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The effects of long-term inorganic nutrient additions on benthic biofilm structure and functions in completely covered, shaded and non-shaded streams: using indoor flume experiment

Thesis submitted for the award of the title

"Master of Science"

By

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Dedication

I dedicate this dissertation to my girlfriend, Katharina who has been with me during the entire research period. This work is also dedicated to my parents, Joshua and Jane who have always showed me unconditional love and whose good examples have taught me to work hard for things I aspire to achieve.

Summary

The pressures of agricultural development, population growth and urbanization have been cited as drivers for land use transformations in recent decades. The impacts for land use transformations on running waters could involve multiple physical and chemical factors acting at varying scales. Riparian degradation and nutrient loadings can influence stream benthic biofilm community structure and ecological functions. The main objective of this thesis study was to investigate the influence of nutrient and light on benthic stream processes. The study focused specifically on the roles and interactions between algae and bacteria. Two experiments were carried out at WasserCluster biological station, Lower Austria, in an indoor air-conditioned room at constant temperature of 20 ± 1ºC. First experiment "dark flumes" ran for 5 weeks. However, second experiment lasted 8 weeks with "shaded flumes" lasting 5 weeks thereafter, shaded flumes were exposed to high-light for further 3 weeks and the period named "light flumes". The two experiments were carried out between September 2016 and February 2017. The facility consisted of 16 flumes in 4× 3 factorial design (four nutrient levels, 3 light conditions, with 4 replicates per treatment) under re-circulatory system. Benthic biofilm was grown on glass slides (rock surrogates), sediments (sand) and leaf-discs. Experiment was carried out under four nutrients enrichments: low (350 µg/L NO_3-N: 15 µg//L NH_4-N: 10 µg//L PO_4-P), moderate (1,300 µg/L NO_3-N: 50 µg//L NH_4-N: 40 µg//L PO_4-P), highly enriched (5,200 µg/L NO_3-N: 200 µg//L NH_4-N: 160 µg//L PO_4-P) and saturated systems (10,000 µg/L NO_3-N: 2,000 µg//L NH_4-N: 2,000 µg//L PO_4-P). The enrichment levels represented forest, pasture, mixture of pasture and cropland and cropland respectively. The land use types have been reported due highly correlated with nutrient enrichment levels. Nutrient levels were comparable to field estimates from Lower Austria. Statistical analysis showed that light had high influence on algal development and enzyme activities. Light flumes had high algal biomass and enzymes activities with similar algal pigments across the nutrient enrichments. However, nutrient enrichment influence enzyme activities but not algal biomass and leaf decomposition. In contrast, N-NH_4 uptake under dark conditions was highest in saturated flumes. Similarly, P-PO_4 was highest in saturated flumes under light conditions. The study concluded that nutrient enrichment influenced enzyme activities but not algal biomass. The results support prediction that fluctuations in PAR could be one of the main factors influencing algal development and ultimately bacterial heterotrophic decomposition. Therefore, open canopy streams can be regarded as zones for organic matter decomposition through indirect

influence on extracellular enzyme activities and priming. Also, riparian management is key to benthic ecosystem processes in streams.

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1.0 Introduction

Benthic biofilm communities, heterotrophs and autotrophs, are key elements in the degradation of organic matter, primary production and the release of energy in aquatic ecosystems (Romani *et al.,* 2004). Biofilms occupy the base of food webs, and thus are important sources of energy to higher trophic levels in the food chain. The structure and functions of heterotrophs and autotrophs in the biofilm matrix often depend on the availability of physical and chemical factors, notably light, oxygen and nutrients (Romani *et al.,* 2004; Francouer, 2001). Significant algal-bacterial interactions may exist when algal exudates are abundant, while interactions are usually low when allochthonous organic matter satisfies the heterotrophic carbon demand (Ylla *et al.,* 2007). For instance, mutual algal-bacterial relationship may exist when microbial community use dissolved organic carbon (DOC) produced by periphyton algae through photosynthesis (Rier and Stevenson, 2001), while algae may also use nutrients recycled by microbes (Rier and Stevenson, 2001). However, competitive interactions may exist when decomposers compete with producers for inorganic nutrients in a nutrient limiting environment as microbial decomposers have higher growth rates than algae (Ylla *et al.,* 2007; Danger *et al.,* 2013).

Biofilm communities are constrained by limiting nutrients such as nitrogen and phosphorus (Rier *et al*., 2014). Autotrophic and heterotrophic microbes utilise dissolve nutrient in streams for both primary and secondary production as well as growth (Sabater *et al.,* 2000). Therefore, availability of nutrient influence stream biota and uptake rate (Niyogi *et al.,* 2004). Anthropogenic nitrogen and phosphorus loadings to streams, mainly from agricultural development and sewage discharge, has the potential to shift the ecosystem from inorganic nutrient limitation to nutrient saturation and carbon flux (Larned, 2010; Rier *et al.,* 2011). Human population growth and the need for better life has exacerbated destruction of natural systems with consequential increase of nutrients to aquatic systems. Increased input of inorganic nutrients coupled with light availability can result to nuisance growth of algae and degradation of ecosystem health and subsequent loss of ecosystem services (Rier *et al*., 2014). Increase in inorganic nutrients can increase algal and bacterial biomass and changes in community composition (Rier and Stevenson, 2002; Romani *et al.,* 2004; Rier *et al*., 2014). Benstead *et al*., (2009) demonstrated that changes in composition and function of biofilm communities in light-limited streams can result to loss of stored organic matter through increased microbial respiration.

There is consistent correlation between extracellular enzyme activities produced by microbial heterotrophs and particulate organic matter (POM) in freshwater environments (Artigas *et al.*, 2008). Therefore, extracellular enzyme activities might be used as reliable indicators of microbial decomposition, nutrient and organic matter availability (Artigas *et al.*, 2008; Mineau *et al*., 2014; Sinsabaugh *et al*., 2014). The activities of extracellular enzymes can dynamically respond to shifts in inorganic nutrient and substrate demand of microbial cells (Artigas *et al.*, 2008). The activity of phosphatase indicates the ability of most bacteria, cyanobacteria, fungi and some algae to transform organic phosphorus into orthophosphate (Romani and Sabater, 2001) by cleaving phosphate from large organic molecules to obtain P-inorganic nutrient and small organic molecules (Rier *et al.*, 2014). Therefore, phosphatase represents the P demand of the benthic community and has been shown to decrease in phosphorus enriched streams (e.g. Sabater *et al.*, 2011). The peptidase activity is related to microbial decomposition of proteinaceous compounds from within the biofilm (Francoeur and Wetzel, 2003; Romani *et al*., 2004). Therefore, higher peptidase activities are most likely when autotrophic development is favoured. β-glucosidase hydrolyses glucose from cellobiose and is involved in the degradation of cellulose (Francouer and Wetzel, 2001; Rier *et al*., 2014). Xylosidase hydrolyses xylose and is involved in hemicellulose degradation (Rier *et al*., 2014). Enzymes active in the mineralization processes tend to increase with enrichment due to increased bacterial activities (Sabater *et al.*, 2011).

Forested streams, where allochthonous leaf litter is the main source of energy and nutrients, have contributed to knowledge about the structure and functioning of detritus based systems (Danger *et al.*, 2013). Oligotrophic conditions prevail in detritus-based ecosystems with probability that even low densities of primary producers may enhance allochthonous organic matter decomposition through priming (Danger *et al.*, 2013). Primary producers could also alter detritus elemental and biochemical quality through production of labile DOC which might affect organic matter processing through priming. Submerged leaf litter is colonized and processed by bacteria and aquatic hyphomycetes (fungi) (Artigas *et al*., 2008), though, fungi is the dominant in biomass and litter-processing efficiency (Hieber and Gessner, 2002). Danger *et al.*, (2013), observed that algal-generated DOC exudates were incorporated into heterotrophic bacterial and fungal biomass associated with leaf litter, which probably enhanced microbial growth rates and subsequent leaf litter decomposition.

Population growth, agricultural development and urbanization have driven extensive land use transformation globally in recent decades (Achara, 2012). The impacts of anthropogenic

activities on running waters are diverse and complex, involving multiple physical and chemical factors acting at varying scales s (Allan, 2004; Maloney and Weller, 2011; Riseng *et al*., 2011). Changes in physical and chemical conditions of stream ecosystems often impair habitat quality and alter resource availability for biologic communities, causing shifts in trophic structure and composition (Diana *et al*., 2006; Johnson & Host, 2010; Riseng *et al.*, 2011). For instance, human activities can alter availability of nutrients and light in lotic systems (Von Schiller *et al.*, 2007). Streams draining catchments subjected to land use changes derived from human activities show higher nutrient concentrations and often also changes in stoichiometry ratios (Bennet *et al.*, 2001). Similarly, light regimes, to which streams are exposed, are substantially altered through removal of riparian vegetation for agriculture or urban development (Von Schiller *et al.*, 2007). These changes may affect structural and functional properties of biofilms which can be reflected at the whole stream ecosystem (Von Schiller *et al.*, 2007).

To better understand how land use activities, like agricultural development, influence stream processes and functions, it is important to determine how stream ecosystems respond to different levels of nutrient enrichment, representing different land use activities, and different light availability, representing different management of riparian vegetation. Also, there is need to investigate effect of multiple stressors on stream ecosystem as currently there are several studies which have investigated the effects of nutrient or light as single factors (Carr *et al.*, 2005; Bernot *et al.*, 2006; Sabater *et al.*, 2011). However, less is known about multiplicative effects when more than single stressors exist in the environment. In this study, three stressors (nutrient, light and substrata) are investigated synergistically. Stressors defined in this study are expected to have strong influence under heterotrophic and autotrophic conditions. The study will be undertaken using indoor flume experiment. Flume is human-made channel for conveying water to the desired location whose walls are raised above the surrounding (Wikipedia).

1.1 Background information on flume experiment studies with nutrient enrichment

Flumes have been extensively used in sedimentary geology and civil engineering, however, their use in benthic biology resulted from recognition that a variety of processes of biological interest could be simulated in flumes (Nowell *et al*., 1987). Earlier studies on flume experiments focused on technique, details of design and theoretical and experimental considerations for simulation of benthic environment (Arthur, 1987). Over the years, studies on flume experiments have increased rapidly to simulate stream ecosystem processes, but the main focus has been on physical factors, for example, hydrology and substratum type, with

little attention on the chemical variability within the stream (Thomson *et al*., 2004; Jonsson *et al*., 2006) but see (Romani *et al.,* 2004; Rier *et al.,* 2014). Flume experiments have enabled ecologists to study biofilms in finer details under controlled conditions which is not possible in the natural environment (Wagner *et al.*, 2015).

Early research on nutrient enrichment assumed that benthic algal growth was not affected by stream nutrient enrichment because of restrictions by other physical, chemical and biotic factors (Smith *et al.,* 1999). However, recent scientific studies have suggested that benthic algal growth is enhanced by nutrient enrichment. Elwood *et al.,* 1981 observed significant increases in benthic biomass along with increased decomposition after using phosphorus to enrich an oligotrophic stream in Tennessee. Horner *et al*., 1990, working in laboratory stream channels, found phosphorus concentrations to be a significant factor for periphyton growth. Recent studies continue to agree with these observations (Dodds *et al*., 2002; Ylla *et al.*, 2007; Schiller *et al*., 2007).

1.2 Background information on benthic biofilm studies

Benthic biofilms have been used for many decades as model systems for study of phototropism, light limitation and other problems in ecophysiology (Larned, 2010). The earliest studies were on seasonal variation in growth and composition of stream and pond algae (Brown, 1908). The role of abiotic factors on the composition and abundance and use of benthic algae as indicators of stream health emerged in 1940s (Butcher, 1947). Modern concepts in biofilm ecology (manipulative experiments, multiple response and explanatory variables) began in the 1950s with focus on freshwater ecosystems and evaluation of human impact on the environment (Odum, 1956). Advancements in technology facilitated research in stream metabolism and organic matter degradation (Odum, 1956), limitation of light on primary production and nutrient uptake (McConnell and Sigler, 1959; Whitford and Schumacher, 1961).

Benthic biofilm ecology studies continued to grow with new conceptual models on the response of biofilms to external environment (Arnon *et al*., 2007) and detailed analysis of biofilms communities' structure and function has also increased with technological advancements. Currently, there are numerous literature reviews on physical and chemical perturbations on biofilm biomass and potential effect to higher trophic level. (e.g., Larned, 2010).

1.3 General objective of the study

The main objective of the study was to investigate the influence of nutrients and light on benthic stream processes. The study focused specifically on the biomass and composition of benthic microalgae and the activity of the microbial community.

1.4 Specific research questions

How does nutrient enrichment and light influence the following structural components and processes in stream ecosystems?

- Algal biomass
- Algal community composition
- Activities of extracellular enzymes
- Nutrient uptake
- Leaf decomposition

1.5 Hypotheses of the study

The study relied on the following hypotheses:

Effects of nutrient enrichment:

a. Nutrient enrichment will have positive effects on algal biomass and on leaf decomposition, but will change the community composition of the benthic algae towards dominance of chlorophyta (green algae).
b. β-glucosidase, leucine-aminopeptidase and β-xylosidase activities will increase with increased nutrient enrichment due to increased bacterial mineralization. However, phosphatase will decrease with increased nutrient enrichment due to increasing phosphorus supply of the biofilm community from the water column.
c. Nutrient uptake will show a saturation curve, with increasing nutrient enrichment and an asymptote point after saturation, when the benthic communities cannot adjust their biomass further to increased nutrients.

Effects of light:

d. Light will have positive effects on algal biomass, nutrient uptake (due to higher algal demand), enzymatic activities and leaf decomposition (due to priming effects).

Effects of nutrient enrichment and light:

e. Light will be the dominant factor and will control biomass and activities independent of nutrient levels.

2. Materials and methods

2.1. Flume set-up

Each flume was made of PVC rain-gutter pipes measuring 120 cm long, 6.5 cm wide and 5 cm deep. A circular water flow was established where a rotatory pump (EDEN 126, Italy) discharged water from a supply container into the respective flume from where the water flowed back again via a pipe into the container. Flow velocity was kept below 10 - 15 cms^{-1} to minimise hydrodynamic shear stress which could influence biofilm architecture, taxonomic composition, and nutrient recycling (Bondar-Kunze *et al.*, 2016). To stabilize the hydrodynamic environment, plastic sponges were placed at the inlet of each flume. Each flume was divided into two transects and at each sampling date, one sample was taken from each transect to ensure representative sampling. Also, to avoid introduction of micro-invertebrates, flumes were supplied with groundwater from a well. A layer of 1 cm sand was used to cover the bottom of each flume and glass slides were used as inlay (as rock surrogates) for biofilm colonization.

Stones were randomly collected from the Oberer Seebach, Lower Austria, and placed into the flumes for inoculation (three stones per flume) during the colonization phase. Stones were removed after two weeks of colonization. The experimental phase with the addition of nutrients started immediately after the colonization phase. Also, 7 leaf-discs were placed under the glass slides at the start of every experimental phase and sampling of the leaf-discs was carried out at the end of the experiments.

2.2. Study design

Two experiments with nutrient enrichment were carried out in the laboratory flumes at WasserCluster Lunz am See, Lower Austria, between September 2016 and February 2017. The first experiment was carried out under complete darkness, “dark flumes”. The second experiment established shaded conditions, “shaded flumes” at the beginning and high-light conditions, “light flumes”, during the second part.

The first experiment ("dark flumes") was conducted under complete darkness for 5 weeks experimental phase (after 2 weeks of colonization) to exclude algal growth. For this reason, all flumes were covered by a dark nylon mesh to prevent or reduce algal growth.

For the second experiment, all flumes were exposed to a 14/10 h day/night cycle. Daylight was supplied by four fluorescent daylight lamps (38 W, Biolux OSTRAM, Germany), resulting to 100 µmol $m^{-2}s^{-1}$ photosynthetic-active radiation (PAR). The experiment was intended to analyse the interaction of heterotrophs and autotrophs by enabling algal growth. However, despite the light, there was hardly any algal growth detectable after 5 weeks' experimental phase. Due to time limitations, it was not possible to conduct a complete third experiment under improved light conditions. Nevertheless, lamps were exchanged with other fluorescent daylight lamps (36W, Philips; The Netherlands), which had proven to support algal growth efficiently in a prior experiment, and the experiment was prolonged for further 3 weeks. The additional "light phase" was intended to simulate sudden improved light availability for the benthic community, which may happen e.g. after removal of riparian vegetation. In order to distinguish between the two experiments and the two phases in the second experiment, the first experiment will be termed as "dark flumes", the first phase in the second experiment will be termed "shaded flumes", and the second phase as "light flumes".

During each experiment, four levels of nutrient enrichment (Table 1) were established in the flumes, with four replicates per treatment, amounting to 16 flumes in total. The different enrichment levels represented nutrient concentrations in streams flowing through catchments with different land use types. Nutrient loadings in streams have been reported to be strongly correlated with the land use type in the area which ultimately influence stream ecosystem processes (Castillo, 2010). Usually, streams bordering forests are expected to have low nutrient loadings while streams next to agricultural areas are expected to have the highest nutrient loadings, mainly from agricultural activities, for instance, the application of fertilisers (Castillo, 2010). The amount of nutrient enrichment used in this study was defined by recorded levels from land use types in Lower Austria (G. Weigelhofer personal communication, September 2016). For the low treatment, we used average concentrations found in pristine forested streams in Lower Austria, with the exception of nitrate which is usually around 900-1000 μgL^{-1} in these streams. This type was expected to represent nutrient-limiting conditions. The moderate and high enrichment levels represented concentrations in streams with pasture and mixed pasture/cropland use, respectively. In the "saturated" flumes, we tried to establish

saturated conditions by aiming at the highest nutrient concentrations ever found in streams in Lower Austria. Such streams are exclusively surrounded by cropland there.

Table 1 Experimental design for the four nutrients enrichment levels

μgL^{-1}	Low	Moderate	High	Saturated
NO_3-N	350	1300	5200	10000
NH_4-N	15	50	200	2000
PO_4-P	10	40	160	2000

2.3 Sampling scheme and frequencies

The sampling scheme for each experiment is shown in Table 2. Every experiment had two parts, a colonization and an experimental phases. The colonization took 2 weeks for both experiments and 0.95 mg/L of P-PO_4 was added to each flume at the start of the process to stimulate growth. The colonization of the first experiment lasted from 27th September 2016 to 11th October 2016, when the experimental phase started with nutrient additions for 5 weeks. Sampling during the first experiment was done between weeks 3 and 5 for slides and 4 and 5 for sediments (Table 2).

The colonization for the second experiment lasted from 22nd November to 6th December 2016. The following experimental phase lasted for 8 weeks in total, but was divided into a shaded phase for 5 weeks and a light phase for further 3 weeks (Table 2). Sampling was done between weeks 2 and 5 during the shaded phase and 1 and 3 during the preceding light phase.

At each sampling date, 2 replicates per flume of both glass slides and sand were taken for chlorophyll-*a* concentrations, pigment analyses and enzymatic activities. Extracellular enzyme activities were analysed in the fresh samples, while chlorophyll *a* samples (plus pigments) were frozen at -20^{o}C for later analysis.

Nutrient uptake measurements were carried out twice at the end of both experiments.

Leaf discs were exposed at the beginning of each experiment and were taken out of the flumes at the end of the dark and the shaded phase for weight loss analysis, respectively. In addition to weight loss, leave discs were also analysed as to enzyme activities. During the light phase, additional leaf discs were exposed in the flumes, which were, however, only analysed for enzymatic activities at the end of this phase.

During each experiment, biofilm surface area was determined of each sample for subsequent estimation of algal biomass and enzyme activities. Biofilm surface areas were calculated as the surface area in the substrata available for colonization (Artigas and Sabater, 2004). Glass slides had a surface area of 13.75 cm^2, leaf-discs had 4 cm^2 and sediments were sampled with a cylindrical tube 7.07 cm^2.

Table 2 Sampling schedule for parameters and sampled substrates during the different experiments and phases

Time	Chlorophyll *a*	Pigment diversity	Extracellular enzymes	Leaf decomposition	Nutrient uptake
Dark Flumes:					
Week 3	Slides	Slides	Slides		
Week 4	Sediments	Sediments	Sediments		
Week 5	Slides, sediments	Slides, sediments	Slides, sediments, leaf discs	x	x
Shaded Flumes:					
Week 2	Slides, sediments	Slides, sediments	Slides, sediments		
Week 4	Slides, sediments	Slides, sediments	Slides, sediments		
Week 5	Slides, sediments	Slides, sediments	Slides, sediments, leaf discs	x	x
Light Flumes:					
Week 1	Slides, sediments	Slides, sediments	Slides, sediments		
Week 3	Slides, sediments	Slides, sediments	Slides, sediments, leaf discs	x	x

2.4 Nutrient control

During the experimental periods of the study, 4.5 liters of water were added to each flume at the start of every experiment and NH_4-N, NO_3-N and PO_4-P were added according to the levels given in Table 1. Similarly, about 2.5 liters were replaced after every 4^{th} day during the entire time of the study period. After each water replacement, nutrient concentrations in the flumes were checked and nutrients were added again to achieve the desired levels in the flumes (Table 1). In addition, PO_4-P was controlled on a daily basis. For nutrient analyses, water was sampled with a syringe from each flume and was filtered using Whatmann GF/F filters. For analyses of PO_4-P, indophenol-blue method was used according to standard procedures (ALPHA 2005).

NH_4-N and NO_3-N were analyzed via Continuous Flow Analyzer (CFA, Systea Analytical Technology)

2.5 Laboratory analyses

2.5.1 Benthic algal community and biomass

The biomass and pigment diversity which characterize algal communities (Sabater *et al*., 2003) were measured during the study period. Algal biomass accumulation was assessed through chlorophyll *a* analysis in sediments and slides. Similarly, algal pigment diversity was assessed through HPLC analysis of slides and sediments samples during the second experiment. The first experiment had no detectable algal development and was excluded from the analysis.

2.5.1.1 Algal biomass on slides

For each sampling date, two glass slides were collected from each flume and placed into 50 mL centrifuge tubes and transported to the laboratory. The biofilms in each slide were scrapped off using sterile razor blades into clean 50 mL centrifuge tubes according to Steinman *et al*., (1996). Then, 15 mL autoclaved Well-water was added and the sample was vortexed for about 30 seconds. 2 mL of the samples were used for enzyme activity analysis and 10 mL for chlorophyll *a* analysis and 3 mL for bacterial abundances. Samples for chlorophyll *a* analysis were filtered through GF/C filters (Whatman) and the filters were frozen at -20^{o}C until analysis was done. During analysis, filters were cut into pieces and placed in glass vials and 7 mL of acetone was added. To ensure the samples were completely homogenized, each sample was sonicated (30 + 30 seconds, 1sec. /1sec., 30 amplitude; Digital Sonifier, W-250D, Branson, USA). The samples were kept in an airtight box for 24 h at 4^{o}C overnight in the dark for acetone extraction. The samples were then centrifuged for 10 min at 2500 rpm before supernatants were decanted with pipette and fluorescence was rapidly measured with the fluorometer (Hitachi, Japan) at 440nm excitation and 660nm emission wavelengths. Chlorophyll *a* was determined according to Steinman *et al.,* 1(996) using the equation

$$\text{Chla } (\mu g l^{-1}) = \frac{(((1.0935.em) - 0.1673) * vol.extract(L))}{V}$$

Where, V = volume of filtered sample [L] / sediment dry weight [g] / area [cm^2]
em = absorbance, vol.extract = volume of acetone used in the sample.

Chlorophyll *a* was determined by subtracting the second measurement (Phaeophytin) from the first measurement (Chlorophyll *a* + Phaeophytin). Second measurement was obtained by

adding 100µL 0.1N Hydrochloric acid to the samples in the cuvette and gently agitating. Hydrochloric acid converted chlorophyll a to phaeophytin in the second measurement.

2.5.1.2 Algal biomass in sand samples

For sediment sampling, 2 replicates were taken from each flume using a cylindrical tube. The sand samples were directly transferred to 50 ml plastic tubes and supernatants were carefully removed. 3 g of the samples were used for analysis of enzyme activities, 1g bacterial abundance and 10 g for chlorophyll *a* analysis. Samples for chlorophyll *a* analysis were then frozen at -20^{o}C until analysis was done. On the day of analysis, frozen samples were unfrozen for one hour at room temperature and oven-dried at 70^{o}C for 12 hours to obtain sediment dry weight. Then, 10 mL 90 % acetone were added to each dried sample. To ensure complete extraction of chlorophyll, samples were sonicated (2 + 2 minutes, 1sec. / 1sec., 65 amplitude; Digital Sonifier, W-250D, Branson, USA). Then, the samples were put in airtight box for 24 h at 4^{o}C for acetone extraction. After that, samples were centrifuged at 2500 rpm for 10 minutes and measurements were made with the fluorometer at 440nm/660nm wavelengths. Similar equation as one described above for slides' algal biomass was used to estimate chlorophyll *a* concentrations in the sediments.

2.5.1.3 Pigment diversity in slides and sediment samples

For pigment diversity analysis, 1.2 mL of acetone extracts samples for chlorophyll *a* measurements were filled in HPLC vials. The acetone extracts were from both slides and sediments samples. The samples were then kept in small plastics box and frozen at -80^{o}C to minimise pigment degradation before analysis via high-performance liquid chromatographic (HPLC) (HPLC system, Shimadzu).

2.5.2 Extracellular enzyme activities

Colonized glass slides, sediments and leaf-discs were measured separately for enzyme activities under spectrophotometer with a 96-well microplate reader (Varioskan Flash; Thermo Fisher Scientific, USA). On every sampling date, the activities of four hydrolytic enzymes (β-glucosidase, β-xylosidase, leucine-aminopeptidase and phosphatase) involved in the degradation of organic matter (OM) were measured on each substratum (slides, sediments and leaf-discs; 2 replicates per substratum). Phosphatase (EC 3.1.3.1-2), β-glucosidase (EC 3.2.2.21) and xylosidase (EC 3.2.1.37) were measured using methylumbelliferyl (MUF)-linked substrates. However, leucine-aminopeptidase (EC 3.4.1.11) was measured with minomethylcoumarin (AMC) substrates (Romani & Sabater, 2001). The blanks for each

enzyme activity on the substrata were also analysed. The substrates were prepared in advance and frozen at -20^{o}C before date of analysis. On, the day of analysis, the substrates were defrozen at room temperature 1 hour before additions in the samples were made.

Enzyme activities were determined with fresh samples on every sampling date. The MUF and AMC standard rows were prepared fresh a day before analysis. The assayed extracellular enzyme activities were associated with the use of algal exudates and as well as decomposition of leaf-discs by microorganisms (Sabater *et al*., 2011).

2.5.2.1 Enzyme activities on glass slides

For glass slides, 2 mL of the 15 mL biofilm suspension (described above in chlorophyll *a* section), were transferred into15 mL Eppendorf tubes and samples were homogenized by vortex for about 30 seconds. 400 µL from each sample within the treatments was pipetted into 15 mL tube to prepare homogenate for each treatment as a base for the standard row. 200 µL of homogenate per treatment was pipetted into the wells. Then, 200 µL of the samples were pipetted into the Wells on the plates' sections designated for phosphatase, β-glucosidase, β-xylosidase and leucine-aminopeptidase. Immediately afterwards, 50 µL of MUF and AMC standard concentrations were added on homogenate samples on Wells. Similarly, MUF-Glucosidase, MUF-Phosphatase, MUF-Xylosidase and AMC-Leucine-aminopeptidase substrates added into the samples in the Wells. Fluorescence was measurement within 5 minutes of substrate addition at 365 nm excitation and 450 nm emission wavelength for MUF substrates and 380 nm and 440 nm (excitation/emission) for AMC substrates. The samples were incubated for another 1 hour before a second reading was made within similar fluorescence and excitation wavelengths. Activity was calculated as either µmolMUF for MUF substrates or µmolAMC for AMC substrates per unit of time and surface area according to Artigas and Sabater (2004).

2.5.2.2 Enzyme activities on sediments

For sand, 3 g of sediments from each of 2 replicates per flume were weighed into 15 mL Eppendorf tube. The samples were carried to the lab and 5 mL of autoclaved water was added. The samples were then homogenized via vortex for approximately 1 minute. Thereafter, the samples were pipetted into the plates' Well and measurements made using similar procedure as described above for glass slides.

2.5.2.3 Enzyme activities on leaf-discs

Enzymatic activities on leaf-discs were determined by carefully retrieving 2 pieces (replicates) of leaf-discs from each flume into 15 mL Eppendorf tubes. The samples were carried to the lab and 5 mL of autoclaved water was added to each sample. The samples were then homogenized via vortex for approximately 1 minute. Similar procedure as for glass slides (described above) was followed to measured activities on the leaf-discs. Leaf-discs were sampled only at the ends of dark flumes, shaded flumes and light flumes periods.

2.5.3 Bacterial abundances

Due to time limitations, samples for bacterial abundances were fixed with 37% formaldehyde and stored at 4°C in the dark for later analyses. Thus, no data on bacterial abundances are shown in this thesis.

2.5.4 Leaf decomposition

To test for leaf decomposition (combination of physical breakdown, leaching of dissolved components and microbial decomposition), Hazelnut (*Corylus sp.*) was used. Hazelnut (*Corylus sp.*) leaves were harvested in fall and stored in the fridge before being used in the study. Hazelnut is the most common tree around the institute and dominates the riparian vegetation (Omondi, personal observation). Several leaf-discs measuring 2×2 cm were made and average weight for every leaf-disc was determined by weighing 40 leaf-discs and oven-dried at 60 ^{o}C for 8 hours and average dry weight was calculated. The average value was used as the initial dry weight of each leaf-discs at the start of each experiment. To have the discs anchored in the flumes, 7 leaf-discs were placed under the glass slides to avoid being washed by current at the beginning of 'dark phase' and second experiment. At the end of the experiments, 3 leaf-discs were carefully retrieved from the flumes into 50 mL vials and transported to the laboratory. In the laboratory, the leaf-discs were carefully scratched and rinsed with water to removed biofilm attachments. Afterwards, the leaf-discs were oven dried at 60 ^{o}C for 8 hours before final dry weight was determined for the discs from each treatment. Decomposition rate was expressed as the decay rate coefficient, *k*, per day (kd^{-1}) according to Edmonds (1991) using the equation $y = e^{-kt}$, whereby y = final dry weight/ initial dry weight, and t = time in days.

2.5.5 Nutrient uptake

Nutrient uptake was measured by addition of PO_4-P, NH_4-N and NO_3-N pulses at the end of the first and second experiment according to concentrations in Table. After additions, 14 mL

of water samples were taken after 30 min, 1 hour and 2 hours and 3 hours into 15 mL Eppendorf tubes and kept in the fridge at 4^{o}C until analysis. The samples were measured using a continuous flow analyser (CFA, Systea Analytical Technology). Uptake rates were calculated through regression coefficient analysis of concentrations in the flumes through the sampling intervals.

2.6 Statistical analysis

Normal distribution of the data was tested by Kolmogorov-Smirnov test. Subsequently, 2-way ANOVA was performed to determine influence of nutrient enrichment and light on algal biomass, algal pigment composition, enzyme activities and leaf decomposition. Also, Spearman non-parametric correlation was performed to assess the influence of algal biomass on enzyme activities. All the analyses were considered significant at $p < 0.05$. Statistical analysis was performed using IBM SPSS statistics 21. (SPSS Inc., Chicago, Illinois, USA). All the graphs were plotted by Sigma plot version 11 from Systat software, inc., San Jose California, USA.

3.0 Results

3.1 Algal biomass in dark, shaded and light flumes

Even after 5 weeks, dark flumes had algal biomass below detection limit. Therefore, dark flumes were excluded from further statistical analyses regarding both chlorophyll-a concentrations and pigment diversity.

In order to show the effects of improved light availability on an already established biofilm community, only the last week of the shaded phase (week 5) was compared with the last week of the light phase (week 3). Comparisons of week 5 in the shaded flumes and week 3 in the light flumes showed that light significantly enhanced algal biomass, while nutrient enrichment had no significant effects on algal biomass in either the shaded or the light flumes (Figure 1&2; Table 3). For glass slides, shaded flumes had mean chlorophyll *a* of 1.17 ± 0.32 g/cm^2 in week 5, while light flumes had 3.89 ± 0.44 µg/cm^2 in week 3. Similarly for sand, shaded flumes had mean chlorophyll *a* of 0.20 ± 0.004 g/cm2 in week 5, while light flumes had 0.41 ± 0.005 gcm^{-2}in week 3. While there was a clear increase in algal biomass between week 2 and 5 in the shaded flumes, there was no observable difference in algal biomass between week 1 and 3 in the light flumes (Figures 1b & 2b).

Table 3 2-way ANOVA for effects of nutrient enrichment and light on algal biomass in shaded and light flumes (n =32 per experiment).

Substrata	Source	df	F	Sig.
Slides	Model	7	3.31	0.057
	Intercept	1	68.95	**<0.001**
	Nutrient enrichments	3	0.20	0.896
	Light	1	19.95	**0.002**
	Nutrient enrichments * Light	3	0.89	0.489
Sediments	Model	7	2.07	0.164
	Intercept	1	80.60	**<0.001**
	Nutrient enrichments	3	1.49	0.289
	Light	1	9.70	**0.014**
	Nutrient enrichments * Light	3	0.12	0.949

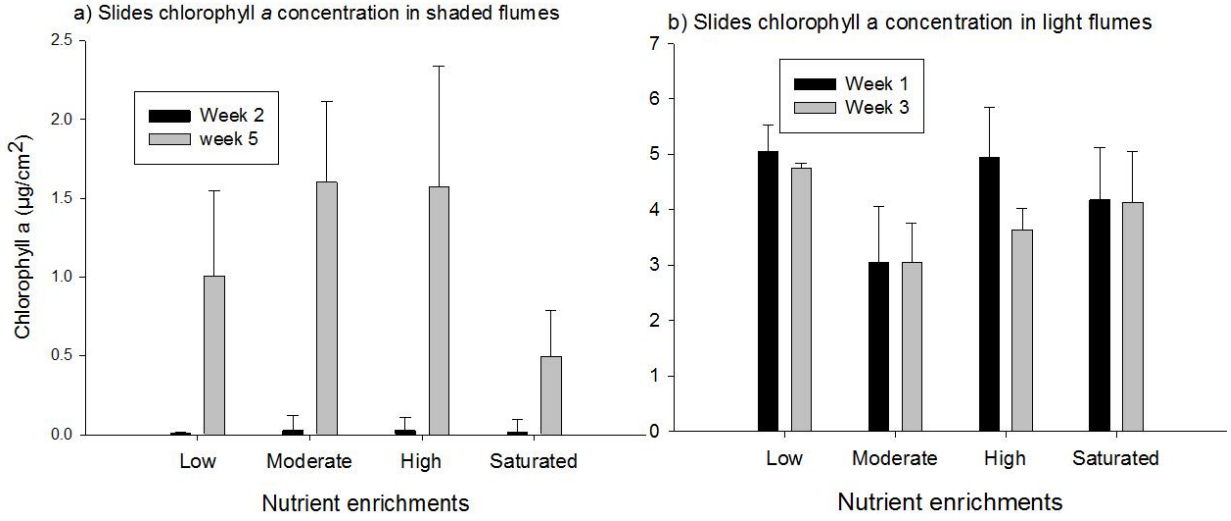

Figure 1 Chlorophyll - *a* concentration on glass slides in flumes with different nutrient enrichment levels during shaded conditions and under optimal light conditions in experimental flumes (mean +/- standard deviation; n = 8).

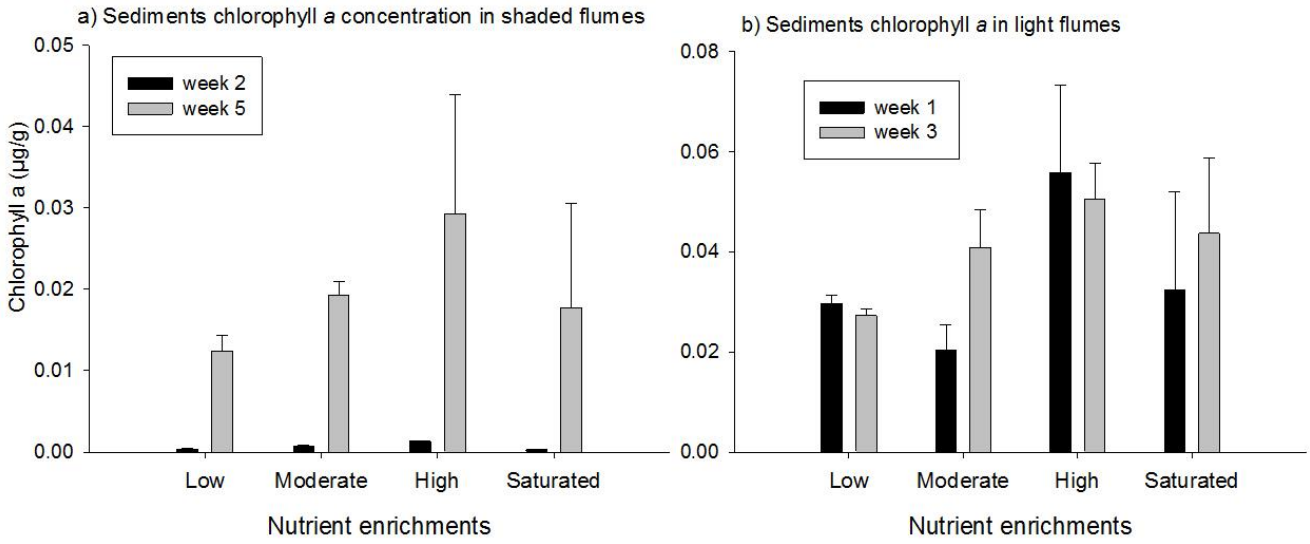

Figure 2 Chlorophyll - *a* concentration on sediments in flumes with different nutrient enrichment levels during shaded conditions and under optimal light conditions in experimental flumes (mean +/- standard deviation; n = 8)

3.2 Pigment diversity

Generally, algal pigments were similar throughout the study period (Figures 3, 4, 5 & 6). However, chlorophyll *a* dominated algal pigments during the whole study period (Figures 3, 4, 5 & 6). Nutrient enrichment did not have influence on algal pigment composition (Table 4). Light slightly decreased chlorophyll *a* and Fucoxanthin pigments in light flumes (Figure 5 & 6). However, the composition of algal pigments in light flumes was similar to that in shaded flumes with chlorophyll *a* being the most abundant (Figures 5 & 6). Thus, there was no difference in algal composition between shaded and light flumes (Figures 3, 4, 5 & 6). Pooled values of light and nutrients did not have significant effect on algal pigment composition. The pigments are indicators of algal groups within the flumes during the study period. For instance, photosynthetic pigments chlorophyll *a*, *c* and accessory pigments fucoxanthin neoxanthin and violaxanthin are indicators for diatoms (Bonilla *et al.*, 2005). Similarly, occurrence of green algae were shown by photosynthetic pigments of chlorophyll *a*, *b* with carotenoid and xanthophylls as accessory pigments (Przytulska *et al*., 2016). Also, cyanobacteria presence was shown by chlorophyll *a* and *c* as photosynthetic pigments and β-carotene as accessory pigment (Takaichi, 2011).

Table 4 2-way ANOVA for effects of nutrient enrichment and light on algal pigment composition in shaded and light flumes (n =32 per experiment)

Source	Dependent Variable	df	F	Sig.
Model	Chlorophyll a	7	1.19	0.403
	Chlorophyll b	7	1.66	0.247
	Chlorophyll c	7	2.07	0.165
	Fucoxanthin	7	8.83	0.003
	Diadinoxanthin	7	1.83	0.207
	Beta carotene	7	0.18	0.981
	Violaxanthin	7	0.18	0.981
Intercept	Chlorophyll a	1	400.03	<**0.001**
	Chlorophyll b	1	178.96	<**0.001**
	Chlorophyll c	1	211.56	<**0.001**
	Fucoxanthin	1	1110.44	<**0.001**
	Diadinoxanthin	1	249.42	<**0.001**
	Beta carotene	1	150.50	<**0.001**
	Violaxanthin	1	487.10	<**0.001**
Light	Chlorophyll a	1	5.491	**0.047**
	Chlorophyll b	1	3.84	0.086
	Chlorophyll c	1	0.78	0.401
	Fucoxanthin	1	48.154	<**0.001**
	Diadinoxanthin	1	3.07	0.118
	Beta carotene	1	0.30	0.600
	Violaxanthin	1	0.19	0.673
Nutrient enrichments	Chlorophyll a	3	0.47	0.714
	Chlorophyll b	3	0.27	0.846
	Chlorophyll c	3	2.60	0.124
	Fucoxanthin	3	3.14	0.087
	Diadinoxanthin	3	1.65	0.254
	Beta carotene	3	0.19	0.899
	Violaxanthin	3	0.22	0.882
Light * Nutrient enrichments	Chlorophyll a	3	0.48	0.707
	Chlorophyll b	3	2.32	0.152

	Chlorophyll c	3	1.97	0.198
	Fucoxanthin	3	1.41	0.309
	Diadinoxanthin	3	1.60	0.264
	Beta carotene	3	0.13	0.937
	Violaxanthin	3	0.15	0.927

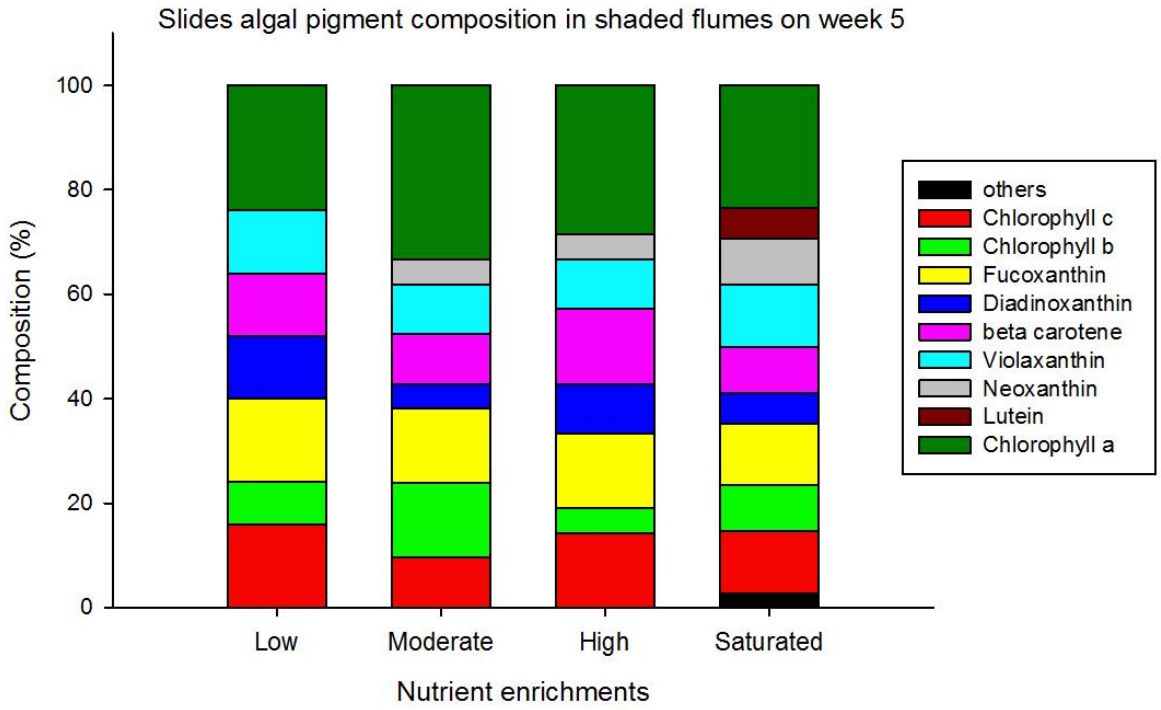

Figure 3 Algal pigments composition on slides at the end of shaded flumes experiment in 5th week for low, moderate, high and saturated nutrient levels.

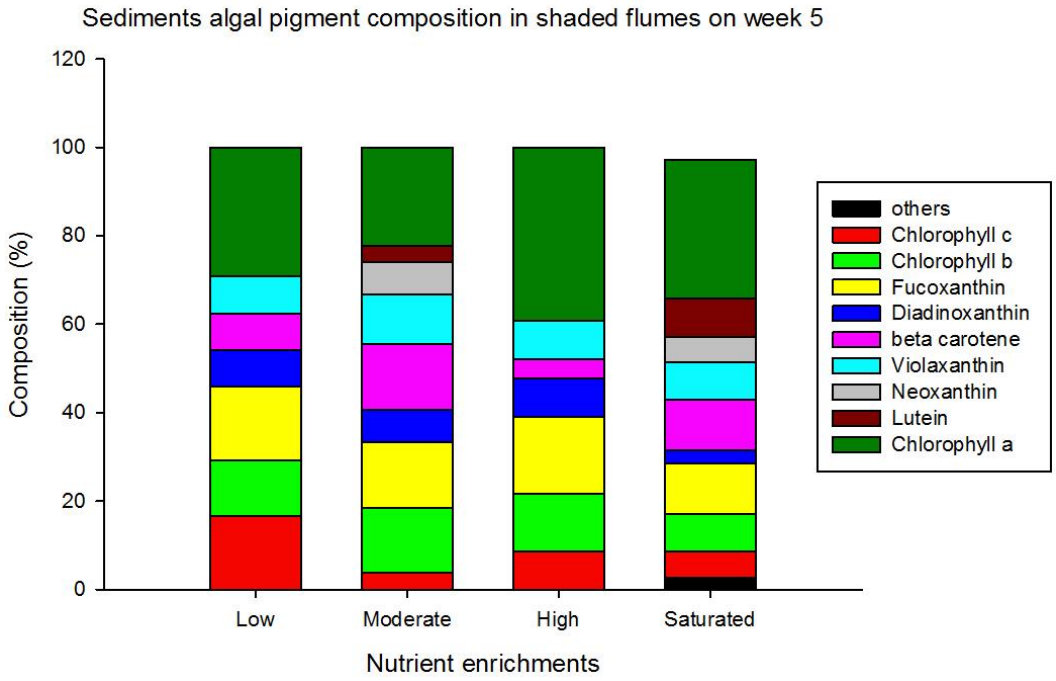

Figure 4 Algal pigments composition on sediments at the end of shaded flumes experiment on 5th week for low, moderate, high and saturated nutrient levels.

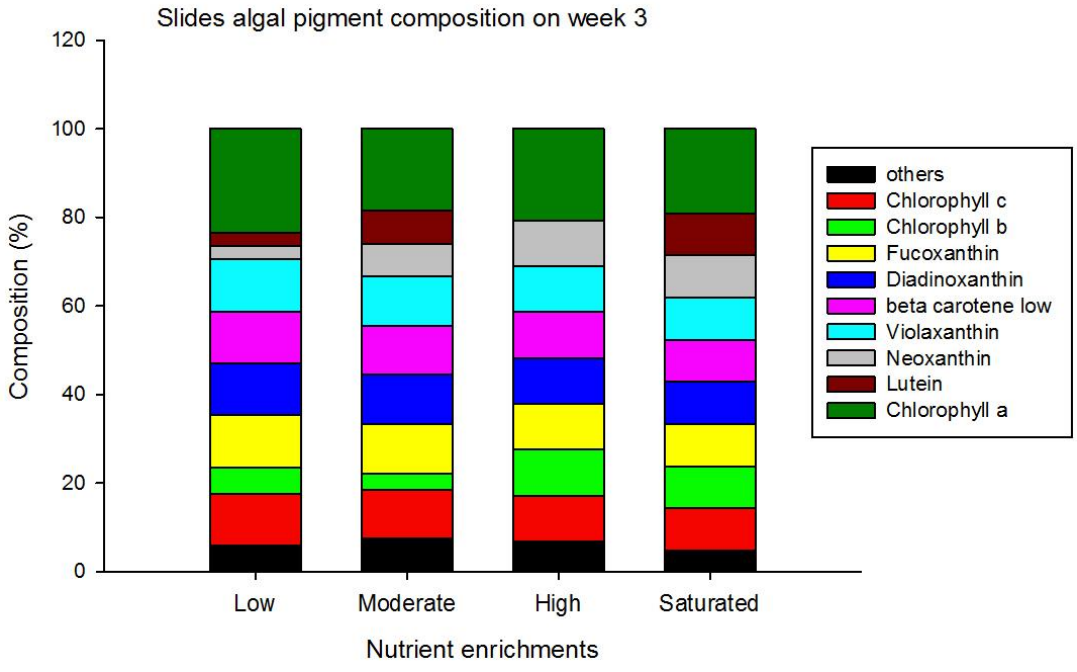

Figure 5 Algal pigments composition on slides at the end of light flumes experiment on 3rd week of light flumes experiment for low, moderate, high and saturated nutrient levels.

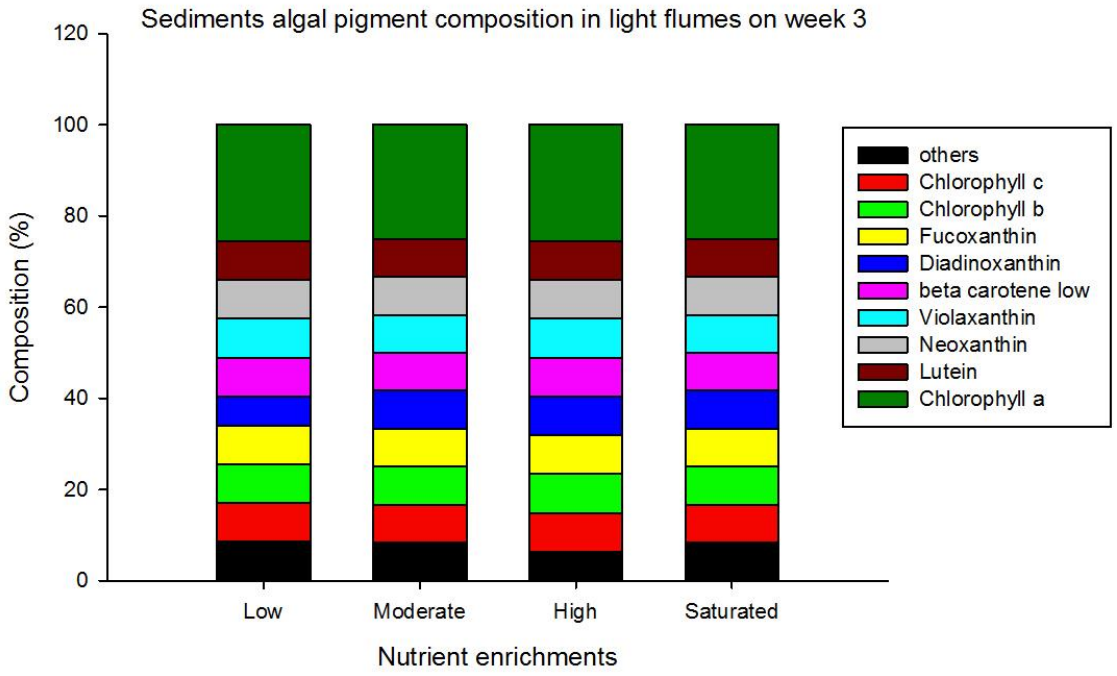

Figure 6 Algal pigments composition on sediments at the end of light flumes experiment on 3rd week of light flumes study for low, moderate, high and saturated nutrient levels.

3.3 Enzyme activities

3.3.1 Comparison of dark and shaded flumes

The statistical comparison of dark and shaded flumes was performed only with data from week 5, as they were supposed to represent a fully developed benthic community in both experiments. For slides, nutrient enrichment had no influence on phosphatase and β-glucosidase activities (Figure 7a & 8a; Table 5). However leucine-aminopeptidase decreased with nutrient enrichments in dark flumes, while β-xylosidase was lowest in saturated flumes on week 5 (Figure 7b; Table 5). Though not statistically tested, there was also observed increase in leucine-aminopeptidase with nutrient enrichment in shaded flumes on week 2 (Figure 8a). Also, phosphatase activity was highest in moderate flumes on week 2. For sediments, there was no influence of nutrient enrichment on enzyme activities (Figure 9 &10; Table 5). For leaf-discs, leucine-aminopeptidase was highest in highly enriched flumes in dark flumes (Figure 11). There was no influence of nutrient enrichment on enzyme activities in shaded flumes (Figure 12; Table 5).

Light had significant influence on activities of all enzymes studied on slides (Table 5). For instance, phosphatase activity increased in shaded flumes compared to dark flumes. In contrast, β-glucosidase, β-xylosidase and leucine-aminopeptidase decreased in shaded flumes compared

to dark flumes (Figures 7a&8a). For sediments light decreased activity of β-xylosidase and increased leucine-aminopeptidase activity in shaded flumes (Table 5). Pooled values of nutrients and light had no influence on enzyme activities on sediments, however for slides, β-glucosidase and β-xylosidase activities were enhanced by nutrients and light (Table 5).

Table 5 2-way ANOVA for effects of nutrient enrichment and light on the activity of extracellular enzyme in dark and shaded flumes (week 5) (n =32 per experiment)

Substrata	Source	Dependent Variable	df	F	Sig.
Slides	Model	Phosphatase	7	6.86	**0.007**
		β-glucosidase	7	2.267	0.137
		β-xylosidase	7	62.57	**<0.001**
		Aminopeptidase	7	10.77	**0.002**
	Intercept	Phosphatase	1	34.76	**<0.001**
		β-glucosidase	1	31.86	**<0.001**
		β-xylosidase	1	346.93	**<0.001**
		Aminopeptidase	1	131.50	**<0.001**
	Nutrient enrichments	Phosphatase	3	2.63	0.122
		β-glucosidase	3	0.92	0.474
		β-xylosidase	3	36.53	**<0.001**
		Aminopeptidase	3	5.22	**0.027**
	Light	Phosphatase	1	32.13	**<0.001**
		β-glucosidase	1	10.16	**0.013**
		β-xylosidase	1	245.29	**<0.001**
		Aminopeptidase	1	40.12	**<0.001**
	Nutrient enrichments	Phosphatase	3	2.67	0.118
	* Light	Glucosidase	3	0.98	0.448
		β-glucosidase	3	27.71	**<0.001**
		β-xylosidase	3	6.55	**0.015**
		Aminopeptidase	3	3.17	0.131
Sediments	Model	Phosphatase	6	4.49	**0.035**
		β-glucosidase	6	1.17	0.414
		β-xylosidase	6	2.35	0.144
		Aminopeptidase	6	18.23	**0.001**
	Intercept	Phosphatase	1	96.24	**<0.001**

		β-glucosidase	1	54.25	**<0.001**
		β-xylosidase	1	18.04	**0.004**
		Aminopeptidase	1	115.01	**<0.001**
	Nutrient enrichments	Phosphatase	3	1.30	0.347
		β-glucosidase	3	1.99	0.205
		β-xylosidase	3	1.50	0.295
		Aminopeptidase	3	1.50	0.295
	Light	Phosphatase	1	17.26	**0.004**
		β-glucosidase	1	0.17	0.690
		β-xylosidase	1	4.54	0.071
		Aminopeptidase	1	87.50	**<0.001**
	Nutrient enrichments	Phosphatase	2	0.07	0.984
	* Light	β-glucosidase	2	0.51	0.622
		β-xylosidase	2	3.55	0.086
		Aminopeptidase	2	2.16	0.186
Leaf-discs	Model	Phosphatase	7	2.71	0.093
		β-glucosidase	7	1.42	0.316
		β-xylosidase	7	1.48	0.296
		Aminopeptidase	7	3.72	**0.043**
	Intercept	Phosphatase	1	32.65	**<0.001**
		β-glucosidase	1	31.14	**0.001**
		β-xylosidase	1	10.04	**0.013**
		Aminopeptidase	1	115.10	**<0.001**
	Nutrient enrichments	Phosphatase	3	1.16	0.385
		β-glucosidase	3	2.56	0.128
		β-xylosidase	3	1.32	0.334
		Aminopeptidase	3	2.97	0.097
	Light	Phosphatase	1	14.28	**0.005**
		β-glucosidase	1	0.91	0.367
		β-xylosidase	1	3.75	0.089
		Aminopeptidase	1	9.21	**0.016**
	Nutrient enrichments	Phosphatase	3	0.41	0.751
	* Light	β-glucosidase	3	0.44	0.732

	β-xylosidase	3	0.89	0.489
	Aminopeptidase	3	2.63	0.122

3.3.2 Comparison of shaded and light flumes

As for chlorophyll-a concentrations, only data from week 5 in shaded flumes were compared statistically with those from week 3 in light flumes for enzyme activities (Table 6). For slides, there was no significant influence of nutrients on enzymatic activities (Table 6). However, light increased activities of β-xylosidase and β-glucosidase in light flumes compared to shaded flumes (Figure 13b; Table 6). On 1st week of light flumes, there was no activity recorded for phosphatase, β-glucosidase, and, β-xylosidase in highly enriched and saturated flumes, instead, leucine-aminopeptidase dominated the activities (Figure 13a). For sediments, leucine-aminopeptidase increased with nutrient enrichment in light flumes on week 3 (Figure 14b; Table 6). Higher light exposure had significant increase on activities of all enzyme activities in light flumes compared to shaded flumes (Figure 14b; Table 6). For leaf-discs, nutrient enrichment increased β-xylosidase activity in light flumes (Figure 15; Table 6). Increased light availability significantly enhanced all enzymes studied in light flumes (Figure 15; Table 6).

Table 6 2-way ANOVA for effects of nutrient enrichment and light on the activity of extracellular enzyme in shaded and light flumes (week 5 vs 3) (n =32 per experiment)

Substrata	Source	Dependent Variable	df	F	Sig.
Slides	Model	Phosphatase	7	0.914	0.541
		β-glucosidase	7	0.992	0.498
		β-xylosidase	7	1.726	0.230
		Aminopeptidase	7	3.715	**0.043**
	Intercept	Phosphatase	1	12.820	**0.007**
		β-glucosidase	1	16.343	**0.004**
		β-xylosidase	1	18.675	**0.003**
		Aminopeptidase	1	17.910	**0.003**
	Nutrient enrichments	Phosphatase	3	0.698	0.579
		Glucosidase	3	0.495	0.696
		β-glucosidase	3	0.293	0.830
		β-xylosidase	3	1.550	0.275
		Aminopeptidase	3	2.74	0.138

	Light	Phosphatase	1	0.311	0.592
		β-glucosidase	1	3.344	0.105
		β-xylosidase	1	10.737	**0.011**
		Aminopeptidase	1	16.820	**0.003**
	Nutrient enrichments *	Phosphatase	3	1.331	0.331
	Light	β-glucosidase	3	0.706	0.575
		β-xylosidase	3	0.155	0.924
		Aminopeptidase	3	1.511	0.284
Sediments	Model	Phosphatase	6	3.890	**0.049**
		β-glucosidase	6	6.564	**0.013**
		β-xylosidase	6	11.373	**0.003**
		Aminopeptidase	6	15.46	**0.001**
	Intercept	Phosphatase	1	15.25	**0.006**
		β-glucosidase	1	34.93	**0.001**
		β-xylosidase	1	62.40	**<0.001**
		Aminopeptidase	1	75.29	**<0.001**
	Nutrient enrichments	Phosphatase	3	2.44	0.149
		β-glucosidase	3	2.86	0.114
		β-xylosidase	3	3.13	0.097
		Aminopeptidase	3	9.57	**0.007**
	Light	Phosphatase	1	12.38	**0.010**
		β-glucosidase	1	16.36	**0.005**
		β-xylosidase	1	40.38	**<0.001**
		Aminopeptidase	1	29.37	**0.001**
	Nutrient enrichments *	Phosphatase	2	2.83	0.126
	Light	β-glucosidase	2	3.26	0.100
		β-xylosidase	2	3.76	0.078
		Aminopeptidase	2	3.45	0.091
Leaf-discs	Model	Phosphatase	7	6.01	**0.011**
		β-glucosidase	7	7.19	**0.006**
		β-xylosidase	7	12.90	**0.001**
		Aminopeptidase	7	14.17	**0.001**
	Intercept	Phosphatase	1	114.67	**<0.001**

	β-glucosidase	1	63.93	**<0.001**
	β-xylosidase	1	105.27	**<0.001**
	Aminopeptidase	1	170.94	**<0.001**
Nutrient enrichments	Phosphatase	3	1.93	0.203
	β-glucosidase	3	0.63	0.614
	β-xylosidase	3	9.80	**0.005**
	Aminopeptidase	3	3.58	0.066
Light	Phosphatase	1	33.62	**<0.001**
	β-glucosidase	1	47.80	**<0.001**
	β-xylosidase	1	44.42	**<0.001**
	Aminopeptidase	1	82.49	**<0.001**
Nutrient enrichments *	Phosphatase	3	0.88	0.490
Light	β-glucosidase	3	0.22	0.879
	β-xylosidase	3	5.50	**0.024**
	Aminopeptidase	3	1.98	0.196

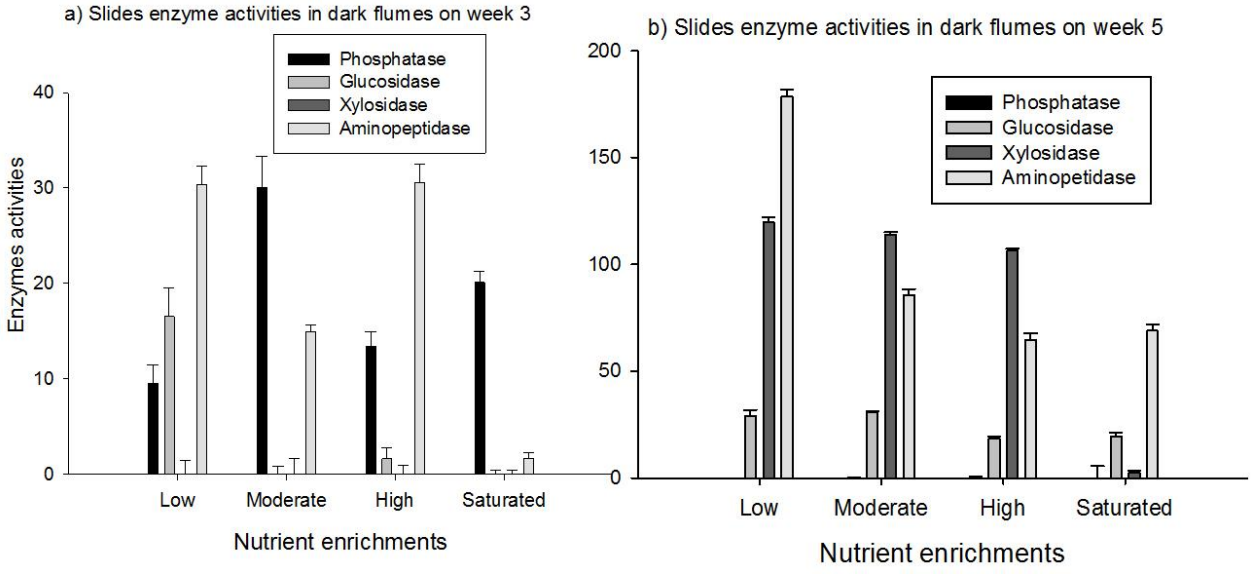

Figure 7 Enzyme activities on slides during dark flumes period of the study (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF $cm^{-2}h^{-1}$ while leucine-aminopeptidase is in µmol AMC $cm^{-2}h^{-1}$)

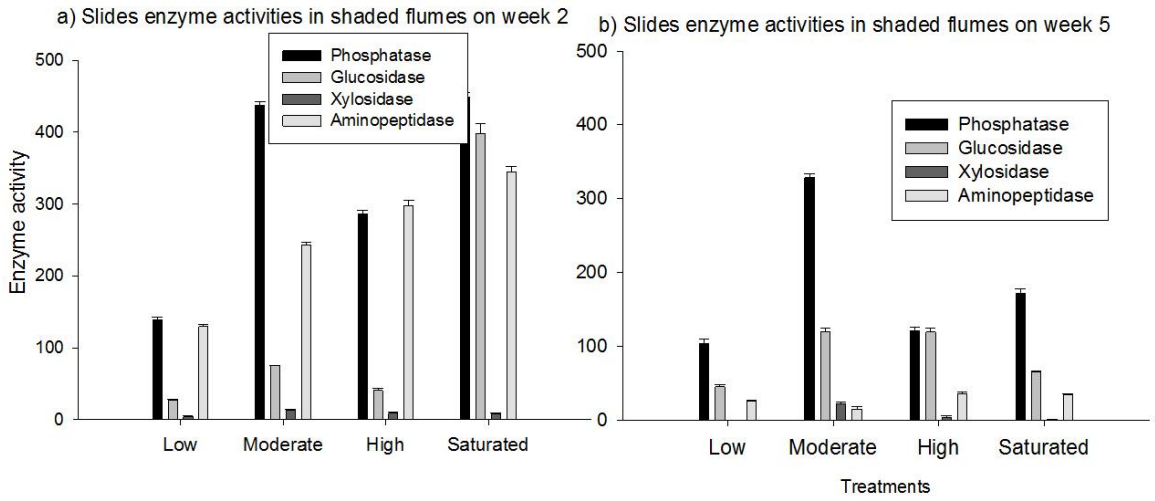

Figure 8 Enzyme activities on slides during shaded flumes period of the study (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF $cm^{-2}h^{-1}$ while leucine-aminopeptidase is in µmol AMC $cm^{-2}h^{-1}$)

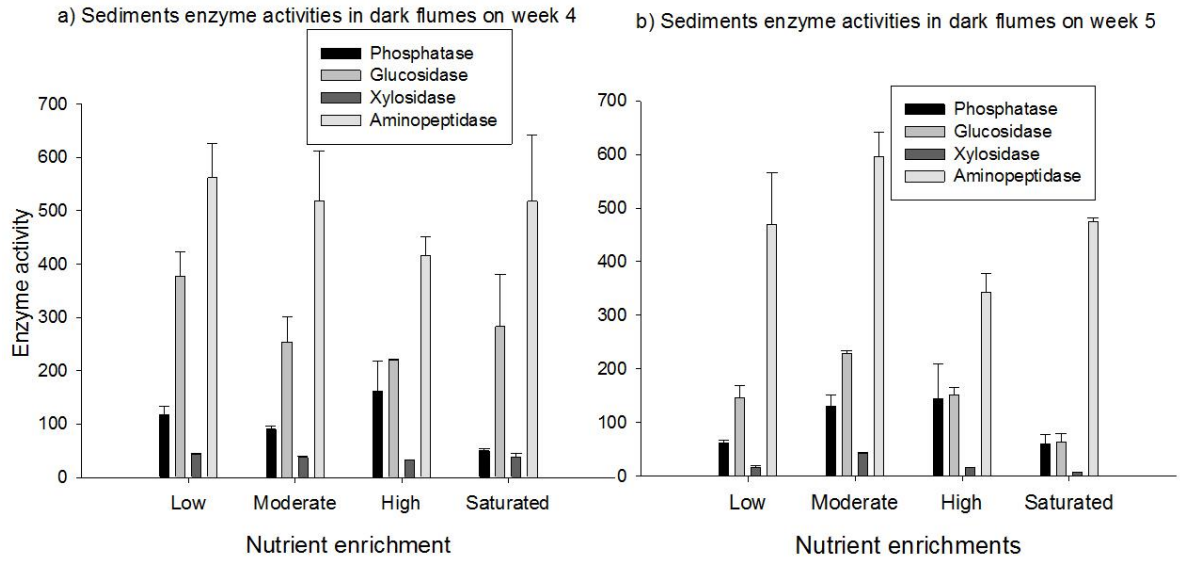

Figure 9 Enzyme activities on sediments during dark flumes period of the study (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF gh^{-1} while leucine-aminopeptidase is in µmol AMC gh^{-1})

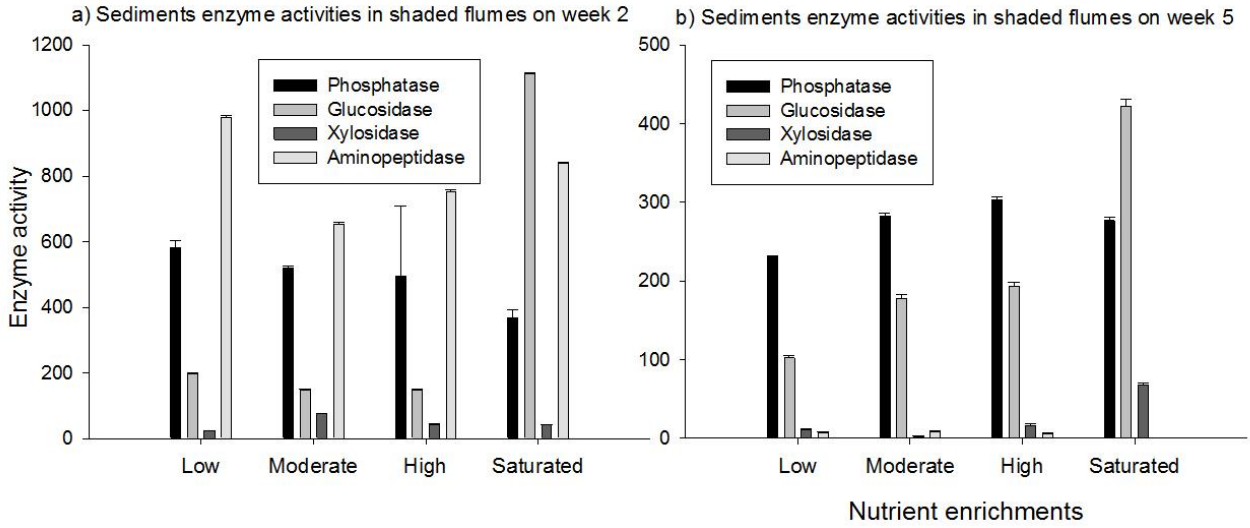

Figure 10 Enzyme activities on sediments during shaded flumes period of the study (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF gh^{-1} while leucine-aminopeptidase is in µmol AMC gh^{-1})

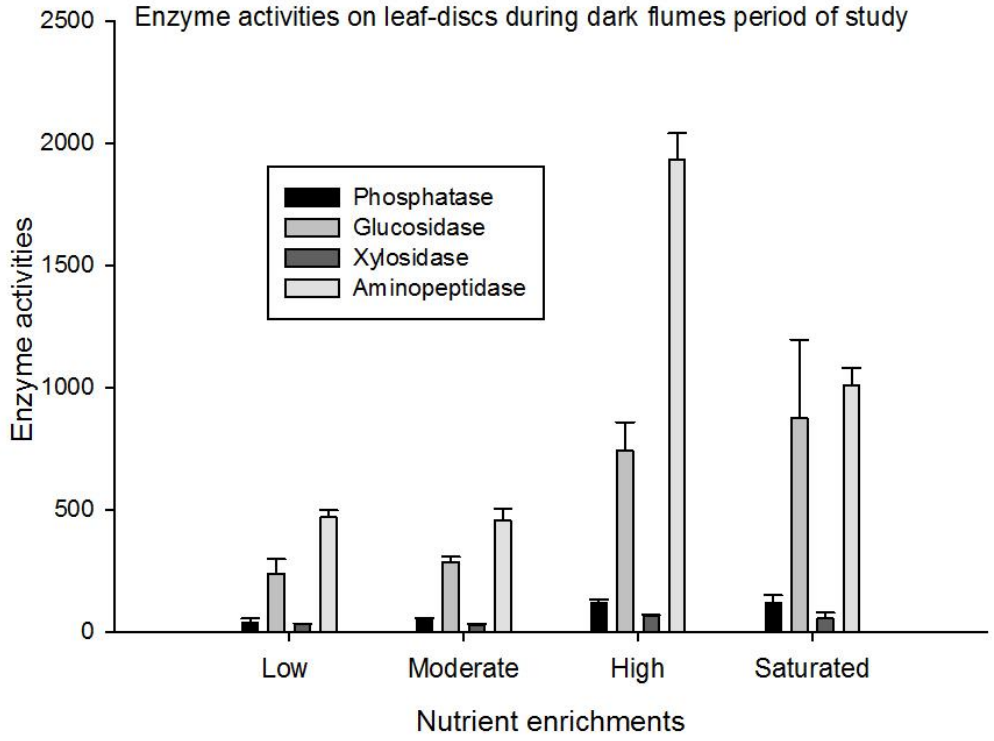

Figure 11 Enzyme activities on leaf-discs during dark flumes study period (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF $cm^{-2}h^{-1}$ while leucine-aminopeptidase is in µmol AMC $cm^{-2}h^{-1}$)

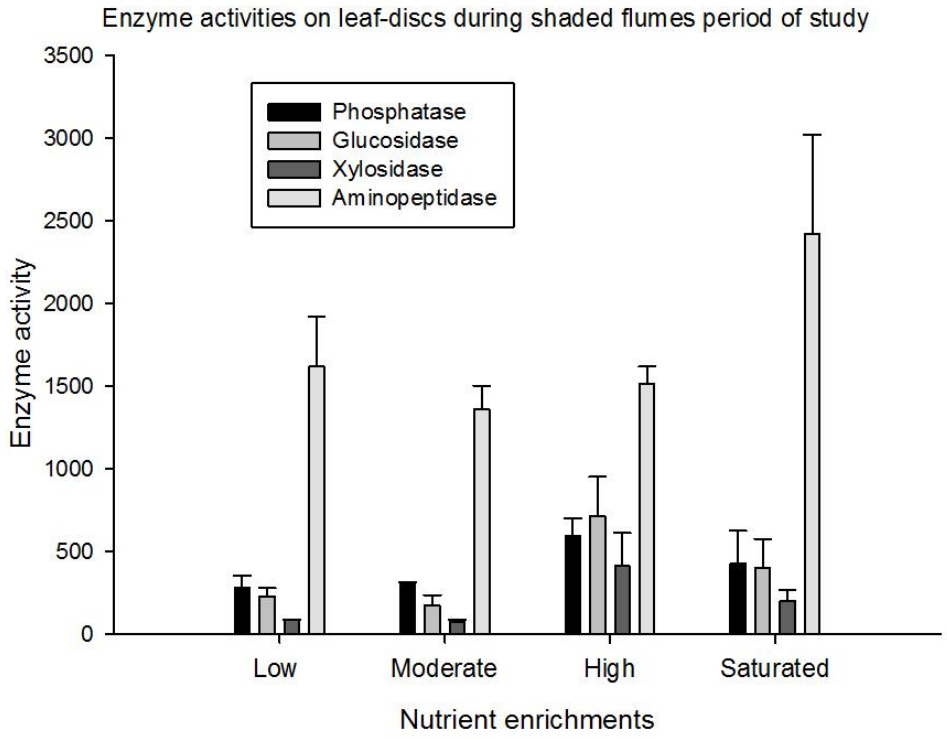

Figure 12 Enzyme activities on leaf-discs during shaded flumes study period (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF $cm^{-2}h^{-1}$ while leucine-aminopeptidase is in µmol AMC $cm^{-2}h^{-1}$)

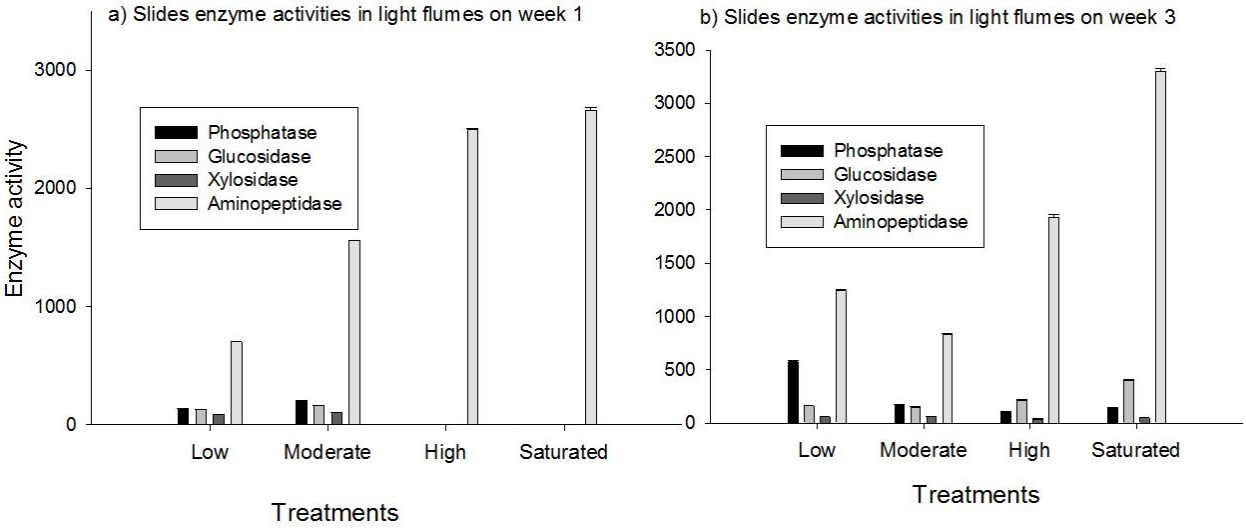

Figure 13 Enzyme activities on slides during light flumes period of the study (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF $cm^{-2}h^{-1}$ while leucine-aminopeptidase is in µmol AMC $cm^{-2}h^{-1}$)

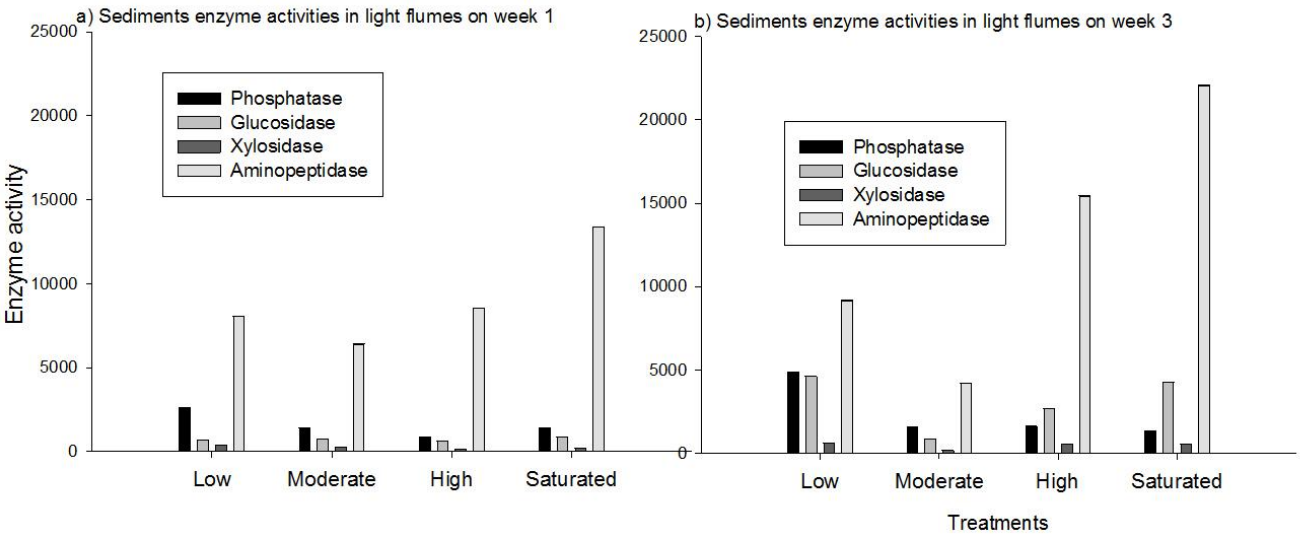

Figure 14 Enzyme activities on sediments during light flumes period of the study (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF gh^{-1} while leucine-aminopeptidase is in µmol AMC gh^{-1})

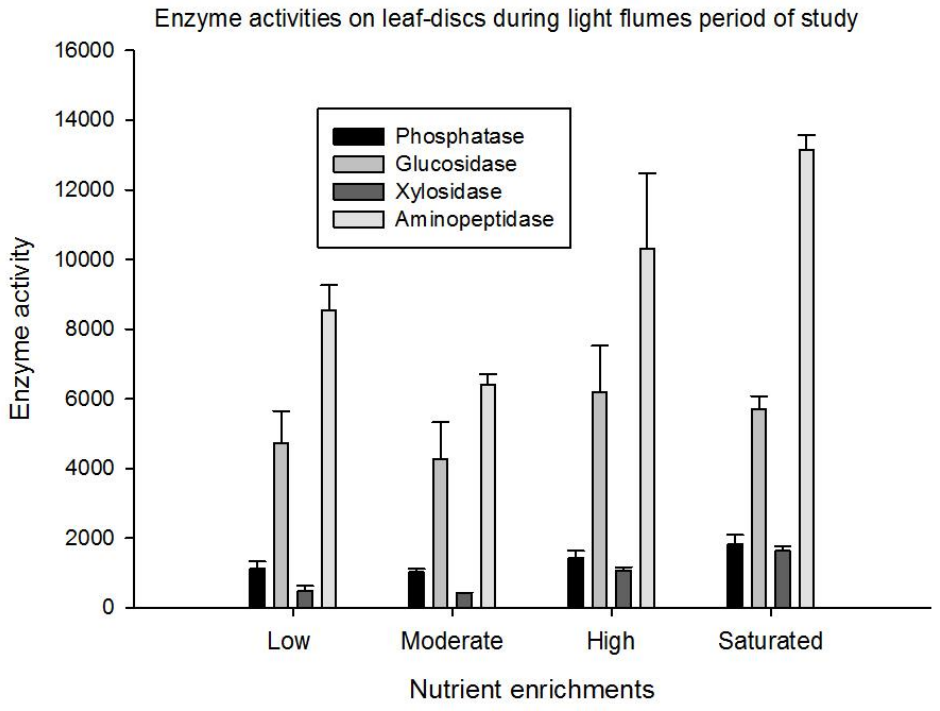

Figure 15 Enzyme activities on leaf-discs during light flumes study period (phosphatase, β-glucosidase and β-xylosidase are given in µmol MUF $cm^{-2}h^{-1}$ while leucine-aminopeptidase is in µmol AMC $cm^{-2}h^{-1}$)

3.3.3 Influence of algal biomass on enzyme activities

There was correlation between enzyme activities and algal biomass (Table 10). For instance in slides, β-xylosidase and leucine-aminopeptidase had significant correlation with algal biomass. Similarly for sediments, β-glucosidase, β-xylosidase and leucine-aminopeptidase had positive correlation with algal biomass (Table 7).

Table 7 Spearman's non-parametric correlation between enzyme activities and algal biomass in each treatment

Substrate	Enzyme	N	p-value
Slides	Phosphatase	38	0.068
	β-glucosidase	38	0.164
	β-xylosidase	38	**0.003**
	aminopeptidase	38	**<0.001**
Sediments	Phosphatase	32	0.080
	β-glucosidase	32	**0.011**
	β-xylosidase	32	**0.005**
	aminopeptidase	30	**<0.001**

3.5 Leaf decomposition

Nutrient enrichment and light did not enhance leaf decomposition (Table 8). Similarly, nutrient and light pooled together failed to have influence on leaf decomposition (Table 8). There was no difference in leaf decomposition within nutrient levels with light (Figure 19).

Table 8 2-way ANOVA for effects of nutrient enrichment and light on leaf decomposition in dark and shaded flumes (week 5) (n = 32 per experiment)

Source	df	F	Sig.
Model	7	1.147	0.421
Intercept	1	96.975	**<0.001**
Nutrient enrichments	3	1.539	0.278
Light	1	0.001	0.973
Nutrient enrichments * Light	3	1.138	0.391

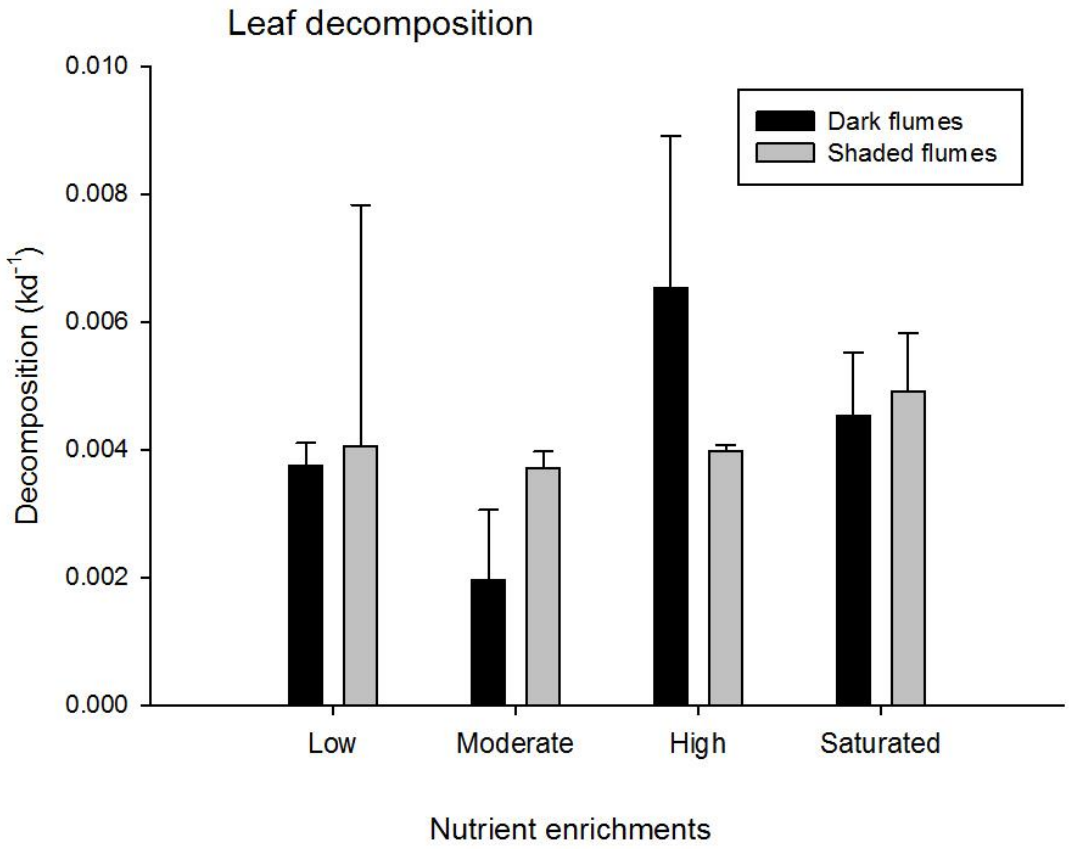

Figure 16 Mean (+ SD) leaf-discs decomposition in dark and shaded flumes. The units are given in decay coefficient per day (kd^{-1})

3.6 Nutrient uptake

$N\text{-}NO_4$ decreased with time in the dark flumes which demonstrated uptakes in the flumes (Figure 17b). However, $N\text{-}NO_3$ and $P\text{-}PO_4$ concentrations in the dark flumes were relatively stable after pulse additions (Figure 17a&c). Also for light flumes, $N\text{-}NO_3$ and $N\text{-}NO_4$ were relatively stable while $P\text{-}PO_4$ concentrations decreased with time suggesting uptake of $P\text{-}PO_4$ by the biofilms (Figure 18). Similar pattern explained for nutrient concentration was found in nutrient uptake rates. For instance, the results showed that $N\text{-}NH_4$ and $P\text{-}PO_4$ were assimilated by biofilms while $N\text{-}NO_3$ accumulated in the flumes during the study period for both dark and light flumes. (Table 10&11). Nevertheless, dark flumes had more $N\text{-}NH_4$ uptake than $P\text{-}PO_4$ and the uptakes were mostly in highly enriched and saturated flumes (Table 10). Similarly, light flumes showed higher $P\text{-}PO_4$ in saturated flumes compared to other enriched flumes but the highest value for $N\text{-}NO_4$ was recorded in highly enriched flumes (Table 11).

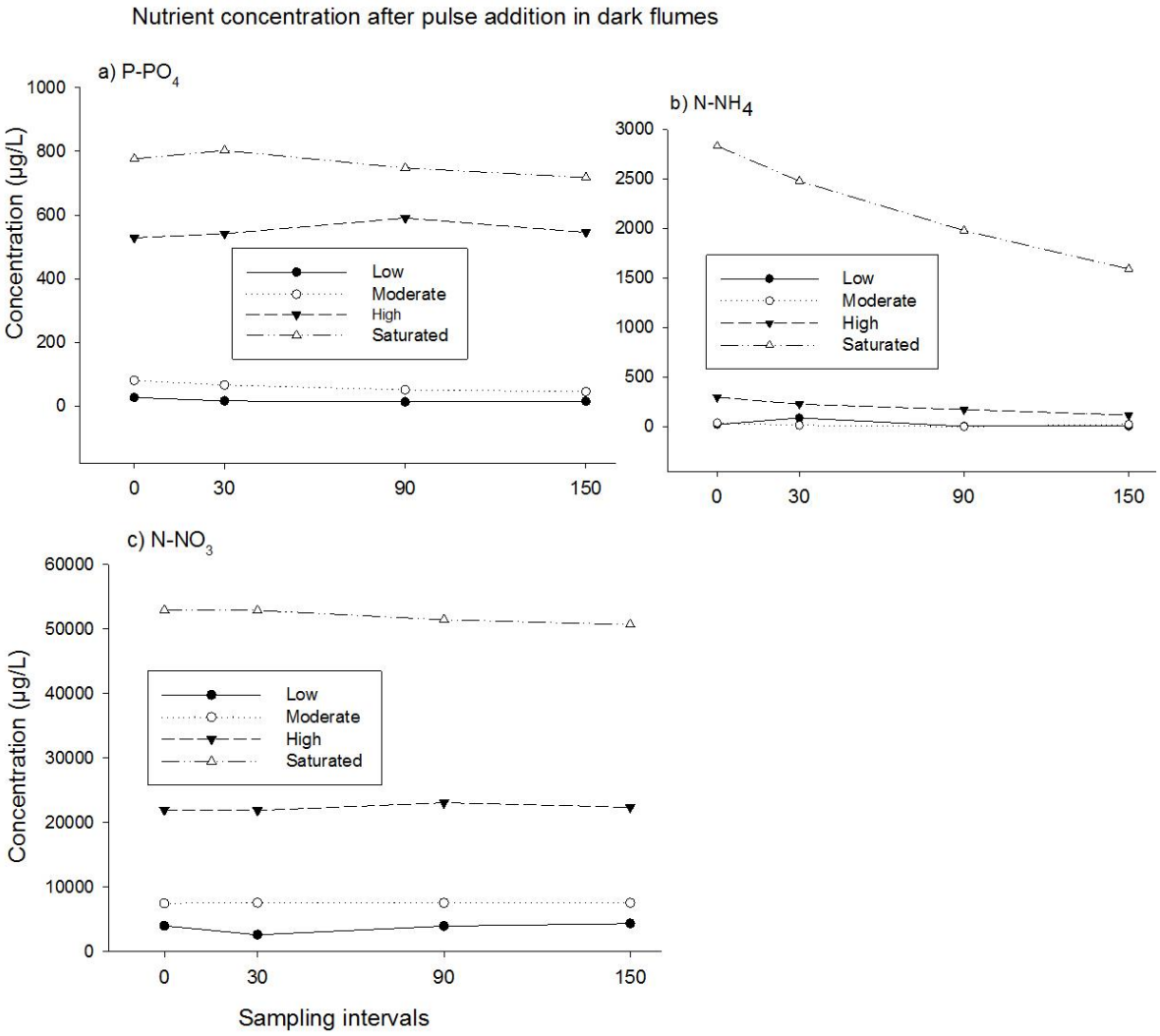

Figure 17 Nutrient concentrations in dark flumes after addition of pulse

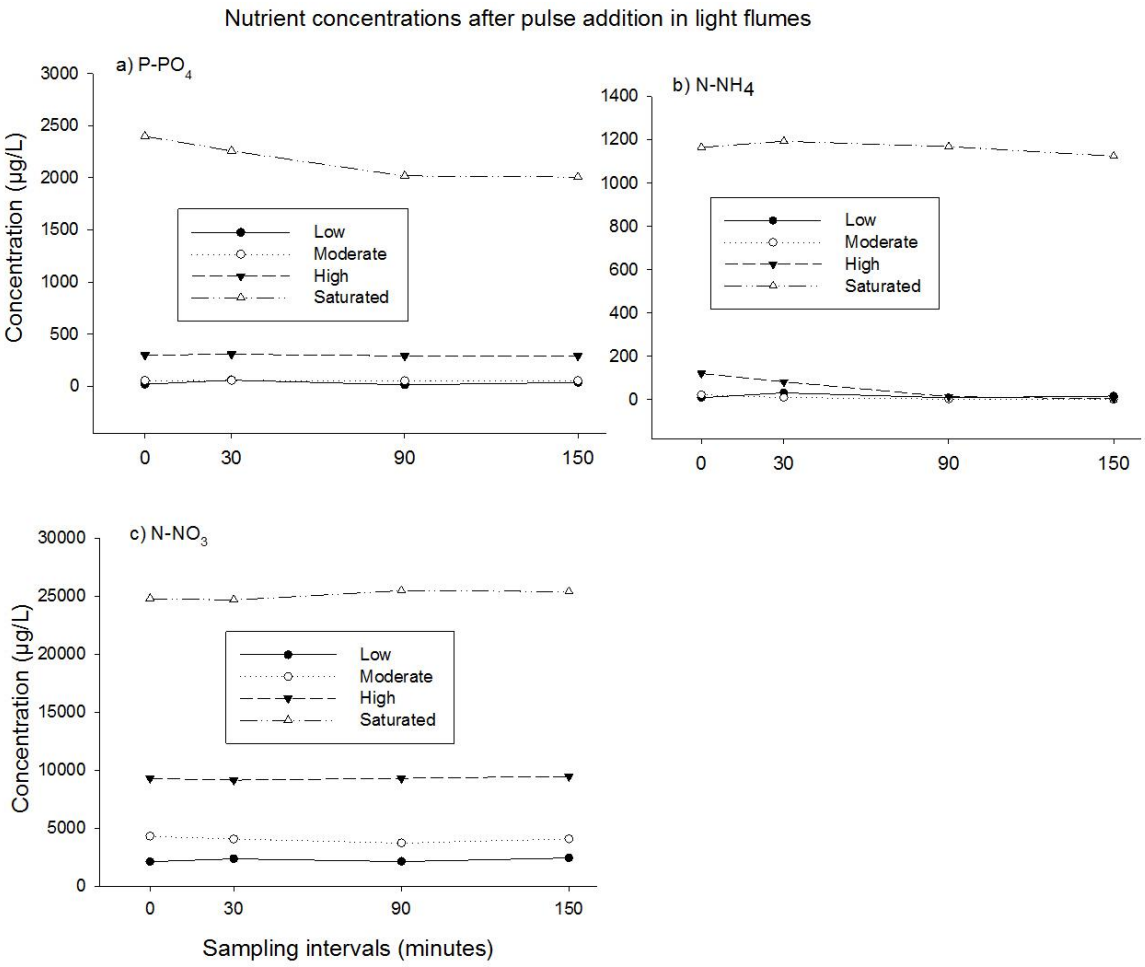

Figure 18 Nutrient concentrations in light flumes after addition of pulse

Table 9 Nutrient uptake rates ($\mu gL^{-1}min^{-1}$) in dark flumes (week 5) (n = 16 per experiment)

Light condition	Nutrient levels	N-NH4	N-NO3	P- PO4
Dark flumes	Low	**-0.32**	5.99	**-0.068**
Dark flumes	Moderate	**-0.084**	0.32	**-0.23**
Dark flumes	High	**-1.13**	4.84	0.17
Dark flumes	Saturated	**-8.16**	16.18	**-0.49**

Table 10 Nutrient uptake rates ($\mu gL^{-1}min^{-1}$) in light flumes (week 3) (n = 16 per experiment)

Light conditions	Nutrient levels	N-NH4	N-NO3	P-PO4
Light flumes	Low	**-0.017**	1.31	**-0.016**
Light flumes	Moderate	**-0.13**	1.71	**-0.011**
Light flumes	High	**-0.80**	1.59	**-0.081**
Light flumes	Saturated	**-0.33**	5.17	**-2.67**

4.0 Discussion

4.1 Influence of nutrient enrichment on benthic biofilm community

Previous studies have reported nutrient enrichment, especially the limiting nutrients (N and P) as key factors influencing ecosystem structure and function through increased primary production (Romani *et al*., 2004; Carr *et al*., 2005). In our results, enzyme activities, algal biomass and composition was not influenced by nutrient enrichment. The results were in agreement with the study done by Sabater *et al.,* (2011) where nutrient enrichment did not influence benthic biofilm. This results demonstrates that nutrients as single factors have less influence on the structure of benthic algal communities.

4.2 Influence of light on benthic biofilm community

Results from the study revealed that light reaching the stream bed is the most important determinant of ecosystem function. Enzyme activities and algal biomass were consistently higher with light and highest values were obtained in light flumes. However, it takes long time under shaded conditions until some algae can develop, but it takes less than a week under optimal light conditions until visible algal biomass is developed which remains stable with light availability.

Also results show that microbial enzyme activities and algal biomass are tightly coupled to light. The pattern observed is consistent with previous studies (Tank and Todd, 2003; Francoeur and Wetzel, 2003; Francoeur *et al*., 2006; Rier *et al*., 2007; Artigas *et al.,* 2008) which found enzyme activities and algal development to be highly light dependent. For instance, Tank and Dodds, (2003) found the highest response of algal biomass in an open canopy streams. Even though all enzyme activities were enhanced with light, β-glucosidase, β-xylosidase and leucine aminopeptidase indicated the use of algal exudates by bacteria (Romani *et al*., 2004; Artiga *et al*, 2008), since bacterial enzyme production can be stimulated by active photosynthesis (Espeland *et al*; 2001).

4.3 Effects of nutrient enrichment and light on benthic biofilms

Slides on, week 4 shaded flumes, and week 1 and 3 of light flumes showed increased leucine-aminopeptidase with nutrient enrichments. The pattern could have been due to rapid utilisation of peptide molecules accumulated through increased benthic algal biomass from photosynthesis enhanced by light (Romani *et al*., 2004; Artigas *et al*., 2008 and Sabater *et al*., 2011). Also, the increase of β-glucosidase with nutrient enriched on week and 5 could have been due to increased carbon demand from increased bacterial growth with nutrient

enrichment. In contrast, phosphatase showed decreased activity with nutrient enrichment on week 1 and 3. This was expected as availability of inorganic phosphorus in water makes phosphatase activity unnecessary since biofilms could obtain phosphorus from the water column (Romani and Sabater, 2001; Romani *et al.*, 2004; Rier *et al*., 2014). Also, leucine-aminopeptidase increased exponentially with nutrient enrichment on week 1 and 3 of the study.

4.4 Possible influence of algae on organic matter decomposition (enzyme activities)

Biological decomposition of organic matter is known to be mainly heterotrophic process since the process relies mostly on heterotrophic microbes (fungi and bacteria) (Rier *et al*., 2007). However, results from this study demonstrated coupled interactions between heterotrophic decomposition and photoautotrophic processes. For instance, on slides β-xylosidase and leucine-aminopeptidase activities were correlated with algal biomass. Similarly for sediments, β-xylosidase, β-glucosidase and leucine-aminopetidase were correlated with algal biomass. Moreover, most enzyme activities studied were highest in light flumes when algal biomass was highest. This pattern could indicate a possible bacterial-algal interaction in which algal biomass accumulation stimulate bacteria to release enzymes related to use of algal exudates (Romani *et al*., 2004; Rier *et al*., 2007). Of the extracellular enzymes studied, phosphatase could be produced both by algae and bacteria, however, β-glucosidase, β-xylosidase, and leucine-aminopeptidase are only known to be produced by heterotrophic bacteria (Rier *et al*., 2007). Therefore, correlation of β-glucosidase, β-xylosidase and leucine-aminopeptidase with algal biomass indicated that heterotrophic metabolism was enhanced in the presence of algae. Even though we did not measure bacterial density, a more dense biofilm mats observed in light flumes might have provided surface area for bacterial colonization, and these surfaces could have potentially increased bacterial density, especially in the light flumes (Rier and Stevenson, 2001; Rier *et al*; 2007). Subsequently, higher bacterial density could have increased heterotrophic decomposition of organic matter which have been shown by higher enzyme activities especially in light flumes. Furthermore, increased heterotrophic bacterial activities (enzyme production) observed in the light flumes might have been a direct response from bacteria to algal release of organic compounds that required extracellular degradation before being transported across cell membrane (Romani and Sabater, 2001).

Therefore, the study supports the hypothesis that stream algae have positive influence on hydrolytic enzymes (Phosphatase, β-glucosidase β-xylosidases and involved in the

mineralization processes within stream microbial communities (Romani and Sabater, 2001; Rier et al., 2007).

4.5 Leaf-discs' enzyme activities and decay coefficient estimation

Microorganisms mediate decomposition of leaf-litter through release of extracellular enzymes (Smart and Jackson, 2009). However, leaf breakdown in aquatic ecosystems is known to be N and P limited (e.g., Robinson and Gessner, 2000). Measuring decay coefficient (kd^{-1}) of the leaf-discs revealed insignificant difference in leaf decomposition with nutrient enrichment during the experimental period. Mathuriau and Chauvet, (2002) found that initial breakdown of leaves in streams is carried out by aquatic hyphomycetes (fungi). Therefore, it's possible that decomposition of leaf-discs were low due to low biomass of aquatic hyphomycetes, though, fungal biomass was not measured in the study. Aquatic hyphomycetes are crucial in leaf decomposing process as it breaks down lignified carbohydrates which offer natural protection of polysaccharide components against enzyme degradation (Griffin, 1994).

Also, physical properties of leaves might have contributed to low decomposition rates. For instance, amount of waxy cuticles have been reported to be a major determinant to leaf decomposition (Artigas and Sabater, 2004). According to Berg (1986), the decomposition of fresh leaves starts with the fresh non-lignified carbohydrate parts, whereas later stages are characterised by mineralization of more recalcitrant fractions of lignified carbohydrates. Artigas and Sabater, (2004) found that leaf decomposition begin with cellulose decomposition followed by lignified compounds.

4.6 Algal composition

There was no observable shift in algal diversity with nutrient enrichments during the study period. This could also be explained by low uptake rates within the nutrient levels. However, frequencies of chlorophyll *a, c* and accessory pigments fucoxanthin neoxanthin and violaxanthin during the study shows that diatoms were probably the most dominant algal group during the study period (Bonilla, 2005). Green algae were probably second algal group dominated flumes due to frequencies of chlorophyll *a, b* and carotenoid and xanthophylls as accessory pigments (Przytulska *et al*., 2016).Cyanobacteria with chlorophyll *a* and *c* as photosynthetic pigments and β-carotene as accessory pigment (Takaichi, 2011) could have been third in dominance. Nutrient enrichment was expected to shift community towards green algae dominance which assimilate nutrient faster and can also overgrow first colonizers (Passy and Larson, 2011; Bondar-Kunze *et al.*, 2016). Instead, diatoms known to be first colonizers

and have Dominated algal groups. The dominance of diatoms could lower irradiance in the flumes compared to natural systems. This also explains why there was no shift in algal community structure during the study period, and. However, low light irradiance could have inhibited dominance of green algae low composition of cyanobacteria recorded could have been due to lower temperature levels as cyanobacteria require above 25ºC for growth rate to competitive with diatoms (Sutula, 2015). Also, water column instability due to continuous flows and shorter residence time could have contributed to low cyanobacteria composition in the flumes (Journey *et al.,* 2013).

4.7 Nutrient uptake

Contrary to our expectations, nutrient uptake was very low. However, dark flumes had higher $N\text{-}NH_4$ compared to PO_4. This shows that the biofilms in the dark flumes had more demand for $N\text{-}NH_4$. This could have been due to low photosynthesis which is a characteristic of biofilms in dark conditions (Romani *et al.,* 2004), this might have created imbalance of N and P in which P was higher in concentrations (Dodds and Smith, 2015) and therefore the need to increase uptake of $N\text{-}NH_4$. However, in light flumes, uptake rates were lower but PO_4 had highest value indicating a possible higher N in the system (Dodds and Smith, 2015). This results could also be explained by enzyme activity results. Phosphatase activity decreased in light flumes while leucine-aminopeptidase increased. Meaning, phosphatase activity decreased due to uptake from water column (which has been found out here) while leucine-aminopeptidase activity increased to acquire more N to balance possible demand for N and P by the biofilms.

5.0 Conclusion

The master thesis addressed the response of benthic biofilms to nutrients and light. Analysis of results showed that benthic biofilms are more sensitive to light than nutrients. This is as a result of coupled increase in algal biomass and enzymes activities with light. Hence, more enzyme activities and algal biomass were measured in the light exposed flumes. Algal groups did not change in diversity with nutrient levels and light. On the other hand, nutrient enrichment only showed influence on enzyme activities only at some days of study. Nutrient enrichments did not show significant difference from each other with algal biomass, and leaf-decomposition. For nutrient uptake, NH_4 uptake increased under dark conditions while PO_4 increased under light conditions. Also, leaf decomposition is not nutrient and light dependent.

6.0 Relevance of the study

The results from the study shows light is the most important environmental variable regulating streams processes. Light is an important factor since it determines proportion of photosynthetic organisms in benthic biofilms. Biofilms in turn are involved in degradation of organic matter and nutrient cycle which regulates energy flow in the streams. Therefore, riparian management is key to stream benthic ecosystem processes. Deforestation of riparian vegetation has the effect of increasing photon flux and subsequent primary production. Increased primary production ultimately leads to increased algal biomass possibly bacterial abundance. Such systems have higher capacity for organic matter decomposition through enzyme activities. Hence, clearing of riparian vegetation by farmers farming adjacent to the rivers should be carried out in spring and summer when there is enough light for biofilms growth and efficient mineralization of organic matter which end up in streams through run-offs. Also, in small streams completely covered by tree canopy, high loads of terrestrial organic matter should be discouraged from activities in the nearby catchments due to low capacity of the streams to mineralize the terrestrial organic matter under dark conditions.

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