EFFECTS OF THE PESTICIDES CAPTAN, DELTAMETHRIN, ISOPROTURON, AND PIRIMICARB ON THE MICROBIAL COMMUNITY OF A FRESHWATER SEDIMENT

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Abstract—In three microcosm experiments, we exposed microbial communities of a natural sediment to environmentally relevant concentrations of the fungicide captan, the herbicide isoproturon, and the insecticides deltamethrin and pirimicarb. Exposure concentrations were estimated negligible concentrations (NCs), maximum permissible concentrations (MPCs), and 100 times MPC (100MPC). Experimental endpoints were microbial community respiration and biomass, bacterial activity, and denitrification. All four pesticides inhibited bacterial activity by 20 to 24% at MPC, which corresponded to concentrations in the range of µg/kg dry-weight sediment. Treatments with deltamethrin and isoproturon showed inhibiting effects on bacterial activity at NC exposures. Surprisingly, for captan, deltamethrin, and isoproturon, this inhibiting effect was not observed at 100MPC treatments. Microbial biomass was negatively affected in MPC treatments with deltamethrin and in NC treatments with isoproturon. The tested pesticides did not affect community respiration and denitrification rates. These results show that exposure to the tested pesticides may induce toxic responses in sediment microbial communities at concentrations that are predicted to be environmentally safe.

Keywords—Pesticides Microbial communities Natural sediment Bacterial activity Denitrification

INTRODUCTION

Pesticides are extensively used to control unwanted organisms occurring on crops and wood, to control weeds on railways and roads, and to protect humans and livestock from insect-borne diseases. Rapid development in the agrochemical field has supplied us with pesticides with high selectivity and specificity toward certain species while reducing toxicity to nontarget organisms [1]. Currently used pesticides are also more readily degraded than the first generation of persistent halogenated compounds. However, these pesticides are frequently transported from the site of application and enter aquatic ecosystems through runoff, soil erosion, or spray drift [2,3] and may accumulate in aquatic sediments [4–6].

Aquatic sediments provide a habitat for many organisms that play key roles in aquatic ecosystems [7]. Sediment microbial communities, including benthic algae, bacteria, fungi, and protozoans, form the base of aquatic food webs and mediate important ecosystem functions like nutrient turnover and contaminant fluxes. Consequently, negative effects on sediment microbes from pesticide exposure may seriously affect ecosystem function. Although a number of studies have addressed pesticide effects on single species of algae or bacteria (see DeLorenzo et al. [8] for review), few studies have addressed the interactions between pesticides and natural aquatic heterotrophic microbial communities [9–12]. For example, Svensson and Leonardsson [10] showed that relatively low concentrations of the fungicide fenpropimorph inhibited denitrification in a eutrophic lake sediment. Furthermore, DeLorenzo et al. [12] showed both direct effects of endosulfan on bacterial abundance and indirect effects of atrazine on bacterial abundance and productivity mediated by the inhibition of phototrophic microbes.

Pesticides have different modes of action that are largely specific for their target organisms but may have more general toxic effects on microbes; that is, they can interfere with respiration, photosynthesis, and biosynthetic reactions as well as cell growth, division, and molecular composition [8]. Considering the enormous diversity of bacteria and their importance in ecosystem processes, it is remarkable that the vast majority of toxicity studies are done with only one single species, that is, with Photobacterium phosphoreum in the Microtox® bioassay (Microbics, Carlsbad, CA, USA) [13]. Although Microtox may be a powerful tool to quantify toxic effects of contaminants on heterotrophic bacteria, it fails to detect community-level effects of contaminant exposure. Conversely, a number of techniques used in sediment microbial ecology (e.g., determination of bacterial activity using radiotracers, microcalorimetry, and isotope pairing) allow for the measurement of ecologically relevant endpoints for heterotrophic bacteria in natural sediments and biofilms and should be applied in ecotoxicology.

In the present study we quantified the effects of four common pesticides on microbial community respiration, microbial biomass, bacterial activity, and denitrification, representing endpoints at different levels of sediment microbial organization. We used a microcosm approach with natural sediment and exposure concentrations that reflected ecotoxicologically relevant concentrations. The pesticides chosen in this study, deltamethrin, pirimicarb, isoproturon, and captan, are functionally different, which allowed us to detect possible differences in toxic effects on sediment microbes, depending on each pesticide’s toxic mode of action.

MATERIALS AND METHODS

Test sediments

Profundal sediment (12–16 m) was sampled on three occasions in Lake Erken, a mesotrophic lake that is relatively...
Effects of pesticides on sediment microbes

Environ. Toxicol. Chem. 23, 2004 1921

Table 1. Pesticides used in experiments, their active ingredients (International Union of Pure and Applied Chemistry names), partition coefficients in octanol and water (log $K_{ow}$), and the applied nominal exposure concentrations (negligible concentration [NC], maximum permissible concentration [MPC], and MPC times 100 [MPC100]) in sediment (effects on bacterial activity and denitrification) and water (effects on microbial biomass and respiration); DW = dry weight

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Active ingredient</th>
<th>Log $K_{ow}$</th>
<th>NC° ($\mu$g/kg DW mg/L)</th>
<th>MPC° ($\mu$g/kg DW mg/L)</th>
<th>100MPC° ($\mu$g/kg DW mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captan</td>
<td>N-trichloromethyl-thiocyclohex-4-ene-1,2-dicarbamide</td>
<td>2.8</td>
<td>0.013</td>
<td>1.3</td>
<td>130</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>(3)-a-cyano-3-phenoxyl-benzyl-(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropanecarboxylate</td>
<td>4.6</td>
<td>0.013</td>
<td>1.3</td>
<td>130</td>
</tr>
<tr>
<td>Isoproturon</td>
<td>3-(4-isopropylphenyl)-1,1-dimethyleurea</td>
<td>2.5</td>
<td>0.053</td>
<td>5.3</td>
<td>530</td>
</tr>
<tr>
<td>Pirimicarb</td>
<td>2-dimethylamino-5,6-dimethylpyrimidine-4-yl-di-methylcarbamate</td>
<td>1.7</td>
<td>0.022</td>
<td>2.2</td>
<td>220</td>
</tr>
</tbody>
</table>

*From Tomlin [16].
*B From Crommentuijn et al. [14].

unaffected by agricultural activities, situated 60 km north-northeast of Stockholm, Sweden (59°51’N, 18°35’E). For the first experiment (pesticide effects on respiration and microbial biomass), sediment was collected with an Ekman grab (Wildco Wildlife, Saginay, MI, USA). After removing the overlying water, the top 2 cm of this sediment were sampled with a spoon and brought to the laboratory and kept cold under aerated water until the start of the experiment. For the other two experiments (pesticide effects on bacterial activity and denitrification), sediment was collected with a core sampler (inside diameter 6 cm), and intact cores were brought to the laboratory and kept cold under constant aeration of the overlying water. Also here, the top 2 cm of sediment were used in the experiments. Sediments were stored no longer than two weeks.

Analyses of physicochemical characteristics of the experimental sediments showed that water content of the sediment (oven dried at 105°C overnight) varied between 91.5 and 92.2% and that organic matter content (loss on ignition at 550°C overnight; Nabtherm oven Mod N54E, Lilienthal, Germany) ranged between 19.0 and 19.8%. Carbon content ranged between 9.5 and 9.8%, and nitrogen content was 1.3% for all samples (both determined with an elemental analyser, LEKO CHNS-932, LEKO, St. Joseph, MI, USA).

Pesticides and pesticide exposure

Ecotoxicologically relevant exposure concentrations were chosen from a study by Crommentuijn et al. [14], who assessed the potential environmental hazard for approximately 150 organic substances and pesticides. Crommentuijn et al. [14] present maximum permissible concentrations (MPCs; the highest permissible concentration of a single substance before ecotoxicological effects can be expected) and negligible concentrations (NCs), defined as 1% of the MPC. The NC also accounts for possible synergistic effects of organic contaminants. The MPCs for water exposures given in Crommentuijn et al. [14] were derived from measured no-observed-effect concentration and in some cases from only a few chronic or acute tests. Crommentuijn et al. [14] point out that no ecotoxicologically relevant data are available for sediment. Consequently, reported MPCs for sediment exposures were instead derived from MPCs in water using equilibrium partitioning, resulting in MPCs with great uncertainty.

For our experiments, we selected four functionally different pesticides that are commonly used within the European Union and for which MPCs had been calculated: captan, deltamethrin, isoproturon, and pirimicarb (Ehrenstorfer reference substances, Augsburg, Germany, determined purity ≥98% for all four). The fungicide captan belongs to the class of carbamates. The exact mechanism(s) by which captan and its analogs exert their cellular toxicity is not known, but several mechanisms are possible [1]. The pyrethroid insecticide deltamethrin impedes the proper functioning of the sodium channels in cell membranes and interacts with gamma aminobutyric acid receptors, thus causing a disruption of nerve signals [1]. Isoproturon is a phenylurea herbicide that inhibits the photosynthetic electron transportation system in plants [15], but it is also directly or indirectly toxic to a wide variety of organisms [16]. Pirimicarb is an insecticide that disables the nervous system by the inhibition of acetylcholinesterase [1]. Our test concentrations for all pesticides were 0 (controls), NC, MPC, and 100 times the MPC (100MPC) (Table 1).

Microbial respiration and biomass

We tested the effects of pesticide exposure on sediment microbial respiration (CO₂ production) and biomass (adenosine triphosphate [ATP] content) in microcosms (n = 4). The experimental setup and methods used were based on the International Toxicity Ring-Test [17], but with some minor alterations for respiration measurements (see Goedkoop et al. [18]). Microcosms were established by transferring 35.0 ± 0.10 g of well-stirred wet sediment to transparent glass vials (175 ml, bottom area 28 cm²), resulting in a sediment layer of approximately 1 cm. Then 140 ml of aerated, glass-microfiber-filtered (Whatman International, Maidstone, UK) Lake Erken (Stockholm, Sweden) water were carefully added (without resuspending the sediment), and the vials were sealed with a metal, polyethylene-lined lid. Microcosms were allowed to acclimate for 8 d in a climate room at 20°C and were aerated 15 min/h with CO₂-free air (airflow 3.0 L/min) using capillary tubing and aquarium pumps (Fig. 1). Ingoing air was stripped of CO₂ in a 160-ml plexiglas tube containing soda lime pellets (Merck, Darmstadt, Germany) and a 340-ml gas-washing bottle containing 100 ml NaOH (5M). In two subsequent 100-ml E-flasks containing deionized water, the air was washed to avoid changes in pH in the microcosms. The pH of this water was checked regularly with a pH indicator strip and was
changed if pH exceeded 8. The air outlet of each microcosm was equipped with a 30-ml CO₂ trap consisting of glass vials containing 5.0 ml of 0.2 M NaOH pro analysi (p.a.).

The experiment was performed in a climate room at 20.2 ± 0.3°C with a 16:8-h light:dark regime using a 22-W lamp as an indirect light source. Temperature and proper function of the aeration were checked daily. To simulate natural sedimentation of organic material and avoid carbon limitation of sediment microbes, fine particulate fish food (TetraPhyll®, TetraWerke, Melle, Germany, 600 μg/microcosm) was added to the microcosms at two occasions, 7 d before and on the same day the pesticides were added. On day 0, equal volumes of stock solutions of the four pesticides, dissolved in ethanol (99% p.a.), were added to the overlying water to achieve final exposure concentrations. The equivalent volume of ethanol was added to the controls. Six blanks, consisting of vials containing only filtered Lake Erken water (without sediment), were also included to correct for CO₂ originating from the overlying water.

Respiration was measured on days −2, −1, 0, 1, 2, 4, 8, 13, and 16. The microcosms were aerated continuously for 45 min to transfer accumulated CO₂ in the microcosms to the CO₂ traps. Then CO₂ traps were uncoupled from the microcosms, and the small inlets and outlets in the lid of each CO₂ trap were immediately sealed with tape. Respiration was determined by titrating the 0.2 M NaOH in the CO₂ traps with 0.1 M HCl [19] directly in the CO₂ traps within 1 h after removing the CO₂ trap. Phenolphtalein (Merck) was used as an indicator, and 2 ml of 1 M BaCl₂ (p.a. Merck) were added to bind the produced CO₃²⁻ ions. The mean respiration of the blanks was subtracted from that in microcosms. Respiration rate was expressed as micrograms of CO₂ produced per gram wet-weight sediment and hour.

Sediment microbial biomass was determined as the sediment ATP content [20]. Measurements were done according to Lundin [21]. The 1.0-g aliquots of the surface sediment layer (~2 mm) of each microcosm were sampled at the end of the respiration experiment (on day 16) and transferred to 50-ml polypropylene centrifuge tubes (Elkay Products, Shrewsbury, MA, USA). For ATP extraction, 10.0 ml of 10% trichloracetic acid (TCA; Merck) containing 4 mM ethylendiamine tetraacetic acid (EDTA; Merck) were added to sediment samples. To disrupt bacterial cell membranes, the samples were vortexed for 1 min and stored for 2 d at 4°C. The samples were then centrifuged (10 min, 1,650 g), and the supernatant, containing extracted ATP, was further processed. For luminiscence measurements, 0.8 ml of cold Tris-EDTA buffer (0.1 mM Tris [hydroxymethyl] aminomethane, 2 mM EDTA, pH 7.75 [BioThema®, Stockholm, Sweden, product no. 21-501]) were added to 5-ml luminometer measuring tubes and allowed to adapt to room temperature. To this buffer, 10 μl of the supernatant and 0.2 ml of a reagent solution (lyophilized reagent containing β-luciferin and luciferase, BioThema, product no. 11-501) were added, and light energy was measured with a luminometer (LKB-Wallac 1250). Finally, 10 μl of an ATP standard (10⁻³ moles ATP/L, BioThema, product no. 45-051) were added to the tubes and the measurements repeated. Microbial biomass was expressed as nanograms of ATP per gram wet-weight sediment.

Denitrification

In our second experiment, we studied the effects of pesticide exposure on sediment denitrification rates (n = 4). Lake Erken water was filtered with glass microfibers (Whatman International) and saturated with N₂ gas overnight to reach anoxia. The K¹⁵NO₃ (p.a. 99%, IsoChem, Berkshire, UK) was added to the anoxic lake water to a final concentration of 1 mg NO₃⁻/L. To avoid carbon limitation in the microcosms, sodium acetate (NaCH₃COO, p.a. Merck) was added to the water to a final concentration of 1 mg C/L. Sediment aliquots of 6 g were spiked with pesticide stock solutions in dimethyl sulfoxide (DMSO; p.a. Merck) to achieve final exposure concentrations (NC, MPC, 100MPC) and were thoroughly mixed. Control sediment received an equal volume of DMSO and was treated similarly. Microcosms were established by transferring 0.50-g subsamples of the sediment aliquots to 12-ml glass exetainers (Labco Limited, High Wycombe, UK) with screw caps equipped with a semipermeable membrane. The anoxic water containing ¹⁵NO₃ was transferred to the exetainers under minimal air exposure using a 50-ml syringe, and the exetainers were sealed airtight. Exetainers were then mounted horizontally on a shaking table in the dark at 10°C for 12 and 24 h, respectively. Incubations were terminated by injecting 250 μl of 50% ZnCl₂ (p.a. Merck) through the semipermeable membrane. Samples were stored for two weeks at 4°C prior to analysis.

Denitrification rate was quantified by using the ¹⁵N-isotope pairing technique according to Nielsen [22], with a few modifications by Svensson [23]. Briefly, first 2 ml of the water phase in each microcosm were replaced by helium, using a syringe. After vigorous shaking, 50 μl of the gaseous phase from each microcosm were injected into a gas chromatograph connected to an isotope-ratio mass spectrometer (Hewlett-Packard 4100 gas chromatography/mass spectrometry, Avon-
Effects of pesticides on sediment microbes

Environ. Toxicol. Chem. 23, 2004 1923

dale, PA, USA) to detect 15N-labeled dinitrogen pairs (14N15N and 15N15N) formed by denitrification [22]. To calculate the denitrification rate, we used the ratio between single-labeled (14N15N) and double-labeled (15N15N) dinitrogen pairs, which indicates the rates of denitrification of 14NO3 (formed by nitriﬁcation) and 15NO3 (in the water phase), respectively (for details, see Nielsen [22]). We corrected for background levels of the different N2 fractions in Lake Erken by analyzing three lake water samples. Finally, total denitrification rate was expressed as nanomoles of N2 produced per gram wet-weight sediment and hour.

Bacterial activity

In our third experiment, we studied pesticide effects on bacterial activity in small microcosms (n = 3). Sediment pesticide exposures were made in glass vials by spiking sediment aliquots of 3 g with pesticide stock solutions (with DMSO) to achieve the experimental exposure concentrations (NC, MPC, 100MPC). Control sediment received an equal volume of DMSO. The sediments were thoroughly mixed, and 0.25-mg subsamples were transferred to our experimental microcosms (10-ml Oak-Ridge tubes, Nalgene, Rochester, NY, USA). Since preliminary experiments with Lake Erken sediment have shown that the sediment microbes are limited by the availability of low-molecular C, sodium acetate (NaCH3 COO, p.a. Merck) was added to each microcosm to a ﬁnal concentration of 1 mg C/L. Pesticide exposure of the sediment was performed for 1 and 8 h in the dark at 15°C.

Bacterial activity was determined as 3H-leucine incorporation rates according to van Duyl and Kop [24], which is a modiﬁcation for sediment samples based on Simon and Azam [25]. The appropriate ﬁnal concentration of leucine (1.36 μmol/g) had been determined by an isotope saturation test with Lake Erken sediment. Because of the high labeling of bacterial protein, radioactive leucine (L-[4,5-3H]-leucine, 148 Ci/mmol, Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) was diluted with nonradioactive leucine (l-leucine, biopur 99%, Merck) of the same concentration to a ﬁnal solution consisting of 15% radioactive and 85% nonradioactive leucine. After 1 or 8 h of pesticide exposure, 50 μL of the leucine solution were added to each microcosm. Sediment slurries were then gently shaken and incubated in a water bath at 15°C for 1 h. Incubations were terminated by adding 1 mL of 80% ethanol. Three blanks, where bacterial activity was stopped with 80% ethanol immediately after 3H-leucine addition, were also included to determine the adsorption of the isotope to sediment particles. Samples were stored in a refrigerator and further processed within 2 to 3 d.

Samples were processed by rinsing the sediment twice with 5 mL of 80% ethanol and four times with 2 mL of 5% ice-cold TCA to remove excess label. Sediment was resuspended, the tubes were centrifuged (20 min, 10,000 g) in a cooled centrifuge, and the supernatant was discarded. In the next step, the samples were hydrolyzed in 2 mL of 2 M NaOH (2 h, 100°C water bath), which precipitates cold TCA-insoluble macromolecules (proteins) [26]. After cooling, the samples were again centrifuged (as previously), and 250 μL of the supernatant were transferred to scintillation vials. Ten milliliters of scintillation cocktail (Optiphase Hisafe 2, Wallac) were added, and radioactivity was measured by liquid scintillation counting (LKB-Wallac, 1217 Rackbeta, Turku, Finland). Scintillation counts were corrected for quenching using internal standard ratios. Bacterial activities were expressed as picomoles of leucine incorporated per gram wet-weight sediment and hour.

Statistical analysis

All data were log10 transformed and analyzed with StatView 5.0 for Macintosh (SAS Institute, Cary, NC, USA) with alpha (α) set at 0.05. Effects of pesticide exposure and exposure time were analyzed by analysis of variance (ANOVA) and Bonferroni/Dunn tests for pairwise comparisons (no signiﬁcance unless p < 0.0083).

RESULTS

Respiration and microbial biomass

Mean respiration varied substantially over the course of the experiment, and an effect of time was observed for all four pesticides (two-way ANOVA, p ≤ 0.03). Interestingly, pesticide exposure did not affect respiration (two-way ANOVA, captan p = 0.32, deltamethrin p = 0.14, isoproturon p = 0.15, pirimicarb p = 0.08). Mean respiration across all treatments and time intervals varied from 26 ± 6.0 to 107 ± 29 μg CO2/(g·d) (Fig. 2). Respiration rates during the pretreatment period (day 0 to day 0) showed declining time trends and were very low at the start of our experiment, 4.0 ± 2.5 to 45 ± 7.3 μg CO2/(g·d) in all microcosms. However, after the second addition of organic material on day 0, respiration rapidly increased to between 28 ± 6.0 and 60 ± 23 μg CO2/(g·d) recorded on day 1 (Fig. 2). Respiration showed similar temporal trends in all pesticide treatments, except those with deltamethrin, that is, initially relatively stable values, higher respiration between days 4 and 8, and again lower values between days 8 and 16. Pairwise comparisons showed that mean respiration in captan and pirimicarb treatments was higher between days 4 and 8 than during all the other intervals (p ≤ 0.0033). In treatments with isoproturon, respiration over the interval from days 4 to 8 was higher than that found for all other intervals except days...

Fig. 3. Mean microbial biomass adenosine triphosphate (ATP; as ng ATP/g) in controls (□) and treatments with negligible concentration (■), maximum permissible concentration (MPC; ◊), and 100MPC (△) for captan, deltamethrin, isoproturon, and pirimicarb. Letter codes denote significant differences between means (Bonferroni/Dunn p, 0.0083); that is, treatments having no letters in common are significantly different. Error bars denote ±1 standard deviation; WW = wet weight.

Fig. 4. Mean denitrification rate (as nmol N₂/g·h) in sediment treated with captan (A), deltamethrin (B), isoproturon (C), and pirimicarb (D) after 12- and 24-h exposures, respectively, at negligible concentration (□), maximum permissible concentration (MPC; ◊), and 100 times MPC (100MPC; △) and in controls (□). Error bars denote ±1 standard deviation.

Fig. 5. Mean bacterial activity (³H-leucine incorporation, as nmol/g·h) in sediment treated with captan (A), deltamethrin (B), isoproturon (C), and pirimicarb (D) after 1- and 8-h exposures, respectively, at negligible concentration (□), maximum permissible concentration (MPC; ◊), and 100 times MPC (100MPC; △) and in controls (□). Error bars denote ±1 standard deviation. * indicates that data were omitted (see text).

Denitrification

None of the four pesticides affected denitrification (two-way ANOVA, p > 0.05) in our experiment. Denitrification in MPC treatments with pirimicarb tended to be lower than that of controls after 24 h, although this difference was not statistically significant (p = 0.02) (Fig. 4). Similarly, denitrification in NC treatments with deltamethrin was lower than in MPC treatments, albeit not significantly. Denitrification rate ranged from 14 ± 2.5 to 20 ± 2.9 nmol N₂/(g·h) for treatments with captan, from 14 ± 4.0 to 19 ± 1.5 nmol N₂/(g·h) for those with deltamethrin, from 15 ± 4.0 to 21 ± 3.5 nmol N₂/(g·h) for those with isoproturon, and from 13 ± 2.9 to 18 ± 5.5 nmol N₂/(g·h) for treatments with pirimicarb. Denitrification measurements showed relatively large variation, with coefficients of variation ranging between 2.4 and 38.2% across all treatments.

Bacterial activity

Bacterial activity (³H-leucine incorporation) was negatively affected by all four pesticides (two-way ANOVA, captan p = 0.0006, deltamethrin p < 0.0001, isoproturon p < 0.0001, pirimicarb p = 0.004) (Fig. 5). Bacterial activity determined after 8 h of exposure was lower than that in controls in MPC treatments with captan and pirimicarb in both NC and MPC treatments with deltamethrin and in all treatments with isoproturon (p ≤ 0.0083). For example, in pirimicarb treatments with MPC after 8 h of exposure, bacterial activity was 250 ± 25 pmol/(g·h) compared to 328 ± 18 pmol/(g·h) in controls, corresponding to a relative decrease of 24%. The lowest bacterial activities in captan and deltamethrin treatments after 8
h were also observed in MPC treatments, 258 ± 20 and 262 ± 16 pmol/(g·h), respectively, which corresponds to relative decreases of 21 and 20% compared with controls. In deltamethrin and pirimicarb treatments, this decreasing trend across the exposure gradient was interrupted at 100MPC, where mean bacterial activity was similar to that in controls but significantly higher than that in MPC treatments ($p < 0.0001$ and $p = 0.0083$, respectively). In 100MPC treatments with captan, bacterial activity was intermediate to that in controls and MPC treatments, while isoproturon instead showed the lowest activity, 235 ± 13 pmol/(g·h), at 100MPC after 8 h of exposure, that is, a 28% decrease relative to controls.

In treatments that were exposed for 1 h with captan and deltamethrin, patterns in bacterial activity were similar to those found after 8 h, with negative effects found in MPC treatments. On average, bacterial activity in these treatments declined by 29% relative to the controls. Also similar to the trend observed for the 8-h exposures, bacterial activity in 100MPC treatments with captan and deltamethrin were again similar to those in controls. Data from the MPC exposures with isoproturon calculated after 1 h were unreasonably low ($-6.6 ± 3.7$ pmol/[g·h]) and therefore were omitted from the data set. Furthermore, one of the isoproturon 100MPC replicates showed only one-third of the activity of the mean of the other two replicates, which resulted in the large standard deviation observed for this treatment.

**DISCUSSION**

Our results show that exposure to pesticides at environmentally relevant concentrations can affect microbial communities of natural sediments. Bacterial activity was inhibited by all four pesticides, whereas microbial biomass was negatively affected only in treatments with deltamethrin and isoproturon. This shows that insecticides and herbicides that are designed to affect specific functions of their target organisms also may have a direct or indirect toxic effect on nontarget organisms, such as heterotrophic microbes. Negative effects were frequently found in NC or MPC exposures, implying that important bacteria-mediated processes in aquatic ecosystems, such as decomposition of organic material, self-purification, and nutrient regeneration, may be affected at concentrations that are considered environmentally safe [14]. Hence, NCs and MPCs for organic contaminants determined for higher-level organisms or by modeling may be too low to be applied to heterotrophic microbes.

The observed effects on bacterial activity and microbial biomass did not show a consistent, inverse relationship with pesticide concentration. For three of four pesticides, mean bacterial activity was lower in MPC treatments than in controls but did not differ from controls at the highest concentration tested (100MPC) (Fig. 5). Similarly, in treatments with isoproturon, mean ATP content was lower at NC than at MPC and 100MPC (Fig. 3). A number of studies report both inhibitory and stimulatory effects of organic contaminants on microbial communities in soils [27–29] and aquatic environments [9,12,30]. Our observed inhibition of bacterial activity and decrease in living biomass at relatively low exposure concentrations are supported by several studies [9,12]. The decrease in bacterial activity in all isoproturon treatments after 8 h is also in agreement with Tixier et al. [31], who reported marked biological effects of isoproturon on bacteria in a Microtox test. Other herbicides belonging to the sulfonylurea group are also known to inhibit microbial activity of the most sensitive species at nanomolar concentrations [30]. Further, in accordance with our results, Groth Petersen et al. [9] found decreases in total DNA content after additions of low and intermediate concentrations of zinc pyrithione (ZPT) and increases in total DNA content and sediment bacterial production ($^{14}$C-leucine incorporation) at the highest concentration of ZPT. These studies show that the interactions between pesticides and microbes can be highly complex and do not always allow for straightforward interpretations.

Stimulatory effects of pesticide exposure on microorganisms are frequently attributed to decreased nutrient competition. Pesticide exposure may poison some groups of microorganisms, while others are favored by decreased competition for energy/nutrients and nutrient release from decaying microorganisms [27,32]. Furthermore, some microbes may experience a competitive advantage by their capability of using pesticides as an energy source [33]. Consequently, pesticide exposure may induce shifts in the dominance of certain groups of microbes in microbial communities, as is supported by molecular fingerprinting methods [9,33]. These pesticide-induced structural changes, however, should not necessarily be accompanied by functional changes. A loss of ecosystem function will occur only if tolerant microorganisms are incapable to compensate for specific functions performed by sensitive groups.

An alternative explanation for the observed increase in activity at high pesticide exposure could be that sediment microbes experienced a toxic effect in MPC and/or NC treatments and responded by decreasing their activity. However, at 100MPC, sediment microbes may have been stressed and instead increased their activity in response. Pell et al. [28] suggested that a toxic response in microorganisms could cause, for example, increased uptake of energy or uncoupling of energy-yielding processes, which in turn increases their activity. This response might be as severe as a decrease in activity since it may cause mortality after prolonged stress. Pell et al. [28] further suggested that stimulatory effects might be due to stress-induced cell growth and an increase in cell numbers.

Although pesticide exposures showed effects on both bacterial activity and biomass, no effects on respiration were observed. This may seem surprising since bacteria represent a large share of the overall respiration of sediment communities [34]. Possibly the lack of effects on respiration may be a consequence of our experimental setup. In the respiration experiment, we spiked the overlying water phase in our microcosms according to the proposed Organization for Economic Cooperation and Development (Paris, France) Guideline for toxicity tests [17]. Most likely, the binding of test compounds to the sediment resulted in high concentrations in the surface layer of sediment and lower (or even undetectable) concentrations in deeper sediment. Therefore, possible effects on respiration in the surface sediment layer may have been masked by the absence of effects in deeper sediment. This conjecture is further supported by the fact that we did find effects on microbial biomass (ATP) in treatments with deltamethrin and isoproturon in the top 2 mm of the sediment in the same microcosms. Furthermore, the relatively large variation in respiration among replicates resulted in relatively low statistical power for this endpoint, and a loss of test compound from the aerated water phase (see Goedkoop and Peterson [35]) may have contributed to the lack of effect on respiration.

Respiration was low after 8 d of pretreatment but recovered rapidly after the addition of organic matter on day 0 and de-
clined again after day 8, probably because of lack of low-molecular carbon that can easily be used as substrate by bacteria [36]. Also, overall denitrification rates in our experimental sediment were relatively low (see Svensson and Leonardsson [10]), showed relatively large variation, and did not allow us to detect any effects on denitrification. Considering that surplus nitrogen was added and that anoxic conditions were created, we speculate that denitrifying bacteria were carbon limited in our tests despite the additions of organic matter. Carbon limitation is an important rate-regulating factor for many sediment microbial processes [28,37], and bacterial demands for nutrients have been reported to increase at low temperatures [38], as was the case in our incubations. Hence, if we are interested in responses of microbial communities to contaminants, it is vital to avoid that energy/nutrient limitation will mask possible treatment effects on microbes.

In our experiments we used single-pesticide exposures, but under in situ conditions, different, synergistic effects of organic contaminants may act on microorganisms. Besides, under field conditions, pesticides may have much longer degradation times than are predicted from laboratory studies (e.g., because of lower temperature) and thus pose a risk to nontarget organisms for a considerable time. Furthermore, indirect negative effects of pesticides on sediment microbes may result from direct effects on aquatic invertebrate populations (mainly insects). Reductions in invertebrate populations would result in a lower bioturbation of the sediment, in decreased bacterial activity [39] and denitrification [40], and ultimately in slower mineralization and recycling of organic matter [7]. Even marginal direct or indirect effects on microbial processes may have a far-reaching impact on ecosystem functions since they act over large spatial scales.

Effects on bacterial activity and microbial biomass were found at NCS and MPCs that are predicted to be safe to the environment [14]. However, calculations of these environmentally safe concentrations are, at best, based on toxicity concentrations determined for a few aquatic organisms (e.g., Daphnia sp. or a cyanobacterium) and not on tests with natural microbes. In most cases, NCS and MPCs for sediments are entirely extrapolated from water exposures because of the lack of toxicity data for sediments. The discrepancies between postulated safe concentrations and the results in our study stress the need for toxicity tests with natural microbial communities. Insights into the importance of heterotrophic microbes in aquatic ecology and the development of techniques to measure a vast array of microbial processes call for the application of these techniques in aquatic ecotoxicology.

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