Student thesis series INES nr 336

Impact of photo-chemical processing of dissolved organic carbon on the bacterial respiratory quotient in aquatic ecosystems

Lina Allesson

2015

Department of
Physical Geography and Ecosystems Science
Lund University
Sölvegatan 12
S-223 62 Lund
Sweden

Lina Allesson (2015). *Impact of photo-chemical degradation of dissolved organic carbon on the bacterial respiratory quotient in aquatic ecosystems*
Master degree thesis, 30 credits in *Physical Geography and Ecosystem Analysis*

Department of Physical Geography and Ecosystems Science, Lund University

Level: Master of Science (MSc)

Course duration: *January* 2014 until *June* 2014

Disclaimer

Impact of photo-chemical processing of dissolved organic carbon on the bacterial respiratory quotient in aquatic ecosystems

---

Lina Allesson

Master thesis, 30 credits, in *Physical Geography and Ecosystem Analysis*

Supervisor: Martin Berggren

Department of Physical Geography and Ecosystem Science

Exam committee:

Lena Ström, Department of Physical Geography and Ecosystem Science

Harry Lankreijer, Department of Physical Geography and Ecosystem Science

Abstract

Many studies assume a respiratory quotient (RQ = CO_2 produced per O_2 consumed, by moles) of ~1 to calculate planktonic bacterial respiration rates from measured O_2 consumption rates. However, the theoretical value of RQ varies with the elemental composition of the compound being decomposed. Photo-chemical oxidation of DOC with ultraviolet (UV) light results in oxygen-rich organic acids which, theoretically, should lead to elevated RQ. In this study samples of both UV light irradiated and non-irradiated water were incubated in the laboratory and the bacterial RQ was monitored with optic gas-pressure sensors. The water samples used were from the humic lake Övre Björntjärnen in the north of Sweden and Leonardite-extracted humic acid solutions In irradiated samples, the RQs frequently exceeded 1 and generally were significantly higher than in the non-irradiated samples. Additionally, enrichment with inorganic nutrients (N+P) to humic acids extract consistently increased the RQs in bioassays. In non-irradiated humic acid solutions, both nutrient enriched and non-enriched, RQ was lower than 1. This study shows that bacterial RQ varies depending on the state of oxidation of the DOC and the access to nutrients in the water. The results imply that RQ can be systematically higher than 1 when the bacterial metabolism is to a large extent based on photo-chemically produced substrates. The use of an assumed RQ of 1 may both underestimate and overestimate bacterial respiration in aquatic ecosystems.

Keywords: Physical geography, biogeochemistry, bacterioplankton respiration, photo-oxidation, degradation, nutrients

Sammanfattning

I många studier antas en respirationskvot (CO_2 producerat per O_2 konsumerat, i mol) på ~1 för att beräkna bakterierespiration från uppmätt O_2 konsumtion. Det teoretiska värdet på RQ varierar dock med elementärsammansättningen hos preparatet som bryts ner. Fotokemisk oxidering av upplöst organiskt kol med ultraviolett (UV) ljus resulterar i syrerika organiska syror som, teoretiskt, borde leda till ökad RQ. I denna studie inkuberades UV ljus belysta och obelysta vatten prov från den humusrika sjön Övre Björntjärnen in norra Sverige samt av Leonardit extraherade humussyrelösningar i laboratoriet och RQ bevakades med hjälp av optiska gastrycksensorer. RQ översteg frekvent 1 i belysta prov och var generellt signifikant högre än i obelyst vatten. Dessutom gav berikning av humussyraextrakt med oorganiska näringsämnen (N+P) konsekvent ökat RQ i bioassayer. I de obelysta proverna med humussyralösning var RQ lägre än 1, varken i de näringsberikade proverna eller i de som inte var berikade. Denna studie visar att bakteriers respirationskvot varierar beroende på det upplösta kolets oxidationsgrad och tillgången till näringsämnen i vattnet. Resultaten tyder på att RQ can vara systematiskt högre än 1 när bakteriell metabolism till stor del är baserat på fotokemiskt producerade substrat. Användningen av det antagna värdet 1 kan både leda till under- och överskattning i bakteriarespiration i akvatiska system.

Contents

1. Introduction

In recent years the view on the role of inland waters in the global carbon cycle has changed from that of a passive pipeline of carbon transport toward the oceans, to being an active part of the cycling (Cole et al., 2007). Several processes in freshwater systems contribute to carbon degradation and most lakes are supersaturated with carbon dioxide (CO_2), leading to emissions of CO_2 to the atmosphere. Organic carbon enters the waters from the surrounding terrestrial ecosystems, mainly as dissolved organic carbon (DOC), and is assimilated by bacterioplankton that mineralize it to CO_2 (Biddanda et al., 1994). This bacterial respiration (BR) is an important part of carbon processing in freshwater, but it is also a process of global significance; in oceans and inland waters combined, BR is probably the largest single sink of organic carbon on Earth (del Giorgio and Williams, 2005).

However, there are still methodological uncertainties in the measurement of BR leading to a lack of understanding of the carbon cycle (Humborg et al., 2010; Berggren et al., 2012). Often when BR is assessed the O_2 consumption is measured rather than the CO_2 production since the oxygen techniques are more developed (del Giorgio and Cole, 1998: Staerr et al., 2010). Thus, deriving the CO_2 production rates normally requires the use of a conversion factor: the bacterial respiratory quotient (RQ: moles produced CO_2 per moles consumed O_2) (Dilly, 2001). The RQ is commonly assumed to be 1.0 based on the stoichiometry of complete oxidation of glucose (e.g. Biddanda et al., 1994). However, bacterioplankton consume a large variety of compounds containing various amounts of oxygen (O) and hydrogen (H). Degradation of more oxidized compounds (with relatively low H content and high O content) theoretically gives a relatively higher RQ since less O_2 needs to be taken from the surrounding for complete oxidation (Berggren et al., 2012).

Phytoplankton produce autochthonous DOC through *in situ* primary production. This DOC often has low oxygen content giving a theoretical RQ of 0.7-0.8 for complete oxidation (Hedges et al., 2002; Berggren et al., 2012). Allochthonous DOC is produced in terrestrial ecosystems and transferred into the waters. Such DOC consists mostly of high molecular weight (HMW) humic compounds (Sondergaard and Middelboe, 1995; Bertilsson and Tranvik, 1998). Complete oxidation of humic substrates gives an RQ of about 0.9 (Dilly, 2001). However, bacterioplankton

do not typically make use of the bulk humic DOC, but instead use small selected fractions that are easy to assimilate. Further, all substrates that are assimilated are not directly oxidized by the bacterioplankton but rather used for production of biomass (Biddanda et al., 1994; del Giorgio and Cole 1998), potentially increasing the RQ (Dilly et al., 2003). Thus, the theoretical RQ for complete oxidation of bulk DOC pools may inform little about the actual RQs.

Chromophoric dissolved organic matter (CDOM) contains aromatic molecules that are highly photo-reactive. Allochthonous DOC to a larger extent than autochthonous is a source of CDOM (Sulzberger and Kaiser, 2009). UV-light irradiation leads to photo-oxidation of the DOC either to CO_2 or to photo-products that are partially oxidized, and thus assimilated by bacteria at a theoretically higher RQ (Bertilsson and Tranvik, 2000; Berggren et al., 2012). In lakes dominated by terrestrially derived DOC, RQ values systematically exceeding 1 have been observed. If this deviation from 1 is not taken into account in O_2-inferred BR assessments, the consequence would be underestimations of BR and its contribution to the CO_2 efflux (Berggren et al., 2012).

By the use of an assumed value (often 1) of RQ, the derivation of BR thus may include large errors. Since the RQ has been shown to vary with the composition of the DOC (Cimberlis and Kalff, 1998; Berggren et al., 2012), propagating through to the BR assessment in the ecosystems there is a need for a better understanding of the bacterial RQ. It seems as if using a fixed value of RQ gives a rather conservative view of the metabolism and the role of the bacteria, specifically in systems with high levels of allochthonous DOC.

In this thesis, the impact of UV radiation on the RQ is therefore tested. Considering the known effect of photo-processing on the oxidation state of DOC (see above), it was hypothesized that photo-chemically processed DOC is 1) used by the bacteria at an RQ > 1 and; 2) used with a higher RQ compared to non-irradiated DOC. Even if there are theoretical explanations to why the RQ should increase after irradiation (Berggren et al., 2012), the significance of this effect is unknown, since empirical evidence is lacking. To test the hypothesis, water samples with humic-rich DOC were irradiated with ultraviolet (UV) light and incubated in a climate chamber at constant temperature (20 °C). These irradiated samples were compared to dark control samples

with non-irradiated water. In order to obtain the RQ, the rates of O_2 and CO_2 were measured simultaneously. Additionally the potential influence of inorganic nutrients on RQ was controlled for by performing the same experiments with nutrient enriched and with nutrient poor water samples.

Three different types of samples were used in the experiments: i) a lake water sample with natural water from an unproductive lake, ii) a sample with standard leonardite extracted humic acids, and iii) a sample with standard leonardite extracted humic acids and added nutrients (N +P).

2. Background

In this chapter the carbon cycling in inland waters and the mechanisms behind DOC degradation and mineralization to CO_2 are thoroughly introduced. An introduction to the bacterial RQ and its importance for estimating the amount of carbon passing through the bacteria and thus the ecosystem will also be presented.

2.1 Role of inland waters in the global carbon cycle

Inland waters cover about 3.5% of the continental area globally, with highest concentrations in boreal and arctic latitudes (Verpoorter et al., 2014). Carbon is present in freshwater systems in different forms, mainly DOC, and dissolved inorganic carbon (DIC), but also particulate organic carbon (POC). The DOC has either autochthonous, i.e. produced *in situ*, or allochthonous origin, i.e. enters the ecosystems from the catchments, primarily from litter and humus (Giesler et al., 2007). DIC of allochthonous origin includes dissolved CO_2 from soil respiration and carbonate species from weathering. Studies show that only about half of all carbon entering the inland waters reaches the oceans while the rest is processed within the systems (Cole et al., 2007), and thus the role of inland waters in the global carbon cycle has been reevaluated. The latest assessment report (AR5) from the intergovernmental panel on climate change (IPCC, 2013) includes the carbon processing in inland waters as a significant part of the cycling on a global scale.

Carbon enters terrestrial ecosystems via sequestration of atmospheric CO_2 in the photosynthesis. The carbon that is not returned to the atmosphere via heterotrophic and autotrophic respiration or via other ways of abiotic oxidation, e.g. through fires or photo-oxidation, has two possible fates: storage within or export from the ecosystem (Cole et al., 2007). The relative importance of DOC, DIC and POC input to inland waters varies, depending on the geographical location and thus on climate, physical and biochemical properties of the soil, and land use. DIC is the dominant form of aquatic carbon in temperate regions and in boreal forests in carbonate terrain where soil respiration, carbonate weathering, and groundwater flow are substantial. In tropical regions and boreal forests in non-carbonate terrain; DOC is dominating (IPCC, 2013). The amount of terrestrial carbon input to freshwater systems is estimated to at least 1.9 Pg C yr^{-1}, which is a

rather conservative estimation not including inundated flood plains. About half of this reaches the oceans and the rest is either buried in sediments within the aquatic ecosystems (10%) or reemitted to the atmosphere as CO_2 (40%) (Cole et al., 2007; Battin et al., 2009).

The majority of lakes are supersaturated with CO_2 and hence have a partial pressure of CO_2 (pCO_2) larger than the overlaying air resulting in emission of CO_2 to the atmosphere (del Giorgio et al., 1997; Jonsson et al., 2003; Raymond et al., 2013). IPCC (2013) estimates the CO_2 emissions from freshwater systems to about 1 Pg C yr^{-1}. Even if conservative, CO_2 outgassing of this magnitude is of the same order as estimations of net carbon sequestration on continents and in oceans (about 2.2 Pg C yr^{-1} each) (Cole et al., 2007; Battin et al., 2009; Tranvik et al., 2009). In a summary of published estimations of CO_2 efflux from inland surface waters to the atmosphere, Aufdenkampe et al (2011) presented a median value of 1.2 Pg C yr^{-1} ranging between 0.75 – 1.4 Pg C yr^{-1} on a global scale. The fluxes from streams and rivers are specifically large regarding their small surface area (about 0.3% of the continental area). More than half of the CO_2 evasion from inland waters is released from streams and rivers (Aufdenkempe et al., 2011; Raymond et al., 2013). If including CO_2 efflux from inland waters in the continental carbon balance, some boreal forests with relatively large areas covered by lakes and streams may shift from net sinks to net sources of CO_2 to the atmosphere (Algesten et al., 2003).

Additionally, the annual sedimentation rates in inland waters are of the same order of magnitude as in marine ecosystems. The range of global carbon burial in freshwater sediments is 0.2 -0.6 Pg C yr^{-1} to be compared to 0.2-0.5 Pg C yr^{-1} in coastal ocean sediments and ~1.2 Pg C yr^{-1} in marine sediments (Cole et al., 2007; Tranvik et al, 2009; Regnier et al, 2013).

The conservative approximations of CO_2 efflux from inland waters used by e.g. IPCC (2013) are a consequence of the uncertainties included in the modeling of the global carbon cycling. Such uncertainties include the estimations of the areal extent of inland waters; the amount of lateral flow of organic carbon between ecosystems and the carbon assimilation mechanisms of bacteria and thus the amount of CO_2 in water; and the gas transfer velocity, a physical parameter that

determines the rate of gas exchange (Raymond and Cole, 2001; Cole et al., 2007; Alin et al., 2011; Raymond et al., 2013).

Primary producers in freshwater systems take up the aquatic CO_2, when producing autochthonous DOC. Primary production is strongly regulated by the access to nutrients, the insolation, and the latitude. The estimated global gross primary production (GPP) in lakes of 0.65 Pg C yr^{-1} represents only a small fraction of the total global GPP of 100-150 Pg C yr^{-1} (Cole et al., 2007). In productive, eutrophic lakes the amount of autochthonous DOC is likely exceeding allochthonous DOC (Berggren et al., 2010; Berggren et al., 2012). Photosynthesis might dampen the CO_2 emissions from the waters some but not to the extent that the global CO_2 supersaturation of lakes is affected significantly. The allochthonous carbon has a greater effect on the gas balance in the waters and in order to compensate the GPP would need to be a several fold larger. The concentration of allochthonous DOC in aquatic ecosystems is often strongly positively correlated to the pCO_2 at the surface (Hope et al., 1996; Sobek et al., 2005) and both tend to be high in unproductive systems (Jonsson et al., 2003).

2.1.1 Sources of CO_2 supersaturation in freshwater systems

The CO_2 supersaturation observed in inland waters is caused by a combination of DIC input from soil respiration or weathering, BR and sediment respiration. In streams and rivers the DIC input is the dominating cause to CO_2 supersaturation, while respiration is the main contributor to the same in lakes (Cole et al., 2000; Sobek et al., 2005; Humborg et al., 2010). Many lake ecosystems are net heterotrophic, i.e. the net in-lake respiration is larger than the net in-lake production, leading to a net release of CO_2 to the atmosphere. The BR increases with the amount of DOC and thus lakes with higher DOC concentrations should to a larger extent be CO_2 supersaturated (Sobek et al., 2005; Jonsson et al., 2007). In humic waters, the bacterioplankton consume allochthonous DOC and is, therefore, less dependent on DOC from primary production (e.g. Berggren et al., 2010). Bacterioplankton are good competitors compared to primary producers and in oligotrophic waters the competition for nutrients can thus lead to an increase in net heterotrophy in humic waters and consequently in elevated CO_2 release from the ecosystem to the atmosphere (Jansson et al., 2001).

Photo-chemical reactions might result in either abiotic mineralization of DOC to CO_2 or in partial-oxidation of the compounds. The photo-transformed compounds are often assimilated at lower bacterial growth efficiencies (BGE) and higher respiration rates. This together with the photo-mineralization in turn increase the amount of DOC that is returned to CO_2 at the expense of production of new bacterial biomass (Pullin et al., 2004).

2.2 DOC processing in freshwater systems

The carbon in aquatic ecosystems may be processed in various ways. If not degassed to the air, CO_2 may be hydrated and by that enter the carbonic system forming carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. The CO_2 thus acts as an acid affecting the pH of the system (e.g. Stumm and Morgan, 1996).

The main process causing of the CO_2 supersaturation in lakes is mineralization of DOC to CO_2 (Sobek et al., 2005). Mineralization takes place through heterotrophic and sediment respiration and through photo-chemical oxidation of the compounds. The relative importance of the different mineralization processes varies depending on the depth of the lake, the temperature and DOC abundance and quality differences within the lake and between shallow and deep sediments (Algesten et al., 2005). In the water column, the bacterial respiration is the dominant source of DOC mineralization to CO_2 (del Giorgio et al., 1999; Jonsson et al., 2001). However, in shallow lakes where much of the sediment is in contact with the upper mixed water the sediment respiration may play a larger or equal role compared to the bacterial respiration than in deeper lakes (Jonsson et al., 2001). The emissions vary over the year. Productive lakes can be net sinks of CO_2 during summer stratification and high solar irradiation, and turning sources as mixing of the layers take place together with decreasing amounts of incoming solar radiation (Cole et al, 1994; Sobek et al., 2005). Unproductive lakes rich in humic substances are often CO_2 sources the whole year (Sobek et al., 2005).

DOC in the water column may be flocculated and sink to the sediments as POC followed by sediment respiration. The carbon content in lake sediments has been estimated to 280 Pg C (Cole

et al., 2007). In sediments both aerobic and anaerobic respiration may take place, leading to mineralization to CO_2 and methane (CH_4) respectively. In shallow sediments, CH_4 is more likely to avoid oxidation and escape to the atmosphere.

2.2.1 Photo-degradation of DOC

As the DOC absorbs sunlight, a number of reactions take place resulting in the degradation of the compounds with effects such as bleaching, size redistribution and partial oxidation or complete abiotic mineralization of the DOC to CO_2 or CO (Tranvik, 1988; Bertilson and Tranvik, 2000; Brinkmann et al., 2003). CDOM containing lignins and other aromatic molecules originating mainly from plants are particularly photo-reactive (e.g. Sulzberger and Kaiser, 2009; Benner and Kaiser, 2011) implying that terrestrial ecosystems are important sources of CDOM. Algal-derived DOC also contains aromatic compounds but to a lower extent (Sulzberger and Kaiser, 2009).

Photo-chemical reactions of DOC takes place in the water column and is dependent on solar irradiation and the amount and age of the CDOM since high levels of CDOM shade the underlying water. Photo-chemical mineralization stands for at least 10% of the mineralization of DOC in inland waters (Granéli et al., 1996; Kortelainen al., 2006; Tranvik et al., 2009), and ca 10% of the total global emissions of CO_2 from inland waters to the atmosphere (Koehler et al, 2014). Additionally, photo-chemical reactions partially oxidize and degrade the DOC compounds, turning them into low molecular weight (LMW) compounds of high oxygen content (Vähätalo et al., 2003; Cory et al., 2014). The importance of complete or partial oxidation is dependent on the aromaticity of the compound, with more aromatic compounds more likely to undergo complete photo-oxidation (Cory et al., 2014). Mineralization of DOC is often as important as or more important than degradation to LMW DOC through photo-oxidation (Köhler et al., 2002; Anesio and Granéli, 2003; Vähätalo et al., 2003; Pullin et al., 2004). In freshwater systems in the arctic region dominated with allochthonous DOC the importance of partial photo-oxidation was recently shown to be equally important as photo-mineralization (Cory et al., 2014)).

The UV radiation induced transformation of DOC can occur through different pathways. If the radiation is absorbed by the CDOM itself, the molecule is excited, and an electron may be emitted in the deexcitation (Bruccoleri et al., 1993). The hydrated electrons react in various ways leading to the formation of organic radicals that further react with the DOC. The final results are DOC compounds of high oxygen content and often lower molecular weight (Zhou and Mopper, 1997; Brinkmann et al., 2003; Sulzberger and Kaiser, 2009). This direct photo-transformation of CDOM is dependent on the absorptivity of the DOC. The absorptivity is a property of the CDOM controlled by its aromaticity and thus by the chemical bindings (Benner and Kaiser, 2011) and by the age and origin of the DOM (Porcal et al., 2013). In an ecosystem the absorptivity of the DOC pool is a function of both the absorption properties of the CDOM compounds and the concentration of the CDOM in the system (Köhler et al., 2002; Sulzberger and Kaiser, 2009). Non-coloured DOC does not absorb UV-irradiation but may still be transformed by indirect processes induced by photo-chemical reactions such as the formation of the hydroxyl radical (Brinkmann et al., 2003; Pullin et al., 2004), hydrogen peroxide (H_2O_2) (Farjalla et al., 2001; Anesio et al., 2005) or other reactive species (Sulzberger and Kaiser, 2009; Benner and Kaiser, 2011). As the CDOM molecules absorb the UV radiation they prevent it from reaching further down in the water column making the photo-chemical reactions mainly take place in the surface layer (Granéli et al., 1996; Köhler et al., 2002; Anesio and Granéli, 2003).

The photo-chemical reactions following UV-light irradiation on CDOM result in the loss of colour intensity of the compounds (bleaching), lowering their aromaticity (Farjalla et al., 2001, Köhler et al., 2002; Brinkmann et al., 2003; Sulzberger and Kaiser, 2009; Pace et al., 2012). Photo-bleaching of CDOM in the UV-region has the consequence of increased UV penetration in the water column making the photo-chemical reactions happen at greater depths enhancing the exposure to UV radiation (Brinkmann et al., 2003; Benner and Kaiser, 2011). Photo-bleaching is often followed by a decrease in molecular weight of the DOC (Köhler et al., 2002; Brinkmann et al., 2003). As the DOC is degraded into compounds of lower molecular weight and lower aromatic content they lose absorptivity resulting in DOC that is less photo-reactive (Köhler et al., 2002; Porcal et al., 2013).

2.2.1.1 Environmental factors influencing photo-chemical reactions

The photo-degradation rate varies seasonally from being highest in spring just after snowmelt to reaching a minimum toward late summer-autumn then increasing again during autumn. This variation is probably due to input of newer DOC with the spring flood and with rain events (Benner and Kaiser, 2011; Porcal et al., 2013). Hence, the variations in photo-reactivity over the year follow the hydrographic variations (Porcal et al., 2013). Besides from properties of the DOC, the photo-transformation of DOC is regulated by the amount of UV radiation hitting the compounds (e.g. Farjalla et al., 2001), depending on geographical location, climate and time of the year, as well as by environmental factors.

One such environmental factor is the concentration of dissolved metal ions in the water. Iron is one such metal, absorbing UV light and working as a catalyst for photo-oxidation of humic substance (Stumm and Morgan, 1995; Bertilsson and Tranvik, 2000). In UV-irradiated water with high iron concentration, Bertilsson and Tranvik (2000) observed both elevated DIC production rates and enhanced photo-bleaching efficiency. In contrast, Gao and Zepp (1998) did not find any relationship between photo-bleaching of CDOM and higher iron concentrations.

The UV-light gives rise to a reduction of Fe(III) to Fe(II) that through the photo-Fenton reaction reacts with the H_2O_2 photo-product forming hydroxyl radicals ($OH\cdot$). The $OH\cdot$ in turn is highly reactive and its major sink in aquatic ecosystems is DOC, forming LMW acids and DIC in the reactions or in other ways modifying DOC compounds (Brinkmann et al., 2003; Pullin et al., 2004). Free radicals can also be formed through decarboxylation as organic iron-carboxylate complexes (Fe-CDOM) undergo photolysis (Gao and Zepp, 1998). By the formation of radicals, UV irradiation indirectly also leads to degradation of non-coloured DOM.

Complexes of copper can also be reduced by sunlight from Cu(II) to Cu(I), in this process radicals that can catalyze photo-degradation of DOC are produced (Sykora, 1997). However, Brinkmann et al. (2003) found the addition of copper to water samples reducing the photo-bleaching of the DOM. This reduction in bleaching can be explained by the quenching effect of Cu(II) on luminescence, shortening the lifetime of excited states of the DOC, a fundamental

condition for potential photo-chemical reactions (Frimmel et al., 1987). Other photo-chemical processes may also produce $OH^{\cdot}$ in natural waters; such as nitrite/nitrates photolysis and direct photolysis of CDOM (Vaughan and Blough, 1998).

The pH of aquatic systems also plays a major role in the photo-transformation of DOM. Several studies have shown that the loss of DOC to DIC is larger at lower pH (Anesio and Granéli, 2003; Molot et al., 2005; Porcal et al., 2013), while others have seen the opposite effect (Gao and Zepp, 1998; Bertilsson and Tranvik, 2000). One explanation of increased mineralization at low pH may be that the $OH^{\cdot}$ production by the photo-Fenton reaction plays a major role at pH in the acidic range, decreasing as the pH increases (Molot et al., 2005). Porcal et al. (2013) also show a negative relationship between pH and iron concentration in their study on how the photo-chemical properties of DOC varies seasonally.

An increase in photo-reactivity may explain observed increases in DOC loss under acidified conditions, but perhaps not fully. Some studies suggest that as the pH sinks, and hence the amount H^{+} ions increase, the dissociation and solubility of the humic compounds are inhibited, leading to flocculation (Krug and Frink, 1983; Chin et al., 1994) or to increased nutrient availability, followed by enhanced microbial degradation (Anesio and Granéli, 2003 and references therein).

Additionally, the photo-bleaching of CDOM seems to increase as the pH increases to the alkaline values (Bertilsson and Tranvik, 2000; Brinkmann et al., 2003; Pace et al., 2012). Pace et al. (2012) found a significant increase in absorptivity with increased pH, while losses of DOC were low in all cases and independent of pH. As the pH increased, the molecular size increased and the structure of the molecules went from being small and rounded at low pH to larger plaque-like at higher pH. The shift in size and shape might be a reason to an increase in absorptivity at more alkaline pH; as the molecules expand more chromophores are exposed to light (Pace et al., 2012). In contradiction Anesio and Granéli (2003) found both higher photo-mineralization and photo-bleaching at lower than at higher pH.

2.2.2 Bacterioplankton respiration and metabolism

In the assimilation of organic carbon the heterotrophic bacteria carry out two major processes: they produce new bacterial biomass through bacterial production (BP) and they mineralize organic carbon into CO_2 though BR (Biddanda et al., 1994). The productivity is well studied while there is a lack of knowledge about the respiration.

The total amount of carbon assimilated by bacterioplankton is the sum of the two processes mentioned above, i.e. BR+BP (del Giorgio and Cole, 1998). BGE is the quantity of biomass produced per unit assimilated substrate; $BGE = \frac{BP}{BP+BR}$ (e.g. Jansson et al., 2006). The energy demand in bacterial metabolism is divided between the demand for production of new biomass and the demand for maintenance of cellular functions. These different demands are not necessarily coupled but the coupling between them varies with environmental factors. Del Giorgio and Cole (1998) show that there is an increase in BGE with productivity in aquatic systems. In unproductive, oligotrophic systems the maintenance of cellular functions may cost more energy due to e.g. the need of producing exo-enzymes to break down substrates to a more bioavailable form (del Giorgio and Cole, 1998; Cimberlis and Kalff, 1998). This higher energy demand inhibits the demand of energy for growth and thus resulting in a lower BGE. A limiting factor of BGE is therefore the quality of the DOC rather than the supply. The DOC pool in more productive systems consists of compounds of higher quality and there are more nutrients available (Mills et al., 2008), resulting in an increase in BGE. Hence, limiting factors of BGE are combinations of DOC quality and age, nutrient availability and the particular energy demands of the system (del Giorgio and Cole, 1998; Berggren et al., 2009).

2.2.2.1 Bioavailability of DOC

The main limiting factors of BR are the amount (Sobek et al., 2005; Jonsson et al., 2007) and the bioavailability of the DOC, although the absolute rates of BR can also be controlled by temperature and by access to inorganic nutrients (Cimberlis and Kalff, 1998: Jansson et al., 2006; Mills et al., 2008). The bioavailability of the DOC is dependent on its origin and its molecular structure (del Giorgio and Davis, 2003). In productive ecosystems, the major source of DOC is

from primary production of phytoplankton (Berggren et al., 2010; Berggren et al., 2012). This autochthonous DOC is labile and consists of LMW compounds such as amino acids and carbohydrates (Sundh 1992) that is easily metabolized by heterotrophic bacteria (del Giorgio and Davis, 2003; Farjalla et al., 2009).

In unproductive lakes and streams, the autochthonous carbon fixation rate is often lower than the loading of allochthonous DOC, and thus major source of carbon comes from the surrounding terrestrial ecosystems (Tulonen, 2004; Berggren et al., 2010). The allochthonous DOC comes primarily from litter and humus (Giesler et al., 2007) containing of partially degraded HMW compounds, not directly available to the bacterioplankton (Sondergaard and Middelboe, 1995; Bertilsson and Tranvik, 1998). However, some allochthonous DOC originates from root exudates (Jones, 1998) consisting of LMW organic acids. The proportion of this LMW DOC from soils is not large though, generally not exceeding 5% of the DOC (Giesler et al., 2007).

The allochthonous DOC is nevertheless an important source of energy for bacteria in unproductive ecosystems where the primary production from phytoplankton is too low to support the bacterial carbon demand (Jansson et al., 2000; Kritzberg et al., 2006). In humic lakes the bacterial biomass correlates positively with the amount of allochthonous DOC (Bergström and Jansson, 2000; Jansson et al., 2000) and according to Kritzberg et al. (2006) this DOC can make up a large share of the bacterial carbon content. Part of the HMW DOC is degraded through extra-cellular enzymes of the bacteria that cleave the macro molecules into smaller ones (e.g. Sondergaard and Middelboe, 1995; del Giorgio and Davis, 2003) or through the exposure of ultra violet (UV) radiation on DOC, transforming the substrate into LMW carboxylic acids (e.g. Bertilsson and Tranvik, 1998) or carbonyl compounds (Zhou and Mopper, 1997). Further, Berggren et al. (2010) suggest that part of the DOC from terrestrial systems might be more labile than previously thought. In their study, they found the LMW allochthonous DOC to be readily available to the bacterioplankton to metabolize. Especially acetate stimulated high rates of production. Large amounts of LMW DOC was found during and after episodes of high inflow of fresh DOC from the surroundings, such as spring flood, and during these events the bacterial carbon demand was fully met by LMW DOC.

2.2.2.2 Changes in bioavailability due to partial photo-chemical degradation

Depending on the chemical composition and the origin of the DOC; photo-chemical reactions may increase or decrease its bioavailability (Vähätalo et al., 2003; Anesio et al., 2005; Sulzberger and Kaiser, 2009). After processing by photo-chemical reactions algal derived LMW DOC might turn more recalcitrant (Tranvik and Kokalj, 1998; Sulzberger and Kaiser, 2010) while photo-transformation of HMW DOC often makes it more bioavailable (Bertilsson and Tranvik, 1998; Vähätalo et al., 2003; Sulzberger and Kaiser, 2010). Tranvik and Kokalj (1998) suggest that the decrease in bioavailability of the algal derived DOC is due to photo-produced radicals reacting with the humic matter making it include the labile DOC to their chains. Thus, they argue that the main process is not a direct photo-transformation but a secondary one and that the more recalcitrant DOC is formed through photo-reactions in presence of HMW DOC.

The increase in bioavailability through photo-reactions with recalcitrant DOC of high humic content is a result of the HMW DOC compounds degrading into more labile substrates with chemical composition similar to the autochthonous LMW DOC. Typically such substrates consist of carboxylic acids and carbonyl compounds readily assimilated by the bacterioplankton (Zhou and Mopper, 1997; Bertilsson and Tranvik, 1998). Although at a lower rate, the photo-products may in turn be further oxidized through photo-chemical reactions when irradiation continues (e.g. Porcal et al., 2013). Several studies have shown an overall decrease in DOC concentration and an increase in the production of LMW compounds after exposure to UV radiation (e.g. Bertilsson and Tranvik, 1998; Köhler et al., 2002; Anesio and Granéli, 2003; Pullin et al., 2004). Together with increased bioavailability of the DOC, photo-mineralization of dissolved organic nitrogen and phosphorous may take place, making it bioavailable (Vähätalo et al., 2003), hence facilitating and increasing the microbial degradation further.

Even if the utilization of DOC and the bacterial carbon production increase after exposure to UV radiation (Köhler et al., 2002; Vähätalo et al., 2003; Anesio et al., 2005) the BGE has been observed to drop, and the microbial respiration increases, producing more CO_2 (del Giorgio and Cole, 1998; Vähätalo et al., 2003; Pullin et al., 2004). The reason for this is probably that the photo-transformation of DOC results in compounds that are highly oxidized and therefore of

lower energy content compared to the microbial biomass (del Giorgio and Cole, 1998; Pullin et al., 2004). Studying the photo-production of carboxylic acids in lakes, Bertilsson and Tranvik (1998) found that formic acid was almost exclusively respired to CO_2 while acetic- and malonic acid was to a substantial part incorporated into biomass. The oxidized products may thus be incorporated less efficiently by the bacteria (Farjalla et al. 2001). In contrast Anesio et al. (2005) observed an increase in BGE in irradiated water samples; they used highly recalcitrant humic water and suggest that the irradiation increases the nutritional value of this particular DOC pool.

Consequently, also the BGE is dependent on the photo-produced substrate and on the origin and chemical composition of the DOC exposed to UV radiation. Investigating only the amount of carbon assimilated by the bacteria is not sufficient; to estimate the impact of photo-transformation of DOC on bacterial activity the change in both bacterial carbon production and respiration must be considered (Anesio et al., 2005). Even if the BGE is lower at assimilation of many photo-transformed DOC compounds; the bacterial carbon and energy demand can be completely or almost completely met by the use of them (Bertilsson and Tranvik, 1998).

2.2.2.3 Respiratory quotient in bacterioplankton

The importance of BR and its relation to other processes generating CO_2 includes many uncertainties (Humborg et al., 2010; Berggren et al., 2012) and there is therefore a lack of complete understanding of the carbon cycling in aquatic ecosystems (Berggren et al., 2012).

One such uncertainty is the bacterial respiratory quotient (RQ) defined as the ratio of moles of CO_2 produced to moles of O_2 consumed (Dilly, 2001) in the oxidation of DOC. As the techniques of measuring O_2 concentrations are more developed than the ones measuring CO_2 concentration; the consumption of O_2 or dissolved oxygen (DO) is generally measured (del Giorgio and Cole, 1998; Staerr et al., 2010; Mc Nair et al., 2013) and the RQ is thus needed for the conversion to CO_2 production rates (Berggren et al., 2012). In studies, a value for RQ within a range of 0.7-1.2 based on somewhat arbitrary assumptions is frequently used (Cimberlis and Kalff, 1998; Berggren et al., 2012). Most often an RQ of 1.0 is assumed (e.g. Biddanda et al., 1994; del Giorgio and Cole, 1998). These assumptions may or may not lead to large errors in the

approximated BR value. Berggren et al. (2012) suggest that the uncertainties are larger than believed so far and that the RQ is often underestimated which propagates through the estimation of the BR contribution to CO_2 efflux and thus the role of the bacteria in this process. Del Giorgio and Cole (1998) argue that this is a minor source of error compared to other problems within the conversion from O_2 consumption to BR and BP. There is anyhow a lack of empirical studies of the bacterial RQ in aquatic ecosystems.

Theoretically, the RQ can be obtained by analysis of the stoichiometry of complete oxidation of compounds. One example is glucose:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$,

where a complete oxidation would give an RQ of 1. Whereas, in the complete oxidation of oxalic acid, the RQ is 4:

$C_2H_2O_4 + 0.5O_2 \rightarrow 2CO_2 + H_2O$.

The RQ is dependent on the oxygen content of the substrate mineralized. If the oxygen content is low, the RQ thus becomes low since more O_2 from the surroundings is needed for oxidation and vice versa (Dilly, 2001). Plankton consist of reduced and oxygen poor material, and hence the RQ is low (in studies set to 0.7) (Hedges et al., 2002; Berggren et al., 2012). Complete oxidation of humic acids from soils gives an RQ of 0.9 (Dilly, 2001). However, humic acids are often of HMW and thus too complex to be degraded biologically (Sondergaard and Middelboe, 1995; Bertilsson and Tranvik, 1998) and the bacterioplankton therefore use further degraded DOC more easily assimilated by the cells (Berggren et al., 2010). This LMW DOC is partially oxidized and therefore of higher oxygen content than the non-degraded compounds, leading to a larger RQ (Berggren et al., 2012).

It cannot be taken for granted that all DOC will be oxidized, but is rather used for growth or enzyme production in turn leading to an elevated RQ (Dilly, 2003; Berggren et al., 2012). Biomass includes substrates of low oxygen content and production of these does therefore not require as much O_2 consumption as catabolic respiration (Dilly, 2003). Additionally, the bacteria may selectively use part of the substrate without mineralizing it completely.

Access to nutrients increases the BGE as more carbon is incorporated into the biomass (del

Giorgio and Cole, 1998; Cimberlis and Kalff, 1998). Anabolic respiration is performed at higher RQs than catabolic (Dilly, 2003), and thus an increase in BGE due to nutrient availability should also increase the RQ in the system. Reduced compounds are more energy rich than oxidized and are therefore assimilated at higher BGEs (del Giorgio and Cole, 1998). Even if growth leads to elevated RQ levels, degradation of reduced compounds starts at such low RQ levels that the elevated RQs are still relatively low compared to degradation of oxidized compounds at low BGEs. A positive correlation between BGE and RQ is hence not observed in nature. Additionally, in nutrient poor systems the maintenance costs are higher and the bacteria cannot afford to break down energy poor, oxidized compounds resulting in selective degradation of reduced compounds at low RQs (Cimberlis and Kalff, 1998).

2.2.2.3.1 RQs in natural ecosystems

Lakes receive large amounts of allochthonous DOC from litter and humus. This DOC is mainly of HMW and needs to be partially degraded in order to be available to bacterioplankton assimilation. Depending on the productivity, in the lake the main source of substrate for the bacteria is either autochthonous or allochthonous. Berggren et al. (2012) found a relation between the degree of supersaturation of CO_2 in a lake and the RQ in the system. If the lake was CO_2 supersaturated and hence had a CO_2 efflux to the atmosphere the RQ tended to be higher (mean RQ = 1.35 for 61 observed lakes) than in the case where the lake was O_2 supersaturated (mean RQ = 0.81 for 11 observed O_2 supersaturated lakes). In the CO_2 supersaturated lakes, the most abundant substrates were of HMW DOC with high photo-chemical reactivity. The highest RQs were found for the bacterial communities which had the highest capacity to degrade highly oxidized organic acids. In O_2 supersaturated lakes the main DOC pool contains planktonic material of lower oxygen content explaining the lower RQ obtained (Hedges et al., 2002). The study shows a mean contribution of BR to CO_2 efflux of 69% while assuming an RQ of 1 the same contribution would be 52% in larger systems (> 0.1 km^2). In the smaller systems (< 0.1 km^2) the same difference in BR contribution to CO_2 efflux was 50% and 32% respectively.

The factor that seems to be the dominating control of RQ is the origin and the composition of the DOC rather than the amount (Cimberlis and Kalff, 1998; Berggren et al., 2012). This indicates

that in humic lakes where the bacteria consume the photo-chemically degraded allochthonous DOC of high oxygen content, the RQ ought to be higher than in more productive lakes where the main source of DOC is autochthonous. In turn leading to more CO_2 emitted from these lakes to the atmosphere. The rate of photo-chemical degradation and hence the amount of UV radiation on the system should thus also be a controlling factor of RQ (Berggren et al., 2012).

In marine ecosystems, the RQ is generally lower than in freshwaters, since the amount of allochthonous DOC is not comparable to that in freshwater systems and the food web has a different composition. Robinson et al. (2002) for instance found a mean RQ of 0.8 for 11 study sites. Studies of the photosynthetic quotient (PQ = O_2 consumption / CO_2 production by moles) for production of different algae substrates in marine systems give a range of PQ of $1.03 < PQ > 1.4$ (Robinson and Williams, 1999 and references therein). A subsequent RQ range for consumption of the same substrates is $0.667 < RQ > 0.97$ (Robinson and Williams, 1999). It has been shown that in eutrophic, upwelling regions in marine systems, the BP can be up to four times the BR while the opposite ratio can be noted in oligotrophic regions (Robinson et al., 2002). According to the argument above that anabolic processes have different stoichiometry (Dilly, 2003), a subsequent difference in RQ should be observable. In a study of plankton production and respiration along an off-shore transect in the Indian Ocean, Robinson and Williams (1999) found RQs varying between 0.4 and 5. The highest values of RQ were found at off-shore, net heterotrophic stations with low phytoplankton biomass, these high RQs could not be accounted for by stoichiometry of organic metabolism.

Total respiration in aquatic sediments is a combination of respiration of several organisms such as larvae, fungi, and algae but primarily it is bacterial (Hargrave, 1972). Below the top 1.8 mm, sediments are generally anaerobic and hence mineralization with alternative electron acceptors other than oxygen takes place (Jörgensen and Revsbech, 1985). Anaerobic decomposition of organic matter through pathways of manganese (Mn^{4+}), iron (Fe^{3+}), nitrate (NO_3^-) and sulfate (SO_4^{2-}) respiration stands for a substantial part of the metabolism (Jörgensen and Revsbech, 1985; Boucher et al., 1994). As a consequence, the apparent RQ of substrate degradation in sediments is elevated compared to degradation of the same substrates in oxygen-rich environments. In order to estimate the community RQ in sediments both aerobic and anaerobic

respiration must be taken into account as they take place simultaneously (Boucher et al., 1994). Anaerobic respiration in sediments release CH_4 that can either escape to the atmosphere if the sediments are shallow or it becomes oxidized in the water column (Algesten et al., 2005). Since CH_4 does not contain oxygen, its oxidation yields a low theoretical RQ (0.5 for complete oxidation).

In soils, organic carbon in the form of litter and plant exudates are transformed and mineralized by heterotrophic microorganisms through respiratory processes. For complete oxidation of substrates like glucose under aerobic conditions, the RQ is 1.0 (Dilly, 2003). In field measurements of soil respiration, the evolution of CO_2 is frequently measured while in laboratory studies CO_2 production and/or O_2 consumption rates may be measured. As in freshwater systems an RQ of 1.0 is often assumed in estimations of soil respiration from O_2 consumption data. However, the substrates assimilated by microbiota in soils are of various quality and origin and may be either oxidized or reduced resulting in RQs above or below 1.0 respectively (Dilly, 2001). Environmental and nutritional factors may retard the complete oxidation and affect the RQ. In anaerobic environments, alternative electron acceptors such as nitrate NO_3^- or Fe^{3+} may couple to the substrates resulting in higher RQs than when O_2 is abundant. If on the other hand ammonium (NH_4^+) is abundant; nitrification may take place in turn lowering the RQ. Additionally, increased temperatures to 20 ºC may have a negative effect on RQ, indicating favorable conditions for assimilation of cellulose rather than lignin at lower temperatures and vice versa (Chapman and Thurlow, 1998).

2.3 Climate change affects the carbon cycling in inland waters

Increased CO_2 levels to the atmosphere have been observed since the industrial revolution as a result of fossil fuel burning and other human activities. The enhanced greenhouse effect coming with this leads to perturbation of the energy balance, in turn changing the climate with a global warming, a shift in precipitation patterns, and extreme weather events (IPCC). The water cycle is sensitive to climate change and the carbon fluxes in water bodies will therefore respond to the same (Battin et al., 2009). An increased productivity in terrestrial ecosystems together with

heavier rainfalls and storms will lead to a rise in export of DOC to and nutrient loadings in inland waters (Christoffersen et al., 2006; Larsen et al., 2011).

Increased temperatures in lakes have been observed to increase pCO_2 as an effect of increased respiration, specifically in lakes with low abundance of primary producers. Respiration rates tend to increase more than primary production rates with temperature and warm lakes metabolize a considerably larger portion of allochthonous DOC than cool ones (Kosten et al., 2007). Discharge events refresh the allochthonous DOC pool in lakes and higher inflow:evaporation ratios also give an elevated pCO_2 (Jonsson et al., 2003; Kosten et al; 2007) with increased mineralization rates at longer residence times (Larsen et al., 2011).

Photo-chemical processes are highly dependent on climatic and environmental factors. One such is obviously the amount of incoming solar radiation; an overcast sky inhibits the radiation from reaching the surface lowering the rate of the photo-degradation. The amount of photo-chemically reactive DOC in the waters is another; increased concentrations of CDOM will lead to shading of the underlying DOC and photo-degradation only taking place in the surface layer (e.g. Granéli et al., 1996). The hydrology also plays an important role; a longer residence time facilitates the DOC to photo-react.

Reduced ice-cover times of lakes and thawing soils due to global warming will particularly affect aquatic ecosystems in boreal and arctic zones with longer productive seasons and increased carbon inputs (Cory et al., 2014). Increased input of photo-labile, fresh DOC make photo-degradation rates in these regions expected to increase (Porcal et al., 2013; Cory et al., 2014). In arctic tundra regions, rivers and lakes are often shallow, and the UV-light penetrates through the whole water column making photo-reactions take place in the entire water bodies. The potential for increased stratospheric ozone in arctic regions makes the photo-oxidation particularly important (Cory et al., 2014).

2.4 Measuring and modeling bacterial respiration

The techniques for measuring O_2 concentrations in water are more developed than the ones measuring CO_2, and therefore the consumption of O_2 or dissolved oxygen (DO) is generally measured when estimating bacterial respiration in aquatic ecosystems (del Giorgio and Cole, 1998; Staerr et al., 2010; Mc Nair et al., 2013). Measuring DO can be performed by titration, e.g. the automatized Winkler titration described by Williams and Jenkinson (1982). This method requires large volumes of water samples and does not supply continuous measurements, for changes over time the titration must therefore be repeated. Recently methods of measuring diel oxygen, i.e. "free water" changes in DO over 24 h, are frequently used. Sondes with electrodes or fiber optic sensors can be placed in the water, measuring and recording the DO concentration continuously at desired time intervals. The change in DO in each time step is due to two processes: net ecosystem production (NEP) and diffusive exchange with the atmosphere. The coefficient of gas exchange is modeled for a given temperature and wind speed and the diffusive exchange with the atmosphere can be calculated and withdrawn from the DO, obtaining the NEP. NEP in turn is the difference between gross primary production and total ecosystem respiration ($R_{day} + R_{night}$) (Odum, 1956; Cole et al., 2000; Staerr 2010).

In the conversion from O_2 consumption to CO_2 evolution, the RQ is crucial. By knowing that the gross primary production (GPP) is zero at night and assuming that BR during the day is the same as during the night, the GPP and BR is obtained through the variations in the concentrations of DO over 24 h (Cole et al., 2000; Staerr, 2010). The calculations however include a number of assumptions and errors may propagate through them. One example is the assumption about the BR being equal at all hours; the BR has been shown to be higher at daytime than at nighttime (Cole et al., 2000). Additionally, measuring only O_2 consumption rates, possible anaerobic respiration is missed.

Measuring dissolved CO_2 usually requires equilibrating the pCO_2 in the water with closed loop of air, measuring the equilibrated gas sample with an infrared gas analyzer (IRGA). Water must be exchanged during equilibration and is pumped through the system (Carignan, 1998; Hanson et

al., 2003). In collected water samples dissolved CO_2 can be measured by the headspace method; equilibrating a volume of water with a volume of air, analyzing the equilibrated gas with an IRGA or a gas chromatograph (GC). However, the techniques of measuring dissolved CO_2 are so far more expensive than the ones measuring DO.

Other, indirect measuring techniques, of CO_2 concentration in water include pH measurements or measurements of total inorganic carbon (TCO_2). The TCO_2 measurements have generally been performed by coulometric titration as described by Johnson et al. (1985). Continuous pH measurements can be performed both *in situ* and in collected water samples in laboratory studies. From the TCO_2 and pH measurements, the CO_2 concentration can be obtained, knowing the alkalinity of the system.

2.4.1 Alkalinity

The alkalinity of an aqueous solution is a measure of its resistance to acidification, i.e. its ability to tolerate an increase in H^+ without a lowering of the pH. In other words it is the acid neutralizing capacity, in the carbonate system referring to the proton condition of H_2CO_3, CO_2(aq) and H_2O (eq. 1) i.e. the deficiency of H^+ with respect to the zero proton level at the CO_2 equivalence point, at pH 4.5 (Cai et al., 1998):

$$[Alk] = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+] \quad (1)$$

For ionic equilibrium, the alkalinity can be defined as conservation of charge. The unit of alkalinity is M (molar concentration; moles of protons per liter) or equivalents per liter; one equivalent is here one mole of charge. The alkalinity of a carbonate bearing water can be obtained by titration with a strong acid to an equivalence point representing the equivalent sum of bases titratable with the acid. Thus alkalinity for aqueous carbonate systems is a measure of the amount of strong acid needed to reach the same pH as for a pure molar solution of H_2CO_3 and CO_2(aq) (Stumm and Morgan, 1996).

If the alkalinity of an aqueous solution is known, the carbonate system can be modeled. As CO_2 enters the system through BR, several reactions affecting the pH might take place. Part of the

dissolved CO_2 reacts with the water molecules forming carbonate acid (H_2CO_3) in turn giving rise to HCO_3^-, CO_3^{2-} and H^+ (Stumm and Morgan, 1996):

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$
$$H_2CO_3 \rightleftarrows HCO_3^- + H^+$$
$$HCO_3^- \rightleftarrows CO_3^{2-} + H^+$$

Or in case of low pH:

$$H_2CO_3 \rightleftarrows CO_3^{2-} + 2H^+$$

The H^+ ions lower the pH at the same time as it is increased due to the HCO_3^- and the CO_3^{2-} ions. Thus depending on the alkalinity the net change in pH is either increased or decreased. Addition of CO_2 to the water does not affect the alkalinity since the reactions produce the same amount of negative and positive charges (Stumm and Morgan, 1996).

By monitoring the pH the rate of CO_2 produced can be modeled. If the alkalinity is high; a larger amount of CO_2 needs to be added to the system in order to change the pH significantly, hence more CO_2 is entering the carbonate cycle. If, on the other hand, the alkalinity is low, the pH drops faster with only a relatively small amount of CO_2 addition and the CO_2 stays as CO_2 to a larger portion.

Part of the CO_2 produced in the mineralization reacts with the water and carbonate ions, forming bicarbonate and hydrogen ions that contribute to a lowering of the pH in the ecosystem (Steel et al., 2013). These reactions are lowering the apparent RQ of the system (Berggren et al., 2012).

3. Methods

In order to investigate the influence of photo-chemical transformation of DOC on bacterial RQ incubations of samples with irradiated water were compared to dark controls. By adding inorganic nutrients to one of the samples the nutrient effect on the RQ was controlled for.

3.1 Sample preparation

To be sure that the compounds were photo-chemically reactive, samples of humic content were chosen. Three different types of samples were used: i) a lake water sample with natural water from an unproductive lake, ii) a sample with standard leonardite extracted humic acids, and iii) a sample with standard leonardite extracted humic acids and added nutrients (N +P). Leonardite is the world's largest source of commercially produced humic acids, providing a highly standard form of HMW humic compounds. It has been used for several decades within humic acid research, as it generates results that can be replicated. However, while leonardite provides the best, i.e. most stable, standard, it should be noted the humic acids that occur in natural waters are diverse, and to various degrees structurally different than leonardite-extracted acids (Malcolm and MacCarthy, 1986). Therefore, experiments were also performed with natural lake water.

The lake Övre Björntjärn is situated at 71˚16' N, 14˚44' E and is surrounded by coniferous forest and mires. It is a rather unproductive lake with a mainly allochthonous DOC pool (Jansson et al., 2001). Water was collected at the epilimnion in the end May of 2014, at this time of the year the thermal stratification is stabilizing after the frozen months (November – early May) (Jansson et al., 2001) and there is an input of fresh DOC from the snowmelt (SMHI). Övre Björntjärn is highly supersaturated with CO_2; leading to a low pH of close to 5 in the sample collected. The supersaturation was evened out through bubbling of the sample with synthetic air (O_2 and N_2) and the pH consequently increased to around 6.8.

The samples with humic acid substrate were prepared in washed plastic bottles. 1 l of deionized water was mixed with 1 ml substrate (Grandma Enggy's H-2, Advanced Nutrients, CA) and 50 ml L16 solution (Lindström, 1991), containing vitamins and minerals but no nutrients. The humic acid substrate does not contain vitamins and minerals essential to the bacteria, explaining the need for L16 solution. To one of the mixes 500 µg N:1 l water and 50 µg P:1 l water (5 ml of

100 mg l^{-1} NH_4NH_3 and 5 ml of 10 mg l^{-1} Na_2HPO_4) was added. These samples were labeled 'HS' and 'HS (N+P)' for the one without and with added nutrients respectively. As argued earlier the molecules most likely to undergo photo-reactions are the ones containing humic substances, which is why the humic acid substrate was suitable to use. Even if these samples are not of natural water, they contain similar type of humic acids as natural water. Working with a standard substrate of this kind there is some control of which compounds are assimilated by the bacteria, and the experiment can easily be replicated. The effects of photo-chemical degradation of humic acids and/or nutrient addition on RQ can be studied knowing that no other substances are present and that the dark control has not been previously exposed to UV-light in nature. It must however be borne in mind that this is not representing a natural system.

3.2 Measurements

3.2.1 Incubation and irradiation

In 100 ml quartz flasks, two optic sensors, one measuring O_2 and the other pH were stuck onto the inside of the neck opposite one another. Quartz was used for its purity and high UV transmission. All samples were sterilized through a 0.2 µm pore size filter (Sartorius Stedim Biotech S.A.) and bubbled for 10 min with synthetic air to get rid of any overpressure in CO_2. Before starting the incubations, 1 ml inoculum of fresh water bacteria (from an unfiltered mix of water from Övre Björntjärn some other brown lakes and streams in the vicinity) was added to the filtered samples. In order to avoid diffusion of CO_2; no head space was left and the flasks were closed with NS grinded glass stoppers and covered with parafilm.

Incubations were carried out in a climate chamber at a temperature of 20 ˚C (±0.1 ˚C). Bacterial O_2 consumption was measured continuously every 5 min as DO concentrations with an 'oxy 10' system (O_2 detection limit: 0.0015 mg l^{-1}; precision: ±0.025; VPreSens, Regensburg, Germany). The bacterial CO_2 production was measured through the change in pH, monitored by the use of a SensorDish Reader (SDR: pH detection limit: 0.05; precision: ±0.1 pH; PreSens, Regensburg, Germany); this device cannot measure pH below 5. Both devices were calibrated for fresh water by the manufacturer and contained software for logging on computer. All incubations were performed during at least 72 h.

Irradiation of samples with artificial UV light was carried out during 48 h in order to be sure that a sufficient portion of the DOM was photo-chemically transformed.

3.2.2 Experimental design

To investigate whether there was an effect on bacterial degradation of photo-transformed DOC compounds on RQ, incubations were performed on both irradiated and non-irradiated samples. The three different types of samples (lake, HS, and HS (N+P)) were incubated and irradiated simultaneously, and in this way external impacts like temperature deviation in the climate chamber could be avoided. Besides incubation of irradiated water and dark controls, samples were first incubated with bacteria and then irradiated halfway through the experiment in one of the treatments, being incubated both before and after, with the aim to investigate whether a shift in RQ could be obtained as a result of photo-chemical transformation of the DOC. The possibility of RQ varying over time as a consequence of microbial transformation of the DOC or due to some other shift in the bacterial community or in the bioavailability of the DOC and not due to photo-chemical reactions cannot be ruled out. The measurements were divided into three different treatment combinations:

1. After filtering and oxygenation, 1 ml of inoculum was added to the samples, which then were incubated in the climate chamber during 72 h. The samples were then moved from the chamber and irradiated with artificial UV light during 48 h. After the irradiation, the samples were again oxygenated to get rid of the overpressure in CO_2 produced by photo-mineralization. Since the UV irradiation has a damaging effect on the bacteria (Anesio and Granéli, 2003) the samples were inoculated with another 1 ml of fresh water bacteria. Finally, they were placed in the climate chamber for another 72 h of continuous measurements.

2. Following filtering the water samples were first placed under the UV lamp for 48 h and then oxygenated and inoculated with 1 ml of fresh water bacteria. Finally, the flasks were incubated during 72 h in the climate chamber.

3. The third treatment represented a non-irradiated control. Thus, the samples were filtered and oxygenated, and inoculum was added. They were then incubated in the climate chamber for 168 h, i.e. the same amount of time as the sum of the three parts of treatment 1. The first 72 h of incubation was considered equivalent to the first 72 h in treatment 1, hence measurements were only performed during the following 48 + 72 h. The bacterial activity produces CO_2 and might lead to overpressure of this, therefore, and in order to have similar procedure as in the other treatments (except for the irradiation) the samples were oxygenated after 120 h (Figure 1).

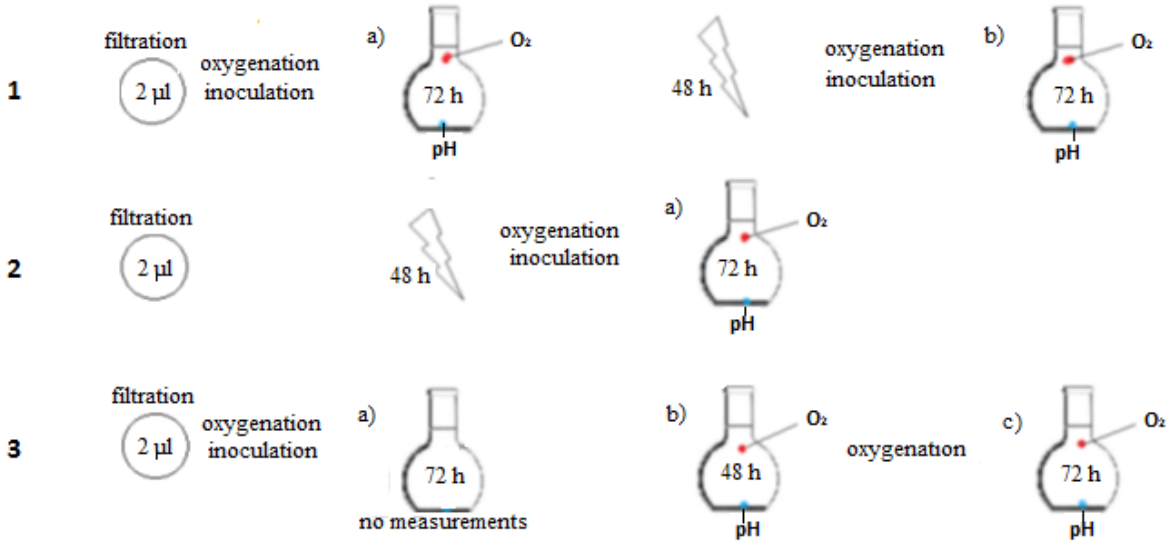

Figure 1. The study was divided into three treatment combinations. Before starting each treatment the samples were sterilized and before each incubation period they were oxygenated and inoculum was added. 1) in the first treatment the samples were incubated for a) 72 h followed by 48 h of UV-irradiation then again followed by b) 72 h of incubation of the irradiated samples. 2) the second treatment was started with 48 h of irradiation followed by a) 72 h of incubation. 3) in the third treatment, the samples were incubated for 192 h and oxygenated after 120 h. During all incubations but the first a) 72 h of treatment 3, oxygen concentration, and pH were monitored.

3.3 Modeling the carbonic system

3.3.1 Titration

To obtain the alkalinity of the water samples, a simple titration method was used. To 100 ml of the sample 0.1 M HCl was added until the pH reached 4.5. At pH 4.5 CO_3^{2-}, HCO_3^- and OH^- ions have been neutralized and converted to H_2CO_3. The three ions are the main forms of alkalinity and hence with them neutralized the buffering capacity is gone. Below this endpoint in pH the water is less able to neutralize the HCl, and the relationship between the added acid and the pH change of the sample becomes direct.

The volume of the added acid divided with the total volume of the sample plus the acid is then direct proportional to the alkalinity in terms of the concentration of carbonate ions:

$$\frac{V_{0.1\ M\ HCl}(ml) \cdot 0.1(M)}{V_{sample}(ml) + V_{0.1\ M\ HCl}(ml)} = alkalinity\ (M) \quad (2)$$

Titration was performed before and after the incubations to make sure that the CO_2 modeling was based on the correct alkalinity.

3.3.2 Calculations

Knowing the alkalinity and the pH of the water; the CO_2 concentration can be calculated. Since the system is closed, any change in pCO_2 comes from mineralization to CO_2. The solute components in aqueous carbonate solutions are: CO_2, H_2CO_3, HCO_3^-, CO_3^{2-}, H^+, and OH^-. The hydration equilibrium of CO_2 is defined as: $CO_2(aq) + H_2O = H_2CO_3$ and lies rather far to the left, as a result the greater fraction of un-ionized CO_2 is in the form $CO_2(aq)$. For the total analytical concentration of dissolved CO_2 one can write:

$[H_2CO_3^*] = [CO_2(aq)] + [H_2CO_3]$, for both hydrated and non-hydrated CO_2 where the last term is negligible. All equations are derived from Stumm and Morgan (1996).

Five equilibrium constants (eq. 3-7) to the equilibrium equations are needed in order to find the concentrations of the different species, the constants are experimentally determined and of high levels of accuracy and vary with temperature:

$$K = \frac{[CO_2(aq)]}{[H_2CO_3]} \tag{3}$$

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3^*]} \tag{4}$$

$$K_{H_2CO_3} = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} \tag{5}$$

$$K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \tag{6}$$

$$K_W = [H^+][OH^-] \tag{7}$$

The concentration condition of total inorganic carbon (C_T) looks as follows:

$$C_T = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}] \tag{8}$$

And the ionization fractions of the DIC species are:

$$[H_2CO_3^*] = C_T\alpha_0 \text{ , where } \alpha_0 = \left(1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}\right)^{-1} \tag{9}$$

$$[HCO_3^-] = C_T\alpha_1 \text{ , where } \alpha_1 = \left(\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}\right)^{-1} \tag{10}$$

$$[CO_3^{2-}] = C_T\alpha_2 \text{ , where } \alpha_2 = \left(\frac{[H^+]^2}{K_1K_2} + \frac{[H^+]}{K_2} + 1\right)^{-1} \tag{11}$$

Hence, the concentrations are strongly dependent on the pH in the solution. As DOC is mineralized into CO_2, forming carbonate species, take place depending on pH and alkalinity. The total CO_2 addition gives the concentration partitioning of DIC as C_T (8) above. Using Henry's law describing the equilibrium distributions of volatile species the hydration equilibrium of CO_2 can be expressed by the mass law relationship:

$$[H_2CO_3^*] = K_H pCO_2 \tag{12}$$

K_H the Henry's law constant for the equilibrium expressed in M atm^{-1}.

As described earlier the alkalinity of a carbonate solution is given by (eq. 1) above. This can be rewritten with ionization fractions equations (10) - (11) as:

$$Alk = C_T(\alpha_1 + \alpha_2) + [OH^-] - [H^+] \quad (13)$$

But $[H_2CO_3^*] = C_T\alpha_0 = K_H pCO_2$ and $K_W = [OH^+][H^+]$

$$\rightarrow Alk = \frac{K_H pCO_2}{\alpha_0}(\alpha_1 + 2\alpha_2) + \frac{K_W}{[H^+]} - [H^+] \quad (14)$$

Rearranging gives the expression for pCO_2:

$$pCO_2 = \frac{\alpha_0\left(Alk + [H^+] - \frac{K_W}{[H^+]}\right)}{K_H(\alpha_1 + 2\alpha_2)} \quad (15)$$

Finally putting equation (15) into Henry's law for CO_2:

$$[H_2CO_3^*] = \frac{\alpha_0\left(Alk + [H^+] - \frac{K_W}{[H^+]}\right)}{\alpha_1 + 2\alpha_2} \quad (16)$$

3.4 Data treatment and statistics

3.4.1 RQ

The respiration quotient is a measure of how many units of O_2 is needed to produce one unit of CO_2. Therefore, the logged concentration of CO_2 can be plotted against the logged concentration of O_2 and the slope of the regression line represents the RQ. Hence:

$$RQ = \frac{\Delta CO_2}{\Delta O_2} \quad (17)$$

3.4.2 Unit conversion of O_2

The concentration of CO_2 is given in µmole l^{-1} in the calculations while the software to the oxy-10 gives the O_2 concentration in mg l^{-1}; it must therefore be converted to the same unit in order to obtain the RQ. The molar mass of O_2 is 32 g $mole^{-1}$, thus:

$$mg(O_2)l^{-1} = \frac{1}{32 \cdot 1000}\ \mu mol(O_2)\ l^{-1} \quad (18)$$

Datasets contained O_2 and CO_2 data for every 5 min during the incubations. In order to make it more manageable averages for every hour was calculated.

3.4.3 Analysis of statistical significance

Statistical significance analysis was carried out in SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA). The standard error of regression lines was obtained using the LINEST function in Microsoft Excel (Microsoft, MA, USA).

3.4.4 Comparison of regression lines

To investigate the effect of the UV treatment on RQ, the slopes of irradiated samples were compared to the ones of non-irradiated samples. This comparison was performed using Analysis of Covariance (ANCOVA). If there is an interaction between the treatment, in this case UV irradiation, and the covariate, here O_2 consumption, the slopes of the regression lines are different. With a p value of $p < 0.05$, the difference in slopes were considered significant.

As incubations started, some time was needed for equilibration of temperature between the samples and the surroundings in the climate chamber. The first hours of the incubations before linearity was reached were not considered in the analysis. The slopes of irradiated water were compared to each slope of non-irradiated water; resulting in three controls to every sample.

3.4.5 The role of nutrient addition

To further analyze how the RQ differed between a humic acid solution with inorganic nutrients (N + P) added and the same mixture with no nutrients; the RQs of each time step (averages of an

hour) were compared. The non-parametric Mann-Whitney test was used, since the data was not normally distributed. The differences were considered significant for α-values of $\alpha < 0.05$.

3.5 Uncertainty analysis

As a consequence of the treatments the alkalinities of the humic acid substrate samples dropped from 291 µeq l^{-1} to 216 µeq l^{-1} and from 304 µeq l^{-1} to 236 µeq l^{-1} for the HS and HS (N+P) samples respectively from the start of the treatments to the end, while the alkalinity of the lake water remained the same.

The RQs were obtained from CO_2 concentrations calculated with different values of the alkalinity. Four different scenarios of alkalinity decrease were analyzed: 1) the alkalinity dropped immediately and the lower value (HS (N+P): 236 µeq l^{-1}; HS: 216 µeq l^{-1}) was used to obtain CO_2 evolution in all treatments; 2) the alkalinity did not drop until the end of the incubations and the higher value (HS (N+P): 304 µeq l^{-1}; HS: 291 µeq l^{-1}) was used throughout the treatments; 3) the alkalinity declined linearly resulting in one specific value for calculation of CO_2 at every measurement point (averages of an hour); 4) the alkalinity declined gradually during incubations but not linearly, in this scenario the higher value was used for calculations of CO_2 rates during the first 72 h (1a), the lower for the last 72 h (1b; 2a; 3c), and the mean value of the two measured alkalinities (HS (N+P): 268 µeq l^{-1}; HS: 254 µeq l^{-1}) was used for the middle 48 h of the dark incubation (3b).

4. Results

The DOC concentrations in the water samples used were relatively high: 24.7 mg l^{-1} for the two humic acid solutions and 21.7 mg l^{-1} for the lake water sample. The datasets from the incubations were all large enough to give significant results even if some of the datasets missed data due to the loss of contact between the sensor and the measuring device.

4.1 Irradiation impact on bacterial RQ

In order to investigate the irradiation impact on the RQ the CO_2 production was plotted against the O_2 consumption for each treatment step; the slope represents the RQ. The datasets of irradiated water (treatment 1b with biological incubation prior to irradiation; and treatment 2a) starting with irradiation) were compared to the datasets of non-irradiated water (treatment 1b); treatment 3a and; treatment 3b.

4.1.1 Lake

The time needed to obtain linearity between O_2 consumed and CO_2 produced was around 20 h from when the incubations started. The bacterial community was assumed to be damaged during the irradiation and therefore another 1 ml of fresh water inoculum was added to the samples before restarting the incubations. There was then another 20 h needed to obtain linearity. Due to this the datasets contained data from at least 50 h which was considered a significant amount.

The coefficients of determination (r^2) of the regression lines were between 0.88 and 0.98 and the standard errors (s.e) were between 0.0067 and 0.098 (Table 1)

The RQs in the samples were above 1 in all cases but the dark control treatment 3b. When the lake water was irradiated without prior incubation (treatment 2a), the RQ was high (RQ = 3.5: Table 1). The slopes were compared through an ANCOVA analysis showing a significant interaction between the treatment and the covariate, and hence a difference in slopes, for the comparison of treatment 1a with treatment 3b non-irradiated sample; while no significant differences in slopes were observed in the comparison with treatment 1b and treatments 1a and

3c (Figure 2; Table 1). Comparisons between treatment 2a and the dark controls showed significant differences in slopes in all three cases (ANCOVA: Table 1).

The lake water experiment

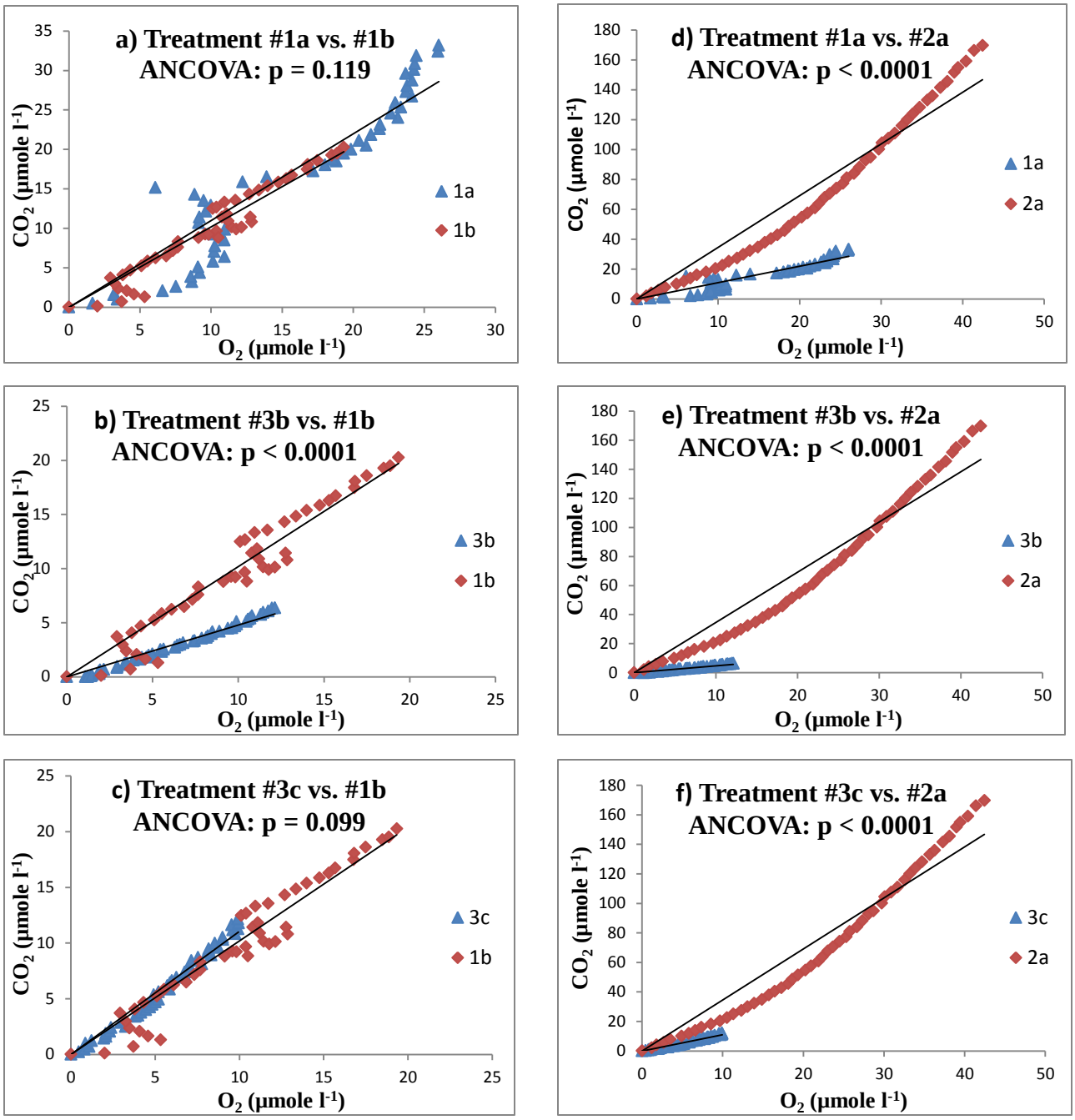

Figure 2. Comparison of the slopes (RQs) in the lake water experiment. To the left (a-c) the RQ in the irradiated water from treatment 1b) (red diamonds) that was incubated for 72 h before irradiation ($y = 1.02x$; $r^2 = 0.93$) is compared to the non-irradiated controls (blue triangles). The controls are in (a) treatment 1a ($y = 1.1x$; $r^2 = 0.88$); (b) Treatment 3b ($y = 0.48x$; $r^2 = 0.97$) and (c) treatment 3c ($y = 1.1x$; $r^2 = 0.98$) incubations respectively. To the right (d-f) the same comparisons are seen for the sample irradiated without previous incubation (treatment 2a: $y = 3.5x$; $r^2 = 0.94$). Note the axes.

Table 1. Information of the slopes of linear regression representing the RQ in the lake water at the different experimental steps.

	RQ	r^2	s.e.
irradiated water:			
treatment 1b	1.02	0.93	0.04
treatment 2a	3.5	0.94	0.098
non-irradiated water:			
treatment 1a	1.1	0.88	0.06
treatment 3b	0.48	0.97	0.0067
treatment 3c	1.1	0.98	0.021

4.1.2 Nutrient enriched humic acid solution

In the HS (N+P) mix some 20 h were needed to obtain linearity between O_2 consumed and CO_2 produced after the start of incubations. The same applied for incubation of both non-irradiated and irradiated samples with new inoculum. The bottles used were rounded, and movement in relation to the SDR plate could not be completely avoided. The pH sensor therefore lost contact after a few hours in treatment 1b, leading to only 13 h of data. All datasets were considered large enough to give reliable results.

The two datasets of irradiated water were compared to the three datasets of water that was not irradiated in the same way as the lake water samples. The RQ in irradiated samples was well above 1 (RQ = 1.8 and RQ = 2.4 in treatments 1b and 2a respectively). All three RQs in non-irradiated samples were below 1 (Figure 3; Table 2).

The slope and hence the RQ of the irradiated samples was significantly different from all three controls with $p < 0.0001$ (ANCOVA: Figure 3; Table 2).

The HS (N+P) experiment

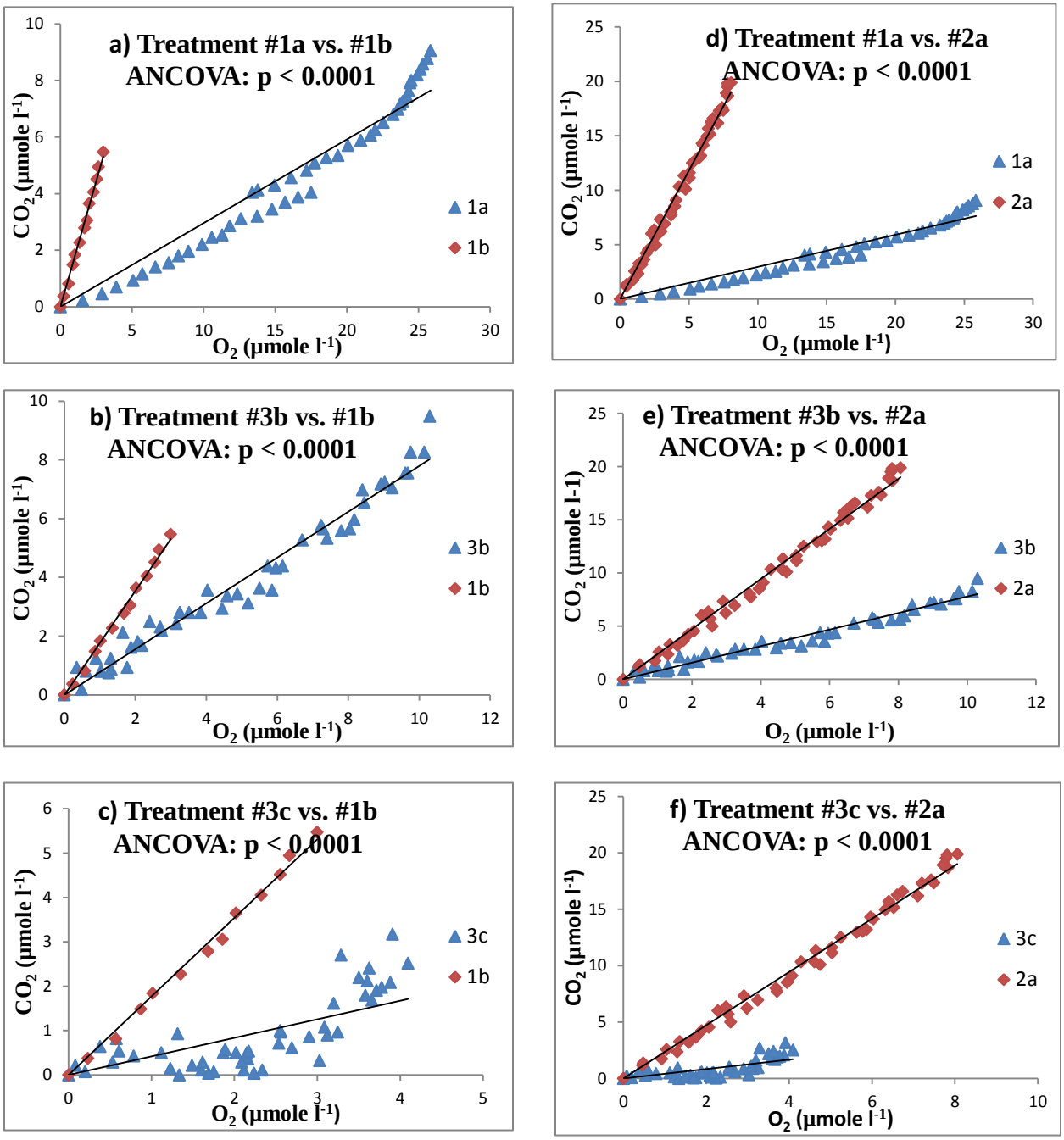

Figure 3. Comparison of the slopes (RQs) in the HS (N+P) experiment. To the left (a-c) the RQ in the irradiated water (red diamonds) that was incubated before irradiation, treatment 1b ($y = 1.8x$; $r^2 = 0.99$) is compared to the non-irradiated controls (blue triangles). The controls are in (a) treatment 1a ($y = 0.3x$; $r^2 = 0.94$); (b) treatment 3b ($y = 0.8x$; $r^2 = 0.97$) and (c) treatment 3c ($y = 0.4x$; $r^2 = 0.52$) respectively. To the right (d-f) the same comparisons are seen for the sample irradiated without previous incubation (treatment 2a: $y = 2.4x$; $r^2 = 0.99$). Note the axes.

Table 2. Information about the slopes of linear regression in the different experimental steps for the HS (N+P) water sample.

	RQ	r^2	s.e.
irradiated water:			
treatment 1b	1.8	0.99	0.039
treatment 2a	2.4	0.99	0.034
non-irradiated water:			
treatment 1a	0.3	0.94	0.0097
treatment 3b	0.8	0.97	0.020
treatment 3c	0.4	0.52	0.038

4.1.3 Humic acid solution without addition of inorganic nutrients

Linearity between O_2 consumption and CO_2 production was obtained faster in the HS water than in the lake and the HS (N+P) water samples; 10-15 h in non-irradiated samples and almost immediately (after only about 5 h) in the irradiated samples. This means that datasets of 50 h – 69 h were obtained.

The RQ of the non-irradiated water of treatment 1a was low (RQ = 0.16). After irradiation it increased to 0.85 (treatment 1b). When more time had passed in the dark control incubations, the RQ increased (treatments 3b and 3c). The irradiated sample in treatment 2a was similar to the one in treatment 1b (Figure 4).

Comparison of the slopes using ANCOVA showed significant interaction between the treatment and the covariate (UV irradiation), this applied for both irradiated samples (treatments 1b and 2a) and all three non-irradiated controls (Table 3).

The HS experiment

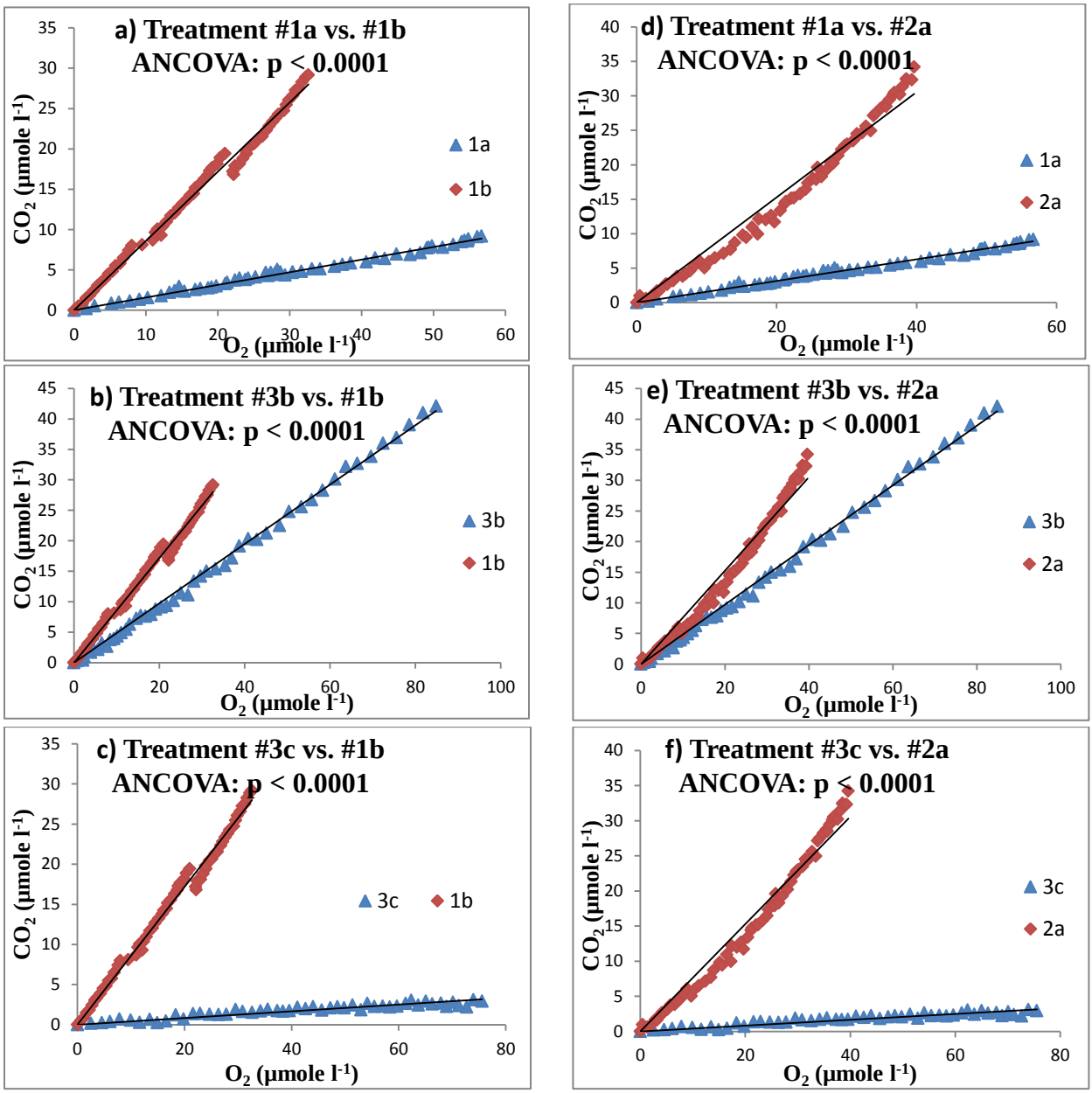

Figure 3. Comparison of the slopes (RQs) in the HS experiments. To the left (a-c) the RQ in the irradiated water from treatment 1b (red diamonds) that was incubated before irradiation (y = 0.9x; r^2 = 0.99) is compared to the non-irradiated controls (blue triangles). The controls are in (a) treatment 1a (y = 0.2x; r^2 = 0.99); (b) treatment 3b (y = 0.5x; r^2 = 0.99) and (c) treatment 3c (y = 0.04; r^2 = 0.86) incubations respectively. To the left (d-f) the same comparisons are seen for the sample irradiated without previous incubation (treatment 2a: y = 0.8x; r^2 = 0.97). Note the axes.

Table 3. Information of the slopes of linear regression for the HS water sample in the different experimental steps

	RQ	r^2	s.e.
irradiated water:			
treatment 1b	0.9	0.99	0.010
treatment 2a	0.8	0.97	0.013
non-irradiated water:			
treatment 1a	0.2	0.99	0.0019
treatment 3b	0.5	0.99	0.0032
treatment 3c	0.04	0.86	0.0018

4.2 Inorganic nutrient effects on bacterial RQ

The samples with the humic acid substrate were equal apart from the inorganic nutrient (N+P) addition in the HS (N+P) sample. Comparisons in the different treatment steps between these two show the nutrient effect on RQ. In the samples with added nutrients (N and P) the bacterial RQ was consistently higher than in the samples without any nutrient enrichment (Figure 4 and 5). The median RQs from the different treatments were significantly higher for the water with N and P enrichment than without; Mann-Whitney test gave $\alpha < 0.001$ for all treatments. The variations within each incubation were smaller in the samples that were incubated before irradiation (treatment 1) as well as in the samples without any addition of nutrients (HS). The RQs in the non-irradiated samples were generally lower than in the irradiated samples (as shown above).

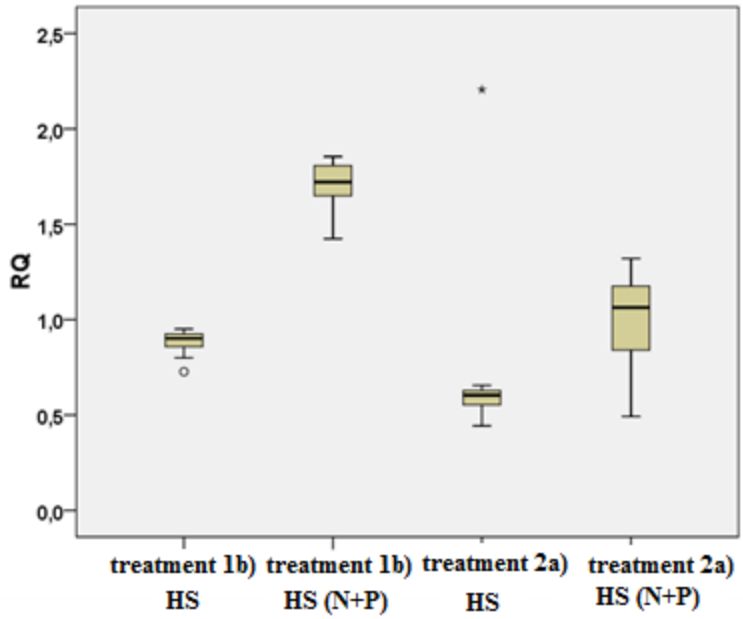

Figure 4. Box-and whisker plot of the RQs in the irradiated humus substrate samples. In treatment 1b (HS: n = 63; HS (N+P): n = 13) the water samples were incubated for 72 h before UV treatment followed by further incubation, whereas in treatment 2a (HS: n = 67; HS (N+P): n = 65) incubation was only performed after incubation. The RQs in the samples with nutrient addition (HS (N+P)) showed significantly higher values than those without (HS) in both cases ($\alpha < 0.001$). Nutrient enriched samples showed higher within incubation variations in RQ.

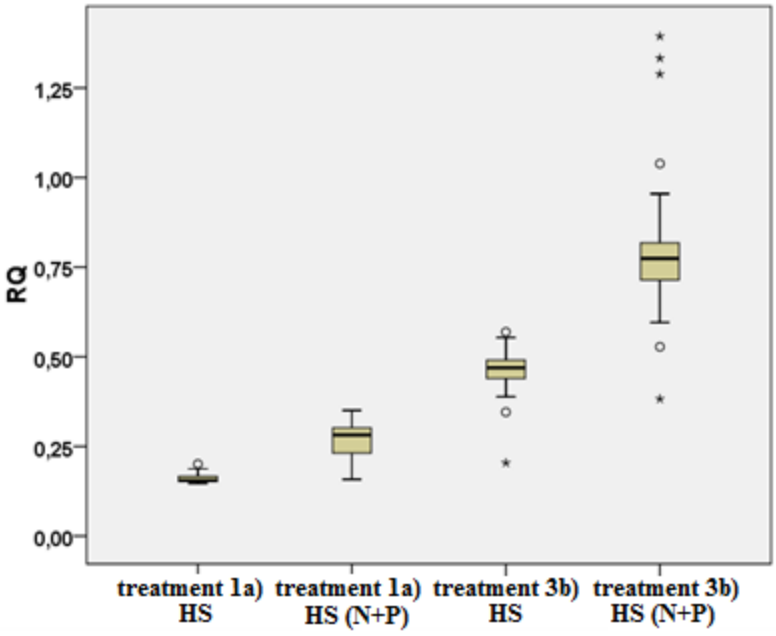

Figure 5. Box-and whisker plot of the RQs in the non- irradiated humus substrate samples. The RQs in the samples with nutrient addition showed consistently higher values than those without ($\alpha < 0.001$). The variations in RQ within the incubations were larger in the nutrient-enriched samples. The RQs were lower in treatment 1a (HS: n = 58; HS (N+P): n = 45), increasing in treatment 3b (HS: n = 48; HS (N+P): n = 47).

4.3 Uncertainty analysis

The obtained RQ values in treatments 1b; 2a; 3c were about 25% higher when using the higher alkalinity compared to when using the lower and in treatment 1a the RQ was 50% higher when modeled with the higher alkalinity than with the lower. Modeling the CO_2 evolution with the linearly decreasing alkalinity gave consistently lower values of RQ than the other scenarios (Tables 4 and 5). In scenario 3 the regression lines of the CO_2 vs. O_2 plots bended downward towards the end of all treatments resulting in non-linearity (data not shown). The RQs calculated from alkalinity declining according to scenario 4 was used in this study.

Table 4. RQs in the HS (N+P) experiment with CO_2 production calculated with different alkalinities.

	Treatment 1a	Treatment 1b	Treatment 2a	Treatment 3b	Treatment 3c
RQ (304 µeq l^{-1})	0.4	2.3	3.1	0.8	0.7
RQ (268 µeq l^{-1})				0.8	
RQ (236 µeq l^{-1})	0.2	1.8	2.4	0.6	0.4
RQ (Linear decrease in alkalinity)	0.2	1.7	2.2	0.6	0.4

Table 5. Possible RQs in the HS experiment as the alkalinity is assumed to drop at different instances during incubations.

	Treatment 1a	Treatment 1b	Treatment 2a	Treatment 3b	Treatment 3c
RQ (291 µeq l^{-1})	0.2	1.0	1.0	0.5	0.05
RQ (254 µeq l^{-1})				0.5	
RQ (216 µeq l^{-1})	0.1	0.9	0.8	0.4	0.04
RQ (Linear decrease)	0.09	0.7	0.7	0.2	0.008

5. Discussion

It was hypothesized that UV-irradiation on water samples with DOC of humic content would give rise to elevated bacterial RQs compared to non-irradiated controls, and further that the RQs would exceed the often assumed value of 1.0. Additionally it was thought that addition of inorganic nutrients would have a positive effect on the bacterial RQ. The overall results of this study were in agreement of the hypotheses. It is here argued that the use of a fixed value of RQ in estimations of bacterial respiration should be reevaluated.

5.1 UV irradiation effect on RQ

As a consequence of the photo-chemical processing of the DOC during light treatment, the results showed elevated bacterial RQs in all cases but one. These results indicate that the role of bacteria in the CO_2 balance of aquatic ecosystems should be reconsidered. In both the natural water and in the nutrients enriched humic acid solution the RQ exceeded the commonly used 1.0 by far after irradiation, especially irradiation of non-preincubated samples (treatment 2a; Figures 1d-f and 2d-f). The lake water sample and the HS (N+P) sample had RQs exceeding 4 and 2 respectively when irradiated without prior incubation. Deriving BR from O_2 consumption with RQ = 1.0 instead would consequently lead to an underestimation by more than half. In both humic acid substrate samples, a shift from low RQ system (treatment 1a) to high RQ system treatment 1b) was observed following UV irradiation, indicating a shift in the DOC pool. A corresponding shift was not observed in the lake water sample and probably the DOC compounds in the lake had been exposed to sunlight and hence undergone some previous partial degradation. In both treatments 1a and 1b the RQ in the natural water exceeded 1.0 (Figure 1; Table 2).

Irradiating a 100 ml sample during 48 h with UV-light degrades most of the photo-reactive DOC, and the bacterial community can selectively assimilate photo-products nearly exclusively. In an ecosystem, however, the UV-light does not hit all molecules and since the bacterioplankton consume a large variety of DOC with different origin and ages, the RQ on an ecosystem level would not reach as high as observed in the water samples in this study. Berggren et al. (2012) found a mean RQ of 1.35 in a large number of unproductive lakes supersaturated with CO_2.

It is well known that photo-chemical reactions mineralize and break down DOC into CO_2 and smaller compounds (e.g. Tranvik, 1988; Brinkmann et al., 2003). As studies show: the photo-produced compounds to a large part are carboxyl acids and carbonyl compounds (Zhou and Mopper, 1997; Bertilsson and Tranvik, 1998) containing more oxygen than the original HMW DOC compounds. This higher oxygen content may explain the elevated RQs in the irradiated samples. The organic acids in the samples were not analyzed in the present study, but previous studies give enough reason to make this assumption (e.g. Zhou and Mopper, 1997).

There was a time lag of about 10-20 h before linearity was obtained between the CO_2 production and the O_2 consumption from when the water samples were first incubated. Before stabilizing the RQs were lower which might be explained by the bacterial community needing to establish (Amado et al., 2014). During establishment more energy/carbon is incorporated in the biomass of the microorganisms leading to a lower CO_2 production rate and lower RQs (e.g. del Giorgio and Cole, 1998), the O_2 consumption rate was however stable from start. After some 10-20 hours had passed the RQs increased with a strong correlation between CO_2 and O_2. The carbonic system is known to be slow (Stumm and Morgan, 1996), which might be an additional explanation the longer time before stabilization of the CO_2 production rates.

The RQs in the humic acid substrate samples were low but with a strong correlation between CO_2 production and O_2 consumption (RQ = 0.2 and RQ = 0.3 for the non-nutrient enriched and the nutrient enriched respectively) in the first 72 h of the dark treatment (1a), increasing in the following 48 h (3b) (Figures 1-3; Tables 2 and 3) reaching 0.5 (HS) and 0.8 (HS (N+P)). Thus, there was a time lag in reaching maximum RQ of about 72 h for both the HS and HS (N+P) samples. The humic acids in the humic acid substrate samples are of high molecular weight and might need to be partially degraded before the bacterioplankton can assimilate them. The process of producing exo-enzymes and breaking down the compounds to a more bioavailable form is energy demanding (del Giorgio and Cole, 1998; Cimberlis and Kalff, 1998). This cost in energy could be an explanation to the long time lag in the stabilization in bacterial community and thus RQ.

Chemical stoichiometry suggests that the RQ should not be lower than 0.5 (complete oxidation of CH_4) or 0.7 (complete oxidation of fatty acids) and the values observed during the establishment phase were surprisingly low. The low RQs indicate that the DOC is not completely oxidized. The bacteria might partially oxidize the compounds in a similar way as the UV light does, which would result in low RQs, and thus elevate the oxidation state of the DOC pool. However, these partially oxidized compounds would be assimilated by other bacteria in turn, at an elevated RQ. Berggren et al. (2012) found RQs below the stoichiometrically possible values in lakes of high pH, arguing that the alkaline water gave rise to precipitation of $CaCO_3$ and part of the CO_2 produced was therefore not registered. However, the pH in the water samples in this study were never in the alkaline range and probably the low RQs were a consequence of anabolism and degradation of reduced compounds. The values are still lower than what can be thoroughly explained and even if the method is well tested and the sensors sensitive to changes the possibility that there was something wrong with the measurements in this treatment cannot be ruled out.

After the UV-treatments there was again a time lag of 10-20 h before a stable relation between CO_2 production and O_2 consumption was reached with a relatively steep slope compared to dark controls. Different bacteria break down different substrates, and it might take time for the bacterial community to reform (Judd et al., 2007). If only part of the community in the inoculum is adapted to the organic acids, this part needs to multiply before degradation can take place. Apart from the establishment of bacterial community this immediate negative effect on RQ can be due to photo-production of reactive oxygen species, inhibiting the microbial activity (Farjalla et al., 2001; Anesio et al., 2005; Amado et al., 2014). The half-life of such species is in the order of hours and the repression thus declines rather soon (Tranvik and Kokalj, 1998; Pullin et al., 2004). Anesio et al. (2005) saw a decrease in BP after 5 h of incubation with irradiated DOC while after a few days it was enhanced compared to non-irradiated controls. They also found a correlation between this initial negative effect and the production of hydrogen peroxide (H_2O_2). In accordance with this Amado et al. (2014) observed a delay in bacteria reaching maximum BP rates in irradiated samples compared to dark treated controls as an effect of radical oxygen species. In the current study, the BP was however not measured and a direct comparison cannot

be made but it might show how photo-chemical reactions impact the use of carbon by bacterioplankton.

Except for the lake water in treatment 1, where the samples were first biologically incubated in the dark before 48 h of UV-light exposure, all RQs increased in UV-treated waters. Compared to the controls, the CO_2 production rates were in most cases higher in the irradiated samples, indicating enhanced BR. The O_2 consumption rates did not follow a clear pattern between irradiated samples and dark controls; it both increased and decreased following UV-treatment. The portion of carbon used for BP compared to BR is dependent on the compound being degraded. In their study (1998) Bertilsson and Tranvik found an almost complete bacterial mineralization of formic acid (98%) into CO_2 while only some 20% of the assimilated malonic and acetic acid carbon was mineralized into CO_2. HMW DOC is often oxidized into such carboxylic acids.

In accordance with this several studies have found an increase in BR after photo-chemical degradation of the DOC (Vähätalo et al., 2003; Pullin et al., 2004; Anesio et al., 2005), while Farjalla et al (2001) did not see any difference in BR between UV-treated samples and dark controls for most irradiation times but in a few exceptions where they also found increased BR following light exposure. On the contrary, Amado et al. (2014) observed lower BR rates in exposed samples compared to dark controls. However, they did not measure the CO_2 production rates directly but the O_2 consumption rates with an RQ of 1.0 for conversion in all treatments. Had they used elevated RQs for irradiated samples, the obtained BR would have been enhanced.

The majority of studies on bacterial activity in waters with photo-chemically degraded DOC measure BP and not BR. Many observe an increased BP, especially on longer timescales (e.g. Anesio et al., 2005; Judd et al., 2007; Amado et al., 2014) but if BR is not measured, the BGE cannot be evaluated. If the respiration increases more than the production of biomass, the growth efficiency may still drop. In studies monitoring both BR and production the BGE have been observed to both increase and decrease following photo-chemical processing, depending on the source of the DOC being degraded (Bertilsson and Tranvik, 1998; Pullin et al., 2004; Anesio et al., 2005; Cory et al., 2014). BGE is decisive for estimating the role of bacteria in carbon flow

through the ecosystem (del Giorgio and Cole, 1998). By using an RQ of 1.0 when it in reality is higher the BR is underestimated and so also the BGE and thus more carbon passes through the bacterioplankton than thought. Such consistent underestimation of the amount of carbon passing through the bacteria leads to errors in estimating and modeling the carbon cycle. The carbon cycle of inland waters has been shown to have an important role in the global carbon cycle and models of this kind are needed for understanding and estimations of carbon fluxes between ecosystems and between the biosphere and the atmosphere. In the extension climate projections become affected since the global carbon cycle in turn has a decisive role in these with CO_2 and CH_4 and their properties as greenhouse gasses (e.g. IPCC, 2013).

5.2 Nutrient effect on RQ

The only difference between the two mixes of humic acid substrate was that one of them was enriched with inorganic nutrients (N and P). The results show consistently higher RQs in nutrient enriched samples (Figures 4 and 5). The maximum RQ in the non-nutrient enriched sample (HS) was around 0.5 which was unexpectedly low. RQs as low as observed in the dark treatments of the HS sample are not expected to be observed in natural ecosystems. The sample was mixed from leonardite extracted humic acids and hence not natural, it is therefore difficult to foresee how the bacterial metabolism will work. The RQs in the irradiated HS samples were never above 0.85 in water both irradiated with and without incubation prior to UV-treatment. With nutrient addition (HS (N+P)), the RQs were elevated compared to HS; reaching more expected values but below 1 in non-irradiated samples (RQ = 0.8). The non-irradiated humic acid substrate samples contain HMW DOC compounds of low bioavailability and lower oxygen content than the irradiated and thus photo-oxidized samples explaining the RQ < 1.0. Reduced compounds are more energy rich than oxidized and at growth the bacteria therefore prefer to assimilate those. This is performed at lower RQs than at assimilation of oxidized compounds but still elevated compared to assimilation at only survival and no growth, explaining the elevated RQs in nutrient enriched samples compared to non-enriched. In the final 72 h of the dark treatment incubation (3c), the RQs declined, implying that the activity in the water dropped as a result of substrate depletion. The decline in RQ was drastic, approaching zero in the nutrient poor system where depletion of substrate has a larger impact on the microbial activity. If the incubations had not

been stopped after 72 h, the RQ in the nutrient enriched sample would probably have continued to drop. A corresponding increase in RQ as a result of bioavailable substrate and/or nutrients addition, together with a subsequent decrease as the substrates/nutrients were consumed was observed for soil microbiota by Dilly (2003). Even if the samples used in the present study are unnatural it seems fairly clear that the nutrient supply has an effect on the bacterial RQ and that the low RQs in the HS samples should be explained by the lack of nutrient access.

Nutrient availability is crucial for DOC assimilation by bacterioplankton and BGE has been shown to increase as the C: N ratio of the substrate decreases (Cimberlis and Kalff, 1998 and references therein). Thus, not only the DOC stock determines the BR and BP but to a large extent the access to nutrients controls the metabolism. As argued earlier anabolic metabolism gives rise to higher RQs than catabolic since biomass contains reduced compounds. Many studies have shown phosphorous to be the major nutrient limiting bacterial growth in freshwater systems, mainly due to the relatively high P content in bacterial biomass (e.g. Schindler, 1977; Cimberlis and Kalff, 1998) and a higher bacterial activity with P addition was therefore expected. However, others have observed the P limitation to decrease as the DOC concentration increases (Jansson et al., 2001), which may be explained by the binding of inorganic P to humic substances (Jones, 1992). In the current study both N and P was probably limiting since the substrate used was of humic acids without added inorganic nutrients.

The addition of nutrients increased the RQ but not the respiration rate; instead the lower RQ in the non-nutrient enriched samples was an effect of higher O_2 consumption rates (data not shown). In accordance with this Cimberlis and Kalff (1998), found increased O_2 consumption per cell with increased C: N and C:P ratios and a subsequent increase in RQ with enhanced P concentrations. Additionally, the oxygen consumption per cell declined as the number of cells increased, suggesting elevated maintenance costs in nutrient-poor waters. This explains only part of the increased O_2 consumption in the present study; the O_2 consumption rate was higher in non-nutrient enriched samples in absolute numbers and not per cell. Presuming fewer cells in non-enriched samples the difference in O_2 consumption per cell between enriched and non-enriched samples might be too large to be easily explained. In nutrient-poor systems, the bacteria cannot afford to use energy to break down and assimilate HMW compounds for growth but

prefer to degrade simpler compounds yielding energy for survival. In a system with low nutrient availability and therefore few cells, the competition for substrate of high energy content is lower, and the bacterial community may selectively consume nearly exclusively simple LMW compounds. These compounds are to a large extent reduced; hence in order to mineralize the DOC the O_2 consumption from the surrounding is high.

With abundant inorganic nitrogen; nitrate (NO_3^-) can act as an alternative electron acceptor to DOC and hence the substrate couples to this instead, uncoupling the relationship between the oxygen consumption and BR (Cimberlis and Kalff, 1998). The uncoupling between O_2 consumption and CO_2 production leads to a higher RQ with N abundance, again complicating the use of a theoretical value of 1.0 (Cimberlis and Kalff, 1998; Dilly, 2003). NO_3^- as alternative electron acceptor is mostly a pathway in anaerobic environments or environments with O_2 limitation, with O_2 abundance aerobic respiration will take place. However, in natural ecosystems the oxygen concentration fluctuates with microzones of low oxygen and the bacterial community is therefore made up of both aerobes and anaerobes (Cimberlis and Kalff, 1998 and references therein). In sediments, bacteria have been observed to respire nitrate in the presence of oxygen (Carter et al., 1995). There is thus a possibility that other respiratory mechanisms than aerobisis took place, uncoupling the O_2 consumption and the CO_2 production in the nutrient-enriched samples, even if the aerobisis is most probably dominating.

It seems clear from the results that the addition of inorganic nutrients has an elevating effect on the bacterial RQ. Elevated RQs as a result of addition of nutrients have been observed in both soils and in freshwater incubations (Cimberlis and Kalff, 1998; Dilly, 2003). Nutrient supply in an ecosystem thus impacts the amount of carbon passing through the bacteria and depending on whether the supply is limited or not the use of an RQ of 1.0 might lead to over or underestimation of BR. However, the humic acids samples used in this study are leonardite extracted for agricultural use mixed with deionized water. Even if the mix contains similar humic acids as allochthonous DOC in aquatic ecosystems; a direct comparison to natural waters and conclusions of the bacterial behavior must be drawn with precaution.

5.3 Weaknesses of the method

The results should be considered reliable considering that both the UV-treatment and the addition of inorganic nutrients had a clear effect on the RQ. The control of the surroundings is an advantage of laboratory analyses: the temperature can be held constant during incubations, and there is no diffusion of gas or input of new species. Specific mechanisms in an ecosystem can thus be investigated, which cannot be studies in field. The high RQs observed in this study as a result of bacterial assimilation of photo-oxidized compounds will not be observed on an ecosystem level but rather in some microzones depending on the light attenuation and the origin of the DOC being photo-chemically processed. The measuring devices used were of high accuracy (±0.1 pH; ±0.025 mg O_2 l^{-1}) and potential uncertainties were negligible for the scope of this study (PreSens, Regensburg, Germany). Due to the large datasets and the accuracy of the measuring devices the results are quite robust and cannot be ignored. The mechanisms behind the high RQs observed are present in natural waters as well. There were however some weaknesses included in the method.

The coefficients of determination were generally high in all treatments, indicating a strong correlation between the CO_2 production and the O_2 consumption rates. However, some of the slopes appear zigzagging (lake water experiment, treatment 1a, and 1b; Figure 2) and some as if they make a jump (HS sample treatment 1b) at some instance. These patterns might be a consequence of either gas bubbles getting stuck on the sensors or of opening of the door to the climate chamber, resulting in temperature deviations inside. If the temperature changes, the solubility of the gasses changes; both CO_2 and O_2 solubility decreases with increased temperatures. Such effects could be avoided by simply not opening the door together with maintaining a room temperature similar to the one in the climate chamber if opening is inevitable.

When the lake water arrived from the field, and the humic acid solutions were mixed the samples were filtered and incubations were initiated, one treatment combination at a time. The rest of the water was then stored in a refrigerator at just above 0 ^{o}C. The aging effect of the DOC during storage was not taken into account in the analysis of the results. The sterilization should rule out

bacterial degradation, but there might be chemical effects on the compounds as a consequence of rapid temperature changes. This effect should however not be large and perhaps does not affect the results noticeably. To avoid such a consequence, a recommendation would be to perform the incubations directly after sampling.

The already mentioned approximations needed to calculate the CO_2 concentrations from the pH measurements could be a source of uncertainty. The potential biases and errors in the approximations propagate through to the CO_2 production rates may result in an overestimation (Raymond et al., 2013). However, the approximations included are well tested and known to be good (Stumm and Morgan, 1996) and errors propagating through to the CO_2 production rates are the same in all datasets and the observed effects on RQ of irradiation and nutrient addition remain. Even if the dissolved CO_2 would have been measured, the CO_2 production needs to be recalculated to TCO_2 (CO_2 + HCO_3^- + CO_3^{2-}), accounting for the carbon respired that is transformed into carbonates. Approximations are thus inevitable.

5.3.1 Alkalinity decrease in the humic acid solutions

The buffering system in natural waters is mostly made up of the aqueous carbonate system but organic matter may contain weak bases contributing to the total alkalinity of aqueous systems, especially in freshwater and estuaries (Cai et al., 1998; Hunt et al., 2011). The titration end point for such organic matter alkalinity is not clear, depending on the quantity and quality of the DOC compounds and is obtained through a Gran plot (Gran, 1952), it lies typically between $2 < pH > 4$ (Hunt et al., 2011). There is thus a possibility that the alkalinity is underestimated in the present study, where a titration endpoint of pH 4.5 was used. If so the CO_2 production rates would be higher than the ones observed as well as the RQs. The results of RQ variations as a consequence of UV-irradiation and nutrient enrichment remain regardless of the potential underestimation of alkalinity.

As the humic acid solutions were not of natural water, there is a chance that a larger part of their buffering system was made up by the DOC (Cai et al., 1998). A change in its molecular pool could therefore also change the equilibrium of its acid-base system. The same decrease in

alkalinity occurred whether the water was irradiated or not, indicating that it was the large compounds that made up the buffering system and as they broke down the alkalinity decreased. Since the alkalinity was measured only before and after treatments, it could not be determined when the decrease took place. This could, and probably did, lead to over- or underestimations of the alkalinity during some of the experimental steps, propagating through to subsequent over- or underestimations in CO_2 evolution.

The first scenario in the uncertainty analysis would probably result in a fairly large underestimation of RQ, especially at the beginning of the incubations (treatment 1a). The second scenario would thus give rise to a corresponding overestimation of RQ, especially towards the end of the incubations (treatments 1b; 2a; 3c). The alkalinity hence has a strong influence on the result in the computations and the change in alkalinity was probably the largest source of uncertainty in the study.

In the computation of the CO_2 evolution with a linearly decreasing alkalinity according to scenario 3, the decrease in alkalinity at times outweighed the decrease in pH, explaining the regression lines in the CO_2 vs. O_2 plots bending downward towards the end of treatments. If the decrease in alkalinity was a result of degradation of DOC compounds it is probably not likely that the decrease was linear from the beginning due to the need for the bacterial community to establish before degradation at a larger scale can take place.

The fourth scenario takes into account that the alkalinity was highest at the beginning of the dark treatment and gradually decreased to become lowest at the end of the treatments but not when the decrease occurred or how much at a time. Since the decrease according to this scenario decreases in two steps, the CO_2 production was probably somewhat overestimated towards the end of treatment 1a, resulting in overestimation of RQ. Correspondingly the CO_2 rates of treatments 1b, 2a, and 3c were most likely underestimated at the beginning giving a subsequent underestimation in RQ.

Regardless of the change in alkalinity the overall results of the study are the same, the RQs are still increasing as a result of photo-chemical degradation of the DOC and addition of inorganic

nutrients. An overestimation of RQ in treatment 1a and an underestimation of RQs during treatments 1b; 2a; and 3c give a smaller difference in RQ than without over- or underestimations. In order to investigate the buffering system further alkalinity measurements needs to be repeated throughout the treatments.

5.4 Future directions

The results in this study are clear, indicating that the bacterial RQ in aquatic ecosystems most likely exceeds 1.0 at times, especially in waters with humic DOC exposed to solar radiation. With these results, it becomes clear that the BR, and thus the amount of carbon passing through the bacterioplankton in freshwater systems are often underestimated when using a fixed RQ of 1.0. Hence, the bacterial contribution to pCO_2 is underestimated. This especially has consequences when modeling the global carbon cycle and the contribution of inland waters to this. Such modeling is crucial when extrapolated to future scenarios of atmospheric CO_2 levels and global warming. More research on bacterial metabolism in decomposition of compounds of different origin, age, and oxidative state is needed.

The RQ values in the dark control incubations in this study varied somewhat over time and it would be interesting to perform longer incubations on both irradiated and non-irradiated samples to investigate whether the maximum values were obtained or if RQ would increase further and at what time it would potentially drop.

The inorganic nutrient effect is also clear even if the mechanisms behind the results remain somewhat unclear. RQs were systematically higher in nutrient enriched samples and in nutrient-poor samples the RQs were surprisingly low. Elevated RQs in nutrient-enriched water compared to nutrient poor did not reach as high as 1.0 in non-irradiated samples, indicating selective assimilation of reduced, energy rich compounds. Hence in nutrient rich ecosystems with low photo-chemical activity the same use of an RQ of 1.0 may overestimate the role of the bacteria to the pCO_2 instead. A corresponding overestimation should be expected in both high and low photo-chemically active nutrient poor systems.

Further studies of the impact of inorganic nutrients are therefore also suggested. In this study, inorganic nutrients were added to a humic acid solution and the control was hence nearly free from nutrients. To further analyze the role of nutrients on RQ in aquatic ecosystems, a corresponding study to the present one, with nutrient addition to natural waters would be suggested together with analysis of the inorganic nutrients in the water. UV-irradiation might mineralize organic nutrients to inorganic form, and analysis of such processes and their consequences for BR could be included.

BR in aquatic ecosystems is not thoroughly studied and with that there is a lack of understanding of the mechanisms in carbon assimilation and mineralization of bacterioplankton. More studies of these mechanisms should be performed. In this study it was assumed that the elevated values of RQ following UV-treatment was due to assimilation of oxygen rich photo-produced carboxyl acids, this assumption could be investigated by analyzing the organic acids in water samples together with treatments similar to the ones in this study.

Most studies investigate changes in bacterial production as a consequence of UV-irradiation or nutrient addition. In order to investigate the BGE and hence the amount of the assimilated carbon used for production, both BP and BR must be measured. If the BR is obtained from measurement of O_2 consumption the use of a correct value of RQ is crucial.

6. Conclusions

The results indicate variability of the bacterial RQ depending on several factors. Photo-chemical degradation of DOC produces compounds of high oxygen content leading to enhanced RQ compared to assimilation of reduced compounds. The RQs of irradiated samples were with only one exception always elevated compared to non-irradiated samples. The highest RQs were found in samples that underwent UV-treatment without prior incubation; indicating that the photo-reactive DOC pool was larger at the irradiation step in these samples than in the samples that were incubated first and then irradiated. In the latter case, some photo-reactive species were thus probably degraded by the bacteria before irradiation.

The RQ also seems to be controlled to a part by the access to inorganic nutrients. The explanation of how it is controlled is however not clear but nutrient limitation affects bacterial assimilation of DOC and which compounds are energetically sustainable to use. The consistently higher RQs in nutrient-enriched samples were distinct and the conclusion that nutrient availability has an essential role in the bacterial mineralization of DOC to CO_2 and the amount of carbon passing through the bacteria can be drawn.

Assuming a fixed, and underestimated RQ propagates through to the BR and to the amount of carbon passing through the bacteria in the ecosystems. This study underlines the lack of knowledge about and the complexity surrounding bacterial metabolism and factors affecting it. Knowledge of the bacterial role in mineralization processes is crucial when modeling the carbon cycle today and extrapolating it to future scenarios with the oncoming climate change. Further research in the area is thus to be recommended.

Acknowledgements

First and foremost, I would like to thank Martin Berggren for his great support in performing this study and writing the thesis, for always replying quickly, and for your enthusiasm about the subject that is really contagious. A big thanks to Marcin Jackowicz-Korczynski, Ana Soares and Julia Jacobsson for technical support in the laboratory and with software. And Julia, thanks for being my personal key. Thank you also Balathandayuthabani Panneer Selvam for donating me some of your water. I also owe my gratitude to Yves Prairie for giving me calculation sheets, facilitating the calculations. Thanks to Anna and Linnea for discussion of results and analyses and fika. And Jonas, thank you for everything.

References

Algesten G, Sobek S, Bergström A-K, Jonsson A, Tranvik LJ, Jansson M (2005). Contribution of sediment respiration to summer CO_2 emission from low productive boreal and subarctic lakes. Microb. Ecol. 50: 529-535.

Alin SR, Rasera MdFFL, Salimon CI, Richey JE, Holtgrieve GW, Krusche AV, Snidvongs A (2011). Physical controls on carbon dioxide transfer velocity and flux in low-gradient river systems and implications for regional carbon budgets. J. Geophys. Res. 116.

Amado AM, Cotner JB, Cory RM, Edhlund BL, McNeill K (2014). Disentangling the interactions between photochemical and bacterial degradation of dissolved organic matter: aminoacids play a central role. Microb. Ecol. 11(3).

Anesio, AM, Granéli W (2003). Increased photoreactivity of DOC by acidification: implications for the carbon cycle in humic lakes. Limnol. Oceanogr. 48:735–744.

Anesio AM, Granéli W, Aiken GR, Kieber DJ, Mopper K (2005). Effect of humic substance photodegradation on bacterial growth and respiration in lake water. Environ. Microbiol. 71(10): 6267-6275.

Aufdenkampe AK, Mayorga E, Raymond PA, Melack JM, Doney SC, Alin SR, et al. (2011). Front Ecol. Environ. 9(1): 53-60.

Battin TJ, Luyssaert S, Kaplan LA, Aufdenkampe AK, Richter A, Tranvik LJ (2009). The boundless carbon cycle. Nat Geosci 2: 598–600.

Benner R, Kaiser K (2011). Biological and photochemical transformations of amino acids and lignin phenols in riverine dissolved organic matter. Biogeochemistry 102: 209-222.

Berggren M, Laudon H, Jansson M (2009). Aging of allochthonous organic carbon reguates bacterial production in unproductive boreal lakes. Linmol. Oceanogr. 54(4): 1333-1342.

Berggren M, Laudon H, Haei M, Ström L, Jansson M (2010). Efficient aquatic bacterial metabolism of dissolved low molecular weight compounds from terrestrial sources. ISME J 4: 408–416.

Berggren M, Lapierre JF, del Giorgio PA (2012). Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients. ISME 6: 984-993.

Bertilsson S, Tranvik LJ (1998). Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. Limnol Oceanogr 43: 885–895.

Bertilsson, S, Tranvik LJ (2000). Photochemical transformation of dissolved organic matter in lakes. Limnol. Oceanogr. 45:753–762.

Biddanda B, Opsahl S, Benner R (1994). Plankton respiration and carbon flux through bacterioplankton on the
Louisiana shelf. Limnology and Oceanography 39 (6): 1259–1275.

Boucher G, Clavier J, Carrigur C (1994). Oxygen and carbon dioxide fluxes at the water-sediment interface of a tropical lagoon. Mar. Ecol. Prog. Ser. 107: 185-193.

Brinkmann T, Sartorius D, Frimmel FH (2003). Photobleaching og humic rich dissolved organic matter. Aquat. Sci. 65: 415-424.

Bruccoleri A, Pant BC, Sharma DK, Langford CH (1993). Evaluation of primary photoproduct quantum yields in fulvic acid. Environ. Sci. Technol. 27:889-894.

Cai WJ, Wang Y, Hodson RE (1998). Acid-base properties of dissolved organic matter in the estuarine waters of Georgia, USA. Geoch. Of Cosmoch. Acta 62(3): 473-483.

Carignan R. (1998). Automated determination of carbon dioxide, oxygen, and nitrogen partial pressures in surface waters. Limnol. Oceanogr. 43: 969–975.

Carter JP, Hsiao YH, Richardson DJ (1995). Soil and sediment bacteria capable of aerobic nitrate respiration. Appleid and Environ. Microbiol. 61(8):2852-2858.

Chapman SJ, Thurlow M (1998). Peat respiration at low temperatures. Soil Biol. Biochem. 30: 1013-1021.

Chin YP, Alken G, O'Loughlin E (1994). Molecular weight, polydispersity, and spectroscopic properties og aquatic humic substances. Environ. Sci. Technol. 28:1853-1858.

Chrisftoffersen K, Andersen N, Sondergaard N, Liboriussen L, Jeppesen E (2006). Implications of climate-enforced temperature increases on freshwater pico- and nanoplankton populations studied in artificial ponds during 16 months. Hudrobiologia 560: 529-266.

Cimbleris ACP, Kalff J (1998). Planktonic bacterial respiration as a function of C : N : P ratios across temperate lakes. Hydrobiologia 384: 89–100.
Cole J J, Caraco N F, Kling G,Kratz T K (1994). Carbon dioxide supersaturation in the surface waters of lakes. Science 265: 1568-l 570.

Cole JJ, Pace ML, Carpenter SR, Kitchell JF (2000). Persistence of net heterotrophy in lakes during nutriens addition and food web manipulations. Limnol. Oceonagr. 45(8):1718-1730.

Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, et al. (2007). Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosyst. 10: 171-184.

Cory RM, Ward CP, Crump BC, Kling GW (2014). Sunlight controls water column processing in arctic fresh waters. Nature 343: 925-928.

del Giorgio PA, Cole JJ (1998). Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics 29: 503–541.

del Giorgio PA, Cole JJ, Cimbleris A (1997). Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. Nature 385: 148–151.

del Giorgio PA, Cole JJ, Caraco NF et al. (1999) Linking planktonic biomass and metabolism to net gas fluxes in northern temperate lakes. Ecology 80: 1422–1431.

Del Giorgio PA, Davis J (2003). Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 399–424. In S. E. G. Findlay and R. L. Sinsabaugh [eds.], Aquatic ecosystems: Interactivity of dissolved organic matter. Academic Press.

del Giorgio P.A. & Williams P.J. (eds.) (2005). *Respiration in aquatic ecosystems*. Oxford University Press.

Dilly O (2001). Microbial respiratory quotient during basal metabolism and after glucose amendment in soils and litter. Soil Biol Biochem 33: 117–127.

Dilly O (2003). Regulation of the respiratory quotient of soil microbiota by availability of nutrients. FEMS Microbiol. Ecol. 43: 375-381.

Farjalla VF, Anesio AM, Bertilsson S, Granéli W (2001). Photochemical reactivity of aquatic macrophyte leachates: abiotic transformations and bacterial response. Aquat. Microb. Ecol. 24:187–195.

Farjalla VF, Amado AM, Suhett AL, Meirelles-Pereira F (2009). DOC removal paradigms in highly humic ecosystems. Environ. Sci. Pollut. Res. 16: 531-538.

Frimmel FH, Bauer H, Putzlen J (1987). Laser flesh photolysis of dissolved aquatic humic mmaterial and the sensitized production of singlet oxygen. Environ. Sci. Technol. 21(6): 541-545

Gao H, Zepp RG (1998). Factors influencing photoreactions of dissolved organic matter in a coastal river of the southeast United States. Environ. Sci. Technol. 32: 2940-2946.

Giesler R, Högberg MN, Strobel BW, Richter , Nordgren A, Högberg P (2007). Production of dissolved organic carbon and low-molecular weight organic acids in soil solution driven by recent tree photosynthate. Biogeochemistry 84: 1-12.

Gran G (1952). Determination of the equivalence point in potentiometric titrations. Part II. Analyst 77: 661-671.

Granéli W, Lindell M, Tranvik LJ (1996). Photo-oxidative production of dissolved inorganic carbon in lakes of different humic content. Limnol Oceanogr 41: 698–706.

Hanson PC, Bade DL, Carpenter SR (2003). Lake metabolism: Relationships with dissolved organic carbon and phosphorous. Limnol. Oceanogr. 48(3): 1112-1119.

Hargrave BT, Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. Limnol. Oceanogr. 17(4): 583-596.

Hedges JI, Baldock JA,Gélinas Y, Lee C, Peterson ML, Wake ham SG (2002). The biochemical and elemental compositions of marine plankton: A NMR perspective. Marine Chem. 78: 47-63.

Hope D, Kratz TK, Riera JL (1996) Relationship between pCO2 and dissolved organic carbon in Northern Wisconsin lakes. Journal of Environmental Quality, 25, 1442–1445.

Hunt CW, Salisbury JE, Vandemark D (2011). Contribution of non-carbonate anions to total alkalinity and overestimation of pCO_2 in New England and New Brunswick rivers. Biogeosci. 8: 3069-376.

Humborg C, Mörth CM, Sundbom M, Borg H, Blenckner T, Giesler R et al. (2010). CO2 supersaturation along the aquatic conduit in Swedish watersheds as constrained by terrestrial respiration, aquatic respiration and weathering. Global Change Biol 16: 1966–1978.

IPCC (2013). Climate change 2013: *The physical science basis. Contribution of working group 1 to the fifth assessment report of the Intergovernmental Panel on Climate Change* [Stocker TF, Qin D, PlattnerG-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM. (eds.)] Cambridge University Press, Cambridge United Kingdom and New York, NY, USA, 1535 pp.

Jansson M, Bergström A-K, Blomqvist P, Drakare S (2000). Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. Ecology 81(11): 3250-3255.

Jansson M, Bergström A-K, Drakare S, Blomqvist P (2001). Nutrient lilmitation of bacterioplankton and phytoplanktob in humic lakes in northern Swden. Freshwater Biology 46. 653-666.

Johnson KM, King AE, Siedburth JMcN (1985). Coulorimetric TCO2 analyses for marine studies; an introduction. Mar. Chem. 16: 61-82.

Jones RI (1992). The influence of humic substances on lacustrine planktonic food chains. Hydrobiologia 229:73–91.

Jonsson A, Karlsson J, Jansson M (2003). Sources of carbon dioxide supersaturation in clearwater and humic lakes in northern Sweden. Ecosystems 6: 224-235.

Jonsson A, Åberg J, Jansson J (2007). Variations in pCO_2 during summer in the surface water of an unproductive lake in northern Sweden. Tellus 59B: 797-803.

Judd KE, Crump BC, Kling GW (2007). Bacterial responses in activity and community composition to photo-oxidation of dissolved organic matter from soil and surface waters. Aquat. Sci. 69: 96-107

Jörgensen BB, Revsbech NP (1985). Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnology and Oceanography 30: 111-122.

Koehler B, Landeius T, Weyhenmeyer GA, Machida N, Tranvik LJ (2014). Sunlight-induced carbon dioxide emissions from inland waters. Global Biogeochem. Cycles. 28: 696-711.

Kortelainen P, Rantakari M, Huttinen JT, Mattisson T, Alm J, Juutinen S, et al. (2006). Sediment respiration and lake trophic state are important predictors of large CO2 evasion from small boreal lakes. Global Change Biology (2006) 12: 1554–1567.

Kosten S, Roland F, Da Motta Marques DML, Van Nes EH, Mazzeo Nsternberg LdSL, et al. (2007). Climate-dependent CO_2 emissions from lakes. Global biogeochem. Cycles 24.

Kritzberg ES, Cole JJ, Pace MM, Granéli W (2006). Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: results from whole-lake ^{13}C addition experiments. Ecosyst. 9: 489-499.

Krug EC, Frink CR (2010). Acid rain on acid soil: A new perspective. Science 221: 520-525

Köhler S, Buffam I, Jonsson A, Bishop K (2002). Photochemical and microbial processing of stream and soil water dissolved organic matter in a Boreal forested catchment in northern Sweden. Aquat. Sci. 64: 269-281.

Larsen S, Andersen T, Hessen DO (2011). Climatechange predicted to cause severe increase of organic carbon in lakes. Global change boil. 17: 1186-1192.

Malcolm RL, MacCarthy P (1986). Limitations in the use of commercial humic acids in water and soil research. Environ. Sci. and Technol. 20: 904-911.

McNair JN, Gereaux LC, Weinke AD, Sesslemann MRKendall ST, Biddande BA (2013). New methods for estimating components of lake metabolism based on free-water dissolved-oxygen dynamics. Ecol. Mod. 263:251-263.

Mills MM, Moore CM, Langlois R, Milne A, Achterberg E, Nachtigall K, et al. (2008). Nitrogen and phosphorous co-limitation of bacterial productivity and growth in the oligotrophic subtropical North Atlantic. Limnol. Oceanogr. 53(2): 824-834.

Molot LA, Hudson JJ, Dillon PJ, Miller SA (2005). Effect of pH on photo-oxidation of dissolved organic carbon by hydroxyl redicals in a coloured, softwater stream. Aquat. Sci. 67: 189-195.

Odum HT (1956). Primary production in flowing waters. Limnol.Oceanogr. 1:102-117

Pace ML, Reche I, Cole JJ, Fernández-Barbero A, Mazuecos IP, Prairie YT (2012). pH change induces shifts in the size and light absorption of dissolved organic matter. Biogeochem. 108: 109-118.

Porcal P, Dillon PJ, Molot LA (2013). Seasonal changes in photochemical properties of dissolved organic matter. Biogeosci. Disc. 10: 5977-6006.

Pullin MJ, Bertilsson S, Goldstone JV, Voelker BM. (2004). Effects of sunlight and hydroxyl radical on dissolved organic matter: bacterial growth efficiency and production of carboxylic acids and other substrates. Limnol Oceanogr 49: 2011–2022.

Raymond P A, Cole JJ (2001). Gas exchange in rivers and estuaries: Choosing a gas transfer velocity. Estuaries 24(2): 312 – 317.

Raymond PA, Hartman J, Lauerwald R, Sobek S, MvDonald C, Hoover M, et al. (2013). Global carbon dioxide emissions from inland waters. Nature 503: 355-359.

Regnier P, Friedlingstein P, Ciais P, Meckenzie FT, Gruber N, Janssens IA, et al. (2013). Anthropogenic perturbation of the carbon fluxes from land to ocean. Nat. Geosci. 6: 597-605.

Robinson C, Williams PJ.leB (1999). Plankton net community production and dark respiration in the Arabian sea during September 1994. Deep Sea Research Part II 46: 745-765.

Robinson C, Serret P, Tilstone G, Teira E, ZubkovMV, Rees AP, et al. (2002). Plankton respiration in the eastern Atlantic ocean. Deep-Sea Research I 49:787-813.

Tranvik LJ (1988). Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb. Ecol. 16: 311–322.

Tranvik LJ, Downing JA, Cotner JB, Loiselle SA, Striegl RG, Ballatore TJ et al. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. Limnol Oceanogr 54: 2298–2314.

Tranvik LJ, Kokalj S (1998). Decreased biodegradability of dissolved organic carbon of phytoplankton origin due to interactive effects of UV radiation and humic matter. Aquat. Microb. Ecol. 14:301–307.

Tulonen T (2004). Role of allochthonous and autochthonous dissolved organic matter (DOM) as a carbon source for bacterioplankton in boreal humic lakes. PhD thesis. Faculty of Biosciences University of Helsinki, Finland.

Schindler DW (1997). Evolution of phosphorous limitation in lakes. Science 195: 260-262.

Sobek S, Tranvik LJ, Cole JJ (2005) Temperature independence of carbon dioxide supersaturation in global lakes. Global Biogeochemical Cycles, 19, GB 2003, doi:10.1029/2004GB002264.

Sondergaard M, Middelboe M (1995) A cross-system analysis of labile dissolved organic carbon. Mar Ecol Prog Ser 118:283–294.

Staerr PA, Bade D, Van de Bogert MC, Koch GR, Williamson C, Hanson P, et al. (2012). Lake metabolism and the diel oxygen technique: State of the science. Limnol. Oceanogr. 8: 628-644.

Steel KM, Alizadehhesari K, Balucan RD, Basic (2013). Conversion of CO_2 into mineral carbonates using a regenerable buffer to control solution pH. Fuel 111:40-47.

Stumm W, Morgan JJ (1996). *Aquatic Chemistry - Chemical Equilibria and Rates in Natural Waters*, 3rd edn. John Wiley & Sons: New York, USA.

Sulzberger B, Durisch-Kaiser E (2009). Chemical characterization of dissolved organic matter (DOM): A prerequisite for understanding UV-induced changes og DOM absorption properties and bioavailability. Aquat. Sci. 71: 104-126.

Sundh I (1992). Biochemical composition of dissolved organic carbon released from natural communities of lake phytoplankton. Arch. Hydrobiol. 125: 347–369.

Sykore J (1997). Photochemistry of copper complexes and their environmental aspects. Coord. Chem. Rev. 159: 95-108.

Vaughan PP, Blough NV (1998). Photochemical Formation of Hydroxyl Radical by Constituents of Natural Waters. Environ. Sci. and Techn. 32: 2947-2953.

Verpoorter C, Kutser T, Seekell DA, Tranvik LJ (2014). A global inventory of lakes based on high-resolution satellite imagery. Geophys. Res. Lett. 41: 6396-6402.

Vähätalo AV. Salonen K, Münster U, Järvinen M, Wetzel RG (2003). Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. Arch. Hydrobiol. 156(3): 287-314.

Williams PJL, Jenkinson NW (1982). A transportable microprocessor controlled Winkler titration suitable for field and ship-board use. Limnol Oceanogr 27: 576-584.

Williams P J Le B, Robinson C (1992). Analysis of oxygen-based productivity measurements through phase I of the EROS 2000 programme. CEC Water Pollution Reports 28: 151-162.

Zhou X, Mopper K (1997). Photochemical production of low-molecular-weight carbonyl compounds in seawater and surface microlayer and their air-sea exchange. Marine Chemistry 56: 201-213.

Institutionen för naturgeografi och ekosystemvetenskap, Lunds Universitet.

Student examensarbete (Seminarieuppsatser). Uppsatserna finns tillgängliga på institutionens geobibliotek, Sölvegatan 12, 223 62 LUND. Serien startade 1985. Hela listan och själva uppsatserna är även tillgängliga på LUP student papers (www.nateko.lu.se/masterthesis) och via Geobiblioteket (www.geobib.lu.se)

The student thesis reports are available at the Geo-Library, Department of Physical Geography and Ecosystem Science, University of Lund, Sölvegatan 12, S-223 62 Lund, Sweden. Report series started 1985. The complete list and electronic versions are also electronic available at the LUP student papers (www.nateko.lu.se/masterthesis) and through the Geo-library (www.geobib.lu.se)

300 Johan Westin (2014) Remote sensing of deforestation along the trans-Amazonian highway

301 Sean Demet (2014) Modeling the evolution of wildfire: an analysis of short term wildfire events and their relationship to meteorological variables

302 Madelene Holmblad (2014). How does urban discharge affect a lake in a recreational area in central Sweden? – A comparison of metals in the sediments of three similar lakes

303 Sohidul Islam (2014) The effect of the freshwater-sea transition on short-term dissolved organic carbon bio-reactivity: the case of Baltic Sea river mouths

304 Mozafar Veysipanah (2014) Polynomial trends of vegetation phenology in Sahelian to equatorial Africa using remotely sensed time series from 1983 to 2005

305 Natalia Kelbus (2014) Is there new particle formation in the marine boundary layer of the North Sea?

306 Zhanzhang Cai (2014) Modelling methane emissions from Arctic tundra wetlands: effects of fractional wetland maps

307 Erica Perming (2014) Paddy and banana cultivation in Sri Lanka - A study analysing the farmers' constraints in agriculture with focus on Sooriyawewa D.S. division

308 Nazar Jameel Khalid (2014) Urban Heat Island in Erbil City.

309 Jessica, Ahlgren & Sophie Rudbäck (2014) The development of GIS-usage in developed and undeveloped countries during 2005-2014: Tendencies, problems and limitations

310 Jenny Ahlstrand (2014) En jämförelse av två riskkarteringar av fosforförlust från jordbruksmark – Utförda med Ekologgruppens enkla verktyg och

erosionsmodellen USPED

311 William Walker (2014) Planning Green Infrastructure Using Habitat Modelling. A Case Study of the Common Toad in Lomma Municipality

312 Christiana Marie Walcher (2014) Effects of methane and coastal erosion on subsea-permafrost and emissions

313 Anette Fast (2014) Konsekvenser av stigande havsnivå för ett kustsamhälle- en fallstudie av VA systemet i Beddingestrand

314 Maja Jensen (2014) Stubbrytningens klimatpåverkan. En studie av stubbrytningens kortsiktiga effekter på koldioxidbalansen i boreal barrskog

315 Emelie Norhagen (2014) Växters fenologiska svar på ett förändrat klimat - modellering av knoppsprickning för hägg, björk och asp i Skåne

316 Liisi Nõgu (2014) The effects of site preparation on carbon fluxes at two clear-cuts in southern Sweden

317 Julian Will (2014) Development of an automated matching algorithm to assess the quality of the OpenStreetMap road network - A case study in Göteborg, Sweden

318 Niklas Olén (2011) Water drainage from a Swedish waste treatment facility and the expected effect of climate change

319 Wösel Thoresen (2014) Burn the forest - Let it live. Identifying potential areas for controlled forest fires on Gotland using Geographic Information System

320 Jurgen van Tiggelen (2014) Assimilation of satellite data and in-situ data for the improvement of global radiation maps in the Netherlands.

321 Sam Khallaghi (2014) Posidonia Oceanica habitat mapping in shallow coastal waters along Losinj Island, Croatia using Geoeye-1 multispectral imagery.

322 Patrizia Vollmar (2014) The influence of climate and land cover on wildfire patterns in the conterminous United States

323 Marco Giljum (2014) Object-Based Classification of Vegetation at Stordalen Mire near Abisko by using High-Resolution Aerial Imagery

324 Marit Aalrust Ripel (2014) Natural hazards and farmers experience of climate change on highly populated Mount Elgon, Uganda

325 Benjamin Kayatz (2014) Modelling of nitrous oxide emissions from clover grass ley – wheat crop rotations in central eastern Germany - An application of DNDC

326 Maxime Rwaka (2014) An attempt to investigate the impact of 1994 Tutsi Genocide in Rwanda on Landscape using Remote Sensing and GIS analysis

327 Ruibin Xu (2014) Spatial analysis for the distribution of cells in tissue sections

328 Annabelle Finck (2014) Bird biodiversity in relation to forest composition in Sweden

329 Tetiana Svystun (2015) Modeling the potential impact of climate change on the distribution of Western Corn Rootworm in Europe”

330 Joel Forsmoo (2014) The European Corn Borer in Sweden: A Future Perspective Based on a Phenological Model Approach

331 Andrew Ekoka Mwambo (2015) Estimation of Cropland Ecological Footprint within Danish Climate Commissions 2050 Scenarios for Land use and Bioenergy Consumption

332 Anna Lindstein (2015) Land- atmosphere exchange of carbon dioxide in a high Arctic fen: importance of wintertime fluxes

333 Karla Susana Markley Vergara (2015) Present and near future water availability for closing yield gaps in four crops in South America

334 Klara Århem & Fredrik Fredén (2015) Land cover change and its influence on soil erosion in the Mara region, Tanzania: Using satellite remote sensing and the Revised Universal Soil Loss Equation (RUSLE) to map land degradation between 1986 and 2013

335 Fei Lu (2015) Compute a Crowdedness Index on Historical GIS Data- A Case Study of Hög Parish, Sweden, 1812-1920

336 Lina Allesson (2015) Impact of photo-chemical processing of dissolved organic carbon on the bacterial respiratory quotient in aquatic ecosystems