by the persistence of intraguild prey species in productive environments (Holt and Polis 1997, Amarasekare 2007). This has recently inspired a series of theoretical papers analyzing possible mechanisms that allow coexistence of intraguild predators and their prey (Křivan and Diehl 2005, Finke and Denno 2006, Janssen et al. 2007, Amarasekare 2008, Abrams and Fung 2010, Urbani and Ramos-Jiliberto 2010, Hin et al. 2011). However, only very few studies have tested these mechanisms experimentally (e.g., Amarasekare 2007). Here, we experimentally explore several potential mechanisms to find a suitable explanation for the observed lack of biological control of the intraguild prey population at high nitrogen loads. Detailed descriptions of the experimental methods can be found in the Appendix.

Genetic diversity within the prey.—Genetic variation within the prey population can result in rapid prey evolution (Yoshida et al. 2003), and may select for predator-resistant genotypes that allow persistence of the prey at high predator densities (Meyer et al. 2006). We therefore tested for genetic variation in the *Microcystis* population using denaturing gradient gel electrophoresis (DGGE) of three different parts of the internal transcribed spacer (ITS) between the 16S and 23S rRNA coding regions. This approach allows detection of different *Microcystis* strains at high resolution (Janse et al. 2003, Kardinaal et al. 2007*a*). Samples from our chemostat resulted in only one band in the DGGE gel, confirming the presence of only the strain PCC 7806 in the chemostats (Fig. 6). Hence, we did not detect genetic variation within the *Microcystis* population, at least not at the ITS region. We note that some degree of mutation-derived genetic variation might also occur within a clonal culture and would escape detection by

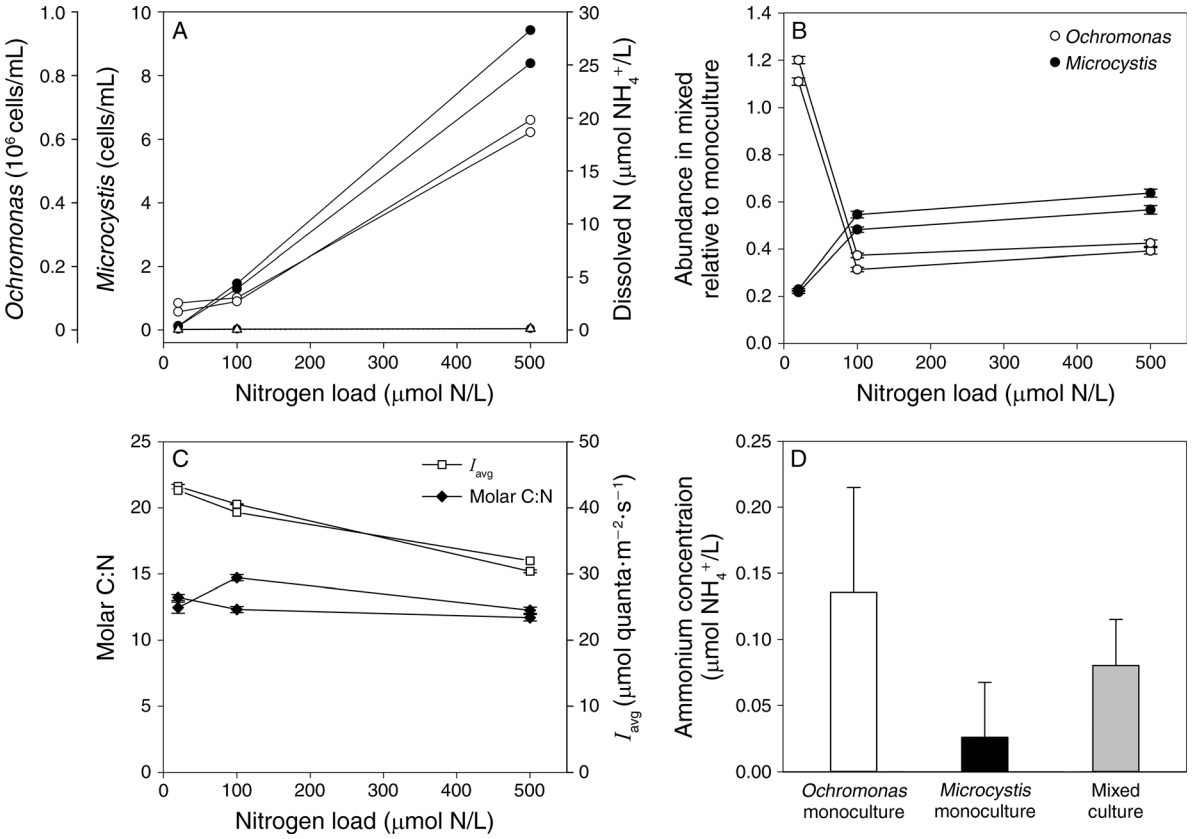

FIG. 5. Steady-state characteristics of the intraguild predation experiments plotted as function of the nitrogen load. (A) Steady-state abundances of *Ochromonas* (open circles) and *Microcystis* (solid circles) in the intraguild predation experiments. The open triangles show the steady-state concentration of dissolved ammonium. (B) The abundances of *Ochromonas* and *Microcystis* in the intraguild predation experiments relative to their abundances in monoculture. (C) Depth-averaged light intensity (I_{avg}) and molar C:N ratio of the species mixture in the intraguild predation experiment. (D) Steady-state dissolved ammonium concentrations of the *Ochromonas* and *Microcystis* monocultures compared to the intraguild predation experiment. The bars show the means (±SD) calculated over all three nitrogen loads using 12 replicate measurements per steady-state culture.

DGGE of the ITS region. Yet, it seems unlikely that de novo mutations during the experiments can explain our results, because the similarity of the trajectories in the different chemostats indicates that this would require similar mutations at almost the same time points in several independent chemostats. Hence, although we cannot exclude the possibility of prey evolution in our chemostats, it does not seem to be a very plausible explanation of our results.

Inducible defenses within the prey.—Predator resistance can also be induced as a phenotypic response of the prey to enhanced predation pressure. Such inducible defenses can prevent oscillations in predator-prey systems (Verschoor et al. 2004) and may enable intraguild prey to coexist with their intraguild predators at high levels of productivity (Abrams and Fung 2010, Urbani and Ramos-Jiliberto 2010). We used a standard protocol to test for inducible defenses (Hessen and Van Donk 1993), in which we exposed the intraguild prey *Microcystis* to the filtrates of other *Microcystis* cultures grown either with or without *Ochromonas* as grazer. In case of an inducible defense, one would expect a lower grazing rate on the "induced" *Microcystis*. However, grazing rates were about 20 % higher on the induced *Microcystis* than on the control (Student's t test, $t_4 = -7.53$, $P < 0.005$; data not shown). Thus, there was no evidence for an inducible defense against grazing in *Microcystis*.

Changes in nutritional quality of the prey.—The nutritional quality of prey species may vary along nutrient gradients (Sterner and Elser 2002, Andersen et al. 2004). Because predatory chrysophytes can alter their grazing rates in response to changes in the nitrogen content of their prey (John and Davidson 2001), we tested whether nitrogen deprivation of the intraguild prey *Microcystis* affected the performance of its predator *Ochromonas*. The results show that nitrogen deprivation caused a clear decrease of the cellular nitrogen content of *Microcystis* (Fig. 7A). Although this did not lead to a decrease in grazing rate (Fig. 7B), it strongly suppressed the growth rate of *Ochromonas* on N-deprived *Microcystis* cells (Fig. 7C). This suggests, however, that *Ochromonas* will be a weaker predator at low nitrogen loads, whereas our chemostat experiments

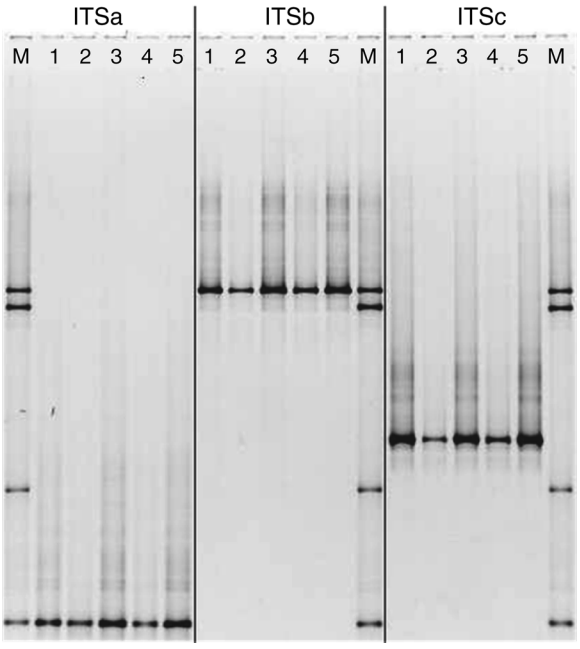

FIG. 6. DGGE-gels for the ITSa, ITSb, and ITSc regions of *Microcystis* sampled from the experiments performed at high nitrogen load of 500 μmol ammonium/L. The lanes represent the following samples: M, marker; 1, day 48 of monoculture; 2, day 9 of mixed culture; 3, day 24 of mixed culture; 4, day 78 of mixed culture; 5, day 165 of mixed culture.

indicated a reduced predation pressure at high nitrogen loads. Hence, it seems unlikely that such a change in nutritional quality of *Microcystis* can explain the observed patterns in population abundance.

A shift toward competition for light.—An increase in nutrient load allows accumulation of higher biomass, which reduces light availability due to self-shading, and may shift the species interactions from competition for nutrients to competition for light (Passarge et al. 2006, Brauer et al. 2012). Assuming that the autotroph *Microcystis* is the better competitor for light, this may tend to favor *Microcystis* at high nitrogen loads. Light limitation might furthermore result in lower grazing rates by the mixotroph (Li et al. 1999), and thus reduce the grazing pressure on *Microcystis*. However, the linear increase in population density with nitrogen load provides strong evidence that nitrogen was the limiting factor across all nitrogen levels that we applied (Fig. 3A). This is further supported by the consistently high C:N ratio of *Microcystis* of ~17 (Fig. 3B). This high C:N ratio is typical for nitrogen-limited growth of *Microcystis*, while it is known to have a much lower C:N ratio of 6–7 under light-limited conditions (Van de Waal et al. 2009). The depth-averaged light intensity was slightly reduced in the chemostats receiving the highest nitrogen load (Figs. 3B and 5C), but this mild degree of self-shading seems insufficient to shift the species interactions to competition for light. For comparison, dense chemostat cultures with lots of self shading reduce I_{avg} to much lower values under light-limited conditions (e.g., Huisman et al. 1999, Passarge et al. 2006, Kardinaal et al. 2007*b*, Van de Waal et al. 2009). As a final piece of evidence to corroborate the absence of light limitation, we increased the light supply (I_{in}) for the chemostats with the highest nitrogen load to reach the same depth-averaged light intensity as in the chemostats with lower nitrogen loads. The time point at which the light supply was increased is indicated by the vertical dotted line in Fig. 4E and F. The results show that an increase in the light supply did not affect the steady-state abundances of the species. Hence, we conclude that even the chemostat experiments at the highest nitrogen load were nitrogen limited rather than light limited. A shift toward competition for light is therefore unlikely to explain the observed increase of the *Microcystis* population with nutrient enrichment.

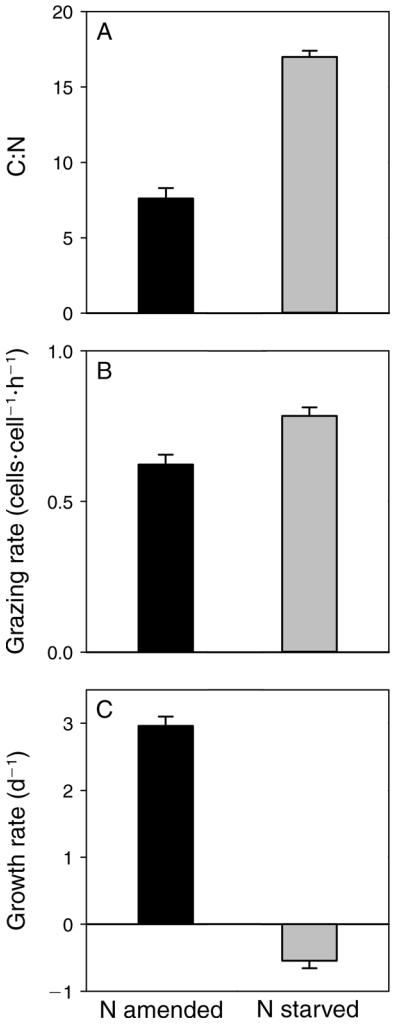

FIG. 7. Differences in nutritional quality of N-amended vs. N-starved cells of *Microcystis*. (A) C:N ratios of *Microcystis*. (B) Grazing rates of *Ochromonas* on *Microcystis*. (C) Growth rates of *Ochromonas* on *Microcystis*. Error bars show standard deviations of triplicate measurements.

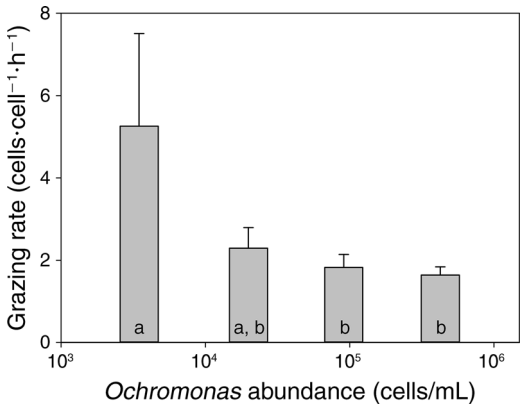

FIG. 8. Grazing rates of *Ochromonas* on *Microcystis* as function of *Ochromonas* abundance. Error bars show standard deviations of triplicate measurements. Bars with different letters are significantly different (ANOVA after log-transformation of data to improve homogeneity of variance: $F_{3,8} = 8.03$; $P < 0.01$).

Intraspecific interference within the predator population.—Grazing rates of the predators may depend not only on prey abundance, but may also change with the abundance of the predator. For instance, grazing rates may decrease with increasing predator abundances due to intraspecific interference within the predator population, which may allow coexistence of the intraguild predator and prey at high levels of productivity (Amarasekare 2008). In mixotrophic populations, physical interactions among the individuals might interfere with the ingestion of prey, while nutrient uptake by mixotrophs is less likely to be affected by interference. Because we observed physical interactions among *Ochromonas* individuals under the microscope, we tested for intraspecific predation interference within *Ochromonas* by investigating the dependence of its grazing rates on its own population density. This confirmed that grazing rates decreased at high abundances of *Ochromonas* (Fig. 8). These results suggest that intraspecific predation interference might explain the reduced predation pressure of *Ochromonas* on *Microcystis* at high nitrogen loads.

To explore this possible explanation in further detail, we rewrote the type III functional response to account for intraspecific interference among the predators (DeAngelis et al. 1975, Skalski and Gilliam 2001)

$$f_M(A,M) = \frac{f_{\max}(A/k_M)^b}{1 + (A/k_M)^b + cM}. \quad (9)$$

The functional response thus becomes a function $f_M(A,M)$ of the abundances of both the autotroph and mixotroph. The parameter c determines the strength of the interference. We fitted c by minimization of the residual sum of squares using the Gauss-Marquardt-Levenberg algorithm in the software package PEST (Watermark Numerical Computing, Brisbane, Australia). All other parameters remained the same as in Table 1. Compared to the original model without predation interference (Fig. 9A), the incorporation of predation interference resulted in a much better fit of the model to our experimental data (Fig. 9B). In particular, the model predicts that predation interference will enable a strong increase of the autotroph *Microcystis* with increasing nutrient loads, in line with our observations. Therefore, interference within the intraguild predator population offers a possible explanation for the discrepancy between the experimental results and the theoretical expectations.

DISCUSSION

Our experimental results show that the mixotroph *Ochromonas* effectively suppressed the toxic cyanobacterium *Microcystis* at low nutrient loads, but not at high nutrient loads. Instead, *Microcystis* and its mixotrophic predator coexisted across the entire productivity range applied in our experiments. Based on intraguild predation theory, a necessary (but not sufficient) condition for coexistence is that the autotrophic prey *Microcystis* is the better competitor for the shared resource (Holt and

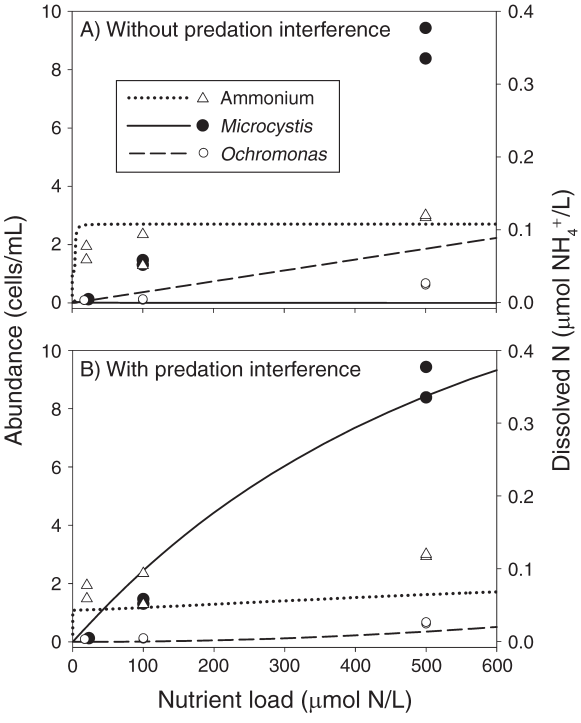

FIG. 9. Model predictions of the intraguild predation model (A) without predation interference and (B) when intraspecific interference among the predators is included (Eq. 9). The graphs show steady-state abundances of the autotroph *Microcystis*, the mixotroph *Ochromonas*, and the dissolved ammonium concentration as a function of the nutrient load. Symbols represent experimental measurements, while lines give model predictions. Parameter values are the same as in Fig. 1 (see Table 1). The interference parameter c takes a value of 2.6×10^{-4} L/cell. In some cases, the measurements from the two independent species mixtures were too similar to be visibly distinguishable in the graph.

Polis 1997). Indeed, *Microcystis* had a lower critical ammonium concentration (i.e., a lower R^*) than *Ochromonas* during autotrophic growth (Fig. 3C), and hence is the better competitor for ammonium (Tilman 1982, Smith and Waltman 1994). The lower competitive strength of *Ochromonas* might reflect the high costs of mixotrophic growth, which requires investments into the biochemical machinery for both autotrophic and heterotrophic nutrition (Raven 1997).

Our results contradict the model prediction that the intraguild prey would be more strongly suppressed with increasing nutrient load, which is widely regarded as one of the key predictions of intraguild predation theory (Holt and Polis 1997, Diehl and Feissel 2000, Mylius et al. 2001). Its function as intraguild predator enabled *Ochromonas* to strongly suppress *Microcystis* at the lowest nitrogen load. However, although *Ochromonas* increased in abundance with a further increase in nitrogen load, this did not result in a stronger suppression of its prey. Instead, the autotrophic prey *Microcystis* increased in abundance with increasing nutrient load, both in absolute terms and relative to its abundance in monoculture (Fig. 5A, B). The ability of *Ochromonas* to suppress *Microcystis* therefore declined at high nitrogen loads.

Motivated by earlier studies aiming to explain the frequently observed coexistence of intraguild prey and predator species (e.g., Liess and Diehl 2006, Amarasekare 2008, Abrams and Fung 2010, Urbani and Ramos-Jiliberto 2010), we explored a number of potential explanations for our experimental results. One interesting aspect of our experiments is that the intraguild predator *Ochromonas* uses a type III functional response (Wilken et al. 2010), whereas most previous theory developed for intraguild predation assumed a type I or type II functional response (e.g., Holt and Polis 1997, Diehl and Feissel 2000; but see Gismervik and Andersen 1997). The type III functional response indicates that *Ochromonas* switches to autotrophic growth when prey becomes rare, which reduces the per capita mortality rate of the prey species. As a consequence, a type III functional response enables persistence of the intraguild prey irrespective of the nutrient load. Yet, despite its persistence, the model predicts that incorporation of the type III response will still lead to a reduction in prey abundance with increasing nutrient load (Fig. 1). Hence, the type III response is not sufficient to explain the experimental results. Furthermore, we did not find experimental evidence for several other possible explanations for the observed pattern, such as genetic diversity within the prey, inducible defenses, a reduced nutritional quality, or a shift from competition for nutrient to competition for light. However, we did find experimental support for intraspecific interference within the *Ochromonas* population, which reduced the grazing rate upon *Microcystis* when *Ochromonas* became more abundant.

Intraspecific interference can suppress the extent to which predator populations increase with nutrient enrichment and has been reported by several zooplankton studies. For example, chemical interference is believed to cause intraspecific density dependence in small cladocerans, while cannibalism has been noted on naupliar stages of copepods (Declerck et al. 2003, Basedow and Tande 2006). Intraspecific interference among the intraguild predator would explain several of our findings. First, given the high ingestion rates that *Ochromonas* can achieve when grazing on *Microcystis*, it should be able to control *Microcystis* growth even at the abundances reached in our experiment. The inability to control *Microcystis* more strongly can therefore not simply be ascribed to an inability of *Ochromonas* to reach higher abundances; something must have suppressed its grazing rate. Second, at the higher nutrient loads, *Ochromonas* reached lower abundances as intraguild predator in the species mixture than as autotroph in monoculture (Fig. 5B). This is rather counterintuitive, because one would expect that addition of prey to an autotrophically growing mixotroph should increase its population abundance, especially if the mixotroph has a preference for heterotrophic growth. Our interpretation of this counterintuitive result is that (1) *Microcystis* is the better competitor for ammonium and thereby suppresses opportunities for autotrophic growth of *Ochromonas*, while (2) at high population abundances *Ochromonas* cannot graze efficiently on *Microcystis* due to intraspecific interference. Third, model simulations show that predation interference indeed leads to a relatively strong increase of the autotroph with increasing nutrient loads, in line with our experimental results. Therefore, interference within the intraguild predator population offers a plausible mechanism for the inability of *Ochromonas* to suppress *Microcystis* at high nutrient loads.

However, the list of mechanisms we tested experimentally is not exhaustive and we can therefore not exclude other factors that were not investigated. For instance, the temporal dynamics suggest a slow decrease in grazing pressure before *Microcystis* abundances started to increase again in the mixed cultures (Fig. 4). It could well be that, due to genotypic or phenotypic changes, *Ochromonas* might have inactivated part of its cellular machinery for heterotrophic metabolism once *Microcystis* was suppressed to sufficiently low abundances. That is, *Ochromonas* might have shifted its trophic position from an intraguild predator to a predominantly autotrophic competitor during the experiment. Since *Ochromonas* is a weaker competitor for ammonium than *Microcystis*, this might explain the initial decline and subsequent partial recovery of the *Microcystis* populations observed in several of the experiments (Fig. 4B, D, F). While evolution of the nutritional strategy of mixotrophs has been shown experimentally (Reboud and Bell 1997, Bell 2013), we have not developed new theory or ran additional

experiments to investigate such alternative explanations in further detail.

Natural communities offer a much more complex setting than our experimental system. The abundance of *Ochromonas* is typically lower in natural communities than in our chemostat experiments (Van Donk et al. 2009), which will most likely reduce the importance of predation interference due to lower encounter rates among *Ochromonas* individuals. However, other factors preventing control of *Ochromonas* over its intraguild prey might become more important in natural waters. For instance, although our *Microcystis* strain remained single-celled, *Ochromonas* is known to induce colony formation in some other strains of *Microcystis* (Burkert et al. 2001), and the large *Microcystis* colonies often observed in eutrophic lakes offer efficient protection against grazing by *Ochromonas* (Yang and Kong 2012). In addition, grazing by protists can act as selection factor on *Microcystis* populations, and evolution toward less edible strains is likely to occur in natural populations (Van Wichelen et al. 2010). Furthermore, in their natural habitat *Ochromonas* and *Microcystis* will not interact in isolation. *Ochromonas* can utilize alternative prey organisms, such as heterotrophic bacteria (Wilken et al. 2013*b*), while both species are subject to predation by and competition with other species. All these aspects may affect the abundance patterns of *Microcystis* and *Ochromonas* along natural productivity gradients.

Nutrient enrichment of aquatic ecosystems, through agriculture, urbanization, and other human activities, has increased the risk of toxic cyanobacterial blooms (Chorus and Bartram 1999, Huisman et al. 2005, Paerl and Huisman 2008). To what extent can mixotrophic species like *Ochromonas* suppress or even prevent cyanobacterial bloom development? Our experiments showed that *Ochromonas* initially reduced the abundance of the toxic cyanobacterium *Microcystis* by more than 97%. After several weeks, however, *Microcystis* partially recovered to about 20% of its original monoculture abundance at low nitrogen loads, and to about 50–60% of its monoculture abundance at high nitrogen loads. Furthermore, in previous experiments we showed that *Ochromonas* cannot grow on nitrate, and therefore *Ochromonas* is capable to suppress *Microcystis* populations much more strongly with ammonium than with nitrate as nitrogen source (Wilken et al. 2014). Taken together, these findings suggest that mixotrophic chrysophytes like *Ochromonas* are most effective as biological control agents of toxic cyanobacteria in relatively oligotrophic waters with ammonium as the dominant nitrogen source. In eutrophic waters, where the problem of cyanobacterial blooms is generally most severe, a newly invading *Ochromonas* population may have a transient effect but will ultimately have less control over cyanobacterial populations.

In conclusion, while intraguild predators may act as effective biological control agents against pest species, our study highlights the potential of several mechanisms to reduce this control. Understanding the relative importance of these mechanisms is crucial to make predictions about the possibilities for biological control in specific communities. The mixotroph *Ochromonas* is capable of strongly suppressing toxic cyanobacteria at low nutrient loads, but intraspecific predation interference is one of the mechanisms preventing its control over toxic cyanobacteria at high nutrient loads. Hence, although *Ochromonas* does reduce the harmful cyanobacterium *Microcystis* to some extent, it is unlikely to be a very effective biological control agent against toxic cyanobacterial blooms in eutrophic waters.

Acknowledgments

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Supplemental Material

Appendix

Detailed methods of additional experiments to explain the lack of biological control at high nitrogen loads (*Ecological Archives* A024-073-A1).