Rapport de stage individuel

5ème année

Bilan trophique et flux de carbone des mares temporaires forestières du plateau landais

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Presentation of the host institution

The host institution for my 5A internship is the Ecole Nationale Supérieure en Environnement, Géoressources et Ingénierie du Développement durable (ENSEGID). This is one of the 9 public engineer schools of Bordeaux INP that train engineers in several fields as environment and water resource management and geology, since 2011. This institution has several main missions that are education and research realised by researchers and teacher researchers. These researches are conducted to answer to specific needs of firms, work-study programs, and territorial communities.

These researches are realised thanks to a high scientific and technological level, and combined geosciences and georesources with the management of societal issues, and the knowledge of economic notions, societal responsibilities and sustainable development.

The Ecole Nationale Supérieure en Environnement, Géoressources et Ingénierie du Développement durable rallied researchers, teacher researchers, doctoral students, postdocs and several trainees, as well as a technical staff including engineers and technicians. In this institution, a scientific project is realised as the project REZIN.

Presentation of missions

During this internship, my mission took part of the project REZIN, i.e. Restauration de zones humides en amont du bassin versant des lacs médocains. In the context of global warming and the increase of summer temperatures, this project globally studies the impact of a decrease of water table level, mainly on ecosystems metabolism and carbon fluxes, around Hourtin, in the largest artificial coniferous forest mass in France, formerly swamp (Jolivet et al., 2007), and studies the impact of these areas on global carbon cycle. This project tries to find solutions to restore its hydrological functions, increase its quality in terms of physical and biogeochemical aspects, to protect wetlands and to restore ecological and hydroclimatic functions.

Within this project, I especially studied gas fluxes and trophic features at the level of twelve small ponds in Landes Plain in comparison to results reported for other ponds in bibliography. Carbon dioxide and methane fluxes were basically studied in comparison with the trophic level of these ponds, in order to analyse the effect of chemical conditions of water on gas fluxes. To do so, monthly samplings were realised and analysed on twelve ponds to determine gas fluxes and the overall trophic level of these ponds. Then, bibliographic data were collected in a database and used to determine the different types of responses in terms of carbon flux according to trophic parameters. Data of samplings were compared to bibliographic data to determine similarities and differences. A meta-analysis was realised to determine the possible link between trophy of a pond and gas fluxes with a high statistical significance.

In Annexe can be found an annexe (Annexe I) that refer to a tool used during this internship. This mission leads to the redaction of a scientific rapport and to a compilation of data collected for the meta-analysis (references in Annexe II). Moreover, the result of this work will be used for realising further analyses within the research project, according to all results (Annexe III, IV, V and VI).

Presentation of the progress of the mission

My internship lasts six months from 7th February to 5th August. The goal of this internship was to determine the relation between gas fluxes, i.e. carbon dioxide and methane concentrations and fluxes, and trophic features of water from ponds and physical parameters as pH and temperature. These analyses allow to specify the nature of the relationship and the need of restoration of these environments. During this period, my mission was separated in different parts, that correspond globally to the successive steps of scientific reflexion: these steps are presented in Table 1.

Table 1: Different steps of my internship and average time that each step took

Part of the mission	Average time
Bibliography and data harvesting	6 weeks
Field prospecting	3 weeks
Test experiment to establish and calibrate the protocol	2 weeks
Laboratory work	1 week
Processing data	4 weeks
Analysis of data	4 weeks
Redaction of the rapport and preparation of the oral defence	6 weeks

During my internship, each week, I discuss about each step of my mission with my internship supervisor. These discussions were useful to help me to continue my work on the right way and to concentrate on the relevant elements of my mission.

Introduction

The context of global warming and an increase of temperature during summer should lead to long-phase-decreased of the water table level. This is highlighted by the fact that, for 25 years, there is a decrease of piezometric levels and water level in headwater wetlands, called in this work temporary ponds (BRGM, 2015 and PNR des Landes de Gascogne, 2019). Moreover, an increase of water withdrawals for human usage as drinking water and agriculture is to predict (SIAEBVELG, 2020). Following long episodes of drought, net ecosystem metabolism should be modified according to air exposition time and the submersion time, to the day/night cycle and to annual variations. In this context, this work concentrates on temporary ponds that are ideal targets to study the effects of different parameters that can be modified due to climate change on ecosystems metabolism around these ponds, according to air exposition and the submersion time.

Wetlands realise many ecosystems services for humans. First, wetlands contribute to the climate regulation by assimilate carbon for 36% by the aerial biomass, 10% underground and in the organic matter and 54% in the soil organic carbon. This assimilation is double in forest wetlands (Bartholomée et al., 2018). Moreover, these areas provide hydrological service as flood controls, water table recharges, low water support, production of oxygen, important carbon storage and filter water and increase water quality (Fabre, 2019; Li et al., 2017 and SIAEBVELG, 2020).

These kinds of ponds can have different origins that are still discussing in scientific society: karstic origin, periglacial origin with melting ice lenses, and wind origin as for dunes creation (Merlet et al., 2007). However, many scientists are according to favours the melting ice lens origin (Berteaud et al., 2020), more than 6200 years ago (Texier et al., 2001). The supply of water in temporary ponds has been highlighted by the similarity between physico-chemical parameters of both water, by accumulation of pollutants from the watershed (BRGM, 2015 and Lischeid et al., 2018). However, this conclusion was realised, based on one measurement on five temporary ponds and cannot constitute a trophic balance as it is not annual measurements.

The temporary character of these ponds presents some specificities with regards to chemical parameters, thanks to episodic hypoxic periods. On the one hand, these periods allow denitrification and enhance phosphorous mobilisation that boosting primary production (Lischeid et al., 2018). Moreover, it leads to sulphate reduction and carbonate mineral dissolution (Lischeid et al., 2018). On the other hand, an inundation should reduce carbon dioxide flux (by three, according to Palmia et al., 2021 (416.5 to 158.2 $mmol.m^{-2}.d^{-1}$)), by limited bacteria activities, reduce methane emissions, increase redox potential and mobilise organic matter (Hamilton et al., 1994; Marcé et al., 2019 and Reverey et al., 2016). Third, a dry sediment releases a lot of CO_2 and little methane, only few days after there is no water, due to the increasing of aerobic bacteria activities and to the decreasing of anaerobic bacteria activities (Marcé et al., 2019). These dry phases have also a role in the conservation of temporary ponds by allowing mineralisation that evacuate a part of organic matter, but force species to adapt to these variable conditions (Reverey et al., 2016). Moreover, it appears that these temporary ponds have a low nitrogen nutriment stock (Jolivet et al., 2007). Finally, chemical parameters in water are also induced by the presence of phytoplankton: the higher concentration is, the higher the concentration of methane is (Holgerson et al., 2015).

Chemical parameters of water can also be induced by physical parameters. Indeed, an augmentation of temperature leads to an augmentation of carbon dioxide flux (Das et al., 2021 and Xiao et al., 2015).

Moreover, a reduction of pH leads to a release of phosphorous (Reverey et al., 2016). The surface and the ratio between surface and depth are factors of water chemical parameters, more precisely for carbon dioxide and methane concentration: the higher surface is, the higher and the most stable these concentrations are (Ferland et al., 2015; Holgerson et al., 2016 and Holgerson et al., 2017).

Vegetation has an important impact on water chemistry: a leaf can influence temperature with shadow, presence of plants leads to an increasing of dissolved oxygen, a decrease of phosphorous and nitrogen concentrations and a production of organic matter according to its species (Hornbach et al., 2020 and Li et al., 2017); submerged plants typically lead to radial oxygen loss, and thus sediment oxygenation, which in turn stimulates methanotrophy (Ribaudo et al., 2011). Moreover, plant's respiration release CO_2 and photosynthesis capture CO_2 (Joyard et al., 2021). Then, for example, siliceous algae influence concentration of Mg^{2+}, sulphate, silica, dissolved organic carbon and pH (Proult, 2014). Moreover, it has been proved that S*phagnum* has an important role in the acidity of water, added to polluted precipitation and bacteria activities which metabolise sulphur from SO_4^{2-}, increase by drought (Bergman et al., 1998 and Clymo et al., 1964). First, *Sphagnum*, when it dies, release -COOH groups that react with the environment and, thanks to the layer of sand (200 to 600 µm) which compose siliceous soil, that characterise the studied site, release H^+ (Canredon et al., 2019; Clymo et al., 1964 and Conseil Général des Landes, 2012). Secondly, sphagnum's cells are composed of molecules that imposes a pH of 3.3. With the difference between internal cell environment and exterior environment, there is exchanges and H^+ are released in water. After this evacuation, there is production of other H^+ and this process is repeated (Clymo et al., 1964).

The second main element that plays a direct role in the water chemical is the presence of bacteria and its activities, influence by physical and chemical parameters of the environment. Thus, methane is released by anaerobic fermentation (Joyard et al., 2021). Bacteria's activities are multiple and induce many effects as nitrification, favoured when there is less water and more oxygen (Palmia et al., 2021 and Reverey et al., 2016). On the contrary, ammonium is more and more available with the presence of water and less oxygen (Palmia et al., 2021). Moreover, bacteria reduce nitrate ions thanks to dissolve and particular organic carbon (Fabre, 2019). All these elements added to a high iron oxide content, a low degradation rate of cellulosic molecules and lignin from helophytes, favour organic matter, carbon and phosphorous storage (Grasset et al., 2011; Pankratov et al., 2009; SIAEBVELG, 2020 and Sobek et al., 2014).

In France, especially in the region Nouvelle-Aquitaine, the decrease of water table level, add to the degradation since the XIXe century of wetlands by draining, will impact the infilling of temporary ponds (Bartholomée et al., 2018 and PNR des Landes de Gascogne, 1995). According to the Conseil Général des Landes, 434 temporary ponds have been identified in the department (Conseil Général des Landes, 2011). These temporary ponds are located in wetland and bog forestry areas, that its surface has been reduced by a half between 1960 and 1990. Between 1950 and 2018, 38.7% of temporary ponds have disappeared (Berteaud et al., 2020).

These kinds of environment represent essential habitat for patrimonial and rare vertebrate, invertebrate and vegetal species such as *Caropsis verticillatoinundata*, *Baldellia ranunculoides*, *Drosera intermedia*, *Rhynchospora fusca*, *Triturus marmoratus*, *Emys orbicularis* and *Leucorrhina albifrons* (Jacquemart et al., 2004) (Figure 1).

Figure 1: Patrimonial species (Drosera intermedia, Emys orbicularis and Leucorrhinia albifrons from left to right)
Sources: https://inpn.mnhn.fr/photos/uploads/webtofs/inpn/2/217892.jpg ;
https://inpn.mnhn.fr/photos/uploads/webtofs/inpn/4/375224.jpg ;
https://upload.wikimedia.org/wikipedia/commons/a/a9/LeucorrhiniaAlbifrons2.jpg

Thanks to all previous elements detailed in this part, it is obvious that there are many conservation challenges against effect of climate change, planning works for hunting, for pine cultivation and for pine wastes, drain and tempest, and to preserve the patrimonial and ecological, as ecological corridors, aspects of this area (Berteaud et al., 2020). This information allows to establish management and action planning by the different actors. Moreover, this place is the location of many activities link to tourism, to pine forestry (80% of the surface), and cereal agriculture, that lead to nitrate and pesticide contamination (BRGM, 2015 and SIAEBVELG, 2020). All of these activities need a precise management of this area to avoid its degradation and restore it. These plannings depend on some documents and classification as Schéma d'Aménagement et de Gestion de l'Eau (SAGE), Directive-cadre sur l'Eau, the Parc Naturel Régional du Médoc charter, Zone Naturelle d'Intérêt Ecologique, Faunistique et Floristique (ZNIEFF) « Landes médocaines entre Hourtin, Carcans et Saint-Laurent » and department scheme of sensitive natural areas, which gives funding for actors (Conseil Général des Landes, 2012 and Département des Landes, 2015). Then, there are several institutions that play a role in the application of these measures and try conciliating the advantage of as many ecosystems services as possible, as the Agence de l'eau Adour Garonne, syndicates, department of Landes, townships, Conseil Général des Landes, Chambre d'Agriculture, National Forest Office (Bartholomée et al., 2018; Conseil Général des Landes, 2012 and SIAEBVELG, 2020). Some planning has been realised since the 1990s. Indeed, stripping, i.e. remove bog layers, make by vegetation, to extract mineral soil, are regularly performed on *Molinia*, which compose 80% of the surface of the wetland, to allow other rare and patrimonial species to develop (Conseil Général des Landes, 2011 and Manneville et al., 2020). Advantages compared to pasture are that vegetal biomass is exported and the cost of maintenance is lower (Jacquemart et al., 2004).

Currently, the ecological functioning of temporary ponds of the Landes Plain is generally unknown, especially with regards to carbon flux (Conseil Général des Landes, 2011 and Holgerson et al., 2015). However, recent studies small temporary ponds play a major role in carbon flux as carbon sink (Holgerson et al., 2016). The bibliography reveals that, among different types of ponds, there are similarities with regard to ecological functioning. Moreover, many studies highlight that there is a possible link between eutrophication and increasing carbon emissions and try to precisely unravel the environmental drivers triggering the degree of emission (Huttunen et al., 2003). In this context, this work highlights specificities and similarities among investigated ponds and in comparison to other studies in literature. The second aim is to highlight the possible link between trophy of water and carbon fluxes. Based on these elements, some questions can be asked: firstly, do the trophic degree of investigated sites of Landes Plain can be compared to bibliographic study sites? In which measure the trophic degree influence the carbon concentrations and fluxes (as carbon dioxide and methane) in the investigated sites and in the literature? To answer to these problematic, this work is divided in three parts. First, a meta-analysis is realised to collect trophic features and carbon fluxes of different classes of ponds from many scientific papers. In parallel, a monthly monitoring of trophic features and carbon fluxes is realised on a set of temporary ponds. These trophic features will be studied in the first part of this work. Secondly, a comparison between carbon fluxes' and concentrations' results from literature and the in-situ study is realised. Thirdly, this work studies how trophic parameters influence carbon dioxide and methane fluxes and concentrations.

Materials and Methods

Data collection from seasonal monitoring of temporary ponds

Study sites

In this work, twelve temporary ponds are studied located in Landes Plain, near the city of Hourtin, surrounded by pines culture (Figure 2). The area receives about 900 mm of water per year (Buquet, 2017). There is an oceanic temperate climate and a mean of 13.5°C over the year. It is exposed to the dominant North-West-West wind characterising the area (BRGM, 2015).

The studied sites are embedded within a larger set of temporary ponds, which are small, natural, acid and oligotrophic, sensible to eutrophication, freshwater ponds, supplied by water table of a thickness from 10 to 130 m, with a low slope, specific from Landes de Gascogne, and have a high patrimonial and ecological interest (Jolivet et al., 2007). There is a high iron oxide content in these ponds (SIAEBVELG, 2020). From the few existing knowledge, the temperature of water is high, and, thanks to a low mineralisation, the conductivity is low (BRGM, 2015). The average surface of investigated ponds of less than one hectare in this area is around 2870 m² (Conseil Général des Landes, 2011), for a diameter inferior to 100 m and a depth from 0.5 to 2 m (Jolivet et al., 2007 and Olivet et al., 2007). There is a uniformity of the sand layer all around the territory. These temporary ponds are located in low-slope area and its main source is water from the superficial aquifer. The water table flush in winter and is low in autumn (Conservatoire Régional d'Espaces Naturels d'Aquitaine, 2008).

Figure 2: Localisation of the 12 study sites

Source: Alexandre Fouache based on Google Satellite data

Samplings

For each of the twelve investigated sites, samplings were realised monthly, divided in two parts: water collecting in water column and flux measurements. Moreover, other additional information is compiled, indicated in Annexe I.

Each step of water collecting is realised three times, with water collecting from three different points of the temporary pond (spatial replicates). Water from all the twelve temporary ponds is sampled. Material is rinsed two times with water collected before the process to sampling manipulation. Before any manipulation, pH, conductivity, temperature and O_2 saturation and content are measured thanks to a multiprobe Multi 3630 IDS (SET G) (Figure 3). After processing these steps, several manipulations are realised to collecting water to analyse further chlorophyll-a, dissolved nutrient, methane concentrations, dissolved inorganic phosphorous and alkalinity.

Figure 3: Installation for water collecting
Source: Alexandre Fouache, 2022

First, thanks to a plastic syringe (TERMO SYRINGE, 50 mL) and a plastic tube, a glass serum bottle is filled thrice its volume with water that is poured without swirl. Then, this bottle is sealed with a rubber septum and a crimped metal capsule. This bottle is conserved protected from light. This step is realised once a site.
Secondly, with a 50 mL syringe and a filter Minisart (cellulosic acetate, 0.2µm), a tube of 15 mL is filled with filtered water, for nitrogenous forms (NO_3^-, NO_2^- and NH_4^+) and for dissolved silica (Si_d). This tube is conserved in a freezer.
Thirdly, with the same syringe and the same filter, a second tube of 15 mL is filled with filtered water, for dissolved inorganic phosphate (DIP or PO_4^{3-}), sulphate (SO_4^{2-}) and dissolved metal (Fe_d and Mn_d). With a micropipette (Eppendorf Research plus, 15081BA), 10 µL of HCl is added in the tube. This tube is conserved in a fridge.
Fourth, water for dissolved organic carbon (DOC) is sampled. A glass syringe and a metal filter holder are used to avoid contamination. A GFF filter (0.7 µm, 25 mm diameter, burnt at 500°C), conserved in an aluminium sheet, is placed in the filter holder thanks to a clamp. After placed the metal filter holder on glass syringe, filter 25 mL of water in a glass bottle (cleaned with detergent and burnt at 500°C). Thanks to a micropipette, 50 µL of HCl is added in the bottle. This bottle is conserved in a fridge. This step is realised once a site.
Finally, thanks to the 50 mL plastic syringe and a plastic filter holder and a filter Whatman, 100 mL of water is filtered in a plastic bottle of 125 mL, for the alkalinity. Then, the first time this manipulation is realised on a site, the filter is conserved on a tube, conserved in the freezer, to analyse chlorophyll-a in water.

Concerning the flux measurements, there are represented by a gradient thanks to the diffusive law of Fick (Xiao, 2015), and carbon dioxide basic concentration in air is of 400 ppm (Joyard et al., 2021). Carbon dioxide fluxes are measured thanks to a closed flux chamber and a CO_2 probe, Aeroqual S500L 2411201-7046. A protocol has been established during this work. However, there are many issues during the trainee about these measurements. Indeed, there are problems about incubation time, volume of air chambers and Air-Water interface surface. Thus, after many tries on sites, these problems have finally been solved.

Finally, carbon dioxide fluxes are measured on six temporary ponds (14, 94, 150, 162, 165 and 246), at the Air-Water interface, and at the Air-Soil interface, in the flood zone. However, the work on Air-Soil interface is not described here (Annexe III). Two probes linked to different flux chambers, thanks to hydraulic tubes, measure at the same time fluxes at the Air-Water interface, during 30 minutes on a surface of 0.134 m² (33.5 x 40 cm). The rectangular aquatic flux chamber volume is 4.5L (0.042 m height). It floats thanks to a polystyrene plaque to which it is stuck with silicon. Volume in hydraulic tubes is negligible. These flux chambers are placed at two different places, at most on the middle of the pond (Figure 4). To avoid any bubble gas evacuation, once flux chambers are put on the site, operators do not touch it, and nobody walks around it. In this work, flux data were collected on June. Data collecting in July are not used because of another problem of leak.

Figure 4: Installation for flux measurements (aquatic chamber)
Source: Alexandre Fouache, 2022

The flux is calculated by using the slope of the relation between the concentration of carbon dioxide in the flux chamber and the time of incubation in hour, and then converted in desired units :
$\mathbf{F = 1.8*V*P/s}$ (with F = CO_2 flux in $mg.m^{-2}.h^{-1}$, V = volume of chamber in m^3, P = slope and s = surface in m²).

Manipulation

Water samplings realised during field prospecting lead to analyses in the laboratory to determine some chemical parameters for each sample. Thus, following dissolved compounds are measured: phosphates, dissolved siliceous, nitrites, nitrates, ammonium, dissolved iron, dissolved manganese, sluphate, sulphides, bromide, dissolved organic nitrogen and dissolved inorganic phosphorous. These chemical parameters are realised by colorimetry with spectrophotometry. This technique consists of inducing the formation of coloured chemical complex by adding one or several reagents to the sample. The intensity of the coloration is measured thanks to a spectrophotometer, calibrated thanks to standard range and rules to a specific wave length. The sample is placed on a translucid cuvette, crossed by a light beam in the spectrophotometer: the most the sample is coloured, the most the light will have difficulties to cross the sample. This difference of lighting intensity corresponds to the absorbance of samples. The intensity of colouration is proportional to the concentration of a certain element. So, it is possible to determine this concentration thanks to the Beer-Lambert Law:

$$\mathbf{A = \xi \times L \times C}$$

A is the absorbance of the solution, without unity
ξ is the coefficient molar extinction in L.mol^{-1}.cm^{-1}
L is the length of the cuvette crossed by light, in cm
C is the molar concentration in mol.L^{-1}

Sample's concentration is determined thanks to a previously made calibration curve against the absorbance values of a standard range. As I only participate a little bit to these colorimetric measurements, the precise protocol will not be described more than previously presented.

Then, the second phase of manipulation consists of measure alkalinity in the sample. Total alkalinity is defined by the difference between the sum of cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, NH_4^{+}, Fe^{2+} and Mn^{2+}) and the sum of anions (SO_4^{2-}, Cl^{-} and NO_3^{-}) that intervene in strong acid/strong base couples (Buquet et al., 2017). This manipulation is realised by titration. A titrator Metrohm 716 DMS Titrino is used (Figure 5). After the purgation of the system to avoid any contamination in samples and calibrate the system, a beaker is filled with 50 mL thanks to a graduated pipette of 50 mL (MBL, Brand ISO 648, Silver brand eterna, +-0.07 mL). A magnetic stirrer (728 Stirrer) is added in the beaker. After diving the probe in the solution, the system is started. The titrator automatically add a solution of HCl (0.1 mol.L^{-1}) and detect equivalences. These equivalences are saved and use to determinate alkalinity. Some drops of methyl orange are added in the solution before the manipulation to control equivalence.

Thanks to the measurement of alkalinity, it is possible to calculate the concentration of carbon dioxide in water. In database from field prospecting, this concentration is calculated from the alkalinity. This calculation leads to some issues. Indeed, in this kind of pond where pH is low (inferior to 6) and where DOC values are elevated, the calculation of concentration of carbon dioxide is not accurate (Abril et al., 2015). So, carbon dioxide calculated concentrations are not used in this work, as my colleagues are working on a protocol for directly measuring CO_2 by gas chromatography.

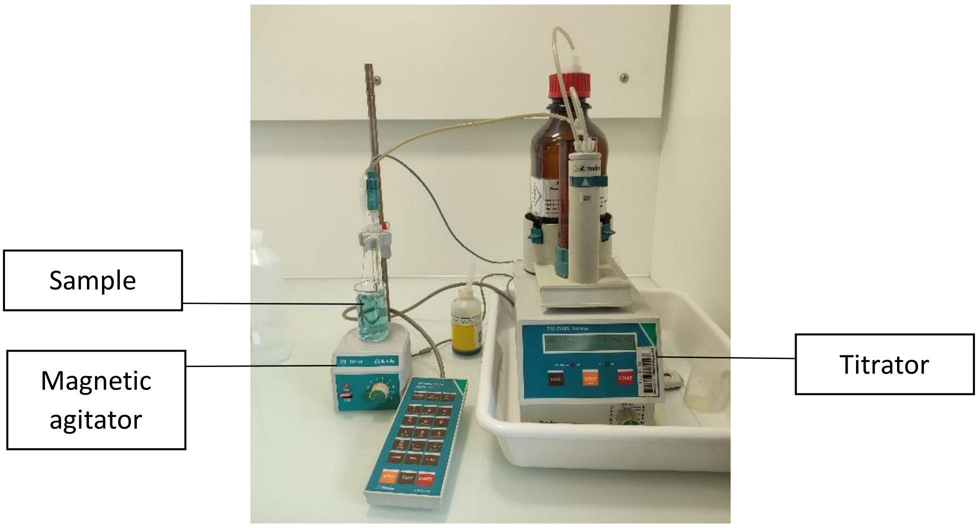


Figure 5: Alkalinity measurement manipulation
Source: Alexandre Fouache, 2022

The next step of sampling analyses is to analyse methane concentration in water. This concentration is measured thanks to a gas chromatograph Agilent 4890D (Figure 6). The protocol Head space is used. First, the sample is weighed. Thanks to a needle inserted in the rubber stopper and a needle linked to a syringe that contains dinitrogen, a gas bubble of dinitrogen (10 mL) is realised in the sealed glass bottle. By the augmentation of pressure, a part of the sample is empty from the needle alone. Secondly, the sample is weighed. Then, gas from the liquid phase is transferred in the gas phase by vigorously shaking the bottle for at least 60 seconds. Then, a part of the gas is taken with a syringe and a needle and injected in the gas chromatograph. The difference of pressure and injection of gas lead to migration of gas on the chromatograph, carry by helium, which analyses gas composition by volatile component separation. The analysis is realised by injecting at the beginning and at the end of the measurement 0.5 mL of standard concentration of methane, in order to assure that the signal from the machine is stable. Between that, two samples can be analysed, twice each, by injecting 0.5 mL of gas contained in the bottle. Output data are converted to get a concentration of methane in the sample, thanks to a worksheet.

These concentrations of methane are used later to determine methane diffusive fluxes. Indeed, thanks to wind speed, concentration of methane in water and in air, it is possible to estimate these fluxes accurately (for detailed calculations, see Ribaudo et al., 2018). To compare data, methane diffusive fluxes from June are used.

Figure 6: Gas chromatograph installation
Source: Alexandre Fouache, 2022

Finally, the analyse chlorophyll-a concentration is realised in two days. The first day, 10 mL of acetone (90%) is added to the samples thanks to a micropipette. The acetonated filter, which filtered a known volume of water, is placed on a fridge until the next day, without light. The second day, after 12 hours sample is placed on a centrifuge (3000 turn by min during 10 min, Jouan C412) to disintegrate the filter. 3 mL of the supernatant is analysed to the spectrophotometer (Thermo scientific, Helios Epsilon) in a quartz cuvette on four wave lengths: 750, 664, 647 and 630 nm. The calibration is realised with acetone at 90%. Then, the concentration of chlorophyll-a is calculated thanks to an internal worksheet (Lorenzen, 1967).

Statistical analysis: classification of ponds

A principal component analysis PCA is realised on Excel thanks to Xlstat. This PCA allows to classify the twelve temporary ponds in different classes according to its trophy. This PCA allows to highlight differences or similarities between the twelve studied sites. For this statistical analysis, data from the twelve ponds are used, collected between November, 2021 and July, 2022.

Data collection from literature (meta-analysis)

By searching information about ponds, its trophy and carbon fluxes for the state-of-the-art and the meta-analysis, articles from the database of the ENSEGID and from website Web of Knowledge were used to establish this review and this meta-analysis. There are number of articles found on the platform Web of Knowledge by using different keywords (Table 2). Only research with at least one result is shown. All articles that have been read and information linked to the subject were selected to help to go further in analyses.

Table 2: Number of results of articles found in Web of Knowledge by searching some keywords on 04-2022

Keyword 1	2	3	4	5	Number of results
Dry inland water	Oligotrophic	Carbon			3
Dry inland water	Mesotrophic	Carbon			1
Dry inland water	Eutrophic	Carbon			4
Dry inland water	Oligotrophic	Phytoplankton			8
Dry inland water	Mesotrophic	Phytoplankton			4
Dry inland water	Eutrophic	Phytoplankton			9
Meta-analysis	Freshwater				711
Meta-analysis	Carbon				3350
Meta-analysis	Freshwater	Carbon			66
Meta-analysis	Freshwater	Carbon	Pond		3
Origin	Carbon	Organic	Dissolve	Water	9
Origin	Carbon	Organic	Particular	Water	262
Pond	Temporary	Oligotrophic	Carbon		3
Pond	Vernal	Oligotrophic	Carbon		1
Pond	Temporary	Eutrophic	Carbon		4
Pond	Vernal	Eutrophic	Carbon		1
Pond	Hydroperiod	Eutrophic	Carbon		1
Pond	Temporary	Oligotrophic	Phytoplankton		4
Pond	Temporary	Eutrophic	Phytoplankton		11
Pond	Vernal	Eutrophic	Phytoplankton		1
Small lake	Temporary	Oligotrophic	Carbon		4
Small lake	Vernal	Oligotrophic	Carbon		1
Small lake	Temporary	Eutrophic	Carbon		6
Small lake	Vernal	Eutrophic	Carbon		1
Small lake	Temporary	Oligotrophic	Phytoplankton		1
Small lake	Vernal	Oligotrophic	Phytoplankton		1
Small lake	Temporary	Mesotrophic	Phytoplankton		1
Small lake	Vernal	Mesotrophic	Phytoplankton		1
Small lake	Temporary	Eutrophic	Phytoplankton		9
Small lake	Vernal	Eutrophic	Phytoplankton		3
Vernal pool	Oligotrophic	Carbon			1
Vernal pool	Eutrophic	Carbon			1
Vernal pool	Oligotrophic	Phytoplankton			1
Vernal pool	Mesotrophic	Phytoplankton			1
Vernal pool	Eutrophic	Phytoplankton			2

Concerning data for meta-analysis, 33 papers have been selected for being linked to the subject of this work (Annexe II). One paper corresponds to one study that regroup data from one or several sites.

Each separate data from one site has been collected in the database. However, face to issues of certain dataset that already are means of single data but does not present standard deviation and/or number of replicates, each of these data is considered as single data. Moreover, when several measurements have been realised across time on the same site, all these data have been taken into account; nevertheless, it has to be noticed that most of the studied have been performed during summer (between April and September). These 33 articles have been selected thanks to different parameters according to study sites and measurements. First, study sites must be inundated, at least during a part of the year. These areas have been divided in 3 types for the analyses: natural ponds (19 papers), temporary ponds (11 papers) and artificial ponds (5 papers). Secondly, the paper needs to contain carbon dioxide and/or methane fluxes and/or concentration. Thirdly, water points need to represent a small surface inundated - comparable to study sites in this work – between 10 m² and 20000 m². Data are extracted from tables and graphs. Thus, for each paper, following data has been extracted: date of sampling, carbon dioxide and methane concentrations and fluxes, pCO_2, carbon fluxes, surface, depth, water temperature, conductivity, pH, O_2 saturation and concentration, DOC, total organic carbon (TOC), alkalinity, ammonium, nitrate and chlorophyll-a concentrations, organic matter, total phosphorous, DIP, sulphate and Fe^{2+} concentrations, total nitrogen, calcium, potassium, magnesium and sodium concentrations.

Meta-analysis: an overview of the database

Several statistical tests are realised in this work in the context of meta-analysis. These analyses are processed on Microsoft Excel 365. First, about carbon fluxes and concentrations and trophic features, differences are studied between different types of ponds (natural (NP), artificial (AP), temporary ponds (TP) from literature and investigated sites (IP)) thanks to a Student T-test (bilateral, heteroscedastic), in a confidence interval of 95 and 99%. H0 is that samples are significatively equivalent. H1 is that samples are significatively different.

Secondly, correlation matrix, based on correlation coefficient *r*, is realised to have an overview on possible links between parameters (Zar, 2021). To realise this matrix, logarithm of all values is calculated. Indeed, this transformation allows to linearise every data comparison. Then, these correlation coefficients are used to determine the significance of relation between trophic features (parameters and concentrations) and flux values. This significance is defined in a confidence interval of 95%.

Results

Trophy of investigated ponds

Classification of investigated sites

The PCA has been realised by using data from the twelve temporary ponds, from November to July. Parameters were selected by its relevance and by data harvesting, because a PCA need a database without any lack of information. So, parameters that have been selected are conductivity, oxygen saturation, pH, DIP, NO_3^-, NH_4^+, DOC, chlorophyll-a concentration and CH_4 concentrations. Thus, PCA represents principal parameters of the database that can define the different axes, and highlights the temporary ponds with similarities and differences (Figure 7).

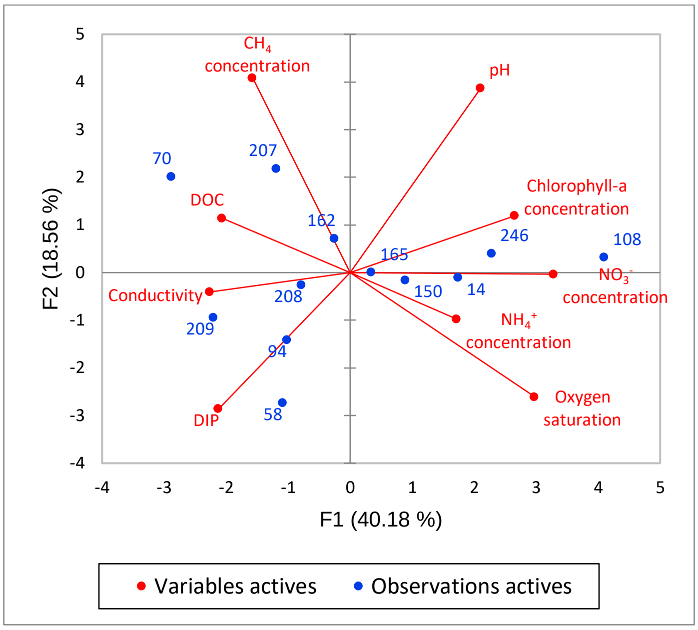


Figure 7: PCA of field prospecting database

The two axes conserved 58.75% of the basic data. This analysis highlights that, mainly, conductivity, DOC, chlorophyll-a and NO_3^- concentration explain well the X axis and CH_4 concentration and pH explain the best the Y axis. About investigated ponds, PCA highlights that many temporary ponds should present similar responses (14, 94, 150, 162, 165, 208). Temporary ponds 108 and and 246 could be classified in a group that present an important concentration of NO_3^-. Thirdly, pond 58 presents a low pH. Pond 207 presents a high methane concentration. Finally, two ponds can be classed independently: 70, that present a high concentration of DOC and 209, that present a high conductivity.

More accurately, to predict the potential effect of temporary ponds on the carbon cycle, it is important to study the trophy of investigated ponds across time.

Trophy of investigated sites

In this work, the used measurements are realised between November 2021 to July 2022, according to the measured parameter, on the twelve ponds. There are several different responses. Temperature of water increase from November to July (between 10 and 30°C more in 8 months). First, there are parameters that are stable across time as pH, DOC, O_2 concentration, conductivity and SO_4^{2-} concentration. Moreover, there is an important range between the different temporary ponds (from 3.88 to 7.35 for the pH and from 0.85 to 33.6 ppm for the DOC).

Then, there are some parameters that fluctuate most between each month. This is the case for DIP, NO_2^- and alkalinity, for example. These two first parameters present a maximum in January-February and in April for some temporary ponds (58, 95, 108, 162, 207, 208 and 209). These variations are also presented for other parameters as Fe^{2+}, NO_3^- and Sid that increase during winter before reducing (from 1.59 to 28.51 µmol.L^{-1} for Fe2+) (Annexe IV). Moreover, NH_4^+, methane concentration and chlorophyll-a seems to be maximum in summer and in autumn and is minimum during winter and spring (from 0.57 to 51.65 µg.L^{-1} for temporary pond 108 in terms of chlorophyll-a) (Figure 8).

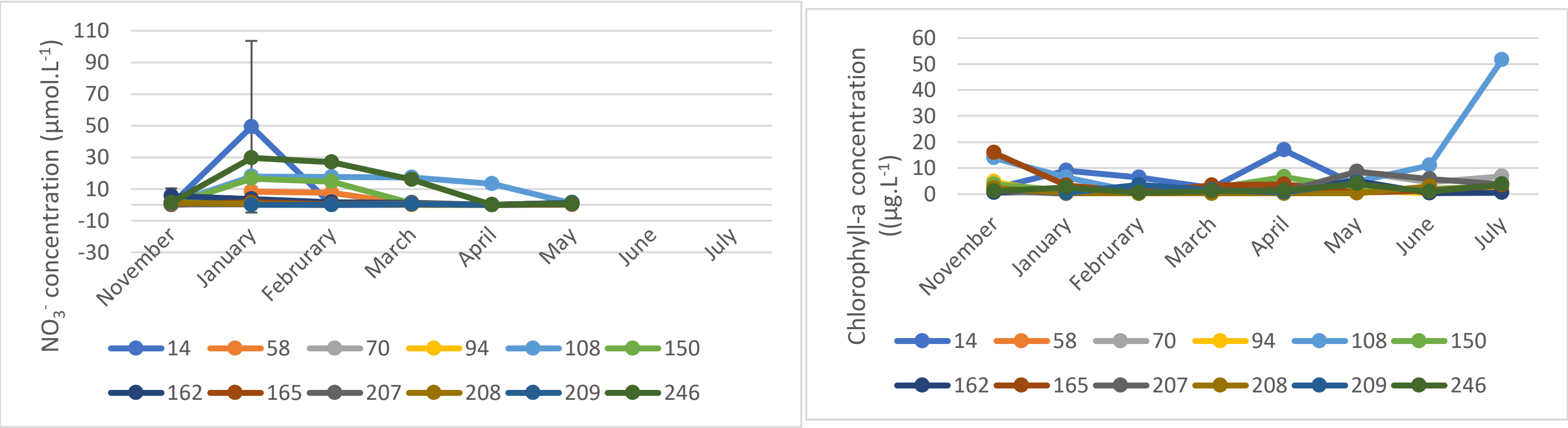


Figure 8: NO_3^- and chlorophyll-a concentrations in investigated ponds across time

Comparison between trophy of investigated sites and ponds studied in the literature

Thanks to data previously collected, from April to June, and data collecting for the meta-analysis, it is possible to determine similarities and differences between different types of ponds (natural, artificial and temporary ponds) to know if it is possible to predict responses in terms of carbon fluxes in investigated sites (Annexe V). This analysis allows to highlight which kinds of ponds are different, and according to which feature (Table 3).

Table 3 : Results of t-test about trophic features from spring and summer data

In light grey : significative difference in a confidence interval of 95%

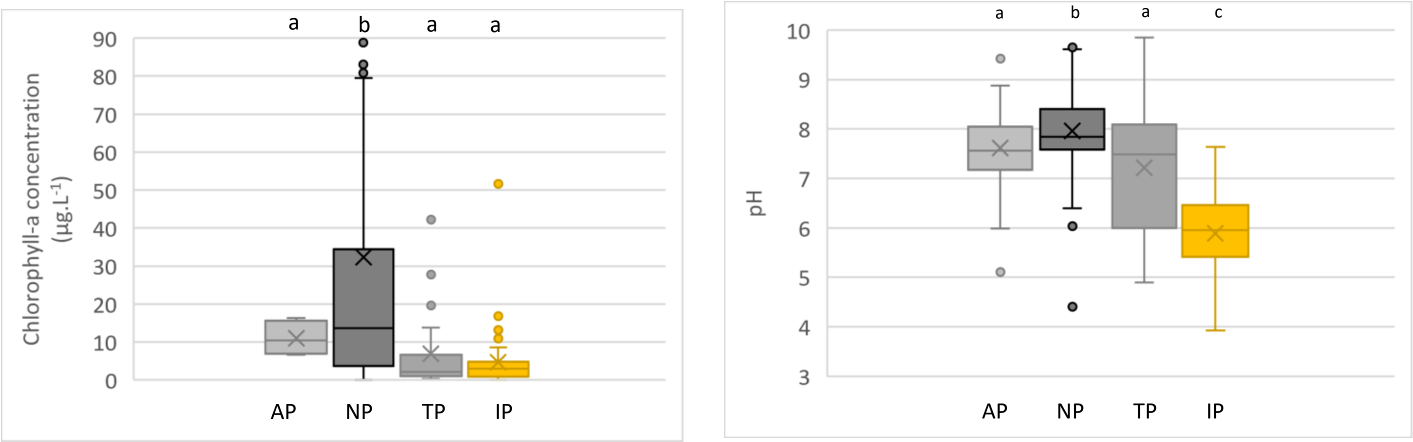
In dark grey : significative difference in a confidence interval of 99%

AP = Artificial ponds, NP = Natural ponds, TP = temporary ponds and IP = Investigated ponds

	Temperature	Conductivity	pH	O_2 saturation	O_2 content	DOC	NH_4^+	NO_3^-	Chlorophyll-a	DIP	SO_4^{2-}
AP-NP	0.000	0.001	0.000	n.a.	0.000	0.007	0.290	0.000	0.000	0.012	0.273
AP-TP	0.002	0.226	0.084	n.a.	0.000	0.848	0.478	n.a.	0.208	0.873	0.000
AP-IP	0.000	0.000	0.000	n.a.	0.000	0.232	0.130	0.398	0.061	0.001	0.000
NP-TP	0.000	0.000	0.002	0.000	0.074	0.005	0.585	n.a.	0.000	0.124	0.203
NP-IP	0.046	0.000	0.000	0.086	0.051	0.000	0.526	0.000	0.000	0.060	0.254
TP-IP	0.000	0.028	0.000	0.000	0.286	0.322	0.982	n.a.	0.345	0.041	0.000

This statistical analysis highlights that there are mainly differences between each type of pond. However, there are some similarities, mainly in terms of nitrogenous features (TP and IP are similar at 98.2%). Moreover, investigated sites present many differences with temporary, artificial and natural ponds from the literature (Figure 9).

DIP data from literature are not enough to be relevant in this work.



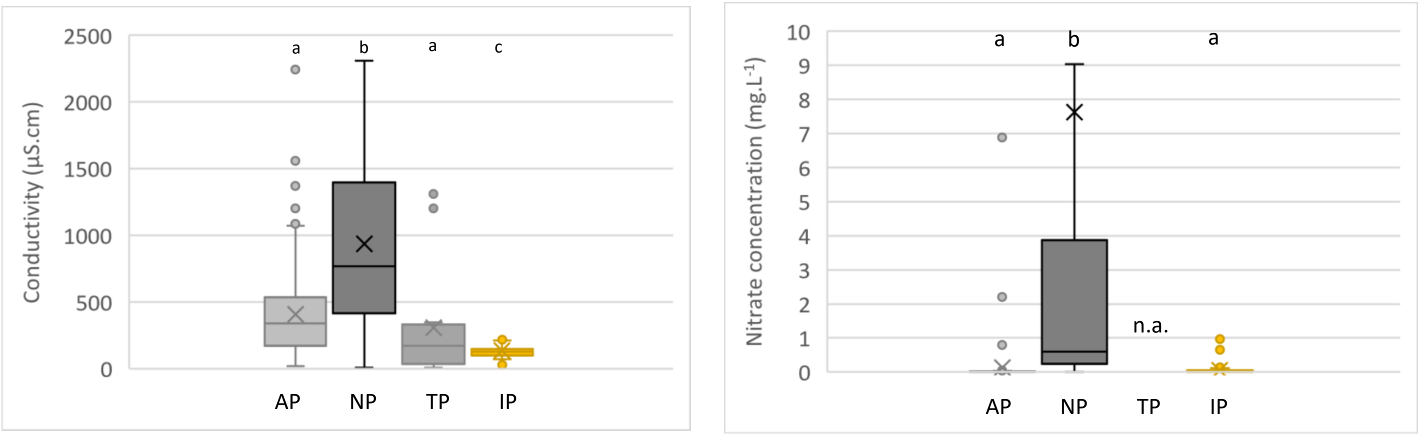


Figure 9 : Trophic features according to type of pond
AP = Artificial ponds, NP = Natural ponds, TP = Temporary ponds, IP = Investigated ponds
a, b, c = significance equality

Carbon flux in ponds

Flux CO_2 from investigated sites and literature

The meta-analysis reveals three types of ponds: natural ponds (n=545), that are non-human-influenced ponds and that are always in water, artificial ponds (n=195), that are human-modified ponds, and temporary ponds (n=59), that correspond to the class of study sites in this work. In the bibliography, the collection of data about carbon dioxide allows to highlight some difference and similarities between the three types of ponds (Table 4).

Table 4 : Results of t-test about carbon dioxide
In dark grey : significative difference in a confidence interval of 99%
AP = Artificial ponds, NP = Natural ponds, TP = temporary ponds, IP = Investigated ponds

	CO_2 concentration	CO_2 flux
AP-NP	0.000	0.000
NP-TP	0.126	0.000
AP-TP	0.000	0.008
AP-IP		0.000
NP-IP		0.285
TP-IP		0.000

This analysis highlights ponds are significantly different in terms of CO_2 concentrations and fluxes, except between natural ponds and temporary ponds and natural ponds and investigated ponds according, respectively, to CO_2 concentration and flux (12.6 and 28.5% of similarities) (Figures 10 and 11).

Thus, there are globally significant differences between each type of ponds, in terms of dioxide carbon fluxes and concentrations. However, some tendencies can be observed. Indeed, results from metanalysis show that temporary ponds are more variable than other ponds according to these parameters. These elements can be compared to the measurement on investigated ponds. To get values of flux, concentration in ppm were converted in $mg.m^{-3}$ by multiply the value in ppm by 1.8 (Terrie, 2006). Thus, on June 2022, investigated ponds present a mean of 7.9 $mmol.m^{-2}.d^{-1}$ (se = 4.4)

(Table 5) (equivalent to 95.1 mgC.m^{-2}.d^{-1}). Thus, two groups of responses can be highlight. Temporary ponds 162 and 165 present a higher carbon dioxide flux than others (respectively 21.4 and 43.8 mmol.m^{-2}.d^{-1}). Ponds 94, 150 and 246 present a carbon flux lower, but still positive (respectively 4.1, 2.9 and 4.8 mmol.m^{-2}.d^{-1}). Then, pond 14 is the exception with a negative flux (-17.8 mmol.m^{-2}.d^{-1}). Finally, data flux measurements are relatively lower than data fluxes from literature, with an accordance with natural ponds' data.

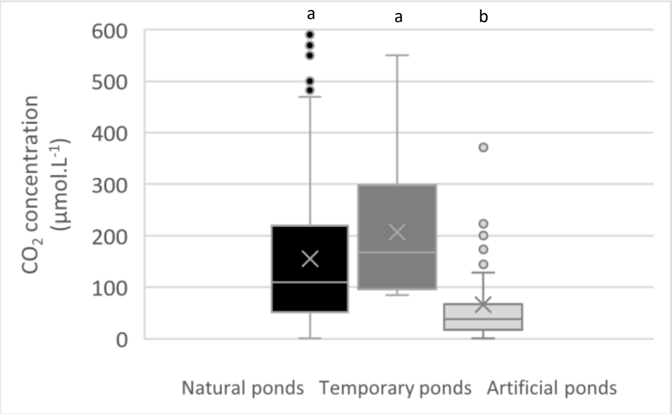


Figure 11 : Carbon dioxide concentration according to ponds' type
a, b = significance equality

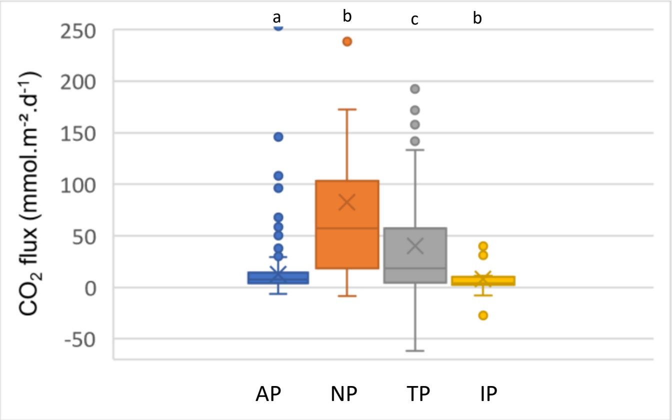


Figure 10 : Carbon dioxide fluxes according to ponds' type
a, b, c = significance equality
AP =Artificial ponds, NP = Natural ponds, TP = Temporary ponds, IP = Investigated ponds

Table 5: CO₂ flux mean values measured by floating flux chambers in 6 temporary ponds in June 2022

Temporary pond	CO$_2$ aquatic flux mean (mmol.m^{-2}.d^{-1})	Standard deviation	Number of replicates
14	-17.8	13.89	2
94	4.1	2.14	4
150	2.9	1.40	4
162	21.4	17.06	4
165	43.8		1
246	4.8		1

Flux CH$_4$ from investigated sites and literature

Then, in the bibliography, the collection of data about methane allows to highlight some difference and similarities between the four types of ponds (Table 6).

Table 6 : Results of t-test about methane
In light grey : significative difference in a confidence interval of 95%
In dark grey : significative difference in a confidence interval of 99%
AP = Artificial ponds, NP = Natural ponds, TP = temporary ponds and IP = Investigated ponds

	CH$_4$ concentration	CH$_4$ flux
AP-NP	0.311	0.079
NP-TP	0.003	0.000
AP-TP	0.309	0.041
AP-IP	0.107	0.072
NP-IP	0.000	0.593
TP-IP	0.000	0.001

This analysis highlights that natural ponds are globally significantly different to temporary ponds. Moreover, investigated ponds are significantly different to temporary ponds (Figure 12).

Moreover, thanks to data collected until June, it is possible to observe that methane concentration and fluxes increase during summer (from 0.64 µmol.L^{-1} to 25.05 between February and June for pond 207, for example) (Figures 13 and 14).

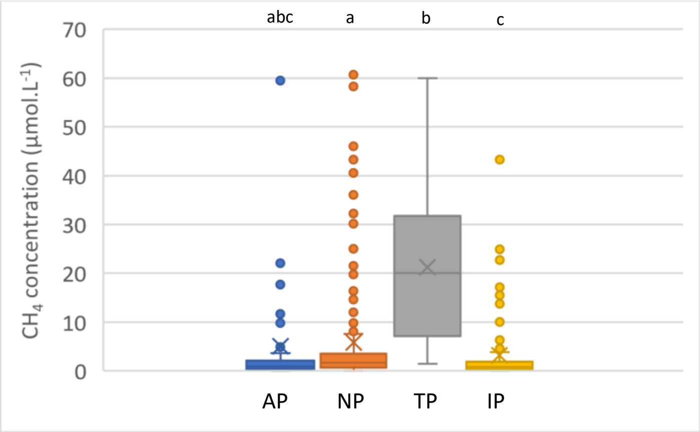


Figure 12 : Methane concentration according to type of pond
AP = Artificial ponds, NP = Natural ponds, TP = Temporary ponds, IP = Investigated ponds
a, b, c = significance equality

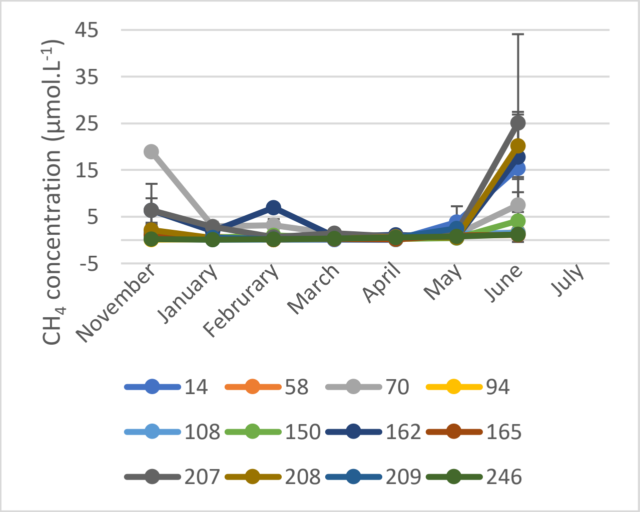


Figure 13 : Methane concentration across time

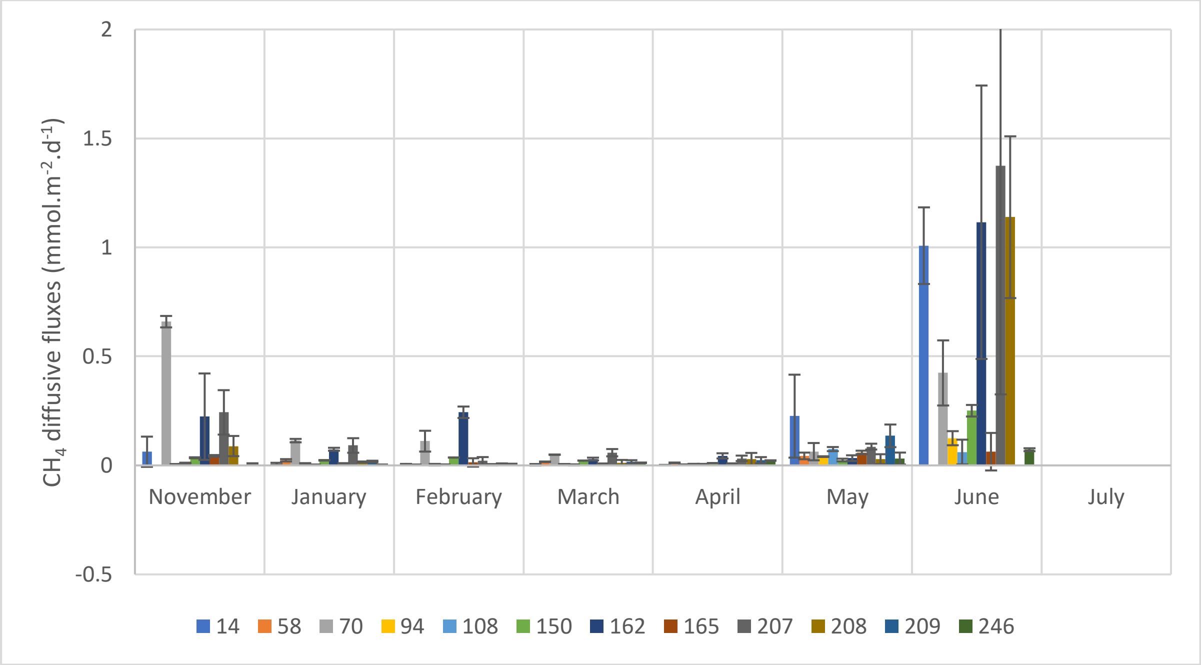


Figure 14 : CH_4 diffusive flux across time

As this concentration allows to calculate methane diffusive flux, it is possible to compare more precisely these results to literature (Figure 15).

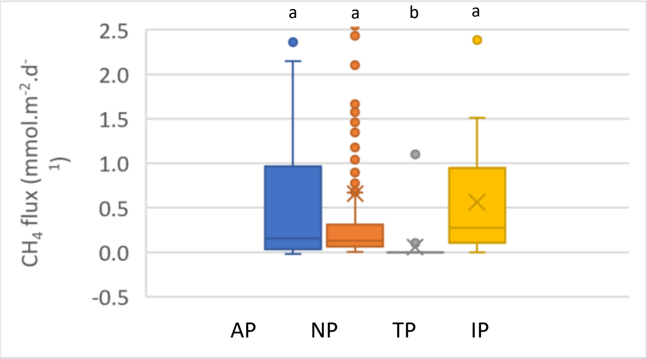


Figure 15 : Methane fluxes according to type of pond
AP = Artificial ponds, NP = Natural ponds, TP = Temporary ponds, IP = Investigated ponds
a, b, c = significance equality

It seems temporary ponds present higher concentrations of carbon, mainly of CH_4 (CH_4 flux's mean of 4.26 mmol.m^{-2}.d^{-1} for temporary ponds, 0.66 for natural ponds and 0.06 for artificial ponds (standard deviations respectively equal to 20.36, 2.44, 0.25, 0.63). These elements can be compared to the measurement on investigated ponds. In June 2022, CH_4 flux of investigated sites is 0.56 mmol.m^{-2}.d^{-1} with a standard deviation of 0.63 (range from 0 to 2.38). So, compared to bibliographic data, the studied ponds are below the majority of values.

In this part, flux parameters on different ponds have been highlighted. In the next part, this work will try to demonstrate the possible existence of a link between trophic parameters and carbon fluxes.

Influence of trophy on flux

Thanks to bibliographic data, many correlations between trophy and carbon-related parameters are highlighted (Table 7). The complete correlation matrix and the number of replicates for each relation is presented on Annexe VI.

This matrix highlights that carbon fluxes and concentrations are linked to several physical and chemical parameters. About physical parameters, carbon fluxes and concentration are mainly negatively correlated to water pH and dioxygen composition (Figures 16 and 17). Indeed, carbon dioxide induces a reduction of pH and, in water, gases are balanced. So, if the concentration of carbon dioxide increase, the concentration of dioxygen will decrease.

Database highlights that carbon parameters are narrowly linked to the majority of chemical parameters. Moreover, methane and carbon dioxide parameters can be influenced by different parameters. For example, on the one hand, total phosphorous influences positively both methane concentration and carbon dioxide flux (Figures 18 and 19).

On the other hand, total nitrate is significantly and positively correlated to carbon dioxide flux and is not significantly correlated to methane concentration (Figures 20 and 21).

So, this study highlights that, to influence the carbon cycle, it is important to control trophic features in general.

Table 7 : Correlation matrix about carbon parameters from bibliographic data

In light grey: significant relation in a confidence interval of 95%

	$[CO_2]$	pCO_2	$[CH_4]$	CO_2 flux	CH_4 flux	C flux
$[CO_2]$	1.00					
pCO_2		1.00				
$[CH_4]$	0.48	0.37	1.00			
CO_2 flux	0.89	0.71	0.30	1.00		
CH_4 flux	0.27	0.65	0.77	0.45	1.00	
C flux	0.12		0.61		0.92	1.00
Surface	-0.17	-0.80	-0.30	-0.06	-0.03	-0.19
Depth	0.18		0.04	-0.09	0.24	0.92
Water T	-0.04	-0.52	0.03	0.03	0.18	0.04
Conductivity	0.07	-0.11	0.07	0.08	-0.06	
Water pH	-0.46	-0.41	-0.09	-0.52	-0.35	
O_2 Saturation	-0.59		-0.46	-0.54	-0.36	
O_2 content	-0.53	-0.79	-0.44	-0.46	-0.38	
DOC	0.31		0.27	0.15	0.10	-0.75
TOC	0.87		0.21		-0.09	
Total Alkalinity		0.00	0.02	-0.01	0.10	
Ammonium	0.41		0.84	0.19	0.15	
Nitrate	0.39		0.04	-0.01	-0.15	
Chl-a	0.10	0.82	0.29	-0.30	0.10	
Total P	0.36		0.57	0.29	0.38	0.79
DIP	0.88		0.26	0.42	0.63	
SO_4^{2-}	-0.10		0.76	0.87	0.45	
Fe^{2+}	0.90		0.99		0.89	
Total N	0.69		0.47	0.65	0.37	
Ca^{2+}					-0.83	
K^+					-0.74	
Mg^{2+}					-0.82	
Na^+					-0.78	

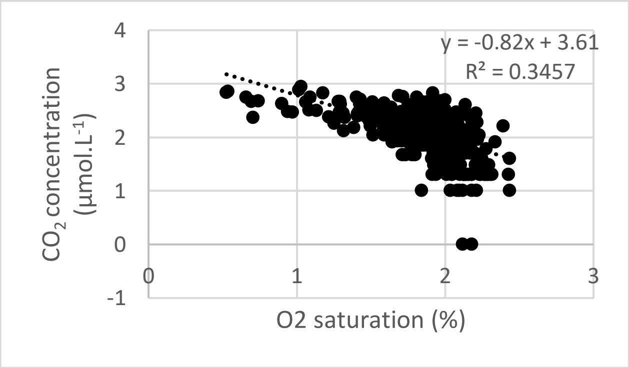


Figure 16 : Carbon dioxide concentration compared to dioxygen saturation

Significantly correlated in a confidence interval of 95%

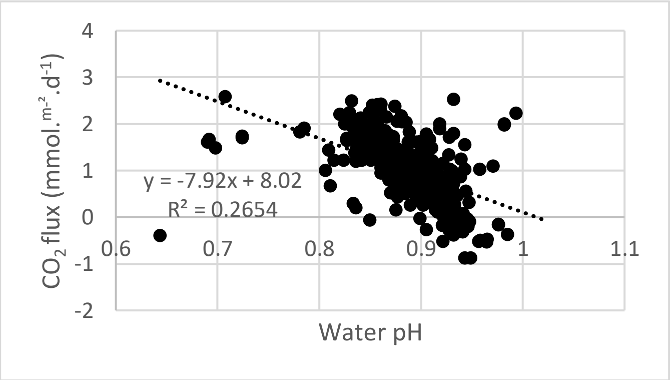


Figure 19 : Carbon dioxide flux compared to water pH

Significantly correlated in a confidence interval of 95%

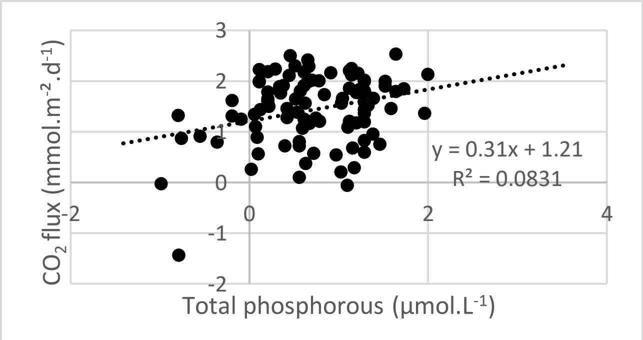


Figure 18 : Carbon dioxide flux compared to total phosphorous concentration

Significantly correlated in a confidence interval of 95%

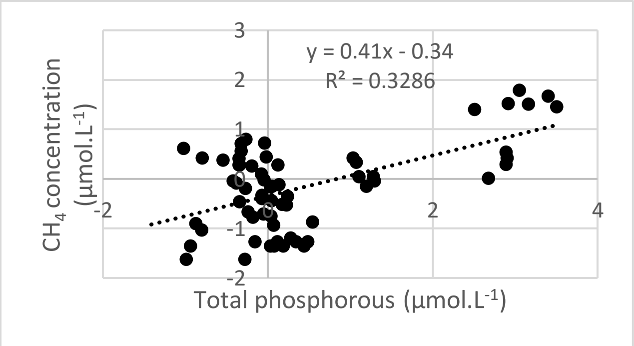


Figure 17 : Methane concentration compared to total phosphorous concentration

Significantly correlated in a confidence interval of 95%

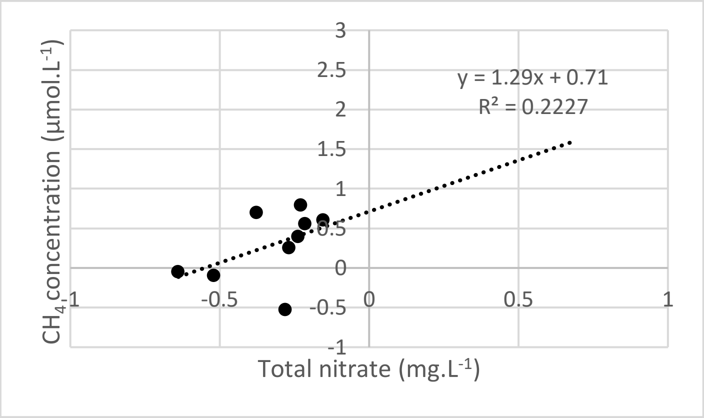


Figure 20 : Methane concentration compared to total nitrate concentration
Non-significantly correlated in a confidence interval of 95%

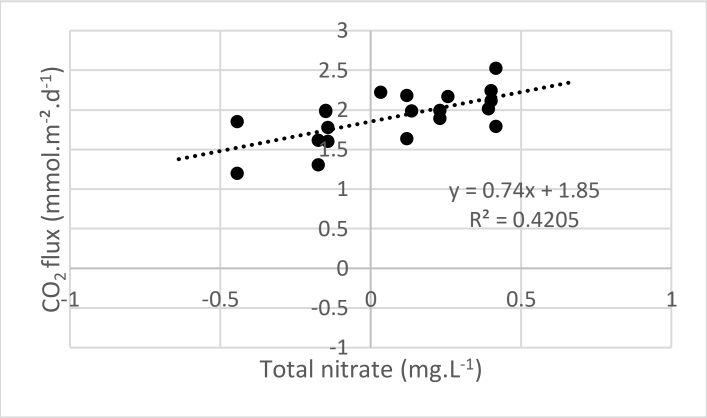


Figure 21 : Carbon dioxide flux compared to total nitrate concentration
Significantly correlated in a confidence interval of 95%

Discussion

In this work, carbon dioxide fluxes have been measured during field surveys and correspond globally to data collected in the meta-analysis. However, measured data need to be extended over the year. Indeed, the mean has been realised for summer data. The influence of plants and living kind is different according to season-induced parameters. Thus, values presented here are a first approach of this problematic.

To know if an area is a sink or a source of carbon, it is needed to compare fluxes on the water-air interface to the sequestration that takes place on the water-soil interface: this is called the net ecosystems flux. In this work, the sequestration of carbon is not measured: it will be measured during next autumn. However, to have a first idea about that, bibliographic data highlight that on temporary ponds, sequestration of carbon is about 85.98 gC.m^{-2}.y^{-1} (se = 10.22, range from 6.84 to 686.2) (235.6 mgC.m^{-2}.d^{-1}) (Bergen et al., 2019 ; Jeffries et al., 2019 ; Kazanjian et al., 2017 ; Macrae et al., 2004 and Taylor et al., 2019) (Figure 22). Moreover, the lower is the surface of a pond, the more there is sequestration (Tranvik et al., 2019). Thanks to data of June from investigated sites, carbon dioxide flux is calculated (95.295 mgC.m^{-2}.d^{-1}, se = 52.5) and methane diffusive flux is calculated (6.749 mgC.m^{-2}.d^{-1}, se = 1.48). Thanks to data from literature, carbon dioxide flux is calculated (281.24 mgC.m^{-2}.d^{-1}, se =25.06) and methane flux is calculated (45.60 mgC.m^{-2}.d^{-1}, se = 10.60).

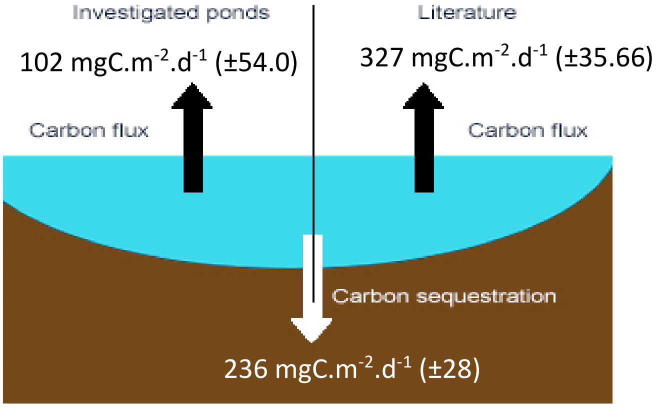


Figure 22: Compilation of carbon fluxes data from field measurement

Secondly, temporary ponds fluxes, from meta-analysis, present a higher dispersion than other types of ponds. This phenomenon can be the result of a less stable area. Indeed, temporary ponds can be influenced by its localisation. In this work, ponds that are near, geographically, tend to present similarities for some trophic features. This is due to the influence of agriculture area that surrounds these ponds and the young hydrographical network that drain water of the area, from East to West (Jolivet et al., 2007). This induces similarities of the direct source of water and nutriments between ponds that are supplied by the ground water. Indeed, nitrate concentration increase in winter, during impoundment, that confirmed this fact. Differences between literature data and data from investigated sites can be explained by the fact that investigated sites are isolated, contrary to those of bibliography, and supply by ground water with low nutriments.

Moreover, carbon dioxide fluxes and methane concentrations are highly correlated to trophic parameters. This is induced by the fact that trophic parameters are used as nutriments for living kinds. Thus, the most there is nutriment, the most activities of plants and bacteria are accelerated. This leads to a production of carbon dioxide during respiration, to a release of methane during bacteria respiration and to a use of carbon dioxide during photosynthesis. This explains the low concentration of methane in investigated ponds. For example, DOC is slow to degrade in peat bog. This fact, coupled with the fact that there is a low concentration of nitrate in investigated ponds, leads to a reduction of fluxes because of limiting factors.

The vegetation has also an important impact on nutriment in water. Agricultural activities lead to a leaching of nutriments in the hydrological network and in the groundwater. Even if investigated ponds are supplied by groundwater, the presence of certain species of plants leads to consumption of precise nutriment, that are used for its development, and act as a filter before reaching ponds. This is confirmed by the relatively low conductivity in investigated ponds, that traduces a low concentration in ions. Thus, an area with divers' plants' species allows a renewal of elements.

PCA highlights that investigated ponds present globally the same trophic conditions and responses. This phenomenon supports that there is an important hydrological network that supplies ground water and influence its composition on a regional scale. In the case of realisation of plannings, it is, thus, needed to realise it at the same scale to reach the objectives. Differences are due to local environmental conditions.

Eventually, parameters that allow to predict carbon greenhouse gases fluxes are trophic features, together. Thus, to preserve and restore these temporary ponds and control carbon fluxes, it is important, in the management program, to realise some samples and measurements on trophic parameters, and to build some plannings to control and regulate these elements. Moreover, as several trophic parameters are linked together, it is possible, with few measurements, to define globally many other trophic features.

In this work, nitrogenous oxide is not measured. However, it is an important greenhouse gas, with effects until three hundred times more than carbon dioxide (Rassamee et al., 2011). Nitrogenous oxide is the product of nitrification by oxidation of NH_4^+ and is used during denitrification that consume dissolved organic carbon to happen. The production of nitrogenous oxide gas is favoured in heterotrophic and anoxic conditions (Rassamee et al., 2011). Moreover, alternation between anaerobic and aerobic condition induces a higher nitrogenous oxide production, that correspond to dry-water alternation on investigated ponds (Rassamee et al., 2011).

Conclusion

To conclude, this work answers to asked questions. First, this work highlight that investigated sites and literature data are different. Then, preliminary results about carbon dioxide flux measurements and methane concentration highlight that investigated ponds present different responses to ponds mentioned in the meta-analysis. Then, investigated ponds present positive flux of CO_2, that traduce efflux from water to the atmosphere. To determine if these temporary ponds are sources or sink of carbon, it is needed to realise carbon sequestration measurement in a further work. The transition zone between herbaceous wet zone and forestry zone favours carbon storage in living-aerial biomass (Bartholomée et al., 2018). So, it could be interesting to realise more accurate flux measurements at the soil-air interface. Third, this work highlights than carbon fluxes and concentrations are closely and mainly positively linked to trophic parameters. However, methane concentration is not influenced by nitrogenous elements. Thus, the most a pond is eutrophic, the higher the carbon dioxide and methane fluxes are (Huttunen et al., 2003).

This information can be used to establish some plannings that would favour the preservation and restoration of these areas and to determine priority intervention sites. Thus, plannings are already realised for several years. For example, a management mode has been established to favour sylviculture and conservation of temporary ponds, and unwooded areas in globality: the goal is to define areas where no deposits must be stored, and where the pine culture cannot be extended. This participates to keep groundwater outcropped and pine can water by this water without risks of filling and drying up. The non-proximity of these cultures allows to limit, in a certain measure, the impact of inputs on water quality (Conseil Général des Landes, 2011). Moreover, diggings are realised very rarely to avoid perturbing the environment, but it is needed to avoid filling by changes that occur during vegetal succession.

This work highlights some elements that could be used in further works. First, a work should be realised more precisely on carbon flux on the soil-atmosphere interface on the transition zone to determine the global impact of these areas on global carbon fluxes. Secondly, some temporary ponds are peaty ponds and others are sandy ponds. It could be interesting to study other temporary ponds from the area to study the influence and responses of each type of pond. To define more precisely if investigated ponds are a source or a sink of carbon, it is important to measure accurately and take into account carbon sequestration. Finally, CH_4 flux measurements should be realised in the next months by sampling air in flux chambers at known time. All these elements will be realised to help actors to preserve these areas with an important ecological role (Figure 23).

Figure 23 : Photo of investigated pond 150
Source : Alexandre Fouache

Reflexive feedback

This internship was very formative. Indeed, the different parts that compose this work are relevant of the process that I followed during these 6 months. Polytech promote greatly scientific works and reflexion. This internship allows me to discover other parts of scientific work and to complete my scientific approach that I started to learn during previous years. Moreover, this work promoted team work and this approach was formative from the point of view of mixing ideas: it is more effective to find solutions to issues.

I apply several skills that I got in Polytech'Tours. First, during these 2 years, I read and resume many scientific papers. This allowed me to read a consequent number of articles during the internship to familiarise with this subject and the studied areas and to collect data that I used for the meta-analysis. Then, I realised statistical tests on data that I got. This step allows to increase my skills in this field, in which I was not so experimented. Indeed, I had not enough basis in that field to apply efficiently some methods as ACP and the usage of R language.

Moreover, the comparison between bibliographic database and field collecting data is not as thorough as I would hope. Indeed, there are many problems about flux measurements as it is explained before in this work. During five months, flux measurements were realised to calibrate the manipulation. These calibrations were needed in the context of the thesis work of my colleague, Romane Darul, that will continue the work realised during the internship. Hopefully, chemical data, excepting flux data, could be used in this work.

If I had one thing to change in this internship is time spend on data collecting from the bibliography. Indeed, this part took several months and weariness was beginning to overtake me: the bibliographic work was limited in time and not according to data collecting. This feeling of no real goal leads to a little decrease of efficiency. Moreover, I need to control my work two or three times to add some data that I did not collect because of a lack of comprehension of the expectation at the conclusion of this work. Indeed, this complete database will also be used in further works.

Moreover, I regret that laboratory works were limited. Indeed, my participation of this part of the work was minimised after samplings because of a lack of space in the laboratory where these manipulations were realised. Thus, I participate a little bit to few of these manipulations, mainly as an observer. So, the majority of data collecting from the field prospecting that I analysed was raw data that qualified person transfers to me.

About my projection in a job, I was always attracted by science and research. My previous internship confirms this wish to continue in the field of research and this internship reinforce this wish. I already inquired about a continuation in a thesis after the getting of my engineer diploma to Mr Rodrigues that I greatly thanks. Moreover, I began to postulate to some thesis offers, eight to be precise, as "Impacts of urban stressors on aquatic plant-fish biotic interactions". However, these candidacies were not successful. I would continue to look for and postulate to theses offers in relation to aquatic ecosystems. Fortunately, I am open-minded: animals, vegetal or sedimentological parts interest me, even if I think I have a preference for animals field and linking these fields to reach conclusions about impacts of parameters on global ecosystems. Eventually, after this project, I know that I will continue to work on the R language, as I begin to learn a little, that is important to master in the research field today.

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Titre : bilan trophique et flux de carbone des mares temporaires forestières du plateau landais

Résumé : In this work, I attempt to highlight relations between carbon fluxes and trophy of temporary ponds and to understand studied temporary ponds from Landes de Gascogne to understand the functioning of these areas and its impact on greenhouse gas fluxes. To achieve it, I realised a meta-analysis on a database collected from several articles and study, during field prospecting, trophy and carbon dioxide fluxes of twelve temporary ponds of the studied area. After many tests on protocols and adjustments, I establish a protocol that allows to measure carbon dioxide fluxes accurately, at the water-air. Thanks to these new-established protocols, preliminary data has been collecting. First, analyses between data from investigated ponds and from literature highlight many differences between functioning. These differences are present both for trophic features and carbon fluxes, as that investigated sites present a low methane concentration. Then, a PCA is realised on studied temporary ponds data. This analysis revealed that investigated ponds present globally similar response and conditions that are explained by the fact that all these ponds are supplied by the same groundwater. Then, the meta-analysis revealed that CO_2 and CH_4 concentrations and fluxes are positively linked to trophic parameters, except nitrogenous forms for CH_4 concentration. This work could be used to establish management gestion plans to restore and preserve these areas that play an important role in carbon fluxes. Indeed, according to preliminary results of this work, these kinds of area should be a sink of carbon.

Mots Clés : temporary ponds, trophy, carbon dioxide, methane, flux

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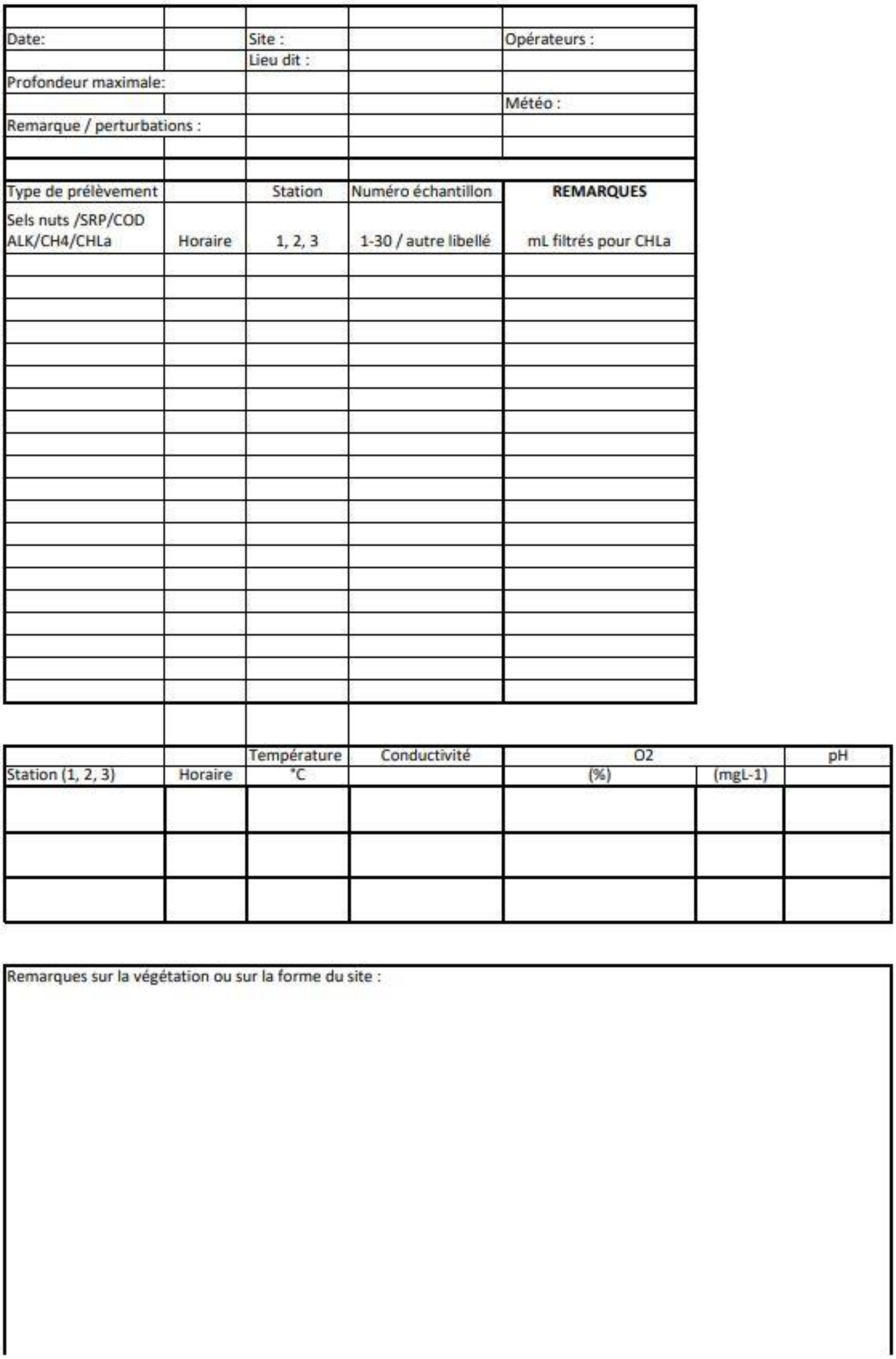
Annexe I: Example of a field sheet



Date:		Site :		Opérateurs :
		Lieu dit :		
Profondeur maximale:				
				Météo :
Remarque / perturbations :				
Type de prélèvement		Station	Numéro échantillon	**REMARQUES**
Sels nuts /SRP/COD ALK/CH4/CHLa	Horaire	1, 2, 3	1-30 / autre libellé	mL filtrés pour CHLa

		Température	Conductivité	O2		pH
Station (1, 2, 3)	Horaire	°C		(%)	(mgL-1)	

Remarques sur la végétation ou sur la forme du site :

Annexe II: References used to realise the meta-analysis

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Riera, J. L., Schindler, J. E., & Kratz, T. K. (1999). Seasonal dynamics of carbon dioxide and methane in two clear-water lakes and two bog lakes in northern Wisconsin, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*(2), 265-274. https://doi.org/10.1139/f98-182

Smith, L. K., & Lewis, W. M. (1992). Seasonality of methane emissions from five lakes and associated wetlands of the Colorado Rockies. *Global Biogeochemical Cycles*, *6*(4), 323-338. https://doi.org/10.1029/92gb02016

Tangen, B. A., Finocchiaro, R. G., & Gleason, R. A. (2015). Effects of land use on greenhouse gas fluxes and soil properties of wetland catchments in the Prairie Pothole Region of North America. *Science of The Total Environment*, *533*, 391-409. https://doi.org/10.1016/j.scitotenv.2015.06.148

Annexe III: Terrestrial chamber experimentations

An important work on carbon dioxide flux measurements at the soil-air interface has been realised in parallel of the work described in this report. Indeed, a decrease of water levels will induce a prolonged emersion of sediments. This annexe will develop the work realised at this interface. Thus, as for aquatic flux measurements, carbon dioxide fluxes are measured on six temporary ponds (14, 94, 150, 162, 165 and 246) at the Air-Soil interface, in the flooding zone. Two probes linked to different flux chamber, thanks to hydraulic tubes, measure at the same time fluxes to Air-Atmosphere, during 30 minutes on a surface of 0.066 m^2 (29 cm of diameter). This circular terrestrial flux chamber volume is around 11L (6L of chamber + 5L induce by an arch that hermitise the chamber). Plastic arches are placed permanently on sites. These arches are replaced by other arches (29 cm of diameter) equipped with join and allow to avoid reactions leading by aerobic new conditions. The top of the arches is around 8 cm above the soil. Terrestrial flux chamber, equipped with a join (2-6mm, Axton, BT202100000007154), is placed on the arch and loaded on its top, by a 5 kg bottle, to create a hermetic chamber. Volume in hydraulic tubes is negligible. These flux chambers are placed at two different places, at most on the opposite side of the pond and on representative areas, according to vegetation cover. To avoid any bubble gas evacuation, once flux chambers are put on the site, operators do not touch it, and nobody walks around it. In this work, flux data were collected in June and some data are not used, because of suspicion of leaks. Data collecting in July are not used because of another problem of leak.

Installation for flux measurement (terrestrial chamber)

These measurements revealed that there is a mean flux of 13.7 $mmol.m^{-2}.d^{-1}$ (sd = 3.92, n = 6) at this interface. This induces that transition zone's soil emit CO_2.

CO_2 flux mean values measured by terrestrial chambers in 4 temporary ponds in June 2022

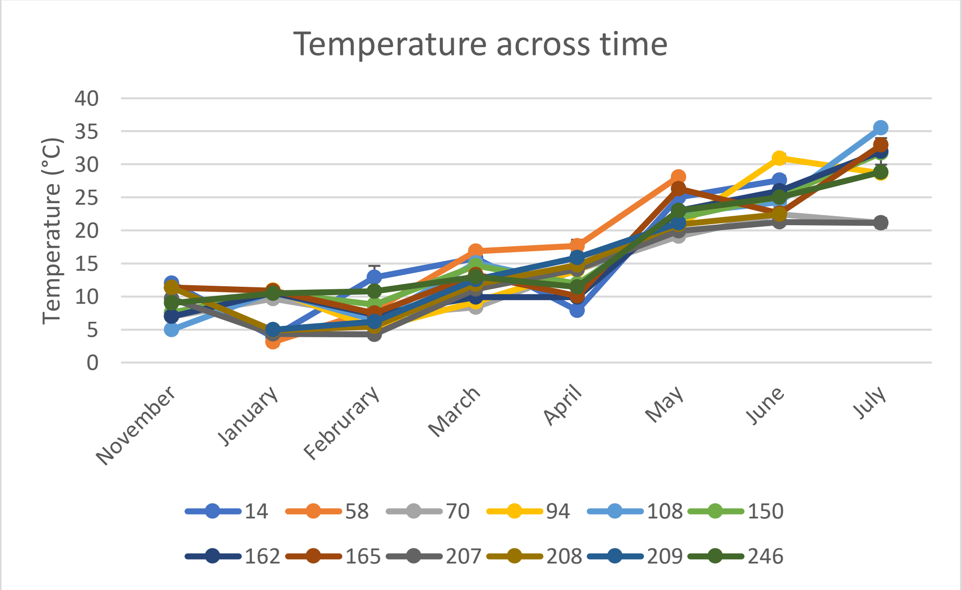
Pond	CO_2 terrestrial measurement flux ($mmol.m^{-2}.d^{-1}$)	Standard deviation	Number of replicats
14	9.0		1
94	8.3	14.4	2
150	13.0	2.31	2
165	18.5		1

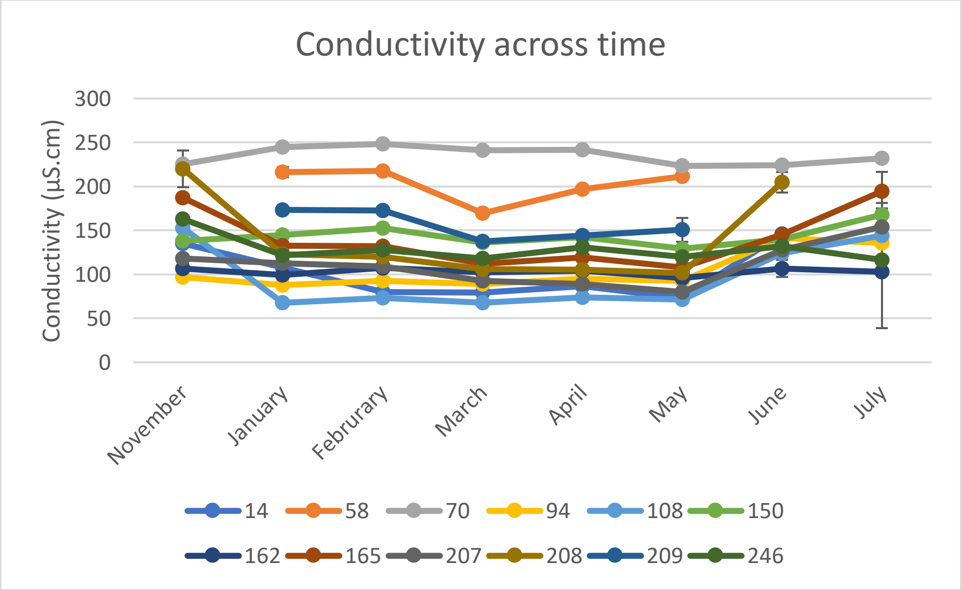
The variability observed in fluxes at the soil-atmosphere interface can be explained by the fact that measurements are realised on heterogeneous areas. Indeed, the presence and density of vegetation have an important effect on carbon dioxide fluxes, due to photosynthesis that uses this molecule:

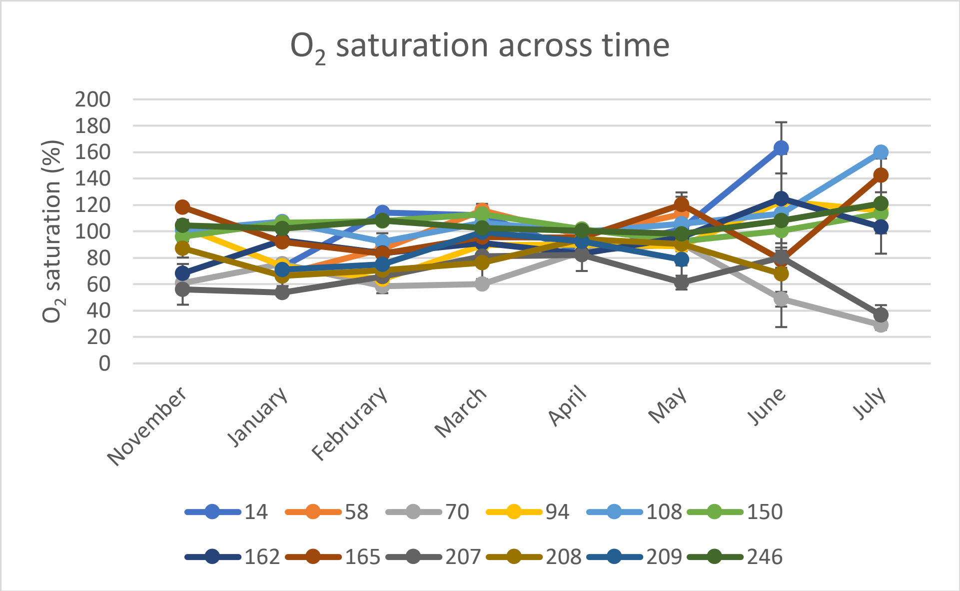
$$H_2O + CO_2 \rightarrow O_2 + CH_20$$

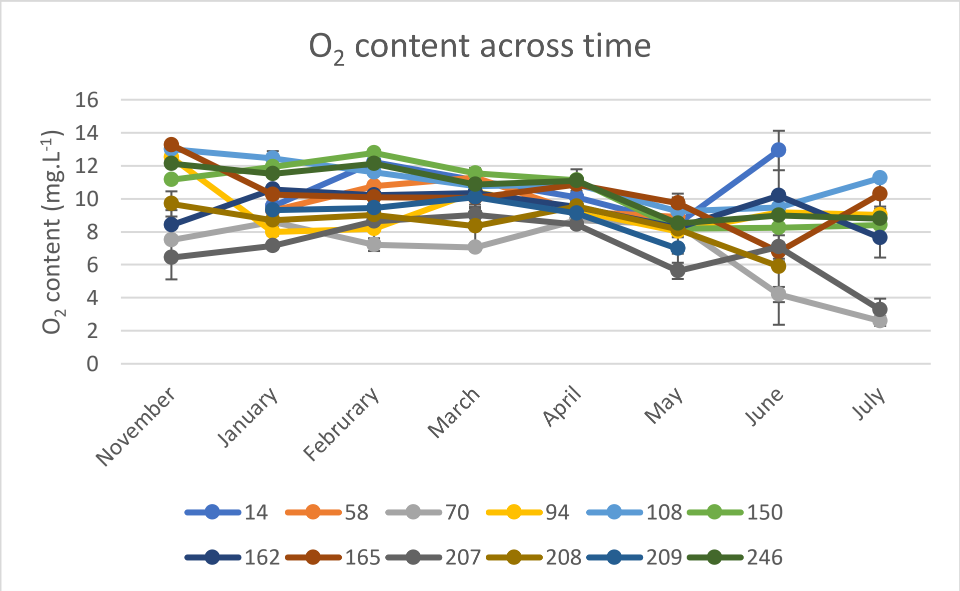
Without plants, bacteria activities and respiration, that produce CO_2, are main reactions.

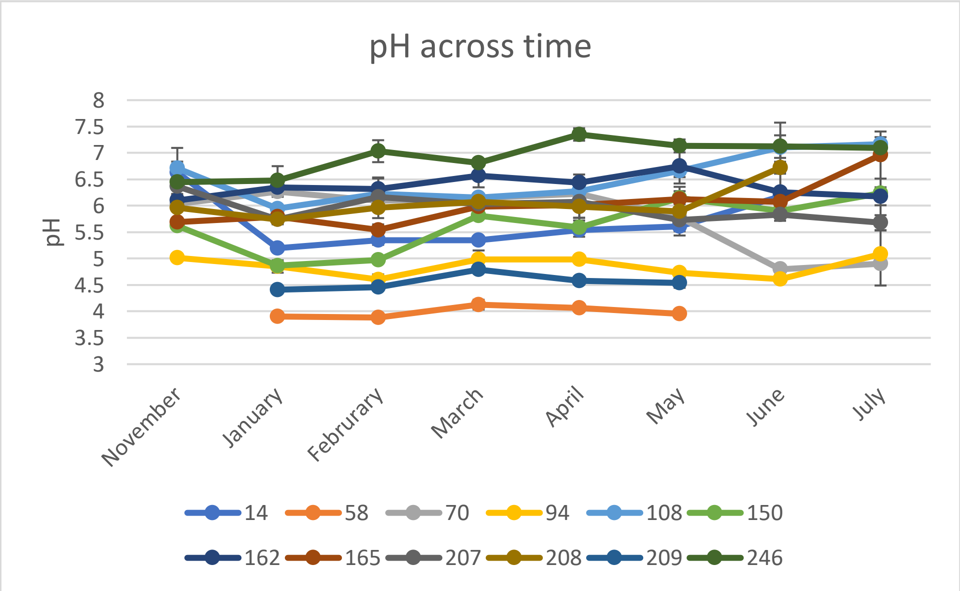
Annexe IV: Graphic of trophy of investigated ponds across time

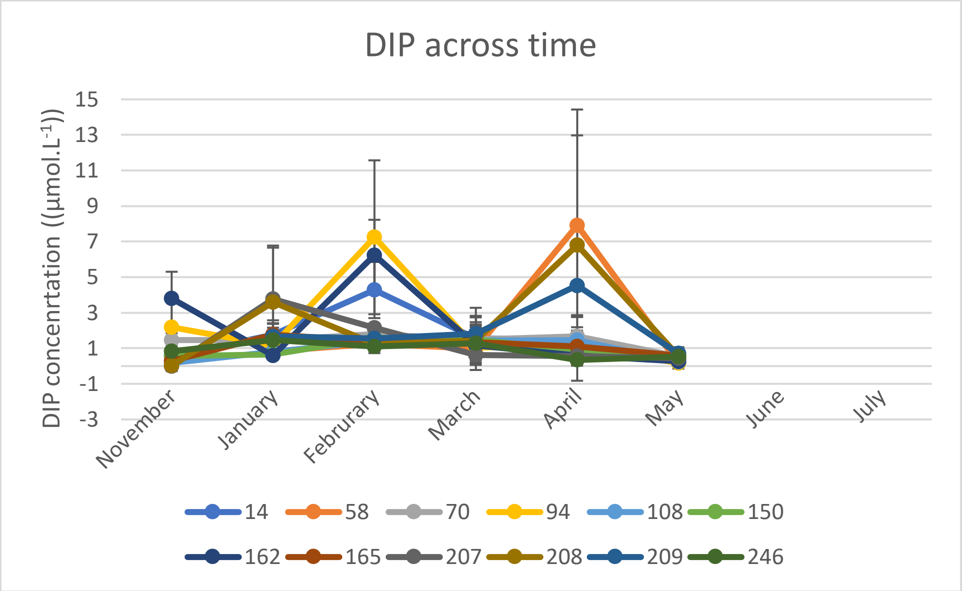


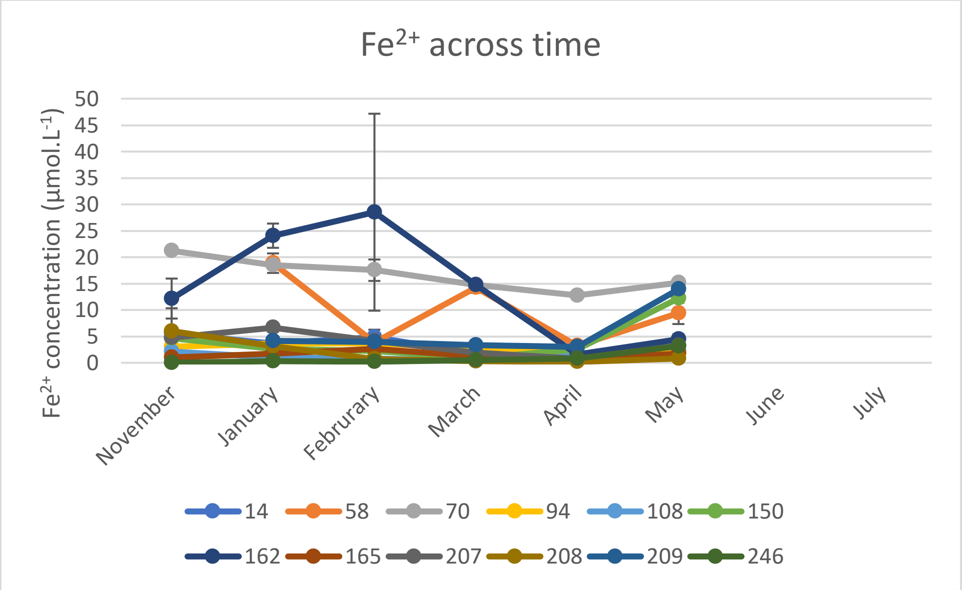


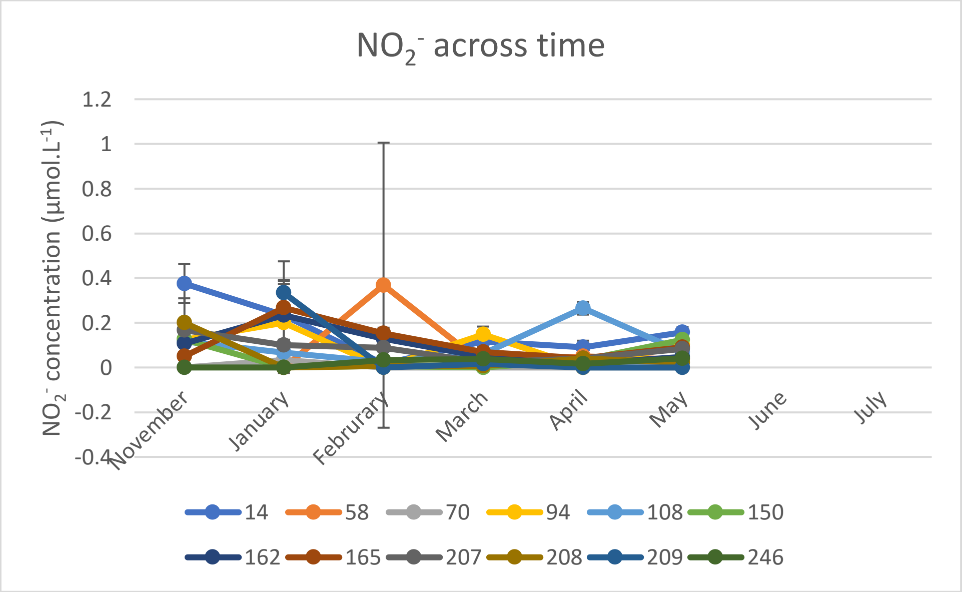

O$_2$ saturation across time
O$_2$ saturation (%)
200
180
160
140
120
100
80
60
40
20
0
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

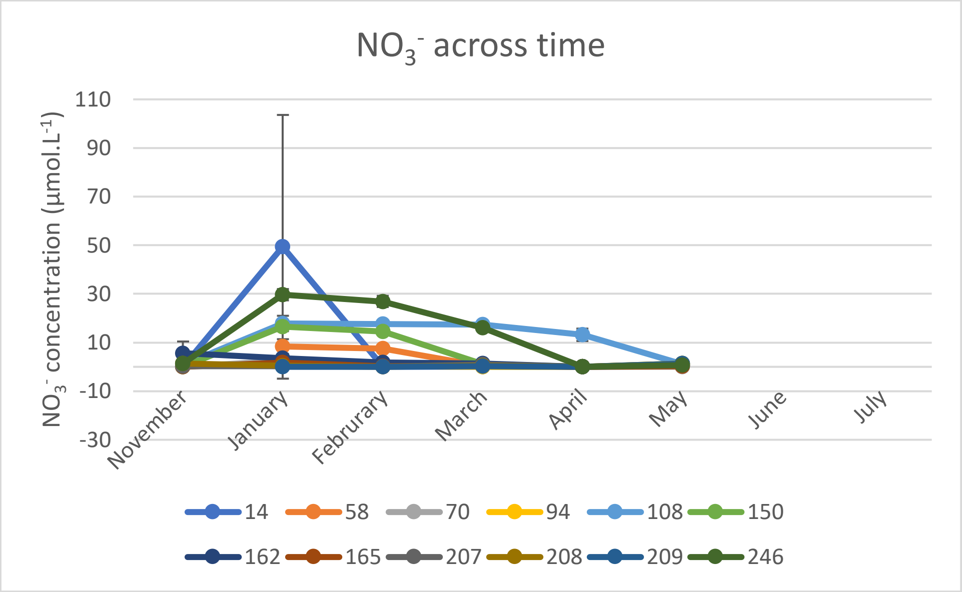

O$_2$ content across time
O$_2$ content (mg.L^{-1})
16
14
12
10
8
6
4
2
0
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

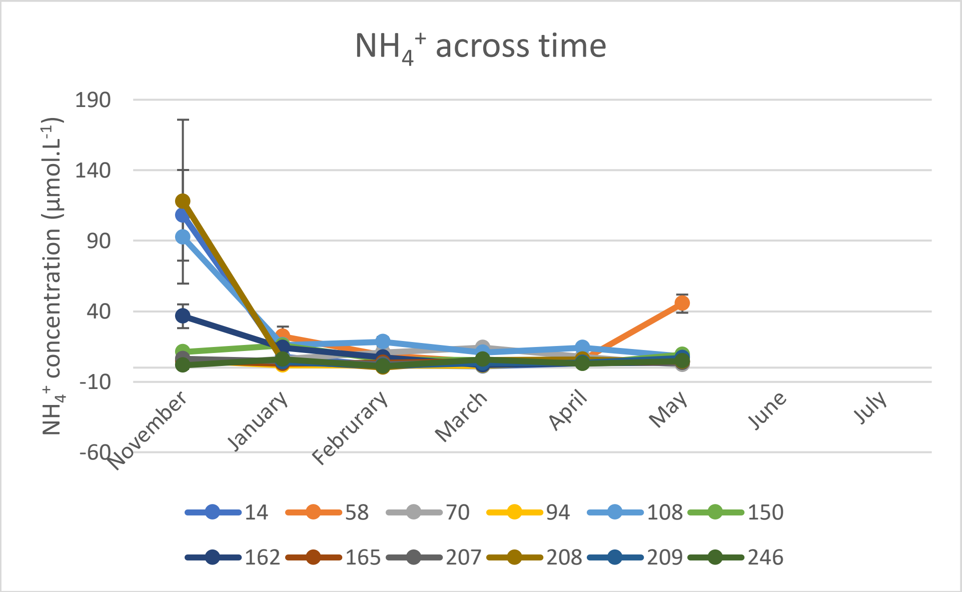

pH across time
pH
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

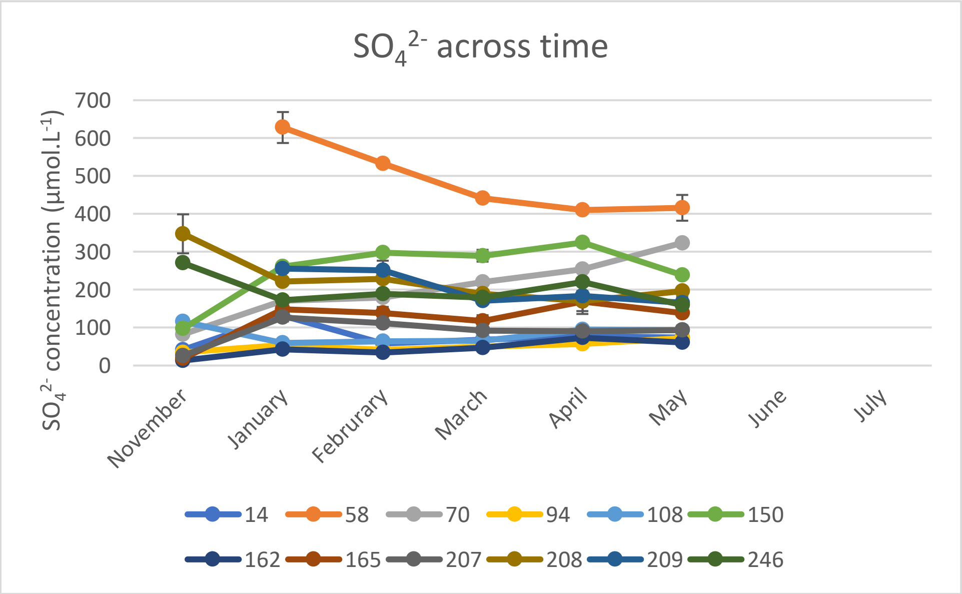

DIP across time
DIP concentration ((µmol.L-1))
15
13
11
9
7
5
3
1
-1
-3
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

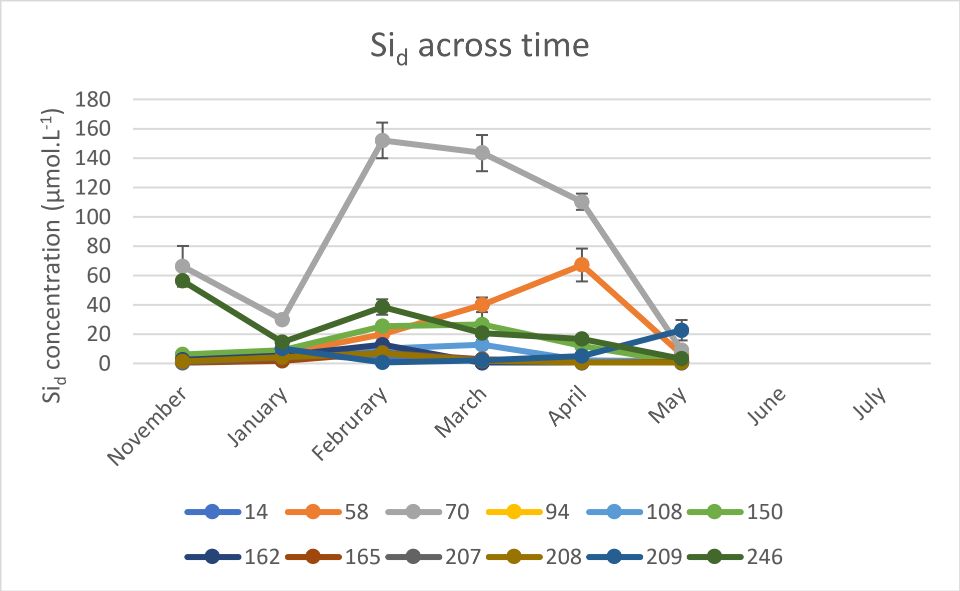

Fe2+ across time
Fe2+ concentration (µmol.L-1)
50
45
40
35
30
25
20
15
10
5
0
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

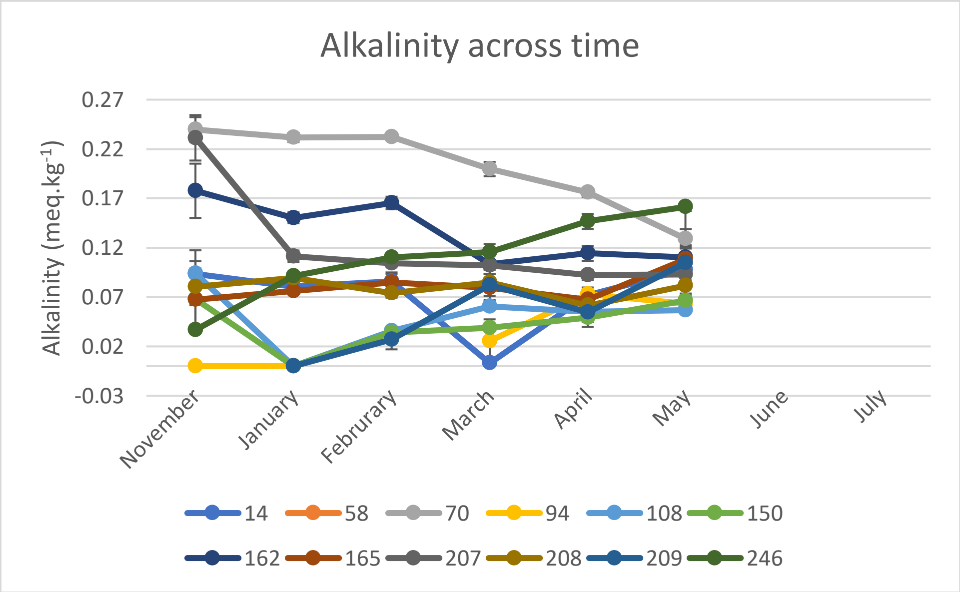

NO2- across time
NO2- concentration (µmol.L-1)
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

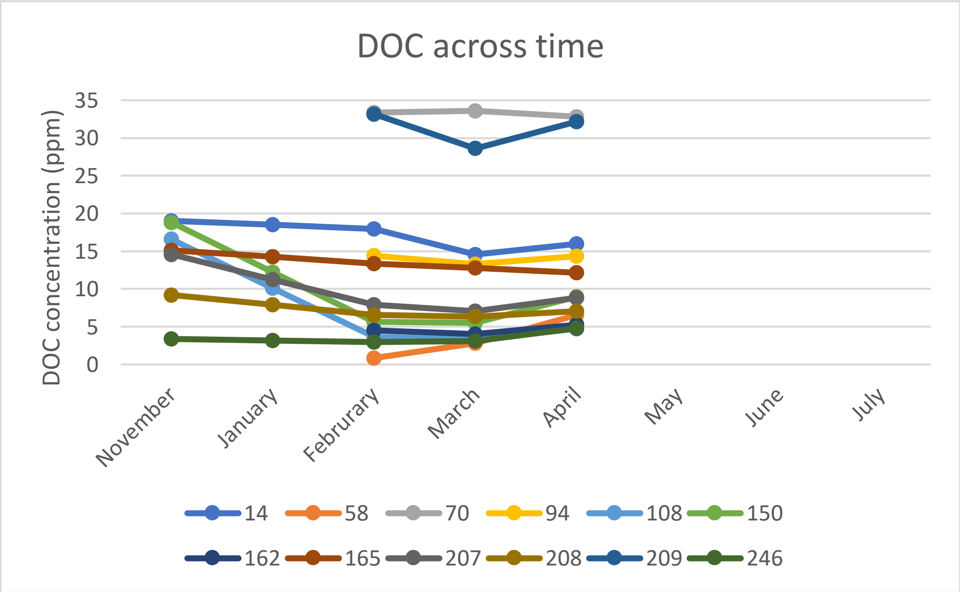

NO$_3^-$ across time
NO$_3^-$ concentration (µmol.L^{-1})
110
90
70
50
30
10
-10
-30
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

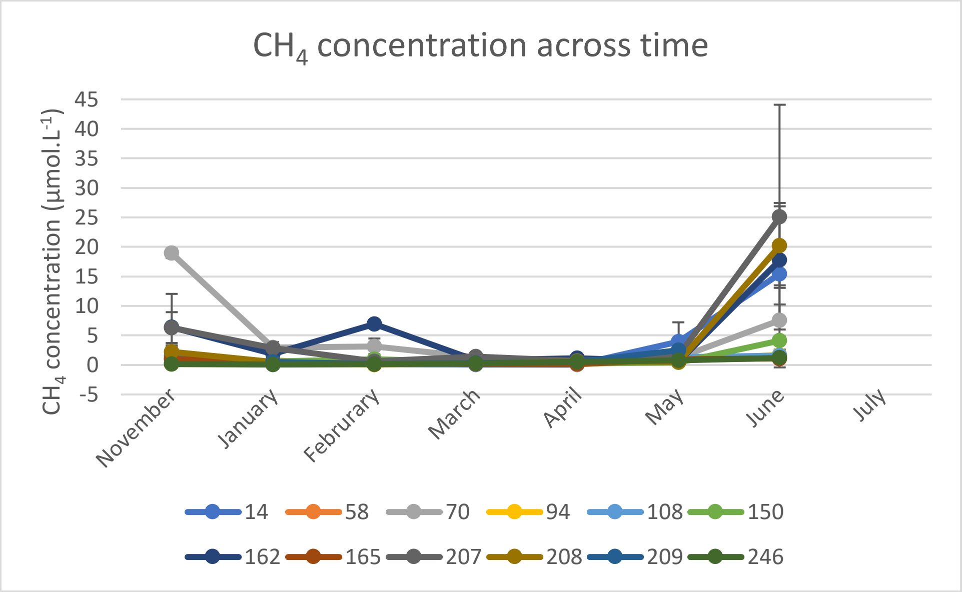

NH$_4^+$ across time
NH$_4^+$ concentration (µmol.L^{-1})
190
140
90
40
-10
-60
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

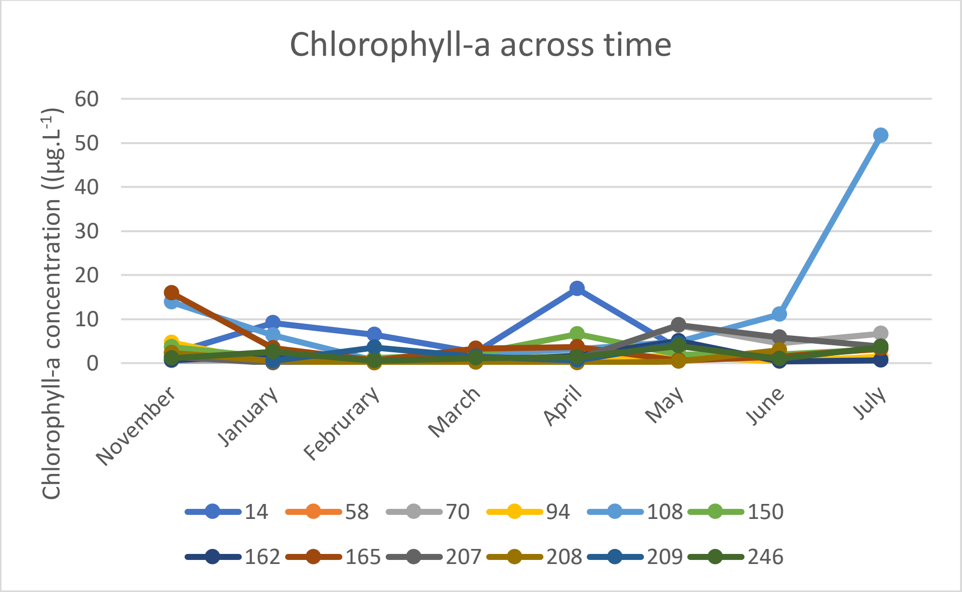

SO$_4^{2-}$ across time
SO$_4^{2-}$ concentration (µmol.L^{-1})
700
600
500
400
300
200
100
0
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246


Si$_d$ across time
Si$_d$ concentration (µmol.L^{-1})
180
160
140
120
100
80
60
40
20
0
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246


Alkalinity across time
Alkalinity (meq.kg^{-1})
0.27
0.22
0.17
0.12
0.07
0.02
-0.03
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

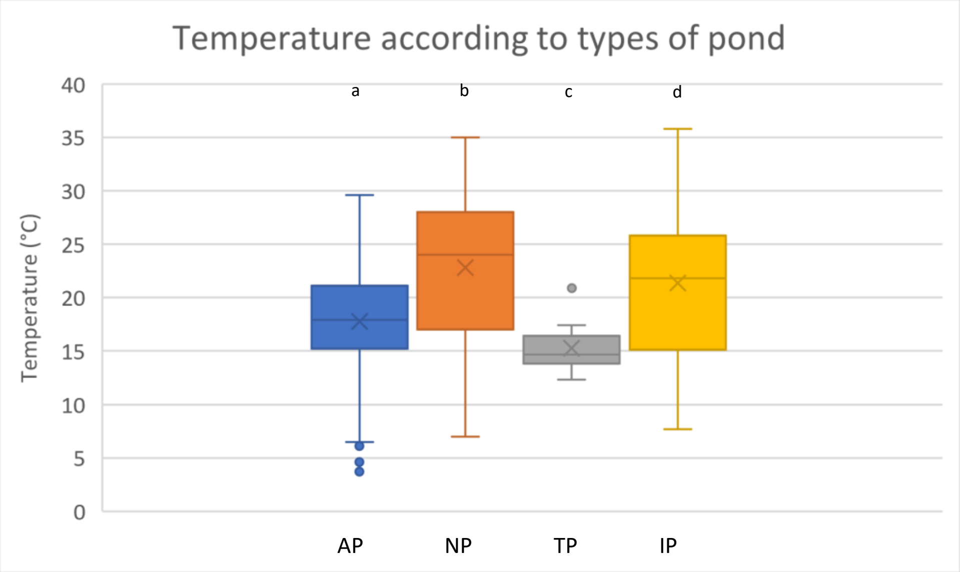

DOC across time
DOC concentration (ppm)
35
30
25
20
15
10
5
0
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

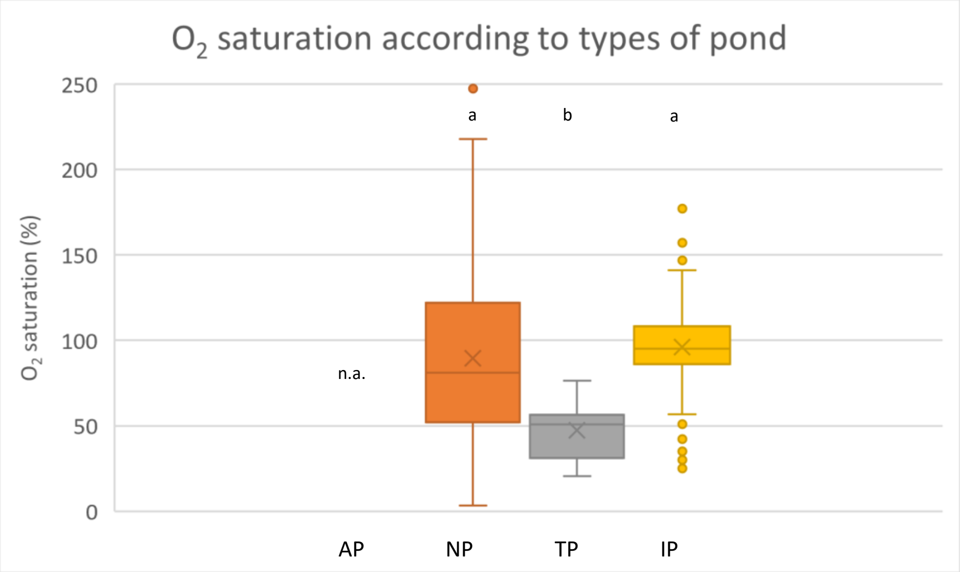

CH$_4$ concentration across time
CH$_4$ concentration (µmol.L^{-1})
45
40
35
30
25
20
15
10
5
0
-5
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

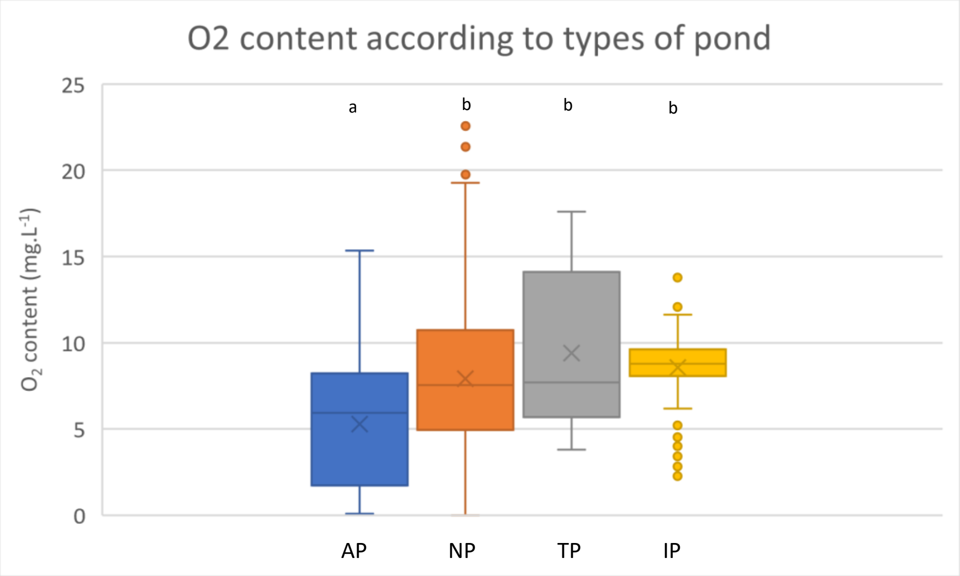

Chlorophyll-a across time
Chlorophyll-a concentration ((µg.L^{-1})
60
50
40
30
20
10
0
November
January
Februrary
March
April
May
June
July
14
58
70
94
108
150
162
165
207
208
209
246

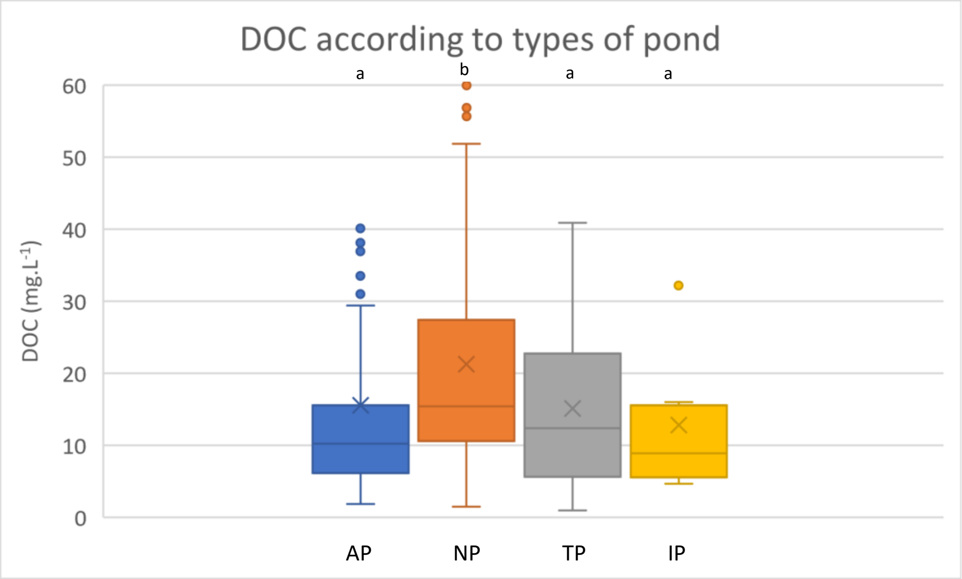
Annexe V: Trophy compared between the type of pond

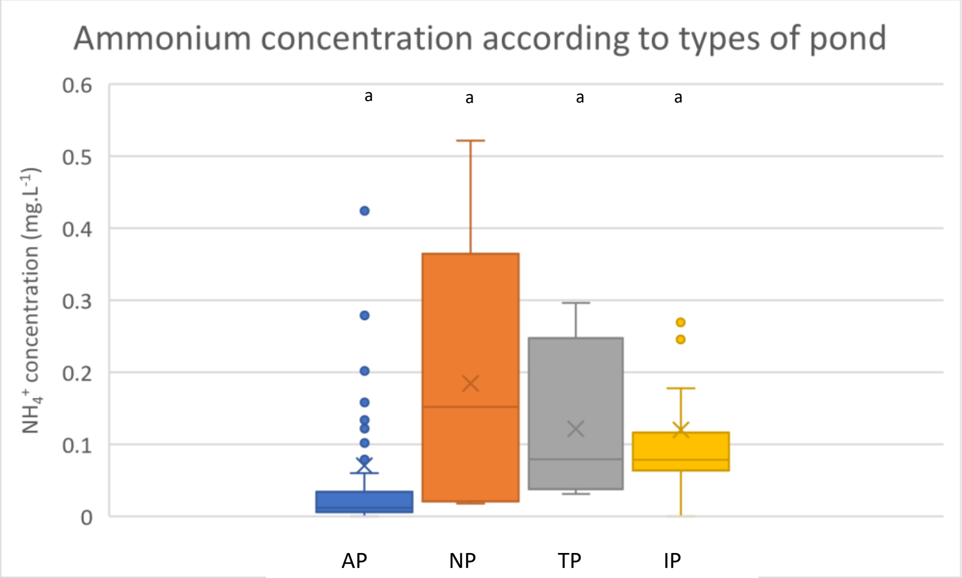
AP = Artificial Ponds, NP = Natural Ponds, TP = Temporary Ponds, IP = Investigated Ponds ; a, b, c, d = significantly similar

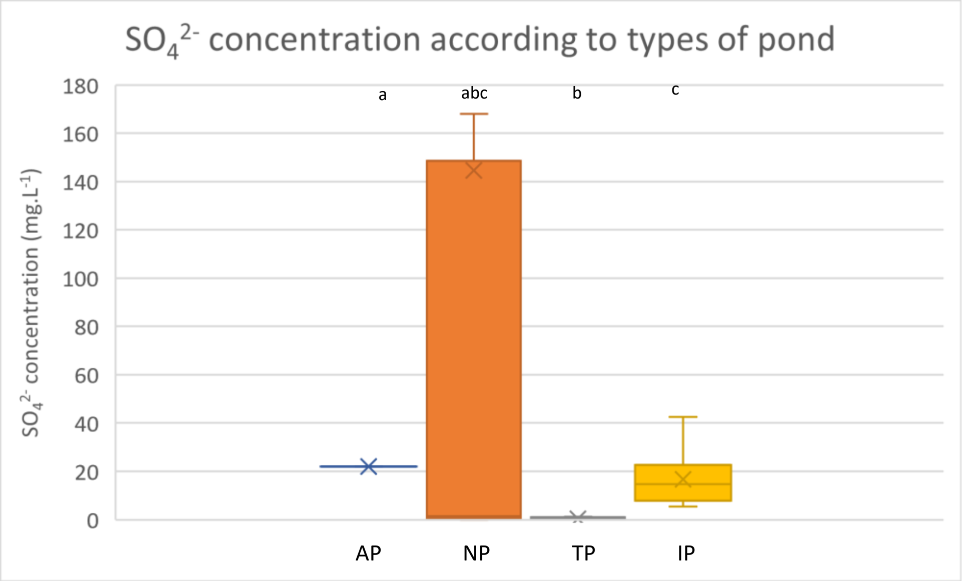


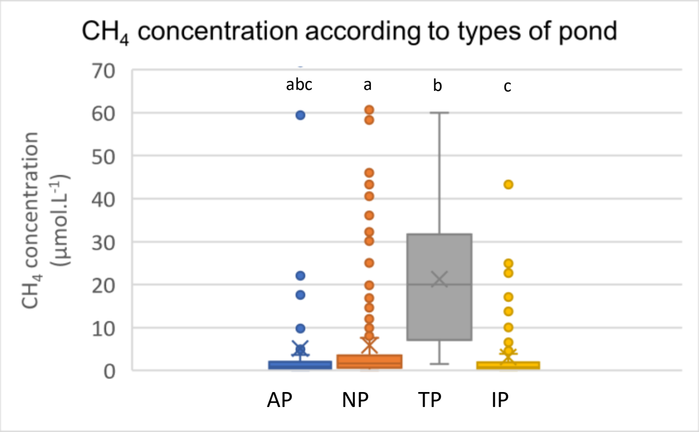


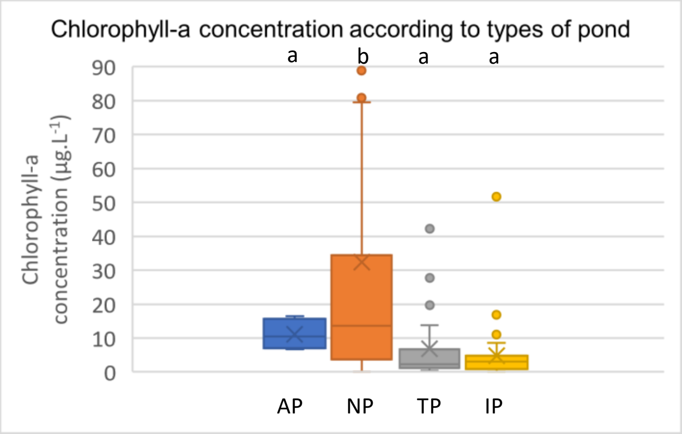


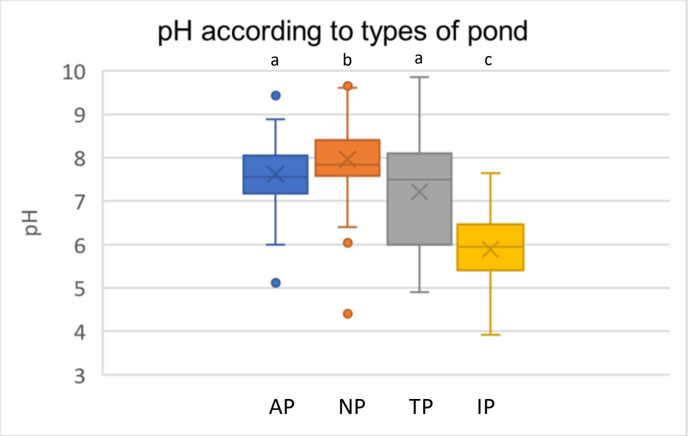

DOC according to types of pond
DOC (mg.L-1)
60
50
40
30
20
10
0
a
b
a
a
AP
NP
TP
IP

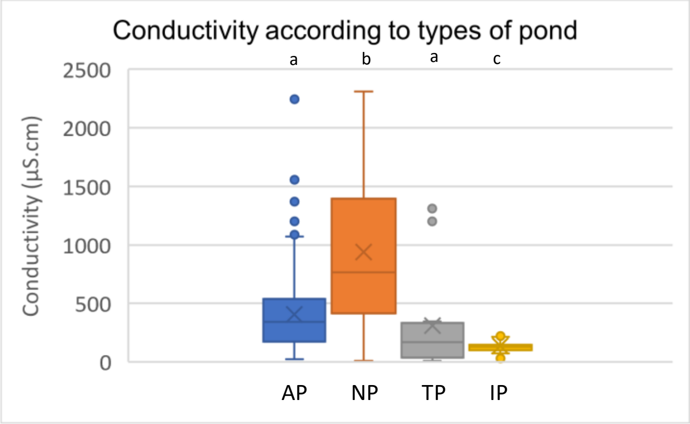

Ammonium concentration according to types of pond
NH4+ concentration (mg.L-1)
0.6
0.5
0.4
0.3
0.2
0.1
0
a
a
a
a
AP
NP
TP
IP

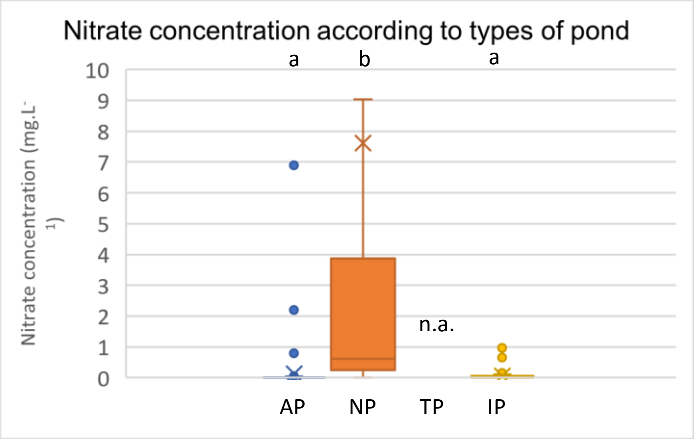

SO42- concentration according to types of pond
SO42- concentration (mg.L-1)
180
160
140
120
100
80
60
40
20
0
a
abc
b
c
AP
NP
TP
IP

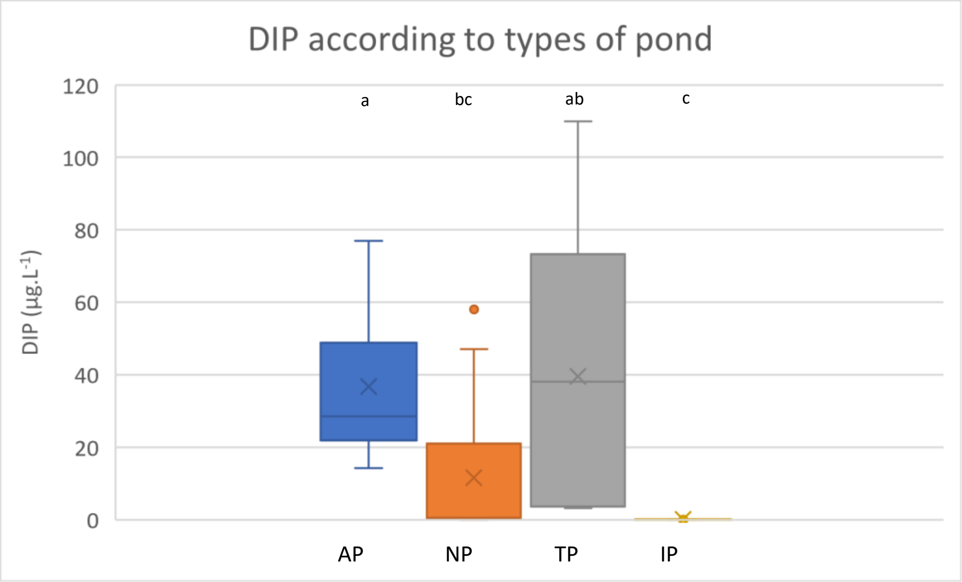

CH4 concentration according to types of pond
CH4 concentration (µmol.L-1)
70
60
50
40
30
20
10
0
abc
a
b
c
AP
NP
TP
IP


Chlorophyll-a concentration according to types of pond
Chlorophyll-a concentration (µg.L-1)
90
80
70
60
50
40
30
20
10
0
a
b
a
a
AP
NP
TP
IP


pH according to types of pond
pH
10
9
8
7
6
5
4
3
a
b
a
c
AP
NP
TP
IP


Conductivity according to types of pond
Conductivity (µS.cm)
2500
2000
1500
1000
500
0
a
b
a
c
AP
NP
TP
IP


Nitrate concentration according to types of pond
Nitrate concentration (mg.L-1)
10
9
8
7
6
5
4
3
2
1
0
a
b
a
n.a.
AP
NP
TP
IP


DIP according to types of pond
DIP (µg.L-1)
120
100
80
60
40
20
0
a
bc
ab
c
AP
NP
TP
IP

Annexe VI: Correlation matrix from bibliographic data and number of replicates for each relation

Correlation matrix from bibliographic data (In light grey : significant relation in a confidence interval of 95%)

	$[CO_2]$	pCO_2	$[CH_4]$	CO_2 flux	CH_4 flux	C flux	Surface	Depth	Water T	Conductivity	Water pH	O_2 Saturation	O_2 content	DOC	TOC	Total Alkalinity	Ammonium	Nitrate	Chl-a	Total P	DIP	SO_4^{2-}	Fe^{2+}	Total N	Ca^{2+}	K^+	Mg^{2+}	Na^+
$[CO_2]$	1.00																											
pCO_2		1.00																										
$[CH_4]$	0.48	0.37	1.00																									
CO_2 flux	0.89	0.71	0.30	1.00																								
CH_4 flux	0.27	0.65	0.77	0.45	1.00																							
C flux	0.12		0.61		0.92	1.00																						
Surface	-0.17	-0.80	-0.30	-0.06	-0.03	-0.19	1.00																					
Depth	0.18		0.04	-0.09	0.24	0.92	0.40	1.00																				
Water T	-0.04	-0.52	0.03	0.03	0.18	0.04	0.01	-0.03	1.00																			
Conductivity	0.07	-0.11	0.07	0.08	-0.06		-0.09	-0.38	0.14	1.00																		
Water pH	-0.46	-0.41	-0.09	-0.52	-0.35		-0.16	-0.34	0.17	0.36	1.00																	
O_2 Saturation	-0.59		-0.46	-0.54	-0.36		0.38	0.34	0.17	0.43	0.52	1.00																
O_2 content	-0.53	-0.79	-0.44	-0.46	-0.38		0.45	0.08	0.05	-0.20	0.46	0.97	1.00															
DOC	0.31		0.27	0.15	0.10	-0.75	-0.09	-0.39	0.18	0.45	-0.06	-0.74	-0.14	1.00														
TOC	0.87		0.21		-0.09		-0.54	0.15	-0.83		0.42	-0.71			1.00													
Total Alkalinity		0.00	0.02	-0.01	0.10		-0.36	0.32	0.24	0.79	0.76	-1.00	0.31	-0.18		1.00												
Ammonium	0.41		0.84	0.19	0.15		-0.45	-0.08	-0.21	0.35	-0.26	0.01	-0.31	0.21		0.52	1.00											
Nitrate	0.39		0.04	-0.01	-0.15		-0.48	-0.13	0.03	0.12	0.12	-0.05	0.19	0.12		0.35	0.32	1.00										
Chl-a	0.10	0.82	0.29	-0.30	0.10		0.44	-0.23	-0.03	0.02	0.03	0.07	-0.03	0.27		-0.38	0.35	-0.29	1.00									
Total P	0.36		0.57	0.29	0.38	0.79	-0.11	0.00	0.01	-0.37	-0.48	-0.82	-0.45	0.24	-0.06	-0.24	0.43	0.05	0.31	1.00								
DIP	0.88		0.26	0.42	0.63		0.32	0.08	0.49	-0.11	-0.18	0.87	-0.52	0.25		-0.41	0.29	-0.13	0.52	-0.73	1.00							
SO_4^{2-}	-0.10		0.76	0.87	0.45		-0.03	0.17	-0.33	0.99	0.71		-0.35	-0.14		0.58	0.27	0.51	-0.02	-0.75	-0.22	1.00						
Fe^{2+}	0.90		0.99		0.89						-0.91			0.84				-0.04		0.89	0.89	-0.43	1.00					
Total N	0.69		0.47	0.65	0.37		0.55	-0.63	0.20	0.25	-0.06	-0.23	-0.06	0.42		-0.14	0.97	0.96	0.32	0.72	-0.49	0.84		1.00				
Ca^{2+}					-0.83					1.00										0.41	0.73	0.96		0.87	1.00			
K^+					-0.74					0.99										0.23	0.65	1.00		0.82	0.97	1.00		
Mg^{2+}					-0.82					1.00										0.39	0.73	0.97		0.87	1.00	0.98	1.00	
Na^+					-0.78					1.00										0.32	0.69	0.99		0.85	0.99	0.99	1.00	1.00

Number of replicates between each parameter (In light grey : significant relation in a confidence interval of 95%)

	$[CO_2]$	pCO_2	$[CH_4]$	CO_2 flux	CH_4 flux	C flux	Surface	Depth	Water T	Conductivity	Water pH	O_2 Saturation	O_2 content	DOC	TOC	Total Alkalinity	Ammonium	Nitrate	Chl-a	Total P	DIP	SO_4^{2-}	Fe^{2+}	Total N	Ca^{2+}	K^+	Mg^{2+}	Na^+
$[CO_2]$																												
pCO_2	0																											
$[CH_4]$	558	5																										
CO_2 flux	350	9	327																									
CH_4 flux	332	5	350	329																								
C flux	5	0	11	1	7																							
Surface	219	4	222	53	62	10																						
Depth	85	0	78	101	121	5	108																					
Water T	450	9	428	457	461	7	149	197																				
Conductivity	98	9	112	126	139	0	109	168	222																			
Water pH	455	9	432	438	425	4	138	197	553	246																		
O_2 Saturation	359	0	322	341	315	4	26	26	362	18	338																	
O_2 content	351	4	316	438	418	1	28	120	460	136	454	348																
DOC	233	0	246	91	109	4	221	165	186	201	244	6	114															
TOC	6	0	6	1	5	4	6	5	5	0	5	5	0	0														
Total Alkalinity	2	4	3	31	14	0	19	11	15	28	35	3	28	28	0													
Ammonium	3	0	3	72	89	0	17	96	95	88	96	3	88	86	0	10												
Nitrate	310	0	305	353	378	0	21	87	390	89	371	305	373	74	0	7	70											
Chl-a	360	4	349	330	310	0	31	24	331	46	365	316	338	77	0	33	10	307										
Total P	59	0	64	101	119	10	49	118	126	120	163	14	111	167	6	23	85	77	74									
DIP	7	0	7	19	29	0	28	25	25	21	28	3	10	15	0	13	17	21	11	15								
SO_4^{2-}	7	0	7	6	18	0	14	14	14	10	14	0	10	12	0	8	9	10	8	12	18							
Fe^{2+}	4	0	4	0	4	0	4	4	4	0	4	0	0	4	0	0	0	4	0	4	4	4						
Total N	9	0	9	20	6	0	10	10	3	22	21	3	18	27	0	19	3	3	30	34	7	4	0					
Ca^{2+}	0	0	0	0	4	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4	4	4	0	4				
K^+	0	0	0	0	4	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4	4	4	0	4	4			
Mg^{2+}	0	0	0	0	4	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4	4	4	0	4	4	4		
Na^+	0	0	0	0	4	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4	4	4	0	4	4	4	4	