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Effects of microbial community coalescence in lake water at ice break-off

Effekter av sammansmältning av mikrobsamhällen i sjövatten under islossning

Christoffer Parrow Melhus

ABSTRACT

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The period of ice break-off in spring is a key event for many biogeochemical processes in lakes globallly. The biogeochemical processes occurring at ice break-off have the potential of influencing characteristics of lakes throughout spring and summer, including algal blooms and greenhouse gas emission. This makes it important to study lakes in the period of ice break-off. At ice break-off, soil bacteria from the catchment area usually enter the lake via spring floods and mix with the bacteria already occurring in the lake water. In this study, the effects of mixing soil- and lake microbial communities during ice break-off-like conditions were tested by performing an experiment under controlled conditions in the laboratory. In the experiment, light, microbial community composition and concentration of soil-derived organic matter were manipulated to simulate different conditions associated with ice break-off. The variables investigated were bacterial activity and functionality, measured as cell abundance and enzymatic activity, as well as primary production and concentration of dissolved organic matter. The results showed that a mix of soil and lake microbial communities had enzymatic activity patterns resembling lake communities, and then shifted to being more similar to soil communities. The experiment also showed that degradation of measured dissolved organic matter was not linked to biotic processes, and that the observed decrease was most likely due to photo degradation. Finally, the experiment showed that primary production, here measured as chlorophyll *a*, was only stimulated by the mixed community with light and added soil dissolved organic matter. The results found in this study are important as they show that microbial communities do alter their function and enzymatic activity based on composition. Furthermore, the result that primary production was only seen in the presence of light, soil-derived organic matter and a mixed community of lake and soil bacteria may be seen as an indication that primary producers in lake ecosystems to some extent depend on the inflow of terrestrial microbes and organic matter. It also possible that the coalescence of microbial communities enables the communities to perform tasks they were unable to prior to coalescence (i.e. perform tasks that allows primary production to take place). These results give the basis for further, more detailed studies.

Keywords: Freshwater lakes, ice break-off, microbial community coalescence, dissolved organic matter, microbial enzymatic activity, primary production, photo degradation.

REFERAT

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Islossningsperioden under våren är en viktig händelse för biogeokemiska processer i många sjöar globalt. Sådana processer som äger rum under islossningsperioden har potentialen att påverka sjöar under vår och sommar, och kan bland annat ha påverkan på algblomningar och utsläpp av växthusgaser. Detta gör islossning i sjöar till ett viktigt forskningsområde. Under islossningen förs vanligen jordmikrober som bakterier från avrinningsområdet till sjön via vårfloden, och blandas med de mikrober som redan finns där. Denna studie undersökte effekterna av att blanda mikrobsamhällen från jord och sjö genom att utföra ett experiment under kontrollerade förhållanden i ett laboratorium. I experimentet manipulerades ljus, sammansättningen av mikrobsamhällen och koncentrationen löst organiskt material från jord, för att simulera förhållanden förknippade med islossning. De undersökta variablerna var bakterieaktivitet och funktionalitet, mätt genom cellantal och enzymaktivitet, såväl som primärproduktion och nedbrytning av löst organiskt material. Resultaten visade att en blandning av jord- och sjöbakterier uppvisade en enzymaktivitet som liknade sjöbakteriers, och sedan växlade till att mer likna jordbakteriers. Nedbrytning av organiskt material kunde inte kopplas till biotiska processer, och den observerade minskningen berodde sannolikt på fotonedbrytning. Slutligen visade mätningar av klorofyll, mätt genom klorofyll *a*, att primärproduktion bara stimulerades av ett blandat mikrobsamhälle med ljus och tillsats av löst organiskt material från jord. Resultaten från denna studie är viktiga då de visar att mikrobsamhällen faktiskt ändrar sin funktionalitet och enzymaktivitet baserat på sammansättning. Vidare kan resultaten att primärproduktion bara förekom i närvaro av ljus, löst organiskt material från jord, samt ett blandat bakteriesamhälle ses som en indikation på att primärproducenter i någon grad är beroende av inflöde av mikrober och organiskt material från jord. Det är också möjligt att sammansmältningen av mikrobsamhällen ger samhällena funktioner de inte var kapabla till var för sig (som att möjliggöra primärproduktion) De erhållna resultaten lägger en grund för fortsatta, mer detaljerade studier.

Nyckelord: Sötvatten, islossning, löst organiskt material, sammansmältning av mikrobsamhällen, mikrobiell enzymaktivitet, primärproduktion, fotonedbrytning.

PREFACE

This work was carried out in spring 2019 at Evolutionsbiologiskt Centrum (EBC), Uppsala University, Sweden. Microbial community coalescence was studied by performing a laboratory experiment specifically investigating the effects of community coalescence in lakes when combined with the different effects expected to be seen at ice break-off in lakes.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Den globala kolcykeln är en viktig modell som visar kolflöden på vår planet, till exempel utväxlingen av koldioxid mellan oceanerna och atmosfären, eller hur mycket koldioxid som tillförs atmosfären genom förbränning av fossila bränslen. Djup förståelse av den globala kolcykeln är viktigt för att vi ska kunna göra korrekta bedömningar och förutsägelser om den globala uppvärmningen. Modellen måste hela tiden utvärderas och förbättras i takt med att nya upptäckter görs. En förändring som skett under de senaste årtiondena är att forskning visat att sjöar och floder har större betydelse i kolets kretslopp än man tidigare trott. Förut trodde man att sjöar och floder fungerade som passiva transportband som förde kol från land till havet, och att allt det viktiga skedde där. På senare tid har forskning visat att sjöar och floder är mycket mer aktivt delaktiga än man trott, och trots att sjöar bara utgör en bråkdel av vattenytan på jorden, släpper de enligt den senaste forskningen ut mer koldioxid till atmosfären än planetens alla oceaner. Det gör sjöar och floder till viktiga forskningsobjekt då vi måste förstå hur de fungerar för att bättre förstå den globala kolcykeln.

En majoritet av alla sjöar i världen befinner sig på sådana breddgrader att de täcks av is under delar av året. Även bilden av frusna sjöar har kommit att ändras. Från att ha betraktats som inaktiva och ”sovande” så får nu sjöar under vintern mer uppmärksamhet av forskare. På land fryser allt vatten under vintern vilket gör att växter utsätts för torka, och de flesta landlevande djur får flytta eller gå i ide för att överleva. I en sjö är läget annorlunda. Även om en sjö ”fryser”, så är det mer korrekt att säga att den täcks av is – för under isen finns fortfarande flytande vatten, och temperaturen är aldrig under noll grader. Detta gör att biologiska processer faktiskt kan ske under vintern, och om istäcket är tillräckligt genomsläppligt för solljus kan även algblomningar ske. Det har även visat sig att processer som äger rum i sjöar under senvinter kan ha stor påverkan på sjön under sommaren.

En särskilt viktig händelse i istäckta sjöar är islossningen under vårvintern. Under vintern har sjön varit isolerad från inflöden av vatten som för med sig organiskt material såväl som organismer från den omgivande marken. Gaser som syre och koldioxid har hindrats från att utväxlas med atmosfären, och istäcket och det mörka vinterhalvåret har saktat in fotosyntesen hos algerna och vattenväxterna. Fotosyntesen startar på allvar när isen försvinner och släpper igenom solen. Vårfloden för med sig organiskt material och mikrober som bakterier från den omgivande marken. Det ger näring åt organismerna i sjön, men i denna studie är omblandningen av mikrobsamhällen särskilt viktig. Detta fenomen kallas på engelska ”community coalescence” och är ett nytt begrepp inom forskarvärlden, myntat 2015. Begreppet rör just sammansmältning av hela samhällen av arter, såsom sker under vårfloden, när samhällen av jordmikrober hamnar i sjön och blandas med sjöns mikrobsamhällen. Sammansmältningen kan ge en hel del intressanta effekter, som att nya arter blir dominerande och att mikrobsamhällens funktioner förändras, och till exempel tar upp eller släpper ut mer koldioxid, eller bryter ner ämnen som inte kunde brytas ner av samhällena innan de blandades.

Detta projekt har undersökt vad som händer under islossningen på sjöar, då solstrålningen ökar och vårfloden för med sig organiskt material och organismer från den omkringliggande marken till sjön. Projektet har undersökt inverkan av ljus, inflöde av kol från mark (s.k. terrestriskt kol) och speciellt blandningen av mikrobsamhällen (”community coalescence”) från mark och sjö. Resultaten styrker forskning som pekar på att islossning är en central period i sjöar. Effekten av ökat ljus, tillsats av organiskt material och blandningen av mikrobsamhällen hade alla tydliga effekter på olika variabler som mättes. Det är sedan tidigare känt att ljus har en dominerande

roll vid nedbrytning av organiskt material i sjöar, och detta kunde bekräftas i studien. Tillsats av organiskt material från marken kring sjön gav en mycket tydlig effekt på mikrobsamhällena, med ökad aktivitet som följd. Vidare visade resultaten att blandade samhällen uppvisar andra aktivitetsmönster och egenskaper än de separata delar som utgör dem. Till exempel var samhällena av sjöbakterier som mest aktiva i början av experimentet, och jordbakterierna som mest aktiva mot slutet. Blandades de två samhällena uppvisade de däremot både en tidig och en sen topp i aktivitet. Detta antyder att de blandade samhällena förmår utnyttja det bästa från båda de samhällen som blandningen innehåller. En annan mycket intressant effekt var att fotosyntes enbart kunde ses i närvaro av blandade mikrobsamhällen, tillsammans med ljus och tillsatts av organiskt material från jord. Detta beror antagligen på att de blandade mikrobsamhällena tillsammans kan bryta ner substrat till näringsämnen som behövs för fotosyntes.

Mikrober är de enda organismer som förmår bryta ner löst organiskt material i sjöar, och många arter är beroende av dem för sin tillväxt. Detta gäller både organismer som äter mikroberna själva, men också alla de organismer som är beroende av att mikroberna frisätter näringsämnen genom sin nedbrytning. Att blandning av samhällen skulle kunna möjliggöra för mikroberna att utnyttja nya nischer och bryta ner nya substrat har stor betydelse ur ekologisk synpunkt. Det kan få effekter långt upp i näringskedjan, och till exempel påverka algblomningar och fiskbestånd. Inte minst kan det påverka sjöars frisättning av koldioxid, vilket är av vikt för den globala kolcykeln och klimatförändringar.

Denna studie öppnar för fortsatta studier av blandade mikrobsamhällen, med andra, mer utförliga och precisa mätmetoder för att bekräfta de fynd och samband som gjorts. Vidare bör forskning undersöka mer konkreta effekter av blandning av mikrobsamhällen. Exempel på detta är att undersöka vilka enzymer som blir mer aktiva, vilka ekosystemfunktioner hos mikrobsamhällena detta påverkar, vilka ämnen som kan brytas ner, och inte minst om det faktiskt stämmer att blandning har effekt på fotosyntes. Detta skulle nämligen antyda att tidig algtillväxt i sjöar under våren i någon utsträckning är beroende av att mikrobsamhällen blandas.

ABBREVIATIONS

A – designation of "aquatic" bacterial communities in this project, meaning bacterial inoculum from Lake Erken.

Bglu – the enzyme β-1,4-glucosidase.

Cello – the enzyme β-D-1,4-cellobiosidase.

cDOM – optically active dissolved organic matter ("coloured dissolved organic matter").

D – designation of treatment "Dark, no DOM addition" in this project.

DD – designation of treatment "Dark, DOM addition" in this project.

DOC – dissolved organic carbon.

DOM – dissolved organic matter, including DOC.

EEA – extracellular enzymatic activity.

L – designation of treatment "Light, no DOM addition" in this project.

LD – designation of treatment "Light, DOM addition" in this project.

Leu – the enzyme Leucine aminopeptidase.

M – designation of "mixed" bacterial communities in this project, meaning from both A and T.

T- designation of "terrestrial" bacterial communities in this project, meaning bacterial inoculum from soil in the Erken catchment area.

Xylo – the enzyme β-1,4-xylosidase.

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1. INTRODUCTION AND BACKGROUND

The global carbon cycle describes the movement of carbon in the world. Examples of such carbon fluxes are human emissions of carbon dioxide from the usage of fossil fuels, and the uptake of carbon dioxide by plants and algae. Deep understanding of the global carbon cycle is crucial to enable predictions of greenhouse gas emissions and its impact on future climate (Tranvik *et al.* 2018).

Over the course of the last century, the view of the role of lakes and rivers in the global carbon cycle has shifted. In the first half of the last century, inland waters were mainly seen as isolated ecosystems or passive transporters of carbon from land to ocean, where the important processes were expected to take place. Today, the view is much more complex. Lakes and rivers are now seen as active contributors to the global carbon cycle through internal processes (Cole *et al.* 2007). Freshwaters receive, process, store and release carbon to a greater extent than previously thought (Tranvik *et al.* 2018).

Much of the carbon within freshwater bodies is in the form of dissolved organic matter (DOM) which is the main source of energy for heterotrophic bacteria (Giorgio & Davis 2003, Rochelle-Newall *et al.* 2014). Changes in composition and concentration of DOM can greatly influence carbon fluxes within the water body (Rochelle-Newall *et al.* 2014). For this reason, understanding the factors controlling DOM-concentration and quality is essential when modelling the global carbon cycle (Rochelle-Newall *et al.* 2014).

DOM is usually defined as organic compounds that pass through a 0.45 µm filter(Zsolnay 2003), and contains a range of organic molecules of different sizes. One way to categorize DOM is to look at its origin. The origin of DOM can greatly influence its composition and characteristics. In large bodies of water such as oceans, DOM mainly originates from primary production within the water body. This kind of DOM is called “autochthonous” DOM and mainly comprises carbohydrates and proteins released from phytoplankton (Carlson & Hansell 2015). It is generally labile and easily degraded by microorganisms (Carlson & Hansell 2015, Berggren & del Giorgio 2015, Moran *et al.* 2016).

In contrast to autochthonous DOM, “allochthonous” DOM refers to DOM introduced externally of the water body, typically from terrestrial sources via inflow from the catchment area. Allochthonous DOM is often the dominating form of DOM in lakes and enclosed waters which have a large catchment area compared to the size of the watershed (Rowe *et al.* 2018). Allochthonous DOM is mainly comprised of plant-derived carbon molecules such as lignin, fulvic and humic acids, and can be harder for microorganisms to degrade (Hessen & Tranvik 1998, Kellerman *et al.* 2015). It is usually brown-coloured, and can be more subjected to photo-degradation or photo-bleaching than autochthonous DOM (Koehler *et al.* 2014).

Despite its low degradability, allochthonous DOM can have a substantial effect on microbial metabolism. Potential reasons for this include: 1) the large quantity of allochthonous DOM,

which compensates for its lower degradability (as allochthonous DOM defines a wide range of substrates, larger quantities are bound to contain more of the easily degraded fractions); 2) photo-degradation, which can transform DOM into more labile substrates; or 3) simply that allochthonous DOM is more labile than previously thought (Berggren *et al.* 2010).

Concentrations of autochthonous and allochthonous DOM may vary over space and time. An example of spatial variation is a higher concentration of allochthonous DOM in inlet waters and in small streams compared to other water bodies, and an example of variation over time is a maximum concentration during spring floods, bringing much allochthonous DOM from the runoff area with the elevated water flows (Vannote *et al.* 1980, Raymond *et al.* 2016, Bernhardt *et al.* 2018). Concentrations of allochthonous DOM have been observed to increase in many streams and lakes in the Northern Hemisphere, a process known as browning of inland waters, which is mainly due to an increased runoff of humic substances from soils in the catchment (Haaland *et al.* 2010). The ratio of allochthonous-autochthonous DOM is important and can influence microbial activity and the fluxes of carbon in the global carbon cycle.

Microbial communities play an important role in the carbon cycle as degraders of DOM (Azam *et al.* 1983; Ducklow 2008). Many microbes use extracellular enzymes to break down large molecules before direct uptake (Arnosti 2011). These excreted enzymes target specific substrates and degrade them into smaller molecules that the bacteria utilize. Typical substrates targeted by these enzymes are different proteins, amino acids, fatty acids and carbohydrates (Moran *et al.* 2016). DOM can be utilized almost exclusively by bacteria, making the study of heterotrophic microbial communities extremely important to understand the carbon cycle (Azam *et al.* 1983, Biddle *et al.* 2006, Azam & Malfatti 2007).

Historically, a predominant view has been that "all microorganisms are everywhere, but the environment selects" (O'Malley 2008, Canfield 2015). This would to some extent imply that microbes are present or not present only because of environmental selection. This has more recently been shown not to be the case, and spatial distribution of microorganisms has become a new research area (Verstraete *et al.* 2007, Ruiz-González *et al.* 2015). This opens up for research investigating when and where microorganisms spread, as well as what mechanisms cause them to spread. More importantly to this project, what effects does this dispersal have, especially when entire communities are dispersed and interact?

This dispersal and mixing of entire communities has been termed "Community coalescence". It also addresses the prospect of the resulting mixed communities interacting in new ways and having distinct properties from the parts it unites (Rillig *et al.* 2015). For example, coalesced communities might be dominated by different species, exert different productivity and be able to perform other processes than prior to coalescence (Székely *et al.* 2013). Aquatic microbial communities are often dominated by bacteria of terrestrial origin, and microbial communities have a clear directional flow pattern, following the flow of water within a runoff area (Crump *et al.* 2012, Rillig *et al.* 2015, Ruiz-González *et al.* 2015). Which microbes dominate can determine community characteristics, for instance, if net flow of carbon goes towards accumulation into biomass or respiration as carbon dioxide (Guillemette *et al.* 2016, Fabian *et*

al. 2017). Thus, it is important to consider microbial processes in order to understand the global carbon cycle and greenhouse gas emissions.

Mixing zones - in which community coalescence can be investigated -are often biogeochemical "hot-spots in space" and "hot moments in time" (e.g. when run-off is high during spring floods), and are correlated to peaks in bacterial productivity and greenhouse gas emissions (Crump *et al.* 2003, McClain *et al.* 2003). Coalescence of communities from different sources, as well as inflow of terrestrial DOM should be particularly pronounced during periods of ice-break in spring since the isolating ice disappears, and water flows are elevated (Crump *et al.* 2003, Denfeld *et al.* 2018). There is an increased need for understanding these processes since ice covers on lakes and rivers are observed to decrease in extent and duration worldwide (Magnuson *et al.* 2000, Benson *et al.* 2012, Knoll *et al.* 2019), yet few studies to date have investigated biogeochemical processes under ice in lake systems (Hampton *et al.* 2015). Processes that take place in boreal lakes in winter and early spring can greatly influence summer characteristics of the lakes (Hampton *et al.* 2017). Both biotic factors - such as plankton population dynamics - and abiotic factors - such as temperature or light conditions - are influenced by ice cover (Gerten & Adrian 2000, Straile 2002, Adrian *et al.* 2006, Hampton *et al.* 2017). Similar to how the view of lakes and rivers in the carbon cycle has shifted, the view of ice-covered lakes is also changing. Winter has been regarded as a mostly dormant period in the lake ecosystem, and less important than open-water season. Now more studies turn attention towards frozen lakes (Salonen *et al.* 2009, Hampton *et al.* 2015, Denfeld *et al.* 2018).

Ice and snow cover reduces light in lakes and affects primary production as well as microbial activity (Garcia *et al.* 2019). Especially important to this project however, is the moment of ice break-off. During winter, the ground in the catchment area is usually frozen, reducing inflow of allochthonous DOM to the lake or stream (Denfeld *et al.* 2018). Thawing and consequent increase in runoff in spring can replace large portions of the water in lakes (Berggren *et al.* 2010) and can account for peaks in terrestrial DOM input (Crump *et al.* 2003, Denfeld *et al.* 2018). Terrestrial microbial communities should thereby also enter the ecosystem, and the period is often, as was stated, associated with peaks in microbial activity (Crump *et al.* 2003). Ice break-off also results in an increase in incoming solar radiation, which has several effects on the lake or stream. One effect of light is increased primary production, which produces autochthonous DOM. Another effect is photo-degradation, which removes DOM from the system, or degrades it into other, possibly more labile, forms (Crump *et al.* 2003, Cory *et al.* 2014, Cory *et al.* 2015). There is also some recent evidence of light affecting microorganisms and their extracellular enzyme activity which this study also aims to investigate (Thomson *et al.* 2017).

Aims:

This project aimed at investigating how community coalescence between microbial communities from a lake and its catchment area influence the abundance of microorganisms, the extracellular enzyme activities of the microorganisms, primary production and

degradation of dissolved organic matter. The effects from increased light levels and addition of soil-derived dissolved organic matter were also investigated.

In order to get a better understanding of microbial communities and carbon processing at lake ice break-off, this study aims to give answers to the following questions:

- Does an increase in light availability at ice break-off influence dissolved organic matter (DOM) concentration, primary production and microbial activity?
- Does the inflow of terrestrial DOM at ice break-off influence dissolved organic matter concentration, primary production and microbial activity?
- Does the coalescence of microbial communities influence DOM concentration, primary production and microbial activity? Is there a difference in the functionality of the mixed microbial community?

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN AND SETUP

In order to better understand how ice break-off influences DOM degradation, primary production and bacterial activity, a laboratory experiment was performed. All of the manipulated factors and measured variables are listed and shortly described here for overview, and explained in detail below.

The manipulated driving variables in the experiment were:

- Temperature – the experiment was performed in a constant-temperature room at 4°C.
- Light - as ice break-off results in increased light levels. This was investigated with one artificial light treatment, and one dark-treatment.
- Allochthonous DOM - inflow of allochthonous DOM should be increased at spring flood. This was applied in the experiment by adding an extract of soil DOM to some treatments.
- Community coalescence – in the same way as allochthonous DOM flows into the lake at ice break-off, the corresponding inflow of terrestrial microbes should also increase, and mix with the communities already present in the lake. Therefore, three microbial communities; aquatic, terrestrial and mixed, were studied. They were extracted from lake water and soil in the catchment area of the same lake.

To measure the effects on DOM concentration, primary production and bacterial activity, the effect variables of the experiment were:

- Fluorescence of cDOM (“coloured dissolved organic matter) – the optically active fraction of DOM, which can be easily measured using a fluorometer. It can be used to approximate DOM concentration.
- Fluorescence of chlorophyll *a* – also possible to measure with a fluorometer, used to approximate primary production. Primary production produces autochthonous DOM – which is important to the study as well as allochthonous (terrestrial) DOM since it can act as food substrate for bacteria and might have different characteristics compared to allochthonous DOM.
- Bacterial cell abundance – the number of cells, indicating if the population decreases or increases.

- Extracellular enzymatic activity – the concentration of enzymes excreted by the bacteria to utilize food substrates, and the rate at which they degrade substrates.

In the experiment focus was on three types of bacterial communities; aquatic lake water bacteria ("A"), terrestrial bacteria from the soil in the catchment area of the lake ("T"), and finally a mix of the two ("M"). All communities were used to inoculate sterile lake water medium in 1 L bottles under the following four treatment conditions (Table 1):

- light, no DOM addition ("L")
- dark, no DOM addition ("D")
- dark, DOM addition ("DD")
- both light and DOM addition ("LD")

The light and DOM manipulations were performed to investigate potential driving variables affecting processes in the lake during ice break-off. The 'light, no DOM addition treatment' (L) focused on the influence of increased light (as when snow- and ice cover is broken up) but without the inflow of terrestrial DOM. The 'dark, no DOM addition' (D) treatment represented a frozen lake with snow cover. Further, the 'dark, DOM addition' (DD) investigated effects of terrestrial DOM without the influence of light. Finally, the treatment with both DOM addition and light (LD) represented conditions during/after ice break, where melting soil in the runoff area enables DOM to enter the lake. Thus, the LD treatment investigated the combined effects of light and DOM. The different treatments were applied to all three communities.

Table 1. Experimental setup. Within the boxes the ID-designation for each set of community and treatment is shown, such as Aquatic community with treatments DOM and Dark, ADD.

Community	**Aquatic**	**Terrestrial**	**Mixed**	**Control (sterile)**
Dark, no DOM	**AD**	**TD**	**MD**	**CD**
Dark, DOM	**ADD**	**TDD**	**MDD**	**CDD**
Light, No DOM	**AL**	**TL**	**ML**	**CL**
Light, DOM	**ALD**	**TLD**	**MLD**	**CLD**
Extra: Light, No DOM, Nycodenz				**CLN**

A sterile control for all treatments was included and each treatment was replicated three times, resulting in a total of 48 bottles.
To check for DOM contamination derived from the Nycodenz extraction procedure used for the terrestrial bacteria (see *2.3 Soil – collection, DOM- and bacterial extraction* below), one single extra control (CLN, table 1) was included. This 49th bottle was prepared using the same extraction techniques as was done for terrestrial (and mixed) communities, but without bacterial inoculum.

Light exposure treatments were performed by using four plant cultivation lamps (Fig. 1) ("LED plant grow bulb PAR20 E12"). The emitted light did not contain wavelengths in the UV-spectra. The light level below the lamps was measured with Hobo UA-002-64 light loggers, which were scattered around the lamps and measured between 1000 and 5500 lux, approximately 3700 lux where the bottles were placed, see Fig. 1 (not shown in table 2). This corresponded well to light levels in surface water below ice without snow cover (Garcia *et al.* 2019).

The measured light levels (table 2) also matched those measured at the sampling site (see *2.2 Lake water –collection, media and aquatic inoculum*). The different measurements of light levels are collected in Table 2 below.

Table 2. Measured light levels (mean values and standard deviation) at the sampling site at Lake Erken and laboratory setup location.

Below snow-free ice at Erken sampling site (lux)	Below snow-covered ice at Erken sampling site (lux)	On top of ice surface at Erken sampling site (lux)	Below the lamps in the lab setup (lux)
Average: 5440 Std: 923	Average: 697 Std: 168	Average: 26560 Std: 3581	Average: 2895 Std: 1374

The light was switched on/off in a 12 h cycle. To compensate for possible spatial differences in light conditions, bottle placement was shuffled when sampled (every second day, see *2.4 Sampling and analysis)*.

The experiment started on 26th of March 2019. Cell abundances in the inocula (see *2.2.2* and *2.3.2* below) were measured (see *2.4.2*), and the volumes to add of the different inocula were calculated with the aim of having similar cell abundances in all treatments. Bacterial inocula for the different communities were added, along with soil DOM extract for the DOM-treatments (see *2.3.1*). As microbial communities were studied, it was crucial to not have them contaminated by other microbes, and all experimental preparations and samplings were done in a sterilized laminar flow bench for this reason. The experiment was conducted in a temperature-controlled room at 4°C and ran for 16 days.

Figure 1. The experimental setup in the 4°c – room. Light treatments are scattered around the lamps; dark treatments are seen in the left of the picture. When the experiment was running, the cardboard box was closed to avoid light contamination.

2.2 LAKE WATER – COLLECTION, MEDIA AND AQUATIC INOCULUM

2.2.1 Collection and collection site

The experiment needed lake water for media in all bottles in the experiment, as well as a source of lake bacteria for aquatic and mixed communities. The choice fell on Lake Erken, since the institute where this experiment was conducted has a monitoring station there.

Lake Erken is a mesotrophic clearwater lake in Uppland in Eastern Central Sweden (coordinates: 59°50'57.6"N 18°35'33.8"E). It has a surface area of 24 km^2 and a water residence time of seven years. It has an average depth of 9 m and the greatest depth is 21 m. Most common soils in the catchment area are brown forest soils and humus podsols (VISS, 2019).

Water was collected from Lake Erken on the 6th of March 2019 when the ice was ca 25 cm thick. After drilling a hole through the ice, 80 l of lake water was collected in 20 l-carboys. Light levels below the ice next to the open hole, as well as below ice in a snow-covered hole, were measured using Hobo UA-002-64 light loggers. The collected water was stored in 4°C in the dark until use in the experiment.

2.2.2 Aquatic bacterial inoculum and media preparation

60 l of the collected lake water was processed (20 l was saved as backup) using tangential flow filtration (TFF). The TFF took place approximately two weeks after collection using a 100 kDa pore size filter (Fig. 2-3). Prior to TFF, the water was passed through a 42 µm plankton net to

remove the largest particles and zooplankton and was collected in sterile carboys. The filtrate from the TFF was collected in sterile carboys and autoclaved (for sterility) to be used as lake medium in all bottles in the experiment. Autoclaving involves heating, and usually changes pH, so after the first autoclaving step, the medium was adjusted to pre-sterilization pH. Then the medium was autoclaved again and stored at 4°C until the experiment was set up.

The TFF returned the retentate from filtration to the feed lake water carboy (see Fig. 3) so that bacteria would accumulate. In this way, the retentate was concentrated from 60 l to 0.9 l and used as the aquatic bacterial inoculum.

At the start of the experiment, bottles purposed for aquatic (A) communities received 32.15 ml of the lake bacterial inoculum. Mixed (M) communities received 30.41 ml (but would also receive inoculum from soil bacteria, see *2.3*). The volumes were based on measurements of cell abundance in the inocula, and the aim was to have similar amounts of bacteria in all the different treatments (where M received inocula from two sources).

Figure 2. Setup of the tangential flow filtration (TFF) of the lake water for media and aquatic bacterial inoculum.

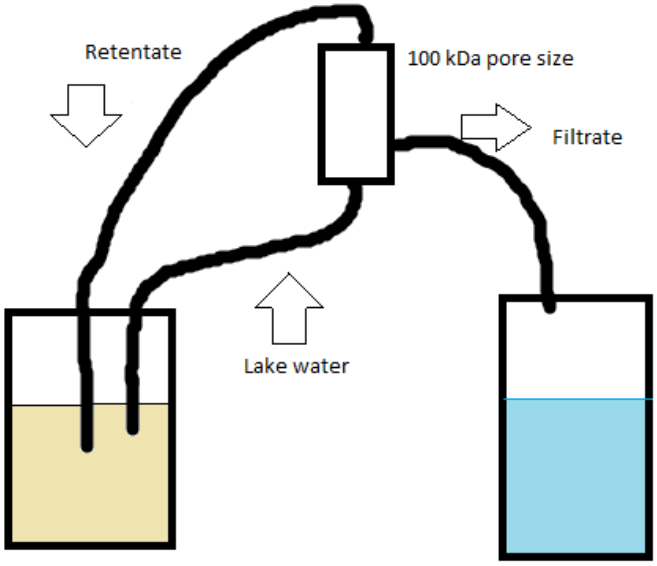

Figure 3. Depiction of the lake water filtration setup for media and Aquatic bacteria inoculum. The carboy to the left contained lake water. The carboy on the right was filled with sterile filtrate to be used as media in all bottles in the experiment. The retentate from the filtration was returned to the lake water carboy with the aim of concentrating lake bacteria there. This was then used as inoculum for aquatic communities.

2.3 SOIL – COLLECTION, DOM- AND BACTERIAL EXTRACTION

2.3.1 Soil for terrestrial communities and DOM extraction

The experiment required soil as a source of terrestrial bacteria and terrestrial DOM. To capture spatial variability of soil characteristics the soil was collected at five different locations around Lake Erken, all being located in the catchment area. The soil was collected on December 18 2018, before the ground had frozen. A total of approx. 5 kg soil was taken from the five locations, from the upper 10 cm soil layer. The soil was stored at -20 °C until the preparation of the soil bacterial inoculum and DOM commenced.

Soil samples were combined and homogenized with MilliQ water to make a 1:3 volume (MilliQ:Soil) slurry. Grass, roots and other large objects were removed manually, and the slurry was shaken at 150 rpm between 2 to 15 h (a first batch for 2 h and a second for 15 h in order to extract more DOM and bacteria). The slurry was then sequentially sieved through 4500 µm down to 42 µm sieves. The remaining slurry was divided between DOM extraction (ca. 4 l) and soil bacterial inoculum preparation (ca. 2 l; described in "Bacterial extraction" below).

Slurry reserved for DOM extraction was centrifuged to remove larger particles prior to another sequential filtration using 20 µm, 1.2 µm and 0.7 µm pore size filters. The final filtration was through 0.1 µm pore size filters to remove microorganisms. The last filtration step was carried out in a laminar flow hood using pre-autoclaved filtration units and filters (Fig. 4). Since the extracted DOM was intended to act as substrate for bacteria, it was very important to maintain sterility in these steps. The final volume of the soil DOM filtrate amounted to 550 ml, and 20 ml were added to each bottle that received a DOM addition.

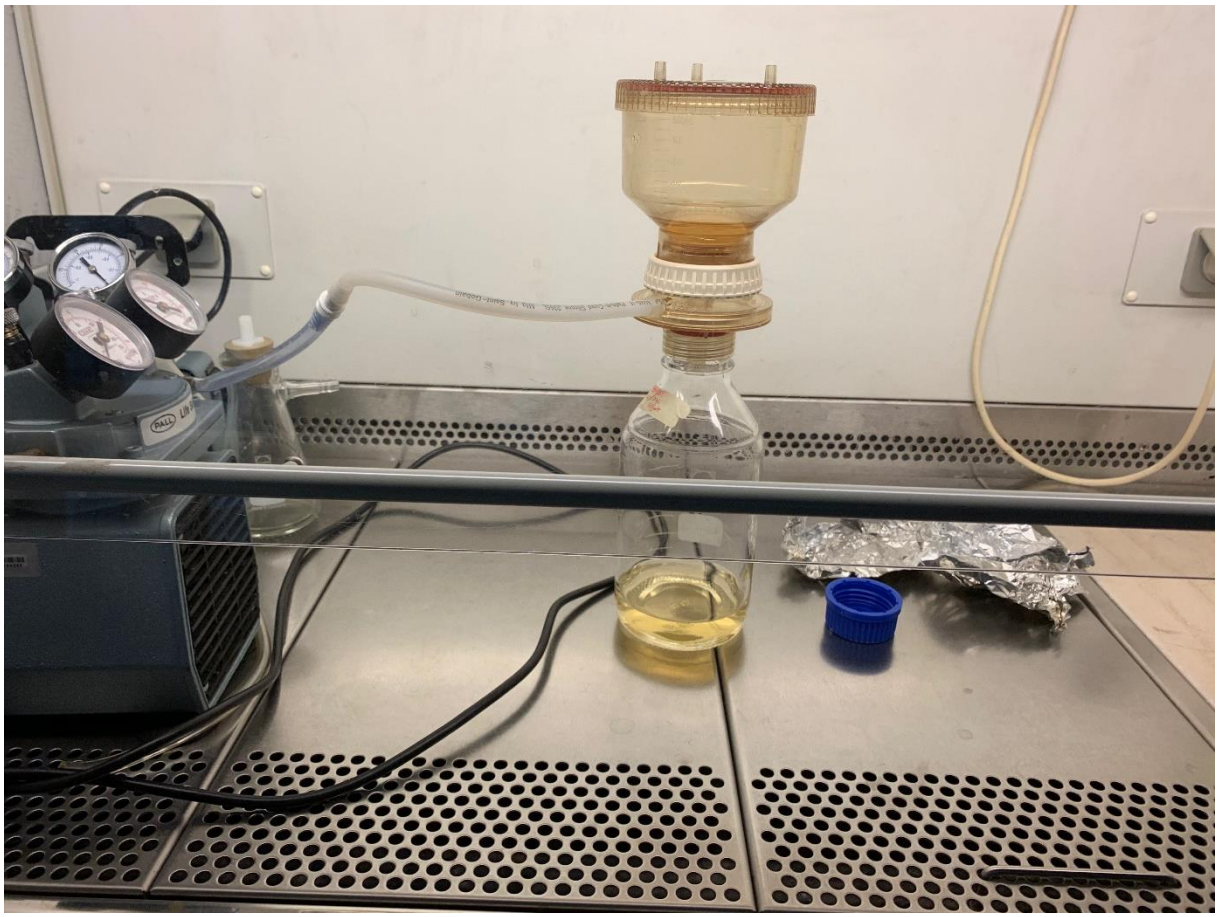

Figure 4. Sterile 0.1 μm pore size filtration of soil DOM for “DOM” treatments, conducted in a laminar flow hood to maintain sterility.

2.3.2 Bacterial extraction

Soil bacteria were extracted using centrifugation and the density gradient Nycodenz (Nycomed Pharma AS, Oslo, Norway) following previously described methods (Bakken & Lindahl 1995, Berry *et al.* 2003, Barra Caracciolo *et al.* 2010). Nycodenz is a nontoxic, non-ionic density gradient medium derived from benzoic acid. The density gradient separates particles and cells depending on buoyancy, thus concentrating cells in one layer while collecting soil particles in another (Bakken & Lindahl 1995). A solution of Nycodenz was prepared in MilliQ using a concentration of 1 g/ml.

A volume of 30 ml of the 42 µm-sieved soil slurry was added to 50 ml Falcon tubes. Then, using a syringe, a 10 ml layer of Nycodenz solution was carefully added below the slurry, pushing up the soil slurry from the bottom and forming a "cushion" of Nycodenz below it (Fig. 5). The tubes were then centrifuged at 2500 x g for 25min. This allowed for soil particles to pass through the Nycodenz layer, while trapping cells on top of the Nycodenz. Afterwards, the cell layer formed on top of the Nycodenz cushion (which was now mixed with soil particles), was extracted with a syringe (Fig. 6). On average, 10ml was extracted from each tube, containing the cell layer and some of the adjacent layers. With several extraction runs, about 700 ml cell extract was obtained. To suppress microbial growth in the extract it was then stored on ice in the 4°C room until start of the experiment. At the start of the experiment, bottles purposed for terrestrial (T) and mixed (M) communities received 29,16 ml soil bacterial inoculum.

Figure 5. A 10 ml Nycodenz "cushion" has been added below 30 ml soil slurry in a Falcon tube. The next step was to centrifuge the tube.

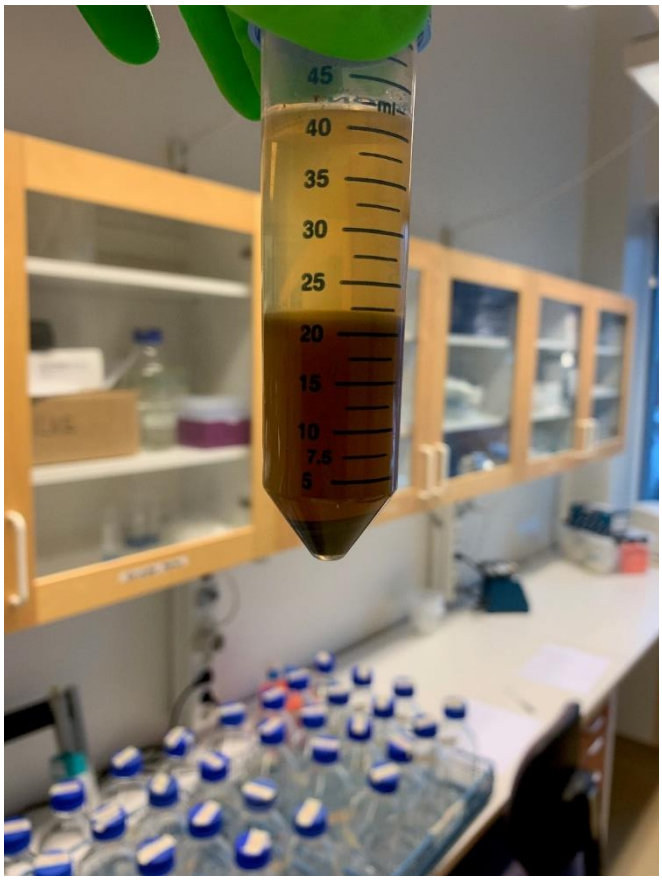

Figure 6. Nycodenz extraction process right after centrifugation. Compare to Fig. 5 - the soil slurry has passed through the nycodenz, and heavier particles can be seen at the bottom. On top of the brown soil-mixed nycodenz layer, just above the 20 ml mark, the thin grey-white layer of bacterial cells can be seen.

2.4 SAMPLING AND ANALYSIS

Experiment bottles were continuously sampled to track bacterial abundance, fluorescence and extracellular enzymatic activity (EEA). Sampling for cell count and fluorescence was done every second day from the start of the experiment (day 0, 2, 4, 6, 8, 10, 12, 14 and 16), and EEA on day 0, 2, 6, 10, 14 and 16. At each sampling day, 1,5 ml from each bottle was collected in tubes for cell counts and enzymatic activity assays, and 3 ml were pipetted directly into cuvettes for fluorescence measurements

2.4.1 DOM and chlorophyll

Fluorescence was measured using a hand-held fluorometer [AquaFluor; Turner Designs] with two channels – channel A for cDOM and channel B for chlorophyll *a*. cDOM is the fraction of DOM that is optically active, and was used as a proxy of DOM-concentrations (Rochelle-Newall *et al.* 2014). Chlorophyll *a* can be used to indicate chlorophyll content and thus primary production.

Dissolved organic carbon (DOC) concentrations of the final samples, as well as the inocula and media in the preparation phase, were measured in a Costech Elemental Combustion System ECS 4010. DOC comprises a large fraction of DOM and can be measured with greater accuracy than cDOM. It was therefore measured to determine carbon concentrations in the soil DOM-extract in order to correctly calculate concentrations in bottles that received that treatment.

In the beginning and at the end of the 16-day long experiment, DOC concentration was also measured using the Costech Elemental Combustion System ECS 4010 as this was deemed to

be more accurate than fluorescence measurements of cDOM. Before the experiment was assembled, the media, different inocula and soil DOM extract had DOC concentrations measured. Based on the volumes added, DOC-concentrations in the different treatments and communities were calculated, and listed in table 3. At the end of the experiment, DOC concentrations in all 49 bottles were measured and averaged, and presented in table 3. This was to compare loss or increase of DOC using a more accurate method than fluorescence measurements of cDOM. However, the process is more complex and time consuming, therefore cDOM measurements were the main method of measurement. This is further discussed in *4 Discussion.*

2.4.2 Cell abundance - Cytoflex.

Cell abundances in the samples were counted using a flow cytometer. It requires the samples to be stained with a dye that colours nucleic acid. This enables the flow cytometer to enumerate cells (which all contain nucleic acid).

A 200 times dilution of Syto13 nucleic acid fluorescent stain (5 µl Syto13 in 1ml MilliQ) was used to stain the samples for cell counts. A volume of 95 µl of each sample was mixed with 5 µl of diluted Syto13 in each well on a 96-well flat bottomed plate. Cells detected in a volume of 50 µl were then enumerated on a flow cytometer (CytoFlex; Beckman-Coulter).

2.4.3 Enzymatic activity assays

Part of the aim of the experiment was to assess bacterial activity. Apart from measuring cell abundances as described above, it can be done by calculating the activity of bacterial extracellular enzymes. Bacteria produce and excrete these enzymes to degrade substrates which they utilize for energy or growth.

Bacterial extracellular enzymatic activity (EEA) was determined using methods described by Hoppe 1983, Fitch *et al.* 2018 and Balmonte *et al.* 2019.

In this study four different enzymes were investigated. They are all used by bacteria to degrade different forms of DOM and included; Leucine aminopeptidase (Leu), β-1,4-glucosidase (Bglu), β-D-1,4-cellobiosidase (Cello) and β-1,4-xylosidase (Xylo). Leucine aminopeptidase is an enzyme widely used by organisms to in the degradation of proteins and amino acids. β-1,4-glucosidase catalyses the hydrolysis of glucosides. β-^{D}-1,4-cellobiosidase catalyse hydrolysis of cellulose into cellobiose. β-1,4-xylosidase is used to utilize xylan, which is a major component in plant cell walls (Chróst 1991).

The activity of the four selected extracellular enzymes was measured using four substrates labelled with one of two fluorophores: Methylcoumarin (MCA) or Methylumbelliferyl (MUF). The enzymes excreted by the bacteria target the added fluorophore-labelled substrates and breaks the bonds between them, which enables the released fluorophore to fluoresce in UV-light, using excitation wavelength 360 nm and emission wavelength 460 nm. This produces a signal that can be measured (Hoppe 1983, Arnosti 1995, 2003, 2011). Fluorescence is then converted to concentration of hydrolysed substrate through a standard calibration curve, which was made at the start of the experiment. For MCA-substrates (used for Leu), this calibration curve is described by equation 1. For MUF-substrates (used for Bglu, Cello and Xylo), the calibration curve is as described by equation 2. Input x is the measured fluorescence and output y is the concentration of the substrate in nmol/l.

$$y = 0.0761x - 61.939 \quad (1)$$

$$y = 0.0644x - 46.492 \quad (2)$$

After samples (see first paragraph of *2.4 Sampling and analysis*) and fluorophore-labelled substrates were added to wells on a 96-well plate, fluorescence was measured on a plate reader (Infinite F200; Tecan), and measured once again after 1 hour had passed. Between measurements, the plates were stored at 4°C. There was one plate for each substrate, for a total of four plates.

When measured fluorescence had been converted to substrate concentration using equation 1 or 2, the activity of the corresponding controls for each treatment was subtracted, after being averaged over the three replicates. This is done in order to remove the autohydrolysis of substrates, so that only the degradation by bacteria is measured. In some cases, this subtraction resulted in negative values, which were set to zero because negative degradation rates indicate no measurable activity. The results were then divided by elapsed time from incubation start to second fluorescence measurement (1 h), which gives a rate of enzymatic activity in nmol/l/h.

2.5 STATISTICAL ANALYSES

Most statistical tests and plots were made with Rstudio. The effect of the treatments (i.e., Light/Dark and DOM/no DOM) between the first and final day (day 0 and day 16) was tested with three-way Analysis of Variance (ANOVA). Differences between the negative controls and the inoculated incubations were assessed using Tukey's HSD post hoc analyses. All of this was done in order to be able to state that a driving variable (i.e. treatment) had 'significant' effect on the effect variable investigated.

Normality of residuals was checked (prior to ANOVA) by a Shapiro-Wilk test. Since Shapiro-Wilks tests are very sensitive, and ANOVAs quite robust, the normality tests were complemented with visual analysis in the cases were Shapiro-Wilks-tests categorised residuals as non-normal.

Nonmetric Multi-Dimensional Scaling (NMDS) (Clarke 1993) was used to explore and visualize the main patterns of variation in enzyme activities between treatments using the activities from all measured enzymes to calculate a distance matrix.

3. RESULTS

3.1 DOC CONCENTRATIONS

Measurements of DOC concentrations in the media, inocula and DOM extracts at the start of the experiment were 31.40 mg DOC l^{-1} in the lake medium and 34.13 mg DOC l^{-1} in the aquatic bacterial inoculum. In the soil DOM extract 99.08 mg DOC l^{-1} was measured, and in the soil bacterial inoculum, concentration was as high as 19 758 mg DOC l^{-1}. Using added volumes, initial concentrations (table 3) were calculated using equation 3.

$$[Initial\ DOC\ concentration\ in\ experimental\ bottles] = ([Added\ media\ volume] * [DOC\ concentration\ in\ media] + [added\ soil\ inoculum\ volume] * [soil\ inoculum\ DOC\ concentration] + [added\ lake\ inoculum\ volume] * [lake\ inoculum\ DOC\ concentration])/[total\ volume\ added] \quad (3)$$

The mean concentrations of DOC measured at the end of the experiment were in the same order of magnitude as those calculated for the initial mixes from the concentration of the medium and added inocula and DOM extract (Table 3). However, concentrations in A and C were about three times lower at the end and in T and M communities, concentrations were slightly higher than in the beginning.

Table 3. Initial and final concentration of DOC in the experiment bottles. Initial values were calculated based on measured DOC-concentrations in everything that was added (equation 3). Final measurements were made for all bottles, and mean values and standard deviation is presented.

	Added lake media (ml)	Added soil inoculum (ml)	Added lake inoculum (ml)	Added soil DOM extract to DOM treatments (ml)	Initial calculated DOC concentration (mg/l) in the experiment bottles	Measured DOC concentration at final sampling day. Mean values of all treatments. Standard deviation of three replicates in parenthesis. (mg/l)
Aquatic	1000	-	32.15	20	34.48	12.71 (0.07)
Terrestrial	1000	29.16	-	20	609.52	666.48 (3.09)
Mixed	1000	29.16	30.41	20	610.56	647.70 (3.27)
Control	1000	-	-	20	33.38	11.99 (0.12)
Control Nycodenz	1000	-	-	-	-	999.00

DOC concentrations measured in the Nycodenz sterile control at the end of the experiment (Table 3) support that the high concentrations in the soil inoculum originated from the Nycodenz and not terrestrial DOM from the soil inoculum. Nycodenz had a tremendous impact on measured DOC concentration (table 3) as the levels were more than a hundred times higher for the inocula extracted with Nycodenz compared to aquatic inoculum (free of Nycodenz). However, the fluorescence measurements of cDOM were not as high. From start to end, measured values of cDOM fluorescence decreased from 0.471 to 0.388 in the

Nycodenz control. Mean values of cDOM fluorescence in the same control treatment without Nycodenz decreased from 0.541 to 0.514.

3.2 FLUORESCENCE

3.2.1 cDOM

Using fluorescence of cDOM to approximate how DOM concentrations change over time as a measure of degradation showed that initial values (day 0 is missing) were around 0.6 with added DOM ('1' and '3' in Figure 7 below). The high standard deviation of control during the first day in treatment Light + DOM was due to a measurement error of one sample. This clear outlier value was therefore removed from further analysis. Treatments without added DOM were slightly lower, around 0.5 at start ('2' and '4' in Figure 7).

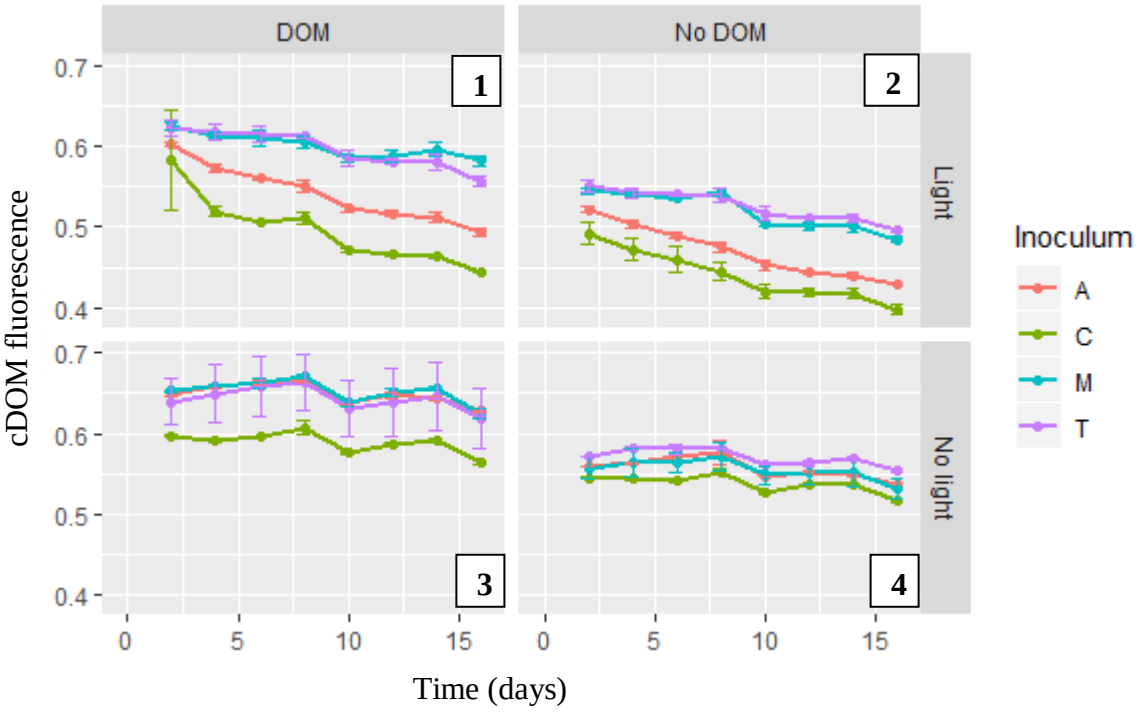

Figure 7. Fluorescence of cDOM from day 2 to day 16 (no measurement was done on day 0). Graphs for the communities are plotted; A for aquatic, M for mixed, T for terrestrial and C for control. Treatments are ordered with DOM-addition on the left hand side, and light treatments on the top.

Concentration of cDOM declined in all communities and treatments during the experiment (Fig. 8). It can clearly be seen that in the light treatments, cDOM decreased more than in dark treatments. The least decline in cDOM in the light treatments was measured for T and M, and the decline was significantly different from the decline in control only for these communities and treatments (marked with "*" in Fig. 8). Furthermore, the least decline in cDOM of all was measured for mixed communities in light + DOM - treatments (MLD).

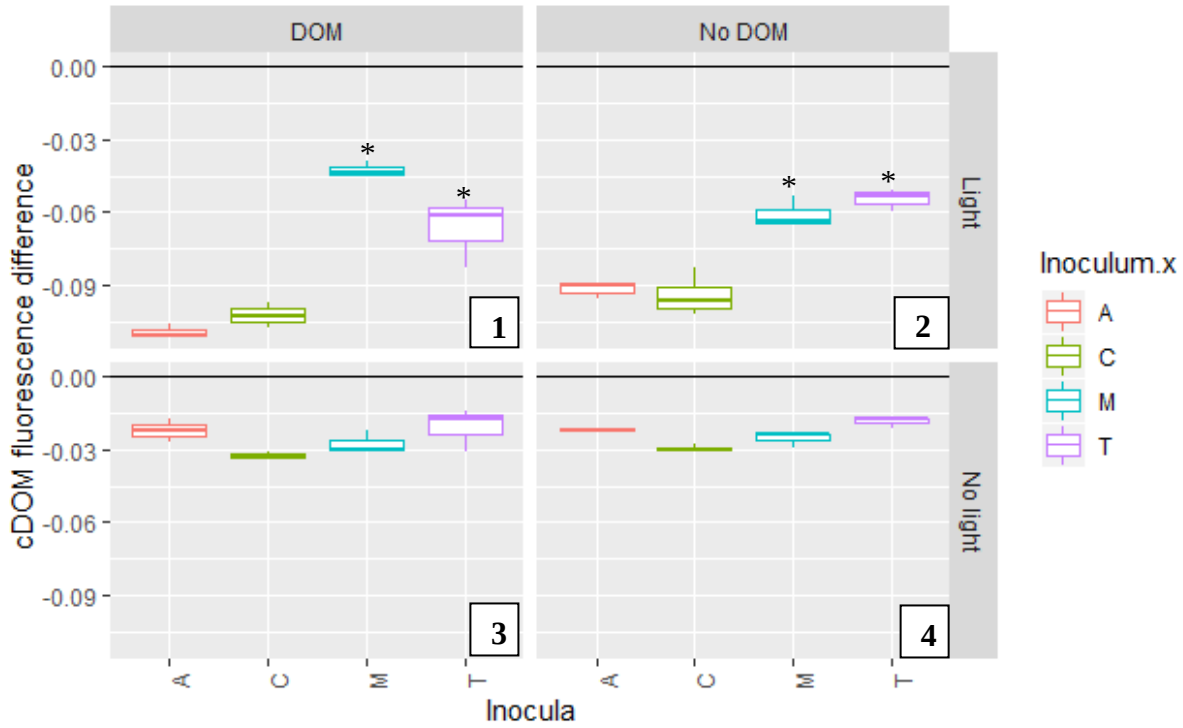

Figure 8. Difference in cDOM fluorescence between day 2 and day 16. Marked with a "*" means it is significantly different to control. Graphs for the communities are plotted; A for aquatic, M for mixed, T for terrestrial and C for control using ANOVA. Treatments are ordered with DOM-addition on the left hand side, and light treatments on the top. The boxplots show mean value and percentiles 25% and 75%.

According to the three-way-ANOVA, all treatments/inocula except DOM and DOM:Light had a significant ($p < 0.01$) effect on the difference in cDOM fluorescence between day 2 and day 16 (table 5). This shows decrease in cDOM is not affected by DOM-addition, but by inocula and light.

Table 5. ANOVA of cDOM difference between first and final day, for all communities except controls. Shown are inocula, treatments, and different combinations, and if they have a significant effect on measured cDOM difference between day 2 and 16. Values marked with green are significant ($p<0.01$).

Response: cDOM fluorescence difference		
	F value	p-values
Inoculum	48.68	<0.001
DOM	1.65	0.211
Light	539.70	<0.001
Inoculum:DOM	6.46	5.69E-03
Inoculum:Light	58.04	<0.001
DOM:Light	0.29	0.597
Inoculum:DOM:Light	7.72	2.59E-03

3.2.2 Chlorophyll a

Using chlorophyll *a*- fluorescence to identify treatments where primary production takes place showed a gradual chlorophyll *a* fluorescence decline in the dark treatments (Fig. 9 and 10). In the light treatments ('1' and '2' in Figure 9 and 10), the pattern was similar, but with one notable exception: M (mixed community) displayed an increase in chlorophyll *a* fluorescence towards the end, which was most pronounced in the light and DOM addition treatment (MLD, '1' in Figure 9 and 10). Starting values were similar for all treatments (not communities), but in light treatments there was a faster decline during the first days (Fig. 9).

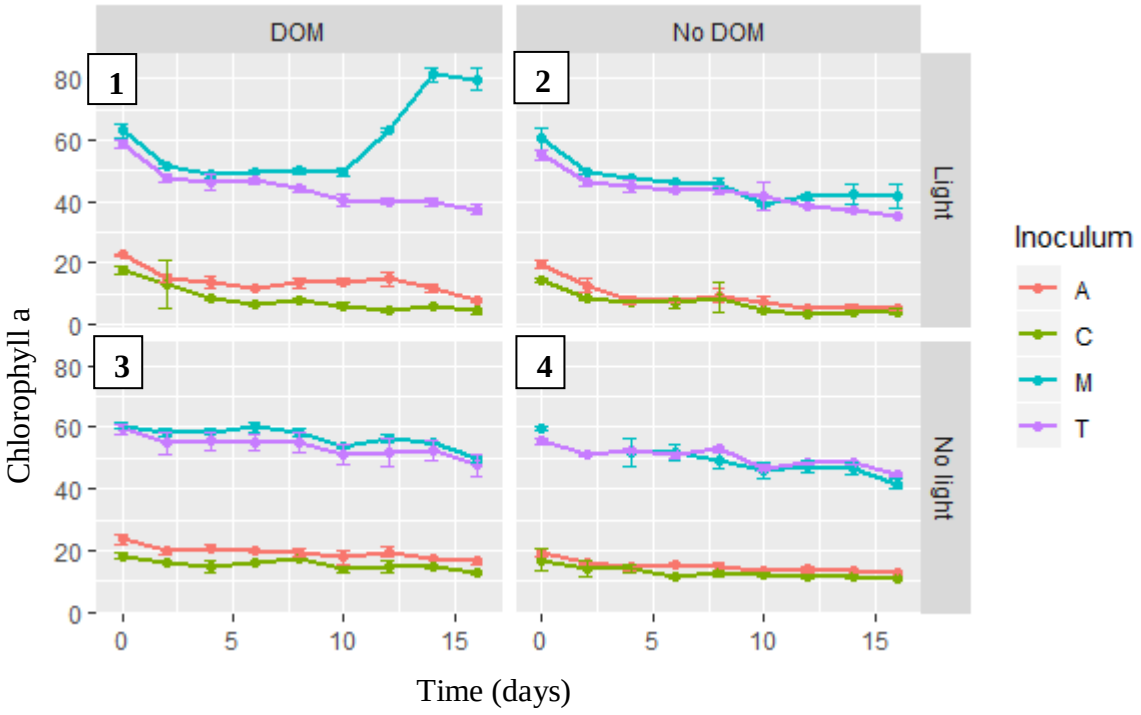

Figure 9. Changes in chlorophyll a fluorescence throughout the experiment. Graphs for the communities are plotted; A for aquatic, M for mixed, T for terrestrial and C for control. Treatments are ordered with DOM-addition on the left hand side, and light treatments on the top.

Changes in A (aquatic communities) were not significantly different from control in any treatment (Fig 10.). Without light, there was no significant difference between control and the other communities, except mixed community with no light and no DOM (MD) which was lower. With light, differences in chlorophyll *a* for M and T communities were significantly lower than control, except M with DOM (MLD) which was significantly higher (Fig. 10).

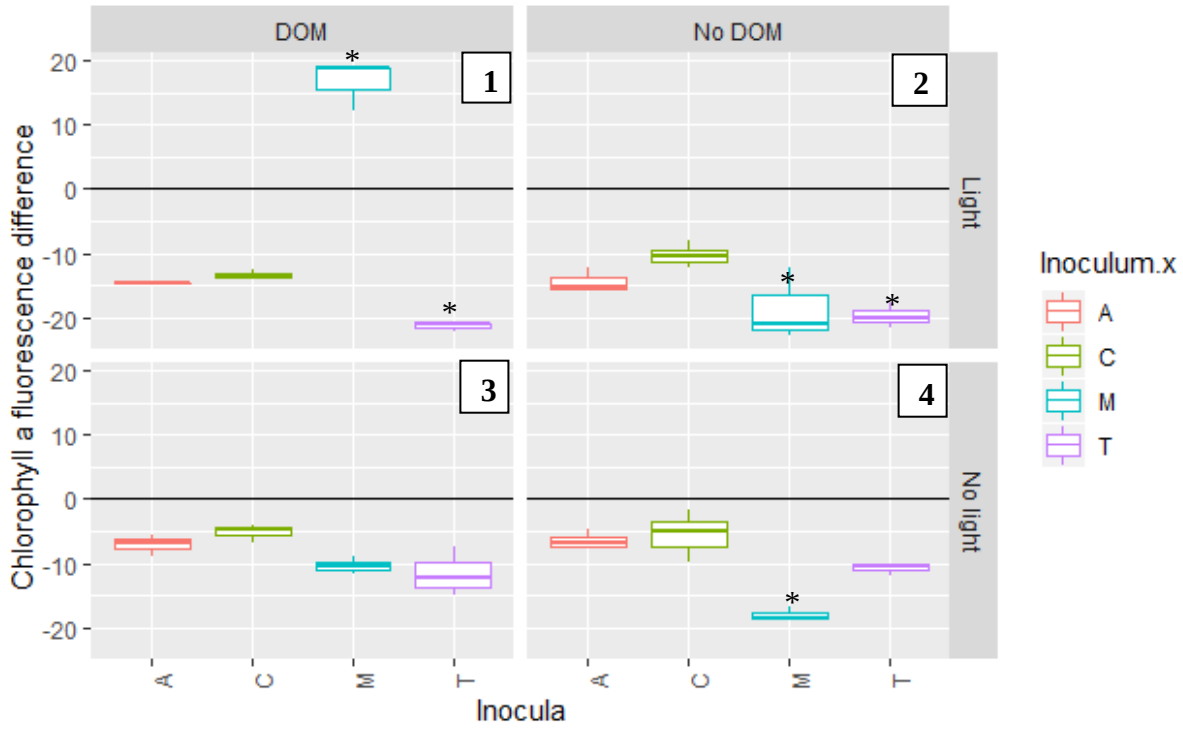

Figure 10. Difference in Chlorophyll a fluorescence between day 0 and day 16. Marked with a "*" means significantly different from control. Graphs for the communities are plotted; A for aquatic, M for mixed, T for terrestrial and C for control using ANOVA. Treatments are ordered with DOM-addition on the left hand side, and light treatments on the top. The boxplots show mean value and percentiles 25% and 75%.

According to the three-way-ANOVA, all treatments and inocula were significant to changes in chlorophyll *a* except Light (table 7). This means changes in chlorophyll *a* depend on light, DOM-addition and inoculum, as well as their interactions.

Table 7. ANOVA of chlorophyll *a* difference between first and final day, for all communities except controls. Shown are inocula, treatments, and different combinations, and if they have a significant effect on measured chlorophyll a difference. Values marked with green are significant ($p<0.01$).

Response: Chlorophyll a fluorescence difference		
	F value	p-values
Inoculum	32.66	1.42E-07
DOM	60.50	5.17E-08
Light	2.37	0.137
Inoculum:DOM	76.54	3.84E-11
Inoculum:Light	73.25	6.05E-11
DOM:Light	28.63	1.72E-05
Inoculum:DOM:Light	30.25	2.76E-07

3.3 BACTERIAL ACTIVITY

3.3.1 Cell abundance

Mean initial cell counts for aquatic (A) communities were 2.5 x 10^6 ml^{-1}, for terrestrial (T) they were 7.3 x 10^6 ml^{-1} and for mixed (M) 8.7 x 10^6 ml^{-1}. The controls were all close to zero throughout the experiment (Fig.11).

The communities as a general pattern stayed within the same order of magnitude of cell abundance, but there were several dips and peaks, especially one prominent dip on day 10 for all communities.

Without addition of terrestrial DOM ('2' and '4' in Figure 11), the aquatic communities slowly increased throughout the experiment, whereas terrestrial communities declined from high cell abundance to being the lowest at the end of the experiment. Mixed community started with the highest abundance and remained the highest throughout the experiment in these treatments.

With addition of terrestrial DOM, all communities grew differently. Aquatic communities, opposite to no-DOM treatment displayed a more rapid initial increase and two distinct peaks, one on day 6 and one on day 14. Mixed communities had lower cell abundance at the end with DOM addition than without, as well as a less pronounced peak on day 14.

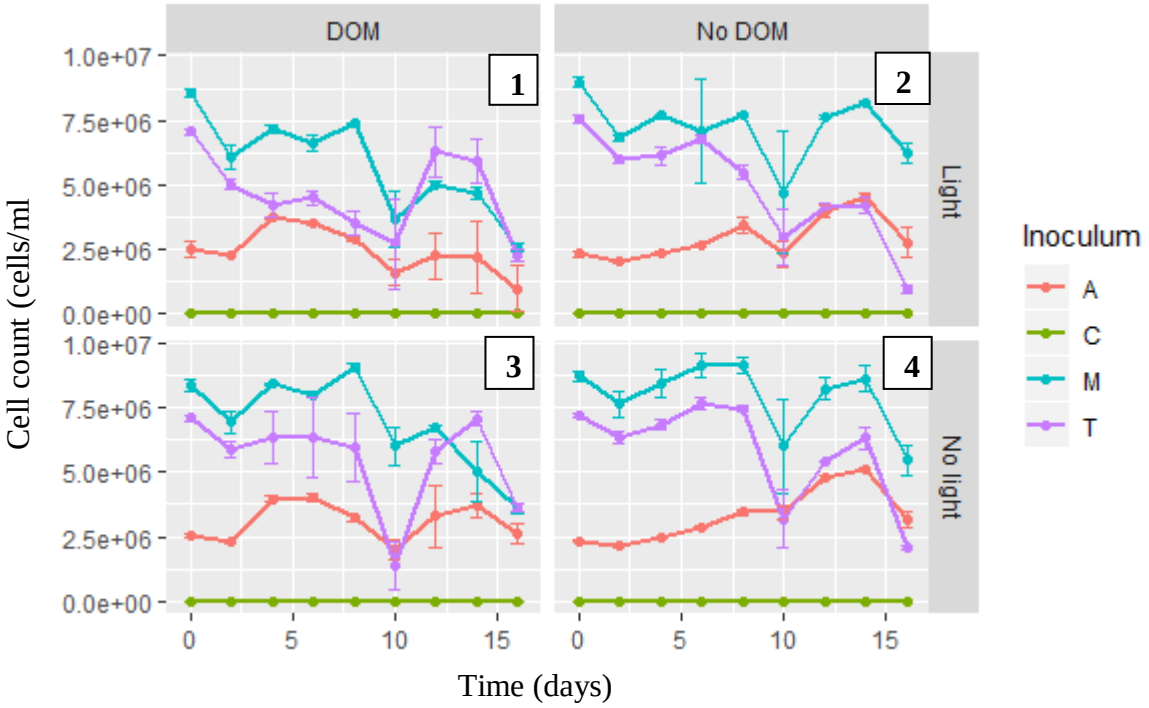

Figure 11. Mean cell counts (cells/ml) and standard deviation displayed for all treatments during 16 days of incubation. Graphs for the communities are plotted; A for aquatic, M for mixed, T for terrestrial and C for control. Treatments are ordered with DOM-addition on the left hand side, and light treatments on the top.

Between day 0 and day 16, all communities decreased except for A and control (Fig. 12). In the cases where A increased, the change was not significantly different from the control. The decrease in mixed and terrestrial communities was significant in all treatments.

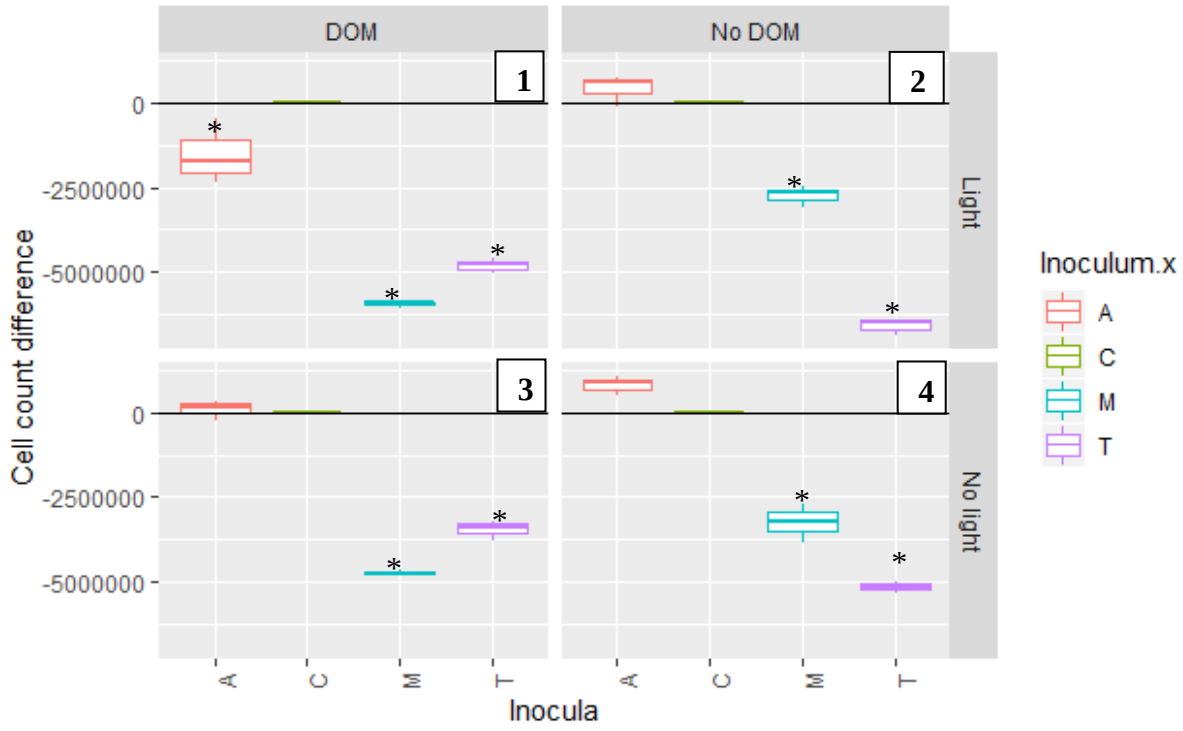

Figure 12. Difference in measured cell abundance between day 0 and day 16 (start and final day). Marked with a "*" means it is significantly different to control. Graphs for the communities are plotted; A for aquatic, M for mixed, T for terrestrial and C for control using ANOVA. Treatments are ordered with DOM-addition on the left hand side, and light treatments on the top. The boxplots show mean value and percentiles 25% and 75%.

According to the three-way-ANOVA, all treatments (DOM, Light) and Inocula (aquatic, mixed, terrestrial), and their interactions had significant effect on the overall difference in cell abundance in the experiment (table 4).

Table 4. Results of an ANOVA analysis of Cell abundance difference between first and final day of the experiment, for all communities except the controls. Shown are results of the inocula, treatments, and combinations of treatments, and whether they have a significant effect on measured cell abundance difference. Values marked with green are significant ($p<0.01$).

Response: Cellcount difference		
	F value	p-values
Inoculum	506.81	<0,001
DOM	22.63	<0.001
Light	45.13	<0.001
Inoculum:DOM	81.57	<0.001
Inoculum:Light	5.01	0.0152
DOM:Light	11.70	0.0022
Inoculum:DOM:Light	3.80	0.0368

3.3.2 Extracellular Enzymatic activities

DOM addition greatly impacted the enzymatic activities (Fig. 13-16) in ways that differed between communities. Addition of DOM led to an increase in extracellular enzymatic activity (EEA) rates in A (aquatic communities) with a peak at day 6 for all enzymes but Xylo. T (terrestrial communities) commonly reached a peak at day 14. M (mixed communities) showed patterns that reflected both the rapid initial increase of A as well as a second peak coinciding with that found for the T, albeit it was in most cases less pronounced.

There was a shift from higher rates in treatments without DOM additions on day 0, to higher rates in treatments with DOM at later time points. This can be clearly seen in Figure 14 but the pattern existed in other figures as well. Figure 18, 21, 24 and 26 in Appendix are bar plots where the y-axis is allowed to vary, which makes comparison between communities and treatments on the same day easier (but makes comparison between days harder). In these figures this pattern (shift in rates from no-DOM to DOM) is easily seen.

For A and M the increase in activity rates in ‘added-DOM’ treatments (LD and DD) occurred already after two days for most enzymes, whereas for T it occurred at day 6 or 10. An exception was for Xylo activity rates, for which the shift occurred later in all communities.

In Appendix, Figures 19, 20, 22, 23, 25, 26, 28 and 29, enzymatic rates have been divided by their respective cell abundances (Fig. 20, 23, 26 and 29 with “varying” y-axis again). These ‘cell-specific’ rates were much higher for A since A had lower cell counts, and lower for M which had the highest cell counts. Otherwise the patterns were similar to those presented in Figures 13-16.

The ANOVAs showed that changes in enzymatic rates between day 0 and day 16 significantly depended on inoculum and DOM-treatment for all enzymes, including the combined effect of the two. Light had no significant influence, except for the enzyme Bglu. The combined effect of Inoculum:DOM:Light was significant to all enzymes except Leu (Tables 8-11).

The Leu rates for community A peaked at day 6, with a higher peak in treatments with added DOM (Fig. 13). T had comparably low rates in the first half of the experiment, then rose to a high peak at day 14 in DOM treatments. M cleary shared both these two peaks, the highest being the one at day 6, but unlike A, M did not decline but rose to a second peak at day 14, much like T.

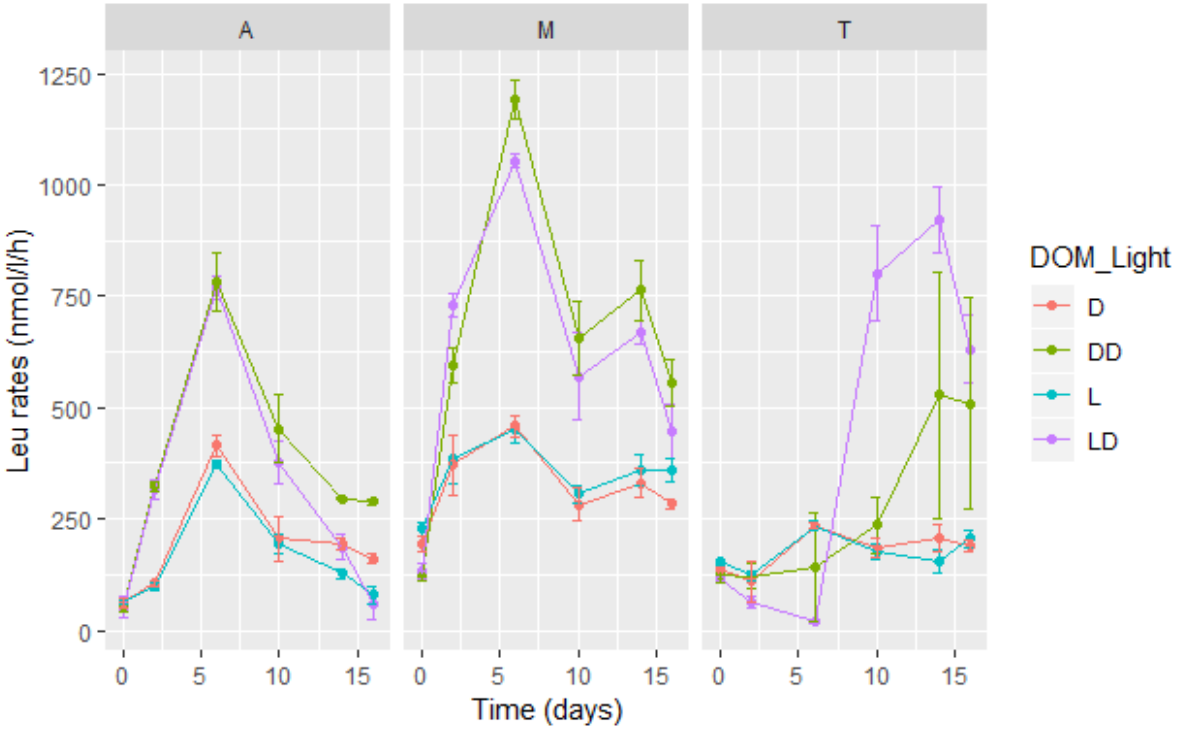

Figure 13. Rates for Leucine aminopeptidase during the first hour after substrate addition. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. D stands for 'dark, no DOM' and DD for 'dark + DOM addition', L for 'light' and LD for' light + DOM addition'.

Table 8. ANOVA of Leu EEA rates difference between first and final day, for all communities except controls. Shown are inocula, treatments, and different combinations, and if they have a significant effect on measured rates of Leucine aminopeptidase difference. Values marked with green are significant ($p<0.01$).

Response: Leu.Diff		
	F value	p-value
Inoculum	14.9475	<0.001
DOM	78.184	<0.001
Light	2.4538	0.130
Inoculum:DOM	12.5066	<0.001
Inoculum:Light	5.3188	0.0123
DOM:Light	1.086	0.308
Inoculum:DOM:Light	3.0295	0.0671

With additions of DOM, Bglu-rates for A had a peak at day 6 and then declined in the treatment LD, but turned upwards again by the end of the experiment for DD (Fig. 14). Treatments without DOM were barely reacting.

EEA rates for T with added DOM-treatments acted much like rates for Leu, with a slow rise to a prominent peak at day 14. Treatments without DOM for T had rates higher than for A, and rose to a peak at day 6, possibly to a second peak at day 16.

M again shared traits with both the other two communities, and the peak at day 6 with DOM additions look very much like the peak in A, except LD did not decline but also rose again at the end. Treatments L and D resembled those in T.

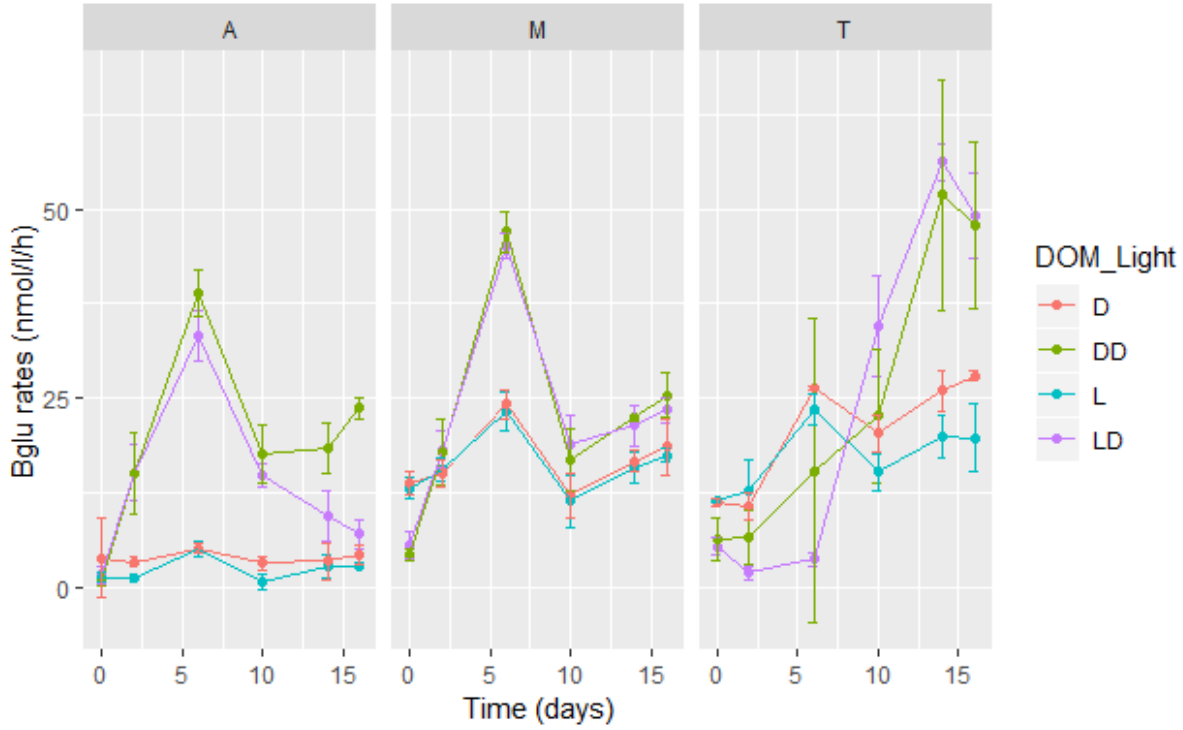

Figure 14. Rates for Beta glucosidase during the first hour after substrate addition. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. D stands for 'dark, no DOM' and DD for 'dark + DOM addition', L for 'light' and LD for' light + DOM addition'.

Table 9. ANOVA of Bglu EEA rates difference between first and final day, for all communities except controls. Shown are inocula, treatments, and different combinations, and if they have a significant effect on measured rates of Bglu difference. Values marked with green are significant ($p<0.01$).

Response: Bglu.Diff		
	F value	p-value
Inoculum	50.2221	<0.001
DOM	128.6505	<0.001
Light	6.6801	0.0162
Inoculum:DOM	9.8852	<0.001
Inoculum:Light	1.3099	0.288
DOM:Light	1.1384	0.297
Inoculum:DOM:Light	6.0386	0.00751

Rates for Cello resembled those for Bglu, but with some more variation (Fig. 15). Rates in treatments with DOM peaked at day 6 for A and M, and at day 14 or day 16 for T. Treatments without DOM had rates that were comparably low for A but also peaking at day 6. Rates for T and M without DOM were similar to each other, and higher than A, peaking at day 6 and 14.

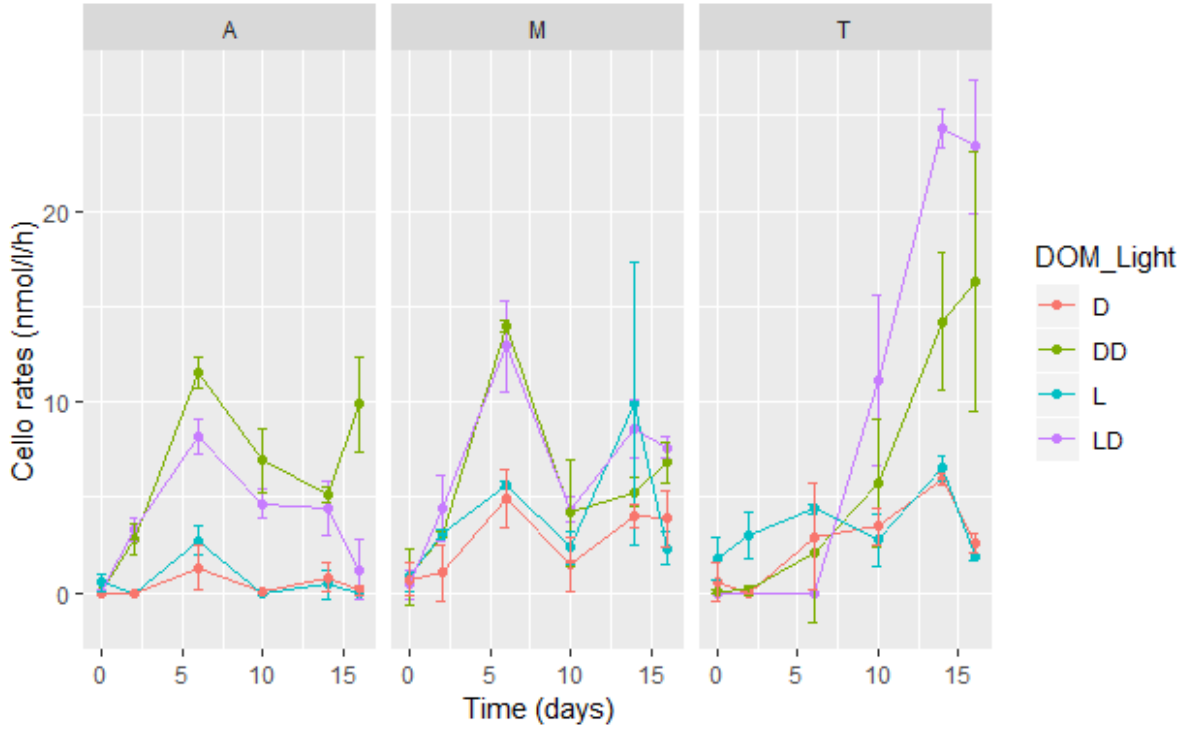

Figure 15. Rates for Cellobiosidase during the first hour after substrate addition. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. D stands for 'dark, no DOM' and DD for 'dark + DOM addition', L for 'light' and LD for' light + DOM addition'.

Table 10. ANOVA of Cello EEA rates difference between first and final day, for all communities except controls. Shown are inocula, treatments, and different combinations, and if they have a significant effect on measured rates of Cello difference. Values marked with green are significant ($p<0.01$).

Response: Cello.Diff		
	F value	p-value
Inoculum	33.8154	<0.001
DOM	140.2483	<0.001
Light	0.94	0.342
Inoculum:DOM	32.5851	<0.001
Inoculum:Light	6.9104	0.00426
DOM:Light	0.614	0.441
Inoculum:DOM:Light	9.4777	<0.001

The fourth and final enzyme had the most varied rates, with high standard deviation and patterns hard to discern (Fig. 16). It can be seen that in A there was very little activity, except for an increased rate in DD (Dark, DOM) at the end. Rates in T rose to a peak on the final day in treatments with added DOM, and for M, all treatments saw slightly higher rates towards the final day. Xylo intially had a dominance in rates by treatment D (dark, no DOM), the only enzyme where this was seen.

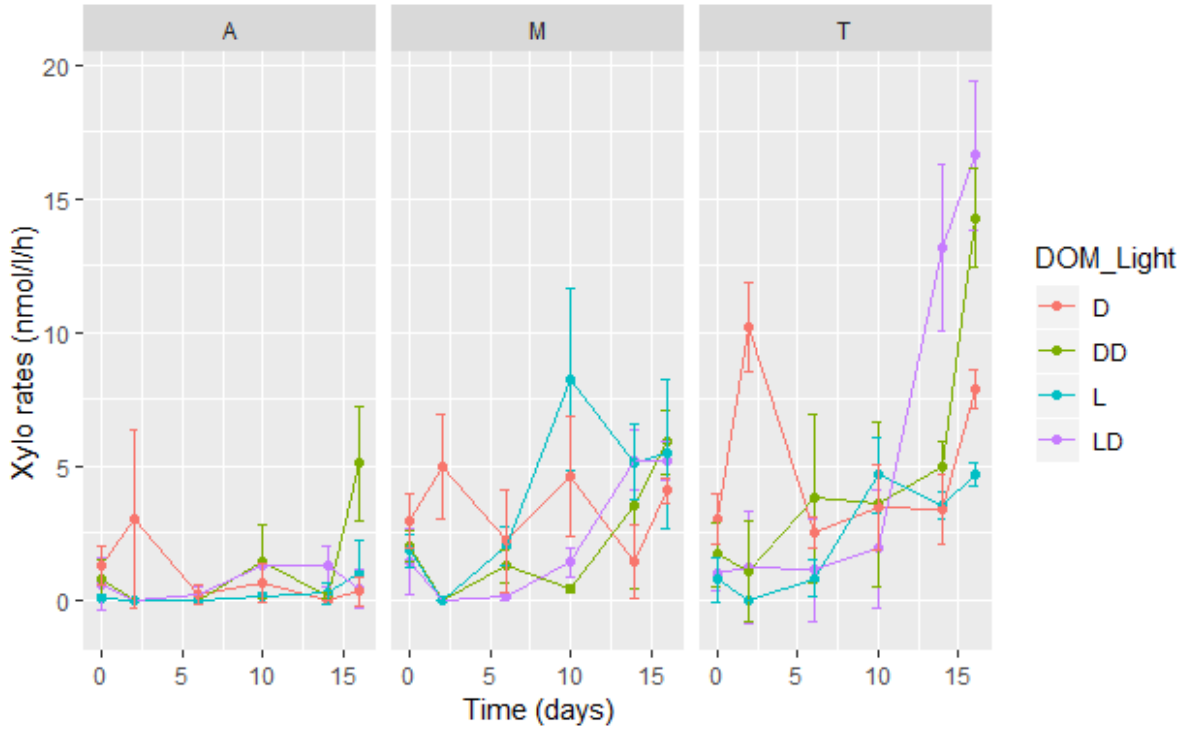

Figure 16. Rates for Xylosidase during the first hour after substrate addition. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. D stands for 'dark, no DOM' and DD for 'dark + DOM addition', L for 'light' and LD for' light + DOM addition'.

Table 11. ANOVA of Xylo EEA rates difference between first and final day, for all communities except controls. Shown are inocula, treatments, and different combinations, and if they have a significant effect on measured rates of Xylo difference. Values marked with green are significant ($p<0.01$).

Response: Xylo.Diff		
	F value	p-values
Inoculum	73.843	<0.001
DOM	59.6314	<0.001
Light	0.3177	0.578
Inoculum:DOM	21.4679	<0.001
Inoculum:Light	2.0316	0.153
DOM:Light	2.0773	0.162
Inoculum:DOM:Light	7.0058	0.00401

3.3.3 NMDS plot of similarity

The following Figure 17 shows plots of Bray-Curtis-based dissimilarity between enzymatic rates of the different communities and treatments, created using non-metric multidimensional scaling (NMDS). Data points closer to each other exhibit more similar activity profiles for the four enzymes measured. This was done in order to visualise patterns seen in enzymatic activities (Fig. 13-16) more clearly, especially how they change over time.

At day 0 in Figure 17 (top left), treatments and communities lack clear groupings by community (inoculum) or by treatment. At day 2, the pattern has changed and there is a grouping of aquatic and mixed communities in DOM treatments (triangles and pluses). This grouping remains until day 6, and during this time, enzymatic activity profiles become more similar by community, evident by more cohesive clustering by inoculum. From day 10 to day 16, enzymatic activity patterns for all treatments with the mixed and terrestrial communities gradually become more similar, whereas those for aquatic communities separate and become dispersed. These results are reflecting patterns observed in section *3.3.2 (EEA)*, characterized by mixed communities sharing an early peak in rates with aquatic, and a later peak with terrestrial communities.

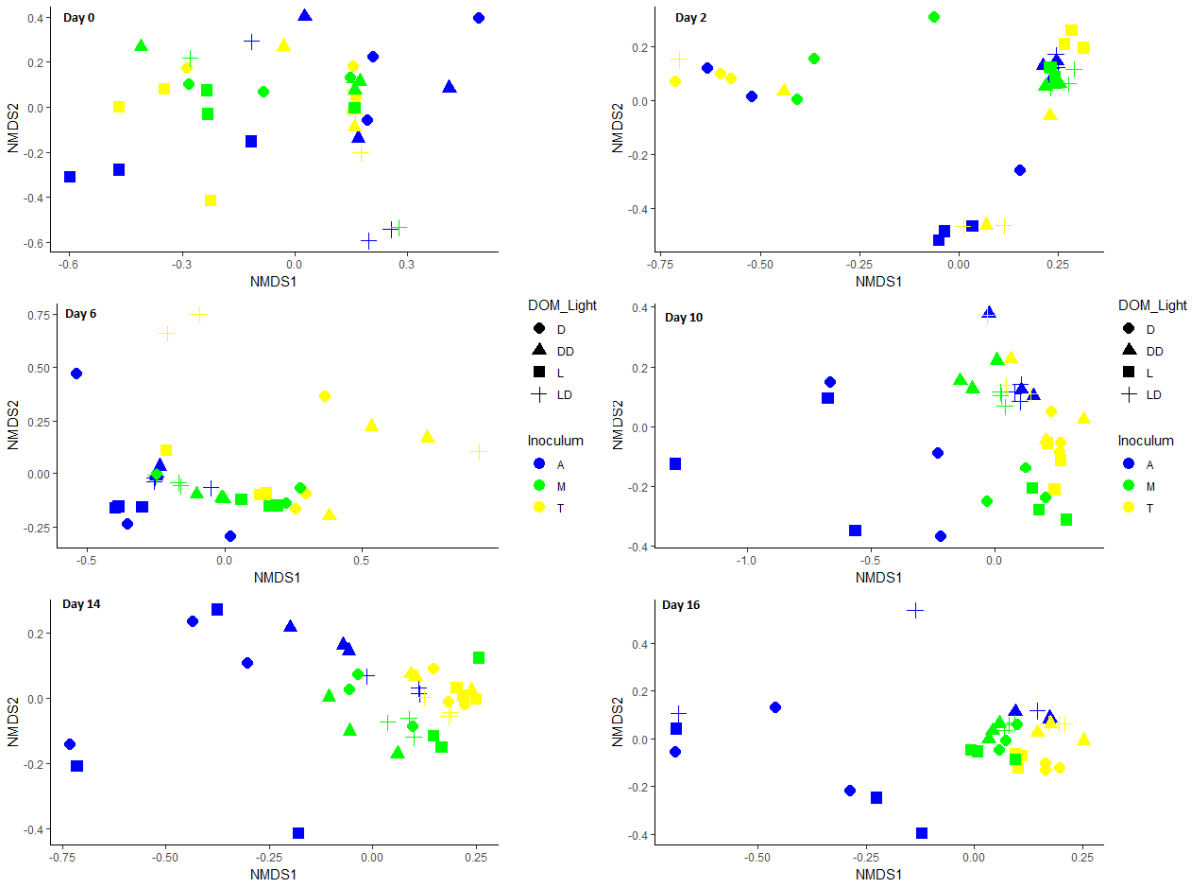

Figure 17. Plots of similarity between enzymatic rates of all the four enzymes. Colour represents community; blue for aquatic, green for mixed and yellow for terrestrial. The shape of the symbols represents treatments; circle is dark, no DOM, triangle is dark+DOM, square is Light, no DOM and plus is Light +DOM.

4. DISCUSSION

This project aimed at investigating how community coalescence between aquatic and terrestrial microbial communities, as well as the addition of allochthonous DOM, influence the abundance of microorganisms, the extracellular enzyme activities of the microorganisms, primary production (chlorophyll *a)* and DOM degradation (cDOM concentrations).

4.1 EFFECTS ON ENZYMATIC ACTIVITY

The goal to investigate whether there was a difference in functionality between coalesced and non-coalesced microbial communities in terms of enzymatic activity succeeded, as it was seen that enzymatic activity rates in mixed communities (M) followed both aquatic (A) and terrestrial (T) activity patterns. The similarity plot (Fig. 17) made clear that there was a shift in mixed communities, from first resembling aquatic and then terrestrial communities pattern of activities.

A clear synergistic effect by community coalescence could not be observed, as the EEA-rates of mixed communities were not always higher (Fig. 13-16), especially when considering cell-specific rates (Figures 19-20, 22-23. 25-26 and 28-29 in Appendix). However, the two peaks in M (mixed) instead of one in A and T (i.e. the non-mixed communities) are still very interesting. An explanation to why rates in M were not higher might be a limit to nutrients needed to create the enzymes, preventing mixed communities from having peaks as high as communities only

having one peak. The question whether these two peaks in M resulted in a higher "total" activity than the single peaks of A and T remain to be answered.

One pattern observed was that initially, treatments without DOM had higher enzymatic activities than treatments with DOM, which then shifted to higher activities in treatments with DOM. The cause of this might be that enzymes in DOM-treatments are degrading the added terrestrial DOM substrates and therefore degrade less of the fluorophore, which would give a lower signal when measuring EEA (Chróst 1991, Arnosti 2011).

A pattern present in all enzymes investigated was that light treatments (especially Light and DOM, LD) had lower rates for A and higher for T. Possibly, there might be an inhibition of EEA by light, even though UV-light was not used in this experiment (Thomson *et al.* 2017). T and M were browner than A due to the terrestrial inocula containing some amount of DOM. This might have shielded enzymes in T and M from some photo degradation or photo inhibition. However, M rates were again somewhere between A and T, and didn't show rates as high as those for T in light treatments, so this phenomenon might also be caused by an actual difference in community. For instance, it might be an indication that A depend more on light degrading DOM into more labile substrates, while T attempts to degrade such substrates using their own enzymes, which would show as a higher rate.

The increase in chlorophyll in the MLD (mixed, light, added DOM) treatment started at day 10 and peaked at day 14, which is interesting since it coincides with the peak in EEA for T and M communities, as well as the second peak seen in all communities' cell abundance. Possibly, organic matter in M bottles were degraded in a first step by aquatic bacteria in the mixed community peaking early, and then further degraded by terrestrial bacteria in the second peak, eventually resulting in products that facilitate algal growth. This result might be supported by the results from EEA where mixed communities peaked twice, following first EEA-pattern of aquatic communities and then shifting to resemble terrestrial communities.

4.2 EFFECTS ON PRIMARY PRODUCTION

One striking result in this study was the increase in chlorophyll *a* in MLD (mixed community, Light and DOM), presented in Figure 9. What made this increase possible? It could be chance that made primary producers or mixotrophs survive in this particular treatment, but there was little variation between the replicates (low standard deviation, Fig. 9), so this is not wholly satisfactory as an explanation. MLD is containing both the most diverse microbial communities and the most beneficial treatment conditions in terms of DOM and light, if one assumes most primary producers benefit from light, active microorganisms and possibly from terrestrial DOM. According to these results, one or the other does not suffice. The same treatments for the other communities show no effect, and M in other treatments does not produce the same results. A slight increase in M with light but without DOM (ML) (Fig. 9) shows that the most crucial combination seems to be M and Light, but the effect on chlorophyll *a* is evidently strongly boosted by addition of terrestrial DOM. An explanation might be a chain of degradation steps releasing nutrients vital to primary producers, where microorganisms from both aquatic and terrestrial communities are required to complete the chain (Lignell *et al.* 2008).

An important question to keep in mind is where the primary producers are coming from. The lake media was sterilised, so all primary producers must come from the microbial inoculas. It could therefore be that the primary producers for instance comes from the aquatic inoculum – but depend on degrading microbes found in the terrestrial inocula.

This result - chlorophyll *a* only really increasing in a mixed community with light and added DOM - might be an important result from an ecological point of view, underlining the importance of biodiversity, and indicating a possible dependence of primary producers on spring runoff and the coalescence of communities, as well as allochthonous DOM. This opens up for further studies to investigate if early algal blooms in lake ecosystems depend on community coalescence.

4.3 EFFECTS ON CELL ABUNDANCE

Cell abundance was slightly higher for dark treatments than for light treatments. Possibly this is because of photo degradation. Light could be exerting a stress on the bacteria themselves through radiation damage (though UV-light which is the most harmful, was not in the spectra of the lamps), or it could degrade their enzymes, as discussed in a previous paragraph. Light could also be degrading the DOM that is their substrate. This somewhat contradicts that light should increase bacterial abundance by degrading DOM into products more easily degraded by bacteria (Lignell *et al.* 2008). It is however a complex matter and other studies suggest the influence of light can make some DOM substrates less labile to bacterial degradation, or result in compounds bacteria are more likely to use for respiration instead of incorporating it into biomass (Cory *et al.* 2014). In both cases, bacterial cell abundance would not increase, but possibly decrease. Another factor that has not been taken into account in this study, is the effect of grazers and viruses. With the filter-sizes used, it is definitely possible for both viruses and grazers to have been present in the inocula, and they may have had a substantial effect on bacterial abundance and productivity (Berdjeb *et al.* 2011). Terrestrial and aquatic bacterial inocula may have had their respective communities of grazers and viruses along with the bacteria in this experiment.

Cell abundances in T and M initially were about three times higher than in A, and both T and M populations declined throughout the experiment whereas population in A increased. This indicates that the high populations of terrestrial and mixed communities were close to- or past-carrying capacity. The increase in A was more rapid with added DOM. This result is possibly explained by research showing that bacteria use allochthonous DOM for biomass production to a greater extent that autochthonous DOM, which is mainly used for respiration (Berggren & del Giorgio 2015, Guillemette *et al.* 2016). Others have described microbial communities being carbon limited in aquatic environments (Lignell *et al.* 2008), and the result could be an indication of carbon limitation, even for A communities and their low cell abundances.

It is a bit surprising that mixed communities' cell abundance declined with the addition of DOM but did not without. DOM is expected to serve as food, and more DOM should enhance population increase, not the opposite (Cory *et al.* 2014, Berggren & del Giorgio 2015). One reason might be that the extracellular enzymes used to degrade terrestrial DOM are very costly to produce, and might reduce population increase, even causing it to decrease (Fitch *et al.* 2018). Also, as was stated, microbes might prefer to use DOM for respiration instead of growth.

4.4 EFFECTS ON DOM DEGRADATION

Fluorescence measurements of cDOM showed strong indications of photo degradation, even though the lamps used did not contain wavelengths in the UV-spectra. Levels of cDOM significantly decreased in all treatments, and more rapidly in light treatments. The strongest decrease was in the controls (Fig. 8), with only one exception (aquatic, which was not significantly different to control). This indicates no measurable degradation of cDOM related to biotic factors took place in this experiment. These results are supported by research stating that photo degradation commonly is dominating DOM degradation (Cory *et al.* 2014). Mixed community with light and added DOM (MLD) was the only set of community and treatments showing strong indications of primary production through measured chlorophyll *a* levels, and in these treatments, decrease in cDOM concentration slowed to a halt towards the end, indicating that MLD through primary production produces carbon at a rate able to compensate for the loss of cDOM to photo degradation. However, the bacteria most likely did degrade DOM, as their enzymatic activities and their response to DOM addition showed. This degraded DOM was probably either in quantities too small to be measured, in form of other substrates and products than cDOM, thus not showing with the measurement methods used, or the degradation products and degraded substrates had similar DOM-content.

4.5 LIMITATIONS, ERRORS AND UNCERTAINTIES

There are a number of limitations that might influence the results of this study. Cell count in the different communities were intended to be similar. They turned out to be the same order of magnitude, but terrestrial and mixed communities were about three times higher than aquatic (Fig. 11). This was discovered when measuring cell abundance on the first sample day, and the cause was likely abundances in the added inocula being too high to be correctly measured, or caused by clustering of cells. Much could still be corrected by for instance considering cell-specific rates for EEA, but cell growth might have displayed different patterns if initial cell abundances had been different. Measured cell abundances throughout the experiment are also subject to uncertainties, as it cannot be excluded that cells clustered or that the measurements for some other reason were inaccurate.

A possible source of some uncertainty in EEA measurements is stated to be an overlap in emission and excitation spectra of the MUF and MCA fluorophores with natural DOM in marine environments, which becomes extra problematic with high DOM contents. (Arnosti 2011). However, EEA-rates were calculated by subtracting the controls (including DOM-treatments), which should eliminate any such effect.

It is stated by the producers (Nycomed Pharma AS, Oslo, Norway) that Nycodenz is inert and shouldn’t react or work as a substrate. The final measurement showed that carbon concentrations in communities with Nycodenz had not degraded but in fact increased (if the measurements and calculations for initial values are correct) so hopefully, as stated by the producers, it is true that Nycodenz does not react or work as substrate. There was a concern that DOM treatments would be overshadowed by high DOM concentrations in terrestrial inocula that came with the Nycodenz extraction, but as the different results showed, there were very clear effects of added DOM.

The fact that Nycodenz gave such a strong signal on DOC measurements made it hard to say much about relative DOC concentrations between inocula. However, this project mostly used fluorescence measurements of cDOM, which did not seem to be affected by Nycodenz at all.

The measurements of DOC are stated to be more accurate than the fluorescence measurements of cDOM, especially as cDOM only includes the optically active fraction. The reason this more accurate method was not used was mainly that the method is more complex and time consuming, and could not be used as often. Another factor is, as was mentioned, that Nycodenz had such a large impact on these measurements, which made the results harder to interpret.

There were also plans to measure particulate organic matter (POM), as well as measuring chlorophyll with more accurate methods. It was also planned to compare DOC measurements with cDOM measurements in an attempt to correlate measured fluorescence of cDOM to actual DOC concentrations. All of this was not done because of lack of time.

5. CONCLUSIONS

From the results of this study it can be concluded that community coalescence does have an effect on microbial activity and functionality. EEA and the similarity plots clearly shows how enzymatic activity rates of mixed communities first resembles aquatic communities, and then shifts to more resemble terrestrial communities. Though it could not be determined if mixed communities grew better or were more efficient at degrading DOM, it seems likely that this ability to shift enzymatic activity pattern gives mixed communities advantages.

One of the most interesting findings in this project was that primary production only developed when a mix of aquatic and terrestrial microbial communities as well as terrestrial DOM were present in lake water. This indicates a dependency of lake ecosystems on community coalescence and the inflow of allocthonous DOM. The timing and extent of spring runoff and dispersal of terrestrial microbial communities and DOM thus may possibly influence the timing and extent of early algal blooms, which in turn have a prominent effect on the ecosystem, possibly for the entire growing season (Kohlbach *et al.* 2016, Hampton *et al.* 2017).

This study expected to see increased degradation of DOM (cDOM) in the coalesced (mixed) communities, but the results of this study clearly show that no biotic degradation of cDOM takes place. Photo degradation was however a major factor in cDOM (and possibly DOM) degradation, as has been stated by other sources.

A return to the questions in *1 Introduction*, and answers:

- *Does an increase in light availability at ice break-off influence dissolved organic matter (DOM) concentration, primary production and microbial activity?*

  Answer: Photo degradation dominated cDOM degradation. Light only had an effect on primary production when combined with mixed community. Light was observed to have varying effects on microbial activity, with some communities having increased enzymatic activities in light treatments and some communities decreased.

- *Does the inflow of terrestrial DOM at ice break-off influence dissolved organic matter concentration, primary production and microbial activity?*

Answer: The addition of terrestrial DOM influenced cDOM concentrations, but did not affect cDOM degradation rates. It did have a large impact on microbial activity with increased rates in most cases. The addition of terrestrial DOM seemed to boost primary production in combination with light and mixed community.

- *Does the coalescence of microbial communities influence DOM concentration, primary production and microbial activity? Is there a difference in the functionality of the mixed microbial community?*

Answer: Coalescence, and biotic factors in general, had no observable effect on cDOM degradation. However, it should be noted that addition of DOM increased enzymatic activities, so most likely DOM was degraded by the bacteria, but not a kind that could be detected with cDOM fluorescence measurements. The coalescence of communities had a large impact on primary production in one case, in combination with light and added terrestrial DOM. There was a large impact on enzymatic activity, especially as mixed communities had two peaks in enzymatic activity rates instead of one as the separate communities had. This indicates a difference in functionality, with mixed communities being able to combine the functions of the communities that comprises it. As results from primary production showed, this possibly gave mixed communities abilities that the separate communities did not have.

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7. APPENDIX

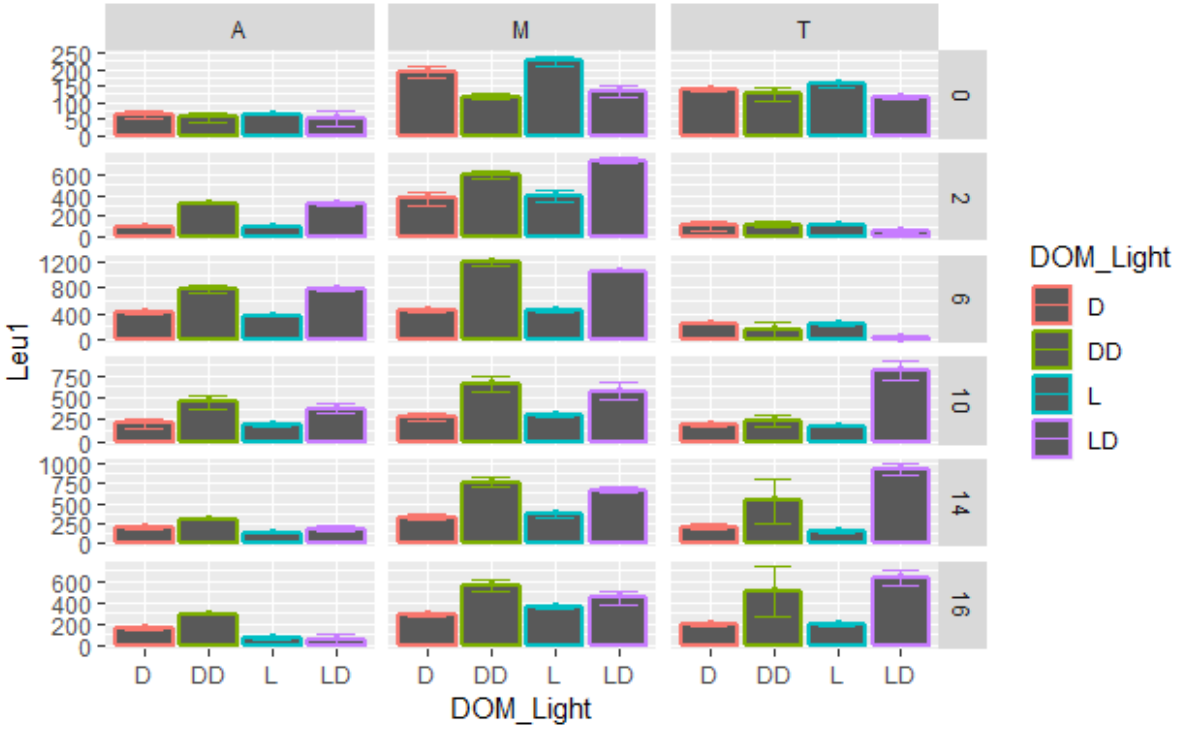

Figure 18. Rates for leucine aminopeptidase the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM). Note that the scale on the left is not fixed.

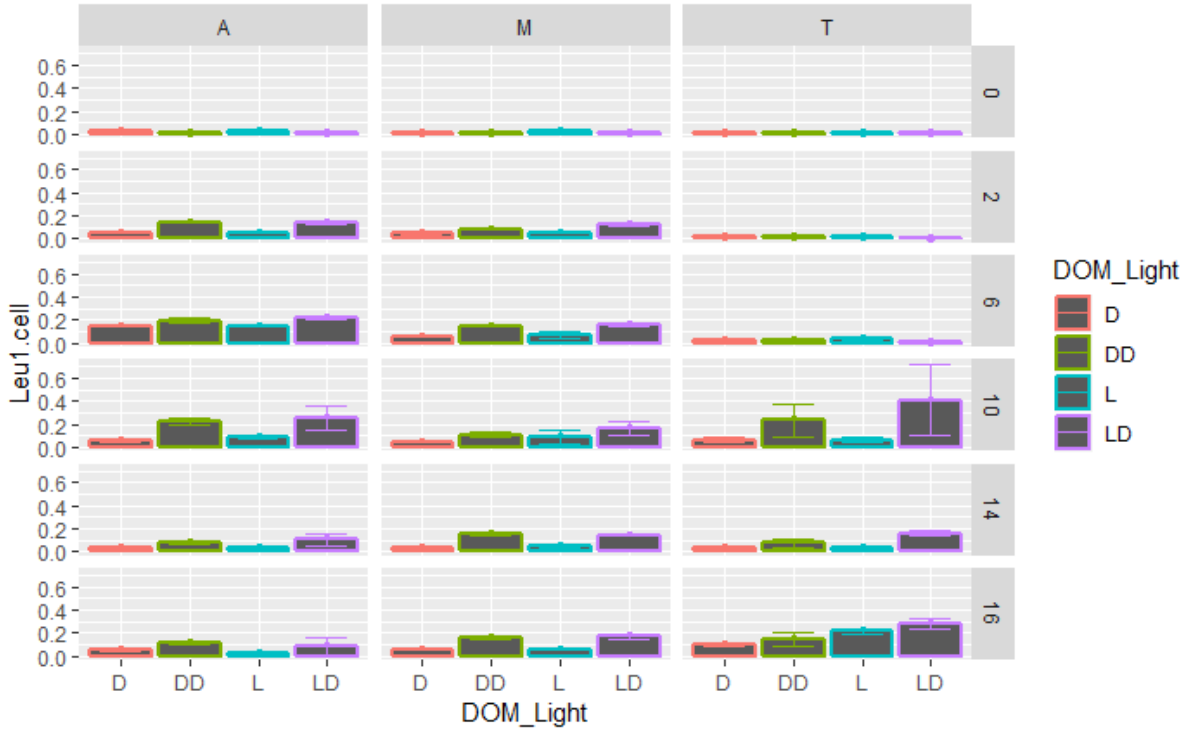

Figure 19. Rates for leucine aminopeptidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM).

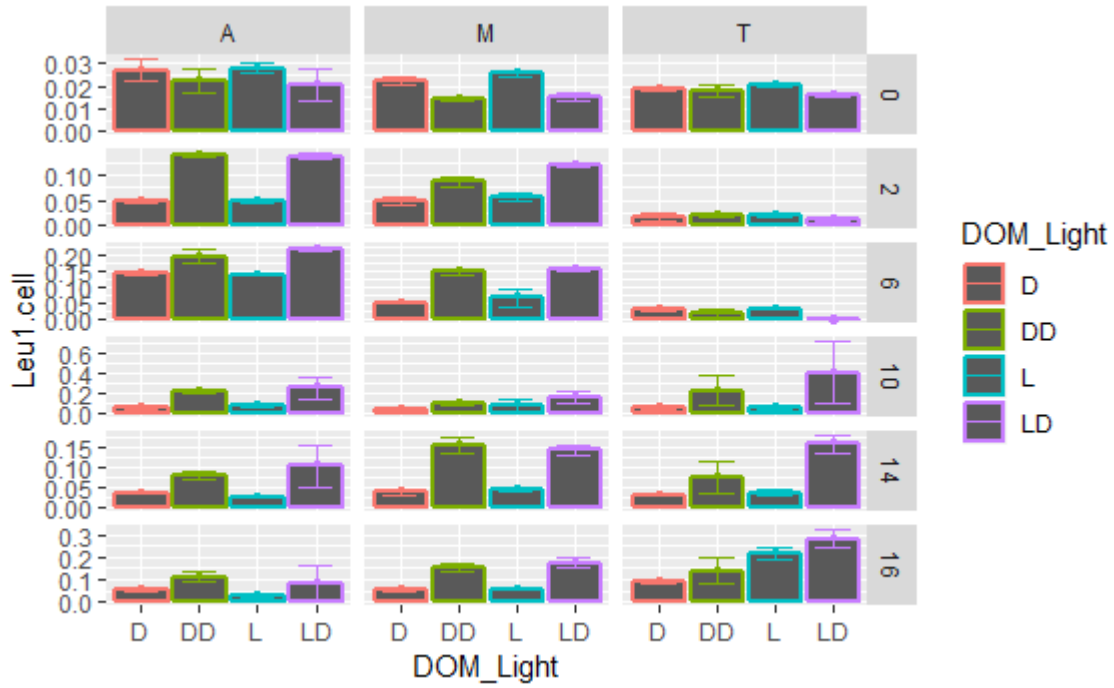

Figure 20. Rates for leucine aminopeptidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM). Note that the scale on the left is not fixed.

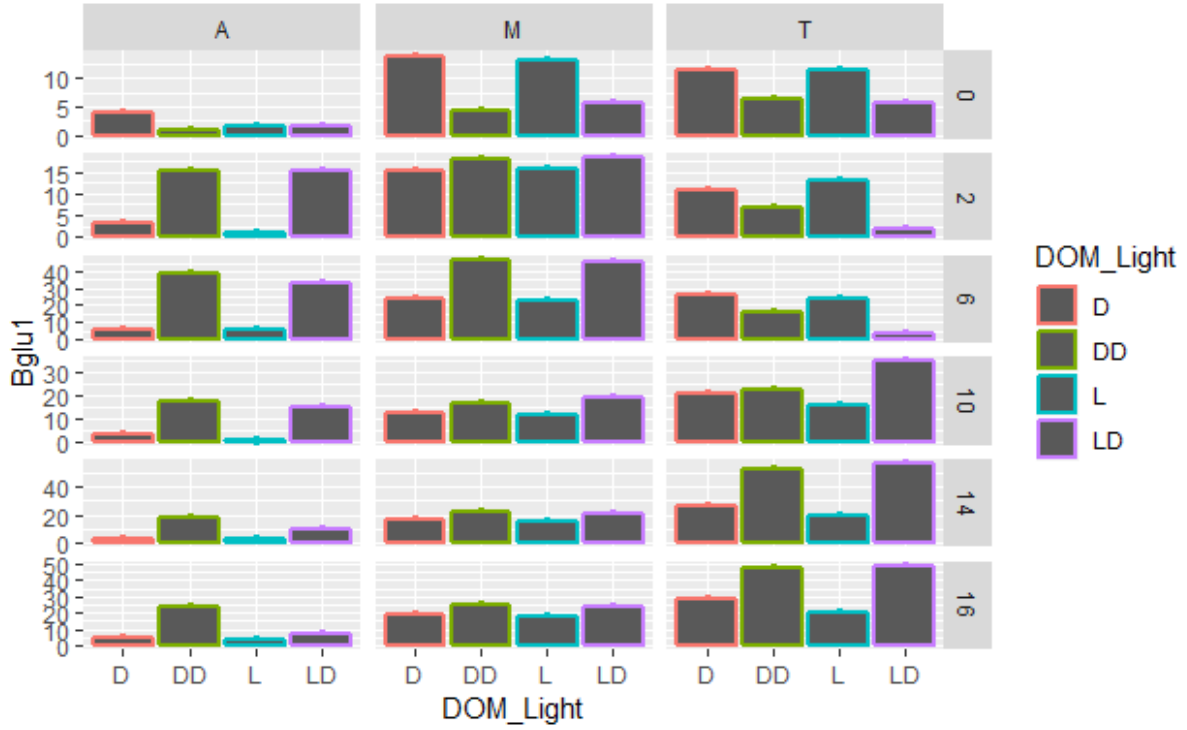

Figure 21. Rates for beta glucosidase the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM). Note that the scale on the left is not fixed.

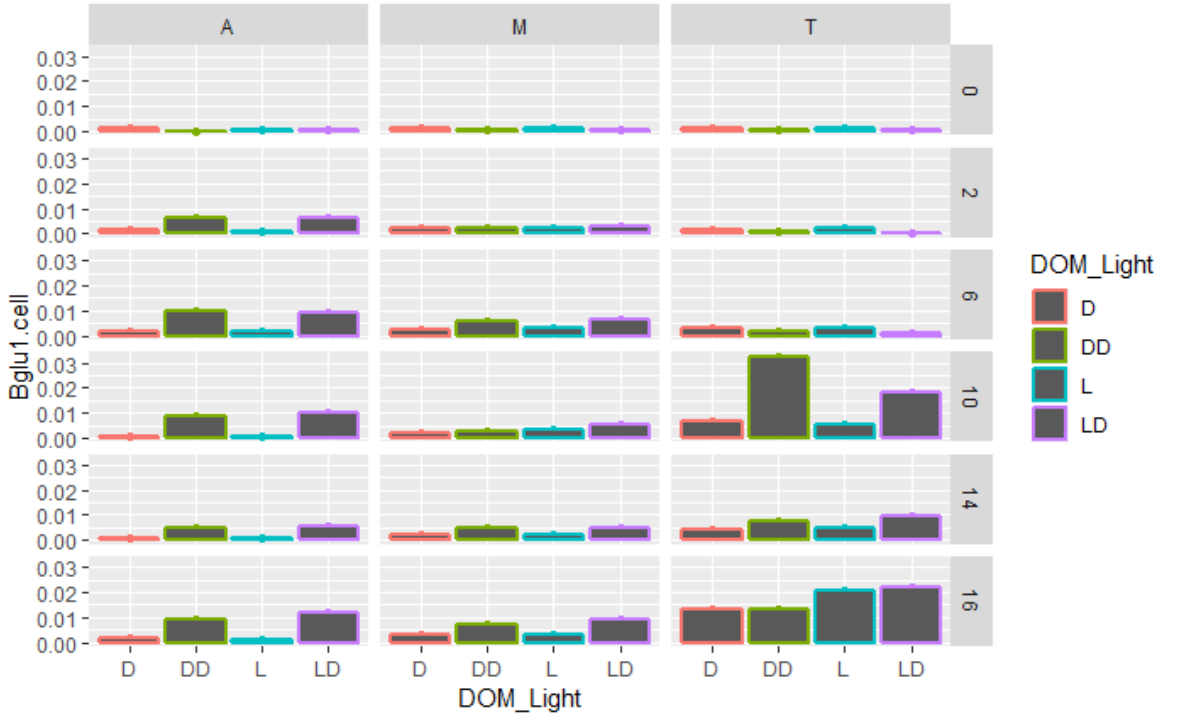

Figure 22. Rates for beta glucosidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM).

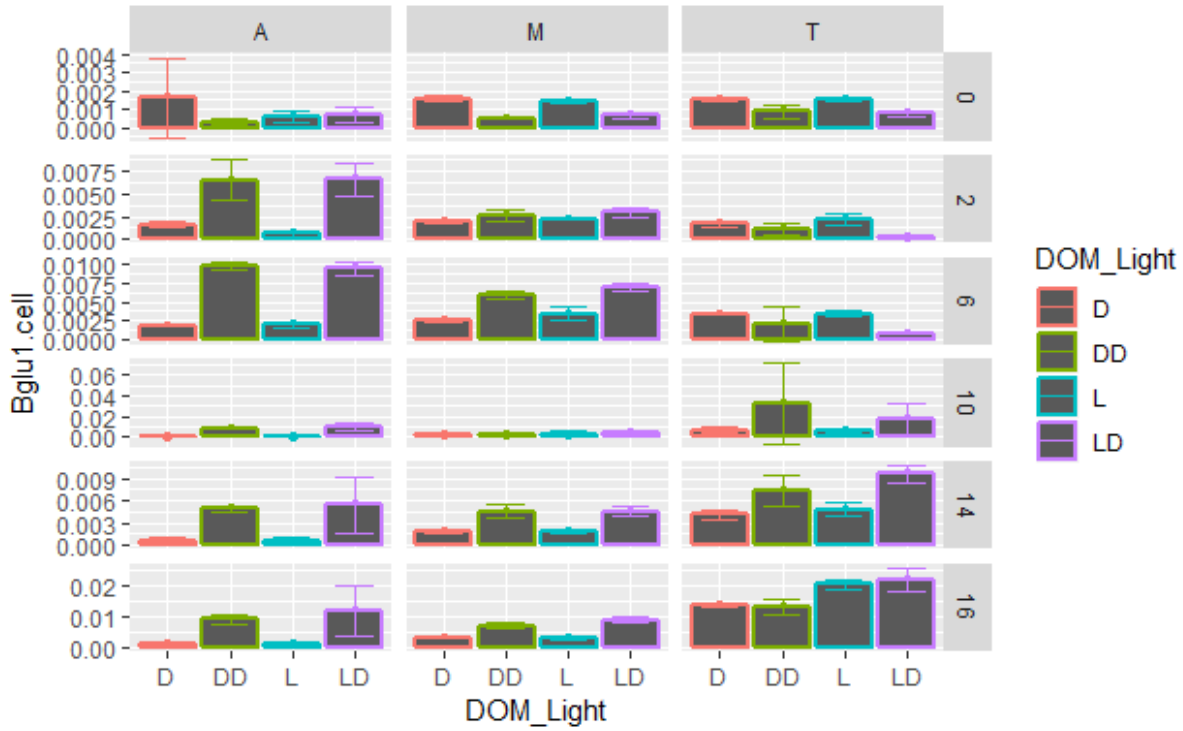

Figure 23. Rates for beta glucosidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM). Note that the scale on the left is not fixed.

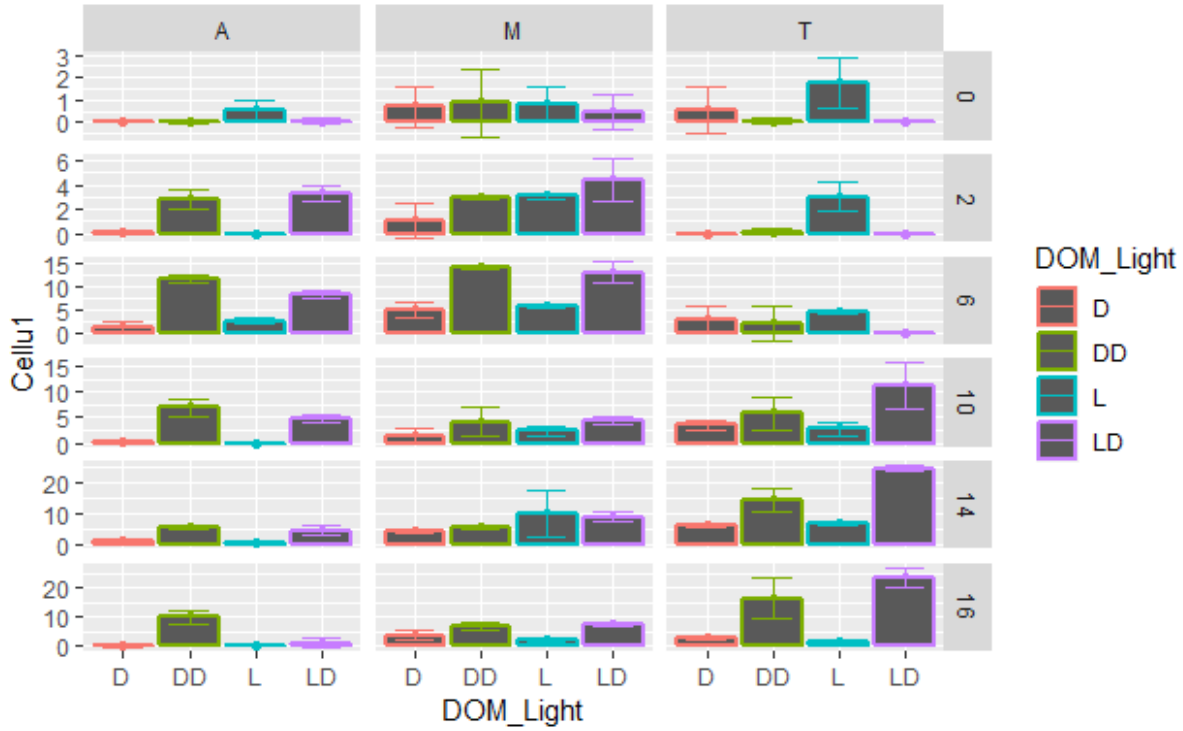

Figure 24. Rates for cellobiosidase the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM). Note that the scale on the left is not fixed.

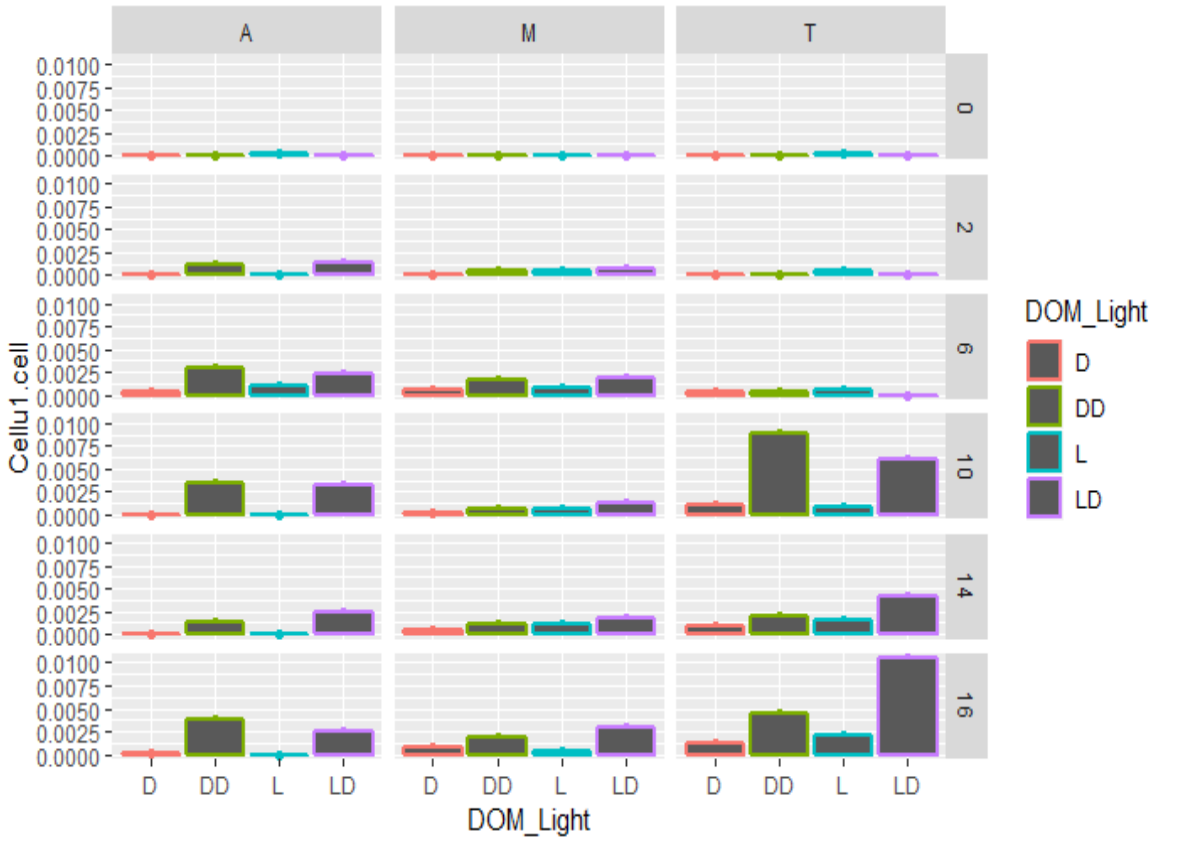

Figure 25. Rates for Cellobiosidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM).

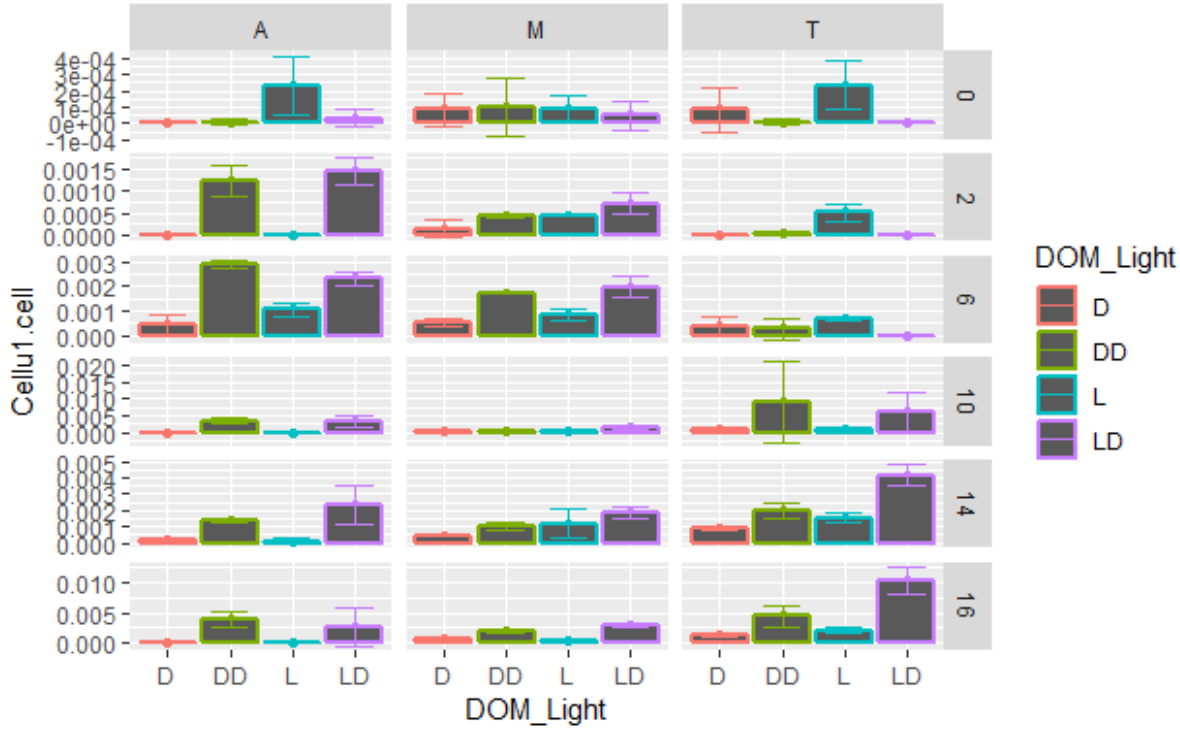

Figure 26. Rates for Cellobiosidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars

on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM). Note that the scale on the left is not fixed.

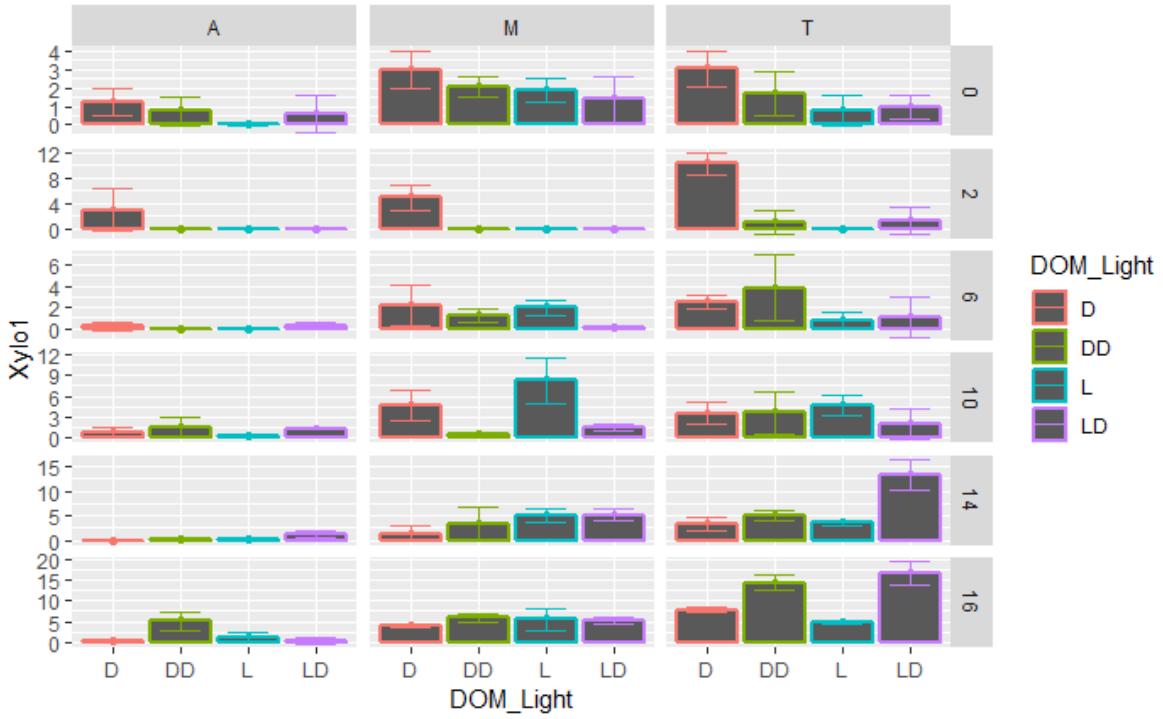

Figure 27. Rates for Xylosidase the first hour after adding fluorophore, with free scale. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM).

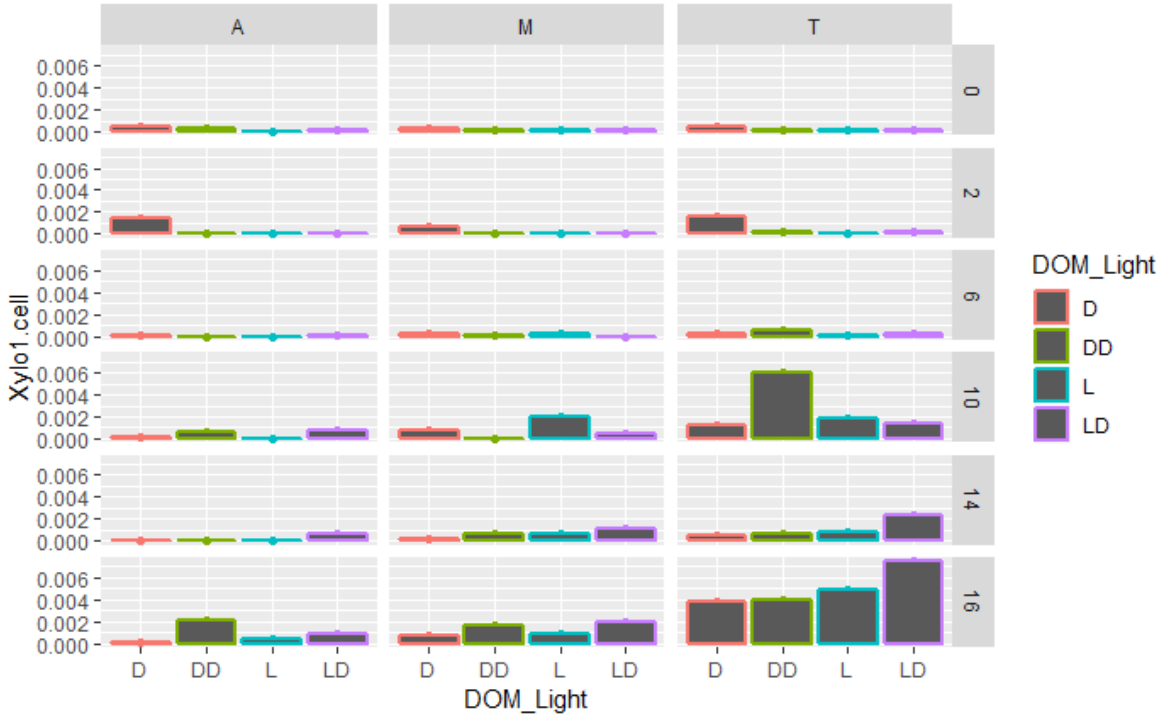

Figure 28. Rates for Xylosidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM).

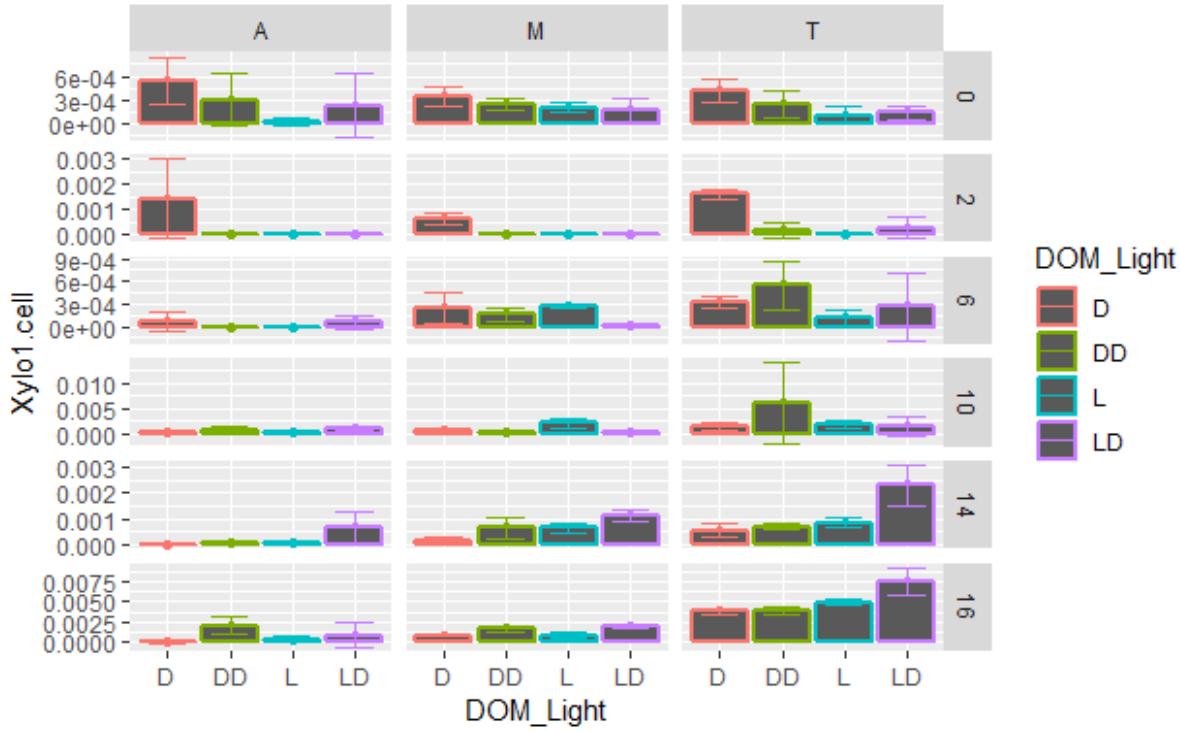

Figure 29. Rates for Xylosidase divided by cell abundances, the first hour after adding fluorophore. Communities are ordered in the three columns, A for Aquatic, M for Mixed and T for Terrestrial. The two bars on the left in each column are dark treatments (D for only dark and DD for dark and added DOM) and bars on the right are light treatments (L for light, LD for light and DOM). Note that the scale on the left is not fixed.