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Variability and regulation of the planktonic respiratory quotient in a eutrophic lake (Lake Vombsjön) in summer 2016

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Abstract

Bacterial respiration and biomass growth are important processes for carbon cycling in freshwater ecosystems and bacterial respiration is often quantified from measured O_2 concentrations by using a respiratory quotient (RQ=O_2 consumed to produce CO_2, in moles) of 1. Recent studies have shown, however, that RQs can vary a lot in different aquatic ecosystems (0.5-5), which may lead to an under- or overestimation of respiration. In this study, we conducted in-situ measurements of O_2 and CO_2 fluxes in a eutrophic lake (Lake Vombsjön) to assess the magnitude and variability of the RQ and bacterial growth efficiency (BGE) during summer 2016. The RQ was mostly <1 and increased with increasing water depth while the BGE was high (0.2-0.5) and decreased with increasing water depth. In both cases, this could be attributed to the preferential use of autochthonous organic matter by bacterioplankton. Still, no coupling between the RQ and BGE was observed. All in all, our observed RQs were much lower than any reported values (between 0.5 and 0.2). No single explaining factor could be found; instead, a combination of high primary productivity, increasing nitrification and denitrification rates over the summer and possibly the occurrence of methane oxidation may be responsible. This indicates that using a theoretical RQ of 1 may lead to an overestimation of bacterial respiration in eutrophic ecosystems and that other metabolic processes (nitrification, denitrification, methane oxidation) should be considered when studying respiration processes in those ecosystems.

Keywords: Physical geography and ecosystem analysis, biogeochemistry, bacterioplankton respiration, eutrophic lake, carbon cycling

Table of contents

List of figures

List of tables

1 Introduction

Metabolism describes the processes involved in the turnover of biomass and energy in an ecosystem (Endquist et al. 2003, Peeters et al. 2016) and is governed by primary production and respiration in aquatic ecosystems. Primary production is the process of fixing inorganic carbon by autotrophs and respiration describes the remineralisation of organic carbon to carbon dioxide by the whole ecosystem community (Cremona et al. 2014). By assessing these two processes, it is possible to gain deeper understanding about energy fluxes within the aquatic food web and how much metabolism contributes to nutrient cycling and carbon (Cotgreave and Forseth 2002). Those fluxes are central to many questions regarding climate change biology and ecology and what role aquatic ecosystems play in the global cycling of carbon (Enquist et al. 2003, Peeters et al. 2016).

Freshwater systems are an important part of the global carbon cycle: while formerly mainly seen as a route for transport of terrestrial carbon to the sea, researchers have acknowledged that lakes act as both sinks and sources of atmospheric carbon (Cole et al. 2007, Tranvik et al. 2009) and that they significantly contribute to global greenhouse gas emissions (Seekell et al. 2014, Downing et al. 2006). Every year, lakes and other inland water bodies release as much carbon dioxide to the atmosphere as is absorbed by the oceans during the same time (Tranvik et al. 2009). Bacterioplankton metabolism is a major driver for carbon cycling in lakes: bacterioplankton mineralise dissolved organic carbon (DOC) and organic matter and use it as an energy source for bacterial respiration (BR) and bacterial production (Jones 1992, Jansson et al. 2000), thus making DOC available for the rest of the food web. Bacterial respiration consumes oxygen to remineralise organic carbon and can contribute to the supersaturation of the lake with CO_2 (Peeters et al. 2016), and thus also CO_2 emissions from lakes to the atmosphere.

Even though we know that BR and other biological processes contribute to the emission of CO_2 from freshwater systems, we do not yet fully mechanistically understand how much and to what extent they do (Berggren 2016). One common approach to analyse bacterial respiration is to measure the O_2 consumption, either in-situ or by bottle incubation in the lab, and then assume a respiratory quotient (RQ) to determine the CO_2 produced (del Giorgio and Williams 2005). The RQ is the ratio of numbers of molecules CO_2 respired to the number of molecules O_2 consumed (Tsutsui et al. 2015) and is usually assumed to be 1 in aquatic systems (del Giorgo and Williams 2005). However, recent studies have shown that the RQ can be 2-4 times higher than that, thus causing us to underestimate the actual CO_2 production in freshwater systems (Berggren 2016). Higher RQs have been linked to the varying quality and composition of DOC, and to oxygen-rich organic acids which are easy to metabolise for bacterioplankton and are available when DOC is degraded by UV light (Bertilsson and Tranvik 2000; Berggren et al. 2012). Especially northern, unproductive lakes that are

dominated by DOC input from the surrounding land may thus have a bigger impact on carbon cycling than previously thought.

While researchers have reported high RQs in northern, oligotrophic lakes (del Giorgio et al. 1997, Cimbleris and Kalff 1998, Berggren et al. 2012), there are also studies in eutrophic and hypertrophic water bodies that have shown high P:R (photosynthesis:respiration) ratios (Hansson et al. 2003, Valdespino-Castillo et al. 2014), pointing towards low RQs in those systems. Part of the organic compounds excreted by phytoplankton and dead phytoplankton organic matter (lipids, fatty acids, proteins) are more reduced and require more O_2 for full oxygenation than glucose (theoretical RQ=1), resulting in an RQ<1 (see Table 1). Still, only Burford and Longmore (2001) have reported a low RQ (~0.5) in a eutrophic system. Berggren et al. (2012) linked low RQs to net-autotrophic lakes (high P:R ratios, high primary production and oxygen saturation) and bacterial communities with a high capacity to metabolise reduced compounds, highlighting the importance of nature of organic carbon pools in lakes as opposed to just nutrient and DOC concentrations. Thus, it stands to reason that an RQ of 1 might be an overestimation of the carbon emissions from eutrophic lakes.

Another key bacterioplankton process in lakes is bacterial production (BP) by which bacteria convert carbon into biomass and make it available to grazers in the food web via the microbial loop (Azam et al. 1983). This available carbon fraction can be as high as carbon from phytoplankton sources and can thus play a major role in food webs in aquatic ecosystems by supplying nutrients to higher trophic levels (Hart and Stone 2000, Li et al. 2014). Together, BP and BR describe the total amount of bacterioplankton assimilated carbon in an ecosystem (del Giorgio and Cole 1998). They can be used to calculate bacterial growth efficiency:

$$BGE = \frac{\mathrm{BP}}{\mathrm{BP} + \mathrm{BR}} \qquad \text{(equation 1)}$$

The BGE indicates the share of the total DOC assimilation that is converted into bacterial biomass (Eichinger et al. 2010). A high BGE indicates that a bigger proportion of carbon is turned into biomass than respired and vice versa. How much carbon is converted into biomass depends on the energy demands within the bacterial metabolism: the demand to produce biomass and the demand to maintain cellular functions. If carbon is not readily bioavailable and has to be broken down by bacteria by energy-consuming processes, e.g. by producing exo-enzymes (del Giorgio and Cole 1998, Cimberlis and Kalff 1998), the demand to maintain cellular functions is higher than the demand to produce biomass, thus inhibiting growth and resulting in lower BGEs. At the same time, the free energy potential of carbon sources is also important for the BGE: energy-rich compounds, like lipids, yield more energy to bacteria than carbohydrates, which are more oxidised. More reduced compounds can thus provide more

energy to be used for cell metabolism than more oxidised compounds, meaning that the BGE may be higher for those compounds.

The BGE in lakes ranges from < 0.1 to > 0.6 and is influenced by many factors, for example temperature, substrate quality and the quantitative availability of DOC to bacterioplankton, which is why it shows such a wide range both between freshwater systems and also within the same sites during the year (Eichinger et al. 2010; Kritzberg et al. 2010; Roland and Cole 1999). BGE increases with increasing lake productivity (Roland and Cole 1999), indicating that organic matter excreted by phytoplankton is an energy-rich carbon source for bacteria and yields a high BGE (del Giorgio and Cole 1998). This is the opposite of RQ, which decreases with increasing productivity due to the reduced nature of phytoplankton organic matter, since more O_2 is required to fully degrade the organic matter. It shows that the BGE and RQ can exhibit different responses to the same organic matter source, though it is important to keep in mind that BGE calculated from BR based on oxygen measurements is dependent on RQ; only BGE based on C unit measurements is independent from RQ.

Another example for this would be the ability of bacteria to readily assimilate small molecules, like oxygen-rich organic acids originating from photochemical degradation of DOC. Those do not yield much energy, however, since they are highly oxidised compounds. So even though little energy has to be expended to assimilate such organic acids, they do not provide much energy for cell growth, resulting in a low BGE. At the same time, the RQ would be high for those compounds, because only little O_2 has to be provided for degradation. Since those organic acids should also be more available in surface waters compared to deeper lake regions due to the UV-light ability to penetrate the water, the BGE should be lower in surface waters and increase with increasing water depth, where slowly sedimentating, phytoplanktonic energy-rich organic matter dominates.

Bacterial respiration, bacterial production and bacterial growth efficiency have often been viewed together in research over the last 15 years. Still, BR is often inferred from oxygen measurements, using an RQ of 1 for conversion, meaning that those processes are rarely viewed uncoupled from each other in the field, e.g. by looking at C-based BGE and measured RQ values. Thus, to gain a deeper insight into how those processes are connected and to which degree they contribute to CO_2 emissions, it is necessary to view them together and as independent of each other as possible. In order to do that, we decided to carry out a seasonal study at Lake Vombsjön in Southern Sweden, both to develop a reliable methodology to measure bacterial respiration in-situ, and to assess the RQ and BGE of the bacterioplankton community in Lake Vombsjön and how they vary during the summer and between depth. We expected the bacterioplankton community to be influenced by seasonal changes, like algal blooms and nutrient availability, and assessed basic water chemistry (POC, DOC, phosphorus and nitrogen concentrations) and which carbon sources were preferably used by the

bacterioplankton community to assess its functional capacities. Microorganisms typically react first to environmental changes and might thus prove useful to explain changes in RQ and BGE that cannot be related to other lake variables.

For that, we expected:

1. The RQ in Lake Vombsjön is smaller than 1, as it is an eutrophic lake.
2. The RQ is higher at the lake surface than at the bottom layer due to photo-chemical breakdown of DOC, thus making it more readily available for bacterial breakdown.
3. The BGE is increasing with increasing water depth, because of decreasing availability of partially broken down DOC material by UV-light and the increase of energy-rich phytoplankton organic compounds sedimentating to the lake bottom.
4. RQ and BGE are negatively correlated, because they can exhibit opposite responses to the same energy sources (organic matter) available.

1.1 Background information: the respiratory quotient (RQ)

The RQ is the ratio of numbers of molecules CO_2 respired to the number of molecules O_2 consumed (Tsutsui et al. 2015). It is used to convert the metabolism of cells and bacteria into carbon units in a number of research fields, like medicine, soil science and biology. With the help of the RQ, researchers can assess whether aerobic (RQ<1) or anaerobic (R>1) occurs and which type of substrate is metabolised: carbohydrates yield an RQ of 1, proteins of 0.9, fats of 0.7, and RQs<0.7 indicate organic acids and RQs>1 oxygen-rich organic acids (Romero-Kutzner et al. 2015). Depending on the research focus, the RQ can be assessed on the cell level, e.g. for a specific substrate, for bacterioplankton or total plankton communities or even on an ecosystem level, like a lake.

In aquatic ecosystems, scientists often monitor the change in oxygen or carbon dioxide concentrations in the water over time using different approaches including chemical analyses, like the Winkler titration (Winkler 1888), or using a membrane inlet mass spectrometer (del Giorgio et al. 2011) and optode and electrode techniques (Marchand et al. 2009). From the change in oxygen concentrations, the carbon dioxide concentrations are then calculated by assuming an RQ of 1 (Demars et al. 2015). This assumption is drawn from the known RQ of glucose (RQ = 1) under aerobic conditions and without other electron acceptors present, like NO_3^- or Fe^{2+} (Dilly 2003). Researchers adjust the assumed RQ to between 0.8 and 1.2 depending on the substrate available in the lake, e.g. at 0.89 (Williams and del Giorgio 2005), though lower RQ-values (e.g. 0.7) can be used to account for the amount of acids occurring in the respiratory substrate (see Table 1). Those RQs are based on a stoichiometric approach.

Table 1: Theoretical respiratory quotients (RQ) based on stoichiometric calculations for different respiratory substrates. Chemical equations for full oxidation of substrates are given for non-mixed substrates (for lipid, palmitic acid is used as an example and pyruvic acid for nucleic acid). All calculations assume that no nitrification takes place. Mixed substrates (algal cell material and planktonic material) are composed of proteins, lipids, carbohydrates and nucleic acids. Sources: Berggren et al. 2012, del Giorgio and Williams 2005, Hedges et al. 2002, Rodriguez and Williams 2001 and Williams and Robertson 1991.

RQ	substrate	chemical equation	CO_2 produced	O_2 consumed
4	oxalic acid	$C_2H_2O_4+0.5\ O_2 \rightarrow 1\ H_2O+2\ CO_2$	2	0.5
1.6	tartaric acid	$C_4H_6O_6+2.5\ O_2 \rightarrow 3\ H_2O+4\ CO_2$	4	2.5
1.33	glycolic acid	$C_6H_8O_7+4.5\ O_2 \rightarrow 4\ H_2O+6\ CO_2$	6	4.5
1.24	nucleic acid	$C_3H_4O_3+2.5\ O_2 \rightarrow 2\ H_2O+3\ CO_2$	3	2.5
1	polysaccharide	$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2+ 6\ H_2O$	6	6
0.97	protein			
0.89	algal cell material			
0.89	planktonic material			
0.7	lipid	$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$	16	24
0.67	saturated fatty acid			
0.5	methane	$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$	1	2

However, there are many bacterial processes taking place in the lake at the same time, like nitrification, denitrification and methane oxidation, as well as photochemical processes, all of which can increase or decrease the RQ (Ribaudo et al. 2017, Cimbleris and Kalff 1998, Berggren et al. 2012). The few reported RQs in aquatic ecosystems vary a lot: while RQs close to 1 have been observed (Prairie et al. 2002, Kortman et al. 2009, Boucher et al. 1994), there have also been incidences of RQs much higher than 1 in northern, oligotrophic lakes (del Giorgio et al 1997, Cimleris and Kalff 1998, Berggren et al. 2012). Those higher RQ values are assumed to be caused by varying quality and composition of DOC, for example when DOC is degraded by UV light to oxygen-rich organic acids, which are easy to metabolise for bacterioplankton (Bertilsson and Tranvik 2000; Berggren et al. 2012).

For RQs lower than 1 there are very few reports. Torgerson and Branco (2008) have observed varying O_2 and CO_2 fluxes that indicate RQs different from 1 in a shallow pond and Burford and Longmore (2001) reported an RQ of roughly 0.5 for the sediment-water exchange in a eutrophic shrimp pond. Other than that, there are only indications, like the studies of Valdespino-Castillo et al. (2014) and Hansson et al. (2003) in eutrophic and hypertrophic ecosystems showing high P:R ratios, which indicate that the RQ in those systems might be significantly lower than 1. This is based on the nature of phytoplankton organic matter, which is made up of nucleic acid, carbohydrates, proteins and lipids. Especially lipids occur in more reduced form than carbohydrates and nucleic acids, meaning that more O2 is required to fully

oxygenate them. As a result, algal cell and planktonic material yield lower RQs than 1, and thus also ecosystems with primarily phytoplanktonic organic matter present might be better represented by an RQ > 1. Empirical correlations between RQ and P:R ratios by Berggren et al. (2012) may indicate that net-autotrophy and the capacity of the bacterial community to metabolise reduced compounds can be linked to low RQs. While this may explain how very low RQs of 0.5 are possible, it is still unclear what causes them.

There are indications from soil science, especially sludge and compost studies, that nitrification decreases the RQ (Tsutsui et al. 2015, Dilly 2003). During nitrification, O_2 is used to oxidise NH_4^+ stepwise to NO_3^-. During this reaction O_2 is consumed, but no CO_2 is produced, meaning that if nitrification occurs parallel to BR, O_2 concentrations will decrease by more than the amount consumed by BR. This results in a seemingly lower RQ for BR, because measuring O_2 and CO_2 does not take the sources or consumers of those gases into account. Kortman et al. (2009) have shown the impact of nitrification on the RQ for an aquatic system, (decrease from RQ=1.51 to RQ=1.14). Most aquatic research that points towards low RQs focuses on sediment and the sediment-water interchange (Sweerts et al. 1991, Ribaudo et al. 2017) or has been conducted in marine ecosystems (Robinson et al. 2002), however, so there is little knowledge about how RQs in the free water column in lakes are affected by nitrification.

All in all, there are very few empirical values available for RQs in aquatic ecosystems, which makes it difficult to draw general conclusions about their range and how reliable those values are. For calculations, an RQ range of 0.8 to 1.2 is assumed to give realistic results in most cases, though abnormal RQs during experiments are acknowledged (del Giorgio and Williams 2005). It should be noted, however, that the effects of nitrification on the RQ are not taken into account when assuming an RQ of 0.8 to 1.2 (del Giorgio and Williams 2005); to account for that, the protein/nucleic acid and carbohydrate/lipid ratios of the substrates need to be known (Rodriguez and Williams 2001).

The uncertainty of RQs in aquatic ecosystems of different trophic states and with pools of DOC poses the question whether calculating CO_2 fluxes from O_2 measurements with an RQ of 1 or close to 1 always gives a true representation of those systems' respiratory processes. Without a correct RQ, it is impossible to know how much bacterioplankton metabolism contributes to O_2 and CO_2 fluxes, making it difficult to correctly quantify and explain those variables. Especially when making statements about carbon emissions from ecosystems and using those results for carbon cycling models on a higher level (regional or global), the model may not be very robust or be able to predict changes in carbon fluxes in a changing climate adequately. The International Panel on Climate Change, the IPCC (2013), states that better understanding of processes involved in climate change generally increases model performance, despite the introduction of more variables and thus sources of errors and

uncertainty. Understanding the biogeochemical processes that cause abnormal RQs in aquatic systems may thus help to better understand the role of freshwaters in the global carbon cycle and their impact on climate change and quantify it more realistically.

1.2 Background information: the bacterial growth efficiency (BGE)

The bacterial growth efficiency is defined as is the quantity of biomass produced per unit assimilated substrate (del Giorgio and Cole 1998) and indicates the share of the total DOC assimilation that is converted into bacterial biomass (Eichinger et al. 2010). The higher the BGE, the higher the share of carbon turned into biomass compared to carbon respired and vice versa. It is calculated from BP and BR as

$$BGE = \frac{\mathrm{BP}}{\mathrm{BP} + \mathrm{BR}} \qquad \text{(equation 1)},$$

with BP values commonly assessed by ^{3}H leucine incorporation and BR values converted from the change of measured O_2 concentrations or from assumed BGE based on BP values (Roland and Cole 1999). The BP, BR and BGE are not necessarily coupled, however, and BGE varies widely across ecosystems from < 0.1 to > 0.6 (del Giorgio and Cole 1998, Roland and Cole 1999). This means that assuming the BGE based on BP and/or BR values and assuming the BR from BGE may not lead to realistic results.

The BGE is influenced by environmental conditions. It changes across types of aquatic ecosystems, increasing from rivers to oceans to lakes to estuaries (del Giorgio and Cole 1998). Within aquatic ecosystems, it increases with increasing productivity (Roland and Cole 1999), which del Giorgio and Cole (1998) have linked to organic matter excreted by phytoplankton acting as an energy-rich carbon source for bacteria. Other researchers have linked BGE to chlorophyll a concentrations (Biddanda et al. 2001), phosphorus availability (Smith and Prairie 2004, Kritzberg et al. 2010), nutrient availability by help of nutrient ratios, e.g. C:N ratio (del Giorgio and Cole 1998, Biddanda et al. 2001) and weakly to temperature (del Giorgio and Cole, Kritzberg et al. 2010). The quantity, quality and age of DOC is also known to influence the BGE (Kritzberg et al. 2010, Berggren et al. 2009) in combination with nutrient availability.

To sum it up, the BGE is dependent on nutrient availability and the quality of DOC, which explains why it increases along a productivity gradient across aquatic ecosystems. Energy-rich autochthonous organic matter in eutrophic may lower the energy demand to retain cell function in bacteria, while humic DOC in oligotrophic lakes may increase this demand,

respectively increasing and decreasing the BGE. It follows that the trophic state of an aquatic ecosystem and the organic matter and DOC quality should be kept in mind when estimating BGE and calculating BGE from C unit measurements should be considered instead, if possible, to view planktonic metabolic processes separately.

2 Materials and methods

2.1 Study site

Lake Vombjsön is a shallow lake in Southern Scania, 20 km east of Lund (55.6784° N, 13.5967° E). The lake has an area of 11.82 km^2, an average depth of 6.6 m (maximum depth 16 m) and lies approximately 20 m above sea level (VISS 2009). The theoretical water retention time is 0.7-0.8 years (VISS 2009). The lake is used for as a drinking water source by Sydvatten (extraction rate ca. 1000l/s), which means that the water level can vary up to 2.5 m (VISS 2009). It is also used for commercial and recreational fishing (Ekologgruppen AB i Landskrona, 2012). The area around Lake Vombsjön is dominated by agricultural landscape (70%), forest (13%) and open fields (10%) (Ekologgruppen i Landskrona AB 2012).

Due to run-off from agricultural fields, Lake Vombsjön is a hypertrophic lake with yearly green-blue algae blooms, often multiple blooms during summer (Ekologgruppen i Landskrona AB 2012; VISS 2009). Algal toxins are commonly found in the water during those times and have led to poisoned drinking water in the receiving municipalities (1994) and mass death of benthic mussels (2009) (Ekologgruppen i Landskrona AB 2012). Despite being relatively deep with 16 metres, Lake Vombsjön usually does not stratify during summer and the water is totally circulated during that time (Gelin 1975), one of the reasons why it retains a stable benthic fish community. We chose this lake as a sample site due to its eutrophic and productive nature and because its depth allows for sampling in shallow and deep water.

According to the authorities, Lake Vombsjön would fail to meet the requirements of the EU Water Framework Directive as of 2015. Both the chemical and the ecological status of the lake were ruled to be unsatisfactory in 2009 and only the chemical status judged to be able to improve to “good” by 2015 (VISS 2009). For the ecological status, it is estimated that Lake Vombsjön will only meet the requirements in 2027 (VISS 2009). The two main problems that Lake Vombsjön faces are morphological changes of the lake and eutrophication (VISS 2009).

2.2 Sampling procedure

We took respiration and water samples twice per month during the summer months June, July, August and September (see Table 2) at 1 and 10 metre water depth. Samples were usually taken at the lake’s deepest point in the middle of the lake (approximately 16 metres under normal water level; we observed fluctuations of 1 to 2 metres from this depth) except for a single instance when weather conditions were too rough; in that case, the next deepest spot close to the northern lake shore was chosen.

Table 2: Overview of sampling dates, sampling locations and maximum water depth during summer 2016 at Lake Vombsjön. Water depth was monitored with an echolot from the boat and used to find approximately the same sampling location during every sampling.

Sampling Date	Sampling location	Maximum water depth [m]
16/6/2016	Middle of lake	14
30/6/2016	Middle of lake	15
18/7/2016	Close to Northern shore	15
28/7/2016	Middle of lake	15
8/8/2016	Middle of lake	15
26/8/2016	Middle of lake	15
8/9/2016	Middle of lake	15
29/9/2016	Middle of lake	14

In the whole water column (surface till maximum depth 15 metres, if available), temperature, oxygen content (saturation and mg/L) and pH were measured with the help of a YSI combination probe. We sampled every half metre from the surface to 3 metres (4 metres from the 4th sampling on) and from there every metre till the lake bottom. The pH probe proved to be unreliable in its measurements, so all reported pH values originate from pH and alkalinity measurements in the laboratory which were conducted on the same day as sampling took place (see 2.5.1).

2.3 Bacterial respiration

The bacterial respiration was measured in a closed system in which the water circulated continuously (see Fig. 1). From the Niskin water sampler at sampling depth (1 or 10 metres), the water was pumped through butyl tubing into a gas exchange membrane and from there back to the water sampler. The gas exchange membrane was connected to an EGM 5 (a CO_2 gas analyser) which logged the CO_2 concentration in 1-second intervals. Dissolved oxygen was measured in the water sampler by a MiniDOT (oxygen logger) at 1-minute intervals. For each sample, we measured for 2.5 hours. The pump, battery, gas exchange membrane and EGM 5 were in a boat from which the water sampler is hanging.

We took respiration measurements in the closed underwater chamber at 1 and 10 metre depth for each sample. The measurements lasted for at least 2 ½ hours to account for equilibration of O_2 and CO_2 concentrations in the chamber due to temperature and pressure adjustments. For 29/9/2016, we also conducted an open chamber measurement at 10 metre depth to assess how well the conditions within the chamber mirrored the conditions in open water.

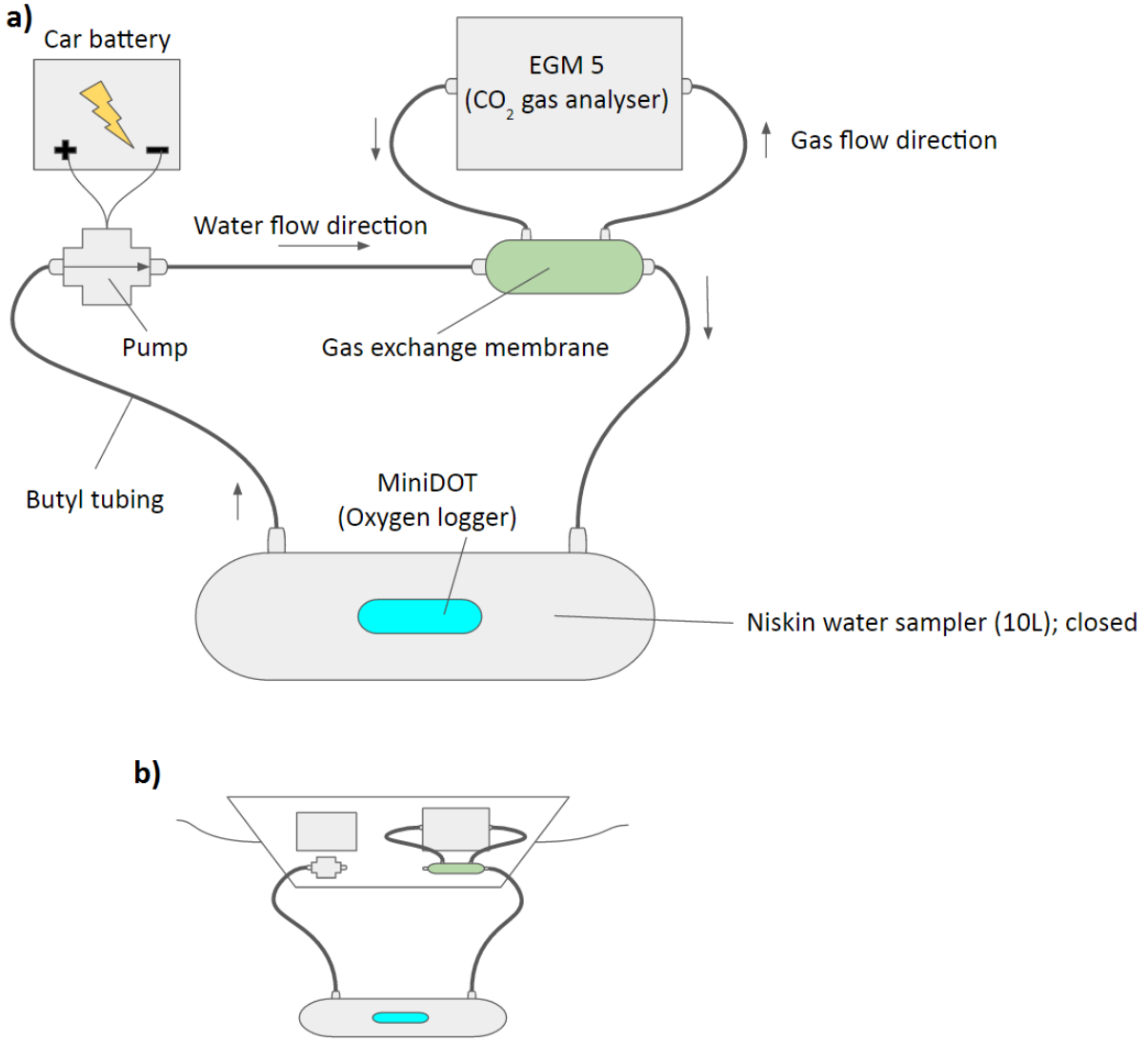

Fig. 1: Experimental set-up of in-situ respiration measurements in detail (a) and shown as used at a lake (b). Arrows between the water sampler, the pump and the gas exchange membrane represent water flow direction, arrows between the gas exchange membrane and the EGM 5 the gas flow direction.

To assess the bacterial respiration from the CO_2 (ppm) and O_2 (mg/L] measurements, both measurements were first converted to µmol/L. The CO_2 (ppm) was converted into total CO_2 production according to Berggren et al. 2012 whose equations are based on Stumm and Morgan (1996) and Oviatt et al. (1986). These calculations accounted for interactions in the carbonate system, namely the share of respired CO_2 that was converted into bicarbonate and thus not recorded by the EGM 5. They were based on temperature and alkalinity measurements from samplings and we assumed that temperature and alkalinity stayed constant in the underwater chamber over the whole measurement period.

Changes in oxygen and carbon dioxide concentrations (in form of dissolved inorganic carbon) were plotted over time. After determining the time point when equilibrium of the gas fluxes

was reached and respiration started to dominate in the chamber, a two-hour period was extracted from that time point onward. From those data sets the concentration change of O_2 and dissolved inorganic carbon (DIC) from zero were plotted against each other. We then used a linear model with a fixed intercept at zero to determine the slope as a measure of the RQ. We used zero as a fixed intercept, because we assumed bacterial respiration to start from zero once the gas fluxes were in equilibrium.

2.4 Bacterial production, functional capacity of the bacterioplankton community and BGE

For bacterial production, the ^{3}H leucine incorporation method was used as described by Tulonen (1993) and modified after Jansson et al. (1996). 1.2 ml of sample water were pipetted into Eppendorf tubes (three tubes per sample plus one control). Then 90 µl of 100% TCA were added to the control tube. After that, 50 µl leucine isotope (20x diluted) were added to all tubes and left to incubate for one hour at in-situ water temperature. The incubation was stopped by adding 90 µl 100% TCA to each tube except the control.

In the lab, samples were centrifuged at 14,000 rpm for 10 minutes and the supernatant sucked out. After that, to get rid of excess isotope, 1.2 ml of 5% TCA was added to the samples, left for 30 minutes, centrifuged again and the TCA discarded. This procedure was repeated twice. After this rinsing, 1.2 ml of scintillation liquid (OptiPhase 3) was added to the samples. 3H activity was then measured on a Tri-Carb 2800 TR liquid scintillation analyser. The leucine incorporation was converted into carbon units according to Azam and Smith, assuming an isotopic dilution factor of 2, using a specific activity of 112 Ci/mmol for leucine and accounting for a counter efficiency of 0.71771 for the scintillation analyser.

In addition to BP, a standard microplate (Biolog EcoPlate) was prepared to observe the capacity of the bacterioplankton community to degrade 31 different substrates (each substrate was replicated three times). Each substrate contains a tetrazolium dye which develops colour while the bacterioplankton respire the substrates (Garland and Mills 1991). 150 µl of sample were added to each substrate well on the microplate and the development of colour measured in a microplate reader (Biolog MicroStation) every one to three days. From the colour development of each well, we calculated the mean for the entire microplate and stopped the measurements once 0.5 was exceeded.

The BGE was calculated according to del Giorgio and Cole (1998) with equation 1

$$BGE = \frac{\mathrm{BP}}{\mathrm{BP} + \mathrm{BR}} \qquad \text{(equation 1),}$$

BP=bacterial production

BR=bacterial respiration

For BR, the measured carbon dioxide values over two hours were used, converted to dissolved inorganic carbon (DIC) and the hourly average used for the BGE calculation.

2.5 Sample analysis

Water samples for analysis were taken with a Ruttner sampler at 1 and 10 metres depth. At least four litres of water were sampled for each depth and later stored in a cool room (4 degrees).

2.5.1 Alkalinity

To calculate alkalinity for each sampling depth, titration was used according to the method described by Snoeyink and Jenkins (1980), though with a different acid concentration and without colour indicator. 0.1 M HCl from a hand pipette was added to 100 ml unfiltered sample water until the pH of the sample water reached 4.5 (range: 4.4-4.5). The sample water was constantly stirred by a magnet stirrer and pH and temperature monitored with a pH 211 Microprocessor pH Meter (Hanna Instruments). This procedure was conducted three times for each sample and the mean result used to calculate the total alkalinity.

$$A = \frac{\mathrm{V(HCl)}\ c(HCl) 1000}{\mathrm{V(sample)}} \qquad \text{(equation 2)}$$

V = volume [ml]
c = concentration [M]
A = alkalinity (mek/L)

2.5.2 Chlorophyll a and particulate organic carbon

For chlorophyll and particulate organic carbon (POC) measurements, 1 litre of sample water was filtered with a GF/F Whatman 0.7 µm filter and the filter frozen for storage. For samples 4, 5, 6 and 8 it was not possible to filter 1 L per filter; instead, the following amounts were filtered: 0.4 and 0.6 L for sample 4 (1 and 10 metres respectively), 0.8 and 0.7 L for sample 5 (1 and 10m respectively), 0.75 L for sample 6 (both depths) and 0.85 L for sample 8 (both depths). After freeze-drying the filters, the ones for chlorophyll-a measurements were sent to Umeå University for analysis, the ones for POC measurements to Hatch Stable Isotope Laboratory at the University of Ottawa for preliminary %C and ^{13}C isotope analysis.

For chlorophyll a analysis, extractions were made from the filter samples in 50 ml EtOH 95% in darkness over 24 hours and afterwards diluted. Pheophytins were then measured by adding 0.008M HCL, left standing for one hour and measured again (Parker et al. 2016) on a Perkin Elmer LS55 (excitation wavelength: 433 nm; emission: 673 nm).

For POC analysis, a sample from the filter was punched out (diameter: 6.2 mm) and weighed into tin capsules. The capsules were then loaded with standards into on Isotope Cube elemental analyser from "elementar" (Germany). The samples were flash combusted with oxygen at about 1800 degrees and carried by helium through columns of reducing and oxidising chemicals to extract N_2, CO_2 and SO_2. Those gases were separated by "trap and purge" of specific adsorption columns in order for the TCD (thermal conductivity detector) to detect each gas separately. The routine analytical precision (2sigma) is +/- 0.1%.

2.5.3 Phosphorus and nitrogen

Sampling water was filtered through a 0.45 µm syringe filter in the field and one 40 ml sample per sampling and depth were acidified and sent to the Erken laboratory at Uppsala University. There, total phosphorus, (soluble reactive) phosphate, total nitrogen, nitrite/nitrate and ammonium concentrations were analysed using continuous flow analysis (CFA) as first described by Skeggs (1957). All samples were run on a SEAL AutoAnalyzer and according to own protocols developed by Erken laboratory; for specific methods and measurement precision, see Appendix, Table S1.

2.5.4 DOC concentration and quality

To analyse the DOC-content, sampling water was filtered through a 0.45 µm syringe filter in the field and two 40 ml filtered samples per sampling and depth were sent to the Hatch stable isotope laboratory (University of Ottawa). The water samples were run on an OI Analytical "TIC-TOC" Analyser (Aurora Model 1030) to determine the organic and inorganic carbon concentration (ppm) and the C13 isotope according to St.-Jean (2003). The TIC-TOC analyser was interfaced to a Finnigan Mat DeltaPlus XP isotope ratio mass spectrometer for analysis by continuous flow. To normalise the data, an internal standard was used. The 2-sigma analytical precision is 2% for the quantitative and +/- 0.2 permil for the isotopes.

To assess the quality of DOC filtered water from the amber vials was used. Absorbance was measured in the lab within the spectrum of 200-800 nm on a UV-2600 Spectrophotometer (Shimazu) with a Beckman cuvette. From those measurements, values for A(254/365) and SUVA(254) were calculated.

$$A(\frac{254}{365}) = \frac{\mathrm{A}(254)}{\mathrm{A}(365)}$$ (equation 3)

$$SUVA(254) = \frac{\mathrm{A}(254)}{\mathrm{DOC}}$$ (equation 4)

A(254) = absorbance at 254 nm
A(365) = absorbance at 365 nm
DOC = dissolved inorganic carbon [mg/L]

2.6 Statistical analysis

Our collected data consisted of two relatively small samples with at most 8 data points: measurements of variables at 1 and 10 metre depth. Differences of variables between depths were analysed with Student's paired t-test, since each observation had a counterpart in the other sample. The distribution of the differences between samples were checked for normality with the Shapiro test (see Appendix, Table S2). If normality was not observed, a paired Wilcox test was used instead. To assess possible relationships between variables and their strength, Pearson coefficients and linear regression analysis was used. The residuals of the regression analysis were tested for normality with the Shapiro test.

The EcoPlate data consisted of multivariate data, so principal component analysis (PCA) was used to explore the data and reduce the 31 measured variables to a few key variables (principal components). As pre-treatment, the data was centered and scaled with the use of z-values. The resulting two main principal components were then used in paired t-tests and linear regression with other variables. All statistical tests were conducted with R.

3 Results

3.1 Ecosystem dynamics

All measured physical, chemical and biological lake factors were similar to earlier studies conducted at Lake Vombjsön (Gelin 1975, Ekologgruppen i Landskrona AB 2012). Temperatures over the summer varied between 16.74 and 21.92 degrees and either stayed relatively constant throughout the water column or declined slowly from the lake surface to the lake bottom (see Appendix, Fig. S2). Oxygen concentrations in the water displayed a similar pattern to temperature, as they declined with increasing depth. Bottom concentrations were usually between 3-6 mg/L except for 28/7/2016 when oxygen was almost completely depleted (0.57 mg/L). The pH was very high throughout the sampling period (8.68 on average) and always varied between 1 and 10 m depth (see Table 3). Alkalinity was high as well, ranging from 2.23 to 3.1 meq/L. It showed no discernible pattern between different depths and corresponding pH values.

Secchi depth was ranged between 2.6 and 0.8 metres over the summer, being lowest in the middle in the summer (see Table 3). Secchi depth corresponded to chlorophyll a concentrations which increased and decreased in similar fashion as the secchi depth (linear regression: R^2=0.5, p-value=0.002**). Chlorophyll a concentrations were always higher at 1 metre depth compared to 10 metre depth (Wilcox test, p-value = 0.004), as were POC concentrations (paired t-test, p-value = 0.01). The POC concentrations were positively coupled to chlorophyll a concentrations (linear regression: R^2=0.77, p-value <0.001***).

Total phosphorus concentrations started out at low levels (3-24 µg/L in June and July) and then increased rapidly during August and September to up to 130 µg/L in (see Table 3). Phosphate followed a similar trend and continuously became a bigger part of the total phosphorus concentration over the summer from roughly 20 per cent in June to 70 per cent in September. Between sampling depths, phosphate concentrations did not vary; total phosphorus concentrations showed differences, being higher at 1 metre compared to 10 m depth (paired t-test, p-value = 0.01).

Opposed to total phosphorus concentrations, the total nitrogen concentrations were at a very high level at the beginning of summer in June (up to 2650 µg/L), then continuously decreased until Mid-July to around 840 µg/L and then stayed in the range between 570 - 820 µg/L for the rest of the summer (see Table 3). The concentrations between 1 and 10 m depth were at similar levels. Nitrite and nitrate concentrations displayed a similar pattern as total nitrogen: they started relatively high (1462 and 1899 µg/L at 1 and 10 m depth respectively) and then decreased steadily until they stabilised between roughly 10 - 60 µg/L from August onwards.

Table 3: Different physical, chemical and biological factors at Lake Vombsjön during summer 2016 at 1 and 10 metre sampling depth. Chl a = chlorophyll a; POC = particulate organic carbon; TP = total phosphorus; PO_4 = phosphate; TN = total nitrogen; NO = nitrate/nitrite; NH_4 = ammonium; DOC = dissolved organic carbon.

Sampling date	depth [m]	pH	alkalinity [meq/L]	chl a [µg/L]	POC [µg/L]	TP [µg/L]	PO_4 [µg/L]	TN [µg/L]	NO [µg/L]	NH_4 [µg/L]	DOC [mg/L]	A(254/365)	SUVA(254)
16/6/2016	1	8.79	2.56	4	28	4	0	2651	1899	35	6.4	7.91	2.74
30/6/2016	1	8.92	3.10	12	53	6	1	2084	1387	9	6.3	7.52	2.74
18/7/2016	1	8.96	2.68	32	52	4	1	1216	680	6	6.3	7.25	2.78
28/7/2016	1	9.31	2.19	60	139	13	4	638	52	8	6.5	7.43	2.63
8/8/2016	1	8.29	2.45	23	64	65	42	826	34	213	6.7	8.33	2.25
26/8/2016	1	9.43	2.23	24	104	63	27	568	13	5	6.5	7.58	2.21
8/9/2016	1	8.62	2.42	30	79	126	83	715	56	58	7.5	8.44	2.02
29/9/2016	1	8.18	2.43	21	62	129	93	725	83	77	7.9	8.53	1.84
16/6/2016	10	8.83	2.45	2	22	11	2	2190	1462	44	6.3	8.09	2.82
30/6/2016	10	8.87	3.10	6	37	3	1	2089	1443	15	6.4	7.43	2.69
18/7/2016	10	9.00	2.73	30	54	5	5	1223	691	10	6.4	7.77	2.67
28/7/2016	10	8.19	2.67	19	54	24	13	1138	330	220	6.3	7.86	2.74
8/8/2016	10	8.21	2.40	17	52	75	40	856	20	236	6.6	8.00	2.17
26/8/2016	10	9.10	2.25	22	75	75	38	579	7	7	6.6	8.05	2.32
8/9/2016	10	8.20	2.42	1	16	131	83	824	63	174	7.0	8.50	2.19
29/9/2016	10	8.11	2.40	14	38	130	92	721	97	72	7.0	8.71	2.11

Ammonium concentrations increased rapidly from almost 0 to 235 µg/L on 8/8/2016 and then ranged between 57 - 173 µg/L during September, being higher at 10 metres compared to 1 metre depth (Wilcox test, p-value = 0.02). It made up only a small part of the total nitrogen concentrations initially (0.4 - 2 per cent during June), but increased during the rest of the summer (8 - 25 per cent). During June and the beginning of July, there was between 40 to 150 times as much nitrate and nitrite than ammonium, but from 28/7/2016, the ratio became almost 1:1.

DOC increased throughout the summer from 6.4 to 7.8 mg/L (see Table 3). The two characteristics of DOC we assessed, the A(254/365) ratio and the SUVA(254), changed over the summer and with changing DOC concentrations. The A(254/365) ratio increased with increasing DOC concentrations from 7.5 to 8.5 (linear regression: R2=0.55, p-value=0.001***) and the SUVA(254) decreased with increasing DOC concentrations (linear regression: R2=0.76, p-value<0.001***).

Table 4: Bacterial production (BP) and the time it took bacteria to degrade substrates on the EcoPlates until a colour development of 0.5 on average.

Depth [m]	Sampling	BP [µgC/d/L]	time on microplates [d]
1	16/6/2016	14.614	46
1	30/6/2016	17.298	33
1	18/7/2016	54.939	15
1	28/7/2016	65.351	36
1	8/8/2016	61.266	22
1	26/8/2016	44.103	18
1	8/9/2016	25.965	5
1	29/9/2016	21.647	5
10	16/6/2016	13.252	46
10	30/6/2016	14.040	33
10	18/7/2016	46.765	15
10	28/7/2016	65.996	13
10	8/8/2016	55.579	19
10	26/8/2016	10.568	7
10	8/9/2016	14.848	5
10	29/9/2016	22.493	5

Bacterial production started out at around 20 µgC/d/L in June, increased to 40-70 µgC/d/L in July and the beginning of August and then decreased again to around 20 µgC/d/L as in the beginning of the summer (see Table 4). It was always higher at 1 metre depth compared to 10 m depth (Wilcox test, p-value = 0.02). It took bacteria a long time to degrade substances in the different wells in the microplates in June (46 and 33 days), while afterwards the time became shorter (15 - 22 days in July and August, 5 days in September). There was no time difference between depths except for 28/7/2016, 8/8/2016 and 26/8/2016 when the microplates with bacteria from 1 metre depth took longer to reach 0.5 than the ones from 10 metre depth. Regarding the interaction of BP with organic matter pools in the lake, there were no interactions with DOC concentrations (linear regression, R^2=0.06, p-value=0.38), but weak, positive relationships with both chlorophyll a (linear regression: R2=0.43, p-value=0.006***) and POC concentrations (linear regression: R^2=0.39, p-value=0.04).

Looking at the EcoPlates (see Fig. 2), it becomes clear that not all substrates were mineralised by bacteria with the same intensity. Sugars, alcohols and amines were the substrates with the highest average values for colour development, while organic acids and polymers almost always exhibit comparatively low values.

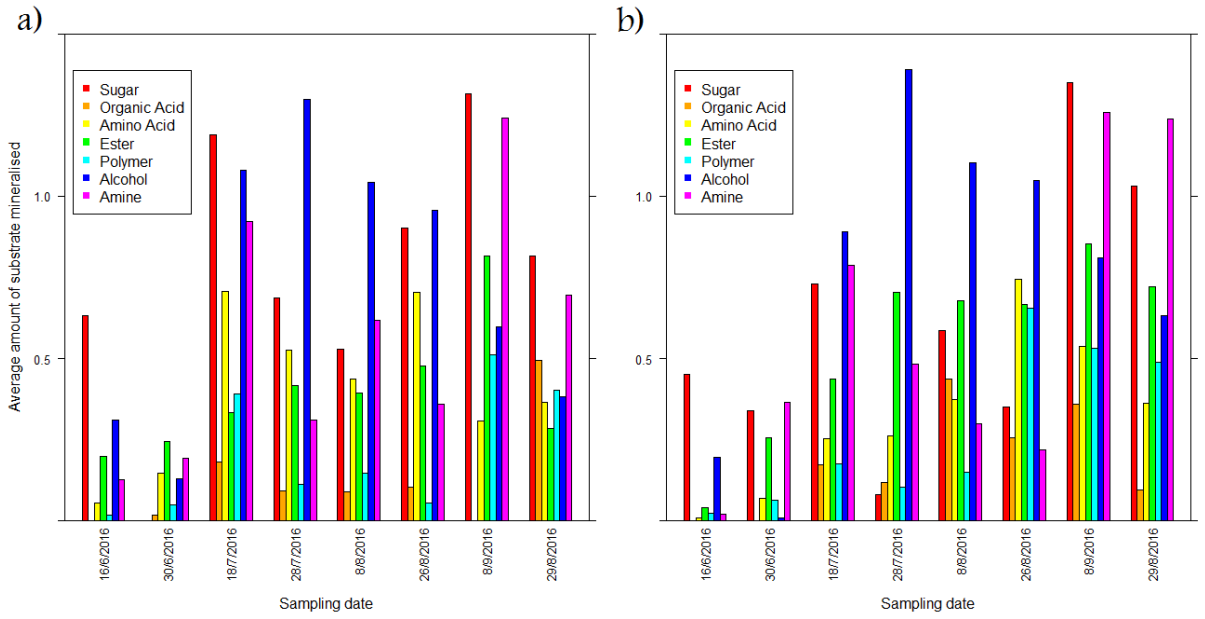

Fig. 2: Average amount of substrate groups mineralised by bacteria in the EcoPlate samples at 1 metre (a) and 10 metre (b) depth. The different substrate groups are sugars, organic acids, amino acids, esters, polymers, alcohol and amines. The number of substrates in each group are 6, 9, 6, 2, 4, 2 and 2 respectively. The samples from 16/6/2016 and 30/6/2016 never reached an overall average of 0.5.

3.2 Magnitude and variability of the RQ

After plotting the changes in O_2 concentrations against DIC concentrations, we could see that O_2 concentrations decreased and DIC concentrations increased during all measurements (see Fig. 3). All in all, the linear model proved a reasonable fit for most of our samplings, with R^2-values between 0.72 and 0.99 (see Table 5). When R^2-values were below 0.9, other factors than change in oxygen concentrations might have influenced the DIC concentrations during those times. Other than that, the linear regression appeared to be a good fit, even though our data did not fulfil all the requirements for linear regression analysis (normal distribution of residuals and homodescity).

Table 5: Regression analysis for RQ calculations. The RQ is represented by the slope of the linear relationship between change in O_2 and DIC concentrations [µmol/L]. The intercept is fixed at zero, assuming that bacterial respiration started from zero after equilibrium between the gas fluxes was reached in the underwater chamber.

Sampling	Depth [m]	p-value (regression line)	slope	confidence interval (slope)		R square	Shapiro test (residuals)
16/6/2016	1	< 0.001	0.44	0.44	0.45	0.99	0.67
16/6/2016	10	< 0.001	0.93	0.87	0.99	0.82	0
18/7/2016	1	< 0.001	1.47	1.16	1.79	0.73	0.22
18/7/2016	10	< 0.001	1.56	1.27	1.85	0.73	0.46
28/7/2016	1	< 0.001	0.13	0.13	0.13	0.99	0.33
8/8/2016	1	< 0.001	0.41	0.37	0.46	0.94	0.02
26/8/2016	1	< 0.001	0.32	0.33	0.33	0.99	0.39
26/8/2016	10	< 0.001	0.49	0.48	0.51	0.97	0.41
8/9/2016	1	< 0.001	0.28	0.28	0.29	0.99	0.95
8/9/2016	10	< 0.001	0.39	0.37	0.4	0.97	0
29/9/2016	10	< 0.001	0.44	0.42	0.46	0.94	0

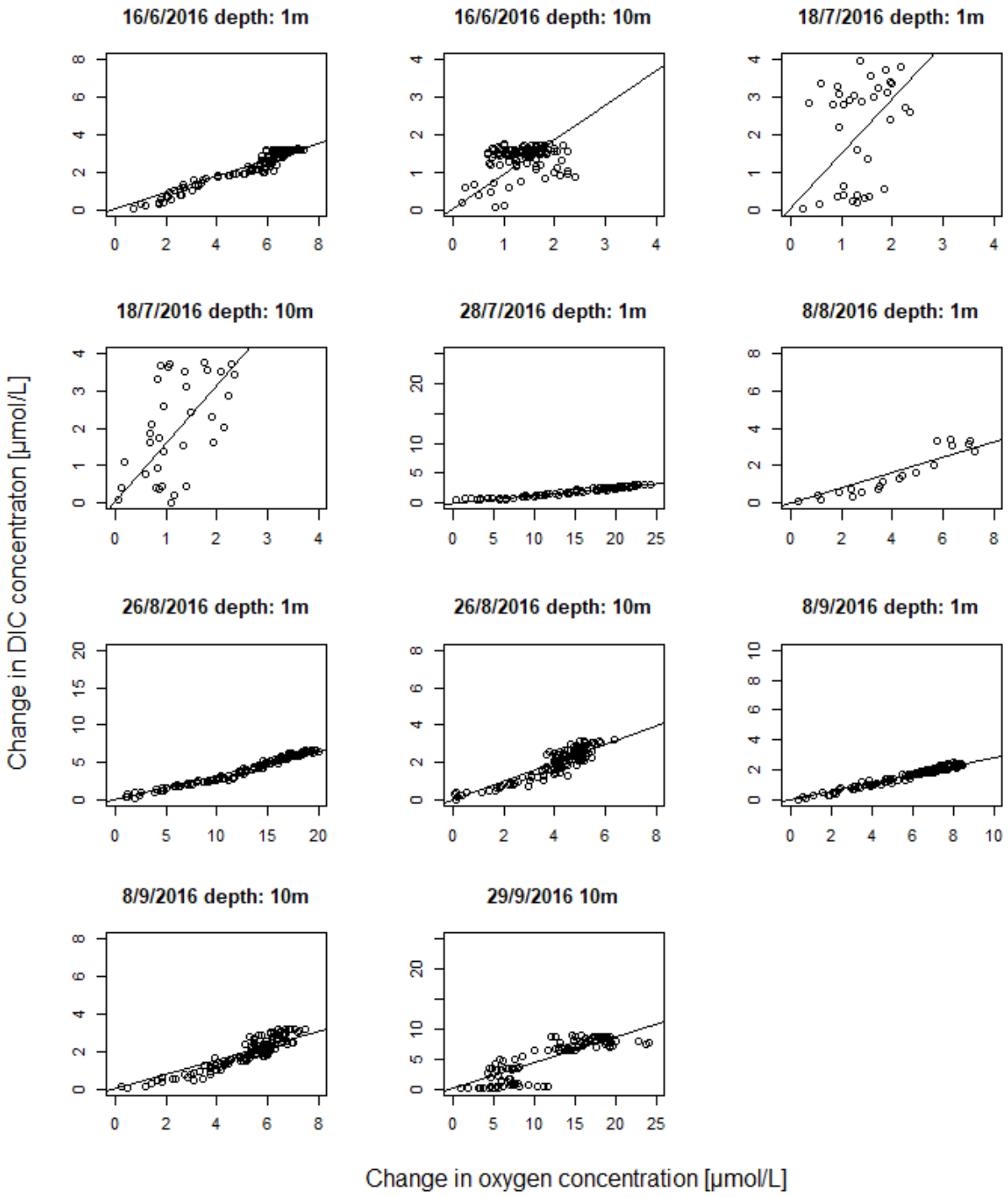

Fig. 3: Changes in oxygen concentrations [µmol/L] plotted against changes in DIC concentrations [µmol/L] in the underwater chamber during summer 2016. A linear regression line was fixed through the data cloud with a fixed intercept at 0; the slope of the regression line denotes the RQ. Data points: 120, except for 18/7/2016 (35 data points), 28/7/2016 (80 data points) and 8/8/2016 (20 data points). 29/9/2016 10 m was an open chamber measurement.

During most samplings, we measured RQs < 1, as we expected, but in two cases the RQs were > 1 and in one almost 1 (see Fig. 4). When we measured over 120 minutes, the RQs varied between 0.28 and 0.5, except for the RQ at 1 metre depth during 16/6/2016 which was 0.93. On 18/7/2016, the RQs for 1 and 10 m depth were much higher (1.47 and 1.56

respectively), though the measurement period was only ¼ of what we measured during other samplings.

In contrast, the 80-minute measurement on 28/7/2016 was extremely low (0.12). The other shorter measurement period of 20 minutes on 28/7/2016 yielded a similar RQ to 16/6/2016 at the same water depth. The open chamber measurement we conducted on 29/8/2016 showed a similar RQ to the two samplings at the same depth before, indicating that our closed chamber measurement mirrors the conditions in the lake relatively well. All in all, even though we often observed RQs < 1, they were much lower than any reported or assumed RQs in the literature.

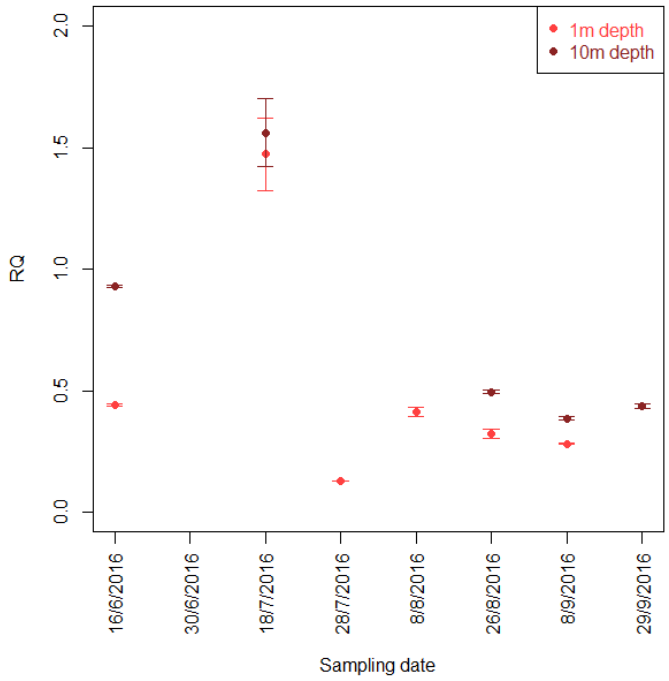

Fig. 4: Respiratory quotients (RQ) at Lake Vombsjön during summer 2016. The RQs and their standard errors are calculated from linear regression models with 0 as a fixed intercept. There are seven RQ values for 1 metre depth and five RQ values for 10 metre depth.

While we expected to observe higher RQs at the water surface than deeper in the water column, the opposite proved to be true: the RQs at 1 metre depth were always lower than the corresponding ones at 10 metre depth, although not significantly (paired t-test, p-value = 0.1). When we had RQs for both 1 and 10 metre depth at the same sampling, they did not differ very much between each other during 18/7/2016 and 8/9/2016 (0.09 - 0.1 difference), while the difference was larger at 26/8/2016 (1.7) and largest at 16/6/2016 (0.48). Our hypothesis that the RQ is higher near the water surface due to more photochemically broken-down DOC available for bacteria does not seem to hold true.

3.3 Magnitude and variability of the BGE

The BGE at Vombsjön varied between 0.15 and 0.5 (see Fig. 5). The BGE was quite stable at 10 metre depth, varying between 0.2 and 0.3, while the values for 1 metre depth showcased both the minimum (0.15) and the maximum value (0.5). In all but one case (16/6/2016), the BGE was higher at 1 metre compared to 10 metre depth, the opposite of what we hypothesised. The difference between depths during the same sampling was at least 0.1 except for 18/7/2016 where the BGE values were almost identical. Even though we could observe this difference in the data, it was not on a significant level (paired t-test, p-value = 0.43).

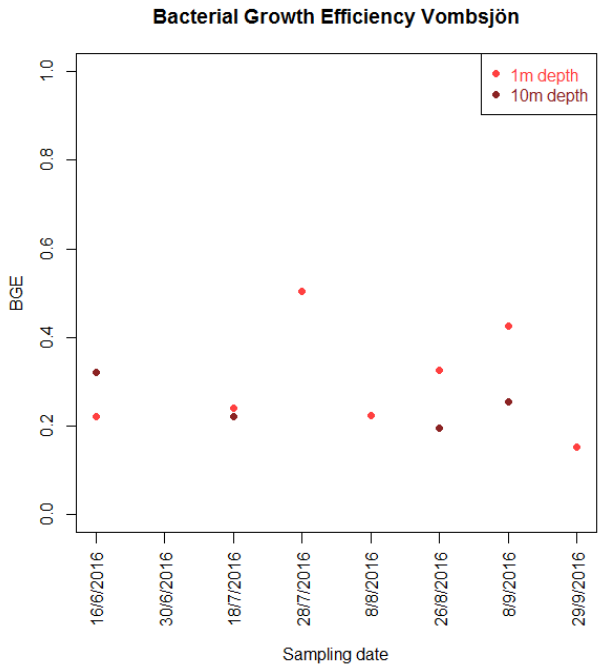

Fig. 5: Bacterial Growth Efficiency (BGE) at Lake Vombsjön during summer 2016. There are five values for 10 metre and seven values for 1 metre depth.

When comparing RQ and BGE with each other, there was no discernible pattern (see Fig. 6) While the highest BGE corresponded to the lowest RQ observed (28/7/2016), this was not the case the other way around. The highest RQ was observed at BGE 0.2 (18/7/2016), which was a similar BGE to when RQ was only 0.5 (8/8/2016 and 26/8/2016). The lowest BGE at 0.15 corresponded to an RQ of approximately 0.5 as well (29/9/2016). Our hypothesis that RQ and BGE would have a negative relationship with each other was thus not supported by our observations.

Statistical analysis revealed a negative correlation between RQ and BGE (Pearson coefficient=-0.37), but linear regression analysis showed only a very weak coupling (R^2=0.13, p-value=0.27). Furthermore, the same DIC consumption values were used for RQ and BGE calculations, meaning that any correlations between the two could be spurious correlations.

Using DIC consumption values based on fixed alkalinity did not improve the robustness of the statistical analysis.

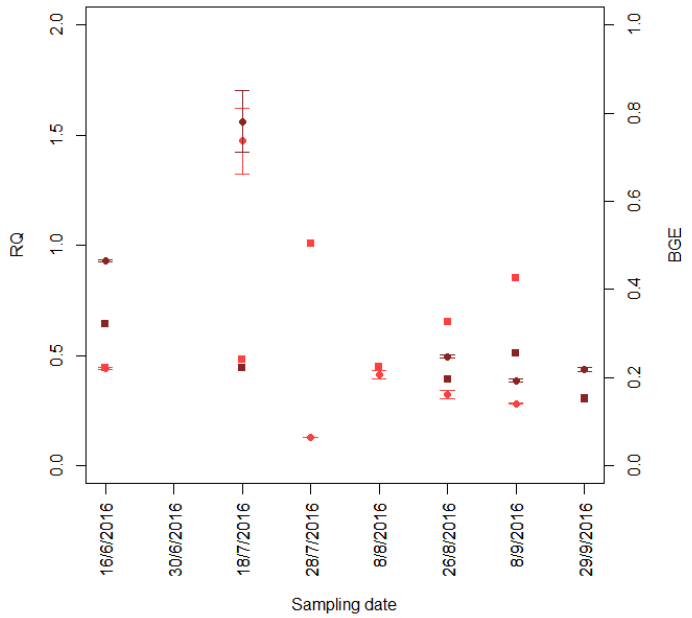

Fig. 6: The RQ (including error bars) and BGE at the same samplings. Red colour denotes values measured at 1 metre depth and brown at 10 metre depth. Circles represent RQs and squares BGEs. For the BGE, there are no standard errors.

3.4 Regulation of the RQ and BGE

Statistical analysis of correlations and following linear regression analysis revealed no strong connection between RQ or BGE with any one measured variable, but two trends (see Table 6). The BGE showed weak positive coupling to POC and chlorophyll a concentrations, and the change in oxygen concentration showed the same weak coupling to POC. RQ did not exhibit any obvious relationships to nutrient concentrations, primary production indicators or bacterial activity. When looking at fractions of the nitrogen concentrations, it was weakly negatively connected to the amount of DIN of TN [%]. The variable showing the most correlation to RQ was the $NO:NH_4$ ratio: the higher this ratio, the higher the observed measured of RQ had been. SUVA(254) also showed a weak positive coupling to RQ, but exhibited a very high standard error (43.5) compared to DIN of TN [%] and $NO:NH_4$ ratio (0.002) and a p-value exactly on the threshold (0.053), which is why it is not considered statistically robust in this context.

No other process related to respiration showed a coupling to those variables. Both DIC production and O_2 consumption were positively correlated with BP and O_2 consumption with POC concentrations, but since the focus of this thesis is on RQ and BGE, those results are not

discussed further. DIC production and O2 consumption also showed insignificant correlations in different direction for the same variable, which we assumed to be a result of the scattering of the data points.

Table 6: Correlation matrix of linear regression results for RQ, BGE, DIC and O with POC, chlorophyll a, DOC, TP, PO_4, TN, NO, NH_4, DIN [%], NO:NH_4 ratio, BP, bacteria time, PC1 and PC2. (+) indicates a positive and (-) a negative relationship, the displayed number is the R^2-value and significant p-values are denoted by *** (0), ** (0.001), * (0.1), and . (0.05). Abbreviations stand for: RQ=respiratory quotient, BGE=bacterial growth efficiency, DIC=change in DIC concentrations (1 hour), O=chance in O_2 concentrations (1 hour), POC=particulate organic carbon, DOC=dissolved organic carbon, TP=total phosphorus, PO_4=phosphate, TN=total nitrogen, NO=nitrate/nitrite, NH_4=ammonium, DIN [%]=percentage of dissolved inorganic nitrogen of total nitrogen, NO:NH_4 ratio= nitrate/nitrite to ammonium ratio, BP=bacterial production, bacteria time=time for bacteria to reduce substrates on EcoPlate to an average of 0.5, PC1=date score of principal component 1, PC2=date score of principal component 2.

Variable	RQ	BGE	DIC production	O^2 consumption
POC	(-) 0.13	(+) 0.44 (*)	(+) 0	(+) 0.34 (.)
chlorophyll a	(-) 0	(+) 0.35 (.)	(+) 0.06	(+) 0.26
DOC	(-) 0.26	(+) 0.03	(-) 0.05	(+) 0
TP	(-) 0.29	(-) 0.01	(-) 0.03	(+) 0
PO_4	(-) 0.22	(-) 0.02	(-) 0.01	(+) 0
TN	(+) 0.1	(-) 0.03	(-) 0.04	(-) 0.19
NO	(+) 0.15	(-) 0.03	(-) 0.03	(-) 0.23
NH_4	(-) 0.09	(-) 0.05	(+) 0.09	(+) 0.14
DIN [%]	(-) 0.41 (*)	(+) 0.1	(-) 0.02	(+) 0.21
NO:NH_4 ratio	(+) 0.73 (***)	(-) 0.05	(+) 0.11	(-) 0.2
BP	(+) 0.02	(+) 0.11	(+) 0.47 (*)	(+) 0.47 (*)
bacteria time	(-) 0	(+) 0.08	(-) 0.03	(+) 0.01
SUVA(254)	(+) 0.36 (.)	(+) 0	(+) 0	(-) 0.07
A(254/365)	(-) 0.15	(-) 0.1	(-) 0.02	(-) 0
PC1	(-) 0.01	(-) 0	(+) 0	(-) 0
PC2	(-) 0	(+) 0.04	(+) 0.04	(+) 0.15

We also performed principal component analysis (PCA) on the EcoPlate data to assess whether the functional capacity of the bacterial community had any influence on the RQ. We found that the new principal components 1 (PC1) and 2 (PC2) explained 73.4 % and 81.3 %

of the total variation in the EcoPlate data for 1 m and 10 m depth respectively. PC1 showed a positive loading to all substrates, especially sugars, alcohols and organic acids, while PC2 showed a positive loading with amino acids and polymers and weaker, negative loading with all other substrates (see Fig. 7). Amines and esters did not contribute much to PC1 and PC2; their arrows in the biplots also stay closer to the centre than all other substrates, meaning that it is difficult to interpret their contribution to the PCs. When viewing all microplate data in a PCA together (see Appendix, Fig. S3, Table S5), the contribution of the less influential substances (esters, amines) to the PCs changed, but the main contributors remained the same (sugars and alcohols for PC1 and polymers and amino acids for PC2).

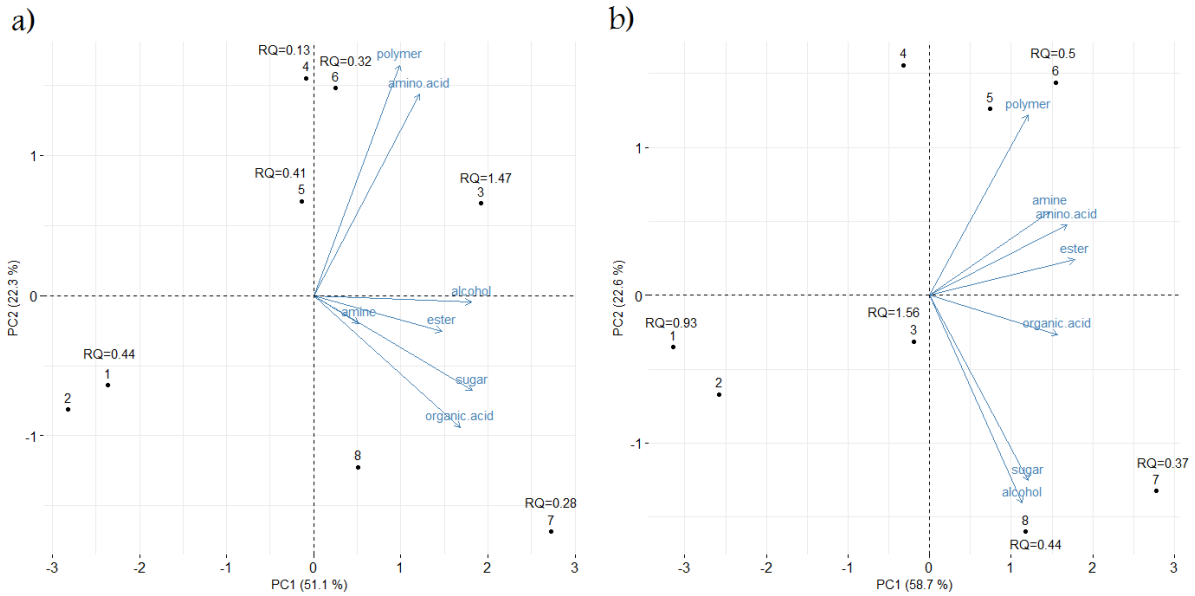

Fig 7: Capacity of bacterioplankton community to degrade algal biomass (PC2) and reduced substances (PC1) over the summer at 1 metre (a) and 10 metre depth (b). Numbers stand for the following: 1=16/6/2016, 2=30/6/2016, 3=18/7/2016, 4=28/7/2016, 5=8/8/2016, 6=26/8/2016, 7=8/9/2016, 8=29/9/2016. Samples with measured RQs feature those RQs above the number. PC1 = principal component 1, PC2 = principal component 2.

Samples for 16/6/2016, 30/6/2016 and 8/9/2016 exhibited the highest scores with PC1 and samples for 28/7/2016 and 26/8/2016 with PC2 (for scores, see appendix, Table S3 and S4). The other three samples (18/7/2016,8/8/2016, 29/9/2016) either scored high on PC1 and PC2 or were sometimes not well explained by any of the two (8/8/2016 at 1 metre depth and 18/7/2016 at 10 metre depth). Comparing the PC1 and PC2 scores to RQ values at the same samplings revealed no relationship (see Table 6); low and RQs occurred at high and low scores for both PCs.

3.5 Sampling method

The underwater chamber set-up was in general able to record O_2 and CO_2 concentrations reliably, but had some issues with leakage and pump function on several occasions (see Table

7). When the set-up was running without problems, we could always observe O_2 concentrations to fall and CO_2 concentrations to increase within the measurement period of 2:30 hours. On occasions of leakage this trend was reversed, showing a decrease of CO_2 and/or an increase of O_2, depending on the size of the leak. The two kinds of leakage we experienced were caused by a fissure in the gas exchange membrane and improperly fixed tubing to the EGM 5. When the pump did not work properly, it usually stopped pumping for extended periods of time, which are visible as spikes in the concentration changes. The open chamber measurement from sample 8 shows abnormal fluctuations over time as well, but this can be attributed to water mixing in the lake and not to leakage or pump problems. Samples from 16/6/2016, 26/8/2016, 8/9/2016 and 29/9/2016 (open chamber measurement) returned usable data points for 120 minutes each (see Table 7). For sample 18/7/2016, 35 minutes of data points were obtained, 80 minutes for sample 28/7/2016 for 1 m depth and 20 minutes at sample 8/8/2016 for 1 m depth.

Table 7: Overview of problems encountered during samplings with the underwater chamber set-up. When samples returned a period of zero minutes for usable data points, all measurement data was unusable and no RQs were calculated for those. For sample 3 (10 metre depth), there is one missing value in the data set.

Date	Problems with underwater chamber set-up	Period of usable data points [minute]
16/6/2016	none	120
30/6/2016	Pump stopped working	0
18/7/2016	Pump stopped working	35
28/7/2016	Pump stopped working (1 metre) Leakage from gas exchange membrane (10 metre)	80 (1 metre depth)
8/8/2016	Leakage from gas exchange membrane	20 (1 metre depth)
26/8/2016	none	120
8/9/2016	none	120
29/9/2016	Leakage between tubing and EGM 5 (closed chamber)	120 (open chamber measurement)

4 Discussion

4.1 Ecosystem dynamics

Lake Vombsjön was highly productive throughout the summer and the bacterial production showed a weak positive relationship to both POC and chlorophyll a, indicating that the BP was driven by autochthonous organic matter rather than allochthonous DOC (Cole et al. 1988). This may explain why DOC and its low molecular weight fraction remain in the water and increase over the summer due to photochemical degradation and continuous circulation.

Out of all measured nutrient concentrations, nitrogen, and within the nitrogen fraction, NH_4, showed the most variation over summer. This may indicate that nitrogen-related processes happen more frequently and become more important to lake metabolism, especially in August and September, like bacteria mineralising NH_4 from sedimentating, dead organic matter (Verdouw and Dekker 1982) or NH_4-release from the sediment under anoxic conditions.

4.2 Magnitude, variability and regulation of the RQ

During most of the summer, Lake Vombsjön exhibits RQ <1 which was likely caused by the high productivity of the lake. Lake Vombsjön has a long history of eutrophication and usually has multiple algae blooms during summer, and our measurements of secchi depth, chlorophyll a, different nutrients and the oxygen profile supported this. In such a eutrophic state, a lot of available DOC is in a reduced state, meaning that bacteria need more oxygen to break it down, explaining why RQs are often <1. An example for this are lipids, like palmitic acid, which requires 23 O_2 for full oxygenation to CO_2 (RQ=0.7), as opposed to 6 O_2 for glucose (RQ=1) (see Table 1).

It should be noted that our calculated RQs are extremely low compared with the traditional assumption (RQ = 1) and values from the literature, where either values around 0.8 - 1.2 or much higher than 1 (1.2 - 4) are reported (Almeida et al. 2016, Jansson et al. 2007, Berggren et al. 2012). RQs as low as 0.5 are rare and only come up in context with eutrophic or hypertrophic lakes (Valdespino-Castillo et al. 2014, Burford and Longmore 2001), whereas RQs <0.5 are not recorded for any aquatic ecosystem. Possible explanations for such low RQs will be discussed in 4.2.1. Another point to keep in mind is that the confidence intervals for some of the RQs are large (see Table 7), which means that they are tricky to interpret and that any conclusions drawn from RQ results should be treated with care.

In comparison, all RQs >1 or close to 1 were measured before chlorophyll a and POC concentrations reached their maximum, which could mean that more photochemically

degraded DOC was used by bacterioplankton during that time and caused higher RQs. An example for this is glycolic acid, which requires 4.5 O_2 for complete oxidation to CO_2 (RQ=1.33), as opposed to 6 O_2 for glucose (RQ=1) (see Table 1). From the PCA analysis, we could observe that the capacity of the bacterioplankton community to degrade different groups of substances changed over the summer. The community went from very low activity to efficient use of algal material (PC2: polymers and amino acids) at the same time when we observed the first high chlorophyll a and POC concentrations, indicating that primary production stimulated bacterial activity during this time. Towards the end of the summer, there is a higher capacity to degrade reduced substances (PC1: alcohols, sugars and organic acids) which might point to more terrestrial and/or partly photochemically degraded DOC becoming a more important energy source again. The RQ does not increase during this period, however, so other processes likely have a bigger influence on them.

As the DOC concentrations did not appear to be a major driver of bacterioplankton metabolism, this might be why we did not observe higher RQs near the water surface than in the deeper water; photochemically degraded DOC compounds were likely not preferably used by bacterioplankton when easily degradable autochthonous DOC was available. Instead, higher RQs occurred in deeper water where respiration processes dominated, not primary production as in the surface water, and where less phytoplankton organic matter in form of chlorophyll a and POC was available for degradation.

4.2.1 Other metabolic processes regulating the RQ

There are several other metabolic processes that occur in lakes that are not accounted for in RQ calculations, but may explain the extremely low RQs that we observed. Two of those might be nitrification and denitrification processes in the water column and sediment. Studies on the RQ in soils and compost have shown that both nitrification and denitrification have the potential to lower the RQ (Dilly 2003, Tsutsui et al. 2015). Nitrification is the stepwise oxidation of NH_4^+ to NO_3^- (see equation 5 and 6). Since measuring O_2 and CO_2 concentrations in the water does not take the sources or consumers of those gases into account, observed RQs might be lowered by nitrification, if it occurs parallel to BR, because seemingly more O_2 is consumed than actually consumed by bacterioplankton during BR.

$2\ NH_4 + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+$ (equation 5)

$2\ NO_2^- + O_2 \rightarrow 2\ NO_3$- (equation 6)

Iriarte et al. (1996) also found that nitrification can increase the pelagic oxygen demand in estuaries, indicating that nitrification may have the same effect on RQ in water ecosystems as in soil or compost. Studies conducted with different kind of soils have reported drops in RQ

of 10-18 per cent (Müller et al. 2004, Dilly 2003) and Kortman et al. (2009) showed an decrease in RQ of 25 per cent that could be accounted to nitrification. In Lake Vombsjön, the NH_4 concentrations increased from 28/7/2016 onwards, the one time when the lake was stratified and experienced severe anoxia at 10 metres and below. That was also the sampling at which we observed the lowest RQ during the whole summer and after that stayed low for the rest of the summer; all RQs higher than 0.5 were measured before. The positive coupling of RQ to the NO:NH_4 ratio supports this, indicating that processes involving NH_4 become more dominant when RQ is low. Another contributing factor might the relatively high water temperature throughout the whole water column (between 17 and 22 degrees over the summer), which positively correlates with the rate of nitrification (Sweerts et al. 1991). So nitrification may lower the RQ by bacteria oxidising NH_4 to NO_3^-, removing oxygen from the water column without the amount of CO_2 increasing at the same rate.

Denitrification is the stepwise reduction of nitrate to nitrogen gas (equation 7) and has indirectly been linked to low RQs by Tsutsui et al. (2015). They have determined nitrification to be the factor driving low RQ, however, and denitrification only being a by-product of increased rates of nitrification, because more nitrate becomes available. Denitrification on its own increases the O_2 concentration in the water, meaning that the RQ increases in response to seemingly less O_2 used by bacterioplankton for BR. If denitrification occurs at Lake Vombsjön, it might thus concentrate to high RQs during 18/7/2016, when a lot of nitrate was present in the water column. If denitrification and nitrification occur at the same time (from 26/8/2016 onwards), the positive effects of denitrification on the RQ are likely less pronounced than the negative effects by nitrification.

$$2\ NO_3^- + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O \qquad \text{(equation 7)}$$

In the lake, denitrification is usually an anaerobic process and thus would not occur in a well-oxygenated lake like Lake Vombsjön; however, there are many nitrifiers found in wastewater treatment plants and natural systems that can use nitrate, nitrite, nitric oxide and nitrous oxide reductase for aerobic denitrification (Ji et al. 2015). Those aerobic denitrifiers can conduct denitrification in oxygen concentrations ranging from 3-5 mg/L, though some can do so under higher concentrations (6-10 mg/L) as well (Ji et al. 2015), which corresponds to the oxygen conditions in Lake Vombsjön.

Anaerobic denitrification can occur in cyanobacterial aggregates suspended in the water column in oxygen-rich waters, if they are >1mm in diameter (Klawonn et al. 2015), because micro-surfaces within the aggregates can become anoxic. So even though Lake Vombsjön exhibits high oxygen concentrations throughout the water column and especially near the lake surface, those anoxic environments may arise within the phytoplankton community. Denitrification rates are tied to the amount of organic matter available; for example,

McCrackin and Elsner (2012) observed an increase of denitrifiers in the lake sediment with higher amounts of organic matter and higher quality of organic matter. Thus, there can be rapid increases of denitrification rates when there is high organic matter input, like after an algal bloom (Wenk et al. 2016). Nitrite and nitrate concentrations have been high at Lake Vombsjön at the beginning of the summer and then there have been several algal blooms, both of which contribute to the rate of denitrification. The RQ was generally higher at higher nitrite and nitrate concentrations, which might indicate that denitrification contributed to higher RQs in the beginning of the summer.

Another oxygen-consuming process in the water column that we have not assessed is methane oxidation. The theoretical RQ for methane, a reduced organic compound, is 0.5 (see, Table 1, del Giorgio and Williams 2005), which roughly corresponds to the RQs that we observe at 10 metre depth. It might thus be possible that methane, in addition to nitrification, contributed to lowering the RQ to less than 0.5, the lowest theoretical possible RQ. Methane can be released from sediments under low oxygen concentrations, which happened at least once at Lake Vombsjön during the summer. Assessing the rate of methane production in the water parallel to respiration measurements might thus provide additional insight as to which processes control RQ.

4.3 Magnitude, variability and regulation of the BGE and relationship to RQ

The overall range of the BGE covered quite a wide spectrum, considering that the BGE varies in freshwaters ranges from 0.1 to 0.7 (Amado et al. 2013, Smith and Prairie 2004). The BGE increases with increasing productivity (del Giorgio and Cole 1998), so since Lake Vombsjön is a very productive lake, higher BGEs between 0.3 and 0.5 do not come as a surprise. Biddanda et al. 2001 have stated that the BGE increases with increasing chlorophyll a values, and although the relationship with chlorophyll a we observed was relatively weak, they may describe part of the BGE variations we observe, especially since POC exhibits the same relationship with BGE, but with a stronger coupling.

This might explain why our BGE values are usually higher at 1 metre compared to 10 metre depth, since primary production is higher close to the water surface where most light is available to phytoplankton. Organic matter excreted by phytoplankton is more available to bacteria there, and the high free energy yield from the complete oxygenation of e.g. palmitic acid (-9800 kJ) compared to glucose (-2872 kJ) contributes to the preferable breakdown of autochthonous carbon and its use for bacterial biomass growth. There are also some bacteria that exhibit higher BGE with increased light exposure (Goméz-Consarnau et al. 2007), so that might contribute to higher BGEs at 1 metre depth, though we cannot say that for sure as we do not know the bacterioplankton community structure.

When viewing BGE and RQ together, we observed the highest BGE and lowest RQ at the same time. In fact, all of the high BGEs fell in the period of the highest POC and chlorophyll a concentrations and often coincided with low RQs, which indicates that mostly reduced algal organic matter was available for bacteria, a situation favouring lower RQs. The highest RQs did not occur at the lowest BGE, however, so the kind and quality of substrate used by bacteria may not be the only factor influencing RQ and BGE patterns. The BGE may be influenced by changing nutrient concentrations and temperature (Smith and Prairie 2004, Biddanda et al. 2001, Lee et al. 2009), while RQ may be influenced by other oxidising and reducing processes in the water column. As the most important drivers of BGE and RQ may thus be very different, this might account for why we see no obvious relationship between the two variables.

4.4 Sampling method

After improving sampling routines and the set-up of the underwater chamber, all mechanical and leakage problems could be solved, as the undisturbed measurement results from the last three samplings show. For future samplings, a more suited pump is being considered, as well as the addition of flow monitors in the tubing to monitor the water circulation in tubes. Another point to keep in mind is to properly check the connection of the tubes between the gas exchange membrane and the EGM 5.

We also conducted a leak test in the laboratory during which we monitored CO_2 and O_2 measurements where we assumed high CO_2 concentrations (approximately 2000 ppm) and low O_2 concentrations (3-4 mg/L). For that, we placed the underwater chamber in a water-filled sink to simulate underwater conditions and used sampling water from lake Vombsjön (sample 8) in order to have the same alkaline conditions as in the field. The CO_2 concentrations in the sampling water were naturally very high (1900 to 2000 ppm); otherwise we would have added carbonised water to increase the CO_2 concentrations artificially. To reduce O_2 concentrations, we added N_2-gas to the circuit, but could not achieve low enough concentrations. Still, as CO_2 leaks easier as O_2 due to its molecular size, we can assume that if there is no leakage of CO_2, O_2 should not leak either. We also compared the change of CO_2 concentrations in the leak test with a leak test with tab water (0 ppm CO2) and concentrations changes (0-40 ppm CO2) we measured in the field (28/6/2016). After initial equilibration, CO_2 concentrations during the leak test with lake water fluctuated around an average value with +/- 2 ppm (see Appendix, Fig. S1), most of which could be attributed to the temperature difference between sampling water (10 degrees) and room temperature (22 degrees) and were not experienced during field measurements (water temperature 17-21 degrees, air temperature 20-23 degrees). We thus concluded that leakage from the chamber set-up was not an issue.

Both the plotted respiration data and the high R^2 values that we observed when assessing the RQ showed that we were successfully able to record bacterial respiration in the underwater chamber for a two-hour period during four samplings. Not all regression analyses were very robust, however, and transforming the data in various ways did not change this fact. Still, the number of sampling points for a two-hour measurement period should be high enough according to the Central Limit Theorem to still consider the linear regression model as an appropriate choice for our data. Regarding the RQs calculated from shorter time periods, they cannot be directly compared to the RQs from the 2-hour period, but they followed the same trend and the linear regression model is relatively robust for them. Still, ideally all RQs should be measured over the same time period so that the initial measurements do not influence the RQ overly much.

4.5 Future directions

It becomes clear that even with in-situ measurements of gas fluxes and empirical RQs, respiration processes in Lake Vombsjön are difficult to interpret and explain. This is especially due to processes outside of photosynthesis and bacterial respiration, like nitrification, denitrification and the release of additional electron acceptors from the sediment, all of which are difficult or time-consuming to quantify in-situ. Those factors have to be taken into account when interpreting resulting RQs from eutrophic systems like Lake Vombsjön in the future. This could be done by assessing the nitrification rate in the water or methane production from the sediment. Oligotrophic ecosystems might not present the same difficulties and could be assessed in the future to compare empirically derived RQs in different trophic systems. Another development could be to construct an automated sampling system to reduce the time-consuming sampling procedure and measure gas fluxes over a longer period of time.

5 Conclusions

Both the RQ and BGE were much more affected by primary production and the highly eutrophic state of Lake Vombsjön than DOC concentrations in the lake. No singular factor could explain the variability of the RQ and BGE over the summer and between water depths, however, which shows that the interactions of BR, BP and BGE are difficult to explain and are still not well understood in aquatic ecosystems.

Theoretical RQs do not take anaerobic respiration into account and assume that no additional electron acceptors, like NO_3 and Fe, occur in the water column. Our results indicate, however, that metabolic processes like nitrification, denitrification and methane oxidation play a bigger role than commonly assumed in the magnitude of the RQ in eutrophic and highly productive lakes like Lake Vombsjön and that they should be taken into account when assessing the magnitude of bacterial respiration in such an ecosystem.

Using a theoretical RQ of 1 to quantify bacterial respiration rates in an eutrophic lake system, like Lake Vombsjön, may overestimate bacterial respiration much more than previously believed. Since many northern, temperate lakes are eutrophic, their role in the global carbon cycle as sources of atmospheric carbon may be wrongly represented on a global scale. It is thus necessary to to gain deeper knowledge and understanding about how bacterioplankton metabolic processes work and interact in eutrophic lakes in order to assess their role in the carbon cycle to date and in a changing climate.

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7 Appendix

Table S1:
Overview of methods for continuous flow analysis (CFA) used by Erken laboratory at Uppsala University. Sample types 1, 2 and 3 stand for freshwater, marine and wastewater samples respectively. For more details about the different methods used, contact Helena Enderskog (Helena.Enderskog@ebc.uu.se)

Vattenanalyser SEAL (mätområde/mätosäkerhet)

Parameter	Analys id	Referens	Provtyp	Mätområde	Mätosäkerhet (ospädda prov)
Fosfatfosfor	EV 41	Method No G-175-96 Rev. 15 för AA3och SS-EN ISO 6878:2005 samt egen metodik	1,2,3	7-500µg/l	± 35 % vid 3-15 µg/l ± 25 % vid 15-100 µg/l ± 23 % vid 100-500 µg/l
Totalfosfor	EV 43	Method No G-297-03 Rev. 1 för AA3 och SS-EN ISO 6878:2005 samt egen metodik	1,2,3	11-1000 µg/l	± 54 % vid 3-15 µg/l ± 18 % vid 15-100 µg/l ± 13 % vid 100-1000 µg/l
Parameter	**Analys id**	**Referens**	**Provtyp**	**Mätområde**	**Mätosäkerhet (ospädda prov)**
Ammoniumkväve	EV 44	Method No G-171-96 Rev 15 för AA3 och SS EN ISO 11732:2005 utg. 1 samt egen metodik	1,2,3	12-500 µg/l	± 38 % vid 5-15 µg/l ± 18 % vid 15-100 µg/l ± 16 % vid 100-500 µg/l
Nitritkväve	EV 45	Method No. G-172-96 Rev. 12 och G-384-08 Rev. 2 för AA3 SS-EN ISO 13395 utg 1 samt egen metodik	1,2,3	4-50 µg/l	± 43 % vid 2,5- 7,5 µg/l ± 30 % vid 7,5- 25 µg/l ± 25 % vid 25-50 µg/l
Nitrit+Nitratkväve	EV 46	Method No G-384-08 Rev. 2 för AA3 och SS-EN ISO 13395 utg. 1 samt egen metodik	1,2,3	7-500 µg/l	± 35 % vid 5-15 µg/l ± 19 % vid 15-100 µg/l ± 17 % vid 100-500 µg/l
Totalkväve	EV 48	Method No G-384-08 Rev. 2 och G-172-96 Rev. 12 för AA3 SS-EN ISO 13395 utg. 1 SS-EN ISO 11905-1	1,2,3	125-2000 µg/l	± 15 % vid 400-2000 µg/l

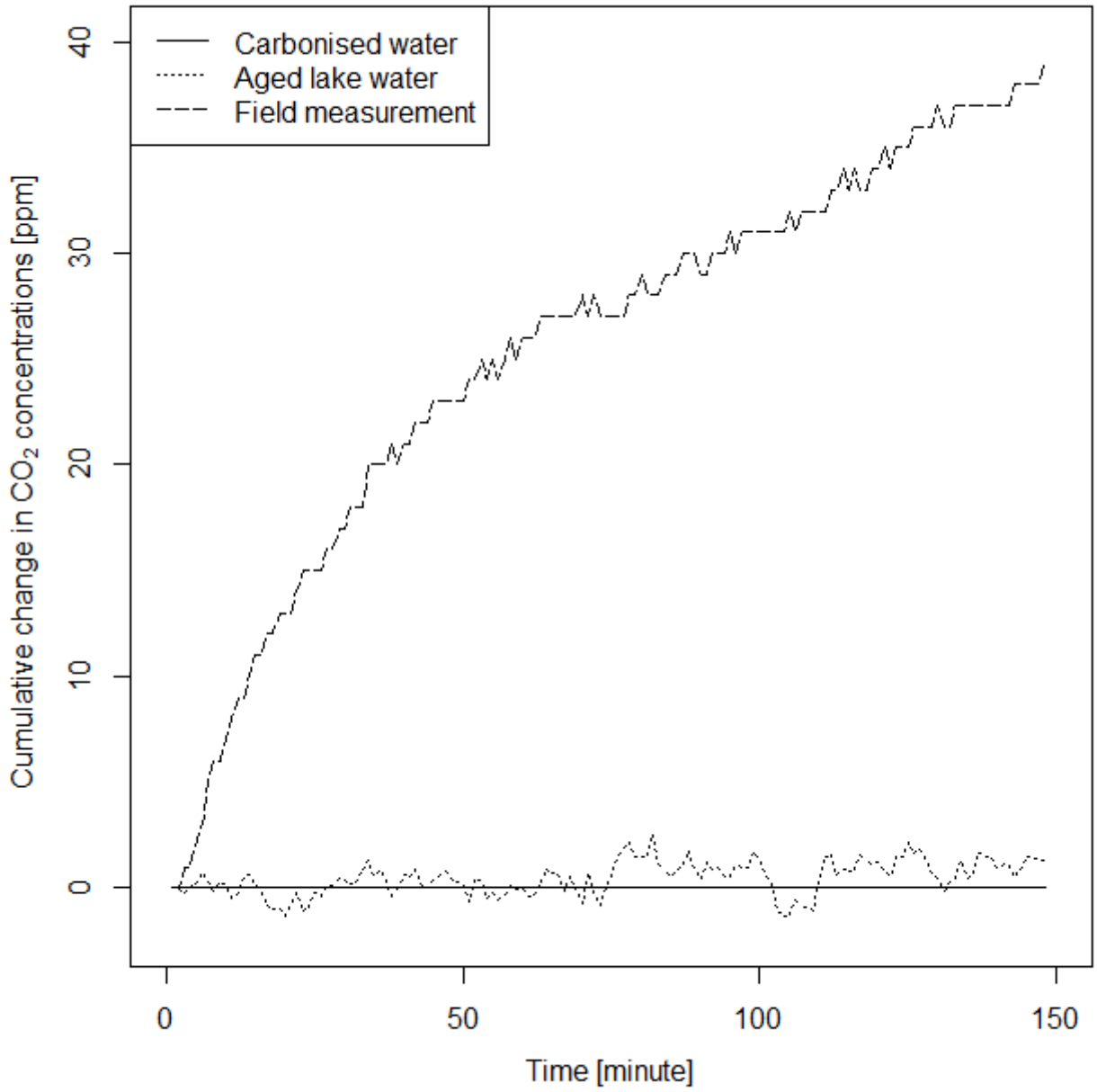

Fig. S1: Cumulative change in CO_2 concentrations [ppm] relative to starting values in the underwater chamber set-up over 150 minutes. The black line shows the change in CO_2 concentrations during a leak test with tab water (CO_2 added by adding carbonated water), the long-dotted black line the change in the field (28/6/2010), and the dotted black line during a leak test with aged lake water. Oxygen concentrations were either decreasing (field), not measured (carbonised tab water) or relatively stable (aged lake water). CO_2 concentrations were at 370-409 ppm (field measurement), 1015 ppm (carbonised tab water) and 1965-1969 ppm (leak test with aged lake water).

Table S2: Results of statistical tests analysing the difference of variables between 1 and 10 metre depths. All variables were assessed with a paired t-test and checked for normally distributed differences with the Shapiro test. If the differences were not normally distributed, a paired Wilcox test was used instead. RQ=respiratory quotient, BGE=bacterial growth efficiency, POC=particulate organic matter, DOC=dissolved organic matter, TP=total phosphorus, PO_4=phosphate, TN=total nitrogen, NO=nitrate/nitrite, NH_4=ammonium, BP=bacterial production, bacteria time=time it took bacteria to degrade substrate on EcoPlate wells to 0.5 on average.

Variables tested	paired t-test p-value	Shapiro test on differences p-value	Wilcox test p-value	1m data compared to 10m data
RQ	0.106	0.07519		
BGE	0.4329	0.6716		
chlorophyll a	0.0538	0.003319	0.003906	greater
POC	0.01422	0.1888		greater
DOC	0.1888	0.06523		
TP	0.01141	0.6571		less
PO_4	0.1509	0.1632		
TN	0.7964	0.04917	0.1953	
NO	0.8755	0.01549	0.5469	
NH_4	0.1396	0.001759	0.01953	less
BP	0.09392	0.009136	0.01953	greater
Bacteria time	0.1611	0.0007537	0.1814	

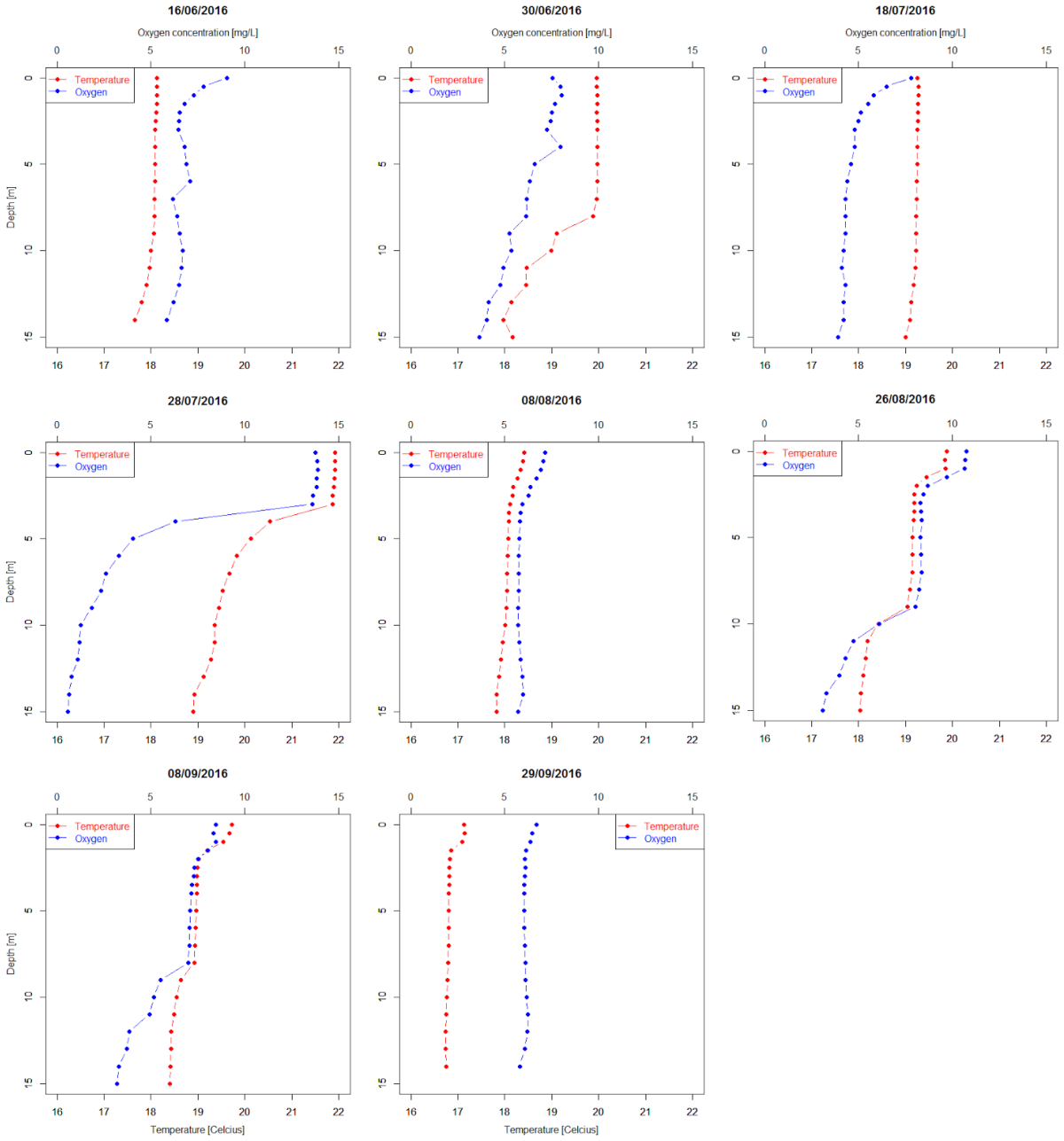

Fig. S2: Temperature and oxygen profile at Lake Vombsjön during sampling in summer 2016. Measurements were taken from the surface to the bottom at 0.5 m intervals until 4 m depth, from there onwards in 1 m intervals. The blue dotted line denotes oxygen concentrations [mg/L] and the red one temperature [degree Celcius].

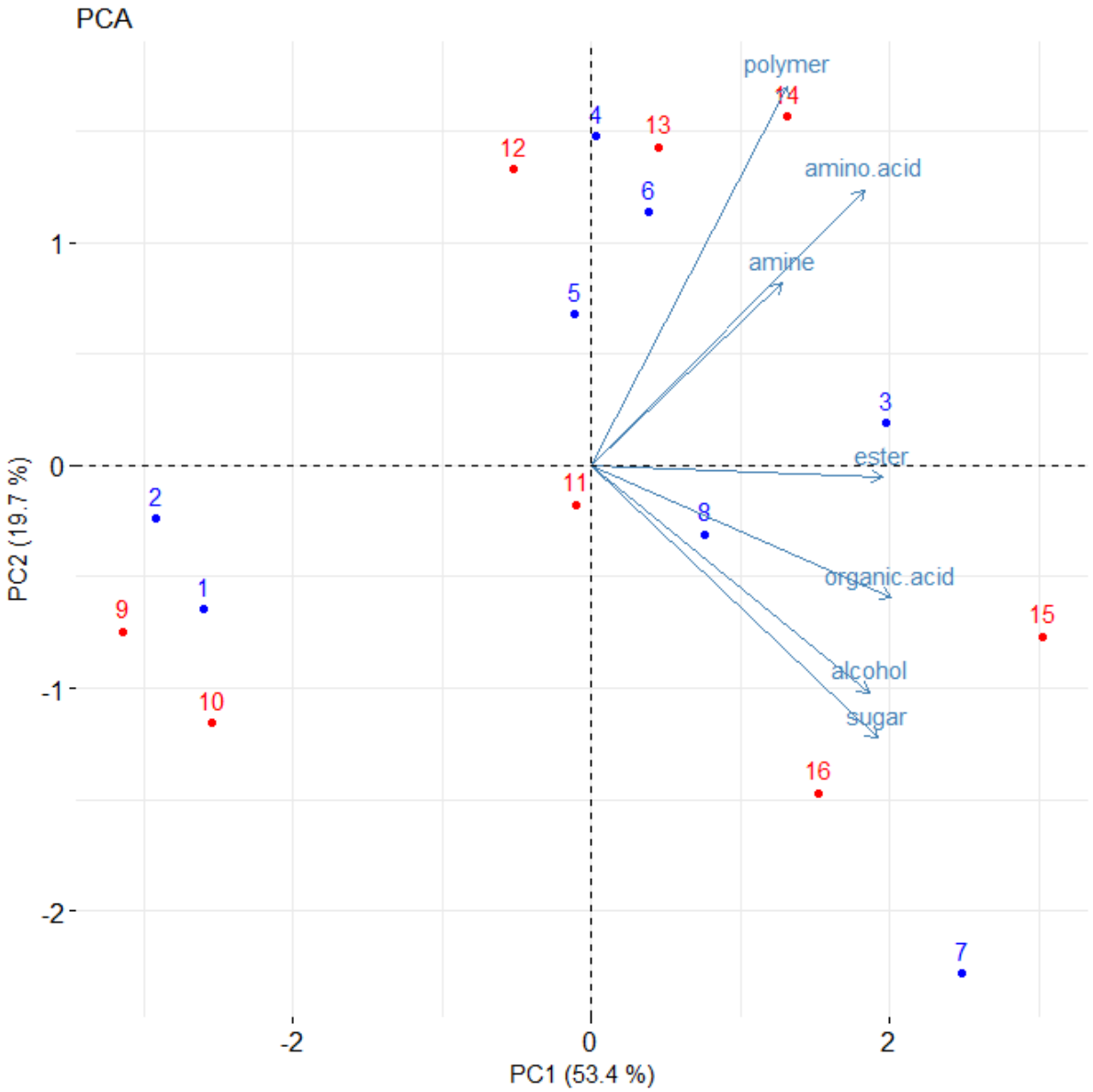

Fig S3: Capacity of bacterioplankton community to degrade algal biomass (PC2) and reduced substances (PC1) over the summer at 1 and 10 metre depth (combined data). Samplings are numbered according to Table 1 for 1 metre depth (blue points and numbers) and from 9-16 for 10 metre depth for the same dates (red points and numbers).

Table S3: Loadings for different substrates, scores for samples and eigenvalues, variance percentage and cumulative variance percentage of all principal components at 1 metre depth. PC1 = principal component 1, PC2 = principal component 2, PC3 = principal component 3.

	Loadings		
Substrate group	**PC1**	**PC2**	**PC3**
sugar	0.4811666	-0.27203871	0.03762558
organic acid	0.445498	-0.37673978	-0.17139622
amino acid	0.3213714	0.57626776	-0.18960579
ester	0.3887898	-0.10181354	0.50130198
polymer	0.2617498	0.6595379	0.06293159
alcohol	0.4780322	-0.01822765	0.04142622
amine	0.1361813	-0.07930366	-0.82236069
	Scores		
Sample	**PC1**	**PC2**	**PC3**
1	-2.3633972	-0.6392583	0.4471822
2	-2.8239909	-0.8138834	0.2875279
3	1.9202857	0.6568715	-0.7278895
4	-0.0829495	1.5528044	0.2471541
5	-0.1396952	0.6739734	-0.2187014
6	0.2536349	1.4811415	0.2360289
7	2.7285538	-1.6863985	1.6287733
8	0.5075585	-1.2252506	-2.3374784

PC	**Eigenvalue**	**Variance percentage**	**Cumulative Variance percent**
PC1	3.57734508	51.1049297	51.1049297
PC2	1.55813406	22.259058	73.36399
PC3	1.29911428	18.5587755	91.92276

Table S4: Loadings for different substrates, scores for samples and eigenvalues, variance percentage and cumulative variance percentage of all principal components at 10 metre depth. PC1 = principal component 1, PC2 = principal component 2, PC3 = principal component 3.

	Loadings		
Substrate group	**PC1**	**PC2**	**PC3**
sugar	0.314647	-0.5247723	-0.3279773
organic acid	0.408013	-0.11103	0.6394447
amino acid	0.438613	0.1987905	0.4532273
ester	0.4608297	0.1003939	-0.2095838
polymer	0.3123186	0.5103658	-0.3866335
alcohol	0.2939389	-0.5882805	-0.1472721
amine	0.3818826	0.2369443	-0.2510506
	Scores		
Sample	**PC1**	**PC2**	**PC3**
1	-3.1467949	-0.35122	0.3279743
2	-2.5841042	-0.6702121	0.3449798
3	-0.1888047	-0.3132764	-0.5966756
4	-0.3158987	1.5573327	-0.857591
5	0.7366821	1.259926	-0.809806
6	1.5439571	1.4391095	1.685559
7	2.7801478	-1.321833	-0.2211047
8	1.1748154	-1.5998267	0.1266642

	Eigenvalue	**Variance percentage**	**Cumulative Variance percent**
PC1	4.107324177	58.67606	58.67606
PC2	1.580157801	22.57368287	81.24974
PC3	0.6971268894	9.958955563	91.2087

Table S5: Loadings for different substrates, Scores for samples and eigenvalues, variance percentage and cumulative variance percentage of all principal components for EcoPlate data at both depths. PC1 = principal component 1, PC2 = principal component 2, PC3 = principal component 3.

		Loadings		
substrate group		**PC1**	**PC2**	**PC3**
sugar		0.4111759	-0.43313826	0.11293309
organic acid		0.4312463	-0.2107673	-0.22332441
amino acid		0.3920467	0.43719757	-0.01465545
ester		0.4195621	-0.01872286	0.42061655
polymer		0.2825922	0.60220845	0.38227092
alcohol		0.3996066	-0.36096704	-0.03377149
amine		0.2751079	0.28900503	-0.78292253
		Scores		
sample	**depth [m]**	**PC1**	**PC2**	**PC3**
1	1	-2.5955537	-0.6446342	-0.080710446
2	1	-2.91850714	-0.2406738	-0.207986357
3	1	1.97255355	0.1874582	-0.265219967
4	1	0.02550386	1.4754776	0.767578002
5	1	-0.1083536	0.6777334	0.561891729
6	1	0.38334911	1.1372062	0.586363055
7	1	2.48646924	-2.2819106	1.248889115
8	1	0.75453869	-0.3106568	-2.610805131
1	10	-3.14246259	-0.7459104	-0.232244458
2	10	-2.5457878	-1.1589023	-0.001636764
3	10	-0.10371188	-0.1769854	0.112764037
4	10	-0.51799275	1.328193	1.278401477
5	10	0.45172425	1.4284508	-0.751047852
6	10	1.31236271	1.5629433	-0.395483258
7	10	3.02561128	-0.7681101	-0.52986876
8	10	1.52025678	-1.4696789	0.519115579
		Eigenvalue	**Variance percentage**	**Cumulative Variance percent**
	PC1	3.73891934	53.413133	53.413133

PC2	1.37799372	19.685625	73.09876
PC3	0.84776997	12.111	85.20976

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