Swedish University of Agricultural Sciences
Department of Aquatic Sciences and Assessment

Multiple pesticide stressors and ecosystem functioning in stream detrital food webs

Mohab Dawoud

2011

Title: Multiple pesticide stressors and ecosystem functioning in stream detrital food webs

Mohab Dawoud

Supervisor:
Associate Professor Brendan M^{c}Kie
Department of Aquatic Sciences and Assessment
Swedish University of Agriculture (SLU)

Professor Willem Goedkoop
Department of Aquatic Sciences and Assessment
Swedish University of Agriculture (SLU)

Examiner:
Associate Professor Stina Drakare
Department of Aquatic Sciences and Assessment
Swedish University of Agriculture (SLU)

30 hp
D level
Independent project/Degree project in Biology
EX0565
Master program in Ecology

Swedish University of Agriculture
Department of Aquatic Sciences and Assessment
2011
http://stud.epsilon.slu.se

SLU

Swedish University of Agricultural Sciences
Department of Aquatic Sciences and Assessment

Contents

Abstract

Streams and rivers are highly susceptible to environmental degradation from agricultural activities, including the clearance of riparian vegetation and the runoff of chemical fertilizers and pesticides. These impacts are likely to increase in the future as agricultural practices intensify to meet the needs of an expanding human population. For example, pesticide application has considerably increased in the last 35 years, with an increased runoff to aquatic ecosystems. Importantly, intensive agriculture often entails the use of multiple pesticides for different purposes (e.g. control of different bacterial, fungal or insect pests). Prediction of the ecosystem effects of the application of multiple pesticides is complicated by the potential both for interactions among the pesticides themselves, and for the pesticides to alter interactions among different organism groups within trophic webs. I investigated the effects of two contrasting pesticides targeting two different organism groups (the insecticide Lindane and fungicide Azoxystrobin) on a stream detrital food web consisting of detritivores (Ispoda: *Asellus aquaticus*) - and microbes (an assemblage of fungal hyphomycetes) consuming leaf litter. I assessed effects of the stressors on ecosystem functioning, quantified as multiple ecosystem process rates. These included leaf decomposition, leaf processing efficiency and detritivore growth rate. Leaf decomposition is a key ecosystem process in the nutrient and energy budgets of forested streams worldwide. Additionally I quantified detritivore mortality and moulting characteristics (frequency and moulting period). Standardized discs of black alder leaves (*Alnus glutinosa L.*) were colonized with a fungal assemblage for use in a microcosm experiment. The fungal assemblage was sourced from a forested catchment characterized by mixed agricultural and forest landuse. Each microcosm contained 20 colonized leaf discs, and 50 mL of standardized artificial fresh water ("M7"). Four pesticide treatments were varied among the microcosms: (i) no presence of pesticides (i.e. controls), (ii) Lindane 5 µg/l (single stressor), (iii) Azoxystrobin 2600 µg/l (single stressor), and (iv) a mixture of Lindane 5 µg/l and Azoxystrobin 2600 µg/l (multiple stressors). Additionally, the presence and absence of the detritivore *Asellus aquaticus* (Isopoda) was varied among the microcosms, to assess the effect of pesticides across multiple trophic levels. I hypothesized that the fungicide and insecticide applied as single stressors will both negatively affect leaf decomposition through negative effects on microbe and detritivore-mediated

decomposition respectively, with additional "knock-on" effects of the fungicide on detritivore leaf processing efficiency and growth due to negative effects on microbial conditioning (microbial "softening" of the litter necessary for detritivore feeding). Consequently, I further hypothesized that two pesticides will interact synergistically negatively to affect leaf processing by the full detrital foodweb, with the strongest effects likely in the pesticide mixture treatment when the detritivores are present.

Pesticides affected ecosystem functioning in my laboratory microcosms, but these effects did not always correspond with expectations based on their target trophic level. The fungicide little affected decomposition mediated by microbes, and the insecticide did not have an overall affect on decomposition mediated by detritivores. However, an important interaction was apparent between the detritivore and pesticide treatments, with the fungicide and mixture treatments reducing decomposition only when the detritivore was present. This indicates the fungicide had significant knock-on effects on the performance of the detritivores, most likely reflecting the importance of microbial "conditioning" (leaf softening) of the detritus for the participation of *A. aquaticus* in the decomposition process. Synergistic interactions between the pesticides were also apparent, with detritivore leaf processing efficiency depressed most strongly when both pesticides were applied together. These effects were not reflected in identical responses for detritivore growth, which may be a consequence of the relatively short experimental period. The mortality rate was higher under the fungicide and mixture treatments, which may reflect reduced resource intake due to fungicide effects on microbial conditioning, toxic effects of the pesticide, or both. Finally, there was evidence that detritivore moulting period (time to first moult) was shortened under the pesticide treatments, which may indicate that detritivores have some capacity to adjust their moulting time to shed exoskeletons contaminated with toxins, particularly under repeated pulses of exposure. My results indicate that changed interactions within food webs can complicate prediction of pesticides effects on ecosystem functioning in streams, and highlight the potential for pesticides to disturb ecosystem structure and function in agricultural areas.

Keywords: Stream ecosystem, decomposition process, leaf litter, Lindane, Azoxystrobin, Asellus aquaticus, aquatic microorganism.

1 Introduction

Impacts of human activities on the world's ecosystems have accelerated rapidly in recent decades, driven both by population growth and the increasing exploitation of natural resources (Vitousek, *et al.,* 1997). This change is particularly evident in the clearance of forest lands for agriculture in many regions of the world, and the increasing use of "intensive" agricultural methods (FAO, 2001; Allen and Barnes, 1985; Simon and Garagorry, 2005). The development of the ''Green revolution'' during the 20th century dramatically raised agricultural production, through the extensive application of fertilizers and pesticides (Tilman, 1998). For example, pesticide application has considerably increased in the last 35 years (FAO, 2002) which in turn intensifies toxic impacts on both soil and water ecosystems (Tilman *et al*., 2002). Agricultural pesticides and fertilizers used in crop production typically transfer to the aquatic community through surface runoff (Richards and Baker, 1993), and leaching from soils, and ground water discharge (Majewski and Capel, 1995). As such, pesticides applied to terrestrial crops can easily contaminate adjacent aquatic environments, with potential consequences for both the structure and functioning of aquatic ecosystems, according to the strength of their affects on different trophic levels. This thesis presents results from an experimental study of the effects of multiple pesticide stressors (a fungicide and an insecticide) on the structure and function of aquatic detrital food webs.

<u>Ecosystem services provided by streams</u>

Ecosystems can be characterized according to both structural and functional attributes (Odum, 1971; McDash, 2001). *Ecosystem structure* refers not only to characteristics of the physical habitat architecture of an ecosystem, but also to the composition and diversity of its biological communities (Risser, 1995; Myster, 2001). *Ecosystem functioning* refers to the efficiency with which an ecosystem processes energy and nutrients, both in production of plant and animal biomass and breakdown and transformation of detritus, and arises from interactions among the diversity of organisms and their environments (Schulze and Mooney, 1994) . Functioning can be quantified as one or more *ecosystem process rates,* such as nutrient storage and recycling rates by aquatic biota (Vanni *et al.*, 2002; Sterner et al., 1997), soil retention facilitated by interactions between plant roots and soil biota (Bardgett & McAlister

1999), water clarification mediated by aquatic algae (Cardinale 2011), and leaf litter decomposition by aquatic microbes and detritivores (Gessner and Chauvet, 2002).

The ecosystem processes that comprise ecosystem functioning further underpin multiple ecological services of importance to humanity. Ecosystem services have been categorized within the framework of the Millennium ecosystem assessment (Ecosystem and Human well-being, 2003) according to supporting value, provisioning value, regulation value, and cultural value. Streams and rivers in particular provide multiple ecosystem goods and services to humanity. For example, supporting services provided by freshwaters include the cycling of nutrients, which underpin biomass production, while regulation services include water purification by microbial detoxification (Trevors, 1989; Okeke *et al.*, 2002). Note that both supporting and regulating services can often be quantified directly as ecosystem process rates (e.g. nutrient uptake rates, chemical detoxification rates). Provisioning services provided by streams include fishing as source of food, and the supply of drinking water, while cultural services comprise the educational, recreational and spiritual values provided by lakes and rivers to humanity (Wilson and Carpenter 1999; Costanza *et al.*, 1997). Threats to ecosystem services arise from human perturbations that either impair underlying ecosystem processes directly (direct impacts on functioning), or else alter community biodiversity and/or composition (ecosystem structural effects) (Jonsson *et al.*, 2002).

Modifications to ecosystem structural components, whether habitat architecture or community composition, often have knock-on effects on ecosystem processes and services, reflecting the strong links between ecosystem structure and function (Tilman, 1997). However, sometimes function can be altered by human impacts even in the absence of structural changes (Bunn and Davies, 2000), where the impact is associated with sub-lethal effects on organisms that compromise their performance and capacity to contribute to ecosystem processes. Equally, changes in community composition may not affect functioning, if unaffected organisms are able to compensate for the roles played by negatively affected organisms in ecosystem processes (Nelson, 2000). This highlights the value of assessing human impacts on both structure and function simultaneously.

Stream detrital food-webs and the effects of pesticides on stream ecosystem functioning

Several studies have measured the integration between the function and structure of the ecosystem by using one or more ecological processes as functional indicators. For aquatic ecosystems, Gessner & Chauvet (2002) suggested that stream ecological integrity under anthropogenic pressure can be quantified both through the assessment of structural integrity (the composition of biological communities, e.g. fish, macroinvertebrates and microinvertebrates), and functional integrity. As a measure of functional integrity, Gessner & Chauvet (2002) suggest focusing on the ecosystem process of leaf litter decomposition. Leaf decomposition is a key ecosystem process in streams and rivers which is regulated by both microbes and invertebrate detritivores. The food webs of forested streams and rivers are based on the allochthonous organic matter inputs produced outside the aquatic community (Cummins, 1975; Wallace *et al.*, 1997; Hall *et al.*, 2000), such as autumn fallen leaves in temperate regions of the world. On entering a stream, autumn shed leaves are exposed to several processes (leaching, conditioning and fragmentation) that convert Coarse Particulate Organic Matter (CPOM) to Fine Particulate Organic Matter (FPOM) and Dissolved Organic Matter (DOM) (Gessner *et al.*, 1999). The process of decomposition begins with the leaching of soluble compounds (Petersen and Cummins, 1974), followed by colonization of microbes, particularly the spores of aquatic hyphomycete fungi (Gessner *et al.*, 1999; Gulis & Suberkropp, 2003). Microbial colonization facilitates leaf degradation through enzymatic release that converts organic matter to CO_2 and biomass (Cummins and Klug, 1979), (Cummins *et al.*, 1980; Gessner *et al.*, 2010). This process, known as “microbial conditioning”, also increases the palatability of the litter (reducing litter toughness and increasing nutritional richness) for detritivores. Invertebrate detritivores in streams are most commonly known as ”shredders’’, and are responsible for the bulk of the physical fragmentation of leaves (Graça *et al.*, 1993; 2001).

Due to the interconnected nature of the detrital food webs, pesticides affecting one trophic level have potential to have “knock-on” effects on other trophic levels. Processes within food webs are potentially structured according two models. In the ‘’bottom-up” model, the diversity, composition and abundance of organisms at intermediate and top trophic levels depend on characteristics of the bottom (producer) level (Polis and Strong, 1996), while in the ‘’top-down model”, top consumers, typically large bodied predators, strongly influence characteristics of lower levels, though not always through direct interactions e.g. where the consumer causes change in the abundance of lower trophic levels (Hairston *et al.,* 1960; Polis *et al.,* 1996). Similarly, pesticides have potential to have top-down or bottom-up effects on processes such as leaf decomposition. Fungicides affecting microbial populations may impair leaf conditioning (Chandrashekar & Kaveriappa, 1989), and hence detritivore feeding activity from the bottom up, whereas insecticides affecting detritivore abundance and feeding rate (Kreutzweiser, 1997) can affect the amount of leaf litter remaining top down. Consequences for ecosystem functioning in turn depend on the importance of the affected trophic level for key ecosystem processes. For example, a fungicide causing strong toxic effects on the microbe trophic level has great potential to be associated with further negative knock-on effects on detritivore leaf processing, due to impaired microbial conditioning (Graça *et al*., 2001; Bärlochar, 1985; Gessner *et al.,* 1999). In contrast, while negative effects of an insecticide on detritivores are likely to impair their leaf-processing capacity, consequences for microbial leaf processing are difficult to predict. Indeed, given that detritivores themselves consume microbes, a negative effect on detritivore feeding activity may even favour greater microbial activity (Graça *et al*., 1993). The study of pesticides affecting different organism groups, and their consequences for ecosystem functioning, can give insight into the relative importance of the affected trophic levels for specific ecosystem processes.

These scenarios become even more complex in the situation where multiple pesticides are applied together. Multiple stressors, including multiple pesticides, have the potential to interact and produce effects that differ from expectations based on the actions of single stressors in isolation (Vinebrooke *et al.,* 2004). For example, microorganisms themselves can often decrease the toxicity level of chemicals by breaking them down or binding them up (DeLorenzo *et al.*, 2001) or degradation in aerobic and anaerobic conditions through microbial utilization of pesticide carbon (Middeldorp *et al.*, 1996), and the application of a fungicide might reduce the capacity of microbes to bind up or detoxify insecticide toxins, thereby increasing the overall impact of the insecticide. Folt et al. (1999) developed the *additive effect model*, which categorizes interactions among stressors as either synergetic effect (increased in stress) or antagonistic effect (decreased in stress) of multiple toxicant pesticides with a similar mode of action. In this model the combination of multiple stressors is greater than (synergism) or less than (antagonism) the sum of individual stressors. Intensive agriculture often entails the use of multiple pesticides for different purposes (e.g. control of different bacterial, fungal or insect pests). The runoff of such a "pesticide cocktail" to streams and rivers may have effects on ecosystem functioning that are difficult to predict, depending both on direct interactions among the pesticides themselves, and the knock-on effects of those interactions within the trophic web.

Microcosm experiment: Treatments & Hypotheses

I investigated the effects of multiple pesticide stressors on stream detrital food webs in a laboratory microcosm experiment. Replicate microcosms, each containing leaf litter colonized with a Swedish fungal assemblage, were subjected to one of four pesticide treatments: (i) *no pesticide* stressor treatment, (ii) the presence of the *fungicide Azoxystrobin* or (iii) the presence of the *insecticide Lindane* (both single pesticide stressor treatments), and (iv) a *multiple pesticide stressor* treatment, with both pesticides applied together. Additionally, the presence of the detritivore *Asellus aquaticus* was varied among treatments. The insecticide was applied at a level that was sublethal for *A. aquaticus* and fungicide was applied at level that was high to microorganisms. I used four response variables to characterize the effects of our pesticide and food web manipulations on mortality and ecosystem functioning:

A) Net Leaf litter decomposition, as a measure of ecosystem functioning

B) Detritivore leaf processing efficiency, characterizing the efficiency of detritivore leaf decomposition relative to detritivore biomass (McKie *et al.*, 2008)

C) Detritivore Mortality rate, to assess variation in mortality under the various pesticide treatments

D) Detritivore Moulting rate, as an additional measure of the stress imposed by pesticides on detritivores. The pesticides used in this study bind strongly to organic substrates (Novak *et al.*, 1995), and the detritivores may be able to respond by increasing their moulting rate, to shed contaminated exoskeletons (Song *et al*., 1997). Alternatively, pesticides may alter moulting rates by directly interrupting the hormonal pathways which regulate the number or timing of moults (An Ghekiere, 2006).

E) Detrivore Growth, as a secondary measure of ecosystem functioning reflecting biomass accrual

Two further measures of ecosystem functioning will also be quantified: fungal biomass (via ergosterol measurement) and fungal spore production. Unfortunately these data were not available at the time of preparation of this thesis, due to circumstances beyond my control (lack of availability of key apparatus and reagents during autumn 2011), but will be included in a future publication.

My research aimed to, a) investigate the effects of two contrasting pesticides whose use is expanding in line with the global intensification of agriculture, on the key ecosystem process of leaf decomposition, and b) use the pesticide manipulations to help clarify the relative importance of top-down (detritivore mediated) and bottom up (microbial-mediated) pathways for the key process of decomposition in streams. I hypothesize that (H.1) the fungicide and insecticide applied as single stressors will both negatively affect leaf decomposition through negative effects on microbe- and detritivore- mediated decomposition respectively, (H.2) detritivore mortality rate will be affected by the presence of Lindane, and possibly also Azoxystrobin, (H.3) detritivore moulting rate will increase under the pesticide treatments, (H.4) detritivores leaf processing efficiency will be negatively affected by Lindane and (H.5) detritivore growth will be affected negatively by the both direct effects of Lindane on detritivore feeding rates, and indirect effects of the fungicide Azoxystrobin on microbial conditioning. Finally, I hypothesize (H.6) that additional “knock-on” effects of the fungicide on detritivore leaf processing efficiency and growth due to negative effects on microbial conditioning will cause the two pesticides to interact synergistically to negatively affect leaf processing by the full detrital foodweb, with the strongest effects likely in the pesticide mixture when the detritivores are present.

2 Materials and methods

Microcosm set up and fungal colonization of the leaf litter

The effects of the fungicide Azoxystrobin and insecticide Lindane on two trophic levels within the detrital food web were assessed in forty 120 ml glass microcosms within a controlled environment room (temperature 11-12 °C during May 2012). The presence of the two pesticides, both alone and together, was varied among the microcosms, with half additionally containing two individuals of the detritivore *A. aquaticus*. Each microcosm contained 50 ml of water and twenty Black alder (*Alnus glutinosa L.*) leaf discs, which had been pre-colonized with a fungal assemblage from a nearby stream. The experiment was terminated after thirteen days, with the pesticide treatments renewed half way through the study period.

Leaf litter and fungal colonization

A. glutinosa leaf litter was collected just prior to abscission by the river Fyrisån, SLU, during October 2010, and subsequently air dried at the laboratory. Prior to the microcosm experiment, these leaves were rewet, and leaf discs were cut using a cork borer (15 mm), ensuring a standardized leaf surface area. The central leaf vein, which is of low nutritional value, was excluded from all leaf discs.

Rather than colonize the leaf discs with hyphomycete fungal spores directly in the field, the discs were colonized from an additional set of pre-conditioned leaves in the laboratory. This was achieved via a two-stage protocol:

(i) An additional set of whole leaves were exposed in a local stream to allow colonization by local fungi. The field colonized litter was later transferred to laboratory aquaria.

(ii) The leaf discs were added to the aquaria, allowing colonization of the discs with spores from the field-conditioned litter.

Laboratory colonization of the leaf discs avoids variability in both fungal community composition and litter decay state potentially associated with microhabitat variability in the field. Thus, compared with colonizing the discs directly in the field, this two-stage process ensures a greater standardization in the condition of the discs prior to the experiment (Ermold, 2009).

Hågaån stream was chosen as the source for the colonizing microbes. It is a sixth order stream located to the southwest of Uppsala at 59.80° 51′ 30″ N, 17.61° 39′ 0″ E (figure 2.1). The Hågaån catchment is characterized by mixed land use, including both forested and agricultural land, and is affected by enrichment of nutrients from the fertilizers from the surrounding organic farms (Bergfur, 2007). Hågaån was chosen as a colonization site for the high diversity and activity of its microbial assemblages, according to previous observations from Ermold (2009).

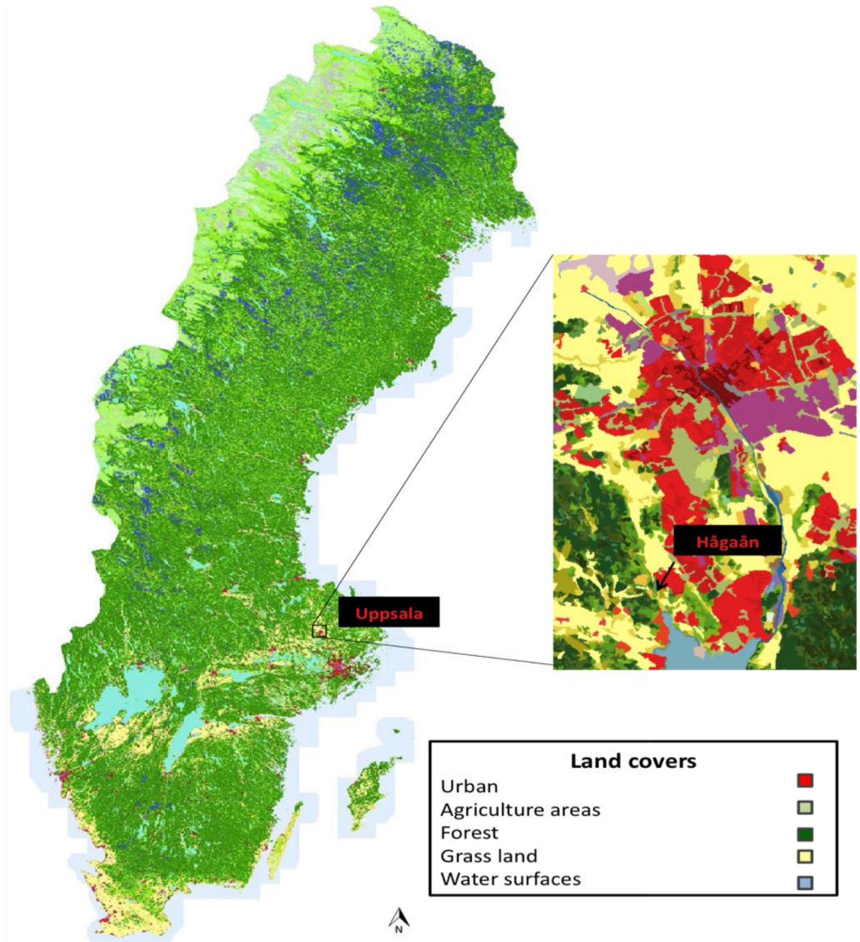


Figure 2.1 Map showing the location of Hågaån stream and the surrounding land covers. Sweden map was obtained from European topic center on spatial information and analysis (*http://sia.eionet.europa.eu/CLC2000/countries/se/full), and Uppsala region map was obtained from Digitala kartbiblioteket (https://butiken.metria.se/digibib/index.php*)

Fungal spore colonization of the experimental leaf discs occurred within a plastic aquarium containing the field colonized leaves. The leaf discs were evenly divided among four 15*15 cm polyamide mesh bags (210 discs per bag), with mesh size 0.5 mm, which were then immersed within the aquarium. The discs were left for fourteen days. In Ermold's (2009) study, this period had been sufficient to achieve a diverse and abundant community of fungi on leaf discs colonized in an identical way from the Hågaån assemblage.

Detritivore collection

Aquatic sowbug (Isopoda: *Asellus aquaticus*) was used as a shredder, due to its status as a common detritivore in the agricultural streams of Europe, Russia and North America (Maltby, 1991; Monahan, 1996). *A. aquaticus* is also used as a water quality indicator for its high chemical pollution tolerance (Slooff, 1983). One week before starting the experiment, a kick sampling method was used for collecting 140 adult and juvenile individuals from the ditches of a pond found in the campus of Swedish university of agricultural Sciences, Uppsala. They were transported to the laboratory in temperature between 11-12 ºC, where they were kept in a plastic aquarium (23 liter) filled with pond water and supplemented with a mixture of autumn shed litter, including *Alnus glutinosa*, *Fraxinus excelsior* and *Populus tremula*, as a natural organic food. Aeration was maintained by three air pumps to keep comfortable conditions for *A. aquaticus* until the starting date of the experiment.

Chemical preparation

Solutions of the two pesticides, the insecticide Lindane and fungicide Azoxystrobin, were prepared from commercially available products (called Gamma-HCH and Azoxystrobin respectively) in M7 medium. M7 medium is standardized water with a defined composition and quantity of elements that is commonly used in laboratory toxicity tests, and was prepared according to the recipe in OECD guideline (annex 2). Three different pesticide stock solutions were prepared, matching the three pesticide treatments applied in the experiment, with acetone (50 µg/mL) used as solvent in all cases (Lindane: 500 µg/ml; Azoxystrobin: 5200 µg/ml; and Combination of Lindane + Azoxystrobin: 500 + 5200 µg/ml). In a previous study (Ermold, 2009), acetone was applied at a higher level (100 µg/ml) than in this study (50 µg/ml), and had no negative effects on the microbes or *A. aquaticus*.

Purposes of Azoxystrobin and Lindane

Pesticide Azoxystrobin commonly sold in Sweden (7.4 ton) and mostly used for agriculture and fruit trees (Kemikalieinspektionen 2010).The agricultural purpose of using Azoxystrobin is to prevent foliar diseases of vegetable and fruit crops by targeting pathogenic fungi (from Ascomycota, Deutermyctoa, Basidomycota and Oomycetes) that cause diseases such as powdery mildow, downy mildow, wheat leaf rust, haustorium (Bartlett *et al.*, 2002). Fungicides from the strobilurin group affect electron transport systems in fungal mytochondira, and interrupt fungal development by disturbing the energy production for spore germination and zoospore motility (Bartlett *et al.*, 2002). In a previous study (Ermold, 2009), Azoxystrobin was shown to have variable effects on microbial community structure and function, depending on characteristics of the source assemblage (Ermold, 2009). Fungal species richness and community composition was strongly affected by Azoxystrobin in a forest assemblage with no history of agricultural disturbance. In contrast, these parameters tended to be affected only at the highest pesticide doses, if at all, for assemblages from agricultural streams (Ermold, 2009). These finding may reflect the composition of the different assemblages, as the agricultural communities were characterized by taxa known to be tolerant of a range of environmental disturbances, though adaptation driven by previous pesticide exposure may also have played a role.

Insecticides from the organochlorine group have toxic effect on the organisms by causing inhibition in the nervous systems (DeLorenzo et al., 2001). Lindane is applied to a wide range of crops, targeting soil-dwelling insects and plant eating worms. Lindane has been banned in Sweden since 1980 (Persistent organic pollutants review committee, 2007), but still persists in Swedish waters as both a legacy of previous use, and resulting from new rainwater deposition arising in surrounding countries.

When applied together, the combination of insecticides and fungicides can have unpredictable effects on the aquatic community (Cuppen *et al.*, 2002; Daam *et al.*, 2010).

In this study, the application of both fungicide (Azoxystrobin) and insecticide Lindane are expected to have strong effects on leaf decomposition due to the simultaneous impairment on the two trophic levels, unless the pesticides interact antagonistically in their effects on functioning. For example, negative effects of Lindane on detritivore feeding might release microbes from detritivore grazing pressure, allowing some compensation for negative effects of Azoxystrobin on the microbial level.

Experimental design & procedures

Pesticide concentration was varied among the microcosms, with four levels of treatment:

1) A control, with M7 medium only, and no pesticides;

2) 50 µl of the Lindane stock solution for a final concentration 5 µg/l;

3) 50 µl of the Azoxystrobin stock solution for a final concentration 2600 µg/l;

4) 50 µl of pesticide mixture for final concentration 5 µg/l+ 2600 µg/l).

The four pesticide treatments were fully-crossed with two *A. aquaticus* presence treatments: absent (no *A.aquaticus* individuals) and present (two adult individuals). The concentrations of the pesticides were at sublethal levels for *A. aquaticus.* The toxic level of Azoxystrobin was determined based on a previous experiment (Ermold, 2009). The sublethal concentration of Lindane was first estimated based on the literature, and then confirmed in a pilot study (Appendix 1). Each pesticide x *A. aquaticus* treatment combination was replicated five times in a controlled environment room, within a temperature between 11 °C and 12 °C. The microcosms (20 colonized leaf discs/microcosm) were placed on a shaker table at an appropriate frequency (50 rpm) to provide aeration and stimulate sporulation (Webster, 1972). The animals had an initial and final photos captured on graphing paper using a 10-megapixel camera.

On the sixth day, the water was decanted from each microcosm and preserved in 50 ml centrifuge tubes in the presence of 2 ml of formalin; tubes were sealed with Parafilm® for later spore counting. The water was then replaced according to the pesticide treatments detailed above.

On the final day of the experiment, the water was decanted from each microcosm and preserved as described above. The leaf discs from each microcosm were randomly divided into two groups of 10. Ten leaf discs were dried in an oven at 50 °C for 3 days and the other ten leaf discs were preserved in freezer for fungal biomass (Ergosterol) analysis. The preserving of leaves and spore water were done for later analysis of fungal biomass and counting of spore production. The animals were preserved in small tubes filled with 70% ethanol.

Measurements

Microcosms containing *A. aquaticus* were checked daily. Dead animals were counted and then picked out and replaced with a new individual using soft forceps. Additionally, moulted exoskeletons were counted and removed daily. The total number of individuals moulting under each pesticide treatment over the experiment was recorded, as was the moulting period (time in the microcosms prior to moulting) for each individual.

The body length was measured for living and dead animals from the head part to the end of the tail part via image analysis software (Image J 1.44P, Wayne Rasband, National institutes of health, USA), and then these measurements were converted to body size via published length-mass relationship equations for Swedish *A. aquaticus* (Reiss *et al.*, 2011).

Leaf mass loss, Leaf processing efficiency and Relative growth rate

1. **Percent of leaf mass loss (LML %):** Initial mass (IM) of the leaf discs was determined based on a random subset of 38 leaf discs which were cut but not used in the experiment. Final leaf mass (FM) was measured directly for the 10 leaf discs per microcosm not allocated for ergosterol analysis. Both IM and FM were quantified on a scale to the nearest 0.01g LML % was then calculated using the following formula

$$LML\% = (IM\text{-}FM/IM)\times 100$$

2. **Leaf processing efficiency (LPE):** LPE quantifies the effeiciency of detritivore leaf breakdown relative to detritivore biomass and percent of leaf mass loss imputable to detritivores ($LML_{Detritivore}\%$) . First, final *A. aquaticus* mass was calculated from the length measures using a published length-mass relationship (Reiss *et al.*, 2011), and then the LPE was measured according to the following calculation.

$$LPE = LML_{Detritivores}\% / \sum M_{Detritivores}^{(0.75)}$$

$LML_{Detrivores}$ was estimated for each microcosm within each pesticide treatment by subtracting the microbial LML (the mean observed in the no-detritivore microcosms for each pesticide treatment) from the observed total LML value. The coefficient 0.75 to the power of M describes a relationship between body size and metabolic rate which applies across most groups of organisms (Brown *et al.*, 2004).

3. **Relative growth rate (RGR)**: RGR was measured by using the following formula:

$$RGR = \ln(W_2) - \ln(W_1)/(T_2 - T_1)$$

W_1 is the initial weight, W_2 is the final weight, T_1 is the initial day and T_2 is the final day. The initial and final weight was measured for the two individuals that stayed alive for the longest period in each microcosms (in most cases > 75% of the study period). This excluded individuals from biomass and growth measurements that had only been present in the microcosms for a short time period before they died.

Statistical analyses

Univariate analysis in SPSS software (version SPSS® 17.0.0, IBM SPSS Inc., IL, USA) was used to assess the effects of the pesticides (four levels: control, Lindane, Azoxystrobin and mixture) and *Asellus* treatments (two levels: present vs. absent) on the response variables (mortality, moulting frequency, moulting period, percent of leaf mass loss, LPE and RGR). For moulting period, there were not sufficient individuals in each pesticide category. Therefore, all individuals moulting under the Lindane, Azoxystrobin and mixture treatments were pooled together as one "pesticide" treatment, and their mean moulting time compared with that of those moulting in the control microcosms. Post-hoc test was performed for the comparison between the factors using Tukey's HSD test.

3 Results

Pesticide effects on the mortality and moulting rates of A. aquaticus

Pesticides increased the mortality of *A.aquaticus* (ANOVA $F_{3,16}$= 19.530, P < 0.001), with greater mortality caused by the mixture and Azoxystrobin treatments than Lindane. There was no mortality in the control (table 3.2). The number of moulting individuals was not affected by pesticides (ANOVA $F_{3,16}$= 1.867, P= 0.176) (table 3.2). However, the time to the first moult was affected by the presence of pesticides (ANOVA $F_{1,10}$= 7.839, P= 0.019), with a shorter moulting period in the presence of pesticide (figure 3.1).

Table 3.1 Analysis of variance of mortality, moulting and moulting period of *A. aauaticus*

	Mortality number			Moulting number			Moulting period		
Factor	DF	F	P	DF	F	P	DF	F	P
Pesticide	3	19.530	< 0.001	3	1.867	0.176	1	7.839	0.019
Residual	16	24.400		16	8.000		10	7.750	

Table 3.2 Effect of different pesticides treatments on the number of mortality and moulting rate of *A. aquaticus* (mean ± standard error)

Treatments	Total Mortality (#individuals)	Mortality/ microcosm (mean ± SE)	Total moulting (#moults)	Moulting/ microcosm (mean ± SE)
Control	0	-	6	1.2 ±0.31
Lindane	2	-	3	0.6±0.31
Azoxystrobin	18	3.6±0.55	1	-
Mixture	25	5.0±0.55	2	-
Total	45	2.25±0.54	12	0.6±0.169

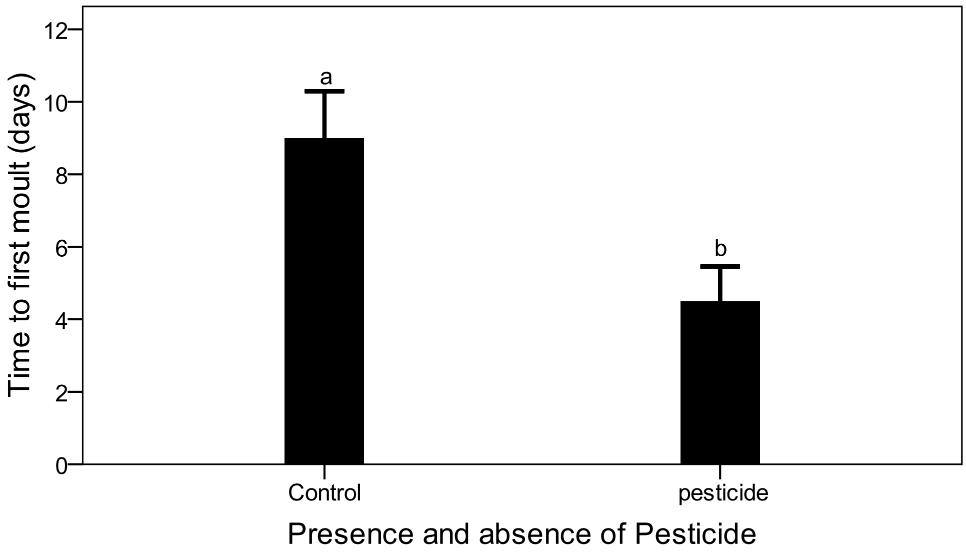


Figure 3.1 Effect of pesticide application on the time to first moult (mean ± 1SE), pooling across pesticide treatment (Lindane, Azoxystrobin and mixture treatments)

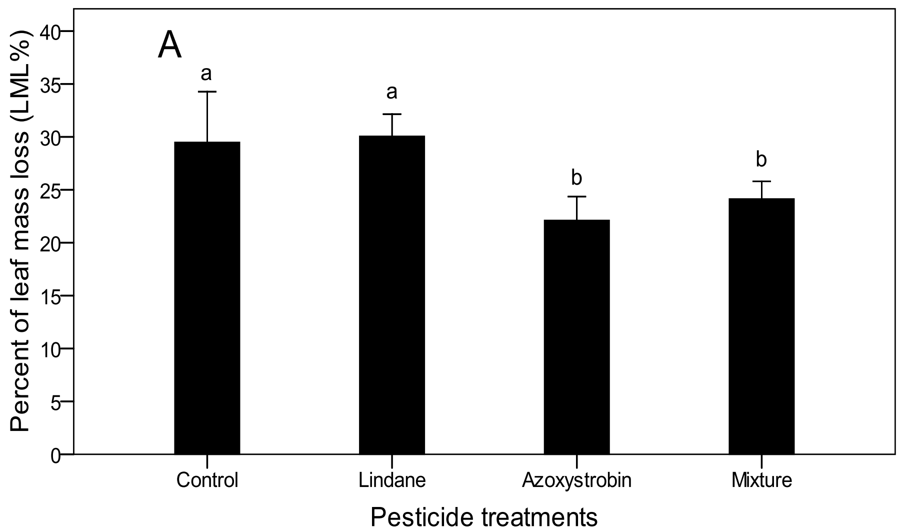
Leaf Decomposition, LPE and RGR

Leaf mass loss was not affected by pesticides at the 5% level of significance (ANOVA $F_{3,36}$= 2.529, P= 0.075), though a strong trend, significant at the 10% level of significance, was apparent for lower decomposition in the Azoxystrobin and mixture treatments (figure 3.2 A). Leaf mass loss was increased by the presence of *A. aquaticus* (figure 3.2 B, $F_{1,38}$ = 10.52, p = 0.003). Additionally, an interaction between *A. aquaticus* and pesticides was apparent ($F_{3,34}$= 3.07, P= 0.041). There was no effect of pesticides on leaf mass loss in microcosms without *A. aquaticus*, but an effect was apparent in the presence of *A. aquaticus,* with reduced decompositon under the Azoxystrobin and mixture but not Lindane treatments (figure 3.2 B).

Leaf processing efficiency was affected by all three pesticide treatments (ANOVA $F_{3,16}$= 4.195, P= 0.023). LPE was lowered by the Lindane and Azoxystrobin treatments relative to the controls by approximately 50%, and was approximately 75% lower in the mixture treatment (figure 3.3). In contrast, relative growth rate of *A. aquaticus* was not affected by the pesticide treatments (ANOVA $F_{3,16}$= 1.381, P= 0.285), averaging 0.003±0.0004 overall (figure 3.4).

Table 3.3 Statistical analysis by using ANOVA model for the percent of Leaf mass loss representing the decomposition process then LPE and RGR of *A. aquaticus*

	Leaf mass loss			LPE			RGR		
Factor	DF	F	P	DF	F	P	DF	F	P
Pesticide	3	2.52	0.075	3	4.195	0.023	3	1.381	0.285
Asellus	1	10.52	0.003						
As*Pest	3	3.07	0.041						
Residual	32	61.184		16	0.094		16	6.65	



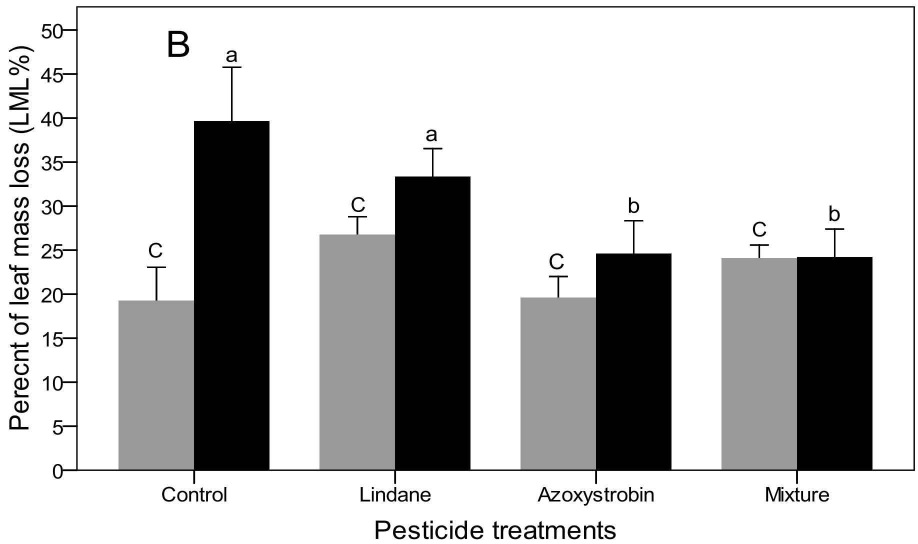


Figure 3.2 Percentage of Leaf mass loss (mean ± 1SE) for the four pesticides treatments. **A**) Total leaf mass loss for each pesticide treatment, pooling across detritivore treatments, **B)** effects of the pesticides separated according to the presence, (black bars) and absence, (grey bars) of *A. aquaticus.*

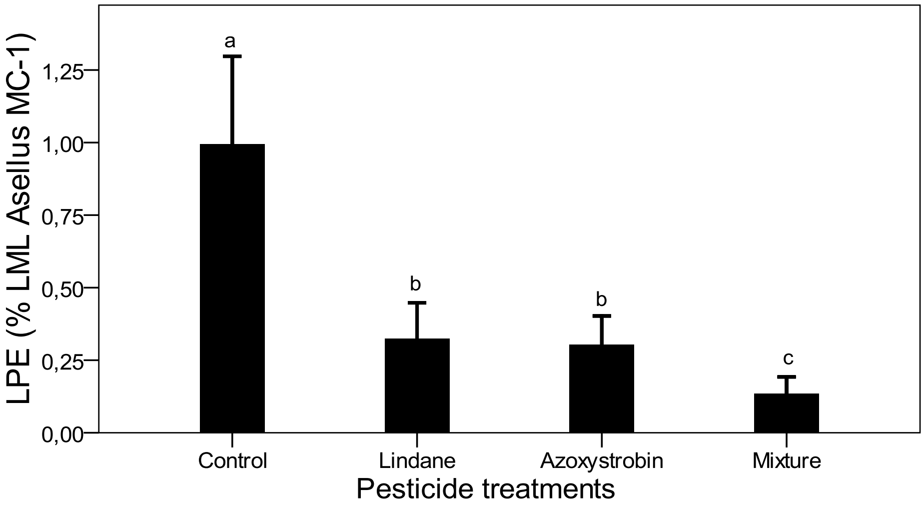


Figure 3.3 Effect of the different pesticide treatments on (mean ± 1SE) detritivore leaf processing efficiency (LPE)

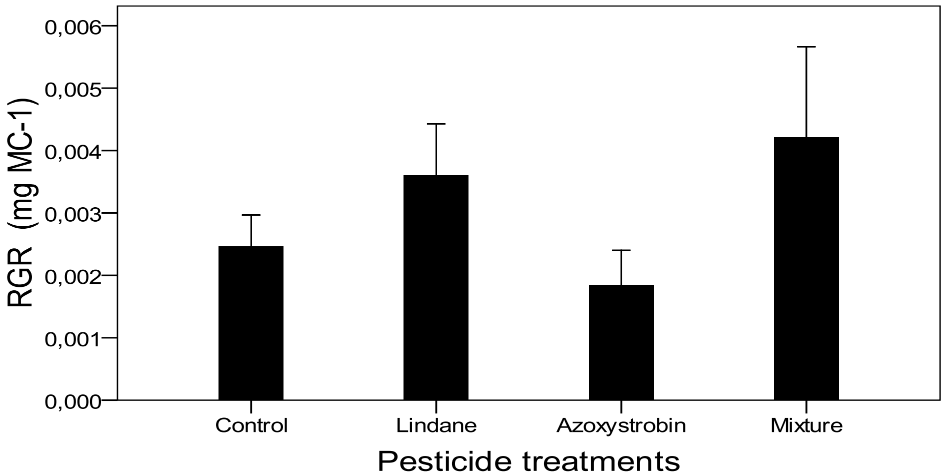


Figure 3.4 Effect of the different pesticide treatments on (mean ± 1SE) relative growth rate (RGR)

4 Discussion

Pesticides affected ecosystem functioning in my laboratory microcosms, but these effects did not match completely with expectations based on their target organism groups. Thus the fungicide Azoxystrobin little affected decomposition mediated by microbes, and the insecticide Lindane did not have an overall affect on decomposition mediated by detritivores. However, Azoxystrobin had important knock-on effects on the performance of the detritivores, with the result that leaf mass loss was reduced more overall by the fungicide than insecticide. Synergistic interactions between the pesticides were also apparent, with detritivore leaf processing efficiency depressed most strongly when both pesticides were applied together, supporting hypothesis H.6. The marked effects of Azoxystrobin, whether applied alone or in mixture with Lindane, most likely reflect the importance of microbial conditioning of detritus for the participation of *A. aquaticus* in the decomposition process. Overall, ecosystem functioning was more strongly affected by the stressor impacting the food web from the bottom up, rather than that applied from the top-down. These results indicate that changed interactions within food webs can complicate prediction of the effects of pesticide stressors on ecosystem functioning in streams

Responses of leaf decomposition process under pesticide treatments

In the absence of pesticides, *Asellus aquaticus* almost doubled decomposition rates compared with the microbe-only controls, reflecting the key role of detritivores in driving bulk fragmentation of leaf litter. Correspondingly, the pesticides had their strongest effects on leaf mass loss when *A. aquaticus* was present, but counterintuitively, these effects were driven more by the fungicide Azoxystrobin than by the insecticide Lindane. The effects of microbes on decomposition is two-fold: (i) the secretion of leaf digestive enzymes converts leaf mass to soluble compounds and fine organic particles directly and (ii) microbes soften and enrich (improve nutrient status) the leaf litter in a process known as “conditioning”, enhancing subsequent feeding activity by detritivores (Graça *et al.* 2001; Gessner *et al.* 1999; Bärlocher 1985).

In this study, Azoxystrobin evidently impaired microbial conditioning, indicated by its negative effects on leaf decomposition in the presence of detritivores, and its negative effect on detritivore leaf processing efficiency (supporting hypothesis H.1). It thus seems surprising that no strong direct effects of Azoxystrobin on microbially-mediated decomposition in the absence of detritivores were apparent. Such effects have been observed in previous studies. For example, Ermold (2009) found reductions in leaf decomposition rate with increasing Azoxystrobin concentration, even in the absence of detritivores, and Dijksterhuis (2011) also observed effects on non-target aquatic fungi, related to variation in their sensitivity to fungicide toxicity levels.

Several factors could help in explaining why I did not find a significant negative effect of Azoxystrobine on decomposition mediated by microbes, in the absence of *A. aquaticus.* Most likely is that the experimental time period was insufficient for effects of the fungicide on microbial performance to be reflected in significantly slowed decomposition rates, though there was an overall non-significant trend for reduced decomposition in the Azoxystrobin treatment relative to the control. It is notable that overall decomposition rates were higher in the study by Ermold (2009), which was run for 5 days longer than mine, and which found significant differences between the Azoxystrobin and control treatments. This suggests that, given more time, my Azoxystrobin and control treatments might have differentiated more clearly. Additionally, other factors may have been less optimal for stimulating microbial activity, and hence hindering a stronger differentiation in the effects of the pesticide treatments. For example greater water nutrients (N and P) and temperatures can stimulate greater microbial activity, and one or both of these parameters were higher in previous studies (Ermold, 2009, Grattan II & Suberkropp, 2001*;* Sridhar and Bärlocher, 2000; Chauvet and Suberkropp, 1998).

However, I also cannot rule out the possibility that the fungicide would never have affected leaf decomposition rates, even if the study had been run for longer. In a previous study (Ermold 2009), fungal assemblages with a previous exposure to agricultural stressors were found to be more resistant to pesticides than those with none. Whilst Hågaån is not an intensively farmed catchment, it does experience agricultural runoff (Bergfur, 2007), and this may have favoured tolerant microbes more resistant to Azoxystrobin. In that case, the effect of Azoxystrobin on *A.*

aquaticus LPE might have arisen from direct toxicity, or a simple aversion to litter with the deposited pesticide, rather than impaired microbial conditioning.

However, it is notable that effects of Azoxystrobin in Ermold's (2009) study were observed at the concentration used in my study, even for impacted agricultural assemblages. Parameters awaiting laboratory analysis from my experiments, namely the sporulation rates and fungal biomass analyses, will help to resolve the question of whether Azoxystrobin truly had no effect on microbial communities, or whether it did affect microbial activity (which would be seen in reduced sporulation and/or biomass), with the experiment simply not long enough to detect an effect on overall decomposition rates.

Lindane strongly affected detritivore LPE which supporting H.4, providing evidence of a sublethal effect on the efficiency of detritivore feeding, relative to their biomass. This effect could arise from several different mechanisms. Lindane binds strongly to organic substrates and biological membranes (Lee et al., 1997). Absorption of Lindane to the body of *A. aquaticus* may well have caused sublethal effects, i.e. the animals to feel less physically fit, impairing resource intake rates. Alternatively, sorption of Lindane to leaves (Bell and Tsezos, 1987) might have reduced leaf palatability, further reducing leaf processing efficiency. Interestingly, a synergistic interaction between Lindane and Azoxystrobin was apparent in their effects on leaf processing efficiency: the reduction in LPE was greater when both pesticides were applied together than when either was applied in isolation. The most likely explanation is that the joint application of Lindane and Azoxystrobin directly affected *A. aquaticus* feeding performance through a combined effect of reduction of microbial conditioning and sublethal toxicity of one or both pesticides on the detritivores' physiological condition, unless the potential sublethal effects of both pesticides were strong enough to induce a change in feeding performance even without an effect on microbial conditioning.

The negative effects of Lindane on detritivore LPE were not reflected in corresponding effects on overall decomposition. This may indicate that microbes were able to compensate for the negative effect on detritivore LPE (Suberkropp *et al.*, 1983).

Given that *A. aquaticus* feeds by scraping at fungal growths on leaf surfaces (Graça *et al.*, 1993), a negative effect of Lindane on *A. aquaticus* feeding would free the microbes from such grazing pressure (Bärlocher, 1980; Graça *et al, 2001*). Additionally, meiofauna also can influence decomposition by preying on microbes, and any negative effects of Lindane on the meiofauna might also have reduced grazing pressure on the microbes (Ribblett, Palmer & Coats 2005).

Detritivore Mortality rate

The lack of a mortality effect of Lindane is not surprising, given I chose a sublethal concentration, which had been confirmed as sublethal in a pilot study. More surprisingly, mortality of *A. aquaticus* was significantly increased in the Azoxystrobin treatment, as well as in the mixture. This could reflect either (i) a direct effect of Azoxystrobin on *A. aquaticus* mortality, (ii) an indirect effect of reduced feeding due either to impaired leaf conditioning or an aversion of *A. aquaticus* for litter with deposited pesticide. Previous studies did not provide sufficient information about the mortality of aquatic invertebrates by Azoxystrobin action. However, another study of a similar fungicide, Carbendazim, reported a decrease in Isopoda abundance at a relatively low dose of 330 µg/l (Cuppen *et al. 2000*), demonstrating that fungicides can induce mortality in Crustacea. Alternatively, assuming the reduction in LPE reflects an overall decrease in resource intake, then the animals may simply have starved to death. The combination of Azoxystrobin and Lindane together may thus have increased the level of toxic stress on *A. aquaticus*, perhaps in combination with dietary stress caused by retarded microbial conditioning, both contributing to elevated *A. aquaticus* mortality rate in mixture treatment.

Detritivore growth rate

Detritivore growth rates should be correlated with their rates of resource intake, or in this case, leaf processing efficiency (McKie *et al*., 2009). However, in this study the strong effects of the pesticides on leaf processing efficiency were not matched by effects on growth, which was not different among pesticide treatments and did not support my hypothesis (H.5). This might reflect the fact that standardization of growth period was difficult to achieve because of the high *A. aquaticus* mortality under the Azoxystrobin and mixture treatments, generating substantial noise in the data. In addition, the short period of the study might not have been sufficient for marked differences in the growth rate to become apparent. In the longer term, impaired feeding by *A. aquaticus* should be expected to impair growth.

Detritivore moulting rate

Even in the absence of mortality, it can be expected that elimination and absorption of the pesticides by *A. aquaticus* will lead to some physiological stress (Thybaud & LeBras 1988). I hypothesized that this might alter detritivore moulting behavior (H.3), if moulting provides a means for eliminating the pesticides (Eijsackers *et al.,* 1978). There was no evidence for an effect of the pesticides on overall skin moulting frequencies. However, of those animals that did moult, the time to first moulting was substantially shortened in the presence of pesticides. This earlier moulting in the presence of pesticides might allow liberation from toxic molecules that attached to the outer body surface. Interestingly, not all animals exposed to pesticides moulted, which may indicate a physiological constraint to this potential stress response. Invertebrate moulting is a complex process controlled by hormonal activity, and varying according to several life history factors, including mating processes, life stage, sex, and animal history. The lack of any moulting response to pesticides among some individuals may indicate that those animals simply were not at a point in their moulting cycle where they could accelerate the moulting process.

Implications and conclusions

This study highlights the potential for pesticides developed to control terrestrial fungal and invertebrate pests to affect non-target organisms in aquatic environments, with knock-on effects on ecosystem functioning. However, this result also highlights the extent to which interactions within affected food webs can complicate the prediction of these effects. In real stream ecosystems, the picture can look even more complicated due to the presence of further food-web connections. For example, few aquatic shredders are obligate leaf feeders, and can switch to alternative food sources (e.g. diatoms) if necessary (Moore, 1975). As such, a negative effect on microbial conditioning in a real stream might not overly compromise survival of detritivores, if alternative resources are available. On the other hand, the negative effect of the pesticides on the ecosystem process (leaf decomposition mediated by detritivores) would remain, and even be strengthened, reflecting both suppression of microbial activity, and switching of the detritivores to an alternative food source.

Such scenarios demonstrate that human disturbances may not always affect ecosystem structure and functioning to the same extent (Dunne *et al.*, 2002).

Additionally, the concentration of pesticides in streams and their effects on ecosystem functioning can vary according to several factors not possible to simulate in the microcosm experiment. These include the amount of pesticide applied to the catchment, the extent of runoff to stream channels, and residence times in the streams. Additionally, geographical location, size of the stream, the amount of leaf litter and other organic substrates, aquatic biodiversity, sediment type, and hydrological cycle all can control the time and the strength of pesticide effect on ecosystem functioning.

Overall, the pesticide having the most consistent effects in this study appeared to be associated with the bottom-up stressor, Azoxystrobin. It is not yet entirely clear that this reflects negative effects on microbial conditioning, but this is the most likely explanation, and will be clarified when data on microbial activity (fungal biomass and sporulation) become available. Assuming these results do relate to reduced microbial conditioning, they highlight the fundamental importance of microbes to the decomposition process because of their role in improvement of the leaf litter for detritivores, even when they do not contribute a large proportion to bulk decomposition.

Finally, results from this study further highlight the threat posed by the intensification of agricultural practices for stream ecosystems. In particular, this study reveals the potential for agricultural to affect the flow of nutrients and energy in streams and rivers, as seen in the effects on leaf decomposition in this study. An impairment of decomposition could cause an increasing in the accumulation of leaf litter at the bed of streams and rivers, and reduce the flow of nutrients from the litter to other organisms, including large predators (Fishes) (Cummins, 1974, Gessner *et al.*, 2010). The potential for pesticides to contribute to further degradation of aquatic ecosystems, impair functioning and threaten services provided by streams and rivers (such as fishing) requires further attention from both scientists and policy makers.

Acknowledgments:

I would like to express my deepest appreciation, to my senior supervisors Brendan McKie and Willem Goedkoop. My special thanks to Brendan McKie for his great support in developing my interest in stream and river ecosystem. He was patient in supporting me in laboratory work and in the process of writing the thesis, and without his guidance and persistent help this thesis would not have been possible. And also, a great thanks to Professor Willem Goedkop for following up my thesis and his measurement for pesticides.

Big thanks to, Matti Ermold who provided me with ideas from his MSc-thesis, and Anna Lundqvist, who freely gave advice on studying pesticides, and provided me with essential articles in my thesis, Märit Petterson assisted in preparation and calculation of the stock solutions of pesticides, Dany Lau, assisted in collection of *Asellus aquaticus*, and Bernadette pree, who helped me to prepare M7 medium – many thanks to all. I also like to express my thanks to Stina Drakare for being my examiner, and Chiho Okuyama for being my opponent and reviewer.

References

Allen, J. C., & Barnes, D. F. (1985). The causes of deforestation in developing countries. *Annals - Association of American Geographers, 75*(2), 163-184.

Bardgett, R. D., & McAlister, E. (1999). The measurement of soil fungal:Bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Biology and Fertility of Soils, 29*(3), 282-290.

Bardgett, R. D., & McAlister, E. (1999). The measurement of soil fungal:Bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Biology and Fertility of Soils, 29*(3), 282-290.

Bärlochar, L. (1985). The role of fungi in the nutrition of stream invertebrates, *Botanical Journal of Linnean Society,* 91 (1-2), 83-94

Bärlocher, F. (1980). Leaf-eating invertebrates as competitors of aquatic hyphomycetes. *Oecologia, 47*(3), 303-306.

Bell, J. P., & Tsezos, M. (1987). Removal of hazardous organic pollutants by biomass adsorption. *Journal of the Water Pollution Control Federation, 59*(4), 191-198.

Bergfur, J. (2007). Seasonal variation in leaf-litter breakdown in nine boreal streams: Implications for assessing functional integrity. *Fundamental and Applied Limnology, 169*(4), 319-329.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology,85*(7), 1771-1789.

Bunn, S. E., & Davies, P. M. (2000). Biological processes in running waters and their implications for the assessment of ecological integrity. *Hydrobiologia, 422-423*, 61-70.

Cardinale, B. J. (2011). Biodiversity improves water quality through niche partitioning. *Nature, 472*(7341), 86-91.

Chandrashekar, K. R., & Kaveriappa, K. M. (1989). Effect of pesticides on the growth of aquatic hyphomycetes. *Toxicology Letters, 48*(3), 311-315.

Chauvet, E., & Suberkropp, K. (1998). Temperature and sporulation of aquatic hyphomycetes. *Applied and Environmental Microbiology, 64*(4), 1522-1525.

Costanza, R., D'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., et al. (1997). The value of the world's ecosystem services and natural capital. *Nature, 387*(6630), 253-260.

Cummins, K. W. (1974). Structure and function of stream ecosystem. BioSience, 24(11), 631-641

Cummins, K. W., Klug, M. J. (1979). Feeding ecology of stream invertebrates. *Annual review of ecology and systematic*, 10, 147-172.

Cummins, K. W., Spengler, G. L., Ward, G. M., Speaker, R. M., Ovink, R, W, Mahan, D. C., Mattingly, R. L.(1980). Processing of Confined and Naturally Entrained Leaf Litter in a Woodland Stream Ecosystem. *Limnology and oceanography*. 25(5), 952-957.

Cuppen, J. G. M., Crum, S. J. H., Van Den Heuvel, H. H., Smidt, R. A., & Van Den Brink, P. J. (2002). Effects of a mixture of two insecticides in freshwater microcosms: I. fate of chlorpyrifos and lindane and responses of macroinvertebrates.*Ecotoxicology, 11*(3), 165-180.

Cuppen, J. G. M., Van Den Brink, P. J., Camps, E., Uil, K. F., & Brock, T. C. M. (2000). Impact of the fungicide carbendazim in freshwater microcosms. I. water quality, breakdown of particulate organic matter and responses of macroinvertebrates.*Aquatic Toxicology, 48*(2-3), 233-250.

Daam, M. A., Satapornvanit, K., Van den Brink, P. J., & Nogueira, A. J. A. (2010). Direct and indirect effects of the fungicide carbendazim in tropical freshwater microcosms. *Archives of Environmental Contamination and Toxicology, 58*(2), 315-324.

Dash, M. C., 2001. *Fundamentals of Ecology*, McGraw-Hill Publishing Company, New Delhi, Second Edition, 519 p.

DeLorenzo, M. E., Scott, G. I., & Ross, P. E. (2001). Toxicity of pesticides to aquatic microorganisms: A review. *Environmental Toxicology and Chemistry, 20*(1), 84-98

Dijksterhuis, J., van Doorn, T., Samson, R., & Postma, J. (2011). Effects of seven fungicides on non-target aquatic fungi. *Water, Air and Soil Pollution,* , 1-5.

Dunne, J. A., Williams, R. J., Martinez, N. D. (2002). Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology letter*, 5(4), 558-567.

Ecosystems and Human Well-being: A Framework for Assessment 2003, Millennium Ecosystem Assessment, Chapter 1, Island.

Eijsackers, H. (1978), Side effects of the herbicide 2,4,5-T on reproduction, food consumption, and moulting of the springtail Onychiurus quadriocellatus Gisin (Collembola). Zeitschrift für Angewandte Entomologie, 85: 341–360.

Ermold M. (2009), Multiple stressor effects on stream fungi communities. *Swedish university of Agriculture*. Dissertation

FAO 2002, World agriculture towards 2015/30, Summary Report. Rome.

FAO**.** 2001. *Global Forest Resources Assessment 2000. Main report.* FAO Forestry Paper No. 140. Rome

Folt, C. L., Chen, C. Y., Moore, M. V., & Burnaford, J. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography, 44*(3 II), 864-877.

Gessner, M. O., & Chauvet, E. (2002). A case for using litter breakdown to assess functional stream integrity. *Ecological Applications, 12*(2), 498-510.

Gessner, M. O., Chauvet, E., & Dobson, M. (1999). A perspective on leaf litter breakdown in streams. *Oikos, 85*(2), 377-383.

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., et al. (2010). Diversity meets decomposition.*Trends in Ecology and Evolution*, *25*(6), 372-380.

Ghekiere, A., Verslycke, T., Fockedey, N., & Janssen, C. R. (2006). Non-target effects of the insecticide methoprene on molting in the estuarine crustacean neomysis integer (crustacea: Mysidacea). *Journal of Experimental Marine Biology and Ecology,332*(2), 226-234.

Graça, M. A. S. (2001). The role of invertebrates on leaf litter decomposition in streams - A review. *International Review of Hydrobiology*, *86*(4-5), 383-393.

Graça, M. A. S., Maltby, L., & Calow, P. (1993). Importance of fungi in the diet of gammarus pulex and asellus aquaticus I: Feeding strategies. *Oecologia*, *93*(1), 139-144.

Graca, M. A. S., Maltby, L., & Calow, P. (1993). Importance of fungi in the diet of gammarus pulex and asellus aquaticus. II.effects on growth, reproduction and physiology. *Oecologia*, *96*(3), 304-309.

Grattan II, R. M., & Suberkropp, K. (2001). Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *Journal of the North American Benthological Society, 20*(1), 33-43.

Gulis, V., & Suberkropp, K. (2003). Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, *48*(1), 123-134.

Hairston, N. G., Smith, F. E., Slobodkin, L. B. (1960). Community Structure, Population Control, and Competition. *The American Naturalist*, 879(94), 421-425.

Hall Jr., R. O., Wallace, J. B., & Eggert, S. L. (2000). Organic matter flow in stream food webs with reduced detrital resource base. *Ecology, 81*(12), 3445-3463.

Jonsson, M., Dangles, O., Malmqvist, B., & Guérold, F. (2002). Simulating species loss following perturbation: Assessing the effects on process rates. *Proceedings of the Royal Society B: Biological Sciences, 269*(1495), 1047-1052.

Kreutzweiser, D. P. (1997). Nontarget effects of neem-based insecticides on aquatic invertebrates. *Ecotoxicology and Environmental Safety, 36*(2), 109-117.

Lee, A. G., Malcolm East, J. and Balgavy, P. (1991), Interactions of insecticides with biological membranes. *Pesticide Science*, 32, 317–327.

Majewski, M.S., and Capel, P.D., 1995, Pesticides in the Atmosphere Distribution, Trends, and Governing Factors: Ann Arbor Press, Chelsea, Michigan, p 228.

Maltby, L. (1991). Pollution as a probe of life-history adaptation in asellus aquaticus (isopoda). *Oikos, 61*(1), 11-18.

McKie, B. G., Woodward, G., Hladyz, S., Nistorescu, M., Preda, E., Popescu, C., et al. (2008). Ecosystem functioning in stream assemblages from different regions: Contrasting responses to variation in detritivore richness, evenness and density.*Journal of Animal Ecology, 77*(3), 495-504.

Middeldorp, P. J. M., Jaspers, M., Zehnder, A. J. B., & Schraa, G. (1996). Biotransformation of α-, β-, γ-, and δ-hexachlorocyclohexane under methanogenic conditions. *Environmental Science and Technology, 30*(7), 2345-2349.

Monahan, C., & Caffrey, J. M. (1996). The effect of weed control practices on macroinvertebrate communities in irish canals.*Hydrobiologia, 340*(1-3), 205-211.

Moore, J. W. (1975), The Role of Algae in the Diet of Asellus aquaticus L. and Gammarus pulex L..*Journal of Animal Ecology*, 44 (3) , 719-730.

Myster, R.W. (2001), What is Ecosystem Structure?. Caribbean Journal of Science, 37 (1-2), 132-134.

Nelson, S. M. (2000). Leaf pack breakdown and macroinvertebrate colonization: Bioassessment tools for a high-altitude regulated system? *Environmental Pollution, 110*(2), 321-329.

Odum, E. P., 1971. *Fundamentals of Ecology*, W. B. Saunders Company, Philadelphia, PA, 574 p.

Okeke, B. C., Siddique, T., Arbestain, M. C., & Frankenberger, W. T. (2002). Biodegradation of γ hexachlorocyclohexane (lindane) and α hexachlorocyclohexane in water and a soil slurry by a pandoraea species. *Journal of Agricultural and Food Chemistry, 50*(9), 2548-2555.

Petersen, R. C., Cummins, K. W. (1974). Leaf processing in a woodland stream. *Freshwater biology*, (4), 343-368.

Polis, G. A., & Strong, D. R. (1996). Food web complexity and community dynamics. *American Naturalist, 147*(5), 813-846.

Reiss, J., Bailey, R. A., Perkins, D. M., Pluchinotta, A., & Woodward, G. (2011). Testing effects of consumer richness, evenness and body size on ecosystem functioning. *Journal of Animal Ecology, 80*(6), 1145-1154.

Ribblett, S. G., Palmer, M. A., & Coats, D. W. (2005). The importance of bacterivorous protists in the decomposition of stream leaf litter. *Freshwater Biology, 50*(3), 516-526.

Richards, R. P., & Baker, D. B. (1993). Trends in nutrient and suspended sediment concentrations in lake erie tributaries, 1975-1990. *Journal of Great Lakes Research, 19*(2), 200-211.

Risser, P. G. (1995). Biodiversity and ecosystem function. *Conservation Biology, 9*(4), 742-746.

Schulze, E. D., Monney, H. A., 1994. Biodiversity and Ecosystem Function, Spring-Verlag Berlin Hedilberg New York, spring study edition, 521P.

Simon, M. F., & Garagorry, F. L. (2005). The expansion of agriculture in the brazilian amazon. *Environmental Conservation, 32*(3), 203-212.

Song, M. Y., Stark, J. D., & Brown, J. J. (1997). Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. *Environmental Toxicology and Chemistry, 16*(12), 2494-2500.

Sridhar, K. R., & Bärlocher, F. (2000). Initial colonization, nutrient supply, and fungal activity on leaves decaying in streams.*Applied and Environmental Microbiology, 66*(3), 1114-1119.

Sterner, R. W., Elser, J. J., Fee, E. J., Guildford, S. J., & Chrzanowski, T. H. (1997). The light:Nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process.*American Naturalist, 150*(6), 663-684.

Suberkropp, K., Arsuffi, T. L., & Anderson, J. P. (1983). Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. *Applied and Environmental Microbiology, 46*(1), 237-244.

Thybaud, E., Le Bras, S. (1988), Absorption and elimination of lindane by*Asellus aquaticus* (Crustacea, Isopoda). *Environmental Contamination and Toxicology,* 40(5), 731-735.

Tilman, D. (1998). The greening of the green revolution. *Nature, 396*(6708), 211-212.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature, 418*(6898), 671-677.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. *Science, 277*(5330), 1300-1302.

Trevors, J. T. (1989). The role of microbial metal resistance and detoxification mechanisms in environmental bioassay research. *Hydrobiologia, 188-189*(1), 143-147.

Vanni, M. J., Flecker, A. S., Hood, J. M., & Headworth, J. L. (2002). Stoichiometry of nutrient recycling by vertebrates in a tropical stream: Linking species identity and ecosystem processes.*Ecology Letters, 5*(2), 285-293.

Vinebrooke, R. D., Cottingham, K. L., Norberg, J., Scheffer, M., Dodson, S. I., Maberly, S. C., et al. (2004). Impacts of multiple stressors on biodiversity and ecosystem functioning: The role of species co-tolerance. *Oikos, 104*(3), 451-457.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of earth's ecosystems. *Science, 277*(5325), 494-499.

Wallace, J. B., Eggert, S. L., Meyer, J. L., & Webster, J. R. (1997). Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science, 277*(5322), 102-104.

Webster, J, Tawfik, F. H. (1997). Sporulation of aquatic hyphomycetes in relation to aeration. *Transactions of the British Mycological Society*, 59(3), 353-364

Wilson, M. A., & Carpenter, S. R. (1999). Economic valuation of freshwater ecosystem services in the united states: 1971-1997. *Ecological Applications, 9*(3), 772-783.

Appendix:

Appendix 1: Pre-test of Lindane on Asellus aquaticus mortality

In a controlled environment room maintained at 11 ^{o}C, twelve microcosms (6 microcosms with Lindane and 6 microcosms without Lindane) were placed on a shaker table (50 rpm) for three days, with each microcosm containing:

M7 medium	50 ml
Leaf discs colonized by microbes	10 leaf discs
6 Lindane/ 6 absence	5 µg/l / 0 µg/l
Asellus aquaticus	2 indvidulas

The *Asellus* individuals in the pesticide treatments were more sluggish, and consistently moved less when disturbed in their microcosms as part of a daily behavioral observation, indicating a sublethal effect on their behavior. However, at the end of the study period, there was no difference in mortality between the controls and Lindane microcosms, and the overall absence of mortality not allowed for statistical analysis

The target sublethal concentration of Lindane was calculated based on the literature by Professor Willem Goedkoop (Goedkoop and Peterson, 2003).