Impacts of agricultural irrigation on nearby freshwater ecosystems: The seasonal influence of triazine herbicides in benthic algal communities

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HIGHLIGHTS

• Agricultural impacts via run off impacted on nearby freshwater algal communities.
• Triazine herbicides impacts changed through the agricultural year.
• Traditional methods do not capture the seasonality of triazine concentrations.
• Algal tolerance informed about the source of herbicides: application vs. background.
• Assessment of herbicide tolerance is a promising monitoring tool.

Abstract

A small hydrological basin (Lerma, NE Spain), transformed from its natural state (steppe) to rain-fed agriculture and recently to irrigation agriculture, has been monitored across four seasons of an agricultural year. The goal of this study was to assess how and whether agricultural activities impacted the nearby freshwater ecosystems via runoff. Specifically, we assessed the toxicity of three triazine herbicides, terbuthylazine, atrazine and simazine on the photosynthetic efficiency and structure of algal benthic biofilms (i.e., phototropic periphyton) in the small creek draining the basin. It was expected that the seasonal runoff of the herbicides in the creek affected the sensitivity of the periphyton in accord with the rationale of the Pollution Induced Community Tolerance (PICT): the exposure of the community to pollutants result in the replacement of sensitive species by more tolerant ones. In this way, PICT can serve to establish causal linkages between pollutants and the observed biological impacts.

The periphyton presented significantly different sensitivities against terbuthylazine through the year in accord with the seasonal application of this herbicide in the crops nowadays. The sensitivity of already banned herbicides, atrazine and simazine does not display a clear seasonality. The different sensitivities to herbicides were in agreement with the expected exposures scenarios, according to the agricultural calendar, but not with the concentrations measured in water, which altogether indicates that the use of PICT approach may serve for long-term monitoring purposes. That will provide not only causal links between the occurrence of chemicals and their impacts on natural communities, but also information about the occurrence of chemicals that may escape from traditional sampling methods (water analysis). In addition, the EC50 and EC10 of periphyton for terbuthylazine or simazine are the first to be published and can be used for impact assessments.

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1. Introduction

Agriculture uses half of the total land in Europe (Stoate et al., 2009). Traditional agriculture has been replaced by intensive works which maximize the impact on the soil and nearby freshwater ecosystems (De Almeida Azevedo et al., 2000; Loos et al., 2009; Arroita et al., 2013). One way to increase crop production is by implementing irrigation, which affects both physical (altering water flow) and chemical (altering nutrient and pollutant concentrations) conditions in rivers by the runoff of excess waters (Abrahao et al., 2011a, 2011b; Merchán et al., 2013). In the Mediterranean climate irrigation is more intense during spring and summer, in those seasons natural rivers have lower flows so the impacts of the runoff waters from irrigation may be maximized.

This study is focused on the impacts of the triazine family of herbicides which is widely used in Europe. Due to environmental concerns, some triazines have been banned (such as atrazine, simazine and propazine European Commission (SANCO/10496/2003-final), 2003;
European Commission (SANCO/10495/2003-final), 2003). However, these herbicides are still detected in the environment together with the triazines in use nowadays as terbutylazine. This is due to their long retention time in the soil and aquifers, which leads to perdu-

The Lerma basin (within Ebro depression in Spain), recently trans-
formed from its natural state (gypsum soils covered by scrubland and steppe-like vegetation) to rain-fed agriculture, has been monitored during a whole agricultural season (one year). The goal of this study was to assess the impacts of triazines from agricultural runoff on the function and structure of algal benthic communities present in the small creek draining the basin (periphyton). In previous years, the creek contained noticeable concentrations of both atrazine and simazine (banned) and terbutylazine (still in use). These data are available at the website of the Ebro Hydrological Confederation (www.chebro.es).

Among the various methods and tools available to assess the impact of pollutants, the Pollution Induced Community Tolerance (PICT) approach offers the means to partially isolate and identify the effects of individual toxicants within an ecosystem subjected to multiple stressors. The ration-

The hypothesis of this work was that the different exposures to the herbicides through the year due to the seasonal agriculture practices would result in changes in the algal community. The sensitivity of the algal communities to the same herbicide in different seasons would depend on the exposure during the growing period. A previous similar seasonal study showed that the structural and functional responses of algal communities to pesticides are likely to reflect past selection pressures (Dorigo et al., 2004).

The sources of herbicides were expected to be: a) the background released from soils (for the two banned herbicides, atrazine and sima-

2.2. Physicochemical water analysis

Water flow (L/s), temperature (°C) and nitrate concentration (NO$_3^-$, mg/L) of the creek were measured in-situ by a water quality sta-

2.3. Algal biofilm sampling and analysis

Benthic algal communities grew on artificial substrates placed on a creek downstream of the basin at different agricultural time periods: pre-herbicide application (autumn and winter), mid-herbicide applica-

2.4. Herbicide analysis

Two different methods were used to assess the herbicide levels in the water: passive sampling with Chemcatcher® devices and discrete water samples. 1 L of water was collected at the end of each sampling period to analyze the concentration of triazines and 10 other pesticides by chromatography (SBSE/CC/MS/HPLC). Moreover, three passive sam-

2.5. Dose–response test in flow-through artificial channels

The tolerance of periphyton (measured as the effect of triazines on the photosynthetic efficiency) against each herbicide was measured in mesocosm (i.e., flow-through artificial channels) by dose–response test. The concentration of herbicide required to reduce 50% and 10% of the photosynthetic performance of the benthic algal community (EC$_{50}$, EC$_{10}$) was used to compare the community tolerance between the dif-

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The toxicity experiments were carried out in flow through methacrylate channels (90 cm long and 10 cm wide) connected to separate water reservoirs. Those reservoirs were submerged in a thermostat bath where the temperature was adjusted to that of the creek (10, 7, 13 and 20 °C for autumn, winter, spring and summer, respectively). Aquarium pumps re-circulated the water from the reservoirs through every channel at 1.3 cm s$^{-1}$. Every reservoir had the same volume (4 L) of buffer solution MOPS 0.01 nM (3-morpholinopropane-1-sulfonic acid) adjusted to a pH of 7.5 using KOH. Light was provided by fluorescence lamps (Blau aquarium TSHO, 39 w/10,000°K, 80 μmol photon m$^{-2}$ s$^{-1}$ at the channel surface).

Every experimental setup for terbuthylazine, atrazine or simazine had 6 channels including one control (herbicide free), and 5 different herbicide doses ranging from 20 nM to 8000 nM. Concentration range had 6 channels including one control (herbicide free), and 5 different surface).

The dose–response curves were decided based on previous short-term experiments to ensure that EC$_{50}$ was within that range (data not shown). The herbicide doses applied in the channels were prepared according to OCDE (Aquatic toxicology test, no. 23). After 20 h acclimation in the channels (i.e. same flowing, temperature and light conditions than during the tests), biofilms were transferred to the experimental channels with the herbicide doses during 2 h. The photosynthetic response of the biofilms to the herbicide exposure was estimated with the Pulse Amplitude Modulated protocol using a Photosynthesis Yield Analyzer (mini-PAM, Walz®). The effective quantum yield was calculated from the steady-state fluorescence (Fs) and the maximum fluorescence (Fm$\prime$) after a saturation light pulse, as ($Fm' - Fs$) / $Fm'$ (Genty et al., 1989). The fluorescence measurements (n = 6) were taken below the channels through the methacrylate in a non-invasive way from. The first measurement was done before inoculating the herbicide in the channel (0 h measurement), the other two after 1 and 2 h of exposure. The measurements were done at the same biofilm positions three times, with similar thickness and Fs estimated by the PAM, to avoid the biases of difference in biofilm thickness. At the end of each experiment water samples from the channels were sent for analyses as described above.

2.6. Data analysis

The dose–response curves at 1 h and 2 h were fitted with R software using a specific developed dose response curve (drc package). This package fits the data to a two parameter log-logistic regression model (Ritz and Streibig, 2005). The upper limit of the curve was normalized to 1, and the lower limit to 0. From the curves we estimated the effective concentrations which reduced the photosynthetic yield of the periphyton by 10% and 50% (EC10 and EC50 respectively). The seasonal EC$_{50}$ values were compared using the CompParm function, included in package drc. This function performs t-test between EC$_{50}$ values. Ratios (EC$_{50}$ case A/EC$_{50}$ case B) were compared with 1 (i.e. the case of no differences between parameters). Those ratios different from 1, with p-values below 0.05 and adjusted using Bonferroni correction for multiple tests, were considered significantly different. The canonical correspondence analysis – CCA – was performed using ade4 package of R software

### Table 1

<table>
<thead>
<tr>
<th>Season</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mg/L)</td>
<td>439.43 ± 45.17</td>
<td>471.01 ± 18.79</td>
<td>466.33 ± 52.83</td>
<td>465.83 ± 12.39</td>
</tr>
<tr>
<td>K (mg/L)</td>
<td>670.86 ± 167.43</td>
<td>604.21 ± 71.44</td>
<td>647.82 ± 45.77</td>
<td>844.18 ± 128.88</td>
</tr>
<tr>
<td>Ca (mg/L)</td>
<td>114.82 ± 31.79</td>
<td>150.71 ± 8.96</td>
<td>142.23 ± 10.78</td>
<td>158.52 ± 5.02</td>
</tr>
<tr>
<td>Mg (mg/L)</td>
<td>101.84 ± 9.97</td>
<td>115.51 ± 5.80</td>
<td>117.70 ± 12.89</td>
<td>127.93 ± 2.47</td>
</tr>
<tr>
<td>S (mg/L)</td>
<td>210.54 ± 27.11</td>
<td>237.99 ± 18.39</td>
<td>240.62 ± 36.54</td>
<td>243.01 ± 18.51</td>
</tr>
<tr>
<td>TDP (μg/L)</td>
<td>7.74 ± 3.57</td>
<td>8.44 ± 1.32</td>
<td>43.81 ± 84.21</td>
<td>115.06 ± 60.86</td>
</tr>
<tr>
<td>TDN (mg/L)</td>
<td>7.74 ± 3.57</td>
<td>8.44 ± 1.32</td>
<td>43.81 ± 84.21</td>
<td>115.06 ± 60.86</td>
</tr>
<tr>
<td>pH</td>
<td>8.50 ± 0.22</td>
<td>8.50 ± 0.28</td>
<td>8.40 ± 0.33</td>
<td>8.20 ± 0.22</td>
</tr>
</tbody>
</table>

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The annual water temperature ranged from 4.5 °C to 22 °C while pH remained constant and slightly basic. Most of the nutrient levels were similar during the four studied periods (Table 1). Only phosphorus concentrations showed an acute increase during summer (×14) compared to autumn or winter (total dissolved phosphorus, TDP, Table 1). The concentrations remained constant and slightly basic. Most of the nutrient levels were similar during the four studied periods (Table 1). Only phosphorus concentrations showed an acute increase during summer (×14) compared to autumn or winter (total dissolved phosphorus, TDP, Table 1). The annual water temperature ranged from 4.5 °C to 22 °C while pH remained constant and slightly basic. Most of the nutrient levels were similar during the four studied periods (Table 1). Only phosphorus concentrations showed an acute increase during summer (×14) compared to autumn or winter (total dissolved phosphorus, TDP, Table 1).

3.2. Herbicide level in the creek

The averaged integrated values of herbicides in the passive sampler disks were calculated as ng/day of exposure while the concentration of herbicides in the water samples were in μg/L (Table 2). The total herbicides included other herbicides that appear in the basin as metholachlor. They belong to other chemical families with a different mode of action than triazines and are out of the scope of this study.

Table 2
Upper part: average of herbicide accumulated in a passive sampler disk per day in ng (SDR-RPD 3M Empore™ disk in a Chemcatcher device). Error terms are SD of three replicate disks. Lower part: concentration in water samples (only one sample was analyzed). Some measurements “<” were under the detection limit (0.001 ng/day for passive samplers and 0.01 μg/L for water samples).

<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Passive samplers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terbutylazine (ng/day)</td>
<td>0.09 ± 0.00</td>
<td>7.79 ± 0.41</td>
<td>0.06 ± 0.25</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td>Atrazine (ng/day)</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>Simazine (ng/day)</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td>Total triazines (ng/day)</td>
<td>0.09 ± 0.00</td>
<td>14.29 ± 1.41</td>
<td>1.19 ± 0.37</td>
<td>1.58 ± 0.30</td>
</tr>
<tr>
<td>Total herbicides (ng/day)</td>
<td>0.15 ± 0.02</td>
<td>15.39 ± 1.83</td>
<td>82.59 ± 19.54</td>
<td>52.49 ± 15.60</td>
</tr>
<tr>
<td><strong>Water samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terbutylazine (μg/L)</td>
<td>0.011</td>
<td>0.11</td>
<td>0.08</td>
<td>0.095</td>
</tr>
<tr>
<td>Atrazine (μg/L)</td>
<td>0.008</td>
<td>&lt;</td>
<td>0.020</td>
<td>0.012</td>
</tr>
<tr>
<td>Simazine (μg/L)</td>
<td>0.022</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>Total triazines (μg/L)</td>
<td>0.041</td>
<td>0.190</td>
<td>0.170</td>
<td>0.198</td>
</tr>
<tr>
<td>Total herbicides (μg/L)</td>
<td>0.190</td>
<td>0.185</td>
<td>0.398</td>
<td>0.041</td>
</tr>
</tbody>
</table>

3.3. Algal biofilm analysis

The algal community was mostly composed of pennate diatoms with negligible presence of other groups (Fig. 3). Generally, two species were dominant: Gomphonema olivaceum (sp1) during autumn and winter (46 and 38% of total individuals, respectively) and Achnanthes minutissima (sp2) during spring and summer (65 and 68%, respectively, Fig. 3).

Toxicity tests

According to the EC50 values, benthic algal communities were most sensitive to terbutylazine followed by atrazine and finally simazine (Table 4). The periphyton tolerance to terbutylazine changed significantly through the seasons (p < 0.05). The summer algal community was 3 times more tolerant than the winter community and two times more than the spring one, based on the EC50 values (Table 4 and Fig. 4). The EC50 values for atrazine and simazine were more constant through the year, i.e., the community tolerance did not present a clear seasonality. Only the EC50 values for atrazine were significantly higher in summer and in the case of simazine significantly lower in spring. The EC10 values in general are shown, aligned with the trends of EC50 (Table 4).

![Fig. 2. Flow and nitrate concentration were registered with an in-situ monitoring system; data plotted are daily averages provided by IGME Institute. The gray-shadowed blocks are the colonization periods of the algal biofilm on the artificial substrata.](https://dx.doi.org/10.1016/j.scitotenv.2014.06.108)
To explore the effect of herbicides on shaping the structure of the benthic algal communities, a multivariate analysis was done. This canonical correspondence analysis (CCA) distributed the species abundance in a multidimensional space according to herbicide tolerance (EC50 values at 1 h and 2 h herbicide exposition) during each season (Fig. 5). Together the first and second axes explained up to 94% of the total variability, meaning that the tolerance level expressed by the communities can be mostly explained by differences in species composition.

4. Discussion

4.1. Seasonal trends in the ecotoxicology of triazines

The sensitivity of benthic algal communities growing downstream of an agricultural basin to an herbicide can change seasonally. Based on the EC50 values the algal community had higher tolerance to terbuthylazine and atrazine during summer period, and lower during the coldest season, winter. The seasonal differences in tolerance are more significant for the herbicide that is currently used in the crops (terbuthylazine), than for those that remain in the environment from past applications (atrazine and simazine).

The algal communities presented significantly different tolerances (EC50) for terbuthylazine between all seasons with the summer algal community being the more tolerant (Table 4). Higher concentrations of terbuthylazine were expected to be found in the creek that season as it is the post-application period of this herbicide. The herbicide applications in the field would lead to a greater exposure of the algal community via run off. In accord with the PICT rationale, the high community tolerance to terbuthylazine in summer may be due to a greater exposure during that season. Therefore EC50 values suggest that there were differential exposures to terbuthylazine through the seasons, even if the methods used for measuring its concentrations in water failed in catching that seasonality.

The already banned herbicides, atrazine and simazine appeared in low concentrations in the creek during the whole year (Table 2). The exposure of the algal biofilm to these herbicides seems to be constant and low along the year, in agreement with the similar EC50 values measured in the algal communities during all seasons. It has been estimated (based on information gathered from farmers and the total surface of corn), that a maximum of 126 kg of terbuthylazine could have been used in the basin during the studied year (1 kg/ha/year).

Some natural and anthropogenic variables co-varying with the herbicide exposure may modulate the sensitivity of the periphyton (Pesce et al., 2008). High temperatures and the higher phosphorus concentrations (Table 1) correlate with the highest EC50 values for terbuthylazine at the summer sampling. High temperatures may have stimulated the photosynthesis (Hancke et al., 2008) helping the community to overcome the herbicide stress. The N/P ratios were lower during the summer period (likely due to the application of fertilizers) due to an extra input of P. In laboratory conditions high P concentrations seem not to

Table 3

<table>
<thead>
<tr>
<th></th>
<th>Chlorophyll a (µg cm⁻²)</th>
<th>Fluorescence (F)</th>
<th>Density (cells·cm⁻²)</th>
<th>Shannon–Wiener index (H)</th>
<th>Simpson index inverse (1/·)</th>
<th>Richness (no. total species)</th>
<th>Non diatom species no. (group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn biofilm</td>
<td>13.61 ± 2.79</td>
<td>949 ± 127</td>
<td>1.64×10⁶ ± 3.85×10⁵</td>
<td>2.05</td>
<td>0.76</td>
<td>23</td>
<td>1 (Cyanobacteria)</td>
</tr>
<tr>
<td>Winter biofilm</td>
<td>12.32 ± 2.30</td>
<td>936 ± 123</td>
<td>3.59×10⁶ ± 6.85×10⁵</td>
<td>1.56</td>
<td>0.68</td>
<td>28</td>
<td>1 (Cyanobacteria)</td>
</tr>
<tr>
<td>Spring biofilm</td>
<td>11.79 ± 2.77</td>
<td>1152 ± 174</td>
<td>5.17×10⁶ ± 2.06×10⁵</td>
<td>1.19</td>
<td>0.54</td>
<td>34</td>
<td>1 (Cyanobacteria) 1 (Chlorophyta)</td>
</tr>
<tr>
<td>Summer biofilm</td>
<td>13.78 ± 3.43</td>
<td>1060 ± 174</td>
<td>4.51×10⁶ ± 7.28×10⁵</td>
<td>1.32</td>
<td>0.52</td>
<td>42</td>
<td>2 (Cyanobacteria) 3 (Chlorophyta)</td>
</tr>
</tbody>
</table>

Fig. 3. Species composition as % of total individuals of benthic algal communities in the four seasons. The species and their respective codes are: sp1 Gomphonema olivaceum, sp13 Nitzschia dissipata, sp2 Achnanthes minutissima, sp14 Gomphonema microspora, sp3 Navicula radiosa, sp15 Nitzschia palea, sp4 Cymbella affinis, sp16 Nitzschia linearis, sp5 Navicula tripunctata, sp17 Gomphonema stauroreformae, sp6 Fragilaria puchella, sp18 Gomphonema angustatum, sp7 Rheicosphenia abbreviata, sp19 Fragilaria capucina, sp8 Navicula lanceolata, sp20 Amphora pediculus, sp9 Nitzschia fonticola, sp21 Surirella brebissonii, sp10 Cymbella microcephala, sp22 Fragilaria subtilissima, sp11 Navicula veneta, and sp23 Melosira varians.

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mask the effect of triazine herbicide on the periphyton (Guasch et al., 2007), whereas opposite results were concluded in field experiments (Pesci et al., 2008).

4.2. Herbicide exposure vs. measured effects

To assess the in situ herbicide concentrations during the study period we applied a combination of direct water analysis and passive sampling (Table 2). However the expected differences in terbuthylazine concentrations through the year were not registered by any method. The measured herbicide concentrations do not correlate with the calculated tolerance values of EC50 for terbuthylazine. As an example, the higher concentration was measured in winter while the higher tolerance was measured in summer, i.e., higher EC50.

We ascribe this discrepancy to the fact that both measuring approaches poorly reflect variability in pollutant water concentrations because of their intrinsic limitations. There are reasons to suspect that herbicide applications in small basins may result in short-time (hours) herbicide discharges, phosphorus inputs (from farm fields) and source of disturbance (Berthon et al., 2011). Conversely, the dominance/absence of a guild can be used to distinguish between different levels and source of disturbance (Berthon et al., 2011). The EC50 concentrations are between 3 and 20 times higher than those of herbicide levels normally found in agricultural watersheds, i.e. from 2 nM to 40 nM (Louchart et al., 2001). The EC10 are higher in summer and lower in winter with intermediates in autumn and winter values during spring and autumn. The changes in the tolerance may be influenced by the combination of factors as: short-time (hours) herbicide discharges, phosphorus inputs (from field fertilization) and high temperatures.

4.3. Impact of herbicides on algal biofilm

The seasonal herbicide physiological changes (i.e. in tolerance to herbicides) were related to changes in the community structure (i.e. changes in algal life forms dominant through the year). The algal species can be classified into: (1) the low-profile guild, consisting of species of short stature, including prostrate, small erect, solitary centric and slow-moving species; (2) the high-profile guild, consisting of species of tall stature including large erect, filamentous, branched, chain-forming, tube-forming, stalked and colonial centric and (3) the motile guild, consisting of fast-moving species (Rimet and Bouchez, 2012).

Species from the high guild disappeared during summer (Gomphonema group, sp1, 18 and 19) together with C. affinis (sp4) and F. capicula (sp19). In fact the abundance of these species was negatively correlated with the EC50 of terbuthylazine and atrazine, i.e. high community tolerances (Fig. 5). Previous studies suggest that each guild responds differently to stress situations; therefore the dominance/absence of a guild can be used to distinguish between different levels and source of disturbance (Berthon et al., 2011). Conversely, the dominant species during spring and summer belonged to the low-profile and motile guild, A. minutissima (sp2), N. lanceolata (sp8), C. microcephala (sp10), Nitzschia palea (sp15), A. pediculus (sp20) and S. brebissonii (sp21). The abundance of these species was positively correlated to the EC50 of terbuthylazine and atrazine i.e. high community tolerances (Fig. 5). The fact that two of these species are known as pioneers, i.e. appearing after chemical disturbances (A. minutissima and N. palea) support the hypothesis of the occurrence of herbicide pollution at these seasons as indicated by the EC50, even if the measured herbicide concentrations do not show it.

4.4. Environmental relevance and application of the results

The EC50 calculated for atrazine is within the range of values previously reported for benthic algal communities (measured as EC50 using the same PAM protocol) which range from 400 to 2500 nM (Guasch and Sabater, 1998; Navarro et al., 2002; Ryan and Prosser, 2013). The EC50 and EC10 values for terbuthylazine or simazine are the first to be published. The EC50 concentrations are between 3 and 20 times higher than those of herbicide levels normally found in agricultural watersheds, i.e. from 2 nM to 40 nM (Louchart et al., 2001). The EC10 are close to peak concentration values measured in similar agricultural basins (Louchart et al., 2001). All these findings, together with the methodological limitations of the traditional continuous monitoring programs, support the use of PICT approaches as a useful and powerful monitoring tool for herbicides.

5. Conclusions

The periphyton presented seasonal variances in its tolerance against terbuthylazine; higher in summer and lower in winter with intermediate values during spring and autumn. The changes in the tolerance may have been influenced by the combination of factors as: short-time (hours) herbicide discharges, phosphorus inputs (from field fertilization) and high temperatures.

Table 4

<table>
<thead>
<tr>
<th>Season</th>
<th>EC50 nM Terbuthylazine</th>
<th>Atrazine</th>
<th>Simazine</th>
<th>EC10 nM Terbuthylazine</th>
<th>Atrazine</th>
<th>Simazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>196 ± 22a</td>
<td>640 ± 51b</td>
<td>1988 ± 172b</td>
<td>24 ± 7</td>
<td>117 ± 19</td>
<td>168 ± 39</td>
</tr>
<tr>
<td>Winter</td>
<td>109 ± 10a</td>
<td>560 ± 33c</td>
<td>2101 ± 113b</td>
<td>25 ± 5</td>
<td>127 ± 16</td>
<td>554 ± 79</td>
</tr>
<tr>
<td>Spring</td>
<td>144 ± 9f</td>
<td>730 ± 48g</td>
<td>1441 ± 73d</td>
<td>35 ± 6</td>
<td>148 ± 24</td>
<td>307 ± 49</td>
</tr>
<tr>
<td>Summer</td>
<td>321 ± 21d</td>
<td>993 ± 46h</td>
<td>1925 ± 127b</td>
<td>69 ± 11</td>
<td>145 ± 23</td>
<td>206 ± 39</td>
</tr>
</tbody>
</table>

Fig. 4. EC50 values for each tested herbicide in the different seasons. The EC50 were normalized by the winter values (set as 1).
The herbicide levels detected in the creek did not correlate with the seasonality of the community tolerance (EC50), as expected based on the PICT concept. However, they were in agreement with the different potential sources of each herbicide: the punctual use during the agronomic year (for terbutylazine) and the gradual and constant background release from soils in which banned herbicides have been stored upon past use (atrazine and simazine). The presence of pioneer species and absence of species from the high-guild during the period of herbicide application (spring and summer), also supported these findings.

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