Effects of a chronic application of the herbicide Afalon (active ingredient linuron) on physicochemical conditions, decomposition of plant litter, and densities of zooplankton and macroinvertebrates were studied in indoor microcosms intended to model drainage ditches. For 28 days, concentrations of 0, 0.5, 5, 15, 50, and 150 µg/L linuron were maintained, each in two replicates. The microcosms were dominated by the macrophyte Elodea nuttallii. The functional response of the ecosystem is discussed in relation to shifts in community structure. Treatment effects of linuron on community metabolism, as a direct effect of the inhibition of the photosynthesis of macrophytes and algae, resulted in a decrease in dissolved oxygen and pH, and an increase in alkalinity and conductivity (NOEC 0.5 µg/L). During the posttreatment period, differences between controls and highest dose fell gradually, but were still significant 7 weeks after the start of linuron application. Decomposition of particulate organic material in litter bags was not affected, despite decreases in DO. The negative effect of linuron on several algae (cryptophytes, diatoms) and the positive effect on the green alga Chlamydomonas resulted in a decrease of several Rotatoria and an increase in Copepoda, and, to a lesser extent, Cladocera. The complete disappearance of the macrophyte E. nuttallii in the 150 µg/L microcosms and a 50% reduction of its biomass in the 50 µg/L microcosms reduced the numbers of the snail Physella acuta, which normally inhabits macrophytes. Artificial substrates indicated a significant increase in the isopod Asellus aquaticus in the 50 and 150 µg/L microcosms during the posttreatment period. This, however, was counteracted by a significant decrease in A. aquaticus at the final harvest. Changes in the ecosystem structure (decline in macrophyte biomass) made the artificial substrates more attractive.

INTRODUCTION

Herbicides are often used in agriculture to reduce or destroy weeds, mainly to avoid competition for nutrients and light between crops and weeds. An undesirable side-effect of the use of these herbicides is that they may enter freshwater ecosystem by spray drift, leaching, run-off, and/or accidental spills. Contamination of surface waters with herbicides has been reported to have direct toxic effects on populations of phytoplankton, epiphyton, and macrophytes. In addition, when these primary producers are affected, indirect effects on ecosystem functioning and animal populations can also be expected (for a review see Brock and Budde (1994) and Kersting (1994)).

In the present study the herbicide linuron (applied as Afalon) was added to microcosms that intended to model stagnant macrophyte-dominated freshwater ecosystems. Within the scope of the present study, a previous paper (part I) dealt with the responses of the primary producers and with the hazard assessment of this herbicide in freshwater ecosystems (Van den Brink et al., submitted).

The first aim of the present paper (part II) is to describe effects of a chronic application of the herbicide linuron on functional aspects such as oxygen metabolism and decomposition. Inhibition of the photosynthesis by linuron and effects on populations of primary producers (Snel et al., submitted; Van den Brink et al., submitted) are hypothesized to cause lower dissolved oxygen concentrations (DO) in the microcosms. A reduced primary productivity will also affect pH, alkalinity, and conductivity. These endpoints have been repeatedly reported as sensitive indicators of metabolic effects of toxicants (Stephenson and Kane, 1984; Brock et al., 1993; Kersting, 1994). DO, pH, alkalinity, and conductivity are often found to be highly correlated, and treatment effects on these functional endpoints can be regarded as a stress syndrome (Giddings, 1982).

A second aim of this paper is to describe the effects of linuron on the secondary producers (zooplankton and macroinvertebrates). A priori, the authors did not expect direct effects on invertebrates, since the EC50 of Daphnia longispina for linuron (360 µg/L; Stephenson and Kane, 1984) is considerably higher than the concentrations used in the current experiment. Similarly, the LC50 values for some macroinvertebrates, such as Dugesia tigrina (10 mg/L), Lymnaea (70 mg/L), and Tubifex (10 mg/L) are too high to expect direct effects (Maier-
Bode and Härtel, 1981). Indirect effects of herbicides on zooplankton and macroinvertebrate populations due to shifts in populations of primary producers, however, appear to be difficult to predict (Hurlbert, 1975; DeNoyelles et al., 1989; Brock and Budde, 1994). A priori, the authors hypothesized either a decrease in zooplankton populations due to an expected decrease in phytoplankton or a shift in dominance from herbivorous taxa to detritivorous taxa. As regards macroinvertebrates, a shift in dominance from herbivores to detritivores was expected to result from the increase in organic matter due to the death of algae and macrophytes (Elodea nuttallii). The loss of the structure provided by the macrophytes was also expected to lead to a dominance of benthic organisms instead of epiphytic organisms.

The third aim of the present paper is to present a synthesis of the impact of herbicide application on the structure and functioning of the freshwater microcosms by incorporating the conclusions of the preceding paper.

MATERIALS AND METHODS

Experimental Design of the Microcosm Study

The construction and properties of the indoor microcosms (length and width 110 cm, depth 70 cm, water depth 50 cm, sediment depth 10 cm), the conditions in the climate room (temperature 20°C, photoperiod 14 hr), and the experimental design have been described in detail by Van den Brink et al. (submitted). Two of the 12 available microcosms served as controls. Of the remaining 10, two microcosms each were loaded with, respectively, 0.5, 5, 15, 50, and 150 μg/L linuron. The linuron concentration in the water column was kept constant for 28 days by adjusting it twice a week. The dynamics of the linuron concentration in the water has been described by Van den Brink et al. (submitted). All microcosms were investigated over a period of 14 weeks: a pre-treatment period of 3 weeks, a treatment period of 4 weeks, and a posttreatment (restoration) period of 7 weeks.

Water Sampling and Water Chemistry Analysis

Dissolved oxygen was measured with a WTW oxygen meter (Oxi 196) and a WTW oxygen probe (EO 196) at a depth of 10 cm at 1-week intervals from Week −3 through Week 11. Oxygen was measured in the morning, at the start of the photoperiod, and in the evening, just before the illumination was switched off. Conductivity and pH were measured with, respectively, a WTW conductivity meter and a Metrohm Herisau pH meter at weekly intervals from Week −3 through Week 11. During the same period, alkalinity was measured weekly in 100-ml samples taken at a depth of 10 cm (titration with 0.05 N HCl until pH 4.2).

Depth-integrated water samples from at least five localities well distributed over each microcosm were taken with a perspex corer (length 40 cm) in Weeks −3, −1, 0.2, 2, 4, 6, 8, and 10 for nutrient analysis. Subsamples of each day and microcosm were pooled and a portion of the well-mixed sample was filtered through prewashed glass fiber filters (Whatman GF/C). Part of the filtered water was transferred to 100 ml iodated polyethylene bottles and stored (−20°C). At the end of the experiment the samples were defrosted and analyzed for ammonium, nitrate, and orthophosphate using a Skalar 5100 Autoanalyzer. Another part of the sample was stored (−20°C) after addition of 1.5 ml HCl. At the end of the experiment the samples were analyzed for Na⁺, K⁺, and Ca²⁺ using an atomic absorption spectrophotometer.

Decomposition Experiment

Decomposition of particulate organic matter (POM) was studied by means of the litter bag technique (Brock et al., 1982). The POM used consisted of E. nuttallii shoots and Populus x canadensis leaves.

The Populus leaves had been leached three times for 2 days to remove the more easily soluble humic compounds. To allow storage of this material, it was dried in an oven for 72 hr at 60°C. Subsamples of Elodea and Populus were subsequently dried at 105°C for 24 hr to establish the 60°C/105°C dry weight ratio.

A 2 g dry weight (dried at 60°C) portion of Elodea or Populus was enclosed in each litter bag. The litter bags consisted of a glass petri dish (diameter 11.6 cm) closed with a cover of stainless steel wire (mesh size 0.7 × 0.7 mm), in which two holes (0.5 cm) had been punched to allow the passage of most invertebrates. The materials used are known to be inert to pesticides. In each model ecosystem two litter bags of each plant type were incubated at the sediment surface for a period of 2 weeks. Whenever a set of litter bags was retrieved on a sampling day, a new set was incubated. At the end of each 2-week decay period, the litter bags were gently washed in the overlying water of each microcosm to remove adhering sediment particles. The contents of the two bags of each plant type from each microcosm were then transferred to a white tray to separate invertebrates from the decomposing material. The plant material was transferred to aluminum foil to determine dry weight (24 hr, 105°C). After identification and counting, the invertebrates were released into their original microcosms.

Sampling of Zooplankton

Zooplankton was sampled from each model ecosystem at 2-week intervals with a perspex corer with a length of 40 cm and a diameter of 4 cm. Several subsamples were collected, regularly distributed over the microcosms, until a 5-L sample was obtained. The total sample from each microcosm was passed over a 55-μm mesh net. This was done over the microcosm to minimize the loss of water. The concentrated sample was fixed with 4 ml 35% formol and supplemented to 100 ml. Zooplankton was identified and counted with a Nikon inverted microscope at a magnification of 100× in a sedimentation cuvet. The density of zooplankton was always relatively
low, which is why total samples were counted. Numbers of zooplankton were divided by five to get the numbers per liter.

Sampling of Macroinvertebrates

Macroinvertebrates were sampled from each model ecosystem at 2-week intervals by means of artificial substrates and litter bags, as described above. In each system three multiplates and two pebble baskets served as artificial sample substrates (for a detailed description see Brock et al., 1992).

On each sampling day, the artificial substrates were gently retrieved from each system, using a net to prevent the escape of swimming invertebrates. Pebble baskets were first washed in a container to remove invertebrates from the substrate. Subsequently, the macroinvertebrates present on the substrates and in the litter bags were collected by handpicking, identified and counted alive, and then released again into the model ecosystems. Data from artificial substrates and litter bags were pooled for further analysis.

At the end of the experiment (Week 13) the macroinvertebrates were quantitatively sampled. First, all macrophytes and artificial substrates were removed (carefully washed to remove all adherent invertebrates), after which the water level in the microcosms was reduced to a depth of 10 cm by means of a siphon. Escape of macroinvertebrates was prevented by leading the water over a net. Escaped organisms were reintroduced into the microcosms. The water in the microcosm was then gently stirred to achieve a random distribution of the macroinvertebrates. A corer with a surface of 30 × 30 cm was placed in each microcosm and the fauna in it was sampled with a small net. Animals were collected by handpicking and identified alive (Turbellaria) or fixed in 4% formol (Oligochaeta) or 70% ethanol (remainder of macroinvertebrates).

Data Analysis

Before analysis, the abundance values of the zooplankton and macroinvertebrates were ln (10x + 1) and ln (2x + 1) transformed, respectively, where x stands for the abundance value. A rationale for these transformations is given in Van den Brink et al. (1995).

NOEC calculations at parameter or species level were obtained using the Williams test (ANOVA) (Williams, 1972). The analyses were performed with the Community Analysis computer program (Hommen et al., 1994).

The response of the communities to the linuron treatment was analyzed using the Redundancy Analysis (RDA) ordination technique. For the theoretical background and technical details of these techniques, see Ter Braak (1987, 1988, 1990). Specific details of the application of RDA in model ecosystem experiments have been described by Van Wijngaarden et al. (1995) and Van den Brink et al. (1996). RDA was used to obtain an overview of the combined effects of time and treatment at the community level. This technique produces a diagram which summarizes the data set, while still indicating species composition for all samples (see, for example, Fig. 3).

RESULTS

Physicochemical Conditions

Figures 1A–1C present the results of the measurements on the DO-pH-alkalinity-conductivity syndrome. After the start of the treatment, DO and pH levels were lower compared to controls at all linuron concentrations except the lowest (Figs. 1A and 1B, Table 1). DO remained above 6 mg/L in all treatments, even at the end of the dark period, when DO levels were generally about 1.0 to 1.5 units lower (results not provided), so that anoxic conditions never occurred in the water column. During the posttreatment period, the 5 and 15 µg/L microcosms regained normal DO and pH levels, whereas those with the two highest concentrations did not reach normal levels within the experimental period.

Corresponding with the decrease in DO and pH, the higher treatment levels led to increases in alkalinity and conductivity (Fig. 1C, Table 1), compared to the controls. At the end of the experimental period, all microcosms except those with the two highest linuron concentrations were not significantly different from control levels, mainly due to a rise in alkalinity levels in the controls.

Application of linuron had no significant treatment effect on the nutrients ammonium and phosphate, while a significant effect was observed for nitrate at the highest linuron concentration from Week 6 onwards (Fig. 1D). In the 150 µg/L microcosms the minerals Na⁺, K⁺, and Ca²⁺ revealed different responses to the linuron application, with a significant positive effect for calcium and potassium, and no effect for sodium.

Decomposition

The application of linuron did not result in significant treatment effects on the decomposition of particulate organic matter (Populus leaves and shoots of E. nuttallii) in litter bags (Fig. 2). The residual dry weights of Populus after decay periods of 2 weeks amounted to 60–70% of the initial dry weight. The residual dry weights of Elodea were distinctly lower and amounted to 50% in Week −1 and to approximately 40% in all other periods.

Zooplankton

The dominant species in the zooplankton samples belonged to the groups of Cladocera, Copepoda, and Rotatoria, while Ostracoda occurred in low numbers. The Cladocera were dominated by Daphnia longispina, Simocephalus vetulus, G. testudinaria, and C. remata, while nauplii were dominant. Rotatoria were the most diverse, with Synchaeta pectinata, Polyarthra remata, and Mytilina bicarinata as the most numerous species. C. vidua was the only representative of the Ostracoda.

A biplot of the redundancy analysis on the zooplankton data set is given in Fig. 3. The diagram summarizes the treatment
effects in the data set, while still indicating the approximate species composition for all samples. The lines represent the course of the various treatment levels in time, so the variation between the replicates has been excluded from the analysis. In the diagram, treatment levels with nearly identical courses of species composition in time lie close together, while treatment levels with very different species compositions lie far apart. For further explanation of the diagram, the reader is referred to Van den Brink et al. (submitted). The variation expressed on the first axis reflects the changes in species composition and abundance in time (the treatment levels move from left to right in time). The clustering of all pretreatment samples (upper left quadrant) indicates minor differences between the microcosms at the start of the experiment. The second axis partly represents the treatment effects. After the start of the linuron application the samples from the 150 μg/L treatment, and, less clearly, the 50 and 15 μg/L treatments, are positioned at the bottom of the diagram, indicating differences in community composition of these microcosms relative to the controls. Synchaeta pectinata, Polyarthra remata, Graptoleberis testudinaria, and Daphnia longispina decreased considerably with time, while most other taxa exhibited increases. In general, the number of taxa increased in the course of the experiment, though most “new” taxa occurred only in low numbers. The copepod taxa Macro-cyclops and nauplii revealed the strongest positive correlation to the treatment, the rotifers Synchaeta pectinata and Polyarthra remata the strongest negative one.

Responses of the main zooplankton groups are presented in Fig. 4 and Table 1. Significant treatment effects of Cladocera could be demonstrated in the posttreatment period in Weeks 7
TABLE 1

NOECs as Calculated by the Williams Test (P ≤ 0.05) for Physicochemical Conditions and Abundances of Zooplankton and Macroinvertebrates for Three Periods: The Pretreatment (Week −3 through −1), Treatment (Week 1 through 4), and Posttreatment (Week 5 through 11) Period

<table>
<thead>
<tr>
<th>Physicochemical</th>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen</td>
<td>50↑</td>
<td>0.5↓</td>
<td>15↓</td>
</tr>
<tr>
<td>pH</td>
<td>50↑</td>
<td>0.5↓</td>
<td>15↓</td>
</tr>
<tr>
<td>Conductivity</td>
<td>—</td>
<td>5↑</td>
<td>5↑</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>—</td>
<td>5↑</td>
<td>5↑</td>
</tr>
<tr>
<td>Nitrate</td>
<td>—</td>
<td>—</td>
<td>15↑</td>
</tr>
</tbody>
</table>

Zooplankton

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocera</td>
<td>—</td>
<td>—</td>
<td>15↑</td>
</tr>
<tr>
<td>Copepoda</td>
<td>0.5↓</td>
<td>—</td>
<td>50↑</td>
</tr>
<tr>
<td>Rotatoria</td>
<td>50↓</td>
<td>5↓</td>
<td>50↓</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>—</td>
<td>—</td>
<td>15↓</td>
</tr>
</tbody>
</table>

Macroinvertebrates

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physella acuta</td>
<td>—</td>
<td>50↓</td>
<td>50↓</td>
</tr>
<tr>
<td>Asellus aquatic</td>
<td>—</td>
<td>—</td>
<td>15↑</td>
</tr>
<tr>
<td>Dugesia</td>
<td>—</td>
<td>—</td>
<td>15↑</td>
</tr>
<tr>
<td>Bithynia</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note. A “—” indicates a NOEC > 150 µg/L.

and 9, when a NOEC of 15 µg/L linuron was observed due to an increase in numbers at the highest concentrations. Copepods demonstrated a significant increase at 150 µg/L linuron during Weeks 5–9 in the posttreatment period. The results for the Copepoda were mainly determined by the changes in abundance of nauplii and, to a much lesser extent, of Macrocyclops.

Rotatoria were most numerous in the pretreatment period (65 individuals per liter), declined seriously during the treatment period, especially at the three highest linuron concentra-

tions, and remained at these levels during the posttreatment period (Fig. 4, Table 1).

Macroinvertebrates

The macroinvertebrates in the model ecosystems were dominated by snails, crustaceans, triclads, and oligochaetes, while leeches and nemerteans were less abundant. Insects, with exception of the phantom midge Chaoborus obscuripes, were very scarce, probably because of their inability to reproduce or oviposit in the climate room after their emergence in the pre-experimental acclimatization period.

Herbivorous snails, mainly living on the macrophytes, were dominated by Physella acuta and Lymnaea stagnalis. The bottom and vegetation dwellers with more differentiated feeding habits were dominated by Bithynia tentaculata and B. leachi. Other snail species were less abundant. All crustaceans in the microcosms were shredders; they included the amphipod Gammarus pulex and the isopods Asellus aquaticus, Pseudosellus meridianus, and P. coxalis. Dugesia tigrina was the most abundant carnivorous triclad, while D. lugubris occurred in much lower numbers. The vegetation inhabiting herbivorous Oligochaeta consisted of Stylaria laevis and Chaetogaster spec., while the detritivorous Dero digitata and Tubificidae were the most numerous benthic taxa. Carnivorous leeches, belonging to the genera Erpobdella, Glossiphonia, and Albuglossiphonia occurred in low densities.

A biplot of the redundancy analysis on the macroinvertebrates data set is given in Fig. 5. The variation expressed on the first axis is mainly related to treatment with linuron, while the second axis is related to changes in species abundances with time. D. lugubris indicated a positive correlation with the treatment, but even before the start of the treatment its abundance was highest in the 150 µg/L microcosms. Adult Physella acuta and their offspring exhibited a negative correlation with the treatment, but even in the pretreatment period this gastropod occurred in high numbers in the 0.5 µg/L microcosms. Adult Physella acuta and their offspring exhibited a negative correlation with the treatment, but even in the pretreatment period this gastropod occurred in high numbers in the 0.5 µg/L microcosms. The 150 µg/L microcosms, and to a lesser extent those with 50 µg/L, demonstrated the most pronounced deviations from the other treatments and controls at the end of the experiment. On the second axis the snail Anisus vortex revealed a strong decline with time, while juvenile Bithynia, Dugesia tigrina, and Chaetogaster indicated an increase in numbers.

The development in time of the most discriminative populations in the RDA-diagram is presented in Fig. 6. Significant negative treatment effects (Table 1) were only found for P. acuta at the highest linuron concentration from Week 3 onwards (Fig. 6A). This negative treatment effect is in accordance with the results of the final sampling (Table 2). A significant positive treatment effect was found for Asellus aquaticus (Fig. 6B) and Dugesia (Fig. 6C), while Bithynia revealed a positive trend (Fig. 6D). The significant treatment effects were usually only found for the highest or two highest linuron concentrations. In most cases, these effects were not confirmed by the final sampling in Week 13; in fact, a significant negative
DISCUSSION

Effects on Ecosystem Functioning

The first response of the microcosms to the addition of linuron was the almost immediate inhibition of the photosynthetic efficiency of primary producers (Snel et al., submitted). This response is in accordance with the well-known inhibition of the photosynthesis of aquatic macrophytes and algae by triazine and phenylurea herbicides (Kemp et al., 1985; DeNoyelles et al., 1989). Corresponding with the inhibition of the photosynthesis, the dissolved oxygen concentration and the pH in the overlying water decreased, while alkalinity and conductivity increased (Figs. 1A–1C). Stephenson and Kane (1984) observed the same effects of a single dose of 1000 μg/L linuron in an enclosure in a small open pond. Other herbicides, such as diquat (Hodgson and Linda, 1984; Draxl et al., 1991) and atrazine (DeNoyelles et al., 1989) were found to result in similar effects on dissolved oxygen concentration, pH, conductivity, and alkalinity, at least temporarily. In the current study, the magnitude of the response was strongly dependent on the linuron concentration applied (NOEC 0.5 μg/L). Over the 4-week treatment period, a more or less stable difference between the different treatment levels was established. The differences for these four variables gradually diminished over the 7-week posttreatment period, but at the end of the experiment, the microcosms with the two highest linuron treatment levels still differed significantly from the controls. The restoration of these variables indicates that, at least at the lower linuron concentrations, the community metabolism regains its “normal” value in the posttreatment period, particularly in those systems in which macrophyte biomass recovered. The slow restoration of DO, pH, alkalinity, and conductivity in the 50 and 150 μg/L microcosms can be explained by the significant decreases in the biomass of E. nuttallii, of 50% and 100%, respectively (Van den Brink et al., submitted). In (control) microcosms, macrophytes formed the bulk of the biomass and can be considered the most important primary producers. The dissolved oxygen, pH, alkalinity, and conductivity syndrome proved to be a very good indicator of the direct effects of linuron. Other herbicides such as atrazine (Neugebaur et al., 1990; Lay et al., 1984), diquat (Strange, 1976), and 2,4-D DMA (Boyle, 1980) have similar effects on this syndrome (see Kersting (1994) for a review).
Despite the lower oxygen concentrations in the overlying water, the decomposition rate of *Populus* leaves and shoots of *E. nuttallii* was not influenced by linuron application (Fig. 2). The dissolved oxygen concentrations above the substrate in all microcosms were probably high enough to prevent anaerobic conditions and an inhibition of microbial activity. In accordance with these results, no evidence could be found in the literature of an inhibitory effect of linuron at concentrations below 150 μg/L on microbial communities in freshwater ecosystems. Despite an increased attractiveness of the artificial substrates for shredders like Amphipoda and Isopoda in the 150 μg/L microcosms (probably due to macrophyte decline), this did not result in an increased decomposition of particulate organic matter in the litter bags. This suggests that the processing of macroscopic detritus, either from terrestrial or aquatic origin, was mainly performed by microorganisms and to a lesser extent by macroinvertebrates. Brock et al. (1993) found a relatively small, though significant, decrease in the decomposition rate of *Elodea* in microcosms in which Amphipoda and Isopoda had been eliminated by the insecticide chlorpyrifos. They also suggested the relative unimportance of shredders in comparison with microorganisms.

**Responses of Invertebrates**

As expected, the zooplankton communities in the microcosms revealed no immediate responses to the application of linuron, though the decline of Rotatoria was relatively fast, indicating a possible role of direct toxicity. A likely explanation for the decrease in Rotatoria, however, is the decrease in the planktonic and epiphytic algae *Chroomonas, Phormidium,*
and Cocconeis and the increase in the flagellate Chlamydomonas (Van den Brink et al., submitted). During the treatment and posttreatment periods the zooplankton communities changed from Rotatoria-dominated to Copepoda-dominated (especially nauplii). Perhaps the selective feeding strategy of nauplii and Macrocylops makes them better grazers of Chlamydomonas in microcosms than the filter-feeding rotifers. Cladocera and Ostracoda hardly changed in abundance (Figs. 3 and 4). In agreement with the results reported, e.g., Gunkel (1983), Hamilton et al. (1989), and Jenkins and Buikema (1990), the size of the dominant zooplankton species increased slightly in response to the treatment with linuron.

As expected, the macroinvertebrates did not indicate a direct response to the linuron application either. The secondary effects of linuron on macroinvertebrate communities were relatively small. The destruction of E. nuttallii biomass at the highest linuron concentration (Van den Brink et al., submitted) caused a severe decline of the snail Physella acuta (Fig. 6A) and its offspring and in the number of egg cases of Lymnaea stagnalis (NOEC 15 µg/L, results not provided). Both species, which normally feed on epiphyton on aquatic macrophytes, probably suffered from the lack of food and oviposition sites on the plants. No total elimination of these snails from the microcosms was observed, as the walls of the aquaria still offered feeding and oviposition sites. The artificial substrates in the microcosms seemed not to be extra attractive to these snails after the decline of Elodea, in contrast to the isopod A. aquaticus and the triclads D. tigrina and D. lugubris, whose numbers increased on the artificial substrates (Figs. 6B and 6C). At the end of the experiment, however, a significant decrease in numbers of A. aquaticus was observed with the help of an absolute sampling method (Table 2). This proves that results on invertebrate dynamics as measured by means of artificial substrates should be interpreted with caution in ecosystems whose structure changes as a result of macrophyte decline due to herbicides. The absolute decrease in A. aquaticus at the end of the experiment is most probably the combined result of habitat destruction (decline of macrophyte biomass due to the herbicide) and severe predation by the triclads. At the end of the experiment, triclads outnumbered the isopods and a decline of triclads would have been expected had the experiment continued.

Normal application of linuron on agricultural fields will not greatly affect species composition of invertebrates in adjacent
aquatic ecosystems. It is only when the exposure concentrations are so high or longlasting that the macrophytes eventually die that this will have serious noxious effects on the invertebrate communities. It is very difficult to predict these secondary effects, as they will depend strongly on the structure of the system before application.

CONCLUSIONS

A synthesis of the overall ecological impact of a chronic application of linuron on the structure and functioning of the microcosms has been visualized in Fig. 7.

As a primary effect of linuron application, the photosynthesis of both algae and macrophytes was inhibited in the 5 μg/L microcosms and at higher concentrations (Snel et al., submitted). This resulted in a decrease in dissolved oxygen and pH.

### TABLE 2

Geometric Mean Numbers/m² of Some Species of Invertebrates at the End of the Experiment in Week 13 in Microcosms Treated with the Herbicide Linuron (n = 2)

<table>
<thead>
<tr>
<th>Treatment level</th>
<th>Control</th>
<th>0.5 μg/L</th>
<th>5 μg/L</th>
<th>15 μg/L</th>
<th>50 μg/L</th>
<th>150 μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. aquaticus</td>
<td>323</td>
<td>247</td>
<td>385</td>
<td>257</td>
<td>676</td>
<td>61*</td>
</tr>
<tr>
<td>G. pulex</td>
<td>193</td>
<td>151</td>
<td>152</td>
<td>94</td>
<td>128</td>
<td>126</td>
</tr>
<tr>
<td>D. tigrina</td>
<td>788</td>
<td>930</td>
<td>649</td>
<td>91</td>
<td>1320</td>
<td>359</td>
</tr>
<tr>
<td>Bithynia</td>
<td>89</td>
<td>20</td>
<td>55</td>
<td>51</td>
<td>122</td>
<td>88</td>
</tr>
<tr>
<td>P. acuta</td>
<td>72</td>
<td>653</td>
<td>100</td>
<td>120</td>
<td>111</td>
<td>9</td>
</tr>
<tr>
<td>Dero</td>
<td>1846</td>
<td>2612</td>
<td>490</td>
<td>649</td>
<td>1667</td>
<td>1775</td>
</tr>
</tbody>
</table>

* Indicates a significant difference (Williams test, P < 0.05).
and an increase in conductivity and alkalinity. The decrease in dissolved oxygen, however, was moderate and did not result in anaerobic conditions in the water column, nor did it affect decay rates of *Populus* and *Elodea* in litter bags or invertebrate species composition. A decrease in invertebrates with a high oxygen demand due to oxygen depletion was reported by Murphy and Barrett (1990). The decreased pH caused a concentration-dependent degradation rate of linuron, with slower degradation at the highest linuron concentration (Van den Brink et al., submitted).

The small, but significant increase in nitrate (and potassium) in the 150 µg/L microcosms can be explained by a reduction of the biomass of the macrophyte *E. nuttallii*, and to a lesser extent by the decline of several planktonic and epiphytic algae. The increase in these nutrients probably resulted in a higher chlorophyll-a concentration, caused by the significant increase in the more tolerant green alga *Chlamydomonas* (Van den Brink et al., submitted). A decrease in the small rotifers in the 50 and 150 µg/L microcosms, perhaps as a combined effect of direct toxicity of linuron and an indirect effect of the decline of several planktonic and epiphytic algae (e.g., the cryptophyte *Chroomonas* and the diatom *Cocconeis*), resulted in an increase in zooplankters such as copepods, especially nauplii, and, to a lesser extent, cladocerans. The increase in these zooplankters was probably also caused by the increase in *Chlamydomonas*, a readily edible chlorophyte (Mitchell et al., 1992).

The decrease in biomass of *E. nuttallii* and its associated periphyton in the 50 and 150 µg/L microcosms negatively affected the grazing snails *P. acuta* and *Lymnaea*. The decline of isopods, especially *A. aquaticus*, at the highest linuron concentrations was only found at the end of the experiment. Prior to that, a positive response was found for this species, probably due to an increased attractiveness of the artificial substrates in the otherwise nearly empty microcosms. No pronounced shift from herbivorous feeding strategies to more detritivorous feeding strategies was found, despite the decline of the macrophytes at the highest linuron concentrations. An increase in detritivorous macroinvertebrates, due to an increase in detritus as a result of macrophyte decay, has been reported by Stephenson and Mackie (1986), Feind et al. (1988), and Murphy and Barrett (1990).

The application of linuron had no significant effect on the decomposition of *Populus* leaves and *Elodea*, suggesting an uninfluenced microbial community.

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**REFERENCES**


EFFECTS OF LINURON ON METABOLISM AND INVERTEBRATES


