Rapport de stage individuel

5ème année

Spatio-temporal dynamics of biodiversity and greenhouse gas emissions in fish ponds

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Table of contents

Introduction

This is the final report of my internship at the Laboratory of Aquatic Ecology, Evolution and Conservation (LAEEC) of the Department of Biology at KU Leuven. The internship took place from March 14th to August 22nd 2022 and is part of my third and last year degree in Freshwater Ecology, Rivers and Wetlands engineering. My research stay at KU Leuven created an unique opportunity to build further on the experiences from my earlier internship at IGB (Leibniz Institute of Freshwater Ecology and Inland Fisheries, March 17th - August 27th 2021) as I joined the team at KU Leuven to support the Belgian team involved in the EU project PONDERFUL.

The overarching aim of PONDERFUL is to develop improved methods for maximising the use of ponds and pondscapes in climate change adaptation and mitigation, biodversity conservation and the provisioning of ecosystem services. The research in PONDERFUL will increase our understanding of the ways in which ponds, as a Nature Based Solution, can help society to mitigate and adapt to climate change, conserve biodiversity and deliver ecosystem services. The project is funded by the European Union and brings together experience from 18 different teams (including KU Leuven) representing nine European states, Turkey and Uruguay.

In January 2022, I contacted Pieter Lemmens who is holding a Post Doctoral researcher position both at IGB and KU Leuven and who I had worked with during my previous internship at IGB. He had indicated me at that time that I was more than welcome to join the Belgian PONDERFUL Team for my final internship. He had also indicated me that a sampling campaign on fish ponds was planned for the coming summer and that we could imagine an internship topic with a special focus on this type of pond system. I enthusiastically took this great opportunity and was part of his team for 5 months and a half.

In contrast to my internship at IGB that largely focussed on the ecological assessement of kettle holes in NE-Germany, my current research visit at KU Leuven mainly targeted the quantification of GHG emission and biodiversity in fish ponds in Belgium. I was involved in several tasks related to the PONDERFUL project, including field work and processing of samples and data. I did not only work with fish ponds, but also contributed to the field surveys of farmland ponds and demo-sites. My work and experiences enhanced my knowlegde on freshwater ecology in general and enabled me to become highly experienced with the multiple practical aspects related to the collection of field data. I also had the opportunity to contribute to the SIL 2022 conference in Berlin with a poster presentation.

This report aims to provide a comprehensive overview of my activities at the Laboratory of Aquatic Ecology, Evolution and Conservation and explains how these relate to the PONDERFUL project. I will present some key findings that are based on my work on fish ponds, and highlight the conclusion of the effect of buffer strips on small lentic waterbodies in Belgium and Germany. The latter builds further on my activities at IGB and was developed in close collabaration with the PhD student Louisa-Marie (KU Leuven/IGB). The document comprises 5 sections. First, I will provide a brief presentation of the Laboratory of Aquatic Ecology, Evolution and Conservation and the Belgian team involved in PONDERFUL. Next, I will provide more information on the PONDERFUL project itself, to subsequently give a detailed overview of my activities and tasks during my internship. In the third section, I will provide an overview of the overall progress of my mission. Then, a large part of this report will be dedicated to the topic of my internship itself i.e. the « spatio-temporal dynamics of biodiversity and green house gas emissions in fish ponds ». Finally, I will shortly present a deliverable that I conducted in continuation of my work at IGB and which was used for making a poster that was presented at the SIL conference in Berlin.

1) The Laboratory of Aquatic Ecology, Evolution and Conservation

1.1) Presentation of the Laboratory of Aquatic Ecology, Evolution and Conservation

The Laboratory of Aquatic Ecology, Evolution and Conservation (LAEEC) is a research group led by Professor Luc De Meester. It is part of the Ecology, Evolution and Biodiversity Conservation Section of the Department of Biology at KU Leuven.

The research group focusses on a wide range of aquatic ecological topics (Figure 1).

What ?

- Analyzing the **structure** and **functioning** of **lakes** and **ponds**
- Study of **biodiversity** and **species composition** in relation to environmental gradients and human impact
- Study of **micro-evolutionary dynamics** in response to environmental gradients (mainly using the waterflea *Daphnia* as a model species)
- Study of **trait evolution** + linking trait responses to population structure and signatures of evolution at the genome level
- Explore **eco-evolutionary dynamics** : how evolution may impact community dynamics at a local and regional scale. With a main focus on strong environmental gradients, including human impact (climate change, pollution)
- Microbial ecological research : how **metacommunity ecology** and eco-evolutionary dynamics may increase our insight into the structure and dynamics of natural microbial communities

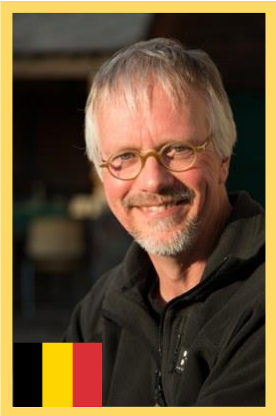
How ?

- Model organisms : ***Daphnia*** and zooplankton (but some studies of the Lab cover the whole range of organisms inhabiting ponds and lakes, from bacteria to fish)
- Field observations and field experiments with large-scale outdoor container experiments, laboratory experiments, resurrection ecology and ecological genomics

Figure 1 : List of the aquatic ecological research topics Luc De Meester Lab is dealing with *(Source : author)*

1.2) Presentation of the Belgian PONDERFUL Team

The Belgian-Ponderful team is highly international and includes people with highly complementary expertises to ensure the success of the project (Figure 2). I was strongly involved in all aspects related to the detailed sampling of more than 105 ponds located in different pondscapes all over Flanders.



Luc De Meester (Professor)

- Head of the Laboratory of Aquatic Ecology, Evolution and Conservation and IGB Director
- Head of PONDERFUL in Belgium

Pieter Lemmens (Post Doctoral researcher at LAEEC and IGB)

- Main investigator for PONDERFUL
- Supervisor of the sampling in Belgium and Germany
- Author of the PONDERFUL sampling protocol used in the 11 countries
- Coordinates the data management, publication and engagement activities

« Core sampling team »

Robby Wijns (PhD student, 1st year)

- Sampled all the different types of pond systems selected for PONDERFUL this year (more than 105 ponds)
- Scheduled and organized the sampling campaign in Belgium for this year
- Supervision of master students in the field
- Zooplankton expertise

Louis-Marie Le Fer (Intern)

- Sampled all the different types of pond systems selected for PONDERFUL this year (more than 105 ponds)
- Supervision of master students in the field
- Driver in the field
- GIS tasks : assessment of the size of the buffer strips around the ponds

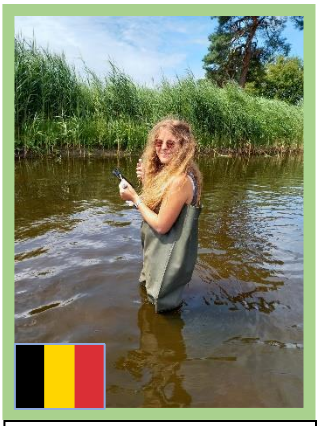
Figure 2 : Organigram visualizing the organisational structure of the Belgian PONDERFUL Team *(Source : author)*

Robby and I had the supervision of four Master students who joined us at different stages in the sampling campaign and made our sampling team highly international (Figure 3).

Haroun Jameel

(Master student)

- University College London (UCL)
- Joined 1 week to sample crater bombs in Tommelen



Ditte Rens

(Master student)

- KU Leuven
- Joined 3 weeks for the GHG and biodiversity assessment of fish ponds

Carlijn Koole

(Master student)

- KU Leuven
- Joined 1 week for the GHG and biodiversity assessment of fish ponds

Péter Imre Fábián

(Master student)

- KU Leuven (Erasmus)
- Joined fieldwork for 3 weeks as part of his internship

Collaborators

Maxime Fajgenblat (PhD student, LAEEC, Data Science Institute (DSI))

- Expertise in statistics
- Contribution to various data analysis related to PONDERFUL

Louisa-Marie von Plüskow (PhD student, IGB Berlin, LAEEC)

- Organized the sampling in Germany for this year
- Contact person when exchanging with the German Team

Figure 3 : Presentation of the four master students we had the supervision of and two main collaborators *(Source : author)*

2) Presentation of my mission as a member of the Belgian PONDERFUL Team

2.1) The PONDERFUL project

The overarching aim of the Horizon 2020 project « PONDERFUL » is to develop improved methods for maximising the use of ponds and pondscapes in climate change adaptation and mitigation, biodiversity conservation and the delivery of ecosystem services. Because of their small size, the significance of ponds has long been underestimated. However, research over the last 10-15 years has shown that, because of their abundance, heterogeneity, biodiversity, naturalness and biogeochemical potency, ponds play a role in catchments, landscapes, and potentially at continental scale which is completely out of proportion to their small size.

The main aims of the research in PONDERFUL is to increase our understanding of the ways in which ponds, as a Nature-Based Solution (NBS), can help society to mitigate and adapt to climate change, protect biodiversity and deliver ecosystem services. The project started in December 2020, and runs for 4 years. The collection of the data through a sampling campaign takes place over 2 years (the project is currently in its second year). The last two years will be dedicated to data analysis, communication of the results and implementation of actions.

The project has five main components :

1) Developing a strategic approach to engagement with stakeholders, to ensure that they are able to effectively implement the benefits of ponds as Nature-Based Solutions

2) Through the generation of extensive new biodiversity and ecosystem services datasets, to better establish the relationship between pond biodiversity and the delivery of ecosystem services

3) Establish models that enable us to test and optimise practical scenarios for the use of ponds and Nature-Based Solutions

4) Create a set of demonstration sites (« demo-sites ») across Europe which show to practitioners and policy makers how ponds can help to mitigate and adapt to the effects of climate change

5) Ensure that the project’s outputs are widely known to policy makes, practitioners and other stakeholders.

The project brings together experienced researchers from nine European states and from Turkey and Uruguay. It is led by the team of the University of Vic (Spain) and in total involves 18 research teams, including KU Leuven (Table 1). The project is built around 6 Work Packages (WPs). The KU Leuven team is involved in all WPs and is coordinator of WP2 (Figure 4).

Table 1 : Table presenting the participants involved in PONDERFUL *(Source : author)*

Country	Participants
Spain	University of Vic
	University of Girona
	Randbee Consultants
Germany	IGB
	Ecologic Institute
	Technical University of Munich
Belgium	**KU Leuven**
Switzerland	Haute Ecole Spécialisée de Suisse Occidentale
UK	University College London
	Bangor University
	Freshwater Habitats Trust
Turkey	Middle East Technical University
Portugal	Interdisciplinary Centre of Marine and Environmental Research
Denmark	Aarhus University
	Amphi International APS
Sweden	Uppsala University
France	Institut Supérieur d'Agriculture Rhône Alpes
Uruguay	Universidad de la Republica Uruguay
Europe	European Pond Conservation Network

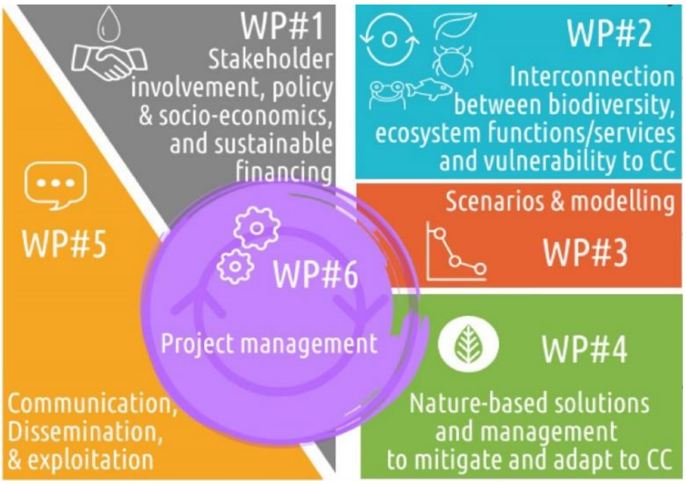


Figure 4 : The 6 Work Packages (WPs) PONDERFUL is built around, in blue the WP2 my internship was largely related to *(Source : ponderful)*

2.2) WP2/WP4 : the framework of my mission

The activities of my internship were largely related to WP2 but I also contributed to the collection of data that will be used in WP4.

The overall objective of WP2 is to determine how biodiversity, ecosystem state and processes and ecosystem services co-vary and interact in ponds and pondscapes across a broad climatic gradient and along landuse gradient. PONDERFUL specifically aims to determine how climate change may alter the interrelationships between biodiversity, ecosystem functions and services. The overall objective of WP4 is to develop efficient and effective Nature Based Solutions (NBS) for climate change adaptation and mitigation through pondscape management as well as tools and guidance for their implementation.

For this purpose, the PONDERFUL consortium is conducting a highly standardized stratified survey for biodiversity and ecosystem services in 30 ponds in eight countries (BE, CH, DK, GE, SP, TR, UK and UY). In each country, ponds were selected along a land use gradient including 3 different types : nature (NA), extensive agriculture (EX), intensive agriculture (IN).

In Belgium, the 30 ponds were selected in 5 different regions in Flanders (Wetteren, Antwerp, Flemish Brabant, Limburg 1, Limburg 2). The number of ponds in each cluster is the same i.e. 6 ponds including 2 ponds of each type of landuse (Figure 5).

The selected set of ponds also includes ponds that are permanent, semi-permanent and temporal.

In all 8 countries (Belgium included), the 30 selected ponds for the stratified survey were sampled last year following a highly standardized protocol.

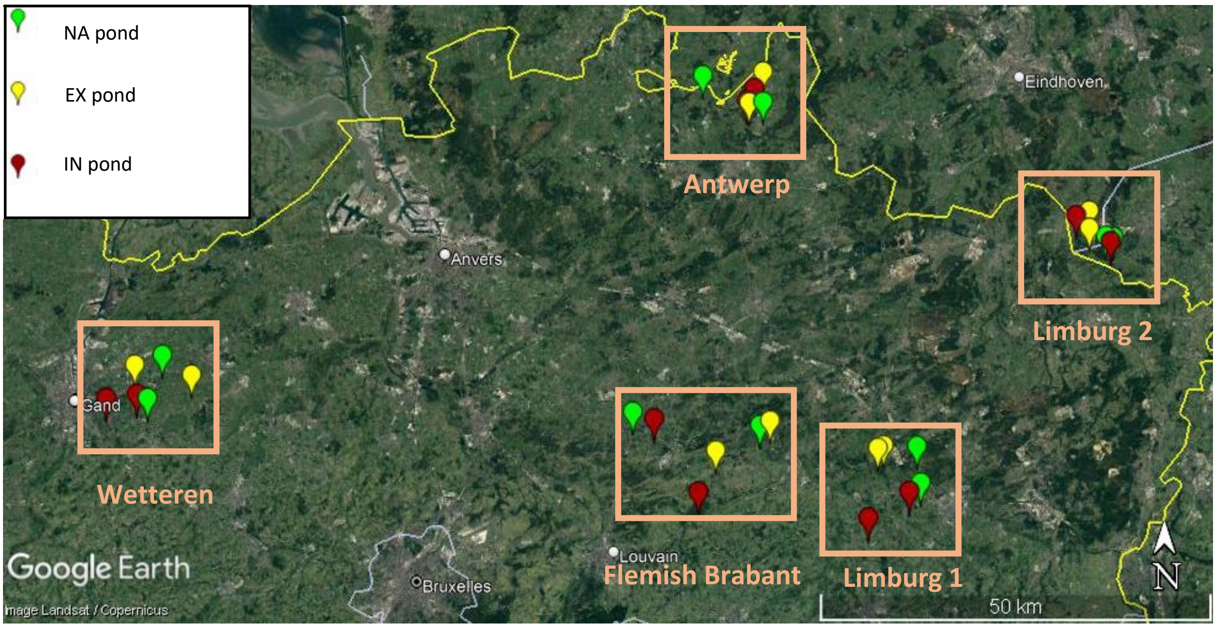


Figure 5 : Locations of the 30 ponds located in 5 different regions in Flanders that have been sampled in 2021 as part of the stratified survey *(Source : author, created with Google Earth)*

For the 2nd year of the project, the practical work involved the collection of data in various types of pond systems (Figure 6). More specifically, it included :

- The resampling of 12 farmland ponds from the stratified survey to assess temporal dynamics in ecological state and GHG emission along a gradient of land use intensity. From the 30 ponds that have been investigated in 2021 as part of the stratified survey, a set of 12 ponds located in two different clusters (Flemish Brabant and Limburg 2) has been selected to assess major local environmental pond conditions, biodiversity and GHG emission. The collected data allow to assess variation between years in relation to land use.

- The sampling of ponds in 3 DEMO-pondscapes

  This activity is part of WP4 which aims to create a network of demonstration (DEMO) sites across 7 European countries (BE, DK, GE, SP, SW, TR, UK) and 1 CELAC country (UY) representing three bioclimatic regions in northern and southern hemisphere, where scenarios of pondscape management can be assessed.

  The Belgian team is conducting research on ponds located in 3 DEMO-pondscapes :

  - Gete Vallei : 19 ponds (selected along a gradient of pond age)
  - Pikhakendonk : 9 ponds (selected along a gradient of pond age)
  - Tommelen : 22 ponds (crater ponds)

  In these DEMO-pondscapes, benefits of pond creation, restoration and management for biodiversity and ecosystem services, as well as for climate change adaptation and mitigation will be assessed and PONDERFUL ideas will be implemented/tested. Sampling ponds along gradients of pond age allows to investigate how pond age affects local environmental pond conditions, biodiversity and community composition, and GHG emission.

- The sampling of 21 fish ponds located in Midden-Limburg

  The investigation of fish ponds in the fish pond system Midden Limburg (NE of Flanders) is part of WP2 and forms a specific case study to assess how management affects the ecological state and GHG by ponds. By sampling the same set in 2021 and 2022, the data will also be used to assess temporal dynamics in relation to fish pond management. A similar study is done by the French PONDERFUL team (ISARA-Lyon) on fish ponds located in the Dombes region.

- The monitoring of 26 newly created ponds in the region of Voeren (at the border between Flanders, Wallonia and Netherlands)

  This pondscape consists of newly created manmade ponds (not older than 1 year) on former agricultural lands. By monthly monitoring the zooplankton community characteristics in this set of newly created ponds, we aim to investigate the short term colonisation dynamics by zooplankon in relation to habitat connectivity and the characteristics of the regional species pool.

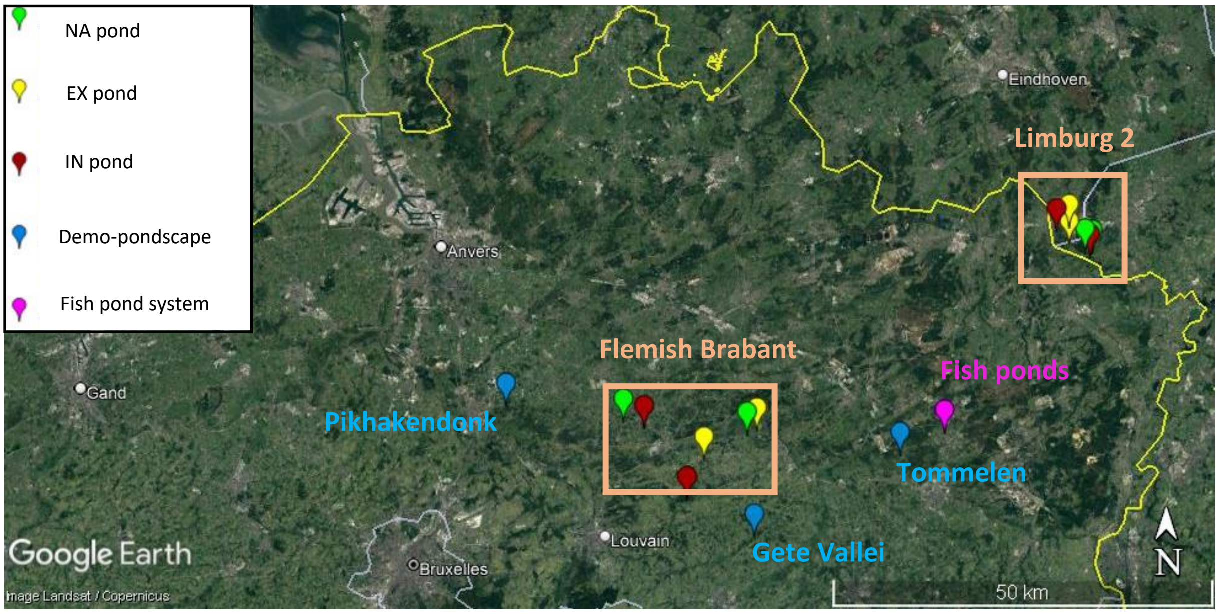


Figure 6 : Locations of the different pond systems we investigated this year in Flanders (Source : author, created with Google Earth)

3) Presentation of the progress of my mission

As indicated above, my internship activities largely contributed to WP2. More specifically, I was intensively involved in various tasks related to the field work. In addition, I carried out further investigations on the spatio-temporal dynamics of biodiversity and greenhouse gas emissions in the fish ponds of Midden-Limburg using the data collected last year. Futhermore, I was able to build forward on the work I had already started in Germany and I quantified the size of the buffer strips around the set of Belgian ponds so that we could investigate the effect of buffer strip size on the ecological state of small ponds in agricultural areas in Belgium and Germany. This resulted in a poster contribution to the SIL 2022 conference in Berlin.

The field work included the sampling of different organism groups (zooplankton, macro-invertebrates, amphibians, fish and macrophytes) and major local environmental pond variables. In addition, we collected samples to quantify GHG emissions, C-sequestration, and decomposition of organic material by the investigated ponds.

The fieldwork was conducted in a specific chronological order (Figure 7). We started beginning of April with the sampling for amphibians and fish by eDNA for the 3 DEMO-pondscapes and subsequently started beginning of May with the first round (Spring) of quantification of gas emissions by the 12 ponds of the stratified survey. Then, mid-May, we conducted the main sampling campaign that involved several organism groups and local environmental pond variables on those same 12 ponds. Throughout the month of June, we sampled the 3 DEMO-pondscapes. Then, the entire month of July was dedicated to the collection of field data in the fish pond system in Midden-Limburg. Finally, mid-August, we carried out the second round (Summer) of quantification of gas emissions by the 12 ponds of the stratified survey.

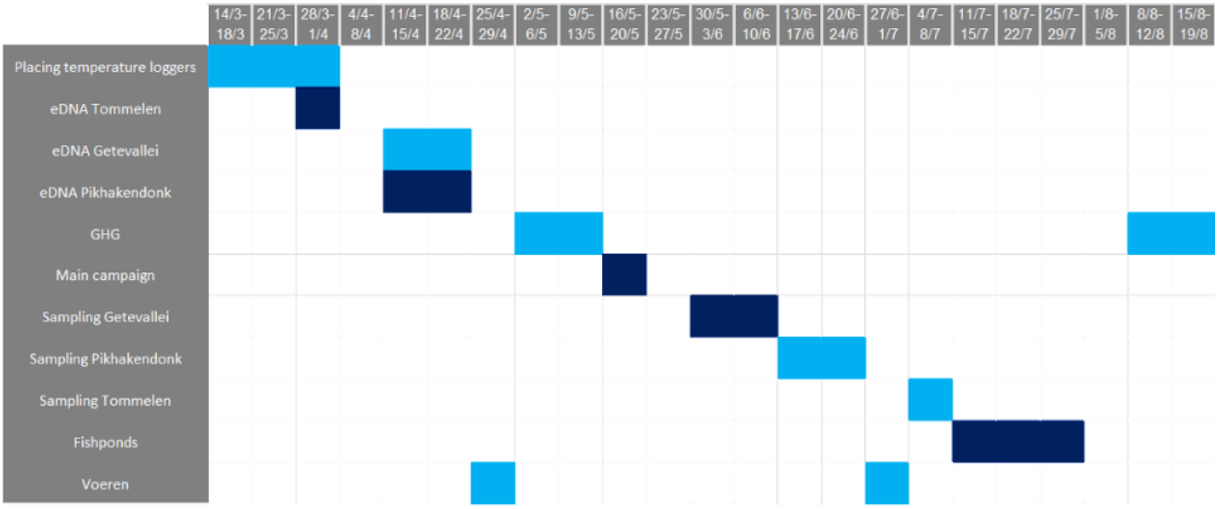


Figure 7 : Gantt chart illustrating the progress of my mission *(Source : Robby Wijns)*

The sampling methods that were used follow a highly standardized protocol. However, the selection of major local environmental pond variables and organism groups to be sampled slightly differed according to the type of pond system. In the table below, I will provide more details on the pond variables and organism groups we sampled for each type of pond system (Table 2).

Table 2 : Table providing a detailed overview of the local environmental variables and the communities that were sampled for each type of pond system included in PONDERFUL *(Source : author)*

Type of pond system	Major local environmental pond variables measured	Organism groups sampled	C-cycle quantification
12 farmland ponds among the 30 selected for the stratified survey and distributed between 2	• Chlorophyll-a (CHLa) /phycocyanine (PC) • pH, water T°, oxygen, conductivity (along a depth profile) • Water column stratification	• Zooplankton : cladocerans and rotifers (quantitative and qualitative sample for genetic analysis)	• C-sequestration (sediment traps) (Figure 8 c) • Decomposition of organic material (Tea Bag Index) • GHG (CO_2, CH_4) emissions (GHG

regions : Flemish Brabant and Limburg 2	(T°loggers at top and bottom) • Total Nitrogen (TN), Total Phosphorus (TP), Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), alkalinity • Total Suspended Solid (TSS) • Water transparency (sneller) • Water depth (different locations) • Thickness of the silt layer • Background information (pond management, surrounding landuse, information on the flora of the immediate surroundings of the pond and of its shoreline	• Macro-invertebrates • Macrophytes • Quantification of fish and amphibian community composition by eDNA was performed last year • Additional community : Phytoplankton (not included in PONDERFUL protocol)	chambers) (Figure 8 d) • Pond metabolism (not for all the 12 ponds) : Gross Primary Production (GPP) and Ecosystem Respiration (ER) are quantified based on a combination of CO_2 concentration (measured with the GHG chambers) and diurnal variation in dissolved oxygen concentration (O_2 logger)
3 DEMO-pondscapes : Getevallei, Pikhakendonk, Tommelen	• CHLa/PC • pH, water T°, oxygen, conductivity (depth profile only for Getevallei and Pikhakendonk) • Water column stratification (T°loggers at top and bottom) (only for Getevallei and Pikhakendonk) • TN, TP • TSS • Sneller • Max depth • Thickness of the silt layer • Assessment of vegetation coverage : submerse, floating, emerse	• Zooplankton (all ponds) : quantitative and qualitative sample • Macrophytes (all ponds) • Macro-invertebrates (only for Tommelen) (Figure 8 a) • Fish and amphibians by eDNA (all ponds in Getevallei and Pikhakendonk + 2 ponds in Tommelen) (Figure 8 b)	• C-sequestration (sediment traps) • Decomposition of organic material (Tea Bag Index) • GHG (CO_2, CH_4) emissions (GHG chambers) • GHG on wet sediments (only on 3 ponds in Pikhakendonk that had dried out) ➔ Only for Getevallei and Pikhakendonk

21 fish ponds in Midden-Limburg (I will provide more details on the sampling methods that were used below)	• CHLa/PC • pH, water T°, oxygen, conductivity along a depth profile • Water column stratification (T°loggers at top and bottom) • TN, TP, TOC, DOC, alkalinity • TSS • Sneller • Max depth • Thickness of the silt layer • Assessment of vegetation coverage : submerse, floating, emerse	• Zooplankton (quantitative sample) • Macro-invertebrates • Macrophytes	• GHG (CO_2, CH_4) emissions (GHG chambers)
26 ponds in Voeren	• CHLa/PC • pH, water T°, oxygen, conductivity • TN, TP • Sneller • Max depth	• Zooplankton (quantitative sample) • Macro-invertebrates	

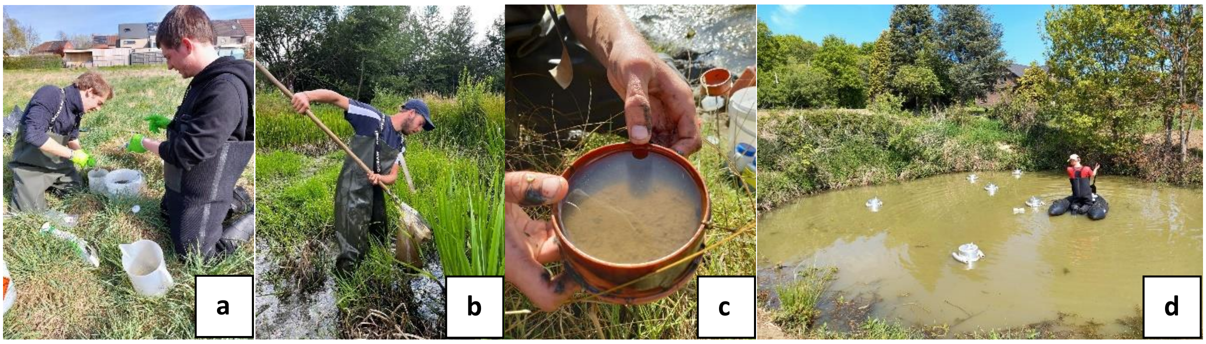


*Figure 8 : **a)** eDNA sampling **b)** macro-invertebrate sampling **c)** collection of a sediment trap **d)** installation of GHG chambers (Source : Haroun Jameel, Louis-Marie Le Fer, Robby Wijns)*

4) Spatio-temporal dynamics of biodiversity and greenhouse gas emissions in fish ponds

4.1) Fish ponds

4.1.1) Purposes of fish ponds

Fish ponds represent a specific type of anthropogenic ponds as they have been created for the cultivation of fish. Large fish pond systems can be found in several European countries, including Belgium, France, Germany, Austria, Hungary and the Czech Republic (Figure 9).

Figure 9 : Aerial picture from our study area : the « Vijvergebied Midden-Limburg » fish pond complex located in North-East Belgium

Although fish ponds are essentially artificial ecosystems, they have become important habitats for biodiversity conservation in human dominated landscapes. Indeed, several studies have clearly shown that fish pond systems can be important surrogate habitats for species that have lost their natural habitats, including several threatened species such as Eurasian Bittern (*Botaurus stellarus*), Little Bittern (*Ixobrychus minutus*), tree frog (*Hyla arborea*) and the European Beaver (*Castor fiber*).

In addition to their socio-economic and ecological value, fish ponds provide numerous other ecosystem services. For example, they provide a regulating service due to their involvement in the carbon cycle. Indeed, several studies have indicated that such water bodies act as GHG sinks as they have a significant contribution to the sequestration of carbon and consequently to climate change mitigation and regulation.

4.1.2) Fish pond management and biodiversity

More recently, there has been a growing awareness of the importance of fish ponds for biodiversity conservation. To obtain high value biodiverse fish ponds, two main management strategies can be implemented (Figure 10).

An important strategy is the draining of ponds. When ponds are drained, a reduction of nutrients can be observed. Ponds are usually drained after the fish are harvested and are kept dry for a certain period of time. These dry soils of the pond beds are ploughed and then used for the plantation of corn or other crops. The growth of crops will result in a decomposition of the organic matter and a removal of the nutrients from the soil (Wezel et al., 2013). This drought period has a positive influence on the growth of submerged macrophytes (Hough et al., 1991). The presence of dense macrophytes will on its turn support a clear-water state with high densities of zooplankton and other organism groups, while phytoplankton densities are kept low (Jeppesen et al., 1997; Scheffer et al., 1993).

Another strategy to promote a biodiverse clear-water state is via fish manipulation (Jeppesen et al., 1997; Scheffer et al., 1993; Søndergaard et al., 1997). In order to enhance the top-down control and predation pressure on phytoplankton, planktivorous fish can be removed or densities of predatory fish can be increased. Both of these strategies will lead to a system with higher zooplankton densities, that on its turn are able to control phytoplankton growth (Jeppesen et al., 1997; Scheffer et al., 1993; Søndergaard et al., 1997).

The dynamic management of pond drainage in combination with the management of the fish stocking density, has resulted in high nature value fish ponds. A study by Lemmens et al. (2013) showed a negative relationship between fish biomass and community biodiversity of several organism groups, including macrophytes and zooplankton. The lowest levels of local and total diversity were observed in the semi-intensive carp-farming ponds, while the no fish ponds showed a high local and total species richness. Thus, frequent draining in combination with low stocking densities seemed to support high local diversity.

However, high levels of biodiversity can only be maintained on the long term by conducting active pond management that is also based on low nutrient inputs (Declerck et al., 2011; Lemmens et al., 2013). Such management practices would basically be assimilated to extensive fish farming.

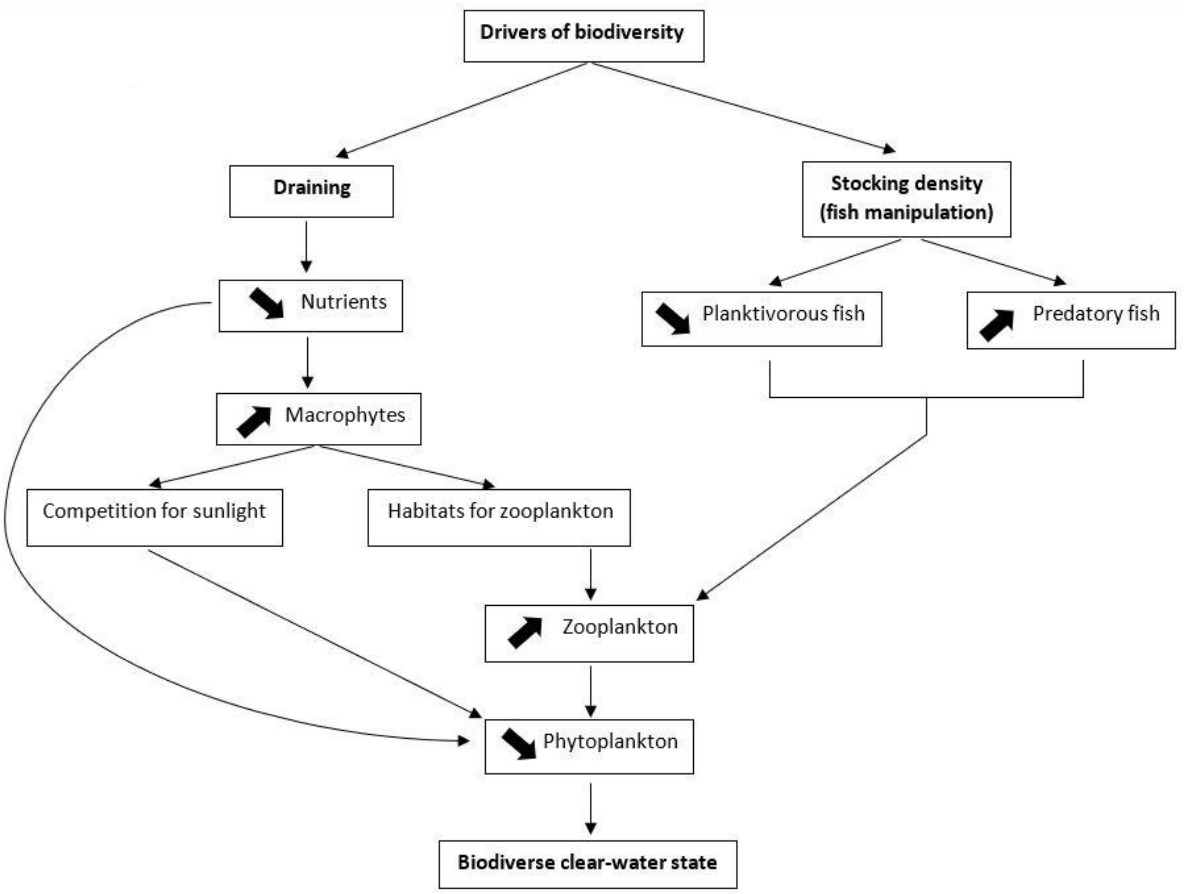


Figure 10 : Scheme of two management practices that can promote a biodiverse clear-water state in fish ponds *(Source: author).*

4.1.3) Fish pond management and greenhouse gas emissions

Management practices can also influence greenhouse gas fluxes in fish ponds.

On the one hand, it has been shown that the carbon storage capacity in the fish ponds depends on the implemented management type with productive fish ponds showing a high absorption capacity (Flickinger et al., 2020). Moreover, the extent to which carbon is stored in pond sediments also depends on the organism that are culture in the pond. For example, fish ponds with common carp (*Cyprinus carpio*) or tilapia were characterized by relatively high rates of carbon sequestration compared to ponds used for the culture of freshwater prawn (Adhikari, 2020).

Considering the capacity of fish ponds to sequester carbon, it is nevertheless important to realize that such waterbodies can also be a source of important greenhouse gas emissions. Indeed, several other studies clearly highlight that fish ponds can have a substantial contribution to greenhouse gas emission and that this is strongly related to management practices (Kosten et al., 2018; Rutegwa et al., 2019). The alternation between draining and rewetting, more in particular, results in higher GHG emissions than in permanently wet ponds.

While carbon sequestration can be seen as an « ecosystem service » of fish ponds, the emission of greenhouse gas is rather an ecosystem « disservice » and the type of implemented pond management can tilt the balance one way or the other.

4.1.4) Aims of the study

Fish ponds are highly dynamic ecosystems in which local environmental conditions and the characteristics of biotic communities can change considerably over time. Earlier studies on the ecology of fish ponds have highlighted the importance of pond management on food web structure and interactions, the ecological status of ponds, and the local and regional biodiversity of multiple aquatic organism groups. However, studies explicitly considering temporal dynamics in the ecological status of fish ponds under different management types remain scarce.

In addition, there is a growing awareness on the potential role of ponds on mitigating and adapting to climate change. With respect to this, insights on the degree of greenhouse gas emissions by fish ponds and understanding how these relate to pond management is highly relevant for the development of effective management programs that aim the conservation of high levels of biodiversity in a rapidly changing environment in the face of climate change.

The present study aims to fill such important knowledge gaps by investigating temporal biotic dynamics in fish ponds and by exploring the factors that drive the extent of greenhouse gas emissions by fish ponds, all this in relation to pond management.

More specifically, we aim to:

1) Investigate the repeatability of local and regional biodiversity patterns in zooplankton and macrophyte communities in relation to pond management.
   Repeatability in biodiversity patterns was investigated by comparing biodiversity patterns obtained from data collected during Summer 2021 on 21 ponds representing three management types (intensive fish farming, juvenile fish farming and ponds without fish) with findings from an earlier study of Lemmens et al. (2013) in the same region with the same fish pond management types, but based on a different set of ponds.

2) Quantify the degree of greenhouse gas emissions by fish ponds, identify the major factors driving greenhouse gas emission, and investigate how this relates to pond management. This was investigated by using the GHG data collected during Summer 2021 on the 21 selected ponds.

4.1.5) Hypotheses

Based on previous studies, the two following hypotheses can be made :

1) We expect to find similar local and regional biodiversity patterns as found in the study of Lemmens et al. (2013), indicating that these patterns can be specifically linked to the pond management type. More in specific, we expect to find the highest local and regional species richness in ponds without fish and the lowest species richness in intensive fish ponds.

2) We expect to find high CO_2 and CH_4 emissions that significantly differ between the different pond management types. We hypothesize that intensive fish ponds that have the highest level of management interventions, such as high inputs of fertilizers and additional fish feeding, will have the highest GHG emission levels (Kosten et al., 2018; Peacock et al., 2019).

4.2) Material and methods

4.2.1) Study area and selection of ponds

The present study was conducted in « Vijvergebied Midden-Limburg », a fish pond system located in the North-eastern part of Belgium (50°59'00.7"N; 5°19'54.2"E and surroundings). This region is part of a larger pond system called "De Wijers", consisting of more than 1000 connected shallow ponds. The ponds are connected via a system of small rivulets. A considerable number of ponds in the region is used for commercial fish farming whereas a growing number of ponds is managed for biodiversity conservation purposes (Lemmens et al., 2013).

21 ponds representing three different pond management types : no fish (NF), juvenile fish farming (JF), intensive fish farming (IF), were selected last year. Ponds within each management type were selected randomly. The original plan of last year was to sample seven ponds from each management type, but due to intermixing ponds during field work last summer, two other ponds were accidentally sampled than those originally selected. As a result, our data set ended up with 6 no fish ponds, 7 juvenile fish farming ponds and 8 intensive fish farming ponds (Figure 11). The three pond management types differ in the purpose targeted, the fish stock management, the draining frequency and the measures that are taken to prevent the immigration and emigration of fish (Table 3).

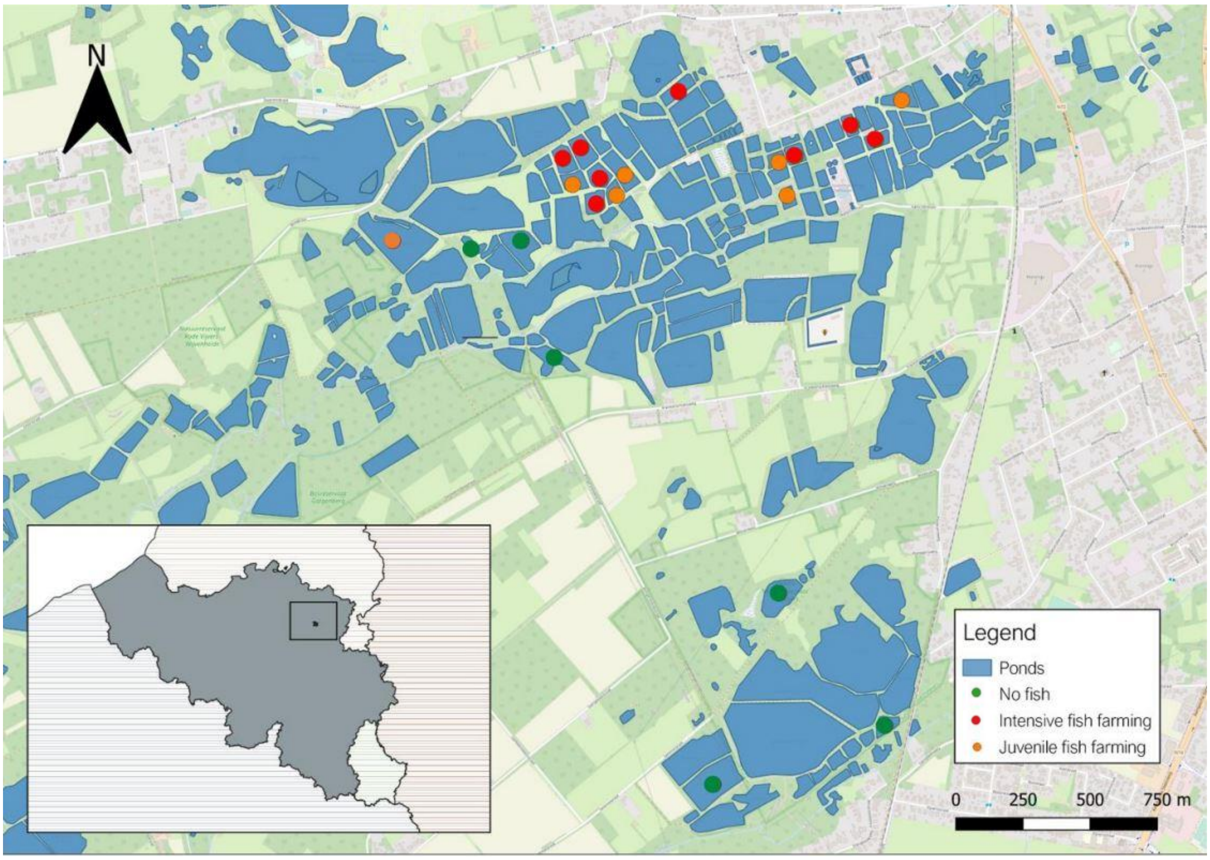


Figure 11 : Overview map of the study area : the fish pond system of « Vijvergebied Midden-Limburg », the dots indicate the 21 selected ponds and the color indicate their management type *(Source : P. Lemmens)*

Table 3 : Detailed comparison between the three pond management types *(Adjusted from Lemmens et al. (2013)).*

Pond management type	Purpose targeted	Fish stock management	Draining frequency	Additional measures to prevent the immigration and emigration of fish
No fish (NF)	Biodiversity conservation	No stocking of fish	Annually drained in autumn/winter to prevent the establishment of fish, refilling in early spring	Use of steel grids on inlets during refilling to maximally prevent the immigration of fish
Juvenile fish farming (JF)	Commercial fish farming	Stocking with low densities of juvenile cyprinids in late spring (May) including ide (Leuciscus idus) in early spring	Annually drained in autumn/winter to harvest the fish, refilling in spring	Use of steel grids on inlets to prevent fish immigration
Intensive fish farming (IF)	Commercial fish farming	Stocking with high density (1000 kg/ha) of common carp	Annually or biannually	Complete or partial coverage of the pond

		(Cyprinus carpio) (and tench (Tinca tinca)) in spring, use of artificial feeds and fertilizers	drained in winter to harvest the fish	with wire netting to prevent bird predation, especially by cormorants

4.2.2) Field data collection

All 21 selected ponds were sampled in Summer 2021 and 2022 (July and August). The collection of the data and samples in the field, as well as the processing in the laboratory afterwards, were done according to a pre-established protocol specifically developed for the PONDERFUL project. Note that only the data from 2021 will be used in the present study as the samples we collected this Summer have not been processed yet.

Major local environmental pond variables

First, a tube sampler was used to collect a depth integrated water sample of approximately 50 liters composed of different subsamples taken at multiple locations within the pond that integrated different depths (littoral and pelagic zone). The water taken at the different locations was pooled in a large recipient (Figure 12 a).

From this depth integrated water sample, in vivo concentrations of chlorophyll A (CHLa) and phycocyanin (PC) were estimated directly in the field using a portable fluorometer and were used as a proxy for the phytoplankton and cyanobacteria biomass respectively (Figure 12 b).

Additionnaly, nutrient concentrations (Total Nitrogen (TN), Total Phosphorus (TP)), alkalinity, Dissolved Organic Carbon (DOC) and Total Organic Carbon (TOC), were measured in the lab from sub-samples of the depth integrated water sample.

The amount of Total Suspended Solid (TSS) in the water was determined in the lab with gravimetrical analyses by filtering a known volume of pond water over a dry and pre-weighted glass fiber filter. This filter was then redried and reweighed, after which the total amount of suspended solids can be determined by the weight difference.

The water transparency can be determined directly in the field using a Sneller tube (Figure 12 c).

The water oxygen concentration and saturation, pH, water temperature and conductivity were measured at a depth of 20 cm below the water surface at a central spot in each pond using a standard electrode.

The thickness of the silt layer was measured based on three measurements at the deepest location of the pond. The thickness of the silt layer can be measured by lowering the measuring stick in the water column until reaching the soft sediment, measuring the depth, and subsequently lowering the stick further until reaching the hard substrate and measuring the depth again. The thickness of the silt layer is the difference between both measurements.

Communities

The zooplankton community (crustacean) was quantitatively sampled by filtering a 40 L subsample from the pre-collected depth integrated water sample through a conical net (with mesh width 53 µm). The sample was then stored in a vial of 50 ml and fixated with glucose saturated formaldehyde (4%) (Figure 12 d).

Macro-invertebrates were sampled using a sweep-net sampling with a kick net (mesh size : 500 µm, 25*18cm frame size). Sweeping was conducted in the different mesohabitats (among the submerged macrophytes, floating-leaved macrophytes and in the littoral vegetation). The sampling was standardized by conducting sweeps of 1 meter during 10 minutes in each pond. The sweeping time in each mesohabitat was in proportion to its occurrence in the pond. After sweeping with the net, all material was pooled. If too many vegetation debris was present in the net, the collected material was carefully washed in pond water in a big recipient. The sample was then fixed with ethanol (70% final concentration) in the field. Organism will be sorted and identified in the laboratory at a later stage.

The macrophytes (submerged, floating and emergent) and helophyte community composition was assessed in the field by scoring the abundances of individual species in each pond following the DAFOR scale (Dominant, Abundant, Frequent, Ocasional, Rare). We used a predefined taxon list to define which plant taxa should be considered during the inventories. The inventory was made for the entire surface of the pond, including the shoreline.

Figure 12 : a) *Collection of a depth integrated water sample using a tube sampler* ***b)*** *Estimation of CHLa and PC concentrations using a portable fluorometer* ***c)*** *Determination of the water transparency using a Sneller tube* ***d)*** *Quantitative sampling of zooplankton (Source : Robby Wijns)*

GHG emissions

The extent of greenhouse gas emissions by the ponds were measured in two different ways to comprise emissions from two main GHG release pathways : via bubble emissions from the sediment, also called ebullition and via diffuse flux between water and air.

To measure the bubble emissions, 10 gas chambers per pond were placed on the water surface at different locations. As GHG emission by ebullition is highly discrete in space and time, we needed to cover different areas of the pond including central and marginal areas (Figure 13 a). The chambers were attached to a brick with a rope to prevent the chambers from floating freely over the water surface. All precautions were taken to avoid sediment disturbance while installing and collecting the chambers. This could have provided unreliable data as an abnormaly high amount of GHG coming up from the sediment would have suddenly accumulated in the chamber. Additionally, the air temperature and pressure were measured at a central location in the pond and once again outside the pond. After 7 days, a 12 ml gas sample was extracted from each chamber and injected into an exetainer vial using a syringe (Figure 13 b). A water depth measurement was also taken under each chamber while collecting the gas samples in order to investigate a potential relation between water depth and GHG emission. The gas samples were then sent to an external lab at Aarhus University in Denmark for the analysis of CH_4, CO_2 and N_2O. To determine the bubble flux per pond, an average was taken from the 10 chambers.

To quantify the diffuse flux, a water sample of 40 ml of both the surface and bottom waters was drawn into a syringe of 50 ml, whereafter 12 ml of air was injected. The sample was then shaken thoroughly for 1 minute and the 12 ml sample was injected into an exetainer vial for the external analysis of the concentrations and partial pressures of CO_2, N_2O and CH_4. In order to measure the dissolved concentration of gases by head space equilibrium, also a 12 ml air sample was taken at each pond. Diffuse fluxes of CO_2, N_2O and CH_4 were determined from the dissolved gas concentrations from the samples and the gas concentration from the atmosphere.

In addition, we determined the degree of water column stratification by taking depth profiles of the pH, conductivity, temperature and oxygen concentration at the deepest spot of the pond. (Figure 13 c). For that, 5 measurements were taken along the depth profile and depth interval depended on the depth of the pond. For example, if the pond was 1 m deep, measurements were taken every 20 cm. Taking depth profile is important as stratification can strongly affect GHG emissions. In addition, the temperature data from two loggers (one near the surface, one at the bottom) were combined to give more information on water stratification. Each logger collected temperature data at 1h time interval. At the same time, the chlorophyll A and phycocyanin concentrations were estimated, and water samples were taken to determine the total nitrogen (TN) and total phosphorus (TP) concentrations, as well as DOC and TOC levels. This was done in the same way as described above.

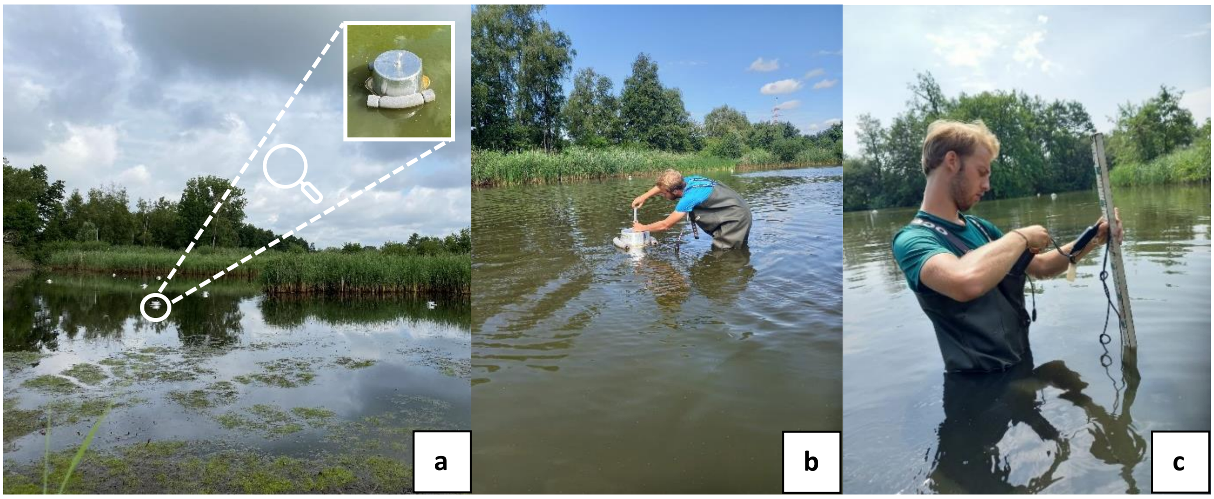


Figure 13 : a) *Overview of a fish pond on which GHG chambers were installed* ***b)*** *Collection of a gas sample after 7 days* ***c)*** *Taking of a depth profile (Source : Robby Wijns, Ditte Rens)*

4.3) Results

4.3.1) Fish pond dynamics in relation to pond management

Dynamics of local environmental pond variables in relation to pond management

The comparison of the local environmental pond variables between the current ponds and the study of Lemmens et al. (2013) show that there is no variation in pond characteristics for the IF and NF ponds. However, a shift of environmental state can be observed for the JF ponds. Indeed, instead of a clear separation between IF ponds and JF ponds, both management types seemed to result in similar local environmental pond conditions in 2021 (Table 4).

Table 4 : Comparison of the local environmental pond variables for each management type between the current ponds and the study of Lemmens et al. (2013) (Source : author)

Pond characteristics \ Management type	NF		JF		IF	
	Lemmens et al. (2006/2007)	2021	Lemmens et al. (2006/2007)	2021	Lemmens et al. (2006/2007)	2021
Nutrient concentrations (TN, TP)	Low	Low	Intermediate	High	High	High
Phytoplankton density (CHLa)	Low	Low	Intermediate	High	High	High
Macrophyte cover	High	High	Intermediate	Low	Low	Low

Local and regional biodiversity patterns in zooplankton and macrophyte communities in relation to pond management

The results reveal a significant effect of pond management type on the local richness of the zooplankton, emergent vegetation and submerged/floating vegetation.

The local zooplankton richness is highest in the NF ponds. While JF ponds in 2006/2007 also have relatively high levels of local zooplankton richness (Figure 14 A), this is not the case in 2021 (Figure 14 B). The zooplankton richness of JF ponds in 2021 is overall low and more similar to IF ponds. (Figure 14 B)

The results for the macrophytes show that the NF ponds in 2021 have the highest local emergent and submerged macrophytes richness and differ significantly from the IF and JF ponds (Figure 15 B/D). The local richness of the emergent and submerged macrophytes in 2006/2007 (Figure 15 A/C) shows similar trends in comparison with the current ponds (Figure 15 B/D), with NF ponds having consistently the highest local richness and IF ponds the lowest.

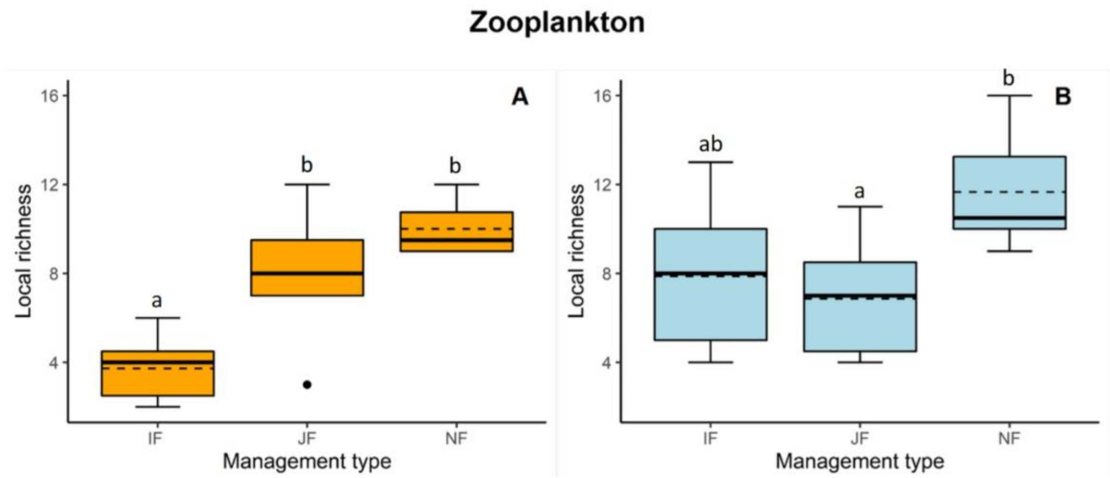


Figure 14 : Boxplots of the local zooplankton richness in function of pond management type. A) *Results from the dataset of Lemmens et al. (2006/2007),* ***B)*** *Results from the current dataset (2021); The boxes represent the 25th and 75th percentiles, the full and dashed lines represent the median (50th percentile) and average respectively. The whiskers indicate the maximum and minimum values; Management types (IF, JF, NF) that have no letters in common (a or b) differ significantly from each other at significance level P = 0.05. (Source : P. Lemmens)*

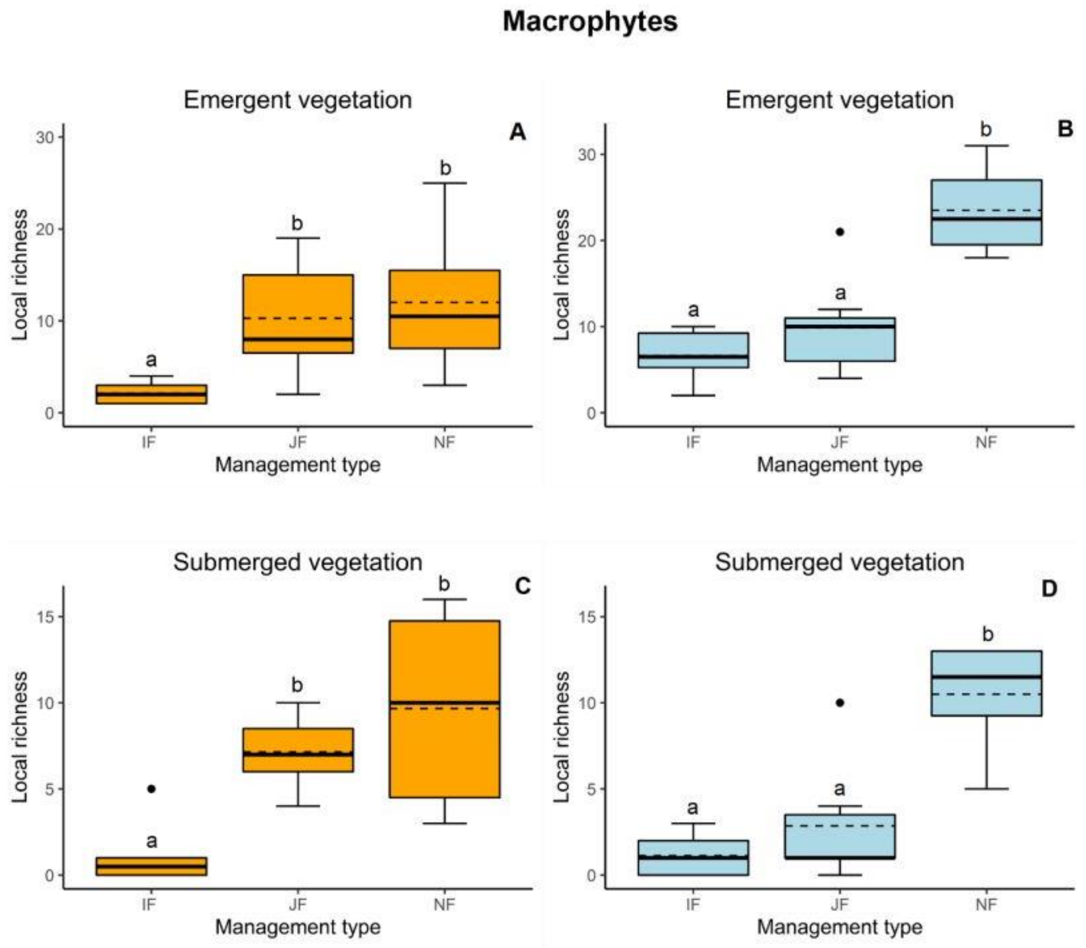


Figure 15 : Boxplots of the local macrophyte richness in function of pond management type. A&C) *Emergent and submerged/floating vegetation from the dataset of Lemmens et al. (2006/2007),* ***B&D)*** *Emergent and submerged/floating vegetation from the current dataset (2021); The boxes represent the 25th and 75th percentiles, the full and dashed lines represent the median (50th percentile) and average respectively. The whiskers indicate the maximum and minimum values; Management types (IF, JF, NF) that have no letters in common (a or b) differ significantly from each other at significance level P = 0.05. (Source : P. Lemmens)*

The accumulation curves representing the number of zooplankton species show consistently the highest regional richness for the NF ponds in both datasets (Figure 16). Compared to the ponds of 2006/2007 (Figure 16 A), the current data show a lower zooplankton regional richness for the JF ponds, and a higher regional richness for the IF ponds (Figure 16 B).

Strong similarities can be found for the regional richness of the macrophytes. The NF ponds show consistently the highest regional richness while the IF ponds show consistently the lowest regional richness (Figure 17). The regional richness for the JF ponds layed consistently between the NF and IF ponds, however, a shift was observed to a lower regional richness for the JF ponds for both the emergent and submerged macrophytes in 2021 (Figure 17 B/D).

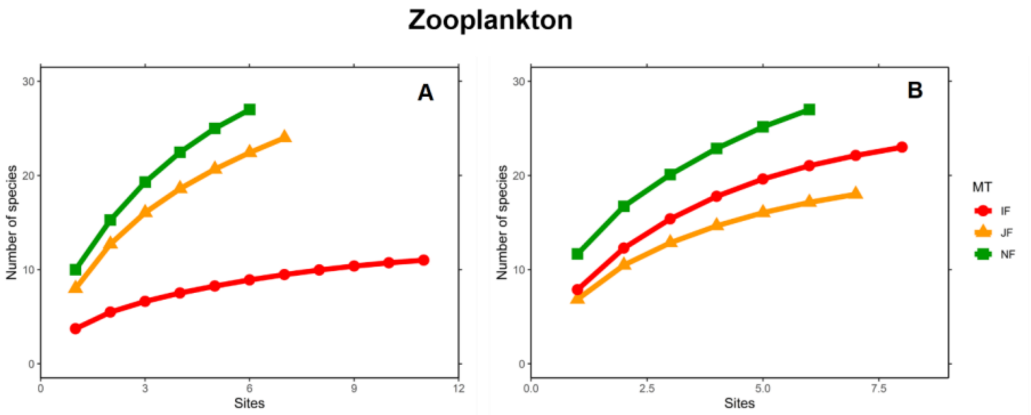


Figure 16 : Accumulation curves of the number of zooplankton species. A) *Results from the dataset of Lemmens et al. (2006/2007),* ***B)*** *Results from the current dataset (2021); The red (circles), orange (triangles) and green lines (squares) represent the IF, JF and NF ponds respectively. (Source : P. Lemmens)*

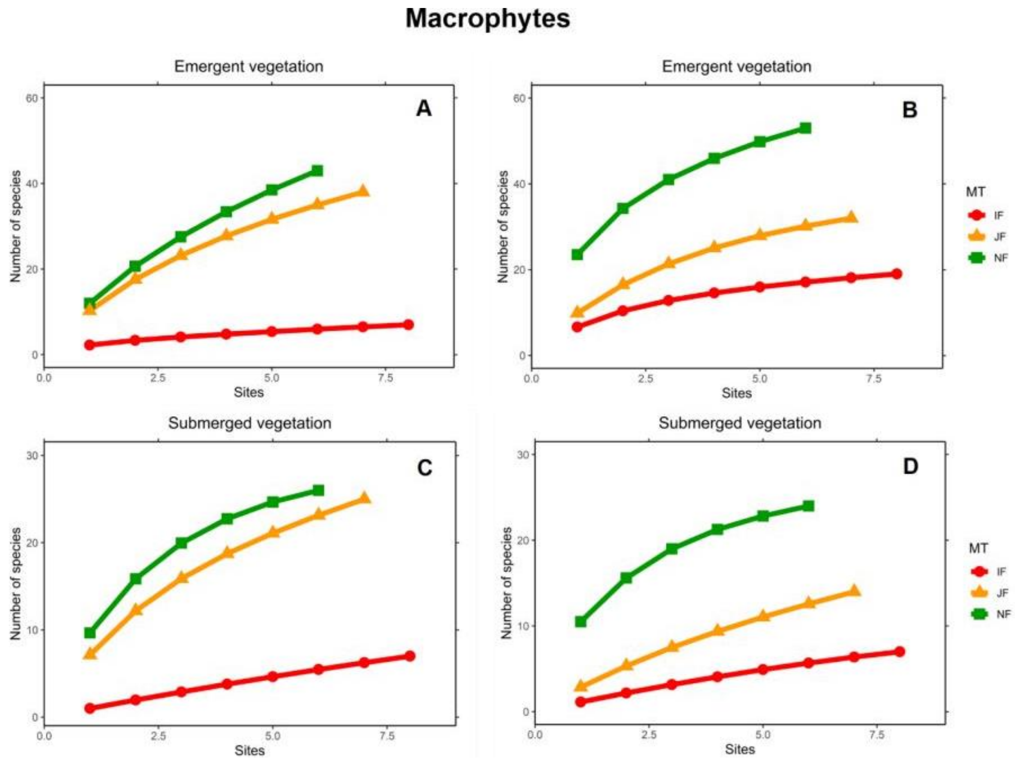
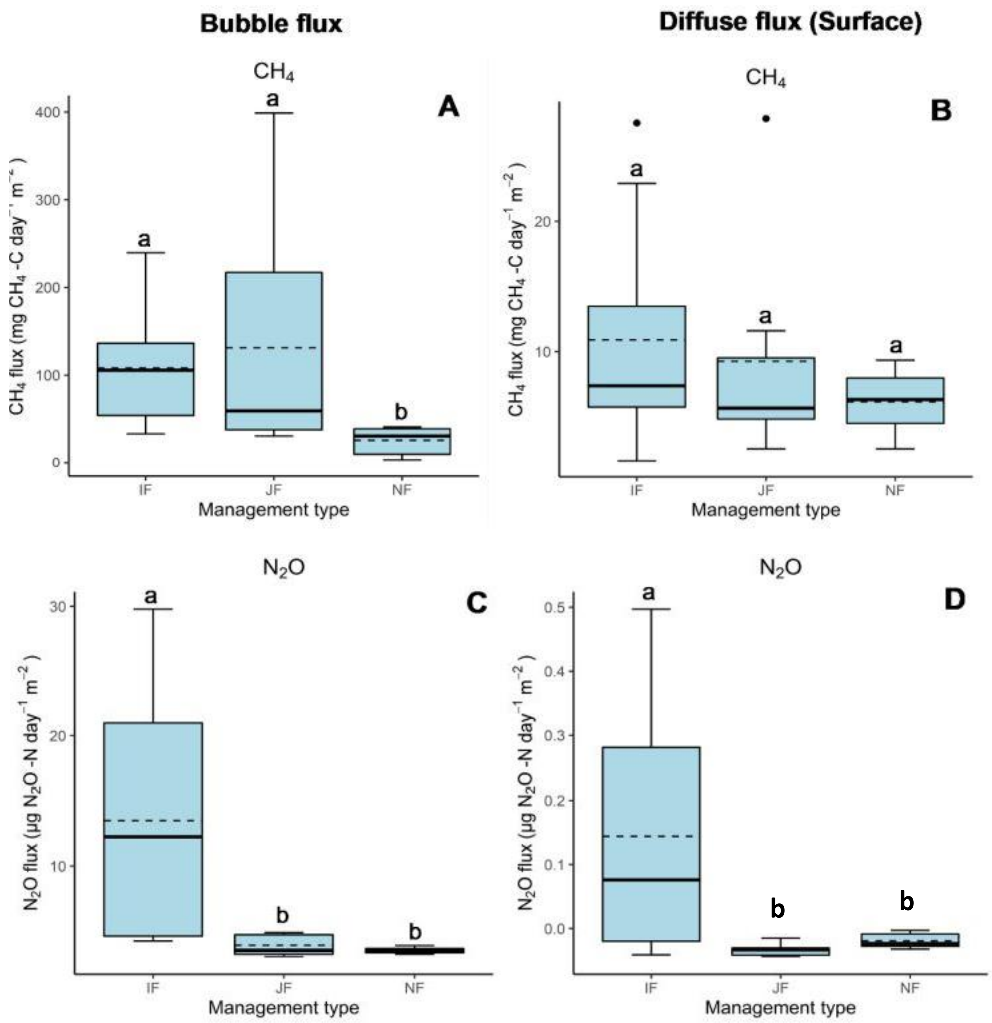


Figure 17 : Accumulation curves of the number of macrophyte species. A&C) *Emergent and submerged/floating vegetation from the dataset of Lemmens et al. (2006/2007),* ***B&D)*** *Emergent and submerged/floating vegetation from the current dataset (2021); The red (circles), orange (triangles) and green lines (squares) represent the IF, JF and NF ponds respectively. (Source : P. Lemmens)*

4.3.2) Greenhouse gas emissions

The results show that gas bubble fluxes vary considerably among ponds. Overall, CH_4 bubble fluxes are much larger than those of N_2O and CO_2 (Figure 18 A/C/E). Our analyses reveal significant differences between the three management types for CH_4 and N_2O but not for CO_2 fluxes. CH_4 fluxes are significantly higher in IF and JF ponds compared to NF ponds (Figure 18 A). IF ponds have significantly higher N_2O bubble fluxes compared to the NF and JF ponds (Figure 18 C). Although CO_2 fluxes tend to be higher in NF ponds than in IF ponds, this difference is not significant (Figure 18 E). The JF management type is characterized by considerable variation among ponds for CH_4 and CO_2 fluxes (Figure 18 A/E). Also, variation in N_2O fluxes among ponds is relatively large in the IF management type (Figure 18 C).

Similar overall patterns can be observed for the diffuse fluxes. A major difference, however, is the order of the fluxes. CO_2 diffuse fluxes (Figure 18 F) are remarkably larger than those of CH_4 and N_2O (Figure 18 B/D). Conversely, N_2O diffuse fluxes are very low compared to other diffuse fluxes (Figure 18 D). JF and NF ponds even show slight negative fluxes of NO_2. A significant difference can be observed between the management types for N_2O diffuse fluxes with fluxes being significantly higher in IF ponds compared to JF and NF ponds (Figure 18 D). On the other hand, no significant differences in CH_4 and CO_2 diffuse fluxes can be highlighted between management types. (Figure 18 B/F)



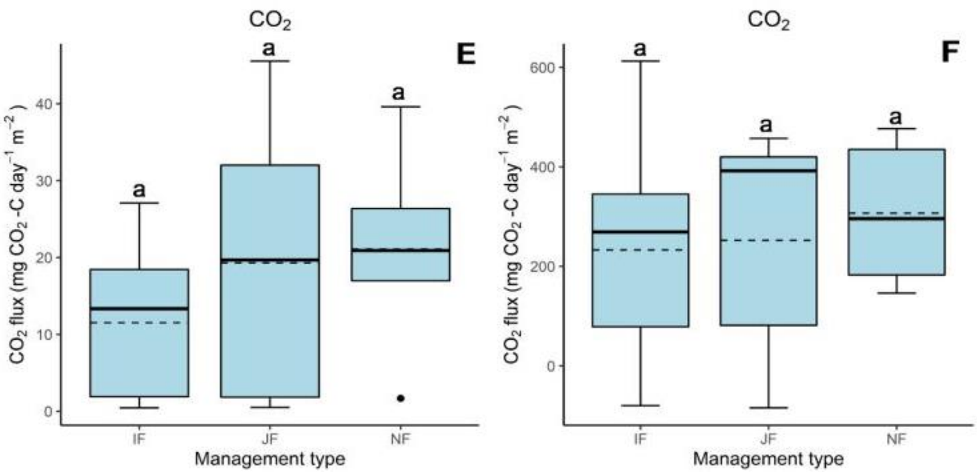


Figure 18 : Boxplots presenting CH_4, N_2O and CO_2 bubble and diffuse fluxes in function of management type. *The left panels present the bubble fluxes of CH_4 **(A)**, N_2O **(C)** and CO_2 **(E)**. The right panels present the surface diffuse fluxes of CH_4 **(B)**, N_2O **(D)** and CO_2 **(F)**. The boxes represent the 25th and 75th percentiles, the full and dashed lines represent the median (50th percentile) and average respectively. The whiskers indicate the maximum and minimum values; Management types (IF, JF, NF) that have no letters in common (a or b) differ significantly from each other at significance level P = 0.05. (Source : P.Lemmens)*

Multiple linear regression identifies CHLa as the only factor having a significant effect on CH_4 bubble flux. Overall CHLa shows a profound positive correlation with CH_4 bubble fluxes (Figure 19 A).

The multiple regression model of the N_2O bubble fluxes shows a significant effect of TN and CHLa (Figure 19 B/C). Both variables are positively correlated with N_2O bubble flux.

Although non-significant, a slight positive correlation can be found between the coverage of emergent vegetation and CO_2 flux. This might help us at a later stage to identify emergent vegetation coverage as a potential driver of CO_2 emissions by fish ponds (Figure 20 A).

Multiple linear regression on diffuse fluxes shows that TN has a significant effect on N_2O diffuse flux. Total nitrogen is positively correlated with N_2O diffuse fluxes (Figure 20 B).

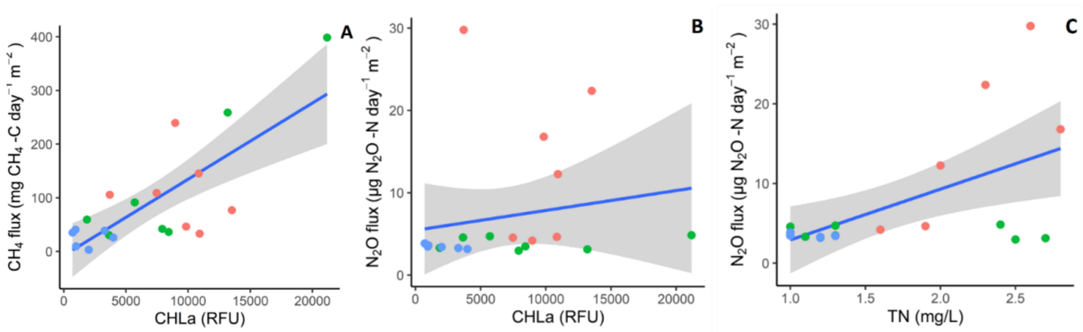


***Figure 19 : Effect plots of bubble fluxes in function of environmental variables**. Effect plots of **A)** CH_4 bubble flux in function of chlorophyll A (CHLa) concentration, **B)** N_2O bubble flux in function of CHLa concentration, **C)** N_2O bubble flux in function of total nitrogen (TN) concentration. The red, green and blue dots present the intensive fish (IF), juvenile fish (JF) and no fish (NF) ponds respectively. 95% confidence intervals are presented. (Source : P.Lemmens)*

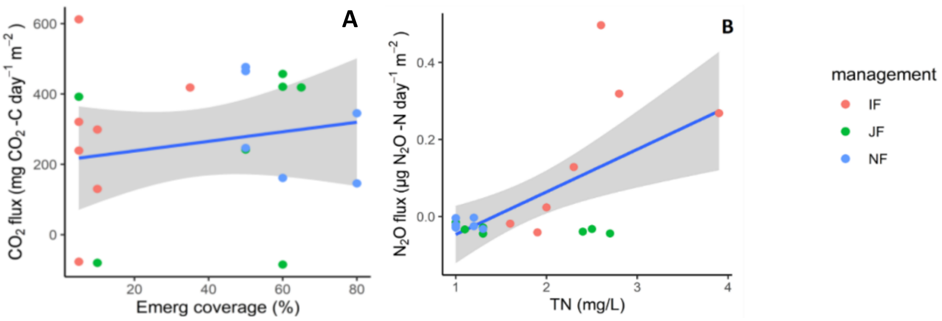


__Figure 20 : Effect plots of surface diffuse fluxes in function of environmental variables__. Effect plots of __A)__ N2O diffuse flux in function of percentage emergent vegetation coverage, __B)__ N2O diffuse flux in function of total nitrogen (TN) concentration. The red, green and blue dots present the intensive fish (IF), juvenile fish (JF) and no fish (NF) ponds respectively. 95% confidence intervals are presented

4.4) Discussion

4.4.1) Local and regional biodiversity patterns in zooplankton and macrophyte communities in relation to local environmental pond variables and pond management

Our study highlighted clear differences between pond management types for local environmental pond conditions and the community composition of zooplankton and macrophytes.

IF ponds were characterized by relatively high nutrients levels, high concentrations of phytoplankton, and typically had a low coverage with aquatic vegetation.

Subsequently, a relatively low local and regional richness in zooplankton and especially emergent and submerged macrophytes was found in comparison with the NF ponds, which is in concordance with the results of Lemmens et al. (2013) and with our hypothesis.

The high phytoplankton densities are linked to increased nutrient levels, caused by both benthivorous fish via resuspension of nutrients, and external nutrient input through fertilizers and fish feeds (Fried et al., 2003; Leibold, 1999; Lemmens et al., 2018; Attayde & Hansson, 2001). In addition, the higher phytoplankton biomass could also partly be the result of top-down control by fish on phytoplankton-grazing zooplankton (Lemmens et al., 2018; Attayde & Hansson, 2001). High benthic fish and phytoplankton densities are indirectly linked to low macrophyte diversities by increasing the water turbidity and thereby reducing the light intensity in the water column (Breukelaar et al., 1994; Duarte, 1995; Xie et al., 2015).

The low zooplankton richness in the IF ponds can importantly be explained by the presence of high fish densities that have a negative effect on the zooplankton diversity via top-down control (Jeppesen et al., 1997; Lemmens et al., 2018; Søndergaard et al., 1997). The loss of macrophytes also has a direct negative effect on the zooplankton richness, since vegetation can positively influence the zooplankton diversity by enhancing the habitat complexity, promoting food availability and providing refuge habitats for prey against predators (De et al., 2019; Jeppesen et al., 1997, 1998a, 1998b).

Conversely, the NF ponds which are characterized by low nutrient levels, no fish stocking and a clear water state, showed a consistently high local and regional richness in zooplankton and emergent and submerged/floating macrophytes, which is in line with our expectations and the results of Lemmens et al. (2013).

Contrary to our expectations, JF ponds showed in 2021 a lower local and regional richness compared to 2006/2007. Instead of a clear separation between IF ponds and JF ponds, both management types seemed to result in similar local environmental pond conditions. We believe that the shift of environmental state of the JF ponds, and the thereby decrease in species richness in these ponds, is most likely the result of an intensification of the juvenile fish farming practices during the last decade.

4.4.2) Greenhouse gas emissions

Our results showed that fish ponds are strong emitters of especially CH_4 and CO_2.

CH_4 emissions were largely coming from direct methane release from the sediment via bubble ebullitive flux. As hypothesized, the highest average daily fluxes were found in the IF and JF ponds. We found that an important driver of CH_4 bubble fluxes is chlorophyll A. Although nutrient levels (TP and TN) were not directly found as significant drivers of CH_4 fluxes in our study, other studies have found an important association between CH_4 emissions and TP levels. The relationship between nutrient status and CH_4 emissions can be linked to an increased primary production with increasing nutrient concentrations, which in turn can promote methanogenesis via higher organic matter content and the formation of anoxic conditions that are needed to generate methane (DelSontro et al., 2016). The association between increased primary production and CH_4 flux can also be reflected in the strong positive correlation we found between CH_4 flux and phytoplankton levels measured as CHLa.

Next, also very high overall CO_2 emissions were observed in our study. In contrast with CH_4, CO_2 emissions were mainly released via diffuse flux. The highest CO_2 emissions were found in the NF ponds. Audet et al. (2020) found a positive correlation between vegetation cover (submerged and emergent) and CO_2 concentration. One of the possible explanations they provide for this rather unexpected pattern is the oxygenation of the sediment by emergent vegetation, which will lead to a higher degradation and release of CO_2. Although non-significant, we also found a slight positive association between the coverage with emergent vegetation and CO_2 flux. Since our NF ponds are characterized by coverage with submerged and emergent vegetation, this positive correlation might partly explain the observed high average CO_2 flux in the NF ponds.

N_2O fluxes in our study were relatively low, especially diffuse fluxes. Nevertheless, we observed significant differences between IF ponds with JF and NF ponds. Although N_2O fluxes in the IF ponds where notably larger than in the other two management types, overall N_2O fluxes were still very low in comparison with CO_2 diffuse fluxes and CH_4 bubble fluxes. The main drivers of N_2O emission that were identified in this study are TN and CHLa. This indicates that N_2O flux is mainly driven by nutrient levels, especially nitrogen, which corresponds to our finding that the nutrient-rich IF ponds have the highest N_2O emissions. Another important finding of this study was the fact that JF and NF ponds even showed slight negative fluxes, indicating that these ponds can act as N_2O sinks.

In general our results show that pond management practices do indeed have an effect on the total amount of greenhouse gasses that are emitted from the ponds through changes in the local environmental pond conditions. This provides the opportunity to use fish ponds as systems that can possibly reduce greenhouse gas emissions by applying appropriate management practices that are especially aimed at keeping the total amount of nutrients in the water at a low level.

4.4.3) Conclusion and future perspectives

Our study highlights the fact that pond management is an important factor driving variation in local pond environmental conditions and in local and regional richness of zooplankton and macrophytes. The intensively managed fish ponds, have a significant lower species richness in comparison to ponds without fish. In contrast to earlier findings, we observed an overall low ecological quality in ponds used for juvenile fish culture, which likely results from an intensification of management practices. Lastly, clear differences in CH_4, CO_2 and N_2O fluxes were observed between the ponds. Overall, fish ponds are strong emitters of CO_2 and CH_4, and greenhouse gas emissions seem to be mainly driven by nutrient related variables.

This study provides important insights for future pond management practices. If we want to obtain high-quality biodiverse ponds, it is essential to minimalize the intensification of management practices to prevent further degradations of ponds. More investments should be made in pond management practices that are aimed at keeping the ponds free of fish. Such management actions would be beneficial for biodiversity conservation and can also play a role in reducing the greenhouse gas emission by ponds.

5) The effect of buffer strip size on the ecological state of small ponds in agricultural areas in Belgium and Germany

In close collaboration with the PhD student Louisa-Marie (IGB-KU Leuven), I was able to build further on the work I had already started in Germany and I quantified the size of the buffer strips around the set of Belgian ponds so that we could investigate the effect of buffer strip size on the ecological state of small ponds in agricultural areas in Belgium and Germany. This resulted in a poster contribution to the SIL 2022 conference in Berlin (Annex 1).

We used 3 criteria for the selection of suitable satellite images. First, we explicitly aimed for satellite images that matched the period of pond sampling as much as possible because the size of the buffer strip surrounding the ponds might vary considerably between years, especially in intensively managed agricultural land. Secondly, we aimed to use images taken in the same season for all ponds in the different regions to avoid biases in land use estimates related to temporal variation. Third, we had to consider the resolution of the images. This is important as we work with relatively small ponds (typically less than 1 ha). We decided to use Google Earth as its satellite images met all these requirements.

When possible, all satellite images that we selected are from the most recent spring possible so that the buffer size measurements are as standardized as possible under the assumption that the buffer size did not change a lot from one spring to another over a 2-year period.

We quantified the size of the buffer strip around each Belgian pond using a standardized approach using 8 cardinal lines. The aim is to evaluate the size of the buffer strip, e.g. the distance to the arable land, along each of the 8 lines. For that, the 8 cardinal lines are placed in a way that they all start from the center of the pond. Each line is numbered from 1 to 8 (always in the same order). Then, each line is measured using the measuring tool available on Google Earth. The measurement starts just after the open water area and ends when arable land is reached. All the lengths are indicated in a dedicated line of an Excel file. Finally, a mean buffer size can be calculated for each pond. We also calculated median and highlighted min and max values. This method was applied to the 30 ponds and the dedicated table was filled in (Table 5). Information concerning the buffer type and the surrounding land use were also collected based on satellite images and observations in the field. The final resulting dataset can be found in Annex 2. This table is a deliverable.

Note that due to some standardization issues encountered last year when positioning and orienting the cardinal lines on the German ponds, we decided to use this time a slightly different approach. For this, a pre-created pattern of the 8 cardinal lines was added as a background on Google Earth for each pond. Then, setting the opacity enabled us to see both the pond and the 8 cardinal lines at the same time. The measuring tool was subsequently used following the cardinal lines. This resulted in a much more standardized method that consequently should bring more accurate measurements. All pond satellite images with the superposed cardinal lines were collected in a table that can be found in Annex 3. This table is a deliverable.

Below, I provided details on the method using one pond as an example (Figure 21).

Figure 21 : Satellite image of the model pond with its superposed cardinal lines to assess its buffer size *(only the red segments are measured) (Source : author, created with Google Earth)*

Table 5 : Line of the Excel file that was filled in for the above-mentionned model pond*. Each line holds information on the buffer size, the buffer type and the surrounding landuse. (Source : author)*

Pond code	Length of the 8 cardinal lines (in meters)								Mean length	Mediane length	Min length	Max length	Buffer type	Type of surrounding landuse
	1	2	3	4	5	6	7	8						
EF1_IN1	8	4.5	6	5	4.5	4.5	9	6.5	6	5.5	4.5	9	Reed belt, trees	Managed grassland/crops, corn field, road

In addition, we developped a new method for buffer strips showing a high variability over time (Figure 23). Indeed, the size of the buffer strip surrounding the ponds might vary considerably between years, especially in intensively managed agricultural land (Figure 22). This is, more in particular, the case for German ponds.

Figure 22 : Satellite images of a German pond located in the region of Müncheberg (NE Germany) presenting a high variability in its buffer a) *2018* ***b)*** *2020 (Source : Google Earth)*

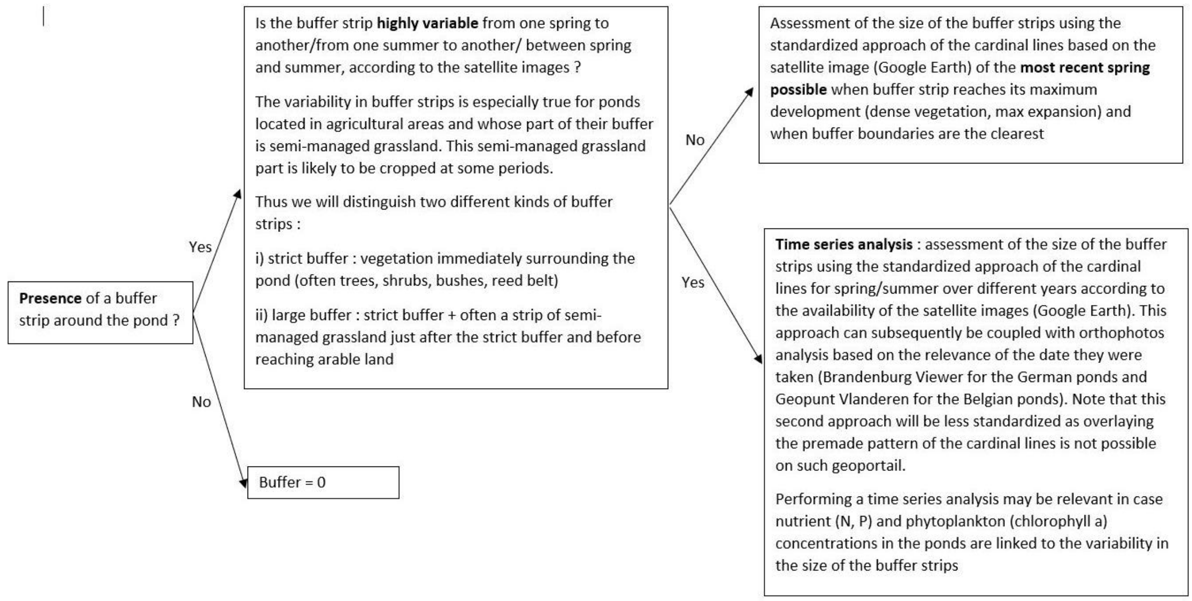


***Figure 23 : Scheme of the method used to assess the size of the buffer strips while taking into account their variability over time** (Source : author)*

The final aim of our study was to explore the potential of buffer strips to reduce the detrimental impact of agriculture on pond trophic state.

For this, we used data on median buffer strip size measured along 8 cardinal lines on 40 ponds located in agricultural settings (18 in Belgium, 22 in Germany). We subsequently attempted to establish multiple correlations between median buffer strip size and various local environmental pond conditions. Other attempts to establish such correlations can be found in Annex 4.

Our results show a negative effect of buffer strip size on variables linked to a turbid ecological pond state : total nitrogen concentration, total phosphorous concentration, turbidity and total suspended solid (Figure 24). No clear evidence for a relationship with phycocyanin and chlorophyll a was found.

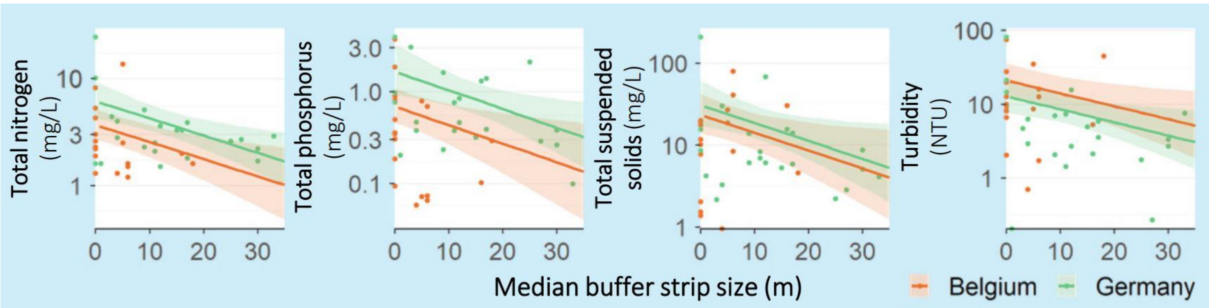


***Figure 24 : Establishment of different correlations linking median buffer size with various local environmental pond variables related to turbid ecological state.** (Source : Maxime Flajgenblat)*

Our preliminary results suggest that even relatively small buffer strips can already be effective in reducing the negative effect of intensive agriculture in close proximity of the ponds.

Conclusion on my internship

My internship at the Laboratory of Aquatic Ecology, Evolution and Conservation as a member of the Belgian PONDERFUL team gave me the unique opportunity to be involved for the second consecutive year in the same project and to experience new aspects of the research process in an international atmosphere.

I was strongly involved in the field data collection of more than 105 ponds of different types across Flanders including farmland ponds, crater ponds, ponds selected along an age gradient and newly created ponds. More specifically, I investigated the temporal dynamics of biodiversity and greenhouse gas emissions in the fish pond system of Midden-Limburg and I attempted to identify the drivers of such dynamics and emissions, and in particular how these relate to the pond management type. I was also able to build further on the work I had already started in Germany and I quantified the size of the buffer strips around the set of Belgian ponds so that we could investigate the effect of buffer strip size on the ecological state of small ponds in agricultural areas in Belgium and Germany.

My internship at the Laboratory of Aquatic Ecology Evolution and Conservation as a member of the Belgian PONDERFUL team was an enriching experience on all practical, professional, personal and social aspects.

From a practical point of view, this internship was an unique experience that enhanced my knowlegde on freshwater ecology in general and more specifically on fish pond systems. It also enabled me to build further on the experiences from my earlier internship at IGB Berlin and to become highly experienced with the multiple practical aspects related to the collection of field data.

From a professional point of view, my internship gave me the opportunity to take a step further in the freshwater research community by attending and even contributing to the SIL 2022 conference in Berlin with a poster presentation that I had developped in collaboration with the PhD students Louisa-Marie from IGB. This enabled me to shed light on the collaboration aspect that I had never experienced so much before. In addition, being involved two years in a row in the same project gave me the opportunity to shed light on most aspects of the research process including the field data collection, the processing of the samples and the data analysis.

More importantly, this internship strongly contributed to my personal development. During my last internship at IGB, I had identified the combination of field work and being part of an international atmosphere as my potential personal definition of « being happy at work ». My internship here at KU Leuven as part of the Belgian PONDERFUL team highly fulfilled my expectations and confirmed the same need. If I was asked to give again my personal definition of « being happy at work », I would give exactly the same.

I am grateful to have been part of PONDERFUL and to have built such strong friendship links with my colleagues. The PONDERFUL experience has made me grow up, both educationally and professionally, but also personally.

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Annex 1: Poster presented at the SIL 2022 conference in Berlin in collaboration with the PhD student Louisa-Marie von Plüskow

The effect of buffer strip size on the ecological state of small ponds in agricultural areas in Belgium and Germany

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Buffer strips to the rescue?

Freshwater ponds strongly contribute to **regional biodiversity** and offer multiple **vital ecosystem services.**

They are **increasingly threatened:** they are strongly influenced by **land use at local scale** and can suffer from **eutrophication** (nutrient overload) due to intensive agriculture.

Could buffer strips (zones of natural vegetation around ponds) help preventing pond eutrophication?

Data collection and analysis

- **40 ponds** in **agricultural settings:** 18 N-Belgium and 22 NE-Germany (Fig 1).
- Data on **pond local environmental conditions.**
- Data on median **buffer strip size** measured along 8 cardinal lines (Fig 2).
- Statistical modelling based on **GLMs** with **gamma distribution.**

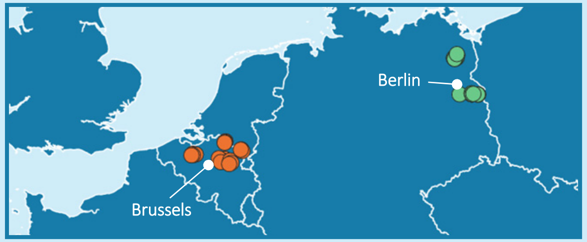

Fig 1. Sampling locations.

Fig 2. Buffer size approach.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No ID 869296

Ponderful
PONDS FOR CLIMATE

Main findings

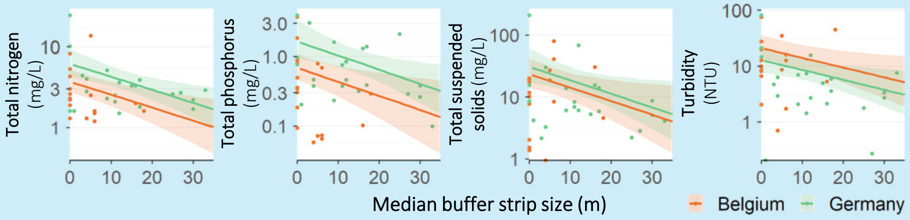

Fig 3. Observed data points (dots) and estimated effects with 95% credible intervals.

High posterior probability of a **negative effect of buffer strip size on variables linked to a turbid ecological pond state** (Fig 3): total nitrogen concentration (99.95%), total phosphorous concentration (99.40%), turbidity (98.65%) and total suspended solids (98.88%). No clear evidence for a relationship with conductivity, total organic carbon, phycocyanin and chlorophyll a (not shown).

Take home message

Relatively small buffer strips can protect ponds against the impact of intensive land use on their ecological state.

Key references

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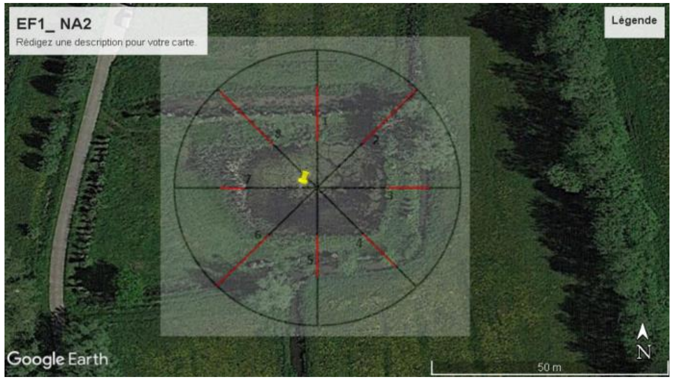
Annex 2. Deliverable 1. Final dataset providing an assessment of the buffer size, the buffer type and the surrounding land use for the set of 30 ponds located in North-East Belgium

Pond cluster	Landuse type	Pond code	Length of the 8 cardinal lines (in meters)								Mean length	Mediane length	Min length	Max length	Buffer type	Type of surrounding landuse
			1	2	3	4	5	6	7	8						
East Flanders	Nature	EF1_NA1	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Forest
		EF1_NA2	12	16.5	8.5	9.5	9	15.5	5	16	11.5	10.75	5	16.5	Trees, shrubs	Semi managed grassland
	Extensive agriculture	EF1_EX1	1	0	0	0	0	0	0	0	0.125	0	0	1	No buffer (except trees for card1)	Managed grassland
		EF1_EX2	0	0	0	7.5	7.5	0	0	0	1.875	0	0	7.5	No buffer (except trees for card4,5)	Managed grassland, horse grazing
	Intensive agriculture	EF1_IN1	8	4.5	6	5	4.5	4.5	9	6.5	6	5.5	4.5	9	Reed belt, trees	Managed grassland/crops, corn field, road
		EF1_IN2	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Managed grassland/cattle, crops (rapeseed field)
Antwerp	Nature	AW1_NA1	0	2.5	6.5	5.5	5	6.5	0	8.5	4.3125	5.25	0	8.5	Yes although very thin and not dense, wetland vegetation (probably juncus)	Pond location: Unmanaged grassland, pond surrondings: forest
		AW1_NA2	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Semi managed grassland, forest
	Extensive agriculture	AW1_EX1	0	7	0	13	7.2	7.5	0	0	4.3375	3.5	0	13	Fragmented buffer	Managed grassland, crops
		AW1_EX2	13	9.5	10.5	11.5	8.5	3.5	5.5	13	9.375	10	3.5	13	Bushes, shrubs	Forest, sandy paths
	Intensive agriculture	AW1_IN1	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Pond location: unmanaged grassland, pond surroundings: crops, forest, -> heterogeneous landscape
		AW1_IN2	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Managed grassland, forest
Limburg 1	Nature	LB1_NA1	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Non/semi managed grassland
		LB1_NA2	11	17	7	4	15.5	12	17.5	13.5	12.1875	12.75	4	17.5	Trees, shrubs, bushes	Unmanaged grassland with trees, shrubs and bushes
	Extensive agriculture	LB1_EX1	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Fruit tree field
		LB1_EX2	2	5	4	3.5	3.5	2.5	2	2.5	3.125	3	2	5	Herbaceous stratum	Managed grassland, crops
	Intensive agriculture	LB1_IN1	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Managed grassland, cattle, corn field, fruit tree field, road
		LB1_IN2	6	4.5	4	3.5	5	6	6.5	7	5.3125	5.5	3.5	7	shrubs, bushes	Semi-managed grassland, cattle grazing, fruit tree field, parking lot
Limburg 2	Nature	LB2_NA1	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Unmanaged grassland, forest
		LB2_NA2	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Semi-managed grassland, forest, cattle grazing
	Extensive agriculture	LB2_EX1	0	0	8	4.5	9.5	6	5	4	4.625	4.75	0	9.5	Only on half of the pond (shrubs, bushes)	Managed grassland, cattle, corn field, forest
		LB2_EX2	20.5	46	13	10	10.5	18	17.5	20	19.4375	17.75	10	46	Very dense buffer (trees, shrubs, bushes)	Managed, semi-managed grassland
	Intensive agriculture	LB2_IN1	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Managed grassland, crops, cattle
		LB2_IN2	15	55.5	17	9	14.5	36.5	16	9.5	21.625	15.5	9	55.5	Fragmented buffer (trees, shrubs and bushes)	Very intensive agriculture, managed grassland, corn fields
Flemish Brabant	Nature	FB1_NA1	7.5	11.5	3.5	3	2.5	3.5	7.5	8.5	5.9375	5.5	2.5	11.5	Shrubs, bushes	Pond location: unmanaged grassland, pond surroundings: agriculture (corn fields), border: tree belt
		FB1_NA2	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Unmanaged grassland, forest
	Extensive agriculture	FB1_EX1	6.5	8.5	6	6	4.5	7	6	5.5	6.25	6	4.5	8.5	Trees, shrubs, bushes	Fruit tree field
		FB1_EX2	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Managed grassland, cattle grazing
	Intensive agriculture	FB1_IN1	3	4	5	7.5	5.5	11.5	10.5	5	6.5	5.25	3	11.5	Trees, shrubs, bushes	In the middle of corn fields
		FB1_IN2	0	0	0	0	0	0	0	0	0	0	0	0	No buffer	Extremely intensive agriculture, diverse crops: corn fields, fruit tree fields, others

Annex 3. Deliverable 2. Table collecting the satellite images with the overlayed cardinal lines that were used to assess the buffer size for the set of 30 ponds located in North-East Belgium

<table>
<tr><th>Pond cluster</th><th>Landuse type</th><th colspan="2">Satellite images</th></tr>
<tr><td rowspan="3">East Flanders</td><td>Nature</td><td>
</td><td>
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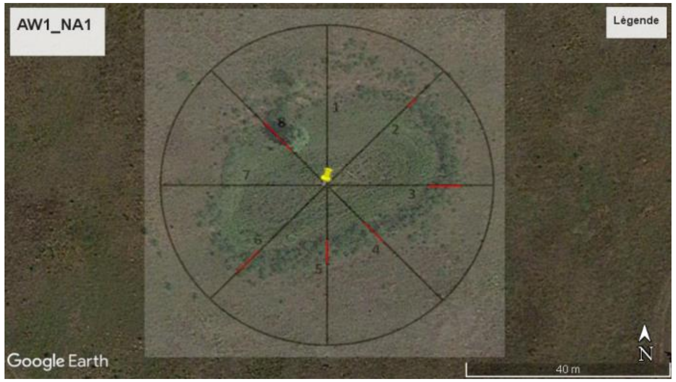
<tr><td rowspan="3">Antwerp</td><td>Nature</td><td>
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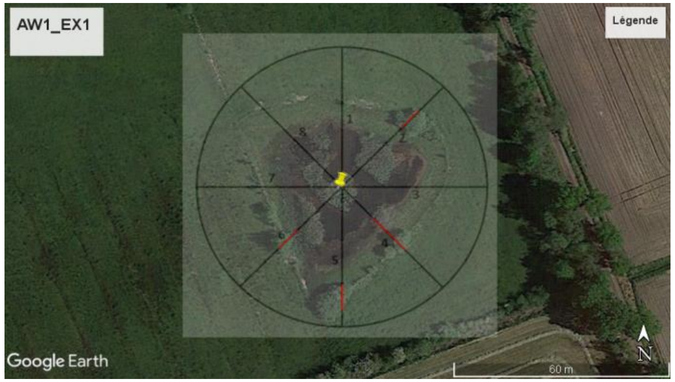
<tr><td>Extensive agriculture</td><td>
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<table>
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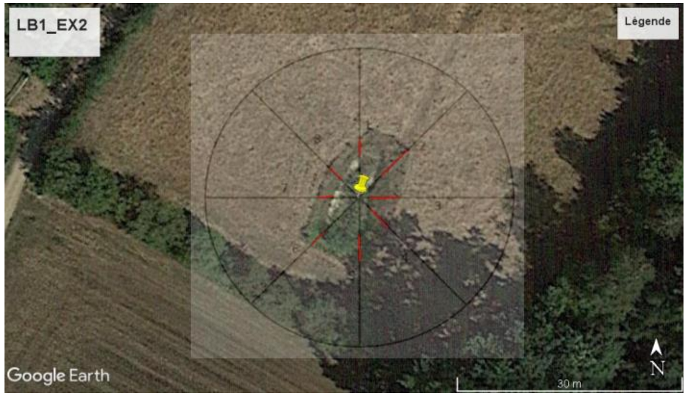
</td><td>
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<tr><td rowspan="3">Limburg 2</td><td>Nature</td><td>
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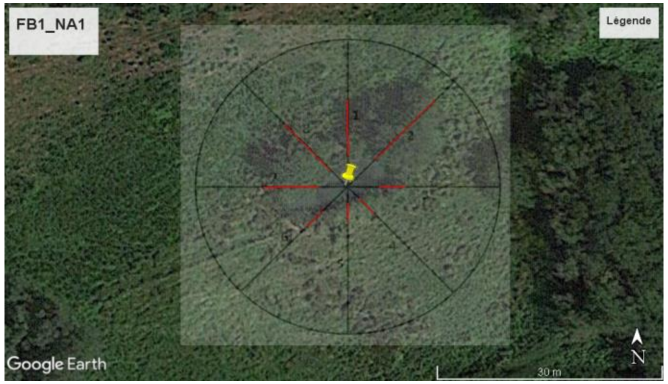
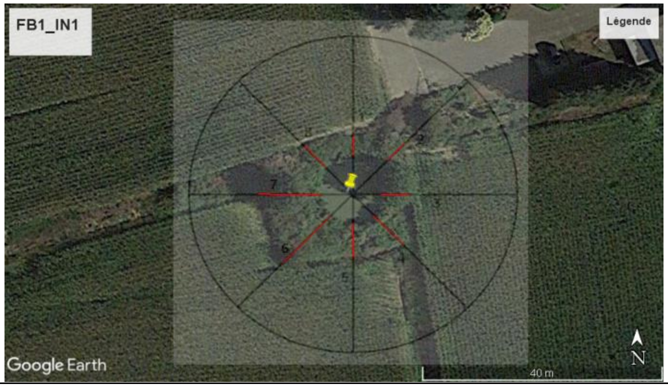
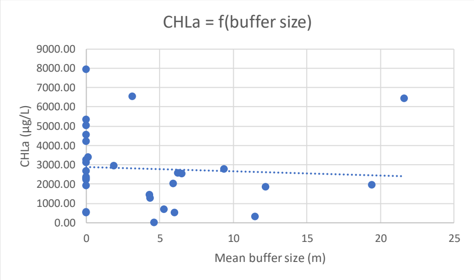
Flemish Brabant	Nature		
	Extensive agriculture		
	Intensive agriculture		

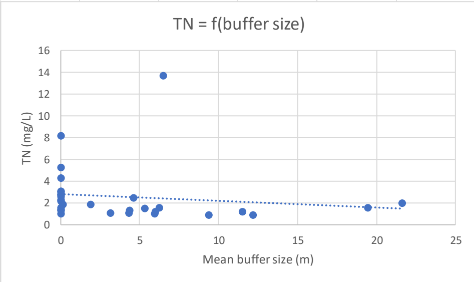
Annex 4. Deliverable 3. Personal attempts to establish multiple correlations between buffer size and local environmental pond conditions

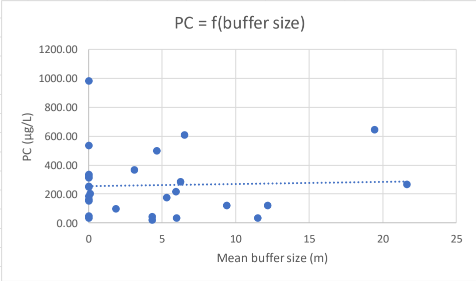
Attempt to establish different correlations between pond chemical variables and the size of the buffer strips

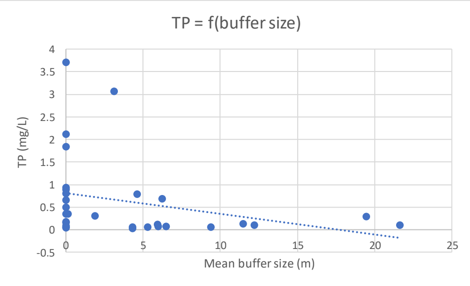
N = 30 ponds from 3 different landuse types (nature, extensive agriculture, intensive agriculture)

Pond cluster	Landuse type	Pond code	TN	TP	CHLa	PC	Mean buffer size
East Flanders	Nature	EF1_NA1	1.6	0.158	2268.59	157.89	0
		EF1_NA2	1.2	0.138	354.33	35.49	11.5
	Extensive agriculture	EF1_EX1	1.9	0.347	3414.88	203.78	0.125
		EF1_EX2	1.9	0.303	2956.36	97.99	1.875
	Intensive agriculture	EF1_IN1	1.2	0.073	545.52	35.25	6
		EF1_IN2	4.3	3.708	3271.31	532.43	0
Antwerp	Nature	AW1_NA1	1.1	0.038	1451.72	40.61	4.3125
		AW1_NA2	3.1	2.125	5361.34	253.81	0
	Extensive agriculture	AW1_EX1	1.3	0.058	1293.32	19.35	4.3375
		AW1_EX2	0.9	0.066	2791.65	117.93	9.375
	Intensive agriculture	AW1_IN1	3	0.893	7971.46	327.75	0
		AW1_IN2	2.6	0.494	2317.19	335.44	0
Limburg 1	Nature	LB1_NA1	2.8	0.936	5059.37	313.00	0
		LB1_NA2	0.9	0.101	1864.24	121.80	12.1875
	Extensive agriculture	LB1_EX1	1.3	0.801	2683.70	248.07	0
		LB1_EX2	1.1	3.069	6555.48	366.36	3.125
	Intensive agriculture	LB1_IN1	5.3	0.184	1951.04	159.37	0
		LB1_IN2	1.5	0.065	702.41	172.33	5.3125
Limburg 2	Nature	LB2_NA1	2.8	0.659	2383.59	152.02	0
		LB2_NA2	1.4	0.048	557.02	34.24	0
	Extensive agriculture	LB2_EX1	2.5	0.794	45.55	498.70	4.625
		LB2_EX2	1.6	0.293	1980.35	643.67	19.4375
	Intensive agriculture	LB2_IN1	2.3	0.356	4579.71	183.11	0
		LB2_IN2	2	0.102	6449.01	265.31	21.625
Flemish Brabant	Nature	FB1_NA1	1	0.114	2059.37	214.22	5.9375
		FB1_NA2	1	0.059	579.69	45.51	0
	Extensive agriculture	FB1_EX1	1.6	0.686	2579.46	284.62	6.25
		FB1_EX2	8.2	1.842	3145.18	979.35	0
	Intensive agriculture	FB1_IN1	13.7	0.072	2556.09	607.01	6.5
		FB1_IN2	2.2	0.094	4241.76	317.10	0



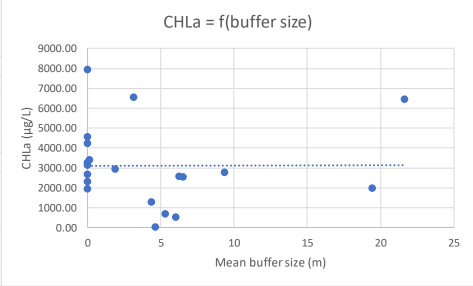


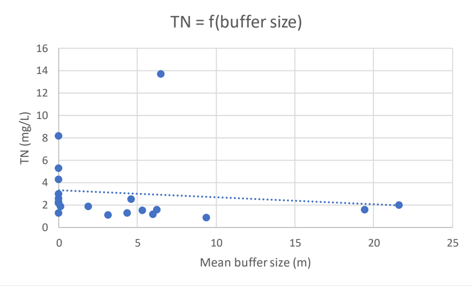


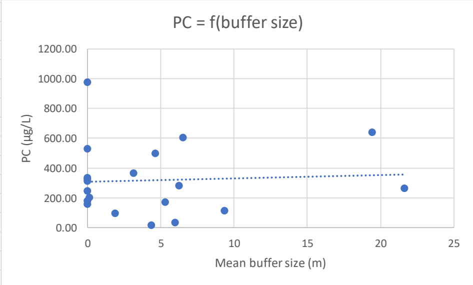


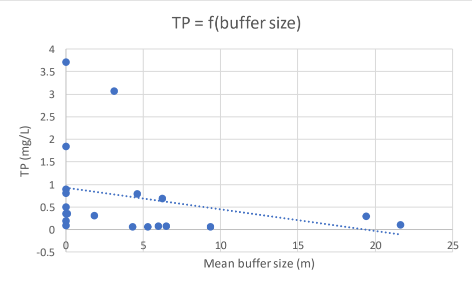
N = 20 ponds from 2 landuse types: extensive agriculture and intensive agriculture

Pond cluster	Landuse type	Pond code	TN	TP	CHLa	PC	Mean buffer size
East Flanders	Extensive agriculture	EF1_EX1	1.9	0.347	3414.88	203.78	0.125
		EF1_EX2	1.9	0.303	2956.36	97.99	1.875
	Intensive agriculture	EF1_IN1	1.2	0.073	545.52	35.25	6
		EF1_IN2	4.3	3.708	3271.31	532.43	0
Antwerp	Extensive agriculture	AW1_EX1	1.3	0.058	1293.32	19.35	4.3375
		AW1_EX2	0.9	0.066	2791.65	117.93	9.375
	Intensive agriculture	AW1_IN1	3	0.893	7971.46	327.75	0
		AW1_IN2	2.6	0.494	2317.19	335.44	0
Limburg 1	Extensive agriculture	LB1_EX1	1.3	0.801	2683.70	248.07	0
		LB1_EX2	1.1	3.069	6555.48	366.36	3.125
	Intensive agriculture	LB1_IN1	5.3	0.184	1951.04	159.37	0
		LB1_IN2	1.5	0.065	702.41	172.33	5.3125
Limburg 2	Extensive agriculture	LB2_EX1	2.5	0.794	45.55	498.70	4.625
		LB2_EX2	1.6	0.293	1980.35	643.67	19.4375
	Intensive agriculture	LB2_IN1	2.3	0.356	4579.71	183.11	0
		LB2_IN2	2	0.102	6449.01	265.31	21.625
Flemish Brabant	Extensive agriculture	FB1_EX1	1.6	0.686	2579.46	284.62	6.25
		FB1_EX2	8.2	1.842	3145.18	979.35	0
	Intensive agriculture	FB1_IN1	13.7	0.072	2556.09	607.01	6.5
		FB1_IN2	2.2	0.094	4241.76	317.10	0



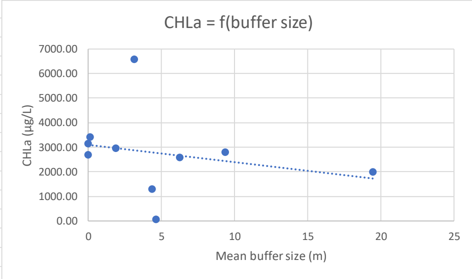


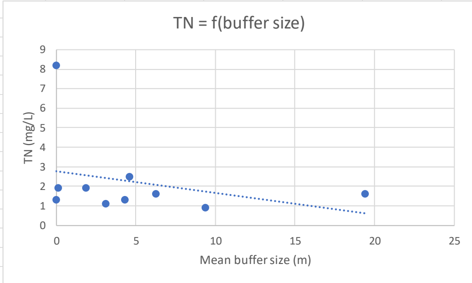


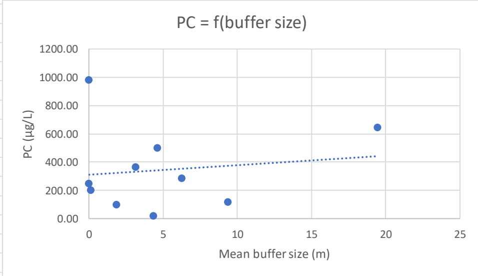


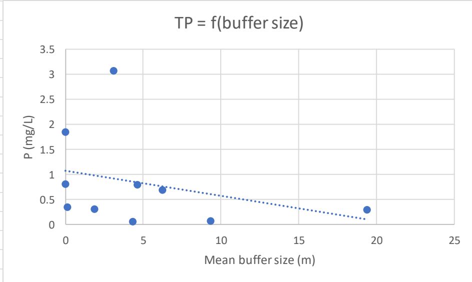
N = 10 ponds from 1 landuse type: extensive agriculture

Landuse type	Pond cluster	Pond code	TN	TP	CHLa	PC	Mean buffer size
Extensive agriculture	East Flanders	EF1_EX1	1.9	0.347	3414.88	203.78	0.125
		EF1_EX2	1.9	0.303	2956.36	97.99	1.875
	Antwerp	AW1_EX1	1.3	0.058	1293.32	19.35	4.3375
		AW1_EX2	0.9	0.066	2791.65	117.93	9.375
	Limburg 1	LB1_EX1	1.3	0.801	2683.70	248.07	0
		LB1_EX2	1.1	3.069	6555.48	366.36	3.125
	Limburg 2	LB2_EX1	2.5	0.794	45.55	498.70	4.625
		LB2_EX2	1.6	0.293	1980.35	643.67	19.4375
	Flemish Brabant	FB1_EX1	1.6	0.686	2579.46	284.62	6.25
		FB1_EX2	8.2	1.842	3145.18	979.35	0



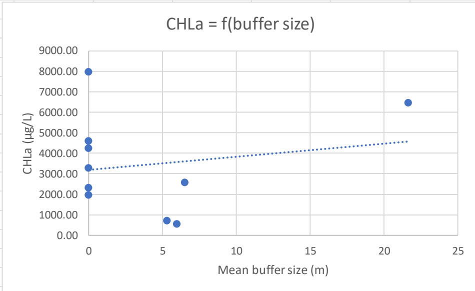


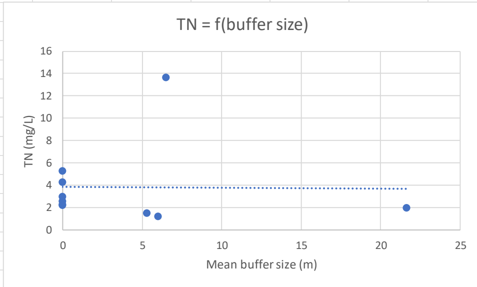


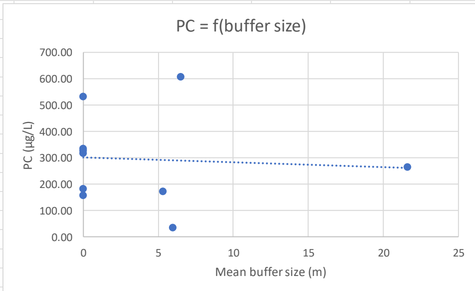


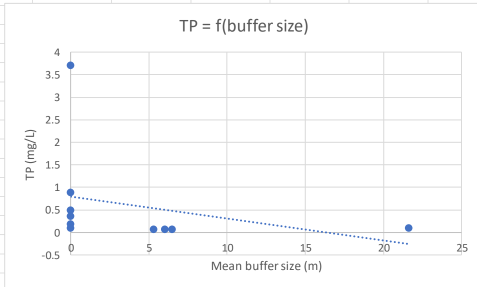
N = 10 ponds from 1 landuse type: intensive agriculture

Landuse type	Pond cluster	Pond code	TN	TP	CHLa	PC	Mean buffer size
Intensive agriculture	East Flanders	EF1_IN1	1.2	0.073	545.52	35.25	6
		EF1_IN2	4.3	3.708	3271.31	532.43	0
	Antwerp	AW1_IN1	3	0.893	7971.46	327.75	0
		AW1_IN2	2.6	0.494	2317.19	335.44	0
	Limburg 1	LB1_IN1	5.3	0.184	1951.04	159.37	0
		LB1_IN2	1.5	0.065	702.41	172.33	5.3125
	Limburg 2	LB2_IN1	2.3	0.356	4579.71	183.11	0
		LB2_IN2	2	0.102	6449.01	265.31	21.625
	Flemish Brabant	FB1_IN1	13.7	0.072	2556.09	607.01	6.5
		FB1_IN2	2.2	0.094	4241.76	317.10	0









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2021-2022

Spatio-temporal dynamics of biodiversity and greenhouse gas emissions in fish ponds

Résumé : This final report aims to give an overview of my internship at the Laboratory of Aquatic Ecology Evolution and Conservation at KU Leuven Department of Biology. During 5.5 months, I was involved in PONDERFUL, a project funded by the European Union which aims to understand to what extent ponds can be used as Nature Based Solutions (NBS) to mitigate and adapt to climate change. I was strongly involved in field data collection of more than 105 ponds of different types across Flanders including farmland ponds, crater ponds, ponds selected along an age gradient and newly created ponds. More specifically, I investigated the temporal dynamics of biodiversity and greenhouse gas emissions in the fish pond system of Midden-Limburg and I attempted to identify the drivers of such dynamics and emissions and in particular how these relate to the type of pond management. Finally, I was able to build further on the work I had already started in Germany and I quantified the size of the buffer strips around the set of Belgian ponds so that we could investigate the effect of buffer strip size on the ecological state of small ponds in agricultural areas in Belgium and Germany.

Mots Clés : PONDERFUL, North-East Belgium, land use gradient, pond age gradient, fish ponds, pond management type, local environmental pond variables, local and regional biodiversity, zooplankton, macrophytes, GHG emissions, bubble and diffuse fluxes, buffer strip size, pond trophic state, SIL 2022 conference

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