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Response of Phytoplankton Community to Low-Dose Atrazine Exposure Combined with Phosphorus Fluctuations

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Abstract The effects of atrazine on a controlled phytoplankton community derived from a natural freshwater wetland exposed to low doses of this photosynthesis-inhibiting herbicide were examined. The community was exposed for 7 weeks to doses of 0.1, 1, and 10 μg L^{-1} atrazine, combined with changes in nutrient concentration, and the photosynthetic activity, biomass, and community structure were noted during the experiment. Responses of the phytoplankton community were examined in terms of photosynthetic activity, biomass, and community structure. Significant effects of atrazine on the phytoplankton assemblage, in terms of primary production and community structure, were highlighted, even at doses as low as 1 and 0.1 μg L^{-1}, when associated with phosphorus fluctuations. The most abundant Chlorophyceae decreased in concentration with increasing atrazine dose, whereas cyanobacteria were more tolerant to atrazine, particularly with increased nutrient supply. The subinhibitory doses of atrazine used in the present study confirmed the higher sensitivity of long-term exposure of multispecies assemblages under resource competition. Our study supports the emerging hypothesis that the increasing prevalence of cyanobacterial blooms in European aquatic systems may result from a combination of unbalanced nutrient enrichment and selective pressures from multiple toxicants.

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Many organic pollutants associated with human activity, such as herbicides, are extensively disseminated in aquatic ecosystems. As most of them persist in the environment at relatively low concentrations, they raise toxicological concerns for ecosystems, particularly when present as mixtures (Schwarzenbach et al. 2006). Experimental studies have shown additive or synergistic effects with mixed concentrations of micropollutants, although none were present at levels permitting detection of the individual effects, and even with mixtures of compounds with different modes of action (Altenburger et al. 2004). However, it is still not known how long-term exposure to organic pollutants, particularly herbicides, even at low doses will affect aquatic communities and, in turn, ecosystem functioning. The toxicological aspect associated with the components and the ecological issue related to the complexity of the interactions in ecosystems both need to be understood when assessing the impact of pollutants in aquatic systems (Schwarzenbach et al. 2006). The interaction between nutrient availability and pollutant exposure is an emerging ecological issue that merits further investigation.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), of the triazine family, is one of the herbicides most widely used in the world, primarily for maize and sorghum crop protection. Despite the recent prohibition of atrazine in several countries in western Europe, including France, it is still the herbicide most frequently detected in surface waters and groundwater (IFEN 2006). The aquatic and terrestrial

toxicity of atrazine and its metabolites has been well documented in bioassays in a large variety of groups of organisms. In 2000, more than 90% of the atrazine ecotoxicology studies collected for the Pesticide Database (613 references in the "Toxicity Studies for Atrazine on Phytoplankton" category) concerned doses $\geq$10 μg L^{-1}, the lowest doses having a nonsignificant effect in toxicity tests. Environmental doses of atrazine reported at most agricultural sites in Michigan did not exceed 2 μg L^{-1}, except for a short period of time after storm events or field application in Spring, (Murphy et al. 2006) and more than 90% of the surface water sampled in western France in 2000 contained <1 μg/L of atrazine (DIREN Bertagne 2001), which is still 10-fold higher than the European standard policy requirement for drinking water (IFEN 2006). The maximum level for drinking water recommended by the World Health Organization is 2 μg L^{-1} (World Health Organization 2000).

Environmentally low doses of atrazine in aquatic systems have been reported to decrease frog populations by disrupting normal sexual development (Hayes et al. 2003). Phytoplankton may also be affected by the photosynthesis-inhibiting effect of atrazine (Leboulanger et al. 2001), with subsequent effects on ecosystem functioning. Low doses of atrazine which were not acutely toxic to phytoplankton in monospecific bioassays were recently suspected to have a significant impact on global ecosystems (Graymore et al. 2001), by reducing the abundance and diversity of herbivorous populations, for example.

Atrazine inputs in surface waters may not just decrease phytoplankton biomass and primary production. If low doses of atrazine are viewed as an additional constraint for the microalgal community, their impact will be compatible with the intermediate disturbance hypothesis of Connell (1978). In this model, which has been successfully adapted and applied to phytoplankton communities (Reynolds et al. 1993; Hambright and Zohary 2000), maximum species diversity is attained at an intermediate degree of environmental disturbance. Environmentally low doses of atrazine, which can be viewed as a sublethal disturbance, may affect the structure of the community.

Experiments with algal communities under constant nutrient concentration are not representative of either limited or excess nutrient fluctuations in natural environments (Jarvie et al. 2006; Donohue and Irvine 2008). Temporal and spatial changes in nutrient concentration may affect the community's tolerance to toxic substances (Navarro et al. 2002). Changes in nutrient availability would modify the phytoplankton "steady-state" assemblage observed in culturing experiments, as defined by Naselli-Flores et al. (2003), while long-term exposure to atrazine would favor stress-tolerant species.

Our objectives in this study, given that nutrient fluctuations were expected to induce transient nonequilibrium dynamics of the community, were to investigate the response of microalgal dynamics to long-term exposure (7 weeks) to increasing atrazine doses, which were non-inhibiting during short-term exposure (a few days). The experiment was performed on a controlled phytoplankton community, sampled from the natural environment and maintained in semicontinuous culture. The dynamics of the phytoplankton community, exposed to a gradient of atrazine doses in the short or long term, were examined from photosynthetic activity (short-term exposure) or from photosynthetic activity, biomass, and community structure (long-term exposure).

Methods

Atrazine Doses

Commercial pure atrazine (Atrazine Pestanal, Riedel-de-Haën) was used for short- and long-term exposure experiments. An aqueous atrazine solution consisting of 1.2 μg mL^{-1} was prepared by dissolving atrazine (2-chloro − 4-ethylamino-6-isopropylamino-*s*-triazine) in distilled water using sonication without any organic solvent.

To ensure that the water used for the experiments was atrazine-free, filtered water samples were tested for absence of atrazine, i.e., for doses below the limit of detection (0.03 μg atrazine L^{-1}) by HPLC. Details of the HPLC method used are given by Alekseeva et al. (2006).

Phytoplankton Community Culture

The natural plankton community was collected by sampling 40 liters of water from a freshwater wetland in Brittany (Pleine-Fougères, France) in autumn 2001. The macrozooplankton community was removed by filtering the water sample through a plankton net of 50-μm mesh, and no individual was recorded after dual optical control in flasks and during counting under microscope. The resulting controlled community was obtained by semicontinuous culture. In accordance with Sommer's work (1995), the competitive exclusion of species was limited by discontinuously diluting (25%) the phytoplanktonic community with fresh medium once a week. The fresh medium input consisted of water collected from the wetland, filtered through 0.2-μm polycarbonate filters to remove all bacterial and phytoplankton cells, stored at 5°C, and supplied with nitrate (final concentration, 350 μg $N\text{-}NO_3^-$ L^{-1}) and phosphate (final concentration, 52.6 μg $P\text{-}PO_4^{3-}$ L^{-1}) before use. Because an equivalent volume was removed for analysis, the total volume remained constant. These semicontinuous cultures were incubated at 20°C, with a photoperiod of 12 h light and 12 h dark. Lighting of $\sim$160 μmol of photons m^{-2} s^{-1}

was provided by cool-white fluorescent lamps. At the beginning of the experiment, the community consisted of eight species (concentration, >100 cells mL^{-1}) and was mainly dominated by two Chlorophyceae: *Oocystis* sp. and *Selenastrum bibraianum* (Table 1).

Short-Term Sensitivity of the Phytoplankton Community to Atrazine

Short-term experiments (48 h of atrazine exposure) were first performed to determine the noninhibiting doses to test in the long-term atrazine exposure experiment. Five nominal atrazine doses (controls and 0.05, 0.5, 5.0, and 50 μg L^{-1}) were tested on the community. Phytoplankton photosynthetic activity was monitored (five replicates per atrazine dose) from the rate of ^{14}C incorporation (Steemann-Nielsen 1952), after 1, 16, 22, 40, and 46 h of atrazine exposure in borosilicate glass vessels. These were incubated with ^{14}C bicarbonates for 1 h in a temperature-controlled incubator, under light and temperature conditions similar to those for the initial phytoplankton community. The EC50 and EC10, which correspond to the inhibitory doses reducing 50% or 10% of the biological activity, respectively, were calculated by adjusting the model of Bailer and Oris (1993; 1997) to the incorporation rates according to atrazine dose.

Design of the Long-Term Atrazine Exposure Experiments

The experiment was designed to test the effects of low-dose atrazine on a phytoplankton community exposed to changes in nutrient concentration during the experiment. The initial community was first starved by reducing nutrient inputs and making it phosphorus deficient a few weeks before starting the long-term experiment. Phosphorus was then resupplied to obtain medium input (52.6 μg $P\text{-}PO_4^{3-}$ L^{-1}) from the day of atrazine input (day 0) onward. The successive constraint factors were atrazine toxicity under nutrient-replete conditions, followed by the combined impact of atrazine toxicity and nutrient limitation when the phytoplankton assemblage reached a new steady state (biomass stabilization). After several weeks, competition for nutrients between species then progressively increased during the long-term test due to the higher nutrient consumption rate with increased biomass.

Long-term experiments (7 weeks of atrazine exposure) were performed in 500-mL borosilicate Erlenmeyer bottles. Two hundred forty milliliters of the phytoplankton community was placed in each of the 16 bottles, with four replicates per dose tested (controls and nominal doses: 0.1, 1.0, and 10 μg atrazine L^{-1}). Semicontinuous culture conditions were maintained (discontinuous fresh input once a week), and similar atrazine doses were added weekly to each medium input to prevent dilution. Given that the atrazine half-life in a water column is between 80 and 150 days (Klassen and Kadoum 1979), the atrazine dose was assumed to remain almost constant throughout the 7 weeks of the experiment, i.e., more than 80% of the initial dose at the lowest half-life calculation (day 48).

The phytoplankton biomass measured by chlorophyll *a* concentration (Lorenzen 1967) and the biological activity measured by carbon dioxide incorporation (Steeman-Nielsen 1952) were examined once a week, whereas the taxonomic composition was determined at the beginning of the experiment, then after 1, 4, and 7 weeks of toxicant exposure. Fluctuations in the biomass of the coexisting species were evaluated by counting cells and colonies under a microscope using a Nageotte chamber and expressing the concentrations as cell units per milliliter for single-cell species and colony cell units per milliliter for colonial species (*Aphanocapsa* sp., *Oscillatoria* sp.).

Table 1 Specific composition of the community at the beginning (initial density) and at the end (final density) of the long-term experiment as a function of nominal atrazine dose (0, 0.1, 1.0, or 10 μg L^{-1})

Class	Genus	Initial density (mL^{-1})	Final density (mL^{-1}) ± standard deviation			
			0 μg L^{-1}	0.1 μg L^{-1}	1.0 μg L^{-1}	10 μg L^{-1}
Chlorophyceae	*Selenastrum bibraianum*	88,600 ± 7,294	6,800 ± 2,000	4,900 ± 1,300	7,500 ± 1,000	5,300 ± 1,300
	Chlorolobion sp.	8,600 ± 1,800	1,600 ± 1,100 a	430 ± 360 b	970 ± 690 b	740 ± 170 b
	Ankistrodesmus falcatus	4,400 ± 1,600	2,700 ± 900	780 ± 520	1,700 ± 1,200	910 ± 670
	Chlorella sp.	2,200 ± 1,000	960 ± 400 a	790 ± 530 a	300 ± 130 b	260 ± 90 b
	Oocystis sp.	153,300 ± 9,700	68,000 ± 12,000 a	21,000 ± 8,000 b	27,000 ± 5,000 b	15,000 ± 6.000 b
	Unidentified chlorophyte	3,500 ± 2,300	2,100 ± 300	1,200 ± 700	1,200 ± 400	930 ± 420
Cyanobacteria	*Aphanocapsa* sp.	500 ± 900	11,000 ± 1,000 a	6,600 ± 2,400 b	8,000 ± 1,200 b	7,400 ± 1,800 b
	Oscillatoria limnetica	1,500 ± 1,300	530 ± 190	110 ± 140	320 ± 200	270 ± 270

Note: *Scenedesmus* sp. was also present, but at a density lower than 150 cells mL^{-1}. For each species, a ≠ b at $p < 0.05$

Dominance within the community was characterized by calculating Simpson's index D of diversity according to Simpson (1949) as follows:

$$1 - D = 1 - \frac{\sum_{i=1}^{S} n_i(_i - 1)}{N(N-1)}$$

This index D varies between 0 and 1 and represents the probability that two cells or colonies randomly isolated from the community belong to the same species. The higher the value $(1 - D)$, the higher the diversity and the lower the dominance with maximal diversity when $(1 - D)$ tends to 1.

Statistical Analysis

The nonparametric two-factor statistical analysis of Friedman was used to test the effects of atrazine doses and time exposures on the rate of carbon dioxide incorporation (for short- and long-term experiments) and the evolution of biomass. A chi-square analysis was applied to test the homogeneity of community structures at different times and doses. The hypothesis that atrazine has no effect on the evolution of species densities was tested in a single-factor (dose) or two-factor (time and dose) analysis of variance. Lastly, a Kruskall-Wallis analysis was applied to test the effects of atrazine on Simpson's index of diversity. In all statistical tests used, the probability level up to which the effect is significant was $p < 0.05$. A factorial correspondence analysis, using software R (Benzécri 1973), was performed to see whether atrazine exposure was a predominant factor responsible for the final composition and structure of the phytoplankton assemblage.

Results

Short-Term Sensitivity of the Community to Atrazine Exposure

Atrazine-treated samples showed significantly lower biological activity than controls ($S = 42.58$, 4 df, $p < 0.001$). Communities exposed to 50 µg L^{-1} atrazine were inhibited after as little as 1 h with a 30% to 50% decrease in carbon incorporation (Fig. 1a). Results with longer exposure times, 16, 22, 40, and 46 h, confirmed this atrazine toxicity level (Fig. 1a). The calculated effective dose of atrazine, which reduced 50% of the activity (EC50), was 60.7 ± 4.0 µg L^{-1} (mean ± standard error), and the EC10, estimated from the different dose-response curves (Fig. 1b; Bailer and Oris model), was about 27.6 ± 4.4 µg L^{-1}. The carbon incorporation of samples treated with

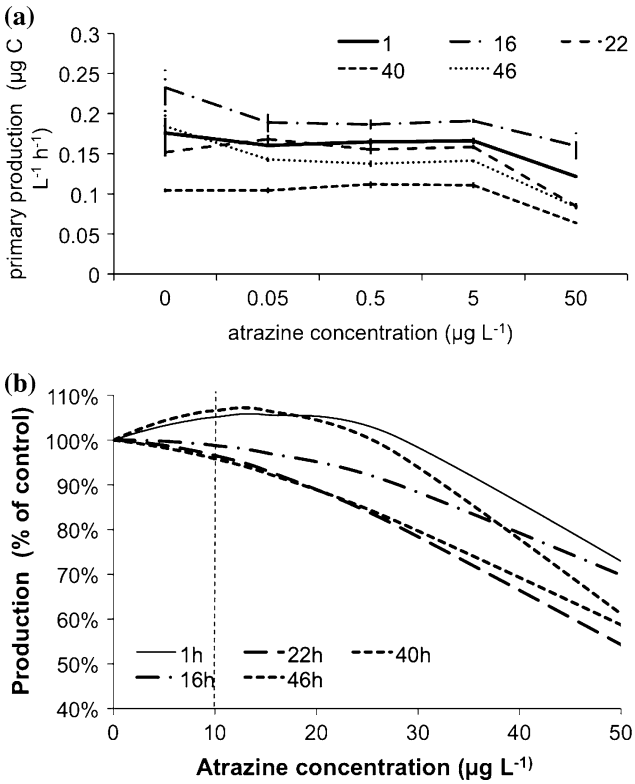

Fig. 1 (**a**) Primary production (carbon dioxide incorporation rates) of the phytoplankton community, exposed for 1, 16, 22, 40, and 46 h to different atrazine concentrations (control series mean ± standard deviation = 2.0 ± 0.1 µgC L^{-1} h^{-1}; $n = 4$). Five replicates were used to adjust the Bailer and Oris model. (**b**) Dose-response curves calculated from the adjustment of the Bailer and Oris' model for the five exposure times. The vertical dotted line shows the highest concentration tested during the long-term exposure experiment

10 µg L^{-1} would probably decrease by <5% during long-term exposure. The European limit for atrazine concentration in drinking water (maximum contaminant level) is 0.1 µg L^{-1}, which is 600 times less concentrated than the EC50 of the tested microalgal community (Fig. 1b). Based on the above results and European water policies, doses ranging from 0.1 to 10 µg L^{-1} were used to examine the long-term effects of atrazine exposure.

Long-Term Atrazine Exposure

Biological Activity and Biomass

The changes in primary production over time, based on the amounts of incorporated carbon dioxide, followed the same pattern regardless of atrazine treatment (Fig. 2). Carbon incorporation, following the resupply of phosphorus on day 0, increased up to the fourth week, then decreased to its initial value. Carbon incorporation by the community after 3 weeks was shown to decrease significantly in comparison

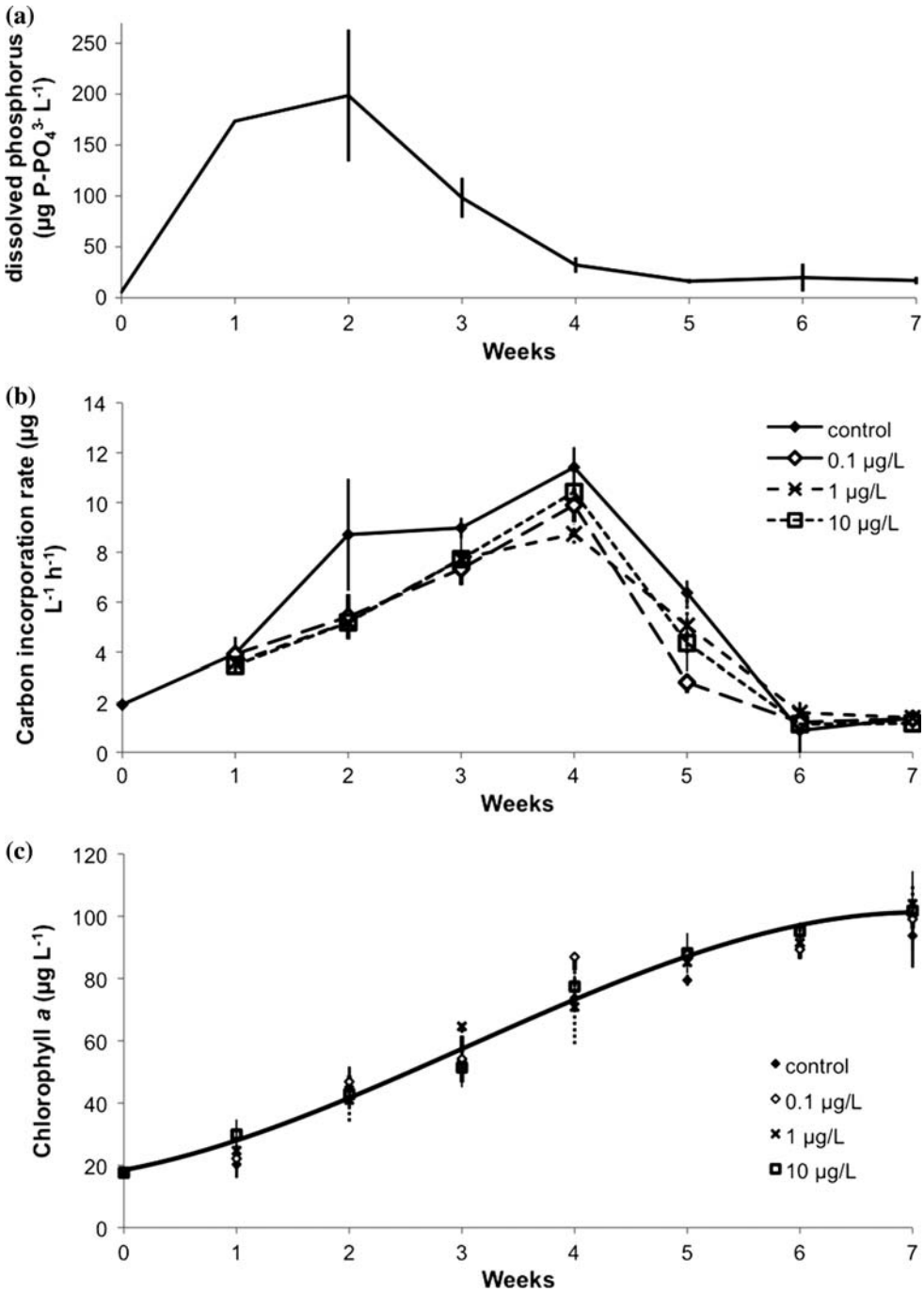

Fig. 2 Temporal pattern of (**a**) the mean phosphorus concentration, (**b**) the carbon incorporation rates (mean values ± standard error; $n = 4$) of the phytoplankton community during the 7 weeks of atrazine exposure as a function of treatments (control and nominal concentration of 0.1, 1.0, and 10 µg L^{-1}), and (**c**) the chlorophyll *a* concentrations. A logistic growth model of Verhulst-Pearl ($r^2 = 0.98$; fitted parameters—initial biomass, 16.0; growth rate, 0.655; maximal capacity K, 103.8) was used to fit the temporal pattern of chlorophyll *a* biomass, all treatments included; no significant differences were observed between treatments

to the control, even with 0.1 µg L^{-1} atrazine (ANOVA and Tuckey comparison tests, $S = 11.61$, 3 df, $p < 0.01$). Chlorophyll *a* concentration increased progressively, according to a logistic function ($R^2 = 0.98$; Fig. 2c). No significant difference was observed between controls and treated communities (Fig. 2c). In the first period, increasing growth rates (C absorption rates) measured on output effluents were significant of nutrient resupply (still available even 1 week after P-enriched medium input), which induced progressive biomass accumulation (chlorophyll *a* abundance). In the second period, i.e., after 4 weeks, the growth rate decreased with accelerated nutrient impoverishment, due to the higher phytoplankton biomass. Whereas biomass production exceeded loss due to weekly culture removal in the first period, it simply compensated this loss during the second period, as the phytoplankton biomass remained almost stable.

Community Structure and Dominance

The major impact on community structure, as shown by comparison of the initial and final densities of enumerated

cells or colonies, has to be attributed to the biomass response following P resupply (Table 1).

Modification of the community structure due to low-dose atrazine exposure was also shown after 7 weeks of exposure, with a clear atrazine dose effect (Fig. 3). The first axis explained more than 61% of the variance of the data set. Going from left to right, the ordination on axis F1 differentiated the control communities from the atrazine-exposed communities, at doses of 10, 1, and even 0.1 μg L^{-1}, although the effect of the 0.1 μg L^{-1} dose showed high variability.

The species contributing to the F1 axis were *Aphanocapsa* sp. (40%), *Oocystis* sp. (31%), and *Selenastrum* sp. (27%). It was concluded that community structure could be significantly affected by 7 weeks of exposure to a dose of atrazine as low as 10 μg L^{-1} or less.

In accordance with the correspondence analysis and depending on the atrazine low dose, significant changes in algal density occurred (Table 1). The density of the small Chlorophyceae *Oocystis* sp. (Fig. 4a) decreased with time and atrazine dose (ANOVA: dose of atrazine, $F = 6.51$, 3 df, $p = 0.001$; time, $F = 85.57$, 2 df, $p < 0.001$). Cell density in the nonexposed community decreased by a factor of four between the beginning and the end of the experiment but decreased almost 10-fold after exposure to 10 μg atrazine L^{-1}. The second dominant Chlorophyceae *Selenastrum* sp. decreased with time, but irrespective of atrazine dose (Fig. 4b). Only the cyanobacteria *Aphanocapsa* sp. (Fig. 4c) increased in density during the 7-week experiment in all atrazine treatments. At the end of the experiment, it was significantly higher in the controls than in the treated samples ($H = 8.18$, 3 df, $p < 0.05$). The density of Chlorophyceae *Chlorella* sp. initially decreased in all atrazine treatments during the first week, then increased until the fourth week and decreased again between the fourth and the seventh week (Fig. 4d). The control contained the highest concentration of cells from the fourth week (Fig. 4d) and the cell concentration at the end of the experiment was significantly higher in the control than in the atrazine treatments ($F = 4.2$, 3 df, $p < 0.05$). The colony density of the cyanobacteria *Oscillatoria* sp. increased during the first week in all treatments, then decreased until the end of the experiment (data not shown). The population density in the controls was higher than in the treated communities (ANOVA: dose of atrazine, $F = 2.88$, 3 df, $p < 0.05$; time, $F = 22.40$, 2 df, $p < 0.001$).

One of the main consequences of low atrazine exposure was a change in dominance and diversity, as demonstrated by changes in Simpson's index of diversity ($1 - D$) (Fig. 5). After 7 weeks of exposure, ($1 - D$) was significantly higher in the community exposed to atrazine than in the controls ($H = 9.2$, 3 df, $p = 0.027$). This higher diversity accounted for a lower dominance in the atrazine treatments. The ($1 - D$) value was proportional to the atrazine dose, demonstrating a consistent dose-response effect.

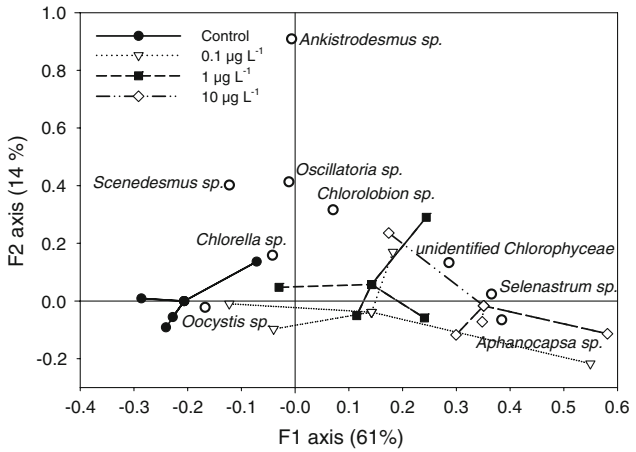

Fig. 3 Correspondence analysis of the phytoplankton communities, after 7 weeks of atrazine exposure (control and nominal concentration of 0.1, 1.0, and 10 μg L^{-1}). Each replicate is connected to the baricenter of the treatment group

Discussion

In the environment, each community has its own particular sensitivity to atrazine, which depends on species composition and pre-exposure to the toxicant (Gustavson and Wangberg 1995). The range of EC50 reported in the literature is large and the values depend on both culture conditions and end points, such as photosynthetic capacity, growth, and biomass. For instance, the EC50 values in laboratory culture are between 42 and 53 μg L^{-1} for *Chlorella vulgaris*, 300 μg L^{-1} for *Scenedesmus quadricauda,* 60 μg L^{-1} for *Ankistrodesmus braunii*, and 776 μg L^{-1} for *Oscillatoria lutea* (Solomon et al. 1996). Although the EC50 values were derived from different end points, most ecotoxicological studies report an EC50 of more than 100 μg L^{-1} for less than 2 days of exposure (Solomon et al. 1996). Depending on the biological parameter of interest, sensitivity may differ for the same species, particularly when the biological parameter is the target of the toxicant. Atrazine, because it inhibits photophosphorylation during photosynthesis, should first influence photosynthetic activity and C absorption rate. In short-term exposure, the EC50 for the primary production as end point was 60.7 ± 4.0 μg L^{-1}, which is in the low range of values reported in the literature (Solomon et al. 1996). In the long-term exposure experiment, a significant effect of the 10 μg L^{-1} atrazine dose on C absorption rate was observed from the first to the last week of the experiment. A maximum 25% decrease in primary production was obtained during long-term exposure, whereas this was less than 5% during short-term exposure.

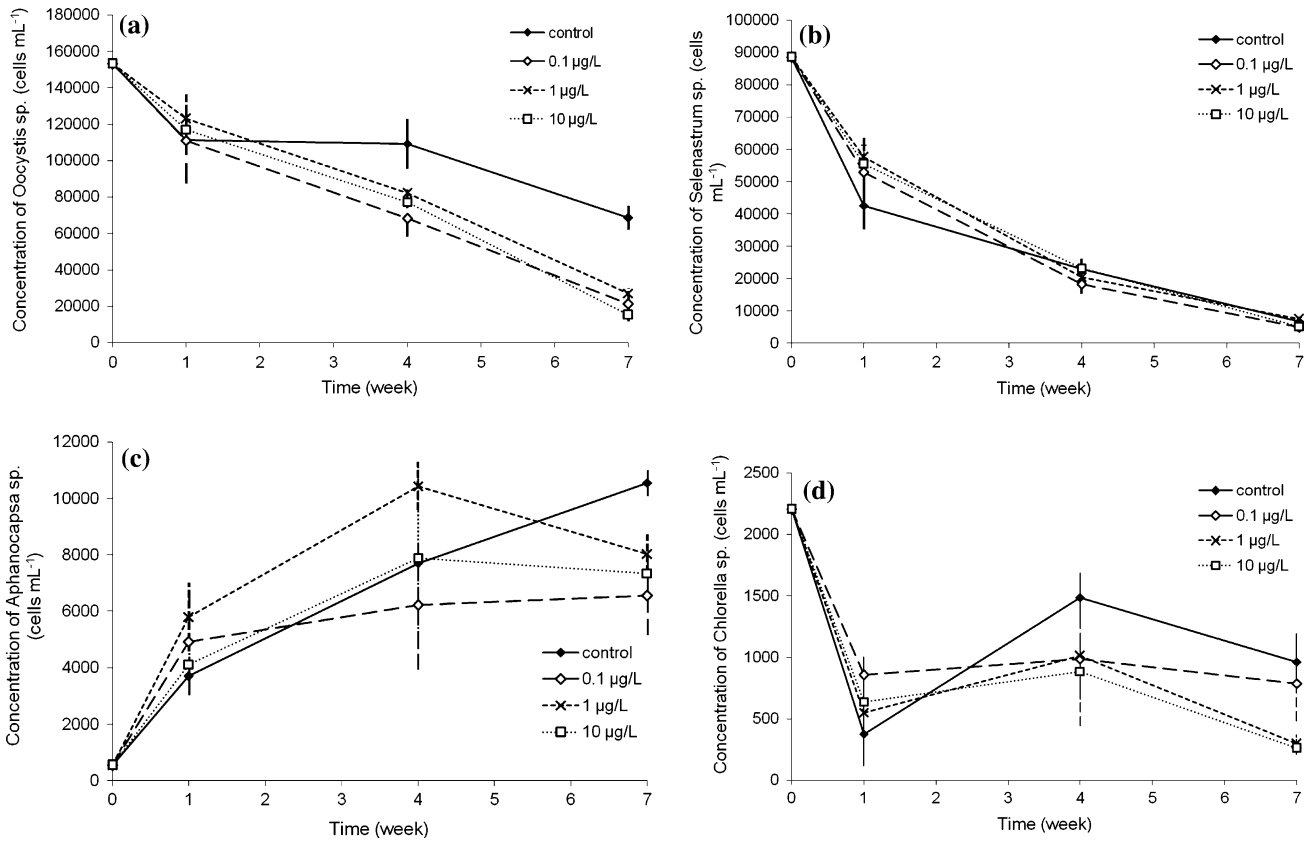

Fig. 4 Concentration of the different species (mean ± standard deviation; $n = 4$): (**a**) *Oocystis* sp., (**b**) *Selenastrum bibraianum,* (**c**) *Aphanocapsa* sp., and (**d**) *Chlorella* sp. during the 7 weeks of incubation for each treatment (control and nominal concentration of 0.1 µg, 1.0, and 10 µg L^{-1})

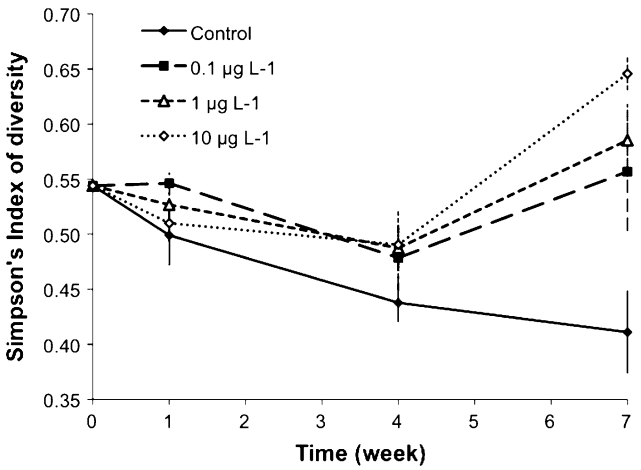

Fig. 5 Changes in Simpson's index of diversity, $(1 - D)$ (mean ± standard deviation; $n = 4$) during the 7 weeks of exposure to the different atrazine treatments (control and nominal concentration of 0.1, 1.0, and 10 µg L^{-1})

Although a significant reduction in carbon incorporation was observed with atrazine exposure, the chlorophyll *a* concentration was multiplied fivefold in all treatments. Considering that carbon incorporation is proportional to organic matter synthesis, the biomass increase would also have been influenced by atrazine dose. Different hypotheses can be evoked to interpret the nonsignificant effect of atrazine on the biomass estimator, i.e., cell content of chlorophyll *a*. First, this result might be due to an adaptative physiological response consisting of an increase in intracellular chlorophyll *a* concentration to compensate the chlorophyll *a* sites inactivated by the atrazine molecule. It has indeed been demonstrated that atrazine can increase the concentration of chlorophyll *a* per cell after 2 weeks of exposure to 10 µg L^{-1} (Gustavson and Wangberg 1995). Second, the impact of atrazine on the structural dynamics of the phytoplankton community might have compensated any changes in biomass, as the intracellular chlorophyll *a* concentration varies between species (Nicholls and Dillon 1978). More probably, the impact of low doses of atrazine on biomass production remained undetectable in comparison to nutrient impact.

All phytoplankton species initially present were maintained throughout the long-term experiment, and different responses, to both nutrient and atrazine combined, were observed, depending on the species. The final nutrient concentrations (350 µg $N\text{-}NO_3^-$ L^{-1} and 52.6 µg $P\text{-}PO_4^{3-}$ L^{-1})

were similar to summer nitrate and phosphate concentrations observed in the wetland, i.e., 230 ± 70 μg $N\text{-}NO_3^-$ L^{-1} and 38.9 ± 3.3 μg $P\text{-}PO_4^{3-}$ L^{-1} respectively. *Aphanocapsa* sp. is characteristic of a shallow nutrient-rich water (Reynolds et al. 2002). This species increased in density in response to initial phosphorus input, from the beginning until the end of the experiment, whereas *Chlorella* sp. increased after the first week, indicating a delayed nutrient effect. The very low atrazine dose of 0.1 μg L^{-1} was sufficient to reduce the cell concentration of *Chlorolobion* sp., *Aphanocapsa* sp., and *Oocystis* sp. deNoyelles et al. (1982) demonstrated the higher sensitivity of *Oocystis* compared with *Cryptomonas* in a natural phytoplankton community exposed to 500 μg atrazine L^{-1} for 19 days. The tendency in other species, such as *Oscillatoria limnetica*, was more complex, with fluctuations in density. This species can be stimulated or inhibited in mixed-species cultures, depending on the temperature and interspecific competition (Bérard et al. 1999). Chlorophytes are known to be more sensitive to atrazine inhibition than diatoms and cyanobacteria (Bérard et al. 2003). Acting as a light limitation, the toxicity of a photosystem II (PSII) inhibitor favored cyanobacteria that have the capacity to adapt to low light through accessory pigment composition (Koenig 1990) or alternative carbon fixation pathways (Egorova and Bukhov 2006). The saturation of PSII by the herbicide may also occur more rapidly in a cell with a lower PSII/PSI ratio. The response of the community tested here is mainly attributed to the relative sensitivities of the component species. The community consisted of small-sized species, with mean biovolumes of 300 or <100 μm^3 (*Oocystis* sp., *Ankistrodesmus* sp., and *Chlorella* sp.). Species sensitivity appeared to be well correlated with the surface/volume ratio of cells (Tang et al. 1998; Lockert et al. 2006), small algal cells with a high ratio accumulating significantly more toxicant per cell than larger ones with a lower ratio. This could be one reason why even low doses of atrazine influenced the phytoplankton community in both long- and short-exposure experiments. The high sensitivity of the initial phytoplankton community may also have resulted from prior low exposure to atrazine in the natural environment. The presence of toxicant over a very long period can indeed induce an increasing tolerance of phytoplankton to PSII inhibitors (Bérard et al. 1998). Seguin et al. (2002) demonstrated an induction of atrazine tolerance in phytoplankton communities in outdoor freshwater mesocosms contaminated with 30 μg L^{-1} atrazine.

The results presented here highlight significant effects of atrazine on the phytoplankton assemblage, in terms of primary production and community structure, even with doses as low as 1 and 0.1 μg L^{-1}, when associated with phosphorus fluctuations. Doses as low as 0.1 μg L^{-1} or higher values were measured in more than 90% of samples from Brittany in 2004 (IFEN 2006). Solomon and al. (1996) considered in their review that atrazine had significant ecological effects at exposures >20 μg L^{-1}. Huber (1993) did not observe atrazine inhibition of photosynthesis below 20 μg L^{-1}, whereas deNoyelles et al. (1982) reported inhibition of some species at doses as low as 1.0 μg L^{-1}. In the present study, the assemblage exposed for 7 weeks to <0.1 μg L^{-1} could be distinguished from assemblages exposed to ≥1 μg L^{-1}, indicating a high sensitivity of the species. Although fluctuations of species abundance reflected mainly a response to the change in nutrient availability, the analyses of community structure changes, i.e., correspondence analysis of the phytoplanktonic communities and Simpson's index of diversity, clearly revealed an indirect impact of low-dose atrazine exposure on community structure. The presence of atrazine in this case should be considered a chronic stress, which removes the most sensitive organisms, thereby liberating space, which can then be colonized by individuals from opportunist species. The reduced dominance observed here might result from the enclosed conditions: the changes would have a different impact if other microalgal propagules and zooplankton were present. Our phytoplankton assemblage contained a limited number of species, so the interaction effects between species may have been underestimated. Changes in phytoplankton community structure induced by long-term exposure to low doses of atrazine, when combined with nutrient fluctuations, may be magnified in natural open systems.

In contrast to a disturbance, i.e., a relatively discrete event in space and time, a background level of atrazine may represent a persistent constraint for the algal communities. Some studies of complex community competition for nutrients have already revealed a higher sensitivity of algae (Seguin et al. 2001; Bérard et al. 2003). Differential sensitivity of microalgae to atrazine could alter species composition (Tang et al. 1997) and bring about changes in community structure without affecting global biomass or growth rate. Based on the resource competition theory we hypothesize that modification of the resource supply rate, by changing selective pressure (Tilman 1982), enhanced community sensitivity to doses as low as 0.1 μg L^{-1}. Only Lampert et al. (1989), studying the D*aphnia* community response in standing water systems, suspected the impact of doses as low as 0.1 μg L^{-1} on phytoplankton. It may be that feedback operates in more complex systems, such as mesocosms, acting through differential grazing pressure, microbial loops, competition with plants for resources, or nutrient recycling, to buffer the microalgal community response. Even if low doses of herbicide have not been demonstrated to have a direct impact on biomass or primary productivity in natural surface water, they are suspected to affect algal community structure and higher food web levels.

In this study we demonstrated changes in both biological activity and community structure of the microalgal population induced by long-term exposure to low doses of atrazine when combined with phosphorus nutrient fluctuations. Differential responses to low doses of toxicants (herbicides, insecticides, and algaecides) in the environment may initiate a shift in algal group structure due to natural sensitivity and the selection of resistant strains. Cyanobacteria and diatoms seem to be more tolerant to atrazine than green algae (Tang et al. 1997; Lockert et al. 2006). One possible change in natural microalgal populations might be the increased dominance of opportunistic resistant species such as certain cyanobacteria. Some strains are less sensitive than green algae to carbamate insecticides (Ma et al. 2006), and mutations conferring copper-resistance represent a survival advantage after copper sulfate treatments (Garcia-Villada et al. 2004). Our study showed that it is important to consider the impact of the interaction between phosphorus nutrient availability and long-term exposure to a low dose of atrazine on phytoplankton communities. It also supports the hypothesis that the increasing prevalence of cyanobacterial blooms in European aquatic systems may result from a combination of impacts due to humans that include unbalanced nutrient enrichment and selective pressures from multiple toxicants.

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