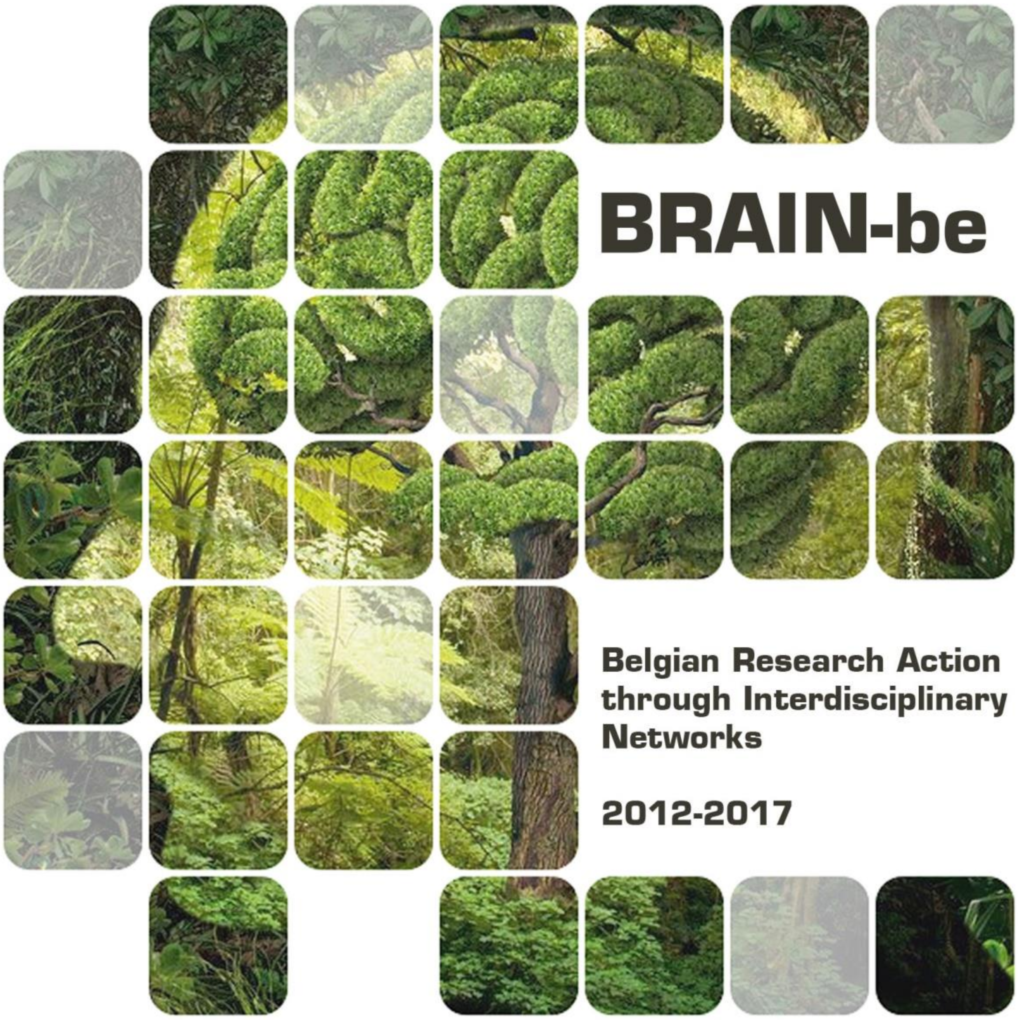

ORCA

A comparative analysis of **OR**ganic and **C**onventional **A**griculture's impact on aquatic biodiversity

Pieter Lemmens (KU Leuven), Rafaela Almeida (KU Leuven), Luc Denys (INBO), Dries Adriaens (INBO), Marie Cours (RBINS), Rein Brys (INBO), Gerald Louette (INBO), Koen Martens (RBINS) and Luc De Meester (KU Leuven)

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Belgian Research Action through Interdisciplinary Networks

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NETWORK PROJECT

ORCA

A comparative analysis of **OR**ganic and **C**onventional **A**griculture's impact on aquatic biodiversity

Contract – BR/175/A1/ORCA

FINAL REPORT

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1. INTRODUCTION

ORCA aimed at documenting the impact of organic and conventional agriculture on aquatic biodiversity of ponds and small shallow lakes in Belgium. The project combined existing datasets with the collection of new data to address one of the primary research domains in current aquatic conservation, i.e. the link between biodiversity and existing agricultural practices. ORCA compared two main approaches to the production of biological resources, organic and conventional agriculture, on aquatic biodiversity in ponds and lakes. The impact of organic and conventional farming has been relatively well documented for terrestrial systems (for example Tsiafouli *et al.*, 2015), but information on the impact on aquatic ecosystems remains limited and scarce. This is surprising since many lentic waterbodies, such as ponds and lakes, strongly contribute to regional biodiversity (Williams *et al.*, 2004; Scheffer *et al.*, 2006) and are highly recognized for the provisioning of multiple vital ecosystem services (Costanza *et al.*, 1997; TEEB, 2010; MEA, 2005).

2. STATE OF THE ART AND OBJECTIVES

Agriculture is one of the most pervasive human activities on earth, impacting key natural resources (Rockström *et al.*, 2009; Steffen *et al.*, 2015), e.g. through eutrophication and the use of pesticides (Smith, Tilman & Nekola, 1999; Beketov *et al.*, 2013). Efficient agriculture is, however, crucial to feed the rapidly increasing human population. There currently is an ongoing debate on the choice of agricultural practices in terms of their impact on food security, ecosystem functioning and biodiversity (Reganold & Wachter, 2016). The impact of organic farming, for instance, is expected to differ from that of conventional farming because of the use of organic fertilizers, the strongly restricted use of a limited set of pesticides, and the implementation of larger buffer zones along more natural elements such as ponds. Moreover, such agricultural practices are highly promoted under the current EU Common Agricultural Policy (2014-2020) as part of the more general 'greening' pillar. An increase in the area and quality of High Nature Value Farmland (HNVF type 2: ponds and other small-scale habitat features within an agricultural matrix) is considered as one of the main objectives of this greening pillar (Oppermann, Beaufoy & Jones, 2012).

To date, most comparative studies of organic and conventional agriculture focus on agricultural fields (Gabriel *et al.*, 2013), on terrestrial ecosystems (Tuck *et al.*, 2014), or on rivers (Thieu *et al.*, 2011), while the numerous small ponds and shallow lakes in agricultural settings are largely ignored, mostly because of their limited size. Yet, they harbor the majority of regional aquatic biodiversity (Williams *et al.*, 2004) and are a key provider of many ecosystem services (Tranvik *et al.*, 2009; Cole *et al.*, 2007; Downing *et al.*, 2008). Moreover, aquatic systems and taxa are amongst the most threatened on earth (IUCN.org, Strayer & Dudgeon, 2010). Ponds have been shown to be excellent sentinels of human impact, especially because they respond to contamination and other disturbances at a very local scale (Declerck *et al.*, 2006), which makes them ideal monitoring systems for local impacts on biodiversity exerted by different types of land use and farming practices. So far, the effectiveness of pond systems to detect and assess environmental impacts on biodiversity has not yet been used in a comparative analysis of organic and conventional agricultural practices, which contrasts strongly with their high potential to contribute to the development of larger areas of High Nature Value Farmland (cf. HNVF type 2: ponds and other small-scale habitat features within an agricultural matrix; Danckaert *et al.*, 2009) and their value for ecosystem services (Hill *et al.*, 2016).

The ORCA project combined existing datasets with newly collected data to:

(1) Assess biodiversity of different organism groups in ponds along strong land-use gradients of both organic and conventional agriculture.
(2) Test the hypotheses that aquatic biodiversity in ponds is higher in:
    a. areas of organic compared to conventional agriculture,
    b. areas of extensive compared to intensive land use (grassland *versus* cropland within each type of agriculture practice).
(3) Test the hypothesis that zooplankton populations are genetically differentiated between ponds in areas with organic *versus* conventional agriculture.

(4) Test the hypothesis that the size of the buffer zone around standing waters impacts local biodiversity, and explore whether this relationship differs between areas with organic and areas with conventional agriculture.

(5) Estimate the effects on aquatic diversity of scenarios of increasing levels of organic farming at the regional scale.

(6) Develop a map of priority areas where a transition to organic agriculture might have the largest impact on biodiversity and ecosystem services.

3. METHODOLOGY

The overall methodology of ORCA comprised three major steps, (1) the collection of new data, (2) the integration of these data in the existing SAFRED data infrastructure, and (3) the analysis of these data. The collection of new data was important to target research questions focussing on the differential effect of conventional and organic farming on biodiversity and local genetic adaptation, whereas the integrated database (SAFRED + ORCA) allowed us to position the data in a broader perspective with respect to land use and the effect of buffer strips. Below we provide a brief overview of the major aspects related to data collection and the integration of these data in the SAFRED database. The methods on the statistical analysis of the data are briefly outlined in the results section.

In contrast to the initial proposal, the obtained data did not allow an adequate estimation of the effect of increasing coverage of organic farming on regional aquatic diversity. This is largely the result from the overall still limited number of ponds located in organic agriculture in Flanders (approximately 0.6% of the ponds) and the geographical clustering of these ponds. Also, we suspect that insufficient time has elapsed since the onset of organic farming to yield marked differences with (often preceding) traditional practices. Consequently, we remain reluctant to propose priority maps showing where converting conventional agricultural practices into organic farming would be most beneficial at the time being. Nevertheless, we were able to position our data in a broader perspective by exploring the effects of overall land use on pond ecology and diversity. In addition and drawing from early ORCA findings, we conducted two experiments that were not included in the original planning. The first experiment explicitly examined the effect of pesticide exposure on zooplankton community characteristics and dynamics. The second experiment aimed at investigating the effect of changing pesticide exposure resulting from a shift from conventional to organic farming. Both experiments provide important new insights on how agricultural activities may affect lentic freshwater ecosystems.

3.1 Field survey

An important part of the ORCA project consisted of the collection of new field data allowing to compare the biodiversity in ponds located within conventional agriculture with that of ponds located within organic agriculture. The selection of suitable ponds was based on existing GIS data of standing water bodies (Packet *et al.*, 2018) and information on land use in terms of farming type (organic or conventional), land-use intensity (cropland versus grassland) and crop type. An inventory of available GIS and land-use data was made at the start of the project. Combining information from multiple GIS layers (annual agricultural parcel registration data of Department of Agriculture and Fisheries; standing water bodies in Flanders (Packet et al. 2018); detailed land-use map of 2014 (Poelmans & Van Daele 2014) allowed us to identify areas dominated by organic versus conventional farming and stratify ponds according to land-use intensity (land use dominated by grassland versus cropland; percentage agricultural land). Based on these analyses, a set of 46 ponds (out of a mere 82 candidates) was selected in areas located within *organic* agriculture, spanning a gradient in surrounding land-use intensity. Multiple preconditions (including position within parcel, size of water body, hydrological connection with running water, proximity of built-up area, location outside nature reserves) were taken into account during the pond selection procedure. Using the same GIS layers, another set of 284 ponds was selected that were all located within *conventional* agriculture. These 'conventional' ponds were paired with the 'organic' ponds based on their distance from the selected organic ponds (within 3 km perimeter) and were then ranked by their similarity to the organic ponds in terms of surrounding land use (100 m buffer). The most similar conventional ponds were the first candidates to form pairs with the organic ponds after field validation preceding actual sampling. This approach resulted in the selection of 30 pairs of ponds (organic-conventional), thus 60 ponds in total. The selection procedure was GIS-data driven. However, the suitability of selected ponds was checked by expert judgement, using aerial images and visual assessment in the field. Because field conditions often tend to differ from desktop estimation, a surplus in the number of ponds in both organic and conventional agriculture was selected to compensate for unforeseen losses of suitable ponds.

A total of 48 farmland ponds (22 ponds in a setting with predominantly conventional agriculture and 26 within organic farming) was surveyed during summer 2017 (Figure 1). Some of the initially selected ponds could not be sampled because 2017 was a dry year in which many ponds dried out in early summer prior to the sampling campaign. In summer 2017 (July-August), we collected data on a broad range of local environmental pond conditions and collected samples from the zooplankton and the macro-invertebrate community in each pond. The aquatic vegetation was inventoried in the field. Sample collection for environmental DNA metabarcoding to screen for the presence and relative abundances of amphibians and fish was performed in 2018 (July).

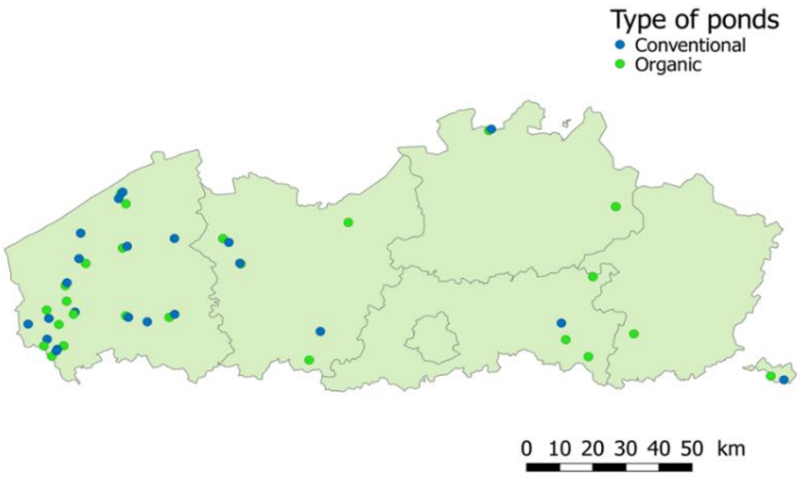


Figure 1. A map of Flanders depicting the sampled ponds in conventional and organic agriculture (blue and green respectively).

Major local environmental variables were quantified following the procedures described by De Bie *et al.* (2012). An assessment of land use in the immediate neighbourhood of the pond was made in the field. Together with *a posteriori* aerial imagery, this information was used to create a GIS layer with details on the size of buffer strips around all investigated ponds and metrics based on the distance to neighbouring agricultural land use. *In vivo* chlorophyll *a* and phycocyanin concentrations were used as a proxy for phytoplankton and cyanobacteria biomass, respectively. Depth-integrated water samples were taken for the measurement of nutrient concentrations in the laboratory. The percentage of the pond area covered with emergent, submerged and floating-leaved vegetation was visually estimated in the field.

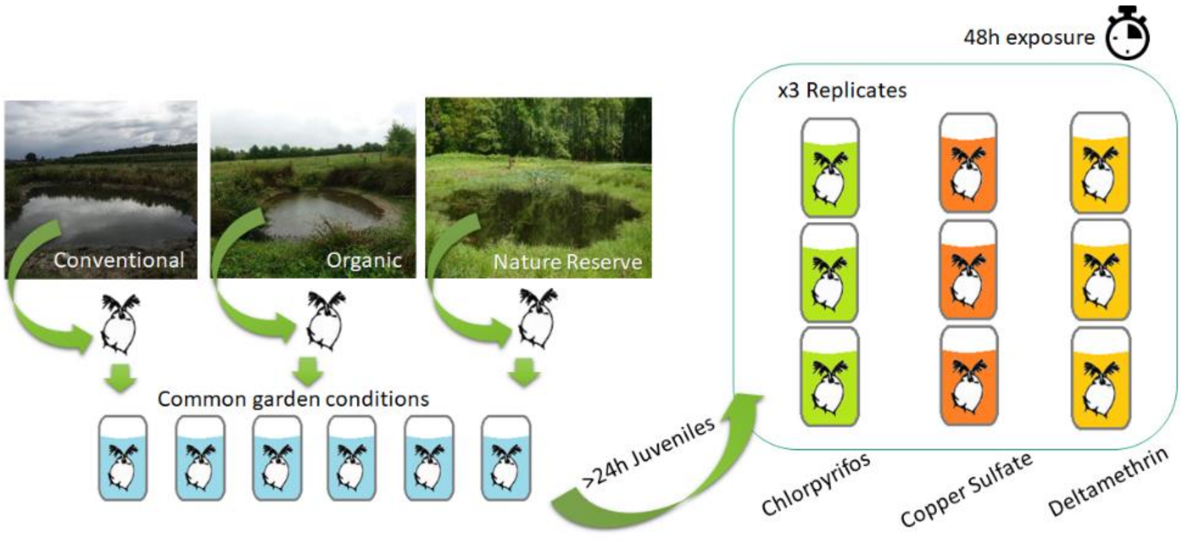
Crustacean zooplankton was sampled by taking depth integrated water samples at 8 different locations in the ponds (4 samples along the bank, 4 samples in the open water area) using a tube sampler, and subsequently filtering the pooled water samples through a conical plankton net (40L, mesh size 64 μm). A tube sampler (7.5 cm cross-section, 2 m length) has the unique advantage to provide depth-integrated samples from the water surface up to very close to the sediment, with the possibility to sample within macrophyte beds. Samples from the macro-invertebrate community were taken in each pond using a sweep net (mesh size: 250 μm). Sweep-net sampling was conducted by striking the net in the open water area, among the submerged macrophytes, floating-leaved macrophytes and in the reed bed. The sampling time allocated to each vegetation type and the open water area was proportional to their estimated percentage cover. Sampling time per pond was adjusted to pond size (with a minimum of 5 minutes). Sampling was conducted by walking around the area and included the whole water column. After sweeping with the net at the different sampling stations, all material was pooled. Data on the macrophyte community were collected from mid-August to September 2017. Two ponds that could not be assessed in 2017 were added in 2018. Separate relevées covered the central part with mainly aquatic vegetation ('water 1'), the marginal helophyte belt ('water2'), marginal areas inundated only during winter ('marginal 1') and those without regular inundation ('marginal 2'). Each zone was delimited by gps. Abundance scores were estimated for all taxa in each relevée and overall percentage cover was noted for the different vegetation strata (filamentous algae, mosses, charophytes, (vascular) submerged rooting plants, submerged floating plants, lemnids, floating-leaved vegetation and helophytes), as well as the percentage plant volume infested (pvi). Sampling for eDNA metabarcoding for fish and amphibians in each of the selected ponds was carried out by taking several subsamples (every 2 m around the pond) that were homogenized into one merged sample per pond. After filtering the water samples, we extracted the fixated eDNA in the lab.

The samples for the quantification of multiple local environmental variables were processed in the laboratory following standard procedures of the ECOCHEM laboratory at RBINS (ECOCHEM, OD Nature, Ostend). Cladocerans were identified to species level and counted. Copepods were grouped into major groups (calanoids, cyclopoids and harpacticoids) and counted. All samples were subsampled and counted to reach at least 300 individuals. If an additional species occurred in the last hundred individuals, an additional 100 individuals were counted. The body length of 15 individuals from each taxon in each sample was measured to determine zooplankton body size. Samples from the macro-invertebrate communities were sorted out and all specimens were sorted into multiple major taxonomic groups (Diptera, Hirudinea, Acari, Crustaceae, Colepterans, Heteropterans and Gastropods). Individuals from each group were identified to the lowest possible taxon level (Diptera, Hirudinea, Acari, Crustaceae). Coleoptera, Hemiptera and Gastropods were determined to species level. All identifications were double checked by taxonomic experts at RBINS. The voucher collection created during the Pondscape project (curated in RBINS) was further extended during the process of organism identification. The community composition of fish and amphibians was analysed based on eDNA extracts collected from water samples from all investigated ponds. Libraries of each sample replicate, including negative extraction controls, have been sequenced single end on a Illumina Hi-Seq platform. Reads were compared to the INBO reference database of all Belgian fish and amphibian species to genetically identify reads during the bio-informatics process. DNA reads have subsequently been analysed bio-informatically using OBI-Tools. These analyses resulted in accurate estimates of relative taxon frequencies per pond. Macrophyte taxa were identified immediately in the field, concomitant with the relevées. No additional sample processing was needed. All newly collected data were digitized and subsequently entered in the INBOVEG vegetation database.

3.2. Common garden experiment testing for differential adaptation of zooplankton to pesticides used in organic and conventional agriculture

We conducted a common garden experiment to investigate adaptation of *Daphnia magna* to the different pesticides that are used in organic and conventional agriculture (Figure 2). For this purpose, individuals from seven different *Daphnia magna* populations were collected in 2018 (between May-June). Three populations were obtained from ponds located in conventional agriculture, two populations were sampled in ponds located in organic farming, and an additional two *D. magna* populations were collected from two ponds located in different nature reserves. Using a common garden, we used these populations to test for differential adaptation to different types of pesticides that are frequently applied in conventional (chlorpyrifos) or organic (deltamithrin and copper sulphate) agriculture. We screened the collected populations for the presence of different genotypes using macrosatellite and kept unique clones in laboratory cultures for multiple generations to prevent potential maternal effects on our result. For each pesticide, individuals from five clonal lineages from each population were exposed for 48h to a range of concentrations and scored for immobilization to determine the Half Maximal Effective Concentration (EC_{50}) for each clone. Each assessment was replicated three times.

Figure 2. Schematic overview of the common garden experiment testing for differential adaptation of Daphnia magna to pesticides frequently used in conventional and organic farming.



3.3. Genomics

Because of the low number of populations of *D. magna* that we could identify in ponds in organic versus conventional agriculture, the population genomics task had to be changed. During the course of spring-summer 2019, we revisited many organic farmland ponds but so far could only collect material from 3 ponds in organic and 5 ponds in conventional farmland. This is insufficient for a detailed genomics analysis, even if we combine them with the clones that we were isolated previously for the pollutant tolerance study. So we decided to empower the genomics study by combining it with genomics on earlier collected *D. magna* populations across Flanders. In the past years, we have collected *D. magna* populations from multiple habitats across a gradient in land use (nature reserves versus agricultural land). We now have full genome resequencing data of 10-20 *D. magna* clones from 20 populations, and we have achieved much progress in the analysis of these data. Once these data are fully analysed they will provide a powerful background against which we can compare the full genome resequencing data on the limited set of populations that we can sample from conventional and organic farm ORCA ponds. The aim is to use the overall data set to carry out a Genome-Wide Association Study (GWAS) allowing us to establish links between genomic information and traits. If that is successful, we will be able to us the genomic information of the ORCA populations to directly see whether some of the functional regionals show genetic differentiation between ponds in organic versus conventional farms. In addition, we will also be able to quantify genetic distances with very high resolution given the fact that we will have resequenced full genomes. In brief, we will have less populations then originally hoped for, but the depth of our genomic analysis will far exceed what we originally aimed for. This approach will lead to some delay, however. In spring 2021 we will again screen all populations to try to add to our set of D. magna clones from ORCA ponds. Isolation of DNA and sequencing will happen in summer and autumn of 2021. By then the data of the benchmark set of 20 populations inclusive the GWAS analysis will be complete, so that we can match the data.

3.4. Integration of novel data in SAFRED infrastructure and the development of trait database for selected organism groups

The integration of the novel data in the existing SAFRED infrastructure comprised multiple steps. First, in order to adequately investigate the impact of land use type and intensity based on the integrated database (newly collected ORCA data and existing SAFRED database), we needed to quantify land-use characteristics of all waterbodies included in the SAFRED database by means of GIS. As a start, contours of the SAFRED ponds were determined based on existing spatial data of the various projects or by matching point coordinates with reference information layers at the time of a projects execution (mainly aerial orthophotographs and topographic maps). These contours were then reviewed by project partners. Concentric ring buffers were calculated around each pond at distances of 50, 100, 200, 500, 1000, 2000 and 3000 metres. Next, source layers with land use information were inventoried for the regions covered by the SAFRED database. These information layers were screened for spatial and temporal scope, spatial and informational resolution, and subdivided according to information type (land use vs land cover). For the layers selected, the land-use classes were translated to a common standard typology. Then, for each layer the proportional area of each land-use type within each buffer was calculated in GIS. As a last step, each pond was matched with the most appropriate land-use layer in terms of information type and temporal correspondence. A match between the time of sampling and the timestamp of the land-use data is crucial for a reliable analysis relating pond biodiversity and environmental data on the one hand and land use on the other hand. Here, a trade-off exists between the optimal temporal match and the need for consistency of landuse categories, both through time and among regions. Indeed, the different projects that contributed to the SAFRED dataset ran from 1996 to 2012 and sampled ponds in the three Belgian regions (Brussels, Flanders and Wallonia) as well as Luxemburg. Various scenarios were developed with emphasis on temporal correspondence rather than consistency among land-use categories spanning the entire dataset. The ORCA project thus allowed us to complement the original SAFRED database, which holds information on pond biodiversity of multiple taxonomic groups along gradients of land use intensity from about 350 standing water bodies in Flanders, with an in depth assessment of land-use characteristics for each pond using a standardized GIS approach. Land use concerning the ORCA ponds was quantified in the same way.

In addition, we also created trait databases (available upon request) for zooplankton, macrophytes and macro-invertebrates (Heteroptera and Coleoptera) based on literature and relevant databases following the structure of the Freshwater Information Platform (http://www.freshwaterplatform.eu/). Trait data have also been collected for diatoms analysed in the Manscape and Pondscape projects. In addition to the analysis of these data as part of ORCA, these trait databases will be important to address a variety of research question beyond the ORCA project.

3.5. Mesocosm experiment testing for the response of zooplankton community structure and dynamics to pesticide application (*additional activity*)

As an addition to the research activities initially proposed in the project application and inspired by the observation that many species are shared among organic and conventional farmland ponds, we carried out an additional experiment in summer 2019 to assess to what extent zooplankton communities respond to exposure to pesticides. We exposed 20 clones of four common cladoceran species (*Daphnia magna, D. pulicaria*, *D. galeata* and *Scapholeberis mucronata*) to three pulses of Chlorpyrifos (Figure 3). All clones from each species were obtained from the same pond ("Langerodevijver") located in a nature reserve area. We used outdoor mesocosms that were monitored for 6 weeks. Each mesocosm contained either a mixture of four different clones of each of the four species, or each species as a monoculture. Five communities x two treatments (control and pesticide treatment) x tree replicates = 30 mesocosms. The animals in the pesticide treatment were exposed to a first chlorpyrifos pulse of 0.3 μg/L, a second pulse of 0.45 μg/L chlorpyrifos two weeks later, and a third pulse of 0.675μg/L chlorpyrifos again two weeks later. Chlorophyll *a* and phycocyanine concentration (as a measure of phytoplankton and cyanobacteria biomass respectively), pH and conductivity were measured twice a week. A weekly sample of the zooplankton community was taken from each mesocosm to assess temporal variation in community composition within and across mesocosms and treatments. Nutrient samples (for the analysis of total nitrogen and total phosphorus) were taken three times during the experiment (start, half-way and end).

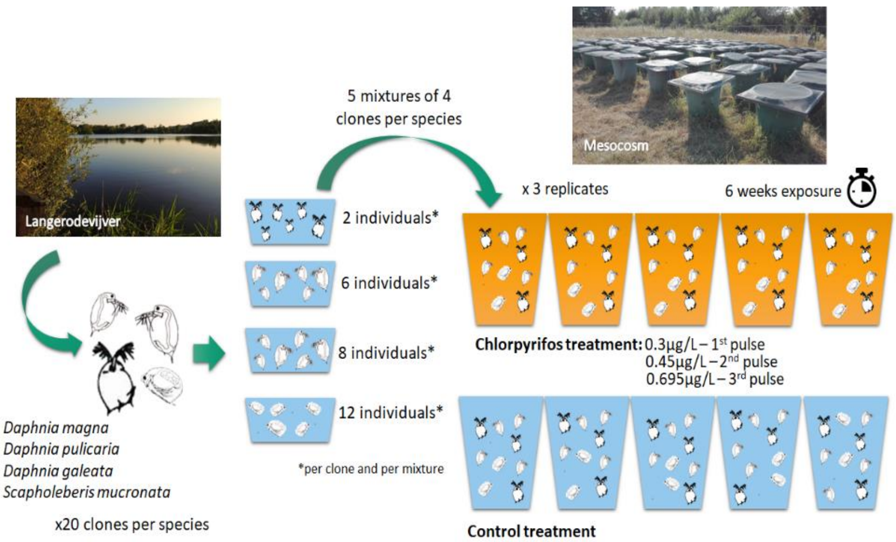


Figure 3. Graphical visualisation of the design of the mesocosm experiment testing for the effect of pesticide exposure on zooplankton community structure and dynamics.

<u>3.6. Experiment investigating the response of *D. magna* to shifts in pesticide application</u> *(additional activity)*

The overall aim of this additional experiment was to assess how pre-exposure and changes in the type of pesticide affect *Daphnia magna* populations. The experiment consisted of two distinct phases, (1) the selection phase, and (2) the new pesticide phase (Figure 4). This design was inspired by the fact that both pesticides used in organic and in conventional agriculture were shown to be toxic to non-target organisms, and the knowledge from literature that adaptation to one pesticide might make populations more vulnerable to other pesticides, especially if the new pesticide has a different mode of action than the old one. Policy-driven, continuous changes in pesticide use might therefore put additional stress on non-target organisms.

In the selection phase, a set of 20 clones of *Daphnia magna* were inoculated in 10L aquaria, forming populations of 100 individuals. These populations were exposed for 3 weeks to either a control treatment or a chlopyrifos treatment. Populations from the chlorpyrifos treatment were exposed to weekly pulses of the chlorpyrifos (0.035 mg/L). After 3 weeks, a 10 day resting period was used to allow populations to recover in densities.

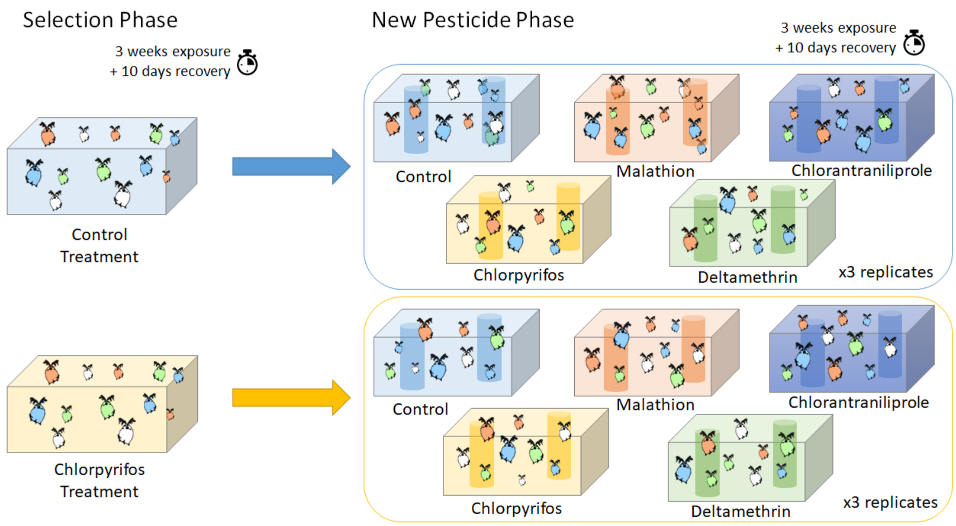


Figure 4. Graphical visualisation of the design of the pesticide shift experiment testing for the effect of pre-exposure to a pesticide and subsequent shift in pesticide exposure on populations of *Daphnia magna*.

In the second phase of the experiment, individuals from the original populations were divided in subpopulation 70 individuals each and subsequently exposed to one of the five different treatments (one control and four pesticide treatments. The pesticide treatments consisted of an exposure to chlorpyrifos, malathion, deltamethrin or chlorantraniliprole. These pesticides were chosen based on their mode of action, which were either identical to chlorpyrifos (in the case of malathion), or different (in the case of deltamethrin and chlorantraniliprole) and because we also wanted to test the specific sequence of a pesticide used in conventional agriculture to a pesticide used in organic agriculture. Similarly to the selection phase, the animals were given weekly pulses of pesticides over a period of 3 weeks. Twice a week, 2 adults and 2 juveniles from each tank were measured and isolated in cylindrical chambers inside the aquaria in order to assess body size, somatic growth rate, number of eggs produced and brood size. Population densities were determined by counting based on video recordings of the *Daphnia* populations.

At the end of this experiment, a standardised grazing experiment was conducted to assess the impact of the pre-exposure and pesticide shift on the grazing efficiency of *Daphnia magna* on phytoplankton. To this end, animals from each aquarium were isolated at the end of the experiment and kept in glass bottles that were subsequently allocated to two treatments, (1) no pesticide treatment, in which they were kept in dechlorinated tap water, or (2) a pesticide treatment, in which they were kept in a solution of the same pesticide they were exposed to during the second phase of the experiment. All bottles were inoculated with a fixed standard amount of the green algae *Acutodesmus obliquus* and kept on a rolling table for 24h. At the end of this period, water samples were collected to measure the algae concentration in each bottle.

3.7 Data management

Data management was an important task in ORCA. A data management plan has been published online on Figshare https://doi.org/10.6084/m9.figshare.7546694.v1 and linked to ResearchGate early in the project (De Wever et al., 2019). The document contains detailed information on the steps that needed to be undertaken to streamline the integration of the newly collected data, facilitate its integration in the SAFRED infrastructure, and prepare the public release of the data after an embargo time of two years starting at the end of the project. RBINS has filed all data bases and prepared them for public release on GBIF. Metadata of the ORCA database will be available through the Freshwater Metadatabase at data.freshwaterbiodiversity.eu/metadb (which is part of the Freshwater Information Platform infrastructure and supports metadata export in EML standard). The ORCA metadata paper is published in the Freshwater Metadata Journal (Cours et al., 2021).

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

4.1. A comparative analysis of the effects of organic and conventional agriculture on pond ecology and biodiversity

A redundancy analysis based on the entire set of local environmental variables yielded no significant difference in local environmental conditions between ponds located in conventional and organic agriculture (RDA: df = 1, R^2adj. = <0.001, p-value= 0.796) (Figure 5). We found that the total amount of agriculture (cropland and agricultural grassland) in the 200m radii around each pond had a significant but small effect on local environmental pond conditions (RDA, R^2adj.=0.078, p-value=0.003). These findings were also confirmed by separate univariate analyses on each of the environmental variables (see annex Figure A1 & Table A1).

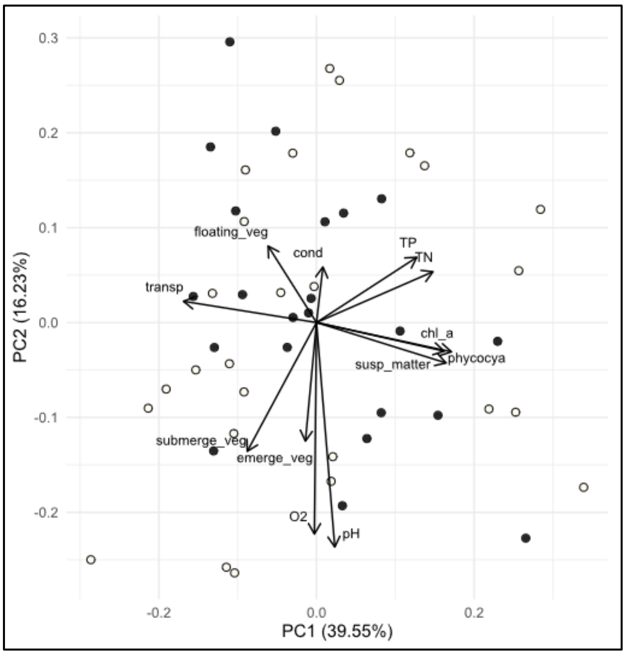


Figure 5. An ordination plot of a Principal Component Analysis based on local environmental pond variables. Arrows represent the environmental variables. Open and closed dots show the individual ponds located in organic and conventional agricultural land respectively.

The effect of agricultural type on aquatic biodiversity was separately investigated for shoreline vegetation, submerged plants, emergent vegetation, zooplankton, gastropods, hemipterans and coleopterans. We considered biodiversity of each considered organism groups at different spatial scales: (1) at the local (pond) level (α-scale), (2) all ponds within each agricultural type (conventional and organic) (γ-scale), (3) among pond differences within each agricultural type (β-scale). Following the approach outlined in Chase et al. (2018), we calculated four different biodiversity metrics for each organism group: species richness (S), total abundance (N), rarefied richness as the expected species richness when sampling a standardized number of individuals (S_N) and the effective number of species derived from the PIE, i.e. the probability of interspecific encounters (Hurlbert, 1971) (S_{PIE}).

Local scale (α-diversity)

Our analysis revealed no clear differences in local diversity (α-scale, for S, S_N and S_{PIE}) between conventional and organic ponds for most of the investigated organism groups (Figure 6 & 7). However, the different components of local diversity (S, S_N and S_{PIE}) of shoreline vegetation were significantly higher in ponds in organic agriculture compared to ponds in conventional agriculture (Table 1). In addition, total species richness of Coleoptera was also higher in organic farms. Abundances of organisms in the all the studied organism groups do not seem to be affected by agricultural type.

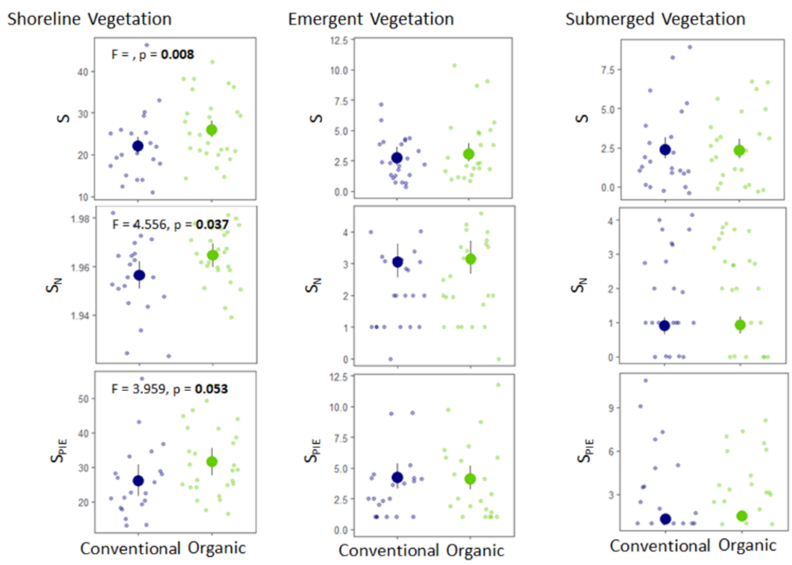


Figure 6. The components of local diversity (S, S_N and S_{PIE}) of shoreline, emergent and submerged vegetation in conventional and organic farmland ponds (blue and green respectively).

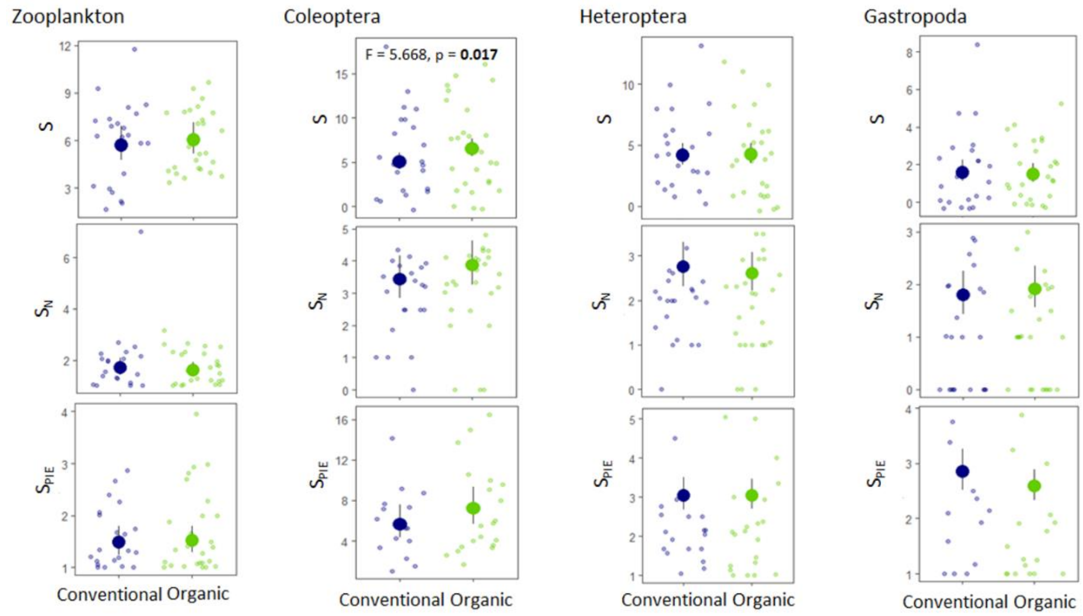


Figure 7. The components of local diversity (S, S_N and S_{PIE}) of zooplankton, coleopterans, heteropterans and gastropods in conventional and organic farmland ponds (blue and green respectively).

Table 1. Results of the linear models for biodiversity metrics (S, S_N and S_{PIE}) and agricultural type. Values of S_N and S_{PIE} were log-transformed. Significant p-values ($p < 0.05$) are given in bold.

	α - scale								
	S			Sn			S_{PIE}		
	DF	χ^2	P-value	DF	F	P-value	DF	F	P-value
Shoreline vegetation	1	7.14	**0.008**	1	4.556	**0.037**	1	3.959	**0.053**
Emergente vegetation	1	0.627	0.429	1	0.167	0.685	1	0	1
Submerged vegetation	1	0.042	0.837	1	0.147	0.704	1	1.201	0.282
Zooplankton	1	0.08	0.778	1	1.026	0.317	1	0.026	0.872
Coleoptera	1	5.668	**0.017**	1	1.411	0.242	1	1.798	0.191
Heteoptera	1	0.126	0.723	1	0.07	0.793	1	0.012	0.912
Gastropoda	1	0.049	0.825	1	0.182	0.672	1	0.421	0.523

Among-site differences (β-diversity)

At the β-scale, the effects of agricultural type vary between the organism groups (Figure 8 & 9). For emergent macrophytes, there is a tendency for higher β-S (Av. Dif = 7.01) in organic ponds, mostly due to higher presence of common species (Av. Dif β-S_{PIE} = 1.82) (Table 2). However, the opposite effect was observed for submerged macrophytes. A significantly higher number of common submerged macrophytes species was found in conventional ponds (Av. Dif. = 3.66, F-stat = 5.16, p-value = 0.025) . For zooplankton, although no clear differences were found for average species richness in the different agricultural types, organic agriculture was found to have a positive effect on both rare and common species when standardized to a common number of individuals (N_{ind} = 5). Organic farming also seemed to have a positive contribution to a higher occurrence of common Heteropteran species.

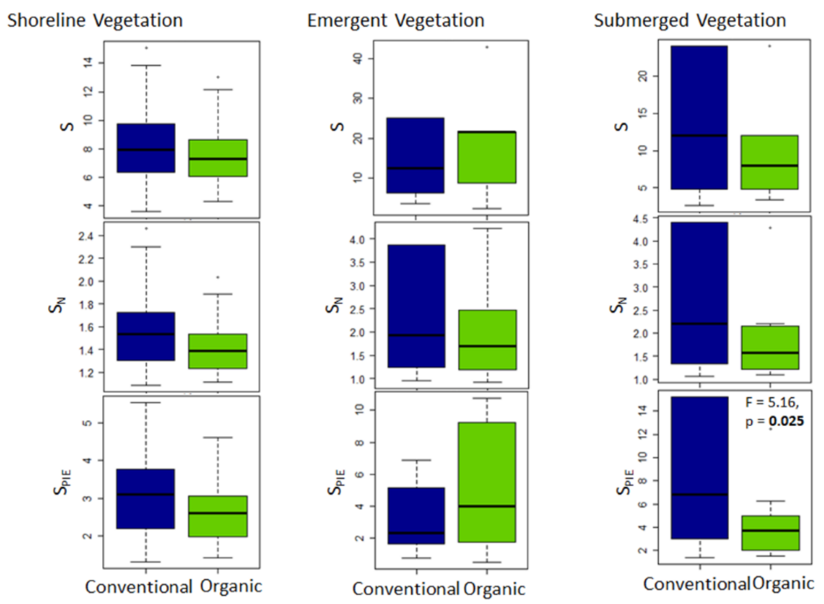


Figure 8. The component of β-diversity (S, S_N and S_{PIE}) of shoreline, emergent and submerged vegetation in conventional and organic farmland ponds (blue and green respectively).

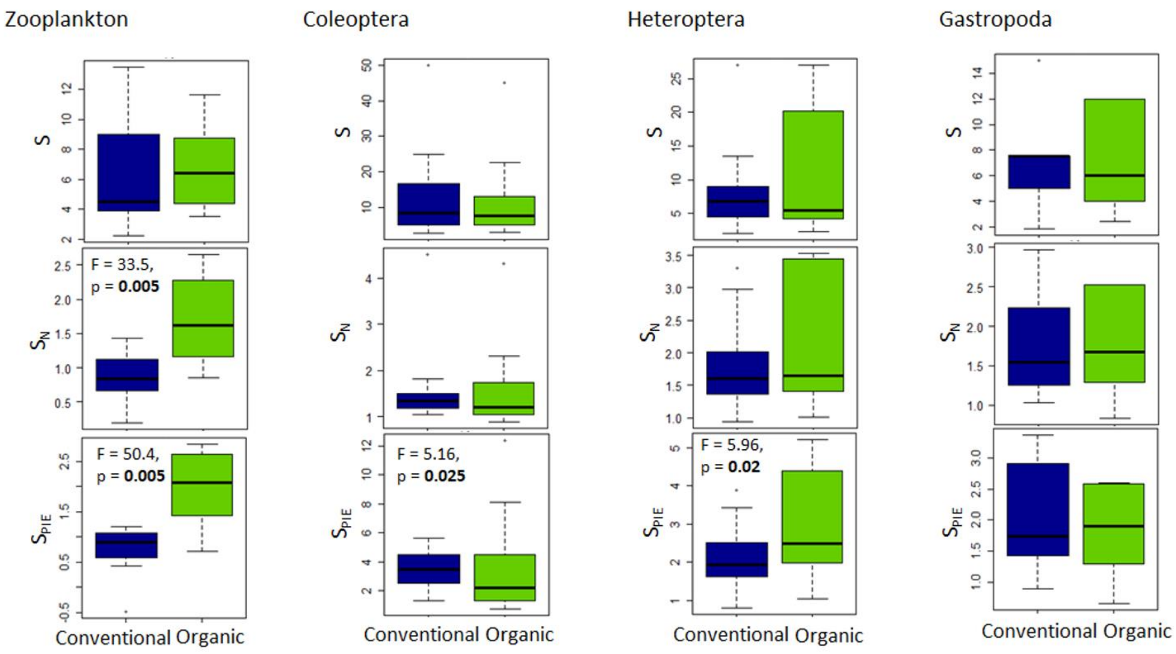


Figure 9. The component of β-diversity (S, S_N and S_{PIE}) of zooplankton, coleopterans, heteropterans and gastropods in conventional and organic farmland ponds (blue and green respectively).

Table 2. Results of the permutation tests for biodiversity metrics (S, S_N and S_{PIE}) and agricultural type. Models were performed for 199 permutations. Significant p-values ($p < 0.05$) are given in bold.

	β - scale								
	S			Sn			S_{PIE}		
	Av. Dif	F	P-value	Av. Dif	F	P-value	Av. Dif	F	P-value
Shoreline vegetation	0.691	0.834	0.35	0.152	3.18	0.075	0.474	2.66	0.085
Emergent vegetation	7.01	3.83	0.075	0.0345	0.009	0.9	1.82	3.41	0.12
Submerged vegetation	3.6	1.89	0.165	0.683	2.58	0.11	3.66	5.16	**0.025**
Zooplankton	0.513	0.365	0.48	0.85	33.5	**0.005**	1.21	50.4	**0.005**
Coleoptera	3.23	0.806	0.29	0.181	0.486	0.485	0.071	0.008	0.955
Heteoptera	3.46	1.78	0.225	0.387	2.25	0.16	0.909	5.96	**0.02**
Gastropoda	0.618	0.163	0.67	0	0	0.965	0.297	0.934	0.36

Regional diversity (γ-diversity)

At the γ-scale, results for effects of agriculture type on biodiversity varied among the different organism groups (Figure 10 & 11). Shoreline vegetation, emergent vegetation and zooplankton communities in organic fields had 16, 18 and 8 more species, respectively. On the other hand, 5 more species of Coleoptera and 3 more of Gastropoda were found in the set of conventional ponds. For the majority of the groups, these variations seem to be due to higher abundance of common species, except for Gastropoda, where we also observed a slight increase in rarified species in conventional ponds.

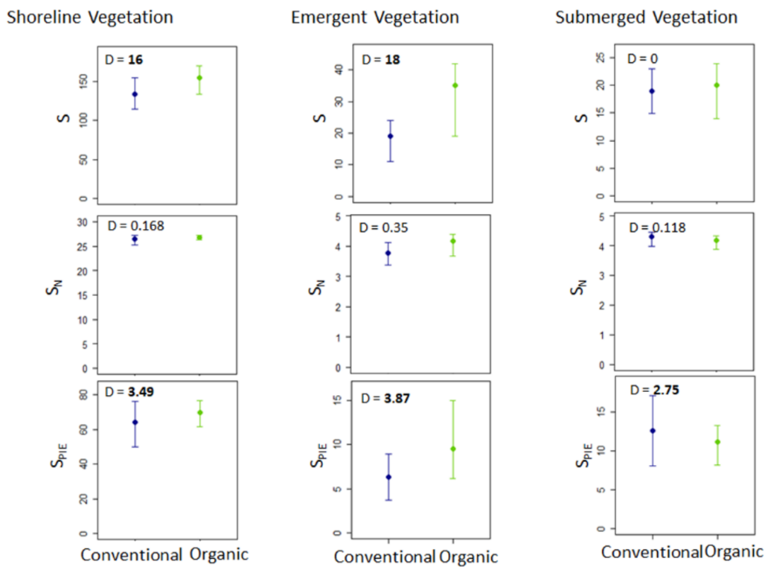


Figure 10. The components of regional diversity (S, S_N and S_{PIE}) of shoreline, emergent and submerged vegetation in conventional and organic farmland ponds (blue and green respectively).

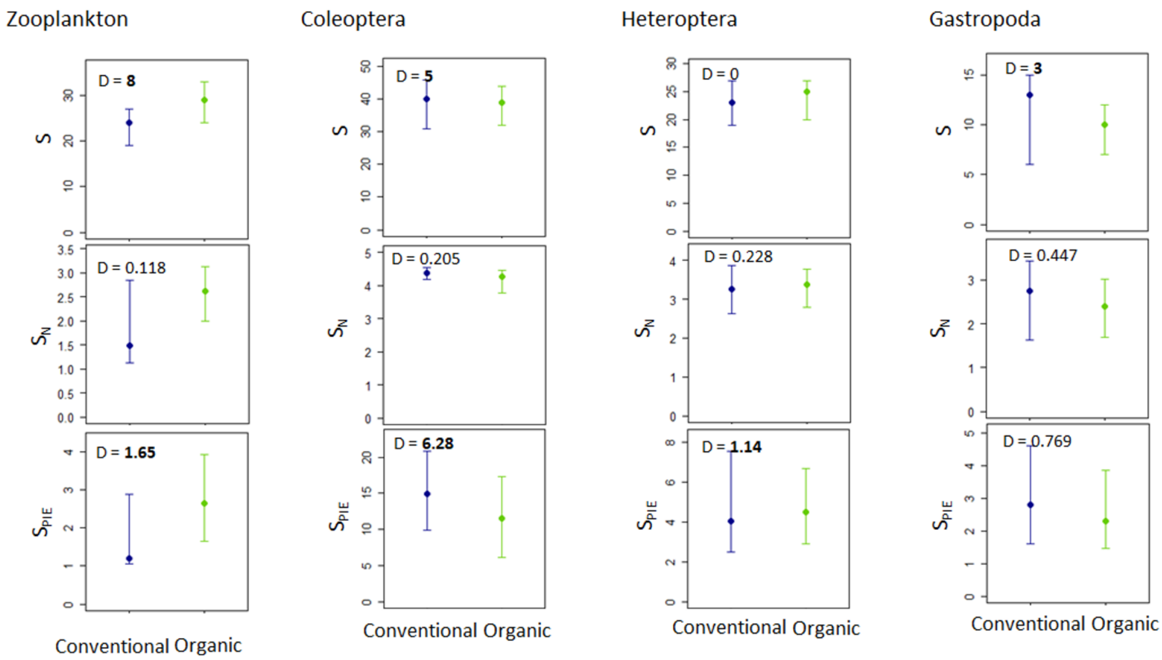


Figure 11. The component of regional diversity (S, S_N and S_{PIE}) of zooplankton, coleopterans, heteropterans and gastropods in conventional and organic farmland ponds (blue and green respectively).

Species accumulation curves show that organic agriculture contributes to a higher regional diversity of shoreline vegetation, emergent vegetation and zooplankton (Figure 12). This effect is, however, not observed for submerged vegetation, coleopteran and gastropods. For heteroptera, the agricultural type does not seem to have an effect on regional diversity.

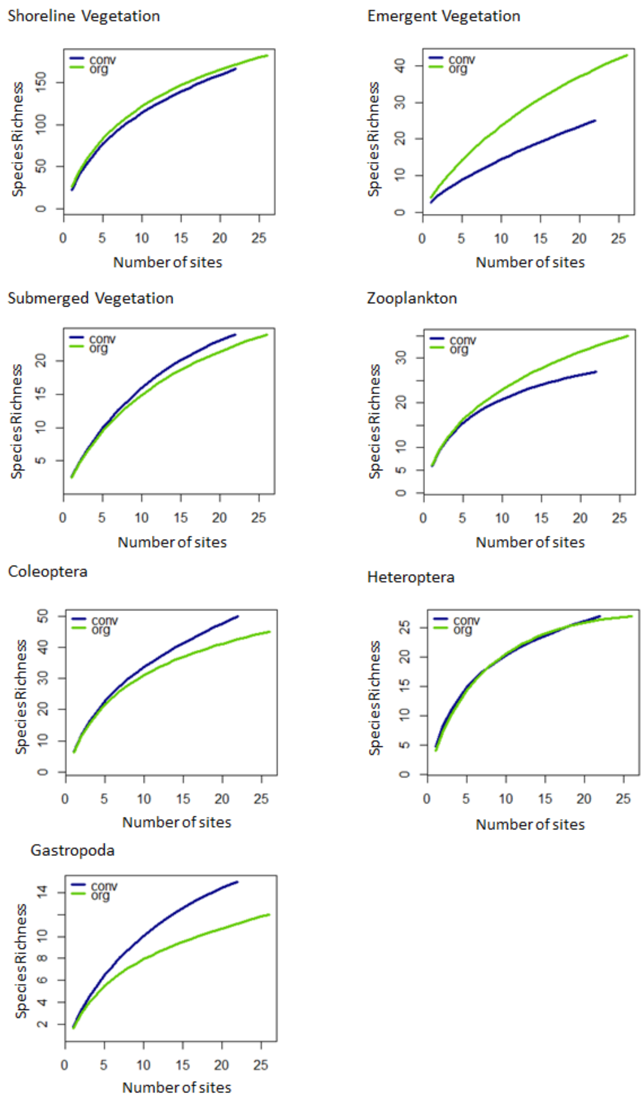


Figure 12. Sample-based species accumulation curves of shoreline vegetation, emergent and submerged vegetation, zooplankton, coleopteran, heteroptera and gastropoda. Curves corresponding to species in conventional agriculture sites are represented in blue and the ones corresponding to organic sites are represented in green.

In summary, our results show that effects of organic agriculture differ among organism groups and spatial scales. Shoreline vegetation and zooplankton are the taxonomic groups for which organic agriculture seems to have a positive effect on biodiversity compared to conventional agriculture. These differences were more evident for β- and γ- than for α-diversity. Our results suggest a moderately positive effect of organic farming on aquatic biodiversity, but the wide variety in biodiversity among ponds irrespective of agricultural type also emphasizes that other factors such as land-use at a larger spatial scale might be important for biodiversity. More detailed analyses that assess the effect of specific land use characteristics on separate biotic organism groups are currently in progress.

4.2. Genetic adaptation to pesticide use in organic and conventional agriculture in an aquatic non-target species

The analysis of the data on immobilisation as a response to exposure to pesticide shows that the median effective concentrations for copper sulfate did not differ significantly between populations from different land use types (conventional agriculture, organic agriculture and nature reserve) (main effect land use type, $F = 1.421$, $df=2$, $p = 0.342$) (Figure 13). In contrast, we observed a significant effect of land use type on the median effective concentrations of deltamethrin ($F=9.071$, $df=2$, $p = 0.033$). Populations from ponds located in organic agriculture have a significantly higher average EC_{50} for deltamethrin compared to populations from ponds located in conventional agriculture or nature reserves.

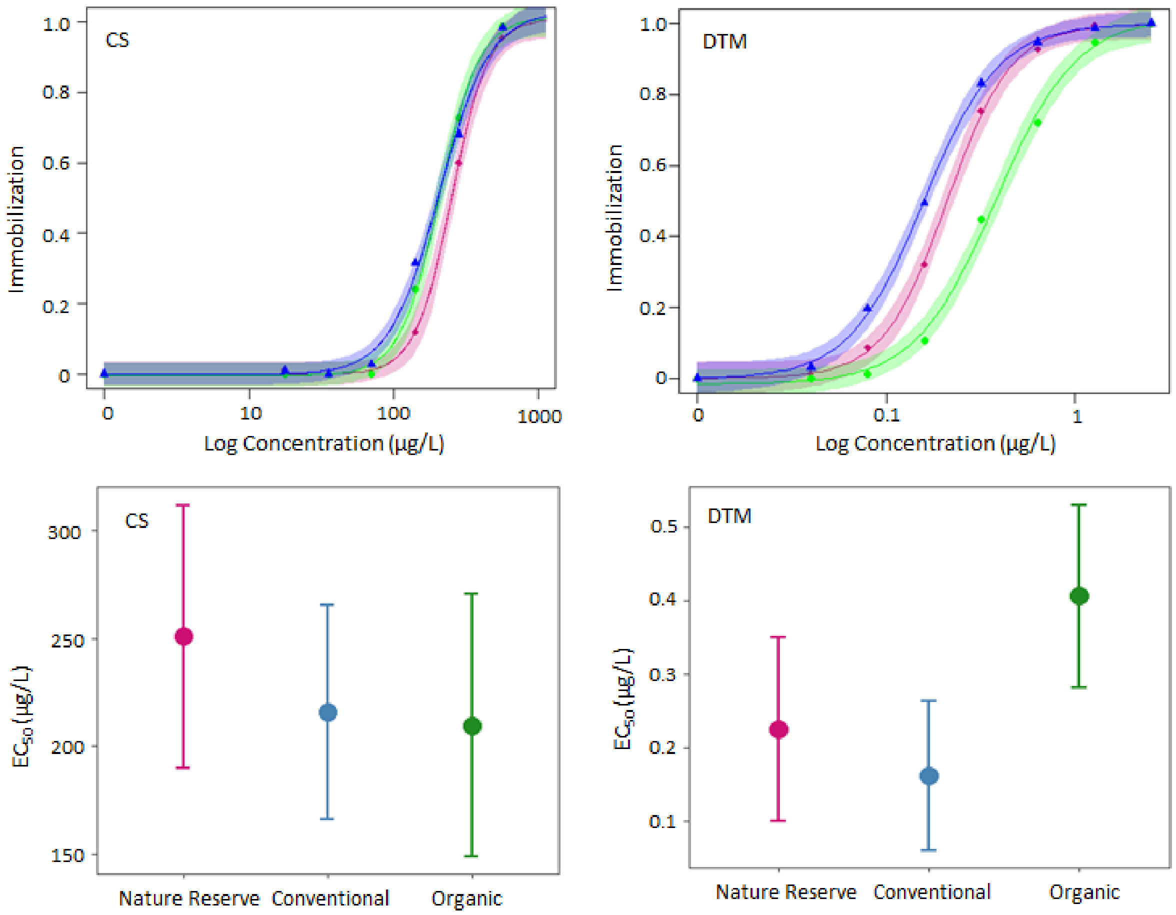


Figure 13. *Upper two panels:* Logarithmic concentration (µg/L) – response (immobilization at 48h) curves and Average EC_{50} values ± s.e. for copper sulfate (CS) and deltamethrin (DTM), for each land use type (nature reserve – pink, conventional agriculture – blue, organic agriculture – green). Shadowed areas in the left panels corresponds to 95% CI. *Lower two panels*: EC50 concentrations for each land use type (nature reserve – pink, conventional agriculture – blue, organic agriculture – green) for copper sulphate (CS) and deltamethrin (DM).

In addition, we found that the resistance to chlorpyrifos was positively related to the percentage of conventional agriculture within the 200 m perimeter the pond. ($F= 4.673$, $df = 1$, $p = 0.038$) (Figure 14).

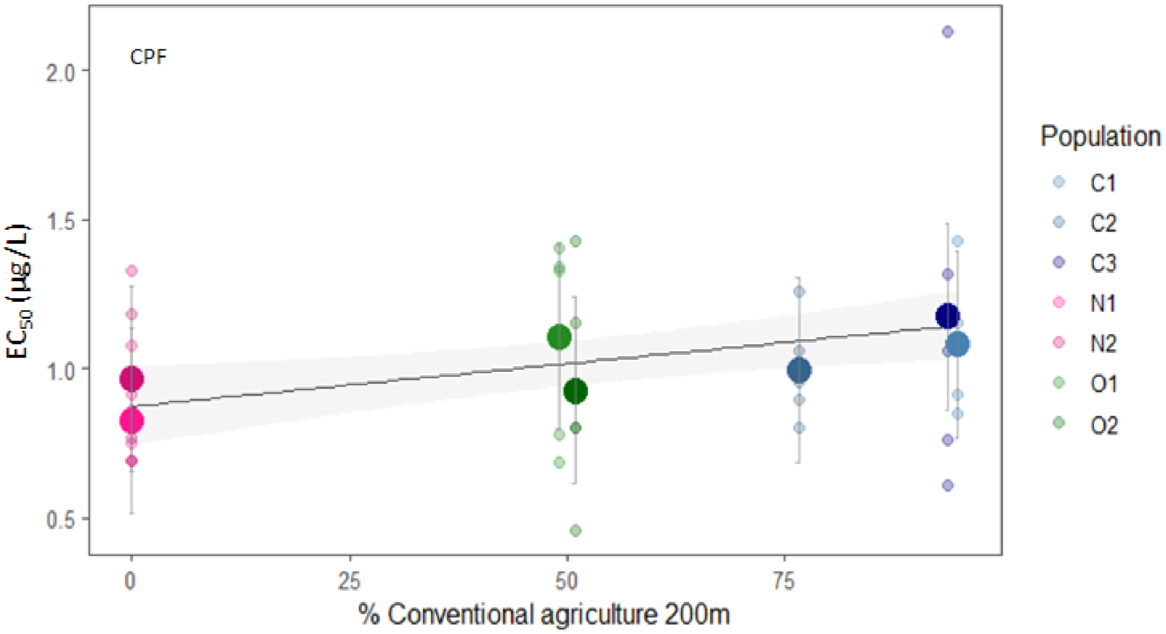


Figure 14. Pesticide resistance expressed as EC_{50} related in response to the percentage of conventional agriculture land in a 200 meter radius around the ponds. Average population EC50 values +- 1 SE are given for the conventional pesticide chlorpyrifos (CPF). Populations from nature reserves are represented in pink, populations in conventional agriculture are shown in blue and populations from organic agriculture are shown in green.

Our results show that *Daphnia magna* populations differentially adapt to pesticide use in organic and conventional farming. Populations become less sensitive to Chlorpyrifos as the intensity of conventional agriculture in the landscape surrounding the ponds increases, whereas populations from areas dominated by organic agriculture are relatively less sensitive to Deltamethrin compared to populations from a conventional agriculture and nature background. These results emphasize that both types of agriculture profoundly impact the genetic features of non-target species, but do so in a different way, reflecting the differences in selection pressure.

4.3. Population genomics

As explained in the methods section, we are currently analysing the full genome resequencing data of 20 populations sampled along a land use gradient. A GWAS analysis will provide an excellent benchmark to analyse the data on the ORCA populations that will be resequenced.

4.4. Analysis of the effect of buffer strips

We used the newly-collected ORCA data to analyse the relation of buffer strips to nutrient concentrations (TN and TP) and the species richness of multiple aquatic organism groups. The newly-collected data include detailed information on the size of the buffer strips within the 10 meter buffer surrounding each pond (see method section), Our analyses revealed no systematic difference in the size of the buffer strips between ponds in conventional or organic farming (ANOVA, df=1, SS=12.1, MS =12.13, F=1.547, p=0.22) (Figure 15). However, GLM analysis indicated that the size of the buffer strip (quantified as median distance between pond and buffer edge along eight cardinal lines) was negatively associated with the concentration of total nitrogen (p = 0.034), irrespective of the type of agriculture (conventional or organic) (Figure 16.). Such a relation was not observed for the concentration of total phosphorus (p=0.273). We observed no systematic effect of buffer strips on the species richness of investigated macrophytes, zooplankton or the different groups of macro-invertebrates.

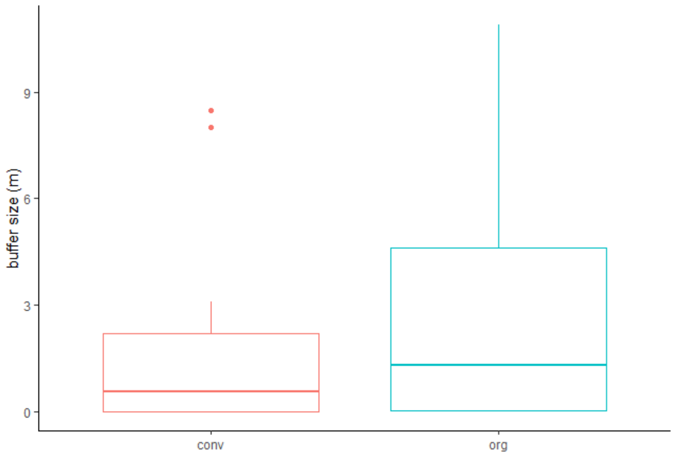


Figure 15. Boxplots showing the buffer size for ponds in conventional and organic agricultural land.

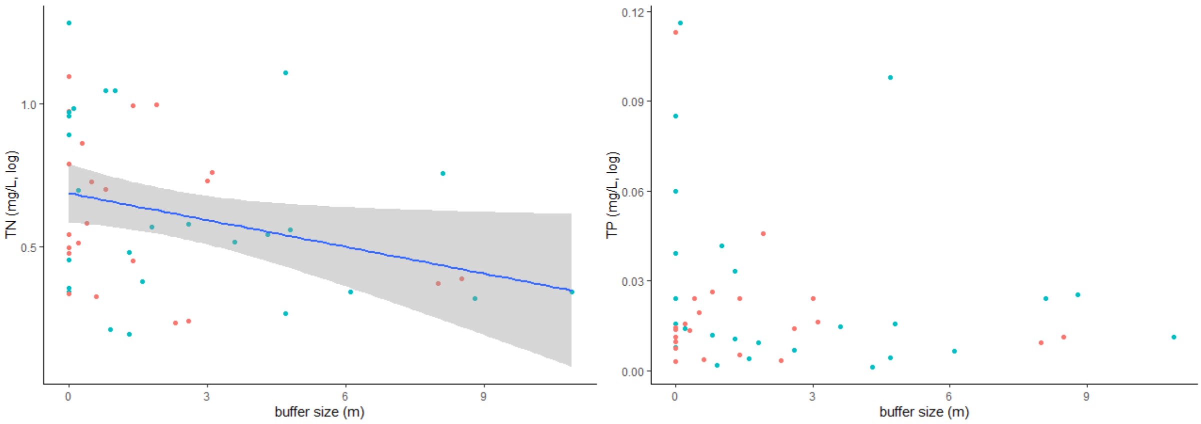


Figure 16. Relation between buffer size (quantified as median distance between pond and buffer edge along eight cardinal lines) and concentrations of total nitrogen (TN, left) and total phosphorus (TP, right). Ponds in organic and conventional agriculture are shown in red and green respectively.

4.5. Response of zooplankton community structure to pesticide application

The effect of pesticide exposure on community biomass dynamics was analysed through a Bayesian Hierarchical Gaussian Process regression model. In both treatments, the largest species *D. magna* dominated the community (Figure 17). We observed a delay in population dynamics of *D. magna* and *D. galeata* in the pesticide treatment. *D. pulex* showed a strong increase in population biomass in the pesticide compared to the control treatment. Our interpretation is that this is due to competitive release from *D. magna* (note the much higher numbers of *D. magna* than of *D. pulex*). By the end of the experiment *D. magna* dominates the communities in the pesticide treatment even more than in the absence of pesticides.

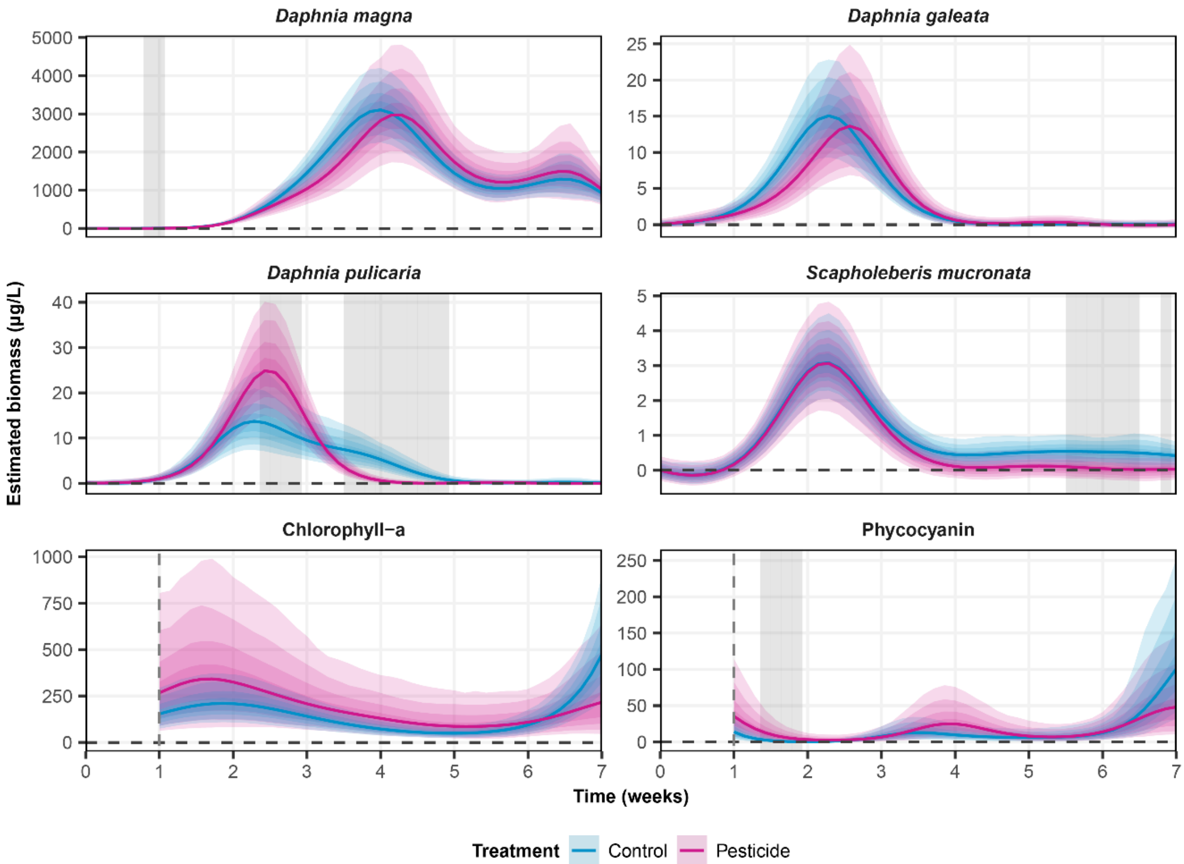


Figure 17. Estimated evolution of the zoo- and phytoplankton biomass through time for the control (blue) and pesticide treatment (pink). These patterns are derived from a Bayesian Hierarchical Gaussian Process regression model that defines the full probability distribution over the evolution functions, while accounting for the hierarchical structure of the data. The full lines indicate the posterior median evolution, while the coloured bands represent the 50, 80, 95 and 99% credible intervals. The shaded zones in grey indicate that the estimated probability of a difference between both treatments is at least 0.95. The vertical dashed lines indicate the pesticide pulses. The chlorophyll-a and phycocyanin curves only start at t=0, as their values was not determined at the time of inoculation (t=-1).

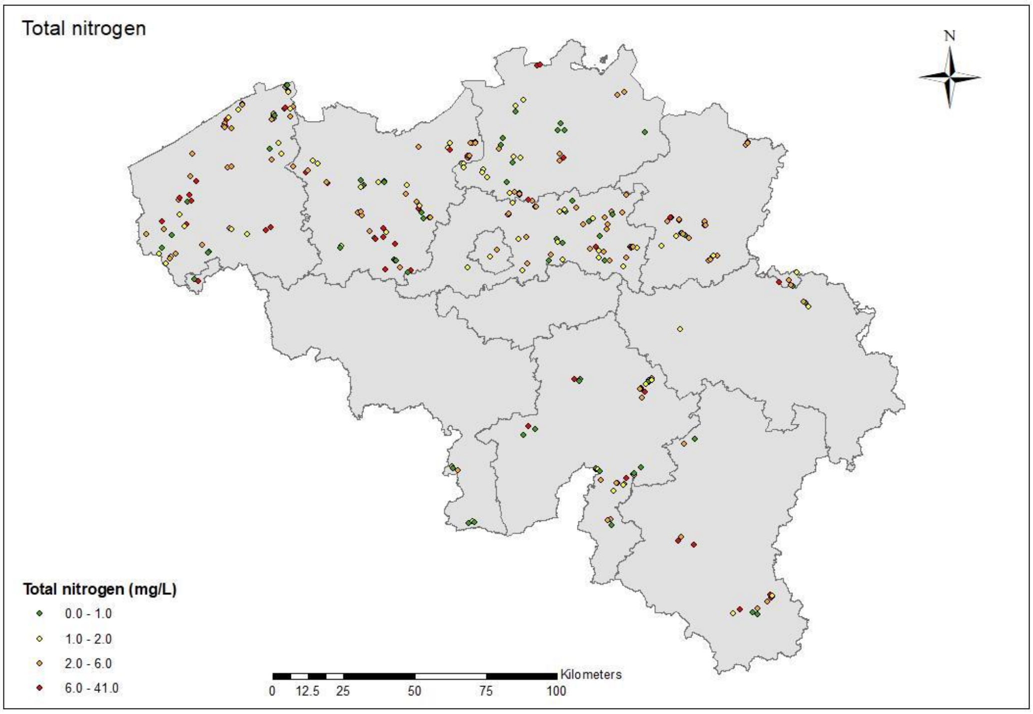
Our results show that pesticide exposure results in the extinction of the less tolerant species and promotes the dominance of *D. magna*, the most tolerant species. Our results emphasize that pesticide exposure not only affects community composition through direct species-specific responses to pesticide, but also by altering interspecific interactions.

4.6. Analysis based on integrated data (SAFRED + ORCA)

The farmland ponds in the integrated database represent a variety of systems that strongly differ in local environmental conditions (Table 3). The majority of ponds is small and relatively shallow. The overall nutrient status of the ponds is rather high and there seems no clear relation between geographical location of the pond and nutrient status (Figure 18). The dominant land use is agriculture (both cropland and agricultural grassland), although a number of ponds is characterised by high degree or urbanisation in close proximity of the pond.

Table 3. Overview of the major local pond characteristics and the dominant land use in the 200 meter surrounding the pond. With respect to the vegetation, 0 = 0% coverage, 1 = 1%-25% coverage, 2 = 26%-50% coverage, 3 = 51%-75% coverage, 4 = 76%-99% coverage, 5 = 100% coverage.

	mean	**median**	**min**	**max**
Total nitrogen (mg/L)	3.69	2.29	0	40.4
Total phosphorus (mg/L)	1.5	0.67	0.02	23.5
Chlorophyll a (µg/L)	163.05	55.59	0.5	3161.4
pH	7.87	7.76	5.4	10.77
Conductivity (µS/cm)	527.81	419	5.84	11787
Maximum water depth (m)	0.84	0.75	0.08	3
Size (m^2)	713	300	8	8100
Water transparency (sneller depth, cm))	19.147	19	1	54
Submersed vegetation cover (0-5)	1.12	0	0	5
Floating vegetation cover (0-5)	1.5	1	0	5
Emergent vegetation cover (0-5)	1.24	1	0	5
Percentage agriculture (%, 200 meter perimeter)	58.16	66.18	0	100
Percentage green (%, 200 meter perimeter)	15.58	5.32	0	99.6
Percentage urban (%, 200 meter perimeter)	24.28	10.9	0	100



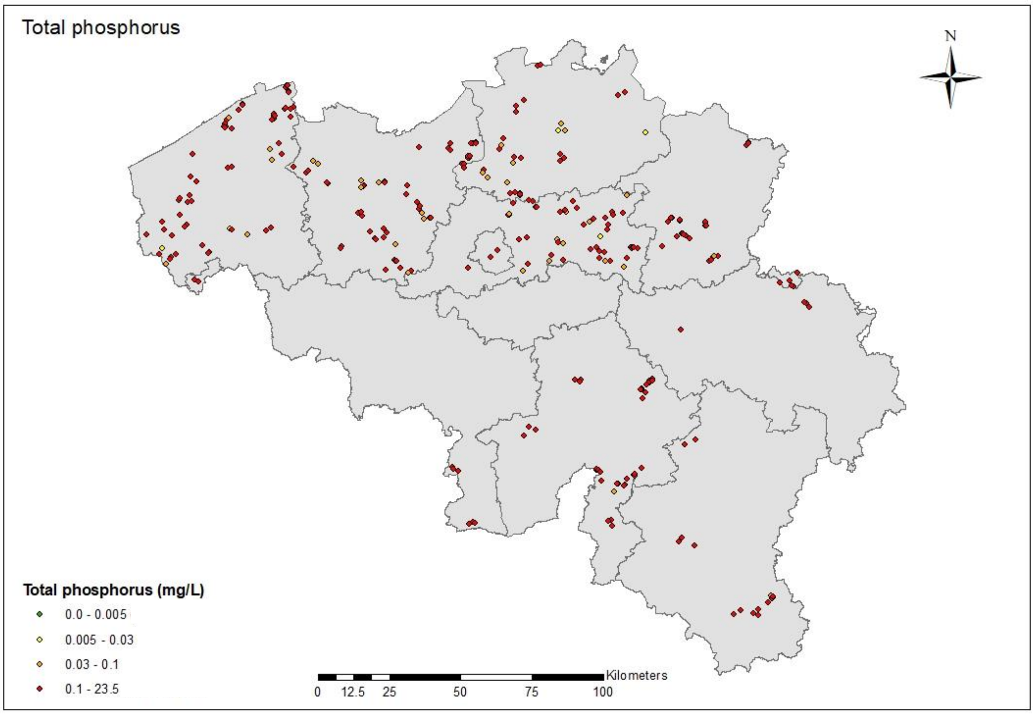
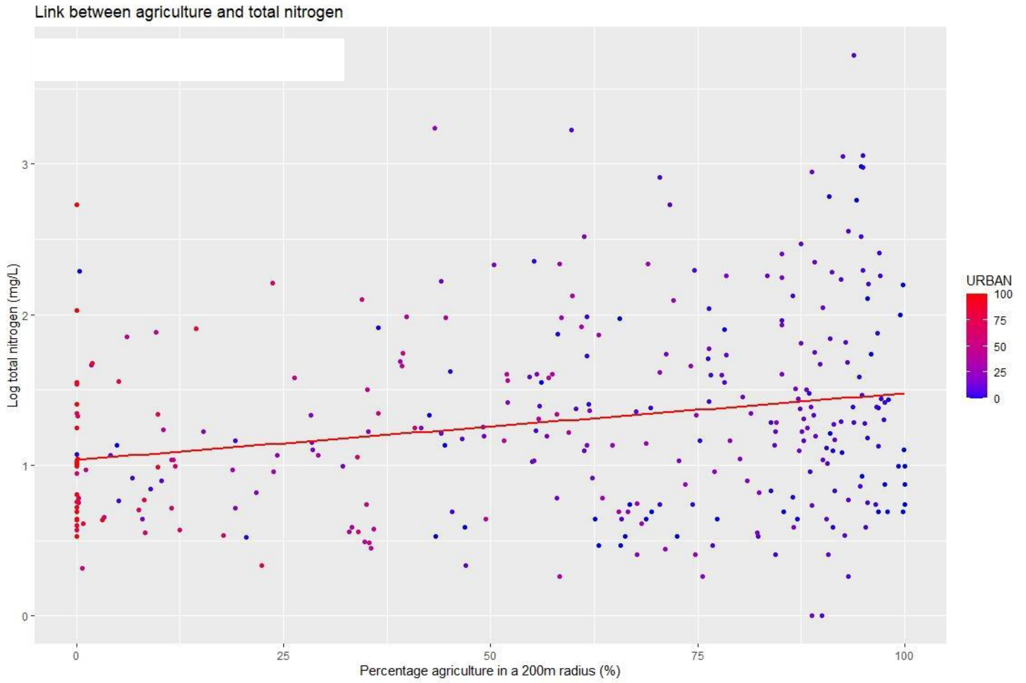


Figure 18. Overview map of the ponds in the integrated database in relation to the concentration of total nitrogen (upper panel) and total phosphorus (lower panel) concentration.

The percentage of agriculture (cropland and agricultural grassland combined) had an overall positive effect on both total nitrogen and total phosphorus concentration (Spearman, R and p value, 0.21, <0.001 and 0.32, <0.001, respectively) (Figure 19.) Interestingly, there is considerable variation in nutrient concentration among ponds located in an urban land use setting.



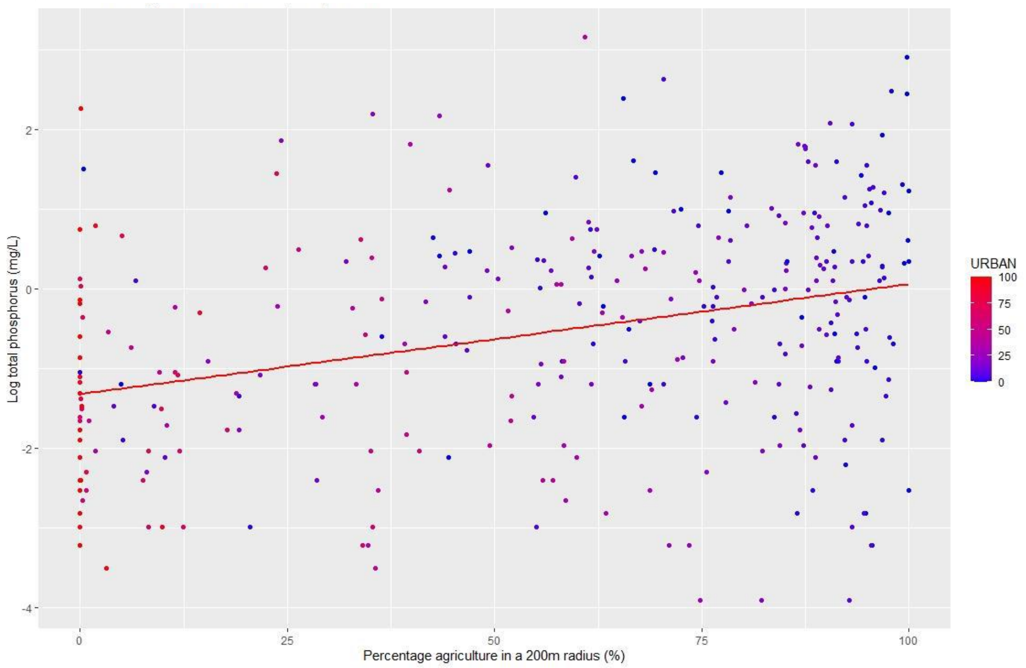


Figure 19. The relation between the percentage agriculture in the 200 meter perimeter around the pond with total nitrogen concentration (upper panel) and total phosphorus concentration (lower panel).

4.7. The effect of land use on pond vegetation characteristics

The ORCA results were positioned in a broader land use perspective by investigating the relation between land use characteristics and variation in macrophyte community composition using a subset of the SAFRED database (134 ponds) that also holds information from ponds in a more pristine and urban land use setting.. We restricted the selection of ponds towards those sampled as part of ORCA and the PONDSCAPE project because both projects shared the same methodology for macrophyte community assessment. The analyses were restricted to true aquatic vegetation (helophyte belt and water). Bank vegetation was thus not considered as it is likely to more prone to local management than to influences from land use surrounding the pond. Taxonomic information was complemented with information on relevant traits athat were collected as part of ORCA.

Our analyses reveal that vascular plants predominate in the investigated macrophyte communities by abundance (mean ± stdev: 90.7 ± 18.2 %) and that hydrophytes slightly outweigh other species. However, variation between ponds is considerably large (57.8 ± 35.8 %). Weighted averaging of indicator values indicates that plant communities consist mainly of species associated with nutrient-rich conditions (Ellenberg N 5.95 ± 0.90; trophic score 2.62 ± 0.19). Non-indigenous species contribute 10.1 ± 12.8 %.

Local macrophyte richness varied from 1 to 23 and is overall low (mean ± stdev: 7.1 ± 4.8). Pearson correlations revealed a positive association between some land-use variables and different measures of diversity (annex Table A2). This relation often becomes more apparent with increasing perimeter size. Species richness and Shannon entropy are negatively correlated with cropland cover at diffent perimeters (100m, 1000m and 3000m). In contrast, Local Contribution to Betadiversity (LCBD) seems positively correlated with cropland cover (Figure 20). Our analyses show that even a limited cover with cropland already has a significant negative effect on local diversity (richness and shannon entropy) (Figure 21). A similar but less profound negative effect of intensive grassland on diversity was observed.

Threshold Indicator Species Analyses were used to investigate to what extent changes in land use in the 100, 1000 or 3000 metre perimeter around the pond can result in changes in macrophyte community composition (Table 3). We found no clear change points for urbanization, infrastructure and intensively managed grassland. At the 100 m perimeter, only intensive agriculture shows a weak effect. The number of significantly decreasing or increasing taxa is low in all cases, preventing robust deductions. The highest number of affected taxa is found for cropland at the 3000 m perimeter (11 decreasing taxa; 7 % of total), intensive agriculture 3000 m perimeter (13 decreasers; 9 %), semi-natural grassland 1000 m perimeter and nature 1000 m perimeter (both 9 increasers; 6%). Less reliable indicators remain with decreasing perimeter size. Even for cropland at the 1000 and 3000 m perimeter, confidence intervals are fairly wide (90 % CI resp. 18.4 and 25.5 % of the complete gradient). Notably, change points become less relaxed for intensive agriculture with increasing perimeter.

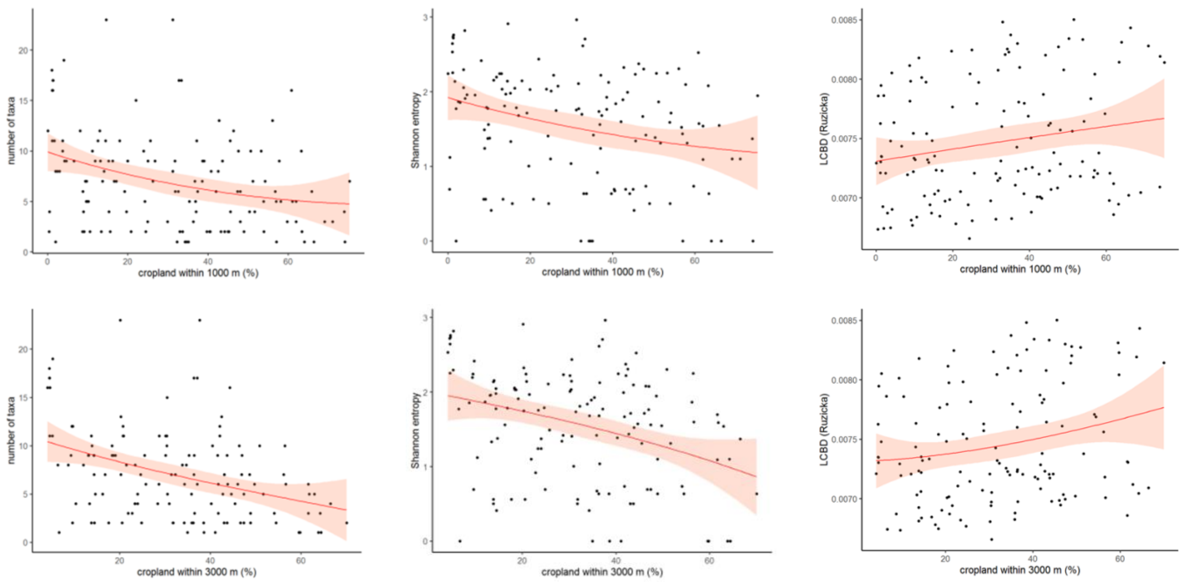


Figure 20. Relation of taxonomic richness (left), Shannon-entropy (middle) and Local Contribution to Betadiversity (right) to the proportion of cropland within the 1000 m (upper) and 3000 m (lower) perimeter around the pond. Loess smoother on 10 data points with 95 % CI.

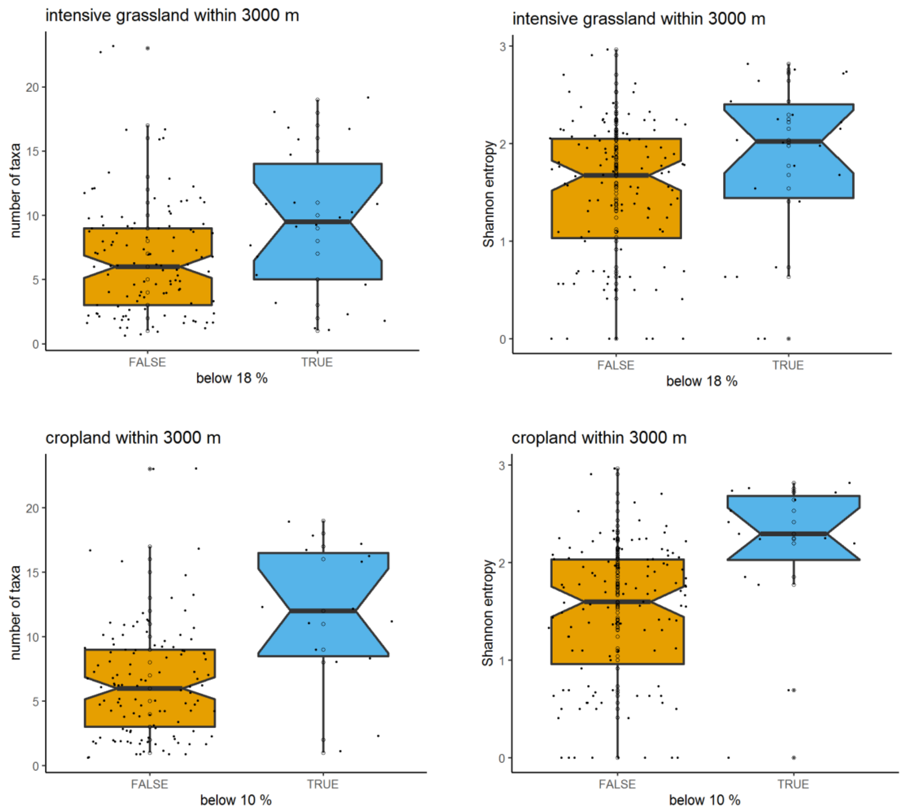


Figure 21. Number of taxa and Shannon entropy for ponds with less or more than 10 % cropland or 18 % intensive grassland within 3000 m. Notched box plots show data points and medians with boxes for 1st and 3rd quartiles, notches at approximate 95 % CI of the median and whiskers up to 1.5 interquartile range.

Table 3. Results of threshold indicator taxa analyses for land-use gradients partim cropland and intensively managed grassland for filtered decreasing taxa (Z-), change points (cp) with confidence limits (CI). CR: cropland, GA: agricultural grassland.

variable	cp (%)	cp CI 0.05 (%)	cp CI 0.1 (%)	cp median 0.5 (%)	cp CI 0.9	cp CI 0.95	number of filtered taxa
CR 1000	1.6	1.5	1.60	6.4	8.5	14.9	7
CR 3000	5.9	4.9	5.2	6.0	14.4	21.7	11
CR+GA 100	72.6	41.7	43.9	68.5	72.6	73.2	6
CR+GA 1000	24.7	22.4	23.8	31.1	51.5	53.0	11
CR+GA 3000	22.7	22.5	22.6	22.7	32.4	45.9	13

The most important changes with increasing agricultural use appear to occur in the helophyte belt where some more ruderal non-clonal taxa (*Lythrum salicaria*, *Persicaria hydropiper*, *Rumex conglomeratus*, *Rumex hydrolapathum*) as well as rhizomatous facies-forming species (*Carex acutiformis*, *Equisetum fluviatilis*, *Juncus effusus*, *Persicaria amphiba*) are already declining well before cropland reaches 10 % (maximum change at c. 6 % cropland) or intensive agriculture attains 30 % (Figures 22 & 23). This proceeds into a more gradual species loss at intermediate levels of intensive agricultural land-use involving e.g. *Glyceria fluitans*, *Potamogeton natans* and *Sparganium erectum*. The very few taxa that seem to be somewhat promoted by a high level of agricultural land-use are *Phragmites australis*, an extremely wide-ranging competitive species, and some tolerant taxa from hard water, rich in electrolytes and nutrients, such as *Stuckenia pectinata* and the algae *Ulva* sp.

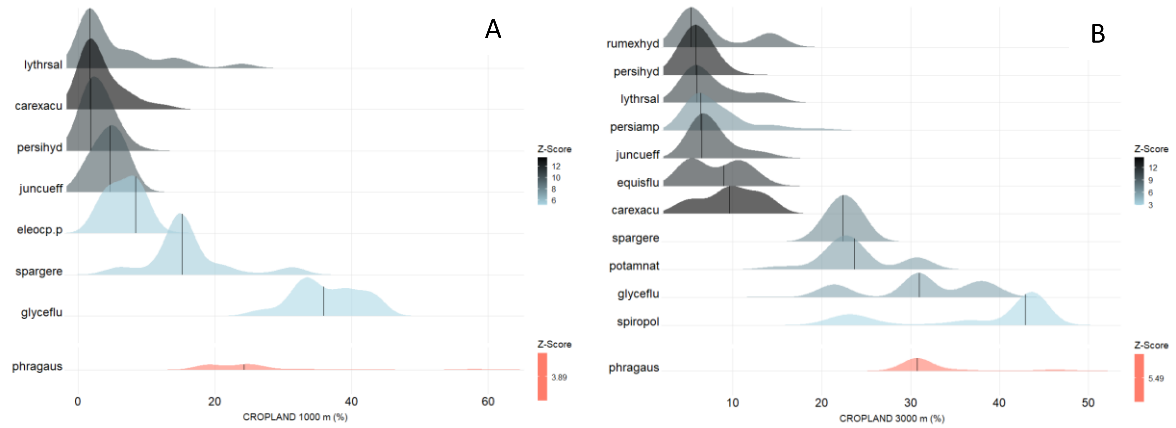


Figure 22. Probability density of change for filtered decreasing (Z-, blue grey) and increasing (Z+, red) taxa along the gradient cropland within 1000 meter perimeter (A) and 3000 meter perimeter (B).

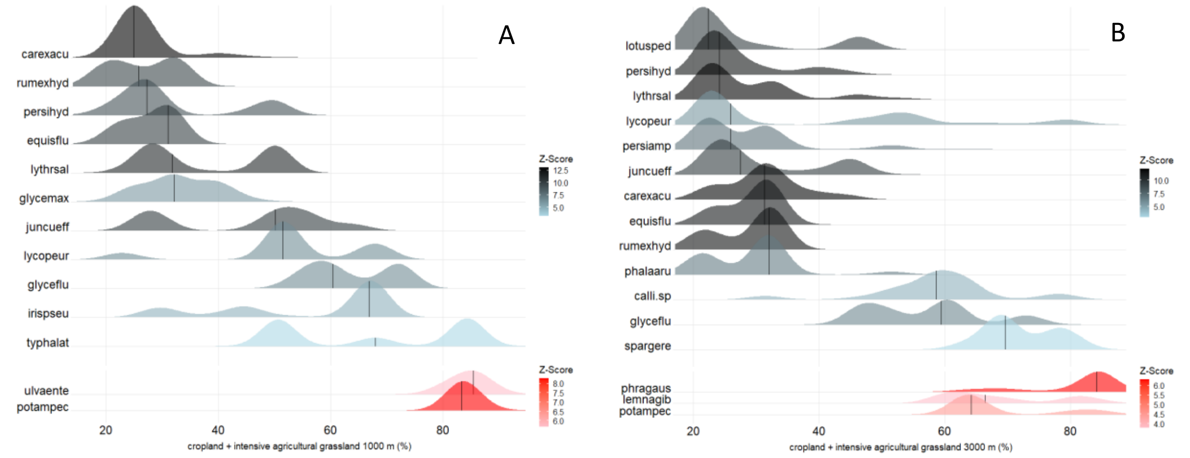


Figure 23. Probability density of change for filtered decreasing (Z-, blue grey) and increasing (Z+, red) taxa along the gradient cropland + agricultural grassland within 1000 m perimeter (A) and 3000 m perimeter (B).

In conclusion, our data show that local diversity of macrophyte species in the set of investigated ponds is overall low. In addition, only a few truly sensitive taxa seem to be present in the surveyed ponds. The major driver for variation in aquatic vegetation community characteristics appears to be nutrient enrichment. Agricultural intensification not only affects species assemblage composition, but also reduces α-diversity, especially when a larger perimeter is considered. This suggests that land use at larger spatial scales is important. A relatively low amount of intensive agriculture in proximity of the pond, e.g. less than 10 % cropland or 30 % intensive agriculture within 3 km perimeter, may already lead to a loss of complexity in the pond vegetation, promoting a more homogeneous, reed-dominated or even helophyte-barren transition from water to land. The resulting loss in plant diversity and change in vegetation structure implies further loss of habitat provision for a range of species associated with this coenocline. Increasing water stress is likely to exacerbate this phenomenon in the future.

4.8. Eutrophication reduces taxonomic and trait diversity of zooplankton in Flemish lentic waterbodies (based on integrated database)

We investigated to what extent eutrophication affects taxonomic and functional diversity of zooplankton in small lentic waterbodies based on the SAFRED integrated database (taxonomy and trait) that has been complemented with novel data collected as part of the ORCA project. We specifically focus on zooplankton as they are the best represented in terms of number of ponds and with respect to the standardization of sampling method and taxon identification.

We selected ponds of similar size and depth (upper threshold values for size and pond depth 0.15ha and 1.2m, respectively) along a gradient of eutrophication from the integrated database. Our final dataset includes a set of 198 small farmland ponds that have been sampled as part of different projects. The degree of eutrophication was assessed for each of these ponds as their scores of the first axes of a PCA ordination based on local environmental variables, which is positively associated eutrophication related variables such as total phosphorus and total nitrogen concentration, chlorophyll a concentration (i.e., algal biomass) and pH (see annex Table A3). We used the first axis of this ordination as a latent variable to capture the degree of eutrophication because (1) eutrophication typically is a multivariate factor comprising multiple aspects of ecosystem organization. Trait richness and trait redundancy were calculated following Laliberté (2010) and Villéger et al. (2008), respectively. The relation of taxonomic richness, trait richness and trait redundancy with eutrophication was examined using linear mixed effect models. The presence of fish was included as a random factor in these models as it is well known that fish can have profound effects on the characteristics of zooplankton communities.

Our analysis shows a significant negative effect of eutrophication on taxonomic diversity, trait diversity and trait redundancy ($r = -0.33$, $p < 0.01$; $r = -0.32$, $p < 0.01$ and $r = -0.30$, $p < 0.01$, respectively), and this relation was very similar for ponds with and without fish (Figure 24). These findings underline that eutrophication of ponds reduces both taxonomic and trait diversity, and results in lower trait redundancy, which on the long term might detrimentally affect ecosystem functioning, especially under conditions of global change requiring ecosystems and communities to respond to changing environmental conditions.

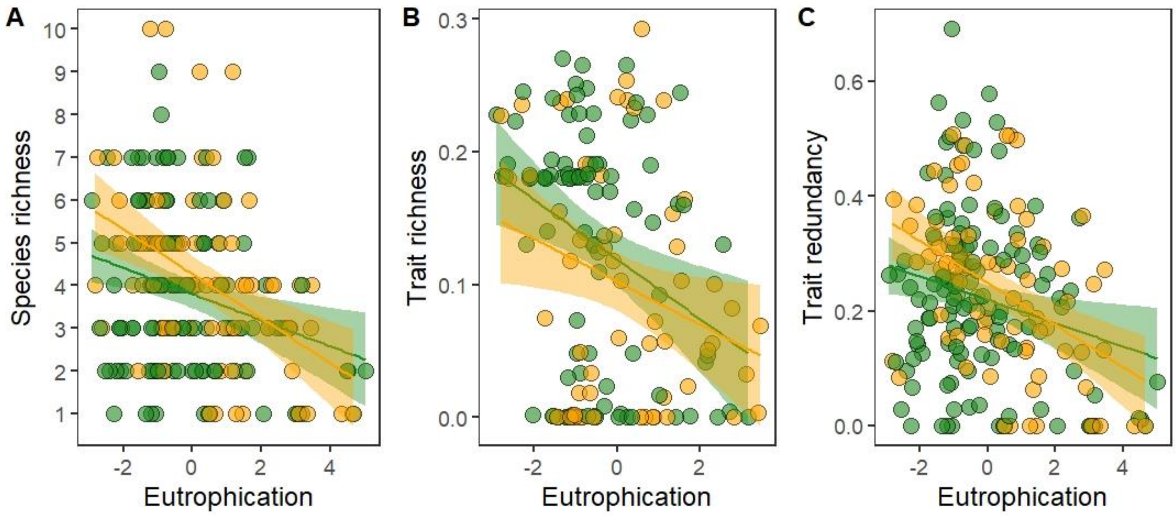


Figure 24. The relation of taxonomic richness (A) , trait richness (B) and trait redundancy (C) in relation to eutrophication for ponds with and without fish (green and yellow, respectively). The lines and their respective shaded region illustrate the best fit with 95% confidence interval.

General findings, conclusions and recommendations.

Our comparative analysis of the effect of organic and conventional agriculture on aquatic diversity shows that differences in terms of taxonomic diversity are modest, but relevant for some organism groups, especially at the regional spatial scale. The diversity of shoreline vegetation and zooplankton seems to benefit most from organic agriculture. Differences in diversity between organic and conventional agricultural ponds were more profound at the β (among ponds) and γ (landscape) scale than at the α- scale (pond level).

The analyses of the integrated database reveals that agriculture in general reduces local diversity and affects species community composition. A relatively low amount of intensive agriculture around the pond already results in a profound loss of complexity in the pond vegetation, which implies further loss of habitat provision for a range of macrophyte associated species. A major driver for variation in zooplankton and aquatic vegetation community characteristics appears to be nutrient enrichment. Nutrient concentrations (total phosphorus and total nitrogen) in Belgian farmland ponds are overall high and positively related to the amount of agriculture in close proximity of the ponds. This likely explains the observed low local diversity of macrophytes and the limited number of truly sensitive taxa in the investigated ponds. Importantly, eutrophication not only reduces taxonomic diversity, but also negatively affects zooplankton trait diversity, resulting in lower trait redundancy. This can undermine ecosystem functioning on the long term, especially under conditions of global change. The creation of relatively small buffer strips around the pond ($<$ 10 meter) can already be effective to mitigate pond eutrophication in agricultural areas.

In addition to differences at the ecosystem and community level, our results show striking differential genetic adaptation of zooplankton to both conventional and organic pesticides. Organic pesticides in general seem to be equally toxic to non-target taxa as conventional pesticides, suggesting the need to be careful in their use. Our results indicate that changing pesticide use may harm non-target species because their populations continuously need to adapt to novel pesticides. One needs to be even more careful when considering that our study species, *D. magna*, is a quite robust species with large population sizes – ecological specialists with typically small population sizes may be more strongly affected, and might be driven to extinction by the use of both conventional as well as organic pesticides.

5. DISSEMINATION AND VALORISATION

All partners actively disseminated project results at several national and international scientific meetings and symposia. Contributions from master students and PhD students were especially encouraged. During the project, we also interacted and disseminated project results with several stakeholders, including representatives of the main agricultural (conventional and organic) organizations, both governmental and non-governmental. Multiple stakeholders, including interested private pond owners, attended the annual stakeholder meetings that have been organized by ORCA. The interaction with agricultural organisations was important to establish contacts with pond owners at the start of the project and allowed us to optimize the final pond selection. In addition, we informed all pond owners individually on the ecological status of their pond using a standard data sheet that summarises the key ecological characteristics of individual ponds (see annex Figure. A2 as example).

A selected list of the scientific dissemination activities that have been undertaken as part of ORCA (in some non-listed talks data of ORCA were included as part of a larger overview on pond biodiversity or land use change effects):

- November 2017, Poster presentation by Cours M. on **Benelux Zoology Congress**. Cours et al., A comparative analysis of organic and conventional agriculture's impact on aquatic biodiversity. Wageningen, The Netherlands
- March 2018, Active participation with lecture of Lemmens Pieter on a **workshop on Agrobiodiversity** on a regional meeting on biodiversity conservation. Mechelen, Belgium (in Dutch).
- March 2018, Oral presentation of S. Delmoitié at regional symposium '**Starters in Natuuronderzoek'**. Een vergelijkende studie naar de impact van biologische en conventionele landbouw op aquatische biodiversiteit. Brussels, Belgium (in Dutch)
- March 2018, Poster presentation of M. Meurisse at regional symposium '**Starters in Natuuronderzoek**'. Integrating taxonomic and functional diversity in community ecology. Brussels, Belgium (in Dutch)
- May 2018, Oral presentation of Lemmens P. on the 8th **European Pond Conservation Network symposium**. Integrated management supports effective and sustainable biodiversity conservation in large pond complexes. Torroella de Montgri, Spain.
- May 2018, Oral presentation of Almeida R.A.. on the 8th **European Pond Conservation Network symposium**. A comparison of the effects of organic and conventional agriculture on biodiversity and the functioning of small aquatic systems. Torroella de Montgri, Spain.
- May 2019, poster presentation of Almeida R.A. on the **SETAC Europe 29th Annual Meeting**. Local genetic adaptation to pesticide use in organic and conventional agriculture
- January 2020, Oral presentation of Almeida R.A. on the **EVENET 10th Annual Symposium** on Eco-evolutionary Dynamics. Local genetic adaptation to pesticide use in organic and conventional agriculture
- Upcoming May 2021. Oral presentation Almeida R.A. on the **EPCN 2021** Virtual meeting. Genetic adaptation to pesticide use in organic and conventional agriculture in an aquatic non-target species
- Upcoming June 2021, Oral presentation by Almeida R.A. on the **ASLO 2021** Virtual Meeting, Response of zooplankton community structure to pesticide application
- Upcoming July 2021, Oral presentation by Almeida R.A. on the **SEFS 12** Symposium for European freshwater sciences. Differential effect of organic and conventional agriculture on pond biodiversity

6. PUBLICATIONS

There are two publication related to the data management activities within ORCA.

1) De Wever, Aaike; Cours, Marie; Milotic, Tanja; Lemmens, Pieter; Adriaens, Dries; Denys, Luc; Martens; Koen & De Meester; Luc (2019): A comparative analysis of ORganic and Conventional Agriculture's impact on aquatic biodiversity (ORCA; BR/175/A1/ORCA) Data Management Plan – Initial version. figshare. Journal contribution. https://doi.org/10.6084/m9.figshare.7546694.v2
2) Cours, M., Lemmens, P., Almeida, R., Brys, R., Denys, L., De Wever, A., Knockaert, M., Leyssen, A., Mergeay, J., Packet, J., Parmentier, K., Schön, I., Venderickx, J., Vercauteren, T., Adriaens, D., Martens, K. & De Meester, L. Metadata description of the ORCA database (ORganic and Conventional Agriculture's impact on aquatic biodiversity). Freshwater Metadata Journal 0: 0-0

At this stage, there are no published scientific peer-review publications yet, but several (see list below) manuscripts are currently being finalized and close to submission.

- The differential effect of organic and conventional agriculture on aquatic biodiversity
- Local genetic adaptation to pesticide use in organic and conventional agriculture in an aquatic no-target species
- The response of zooplankton communities to pesticide exposure
- The response of D. magna to shifts in pesticide application.
- The effect of eutrophication on zooplankton taxonomic and trait diversity.
- The relation between taxonomic and trait diversity of multiple aquatic organism groups in relation to land use and spatial scale.

7. ACKNOWLEDGEMENTS

List of Monitoring Committee members

- Leen Franchois, Boerenbond, Studiedienst, Adviseur water-, natuur- en plattelandsbeleid
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ANNEXES

Boxplots and separate T-test testing for differences in major local environmental variables between ponds located in conventional and organic agriculture.

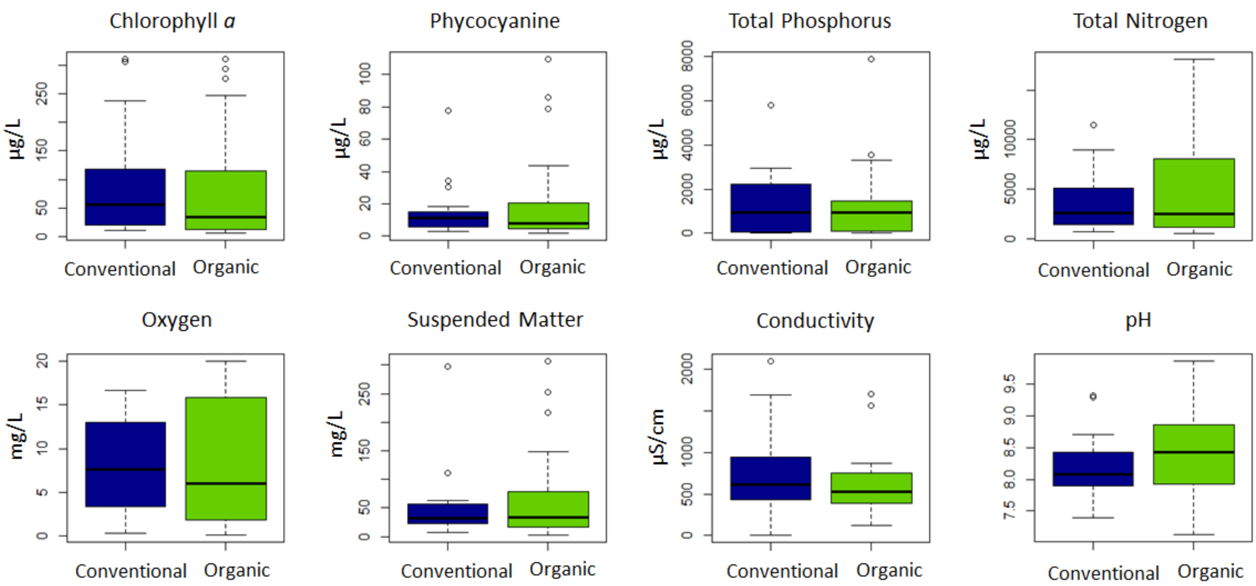

Figure A1 – Boxplots of chlorophyll *a*, phycocyanine, total phosphorus, total nitrogen, dissolved O_2, pH, conductivity and suspended matter) for pond located in conventional and organic agriculture.

Table A1 – Results of t-test on the effect of agriculture type on environmental variables

	t	df	p-value
Chlorophyll a	-0.189	45.651	0.851
Phycocyanine	0.863	40.705	0.393
Total Phosphorus	-0.121	45.943	0.904
Total Nitrogen	0.506	44.458	0.615
Dissolved O_2	0.004	45.395	0.997
pH	1.367	45.135	0.179
Conductivity	-0.969	34.911	0.339
Suspended matter	0.9	45.512	0.373

Table A2. Pearson correlation between the proportion of different land-use types at different spatial perimeters and different measures of diversity metrics ($P < 0.05$).

	Richne	Shannon	$LCBD_{ruz}$
urbanized 1000	0.26	0.18	-
urbanized 3000	0.37	0.30	-
infrastructure 1000	0.24	0.17	-
infrastructure 3000	0.29	0.25	-
cropland 100	-0.33	-0.30	0.20
cropland 1000	-0.3	-0.28	0.20
cropland 3000	-0.38	-0.35	0.23
extensive grassland 100	0.23	0.22	-
extensive grassland 1000	0.31	0.25	-
water 1000	0.29	0.23	-
forest 1000	-	-	-0.24
forest 3000	0.17	-	-0.28
nature 100	0.21	0.18	-
nature 1000	0.23	0.23	-
cropland+intensive grassland 100	-0.29	-0.25	-
cropland+intensive grassland 1000	-0.38	-0.30	-
cropland+intensive grassland 3000	-0.41	-0.36	-
land-use intensity 100	-0.34	-0.32	-
land-use intensity 1000	-0.23	-0.22	0.22
land-use intensity 3000	-	-	0.26

Table A3. The loadings of the environmental variables for the different PCA axes.

Environmental variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
pH	0.25	-0.51	0.56	-0.10	0.18	0.41	0.39	-0.11
Transparency	-0.48	-0.25	0.03	0.14	0.17	-0.20	0.30	0.73
Chlorophyll a	0.45	-0.30	-0.05	0.04	-0.05	0.18	-0.64	0.51
Total Nitrogen	0.29	-0.20	-0.49	-0.46	0.57	-0.28	0.15	-0.04
Total Phosphorus	0.38	0.37	0.02	-0.41	-0.46	0.02	0.43	0.40
Submerged Vegetation	-0.29	-0.03	0.49	-0.66	-0.03	-0.32	-0.37	-0.02
Emergent Vegetation	-0.22	-0.60	-0.39	-0.19	-0.60	0.04	0.06	-0.17
Floating Vegetation	-0.38	0.22	-0.24	-0.34	0.19	0.76	-0.09	0.10

Figure A2. Example of feed-back form to report data to pond owner and local stakeholders.

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KU LEUVEN

Terugkoppeling KU Leuven/KBIN/INBO aquatisch veldonderzoek

Wij danken u voor uw toestemming om uw poel/vijver te onderzoeken binnen het kader van ons wetenschappelijk project. Wij koppelen graag de resultaten van de opgemeten variabelen van uw poel/vijver naar u terug via dit document.

Achtergrond van het onderzoeksproject

De veldstudie waarin uw vijver werd opgenomen kadert in het "onderzoeksproject ORCA", dat gesubsidieerd wordt door het Belgisch Wetenschapsbeleid (Belspo). Binnen dit project onderzoeken wij poelen in landbouwgebied binnen Vlaanderen. Een Nederlandstalig artikel met achtergrond rond de ecologie en het beheer van ondiepe vijvers vindt u in Natuur.Focus 5, 2006 (https://www.natuurpunt.be/sites/default/files/documents/publication/natuur.focus_2006-1_ondiepe_vijvers_en_meren.pdf).

Specificaties van uw vijver/poel

Vijvercode: BW_07411
Adres: [redacted]
Contactpersoon: [redacted]

Opgemeten variabelen en meetwaarden

In onderstaande tabel vindt u een overzicht van de opgemeten variabelen met de meeteenheid en meetwaarde voor uw vijver. De voorlaatste kolom (Mediaan) geeft de mediaan voor de specifieke variabele berekend over alle vijvers van deze veldstudie. De laatste kolom (Min-max) geeft de minimum- en maximumwaarden opgemeten binnen deze veldstudie. In het kader van deze veldstudie werden 48 vijvers bemonsterd.

Opgemeten variabele	Eenheid	Meetwaarde	Mediaan	Min-max
Abiotische variabelen				
Geografische coördinaat: Breedtegraad		51.421849		
Geografische coördinaat: Lengtegraad		4.602937		
Diepte van sedimentlaag	cm	24	29	1-100
Watertransparantie (Sneller's diepte)	cm	5	22	3-44
Conductiviteit	µS/cm	117	557	6-2098
pH	log	8.12	8.27	7.13-9.87
Temperatuur	°C	17.6	20.9	15.9-26.9
Zuurstofgehalte	mg/L	0.22	6.99	0.14-18.71
Zwevende stof	mg/L	216.2	32.40	3.00-306.60
Alkaliniteit	mg/L	208.35	403.95	208.35-727.48
Sulfaten	mg/L	5.53	40.56	5.53-294.52
Opgeloste organische koolstof	mg/L	33.1	21.39	6.05-52.08
Totaal stikstofgehalte (anorganisch	µmol/L	573.81		

Terugkoppeling aquatisch veldonderzoek 1/2

Opgemeten variabele	Eenheid	Meetwaarde	Mediaan	Min-max
+ organisch)			181.92	32.13-1292.45
Totaal fosforgehalte (anorganisch + organisch)	µmol/L	114.82	30.08	0.58-254.89
Nitraat - Nitriet (NOx)	µmol/L	7.22	2.10	0.10-361.12
Nitriet	µmol/L	0.95	0.47	0.07-8.91
Ammonium (NH4)	µmol/L	4.79	3.92	0.85-942.19
Fosfaten (PO4-)	µmol/L	25.63	19.43	0.00-306.87
Hardheid	°fH	-0.2	14.65	-12.5-134.4
Chloriden	ppm	25.54	57.03	24.12-3072.53
Silicaat (Si)	µmol/L	14.81	94.72	1.59-1009.02
Maximale diepte	cm	40	62	11-175
Biotische variabelen				
Concentratie Chlorofyl-a	RFU	8030	937.42	62.86-8040.33
Phycocyanine	RFU	78.96	8.43	1.99-109.43
Biodiversiteit (berekend als totaal aantal soorten)				
Waterplanten (Macrofyten)	Aantal soorten	28	26.5	14-51
Zooplankton (Cladoceren en copepoden)	Aantal soorten	7	6	2-12
Slakken (Gastropoda)	Aantal soorten	1	1	0-8
Kevers (Coleoptera)	Aantal soorten	14	5	0-18
Wantsen (Heteroptera)	Aantal soorten	11	5	0-13
Vissen	Aantal soorten	0	0	0-12
Amfibieën	Aantal soorten	2	3	0-5

Bevindingen

Onze metingen van het water van uw poel/vijver vallen binnen de normale waarden.

Contact

Heeft u nog vragen? Neem dan gerust contact op met Marie Cours via mail (mcours@naturalsciences.be).

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