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The Effect of the Freshwater-Sea Transition on Short-term Dissolved Organic Carbon Bio-reactivity: The Case of Baltic Sea River Mouths

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Abstract

Terrestrially-derived dissolved organic carbon (DOC) is transported to estuarine systems via river runoff, where it is largely degraded by microbial communities. I studied the combined influence of salinity (NaCl), microbial community (marine vs. freshwater) and inorganic nitrogen (N) and phosphorus (P) availability on DOC degradation rates in 10 major rivers in Sweden sampled during summer. The DOC degradation (inferred from dissolved O_2 consumption rates) was determined on filtered river water during one week (7-days) *in vitro* experimental bioassays, applying the factors alone and in all possible combinations. Compared to the controls, addition of salt (10 psu) caused significant decreases in the DOC degradation in most cases, by 21-49%. In two cases, a significant P limitation was found, and a few additional rivers responded positively to P addition, although the effect was not statistically significant. Additions of N had no significant effect on the DOC degradation. Only one river – Pite älv – showed significant interactions effects between N and P, or N and S, but these effects were relatively small. Interactions between P and salt were found in two cases (Helgeån and Nyköpingsån).These interactions were strong and the results suggest that P additions significantly dampened the salt stress. Tests in which the microbial community was manipulated showed that salt stress was similar for both freshwater and marine microbes. In fact bacteria from the coast outside Malmö and ambient river mouth communities showed a similar negative response to salt additions. Finally, possible salt induced flocculation and particle formation was tested. According to results, there was no particle formation that significantly affected the DOC degradation rates. This study highlights the need for further studies of interactions between nutrient and salt concentrations on the fate of the terrestrially-derived DOC in the marine environment.

Keywords: DOC, Reactivity, Degradation, Nutrients, Microbial community, Estuary, Bioavailability.

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Contents

Abbreviations and Acronyms

DOC	Dissolved Organic Carbon
POC	Particulate Organic Carbon
BP	Bacterial Production
BGE	Bacterial Growth Efficiency
BR	Bacterial Respiration
THM	Trihalomethanes
N	Nitrogen
P	Phosphorous
S	Salt
Fe	Iron
M	Microbes
Fm	Freshwater microbes
Mm	Marine water microbes
mL	Milliliter
g	Gram
ANOVA	Analysis of Variance
SPSS	Statistical Package for the Social Sciences
YSI-556	Multi-Parameter Water Quality Meter

Chapter One: Introduction

1.1 Introduction

Dissolved organic carbon (DOC) plays an important role in the global carbon cycle (Hansell & Carlson 2001; Hopkinson et al., 2002), and DOC in aquatic systems constitutes the largest pool of organic carbon on earth (Hopkinson et al., 2002; Guillemette et al., 2011; Koehler et al., 2011). Part of this large reservoir of organic carbon has been produced in the aquatic environment itself (autochthonous DOC), but a significant component originates in terrestrial organic matter (allochthonous DOC), partially decomposed or processed by micro-organisms (Zweifel et al., 1993; Mills et al., 2008; Dinasquet et al., 2013). Although 30-80% of this terrestrially-derived DOC is being degraded during its transit through freshwater systems (Algesten et al. 2004), 2.5×10^{14} g yr^{-1} of DOC is further transported to the coastal oceans on a global scale (Hedges et al., 1997).

The Baltic basin receives a large amount of DOC, mostly originating from surrounding terrestrial sources, transported via river runoff (Omstedt et al., 2012). The DOC is impacting on aquatic ecosystems by causing changes in the bioavailability of toxic compounds, increases in acidity and changes in microbial nutrient uptake and metabolic activities (Stanley et al., 2011). Organic carbon can influence the solubility, mobility and bioavailability of toxic metals, e.g., mercury (Hg), copper (Cu) and lead (Pb) (Ledesma et al., 2012) that cause human health risk (YaBin et al., 2012). A recent study showed that a large quantity of freshwater DOC was both biologically and photo- chemically degradable (Mann et al., 2012). However, the part of the DOC that can be degraded varies greatly between studies (del Giorgio and Davis 2003) and also the availability of inorganic nutrients influence this share. Labile DOC causes oxygen depletion (Gray et al., 2002) that severely effect on the bottom living populations, altering benthic faunal communities and affecting fisheries harvest (Breitburg 2002; Conley et al., 2011).

DOC reactivity and bacterial production, i.e., bacterial growth based on DOC, are limited by the presence of nitrogen (N) and phosphorous (P) in oligotrophic estuarine environments (Mills et al., 2008), but it is unclear if this applies to the Baltic Sea estuarine and coastal water. One laboratory study found that bacterial production increased by 156% and DOC degradation increased by 64% when inorganic nitrogen and phosphorous were added to water from the Baltic

Sea and the Northeast Mediterranean (Zweifel et al., 1993). The potential nutrient limitation of the DOC degradation is important since nutrient concentrations in the Baltic Sea represent an important aspect of environmental change in the region. Sandén and Rahm (1993) demonstrated that in the Baltic Sea, nutrient load has increased during the last century, by 1-4% yr^{-1}. In 2006 around 638,000 tons of nitrogen and 28,400 tons of phosphorous was discharged into the Baltic Sea. The different river catchments including agriculture and industrial discharge contribute around 45% of the nitrogen and phosphorous loads into the Baltic Sea (Knuuttila et al., 2011). However, even in the Baltic Sea, which receives a large amount of inorganic nutrients (Sandén and Rahm, 1993) and organic carbon (Omstedt et al. 2012), our understanding of nutrient influence on DOC degradation is incomplete, especially with regard to possible organic carbon and nutrient co-limitation of bacterial metabolic activity (Jansson et al., 1996; Vrede et al., 2005).

Terrestrially derived DOC can be degraded by heterotrophic bacteria, which can also alter its structure, during river transport before being discharged to the coastal ocean (Raymond and Bauer 2001). It is often assumed that DOC is more susceptible to degradation in the marine environment because saline water offers favorable physical and chemical environment compared to fresh water (Wikner et al., 1999). Estuarine systems receive bacteria from both fresh and marine water sources (Langenheder 2003). Diverse *in situ* bacterial species assemblages are present in the Baltic Sea estuarine environments. During the growing season a large phylogenetic diversity covering α-, β-, γ- *Proteobacteria*, and the *Cyatophaga-Flexibacter-Bacteroides* (CFB) group, might be supported by allochthonous DOC, originating from river and terrestrial sources (Kisand et al., 2002). The salinity changes act as stress factor on bacterial communities that are involved in DOC degradation, but the final outcome of this salt stress on metabolic rates is unclear. Some studies indicate that when riverine bacteria are confronted with estuarine salinity, bacterial respiration increases and, as a consequence, more organic carbon is utilized (Langenheder et al., 2003). Thus, it is important to consider bacterial response to salinity when studying DOC degradation. Additionally, colloidal iron dominated DOC fractions aggregate very quickly when mixing with saline water, subsequently creating a negative effect on DOC degradation (Nowostawska et al., 2008). However, although a few studies have demonstrated that nutrients, salinity, and bacteria community composition are factors that may influence the

DOC degradation (Zweifel 1993; Milles et al., 2008; Krachler et al., 2009; Knuuttila et al., 2011; Koehler et. Al., 2012, Catalan et al., 2013; Asmala et al., 2013), we know less about the interaction effects between salinity and other factors that are mentioned, on DOC bio-reactivity. A better understanding of DOC bio-reactivity is needed in order to predict the effect of the terrestrial DOC on the oxygen concentrations in the coastal zone of the Baltic Sea. This research work will use an experimental approach to assess how nutrient concentrations, salinity and source of bacterial community (inoculum) affect short term DOC bio-reactivity in the fresh-sea water transition zone.

1.2 Aim and Objectives

The overall aim of this project was to explore the effects of nutrients, salt and microbes on DOC degradation with data obtained from short term *in vitro* bioassays.

The specific objectives are:

- To quantify the individual effects of nutrient amendments (N and P, respectively) and salinity on short term DOC bio-reactivity, at the Baltic Sea river mouths.

- To assess the interactive effects of microbial community (freshwater and marine microbes), nutrient availability and salt concentrations on DOC reactivity.

- To investigate the effect of salt stress on DOC degradation, showed by different microbial communities.

1.3 Specific Hypotheses

Hypothesis 1: The bacterial DOC degradation in Baltic Sea river mouths is limited by the ambient concentrations of bioavailable N and P.

Hypothesis 2: The NaCl concentration is a significant regulator of bacterial DOC degradation in the estuarine environments.

Hypothesis 3: Increased salinity induces flocculation and, thus, formation of potentially reactive particles that can influence the fate of the DOC.

Chapter Two: Background and Literature Review

2.1 Dissolved Organic Carbon (DOC)

Dissolved organic carbon (DOC) is generally described as the organic material, or colloidal organic compounds, dissolved in water that is able to pass through a 0.45µm filter (Kolka et al., 2008) or 0.1 to 0.7 µm filter (Asmala et al., 2013). DOC is a complex mixture of compounds based on carbon, hydrogen, oxygen, nitrogen, phosphorous and other elements (Arnosti et al., 2011; Asmala et al., 2013). The main sources of DOC are the organic top soil layers of riparian zones and organic podzol soils of coniferous or boreal forests and wetland ecosystems (Maehder 2012). DOC that is delivered via different pathways and that originates in different sources has different chemical characteristics (Ågren et al., 2008). In natural systems, DOC occurs as non-aromatic compounds derived from macrophytes and algae, but also as aromatic compounds from different origins, like terrestrial plants, dissolved atmospheric dust and soil organic matter (Krupa et al., 2012). Organic carbon occurs because of decomposition of plant and animal remains at various stages, and it is known as a critical substance in water ecosystems (Pace and Cole 2002). A big part of organic carbon input to rivers, lakes and estuaries is transported from the terrestrial source by stream flow, although some carbon also may enter by atmospheric deposition (Bruckner 2012; Wikner et al, 1999). Wetland dominated areas produce DOC that is more aromatic and of higher molecular weight than forest derived DOC. Thus, DOC through different paths and sources has different chemical characteristics (Ågren et al., 2008). Depending on the origin, DOC consists of wide range of molecular sizes and structures, from simple acids and sugars to complex humic substances (Wang et al., 2013). Generally, forested land provides higher concentrations of low molecular weight carbon consisting of organic acids, free amino acids and simple carbohydrates compounds which are more reactive than mire produced organic carbon (Berggren et al., 2010a).

In a study about patterns and dynamics of DOC in boreal streams, it was shown that in a small homogeneous catchment area, DOC variability is regulated by hydrological functioning (Laudon et al., 2011). The same study further demonstrated that in a large catchment area, stream water DOC is regulated by the combined effect of hydrological processes and major landscape types e.g. mires, forest or wetland. The seasonality of terrestrial carbon exports is an important factor that regulates DOC composition, concentrations and that also significantly affects the

susceptibility of the DOC to bacterial degradation in the aquatic environment (Mann et al., 2012; Asmala et al., 2013). For example, one study showed that DOC concentrations increased in autumn, while NO_3 and reactive phosphorous concentration increased in summer (Mulholland and Hill 1997). Another study (Kaiser et al., 2001) revealed that the organic carbon concentration is larger during summer in a forest catchment area. The same study also showed that in winter and spring organic carbon consist of more hydrophobic fraction whereas in summer and autumn organic carbon consists of more hydrophilic fraction. The study further showed that during summer and autumn organic carbon structure is more aromatic and aliphatic whereas in winter and spring organic carbon consists of carbohydrates compounds (Kaiser et al., 2001). This variability of organic carbon concentration and composition and nutrient concentration could impact on DOC degradation.

Color and concentration of the DOC in the water body depend on the residence time of the water and also the drainage area of DOC loading. An aquatic system with a large catchment area, like a river, and relatively short water residence time could be expected to have relatively high DOC concentration because of large loading area and lower photochemical and biological mineralization (Pace and Cole 2002). In general, high concentration of organic carbon in the water body makes its color similar to straw or tea brownish (Bruckner 2012).

The dissolved fraction of organic carbon is often operationally classified as labile (lasting ~0.001 years), semi-labile (lasting ~1.5 years), semi-refractory (lasting ~20 years) refractory (lasting ~16,000 years) and ultra-refractory (lasting ~40,000 years), although all molecules in reality are distributed along a reactivity continuum (Eichinger et al., 2010; Hansell 2013). A large part of the organic carbon is utilized by the bacterial activities, in turn affecting the bio-structure and energy pathways of aquatic ecosystems (Berggren et al., 2010b).

2.2 DOC reactivity

Hansell (2013) defines DOC reactivity as the susceptibility to removal of a DOC fraction by either biotic or abiotic processes, measured by its lifetime. DOC reactivity depends on its source and bioavailable components (Koehler et al., 2012; Asmala et al., 2013; Catalán et al., 2013) and also by a number of environmental factors including temperature, light, microbial composition,

nutrient and oxygen availability, that are the most associated with DOC degradation and removal (Koehler et al., 2012; Ghani et al., 2013).

The DOC reactivity is not identical for all sources of DOC (Guillemete and del Giorgio 2011). The time required to degrade DOC varies from minutes to hundreds of years (del Giorgio and Davis 2003), primarily due to the structural and chemical compositional differences of organic compounds (Arnosti et al., 2011; Guillemette et al., 2011; Hansell 2013) that in turn are affected by the source of the DOC and by aging of DOC in the aquatic environments (Berggren 2009). Although it was previously believed that autochthonous DOC is more reactive than allochthonous DOC, results from recent studies have challenged this view. For example, externally produced allochthonous DOC that consists of aliphatic compounds can be comparatively more degradable than the internally produced autochthonous DOC that consists of more aromatic compounds (Koehler et al., 2012; Catala'n et al., 2013). A substantial part of fresh organic carbon might be consumed by microbes within short period of DOC residence time, but the DOC utilization tends to decline on longer time-scales because bacterial metabolism and DOC age are negatively correlated (Berggren et al., 2009; Mann et al., 2012). A bioassay study showed that increasing retention times of organic carbon leads to decline of bacterial production (BP) and bacterial growth efficiency (BGE) (Berggren et al., 2009); these two factors are positively correlated with the externally produced low molecular weight organic carbon concentration (Berggren 2009).

In the DOC mineralization and transformation processes, the bacteria represents one of the most important biological components (del Giorgio and Cole 1998) that play a key role for cycling of organic carbon in the aquatic systems (Berggren et al., 2010c). Microbial DOC degradation is regulated by the chemical structure and the bioavailability of the DOC (del Giorgio and Davis 2003). Thus, both DOC concentrations and quality are affecting bacterial metabolic process (Berggren, 2009; Eichinger et al., 2010; Asmala et al., 2013). One aspect of bacterial activity that is usually measured is the Bacterial growth efficiency (BGE) (Eichinger et al., 2010). Bacterial production (BP) and bacterial respiration (BR) are linked to BGE (del Giorgio and Cole 1998). The BGE is defined as the amount of bacterial biomass converted per unit of carbon substrate consumed, calculated as BGE=BP/ (BP+BR) (del Giorgio and Cole 1998; Lee et al., 2009).

Increased temperature has a negative effect on BGE (Berggren et al., 2010c). The freshwater environment is less suitable for high BGE than the seawater and nutrient addition could further increase of BGE in the sea (Asmala et al., 2013). Since sea water DOC is exposed to photo-oxidation, salt induced changes and large microbial activity, it stimulates organic carbon reactivity and possibly also BGE (Berggren et al., 2010c; Asmala et al., 2013). In the aquatic systems, bacterial production (BP) and bacterial respiration (BR) are correlated with the DOC concentration. BP can be defined as the synthesis of bacterial biomass which is mainly from organic matter and inorganic nutrients (Ducklow 2000). BR is a way of obtaining energy by oxidizing DOC (Berggren 2009) and typically, by this process bacteria consume a large pool of organic carbon in the aquatic ecosystems and release inorganic carbon to the atmosphere (Ågren et al., 2008a; Lee et al., 2009; Berggren et al., 2012). For the BP, organic carbon compounds with low molecular weight are better substrates than complex organic carbon compounds. Forest is the main contributor to highly bio-available organic carbon that also supports high BP and BGE (Ågren et al., 2008b; Berggren et al., 2007). Increasing allochthonous organic carbon and low molecular weight organic carbon stimulate BR and BP, respectively (Berggren 2009). A laboratory study demonstrated that increasing temperature stimulates bacterial production (BP) and bacterial respiration (BR) linearly, whereas rising inorganic nutrient concentration has not maintained the continuity of the increasing trend of BR and BP. This could be due to a high temperature stimulate respiration and consequently, maintenance cost of bacterial cell is also increased (Berggren et al., 2010).

Furthermore, natural forests and peatlands produce aromatic pool organic carbon, which is less biodegradable than agriculture dominated catchment area (Asmala et al., 2013). In general, DOC fluxes to the water systems are higher from the mires than from forested areas (Ågren et al., 2008a). A major part of this organic carbon that enters surface water is bio-available (Ågren et al., 2008b). The low molecular weight carbon that is exported by forests may have a turnover of a few hours or days as it is degraded by the bacteria (Berggren et al., 2010a). The bioavailability of DOC further depends on the size of organic matter molecules; for example as the large organic molecules become gradually smaller during the continuous degradation, the organic matter bioavailability tends to decrease (Asmala et al., 2013).

2.3 Why do we care about DOC reactivity?

On a global scale, terrestrial sources provide large amounts of organic carbon to the aquatic environments (Berggren 2009) and when degraded the atmospheric greenhouse gas CO_2 is released (Hopkinson et al., 2002). It has recently been calculated that every year inland waters (larger streams, lakes and rivers) emit 2.1 Pg of CO_2 to the atmosphere, which is a relatively high value compared to previous estimations (Raymond et al., 2013). This CO_2 flux could be further increased by the vegetation dominated catchment area because the vegetation covered area is relatively bigger source of organic carbon (Koehler et al., 2012). Dissolved organic carbon plays a crucial role by influencing physical, chemical and biological properties of aquatic systems (Berggren 2009; Pace et al., 2012). Rising DOC concentrations in water bodies affect water chemistry and living organisms of surface waters (Maehder 2012). For example, hypoxia (oxygen depletion) is one of the most widespread and accelerating impacts in the river dominated coastal zone. River runoff carries huge amounts of carbon and nutrients which is a base of hypoxia to coastal water. The lowering of oxygen concentration is hazardous to pelagic and benthic organisms. Hypoxic conditions have expanded since 1960s and it is widespread near the large populous coastal area (Alvisi et al., 2013). In the Baltic Sea coastal areas, 115 sites were identified to have hypoxic condition, which in more than 20% of all known sites for coastal hypoxia globally (Conley et al., 2012). Hypoxia occurs throughout the world of coastal waters where the oxygen concentration is less than 2 mg L^{-1}(Conley et al., 2011).

In many regions of the world, surface water is the main source of drinking water. This important and life giving resource is becoming contaminated by natural and anthropogenic pollutants (Ledesma et al., 2012). Organic carbon is not toxic in itself, but could transport pollutants and toxic compounds which are alarming for drinking water (Erlandsson et al., 2010; Ledesma et al., 2012). Surface water organic carbon concentrations are increasing due to changes of climate, land use and acid deposition (Futter et al., 2011; Ledesma et al., 2012). Organic carbon concentration helps to form a trihalomethanes (THM) compound which is considered as carcinogenic. Increase of surface water DOC concentration is correlated with the decrease of sulfate deposition that could influence on living organisms and on human health (Fawell et al., 2004; Ledesma et al., 2012).

Two factors have been proposed as driving forces of increasing DOC concentration in surface water of Sweden. These are decreasing sulfate deposition and increasing water flow (Ledesma et al., 2012). In a broader aspect, climate change (e.g. changes in temperature and hydrology) and changes in sulfate and chlorine deposition could further increase surface water DOC concentrations (Erlandsson et al., 2008).

Organic carbon is of significance for aquatic systems, carbon budgets, metals, nutrients, organic pollutants, and bioavailability of different components (Erlandsson et al., 2010). Organic carbon contains humic substances that absorb light and decrease the availability of light for the aquatic primary production. Such scenario affects the total food web of aquatic ecosystems. Organic carbon effectively supports bacterial production by providing carbon and energy in the aquatic systems (Berggren et al., 2008). In a small lake in Northern Sweden, terrestrial organic carbon was found to support around 85% of basal production (primary plus bacterial production) and to make up 47% zooplankton biomass, 63% of benthic animals, and 57% fish biomass (Karlsson et al., 2012).

2.4 Salinity effects on DOC degradation

Most often DOC degradation is enhanced by the highly saline environment. On the other hand, in brackish water the DOC degradation rate is non-detectable (Kisand et al., 2008). Saline waters are very fertile systems of bacterial communities where the assimilation of DOC is relatively high (Nydahl et al., 2013), though bacterial response differs based on their source of origin (Langenheder et al., 2003). Impacts of salinity on DOC are expected through the large removal of organic carbon from estuaries and coastal environment, since microbial metabolism is relatively high in the salinity environment (Langenheder et al., 2003; Rocker et al., 2012). Significantly, saline systems are sensitive to various transportation and removal process of DOC, i.e. accumulation of molecules to particulate matter, rapid salt – induced flocculation (Asmala et al., 2013). Morrissey et al., (2013) found that salinity is positively related to bacterial abundance and also linked with community composition. He further showed that salinity has strong relationship with the activity of bacterial specific enzymes that support carbon degradation. Furthermore, soil producing organic matter is accumulated when mixed with the saline water (Morrissey et al., 2013). Another study found that salinity intrusion into the abiotic freshwater sediment reduce methanogenesis with a rapid shift to sulfate reduction , increase microbial

organic matter mineralization, increased nutrient (ammonium, silicate, phosphate) concentration associated with increasing pore water ionic strength (Weston et al., 2006). However, salinity increase might be driven changes in microbial metabolism, nutrient cycling, and community composition of ecosystem that may alter the carbon biogeochemistry and organic matter storage capacity of the water systems (Weston et al., 2006).

2.5 Biogeochemical processes in the estuarine environment

The physical forms of humic substances are affected by the pH gradient. Some molecules 'fold out' and change their diameter. Other molecules, especially large humic acid molecules, flocculate and form particles that may or may not be bioavailable. Additionally, as iron solubility decreases in salt, dissolved complexes between DOC-Fe are affected. This can cause flocculation and physical change of the DOC molecules as well. At the same time, nutrient cycles are affected. For example, in freshwaters, PO_4 is effectively trapped by iron(III). This is known mainly as a sedimentation process, but also in the water column, oxidation of iron(II) to iron(III) can trap PO_4, especially when anoxic waters get oxygenated. However, in saltwater, this trap is not so effective because Iron is less soluble and it especially tends to react with sulfur, forming FeS_2 which does not form complexes with PO_4 (Blomqvist et al., 2004). All of this, in combination with coastal pollution, often contributes to rising P, which is a nutrient that often limits bacterial DOC degradation.

2.6 Nutrients and DOC input to Baltic Sea

The Baltic Sea in northern Europe is semi-enclosed and the largest brackish water body in the world, which is connected to the ocean by means of fresh water (Neumann 2000; Omstedt et al., 2012; Thang et al., 2013). The Baltic is dominated by cultivated landscape, temperate climate, and by evergreen coniferous forest and diversified wetlands in the north, causing variations of water chemistry. The entire aquatic environment is complex; there are many interactions taking place between anthropogenic nutrient loads and physical, chemical and biological processes for example. These characteristics can be represented by the extent of bottom water hypoxia in the coastal basin of the Baltic Sea (Omstedt et al., 2012). Hypoxic conditions in the coastal water have increased during the last decade with the increase of nutrient loads (nitrogen and phosphorous) and with the changes of climate at the same time scale (Hansson and Gustafsson 2011).

The Baltic Sea receives organic carbon from the river discharges, primary production and from the North Sea. A significant amount of nutrients, produced by agriculture and industry, enters to the Baltic Sea through river runoff (Szymczycha et al., 2013). Rivers can have a significant impact on the coastal ocean biogeochemistry by the input of nutrients and carbon. Human activities, for example agriculture, population density (sewage), increase terrestrial nutrient load that might impact on carbon budget (Cunha et al., 2007).

Chapter Three: Materials and Methods

3.1 Study Site Description

Ten rivers were used for this project: Lyckebyån (R1), Helgeån (R2), Nyköpingsån (R3), Motala Ström (R4), Vättern Outlet (R5), Torne älv (R6), Töre älv (R7), Pite älv (R8), Öre älv (9) and Ume älv (10). Rivers R1 to R5 were located in southern Sweden and R6 to R10 were located in northern part of Sweden (Fig. 1). The river catchments are composed and influenced by forest, wetlands, alpine areas, agriculture and urban land use.

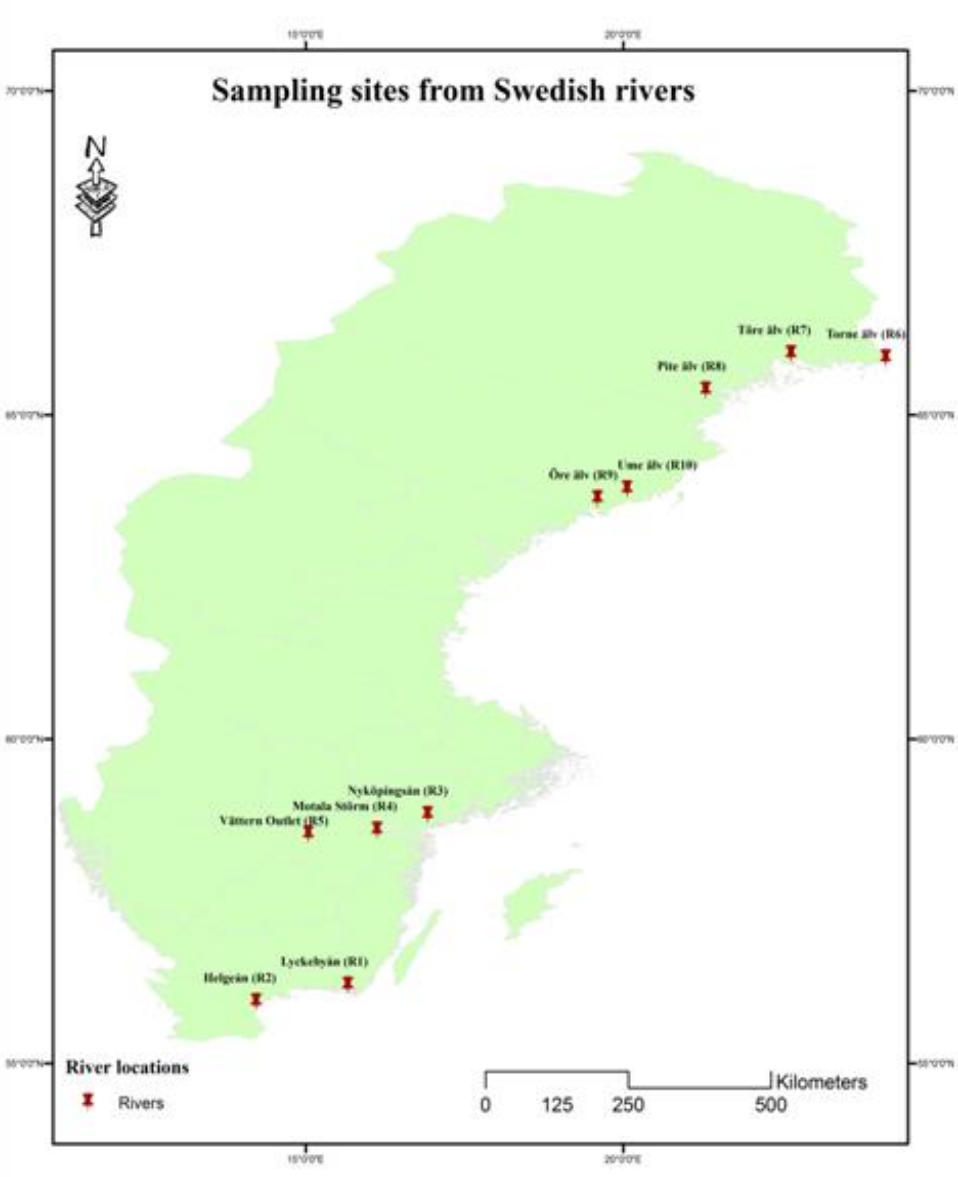

Figure 1: Map showing the location of 10 major rivers in Sweden used for water sampling (see Table 1 for site descriptions).

3.2 Water Sampling

One water sample per river was collected in June and July during summer low flow conditions of 2013. Samples were collected from the flowing water and at ~0.3 m depth under water surface to avoid collecting large materials, by using a bilge pump. Each sample of water was collected in cubitainer plastic containers (size 4 liter) after multiple rinses with sample water. All the collected samples were kept chilled on ice in plastic container until arrival at the laboratory. Samples were transported to the laboratory within 24 hours of sampling in the south and within 48h of sampling in the north. In addition, in situ water temperature and detail water quality/chemistry were measured at each sampling location using a fully equipped YSI-556 multi-probe.

3.3 Water Cultures

Collected water samples were filtered through 1.2 µm Pall A/E glass fiber filters to remove particulate organic carbon and organisms larger than bacteria. The filtration procedure did not change the DOC concentration of the water sample (Wikner et al., 1999). Filtered water was kept in cubitainer plastic container after multiple rinses. The filtered water was used for nutrient and salt treatments.

In order to assess the microbial impact on DOC degradation, some samples were further filtered by using a 0.2 µm membrane filter. It is assumed that above 97% of the particles including bacteria and viruses retained during 0.2 µm filtration (Stockner et al., 1990). This filtration procedure could lead to a minor change of DOC due to voiding from algal cells, but this is ignorable (Tranvik 1988).

3.4 Experimental Set-up

In this project work a total of three types of experiments were performed:

3.4.1 Experiment 1 [E-1]: Nitrogen, Phosphorus and Salt Addition

Nitrogen and phosphorous are considered as factors that could limit bacterial DOC degradation (Vrede 2005). Nitrogen is fundamentally linked to the organic carbon processing because nitrogen is an important component of dissolved organic matter (Ghosh and Leff 2013). For this experiment 1.2 µm filtered water was used. This experiment consisted of five steps.

1. At the beginning of this experiment sample bottles were rinsed multiple times with filtered water. For each site, eight bottles were filled with 100 mL filtered (1.2 µm) sample water.

2. Inorganic nutrients nitrogen (N), phosphorous (P) and salt (S) were added to the treatment bottles in all possible combinations: no addition of nutrients and salt (control), N alone, P alone, NP, S alone, NS, PS, and combination of NPS. Nitrogen (1 mg N L^{-1}) was added in the form of NH_4NO_3, phosphorous (0.1 mg P L^{-1}) in the form of Na_2HPO_4 and salt (10 psu) in the form of NaCl (approximately 10 g NaCl added per L sample water). The added quantities of N and P were enough to cause excess of these nutrients (Berggren et al., 2007).

3. The treatment bottles were well shaken to ensure that all added nutrients and salts were dissolved. This cocktail of water was then poured into O_2 sensor vessels (25 mL each) after multiple rinses using the treatment water. Two replicate sub-samples of each treatment were prepared (Fig. 2) and kept open for a few seconds in room temperature in order to release water bubbles before the cap was sealed. For re-using, treatment bottles (250 mL) were washed by using 95°C warm water.

4. When the cap was sealed, the sensor vessels were incubated in the dark and at room temperature (19-20° C) for one week.

5. During the incubation time (one week), oxygen concentrations were measured manually by using an oxygen logger (Fibox 3). Each O_2 vessel was connected for 10 second to the O_2 logger. Measurement was taken twice per day except weekend day, from start (0 day) day to the end of the incubation. For re-using oxygen sensor vials (25 mL) were washed using ethanol and Millipore –deionized water. Calibration data were fixed before measurements were taken. Finally, the DOC degradation was assessed from the measured oxygen consumption as described below, assuming a respiratory quotient of 1.

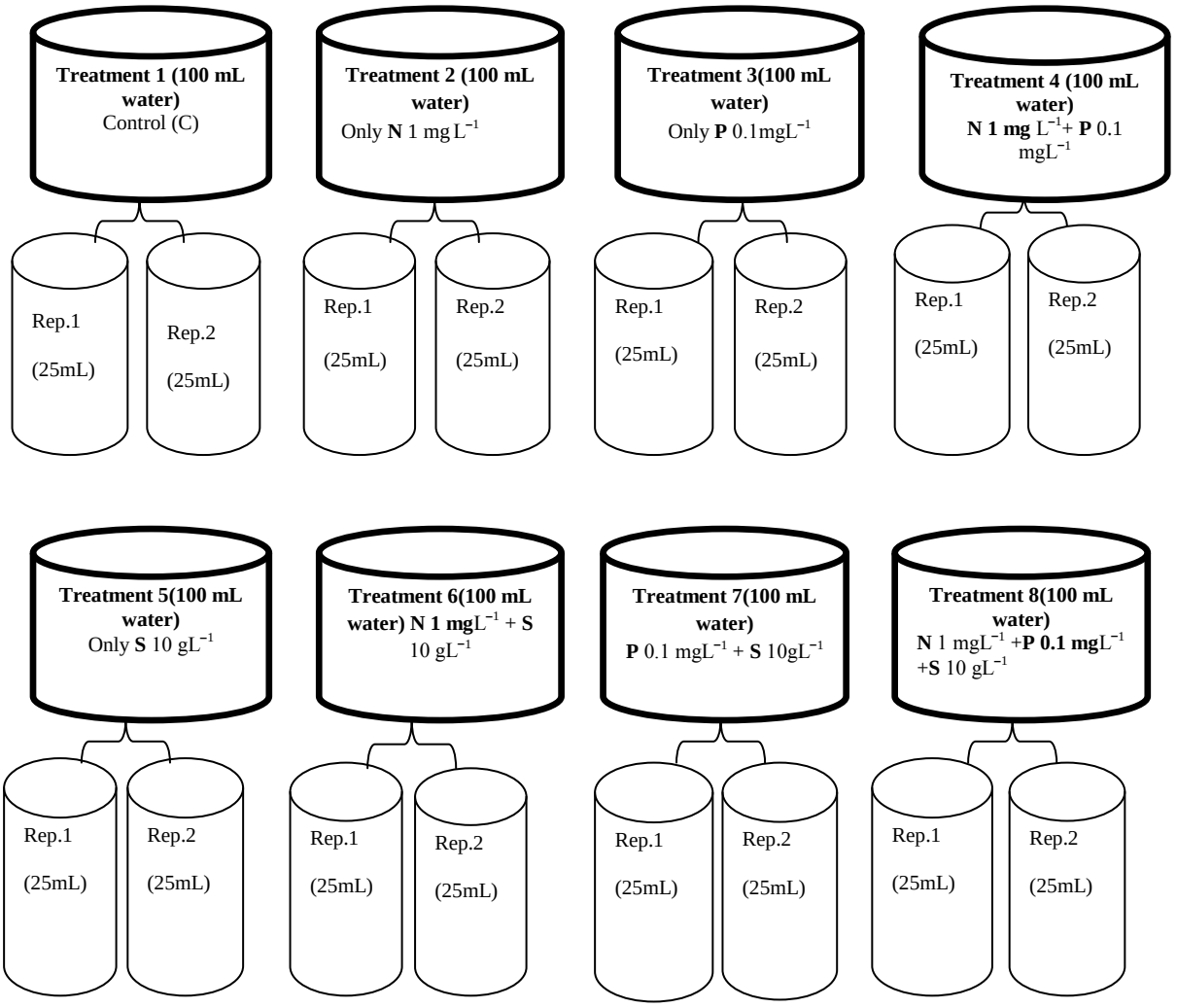

Figure 2[E-1]: General set-up scheme for nutrients (N and P) and salt treatments. N: Nitrogen, P: Phosphorous, and S: Salt. Rep.: Replicate

3.4.2 Experiment 2[E-2]: Microbial Community Test

A large part of DOC degraded by the microorganisms (del Giorgio and Davis 2003). Organic carbon enters the aquatic food web by microbial uptake and is to a large extant returned to the atmosphere as CO_2 (Berggren et al., 2010). Microbial communities are extremely sensitive and respond strongly to slight changes of nutrients, organic carbon, salinity and temperature in both fresh and marine water environment (Compte and del Giorgio 2011). In the aquatic systems the microbial community participate in the organic carbon production, transformation and degradation (del Giorgio and Davis 2003). This experiment was performed by using 0.2 µm

filtered water with either fresh or marine water microbes. This experiment consisted of four steps:

1. At first eight sample bottles were multiple rinsed with filtered water and then four bottles were prepared for freshwater microbial test and another four for marine water microbial test.

2. From the filtered water (0.2 µm sterile-filtered), 100-mL was poured to each treatment bottle. The three factors nutrients (N+P combined as one factor), salt (S) and microbial community (freshwater or marine) were applied in all eight combinations. Nitrogen (1 mg N L^{-1}) was added in the form of NH_4NO_3, phosphorous (0.1 mg PL^{-1}) in the form of Na_2HPO_4 and salt (S) (10g L^{-1}) was added as solid NaCl.

3. In the next step, freshwater microbes (Fm.) were added to four of the treatment bottles and marine water microbes (Mm.) were added to another four treatment bottles. Inoculums with unfiltered river water (1 mL) and Baltic Sea water (1 mL) were added to get fresh versus marine water microbes. Eight combinations of treatments were applied: Only Fm. addition (control), Nutrients (NP combined) + Fm., S+ Fm., Nutrients + S+ Fm., only Mm. addition (control), Nutrients (NP combined) + Mm., S+ Mm., and Nutrients + S+ Mm.

4. Finally, two replicate sub-samples (25 mL each) of each treatment were prepared for one week incubation. Oxygen consumption was measured twice per day during 7 d.

3.4.3 Experiment 3 [E-3]: Test of possible salt-induced formation of reactive organic particles

Increasing salinity has a potential to aggregate organic carbon (Asmala et al., 2013). Riverine organic carbon flocculates rapidly when mixed with estuarine water (Sholkovitz et al., 1978). To assess the particulate organic carbon (POC) formation in salinity environment, 0.2 µm sterile-filtered water were used. This treatment consisted in four steps:

1. Three 250mL sample bottles were multiple rinsed with filtered water and one filled with 200 mL and other each filled with 100 mL sterile-filtered water. Then 2 g and 1 g solid

NaCl as salt was added respectively to each bottle to create the approximate salinity of 10 psu. The water was shaken vigorously for dissolution of salt and then kept for around three hours for POC formation.

2. The water in 200 mL of the sample bottle was again filtered by using 0.2 µm filters to remove potential POC. The other bottles served as a control.

3. The water in 200 mL sample was divided into two treatment bottles, each containing 100 mL water. Unfiltered 1mL freshwater was added as freshwater microbes with one control and with one extra filtering treatment bottles and 1mL Baltic sea water was added as marine microbes with another control and extra filtering treatment bottles.

4. From each treatment bottle two replicate samples were prepared for seven days incubation.

3.5 Data Processing and Calculations

Measured data were stored in the computer as text file and for the calculation and statistical analysis data were converted to Excel. From these raw data, last five values of each day incubation were considered for calculating the average of oxygen consumption. The solubility of O_2 at 10 psu salinity is approximately 5% lower compared to the solubility of O_2 at 0 salinity. To bring the balance, all the values from the salt treatments were multiplied by a conversion factor 0.9427 (USGS 2013). After that, the slope was calculated to get the total DOC consumptions during the incubation time. Slope values indicate the variability of oxygen consumption of the incubated samples.

DOC degradation was determined by the following equation:

$$DOC\ degredation = \frac{\text{Slope value} * 12}{32} \quad \text{.....1}$$

Here 12 is molecular weight of carbon and 32 is molecular weight of oxygen. DOC degradation rates were expressed as mg/litre/day, abbreviated as mg L^{-1} d^{-1}.

3.6 Statistical Analysis

For studying the statistically significant differences between the factors, 3 fixed factor ANOVA (general linear model) were applied. All Statistical analysis in this study were carried out using

SPSS ver. 17.0. The experiment has three factors: Nutrient, salt and microbes. So it seems logical to apply 3-way ANOVA for the appropriate statistical analysis. The raw data were put into a 3-way ANOVA model. The model output was given P values that were significant differences among the treatments to the respective experiments. The statistical out comes were: The differences of marginal mean when factor was added: 1 and not added: 0, is statistically significant or not, on DOC degradation.

The statistical output of each factor and sample, divide the marginal mean when the factor is 1 (1: factor added) by the marginal mean when the factor is 0 (0: factor not added). This gives a measure of the relative impact of factor. For example, a value of a factor 0.5 means that the DOC degradation decreased by half when the corresponding control value is at1. A value of 2 of a factor means the DOC consumption has increased double in response to corresponding control.

Applied multifactor ANOVA is much more powerful and interactions can be evaluated in a way that is needed to address the hypotheses and to reach the aim. When we run a single-factor ANOVA with post hoc test to examine the three or more treatments, a significant result only indicates specific comparisons between all different combinations of treatment. It does not identify factorial design. It could be done through the multiple comparisons among the means of treatment. In this case multi-factor ANOVA is suitable procedure. Furthermore multi factor ANOVA is very useful to have separate *F* ratios for each of the factors (Mckillup and Darby 2010).

A factorial design is much more powerful since it tests the significance of specific factors, applied in all possible combinations. In statistical terms, that gives a lot of degrees of freedom. In the experiment total 4x2 samples in which a certain factor is 'on' and 4x2 samples in which the same factor is 'off', i.e. has not been applied. The big advantage with the multi-way ANOVA is, thus, that it uses the information in all samples when assessing the significance of each factor. Another great advantage with a multi-way ANOVA is that it can evaluate the interactions.

Chapter Four: Result

4.1 River Water Chemistry

The DOC concentration varied between 2.9 and 23.0 mgL^{-1} (Table 1). Among the DOC concentration values, R8 showed the lowest value. In the rivers R2, R6, and R7 the same concentration of PO_4-P (6 µg L^{-1}) was observed. In a significant number of rivers (R3, R6, R7, R9, and R10), no nitrates was observed. The rivers showed TP values of 10-29 µg P L^{-1} and TN of 0.08 - 1.14 mg N L^{-1} (Table 1)

Table 1: Physical and chemical properties of rivers sampled water in 2013

Rivers	[1]DOC (mg L^{-1})	[2]PO_4-P (µgL^{-1})	[3]NH_4-N (µg L^{-1})	[4]NO_3-N (µgL^{-1})	[5]Total P(µgL^{-1})	[6]Total N(mgL^{-1})	pH
Lyckebyån(R1)	23.0	3	20	174	29	1.14	6.7
Helgeån (R2)	18.3	6	14	367	24	1.06	7.4
Nyköpingsån(R3)	16.7	7	7	0	26	0.67	7.6
Motala Ström (R4)	11.3	11	54	270	24	0.43	7.6
Vättern Outlet (R5)	3.7	2	11	123	10	0.35	8.1
Torne älv (R6)	5.6	6	2	0	15	0.17	7.5
Töre älv (R7)	6.3	6	4	0	20	0.60	6.4
Pite älv (R8)	2.9	5	7	4	21	0.08	6.8
Öre älv (R9)	6.5	5	10	0	17	0.36	7.1
Ume älv (R10)	3.8	5	11	0	18	0.09	7.2

[1]DOC: dissolved organic carbon concentration,[2]PO_4: phosphate concentration, [3]NH_4-N: ammonium concentration, [4]NO_3-N: nitrate concentration, [5]Total P: total phosphorous concentration [6]Total N: total nitrogen concentration. DOC concentration data and pH were analyzed in G.G. Hatch Isotope Laboratories at the University of Ottawa, Canada. The nutrient concentration were analyzed at the limnology department of Uppsala university, Sweden.

4.2 DOC Bio-assays

The bio-assay study showed the effect of nutrient and salt on DOC degradation rate per day in every river (Fig. 3). The factors were applied alone and in all possible combinations with the

sample waters. The DOC degradation response to nutrients and salt addition varied between the treatments (Fig. 3). In the different rivers the variability of DOC degradation rate was observed following the nutrient and salt manipulation. In river R1 DOC degradations found to be around 0.03, 0.04, and 0.02 mgL^{-1} d^{-1} due to manipulation of N, P and salt (NaCl) respectively. In case of R10 river DOC degradation rates found to be about 0.06, 0.04, 0.05 and, 0.05 mg L^{-1} d^{-1} for NP, NS, PS and NPS manipulations respectively. The highest amount of DOC reactivity due to nutrient manipulation was obserbed in river R8 following the P treatment, where degradation was enhanced by 33.22% compared to the corresponding control.The DOC reactivity increased by 29. 91% in R5 and 44.94% in R9 compared to the corresponding control due to nutrient NP manipulation (Fig. 3).

Salt treatment systematically showed negative effects on DOC degradation in all of the water samples. A DOC degradation decrease of about 4.64% due to salt manipulation in R3 river in comparison to the control. After calculating the average of all of the nutrient and salt treatments, results showed that only P had positive effect on DOC degradation compared to the treatments in which P was not added.

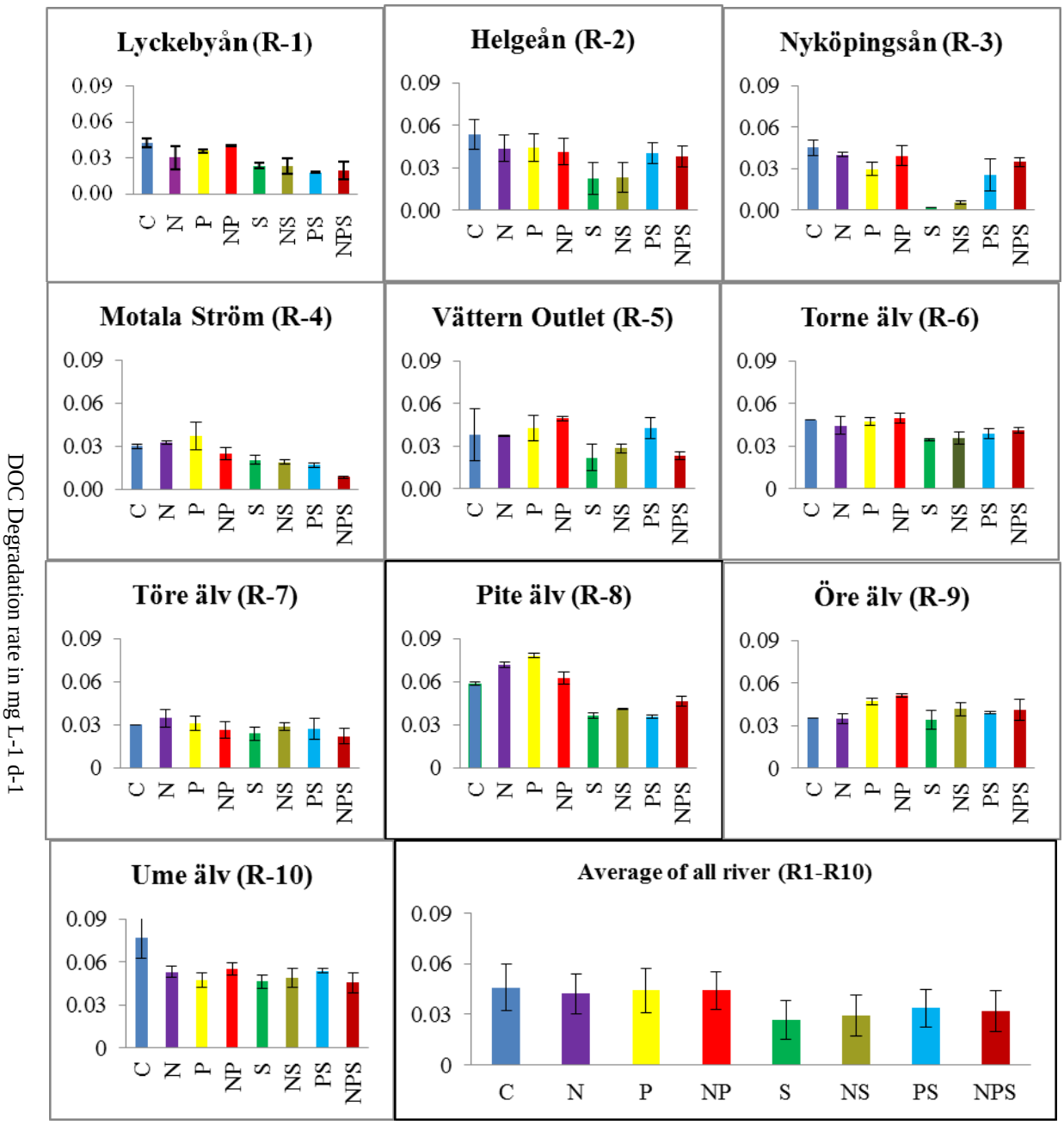

Figure 3: The variability of DOC reactivity based on data from seven days incubations in 10 different rivers (R1-R10)**.**

The y- axis indicate the DOC degradation rate (mg L^{-1} d^{-1}). C: control, N: nitrogen, P: phosphorous, S: salt. Error bars show standard deviations calculated from two replicates.

4.3 Effects of Nutrient and Salt on DOC Degradation

The statistical analysis for N, P and Salt (S) shows that salt had a significant effect on DOC reactivity in most of the rivers only R5, R9 and R10 showed no significant differences (Fig. 4).

However, P shows insignificant values in the majority of the rivers with significant values in R2 and R9. There were no significant differences between rivers under N treatment.

Salt had the strongest negative effect on DOC degradation (Fig. 4, Table 2). In case of river R8, salt decreased the DOC degradation from 0.068 to 0.040 mg $L^{-1}d^{-1}$, which is a 41.18 % decrease compared to control, and that is statistically significant ($F_{1, 8}$ = 298.95, P< 0.001, ANOVA-test).

Nutrient P showed a positive effect on DOC degradation in only two of the rivers (Fig.4, Table 2). In river R2 P increased the DOC degradation from 0.037 to 0.045 $mgL^{-1}d^{-1}$, which is an increase by 24.24% compared to the control, and is statistically significant ($F_{1, 8}$ = 9.80, P< 0.05, ANOVA-test). Also in two cases (R3 and R8), P addition enhanced the DOC degradation and their marginal means difference is near the significant threshold ($F_{1, 8}$ = 5.12, P<0.10 and $F_{1, 8}$ = 5.05, P<0.10, ANOVA test) Fig. 4, Table 2.

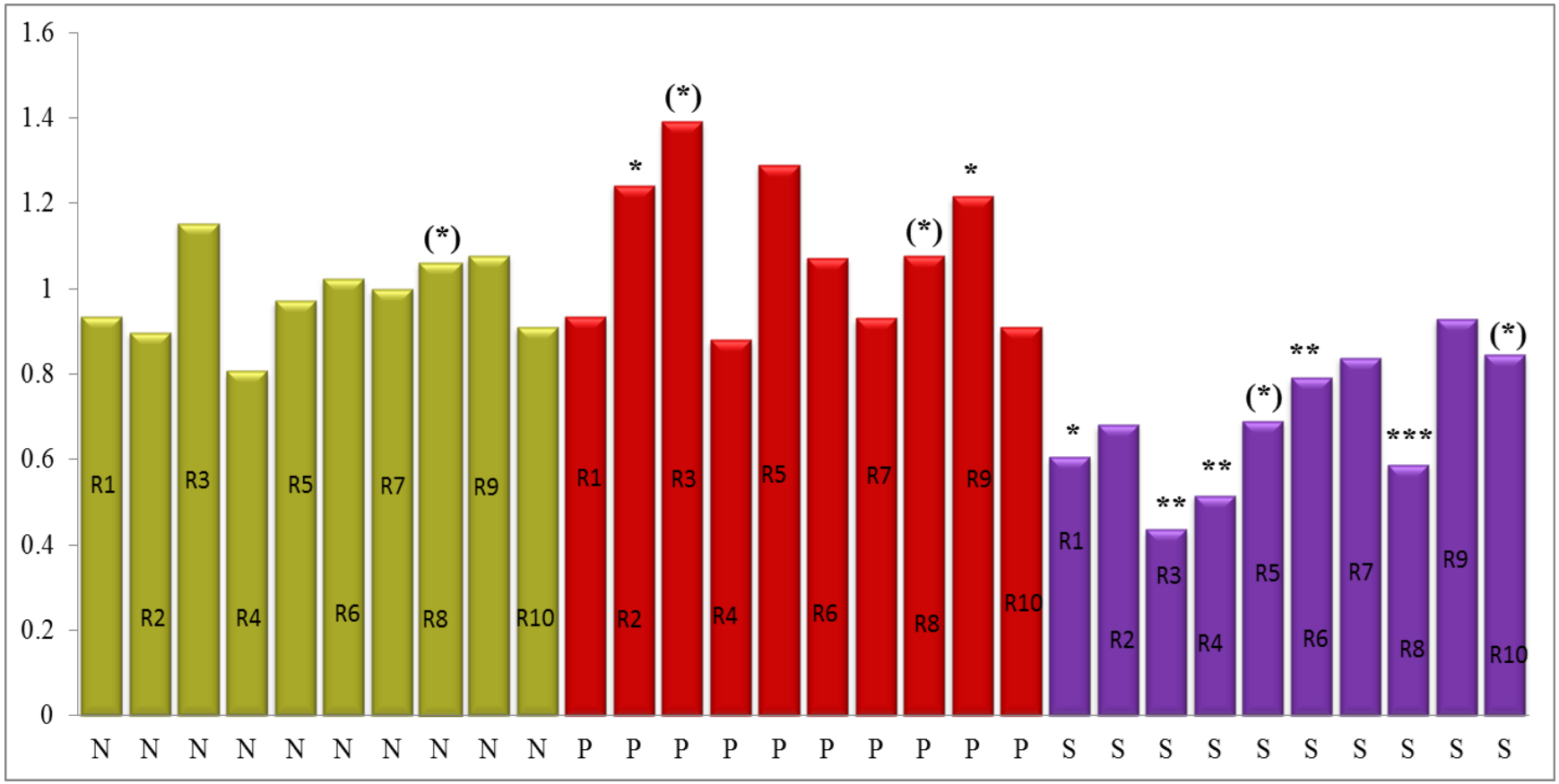

Figure 4: The relative effects of increased N, P and salt on DOC degradation in 10 different rivers (R1-R10).

The relative effect of factors were calculated as divide the marginal mean when the factor is added by the marginal mean when the factor is not added. N: nitrogen, P: phosphorous and S: salt . The degree of significance in the ANOVA analysis are denoted by asterisk simboles. *** for P <0.001, ** for P < 0.01, * for < 0.05 and P (*) <0.10 indicate marginal means near the significant value . All salt significant effects were negative.

4.4 Nutrient and Salt Interaction Effects on DOC Degradation

The significance analysis of the interaction effect showed that N, P and S in all possible combinations have an effect on DOC degradation.

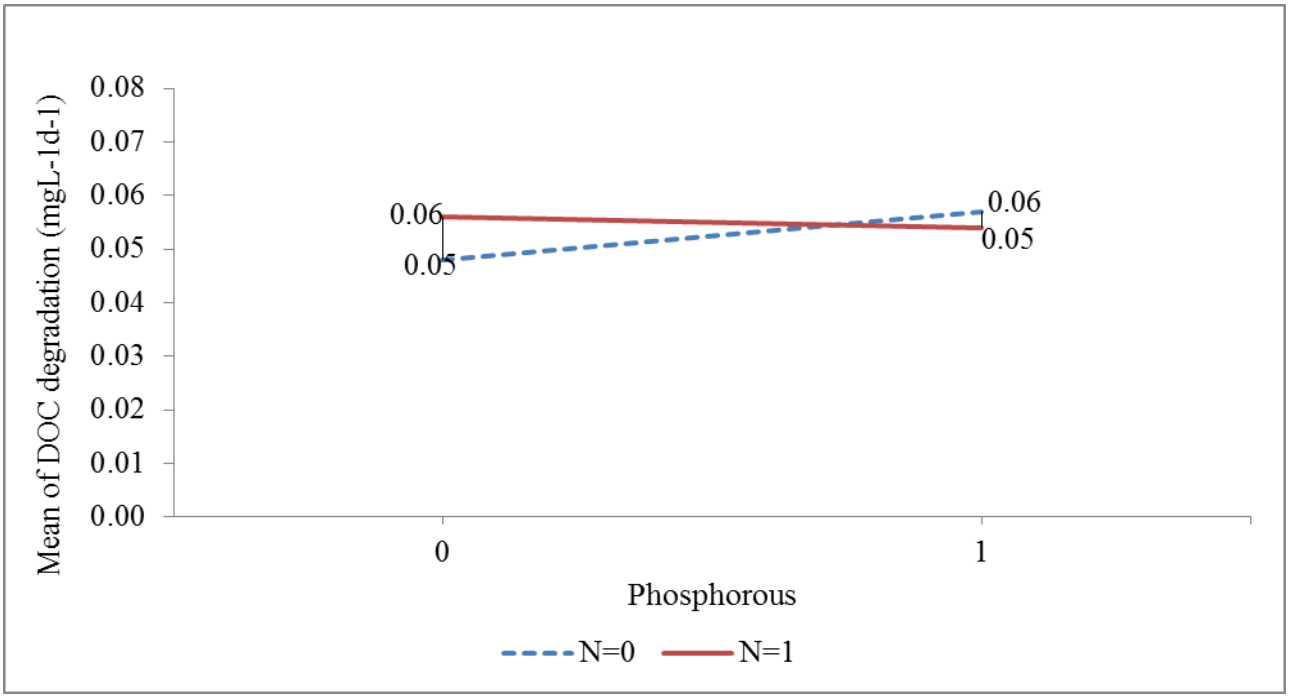

Figure 5: NP interaction effect on DOC degradation in river R8.

N=0: nitrogen not added, N=1: nitrogen added. The x- axis shows phosphorous (P) addition. 0: phosphorous not added, 1: phosphorous added.

Result from ANOVA analysis showed that the combined effect of NP decreased DOC reactivity to river R8 (Fig. 5 and Table 2), which is statistically significant ($F_{1, 8}$ = 11.74, P<0.01, ANOVA -test). In case of R8 river P stimulated DOC degradation from 0.05-0.06 mg $L^{-1}d^{-1}$ that is 15.79% compared to the control. When N was added with P, DOC degradation decreased from 0.06 to 0.05 $mgL^{-1}d^{-1}$ that is by 3.57% decreased compared to the N- effect alone.

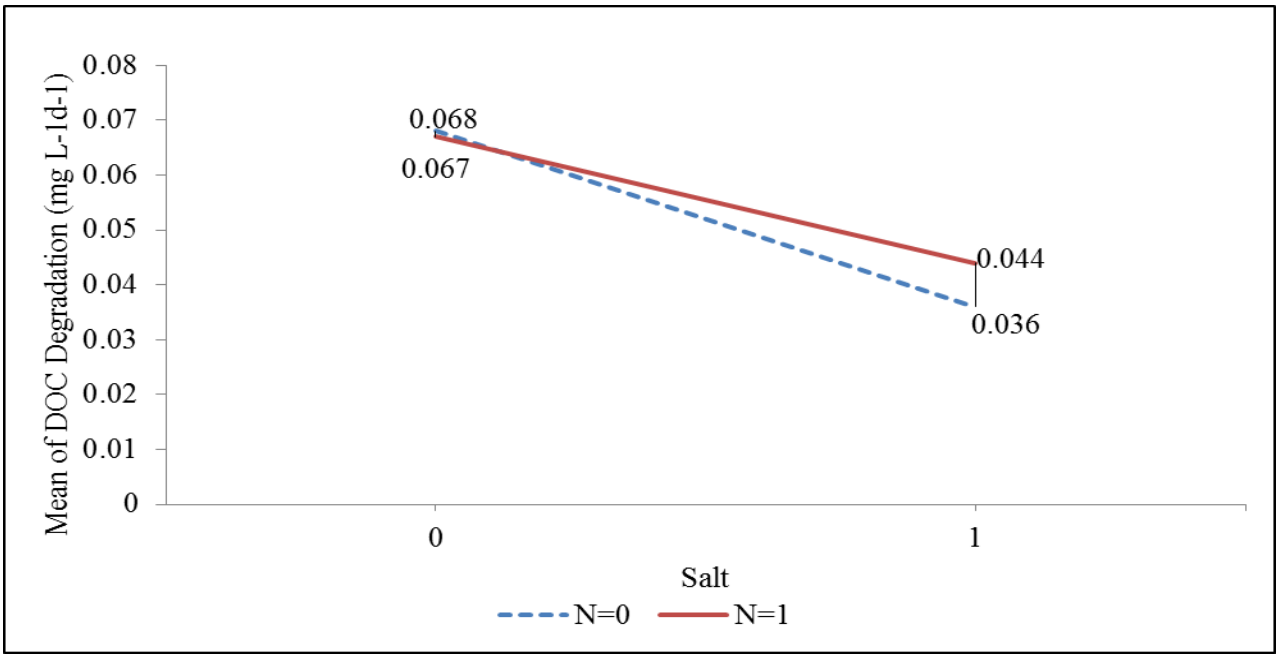

Figure 6: NS interaction effect on DOC degradation in river R8.

N=0: nitrogen not added, N=1: nitrogen added. The x -axis shows salt addition. 0: not added salt, 1: salt added

According to significance analysis the result showed that DOC degradation was affected by NS interaction in only one river (Table 2). Result shows that in river R8 salt decreases the DOC reactivity from 0.068-0.036 $mgL^{-1}d^{-1}$ (Fig. 6), that is 47.06% compared to the control. When N was added with salt, the DOC reactivity was decreased by 34.33% compared to the N effect alone, which statistically significant ($F_{1, 8}$ = 6.98, P< 0.05, ANOVA -test). Here one can notice that salt alone decrease the DOC reactivity more compared to the combined effects of NS.

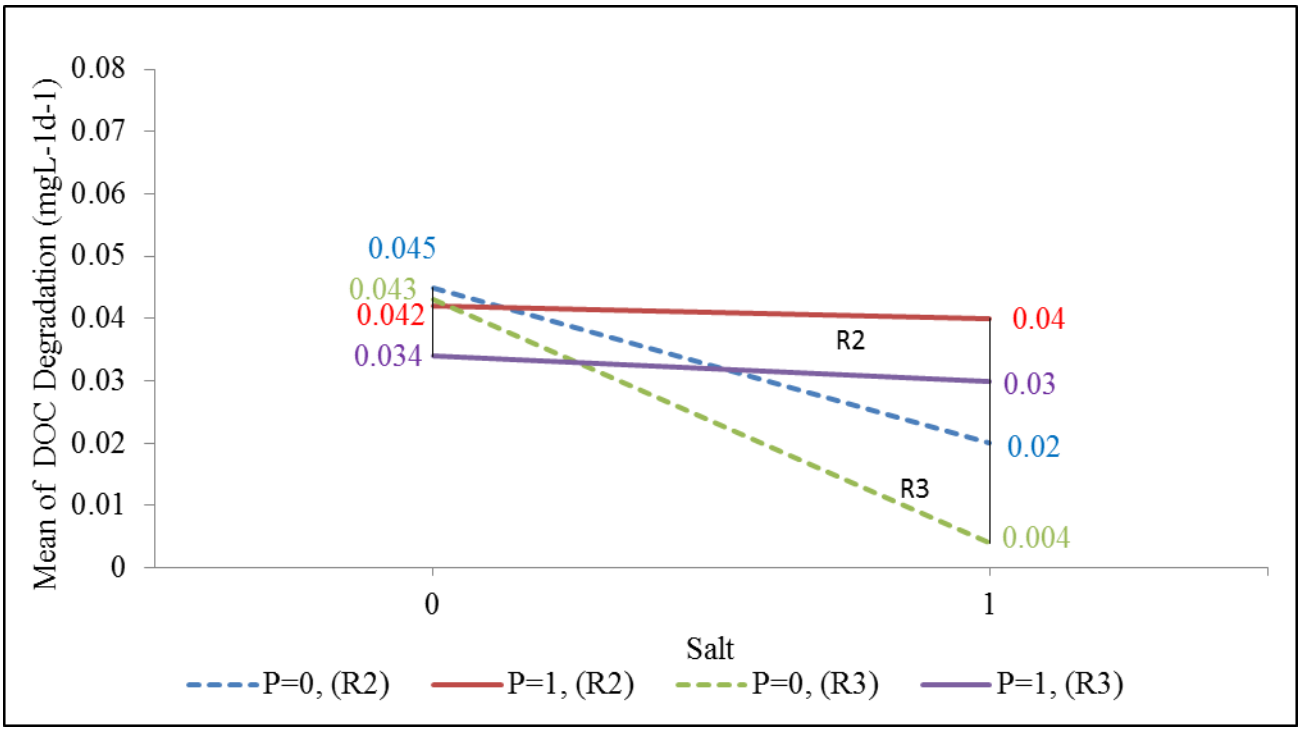

Figure 7: PS interaction effect on DOC degradation in rivers R2 and R3.

P=0: phosphorous not added, P=1: phosphorous added. The x-axis shows, 0: salt not added, 1: salt added.

The PS interaction effect on DOC reactivity was found statistically significant. Two rivers: R2&R3 showed a negative effect on DOC degradation by 4.76% and11.77% respectively following the PS interaction factor compared to P effect alone (Fig. 7, Table 2).The result showed that PS enhanced the DOC degradation compared to salt effect alone, which is an important result.

4.5. Significance analysis of all factors and interactions

Table 2: Significance analysis of all individual factors and in all possible combinations in 10 different rivers (R1-R10).

River No.	Factors and their Interaction						
	N	**P**	**N*P**	**S**	**N*S**	**P*S**	**N*P*S**
R-01	-	-	-	($F_{1, 8}$ = 12.00, *P**)	-	-	-
R-02	-	($F_{1, 8}$ = 9.80, *P**)	-	($F_{1, 8}$ =24.20, *P***)	-	($F_{1, 8}$ = 16.20, *P**)	-
R-03	-	($F_{1, 8}$ = 5.12, *P(**))	-	($F_{1, 8}$ = 29.02, *P***)	-	($F_{1, 8}$ = 18.68, *P**)	-
R-04	-	-	-	($F_{1, 8}$ = 27.14, *P***)	-	-	-
R-05	-	-	-	($F_{1, 8}$ = 4.69, *P(**))	-	-	-
R-06	-	-	-	($F_{1, 8}$ = 17.27, *P**)	-	-	-
R-07	-	-	-	-	-	-	-
R-08	($F_{1, 8}$ = 3.71, *P(**))	($F_{1, 8}$ = 5.05, *P(**)	($F_{1, 8}$ = 11.74, *P***)	($F_{1, 8}$ = 298.95, *P****)	($F_{1, 8}$ = 6.98, *P**)	-	($F_{1, 8}$ = 30.36, *P***)
R-09	-	($F_{1, 8}$ = 7.10, *P**)	-	-	-	($F_{1, 8}$ = 3.93, *P(**))	-
R-10	-	-	-	($F_{1, 8}$ = 3.78, *P(**))	-	-	($F_{1, 8}$ = 4.99, *P*(*))

The analysis were conducted on 3-ways ANOVA. N: nitrogen, P: phosphorous, S: salt. *P**** for <0.001, *P*** for < 0.01, *P* * for P< 0.05 and marginal means near the significant value *P* (*) for <0.10, *P*: significant, - : Nonsignificant > 0.05.

The significance analysis of all of the treatments showed that the majority of the factors (individual and in combination) have an effect on DOC reactivity, except N treatment (Table 2). Salt treatment showed a negative significant effect and P showed the opposite effect on DOC

reactivity. The interaction effect NP and NS showed a negative significant effect in a single river. Another interaction treatment of all factor combined (NPS) showed that the significant effect on DOC degredation is negative.

4.6 DOC Bio-assays with microbial inoculums

Our microbial experiment showed that DOC reactivity is limited by the microbial communities following the different treatments in river R2 and R9 (Fig. 8). The largest increase of DOC degradation was observed in the river R9 following the MN treatment, in which DOC degradation enhanced up to 0.70 fold compared to corresponding control. The DOC degradation was tended to decreased by 8.79 fold following the MNS treatment with the respective control in the river R2, which is the highest decreasing rate between the rivers.

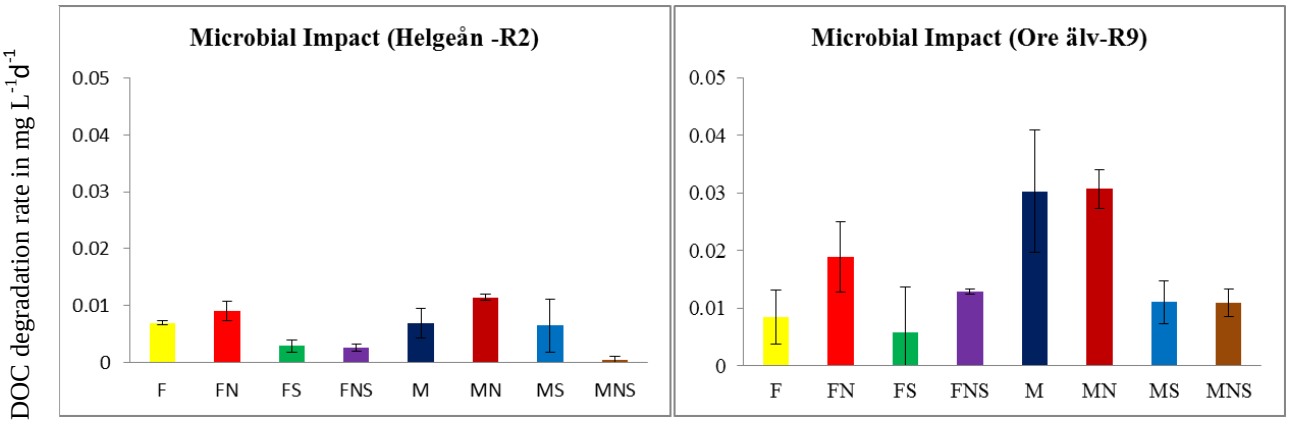

Figure 8: The microbial impact on DOC degradation based on data from seven days incubations (R2&R9).

The y- axis indicate the DOC degradation rate (mg L^{-1} d^{-1}). F: freshwater microbes, N: nutrient (N &P as one factor), M: marine water microbes and S: salt. Error bars represent standard deviations.

4.7 Effect of Microbial Communities on DOC Degradation

According to the statistical analysis nutrient addition had no significant effect on DOC degradation in either the R2 or R9 river (Fig. 9). Salt stress to microbes was siginificant for both of the river experiments (Fig. 9). In R2 and R9 river DOC degradation was dampened by 88% and 50% respectively due to salt manipulation compared to the corresponding control, which is statistically significant ($F_{1, 8}$ = 19.64, *P=<0.01 and* $F_{1, 8}$ = 8.79, *P=<0.05*, ANOVA-test) Fig. 9. In case of river R9, marine water microbes enhanced DOC degradation by 0.75% compared to freshwater microbes, and the marginal means difference was close to satistically significant ($F_{1, 8}$ = 5.13, *P*=<0.10) Fig. 9, Table 3.

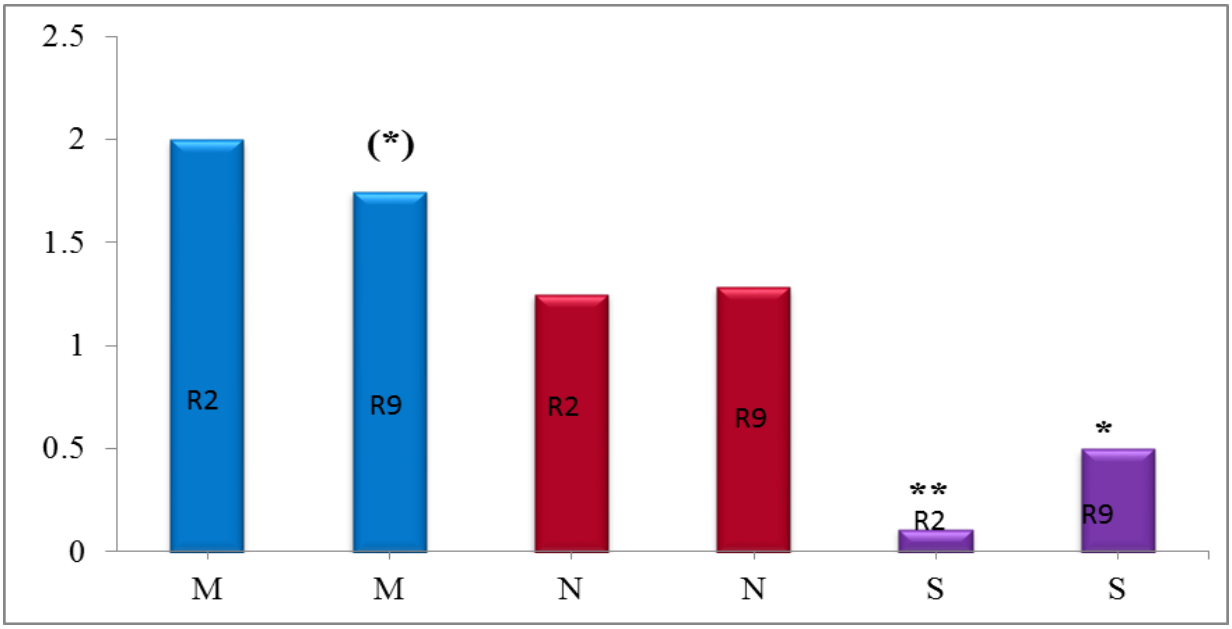

Figure 9: Relative effect of microbial community on DOC degradation in river R2 & R9.

M: microbial comunities, N: nutrient and S: salt. The relative effect of factors were calculated as divide the marginal mean when the factor is 1(1: factor added) by the marginal mean when the factor is 0 (0: factor not added). The degree of significance in the ANOVA analysis are denoted by asterisk symbols. ** for P < 0.01, * for < 0.05 and P (*) <0.10 indicate marginal means near the significant value .

4.8 MS Interaction Effect on DOC Degradation

In general, for rivers R2 and R9, salt addition with microbes (both fresh and marine microbes) had a negative effect on DOC degradation (Fig. 10). The significance analysis showed that in MS interaction effect was negative on DOC degradation compared to fresh water microbes effect in river R9 and marginal mean difference was close to the significance threshold ($F1, 8 = 4.44$, P=<0.10) Fig. 10, Table 3.

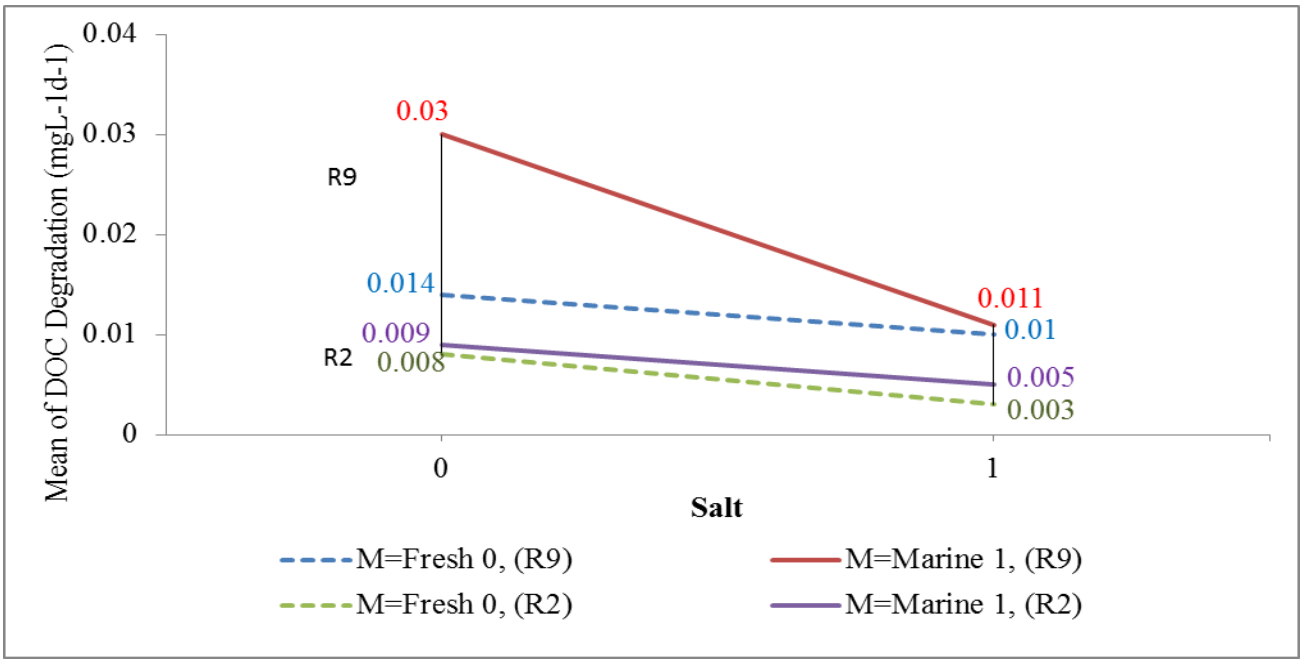

Figure 10: Fresh and marine water microbes impact on DOC degradation (R2 &R9).

The x-axis shows the salt concentration. 0: salt not added and 1: salt added. M=Fresh 0: freshwater microbes added and M=Marin 1: marine microbes added.

Table 3: Significance analysis of microbial community test in rivers R2 and R9.

River No.	Factors and their Interaction						
	M	**N**	**S**	**M*N**	**M*S**	**N*S**	**M*N*S**
R-02	-	-	($F_{1,8}$ = 19.64, *P***)	-	-	-	-
R-09	($F_{1,8}$ = 5.13, *P*(*))	-	($F_{1,8}$ = 8.79, *P***)	-	($F_{1,8}$ = 4.44, *P*(*))	-	-

M: microbial comunities, N: nutrient and S: salt.The degree of significance in the ANOVA analysis are denoted by asterisk simboles.

4.9 Effect of Salt Induced Particle formation on DOC Degradation

The salt induced reactive particles were removed by 0.2 µm extra filtering that could effect on DOC reactivity was compared with the microbial community incubations (Fig. 11). The statistical analysis result showed that there were no particles formed that significantly affected the DOC degradation rates (Fig. 11). However, marginal means difference of extra filtering treatment was close to near the significant threshold ($F_{1,4}$ = 6.15, *P<0.10*, ANOVA-test) Fig. 11.

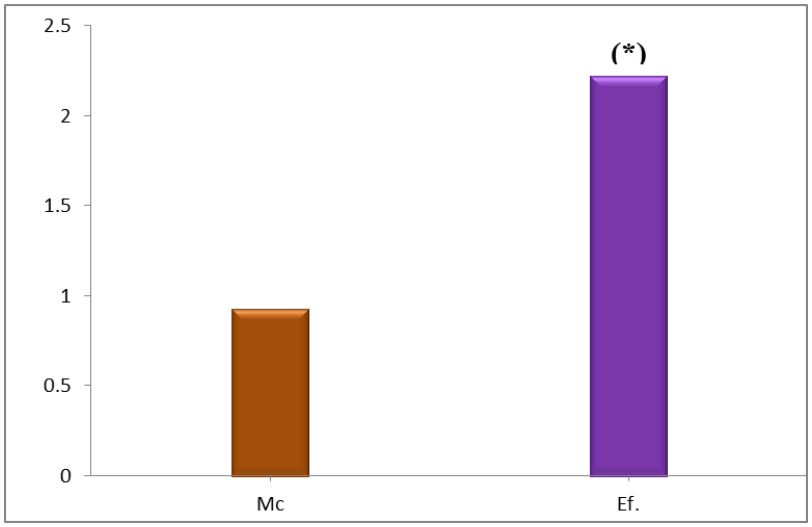

Figure 11: The relative effect of POC formation on DOC degradation in river R9.

Mc: microbial community (fresh vs. marine microbes) in which POC had not been removed by filtering and Ef.: extra filtering sample in which POC had been removed.

Chapter Five: Discussion and Conclusions

5.1 Discussion

In this study a short-term bio-assay approach was applied to indirectly assess DOC degradation in the estuarine system. On shorter time scales (hours to weeks or perhaps months), typically a substantial part of the organic carbon (22 to 26%) was lost by microorganisms, though this percentages vary in different hydrological situation , and bioavailable nutrients influenced the capacity of microbial DOC consumption (del Giorgio and Davis 2003 and Mann et al., 2012). Generally estuarine environments are most suitable for DOC degradation due to accumulation of physiochemical properties and aggregation of microorganisms compared to freshwater conditions (Wikner et al., 1999).

According to the hypotheses, N and P are limiting factors of bacterial DOC degradation, but the results demonstrated that enrichment of N was never a significant factor for determining bacterial DOC degradation (Fig. 4). Bacteria utilize organic carbon through anabolic and catabolic pathways where nutrients play a significant role. Aquatic bacteria use a variety of organic and inorganic nutrient fractions, but it is difficult to establish the exact nutrient availability that cells are exposed to in the natural environment (del Giorgio et al., 2012). So it is argued that in this experiment the ambient availability of bioavailable N is already saturated, so that further additions have no effect. The N effect on DOC degradation may be regulated by the ambient organic matter characteristics. It has been well established that the intrinsic chemical properties of the organic matter is a major influential factor of microbial DOC degradation (Guillemette and del Giorgio 2011), and with this, bio-available nutrients cause an effect on this variability of labile DOC. In this regard, it can be argued that the insignificant effect of N to the DOC degradation is due to the nutrient rich samples. The sample waters were then super saturated with bioavailable N and for this reason extra N addition did not significantly impact the DOC degradation.

The P treatments yielded evidence that P is a limiting factor of DOC degradation. In two cases, Lyckebyån and Öre älv, P addition enhanced the DOC degradation which is statistically significant (Fig. 4).Similarly, in another two rivers Nyköpingsån and Pite älv P manipulation enhanced the DOC degradation and their marginal means difference was nearly significant.

However, the availability of iron (Fe) is a one significant regulator of the P effect on DOC degradation. To precipitate one P molecule, at least two iron atoms are required. In freshwater the ferric iron (Fe^{3+}) concentration is high, which is related to high precipitation rates of P molecules (Blomqvist et al., 2004). Therefore, the addition of P may be precipitated with ferric iron and as a consequence have less chance of the availability of free P molecules in the fresh water. So, iron concentration in the sample water is a potential factor of the hypothetic P effect on the DOC degradation. On the other hand in the marine water iron is precipitate by sulfide and form FeS_2 as a result found free P molecule (Blomqvist et al., 2004), therefore in the estuarine water P addition may be influenced into the DOC degradation. Zweifel et al., 1993 and Wikner et al., 1999 tested the nutrient enrichment impact on DOC degradation and both of them proved that in the estuarine environment DOC degradation was promoted. Here it was assumed that due to using NaCl instead of natural estuarine water results diverged in large extend with Zweifel et al., 1993 and Wikner et al., 1999, findings.

In the saline environment different bacterial species respond differently or a community of bacteria active independently that may influence on DOC degradation. Certain groups of bacteria were stimulated while other groups were repressed along a salinity gradient (Langenheder et al., 2003). It was hypothesized that NaCl is a determinant factor of microbial DOC degradation and we found that adding NaCl significantly dampened the DOC degradation in most cases by ca. 21-49% (Fig.4). In general, increasing salinity is related to an increase in bacterial respiration and as a consequence more carbon is consumed to gain energy and thereafter increase microbial CO_2 flux. Furthermore, it has been shown that estuarine bacteria are less efficient in consuming carbon than riverine bacteria and as a consequence more CO_2 is released in the estuarine environment (Langenheder et al., 2003). This conclusion does not contradict the result in this study. In the experiment 10g NaCl was applied which enhanced the salinity approximately by ca. 10 psu. In this salinity condition may be bacterial GE is higher for both marine and freshwater microbes and as a result bacteria consumed less dissolved O_2. Furthermore, since estuarine brackish water is not only composed of NaCl but also includes sulfate, magnesium, potassium and others dissolved salts in the water (Nowostawska et al., 2008), it may cause the variation of salinity influence on the DOC degradation. If the cultures water could be adjusted to estuarine salinity properties then the role of salinity on the DOC degradation might have followed the

hypothesis. It was assumed that NaCl application instead of synthetic Sea salt might divert the salinity effect on DOC degradation. However, the striking feature of the results is that only NaCl influenced DOC degradation negatively. It has been proven that slight changes in salinity might influence genetic composition and functional performance of microbial communities (Langenheder et al., 2003), is related to microbial DOC degradation. In addition, in the concentration level of salinity, DOC composition is another influencing factor of microbial DOC degradation. DOC consisting of the humic acid, fulvic acid and hydrophilic acid fractions shows the variability of degradation differs between different salinity level (Kisand et al., 2008). The study did not identify the humic substances that is one of the limitations to be more confirmed of DOC degradation. Kisand et al., (2008) found that humic rich DOC degradation is higher (ca. 60%) at the higher salinity (30psu) level than the lower salinity (15 to 5 psu) level. From the results, it could be concluded that the salinity level is a significant regulating factor of DOC degradation but exactly how much salinity will enhance the DOC degradation is unknown. In the north-east and south -west side of the Baltic Sea the salinity ranges are 4 and 15-25 respectively (Jaspers et al. 2011). For the microbial experiment marine microbes were sampled from the Malmö coast and this region is under the salinity range of south-west coast of Baltic Sea. Therefore the assumption is that a 1‰ enhancement of the salinity would not influence microbial DOC degradation. On the other hand 1‰ enhanced salinity may be too high for the freshwater microbial metabolism because riverine freshwater microbes may be too sensitive to the salinity disturbance (Langenheder et al. 2003). Furthermore, humic rich DOC flocculates when mixing with salinity environment (Sholkovitz, et al., 1978). So, salt addition may be caused flocculation of reactive organic carbon which influences to decrease the microbial DOC degradation. Additionally, in the collected sample may be DOC concentration was not much enough that may impact on bacterial metabolism. It was proved that bacterial respiration and abundance enhanced where higher concentration of DOC assimilated in the estuarine systems (Nydahl et al., 2013).

Only in one case -Pite älv- the observed effect of NP or NS interaction decreased the DOC degradation, however the effect was relatively small (Fig.5 and Fig.6). It has been noticed that P addition enhance DOC degradation with more than 15% but interestingly, when N was added with P the degradation rate was dampened only by 3.75%. One study showed that NP addition with DOC elicited bacterial productivity and biomass in the surface Sea water experiment (Mills

et al., 2008) though in this study river water was used. So, from the interaction treatment and considering the previous study it could be concluded that N and P exerts a co-limitation on the DOC degradation. By applying NS factor DOC degradation was decreased by 34.33% compared to the N effect alone. The results indicate that N influence the salt effect because the NS interaction effect was lower compared to the salt effect alone.

In two cases - Helgeån and Nyköpingsån – the PS interaction effect was strong and significant (Fig. 7). These were novel findings- given the application of P with salt which dampened the salt effect on the DOC degradation. The P addition with salt enhances the DOC degradation compared to salt effect alone. In Sea water cultures Zweifel et al. (1993) found that bacteria utilized a P rich substrate more compared to a N rich substrate. On the other hand, in the study P and salt together dampened the degradation compared to P effect alone. In the aquatic systems, iron and phosphate ratio is a critical control for DOC degradation. In most of the freshwater systems have Fe: P>2, that bounds the phosphate molecules and marine water systems has Fe: P<2, that cause incomplete precipitation of phosphate (Blomqvist et al., 2004). These bounded formations and also incomplete precipitation of phosphate might be influenced by DOC degradation.

Possible salt induced flocculation and particle formation result was that no particles was formed that significantly affected the measured O_2 consumption rates. This was showed by a microbial community incubation in which possible particles formed by the salt were removed by 0.2 µm filtration. In a estuarine environment, Nowostawska et al., (2008) found that a large part of colloidal dissolved iron fraction associated with natural organic matter is aggregated within a few seconds of mixing and it means that the aggregation and flocculation process is relatively fast. In the seawater induced aggregation process Mg^{2+} and Ca^{2+} ions influence is higher compared to other cation ions in the estuarine water (Nowostawska et al., 2008). This implies that the application of NaCl may have no or small influence on the aggregation process. However, there is also uncertainty due to the fact that only one river water sample was tested. In addition we could expect the specific role of NaCl in the aggregation process if immediately run the experiment after collecting water sample.

As far as we are aware of, the interactive effect of salinity and nutrient effect on DOC degradation have not been described previously. This and the microbial experiments suggest that DOC degradation was controlled by the P, salt and the interactions between nutrients, especially P, and salt. The results of this study have important implications for our understanding of DOC degradation and the oxygen depletion that is causes in the coastal environment.

5.2 Conclusions

Based on our result, nutrient enrichment experiment showed that only in two cases P enhanced DOC degradation significantly whereas addition of N effect was insignificant. In most cases NaCl have a pronounced negative effect on DOC degradation. This study provides good evidence that nutrient and salinity interaction effect influence also negatively. Salt stress was not unique for fresh or marine water microbes since Baltic Sea marine water microbes also showed a negative response to salt addition. Addition of salt generally aggregate reactive organic carbon but in our one river experiment indicates that no possible particle was formed that may influence on the bacterial oxygen consumption.

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