Effects of Vegetation and Nutrients on Methanotroph Abundance and Methane Emissions from Constructed Wetlands

Klara Li Yngve

Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Natural Resources and Agricultural Sciences • Department of Aquatic Sciences and Assessment
Agriculture Programme – Soil and Plant Sciences
Uppsala 2022

Effects of Vegetation and Nutrients on Methanotroph Abundance and Methane Emissions from Constructed Wetlands

Effekter av vegetation och näringsstatus på mängden metanotrofer och metangasavgång från anlagda våtmarker

Klara Li Yngve

Supervisor:	**Pia Gerenmayeh, SLU, Department of aquatic sciences and Assessment**
Assistant supervisor:	Michael Peacock, SLU, Department of aquatic sciences and Assessment
Assistant supervisor:	Tong Liu, SLU, Department of aquatic sciences and Assessment
Assistant supervisor:	Stefan Bertilsson, SLU, Department of aquatic sciences and Assessment
Examiner:	Marcus Wallin, SLU, Department of aquatic sciences and Assessment

Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Master thesis in Biology
Course code:	EX0898
Programme/education:	Agricultural Programme – Soil and Plant Sciences
Course coordinating dept:	Department of Aquatic Sciences and Assessment
Place of publication:	Uppsala
Year of publication:	2022
Keywords:	Diffusive flux, ebullition, dissolved CH_4, CH_4 oxidation, phosphorous

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Aquatic Sciences and Assessment
Section for Geochemistry and Hydrology and Section for Ecology and Biodiversity

Abstract

Agriculture is the largest anthropogenic source of nitrogen and phosphorous (P) leakage to Swedish coastal seas, causing eutrophication which threatens marine ecosystems. Constructing wetlands is one way to improve nutrient retention in the modern agricultural landscape. However, constructed wetlands can emit substantial amounts of methane (CH_4). Methanotrophs facilitate aerobic oxidation of CH_4, which transforms the CH_4 into less potent carbon dioxide. However, the factors controlling the methanotrophs and their effect on CH_4 emissions are poorly understood. The aim of this thesis was to investigate the effect of vegetation and nutrient status on the CH_4 emissions and abundance of methanotrophs from constructed wetlands in agricultural areas in Sweden. Water samples for DNA extraction and quantification by qPCR and gas samples for concentrations of dissolved CH_4 was taken at 34 wetlands in southwest and middle Sweden. At 5 of the wetlands CH_4 fluxes were measured using floating chamber technique. At each wetland samples were taken at one point with and one without vegetation. In addition, water chemistry and nutrient status were measured.

The results show that vegetation had no effect on the abundance of methanotrophs or on the dissolved CH_4 and CH_4 fluxes. This contradicts previous studies and may be explained by even conditions throughout the wetland and the off-vegetative season. Of the nutrients and hydrochemical factors, P and the increased fraction of P in relation to carbon and nitrogen was significantly correlated to the abundance of methanotrophs, which is in line with previous studies. None of the studied variables were correlated to the dissolved CH_4. The total fluxes corresponded to fluxes reported in previous studies and indicated that ebullitive fluxes can be large even during winter season. Except the relationship between increased abundance of methanotrophs and increased P concentrations, the lack of significant results in this study may indicate that the abundance of methanotrophs, dissolved CH_4 and CH_4 fluxes depend on multiple direct and indirect variables that interactively control CH_4 emissions and the bacterial community involved in CH_4 oxidation.

Keywords: Diffusive flux, ebullition, dissolved CH_4, CH_4 oxidation, phosphorous

Sammanfattning

I Sverige är jordbruket den största mänskliga källan till läckage av kväve och fosfor till kustnära vatten, vilket orsakar övergödning och hotar marina ekosystem. Att bygga anlagda våtmarker är ett sätt att förbättra näringsretentionen i det moderna jordbrukslandskapet. I våtmarker kan det dock bildas och avgå en betydande mängs metangas (CH_4). En särskild grupp av mikroorganismer, så kallade metanotrofer, omvandlar CH_4 till den mindre starka växthusgasen koldioxid genom oxidation, men de faktorer som kontrollerar metanotroferna och deras påverkan på CH_4-avgången är dåligt studerade. Syftet med det här masterarbetet var att undersöka effekten av våtmarksvegetation och näringsstatus på mängden metanotrofer och utsläppen av CH_4 från konstruerade våtmarker i svenska jordbrukslandskap. Vattenprover för DNA-extraktion och kvantifiering av metanotrofer genom qPCR samt gasprover för CH_4 löst i vatten togs på en plats med och en plats utan vegetation i 34 våtmarker i Halland och Mälardalen. I fem av våtmarkerna mättes också metangasavgången med hjälp av flytande kamrar. På alla platser mättes vattenkemi och koncentration av näringsämnen.

Resultaten visar en stor variation inom och mellan våtmarker, men vegetationen hade ingen signifikant effekt på varken mängden metanotrofer, löst CH_4 eller CH_4-avgång. Detta är i motsats till tidigare studier och kan möjligen förklaras av en jämn fördelning av näringsämnen i hela våtmarken samt att proverna togs när det inte var växtsäsong. Av de näringsämnen och vattenkemiska faktorer som mättes visade fosfor (P) samt en ökande mängd P i förhållande till kol och kväve ett signifikant, positivt samband till mängden metanotrofer men inga signifikanta samband till löst CH_4 kunde påvisas. CH_4-avgången som mättes i kamrarna var av samma storleksordning som tidigare studier rapporterat men visade också, tvärtemot vad tidigare studier antytt, att avgången kan vara stor även vintertid. Förutom korrelationen mellan P och metanotrofer antyder avsaknaden av signifikanta resultat och samband i den här studien att mängden metanotrofer och utsläppen av CH_4 inte kan förklaras av en eller ett fåtal faktorer, utan sannolikt beror på flera både direkta och indirekta samverkande faktorer som kontrollerar CH_4-avgång och mikrobsamhällena involverade i CH_4-oxidation.

Populärvetenskaplig sammanfattning

Svenskt jordbruk är viktigt för landets självförsörjning, något som särskilt har uppmärksammats under den senaste tidens pågående klimatkris, pandemi och krig. Samtidigt är jordbruket långt ifrån hållbart och har flera negativa effekter på natur och miljö. Bland annat läcker näringsämnen ut från åkrar och hamnar slutligen i vattendrag och kustnära hav där de orsakar övergödning, vilket gör att många arter som lever i eller nära vattnen riskerar att dö ut. Men om vattnet på sin väg från åker till hav passerar våtmarker, till exempel myrar, grunda sjöar och låglänta områden som svämmas över av vatten vissa delar av året, kan näringsämnena sjunka till botten eller brytas ner av mikroorganismer, vilket renar vattnet och minskar övergödningen. För drygt 200 år sedan bestod en stor del av landskapet i södra och mellersta Sverige av just sådana våtmarksområden. Men under 1800- och 1900-talen, då befolkningen ökade kraftigt och det ofta var hungersnöd, torrlade man stora arealer genom att gräva diken och sänka vattennivån i sjöar. Detta skapade mer jordbruksmark som bidrog till att trygga livsmedelsförsörjningen, men orsakade på lång sikt att den naturliga vattenreningen försämrades. För att kompensera för detta i dagens moderna jordbrukslandskap kan man bygga anlagda våtmarker eller dammar. Med rätt placering och utformning kan de effektivt fånga upp en del av den näring som läcker ut från åkrarna.

Men de anlagda våtmarkerna är inte problemfria. I deras bottnar kan det bildas metan när bakterier bryter ner döda växtdelar som ansamlas där. Metan är en växthusgas som har 25 gånger starkare effekt på den globala uppvärmningen än koldioxid. Den enda process som på naturlig väg kan minska utsläppen av den metan som bildas, är när metan omvandlas till koldioxid av en särskild grupp bakterier som lever i våtmarkernas botten och vatten och kallas metanotrofer. I strävan efter att utforma våtmarker som renar vattnet från så mycket näring som möjligt och samtidigt släpper ut minimalt med metangas är det viktigt att förstå vad som påverkar metanotroferna och deras förmåga att omvandla metan till koldioxid. Syftet med det här masterarbetet var att undersöka om växtligheten samt koncentrationen av näringsämnena fosfor, kol och kväve i anlagda våtmarker spelar någon roll för hur mycket metanotrofer det finns i våtmarkerna och hur mycket metan som släpps ut. Det gjordes genom att ta bakterie- och gasprover från 34 anlagda våtmarker i Halland och Mälardalen, som sedan analyserades på labb. Tyvärr gav resultaten inga tydliga svar. Både mängden metanotrofer och

metangasavgången varierade mycket mellan de olika våtmarkerna och mellan bevuxna och icke bevuxna platser inom samma våtmark, men skillnaderna kunde varken förklaras av vegetationen eller koncentrationen av kol och kväve. Dock pekade resultaten på att mängden metanotrofer kanske gynnas med en ökad halt av fosfor i våtmarkens vatten, och att utsläppen av metan kan vara ganska stora även vintertid trots låga temperaturer, något som tidigare antagits minska metanutsläppen.

Samtidigt som det inte går att dra några säkra slutsatser kring växtlighetens och näringsämnenas påverkan utifrån studiens resultat, pekar just otydligheten på att det är svårt att försöka reglera metanotroferna i en våtmark med hjälp av en eller några få faktorer. Snarare styrs både mängden metanotrofer och deras aktivitet samt utsläppen av metan troligtvis av väldigt många olika, samverkande faktorer. Forskningsfältet kring metanotroferernas funktion och roll för metangasavgång är hitintills ganska begränsat och fler studier behövs för att förstå om och hur metanotrofer kan gynnas för att utforma våtmarker med så liten påverkan på den globala uppvärmningen som möjligt.

Table of contents

List of tables

List of figures

Abbreviations

CH_4	Methane
CO_2	Carbon dioxide
TP	Total Phosphorous
TN	Total Nitrogen
TOC	Total Organic Carbon
V	Vegetated
U	Unvegetated
Chl-a	Chlorophyll a

1. Introduction

Swedish agriculture is crucial for domestic food production and supply, whose importance is particularly stressed in times of societal crises such as climate change, pandemics and war. However, agriculture also contributes to eutrophication of water bodies through nutrient leakage from fields. In fact, agriculture is the largest anthropogenic source of nitrogen (N) and phosphorous (P) loads to our coastal seas (Havs- och vattenmyndigheten 2019). Biogeochemical and physical processes, such as denitrification, sedimentation of particle bound P, chemical sorption and biological uptake occur naturally in wetlands and can retain nutrients and counteract excessive nutrient loads to lakes and seas (Hoffmann et al. 2009). However, during the 19th and 20th centuries, wetlands and shallow lakes were drained to generate more arable land that could feed a growing population and prevent famine, and this largely deprived south and middle Sweden of wetlands (Gunnarsson & Löfroth 2009; Feuerbach & Strand 2010). Not only did this cause poor conditions for nutrient retention; it also led to altered hydrological flows, decreased biodiversity and increased emissions of carbon dioxide (CO_2) (Gunnarsson & Löfroth 2009; Naturvårdsverket 2009).

Building constructed wetlands is one way to improve nutrient retention, water holding capacity, biodiversity and carbon sequestration in the modern agricultural landscape. In Sweden, constructed wetlands can be defined as "areas where either raising the water table (damming) or lowering the ground level (excavating) have led to the new existence of open water surfaces in the landscape, permanently or temporarily during the year" (Strand & Weisner 2013, p. 15). Since the 1990s, different economic subsidy programs have been available for landowners to promote restoration and construction of wetlands and a large number of wetlands have been created in the agricultural landscape (Strand & Weisner 2013). However, wetlands are natural emitters of methane (CH_4), which is the greenhouse gas contributing most to global warming after carbon dioxide (CO_2) (Solomon et al. 2007). CH_4 is produced in the microbial decomposition of organic matter in oxygen free bottom sediments (Cole et al. 2007). High nutrient status and small wetland size, both common properties of wetlands constructed for nutrient retention in agricultural areas, are positively correlated to CH_4 production and release (Grasset et al. 2016; Holgerson & Raymond 2016; Peacock et al. 2021), which makes constructed wetlands to a considerable source of anthropogenic CH_4 emissions.

Methanotrophs is a functional group of bacteria that oxidises CH_4 in their metabolism. They function as a biological filter that can reduce CH_4 release to the atmosphere (Whalen 2005) and may therefore be utilized to decrease the contributions of wetlands to global warming. When wetlands are ice covered during winter, the filter may be particularly effective. This is because the CH_4 are trapped in the water column under the ice and therefore exposed to oxidation for an extended time (see e.g. Sawakuchi et al. 2021). However, more insight is needed to understand what environmental factors and wetland features that affect the abundance and activity of methanotrophs (Samad & Bertilsson 2017). The effects of nutrients, and their relative proportions, on CH_4 oxidation is poorly studied (Veraart et al. 2015) but could be of particular relevance for wetlands that receive high nutrient loads. Aquatic plants constitute an important part of wetland design as they have important functions for nutrient retention and diversity (Jordbruksverket 2004). The effect of vegetation on methanotrophs and CH_4 production, oxidation and release have been studied during growing season in different kind of water bodies with varying and sometimes opposing results (Holzapfel-Pschorn et al. 1986; Segers 1998; van der Nat & Middelburg 1998; Kankaala et al. 2003, 2005; Ström et al. 2005; Fritz et al. 2011; Carmichael et al. 2014; Grasset et al. 2016; Turner et al. 2020; Bodmer et al. 2021). However, no such studies have been conducted on constructed wetlands in Sweden during winter season. The objectives of this thesis were therefore to investigate the effect of vegetation and nutrient status on the CH_4 emissions and abundance of methanotrophs from constructed wetlands in agricultural areas in Sweden during winter season. The thesis was part of a research project called Wetland Toolkit for Hydrological Ecosystem Services (WetKit Hydro-ES, 802-0083-19) at the Swedish University of Agricultural Sciences (SLU), which aims to optimize the design and placement of wetlands in agricultural areas to achieve optimal nutrient retention and biodiversity while at the same time minimize greenhouse gas emissions. The project is financed by the Swedish Environmental Protection Agency.

1.1 Research questions

The following questions were addressed in this project.

1. Are vegetation and nutrient levels affecting the concentrations of dissolved CH_4 and the CH_4 fluxes from the wetlands?
2. Are vegetation and nutrient levels affecting the abundance of methanotrophs in the wetlands?
3. Is there a correlation between the abundance of methanotrophs and the concentration of dissolved CH_4 in the wetlands?

2. Theory

2.1 Methane production, oxidation and emissions

2.1.1 Methanogenesis

The turnover of organic material in wetlands is an important part of the global biogeochemical carbon cycle, and CH_4 is a major product of the carbon metabolism in anoxic wetland sediments. CH_4 is produced by a certain group of microorganisms called methanogens, which belongs to the domain archaea. This process is called methanogenesis and is the final step in a series of reactions that facilitate anaerobic degradation of organic matter (Whalen 2005; Bastviken 2009; Enrich-Prast et al. 2009). Acetate ($CH_3CO_2^-$), hydrogen gas (H_2) and CO_2 are intermediate products formed in the anaerobic degradation and these are utilized by the methanogens as a source of electrons in either an acetate-dependent (equation 1) or H_2-dependent (equation 2) pathway (Whalen 2005; Bastviken 2009).

Equation 1.

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Equation 2.

$$4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O$$

Methanogenesis mainly occurs in sediments since they generally are more anoxic and have a higher content of organic matter. The temperature, amount of organic matter feeding the community and the concentration of O_2 and alternate electron acceptors such as NO_3^-, Mn_4^+, Fe_3^+, and SO_4^{2-}, are the main factors controlling the CH_4 production (Whalen 2005; Bastviken 2009).

2.1.2 CH_4 oxidation

CH_4 is the most reduced organic carbon compound and can in the presence of oxygen be used as an electron and carbon source by methane oxidising bacteria (methanotrophs) (equation 3).

Equation 3.

$$CH_4 + 2\,O_2 \rightarrow CO_2 + 2\,H_2O$$

Methane oxidation is mainly controlled by CH_4 and O_2 concentrations and occur both in wetland water and in oxic parts of sediments (Sundh et al. 2005; Bastviken 2009). CH_4 emissions from wetlands are generally determined by the balance between CH_4 production and oxidation (Whalen 2005). A compilation of 17 studies on methane oxidation rates in lake water columns by Bastviken (2009) showed that 45-100% of the produced CH_4 was oxidized. Hence, methanotrophs works as a biofilter for CH_4 (Whalen 2005).

2.1.3 Pathways for CH_4 emissions

For the CH_4 that is not oxidized, there are four main emission pathways into the atmosphere, summarized by e.g. Bastviken et al. (2004): I) diffusive flux resulting from a concentration gradient between the sediment, water column and atmosphere, II) ebullitive flux resulting from bubbles emerging from the sediment, III) fluxes resulting from CH_4 storages in anoxic water layers that is released during lake turnover and IV) emissions mediated by emerging plant stems, acting as chimneys for CH_4 produced in the sediment. Measurements of the methane emissions can be done in several different ways and include one or more of the pathways (Bastviken et al. 2002). Wetlands are generally shallow and lack seasonal turnovers, thus the flux associated to these are probably less important. In contrast, ebullitive flux in shallow waters is often rapid and goes directly to the atmosphere, with limited impact from oxidation. Therefore, ebullition is important to include in the emission measurements not to underestimate the methane emissions from wetlands (Bastviken et al. 2004). However, ebullitive emissions are highly variable within and between wetlands and to get measurements that reflect the actual ebullition require repeated measurements with many replicates (Bastviken et al. 2002, 2004).

2.2 Effects of vegetation on emissions and methane oxidation

Vegetation can affect the production, oxidation and emissions of CH_4 in several ways. Plants with emerging stems can act as chimneys for CH_4 that is produced in the sediments, providing a direct way to the atmosphere without bypassing the methanotrophic biofilter (Brix 1993; Oliveira-Junior et al. 2018). An important factor for methane production is the availability of substrate, i.e. decayed organic matter. The greater the plant production is, the greater will the production of organic matter be and this will stimulate the CH_4 production if the environment is anoxic

and lack other electron acceptors (Segers 1998; Carmichael et al. 2014; Grasset et al. 2016; Bodmer et al. 2021). This is linked to the nutrient status of the wetland, since higher nutrient status increase the plant productivity and the amount of organic matter (Grasset et al. 2016). In addition, organic exudates from plant roots can stimulate CH_4 production (Carmichael et al. 2014; Bodmer et al. 2021). Plant roots also release O_2 into sediments which both inhibits CH_4 production and stimulate CH_4 oxidation (van der Nat & Middelburg 1998; Ström et al. 2005; Fritz et al. 2011). However, plant root release of oxygen and exudates might also alter the microbial community and thereby outcompete methanotrophs (Turner et al. 2020).

3. Material and methods

3.1 Wetlands and sampling sites

The sampling was carried out in 34 constructed wetlands in agricultural areas, with a catchment area consisting of more than 50 % arable land or pasture. 13 of the wetlands were located in the county of Halland, that generally have coarse soils with high N losses (Figure 1). 21 wetlands were located in the area of Mälardalen (Figure 1), where the soils are dominated by clay and generally have higher losses of P. 13 of the wetlands in Mälardalen were constructed specifically for P retention and will in this thesis be named *P-wetlands*. P-wetlands consist of a deep basin at the inlet that slows down the water flow and increases sedimentation, followed by a shallow, vegetated part. The 21 remaining wetlands will be named *ponds.* Both the ponds and the P-wetlands were long and narrow, a design that improves the nutrient retention (Kynkäänniemi 2014).

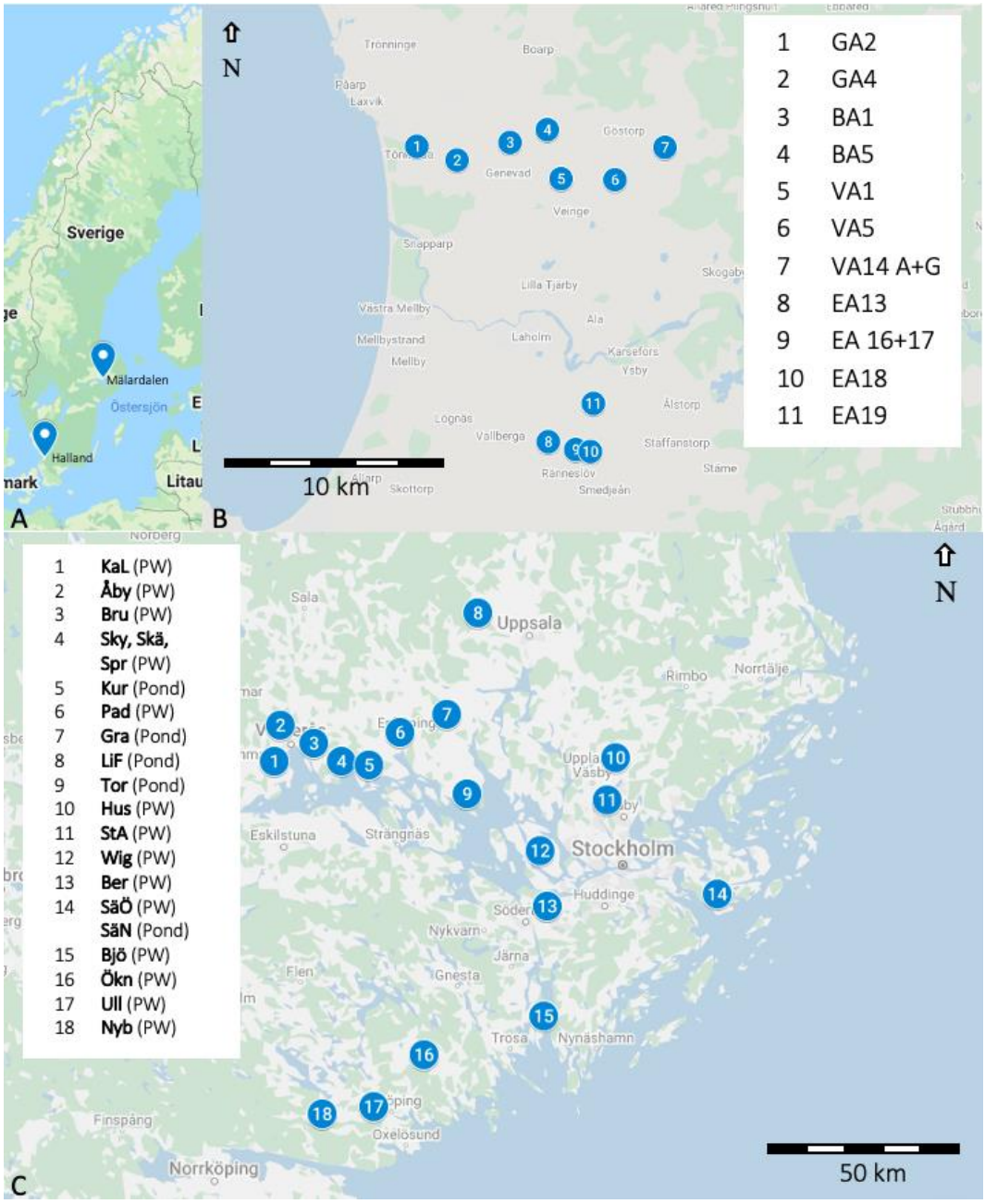

Figure 1. A: The location of Halland and Mälardalen (Kartdata ©2022 Google, INEGI). B: The locations of the Halland wetlands, which were all ponds. The name of each wetland is presented in the legend. VA14 A and VA14 G and EA16 and EA17 are closely located and represented by common numbers on this map (Kartdata ©2022 Google). C: The locations of the wetlands in Mälardalen. The name and wetland type are presented in the legend (PW = P-wetland). Sky, Skä and Spr and SäÖ and SäN are closely located and represented by common numbers on this map. (Kartdata ©2022 GeoBasis-DE/BKG (©2009), Google).

3.2 Sampling points

Sampling was carried out 2022-01-31 - 2022-02-03 in Halland and 2022-02-07 - 2022-02-11 in Mälardalen. At each wetland, samples were taken from one point with open water surface (hereafter referred to as "unvegetated (U) site/sample") and one site with vegetation (hereafter referred to as "vegetated (V) site/sample"). Figure 2 provides a typical example of sampling points in a wetland. It was noted if the vegetation was emergent, floating or submerged. For most wetlands, the vegetated site was close to the edge and the unvegetated site was 2-5 m from the edge. However, at some wetlands the unvegetated samples were taken at an edge were there was no vegetation and the vegetated sample were at some places taken further out in the wetland. In Halland, a stand-up paddleboard was used to reach sampling points too far from the wetland edges to prevent disturbing the sediment. In Mälardalen, most wetlands were covered in a 10-50 cm thick layer of ice. Sampling points far from the edge could therefore be accessed via the ice, and an ice drill was used to access the water. The only wetland that was not ice covered in Mälardalen was Bru, where both sampling points were located at the wetland edges. At Björkhagen and Ökna, the ice was too thin to thread and there was no unvegetated part of the edges. Thus, only the vegetated samples were taken for these wetlands.

Figure 2. A: Example of typical vegetated and unvegetated sampling points in a wetland (SäN, Mälardalen). B: Close-up on a typical vegetated sampling point (SäN, Mälardalen).

3.3 Water characteristics

At each sampling point, water depth (m) was measured with a folding rule. Water temperature (°C), atmospheric pressure (atmospheres), pH, oxidation-reduction

potential (mVpH), dissolved oxygen concentration (% and mg/L) and electric conductivity (uS/cm) were measured with a Hanna HI 9829 Multiparameter (Hanna instruments). Chlorophyll a (µg/L) was measured with a FluoroSense™ handheld fluorometer (Turner designs). Air temperature was measured with the thermometer in the field car, parked closely to the wetlands. The sampling for analysis of TOC, TN and TP in the water was conducted simultaneously. Water samples were taken at the inlet and the outlet of each wetland and sent to the SWEDAC-accredited Geochemical Laboratory at the Swedish University of Agricultural Sciences (SLU) in Uppsala for analysis. For this thesis, the mean value from the inlet and the outlet was used as a proxy for the nutrient status in the whole wetland, thus the TOC, TN and TP values at the vegetated and unvegetated sampling points in each wetland were assumed to be the same.

3.4 Greenhouse gas sampling

3.4.1 Dissolved gas samples

Samples of dissolved CH_4 in the water were taken at all sampling points in all wetlands using a headspace technique (Hope et al. 2004). First, a 60 ml syringe was flushed several times with atmospheric air in the field. 30 ml of atmospheric air from approximately 1 m above the water surface was taken into the syringe. The syringe was then put just under the wetland water surface and 30 ml of water was withdrawn into it. A needle was attached to the syringe and the syringe was vigorously shaken for 60 seconds. Holding the syringe with the needle upright, ca 5 ml of the headspace air from the syringe was discarded trough the needle, and ca 15 ml of headspace air was then injected to a pre-evacuated 12 ml gas-tight glass vial.

3.4.2 Samples for ebullitive and diffusive flux

Samples measuring both the diffusive and ebullitive flux of CH_4 were taken with floating chambers at four wetlands in Halland: GA2, GA4, VA1 and VA5 and at one wetland in Mälardalen: Bru. The chambers were made of circular, shallow plastic buckets with a basis diameter of 31.5 cm and a total volume of 9.56 L. They were covered in aluminium foil to reflect sunlight and minimize heating effects and provided with pool noodles to keep them floating. A closable sampling valve was attached on top of each chamber. Four chambers were placed at the unvegetated sampling point and another four at the vegetated sampling point in each wetland. Where the vegetation was emergent and too high for the chambers to cover it, the chambers were placed in between the vegetation. The chambers were then left for

20-24 hours. After the deployment, a syringe was attached to the sampling valve and flushed several times to mix the air in the chamber. The syringe was then filled with chamber air and the air was injected into a pre-evacuated 12 ml gas-tight glass vial using a needle.

3.4.3 Gas calculations

Both the dissolved gas samples and the samples for gas ebullition were analysed on a Perkin Elmer Clarus 500 gas chromatograph equipped with an electron capture detector at the Department of Soil and Environment at SLU. The concentration of dissolved CH_4 in the glass vials was given in ppm. To convert the concentrations to mg/L, which was the unit used for this thesis, the method by Weiss (1974) and Wiesenburg & Guinasso (1979) was used, which is based on Henry's law and solubility coefficients. The temperature and pressure used for the calculation was measured as described in section 3.3.

The CH_4 fluxes were calculated based on the dissolved CH_4 concentrations according to the method of Bastviken et al. (2004). The diffusive flux from the water surface into the chamber was calculated by equation 4, in which F is the CH_4 flux (moles/m^2/d), k is the piston velocity (m/d), C_w is the aquatic CH_4 concentration (moles/m^3) and C_{fc} is the partial pressure of CH_4 in the chamber (Bastviken et al. 2004).

Equation 4.

$$F = k \times (C_w - C_{fc})$$

However, the diffusive flux is driven by the CH_4 concentration gradient between the chamber and the water and is thus larger in the beginning of the deployment and decreases with time. In order to include this in the calculation, the piston velocity k needed to be solved. This was done by rewriting equation 4 to equation 5, in which P_0 and P_t are the partial pressures of CH_4 at the start and after 24 h, respectively. V is the volume of the chamber (m^3), R is the gas constant (8.314 m^3Pa/K/mol), T is the temperature (K), A is the chamber area (m^2), P_w is the partial pressure of methane in the chamber at equilibrium with C_w (Pa), and K_h is the Henry's Law constant for methane (moles m^3/Pa) (Bastviken et al. 2004).

Equation 5.

$$\frac{(P_t - P_0)V}{RTA} = k(P_w K_h - P_0 K_h)$$

This gives that:

Equation 6.

$$\frac{dP}{dt} = K(P_w - P), \text{ in which } K = k\frac{(K_h RTA)}{V}$$

And the solution for equation 6 is therefore as in equation 7, in which C is a constant determined by setting t = 0.

Equation 7.

$$(P_w - P) = Ce^{(-Kt)}$$

When k was solved, the correct flux into the chambers could be calculated. To further estimate the fractions of diffusive and ebullitive flux from the total flux, the k values for each chamber were transformed to k_{600} values, which allows them to be compared for any gas and temperatures. Chambers receiving ebullition will have high values of k_{600} compared to chambers receiving only diffusive flux. Therefore, the k_{600} value for each chamber was divided by the minimum k_{600} for the same sampling period, and if the ratio >2, it was interpreted as ebullitive flux had occurred. The diffusive flux was then calculated according to equation 1 and subtracted from the total flux, and the remaining fraction was attributed to ebullition (Bastviken et al. 2004).

3.5 Abundance of methanotrophs

3.5.1 Water sampling

At each sampling point in each wetland, an integrated water sample of the water column was taken to analyse the amount of methanotrophic bacteria in the water. At sampling points with a water depth larger than 0.3 m the sample was taken with a Ruttner water sampler with a volume of ca 4 L. At points with a water depth shallower than 0.3 m, the sample were taken with a handheld one litre plastic bailer. Since the aim was to measure the amount of methanotrophs in the water column, care was taken not to touch the bottom sediment with the equipment and contaminate the water with sediment particles. However, the water sight was rarely clear and it was tricky to place the Ruttner water sample precisely above the sediment. Thus, some sediment particles could not be avoided in the samples. When sampled, the water was poured into a 10-litre bucket and mixed with the help of the

bailer. Both the bailer and the bucket were rinsed in the water at the sampling site before sampling. The water was filtered through 0.2 um sterile Sterivex™ filters (EMD Millipore corp., Billercia MA, USA) using a 60 ml syringe. The volume of water pushed through the filter ranged from 25 to 600 ml, depending on how fast the filter clogged. At the first three wetlands (GA2, GA4 and EA13), the filtering was done directly at the site. However, this proved to take too long time in the field and the remaining samples were therefore poured into plastic water bottles that were kept dark at + 4°C for 1-7 days, before filtered in lab. The Sterivex filters were stored at -18°C for 2-14 days before DNA extraction.

3.5.2 DNA extraction

Preparation of filters

The plastic cylinder surrounding the Sterivex filters was cracked open with a pair of tongs. The filters were removed from the cylinder with the help of a disposable razor blade and a pair of tweezers. When removing the filters, they were also cut in half or thirds. All tools and the working area were carefully wiped with 70%-ethanol before and after each sample. The filters were put in sterile 5 ml plastic tubes afterwards (Sarstedt AG & Co. KG, Nümbrecht, Germany) and the DNA extraction continued directly.

Extraction

For the DNA extraction, the DNeasy® PowerSoil® Pro Kit (Qiagen) was used. The protocol for the extraction was followed with exception for step 1-3. Instead, the microbeads from the PowerBead Pro tubes provided in the DNeasy® PowerSoil® Pro Kit was poured into the 5 ml plastic tubes containing the filters, along with 800 ul of Solution CD1. The tubes were vortexed for 5 min at a speed of 2.60 m/s in a Fisherbrand™ Bead Mill 24 Homogenizer. At this point, step 4 in the protocol was reached. The supernatant in the tubes were transferred to clean 2 ml-Microcentrifuge tubes according to the instructions and no further exceptions from the protocol were made. 6 µl of extracted DNA from each sample were diluted in 54 µl DNase/RNase free water and stored in -18°C for 14-28 days before quantitative Polymerase Chain Reaction (qPCR) analysis.

3.5.3 qPCR Analysis

To determine the abundance of methanotrophs in each sample, qPCR with a pair of primers targeting the mxaF-gene was carried out. MxaF was choosen as it covers most of all known methanotrophs (Lau et al. 2013). The sequence for mxaF forward was TGGAACGAGACCATGCGTC, and for mxaF_reverse CATGCAGATGTGGTTGATGC (McDonald & Murrell 1997). A 9-fold dilution

series of known copies of linearized plasmid (pCR4-TOPO, Invitrogen) containing a single copy of mxaF gene (GenBank accession number LT962688.1) was used as a standard curve. To save time all three different qPCR machines at the lab were used: CFX96™ and Connect™ Real-Time system (BIO-RAD) and ARKTIK Thermal Cycler (Thermo scientific). The 20 µl reactions contained 10 µl of 2×Master mix (TATAA SYBR®GrandMaster Mix 625 rxn), 1 µl each of 10 pmol forward and reverse primer and 8 µl of DNA template. For each sample as well as for the linearized plasmid standards, reactions were carried out in technical triplicates. Three step cycling protocols were followed with an initial 7 min denaturation at 95°C followed by 39 cycles of denaturation at 95°C for 40 s, annealing at 60°C for 1 min, and extension at 72°C for 40 s. Fluorescence data was acquired at 72°C after completing each consecutive cycle. After 39 cycles, melting curve analysis was performed by raising the temperature from 55 to 95°C and reading the fluorescence 10 s after every 0.5°C increase in temperature. The qPCR efficiency and dissociation/melting temperature (T_m) were around 81% and 87.5°C, respectively. The coefficient of determination (R_2) was 0.97.

3.6 Statistical analysis

For all statistical analyses in this thesis, the confidence interval was set to 95%, which gives a significance threshold of 0.05. Hence, if a test gave a probability lower than 0.05 for the null hypothesis, it was regarded as significant.

3.6.1 T-tests

T-tests were performed in Microsoft® Excel to determine if there was a statistical difference between the vegetated and unvegetated sampling points regarding the abundance of methanotrophs, the dissolved CH_4 and the total CH_4 fluxes. The t-tests were two sided of the type “two sample equal variance”. The same test was performed in Minitab®19 to reveal if the wetland type (ponds and P -wetland) or the location (Halland and Mälardalen) had an effect on the abundance of methanotrophs and the dissolved CH_4.

3.6.2 Correlation tests

Several correlation tests between different dependent and independent variables were performed (Table 1). All tests were performed in Minitab®19, however the scatter plots visualising the significant correlations in the results were made in Microsoft® Excel. Before running the correlation tests, a Ryan-Joiner normality test was done on the dependent variables to see if the data sets were normally distributed. None of the dependent variables had normally distributed data and thus

the Spearman correlation test was used since it does not require normally distributed data.

Table 1. Summary of the variables tested with the Spearman correlation test. The tests in which methanotrophs and dissolved CH_4 were dependent variables were performed to one independent variable at a time.

Independent variable (x)	Dependent variable (y)
Methanotroph abundance (copies/mL sample)	Total CH_4 fluxes (mg/m^2/day)
O_2 (mg/L), pH, Chlorophyll (µg/L), Total Organic Carbon (TOC, mg/L), Total Nitrogen (TN, mg/L), Total Phosphorous (TP, µg/L), C/N-, C/P- and N/P-values and concentration of dissolved CH_4 (µg/L).	Methanotroph abundance (copies/mL sample)
O_2 (mg/L), pH, Chlorophyll, TOC, TN, TP and C/N-, C/P- and N/P-values and methanotroph abundance (copies/mL sample).	Concentration of dissolved CH_4 (mg/L)

3.6.3 ANOVA -test

To see if the ebullitive fluxes were significantly different between the 5 wetlands and sampling points where it was measured, a One-way ANOVA-test was performed in Minitab®19. The test assumed equal variances and Tukey's comparison procedure was used.

4. Results

4.1 Abundance of methanotrophs

There was a large variation in the abundance of methanotrophs among the wetlands (mean: 712 copies/mL ± 1123), ranging from 57 copies/mL water sample in GA2 U to 8178 copies/mL water sample in Hus U (Figure 3; Table 2). Most wetlands had abundance below 1000 copies/mL except Hus, KaL, Nyb, Åby and VA1. There was no significant difference in the abundance of methanotrophs between all vegetated (mean: 682 copies/mL ± 788) and all unvegetated (mean: 745 copies/mL ± 1409) sampling points (Table 2). However, in GA2 the abundance at the vegetated point was 7 times higher than in the unvegetated point and in Bru, Wig, Kur, EA18 and EA19 it was 1.5 – 3 times higher. Conversely, the abundance was 1.5 – 4 times higher at the unvegetated points in Hus, Pad, Sky, EA13, GA4 and VA5 (Figure 3).

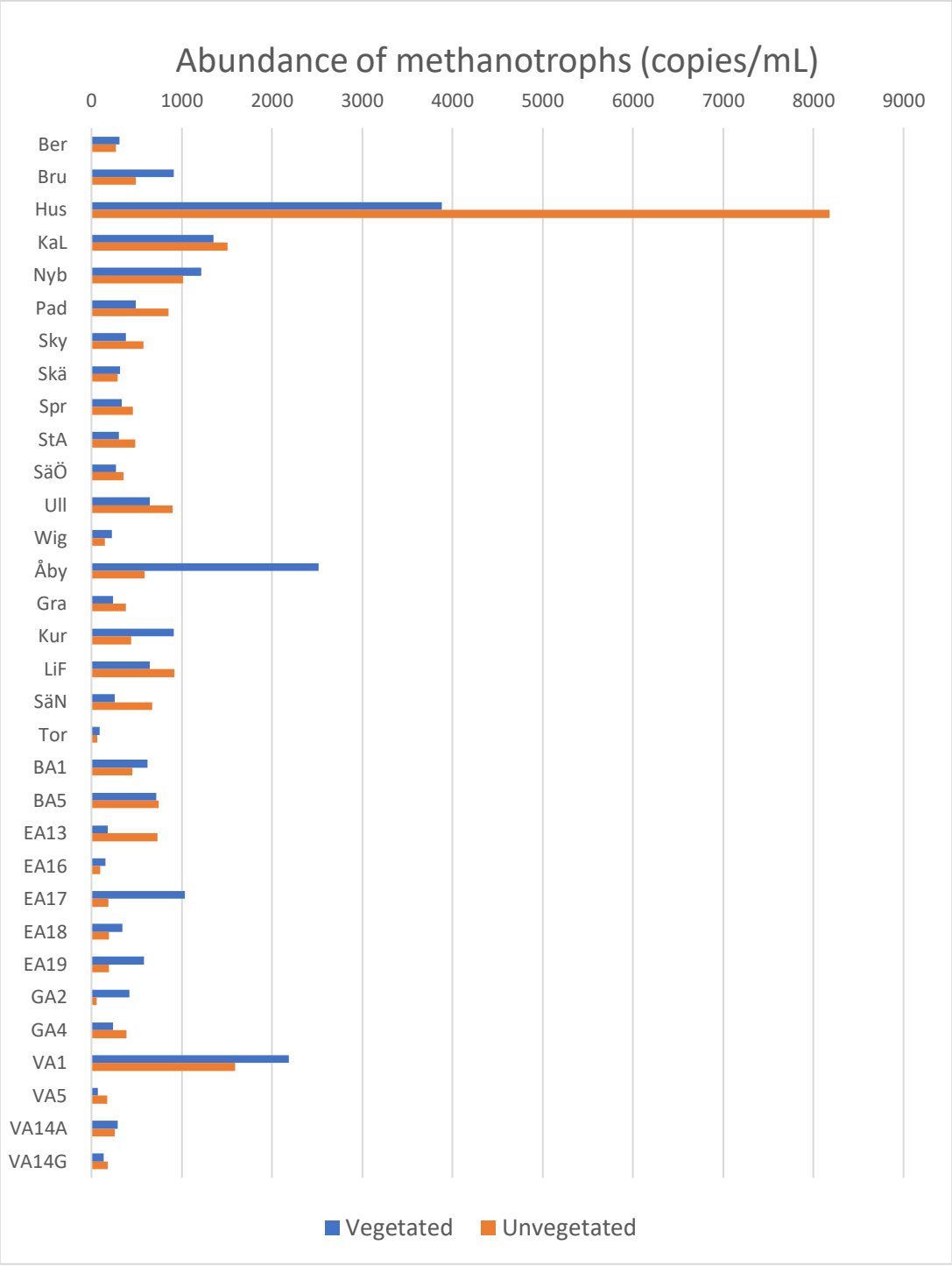

Figure 3. The abundance of methanotrophs at the vegetated and unvegetated site for each wetland. Ber – Ull = P-wetlands in Mälardalen; Wig – Tor = ponds in Mälardalen; BA1 – VA14G = ponds in Halland.

Table 2. The concentrations of dissolved CH_4, the abundance of methanotrophs, type of vegetation at the sampling point (E = emergent, F = floating, S = submerged, N = no veg), dissolved O_2, pH, Chla, nutrients and nutrient ratios. Ber – Ökn = P-wetlands in Mälardalen; Wig – Tor = ponds in Mälardalen; BA1 – VA14G = ponds in Halland.

Site	CH4 (µg/L)	Methanotrophs (copies/mL)	Vegetation	O_2 (mg/L)	pH	Chla (µg/L)	TOC (mg/L)	TN (mg/L)	TP (µg/L)	C/N	C/P	N/P
Ber V	16	308	E	12	8.2	6	14	2	137	6	101	17
Ber U	36	271	N	12	8.1	6	14	2	137	6	101	17
Bjö V	13	382	E	10	6.9	10	15	2	200	6	74	12
Bru V	0	913	E	13	7.9	8	16	3	246	5	66	12
Bru U	2	493	N	13	7.9	5	16	3	246	5	66	12
Hus V	4	3882	E	9	6.6	36	19	3	689	6	27	4
Hus U	40	8178	N	13	5.8	4	19	3	689	6	27	4
KaL V	3	1350	E	13	7.8	5	13	3	266	4	48	11
KaL U	4	1507	N	13	7.8	6	13	3	266	4	48	11
Nyb V	16	1216	E	11	7.3	7	14	2	272	7	53	7
Nyb U	49	1019	N	12	7.5	10	14	2	272	7	53	7
Pad V	2	488	E	12	8.1	32	7	3	41	2	162	68
Pad U	24	851	N	12	8.5	7	7	3	41	2	162	68
Sky V	7	381	E	11	8.0	8	13	1	213	9	63	7
Sky U	12	574	N	11	8.0	6	13	1	213	9	63	7
Skä V	3	317	F	10	7.9	5	17	4	326	4	52	12
Skä U	1	288	N	12	7.9	3	17	4	326	4	52	12
Spr V	12	335	E	11	8.0	7	21	4	389	5	53	11
Spr U	89	456	N	11	8.0	8	21	4	389	5	53	11
StA V	12	304	E	10	7.6	6	17	2	211	8	78	10
StA U	16	483	N	10	7.6	5	17	2	211	8	78	10
SäÖ V	3	269	E	8	6.7	22	12	2	80	5	150	27
SäÖ U	1	356	N	10	6.6	24	12	2	80	5	150	27

Site	CH4 (µg/L)	Methanotrophs (copies/mL)	Vegetation	O_2 (mg/L)	pH	Chla (µg/L)	TOC (mg/L)	TN (mg/L)	TP (µg/L)	C/N	C/P	N/P
Ull V	45	649	E	11	7.3	19	10	3	136	3	72	21
Ull U	9	903	N	12	7.2	6	10	3	136	3	72	21
Ökn V	9	488	S	12	7.6	7	17	3	276	6	60	9
Wig V	5	229	E	12	8.2	7	13	3	134	5	96	20
Wig U	4	146	N	13	8.2	4	13	3	134	5	96	20
Åby V	5	2518	E	12	7.8	7	18	2	82	11	223	20
Åby U	3	592	N	12	8.0	8	18	2	82	11	223	20
Gra V	15	238	E	11	7.7	27	89	13	478	7	186	28
Gra U	6	384	N	15	7.4	3	89	13	478	7	186	28
Kur V	8	911	E	11	7.5	4	11	4	142	3	80	25
Kur U	7	440	N	15	7.4	3	11	4	142	3	80	25
LiF V	65	647	E	7	8.1	23	11	2	68	7	160	23
LiF U	49	919	N	8	7.9	37	11	2	68	7	160	23
SäN V	23	260	E	5	6.7	21	613	7	1465	92	418	5
SäN U	104	672	N	8	6.7	18	613	7	1465	92	418	5
Tor V	10	92	E	8	7.6	7	9	3	98	3	89	31
Tor U	4	63	N	14	7.3	3	9	3	98	3	89	31
BA1 V	1	619	E + S	10	8.0	20	10	10	159	1	61	61
BA1 U	1	450	N	11	7.9	7	10	10	159	1	61	61
BA5 V	11	718	E	11	7.4	5	32	6	268	5	118	23
BA5 U	11	742	N	70	7.1	6	32	6	268	5	118	23
EA13 V	4	184	E	11	7.5	12	8	17	370	0	21	45
EA13 U	3	729	N	13	7.5	4	8	17	370	0	21	45
EA16 V	19	156	E	9	7.3	7	6	13	15	0	367	821
EA16 U	4	97	N	11	7.0	4	6	13	15	0	367	821
EA17 V	7	1034	E	11	7.0	5	6	13	13	0	421	959
EA17 U	6	188	F	12	6.8	5	6	13	13	0	421	959
EA18 V	67	342	E	10	6.7	13	6	13	11	0	559	1171

EA18 U	23	192	N	11	6.7	5	6	13	11	0	559	1171
Site	**CH4 (µg/L)**	**Methanotrophs (copies/mL)**	**Vegetation**	**O_2 (mg/L)**	**pH**	**Chla (µg/L)**	**TOC (mg/L)**	**TN (mg/L)**	**TP (µg/L)**	**C/N**	**C/P**	**N/P**
EA19 V	5	584	S	9	6.8	3	4	11	56	0	67	203
EA19 U	1	191	N	13	7.7	14	4	11	56	0	67	203
GA2 V	2	418	E	11	6.7	43	6	11	55	1	107	203
GA2 U	5	57	N	12	7.0	7	6	11	55	1	107	203
GA4 V	4	242	E	14	8.2	18	8	6	162	1	48	38
GA4 U	5	389	N	23	7.6	5	8	6	162	1	48	38
VA1 V	85	2189	E	1	7.2	6	9	11	193	1	46	56
VA1 U	133	1594	N	13	7.4	3	9	11	193	1	46	56
VA5 V	9	71	F	10	6.6	11	4	12	8	0	512	1452
VA5 U	5	176	N	10	6.8	10	4	12	8	0	512	1452
VA14A V	6	256	E	11	7.4	11	3	9	13	0	243	674
VA14A U	2	294	N	12	6.9	18	3	9	13	0	243	674
VA14G V	2	138	E	11	6.9	13	27	5	216	6	126	21
VA14G U	2	178	N	16	7.3	5	27	5	216	6	126	21

The abundance of methanotrophs was weakly positive and significantly correlated to TOC ($r = 0.257$; $p = 0.037$), TP ($r = 0.331$; $p = 0.007$) and TN ($r = 0.321$; $p = 0.009$) (Figure 4 A, C and D). However, both Hus U and SäN had much higher abundance of methanotrophs and concentrations of TOC and TP, respectively, than the others and could presumably strongly affect the correlations. Nevertheless, when SäN and Hus U were excluded, there was still a correlation between the abundance of methanotrophs and TOC (Figure 4 B) but close to not being significant ($r = 0,249$; $p = 0,049$). For TP, the correlation did not change much ($r = 0,317$; $p = 0,011$). For TN, no wetland was apparently deviating, so only Hus U was excluded and the correlation became significantly negative instead ($r = -0,318$; $p = 0,010$). There was no significant correlation between the abundance of methanotrophs and the concentrations of dissolved CH_4.

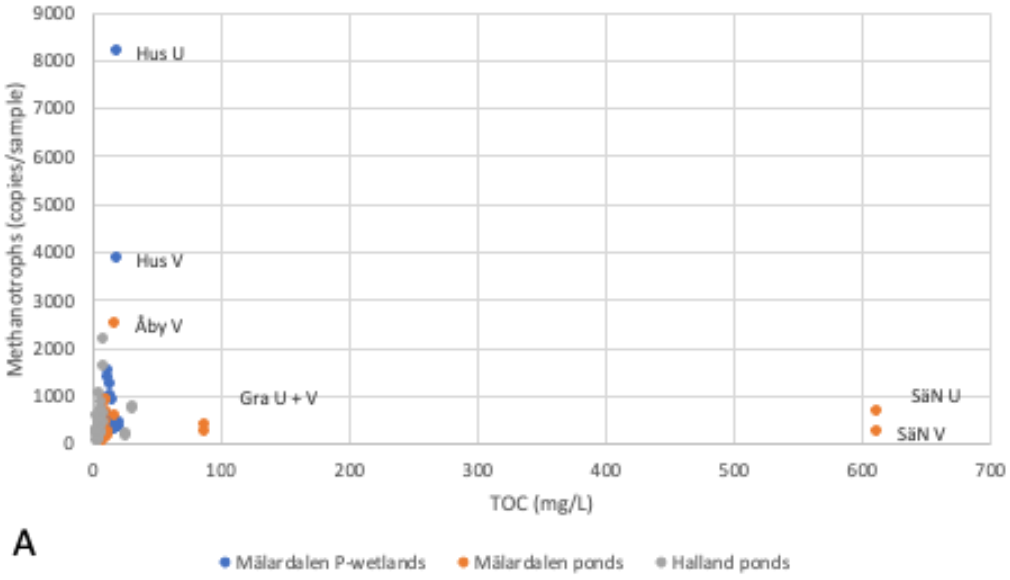

Figure 4 A. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of TOC (r = 0.257; p = 0.037). The same graph, but with Hus U and SäN excluded and with adjusted axes is shown in Figure 4 B to give a better picture of the cluster of data points in the lower left corner.

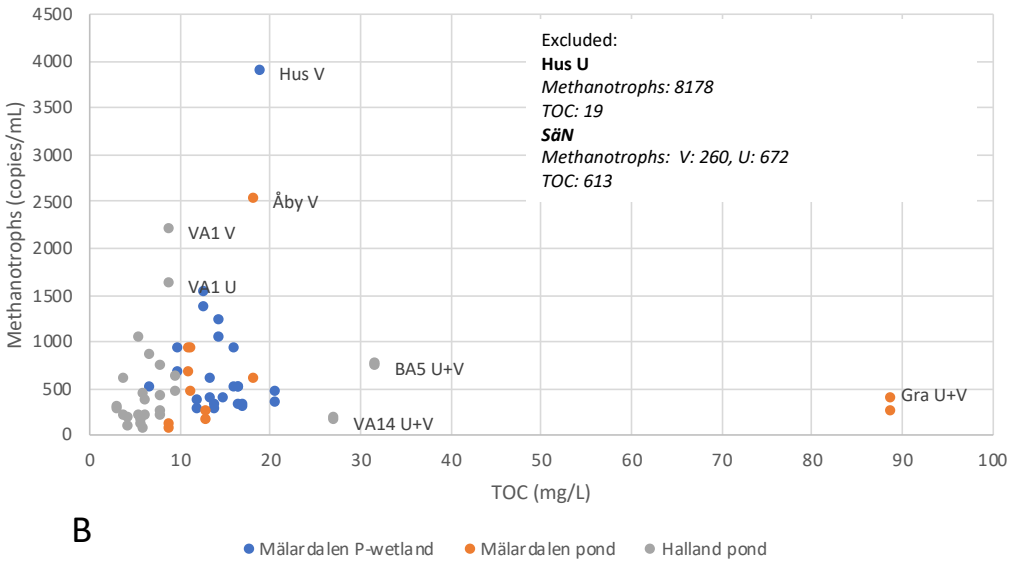

Figure 4 B. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of TOC with Hus U and SäN excluded (r = 0,249; p = 0,049).

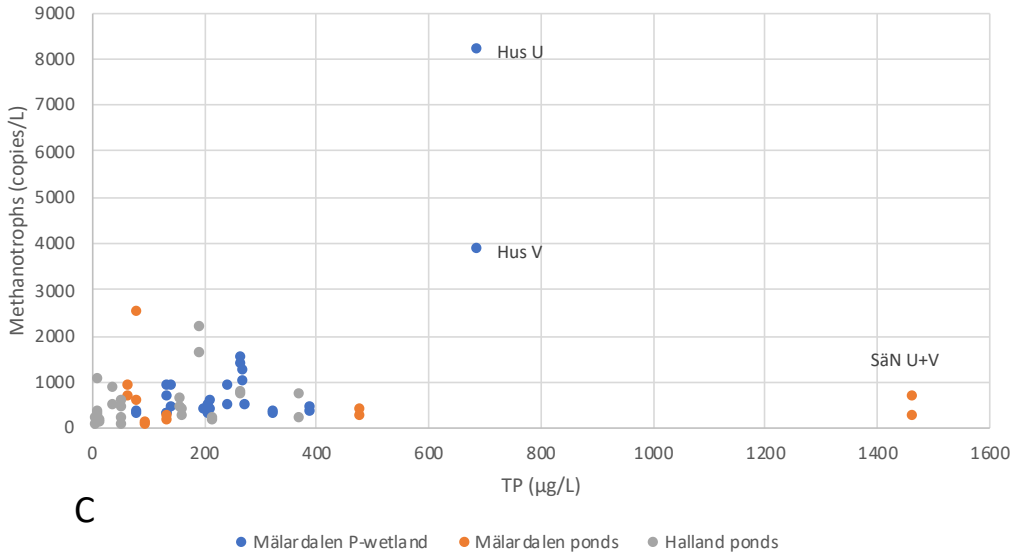

Figure 4 C. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of TP ($r = 0.331$; $p = 0.007$).

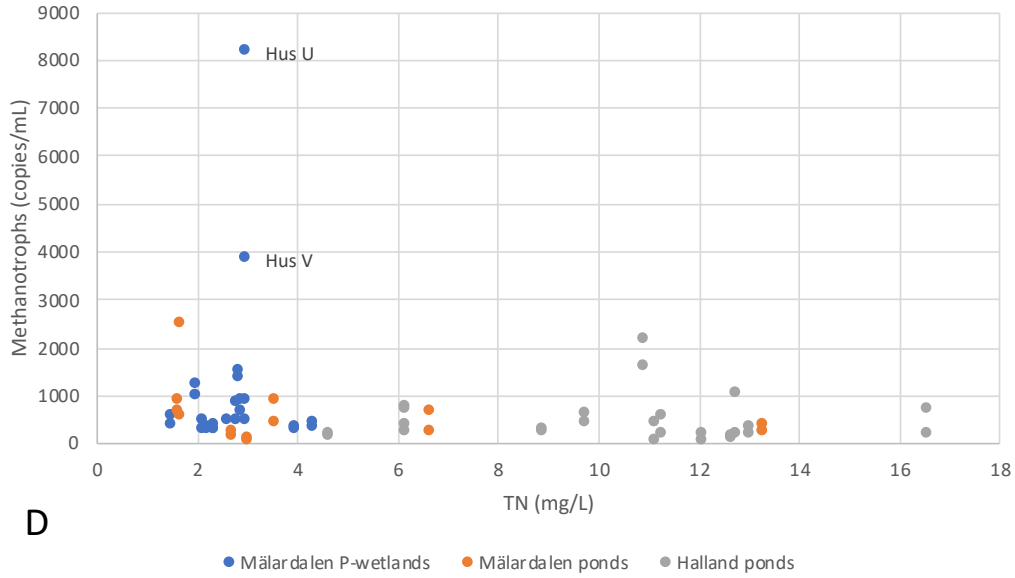

Figure 4 D. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of TN ($r = 0.321$; $p = 0.009$).

There was a weak positive correlation to the C/N-value (r = 0.305; p = 0.013), which means that the more C in relation to N in the water, the higher was the abundance of methanotrophs (Figure 5 A). The correlation was similar when Hus U and SäN were excluded (r = 0.304; p = 0.015) (Figure 5 B). The correlations to the C/P and N/P values were significant and weakly negative (r = -0.387; p = 0.001 and r = -0.383; p = 0.002, respectively) (Figure 5 C and D), which means that the more P in relation to both C and N there was in the water, the higher was the abundance of methanotrophs. When Hus U and SäN were excluded, the results were almost the same (r = -0.362; p = 0.003 for C/P and r = -0.354; p = 0.005 for N/P).

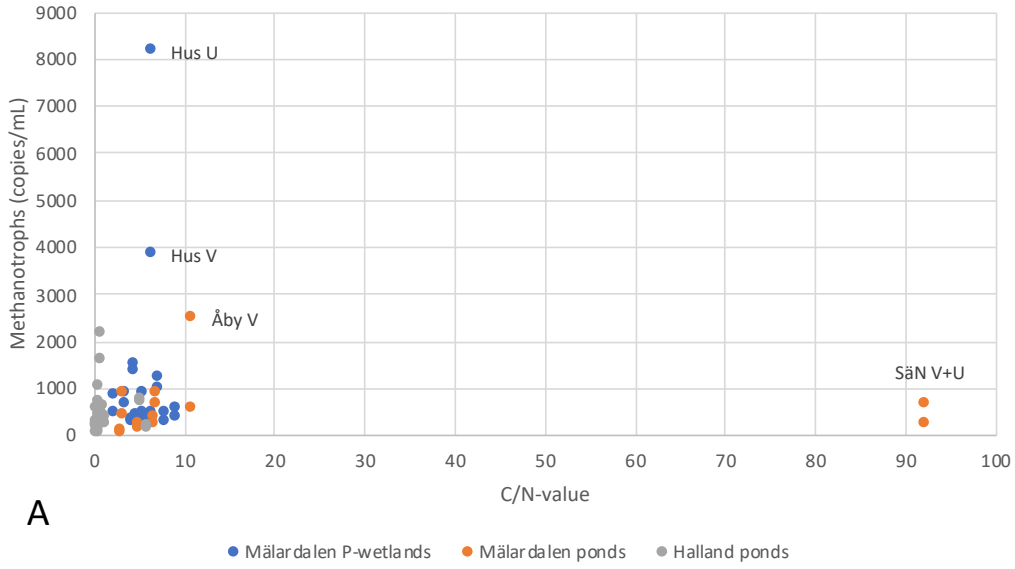

Figure 5 A. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of C/N-value (r = 0.305; p = 0.013). The same graph, but with Hus U and SäN excluded and with adjusted axes is shown in Figure 4 B to give a better picture of the cluster of data points in the lower left corner.

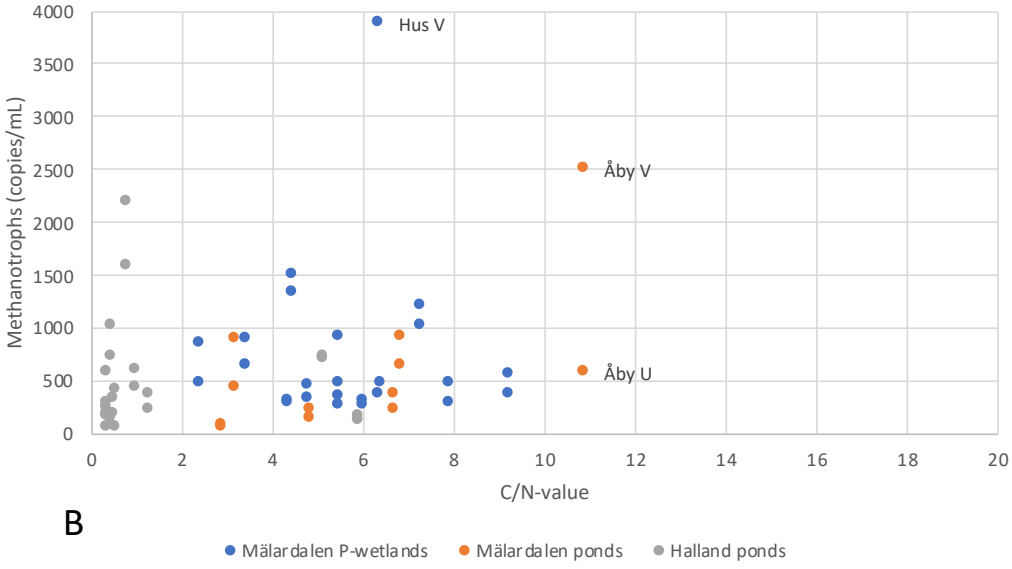

Figure 5 B. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of C/N-value with Hus U and SäN excluded (r = 0.304; p = 0.015).

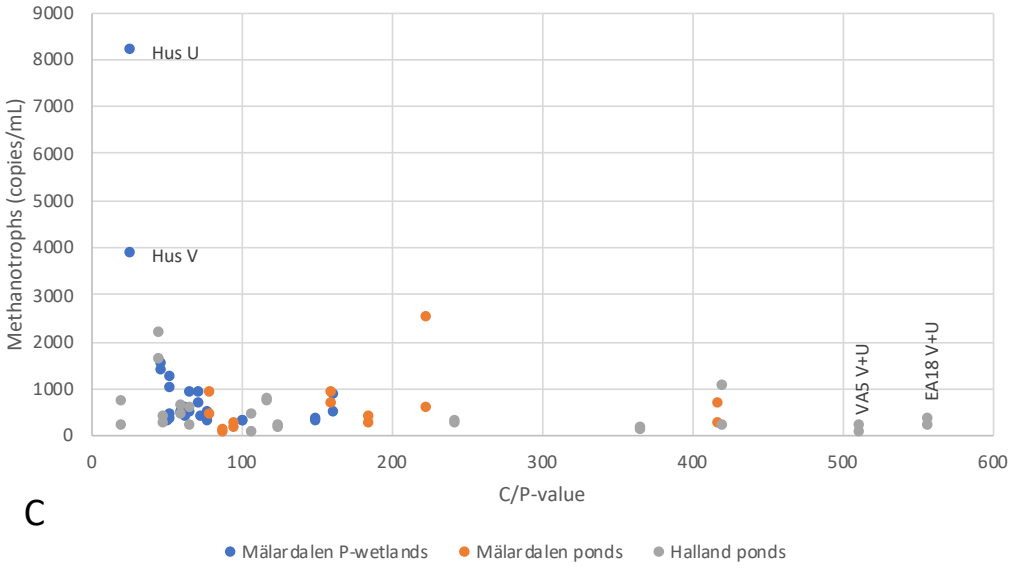

Figure 5 C. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of C/P-value (r = -0.387; p = 0.001).

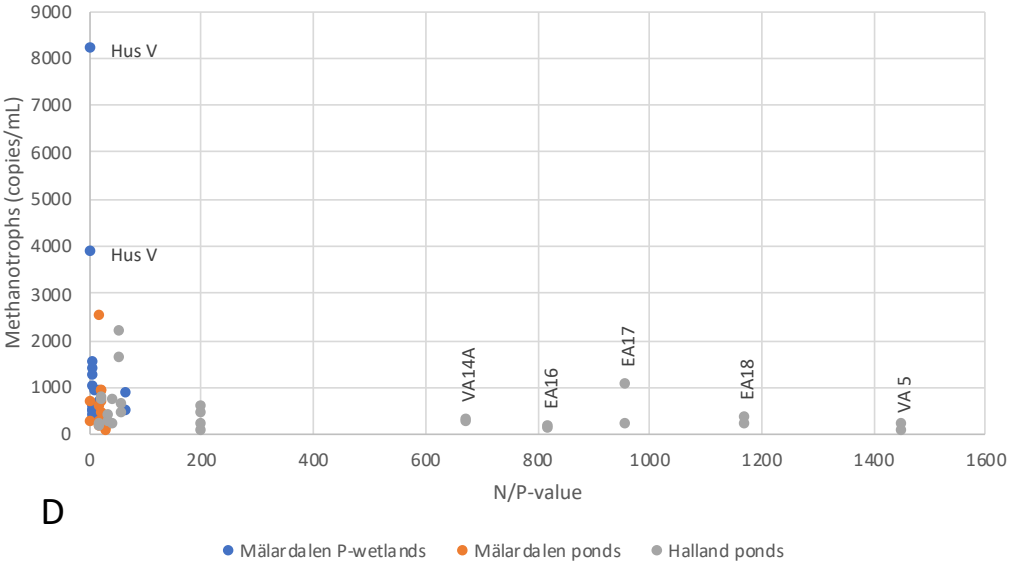

Figure 5 D. The abundance of methanotrophs (copies/mL water sample) as the dependent variable of N/P-value (r = -0.383; p = 0.002).

Hus U and V, that had almost eight and four times higher abundance of methanotrophs than most of the other sites, also had the second highest P concentration in the water among all wetlands, 689 µg/L and the proportion of P compared C and N was also high (Figures 4 C, D and E; Table 2). In addition, pH was 1-2 units lower in Hus than in the other wetlands (Table 2). For the other wetlands with high abundance of methanotrophs (KaL, Nyb, Åby V and VA1) there was no clear differences in pH, Chla or nutrient status compared to the other wetlands (Table 2). SäN, that had much higher concentrations of TOC and TP than the other wetlands, 613 and 1465 µg/L respectively, did not have a particularly high abundance of methanotrophs: V = 260 and U = 272 copies/mL (Figure 4 C; Table 2).

4.2 Dissolved CH_4 concentrations

There was a large variation in the concentration of dissolved CH_4 (mean 17.6 µg/L ± 26.7), ranging from 0.59 µg/L in BA1 U to 133 µg/L in VA1 U (Figure 6; Table 2). The concentrations in VA1, SäN, Spr, EA18, LiF, Nyb, Ull, Hus and Ber were more than twice as high as the mean value for all wetlands (Figure 6; Table 2). VA1 also had high abundance of methanotrophs (V = 2189 copies/mL, U = 1594 copies/mL) (Table 2). There was no significant difference between the vegetated (mean 15.2 µg/L ± 20.3) and unvegetated (mean 20.5 µg/L ± 32.4) sampling points according to the t-test. However, in Pad, Hus, Spr and SäN the unvegetated points had around 11 (26 µg/L), 9 (40 µg/L), 7 (89 µg/L) and 5 (104 µg/L) times higher concentrations respectively, than the vegetated points. For all these wetlands, the

abundance of methanotrophs were also higher at the unvegetated point. In Ber, Nyb, Sky and VA1 the differences were not as large but still 1.5 – 3 times higher (Figure 5). Conversely, the concentrations in Skä, Ull, Gra, Tor, EA18, EA19 and VA14A were 2 – 5 times higher at the unvegetated points (Figure 5).

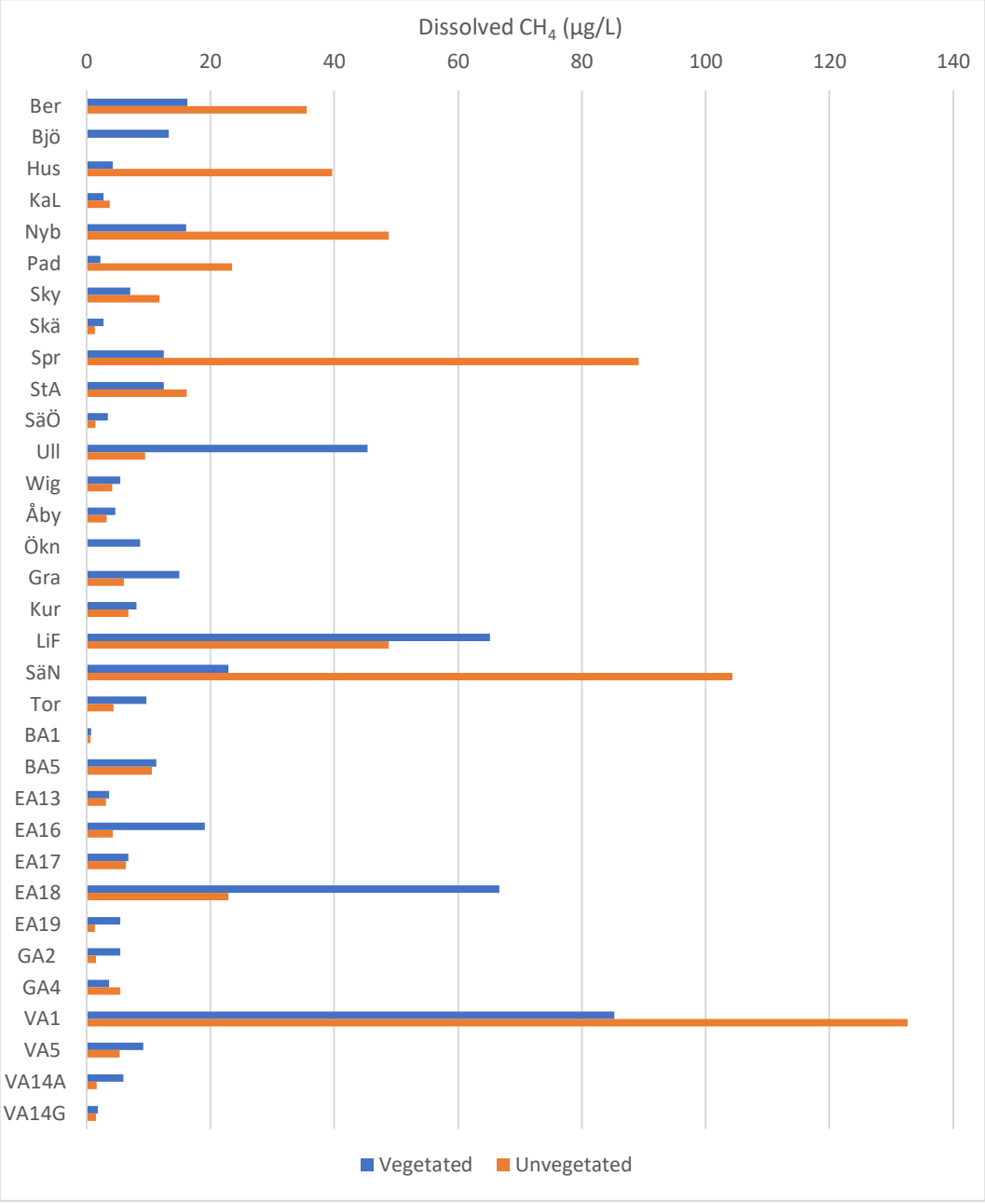

Figure 6. Concentrations of dissolved CH_4 (µg/L) among the wetlands. Ber – Ull = P-wetlands in Mälardalen; Wig – Tor = ponds in Mälardalen; BA1 – VA14G = ponds in Halland.

The concentration of dissolved CH_4 was weakly positive and significantly correlated to the abundance of methanotrophs (r = 0.268; p = 0.031) (Figure 7 A). However, if Hus U (that had much higher abundance of methanotrophs than the other sampling points and could be regarded as an outlier) was excluded, the correlation was not significant (p = 0.057). The correlation between concentration of dissolved CH_4 and C/N-value was also significant and weakly positive (r = 0.264 CH_4, p = 0.032) (Figure 7 B), i.e. the more C in relation to N (higher C/N-value), the higher was the CH_4 concentration. However, when SäN (that had much higher C/N-value, 92.3, than the other sampling points and could be regarded as an outlier) was excluded, the relationship was not significant (p = 0.097). There was no significant correlation between the dissolved CH_4 and the O_2-concentration, pH, chlorophyll, TOC, TN, TP, C/P- and N/P-values.

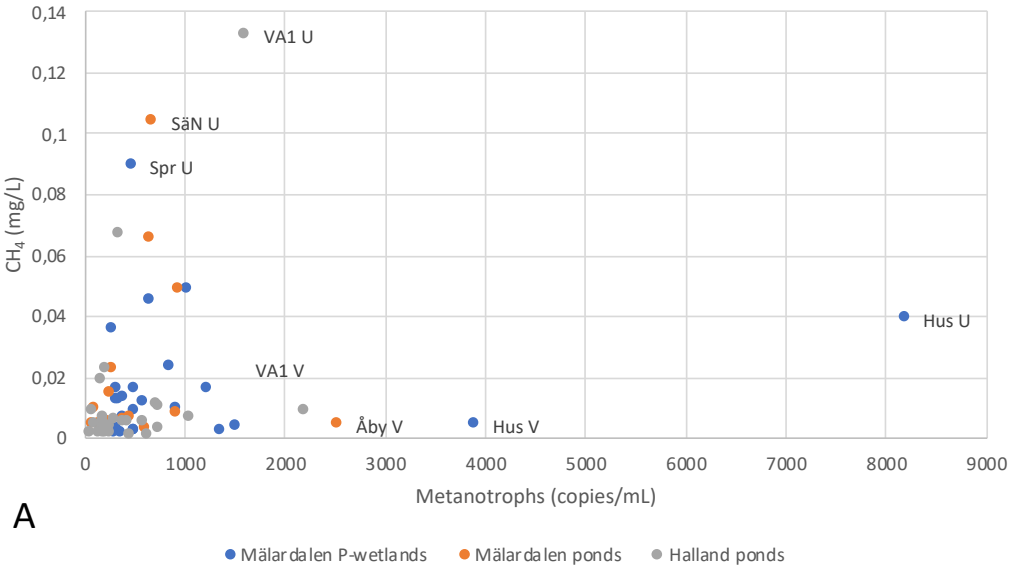

Figure 7 A. Correlation between dissolved CH_4 (mg/L) and the abundance of methanotrophs (copies/mL) (r = 0.268; p = 0.031).

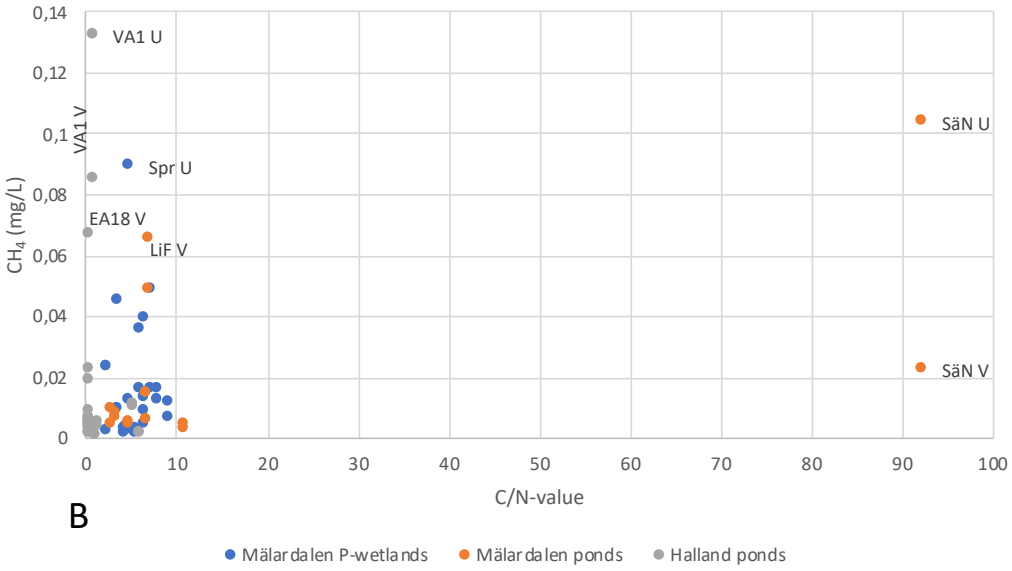

Figure 7 B. Correlation between dissolved CH_4 (mg/L) and the C/N-value (r = 0.264, p = 0.032).

4.3 CH_4 fluxes

There was a large variation in the mean total flux from the wetlands, ranging from 1.02 mg/m^2/d in Bru U to 112 mg/m^2/d in VA1 U (Figure 8; Figure 9; Table 3). The mean total fluxes were significantly higher in VA1 (V = 88 ± 49, U = 112 ± 63 mg/m^2/d) and VA5 (V = 50.3 ± 17.1, U = 40.9 ± 18.3 mg/m^2/d) compared to Bru (U = 1.0 ± 0.9 mg/m^2/d), GA2 (V = 2.1 ± 1.5, U = 2.6 ± 1.2 mg/m^2/d) and GA4 (V = 1.4 ± 1.9, U = 8.1 ± 10.0 mg/m^2/d) (Table 3), but there were no significant differences between the wetlands within VA1 + VA5 and Bru, GA2 and GA4.

There was a large variation in total flux between the chambers at each sampling point, apparent by the large standard deviations presented above and visualised by the red crosses in Figure 9. At all sampling points, the ebullitive flux contributed a major portion of the total flux (Figure 8; Table 3), on average 94.9 % ± 10.4 %. There was no significant difference between the CH_4 fluxes from the vegetated and unvegetated sampling points.

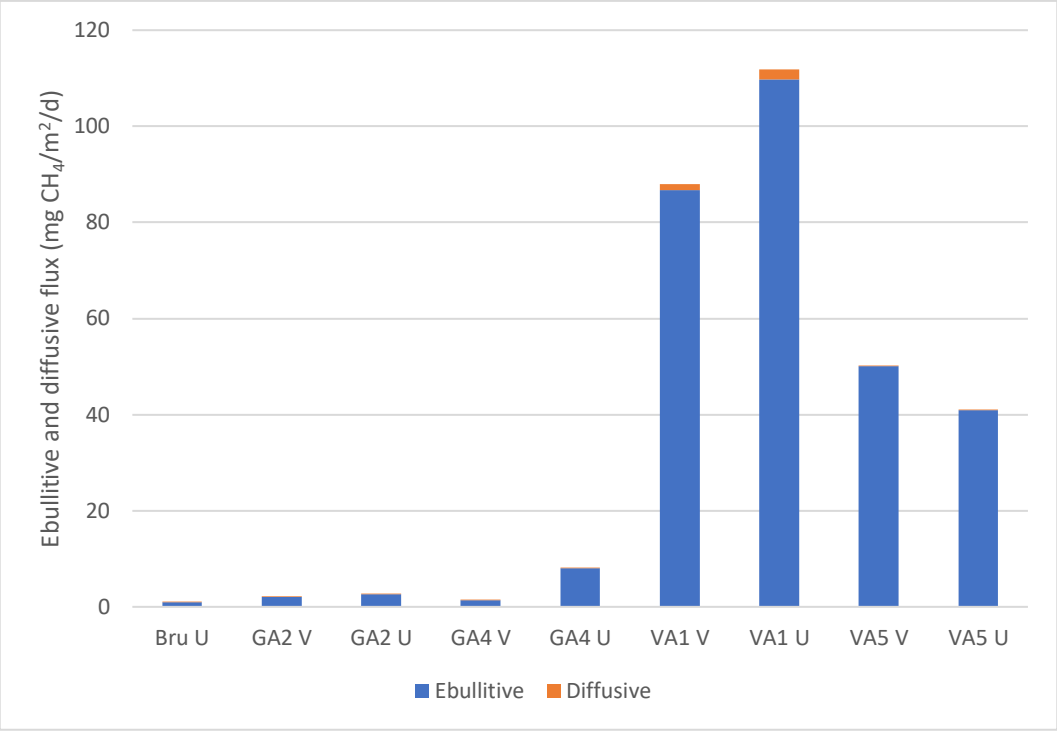

Figure 8. Mean ebullitive and diffusive flux (mg CH_4/m^2/day) from each sampling point.

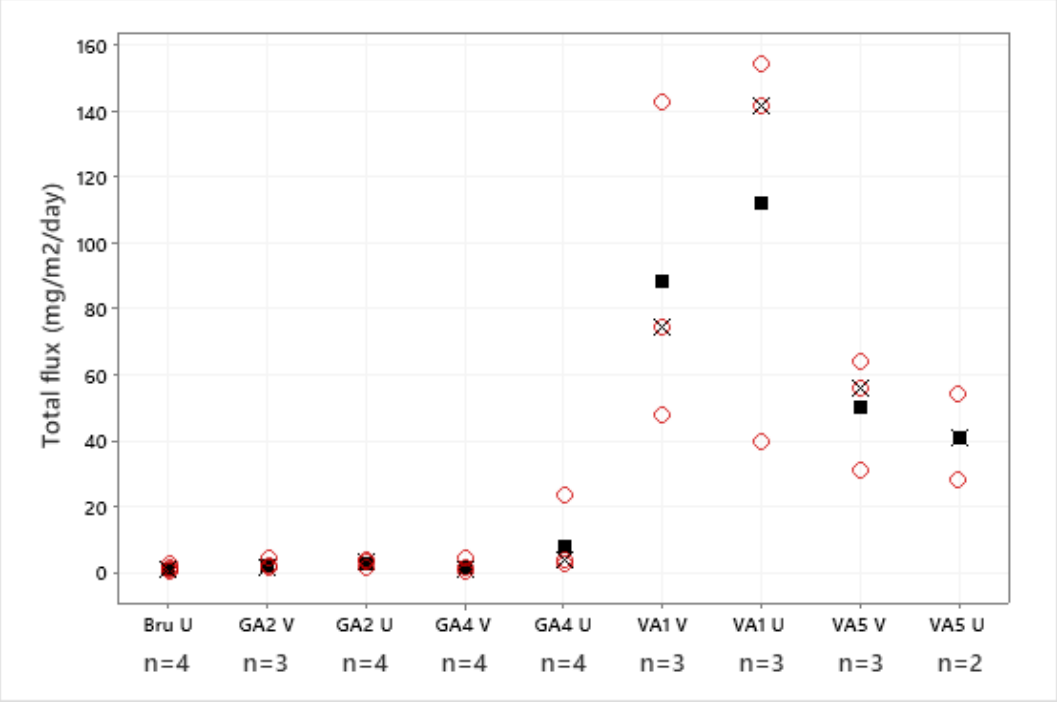

Figure 9. The total flux from each chamber at each sampling point (red circles), mean total flux (black squares) and median total flux (crosses). The number of chambers at each point are presented below the wetland names.

Table 3. Range, median and mean for total flux (mg/m^2/d), mean ebullitive and diffusive flux (mg/m^2/d) and dissolved O_2, pH, Chl-a, nutrients and nutrient ratios for the 9 sampling points where floating chambers were deployed.

	Bru U	GA2 V	GA2 U	GA4 V	GA4 U	VA1 V	VA1 U	VA5 V	VA5 U
Range total flux	0.34 - 2.3	0.97 - 3.8	0.97 - 3.7	0.036 - 4.1	2.1 - 23.0	47 - 142	40.0 - 154	31 - 64	28 - 54
Mean total flux	1.0	2.1	2.6	1.4	8.1	88.0	112	50.3	40.9
Median total flux	0.7	1.5	3.0	0.8	3.6	74.2	142	56.0	40.9
Mean ebullitive flux	1.0	2.0	2.6	1.4	8.0	86.7	110	50.1	40.9
Mean diffusive flux	0.0	0.1	0.0	0.0	0.1	1.3	2.0	0.1	0.1
O2 (mg/L)	13.4	11.1	11.9	13.5	22.7	12.7	1.33	9.6	10.1
pH	7.9	6.7	7.0	8.2	7.6	7.21	7.39	6.6	7.3
Chl-a	5	43	7	18	7.6	6	3	11.0	10
TN (mg/L)	2.9	11.1	11.1	6.1	6.14	10.9	10.9	12.1	12.1
TOC (mg/L)	16.2	5.9	5.9	7.8	7.8	8.8	8.8	4.3	4.3
TP (µg/L)	246	54.6	54.6	162	162	193	193	8.3	8.3
C/N	5.5	0.5	0.5	1.3	1.3	0.8	0.8	0.4	0.4
C/P	65.8	107	107	48.1	48.1	45.6	45.6	512	512
N/P	12.0	203	203	37.9	37.9	56.5	56.5	1452	1452

4.4 No differences between regions and wetland types

There were no significant differences between the P-wetlands and ponds regarding the abundance of methanotrophs ($p = 0.143$) or the concentrations of dissolved CH_4 ($p = 0.518$). The same was true when only the P-wetlands and ponds in Mälardalen were compared. Similarly, there was no difference between the regions, Halland and Mälardalen, neither for the abundance of methanotrophs ($p = 0.300$) nor for the concentrations of dissolved CH_4 ($p = 0.761$).

5. Discussion

5.1 The abundance of methanotrophs

The abundance of methanotrophs ranged from 10^1-10^3 copies/mL. This is in the same range as numbers previously reported in a study by Samad and Bertilsson (2017), where the abundance of methanotrophs in lakes during winter season ranged between 10^2-10^3 copies/mL. Even Hus U, that had much higher abundance of methanotrophs than the other sites, (8178 copies/mL, compared to the respective means of 681 and 745 copies/mL for the vegetated and unvegetated sites), is within this range. This suggests that even though Hus U strongly deviated in this study, it was a reasonable value and cannot be dismissed as an artefact. However, while this study targeted the mxaF gene, Samad and Bertilsson (2017) used a set of primers targeting another gene called pmoA. MxaF encompasses most of all known methanotrophs but it also includes certain groups of methylotrophs (Lau et al. 2013). pmoA targets all known methanotrophs except for the two genera Methylocella and Methyloferula (Lau et al. 2013; Samad & Bertilsson 2017). It seems as if no studies have investigated whether results from qPCR using these different primers are comparable. However, the fact that the abundance of methanotrophs in this thesis was of the same magnitude as in the study by Samad & Bertilsson (2017) may indicate that primers targeting mxaF and pmoA give similar results and are relevant to compare.

The high abundance in the Hus wetland may be explained by the high TP concentration compared to the other sites, 689 µg/L, or by the pH, which was 1-2 units lower at Hus than at most of the other wetlands. In addition, the concentration of dissolved CH_4 at Hus U, 40 µg/L, was well above the mean of all wetlands (7.6 µg/L), which combined with the high TP concentration and low pH may have created good conditions for methanotrophs. Even though the availability of CH_4 has been reported to be a main factor controlling methanotrophs (Sundh et al. 2005; Bastviken 2009), the abundance of methanotrophs was not correlated to the concentration of dissolved CH_4 in this study. The explanation for this may be that other factors, such as temperature, may have had a larger effect that obscured any effects of the CH_4.

5.1.1 Vegetation

The abundance of methanotrophs did not differ significantly between the vegetated and the unvegetated sampling points. This could most probably be attributed to similar hydrochemical and nutrient conditions in the U and V sampling points and the off-season for plant growth. When the plants are inactive, they do not exudate chemicals through the roots and presumably have little or no effect on the soil environment and microbial communities compared to during growing season (Turner et al. 2020). For GA2, however, the abundance of methanotrophs at the vegetated sampling point was 7 times higher than the unvegetated sampling point, which may be explained by the high content of chlorophyll a (43 μg/L) in V compared to the much lower content (7 μg/L) in U. Methanotrophs are generally sensitive to light and more chlorophyll makes the water column darker, which favour methanotrophs (Dumestre et al. 1999; Shelley et al. 2017). Not surprisingly, the Chl-a values were generally higher at the V sapling points, however, no clear correlation with the abundance of methanotrophs except for GA2 could be observed.

5.1.2 Nutrients

The abundance of methanotrophs were positively correlated both with TOC, P and N. However, the correlation with TOC became close to non-significant ($p = 0{,}049$) when outliers were removed, and the correlation to N became negative. Thus, the TOC- and N-correlations were strongly affected by Hus U and SäN. The correlation with P remained significant even after the outliers were removed and may thus be regarded as more likely to be true. The same applies to the correlations with the C/N-, C/P- and N/P-values. This is in line with the result of Denfeld et al. (2016) and Sawakuchi et al. (2021) who investigated the CH_4 oxidation and bacterial community composition in surface water under ice cover during winter for lakes in central Sweden and found that CH_4 oxidation only occurred in the three lakes with highest P concentrations. They concluded that since methanotrophs are considered to be slow growing and thus bad competitors, they may be outcompeted for P by other heterotrophic bacteria if the P concentrations are not high enough (Denfeld et al. 2016). Alternatively, higher P concentrations may indirectly enhance CH_4 oxidation via stimulation of the whole microbial community which causes a release of vitamins and other chemical compounds by other bacteria that can benefit the methanotrophs (Sawakuchi et al. 2021). Similarly, studies from drainage ditch data and various environmental observations reviewed by Veraart et al. (2015) showed a positive correlation between P and CH_4 oxidation. However, in fertilization experiments reviewed in the same study the methanotrophs showed more variable

responses to P. Sundh et al. (2005) have shown that CH_4 oxidation can be positively correlated to the abundance of methanotrophs. Hence, like both Denfeld et al. (2016) and Veraart et al. (2015), the results in this thesis suggest that the relationship between P and methanotrophs may be an important regulator of CH_4 oxidation and thus of CH_4 emissions to the atmosphere. Indeed, this is particularly relevant for wetlands constructed for P retention that have intrinsically high P concentrations. However, nutrient status probably has many indirect effects on CH_4 oxidation, which influence on the results can be especially hard to exclude in field based studies. Hence, the mechanisms involved in P regulation of the CH_4 oxidizing microbial communities need further investigation, both in controlled experiments in lab and mesocosms as well as in further field based studies.

5.2 The concentrations of dissolved CH_4

There was a large variation in the concentration of dissolved CH_4 between the wetlands, ranging from 0.6 to 133 µg/L. The mean of 17 µg/L correspond to the results in Bastviken et al. (2004), in which a range of 1 – 30 µg/L was reported for 16 Swedish lakes. Similarly, Bastviken et al. (2008) presented a range of 8 – 42 µg/L in surface layer water for a relatively eutrophic lake in USA and Denfeld et al. (2016) and Sawakuchi et al. (2021) reported ranges between 1 and 18 µg/L, both from studies of ice-covered lakes in central Sweden. A handful of the wetlands studied in this thesis had dissolved CH_4 concentrations well above the mean and the ranges reported in these previous studies. However, these high concentrations could not be related to the nutrient levels or hydrochemical factors measured in the same wetlands, which was not surprising since the correlation tests mostly resulted in weak and non-significant correlations. Instead, the high concentrations of dissolved CH_4 found in this study may be attributed to the much smaller size of the wetlands compared to the concentrations reported from lakes. This is in agreement with findings that CH_4 emissions per unit area increase with decreasing waterbody size (Peacock et al. 2021). Indeed, Holgerson and Raymond (2016) reported mean dissolved CH_4 concentrations of 120 µg/L for waterbodies smaller than 0.001 km^2 and (Peacock et al. 2021) reported a mean of 300 µg/L for small, artificial waterbodies. Nevertheless, the TP concentrations were generally tenfold higher in the wetlands in this thesis than in the lakes from the studies mentioned above, and the correlation between high nutrient status and increased CH_4 emissions have been demonstrated in previous studies (Beaulieu et al. 2019).

5.2.1 Vegetation

The concentrations of dissolved CH_4 did not differ significantly between the vegetated and the unvegetated sampling points, which, similar to the abundance of

methanotrophs, most probably was due to similar hydrochemical and nutrient conditions in the U and V sampling points and off-season for plant growth. Vegetation may have many different direct and indirect effects on the microbial communities involved in CH_4 production and oxidation (Thottathil et al. 2018), however these effects may be small when the plants do not grow. In addition, other factors affecting methane production and oxidation, such as the low temperatures, may be of greater importance than the effects of plants during winter and thus obscure any differences due to vegetation. At Pad, Hus, Spr and Sän the unvegetated sampling points had much higher CH_4 concentrations than the vegetated. However, there were no clear differences among the measured variables in this study that could explain this gap between U and V in these wetlands.

5.3 The CH_4 fluxes

The large differences in ebullitive flux both between the wetlands and between the individual chambers at each sampling point is in line with results presented in previous studies, demonstrating that ebullition seem to occur randomly and therefore is hard to quantify (Bastviken et al. 2002). Likewise, the low diffusive fluxes correspond to previous findings from winter season sampling, that ranged between 0 (Ollivier et al. 2019) and 5 $mg/m^2/d$ (Stadmark & Leonardson 2005). However, ebullition events have previously been reported do decrease during wintertime due to low temperatures, but the high ebullition events measured in this thesis suggests that the ebullitive fluxes can be large and may contribute to large emissions to the atmosphere even during the cold season. Most studies that measure CH_4 only include diffusive flux, however Peacock et al. (2021) reported a mean annual total flux of 8.5 $g/m^2/year$ for small artificial waterbodies, which equals 23 $mg/m^2/d$, which is within the range observed in the current study. Bastviken et al. (2008) reported a flux of around 4 $mg/m^2/d$ for both ebullitive and diffusive flux for summer season, respectively, which generates a total flux of 8 $mg/m^2/d$.

5.3.1 Vegetation

There were no significant differences between the vegetated and unvegetated sampling points regarding CH_4 fluxes. Nevertheless, previous studies have shown that CH_4 fluxes can be significantly higher from vegetated sampling points compared to unvegetated controls (Ström et al. 2007) and that higher density of vegetation enhances the CH_4 emissions (de Klein & van der Werf 2014). However, these studies included the flux mediated through emergent plant stems, which was not measured in this thesis. If the plant-mediated flux had been included, the difference between vegetated and unvegetated sites may have been larger.

5.3.2 Nutrients

There were no correlations between the flux and the nutrient status, which may be explained by the few sampling points (five wetlands). However, the ebullition is probably more driven by factors in the wetland sediments rather than in the water, and thus correlated to factors that was not measured in this study.

5.4 Conclusion

In this study the wetland vegetation had no effect on the abundance of methanotrophs or on the dissolved CH_4 concentrations and CH_4 fluxes. This contradicts previous studies and may depend on even conditions throughout the wetland and on the off-vegetative season. Of the nutrients and hydrochemical factors, P and the increased fraction of P in relation to N and C were significantly correlated to the abundance of methanotrophs, but no factors were correlated with dissolved CH_4. The total fluxes corresponded to fluxes reported in previous studies and indicate that ebullitive fluxes can be large even during winter season. Overall, the lack of clear and significant results in this thesis indicates that the abundance of methanotrophs, dissolved CH_4 and CH_4 fluxes cannot be explained and monitored by one or a few factors. Instead, multiple direct and indirect variables most likely interactively control CH_4 emissions and the bacterial community involved in CH_4 oxidation. In order to understand how constructed wetlands can be designed to favour methanotrophs and mitigate their effects on global warming, it is important with further studies on the mechanisms of CH_4 oxidation and release, both in lab and mesocosms in which variables can be controlled, and in field-based studies.

References

Bastviken, D. (2009). Methane. I: Likens, G.E. (red.) *Encyclopedia of Inland Waters*. Oxford: Academic Press, 783–805. https://doi.org/10.1016/B978-012370626-3.00117-4

Bastviken, D., Cole, J. & Pace, M.L. & Van de Bogert, M.C. (2008). Fates of methane from different lake habitats: Connecting whole-lake budgets and CH_4 emissions. Journal of Geophysical research, 113 (G2), G0024-n/a. https://doi.org/10.1029/2007JG000608

Bastviken, D., Cole, J., Pace, M. & Tranvik, L. (2004). Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochemical Cycles*, 18 (4). https://doi.org/10.1029/2004GB002238

Bastviken, D., Ejlertsson, J. & Tranvik, L. (2002). Measurement of Methane Oxidation in Lakes: A Comparison of Methods. *Environmental science & technology*, 36 (15), 3354–3361

Beaulieu, J.J., DelSontro, T. & Downing, J.A. (2019). Eutrophication will increase methane emissions from lakes and impoundments during the 21st century. *Nature Communications*, 10 (1), 1375. https://doi.org/10.1038/s41467-019-09100-5

Bodmer, P., Vroom, R. & Stepina, T. (2021). Methane Fluxes of Vegetated Areas in Natural Freshwater Ecosystems: Assessments and Global Significance. 35

Brix, H. (1993). Macrophyte-Mediated Oxygen Transfer in Wetlands: Transport Mechanisms and Rates. *Constructed Wetlands for Water Quality Improvement*. CRC Press

Carmichael, M.J., Bernhardt, E.S., Bräuer, S.L. & Smith, W.K. (2014). The role of vegetation in methane flux to the atmosphere: should vegetation be included as a distinct category in the global methane budget? *Biogeochemistry*, 119 (1), 1–24. https://doi.org/10.1007/s10533-014-9974-1

Cole, J.J., Prairie, Y.T., Caraco, N.F., McDowell, W.H., Tranvik, L.J., Striegl, R.G., Duarte, C.M., Kortelainen, P., Downing, J.A., Middelburg, J.J. & Melack, J. (2007). Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. *Ecosystems*, 10 (1), 171–184

Denfeld, B.A., Ricão Canelhas, M., Weyhenmeyer, G.A., Bertilsson, S., Eiler, A. & Bastviken, D. (2016). Constraints on methane oxidation in ice-covered boreal lakes. *Journal of Geophysical Research: Biogeosciences*, 121 (7), 1924–1933. https://doi.org/10.1002/2016JG003382

Dumestre, J.F., Guézennec, J., Galy-Lacaux, C., Delmas, R., Richard, S. & Labroue, L. (1999). Influence of Light Intensity on Methanotrophic Bacterial Activity in Petit Saut Reservoir, French Guiana. *Applied and Environmental Microbiology*, 65 (2), 534–539. https://doi.org/10.1128/AEM.65.2.534-539.1999

Enrich-Prast, A., Bastviken, D. & Crill, P. (2009). Chemosynthesis. I: Likens, G.E. (red.) *Encyclopedia of Inland Waters*. Oxford: Academic Press, 211–225. https://doi.org/10.1016/B978-012370626-3.00126-5

Feuerbach, P. & Strand, J. (2010). *Water and Biodiversity in the agricultural landscape*.

Fritz, C., Pancotto, V.A., Elzenga, J.T.M., Visser, E.J.W., Grootjans, A.P., Pol, A., Iturraspe, R., Roelofs, J.G.M. & Smolders, A.J.P. (2011). Zero methane emission bogs: extreme rhizosphere oxygenation by cushion plants in Patagonia. *The New Phytologist*, 190 (2), 398–408

Grasset, C., Abril, G., Guillard, L., Delolme, C. & Bornette, G. (2016). Carbon emission along a eutrophication gradient in temperate riverine wetlands: effect of primary productivity and plant community composition. *Freshwater Biology*, 61 (9), 1405–1420. https://doi.org/10.1111/fwb.12780

Gunnarsson, U. & Löfroth, M. (2009). *Våtmarksinventeringen – resultat från 25 års inventeringar. Nationell slutrapport för våtmarksinventeringen (VMI) i Sverige.* (5925). Naturvårdsverket.

Havs- och vattenmyndigheten (2019). *Näringsbelastningen på Östersjön och Västerhavet 2017. Sveriges underlag till HELCOM:s sjunde Pollution Load Compilation*. (2019:20). Havs- och vattenmyndigheten.

Hoffmann, C.C., Kjaergaard, C., Uusi-Kämppä, J., Hansen, H.C.B. & Kronvang, B. (2009). Phosphorus Retention in Riparian Buffers: Review of Their Efficiency. *Journal of Environmental Quality*, 38 (5), 1942–1955. https://doi.org/10.2134/jeq2008.0087

Holgerson, M.A. & Raymond, P.A. (2016). Large contribution to inland water CO2 and CH4 emissions from very small ponds. *Nature Geoscience*, 9 (3), 222–226. https://doi.org/10.1038/ngeo2654

Holzapfel-Pschorn, A., Conrad, R. & Seiler, W. (1986). Effects of vegetation on the emission of methane from submerged paddy soil. *Plant and Soil*, 92 (2), 223–233. https://doi.org/10.1007/BF02372636

Hope, D., Palmer, S. M., Billett, M. F., & Dawson, J. J. (2004). Variations in dissolved CO2 and CH4 in a first-order stream and catchment: An investigation of soil-stream linkages. *Hydrological Processes*, 18(17), 3255-3275. https://doi.org/10.1002/hyp.5657

Jordbruksverket (2004). *Kvalitetskriterier för våtmarker i odlingslandskapet – kriterier för rening av växtnäring med beaktande av biologisk mångfald och kulturmiljö.* (2004:2). Jönköping.

Kankaala, P., Käki, T., Mäkelä, S., Ojala, A., Pajunen, H. & Arvola, L. (2005). Methane efflux in relation to plant biomass and sediment characteristics in stands of three common emergent macrophytes in boreal mesoeutrophic lakes. *Global Change Biology*, 11 (1), 145–153. https://doi.org/10.1111/j.1365-2486.2004.00888.x

Kankaala, P., Mäkelä, S., Bergström, I., Huitu, E., Käki, T., Ojala, A., Rantakari, M., Kortelainen, P. & Arvola, L. (2003). Midsummer spatial variation in methane efflux from stands of littoral vegetation in a boreal meso-eutrophic lake. *Freshwater Biology*, 48 (9), 1617–1629. https://doi.org/10.1046/j.1365-2427.2003.01113.x

de Klein, J.J.M. & van der Werf, A.K. (2014). Balancing carbon sequestration and GHG emissions in a constructed wetland. *Ecological Engineering*, 66, 36–42. https://doi.org/10.1016/j.ecoleng.2013.04.060

Kynkäänniemi, P. (2014). *Small Wetlands Designed for Phosphorus Retention in Swedish Agricultural Areas*. Diss. Uppsala: Dept. of Soil and Environment, Swedish University of Agricultural Sciences.

Lau, E., Fisher, M.C., Steudler, P.A. & Cavanaugh, C.M. (2013). The Methanol Dehydrogenase Gene, mxaF, as a Functional and Phylogenetic Marker for Proteobacterial Methanotrophs in Natural Environments. *PLOS ONE*, 8 (2), e56993. https://doi.org/10.1371/journal.pone.0056993

McDonald, I.R. & Murrell, J.C. (1997). The methanol dehydrogenase structural gene mxaF and its use as a functional gene probe for methanotrophs and methylotrophs. *Applied and Environmental Microbiology*, 63 (8), 3218–3224. https://doi.org/10.1128/aem.63.8.3218-3224.1997

van der Nat, F.-J.W.A. & Middelburg, J.J. (1998). Seasonal variation in methane oxidation by the rhizosphere of Phragmites australis and Scirpus lacustris. *Aquatic Botany*, 61 (2), 95–110. https://doi.org/10.1016/S0304-3770(98)00072-2

Naturvårdsverket (2009). *Rätt våtmark på rätt plats - En handledning för planering och organisation av arbetet med att anlägga och restaurera våtmarker i odlingslandskapet*. (5926). Stockholm: Naturvårdsverket.

Oliveira-Junior, E.S., Tang, Y., van den Berg, S.J.P., Cardoso, S.J., Lamers, L.P.M. & Kosten, S. (2018). The impact of water hyacinth (Eichhornia crassipes) on greenhouse gas emission and nutrient mobilization depends on rooting and plant coverage. *Aquatic Botany*, 145, 1–9. https://doi.org/10.1016/j.aquabot.2017.11.005

Ollivier, Q.R., Maher, D.T., Pitfield, C. & Macreadie, P.I. (2019). Winter emissions of CO2, CH4, and N2O from temperate agricultural dams: fluxes, sources, and processes. *Ecosphere*, 10 (11), e02914. https://doi.org/10.1002/ecs2.2914

Peacock, M., Audet, J., Bastviken, D., Cook, S., Evans, C.D., Grinham, A., Holgerson, M.A., Högbom, L., Pickard, A.E., Zieliński, P. & Futter, M.N. (2021). Small artificial waterbodies are widespread and persistent emitters of methane and carbon dioxide. *Global Change Biology*, 27 (20), 5109–5123. https://doi.org/10.1111/gcb.15762

Samad, M.S. & Bertilsson, S. (2017). Seasonal Variation in Abundance and Diversity of Bacterial Methanotrophs in Five Temperate Lakes. *Frontiers in Microbiology*, 8. https://doi.org/10.3389/fmicb.2017.00142

Sawakuchi, H.O., Martin, G., Peura, S., Bertilsson, S., Karlsson, J. & Bastviken, D. (2021). Phosphorus Regulation of Methane Oxidation in Water From Ice-

Covered Lakes. *Journal of Geophysical Research: Biogeosciences*, 126 (9), e2020JG006190. https://doi.org/10.1029/2020JG006190

Segers, R. (1998). Methane Production and Methane Consumption: A Review of Processes Underlying Wetland Methane Fluxes. *Biogeochemistry*, 41 (1), 23–51

Shelley, F., Ings, N., Hildrew, A.G., Trimmer, M. & Grey, J. (2017). Bringing methanotrophy in rivers out of the shadows. *Limnology and Oceanography*, 62 (6), 2345–2359

Solomon, S., Qin, D., Manning, M., Chen, Z., Marquiz, M., Averyt, K.B., Tignor, M. & Miller, H.L. (red.) (2007). *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, USA: Cambridge University Press. https://www.ipcc.ch/report/ar4/wg1/ [2022-04-27]

Stadmark, J. & Leonardson, L. (2005). Emissions of greenhouse gases from ponds constructed for nitrogen removal. *Ecological Engineering*, 25 (5), 542–551. https://doi.org/10.1016/j.ecoleng.2005.07.004

Strand, J.A. & Weisner, S.E.B. (2013). Effects of wetland construction on nitrogen transport and species richness in the agricultural landscape—Experiences from Sweden. *Ecological Engineering*, 56, 14–25. https://doi.org/10.1016/j.ecoleng.2012.12.087

Ström, L., Lamppa, A. & Christensen, T.R. (2007). Greenhouse gas emissions from a constructed wetland in southern Sweden. *Wetlands Ecology and Management*, 15 (1), 43–50. https://doi.org/10.1007/s11273-006-9010-x

Ström, L., Mastepanov, M. & Christensen, T.R. (2005). Species-specific Effects of Vascular Plants on Carbon Turnover and Methane Emissions from Wetlands. *Biogeochemistry*, 75 (1), 65–82. https://doi.org/10.1007/s10533-004-6124-1

Sundh, I., Bastviken, D. & Tranvik, L.J. (2005). Abundance, Activity, and Community Structure of Pelagic Methane-Oxidizing Bacteria in Temperate Lakes. *Applied and Environmental Microbiology*, 71 (11), 6746–6752. https://doi.org/10.1128/AEM.71.11.6746-6752.2005

Thottathil, S.D., Reis, P.C.J., del Giorgio, P.A. & Prairie, Y.T. (2018). The Extent and Regulation of Summer Methane Oxidation in Northern Lakes. *Journal of Geophysical Research: Biogeosciences*, 123 (10), 3216–3230. https://doi.org/10.1029/2018JG004464

Turner, J.C., Moorberg, C.J., Wong, A., Shea, K., Waldrop, M.P., Turetsky, M.R. & Neumann, R.B. (2020). Getting to the Root of Plant-Mediated Methane Emissions and Oxidation in a Thermokarst Bog. *Journal of Geophysical Research: Biogeosciences*, 125 (11), e2020JG005825. https://doi.org/10.1029/2020JG005825

Veraart, A.J., Steenbergh, A.K., Ho, A., Kim, S.Y. & Bodelier, P.L.E. (2015). Beyond nitrogen: The importance of phosphorus for CH4 oxidation in soils and sediments. *Geoderma*, 259–260, 337–346. https://doi.org/10.1016/j.geoderma.2015.03.025

Weiss, R.F. (1974). Carbon dioxide in water and seawater: the solubility of a non-ideal gas. *Marine Chemistry*, 2 (3), 203–215. https://doi.org/10.1016/0304-4203(74)90015-2

Whalen, S. c. (2005). Biogeochemistry of Methane Exchange between Natural Wetlands and the Atmosphere. *Environmental Engineering Science*, 22 (1), 73–94. https://doi.org/10.1089/ees.2005.22.73

Wiesenburg, D.A. & Guinasso, N.L. (1979). Equilibrium solubilities of methane, carbon monoxide, and hydrogen in water and sea water. *Journal of Chemical & Engineering Data*, 24 (4), 356–360. https://doi.org/10.1021/je60083a006

Acknowledgements

I would like to thank my supervisors, Pia Geranmayeh, Michael Peacock, Tong Liu and Stefan Bertilsson for their guidance and support throughout the project. To Tong Liu, I wish to give a special thanks for spending hours guiding me in the lab; his support and patience made the lab work fun and I learned a lot. I also want to thank research engineer Prune Leroy and the other staff members at the Functional Microbial Ecology Lab at SLU, whose skills and helpfulness were very valuable. Further, I wish to thank Hushållningssällskapet in Halland for providing information and guidance about the wetlands in Halland and for kindly lending me their Stand Up Paddleboard. Last but not least, I would like to thank Johanna Kero for being such a nice and helpful field trip partner, without her company it would have been a challenge to take any samples at all.

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.