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Can we predict community-wide effects of herbicides from toxicity tests on macrophyte species?

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ABSTRACT

Macrophyte communities play an essential role in the way freshwater ecosystems function. It is thus of great concern to understand how environmental factors, especially anthropogenic ones, influence their composition and diversity. The aim of this study was to examine whether the effects of a herbicide mixture (50% atrazine, 35% isoproturon, 15% alachlor) on single macrophyte species can be used to predict its impact at a community level. In a first experiment we tested the sensitivity of six species (*Azolla filiculoides*, *Ceratophyllum demersum*, *Elodea canadensis*, *Lemna minor*, *Myriophyllum spicatum* and *Vallisneria spiralis*) grown separately and exposed to 0.6–600 $\mu\text{g L}^{-1}$ of the herbicide mixture. In a second experiment, conducted in microcosms, we tested the effects of herbicides on macrophyte assemblages composed of the same six species exposed to 0, 6 or 60 $\mu\text{g L}^{-1}$ of the herbicide mixture. Species grown separately exhibited growth inhibition at 60 and 600 $\mu\text{g L}^{-1}$. At 600 $\mu\text{g L}^{-1}$ the sensitivity differed significantly between species. *V. spiralis* was the most resistant species, *C. demersum*, *M. spicatum* and *E. canadensis* exhibited intermediate sensitivities, and *A. filiculoides* and *L. minor* were the most sensitive species. In microcosms, community biomass and Shannon evenness index were reduced after 8 weeks at 60 $\mu\text{g L}^{-1}$. Communities also exhibited changes in their composition: the relative and absolute abundance of *C. demersum* increased at 6 $\mu\text{g L}^{-1}$, while the relative abundance of *V. spiralis* increased at 60 $\mu\text{g L}^{-1}$. These results are in agreement with the individual responses of these species to the herbicides. It is therefore concluded that short-term effects of herbicides on simple macrophyte communities can be predicted from the sensitivity of individual species. However, further investigations are required to examine whether longer term effects can be predicted as well, especially in more complex communities.

1. Introduction

Aquatic macrophytes are key elements of freshwater ecosystems. They provide food, shelter and substrate to various aquatic organisms (Carpenter and Lodge, 1986; Lodge, 1991). Moreover, rooted macrophytes influence flow, sediment stability and organic matter retention, and play an important role in oxygenating the water column and superficial sediment layers (Carpenter and Lodge, 1986; Sand-Jensen, 1998; Clarke, 2002). Macrophytes are also pivotal in sustaining water clarity of shallow standing waters, as they efficiently inhibit phytoplankton development via nutrient competition and release of allelopathic compounds (Scheffer et al., 1993). On the other hand, both native and exotic macrophyte species can proliferate in some instances, raising important ecological and economic concerns (Murphy, 1988; Peltre et al., 2002). For these different reasons, macrophytes are often of great importance

to environmental managers who aim to promote some species and control others, and are therefore eager to understand mechanisms of community structuring (Coops et al., 2002).

In natural conditions, the composition and diversity of freshwater macrophyte communities are ruled by various environmental factors, such as hydraulic disturbances, nutrient richness or light availability (Melzer, 1999; Amoros et al., 2000; Lacoul and Freedman, 2006). Biotic interactions, such as competition or herbivory, also play a role in structuring macrophyte communities (Barrat-Segretain, 2005; Elger et al., 2009). In addition, due to the importance of human impacts on freshwater ecosystems, predictive models of macrophyte community structure must take into account anthropogenic pressures such as river regulation, water eutrophication or contamination by xenobiotics. All these natural and anthropogenic determinants can be seen as environmental filters that select some species, based on their life-history traits, from among a local or a regional pool (Keddy, 1992; Clements and Newman, 2002).

Herbicides are the xenobiotics most likely to impact macrophyte communities. Even if we exclude direct applications of these sub-

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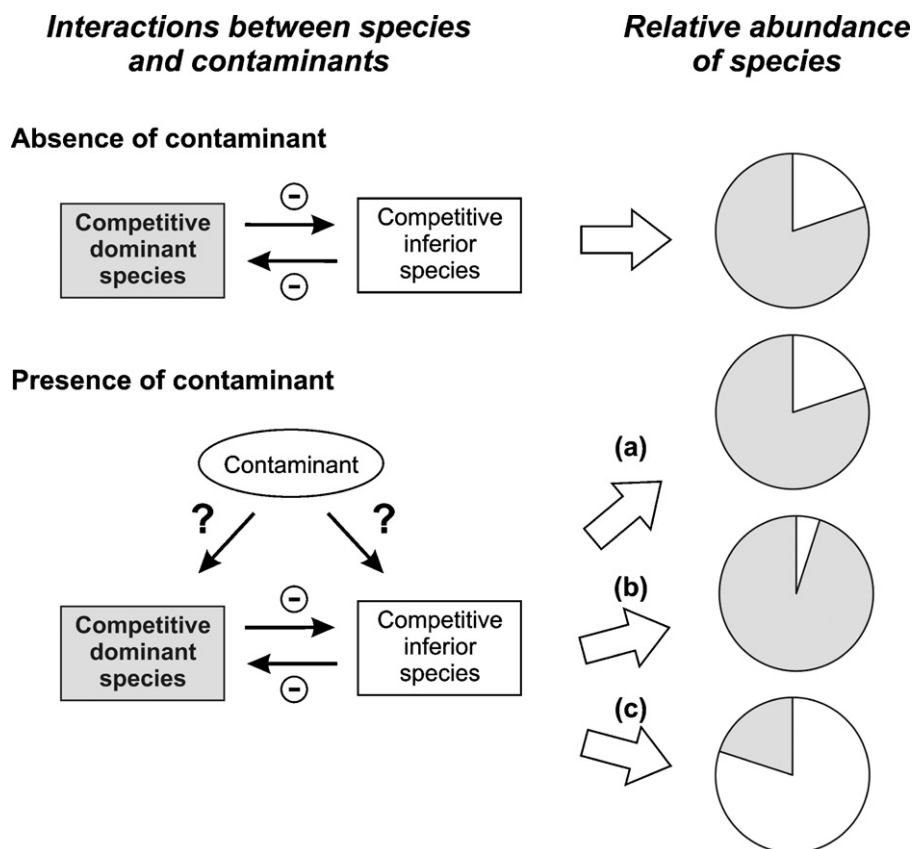


Fig. 1. Effects of a sublethal contaminant in the outcome of competitive interactions between two plant species. In the absence of contaminant we expect the competitively dominant species to be the most abundant in the community. In the presence of contaminant, several outcomes are possible depending on the relative sensitivity of species: (a) if both species are equally sensitive, their relative abundance should remain the same; (b) if the competitively dominant species is less sensitive, its abundance should further increase; (c) if the competitive inferior species is less sensitive, its abundance should increase and possibly lead to a shift in the relative abundance of the two-species (modified, after Rohr et al., 2006).

stances for controlling water plants (Hofstra and Clayton, 2001), herbicides frequently enter freshwater systems and can be present at non-negligible concentrations in water or sediments (Lerch and Blanchard, 2003; Muller et al., 2004). This is particularly the case for pre-emergent herbicides, used in agriculture at a period with low vegetation cover on arable lands, and easily leached to aquatic ecosystems through superficial run-off or drain-water supplies. For instance, in the mid-Garonne River and its tributaries, Devault et al. (2007) found herbicide concentrations above $5 \mu\text{g g}^{-1}$ in sediments (in order of decreasing importance: chloro-s-triazines > substituted phenylureas, chloroacetanilides), and above $2 \mu\text{g L}^{-1}$ in unfiltered water (mainly substituted phenylureas; chloro-s-triazines to a lesser extent). During flood events, herbicide concentrations in water can exceed $10 \mu\text{g L}^{-1}$ (Debenest et al., 2009).

Early investigations have shown that these herbicide concentrations usually found in freshwater ecosystems only have a low inhibitory effect on the growth of common macrophyte species (Jones and Winchell, 1984; Jones et al., 1985). However, because herbicide sensitivity varies across macrophyte species and largely depends on the compound considered (Cedergreen et al., 2004a,b; Fairchild et al., 1998; Lambert et al., 2006), we cannot exclude important community-wide effects of sublethal herbicide concentrations on macrophyte assemblages, such as shifts in the dominance of some species or functional groups of macrophytes. Such shifts might be difficult to predict, as they can result from both direct effects of herbicides on individual species (and therefore be related to the intrinsic sensitivity of the species to the xenobiotics considered) and from indirect effects via the modulation of biotic

interactions involving macrophytes (Rohr et al., 2006). For instance, interspecific competition could be affected by herbicides, due to differential sensitivity among species (Relyea and Hoverman, 2006). This could enhance diversity, if the competitive dominant species is the most sensitive to the herbicides (direct detrimental effect on key species inducing positive indirect effect on other species, *sensu* Rohr et al., 2006). But other trajectories in community structure are also possible (Fig. 1). Despite the need to predict herbicide effects at a community scale, very few studies have assessed the toxicity of such compounds on freshwater plant communities (Coors et al., 2006). Moreover, most of these studies have not examined whether the observed effects could be predicted from individual responses of species to herbicides (but see McGregor et al., 2008), which is crucial information for risk-assessment approaches.

In the present article, we addressed this question through measured growth effects of a herbicide mixture (50% atrazine, 35% isoproturon and 15% alachlor) on six macrophyte species. The choice of compounds and their proportions in the mixture was made so as to represent the three major families of herbicides widely used in agriculture during the last 15 years (i.e. s-triazines, anilides and substituted ureas) (European Commission, 2007). In a first experiment, we tested the effects of a range of concentrations of the herbicide mixture on separately cultivated macrophytes, in order to assess their intrinsic sensitivity. In a second experiment, we studied the response of the species grown together and forming a simple macrophyte community, to the same herbicide mixture.

2. Materials and methods

2.1. Macrophytes

Three species of rooted macrophytes, *Elodea canadensis* Michx., *Myriophyllum spicatum* L. and *Vallisneria spiralis* L., and three species of free-floating macrophytes, *Azolla filiculoides* Lam., *Lemna minor* L. and *Ceratophyllum demersum* L. were studied. The species were chosen to represent natural communities in the mid-part of the Garonne River, SW France, but similar communities can be found elsewhere in Europe. All plants were collected from cut-off channels of this river (Verdun sur Garonne, 43°51'N, 1°15'E) and carried to the laboratory in a cool-box. After collection, species were acclimatized to laboratory conditions for a minimum of 2 weeks prior to experiments. In all bioassays, we used young *V. spiralis* plants without stolons, 12-cm-long shoots of *C. demersum*, *E. canadensis* and *M. spicatum*, fronds of *A. filiculoides* and *L. minor* at the three- to five-leaf stage.

2.2. Herbicides

The herbicide mixture was composed of 50% atrazine, 35% isoproturon and 15% alachlor. Atrazine and isoproturon are photosynthesis inhibitors and alachlor inhibits protein and fatty acid synthesis (Tomlin, 2003). Although banned in France in 2003, atrazine was still present in river sediments several years thereafter (Devault et al., 2007). In addition, a recent study by Barrek et al. (2009) showed that atrazine, alachlor and isoproturon were present in 100%, 95.4%, and 31.8%, respectively, of surface water samples (Rhône-Alpes region, France) at concentrations up to 1.2, 0.6 and 0.6 $\mu\text{g L}^{-1}$, respectively. The herbicide stock solution was prepared by dissolving herbicide crystals (purity >99%, Pestanal, Aldrich) in acetone (pesticide analysis quality, Pestipur, Carlo Erba-SDS). The stock solution was then serially diluted to obtain the herbicide concentrations used in the different experimental treatments.

2.3. Single-species toxicity tests

The setup consisted of 2L glass flasks containing quartz sand (SiO₂ 98.7%, 0.8/1.8 mm diameter, Sifraco, Mios, France) and filled with amended dechlorinated tap-water [resulting concentrations (macronutrients analyzed by high pressure ion chromatography with a Dionex ICS-1000; micronutrients measured using a Perkin-Elmer ELAN 6000 ICP-MS): Ca, 40.00 mg L⁻¹; Na, 19.00 mg L⁻¹; Cl, 8.00 mg L⁻¹; S, 7.00 mg L⁻¹; Mg, 3.50 mg L⁻¹; Fe-EDTA, 1.80 mg L⁻¹; N-NO₃, 1.00 mg L⁻¹; Mn, 0.06 mg L⁻¹; K, 0.04 mg L⁻¹; P, 0.03 mg L⁻¹; B, 0.03 mg L⁻¹; Zn, 0.01 mg L⁻¹; Cu, 0.01 mg L⁻¹; Mo, 0.004 mg L⁻¹]. Each flask received one species represented by one individual, except for *A. filiculoides* and *L. minor* with 3 and 5 fronds, respectively. The experiment lasted 3 weeks. Room temperature was maintained at 21 °C and all plants were cultivated under the same light conditions (12 h:12 h light:dark cycle, artificial light provided by Philips TL5 HO/865 fluorescent tubes, ca. 70 $\mu\text{mol s}^{-1} \text{m}^{-2}$ (PAR) at the water surface). Plants were exposed to 5 concentrations of the herbicide mixture: 0 (control), 0.6, 6, 60 and 600 $\mu\text{g L}^{-1}$ through a single contamination on the first day. The control treatment contained the proportion of acetone (<4% (v/v)) used to dissolve the herbicides for the other treatments. There were 6 replicates for each treatment combination (herbicide concentration \times species). On day 21, macrophytes were harvested, wet-blotted, dried at 70 °C until constant weight and their dry mass (whole plants, including roots) was determined. Conductivity and pH were measured twice a week.

2.4. Community response to herbicides

The microcosms used in this experiment consisted of 100L glass tanks ($L \times W \times H = 49 \text{ cm} \times 38 \text{ cm} \times 50 \text{ cm}$). Experimental conditions were similar to those used in the single-species toxicity test, respecting substrate, water, room temperature, lighting and herbicide mixture. Three herbicide concentrations were used: 0 (control), 6 and 60 $\mu\text{g L}^{-1}$. Each community was composed of 5 *A. filiculoides* fronds, 30 three- to five-leaf stage *L. minor* fronds, 4 *C. demersum* 10-cm-long shoots, 6 *E. canadensis* 12-cm-long shoots, 6 *M. spicatum* 12-cm-long shoots and 6 *V. spiralis* young plants. All plants were collected at the same place and the same day as the plants used in the single-species toxicity test. Individuals of *E. canadensis*, *M. spicatum* and *V. spiralis* were planted in random positions. The density of rooted macrophytes corresponded to 1 plant per 100 cm². The experiment lasted 8 weeks, which was considered to be compatible with establishment of competition between plants, regarding the densities used. In order to avoid phytoplankton proliferation (observed in a pilot experiment) about 30 individuals of *Daphnia* sp. were added to each microcosm. There were 6 replicates for each herbicide concentration. At the end of the experiment, macrophytes were harvested, wet-blotted, dried at 70 °C until constant weight and their dry mass was determined. Conductivity and pH were measured once a week.

2.5. Data analyses

In the first experiment (species sensitivity assessment), the dry mass of species was compared across herbicide concentrations using one-way ANOVA on square-root transformed data (to reach the homoscedasticity assumption), followed by Tukey's honestly significant difference (HSD) test to identify differing concentrations. Then, a growth response was calculated from the dry mass (m) measured for each treatment trial (species i , herbicide concentration j , replicate k) relative to the average dry mass (\bar{m}) measured in the control trials ($j=0$) for the same species:

$$\text{Growth}(\%)_{i,j,k} = \frac{m_{i,j,k}}{\bar{m}_{i,j=0}} \quad (1)$$

Relative growth responses were preferred to absolute biomass values for subsequent analyses, to overcome morphological and growth rate differences between species. These responses were compared across species, separately at the different herbicide concentrations, using one-way ANOVAs (or non-parametric Kruskal–Wallis tests when data could not reach the homoscedasticity assumption), to determine which concentrations induced species-dependent responses (variance heterogeneity across treatments precluded the use of a two-way ANOVA on the complete data set).

For each species, we determined the concentration of herbicides reducing the biomass by 50% (EC₅₀), using a logistic dose–response model:

$$\text{Growth}(\%) = \frac{\text{Growth}(\%)_0}{1 + (\text{Herbicide concentration} / \text{EC}_{50})^n} \quad (2)$$

The parameters EC₅₀ and n were determined for each species, using a non-linear fitting procedure, under the hypothesis $\text{Growth}(\%)_0 = 100\%$.

The relative growth rates (RGRs) of each plant species were determined in the absence of herbicides, according to: $(\ln m_2 - \ln m_1) t^{-1}$, where m_1 and m_2 are initial and final dry masses of a plant sample (initial dry mass was calculated using the water content determined at the end of the experiment) and t is incubation time in days. The possible correlation between mean RGR and herbicide resistance of plant species was then examined using regression analysis.

Table 1

Dry masses after 21 days and relative growth rates (mean \pm SD) of six macrophyte species grown individually in the absence of herbicides (controls).

Species	Dry mass (mg)	RGR (day ⁻¹)
<i>Azolla filiculoides</i>	34.8 \pm 12.1	0.075 \pm 0.007
<i>Ceratophyllum demersum</i>	206.1 \pm 78.8	0.055 \pm 0.012
<i>Elodea canadensis</i>	93.8 \pm 29.1	0.048 \pm 0.018
<i>Lemna minor</i>	38.9 \pm 12.6	0.093 \pm 0.013
<i>Myriophyllum spicatum</i>	99.8 \pm 31.5	0.030 \pm 0.014
<i>Vallisneria spiralis</i>	188.5 \pm 42.5	0.024 \pm 0.007

In the second experiment (herbicide impact at the community level), dry masses of individual species i were used to determine the total community dry mass for the replicate k of each herbicide concentration j , and to calculate the Shannon evenness index (J') as follows (Magurran, 2004):

$$J'_{j,k} = - \frac{\sum_{i=1}^S [(m_{i,j,k}/M_{j,k}) \times \ln(m_{i,j,k}/M_{j,k})]}{\ln S} \quad (3)$$

with S = total number of species (6), m = dry mass of individual species within a community, M = total community dry mass.

Total dry mass, separate dry masses for each plant species and Shannon evenness indices were compared across herbicide concentrations using one-way ANOVA, followed by a Fisher's least significant difference (LSD) post hoc test to identify differing treatments (this test was preferred to the Tukey's HSD test in the present case, because it is less conservative and appropriate for comparing a maximum of 3 treatments). When data did not satisfy ANOVA assumptions, we used the non-parametric Kruskal–Wallis test.

Finally, we used the results obtained in the single-species toxicity tests to calculate the expected composition of macrophyte communities at 6 and 60 $\mu\text{g L}^{-1}$. For this purpose, the final average biomass measured for the different species in the control communities was multiplied by the percent response obtained at the corresponding herbicide concentration in single-species toxicity tests. The similarity between expected and observed communities was globally measured with the Steinhaus's index (Legendre and Legendre, 1998). In addition, for each species, the observed biomass was compared to the expected biomass using a one-sample Student's t -test, with Bonferroni correction.

Statistical analyses were performed using the R package (version 2.10.1.) (The R Foundation for Statistical Computing, Vienna, Austria, © 2009).

3. Results

3.1. Single-species toxicity tests

The final dry mass of species differed significantly depending on the concentration of herbicides (ANOVA after square-root transformation, $F_{4,175} = 12.92$, $P < 0.001$). In the control trials, average (\pm SD) final dry mass ranged between 34.8 \pm 12.1 mg for *A. filiculoides* and 206.1 \pm 78.8 mg for *C. demersum* (Table 1). No significant changes in biomass were observed for concentrations of herbicides below 60 $\mu\text{g L}^{-1}$ (data not shown). For the complete set of species analyzed together, there was a decrease in biomass at 60 $\mu\text{g L}^{-1}$, becoming significantly greater at 600 $\mu\text{g L}^{-1}$ of herbicides (Tukey's HSD test). A similar trend was observed for the pH: pH = 8.6 \pm 0.5 between 0 and 6 $\mu\text{g L}^{-1}$, pH = 8.3 \pm 0.3 at 60 $\mu\text{g L}^{-1}$ and pH = 7.9 \pm 0.2 at 600 $\mu\text{g L}^{-1}$. Differences in relative growth response across species were only significant at 600 $\mu\text{g L}^{-1}$ (Kruskal–Wallis test, $H = 20.97$, $df = 5$, $P < 0.001$). At this concentration, relative growth responses ranged between 3% and 45%, *V. spiralis* being the most resistant species, *M. spicatum*, *C. demersum* and *E. canadensis* exhibiting intermediate sensitivities, and *L. minor* and *A. filiculoides* being the most sensitive

(Fig. 2). The comparison of species sensitivities based on their EC_{50} values gave a different ranking (Table 2). Still, *V. spiralis* was far more resistant than the other species. This was followed by *C. demersum*, *L. minor*, *M. spicatum*, *E. canadensis* and finally *A. filiculoides*. Relative growth rates (RGRs) in the absence of herbicides ranged between 0.024 \pm 0.007 days⁻¹ for *V. spiralis* and 0.093 \pm 0.013 days⁻¹ for *L. minor*. There was a significant negative correlation between RGR and relative growth response of plant species at 600 $\mu\text{g L}^{-1}$ of herbicides (growth (%)) = 0.6493 – 0.2766 ln(RGR), $R = -0.92$, $n = 6$, $P = 0.0084$). A negative relationship was found also between ln(RGR) and EC_{50} , but this was not significant ($R = -0.64$, $n = 6$, $P = 0.1672$).

3.2. Community response to herbicides

Community biomass was significantly influenced by herbicide concentration (one-way ANOVA, $F_{2,15} = 13.50$, $P < 0.001$). This parameter was not significantly different at 0 and 6 $\mu\text{g L}^{-1}$, but did show a significant decrease at 60 $\mu\text{g L}^{-1}$ of herbicides (Fisher's LSD test), being reduced by ca. 30% compared to the control (Fig. 3). Herbicide concentration also had an effect on the Shannon evenness index (one-way ANOVA, $F_{2,15} = 4.56$, $P = 0.028$). This parameter was significantly lower at 60 $\mu\text{g L}^{-1}$ compared to the other treatments (Fisher's LSD test), indicating that species tended to be less evenly abundant as a response to herbicides. Indeed, the relative abundance of species already dominating the community in the absence of herbicides increased under herbicide contamination. The proportion of *C. demersum* was thus increased at 6 $\mu\text{g L}^{-1}$ compared to the control (Tukey's LSD test; Fig. 4), due to an absolute increase of its biomass (data not shown). Similarly, the proportion of *V. spiralis* increased at 60 $\mu\text{g L}^{-1}$ compared to the other treatments. This was due to *V. spiralis* biomass stability, while the other species exhibited a decrease in their biomass. By contrast, the proportion of *A. filiculoides* (already in minority in controls) significantly decreased at 60 $\mu\text{g L}^{-1}$.

The communities observed under herbicide contamination were very similar to what could be expected from the sensitivity of individual species (Fig. 4). The Steinhaus similarity index between observed and expected communities amounted to 96% at 6 $\mu\text{g L}^{-1}$ and to 92% at 60 $\mu\text{g L}^{-1}$. The comparison, for each species, between observed and expected final dry masses did not show any significant difference (t -test after Bonferroni correction).

As in the single-species toxicity tests, the pH was lower at 60 $\mu\text{g L}^{-1}$ than in the control and 6 $\mu\text{g L}^{-1}$ trials.

4. Discussion

Comparison of the results obtained in our single-species toxicity experiment with previously published reports indicates that the EC_{50} values we found are within the ranges reported in the literature (Table 2). We found *A. filiculoides* to be the most sensitive species and *V. spiralis* the least sensitive species to the herbicide mixture, but a ratio of 17 between their EC_{50} values is not very large compared to the variability in sensitivity across species found in other studies conducted on freshwater macrophytes. For instance, Cedergreen et al. (2004b) found a ratio of 56, and Fairchild et al. (1998) a ratio >35. The relative sensitivity of the same species can differ strongly across various studies: while Cedergreen et al. (2004b) found *L. minor* to be significantly less resistant to herbicides than *C. demersum* (which is in agreement with our own results; see Table 2), Fairchild et al. (1998) found exactly the opposite pattern. This is obviously not only related to the mode of action of the herbicides tested, as in the latter study the herbicides used were atrazine and alachlor (two of the compounds included in our mixture). However, interactions between herbicides might explain

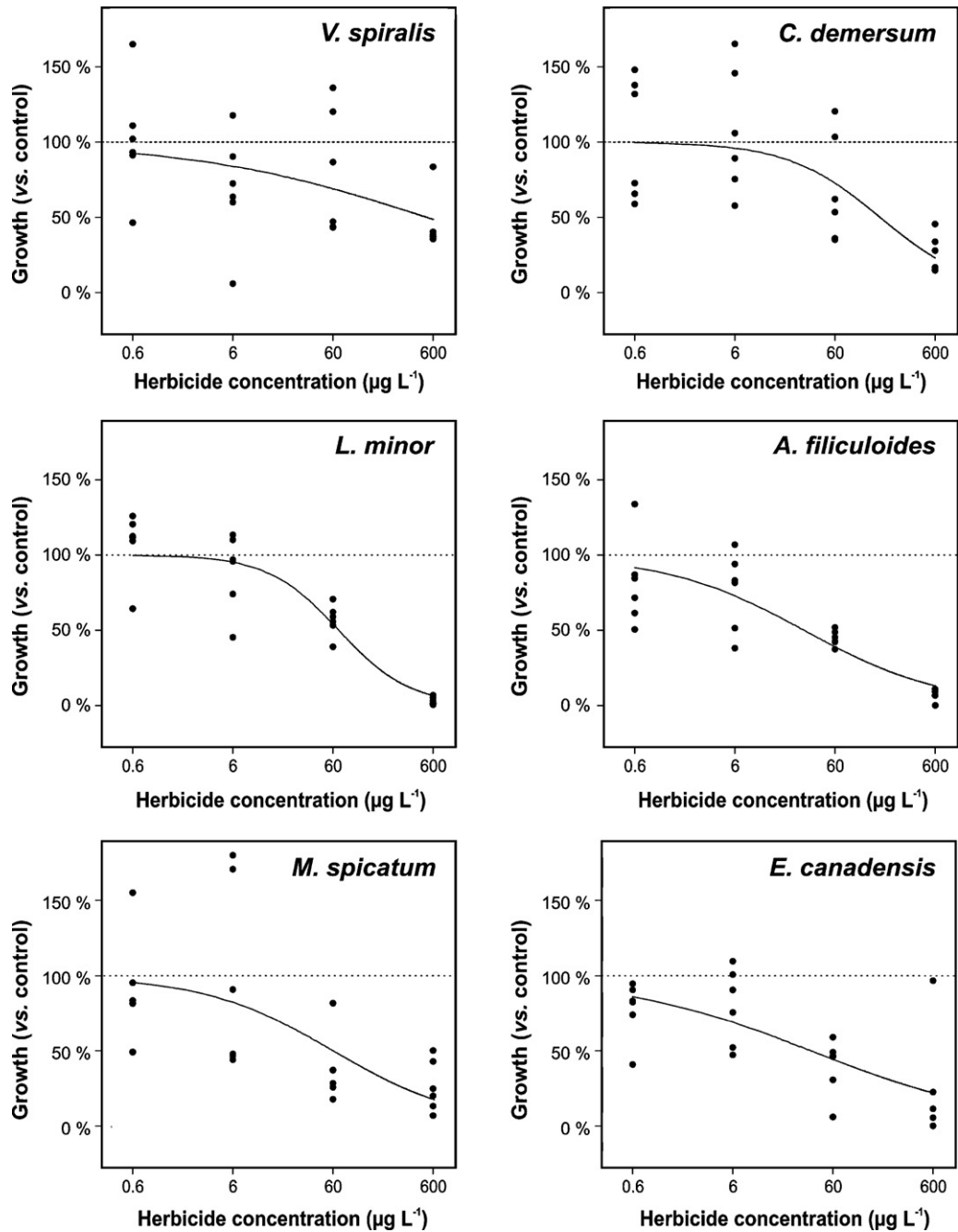


Fig. 2. Growth responses (relative to controls without herbicide) of six macrophyte species grown individually for 21 days at various concentrations of a herbicide mixture (50% atrazine, 35% isoproturon, 15% alachlor).

differential effects of isolated or mixed compounds (Junghans et al., 2006). The experimental conditions (e.g. presence and composition of sediment vs. hydroponic culture, mode of herbicide exposure, experiment duration), but also intrinsic plant factors (e.g. genetic variations between populations, phenotypic plasticity, phenology), might also contribute to such variations. The correlation we found between the growth response of plants to herbicides and their RGR suggests that fast-growing species are more sensitive to the herbicide mixture used than slow-growing species, which has been shown previously for other active compounds such as metsulfuron-methyl (Cedergreen et al., 2004b).

Two of the three compounds used in our study (atrazine and isoproturon) are photosystem II inhibitors, competing with plastoquinone for binding to the D1 protein in the thylakoid membrane

(Trebst, 1987). The third compound (alachlor) inhibits the syntheses of proteins and fatty acids (via the inhibition of elongase and cyclisation enzymes) (Möllers and Albrecht, 1994), and may thus indirectly reduce plant photosynthesis. Photosynthetic inhibition by these herbicides is probably the reason for the pH decrease in our experiments at the highest concentrations, as previously observed by Wendt-Rasch et al. (2003). Such pH variations, however, are unlikely to significantly influence the fitness of the plant species studied as the variations are not very large (less than one pH unit) and many macrophyte species can tolerate a much wider range of pH under natural conditions (Robach et al., 1996).

At high concentrations, the herbicides used in our study can have lethal effects on macrophyte species. However, in field situations, the total concentration of herbicides in surface waters

Table 2
Comparison of herbicide effects on the growth of the macrophyte species studied. Results from the present study are based on a herbicide mixture containing 50% atrazine, 35% isotoproturon and 15% alachlor. Growth EC₅₀ values are model estimates ± standard errors.

Species	Herbicide	Experiment duration	EC ₅₀ (μg L ⁻¹)	Reference
<i>Azolla filiculoides</i>	Atrazine isotoproturon alachlor mixture	21 days	30 ± 12	This study
<i>Ceratophyllum demersum</i>	Atrazine	14 days	22 ± 1	Fairchild et al. (1998)
	Alachlor	14 days	85 ± 7	Fairchild et al. (1998)
	Metsulfuron-methyl	14 days	4.1 ± 3.3	Cedergreen et al. (2004b)
	Atrazine isotoproturon alachlor mixture	21 days	170 ± 92	This study
<i>Elodea canadensis</i>	Atrazine	14 days	21 ^a	Fairchild et al. (1998)
	Alachlor	14 days	>3000 ^b	Fairchild et al. (1998)
	Atrazine isotoproturon alachlor mixture	21 days	36 ± 22	This study
<i>Lemna minor</i>	Atrazine	96 h	92 ± 6	Fairchild et al. (1998)
	Alachlor	96 h	198 ± 60	Fairchild et al. (1998)
	Alachlor	96 h	482 ± 77	Fairchild et al. (1998)
	Metsulfuron-methyl	14 days	0.80 ± 0.15	Cedergreen et al. (2004b)
	Metsulfuron-methyl	14 days	1.13 ± 0.28	Cedergreen et al. (2004b)
	Atrazine isotoproturon alachlor mixture	21 days	70 ± 16	This study
<i>Myriophyllum spicatum</i>	Atrazine isotoproturon alachlor mixture	21 days	59 ± 44	This study
<i>Vallisneria spiralis</i>	Atrazine isotoproturon alachlor mixture	21 days	520 ± 720	This study

^a Value graphically interpolated between control and lowest concentration tested.

^b The EC₅₀ value was greater than the highest concentration tested (i.e. 3000 μg L⁻¹).

seldom exceeds 10 μg L⁻¹, or only during short periods after heavy falls of rain following herbicide spraying (Thurman et al., 1991; Debenest et al., 2009). Hence, the consequences of such herbicide contamination on freshwater macrophytes are usually only

a reduction of photosynthetic activity and/or growth rate, which is not likely to have a strong impact on community structure (Jones and Winchell, 1984; Kemp et al., 1985). However, because different macrophyte species can exhibit different sensitivities (Fig. 2, Table 2), and because they interact in communities, notably competing for light and nutrients and releasing allelopathic compounds (Agami and Waisel, 2002; Barrat-Segretain and Elger, 2004; Wu and Yu, 2004), we cannot exclude that small effects at the plant level induce strong cascading modifications in community structure. This is an important issue that should be taken into account in risk-assessment protocols.

In the present study, species responses in the community experiment were in agreement with the results of the single-species experiment. We can therefore conclude that effects of herbicides on simple macrophyte communities following 8 weeks' exposure can be predicted from the sensitivity of individual species. Our results are in agreement with those obtained by McGregor et al. (2008), who compared the sensitivity of *M. spicatum* and *E. canadensis* to atrazine over 6 weeks in different planting systems (i.e. individuals, populations and two-species communities), and found no significant differences in biomass or relative growth rate between the planting systems.

However, we cannot exclude the possibility of getting different results under other experimental conditions. For instance, higher concentrations of nutrients in water could promote floating species, by increasing competition for light to the detriment of submerged species (Scheffer et al., 2003). In this case, interspecific competition could be affected by herbicides, because of differential sensitivity among species, and potentially lead to a release of competition pressure (Relyea and Hoverman, 2006). This could increase diversity, if the competitive dominant species is the most sensitive to herbicides (Fig. 1c). However, Rohr et al. (2006) suggest that this case, which implies a disproportionately large impact on a competitive dominant, would seldom happen in nature. In addition, other important factors that could also modulate the results of our community experiment are the duration of the monitoring and initial planting densities. Although growth interactions between macrophyte species (including *E. canadensis* and *C. demersum*) have been shown in some studies after a few weeks (Barrat-Segretain and Elger, 2004; Hanson et al., 2006), it is possible that a longer time

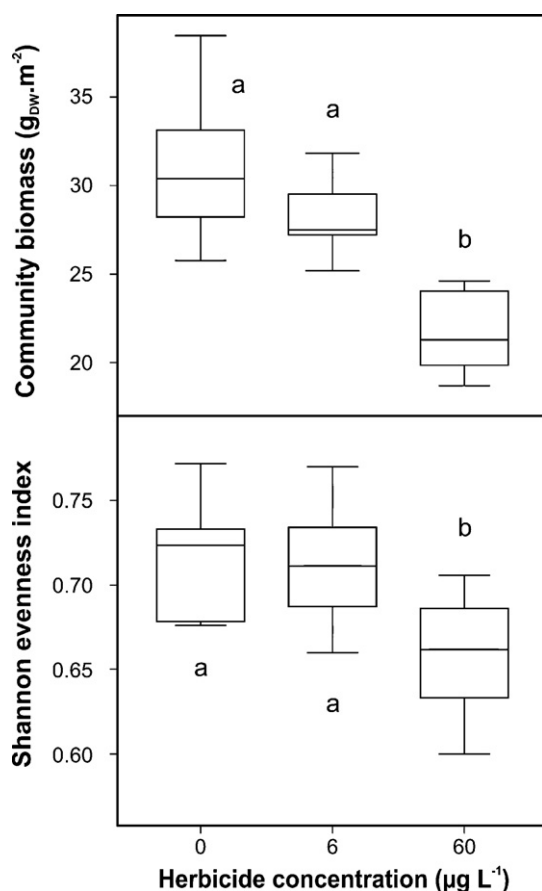


Fig. 3. Macrophyte community structure after 8 weeks at 6 and 60 μg L⁻¹ of a herbicide mixture (50% atrazine, 35% isotoproturon, 15% alachlor). Top: final dry mass of the total community. Bottom: Shannon evenness index. Identical letters indicate treatments that do not differ significantly at $P < 0.05$ ($n = 6$ for all treatments).

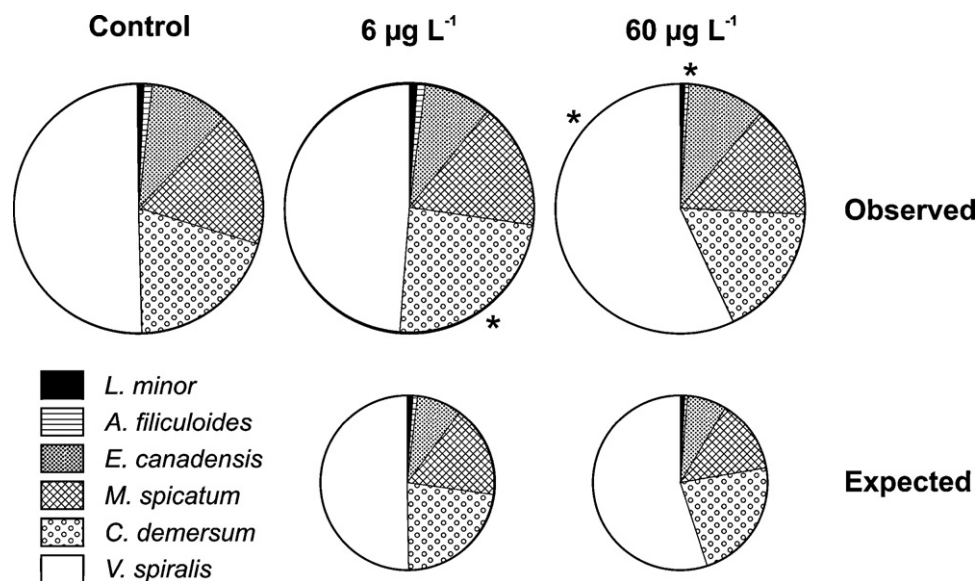


Fig. 4. Relative abundance of macrophyte species in the community at 6 and 60 $\mu\text{g L}^{-1}$ of a herbicide mixture (50% atrazine, 35% isotoproturon, 15% alachlor). Upper row: actual observed abundance in the community experiment (the asterisk indicates a significant difference in the abundance of one species at a given concentration compared to the control; LSD post hoc test, $P < 0.05$). Lower row: expected abundance predicted from the sensitivity of species measured in the single-species experiment.

would have enhanced competitive interactions in our experiment, and that community structure would therefore have followed a different trajectory. For instance, when studying the impact of copper in freshwater mesocosms, Rousset et al. (2007) showed that some effects, including interspecific interactions, appeared only after three months of exposure.

Under natural conditions, other biotic components of the ecosystem could also interfere with the impact of herbicides on plant development. Herbivory, for instance, is an important structuring factor of macrophyte communities (Lodge, 1991; Elger et al., 2009). Contaminants can reduce the density of herbivores or alter their behavior, which might increase plant biomass (Relyea and Hoverman, 2006) and particularly promote palatable species that are usually the most grazed. Therefore, the impact of a contaminant will depend on the density of herbivores, and on their interactions with macrophytes. Similarly, contaminants might alter symbiotic or parasitic interactions in which plants are involved. However, the ultimate consequences of such impacts on plant communities are largely unknown and should be explored both in controlled and natural conditions.

5. Conclusion

Manipulating the presence of species in more or less complex communities is essential to be able to test the role of biotic interactions in xenobiotic ecotoxicity (Rohr and Crumrine, 2005). A major drawback of performing ecotoxicological studies on communities in the absence of corresponding single-species toxicity tests is the inability to discriminate between direct and indirect effects (Rousset et al., 2007). According to Fleeger et al. (2003), microcosm experiments are a very convenient tool for generating and testing hypotheses on the mechanisms by which indirect effects might occur. In the present paper, we proposed a method to compare results obtained from multi- and single-species growth experiments. First results indicate that single-species toxicity tests can be extrapolated to predict impact at a community level, in line with the results previously obtained by McGregor et al. (2008). However, further investigations are required to examine whether longer-term effects can be predicted as well, especially in the case of more complex communities.

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