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IMPACT OF NUTRIENT CONTENT AND ZOOPLANKTON COMMUNITY STRUCTURE ON METHANE EMISSION FROM URBAN PONDS IN ANKARA

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
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BY

FERİDE AVCI

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FOR
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IMPACT OF NUTRIENT CONTENT AND ZOOPLANKTON COMMUNITY STRUCTURE ON METHANE EMISSION FROM URBAN PONDS IN ANKARA

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ABSTRACT

IMPACT OF NUTRIENT CONTENT AND ZOOPLANKTON COMMUNITY STRUCTURE ON METHANE EMISSION FROM URBAN PONDS IN ANKARA

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Master of Science, Biology
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Ponds are globally the most numerous yet neglected freshwaters because of their small size and shallowness. Ponds are important freshwater bodies as they largely contribute to aquatic biodiversity. They can also act as a sink or source of greenhouse gas (GHG) emissions, depending on their condition. Many studies show that CO_2 and CH_4 emissions from shallow freshwater bodies such as ponds are significant. In freshwaters, respiration produces CO_2 when there is oxygen; when there is no oxygen respiration produces CH_4. The lack of oxygen is usually caused by high amounts of organic matters and nutrients in the system.

The research was conducted as a part of EU-H2020 funded large scale consortium project entitled PONDERFUL in 2021 summer in 15 urban ponds in Ankara. The study sites were İmrahor River Valley, Gölbaşı Düzlüğü, and Lake Mogan. There were 7 ponds sampled in İmrahor River Valley, 3 in Gölbaşı Düzlüğü, and 5 in Lake Mogan. This thesis assumed that the amount of nutrients in ponds had strong positive effect on methane emission. Analysis showed that total phosphorus and total nitrogen concentrations in the ponds had strong effect on methane emission. Methane can be oxidized by methane oxidizing bacteria (MOB) when the water is oxic again. Zooplankton are a crucial component of freshwater food webs. Depending on their size and feeding type, they can prey upon other zooplankton, phytoplankton, or bacteria. MOB can be grazed by zooplankton, and this would result in a decrease in methane oxidization. Thus it was predicted in the current study that zooplankton community structure in ponds has an effect on CH_4 emissions through grazing on MOB. Total Cladoceran density in ponds had significant positive effect on methane ebullution from ponds probably through grazing pressure on MOB.

Keywords: Pond, Greenhouse Gas, Methane Emission, Nutrients, Zooplankton

ÖZ

ANKARA’DA ŞEHİR İÇİNDEKİ GÖLCÜKLERDEKİ BESİN TUZU BESİN TUZU MİKTARI VE ZOOPLANKTON KOMÜNİTE YAPISININ METAN EMİSYONU ÜZERİNDEKİ ETKİSİ

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Gölcükler dünyada en çok sayıda bulunan fakat küçük boyutları ve sığlıkları sebebiyle göz ardı edilen tatlı sulardır. Gölcükler su biyoçeşitliliğine büyük ölçekte katkı sağladıkları için önemli tatlı su kütleleridir. Aynı zamanda, durumlarına bağlı olarak, sera gazları için kaynak ya da havza görevi görebilirler. Birçok çalışma, gölcükler gibi sığ tatlı su kütlelerinden çıkan CO_2 ve CH_4 miktarının kayda değer olduğunu göstermiştir. Tatlısularda, oksijen varlığında solunum sonucunda CO_2 üretilirken; oksijen yokken, oksijensiz solunum sonucunda CH_4 üretilir. Oksijen eksikliği genelde sistemdeki yüksek miktarda organik materyal ve besin tuzundan kaynaklanır.

Bu araştırma EU-UFUK2020 tarafından desteklenen geniş kapsamlı konsorsiyum projesi PONDERFUL kapsamında, 2021 yılı yazında Ankara’da şehir içinde kalan 15 gölcükte yürütülmüştür. Çalışma alanları İmrahor Deresi Vadisi, Gölbaşı Düzlüğü ve Mogan Gölü’dür. İmrahor Deresi Vadisi’nde 7 gölcük, Gölbaşı Düzlüğü’nde 3 gölcük ve Mogan Gölü’nde 5 gölcük örneklenmiştir. Bu tez, gölcüklerdeki besin tuzu miktarının metan emisyonu üzerinde yüksek pozitif etkisi olduğunu varsaymıştır. Analizler, gölcüklerdeki toplam fosfor ve toplam nitrojen konsantrasyonunun metan çıkışı üzerinde güçlü bir etkisi olduğunu göstermiştir. Metan, oksijen varlığında metan oksitleyen bakteriler (MOB) tarafından oksitlenebilir. Zooplankton tatlısu besin ağlarında kritik öneme sahip bir canlıdır. Boyutuna ve beslenme şekline bağlı olarak diğer zooplanktonlarla, fitoplanktonla veya bakteriyle beslenebilir. MOB da zooplankton tarafından avlanabilir, bu da metan oksitlenmesinde düşüşe neden olur. Bu sebeple, bu tezde zooplankton komünite yapısının, MOB avlanma miktarını etkileyerek, metan çıkışını etkileyeceği öngörülmüştür. Bu çalışmada, gölcüklerdeki toplam Cladocera yoğunluğu, muhtemelen zooplanktonların MOB ile beslenmesi sebebiyle, metan emisyonu üzerinde gözle görülür pozitif etki göstermiştir.

Anahtar Kelimeler: Gölcük, Sera Gazı, Metan Emisyonu, Besin Tuzu, Zooplankton

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ABBREVIATIONS

NbS: Nature-Based Solutions

GHG: Greenhouse Gases

MOB: Methane Oxidizing Bacteria

CHAPTER 1

INTRODUCTION

1.1 Ponds

Ponds are small water bodies that make up nearly 30% of the global waterbodies by surface area (Malyan et al., 2022). However, since they are very small, they have been neglected by scientists and policy makers until the last 20 years. Ponds are hotspots for freshwater biodiversity and they also contribute highly to terrestrial biodiversity by being water source to many animals, being a sanctuary for many macroinvertebrates' larval stages, and increasing macrophyte biodiversity by creating littoral zones (Hassall, 2014). Ponds, also create dispersal corridors for freshwater species by connecting and creating "pondscapes", which is a network of ponds in association with their surrounding landscapes (Froneman et al. 2001). Ponds are also considered as buffer ecosystems against climate change since they are great contributors of greenhouse gas (GHG) sink, carbon sequestration, nutrient interception, rainfall management etc. Creating and restoring ponds using nature-based solutions (NbS), would contribute to mitigating the effects of climate change or even reduce them (Cereghino et al., 2014).

1.2 Urban Ponds

With the increase in human population, the urban land use also increases. Projections suggest that between 2000 and 2030 there will be at least 185% increase in the extend of urban land (Seto et al., 2012). This will be a serious issue for all biodiversity by decreasing habitat size, decreasing food source, increasing pollution, and more. One of the ecosystems that would be affected the most would be urban ponds. Despite being in close contact to humans, urban ponds can support substantial biodiversity as well as show less homogenization in community structure compared to other urban ecosystems (Hill et al., 2017). Urban ponds can support amphibian, bird, and macroinvertebrate biodiversity (McKinney, 2008). Pondscapes throughout a city can be very important natural corridors for migration and dispersal of aquatic wildlife (Hassall, 2014).

Urban ponds also provide many ecosystem services such as regulation of floods, creation of habitats, enhancing diversity, and regulation of water quantity and quality (Rounsevell et al., 2018). They are also important for learning by contributing to citizen science and being a small observation spot for people curious of ecology. They can be resting spots which is a very important aspect in human psychology and community health (Hassall, 2014).

The biodiversity of urban ponds can be in danger since as urbanization increases, heavy metals can enter to pond systems as a result of industrial activities (Van Ginneken et al., 2007). Road salting is also an important threat for biodiversity since it causes osmoregulation problems for aquatic species (Karraker et al., 2008). Pond loss, caused by filling up the ponds to increase farmland or extend the cities is a great risk for freshwater biodiversity (Gledhill et al., 2008).

1.3 Climate Change and GHG Emissions

Climate change is the long-term changes in weather patterns and temperature which has been increasing since 1800s because of human activities (Allan et al., 2021). These activities are burning of fossil fuels, electricity generation, transportation, deforestation, intense agriculture, and increasing the number of livestock (Rosa & Dietz, 2012).

The main driver of climate change is greenhouse gas emissions. GHG are defined as water vapor, CO_2, CH_4, and NO_2 (Ramanathan & Feng, 2009). When GHG increases in the atmosphere, temperature on earth increases since the heat gets trapped with the GHG layer (Shukla et al., 2019). Global rates of precipitation and evaporation increase with large geographic variations and in turn changing the water cycle of our planet (Manabe, 2019). The rise in temperature and the change in water cycle means intense droughts or flooding events, rising sea levels, and melting of glaciers (Allan et al., 2021).

Freshwaters are considered as one of the main sources of GHG emissions. CO_2 is produced as the product of oxic degradation of the organic matter in the sediment while CH_4 is produced when the degradation of organic matter occurs anaerobically (Kumar et al., 2019). N_2O is produced during microbially mediated nitrification and denitrification (Galloway et al.,2008).

1.4 Methane Emission

Freshwaters are known to be important GHG emission resources to the atmosphere (Bastviken et al., 2011). Main GHGs that are of concern are CO_2, CH_4, and N_2O. Latest studies show that while the absolute CO_2 emissions from freshwaters are 5 to 10 times more than CH_4 or N_2O, 72% of the impact caused by GHG emissions is due to CH_4. The reason for this is that CH_4 is nearly 34 times more harmful as a GHG than CO_2 (Beaulieu et al., 2019).

It has been known that CH_4 production is caused by the anoxic respiration processes in lake sediments and anoxic layer of water column (Jones & Grey, 2011). The CH_4 produced in the anoxic layer can be emitted in three different ways: ebullition, diffusive flux, and plant-mediated flux (Davidson et al., 2018). Plant-mediated flux is the process of CH_4 produced in the sediment being taken up by plant roots and emitted to the atmosphere (Shannon & White, 1994). Ebullition is a highly episodic and spatially heterogeneous process. The changes in hydrostatic pressure and disturbance of sediments can trigger ebullition (DelSontro et al., 2016). Ebullition is estimated to make up around 30% of total CH_4 emissions (DelSontro et al., 2016). Ebullition is the main way of CH_4 to be emitted to the atmosphere as bubbles, directly from the sediment in shallow lakes and ponds. In comparison to ebullition, CH_4 travelling through the water column by diffusive flux can be oxidized by methane oxidizing bacteria (MOB) once it reaches the oxic water column, before it reaches the atmosphere (Jones & Grey, 2011).

On the other hand, there are studies which show that methane production can also occur in the upper oxic layer of lakes and ponds (Perez-Coronel & Beman, 2022). Experiments showed that bacterioplankton in the lake subsurface could produce methane through the decomposition of methylphosphonic acid, as a by-product of the demethylation of phosphonic acids (Khatun et al., 2019). Methane is oxidized by methanotrophs in the presence of oxygen, as it has been seen in the oxic layers above the anoxic layers. Different studies showed that the methane oxidizers occur below the thermocline where there is low light intensity (Grossart et al., 2011). The absence of methane oxidizers in the upper oxic water column allows methanogenesis in oxic conditions. This system was not discovered further in this study.

1.5 Abiotic Factors Effecting Methane Emissions

In a pond, GHG processing occurs more intensively due to small surface area and shallowness, which causes frequent water column mixing (Holgerson & Raymond, 2016). Frequent mixing increases CH_4 saturation in the water and prevents efficient CH_4 oxidation. Shallow depth of ponds also indicates rapid exchange between water surface and atmosphere, giving less time for oxidation process. Furthermore, shallowness also fuels up the sediment with organic matter loads from the water column where less decomposition takes place due to short distance travelling of organic matters. Studies of local systems also shows clearly that there is strong correlation between temperature increase and CH_4 release (Wik et al., 2016). Respiration and methanogenesis in freshwaters respond stronger to the changes in temperature than photosynthesis. As a result, global warming is predicted to increase CO_2 and CH_4 emissions from freshwater bodies. Yvon-Durocher et al. (2011) suggested that increase in the temperature can alter the carbon balance of ponds which will cause an increase in CH_4 emissions. Consequently, there will be a positive feedback mechanism where high temperature will cause high CH_4 emission, and high CH_4 emission will contribute to accelerating warming.

It was shown by the previous studies that CH_4 concentrations in ponds with high TP (Total Phosphorus) and TOC (Total Organic Carbon) content (high nutrient ponds) are higher than low nutrient ponds which indicates that the nutrient level of a pond is an important driver for organic matter production and in turn CH_4 emission (Peacock et al., 2019). The high amount of organic matter accumulated in the sediment, which also means high nutrient levels, are available for bacterial degradation. The decomposition of organic matter causes reduction in the oxygen content and creates anoxic layer in the bottom of the pond. Under anaerobic environment, methanogenic bacteria degrade organic matter and causes methane production (Malyan et al., 2022).

These findings support the idea that the ponds in urban areas can be stronger CH_4 emitters since they can be highly eutrophic or hypertrophic.

With the increasing rate of expansion of urban areas, more ponds being eutrophic-hypertrophic, understanding how the GHG emission processes work in ponds, and restoration of ponds would be critical issues.

1.6 Biotic Factors Effecting Methane Emissions

1.6.1 Methane Oxidizing Bacteria (MOB)

Microbiological oxidation of methane takes place when methane diffuses to an oxic level in the water column. Many strains of bacteria capable of aerobic methane oxidation have been isolated and characterized, and phylogenetically all belong to either the gamma proteobacteria (commonly referred to as “type I” methanotrophs) or the alpha-proteobacteria (“type II” methanotrophs) (Murrell, 2010). Methane oxidizing bacteria (MOB), consumes a big part of the methane which was produced in the sediment or in anaerobic layers of water (Rudd et al., 1974). They also create a route for carbon to reenter to the food-web (Bastviken et al., 2003).

MOB can be encountered in many parts of a lake, depending on the spatial and temporal changes in the environment. During thermal stratification in summer, large amounts of methane can accumulate in the bottom anoxic layers (Fernandez et al., 2014). At this time, the highest MOB activity can be observed at the bottom part of the oxycline, where methane and oxygen gradients meet (Bastviken et al., 2002). MOB niche differentiation can also be determined by competition for oxygen, methane, copper, and iron concentrations (Knief, 2015). Nitrogen availability is also highly correlated with MOB activity (Guggenheim et al., 2020).

1.6.2 Zooplankton

Zooplankton are heterotrophic plankton which are the major primary consumers of many freshwater systems. They are one of the most abundant organisms in freshwaters and they play key roles in food web. They play a role in the aquatic food web by grazing phytoplankton and other smaller food sources, and also by being consumed by fish. Zooplankton can respond rapidly to changes in phytoplankton abundance, nutrient levels, and pollutants which makes them important indicators for limnologists to see the changes in freshwater systems. So, knowing the functioning and structure of zooplankton communities is very important to have a general understanding of a freshwater ecosystem.

The master trait body size is important for determining the functioning of a zooplankton community. Different size groups of zooplankton have different prey size preferences. However, it can be said that larger zooplankton would have larger prey size range since their mesh size would be bigger (Litchman et al., 2013).

Large zooplankton such as *Daphnia* have higher grazing pressure on bacteria than smaller zooplankton such as copepods. When systems dominated by large bodied zooplankton (Cladocera) and small bodied zooplankton (Copepod) are being compared, it is seen that the systems with larger zooplankton have lower bacterial abundance and low diversity compared to the system with small zooplankton (Jürgens,1994).

MOB can be grazed by the zooplankton in the oxic layer. It has been long known that zooplankton can graze on bacteria. If the zooplankton in a pond can reach to the oxic layer by vertical migration, it can feed on MOB. It has also been shown in a whole-lake experiment done by Devlin et al. (2015) that the presence and absence of zooplankton has an effect on MOB and CH_4 flux. In their experiment, a fishless lake was divided into two treatment basins and fish abundance was manipulated on one side. On the side where fish was added, there was high grazing pressure on zooplankton. The decrease in zooplankton population density caused an increase in the abundance of MOB and CH_4 emission rate from that basin decreased about 10 times. Therefore, regulation of CH_4 oxidation by the grazing of zooplankton on MOB can be an important factor that determines the CH_4 emission from ponds to the atmosphere. Kankaala et al. (2007) also showed in a laboratory experiment that the grazing by the large bacterivorous *Daphnia longispina* decreased the MOB abundance in the microbial community.

1.6.3 Macrophyte

Macrophytes are important parts of shallow lake and pond ecosystems (Declerck et al., 2011). They provide shelter and habitat for zooplankton and macroinvertebrates, they create spawning area for fish, and they contribute to the complexity of habitat and overall biodiversity (Jeppesen et al., 1998). High nutrient content causes a decrease in macrophyte coverage through shading by phytoplankton, suspended and inorganic matter (Van den Berg et al., 1999).

Macrophytes also play an important role in the emission of methane by taking the methane produced in the sediment by their roots and carrying it to the atmosphere (Laanbroek, 2010). Sorrell and Boon (1994) studied the importance of methane transport through the macrophytes by using *Eleocharis sphacelata* in a freshwater wetland where the measurements of methane efflux from macrophytes represented 1-15 times the methane release by ebullition from the sediment in the non-vegetated site.

1.7 Aim of This Study and Hypothesis

Within the context of PONDERFUL (Pond Ecosystems for Resilient Future Landscapes in a Changing Climate), a EU- H2020 funded project, within the fourth work package of the project (WP4), urban ponds in Ankara from south of Lake Mogan to Gölbaşı Düzlüğü and through Lake Eymir to İmrahor River Valley ponds were studied (Fig.1).

The relationship between the CH_4 release and nutrient concentrations were investigated in Ankara's urban ponds. The first hypothesis was "Nutrient concentrations in ponds has an effect on CH_4 emissions". The prediction was as the nutrient concentrations in the pond increase, dissolved oxygen concentration decrease. As a result, methane will be produced and emitted.

The second hypothesis was "zooplankton community structure in ponds had an effect on CH_4 emissions through grazing on MOB". The relationship between zooplankton and CH_4 emissions is a rarely explored topic. The research by Devlin et al. (2015), showed that top consumer abundance influences lake methane efflux that put emphasis on this top-down effect on CH_4 emission. The prediction was that in the ponds with higher Cladocera density, a higher grazing pressure on MOB would be created. Thus, more CH_4 emission would be detected. In contrast, in the ponds with high abundance of copepods, lower grazing pressure on MOB would occur, leading to less CH_4 emission.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Area and Design

The study was conducted in Ankara, Turkey. There was a total of 15 ponds. There are 7 ponds which are located in the; South of Lake Mogan, 3 ponds are in Gölbaşı Düzlüğü region, and 5 ponds are in İmrahor River Valley (Fig.1). The ponds were coded as “DP”, meaning “Demo Ponds”. The ponds were numbered from north to south, starting from İmrahor River Valley, followed by Gölbaşı Düzlüğü and Lake Mogan. At the beginning of the study, there were 18 ponds. However; one of the ponds dried out during the sampling, one of them was not considered safe to leave gas chambers in, and gas chambers were removed by the locals from one of the ponds. Thus, the codes of the ponds in this study are DP2, DP4, DP5, DP6, DP7, DP9, DP10, DP12, DP13, DP14, DP16, DP17, DP18, DP19, and DP20.

Samplings protocols followed during the study was developed by PONDERFUL consortium and the details are provided in the materials and methods section. However, it must be mentioned that the sampling protocol didn’t include any bacterial sampling. Thus, there was no data collected regarding MOB. This research is an association study which aims at seeing a connection between CH_4 emissions from a pond by analyzing zooplankton community.

2.2 Physical and Chemical Variables

The depths of the ponds were measured by a depth-meter, and a bathymetry map was created by using Mapit GIS-Map Data Collector application. The areas were calculated by using Google Earth. Conductivity, pH, oxygen concentration, and water temperature were measured at the same point in every 0.5m intervals using a multiprobe YSI. Secchi disc depth was also measured using a 20 cm diameter Secchi disc. A depth integrated water sampling covering the entire water column was carried out where the deepest point of each pond with 0.5 m intervals from water surface to the bottom using Ruttner sampler. Integrated water samples were used for total phosphorus (TP), total nitrogen (TN), total and dissolved organic carbon (TOC, DOC), and chlorophyll-a (Chl-a) analyses. A separate water sample wasn’t taken from the hypolimnion, or close to the sediment since the sampling was done according to the PONDERFUL protocol. Thus, the data from the water column samples were used as a proxy value for the ponds. When there was thermal stratification, samples were collected separately for both layers. When there was thermal stratification, samples were collected separately for both layers.

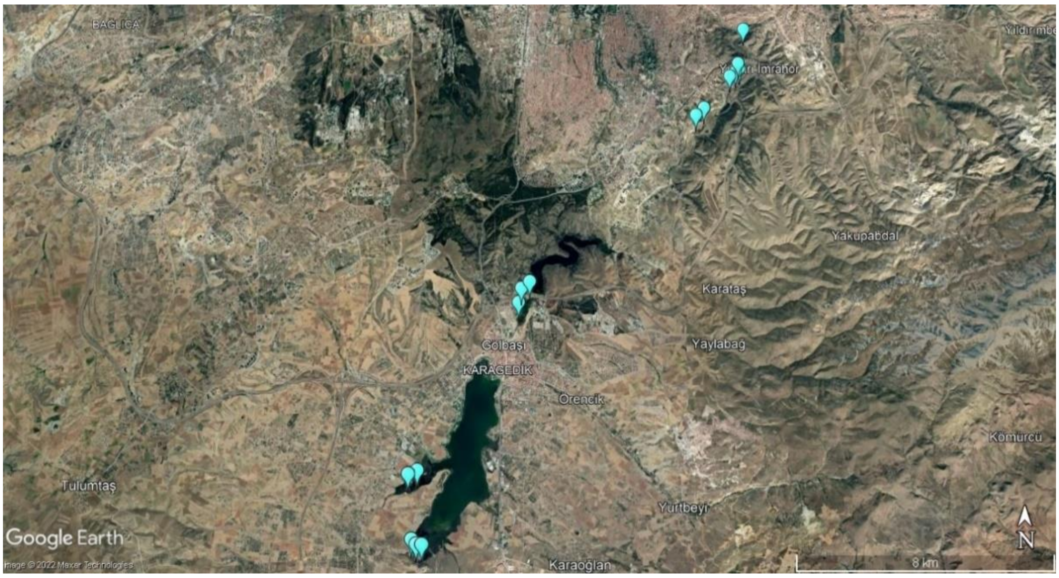


Figure 1: Google Earth image of the research area showing 15 ponds in İmrahor River Valley, Gölbaşı Düzlüğü, and Lake Mogan.

2.2.1 Water Chemistry Analysis

Chl-a analysis was carried out by filtering water from Whatman GF/C glass fibre filters, extracting chl-a with ethanol and then reading the absorbances at 663 nm and 750 nm in a spectrophotometer spectrophotometer (Perkin Elmer Lambda35) (Jespersen & Christoffersen, 1987).

TP was determined by following the molybdenum blue method described by Machereth et al. (1978). TN determination, including ammonium (NH_4^+), and nitrate (NO_3) were done by using an automated wet chemistry analyzer (Baird & Bridgewater, 2017).

TOC and DOC analysis were done in a private laboratory following the TS 8195 EN 1484 standardization methodology (TSE,2000).

2.2.2 GHG Sample Collection

Gas chambers (Fig. 2) were used to measure the diffusive flux and ebullition of greenhouse gases from ponds. To each pond, a minimum of 3 and maximum of 8 chambers were placed, depending on the size of the ponds. One chamber was always put to the deepest point, and other chambers were put in a way to cover different parts of the ponds. For each chamber, the depth of the location they were put were recorded. Gas chambers were put to the ponds between 4-6 August 2021 and collected a week later between 11-13 August 2021. The chambers were kept on the pond surface waters for a week since there needs to be significant air pressure difference between the bottom and top of the water column for ebullition to occur. While collecting the chambers, multiprobe measurements were done at the location of the deepest point. Water temperature, air temperature, and air pressure was also measured (Ponderful, 2020).

2.2.3 Quantifying Diffusive Flux

This was done by measuring the dissolved concentration of gases in the surface waters by head space equilibration. Forty ml of water from the water surface at the deepest point location was taken to a 50 ml syringe slowly to avoid bubbles. Ten ml of air was then inserted to the syringe. The syringe was shaken for 1 minute, then 10 ml of air was ejected to an exetainer. As air was used for head space equilibrium, 10 ml of air was sampled to a separate exetainer at each pond. The exetainers were sent to Aarhus University, Denmark for analysis of CO_2, CH_4, and N_2O concentration and partial pressure.

Diffusive flux calculation was done by calculating the dissolution coefficient based on the water temperature. Then, the total gas concentration in the water was calculated by dividing the total gas in headspace sample and water to 0.05L (the volume of the headspace sample). The concentration of the gas was calculated according to Schmidt number (Wanninkhof, 1992) and the gas fluxes were concentrated by using the k values (gas transfer velocity) (Holgerson & Raymond, 2016).

2.2.4 Quantifying Ebullition

After 7 days, 10 ml of gas, which was collected inside the chamber, was extracted from each chamber with a syringe, and ejected to an exetainer. The exetainers were sent to Aarhus University, Denmark for CH_4 analysis.

The methane concentrations were determined on an Agilent 7890 Gas Chromatograph (GC) system interfaced with a CTC CombiPal autosampler (Ponderful, 2020).

The ebullitive flux was determined by calculating the number of moles in the bubbles by using the air pressure and temperature values. Then, the number of moles per bubbles were divided by the chamber stay days, which was 7, and the chamber surface area (Ponderful, 2020).

In this study, only CH_4 ebullition was considered as the methane emission and diffusive CH_4 data was not taken into account as preliminary data analysis showed that diffusive CH_4 emission data shows no strong correlation with any of the explanatory variables (Fig. 3). Another reason ebullition data was used is because CH_4 emitted by bubbles mean that CH_4 was not converted by any MOB at any level of the water column. Thus, it was chosen as the better suited data to answer our question.

Figure 2: Photo of a gas chamber which is used to collect GHG emitted from ponds. Gas chamber design belonging to Thomas A. Davidson, (2020).

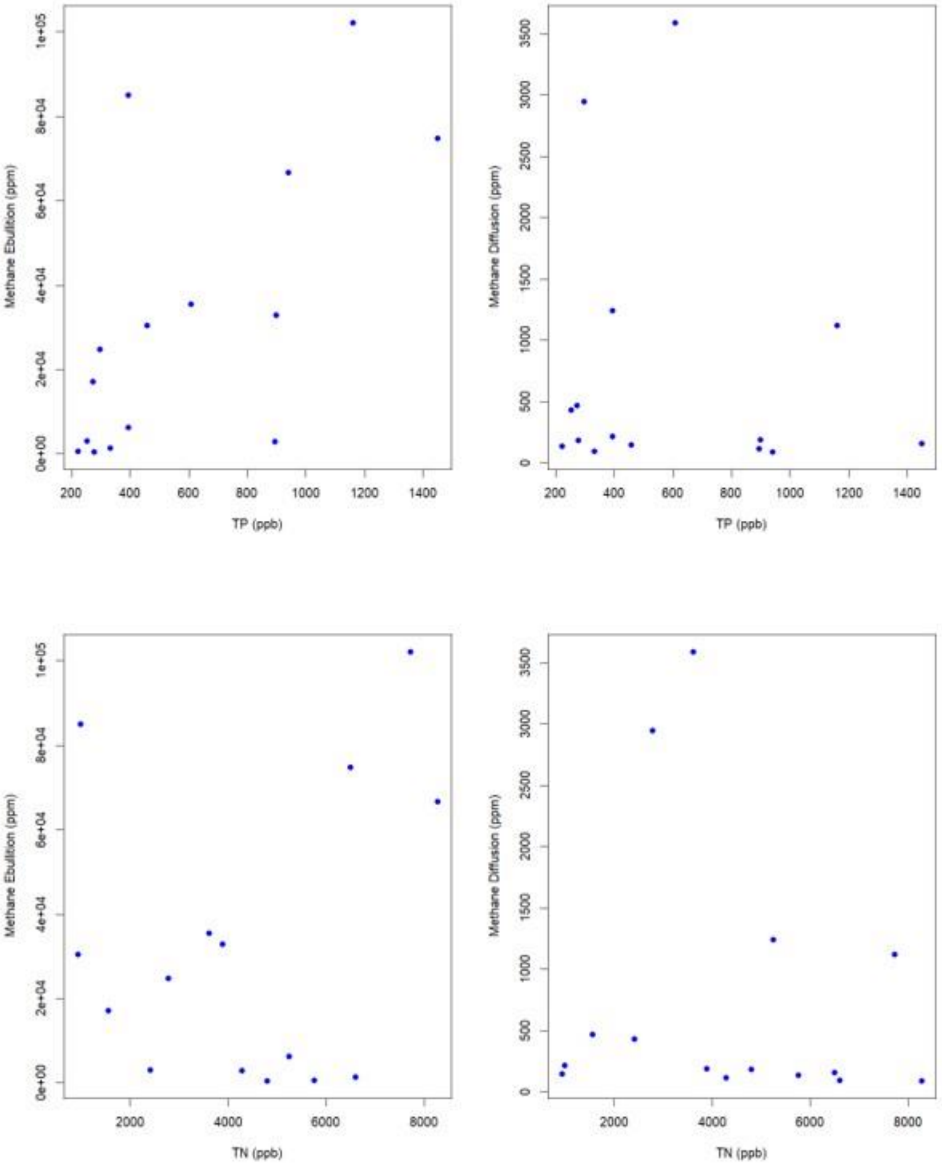


Figure 3: The plots which were generated with the original dataset, showing the relationship between TP and methane ebullition, TP and methane diffusion, TN and methane ebullition, and TN and methane diffusion.

2.2.5 Zooplankton Sample Collection, Identification, and Counting

Ponderful project protocol (2020) was followed for collection, preservation, and counting of zooplankton. Zooplankton samples were collected with tube sampler from 8 different points. A predefined grid was used to decide those 8 locations to make sure that different sub-habitats of the pond area are represented in the sample. Six liters of whole water column was collected in 8 points. The 48 L of water collected from the entire pond, 40L of the water was filtered through a 53 μm conical plankton net and collected in a 100ml amber bottle. The samples were preserved in 4% Lugol (Sigma Aldrich). A month after sampling, a smell from the samples were detected. In order to preserve the samples better, 4% final concentration of formaldehyde saturated with glucose (a small spoon of table sugar) was added. After formaldehyde addition, no more smell was detected. The zooplankton samples were counted using Leica DFC295 digital microscope and the LAS V4.12 software.

For counting, 8-10 ml of subsamples were taken from each bottle with a Pasteur pipette and diluted with distilled water in the counting plate to make the counting easier. Subsampling and counting continued until 300 individuals from each species were counted. The number of individuals were decided as 300 since the samples started deteriorating and glutaraldehyde addition was performed.

Body size of the first 25 individuals first encountered from each taxon were measured. The body size of the copepods was measured from the anterior tip to the end of caudal ramus. The body size of Cladocera was measured from the center of the eye to the base of the tail spine. Ostracoda were heavily deteriorated and unidentifiable. For this reason, they were not identified down to species level. Ostracoda body size measurements were done from the anterior tip of the carapace to the posterior tip. Nauplii and copepodites were counted but they were not measured and identified. Rotifers were not counted. For identification, A Key to the British Freshwater Cyclopoid and Calanoid Copepods (Harding & Smith, 1974) and A Key to the British Species of Freshwater Cladocera (Scourfield & Harding, 1966) were used frequently. The website An-Image-based Key to the Zooplankton of North America (Haney, J.F. et al, 2013) was also a useful reference.

2.2.6 PVI

The PVI% protocol was adapted from (Canfield et al., 1984). Transects across the pond surface were taken with 3 m gaps. The gaps were adjusted depending on the area of the pond. A water rake was randomly thrown to collect macrophytes. The coordinates of collection points and the number of points were recorded. The height of the macrophytes were measured. The density of the macrophyte coverage was detected by water telescope.

2.3 Statistical Analysis

2.3.1 Zooplankton Density Calculation

Zooplankton density calculation was done for each species in each pond. After all the individuals in a sample bottle (100ml) were counted, the number of individuals counted were divided by the total amount of water filtered to collect the sample (40L). This way, the number of individuals of a species in every 1L of the total pond volume was estimated.

Biomass calculation for each zooplankton species was calculated by using standard allometric equations to convert body lengths to biomass (Dumont et al., 1975; McCauley,1984).

2.3.2 Regression Trees

The sample size for this study was very small since there were only 15 ponds which were sampled. The only response variable was methane ebullition. However, there were 27 possible explanatory variables which included abiotic factors (like pond depth, area, TP, TN, DOC) and biotic factors (like Cladocera density, Daphnia density, total number of Copepods). The number of the explanatory variables were too many compared to the sample size. In order to increase the datapoints in the dataset and make correct statistical predictions, bootstrapping the data and creating random forest models from regression trees was the preferred method.

The aim of regression trees is to evaluate every possible explanatory variable in a continuous dataset and create partitions in the dataset in order to create homogeneous subsets in order to create a predictive model. Regression tree analysis creates an “upside-down tree” in order to show these partitions in the dataset. The “root” at the top represents all of the dataset. The root is then split into two branches at a “node”. This split is based on the explanatory variable which results in two subsets with the smallest residual sums of squares for the response variable. After the first split, the same process is repeated for each subset for all of the explanatory variables and more branches are created. Each subset that is created is “purer” than the ones previously created. When a branch is not further split, it is called a “leaf” (Quinn & Keough, 2002).

Random forest model was used to average the estimates from the series of regression trees which were created by using an independent bootstrapped dataset for each tree which is the same size as the original dataset and selected by replacement from the original dataset. After the creation of regression trees with the bootstrapped data, a prediction was formed from each regression tree for new date. The mean of these predicted values from all of the regression trees was the final predicted value.

In order to create a random forest model; hypolimnion percent, DOC, Chl-a, TP, TN, TN/TP ratio, total Cladocera density, total copepod density, Cladocera copepod density ratio, *Daphnia* density, total zooplankton density, total zooplankton biomass, Cladocera biomass, and copepod biomass were chosen as the predictor variables. Density rather than biomass was preferred since the question mainly focused on zooplankton community structure. Chl-a was used as rank data since there was compromised data. Variable importance plots were produced for the generated random forest model.

The first graph shows the percentage increase of the mean squared error (%IncMSE) in the out-of-bag (OOB) subset after permutation. OOB subset is the training set of bootstrapped data which is not used to build a tree. Each predictor in the OOB sample is randomly permuted and passed down the tree to generate an error rate which is the mean square error (MSE) (Liaw & Wiener, 2002). In order to calculate the percentage increase of MSE, the differences between the MSE of the original and shuffled datasets are averaged and normalized by the standard deviation of the differences. High %IncMSE means when the variable is removed from the model, there will be a big change in the model output. If the %IncMSE is low for a variable, that variable has low importance. The second graph shows the increase in node purity (IncNodePurity). IncNodePurity shows the increase in homogeneity in each node of the tree. It is calculated by averaging the total decrease in “impurities” in each node for all of the generated regression trees.

2.3.3 GLM

Since the predicted methane ebullition values created by the bootstrapping in random forest were not normally distributed, generalized linear models (GLM) was the preferred modeling method. Log normal distribution was chosen to allow tails in the data.

There are three properties of GLM; the linear predictor, error distribution, and link function. These properties cause the linear relationships with normal error distribution, and also complex non-linear relationships with alternative error distributions to be modelled. The linear predictor is similar to standard regression, it is always linear. The error distribution explains how the variation in the data is distributed. The link function provides a link between the linear predictor and the response variable (Fox et al., 2015).

With GLM log-likelihood ratio test (LRT) with Chi-squared distribution was used to see the goodness of fit of different models created with the chosen explanatory variables. Backwards selection method was the preferred method for this. The reason backwards selection was chosen was to avoid dealing with the complications that may have risen with the order of the variables.

All analyses were carried out using RStudio 2022.07.1+554 "Spotted Wakerobin" Release.

CHAPTER 3

RESULTS

Table 1 shows that the size of the ponds was ranging from 0.03 hectare to 4.43 hectare. The depths of the ponds were ranging from 0.5m to 6.1m. The largest pond was DP19, which is in the south of Lake Mogan, the smallest pond was DP2 which is in İmrahor Valley region. The shallowest ponds were in the south of Lake Mogan (DP16, DP17, DP18, DP19, DP20). DP16, DP17, DP18, and DP20 also didn't have thermal stratification and in turn there were no hypolimnion. The highest temperature (32.97 ^{o}C) was measured in the shallowest pond, DP17.

Table 1: List of ponds with area, depth, temperature, Secchi depth and hypolimnion percentage of the water column data.

Pond Code	**Area (ha)**	**Depth (m)**	**Temperature (°C)**	**Secchi Depth (m)**	**Hypolimnion Percent**
DP2	0.03	2.40	18.12	0.55	0.75
DP4	0.33	3.50	20.85	0.99	0.50
DP5	0.22	1.90	21.76	1.00	0.75
DP6	0.48	4.00	17.70	0.76	0.57
DP7	0.23	3.80	19.18	0.67	0.86
DP9	2.54	6.10	20.93	0.54	0.83
DP10	0.98	3.50	18.16	0.63	0.83
DP12	0.32	2.20	17.28	0.98	0
DP13	1.00	2.50	18.38	0.47	0.67
DP14	0.44	5.80	13.36	1.37	0.91

Table 1 (continued)

DP16	1.14	0.52	19.77	0.50	0
DP17	0.54	0.26	32.97	0.26	0
DP18	0.46	0.47	21.26	0.39	0
DP19	4.43	1.10	22.55	0.76	0.50
DP20	1.29	0.45	20.55	0.21	0

Table 2 shows the chemical variables, and Chla- and PVI measured in the ponds. The highest TP concentration was measured in DP10 in Imrahor River Valley where also the highest methane ebullition was recorded (Fig. 4). The lowest TP concentration was recorded in Lake Mogan pondscape, in DP16. The highest TN concentration was measured in DP9 in İmrahor River Valley. The lowest Chl-a concentration was measured in DP9. Another pond in İmrahor River Valley, DP5, had the lowest TN and DOC concentrations. Highest Chl-a concentration was measured in DP14 in Gölbaşı Düzlüğü, which had the lowest DO concentration. DP20 in Lake Mogan pondscape had the highest DO and highest PVI. The second lowest methane ebullition was also recorded in this pond. The lowest methane ebullition was recorded in its neighboring pond.

Table 2: List of ponds with, DO (dissolved oxygen (mg/L)), DO (%) (dissolved oxygen percentage), TN, TP, DOC (dissolved organic carbon), Chl-a (chlorophyll-a), PVI coverage and methane ebullition data.

Pond Code	**DO (mg/L)**	**DO (%)**	**TN (ppb)**	**TP (ppb)**	**DOC (mg/L)**	**Chla (µg/L)**	**PVI (%)**	**Methane Ebullition (mmol CH4-C m^{-2} d^{-1})**
DP2	1.7	19.3	2779.77	297.34	8.19	0.08	0	7.276
DP4	2.1	24.7	1548.58	271.30	10.43	187.16	0.07	17.971
DP5	3.7	43.2	932.39	457.69	5.64	0.04	0	22.096
DP6	4.9	55.9	984.66	394.55	6.58	122.26	0	12.535
DP7	5.5	64.5	3890.18	898.29	10.67	138.34	0	17.362
DP9	1.8	21.7	8276.14	940.17	13.55	0.03	0	22.346
DP10	6.5	72.3	6503.02	1449.91	12.40	0.16	0	39.238
DP12	1.3	13.0	3604.92	608.50	10.90	187.68	0	11.881
DP13	2.0	22.1	7730.63	1160.33	18.65	135.88	1.15	35.917
DP14	0.3	3.55	5246.53	393.13	7.53	308.14	0	2.820
DP16	2.9	33.5	5760.26	222.04	62.10	31.49	0	2.435
DP17	2.5	42.0	4286.66	894.30	521.00	26.02	0	0.994
DP18	5.7	68.5	6600.06	332.38	142.00	45.26	0	0.678
DP19	1.7	20.0	2403.41	253.40	32.15	7.16	2.6	0.901
DP20	9.9	115.0	4801.82	277.62	69.50	225.21	28.2	0.348

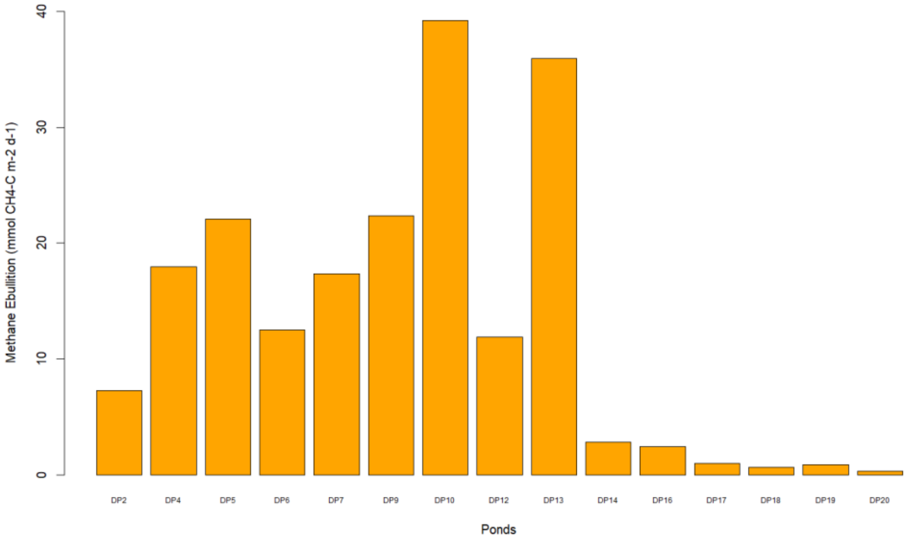


Figure 4: Bar plot showing the amount of methane ebullition (mmol $CH4$-C m^{-2} day^{-1}) from each pond.

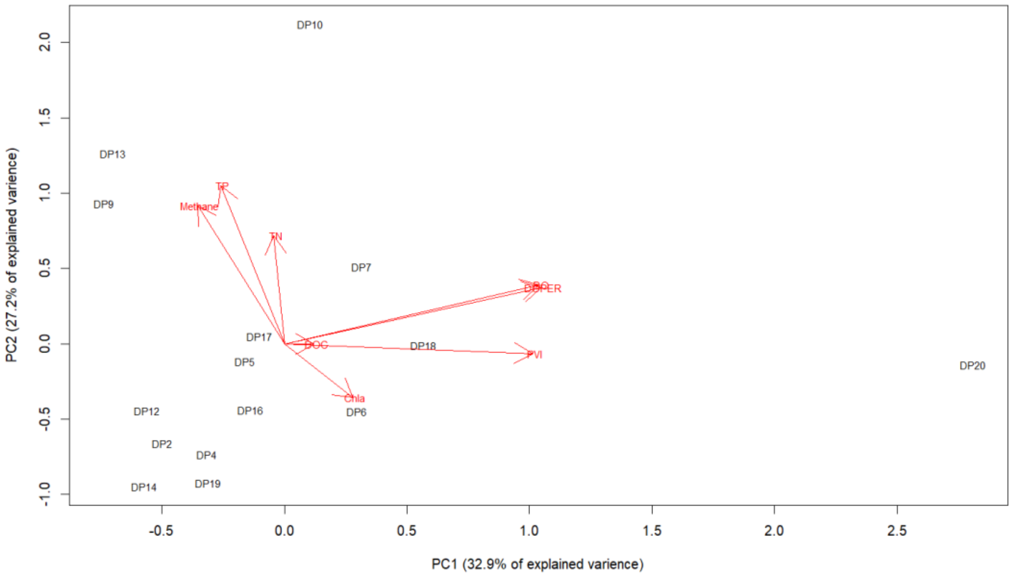


Figure 5: PCA analysis of abiotic and biotic factors (TP, TN, DOC, Chl-a, PVI, DO, DO (%)) and methane ebullition with PC1 explaining 32.9% of variance and PC2 explaining 27.2% of the variance.

The PCA analysis shows that TP, TN concentrations, and methane ebullition were positively correlated while Chl-a can be negatively correlated with methane ebullition. PVI, DO, and Chl-a were positively correlated.

Table 3: Table of zooplankton count data showing the total number of zooplankton, Cladocera, and Copepod species counted; total zooplankton, Cladocera, and Copepod density; and total zooplankton, Cladocera and Copepod biomass in each pond.

Pond Code	Total Num. of Sp.	Clad. Sp.	Cop. Sp.	Total Den.	Clad. Den.	Cop. Den.	Total Biomass	Clad. Biomass	Cop. Biomass
DP2	4	1	2	0.89	0.05	0.79	5.181	2.682	2.499
DP4	8	3	4	41.86	0.67	36.96	16.755	6.527	10.228
DP5	6	2	3	15.8	1.3	6.13	7.621	4.179	3.442
DP6	8	3	4	19.06	0.23	10.44	8.55	1.564	6.986
DP7	9	2	6	11.98	0.21	3.71	10.168	0.84	9.328
DP9	14	8	5	21.6	1.63	2.75	34.974	5.812	29.162
DP10	7	2	4	4.93	0.06	1.14	12.044	0.758	11.286
DP12	4	2	2	4.6	0.45	4	42.253	20.316	21.637
DP13	7	4	3	2.19	0.63	0.48	29.602	22.161	7.441
DP14	5	1	4	7.38	0.02	2.68	12.066	0.542	11.524
DP16	6	0	5	1.74	0	0.76	36.919	0	36.919
DP17	2	0	1	1.76	0	0.03	4.832	0	4.832
DP18	3	0	2	0.8	0	0.52	14.298	0	14.298
DP19	6	1	4	10.42	0.03	4.89	21.898	5.279	16.619
DP20	5	0	3	4.69	0	1.94	10.514	0	10.514

Table 3 shows the result of count data for zooplankton. The highest number of zooplankton species was observed in DP9 which also had the highest number of Cladocera species. The highest total zooplankton density and the highest copepod density were in DP4, which is also in Imrahor River Valley. In four of the ponds in Lake Mogan pondscape had no Cladocera species. Figure 6 shows the number of ponds in which zooplankton species are present. *Cyclops scutife*r was the most common copepod, which was encountered in 14 ponds. Ostracoda, nauplii, Microcyclops rubellus, and copepodid followed it. *Chydorus sphaericus* was the most common Cladocera as it was counted in 8 ponds.

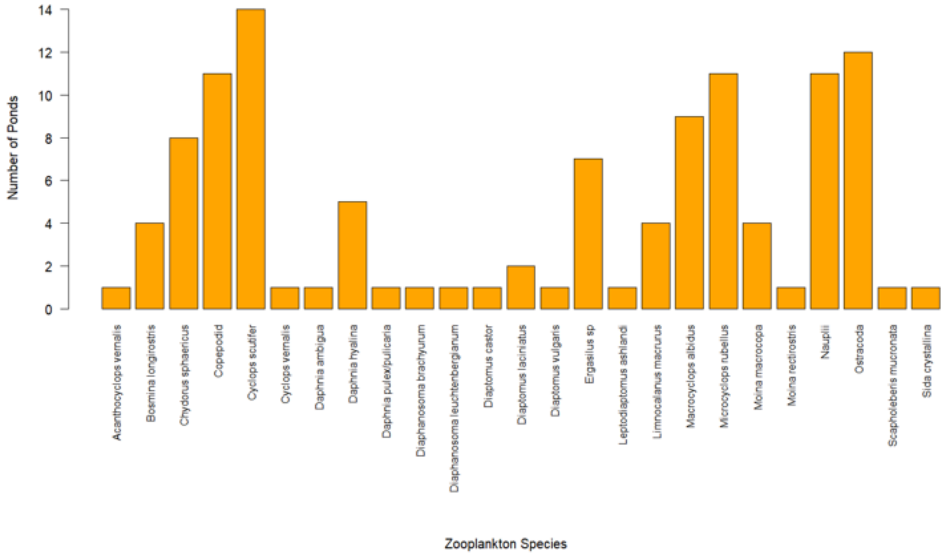


Figure 6: The frequency graph showing the number of ponds at which every zooplankton species is present.

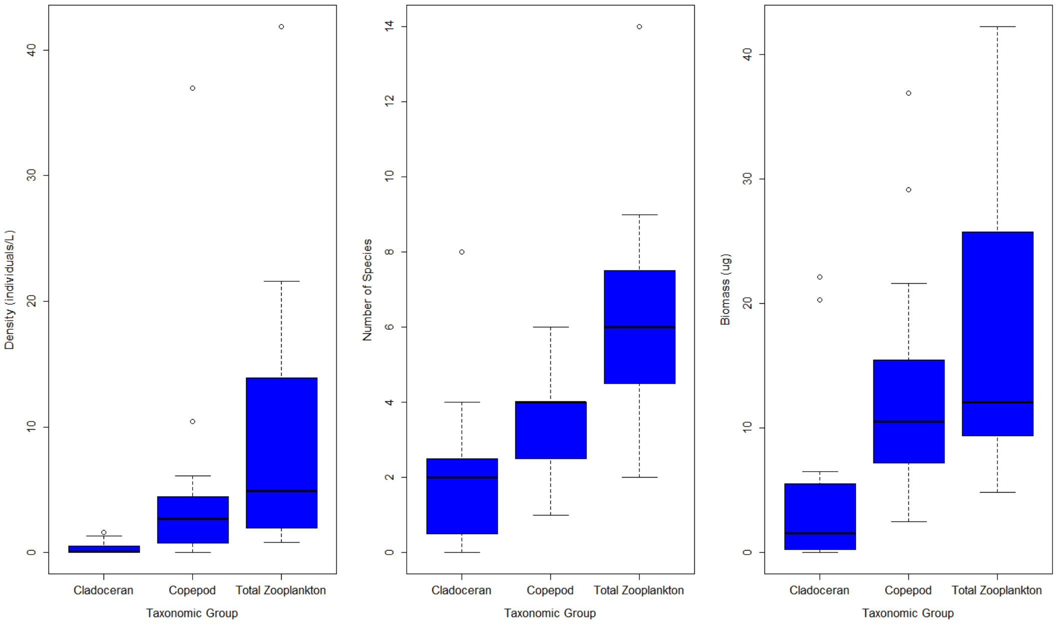


Figure 7: The boxplot on the left showing the Cladocera, Copepod, and total zooplankton density in ponds. The boxplot in the middle showing the total number of Cladocera, Copepod, and zooplankton species. The boxplot on the right showing the Cladocera, Copepod, and total zooplankton biomass in ponds. Total zooplankton category includes Ostracoda and nauplii which were added to density calculations but were not belonging in Cladocera or Copepod order.

Figure 7 shows that the total zooplankton density, total zooplankton biomass, and the number of total zooplankton species has the highest variation between ponds. The Cladocera density has the lowest variation between ponds; ponds have similar Cladocera density. The number of Cladocera species in ponds shows a larger variation than the number of Copepod species. However, the number of copepod species was always higher than the number of Cladocera species found in the ponds. The Cladocera biomass in ponds was also lower than the copepod biomass.

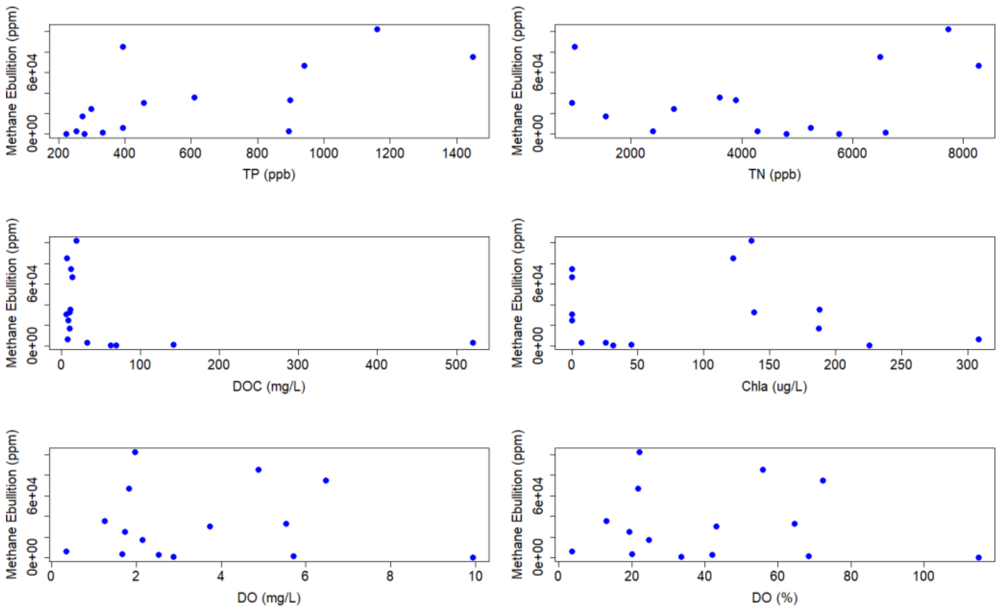


Figure 8: The plots which were generated with the original dataset for TP, TN, DOC, Chl-a, DO, and DO (%) correlation with methane ebullition.

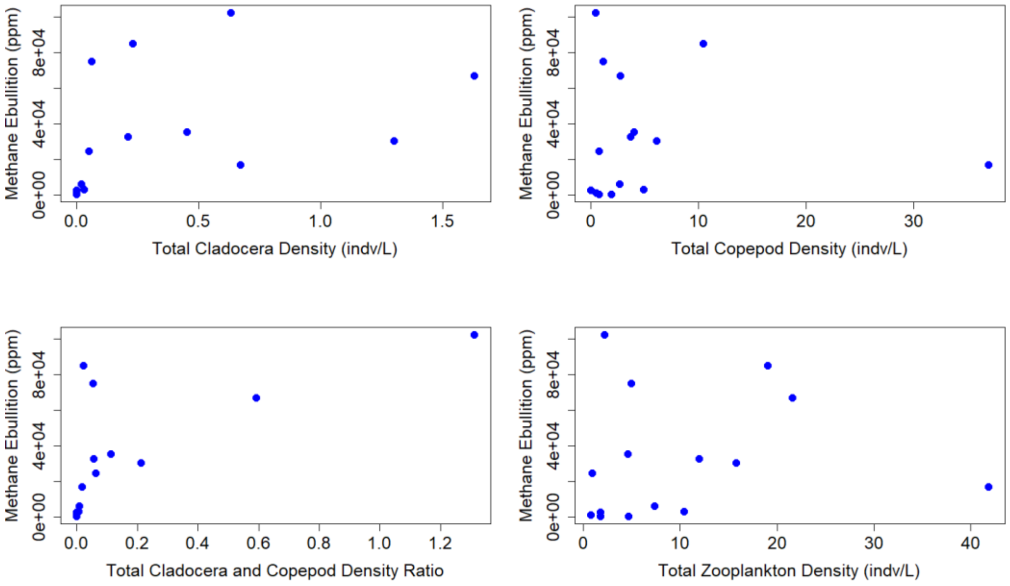


Figure 9: The plots which were generated with the original dataset for total Cladocera density, total copepod density, total Cladocera and copepod density ratio, and total zooplankton density correlation with methane ebullition.

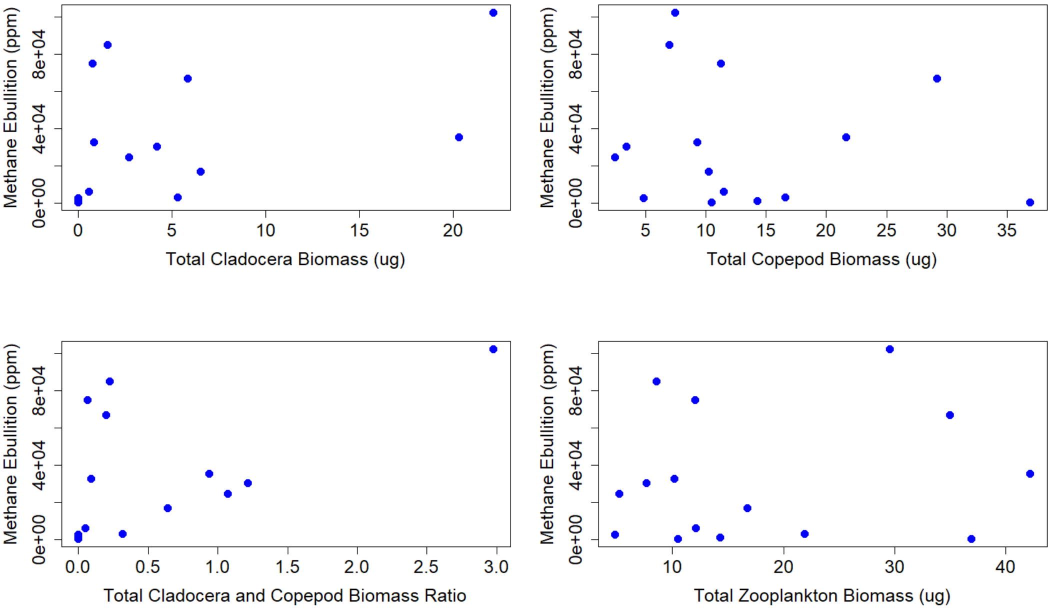


Figure 10: The plots which were generated with the original dataset for total Cladocera biomass, total copepod biomass, total Cladocera and copepod biomass ratio, and total zooplankton biomass correlation with methane ebullition.

Among the plots in Figure 8, TP, TN, DO, and DO (%) show that there were correlation with methane ebullition. DOC and Chl-a didn’t show a clear correlation with methane ebullition. Among the plots in Figure 9, only the total Cladocera density showed correlation with methane ebullition. None of the plots in Figure 10 showed any correlation.

After random forest model was run, variable importance plot was generated (Fig. 11). The variables with the highest %IncMSE and IncNodePurity were chosen as the explanatory variables for GLM since they would be the variables with the highest explanatory power. Thus, Cladocera to copepod density ratio, total Cladocera density, TP, and TN concentrations were picked to run the GLM (Table 4 and Fig.11). The reason these four variables were picked was because all of these variables have %IncMSE higher than 1% and they have the highest IncNodePurity values (Fig. 11, Table 4).

The first GLM (Table 5) showed no significance when all of the variables were in the model. So, backwards selection was done starting from TP and TN interaction effect. After running the GLM and doing backwards selection, it was revealed that total Cladocera density, TP, and TN were the explanatory variables which showed significance for CH_4 ebullition (Fig. 12, Table 6). The final GLM model showed that TP and total Cladocera density have a positive significant effect on methane ebullition while TN shows negative effect (Fig. 12).

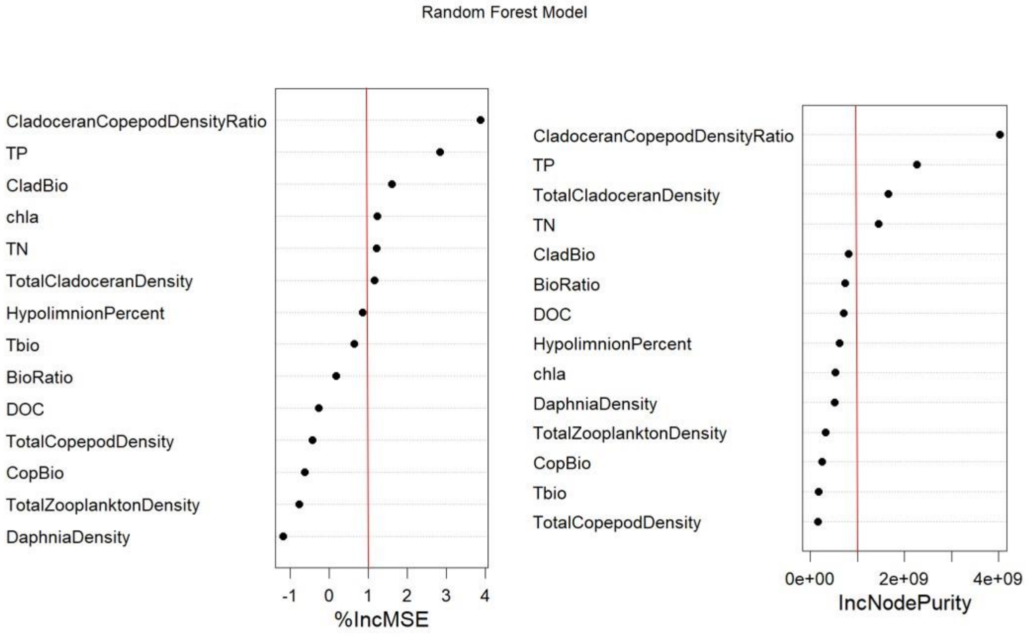


Figure 11: Variable Importance Plot of the random forest model. TP means Total Phosphorus (ppb), TN means Total Nitrogen (ppb), DOC means dissolved organic carbon (mg/L), chla means chlorophyll-a (ug/L), CladBio means Cladoceran biomass, Tbio means total biomass, BioRatio means biomass ratio, and CopBio means copepod ratio.

Table 4: Table showing the %IncMSE and IncNodePurity values of Variable Importance plot.

Explanatory Variables	**%IncMSE**	**IncNodePurity**
HypolimnionPercent	0.8600513	615559600
DOC	-0.2632736	712232600
chla	1.2339800	537900900
TP	2.8391720	2263139000
TN	1.2203590	1447828000
TotalCladoceranDensity	1.1561010	1652395000
TotalCopepodDensity	-0.4294286	158079500
DaphniaDensity	-1.1667450	523510000
TotalZooplanktonDensity	-0.7677670	320505500
CladoceranCopepodDensityRatio	3.8732080	4017824000
BioRatio	0.1768453	737275900
CladBio	1.6094600	820174400
CopBio	-0.6160674	258722700
Tbio	0.6377355	177232200

Table 5: GLM analysis results of predicted methane ebullition in relation to total Cladocera density, Cladocera copepod density ratio, TP, and TN. Every row of the table represents a separate GLM model.

(PredictedMethane~TotalCladoceranDensity+CladoceranCopepodDensityRatio+TP*TN)

	Estimate	**Standard Error**	**t value**	**P value**
(Intercept)	1.01e+01	6.46e-01	15.557	0.0000000822***
TP	1.30e-03	1.20e-03	1.091	0.3036
TN	-9.92e-05	1.39e-04	-0.714	0.493

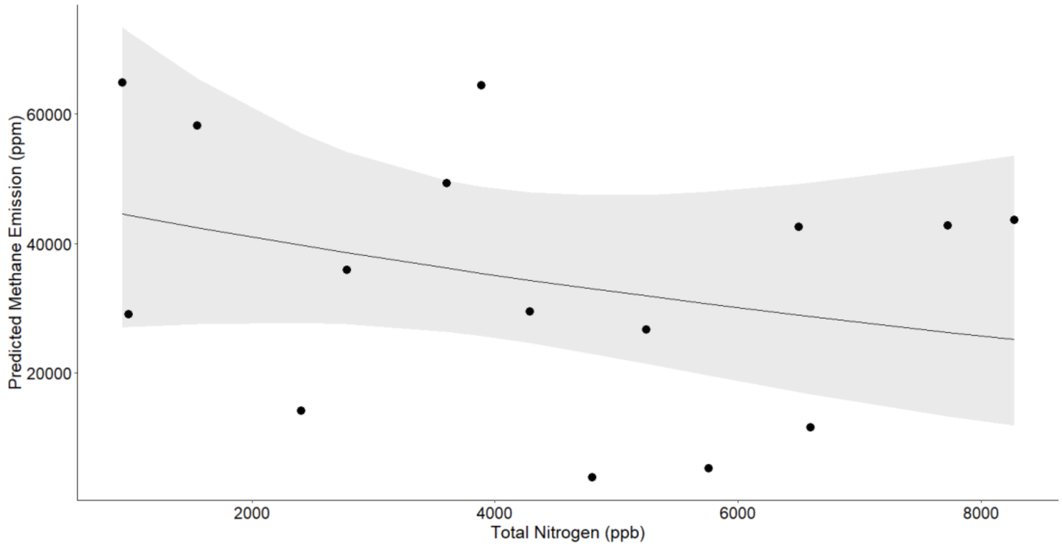
Table 5 (continued)

TotalCladoceranDensity	4.11e-01	1.93e-01	2.128	0.0623.
CladoceranCopepodDensityRatio	5.24e-01	3.74e-01	1.399	0.1952
TP:TN	-9.33e-08	2.16e-07	-0.433	0.6755

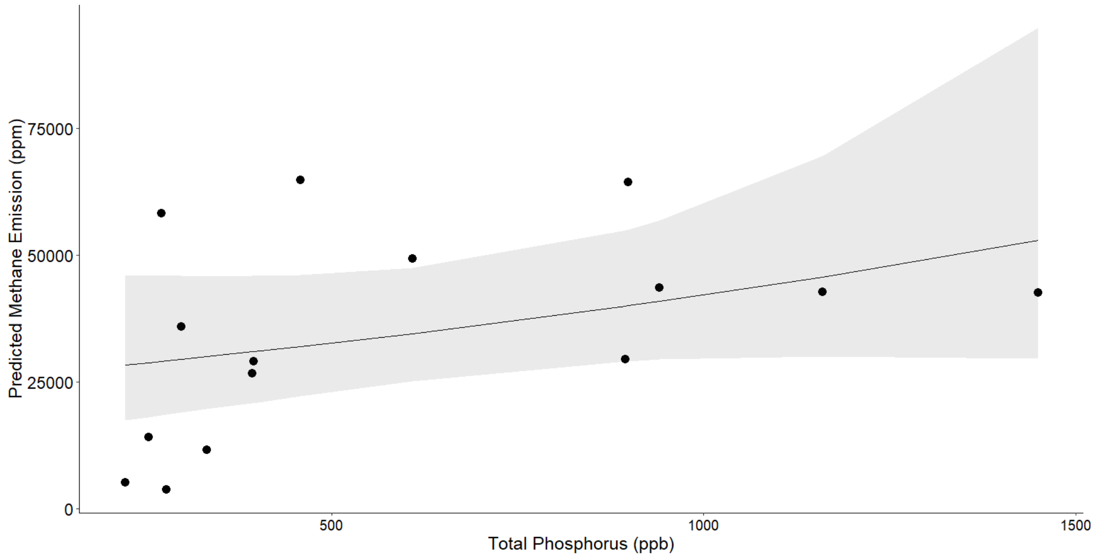
Table 6: GLM analysis results of predicted methane ebullition in relation to TP, TN, and TotalCladoceranDensity. Every row of the table represents a separate GLM model. (PredictedMethane~ TP+TN+TotalCladoceranDensity)

	Estimate	**Standard Error**	**t value**	**P value**
(Intercept)	1.02e+01	2.43e-01	42.043	0.000000000000168***
TP	8.52e-04	3.98e-04	2.139	0.05569.
TN	-1.22e-04	5.32e-05	-2.296	0.04236*
TotalCladoceranDensity	5.17e-01	1.64e-01	3.159	0.00909**

(A)


Predicted Methane Emission (ppm)
60000
40000
20000
2000
4000
6000
8000
Total Nitrogen (ppb)

(B)


Predicted Methane Emission (ppm)
75000
50000
25000
0
500
1000
1500
Total Phosphorus (ppb)

(C)

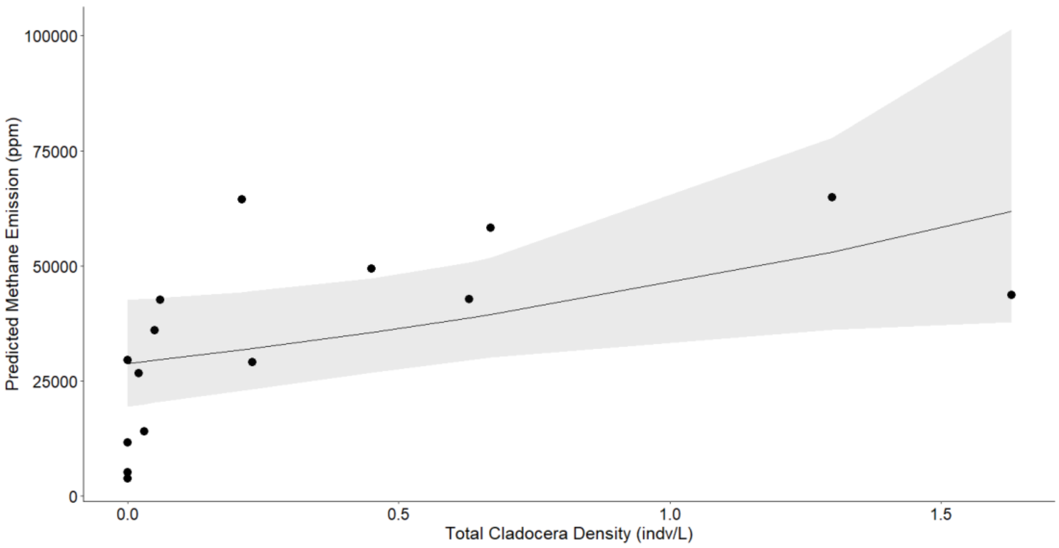


Figure 12: Plots showing the relationship between bootstrapped predicted methane ebullition and TN (A), TP (B), and total Cladocera Density (C) with confidence intervals. (A) shows a negative correlation with methane ebullition while (B) and (C) show a positive correlation.

CHAPTER 4

DISCUSSION

4.1 Methane Emission

In the ponds that were studied, the amount of methane ebullition varied greatly. The lowest range of methane ebullition was recorded in Lake Mogan pondscape. The reason for this may be that the ponds in this pondscape were very shallow and open to wind as its near catchment is not surrounded with trees, preventing an anoxic layer to occur and cause methanogenesis. The highest ebullition recorded was 39.24 mmol m^{-2} d^{-1} in Imrahor River Valley that is very close to the highest methane ebullition recorded from a freshwater pond and it was 40 mmol m^{-2} d^{-1} in Manitoba, Canada (Baron et al., 2022). This shows that the methane ebullition we've recorded was record creakingly high. The methane ebullition in the Imrahor River Valley ponscapes ranged between 7.28-39.24 m^{-2} d^{-1} which is high compared to the other pondcapes measurements of the current study. The methane ebullition recorded in Lake Mogan pondscape was measured closed to the records obtained in from Finnish mesotrophic ponds ranged between 0.22-0.47 mmol m^{-2} d^{-1} (Huttunen et al., 2003).

In Gölbaşı Düzlüğü, ebullition measurements ranged between 2.82-35.92 mmol m^{-2} d^{-1} which is a very large range, considering the fact that ponds with the highest and lowest range were connected. This difference might have been caused by sampling error. During the 7 days that the chambers were in the ponds, the chambers may have been tipped over because of birds or wind, causing the escape of gas and low methane measurements in DP14.

4.2 Abiotic Factors and Methane Emissions

The first hypothesis "Nutrient concentrations in ponds has an effect on CH_4 emissions" can be accepted. The results of the models (Fig. 12 (A) & (B)) show that there was a highly significant association between TP, TN and CH_4 emissions. TP and TN are also known to have a correlation with GHG emissions. In lakes, TP values higher than 100 ppb are considered hypereutrophic (Brönmark & Hansson, 2018). All of the ponds in this study have TP values higher than 100 ppb. TN between 1500-5000 ppb is also a sign of considered eutrophication (Brönmark & Hansson, 2018). Most of the ponds in this study have higher TN values than 1500 ppb.

Nitrate and phosphate can have a negative and positive, respectively effect on CH_4 emissions from pond water. Nitrate can have negative effect on CH_4 production since it can also act as an electron acceptor and inhibit the methanogenesis process (Malyan et al., 2022). This may be the reason why there was a negative relationship between TN and predicted methane emissions (Fig. 12 (A)). Phosphate can have positive effect on CH_4 production since it increases the organic matter in the system, which increases the production of CH_4 by methanogenic bacteria. This was also proven by the results of this study (Fig. 12 (B)). Peacock et al. (2019) also observed that the total phosphorus amount is positively correlated with the CH_4 emission rates from urban ponds. In our ponds, the pond with the highest TP measurement has the highest methane ebullition (Table 2).

Eutrophication is the process of over-production of organic matter induced by nutrients, primarily nitrogen and phosphorus. Chl-a is an important indicator of high productivity and eutrophication (Liu et al., 2010). Eutrophic status, as indicated by concentrations of Chl-a and nutrients in freshwaters, is a major driver of local freshwater GHG emissions (Li et al., 2021). However, although many of the ponds had high Chl-a concentration in this study, there was no correlation observed between Chl-a and methane ebullition.

Studies by van Bergen et al. (2019) show that organic carbon contributes roughly 6% and 20% to total carbon emissions from ponds and reservoirs. Peacock et al. (2019) observed that there was a positive correlation between organic carbon and the rate of CH_4 emissions from the urban ponds. Thottathil et al. (2018) showed that between concentrations of 1.9–11 mg/L, DOC can modulate CH_4 oxidation during the summer stratification. Increasing DOC can enhance oxidation in the upper layers by reducing light penetration to the bottom of the pond where methanotrophic activity occurs, while also decreasing oxygen availability in the deeper layers. However, no correlation between DOC and methane emissions were measured in our pondscapes but likely that we had low number of ponds falling into this category that significant interactions did not emerge.

In shallow lakes and ponds, the dominating primary producers can be phytoplankton or macrophytes. In the case of high nutrients and temperature, it is expected for the ponds to be dominated by phytoplankton (Davidson et al., 2018). In our study, most of the ponds had no macrophyte coverage. However, not all of them had high Chl-a concentrations. On the contrary, the highest Chl-a concentrations were recorded in the ponds with macrophyte coverage, except one pond. The low macrophyte coverage might be caused by high nutrient concentrations (Bucak et al., 2012).

Eutrophication also results in the decrease of the DO concentration in pond water because of decomposition of the organic matter (Deemer et al., 2016). The anoxic conditions promote CH_4 production further since CH_4 is the primary product of organic carbon mineralization under anaerobic conditions (Liikanen et al., 2002). In addition, in eutrophic ponds, algal blooms will decrease the oxygen, reduce the rate of CH_4 oxidation, and increase the diffusive flux of CH_4 (Yan et al., 2017). Pond DP20 in Lake Mogan pondscape has the highest DO and the lowest methane ebullition. It can show that in the presence of oxygen, respiration is carried out with oxygen and no methane is produced (Brönmark & Hansson, 2018). However, in this study, there was no significant correlation observed between DO and methane ebullition. This may have been caused by the possible production of methane in oxic layers in ponds where the rate of the demethylation of phosphonic acids is high (Khatun et al., 2019).

In order to understand the system better and make a stronger argument, phytoplankton counting with the microscope rather than relying on Chl-a data might've been better. Future studies may be planned better by considering the time restriction of analyzing phytoplankton samples.

4.3 Methane Emission and Zooplankton Density

In this study, it was found that total Cladocera density had the highest correlation with CH_4 ebullition (Fig 12, (C)). This could be probably through high Cladocera density exerting high grazing pressure on MOB and in return CH_4 ebullition would be higher in ponds where Cladocera density was high. Thus, the hypothesis pointing at "high Cladocera density causes low CH_4 ebullition likely through strong grazing on MOB" is accepted. This is also found in an experiment conducted by Kankaala et al. (2007) who conducted a bottle experiment to see the grazing effect of *Daphnia longispina* on MOB. The results showed that at higher densities of *Daphnia,* methanotrophic activity was higher. They also measured MOB abundance by molecular analyses and were found that MOB abundance was lower in microbial communities where *Daphnia* biomass was higher.

However, the same results cannot always be observed in nature. In an experiment carried out in 2009 conducted by Jones and Lennon, interactions between *Daphnia* and MOB were studied in a lake in Michigan using isotope analysis and bioassays. They showed no direct interaction between *Daphnia* and MOB, it rather indicated that there were indirect interactions like *Daphnia* grazing on MOB-feeding protists. This could also be the case in above study by Kankaala et al. (2007) instead of a direct effect indirect effect or maybe both might have been important. It is also worth mentioning that in the experiments conducted for observing zooplankton grazing on MOB, *Daphnia* was the model Cladocera. However, in our pondscape, *Daphnia* was not an often-observed Cladocera (Fig. 6). Thus, comparing our results with the experiments in the literature might not be correct.

There was no significant correlation with copepod density and CH_4 ebullition. The reason can be attributed to copepods having lower grazing pressure on bacteria. Jürgens (1974) also showed that in lakes where zooplankton communities were copepod dominated, bacteria biomass, number, and morphological diversity would be higher.

4.4 Limitations of This Study

This study was conducted in order to learn more about the urban ponds in Ankara which were never studied before. All of the data was collected during the summer of 2021. Along the way, methods were modified and schedules were adapted to better work with the conditions of the ponds. The fact that these ponds are now under the attention of scientists and there is future research being planned about these ponds is a huge step. By better studying these ponds, the aim is to restore and manipulate these systems to fight against the threats of climate change as nature-based solutions. Now that these ponds are better known, the future fieldworks can be better planned by keeping in mind the missing points of the current study.

One of the obstacles that were faced during this study was the underestimation of the eutrophication level as well as rubbishes all around the ponds. The ponds had high density of debris caused by mixing, human activity, and cattle getting into the ponds. As a result, a lot of debris was collected during sampling. This caused the fast degradation of zooplankton samples and smell was detected in the samples even though all samples were filled with 4% Lugol iodine solution. To save the samples, 70% glutaraldehyde were added to each sample, then degradation and smelling stopped. However, it was seen that some individuals in the samples were already damaged and they were not identifiable. The sample size decreased and the accuracy of the records of zooplankton content of each pond was reduced. Now that these ponds are better known, filtration and protection of field samples can be better performed during future studies.

Another point that must be considered is the number of ponds and equipment protection. There were 18 ponds selected and sampled in summer 2021. However, we were only able to include 15 of them for this study since one of the ponds were dried, one of them was too deep to be considered as a pond, and in one of the ponds, the gas chambers were thrown out of the pond by the locals. There were also some gas chambers removed or replaced in a few ponds, but since there were multiple gas chambers in each pond, gas sampling was still done successfully. For future studies, the pondscape can be enlarged in order to have more ponds sampled. Also, since the area is now better known, it should be easier to detect the depth of waterbodies in the area and decide which ones are true ponds. Finally, more methods for equipment protection can be developed. These points were important since one of the biggest challenges of statistical analysis for this research was the small sample size. Repeating the same sampling with more ponds and acquiring more samples without losing any gas chamber would result in stronger analysis results and give more confidence.

It should also be kept in mind that this study was conducted with a one-time sampling. Considering the small sample size, it would’ve been statistically better to monitor these ponds throughout the year, or do multiple samplings in preset intervals of time. Ponds are very delicate systems that temperature or DO levels can change even day to day, other than the expected seasonal changes. So, observing the ponds in a time scale, seeing those changes and seeing the changes in GHG emission rates would’ve given more confidence to answer the questions that were asked for this research. The change in zooplankton density and size could also be recorded and the assumptions could be more strongly tested.

The water chemistry samplings should be done twice; while putting the gas chambers and while collecting them. The samples should be taken separately from the different layers of the ponds, rather than collecting one whole water column sample. This way the differences in the chemistry of oxic and anoxic layers can be detected. Zooplankton sampling can also be done separately to see the differences in zooplankton communities of oxic and anoxic layers, and make stronger claims about the grazing effect of zooplankton on bacteria. Sediment sampling should also be done to measure the decomposition rate and methane production in the sediment.

Finally, the processes and food web in a pond can be too complicated and rapidly changing to understand in a single sampling. To observed the structure and the changes in the microbial loop and how this effects CH_4 emissions, experiments where there are controlled variables should be conducted. These can be in-pond bottle experiments where only the changes in microbial loop can be studied, or mesocosm experiments where the abiotic factors and the history of the mesocosms can be controlled and known.

CHAPTER 5

CONCLUSION

In this thesis, the association between nutrient concentrations and methane emission, as well as zooplankton community structure and methane emission from ponds were studied. The data was bootstrapped by using regression tree analysis and creating a random forest model. The explanatory variables chosen from the random forest model were then put to goodness of fit models and eliminated by backwards selection. As a result, the variables that show the most significant association with methane emissions were TP, TN and Total Cladocera Density.

These findings support the idea that nutrient concentrations in ponds, TP and TN, have a significant effect on methane emissions. Additionally, it shows that zooplankton community structure might also have an effect on methane emissions by zooplankton grazing on MOB and changing MOB community.

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