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Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences

Factors driving GHG emissions in kettle ponds in NE Germany

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Submitted by : Nicolas Ignacio Marturano

Student ID: 616674

marturan@hu-berlin.de

First Examiner: PD. Dr. Thomas Mehner

Division of Ecology, Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin.

Department of Fish Biology, Fisheries and Aquaculture. Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany.

Second Examiner: Prof. Dr. Robert Arlinghaus,

Division of Integrative Fisheries Management, Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-Universität zu Berlin.

Department of Fish Biology, Fisheries and Aquaculture. Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany.

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List of abbreviations

Ammonium	NH_4
Calcium	Ca
Carbon dioxide	CO_2
Chlorophyl a	Chl_a
Conductivity	Cond
Dissolved Organic Carbon	DOC
Dissolved Oxygen	DO
Greenhouse Gas/es	GHG/s
Magnesium	Mg
Methane	CH_4
Nitrate	NO_3
Nitrite	NO_2
Nitrous oxide	N_2O
Northeast	NE
Organic matter	OM
Phosphate	PO_3
Phycocyanin	PC
Plant Volum Inhabited	PVI
Potassium	K
Principal Component Analysis	PCA
Sodium	Na
Total Nitrogen	TN
Total Organic Carbon	TOC
Total Phosphorus	TP
Total Soluble Solids	TSS
Turbidity	Turb
Variance Inflation Factor	VIF
Water Temperature	WT

Summary

Freshwater systems are considered important contributors to global greenhouse gas (GHG) emissions. However, most studies so far focus on relatively large systems and typically disregard small ponds (< 0.01 ha) and shallow lakes. This is surprising since some studies have shown that small aquatic systems significantly contribute to global GHG emission. A profound understanding of the factors that determine GHG emission by small ponds is therefore important, especially in the face of climate change. The present study aims to fill the knowledge gap by investigating methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions in a set of 30 kettle hole ponds located in agricultural landscapes in different regions of Northeast (NE) Germany. More specifically, this study aims to assess the effect of hydroperiod on GHG emissions by ponds in relation to local environmental pond conditions, to assess seasonal and interannual variation in GHG emission, and to identify significant environmental drivers for variation in GHG emissions in the set of investigated ponds. For this purpose, we investigated a set of kettle hole ponds located in different regions in NE Germany for major local environmental pond variables and GHG emission during spring, summer and autumn during two subsequent years (2021 and 2022). Our results show that pond hydroperiod affects GHG emission and reveals considerable seasonal variation in the extent GHG emission. Fluxes of CH_4 were higher in permanent ponds compared to temporal ponds, being the opposite for CO_2 fluxes, and emission in summer overall higher than in spring and autumn, except N_2O. We did not observe systematic differences in GHG emission within ponds between years.

Overall, we observed that the investigated ponds acted primarily as CO_2 and CH_4 emitters, while N_2O emissions were comparatively low and even negative. We also observed that the main pathway for CH_4 release was through ebullition, rather than by diffusive fluxes. The emission rates of CH_4 and N_2O seems largely driven by sulphate concentration in the ponds. Additionally, CH_4 emissions were also determined by the concentration of total phosphorus and dissolved oxygen. N_2O emissions were also determined by NH_4, TOC and chloride concentrations. In contrast, we could not identify a significant local pond variable explaining variation in CO_2 emission between ponds.

1. Introduction

Small lentic waterbodies, such as ponds and shallow lakes, can be found in almost all biogeographical regions (Holgerson & Raymond, 2016). It has been estimated that approximately 304 million ponds exist globally, with about 90% of these being smaller than 0.1 ha (Céréghino et al., 2014; Malyan et al., 2022). Global estimates suggest that lakes and ponds jointly account for only 3% of the planet's surface, yet they represent approximately 90% of the standing waterbodies on earth and comprise up to 30% of the standing water surface area (Downing et al., 2006). Despite their relatively small individual size, ponds are important ecosystems because they support global biogeochemical cycles (Cole et al., 2007; Downing et al., 2008; Holgerson & Raymond, 2016), provide vital ecosystem services (IPBES, 2018), and strongly contribute to biodiversity (Davies et al., 2008; Hoverman & Johnson, 2012; Williams et al., 2004). Over the past decade, research on the potential of ponds to mitigate the effects and adapt to climate change is rapidly expanding (Davies et al., 2008; Li et al., 2021; Malyan et al., 2022).

Kettle hole ponds, a specific type of pond, were created at the end of the last glaciation approximately 12,000 years ago and are small depressions in the landscape resulting from the melting of trapped ice following the retraction of glaciers (Bizic et al., 2022). Previous studies by Kalettka & Rudat, (2006) have determined that the density kettle hole ponds in the landscape can reach up to 40 per km^2 in northeast Germany, making them the dominant aquatic landscape element in this region. Water budgets for kettle hole ponds in this region are primarily based on precipitations during winter. As a consequence they experience significant short-term seasonal fluctuations in water level (Kayler et al., 2018).

More recently, there is a rapidly growing interest in the extent to which ponds and lakes can act as sources and sinks of greenhouse gases (GHGs) (Malyan et al., 2022; Taylor et al., 2019). Earlier studies estimated the emission of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) by freshwater systems (i.e., streams, rivers, lakes, and reservoirs) to be around 1.36 Pg C yr-^{1}, 0.96 Pg C yr-^{1}, and 0.10 Pg C yr-^{1} (CO_2-equivalents) , respectively (Bastviken et al., 2011; Soued et al., 2016; Tranvik et al., 2009). Although lakes and ponds can bury considerable amounts of carbon in their sediments (Dean & Gorham, 1998; Mendonça et al., 2017), it has been shown that the majority act as significant sources of CO_2 and CH_4 to the atmosphere (Bastviken et al., 2011; Cole et al., 1994). Indeed, recent studies estimate that the total GHG emission of freshwater systems is close to 31% of the total annual CO_2 emissions from burning fossil fuels (IPCC, 2013). Previous studies have estimated that small water bodies contribute approximately 15% to the total pool of carbon dioxide emissions and 40% of the total methane emissions (Audet et al., 2020; Holgerson & Raymond, 2016; Malyan et al., 2022)

Greenhouse gases such as CO_2, CH_4 and N_2O, absorb and reemit energy in the lower atmosphere, which strengthens the greenhouse effect (Montzka et al., 2011). These three gases are the dominant GHGs and it is widely recognized that they contribute 74.4%, 17.3%, and 6.2% of global warming, respectively (Ritchie et al., 2020). High levels of GHG emissions facilitate and strengthen climate change and are often associated environmental degradation, which is expected to result in significant socio-economic impacts (Dottori et al., 2018). Although CO_2 is the most frequently produced and emitted GHG, also other GHG gases are particularly important. For example, methane is currently the second most prevalent GHG, and is approximately 34 times as potent as greenhouse gas than CO_2 (IPCC, 2013) . CH_4 emissions have increased massively over the past few decades, which has strongly contributed to global warming (Saunois et al., 2016). Another important GHG is N_2O, which has a 298-times greater potential for global warming than CO_2 (IPCC, 2013), regardless having attracted less attention due to its relatively low emissions (Kumar et al., 2019). In general, these three frequently occurring GHGs play significant roles in the greenhouse effect due to their diverse global warming potentials and emission magnitudes (Li et al., 2021).

Although earlier studies have provided crucial insights into the extent to which ponds and lakes contribute to GHG emissions (Cole et al., 2007; Raymond et al., 2013), they are also limited because they typically focus on larger systems and mostly exclude small ponds and small, shallow lakes (Downing, 2010). This largely stems from the fact that small ponds are often difficult to detect on maps when using conventional satellite images. In addition, a vast number of ponds are closely associated to wetlands, which makes it difficult to discern between the two habitats (Holgerson & Raymond, 2016). However, ignoring the effects of small lentic waterbodies on GHG budgets might also profoundly limit our understanding of the role of ponds and lakes on GHG fluxes, and might strongly hamper our insights on their potential role of ponds to mitigate and adapt to climate change (Holgerson & Raymond, 2016; Malyan et al., 2022). Indeed, an increasing number of studies demonstrates that ponds and small lakes possess high concentrations of GHGs in the water column and can emit considerable amounts of CH_4 (Bastviken et al., 2004; Holgerson, 2015; Juutinen et al., 2009; Kankaala et al., 2013) and CO_2 (Downing et al., 2006; Kankaala et al., 2013; Raymond et al., 2013) due to their shallowness, frequent water column mixing, high sediment deposition, and high surface-to-shoreline ratio (Holgerson & Raymond, 2016). Furthermore, lower water levels can also lead to shorter gas transport paths and decreased water hydrostatic pressure, which can increase atmospheric CH_4 emissions and decrease CH_4 oxidation (Sun et al., 2021). In contrast, other studies show that ponds can also act a GHGs sinks (Taylor et al., 2019). Understanding the factors determining whether ponds act as sources or sinks of GHGs is crucial to assess their potential role in mitigating to climate change (Malyan et al., 2022; Taylor et al., 2019).

1.1 Current and future threats to ponds and their implication of GHG emission

Small standing waterbodies belong to the most endangered ecosystems on earth (Carpenter et al., 2011; Gozlan et al., 2019; Sala et al., 2000; Strayer & Dudgeon, 2010), which severely undermines their potential role in mitigating climate change by acting as GHGs sinks.

Habitat degradation and habitat destruction are considered major anthropogenic factors affecting the ecological integrity of ponds (Dudgeon et al., 2006; Strayer & Dudgeon, 2010). Additional concern arises from direct effects from climate change (Biggs et al., 2005; Boothby, 2003; Moss, 2017). In addition to ecological degradation and climate change, land-use intensification has led to dramatic losses of ponds worldwide (Curado et al., 2011; Davidson, 2014). In Western Europe, for instance, approximately 50% of all ponds have been lost over the past century (Hassall, 2014). Intensification of agricultural practices also leads to eutrophication of ponds in agricultural areas, which not only has substantial impact on the water quality and aquatic biodiversity, but has also been linked to enhanced GHG emissions by ponds and strongly altered carbon dynamics (source and/or sink) in ponds (Malyan et al., 2022).

Eutrophication affects many water bodies on Earth, (Li et al., 2021), often leading to a turbid phytoplankton dominated state with low levels of biodiversity and overall low water quality (Le Moal et al., 2019; Scheffer & Carpenter, 2003). Several studies have reported a significant relation between eutrophication of freshwaters and their respective GHG emissions (Huttunen et al., 2003; West et al., 2016), which can be linked to the role played by different abiotic factors (e.g., organic matter, nutrients, oxygen concentration), and their effects on GHG emissions (Huttunen et al., 2003; Li et al., 2021; West et al., 2016). Eutrophication in freshwater systems can promote algal blooms and facilitates a turbid, phytoplankton dominated state through multiple, positive feedback loops (Kéfi et al., 2016). Moreover, some types of algae are known to either produce or convert CH_4 and N_2O, further contributing to GHG emissions (Li et al., 2021).

Pond sediments play a central role in GHG emission as they accumulate organic matter (OM) resulting from autochthonous primary production and run-off from the surrounding terrestrial environment (Malyan et al., 2022). The latter can be especially important in ponds as their high perimeter to surface area ratio may lead to relatively high inputs of terrestrial OM (Hanson et al., 2007), especially under conditions of intensive land use (Turner et al., 2007).

The decomposition of organic material in the pond sediment can lead to significant concentration of GHGs at the water-atmosphere interface (Gorsky et al., 2019; Kumar et al., 2021; Malyan et al., 2016, 2021) and results in reduced oxygen concentrations in the water column. This in turn promotes anaerobic conditions in pond sediments (Malyan et al., 2022). Under anaerobic conditions, methanogenic bacteria consume the available OM and produce CH_4 as a result (methanogenesis) (Bastviken et al., 2004; Holgerson, 2015; Malyan et al., 2016). The same CH_4 produced under anaerobic conditions gets oxidized by methanotrophic bacteria in the upper aerobic zone of the pond, producing CO_2 (Baron et al., 2022; Kumar et al., 2021). The mineralization of the OM thus emits CO_2 or CH_4, depending on an overall aerobic or anaerobic environment. However, there is still a lack of research on sediment CH_4 emissions from aquatic ecosystems, with a limited number of studies focussing on coastal wetlands, aquaculture ponds, reservoirs, and small lakes (Rosentreter et al., 2021). One possible explanation for this knowledge gap could be the lack of observations. Previous studies conducted by Rosentreter et al., (2021) found that aquatic ecosystems on average, produce 53% of all CH_4 emissions worldwide. Furthermore, they registered increasing CH_4 emissions from natural to impacted aquatic systems, and from coastal to freshwater ecosystems. The third significant GHG, N_2O, is typically released from ponds in nitrogen-rich environments as a result of the denitrification process in an anaerobic environment (Kumar et al., 2019). Its emissions are usually much lower than those from CO_2 and CH_4 (Malyan et al., 2022). That could be why research on N_2O emissions by ponds has been far less extensive that research conducted on CO_2 and CH_4 emissions, despite having a far higher global warming potential (Hu et al., 2012). Nevertheless, the importance of this GHG is expected to increase in the future given the ongoing nitrogen enrichment of many freshwater ecosystems (Li et al., 2021).

Other variables influencing GHG fluxes from ponds into the atmosphere are seasonality and pond depth . Seasonal changes in (for example) temperature may lead to changes in metabolic rates and gas concentrations in the water column, which may have a direct effect on GHG fluxes (Prėskienis et al., 2021; van Bergen et al., 2019). Shallow ponds can experience a much faster warm-up of their water column, resulting in increased metabolic rates, bacterial build-up and methanogenetic processes (Prėskienis et al., 2021). These ponds are also more susceptible to wind-induced disturbance of their sediments (Joyce & Jewell, 2003), which may facilitate the emergence of $CH4_4$ bubbles trapped in the sediment. Furthermore, shallow aquatic ecosystems provide produced CH_4 in anoxic sediments with shorter residence times. This in turn results in lower oxidation potential of produced CH_4 (Holgerson & Raymond, 2016). Previous studies by Gorsky et al., (2019) have demonstrated a negative correlation of CO_2 to pH of the surface water and also that CH_4 is negatively correlated to the depth of the pond. For these reasons, preserving the capacity of ponds to act as carbon sinks is an additional challenge for managers and stakeholders (Taylor et al., 2019).

An additional important factor affecting GHG emissions is the hydroperiod of the ponds. Empirical research has shown that both period drying out and hydroperiod length directly affect the extent of GHG emissions (Bolpagni et al., 2017; Gilbert et al., 2017; Jin et al., 2016; Y. Yuan, 2020). This information is important, especially in the face of climate change as current models predict a profound increase in the frequency of drying events in many regions on earth (Pekel et al., 2016). For example, it has been estimated that two thirds of the planet’s first-order mid-latitude streams and one-third of all fifth-order rivers will be characterized by only a temporary water flow (Raymond et al., 2013). Desiccation is expected to affect 18% (around 800,000 km2) of the global surface area covered by inland waters (Pekel et al., 2016). Recent studies have shown that sediments that are exposed to the air following a desiccation event can much stronger contribute to CO_2 emissions to the atmosphere compared to the CO_2 emission from water surfaces during inundated periods (Catalán, Núria et al., 2014; Obrador et al., 2018). Despite carbon cycle processes in both water and submerged sediments have been thoroughly studied, processes occurring in the shallow zone which periodically dries out and becomes wet once again have not been studied in the same in-depth (Jin et al., 2016; von Schiller et al., 2014).

1.2 Research aims and objectives

There is an urgent need for complete and accurate estimations of the extent of GHG emissions by ponds and to gain better insights on the factors underpinning variation in GHG emission by kettle ponds in NE Germany. More specifically, we aim to

1) Assess the effect of hydroperiod and season on GHG emissions by ponds.
2) Identify key environmental pond properties determining GHG emission.
3) Investigate interannual variation in GHG emission.

For this purpose, we sampled 30 kettle hole ponds in four regions in NE Germany, for which we assessed major local environmental pond conditions and the extent of GHG emissions of CH_4, CO_2 and N_2O during spring, summer and fall of two subsequent years (2021 and 2022).

We hypothesize that hydroperiod will have a significant effect on GHG emissions and on environmental pond conditions. We expect higher CH_4 and N_2O emissions from permanent ponds compared to temporal ponds and higher CO_2 emissions from ponds with a temporal one (Catalán al., 2014; Gómez-Gener et al., 2016). We also expect higher nutrient and salt related variables in temporal ponds, given a concentration process. We also expect a significant effect of seasonality on our GHG emissions. We expect higher emissions of all GHGs during summer, given the higher temperatures and other factors which vary seasonally, like primary producer biomass (Audet et al., 2020; Gorsky et al., 2019).

This study part of the EU H2020 funded PONDERFUL (POND Ecosystems for Resilient Future Landscapes in a changing climate, (Grant No. H2020-LC-CLA-2019-2),) initiative that investigates how ponds can be used as nature based solutions to climate change and adaptation solutions, while also aiming to improve biodiversity protecting methods and ecosystem services provision.

2. Methods

2.1 Study design and site description

We selected a total of 30 kettle hole ponds located in four different geographical regions, Schöneiche, Müncheberg, Lietzen and Quillow, in North East Germany (Fig. 1). All regions were located in lowland areas (lower to 800m altitude) and included both permanent (n=13) and temporal systems (n=17). Individual ponds within each region were selected based on their expected hydroperiod (temporal or permanent) and the size of their surrounding terrestrial buffer strip. The majority of ponds were located in intensively managed agricultural land.

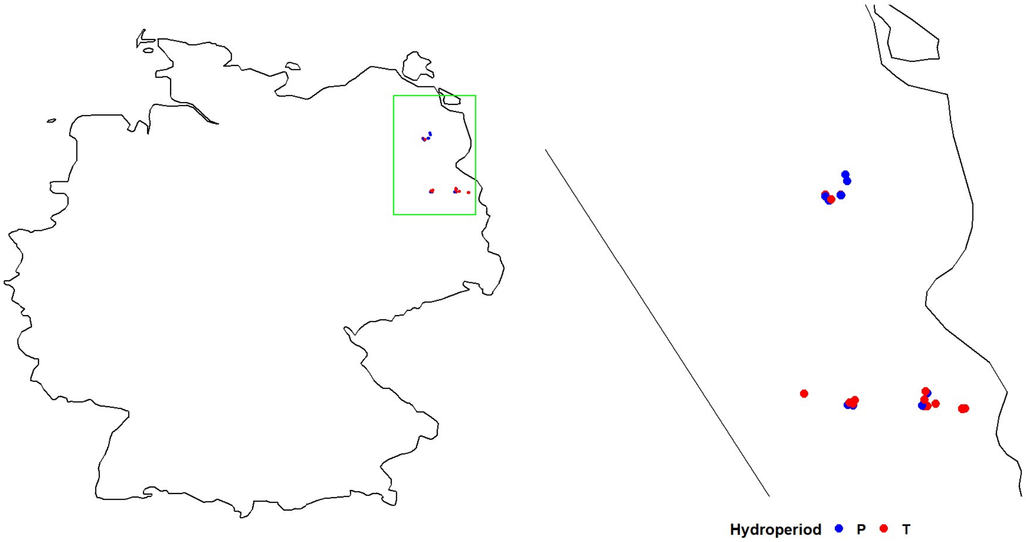


Figure 1: Map of Germany and zoom over area with the studied ponds. Blue dots indicate ponds with Permanent hydroperiod and red dots indicate ponds with Temporal hydroperiod.

2.2 Data collection

All selected ponds were visited in spring, summer and autumn of 2021 to collect information on key local environmental pond conditions, hydroperiod and GHG emissions. A subset of 12 ponds (8 in Quillow and 4 in Müncheberg) have also been investigated in 2022 to investigate interannual variation in GHG emissions.

2.2.1 Physical, chemical and biological variables

Day-time oxygen concentration and saturation, pH, water temperature and conductivity were measured *in situ* at a central spot in each pond (or in middle of the largest open water patch in case of macrophyte covered ponds) at the depth of 20cm below water surface using standard electrodes.

A depth-integrating water sample (approximately 10 L) covering the entire water column was taken with a tube sampler at a central spot in the pond for the quantification of suspended matter, water turbidity, concentrations of total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (TN), Ammonia (NH_4), nitrite (NO_2), nitrate (NO_3), total phosphorus (TP), phosphate (PO_4), sulphates, chlorides, alkalinity, calcium (Ca), magnesium (Mg), sodium (Na) and potassium ions (K). Anions and cations were only sampled and quantified during Spring 2021 and 2022. Water turbidity was measured directly in the field from the water sample using a portable fluorometer (AlgaeTorch, bbe©). Subsequently, subsamples were taken and poured into several vials for further analysis at the laboratory of the Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin. At the same time, we took a depth-integrated water sample from the deepest part of the pond for *in situ* measurement of the concentration of *in vivo* chlorophyll A (Chl_a) and Phycocyanin (PC), as proxy for phytoplankton and cyanobacteria biomass respectively, using a portable fluorometer (AlgaeTorch, bbe©). In macrophyte-covered ponds, the water samples were taken in the middle of the largest open water patch without vegetation. Contact with the pond sediment by the tube was avoided at any time by sampling just above the sediment surface. If the sediment in the open water zone was covered with macrophytes, only water from the surface to 20 cm above the plants was used. In the case of very shallow ponds (less than 20 cm), the water collection was done using a 1 L beaker.

Plant Volume Inhabited (PVI) was estimated by measuring water depth and height of the macrophyte vegetation along two perpendicular transects at 2 meter intervals in each pond. Measurements for this parameter were carried out only on Spring and Summer 2021.

The hydroperiod of all ponds was assessed visually. Additionally, a more in-depth assessment was carried out using a set of two temperature loggers. One logger was placed at the bottom, at the deepest part of the pond, and the other one was set in the shade outside the pond. All temperature loggers were collected in late autumn and the hydroperiod length was determined by comparing the daily temperature profiles of the logger inside and outside the pond. From the moment when both temperature profiles were similar, we assumed the pond to be dry.

2.2.2 GHG sampling

GHG were sampled in all ponds during summer and autumn in 2021 and in spring 2022. In addition, a subset of 12 selected ponds (Fig 2) were resampled during summer and autumn 2022 to investigate the interannual variation in our different fluxes.

The sampling was carried out according to the two main pathways for GHG; (1) the release or consumption by diffusion across a concentration gradient between water and air (dissolved fluxes); and (2) the release as bubbles from the sediment, also termed ebullition. The bubble release is largely comprised by methane, thus we assumed the ebullition flux to be composed only by this gas (bubble fluxes).

Not all ponds were sampled in every season, since some of them dried out in summer and/or autumn. Therefore, only the remaining standing ponds were sampled (Figure 2).

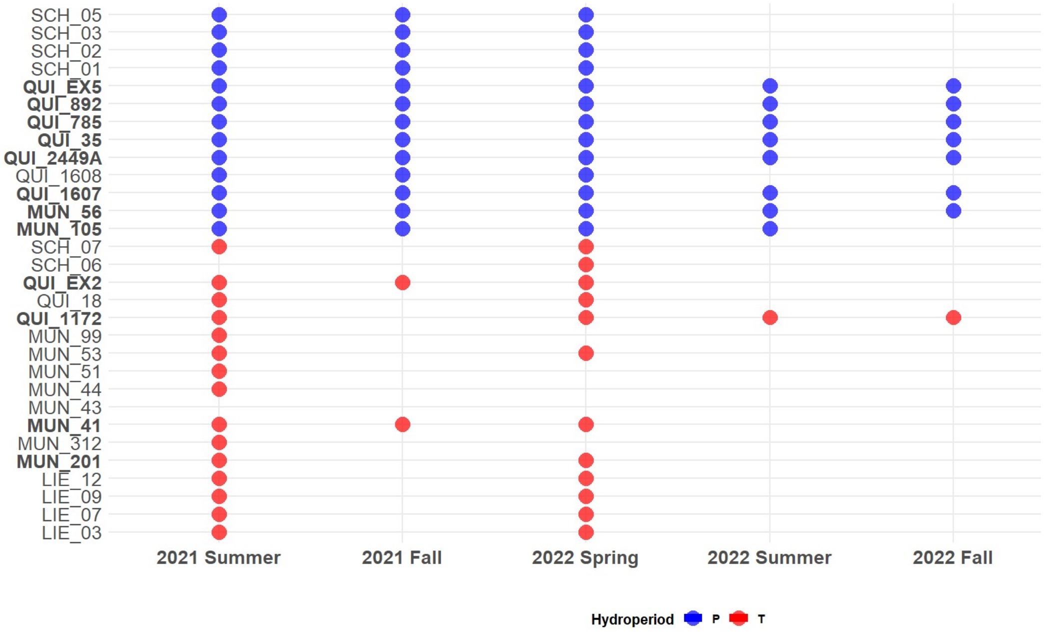


Figure 2: Sampled ponds across both years and seasons. Bold labels in y-axis correspond to the 12 ponds that have been resampled in 2022. Blue points indicate permanent ponds that permanently hold water while red points correspond to temporal ponds that periodically dry out.

2.2.2.1 Quantifying Dissolved flux

Dissolved concentrations of CH_4, CO_2 and N_2O were quantified using by the head-space equilibrium method. For this purpose, we collected a 40 mL water just below the surface of the water column using a 50 mL syringe, injected 12 mL of air and subsequently shake the syringe for 1 minute. After that, 10 mL of air from the syringe was injected into an exetainer vial for the analysis of CH_4, CO_2 and N_2O concentrations in the laboratory. At the same time, we also collected 10 mL air sample net to the pond. The measurement of GHG concentrations in both samples allowed us to calculate dissolved fluxes of CH_4, CO_2 and N_2O for each pond.

Two samples per pond were obtained during each season, with a seven day interval between each sampling. In further analysis, we combined the data from both measurement by taking the mean concentrations.

2.2.2.2 Quantifying bubbles emission

Ebullition flux of CH_4 was measured using multiple floating chambers (up to eight, depending on pond size, with a minimum of 3 chambers) in each pond over a period of 7 days. Since ebullition is highly discrete in space and time, it was required to cover different areas of the pond that had varying water depths, to capture spatial variability within each pond. The placement of the chambers followed an even distribution along the pond, including marginal and central areas of it. Water column depth was measured at the location of each chamber. After 7 days a 10 ml gas sample was extracted from each chamber and injected into an exetainer vial. All gas samples were sent to Aarhus University in Denmark for the analysis of CH_4, CO_2 and N_2O. In further analyses, we combined the measurement of all chambers, for each pond, by taking the mean concentration.

2.3 Data Analysis

2.3.1 Exploration of variability in local environmental pond conditions

The variation in local environmental pond conditions in relation to geographical region and hydroperiod (temporal vs. permanent) was visually explored using on ordination plot of a Principal Component Analysis (PCA) based on standardized physico-chemical and biological data from spring 2021. We formally tested for the effect of hydroperiod and region on the entire set of $\log_{10}$-transformed local environmental pond variables using a PERMANOVA analysis for the data from Spring 2021. Subsequently, we tested for the effect of hydroperiod on each environmental variable separately using multiple Two Sample T-tests and Wilcoxon Signed-Ranked tests. In addition, we tested for an effect of season, hydroperiod and their interaction on each environmental variable separately that were sampled during both in spring and summer 2021 (Water temperature, TN, TP, TOC, DOC, Chl_a, PC, conductivity and depth) using multiple linear mixed effects models. Finally, we conducted a more in depth analysis on the effect of season by only considering permanent ponds as they have data from spring, summer and fall. For this purpose we conducted multiple separate One Way Repeated Measurements ANOVAs and Friedman tests, with subsequent post-hoc analyses (Tukey or Dunn tests according to the assumptions that were met). Data from spring 2022, summer and fall 2021 was used and we tested only the variables that were consistently sampled across the three seasons: water temperature, TN, TP, TOC, DOC, Chl_a, PC, conductivity and water column depth.

We also tested for an effect of interannual variation for individual environmental variables using several Paired t-tests and Wilcoxon-Signed Rank tests, using data from spring 2021 and 2022. In the case of water temperature, we possessed the measurements for each season for both years, so we tested for an effect of interannual variation on this variable between summer 2021 and 2022 and between fall 2021 and 2022, using Paired t-tests.

2.3.2 Patterns of variation in GHG emissions between ponds located in different regions.

We formally tested for an effect of region on the emission of CH_4 (ebullition and dissolved), CO_2 and N_2O using multiple separate Kruskal-Wallis tests. Spring 2022, summer and fall 2021 data was used. This non parametric test was chosen for all GHG emissions, in order to tackle the different sample sizes for ponds in each Region, the non-normal distribution of the variables (Shapiro-Wilk test, $p < 0.05$) and heteroscedasticity of variables (Levene-test, $p < 0.05$).

2.3.3 Effect of Hydroperiod and Season on GHG emissions

In order to tackle the inherent complexity of our data, we created several linear mixed effects models to test for an effect of hydroperiod, season and their interaction on the emission of GHGs. Emission data from summer 2021 and spring 2022 was used, due to the fact they had the most balanced sample sizes for each group (Fig 2).

Secondly, we also tested for an effect of Season on GHG emissions by only considering **permanent ponds**, and using a separate One-Way Repeated Measures ANOVAs or Friedman tests for CH_4, CO_2 and N_2O fluxes. Further post hoc analyses were performed on significant results.

2.3.4 Identify key environmental pond properties determining GHG emission

We identified relevant environmental pond properties for observed variation in GHG emission between ponds using multiple linear regression models based on the AICc model selection procedure (Hurvich & Tsai, 1989). These environmental variables were preselected based on ecological knowledge. Data from summer 2021 was used to carry out the regressions. We assumed salt-related variables to be similar throughout the seasons, thus using the spring 2021 data of these variables for our model. Multicollinearity among predictor variables was tested using the Variance Inflation Factor (VIF). In all cases, VIF-values did not exceed 3.7, which is a typical threshold value which indicates problems with collinearity (e.g. VIF values above 5 O'brien, 2007). Dissolved CH_4 and N_2O fluxes were $\log_{10}$-transformed prior to the statistical analysis to improve normality of residuals and homoscedasticity of variances. In the case of CH_4 bubble flux, a square-root transformation was applied to achieve a normal distribution of residuals and improve homoscedasticity of variances. Dissolved CO_2 flux, was previously cubic-root transformed for the same purposes.

2.3.5 Effect of interannual variation on GHG emission

We tested for an effect of interannual variation on GHG emissions by comparing data from summer and fall 2021 with data from summer and fall 2022 using separate Paired t-tests and Wilcoxon-Signed Rank tests. Only data from ponds sampled in both years were used (Fig 2).

All statistical analyses were conducted in R version 4.3.1 (R Development Core Team, 2023) using the "PCA", "adonis2", "t.test", "wilcox.test" "kruskal.test", "aov", "friedman.test", "shapiro_test", "levene_test", "emmeans", "dunn.test", "lmer" "lm", "ols_vif_tol", "ols_test_breusch_pagan", "raintest" and "AICc" functions using the "FactoMineR", "vegan", "stats", "rstatix", "lme4", "ememeans", "dunn.test", "olsrr", "lmtest" and "AICcmodavg" libraries.

3.Results

3.1.1 Exploration of variability in local environmental pond conditions

The entire set of ponds included 17 temporal and 13 permanent ponds. All investigated ponds were relatively small (0.01 ha to 0.48 ha) and shallow (0.05 m – 2.5 m mean depth). The trophic status of the studied ponds ranged from eutrophic to hypertrophic (with mean TN and TP concentrations of 3.58 mg L^{-1} and 0.92 mg L^{-1}, respectively). The first two dimensions of the PCA ordination plot based on standardized local environmental pond variables jointly comprised 40% of the total variation in local pond conditions (Fig. 3). The first was positively correlated to salt associated variables (including sulphates, Ca, Mg, conductivity, chlorides, alkalinity and Na) and to organic carbon content (both TOC and DOC). The second axis seems to be associated to eutrophication related parameters such as nutrient concentrations (NH_4, PO_4, TP and TN) and phytoplankton biomass (both Chl_a and PC). Interestingly, the first axis seems to differentiate permanent ponds from temporal ponds, while the importance of region on variation in local environmental ponds conditions seems to be less profound.

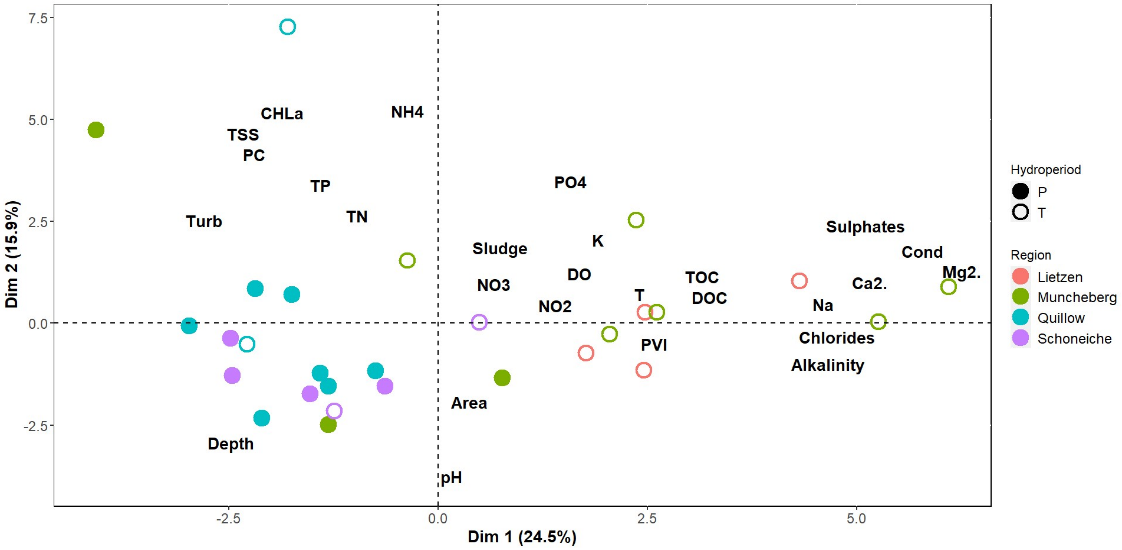


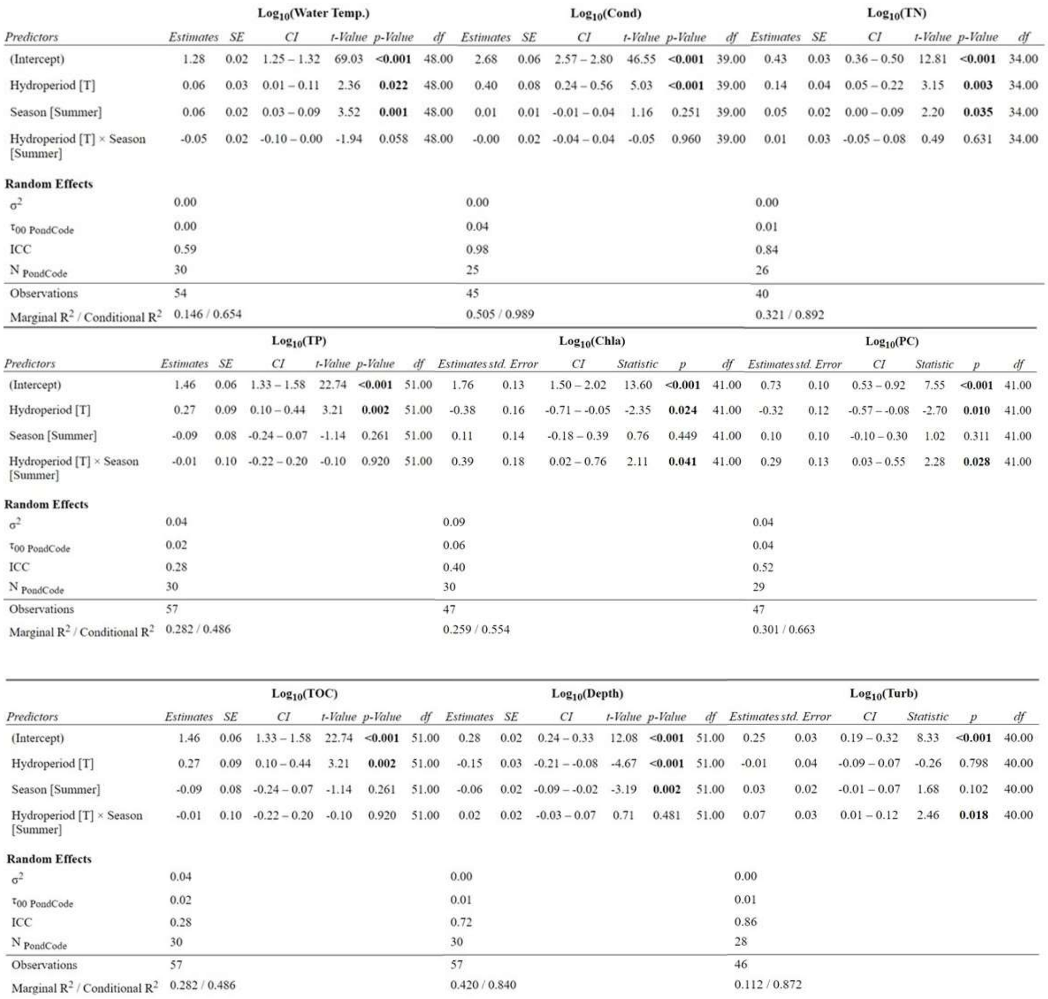
Figure 3: PCA ordination plot based on standardized spring local environmental variables. Filled and open dots represent the investigated permanent and temporal ponds respectively. Colours indicate in which region individual ponds are located.

3.1.2 Effect of Hydroperiod and Season on local environmental pond conditions

PERMANOVA analyses revealed overall significant differences between permanent and temporal ponds (p = 0.001) and between regions (p = 0.003) with respect to multiple major local environmental pond variables (Supplement: Table 1). Additional Two Sample T-tests and Wilcoxon Signed-Rank tests revealed significant differences between permanent and temporal ponds for potassium, sulphates, calcium, conductivity, water temperature, DOC, TOC, depth, and TN (only during summer), Chl_a and PC ($p < 0.05$) (Supplement: Table 2). Overall, temporal ponds were characterized by relatively higher cation and anion concentrations, higher water temperatures and higher nutrient concentrations compared to permanent ponds. Compared to temporal ponds, permanent ponds were deeper and had higher Chl_a and PC concentrations (Supplement: Fig 1).

Linear mixed effects models revealed significant differences between permanent and temporal ponds with respect to water temperature ($p = 0.180$), conductivity ($p = 4.98e-07$), TN ($p = 0.002$), Chl_a ($p = 0.001$), PC ($p = 0.007$), TOC ($p = 0.001$) and water column depth ($p = 2.97e-06$), but not for TP ($p = 0.848$) or turbidity ($p = 0.927$) (Table: 1). Concerning DOC, we were unable to create a model without singular fit issues. Water temperature and TN were higher in temporal ponds (average difference of 2.2 °C and 1.63 mg L^{-1} between hydroperiods respectively) and were typically higher in summer (average difference of 1.4 °C and 0.6 mg L^{-1} between seasons respectively) (Supplement: Fig. 1 and 2). Conductivity and TOC were higher in temporal ponds compared to permanent ponds, but did not differ between seasons. Spring Chl_a and PC concentrations were higher in permanent ponds than in temporal ponds, but not in summer where concentrations of both Chl_a and PC were higher in temporal ponds (interaction effect). Water column depth was overall lower in temporal ponds than in permanent ponds and was significantly lower in summer compared to summer.

Table 1: Results of linear mixed models analyses on the different environmental parameters between Hydroperiod and Season. Spring and Summer 2021. SE= Standard Error, CI = Confidence interval, σ^2 = variance of random effect, t_{00} = variance of intercepts, ICC = Intra-Class Correlation.



	Log_{10}(Water Temp.)						Log_{10}(Cond)						Log_{10}(TN)					
Predictors	*Estimates*	*SE*	*CI*	*t-Value*	*p-Value*	*df*	*Estimates*	*SE*	*CI*	*t-Value*	*p-Value*	*df*	*Estimates*	*SE*	*CI*	*t-Value*	*p-Value*	*df*
(Intercept)	1.28	0.02	1.25 – 1.32	69.03	**<0.001**	48.00	2.68	0.06	2.57 – 2.80	46.55	**<0.001**	39.00	0.43	0.03	0.36 – 0.50	12.81	**<0.001**	34.00
Hydroperiod [T]	0.06	0.03	0.01 – 0.11	2.36	**0.022**	48.00	0.40	0.08	0.24 – 0.56	5.03	**<0.001**	39.00	0.14	0.04	0.05 – 0.22	3.15	**0.003**	34.00
Season [Summer]	0.06	0.02	0.03 – 0.09	3.52	**0.001**	48.00	0.01	0.01	-0.01 – 0.04	1.16	0.251	39.00	0.05	0.02	0.00 – 0.09	2.20	**0.035**	34.00
Hydroperiod [T] × Season [Summer]	-0.05	0.02	-0.10 – 0.00	-1.94	0.058	48.00	-0.00	0.02	-0.04 – 0.04	-0.05	0.960	39.00	0.01	0.03	-0.05 – 0.08	0.49	0.631	34.00
Random Effects																		
σ^2	0.00						0.00						0.00					
$\tau_{00\ PondCode}$	0.00						0.04						0.01					
ICC	0.59						0.98						0.84					
$N_{PondCode}$	30						25						26					
Observations	54						45						40					
Marginal R^2 / Conditional R^2	0.146 / 0.654						0.505 / 0.989						0.321 / 0.892					

	Log_{10}(TP)						Log_{10}(Chla)						Log_{10}(PC)					
Predictors	*Estimates*	*SE*	*CI*	*t-Value*	*p-Value*	*df*	*Estimates*	*std. Error*	*CI*	*Statistic*	*p*	*df*	*Estimates*	*std. Error*	*CI*	*Statistic*	*p*	*df*
(Intercept)	1.46	0.06	1.33 – 1.58	22.74	**<0.001**	51.00	1.76	0.13	1.50 – 2.02	13.60	**<0.001**	41.00	0.73	0.10	0.53 – 0.92	7.55	**<0.001**	41.00
Hydroperiod [T]	0.27	0.09	0.10 – 0.44	3.21	**0.002**	51.00	-0.38	0.16	-0.71 – -0.05	-2.35	**0.024**	41.00	-0.32	0.12	-0.57 – -0.08	-2.70	**0.010**	41.00
Season [Summer]	-0.09	0.08	-0.24 – 0.07	-1.14	0.261	51.00	0.11	0.14	-0.18 – 0.39	0.76	0.449	41.00	0.10	0.10	-0.10 – 0.30	1.02	0.311	41.00
Hydroperiod [T] × Season [Summer]	-0.01	0.10	-0.22 – 0.20	-0.10	0.920	51.00	0.39	0.18	0.02 – 0.76	2.11	**0.041**	41.00	0.29	0.13	0.03 – 0.55	2.28	**0.028**	41.00
Random Effects																		
σ^2	0.04						0.09						0.04					
$\tau_{00\ PondCode}$	0.02						0.06						0.04					
ICC	0.28						0.40						0.52					
$N_{PondCode}$	30						30						29					
Observations	57						47						47					
Marginal R^2 / Conditional R^2	0.282 / 0.486						0.259 / 0.554						0.301 / 0.663					

	Log_{10}(TOC)						Log_{10}(Depth)						Log_{10}(Turb)					
Predictors	*Estimates*	*SE*	*CI*	*t-Value*	*p-Value*	*df*	*Estimates*	*SE*	*CI*	*t-Value*	*p-Value*	*df*	*Estimates*	*std. Error*	*CI*	*Statistic*	*p*	*df*
(Intercept)	1.46	0.06	1.33 – 1.58	22.74	**<0.001**	51.00	0.28	0.02	0.24 – 0.33	12.08	**<0.001**	51.00	0.25	0.03	0.19 – 0.32	8.33	**<0.001**	40.00
Hydroperiod [T]	0.27	0.09	0.10 – 0.44	3.21	**0.002**	51.00	-0.15	0.03	-0.21 – -0.08	-4.67	**<0.001**	51.00	-0.01	0.04	-0.09 – 0.07	-0.26	0.798	40.00
Season [Summer]	-0.09	0.08	-0.24 – 0.07	-1.14	0.261	51.00	-0.06	0.02	-0.09 – -0.02	-3.19	**0.002**	51.00	0.03	0.02	-0.01 – 0.07	1.68	0.102	40.00
Hydroperiod [T] × Season [Summer]	-0.01	0.10	-0.22 – 0.20	-0.10	0.920	51.00	0.02	0.02	-0.03 – 0.07	0.71	0.481	51.00	0.07	0.03	0.01 – 0.12	2.46	**0.018**	40.00
Random Effects																		
σ^2	0.04						0.00						0.00					
$\tau_{00\ PondCode}$	0.02						0.01						0.01					
ICC	0.28						0.72						0.86					
$N_{PondCode}$	30						30						28					
Observations	57						57						46					
Marginal R^2 / Conditional R^2	0.282 / 0.486						0.420 / 0.840						0.112 / 0.872					

One Way Repeated Measurement ANOVAs and a Friedman test showed that pH, TOC, DOC, and water temperature significantly differed between seasons in permanent ponds ($p < 0.05$), while DO, conductivity, TN, TP, turbidity, Chl_a and PC did not ($p > 0.05$) (Supplement: Table 3). Tukey post hoc analyses revealed that pH was lowest during summer, with average values of 7.53, 7.57 and 6.5, in spring, fall and summer respectively (Supplement: Fig 3 and Table 4), DOC was higher in spring (mean DOC 26.3 mg L^{-1}) and did not differ between summer and fall (mean values of 18.8 mg L^{-1} and 16.0 mg L^{-1} in summer and fall respectively). An additional Tukey post-hoc analysis revealed higher TOC concentrations in spring and summer and lower in fall (mean TOC 29.5 mg L^{-1}, 24.3 mg L^{-1} and 19.5 mg L^{-1}, respectively). Lastly, a Dunn test showed that water temperature was significantly different across every season, with the highest temperatures registered in summer (21°C), followed by spring (18.3 °C) and fall being the coldest season (14.2 °C) (Supplement: Fig. 3 and Table 4).

Separate Paired T-tests and Wilcoxon Signed-Rank tests revealed that the only variable that significantly differed between 2021 and 2022 was magnesium concentration ($p < 0.05$) (Supplement: Fig. 4, 5 and Table 5).

3.2 Patterns of variation in GHG emissions between ponds located in different regions.

3.2.1 CH_4 Ebullition Flux

The investigated ponds were net emitters of CH_4 ebullition fluxes in all regions. CH_4 fluxes varied considerably between ponds in all regions (range from 1.8 to 303.1 mg CH_4-C d^{-1} m^{-2} in Lietzen, from 0.3 to 374.4 mg CH_4-C d^{-1} m^{-2} in Müncheberg, from 0.9 to 349.4 mg CH_4-C d^{-1} m^{-2} in Quillow and from 0.9 to 628.1 mg CH_4-C d^{-1} m^{-2} in Schöneiche (Fig. 4), but Kruskal-Wallis tests revealed no systematic differences in CH_4 ebullition between regions in any of the seasons (Spring: H(3) = 2,99, p = 0.388; Summer: H(3) = 0.65 , p = 0.889; Fall: H(2) = 0.29, p = 0.868).

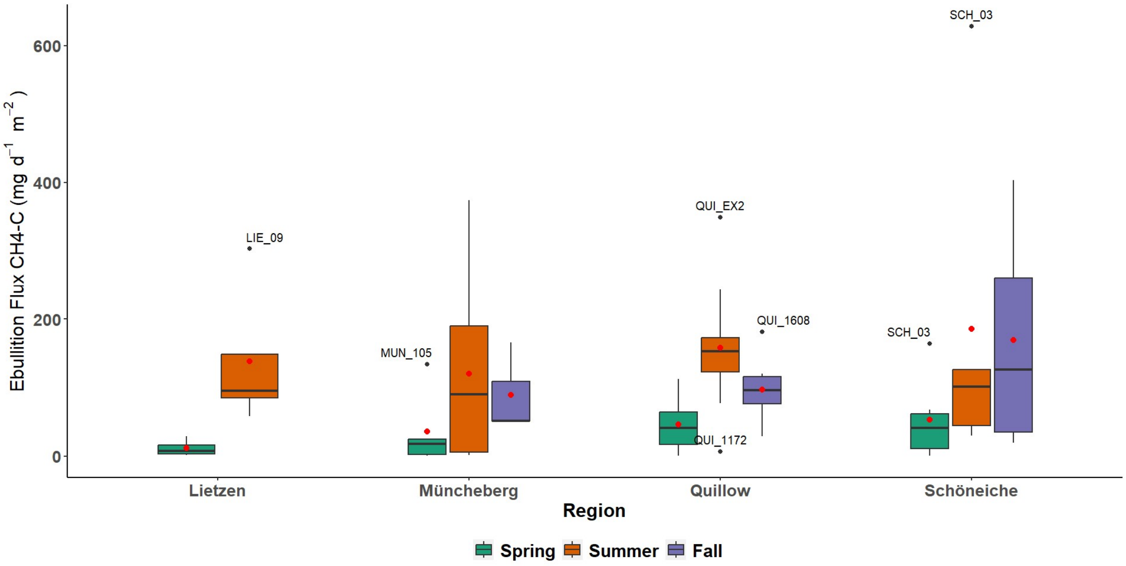


Figure 4: Boxplots showing ebullition CH_4 fluxes quantified in spring, summer and autumn from ponds located in Lietzen, Müncheberg, Quillow and Schöneiche. Boxes represent first and third quartiles and whiskers are minimum and maximum, black horizontal lines and red points represent the median and the mean respectively. Black dots show outliers and are annotated with the name of the pond they belong to.

3.2.2 Dissolved Fluxes

Dissolved fluxes of CH_4, CO_2 and N_2O varied between ponds (-0.01 mg CH_4-C d^{-1} m^{-2} to 677.8 mg CH_4-C d^{-1} m^{-2} ; -294.7 mg CO_2-C d^{-1} m^{-2} to 15,009.8 mg CO_2-C d^{-1} m^{-2} ; -0.1 mg N_2O-N d^{-1} m^{-2} to 1.1 0.1 mg N_2O-N d^{-1} m^{-2}) and season (mean spring fluxes of 8.7 mg CH_4-C d^{-1} m^{-2}, 1143.0 mg CO_2-C d^{-1} m^{-2} and 0.08 mg N_2O-N d^{-1} m^{-2}; mean summer fluxes of 66.6 mg CH_4-C d^{-1} m^{-2}, 3354.0 mg CO_2-C d^{-1} m^{-2} and -0.06 mg N_2O-N d^{-1} m^{-2}; mean fall fluxes of 113.0 mg CH_4-C d^{-1} m^{-2}, and 3307.0 mg CO_2-C d^{-1} m^{-2} and -0.06 mg N_2O-N d^{-1} m^{-2}) (Fig. 5), but did not differ significantly between regions (Kruskal-Wallis tests, Supplement: Table 7).

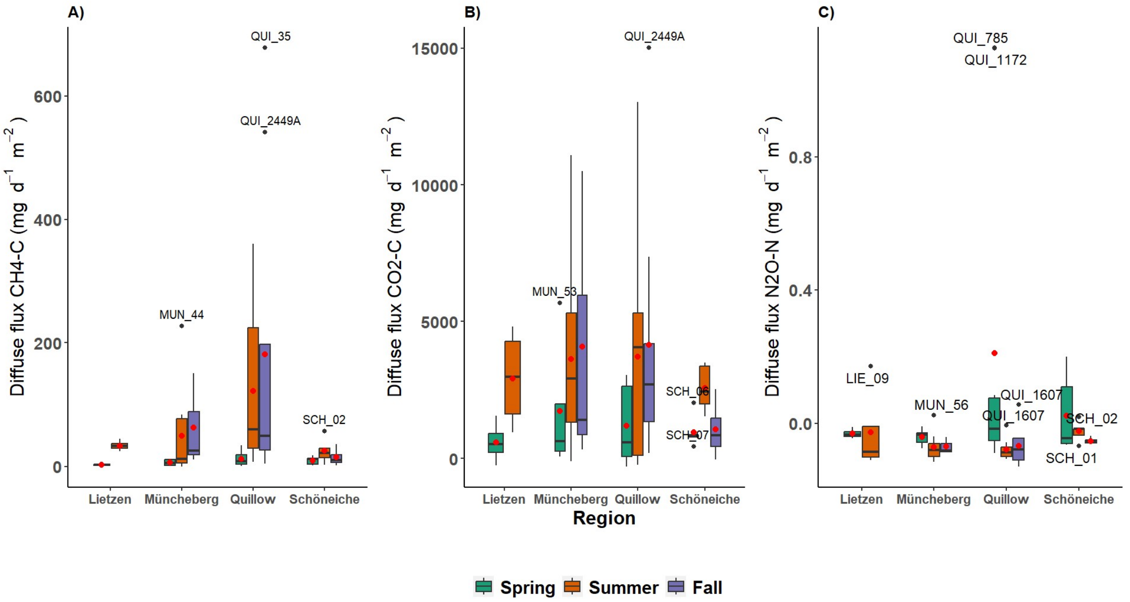


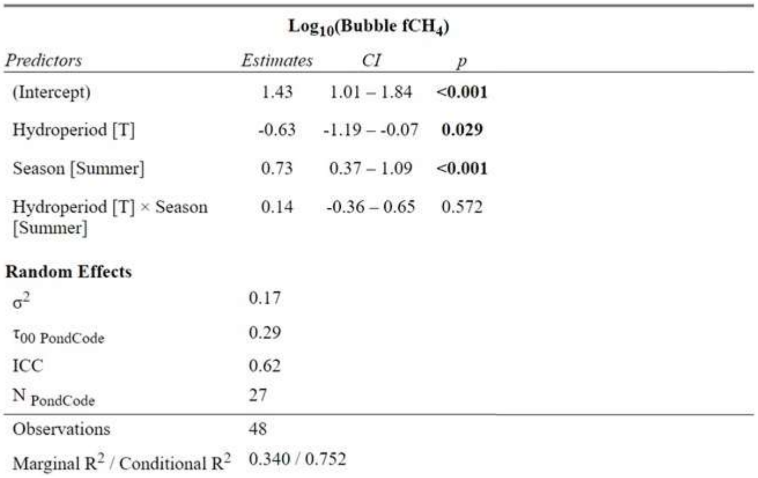
Figure 5: Boxplots showing A) Dissolved A) CH_4 B) CO_2 and C) N_2O fluxes, quantified in spring, summer and autumn from ponds located in Lietzen, Müncheberg, Quillow and Schöneiche. Boxes represent first and third quartiles and whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers and are annotated with the name of the pond they belong to.

3.3 Effect of Hydroperiod and Season on GHG emissions

3.3.1 CH_4 Ebullition flux

A linear mixed effect model significantly revealed (p = 0.022) that CH_4 bubble emissions were higher in permanent ponds compared to temporal ponds, and were higher in summer than in spring. There was no significant interaction effect between hydroperiod and season (Table: 2). Overall, average emissions of CH_4 were two times higher in permanent ponds than in temporal ponds and three times higher during summer compared to spring (Fig. 6A). The highest emissions were systematically observed in a permanent pond located in Schöneiche (pond “SCH_03”).

Table 2: Linear mixed effects model of $\log_{10}$-transformed ebullition CH_4 flux. SE= Standard Error, CI = Confidence interval, σ^2 = variance of random effect, t_{00} = variance of intercepts, ICC = Intra-Class Correlation.



	$\mathbf{Log_{10}(Bubble\ fCH_4)}$		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.43	1.01 – 1.84	**<0.001**
Hydroperiod [T]	-0.63	-1.19 – -0.07	**0.029**
Season [Summer]	0.73	0.37 – 1.09	**<0.001**
Hydroperiod [T] × Season [Summer]	0.14	-0.36 – 0.65	0.572
Random Effects			
σ^2	0.17		
$\tau_{00\ PondCode}$	0.29		
ICC	0.62		
$N_{PondCode}$	27		
Observations	48		
Marginal R^2 / Conditional R^2	0.340 / 0.752		

A more detailed One-Way Repeated Measures ANOVA analysis on the effect of season based on permanent ponds only, revealed overall significant effects of season on CH_4 bubble emissions (F(2, 20) = 25.26, p < 0.001) (Supplement: Table 8). A complementary Tukey post hoc test indicated that methane bubble emissions were highest in summer, followed by fall and lowest in spring (mean CH_4 emission: 149.0 mg CH_4-C d^{-1} m^{-2}, 115.0 mg CH_4-C d^{-1} m^{-2} and 40.2 mg CH_4-C d^{-1} m^{-2} in summer, fall and spring respectively) (Fig 6B, Supplement: Table 9).

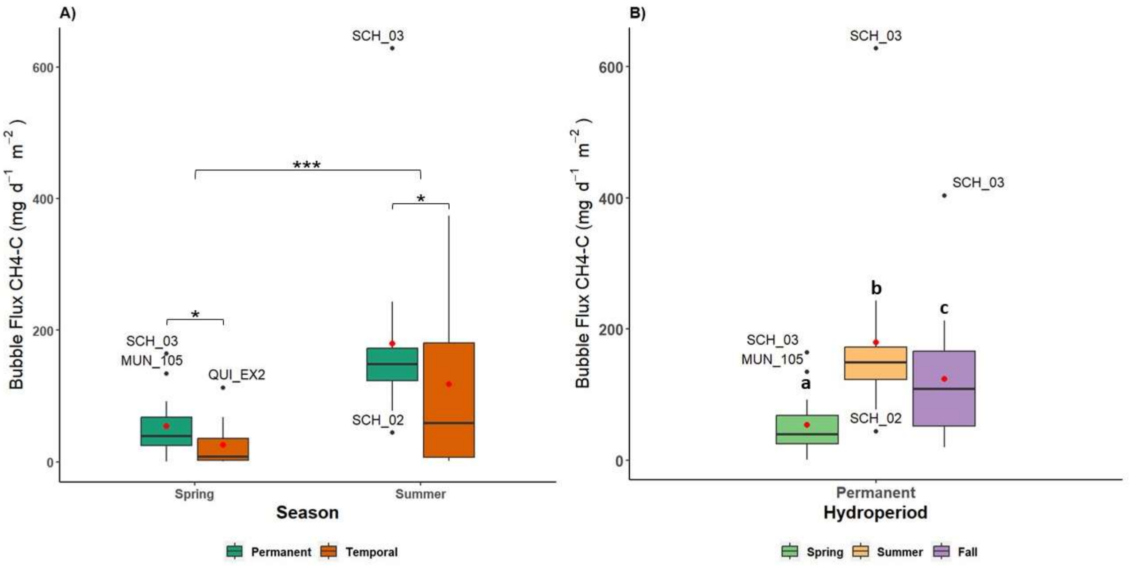


Figure 6: Boxplots showing A) Ebullition CH_4 fluxes in spring 2022 and summer 2021 for permanent and temporal ponds, B) Ebullition CH_4 fluxes for permanent ponds in spring 2022, summer 2021 and fall 2021. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers and are annotated with the name of the pond they belong to.

3.3.2 Dissolved fluxes

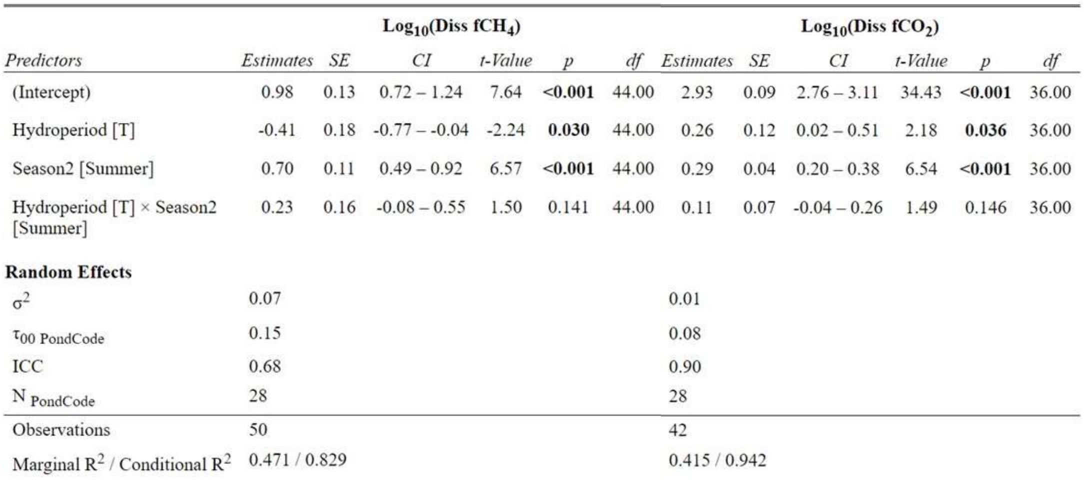
The majority of investigated ponds acted as net emitters of CH_4 and CO_2, while they largely acted as sinks for N_2O (Fig 7A). The carbon emission in the form of CO2 was considerably higher (41 times) than dissolved emissions of CH_4.

Linear mixed effects model significantly showed (p = 0.025) that dissolved CH_4 fluxes were significantly different between seasons, and between permanent and temporal ponds. We also did not observe a significant interaction effect between season and (Table 3). CH_4 emissions were systematically higher in summer than in spring (mean CH_4 emission: 66.6 mg CH_4-C d^{-1} m^{-2} and 8.7 mg CH_4-C d^{-1} m^{-2} in summer and spring respectively) (Fig. 7A). Similarly, emissions in permanent ponds were systematically higher (mean CH_4 emission: 41.5 mg CH_4-C d^{-1} m^{-2} and 36.0 mg CH_4-C d^{-1} m^{-2} in permanent and temporal ponds respectively). The three highest CH_4 emissions in spring and summer were consistently recorded in the same set of ponds (“QUI_EX2”, “QUI_1608” and “QUI_35”).

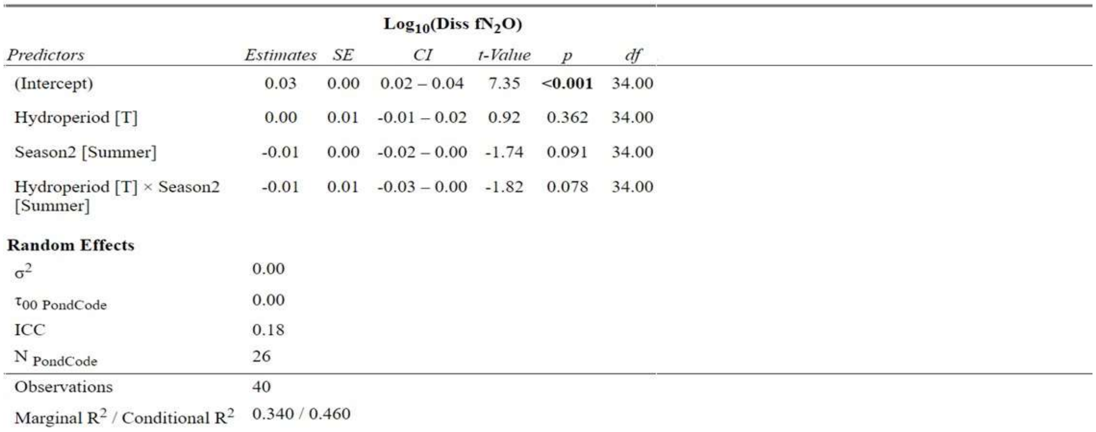
Linear mixed effects model for dissolved CO_2 fluxes was significant (p = 0.029) in exhibiting an effect of season and hydroperiod, but no interaction effect between season and hydroperiod (Table 3). In contrast to CH_4 fluxes, CO_2 flux emissions were higher in temporal ponds compared to permanent ponds (mean CO_2 emissions: 2838.0 mg CO_2-C d^{-1} m^{-2} and 1744.0 mg CO_2-C d^{-1} m^{-2} in temporal and permanent ponds respectively). Similarly, CO_2 emissions were higher during summer and in this case they tripled spring CO_2 emissions (mean CO_2 emissions: 3354.0 mg CO_2-C d^{-1} m^{-2} and 1143.0 mg CO_2-C d^{-1} m^{-2} in summer and spring respectively). For both temporal and permanent ponds, CO_2 flux emissions were higher in summer than in spring (FIG 7B). During summer, temporal ponds emitted on average 1291 mg CO_2-C d^{-1} m^{-2} more than permanent ponds and in the case of spring the average difference was of 708 mg CO_2-C d^{-1} m^{-2}.

A linear mixed effects model suggest that variation in dissolved N_2O fluxes were not significantly explained by hydroperiod, season or their interaction (p = 0.355), although N_2O fluxes tended to be slightly higher in spring compared to summer. Importantly, both permanent and temporal ponds tended to act as sinks of N_2O during summer, with average fluxes of -0.06 mg N_2O-N d^{-1} m^{-2} in , while they seemed to act as net emitters during spring with average fluxes of 0.08 mg N_2O-N d^{-1} m^{-2} (Fig. 7C).

Table 3: Linear mixed effects model of log_{10}-transformed dissolved CH_4, CO_2 and N_2O fluxes. SE= Standard Error, CI = Confidence interval, σ^2 = variance of random effect, t_{00} = variance of intercepts, ICC = Intra-Class Correlation.



	Log_{10}(Diss fCH_4)						Log_{10}(Diss fCO_2)					
Predictors	*Estimates*	*SE*	*CI*	*t-Value*	*p*	*df*	*Estimates*	*SE*	*CI*	*t-Value*	*p*	*df*
(Intercept)	0.98	0.13	0.72 – 1.24	7.64	**<0.001**	44.00	2.93	0.09	2.76 – 3.11	34.43	**<0.001**	36.00
Hydroperiod [T]	-0.41	0.18	-0.77 – -0.04	-2.24	**0.030**	44.00	0.26	0.12	0.02 – 0.51	2.18	**0.036**	36.00
Season2 [Summer]	0.70	0.11	0.49 – 0.92	6.57	**<0.001**	44.00	0.29	0.04	0.20 – 0.38	6.54	**<0.001**	36.00
Hydroperiod [T] × Season2 [Summer]	0.23	0.16	-0.08 – 0.55	1.50	0.141	44.00	0.11	0.07	-0.04 – 0.26	1.49	0.146	36.00
Random Effects												
σ^2	0.07						0.01					
$\tau_{00\ PondCode}$	0.15						0.08					
ICC	0.68						0.90					
$N_{PondCode}$	28						28					
Observations	50						42					
Marginal R^2 / Conditional R^2	0.471 / 0.829						0.415 / 0.942					



	Log_{10}(Diss fN_2O)					
Predictors	*Estimates*	*SE*	*CI*	*t-Value*	*p*	*df*
(Intercept)	0.03	0.00	0.02 – 0.04	7.35	**<0.001**	34.00
Hydroperiod [T]	0.00	0.01	-0.01 – 0.02	0.92	0.362	34.00
Season2 [Summer]	-0.01	0.00	-0.02 – 0.00	-1.74	0.091	34.00
Hydroperiod [T] × Season2 [Summer]	-0.01	0.01	-0.03 – 0.00	-1.82	0.078	34.00
Random Effects						
σ^2	0.00					
$\tau_{00\ PondCode}$	0.00					
ICC	0.18					
$N_{PondCode}$	26					
Observations	40					
Marginal R^2 / Conditional R^2	0.340 / 0.460					

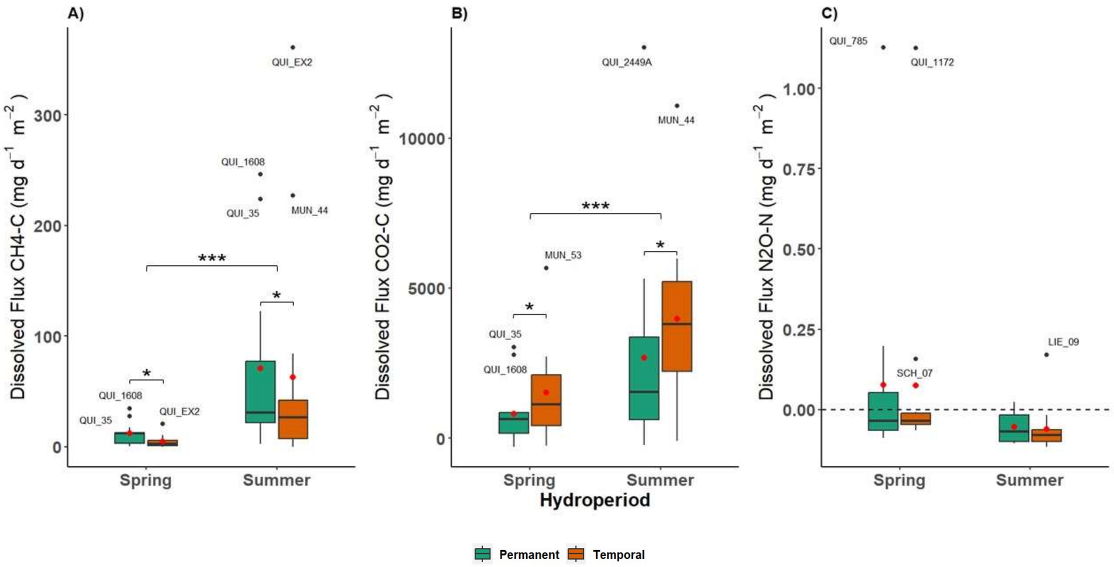


Figure 7: Boxplots showing the diffusive fluxes of A) CH_4, B) CO_2 and C) N_2O for permanent and temporal ponds. Boxes represent the first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Full black points dots outliers and are annotated with the name of the pond they belong to.

A more detailed Friedman analysis and complementary Dunn tests for dissolved CH_4 emissions based only on permanent ponds revealed a significant effect of season on dissolved methane flux ($\chi^2(2) = 9.38$, $p < 0.01$) and that during summer CH_4 emissions were higher in summer and autumn compared to spring, and that summer and autumn emission did not differ (median CH_4 emission: 31.0 mg CH_4-C d^{-1} m^{-2}, 25.9 mg CH_4-C d^{-1} m^{-2} and 12.2 CH_4-C d^{-1} m^{-2} in summer, fall and spring respectively) (Fig 8A, Supplement: Table 8 and 9). It is worth noting that the highest recorded emissions were consistently found in ”QUI_35” for all seasons and “QUI_1608” for Spring and Summer.

A One-Way Repeated Measures ANOVA and post-hoc Tukey tests revealed higher CO_2 fluxes during summer compared to spring, while fall did not significantly differ from the other two seasons. ($F(2, 16)= 4.28$, $p < 0.05$) (Supplement: Tables 8 and 9). We registered the highest CO_2 daily fluxes during summer and fall, with values ranging from -230 mg CO_2-C d^{-1} m^{-2} to 13018 mg CO_2-C d^{-1} m^{-2} and -30.3 mg CO_2-C d^{-1} m^{-2} to 15010 mg CO_2-C d^{-1} m^{-2}, respectively (Fig. 8B). On the other hand, spring CO_2 dissolved flux ranged between -295 mg CO_2-C d^{-1} m^{-2} and 3039 mg CO_2-C d^{-1} m^{-2}.

When it comes to only permanent ponds, a Friedman test concluded that N_2O emissions did not significantly differ across seasons ($\chi^2(2) = 0.17$, $p > 0.05$) (Supplement: Table 8). Nevertheless, observed dissolved N_2O fluxes reached maximum values during spring (1.13 mg N_2O-N d^{-1} m^{-2}), being 46 times higher than those reported in summer (0.02

mg N_2O-N d^{-1} m^{-2}) and 18 times higher than the reported maximum value in fall (0.06 mg N_2O-N d^{-1} m^{-2}) (Fig. 8C). As a whole, permanent ponds acted as sinks of N_2O across all seasons, with median fluxes ranging from -0.07 mg N_2O-N d^{-1} m^{-2} in summer to -0.03 mg N_2O-N d^{-1} m^{-2} in spring.

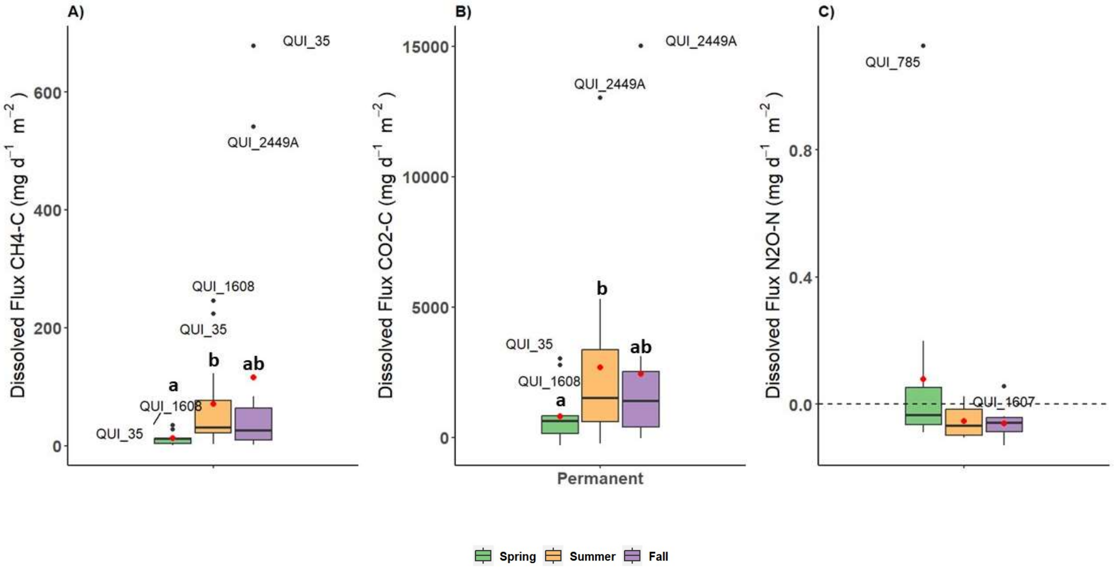


Figure 8: Boxplots showing the diffusive fluxes of A) CH_4, B) CO_2 and C) N_2O for permanent ponds measured in spring 2022, summer and fall 2021. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers and are annotated with the name of the pond they belong to.

3.4 Key environmental pond properties determining GHG emission

3.4.1 CH_4 Ebullition flux

Multiple models were selected to predict CH_4 bubble flux in the set of studied ponds based on local environmental pond variables (Supplement. Table 10). Overall, it seems that nutrient concentrations (TN and/or TP), DO, pH and sulphates are important variables driving variation in CH_4 bubble fluxes. The best fitting model differed from the second best model by including TP instead of TN as an explanatory variable. The third best model included both TN and TP as predictor variables. Our analysis reveals that sulphate concentration in the water column had a profound negative effect on CH_4 bubble emissions in all models ($p < 0.001$). In our best model, both DO and TP were positively correlated to the CH_4 bubble emissions ($p < 0.05$).

3.4.2 Dissolved Fluxes

Multiple models were selected to predict CH_4 dissolved fluxes (Supplement: Table 11). All in all, pH, TP, sulphates, potassium and DOC are relevant variables driving variation in methane dissolved fluxes. The best fitting model differed from the second model by excluding DOC as an explanatory variable. The third best fitting model differed from the second by excluding sulphate as an explanatory variable. The fourth best fitting model differed from the third best one by using only pH and TP as explanatory variables. Our analysis shows that pH of ponds had a substantial positive effect on dissolved methane emissions in all models ($p < 0.01$). In our best fitting model, TP was positively correlated to dissolved CH_4 emissions ($p < 0.001$), while sulphate concentration in the water column was negatively correlated to this flux ($p < 0.01$).

None of the predictor variables significantly explained dissolved fluxes of CO_2 (Supplement: Table 12).

Multiple models were selected to predict N_2O dissolved fluxes (Supplement: Table 13). Altogether, NH_4, TOC, sulphates, chlorides, water column depth and PO_4 are important variables influencing variation in dissolved N_2O fluxes. The best fitting model differed from the second best fitting model by including water column depth as an explanatory variable. The third best fitting model differed from the second one by excluding chloride concentration as an explanatory variable. The fourth model differed from the third best fitting model by including PO_4 as an explanatory variable. These analyses showed a profound negative effect of TOC on nitrous oxide fluxes in all selected models ($p < 0.01$), while NH_4 and sulphate concentrations in the water column proved to have a positive effect ($p < 0.01$). In our best fitting model, chloride concentration was negatively correlated to dissolved nitrous oxide emissions and sulphate concentration was positively correlated to this flux ($p < 0.05$).

3.5 Impact of interannual variation on GHG emissions

3.5.1 CH_4 Ebullition flux

Our analysis reveals that mean CH_4 bubble fluxes in summer and autumn did not differ between sampling years (2021 and 2022) (Fig. 9) (Paired T-test, $t_{summer}(7) = 0.32$, $p > 0.05$; $t_{fall}(6) = 1.67$, $p > 0.05$) (Supplement: Table 14).

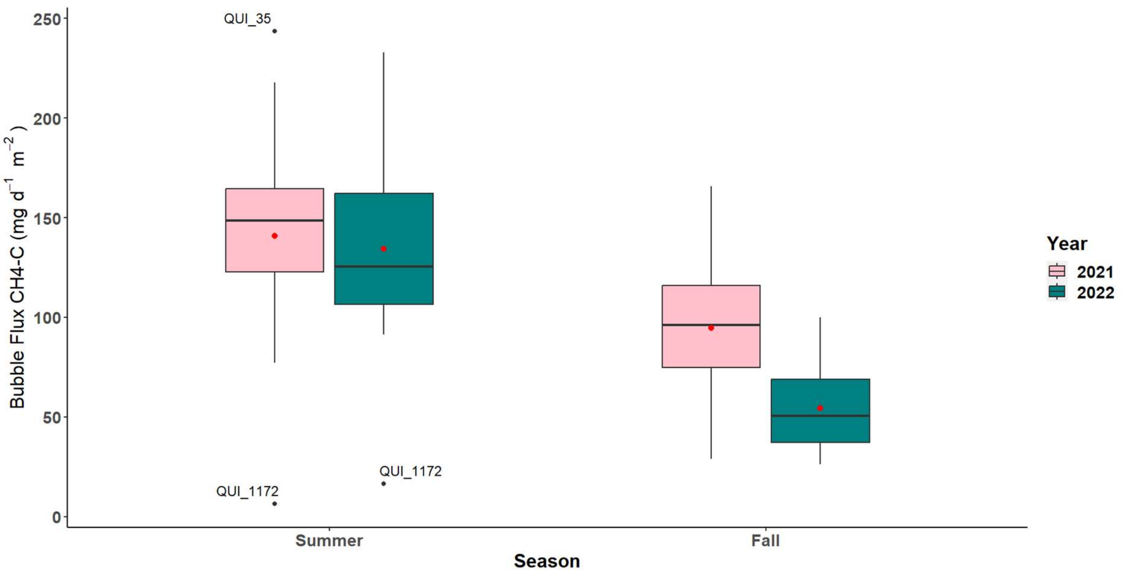


Figure 9: Boxplots showing ebullition CH_4 fluxes measured in summer and fall, 2021 and 2022. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Full black dots show outliers and are annotated with the name of the pond they belong to.

3.5.2 Dissolved fluxes

Dissolved CH_4 fluxes did not differ systematically between sampling years (Paired T-test, $t_{summer}(5) = -0.38$, $p > 0.05$; $t_{fall}(5) = 0.84$, $p > 0.05$) (Supplement: Table 14). Interestingly, CH_4 fluxes were considerably more variable between ponds in 2021 compared to 2022 (Fig 10A). The same pond (“QUI_35”) had the highest CH_4 dissolved flux in both years.

Wilcoxon Signed-Rank tests revealed that CO_2 fluxes did not significantly differ between sampling years ($W_{summer} = 19$, p = 0.734; $W_{fall} = 23$, p = 0,156). During 2021 the pond “QUI_2449” had the highest CO_2 dissolved fluxes summer and fall ,which were five or even seven times higher than the average CO_2 dissolved flux for all ponds (Fig. 10B). In the following year pond “QUI_1172” had the highest fluxes, being five-fold the average summer mean and three-fold the average fall mean.

We did not observe a systematic difference in N2O flux emission between sampling years ($W_{summer} = 17$, p = 0.945; $W_{fall} = 7$, p = 0.267) (Figure 10C). Overall, the investigated ponds acted as N2O sinks in both years and only four ponds acted as emitters of N2O.

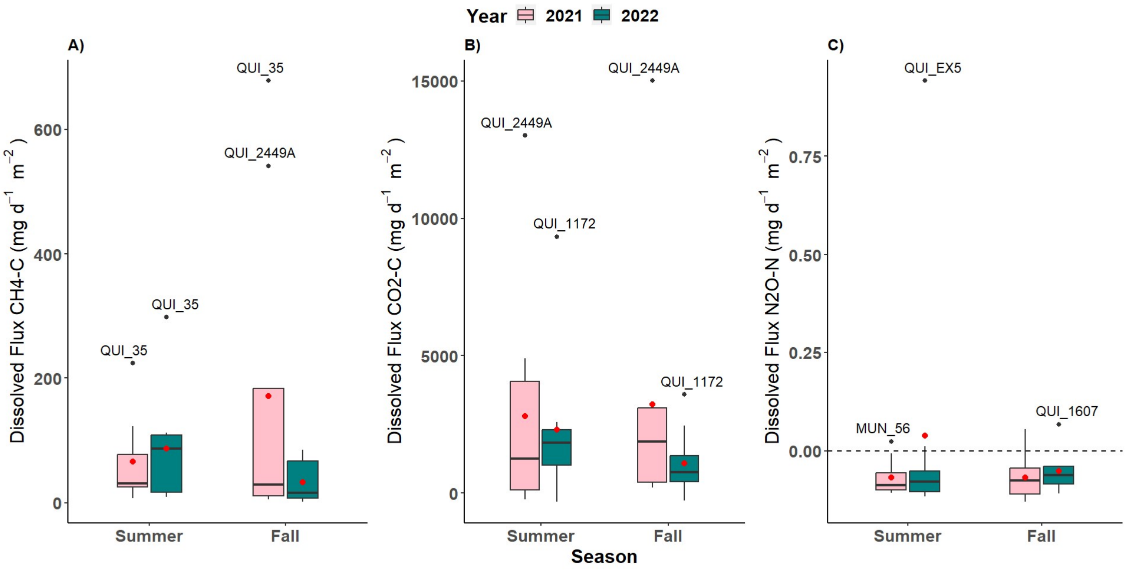


Figure 10: Boxplots showing the diffusive fluxes of A) CH$_4$, B) CO$_2$ and C) N$_2$O measured in summer and fall, 2021 and 2022. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers and are annotated with the name of the pond they belong to.

4. Discussion

The present study investigates local environmental conditions of ponds located in different regions in NE Germany and aims to assess the effect of hydroperiod, season and local pond conditions on the extent of GHGs emissions (CH_4, CO_2 and N_2O) by ponds, and to assess how this relates to interannual variation in GHG emission. Our results show that permanent ponds were characterized by higher ebullition and dissolved CH_4 emissions, while temporal ponds had higher CO_2 emissions. Our findings also show that during summer emissions of CH_4 and CO_2 were highest. This study also reveals that N_2O fluxes were not significantly affected by either hydroperiod or season. In addition, CH_4 and N_2O fluxes were strongly affected by sulphates, DO, TP and pH and by NH_4, TOC, sulphates and chloride concentration respectively. We did not observe systematic differences in GHG emissions between 2021 and 2022.

1. Overall patterns of GHG emissions

We observed considerable variation in GHG emissions between ponds, but most ponds acted as emitters of CH_4 (both ebullition and dissolved) and CO_2 and as sinks for N_2O.

In our study, we found average CH_4 fluxes of 99.3 mg CH_4-C d^{-1} m^{-2} and 56.6 mg CH_4-C d^{-1} m^{-2} for ebullition and dissolved fluxes respectively. Methane emissions were sensibly lower than those reported by Herrero Ortega et al., (2019) in urban ponds in Berlin. This author reported an average ebullition flux of 300 mg d^{-1} m^{-2} and mean dissolved fluxes of 120 mg d^{-1} m^{-2}, while the corresponding fluxes in our study were about a third and half respectively. We also registered average dissolved CO_2 fluxes of 2358.8 mg CO_2-C d^{-1} m^{-2}. These results are sensibly higher than the 628 mg CO_2-C d^{-1} m^{-2} reported by Audet et al., (2020) in Danish urban ponds. Lastly, Singh et al., (2005) reported much higher average N_2O emissions than those observed in our study. These authors reported average nitrous oxide emissions of 0.51 mg N_2O-N d^{-1} m^{-2} in an urban pond receiving domestic and agricultural runoff in Ujjain City in India, while in our study we found average N_2O dissolved fluxes of -8.3 e^{-3}.

Our findings provide strong evidence that the main pathway for methane emissions in ponds happens through ebullition, showing substantially higher fluxes than the diffusive pathway. The observation that ebullition represented more than 60% of total CH_4 emissions in our ponds is partially in line with earlies studies. Partially in line with our study, Herrero Ortega et al., (2019) reported that ebullition contributed 80% of total methane emissions, or Casper et al., (2000), who reported that 96% of the total CH_4 emissions from ponds occurred through ebullition pathway.

During our study we did not find a significant effect of Region on our GHGs fluxes.

4.2 The effect of Hydroperiod and temporal variation on GHG emissions

The observed differences in GHG emissions between permanent and temporal ponds can be linked to multiple factors, including differences in local environmental pond conditions between hydroperiod groups. We expect that the observed differences most likely result from differences in water column depth, water temperature, TN, TOC, DOC, phytoplankton biomasses, sulphate, calcium and potassium concentrations.

The importance of the water column depth on GHG emissions in ponds has been demonstrated in earlier studies (Blodau & Moore, 2003; Macdonald et al., 1998; Moore & Dalva, 1993; Yang et al., 2013 and Yuan, 2020). Higher water levels and increased water permanence would enhance CH_4 and N_2O emissions by preventing sediments exposure to the atmosphere and thus getting oxidized (Catalán al., 2014; Gómez-Gener et al., 2016), while also creating suitable anoxic sediment conditions that promote methanogenesis (Y. Yuan, 2020) and denitrification (Kumar et al., 2019; Marotta et al., 2014). In our study, permanent ponds were significantly deeper, offering a larger water column over the sediments which could promote anaerobic conditions and enhance methanogenesis (Bastviken et al., 2004; Holgerson, 2015; Malyan et al., 2016). Contrary to permanent ponds, we observed lower water column depths in temporal ponds. A decreased water column depth would have an opposite effect on dissolved CO_2 emissions by facilitating the exposure of pond sediments to an aerobic environment and thus facilitating oxidation that typically results in enhanced CO_2 flux emissions.

Another factor that can explain differences in GHG emission between temporal and permanent ponds might be water temperature (Audet et al., 2020; Baron et al., 2022; Bergen et al., 2019; DelSontro et al., 2016; Marotta et al., 2014; Peacock et al., 2021; Prėskienis et al., 2021; Wang et al., 2021). In our study, temporal ponds had on average higher water temperatures compared to permanent ponds. Not only does higher water temperature decrease gas solubility in the water column, but it also increases metabolic activity in ponds (Audet et al., 2020; Peacock et al., 2021; Wang et al., 2021), yielding increased GHG emissions through these two pathways. Furthermore studies by Baron et al., (2022) and Bergen et al., (2019) have shown that for every 1°C increase in temperature, CH_4 ebullition fluxes increase by 11%. In our study, we registered mean water temperatures 2.2 °C higher in temporal than in permanent ponds.

The registered differences in GHG emissions between hydroperiod groups can be also attributed to nutrient concentrations in ponds (e.g. TN, TP, TOC and DOC) (Gorsky et al., 2019; Hao et al., 2021; Köhn et al., 2021; Kumar et al., 2019; Obrador et al., 2018; Peacock et al., 2019, 2021; Wang et al., 2021; Webb et al., 2019; S.-S. Yang et al., 2015; Zheng et al., 2022).

The observed positive effect of increasing nutrient concentrations on GHG emission can likely be explained by the overall higher primary production in ponds, which can increase the amount of carbon for bacteria in both the sediments and the water column (Audet et al., 2020; Gorsky et al., 2019; Köhn et al., 2021; Kumar & Sharma, 2016; Obrador et al., 2018; Peacock et al., 2019; Webb et al., 2019). In addition, nitrogen-rich environments enhance both nitrification and denitrification pathways, having CO_2 emissions as one of the by-products (Webb et al., 2019). In our study, TN concentrations were higher in temporal ponds compared to permanent ponds. Additionally, we registered over double mean TOC and DOC in temporal ponds. Therefore, these factors would explain the higher dissolved CO_2 fluxes temporal ponds. Moreover, mean TP levels in our study were quite high (0.8 mg L^{-1}) and despite not being significantly different between hydroperiod groups, they could enhance both CH_4 and dissolved CO_2 fluxes.

However, despite the higher nutrient concentrations and water temperature in temporal ponds, we registered higher phytoplankton biomasses in permanent ponds during spring. Hence, it could be assumed that during this season other underlying processes determine the overall phytoplankton biomasses. These other factors could be higher photosynthetic performance (i.e. due to lower irradiance inhibition) (Yang et al., 2020) the presence of more competitive phytoplankton species (i.e. in nutrient acquisition) (Sommer et al., 2016), lower grazing pressures and/or increased resistance to it (Lürling, 2021), favouring CH_4 fluxes in permanent ponds.

Salt-related variables like sulphate, calcium and potassium concentrations in the water column can have profound impact on GHG emissions in ponds (Jagadeesh Babu et al., 2006; Malyan et al., 2016; Zhang et al., 2021). Sulphate, has an indirect impact on methanogenesis. Both methanogenic and sulphate-reducing bacteria utilize identical carbon sources as labile carbon (Malyan et al., 2016), resulting in competition between these communities for the available organic matter (Baron et al., 2022). Calcium, specifically Calcium Super Phosphate, a commonly used fertilizer (Zhang et al., 2021), has been found to inhibit methanogenesis in rice paddle systems though the addition of sulphate, which favours sulphate-reducing bacteria, but also through the shift in methanogenic communities to less efficient genera, like *Methanobrevibacter* (Miller et al., 2000; Mountfort et al., 1982). It is worth to note that majority of the sampled ponds in our study are located in agricultural areas. Earlier studies conducted Jagadeesh Babu et al., (2006) on flooded rice fields and pig manure composting, demonstrated that potassium not only prevents a drop in redox potential, reducing the contents of other active-reducing substances, like Fe^{2+} (favouring an aerobic environment), but also inhibits methanogenesis while stimulating methanotrophic bacteria (Trolldenier, 1973). In our study, we observed that temporal ponds possessed seven and over three times higher sulphate and calcium concentrations respectively, and 45% higher potassium concentrations. Even more, our selected models for CH_4 (ebullition and dissolved fluxes) have shown profound negative impact of sulphate concentration on this GHG flux. As a result, the combination of these factors could lead to higher dissolved CO_2 emissions in temporal ponds and lower CH_4 emissions also in temporal ponds.

Contrary to our expectations, dissolved N_2O did not significantly differ between hydroperiods. We would have expected higher N_2O fluxes in permanent ponds, given the higher water levels (Kumar et al., 2019; Marotta et al., 2014), which would favour the occurrence of anaerobic conditions in sediments, creating a suitable environment for denitrification (Kumar et al., 2019; Marotta et al., 2014). Nitrous oxide emissions are mainly produced through this process, where nitrogen compounds like NO_3 and NH_4 are substrates (Ma et al., 2018; Zheng et al., 2022). Thus, the higher TN concentrations registered in temporal ponds could in turn overrule the negative effect of a lower water level column and thus contribute to creating similar fluxes between hydroperiod groups. On top of this, higher sulphide concentrations are associated with higher N_2O concentrations (Sørensen et al., 1980). The inhibitory effect of sulphide on the conversion of N_2O to N_2, derived from the reduction of sulphates. Therefore the higher concentrations of sulphates in temporal ponds, which would later become reduced to sulphide by sulphate-reducing bacteria, could also play a role in neutralizing the effects of higher water levels found in permanent ponds. In addition, the higher water temperature registered in temporal ponds could increase overall microbial activity, increasing denitrification rates, contributing to overrule the effects of a lower water column (Khoiyangbam & Chingangbam, 2022; Marotta et al., 2014; Peacock et al., 2019).

The observed differences in GHG emissions between seasons could be linked to multiple factors, including differences in local environmental pond conditions between seasons. We expect the registered differences to likely result from differences in water column depth, favouring anaerobic or aerobic environments (Blodau & Moore, 2003; Catalán et al., 2014; Gómez-Gener et al., 2016; Macdonald et al., 1998; More & Dalva, 1993; J. Yang et al., 2013; Y. Yuan, 2020), water temperature, regulating overall gas solubility in water and also microbial activity (Audet et al., 2020; Baron et al., 2022; Bergen et al., 2019; DelSontro et al., 2016; Marotta et al., 2014; Peacock et al., 2021; Prėskienis et al., 2021; Wang et al., 2021), TN and phytoplankton biomasses, regulating available carbon sources for bacteria and also modulating nitrification and denitrification pathways (Audet et al., 2020; Gorsky et al., 2019; Köhn et al., 2021; Kumar & Sharma, 2016; Obrador et al., 2018; Peacock et al., 2019; Webb et al., 2019). Additionally, a lower water column depth can increase sediment's exposure to wind induced turbulence, which triggers the release of methane bubbles trapped in the sediments (Ferencz et al., 2018; Joyce & Jewell, 2003). During summer, ponds were significantly shallower, had higher water temperatures and higher TN concentrations. During summer, we also registered higher phytoplankton biomasses (in temporal ponds). Thus, these differences could explain the higher CH_4 and CO_2 emissions registered during this season. During fall, we continued registering high CO_2 and CH_4 emissions, despite having lower water temperature and TOC concentration than during spring. This could stem from the increased phytoplankton biomass generated during summer which later on sediments to the bottom of the ponds, creating a convenient environment for CH_4 and CO_2 emissions during fall, despite the more suitable spring conditions (Audet et al., 2020; Gorsky et al., 2019; Köhn et al., 2021; Kumar & Sharma, 2016; Obrador et al., 2018; Peacock et al., 2019; Webb et al., 2019). Furthermore, the decreasing area of all ponds during fall, related to drying-up events (main precipitation period occurs during winter), could expose more sediments to the air, resulting in higher oxidation rates and CO_2 emissions (Catalán al., 2014; Gómez-Gener et al., 2016).

Against our expectations, we did not find an effect of Season on N_2O emissions. We would have expected higher N_2O fluxes in during summer, mainly due to the higher water temperatures (Khoiyangbam & Chingangbam, 2022; Marotta et al., 2014; Peacock et al., 2019) and higher TN concentrations, increasing substrates for denitrification (Khoiyangbam & Chingangbam, 2022; Tian et al., 2012). Nevertheless, the negative impact of a lower water column depth (favouring an aerobic environment) could have counteracted the enhancing effect of these other variables, resulting in no overall differences in N_2O fluxed between seasons.

In addition, we found no significant differences in emissions between the 2021 and 2022. This is to be expected given most of the environmental pond conditions did not significantly change between years.

4.3 Key environmental parameters effect on GHG emissions

Our multiple regression models revealed a positive correlation between ebullition and dissolved CH_4 fluxes with TP, DO and pH and a negative correlation to sulphate concentration. In contrast to our expectations, DO seemed to have a positive effect on methane fluxes. Previous studies by Wilmoth et al., (2021) in wetlands found that peat samples exposed to oxygen produced dramatically higher methane levels by the end of an anoxic incubation phase, when compared to samples continuously kept in anoxic environments. Other authors found that a vital determinant of overall decomposition efficiency is the initial conversion of carbon present in large polymeric compounds into more bioavailable forms (Lehmann & Kleber, 2015; Megonigal et al., 2003; Wakeham & Canuel, 2006). The inefficient catabolizing of these large compounds due to the lack of oxygen and the proper microbial enzymes is common in oxygen-deprived systems (Fenner & Freeman, 2011; Wakeham & Canuel, 2006). Posterior anaerobic hydrolysis of these more bioavailable polymers into simpler monomers and the fermentation of monomeric carbon, results in a higher concentration of substrates that can enhance methanogenesis (Megonigal et al., 2003). Therefore, temporarily increasing DO concentrations in the sediments could promote the breakdown of complex carbon structures into more bioavailable forms and once anoxic conditions return, methanogenesis becomes enhanced. The positive effect of pH could be explained given the maximum CH_4 yield varies between pH from 6.5 to 8.2, with the optimum pH for methanogenesis being between 7.0 and 7.2 (Lai et al., 2009). During summer, the average pH of our set of ponds was of 6.7, therefore an increase in pH would yield higher methanogenetic rates.

None of the multiple linear regression models were able to significantly correlate environmental conditions to dissolved CO_2 fluxes. We believe this to be due to the high variability in measurements from this flux between ponds.

Our multiple regression models for dissolved N_2O fluxes revealed a positive correlation with NH4 and sulphate concentrations, and a negative correlation with TOC and chloride concentration in the water column. Contrary to our expectations, our selected model showed that TOC had a negative effect on nitrous oxide fluxes. Nevertheless, higher concentrations of available organic matter do not necessarily translate into higher nitrous oxide fluxes. Parameters such as the C/N ratio of the organic matter and the nature of the carbon source itself have an important effect on nitrous oxide production (Kargi, 2003). Previous studies in wastewater treatment by Elefsiniotis & Wareham, (2007) have shown that denitrifying bacteria favour volatile fatty acids and not organic complex molecules as carbon sources. Other studies by Bremmer, (1977) revealed that depending on the carbon source used, denitrification rates may be similar, but the overall nitrous oxide produced can be sensibly different. Thus, the very nature of the available TOC concentrations could not be suitable for the denitrification pathway and inhibit nitrous oxide emissions rather than promote it. Finally, our selected model for nitrous oxide fluxes showed that chloride concentrations exhibited a negative correlation to this flux. Chloride has been found to reduce nitrification rates in soils in several studies (Chen & Wong, 2004; Chowdhury et al., 2011; Darrah et al., 1987; Guo et al., 2022; B.-C. Yuan et al., 2007). The main mechanism for nitrification inhibition by chlorides results of increased solute concentration (Darrah et al., 1987; Golden et al., 1981; Souri, 2010) and the strong oxidizing and biocide properties it possesses (Chen & Wong, 2004). Therefore, increasing chloride concentrations in our ponds would effectively inhibit nitrification, in which N_2O accumulates as a by-product of ammonia oxidizing bacteria (Conthe et al., 2019; Kampschreur et al., 2009; Schreiber et al., 2012), and reduce the overall nitrous oxide flux.

4.4 Limitations of the study

Although we found most ponds were net emitters in the case of CO_2 and CH_4, this does not confirm them as net sources or sinks since during this study we did not focus on the entire carbon budget for each pond. Complementing our study with data obtained from sediment traps might offer additional insight into the overall carbon budget of ponds and allow us to classify each pond into carbon sources or sinks. Furthermore, the microbial community of the ponds was not studied, which could have allowed us to clearly identify which groups were present in each pond and result in a better understanding on how the environmental conditions of the ponds would have affected them and the different GHG fluxes. Including the composition of the organic matter present in the water column and sediment of ponds could also provide additional useful insight into the quality of it and how it would respond to physical and biological transformations, with the corresponding effect on overall GHG emissions.

5. Conclusion

The insights gained from this research study can be valuable with respect to future management practices of ponds and effective decision-making in the face of climate change. Based on our results, we especially recommend management strategies that reduce nutrient concentrations in ponds in an effort the reduce GHG emission by ponds. We anticipate that such action also benefit aquatic biodiversity in general. In addition, our result highlight GHG emission by ponds will likely increase under future climate change scenarios as ponds are expected to become increasingly temporal. Future research could focus on the composition and quality of organic matter of sediments which will a key variable in determining overall GHG emissions by ponds, especially in a climate change scenario where pods are expected to become increasingly temporal.

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Supplement

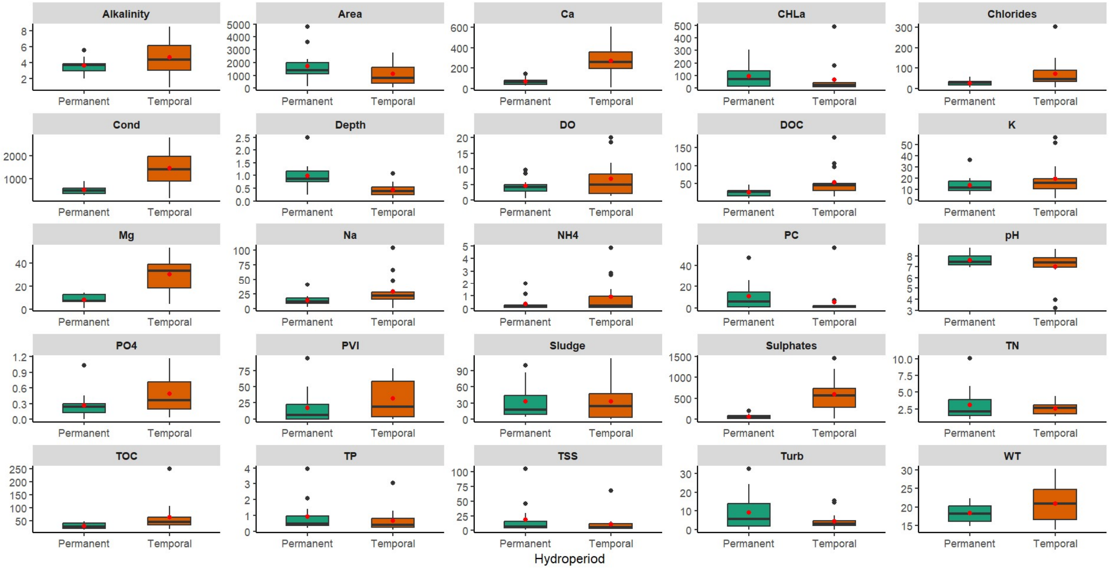

Figure 1: Boxplots showing various ecological variables for permanent and temporal ponds, sampled during spring 2021. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers.

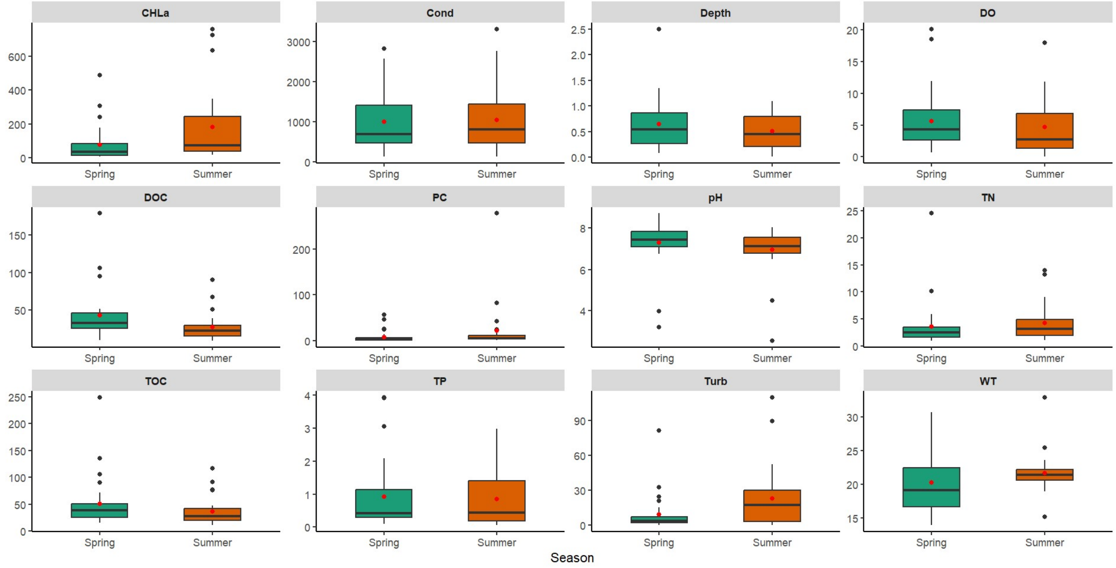

Figure 2: Boxplots showing various ecological variables for ponds, sampled during spring and summer 2021. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers.

Table 1: Results of the effect of hydroperiod and region on the entire set of environmental variables. Data from Spring 2021.

PERMANOVA						
Factor	**Variable**	**Df**	**Sum Sq**	**R^2**	**F**	**Pr(>F)**
Hydroperiod	Hydroperiod	1	0.08	0.194	6.263	**0.001**
	Residual	26	0.31	0.806		
	Total	27	0.39	1.000		
Region	Region	3	0.10	0.264	2.870	**0.003**
	Residual	24	0.29	0.736		
	Total	27	0.39	1.000		

Table 2: Test results for differences in several environmental variables between temporal and permanent ponds. Spring 2021.

Panel A

Two Sample T-test

Variable	**t**	**Df**	**p-Value**	**95%CI**	**Mean Diff**
Log$_{10}$(K)	-2.39	23	**0.026**	-0.31 – -0.22	-0.16
Log$_{10}$(Water Temp.)	-2.67	21	**0.014**	-0.15 – -0.02	-0.08
Log$_{10}$(DOC)	-2.47	23	**0.022**	-0.43 – -0.04	-0.23
Log$_{10}$(TOC)	-2.48	21	**0.022**	-0.42 – -0.04	-0.23
Log$_{10}$(Depth)	3.85	23	**< 0.001**	0.06 – 0.20	0.13
Log$_{10}$(Turb)	1.10	23	0.281	-0.15 – 0.52	-0.67

Panel B

Wilcoxon Signed-Rank test

Variable	**W**	**p-Value**
Sulphates	8.23	**0.004**
Ca	12.78	**< 0.001**
TN*	5.32	**0.021**
Chl$_a$	4.56	**0.032**
PC	6.73	**0.019**
Cond	28.34	**< 0.001**
TP	0.32	0.947

*Notes: TN was tested for Summer 2021.

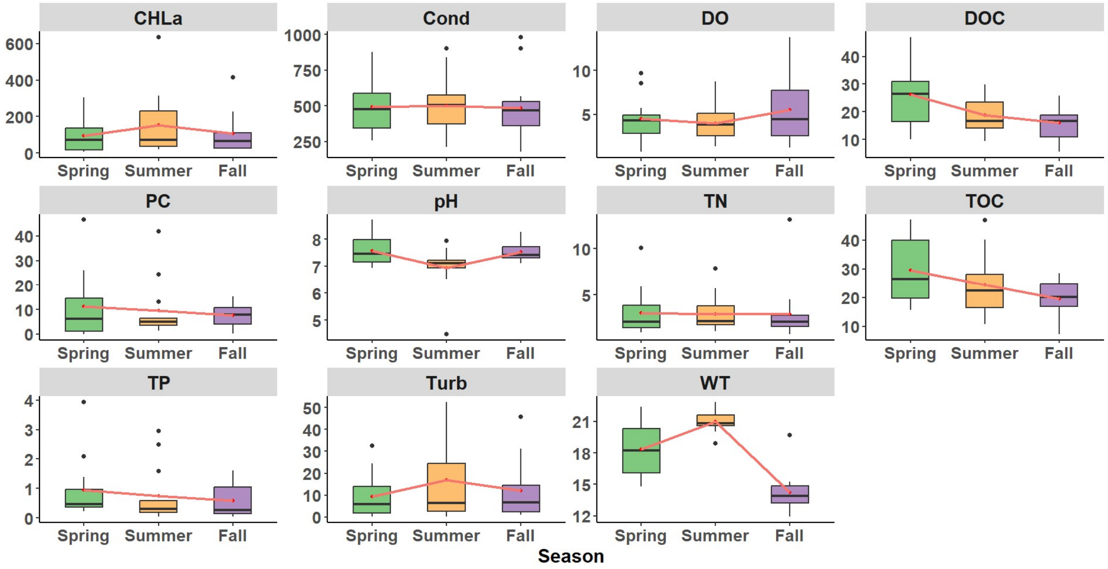

Figure 3: Boxplots showing various local pond ecological variables in permanent ponds, sampled during spring, summer and fall 2021. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers.

Table 3: Test results to check for the effects of Season on several pond environmental conditions in permanent ponds. Spring, Summer and Fall 2021 data.

Panel A

One-Way repeated measures ANOVA

Variable	Variable	Df	Sum Sq	Mean Sq	F-Value	Pr (>F)
Log$_{10}$ (pH)	Season	2	0.01	0.01	4.672	**0.019**
	Residuals	26	0.03	0.001		
Log$_{10}$(TOC)	Season	2	0.20	0.10	6.849	**0.004**
	Residuals	26	0.38	0.01		
Log$_{10}$(DOC)	Season	2	0.28	0.14	10.94	**< 0.001**
	Residuals	26	0.20	0.01		
Log$_{10}$(DO)	Season	2	0.08	0.04	0.805	0.458
	Residuals	26	1.28	0.05		
Log$_{10}$(Turb)	Season	2	0.21	0.11	1.184	0.323
	Residuals	26	2.15	0.09		
Log$_{10}$(Cond)	Season	2	0.01	3.00e^{-3}	0.787	0.466
	Residuals	26	0.13	4.00e^{-3}		
Log$_{10}$(TN)	Season	2	1.20e^{-3}	5-82e^{-4}	0.04	0.961
	Residuals	26	0.38	0.02		
Log$_{10}$(TP)	Season	2	0.03	0.02	1.963	0.161
	Residuals	26	0.20	0.01		
Log$_{10}$(Chl$_a$)	Season	2	0.33	0.17	1.17	0.326
	Residuals	24	3.41	0.14		
Log$_{10}$(PC)	Season	2	0.01	0.01	0.03	0.968
	Residuals	24	4.71	0.20		

Panel B

Friedman Test

Variable	Df	Chi-Squared	p-Value
Water Temp	2	23.804	**< 0.001**

Table 4: Post-hoc analyses to test for pairwise differences between seasons on several environmental variables in permanent ponds. Spring, Summer and Fall data, 2021. F = Fall, Sp = Spring, Su = Summer, LCL = Lower Confidence Interval, UCL = Upper Confidence Interval.

Panel A

Tukey HSD

Variable	Pair	diff	SE	Df	LCL	UCL	p adj.
Log$_{10}$ (pH)	F-Sp	$-1.60e^{-3}$	0.01	24	-0.4	0.03	0.906
	F–Su	0.03	0.01	24	$-1.44e^{-3}$	0.07	**0.049**
	Sp-Su	0.03	0.01	24	$1.53e^{-4}$	0.07	**0.049**
Log$_{10}$(TOC)	**F-Sp**	-0.17	0.05	24	-0.29	-0.05	**0.005**
	F–Su	-0-08	0.05	24	-0.20	0.04	0.158
	Sp-Su	0.09	0.05	24	-0.04	0.21	0.159
Log$_{10}$(DOC)	**F-Sp**	-0.20	0.04	24	-0.31	-0.08	**< 0.001**
	F–Su	-0.07	0.04	24	-0.18	0.05	0.140
	Sp-Su	0.13	0.04	24	0.01	0.24	**0.017**

Panel B

Dunn Test

Variable	Pair	Z	p adj.
Water Temp.	F - Sp	-2.95	**0.005**
	F - Su	-5.08	**< 0.001**
	Sp - Su	-2.13	**0.048**

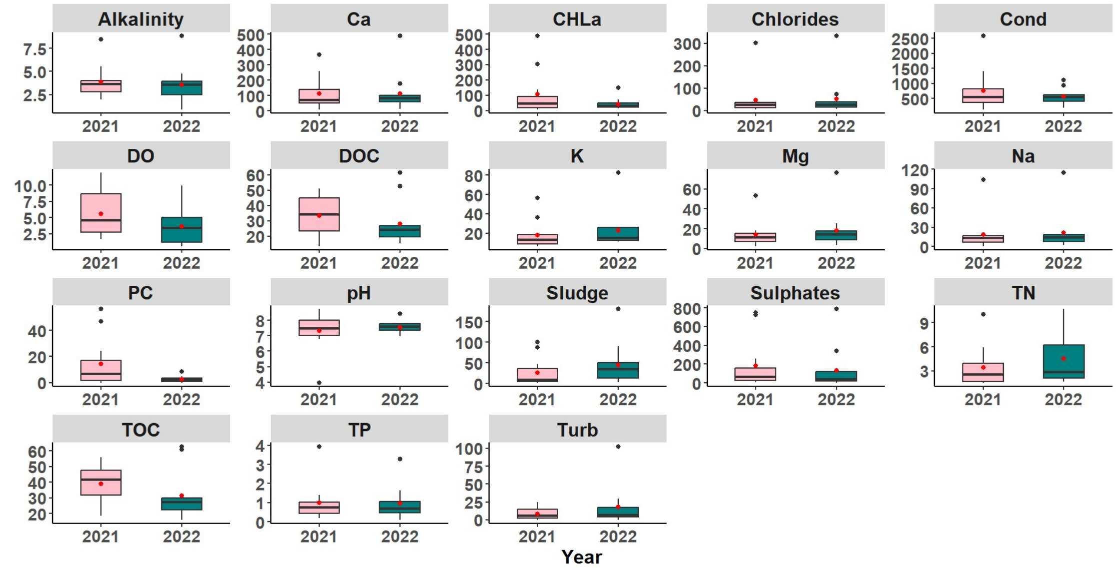

Figure 4: Boxplots showing various ecological variables measured in spring 2021 and 2022. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers.

Table 5: Test results for differences in environmental variables between spring 2021 and 2022. Variables in bold proved to be significantly different between years.

Panel A

Paired T-test

Variable	Pair	t	Df	p-Value	95%CI	Mean Diff
Log$_{10}$(DO)	2021 – 2022	2.01	11	0.070	-0.02 – 0.35	0.17
Log$_{10}$(Turb)	2021 – 2022	-1.70	11	0.118	-0.42 – 0.05	-0.18
Log$_{10}$(Sludge)	2021 – 2022	-2.14	11	0.056	-0.57 – 0.01	-0.28
Log$_{10}$(Cond)	2021 – 2022	0.58	11	0.575	-0.13 – 0.23	0.05
Log$_{10}$(TN)	2021 – 2022	-1.71	11	0.116	-0.20 – 0..03	-0.09
Log$_{10}$(DOC)	2021 – 2022	0.90	11	0.388	-0.11 – 0.25	0.07
Log$_{10}$(Chl$_a$)	2021 – 2022	1.03	11	0.323	-0.21 – 0.58	0.18
Log$_{10}$(PC)	2021 – 2022	2.10	11	0.060	-0.02 – 0.84	0.41
Log$_{10}$(Sulphates)	2021 – 2022	1.98	11	0.073	-0.02 – 0.29	0.14
Log$_{10}$(Chlorides)	2021 – 2022	-2.04	11	0.066	-0.15 – 0.01	-0.07
Log$_{10}$(Alkalinity)	2021 – 2022	1.27	11	0.231	-0.03 – 0.10	0.04
Log$_{10}$(Ca)	2021 – 2022	-0.74	11	0.473	-0.10 – 0.05	-0.03
Log$_{10}$(Mg)	2021 – 2022	-3.34	11	**0.007**	-0.15 – -0.03	-0.09
Log$_{10}$(Na)	2021 – 2022	-2.15	11	0.055	-0.17 – 0.002	-0.08

Panel B

Wilcoxon Signed-Rank test

Variable	Pair	W	p-Value
pH	2021 – 2022	29	0.756
TP		37	0.910
TOC		57	0.170
K		18	0.110

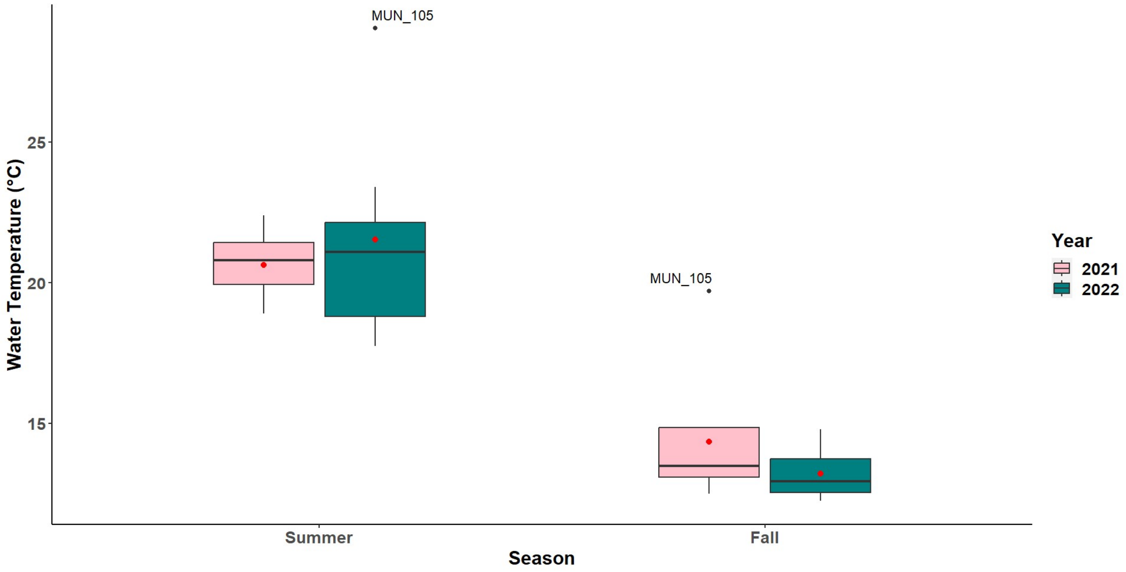

Figure 5: Boxplots showing water temperature measured in Summer and Fall, 2021 and 2022. Boxes represent first and third quartiles; whiskers are minimum and maximum, black horizontal lines and red dots represent the median and the mean respectively. Black dots show outliers and are annotated with the name of the pond they belong to.

Table 6: Results of Paired t-test for differences in water temperature during summer and fall between 2021 and 2022.

Paired T-test

Variable	Pair	t	Df	p-Value	95%CI	Mean Diff
Log_{10}(Water Temp.)	Su.2021 – Su.2022	-0.13	7	0.898	-0.05 – 0.04	$-2e^{-3}$
	Fa.2021 – Fa.2022	0.46	6	0.662	-0.03 – 0.05	0.01

Table 7: Results of Kruskal-Wallis results for the effect of Region on CH_4, CO_2 and N_2O dissolved fluxes.

Kruskal-Wallis Test

Flux	Season	H	Df	p-Value
CH_4	Spring	3.16	3	0.367
	Summer	4.43	3	0.218
	Fall	2.57	2	0.276
CO_2	Spring	1.12	3	0.771
	Summer	0.10	3	0.991
	Fall	1.88	2	0.392
N_2O	Spring	0.86	3	0.834
	Summer	5.69	3	0.128
	Fall	1.52	2	0.468

Table 8: Test results to check for the effects of Season on emission fluxes in permanent ponds. Summer and Fall 2021, Spring 2022.

Panel A

One-Way repeated measures ANOVA

Flux	Variable	Df	Sum Sq	Mean Sq	F-Value	Pr (>F)
Sqrt(CH_4 Bub)	Season	2	208.77	104.39	25.26	**$3.37e^{-06}$**
	Residuals	20	82.64	4.13		
CO_2 Diss	Season	2	$730e^4$	$365e^4$	4.28	**0.033**
	Residuals	16	$137e^5$	$853e^3$		

Panel B

Friedman Test

Flux	Df	Chi-squared	p-Value
CH_4 diss	2	9.385	**0.009**
N_2O diss	2	0.167	0.920

Table 9: Results of post-hoc analysis to test for pairwise differences between seasons on emission fluxes in permanent ponds. 2021 Summer and Fall, and Spring 2022 data. F = Fall, Sp = Spring, Su = Summer, LCL = Lower Confidence Interval, UCL = Upper Confidence Interval.

Panel A

Tukey HSD

Flux	Pair	diff	SE	Df	LCL	UCL	p adj.
Sqrt(CH_4 Bub)	**F-Sp**	3.79	0.87	20	1.59	5.98	**< 0.001**
	F–Su	-2.32	0.87	20	-4.51	-0.13	**0.015**
	Sp-Su	-6.10	0.87	20	-8.30	-3.91	**< 0.001**
CO_2 Diss	F-Sp	855	436	16	-309	2019.1	0.135
	F–Su	-390	436	16	-1554	774.2	0.384
	Sp-Su	-1245	436	16	-2409	-80.7	**0.034**

Panel B

Dunn Test

Flux	Pair	Z	p adj.
Dissolved fCH4	F - Sp	1.918	0.083
	F - Su	-0.523	0.901
	Sp - Su	-2.441	**0.022**

Table 10: Results of multiple linear regression analyses on square-root transformed CH4 bubble flux. Summer 2021. The best 3 selected models are shown with their corresponding AICc score. Model in bold indicates best fitting regression model. DO = Dissolved oxygen, TP = Total Phosphorus, TN = Total Nitrogen.

	Coefficients	Estimate	SE	t-Value	Pr(>|t|)	R^2_{adj}	p-Value	AICc
Model 1	Intercept	-8.72	9.23	-0.945	0.357	0.584	**< 0.001**	134.66
	pH	2.51	1.24	2.023	0.057			
	DO	0.69	0.21	3.349	**0.003**			
	TP	1.54	0.72	2.145	**0.045**			
	Sulphates	-0.01	2.00e^{-3}	-5.689	**< 0.001**			
Model 2	Intercept	-3.52	8.35	-0.421	0.678	0.563	**< 0.001**	135.79
	pH	1.76	1.13	1.560	0.135			
	DO	0.66	0.21	3.149	**0.005**			
	TN	0.53	0.28	1.874	0.076			
	Sulphates	-0.01	2.00e^{-3}	-5.429	**< 0.001**			
Model 3	Intercept	-8.31	9.45	-0.879	0.391	0.567	**< 0.001**	138.38
	pH	2.42	1.28	1.886	0.075			
	DO	0.70	0.21	3.305	**0.004**			
	TP	1.15	1.07	1.069	0.299			
	TN	0.21	0.41	0.508	0.618			
	Sulphates	-0.01	2.00e^{-3}	-5.315	**< 0.001**			

Table 11: Results of multiple linear regression analyses on $\log_{10}$-transformed CH4 dissolved flux. Summer 2021. The best 4 selected models are shown with their corresponding AICc score. Model in bold indicates best fitting regression model. TP = Total Phosphorus, TN = Total Nitrogen, K = Potassium salts, DOC = Dissolved Organic Carbon.

	Coefficients	Estimate	SE	t-Value	Pr(>|t|)	R^2_{adj}	p-Value	AICc
Model 1	Intercept	0.05	0.49	0.108	0.915	0.636	**< 0.001**	26.34
	pH	0.21	0.06	3.341	**0.003**			
	TP	0.27	0.07	4.063	**< 0.001**			
	Sulphates	-1.00e^{-3}	0.00	-3.834	**0.001**			
	K	-0.01	0.01	-1.649	0.115			
Model 2	Intercept	-0.14	0.51	-0.272	0.789	0.591	**< 0.001**	29.26
	pH	0.22	0.07	3.147	**0.005**			
	TP	0.23	0.09	2.620	**0.016**			
	Sulphates	-1.00e^{-3}	0.00	-4.017	**< 0.001**			
	DOC	2.00e^{-3}	0.01	0.461	0.650			
Model 3	Intercept	-0.66	0.64	-1.010	0.324	0.296	**0.015**	40.54
	pH	0.27	0.09	3.008	**0.007**			
	TP	0.30	0.11	2.752	**0.012**			
	DOC	-4.00e^{-3}	0.01	-0.614	0.546			
Model 4	Intercept	-0.63	0.63	-0.997	0.330	0.316	**0.006**	37.83
	pH	0.26	0.09	2.989	**0.007**			
	TP	0.26	0.09	2.970	**0.007**			

Table 12: Results of multiple linear regression analyses on cubic-root transformed CO2 dissolved flux. Summer 2021. 3 models are shown.. TP = Total Phosphorus, TN = Total Nitrogen, Chl_a = Chlorophyl a, PVI = Plant Volume Inhabited.

	Coefficient	**Estimate**	**SE**	**t-Value**	**Pr(>|t|)**	**R^2_{adj}**	**p-Value**	**AICc**
Model 1	**Intercept**	10.48	1.46	7.194	**< 0.001**	0.243	0.052	144.00
	TP	-1.09	0.93	-1.168	0.257			
	CHLa	4.04e^{-4}	0.01	0.072	0.944			
	TN	0.64	0.33	1.968	0.064			
	PVI	0.08	0.03	2.562	**0.019**			
Model 2	**Intercept**	12.26	1.45	8.466	**< 0.001**	0.033	0.314	147.51
	TP	-1.06	1.06	-1.002	0.328			
	CHLa	-3.00e^{-3}	0.01	-0.482	0.635			
	TN	0.71	0.37	1.923	0.069			
Model 3	**Intercept**	13.44	1.39	9.647	**< 0.001**	-0.091	0.959	148.35
	TP	0.23	0.87	0.261	0.797			
	CHLa	4.04e^{-4}	0.01	0.067	0.947			

Table 13: Results of multiple linear regression analyses on log_{10}-transformed N_2O dissolved flux. Summer 2021. The best 4 selected models are shown with their corresponding AICc score. Model in bold indicates best fitting regression model. TOC = Total organic Carbon, NH_4 = ammonium.

	Coefficient	**Estimate**	**SE**	**t-Value**	**Pr(>|t|)**	**R^2_{adj}**	**p-Value**	**AICc**
Model 1	**Intercept**	-1.45	0.26	-5.513	**< 0.001**	0.590	< 0.001	37.47
	NH_4	0.23	0.08	2.837	**0.009**			
	TOC	-0.02	4.00e^{-3}	-3.970	**< 0.001**			
	Sulphates	6.12e^{-4}	2.20e^{-4}	2.756	**0.011**			
	Depth	0.38	0.19	1.931	0.067			
	Chlorides	-3.00e^{-3}	1.00e^{-3}	-2.435	**0.020**			
Model 2	**Intercept**	-1.02	0.15	-6.806	**< 0.001**	0.540	< 0.001	38.19
	NH_4	0.23	0.09	2.712	**0.009**			
	TOC	-0.02	4.00e^{-3}	-4.700	**< 0.001**			
	Sulphates	4.33e^{-4}	2.14e^{-4}	2.025	0.055			
	Chlorides	-4.00e^{-3}	1.00e^{-3}	-2.351	**0.028**			
Model 3	**Intercept**	-1.12	0.16	-7.029	**< 0.001**	0.449	< 0.001	40.54
	NH_4	0.29	0.09	3.217	**0.008**			
	TOC	-0.02	4.00e^{-3}	-4.859	**< 0.001**			
	Sulphates	2.48e^{-4}	2.18e^{-4}	1.138	0.267			
Model 4	**Intercept**	-1.07	0.15	-6.926	**< 0.001**	0.498	< 0.001	40.9
	NH_4	0.33	0.09	3.719	**0.009**			
	TOC	-0.02	4.00e^{-3}	-3.712	**< 0.001**			
	Sulphates	2.29e^{-4}	2.08e^{-4}	1.103	0.282			
	PO_4	-0.54	0.30	-1.800	0.086			

Table 14: Results of paired t-test for different fluxes between summer 2021 and 2022 and fall 2021 and 2022. Su.21 = Summer 20221, Su.22 = Summer 2022, Fa21= Fall 2021 and Fall22 = Fall 2022.

Paired T-test						
Flux	**Pair**	**t-Value**	**Df**	**p-Value**	**95%CI**	**Mean Diff**
$Log_{10}(CH_4$ Bub)	Su.21 – Su.22	0.32	7	0.762	-0.15 – 0.19	0.02
	Fa21 – Fa22	1.67	6	0.146	-0.10 – 0.51	0.21
$Log_{10}(CH_4$ Diss)	Su.21 – Su.22	-0.38	7	0.716	-0.70 – 0.51	-0.10
	Fa21 – Fa22	0.84	5	0.438	-0.82 – 1.61	0.40

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Faculty of Life Sciences

Declaration of Originality

Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences

I hereby declare that this thesis is the result of my own work and that I have indicated all sources, including online sources, which have been cited without changes or in modified form, especially sources of texts, graphics, tables and pictures.

I confirm that I have not submitted this thesis for any other examination. I am aware that in case of any breach of these rules procedures concerning plagiarism or attempted plagiarism will be taken in accordance with the subject-specific study and examination regulations and/or the General Admission, Study and Examination Regulations of Humboldt-Universität zu Berlin (ZSP-HU) / Allgemeine Satzung zur Regelung von Zulassung, Studium und Prüfung der Humboldt-Universität zu Berlin (ZSPHU).

Berlin, 12.12.2023