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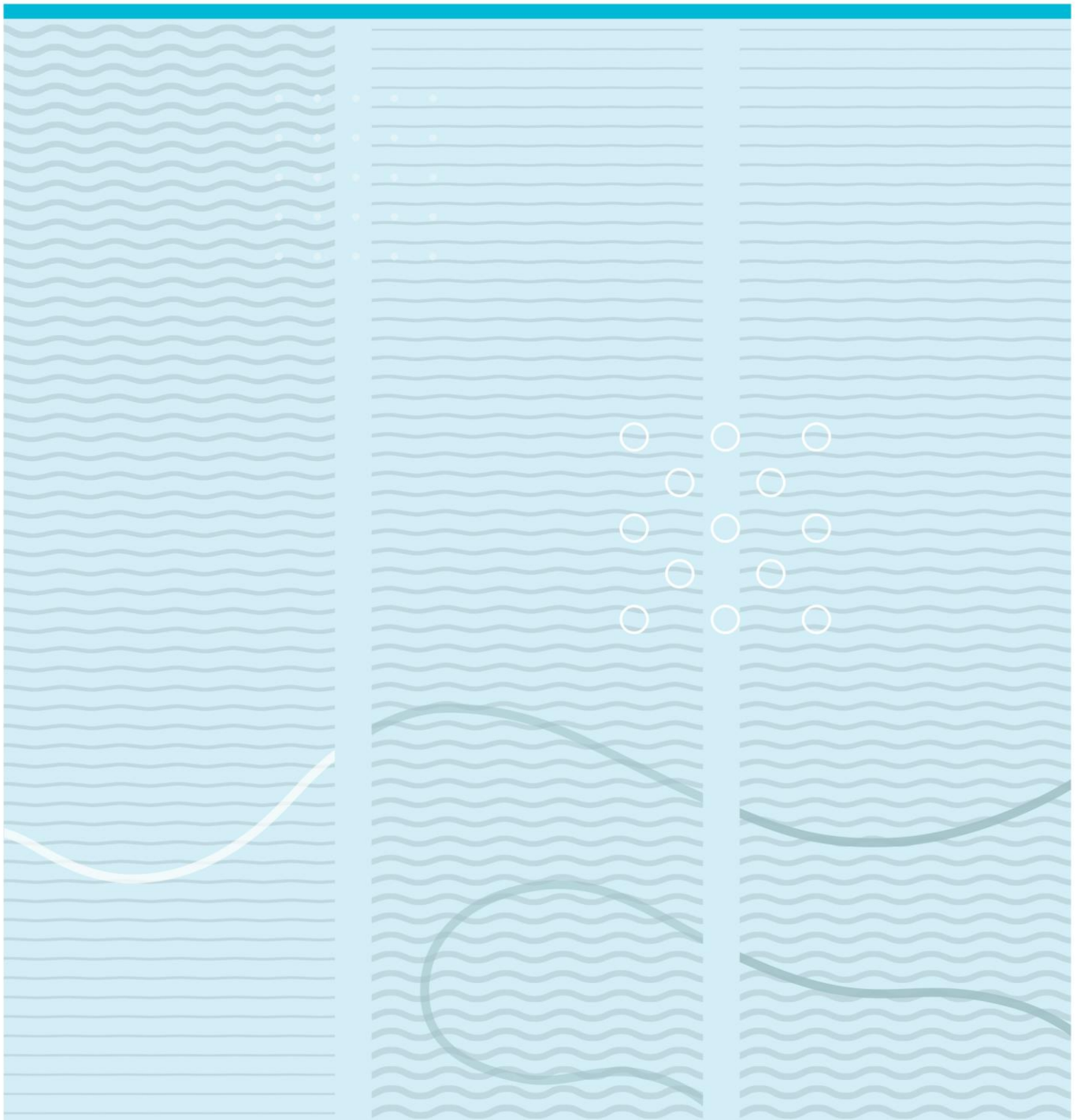
Miguel A. Segarra

The importance of microhabitat for the benthic algae of a Norwegian oligotrophic river:

implications for diversity, biomass and ecological indices

This thesis is worth 60 ECTS credits.

Abstract

Relationships between soft-bodied benthic algae and their habitat have traditionally been studied among different waterbodies, but less attention has been paid to the effects of combined microenvironmental conditions within streams. Soft-bodied benthic algae are considered as good indicators for both trophic and acidification status in Norwegian rivers, but the way microenvironment might influence ecological assessments when using algae have not been investigated yet. Benthic algae and environmental factors from 32 locations along a Norwegian oligotrophic river were analysed in order to 1) explore relationships among important abiotic environmental variables in the river, 2) study changes in biovolume, algal richness and associations among soft-bodied algae in relation to microenvironment, and 3) investigate the effects of environment on ecological indices based on soft-bodied algae at meso- and microhabitat scales. Mesoscale variations in water quality along the river continuum did not influence the ecological indices. Deep and still-water microenvironments were associated to higher resulting values for the periphyton index of trophic status (PIT) and lower values for the acidification index of periphyton (AIP). Algal richness was better predicted by photosynthetic active radiation (PAR), while the combination of PAR and water velocity was suggested in order to explain variations in relative biovolume. Multivariate analyses showed relationships between algal species and different measured microenvironmental variables. The insights from this study suggest that registering microenvironmental factors during benthic algal assessments may be valuable for future improvements of the trophic and acidification indices used in Norway with regard to the EU Water Framework Directive.

Contents

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Nøtterøy, 16.01.2017
Miguel A. Segarra

1. Introduction

1.1. Benthic algae and the environment

Streams are complex dynamic systems which are influenced by multiple environmental variables at different time-space scales. Climate, geology and human activity are important elements determining their ecological traits at a broad scale. Conditions within catchment areas such as topography, slope, vegetation and land use, are decisive when explaining water quality and habitat heterogeneity at local and smaller scales (O'Brien and Wehr 2010). This environmental complexity results in a likewise complex variety of biological processes that vary in time and space. Stream periphyton is one of the biological elements that is profoundly affected by the surrounding environmental conditions. The term periphyton refers to the community of all organisms -including photosynthetic benthic algae and heterotrophic bacteria, protozoa and fungi- that lives on, or in association with the surfaces of submerged substrata (Wetzel 1983). This complex array of organisms constitutes at the same time a suitable habitat and food source for many benthic invertebrates (Lamberti 1996). Benthic communities have a high spatiotemporal variability as a response to the underlying environmental factors, disturbance episodes and algal growth cycles (Biggs and Stokseth 1996). Benthic algae are considered to be the main primary producers of lotic, unshaded environments in temperate regions (Biggs 1996; Lamberti 1996) and they constitute an important carbon source for organisms higher in the food web (Frost et al. 2002). They are therefore an important component of the periphyton communities and are crucial when explaining ecological processes occurring in streams. Our understanding of variations in production and diversity of benthic algae and the environmental factors that control them is however still limited (Biggs and Smith 2002).

Production and taxonomic composition are two central variables when studying benthic algal communities in streams, and they can be analysed at different time-space scales. Some authors have linked the temporal patterns of benthic algal biomass and taxonomical richness to the disturbance regimes in different streams (Biggs and Stokseth 1996; Biggs and Smith 2002; Cardinale et al. 2006). Flood disturbance, as well as the environmental conditions during inter-flood periods, are important factors when

explaining biomass loss and accrual processes. Flood disturbance episodes lead to a net loss of algal biomass due to high water velocities, substratum instability and abrasion of algal communities by suspended sediments. The frequency and intensity of floods determine the settlement efficiency of algae propagules and time for algal accrual, and can be used to explain the prevalent type of biomass accrual cycles in different streams (Biggs 1996). Frequent disturbances can lead to relatively constant low-biomass algal communities as a result of constant sloughing. The algal assemblages dominating in these streams are likely to be constituted by species specialized in overcoming disturbance episodes – i.e. small species such as adnate diatoms that are especially resistant to scour from flood episodes (Cardinale et al. 2006). Streams experiencing a moderate or seasonal frequency of flood disturbances can allow the occurrence of biomass accrual cycles (Biggs 1996). They are likely to be dominated by stalked and filamentous taxa which are highly prone to score, but which are often competitively superior during stable inter-flood periods (Cardinale et al. 2006).

The development of algal communities during inter-flood periods is determined by factors such as temperature, availability of nutrients and light, spatial differences in water velocity and turbulence, loss by grazing, and the growth strategies of individual species (Biggs 1996). In the absence of loss processes such as grazing and floods, autogenic sloughing of mature filaments and mats takes place after the community reaches its biomass maximum (Hill et al. 2009). An idealised biomass curve after a severe flood episode might consist of an initial phase of colonization and exponential growth -accrual phase-, and a following loss phase dominated by death, sloughing, emigration and grazing processes (Figure 1).

Temporal changes in algal taxonomic composition can be explained in concert with the biomass-accrual cycles occurring between disturbance periods. A typical algal succession after a spring flood might start with the development of low-biomass diatom communities, be followed by the progress of cyanobacterial taxa in early summer, and culminate with the growth of patchy communities of large filamentous green algae - peak of biomass- in late summer (Biggs 1996 and literature cited therein).
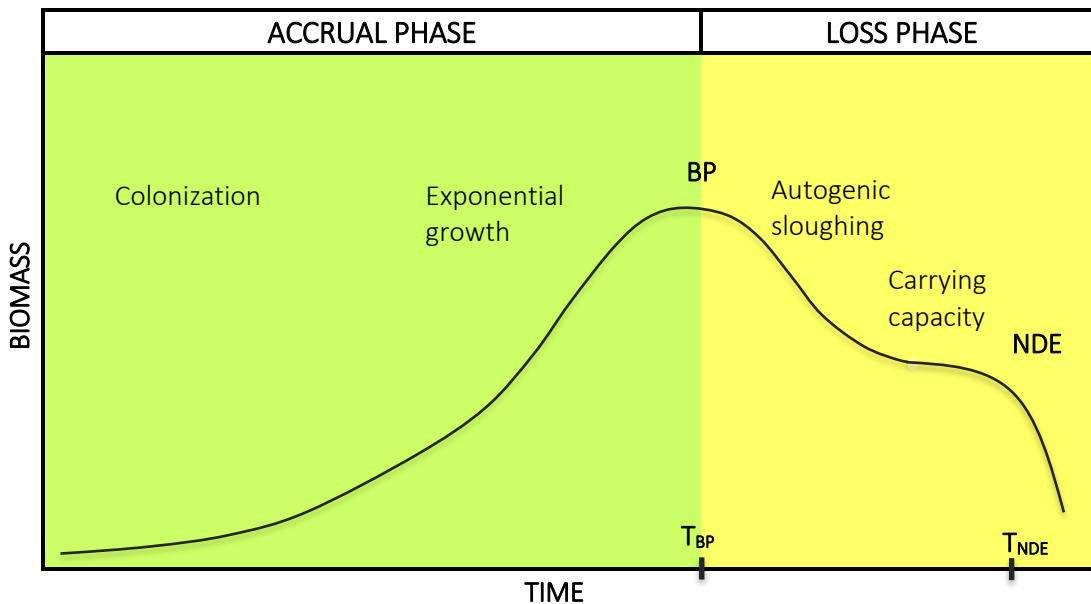
Figure 1. Idealized benthic algal biomass cycle after a disturbance event. BP = biomass peak; T_{BP} = time from disturbance event to biomass peak; NDE = new disturbance event; T_{NDE} = time from disturbance event to new disturbance event. Based on Biggs (1996) and Townsend and Padovan (2005).

A general pattern in spatial distribution of algal biomass within streams consists in higher biomasses on larger and more stable substrata, where algal communities have the possibility to get mature. Algal communities developing on sand and gravel - substrates that are more easily mobilized under small and medium-size floods- get often abraded and set back to early-successional and low-biomass stages (Biggs 1996). Water velocity plays also a defining role in the spatial distribution and abundance of benthic algae both within and between streams (Hart et al. 2013). Higher biomasses formed by filamentous green algae are usually linked to low water velocity habitats in nutrient-rich streams. These algal growths are more limited in habitats with higher water velocities, where the shear stress restricts the accrual of algal biomass. On the other hand, nutrient-poor streams experience higher biomass levels in high-velocity habitats, where there is a continuous input of nutrients and a greater mass transfer of metabolites (Biggs 1996; Stevenson 1996). The most general pattern in taxonomic composition associated to water flow is the transition from high to low profile diatom species with increasing water velocity (Peterson 1996; Biggs et al. 1998; Passy 2007). This relationship has mainly been studied in benthic diatom communities, and there is

still a lack of knowledge regarding water flow and taxonomic composition of soft-bodied benthic algae.

Differences in biomass and taxonomic composition linked to water velocity can also become evident at higher spatial levels. The environmental characteristics of different mesohabitats within a single stream -i.e. riffles, runs and pools- may also result in different shear stress and nutrient mass transfer. In addition, a downstream increase of nutrient concentrations -typical in many unshaded rivers with increasing land use- may result in biomass and algal composition gradients along the streams. At a regional level, differences in flood frequency and intensity, geology and land use are decisive for explaining biomass and taxonomic composition of algal communities (Biggs 1996).

Light is another important abiotic factor influencing benthic algal communities in streams. Because of its crucial role in photosynthesis and algal growth, light is a fundamental variable for explaining variation in biomass and productivity (Hill 1996; Hill et al. 2009). Photosynthesis-irradiance measurements suggest that photosynthesis by benthic algae developing in streams is saturated at irradiances between 100 and 200 μmol photons m^{-2} s^{-1} (Hill et al. 2001; Hill et al. 2009). Nevertheless, streams flowing through undisturbed forests often experience irradiances that are lower than 10 μmol photons m^{-2} s^{-1}, limiting severely the photosynthetic activity of benthic algae (Hill et al. 2009). Regardless the importance of this factor, the relationships between light and algal growth, as well as the synergistic interactions between light and nutrients, are still poorly understood in stream ecosystems (Hill et al. 2009; Hill et al. 2011). In addition to the effects on biomass and productivity, Hill (1996) postulated that different light requirements of distinct algal species may influence the taxonomic composition of periphyton communities at different light conditions. Yet the role of light on taxonomic composition has received very little focus, and 20 years later our understanding of the effects of light availability on algal distribution and algal assemblages in streams is still very limited (Atkinson and Cooper 2016).

In addition to the physical and chemical characteristics affecting algal biomass and taxonomic composition, there are biological factors that also contribute to the heterogeneity of the benthic algal communities. Evolution of different algal taxa has resulted in different ecological strategies to colonise and compete for space. This is

reflected in the distribution and taxonomic composition of algal communities along environmental gradients (McCormick 1996). Grazing by benthic invertebrates and other organisms is another biological factor affecting benthic algae, and it has been primarily linked to reduction in algal biomass (Steinman 1996). Additionally, a meta-analysis carried out by Hillebrand (2008) showed that the presence of grazers is also related to an increased spatial heterogeneity in the distribution of benthic algal biomass. The way grazing might influence the abundance and presence of algal taxa is not well understood, and just a few studies have investigated its effects on community structure (Wellnitz and Rader 2003). The high diversity of grazers and algal taxa that can be found in benthic habitats does not make it easy to generalize about the effects of herbivory on algal taxonomic composition (Steinman 1996).

A complex combination of environmental factors and the effects of their interactions are crucial when explaining the development of algal communities in lotic systems. Nevertheless, studies and models attempting to predict biomass and taxonomic variability have traditionally been based on single factors. These approaches have often proved to be overly simplistic, and multifactorial analyses are necessary to improve our understanding of the processes that explain algal community patterns in streams (Wellnitz and Rader 2003; Cardinale et al. 2006; Hill et al. 2011). A diagram showing relevant factors affecting periphyton and benthic algae is shown in Figure 2.

1.2. Ecological assessments using benthic algae

Species composition and algal biomass are considered good indicators of the ecological status of freshwater and marine water bodies and these elements are used nowadays in environmental assessments in many countries around the world (Stevenson 2014). In the European Economic Area (EEA) member states are required to implement the EU Water Framework Directive (European_Commission 2000) and regularly assess the ecological status of their water bodies by using biological quality elements. In Norway, benthic soft-bodied algae are one of the biological quality elements that are used to assess the status of rivers in relation to eutrophication and acidification (KLD 2006). These environmental impacts are analysed by studying the communities of soft-bodied benthic algae and implementing the Periphyton Index of Trophic status (PIT) (Schneider and Lindstrøm 2011) and the Acidification Index of Periphyton (AIP) (Schneider and

Lindstrøm 2009). Both indices are based on presence-absence data and are defined as the arithmetic mean of the indicator values of the algal taxa that are found at each sampling location. Class boundaries for the ecological status indicated by PIT and AIP are presented in the Norwegian guide for classifying the environmental status in water (Direktoratsgruppa 2013).
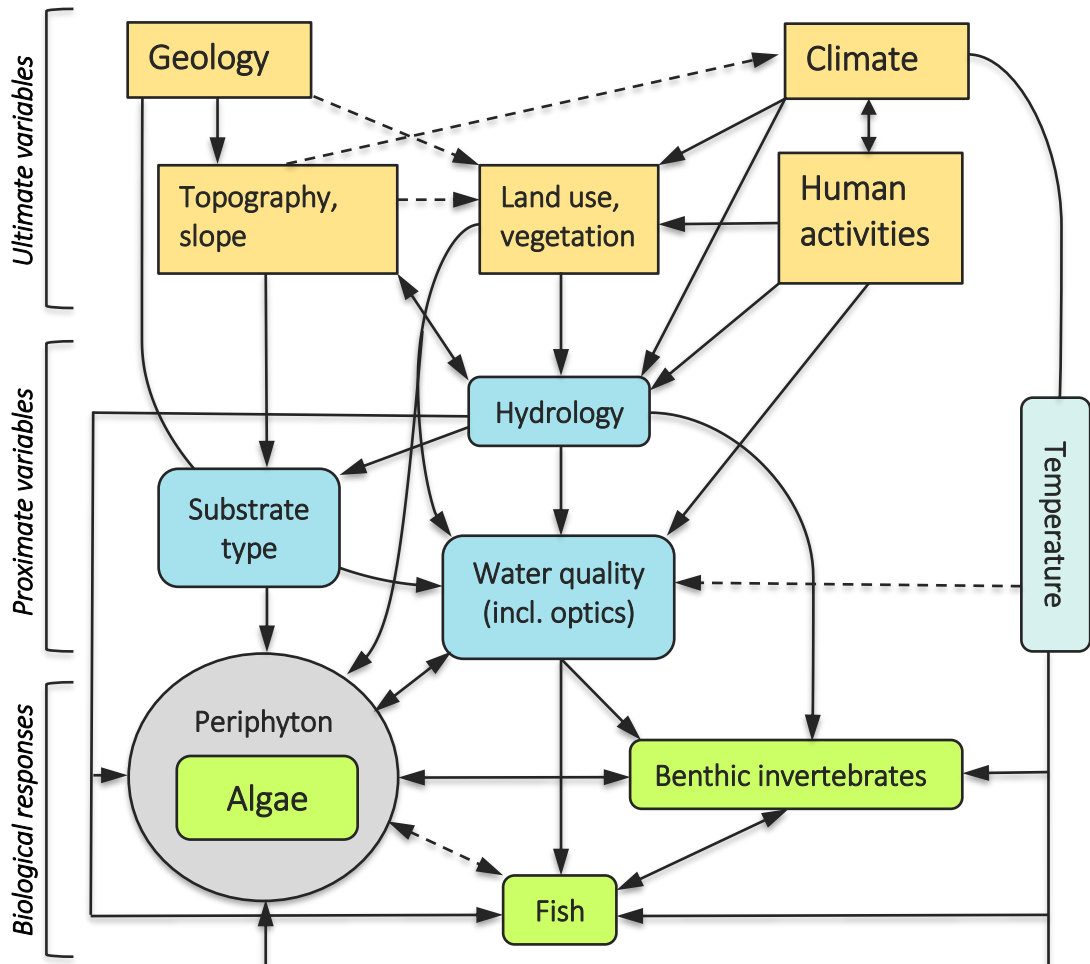
Figure 2. Regional features controlling physicochemical variables in temperate lotic systems, and biological elements affected by resulting environmental conditions. Solid and dashed arrows show strong casual interactions and weaker interactions respectively. Feedback relationships are indicated by double arrows. Modified figure from Biggs (1996).

Soft-bodied benthic algae are good indicators of the trophic and acidification status of lotic systems in Norway, but little is known about the way other factors than water quality may affect benthic algal communities. This study aims to use a multivariate

approach to better understand how environment shapes mature communities of soft-bodied benthic algae in a Norwegian oligotrophic river. Specific study objectives are to (i) study variations in water quality along the river continuum due to increased land use, and possible influences on ecological indices, (ii) explore the relationships among decisive abiotic environmental variables affecting benthic algae at a microhabitat level, (iii) identify the abiotic environmental variables that best explain spatial changes in biomass and algal richness, (iv) investigate the degree in which resulting PIT and AIP values may be altered when obtaining algal samples from specific microhabitats and (v) inspect the possible presence of different algal assemblages and their relationships with the underlying microenvironmental conditions.

2. Methods

2.1. Study site

Periphyton samples were collected from a small oligotrophic river located in Southeast Norway (Nome, Telemark County). This river is a lake-fed stream that originates from Lake Sveigstjønn at an elevation of 166 metres above sea level, and has a length of 2,67 km. The flow direction has a dominant west-to-east component, and flows into lake Damtjønn at 86 meters of elevation. The river is known as *Lona*, but its official name is Gryteåa øvre and its Norwegian water body ID-code is 016-1715-R (Vann-nett 2016).

The bedrock of the study area consists of dioritic to granitic gneiss and migmatite (NGU 2016). The vegetation along the river is dominated by coniferous forest, and there is a smaller area surrounded by deciduous forest towards the downstream end of the river. There are also bog areas surrounding the feeder lake, the central section of the river, and bordering the mouth of the river at its downstream end. Agriculture and farming are also important in the area, and there are several cultivated areas neighbouring the central and downstream stretch of the river (Figure 3).
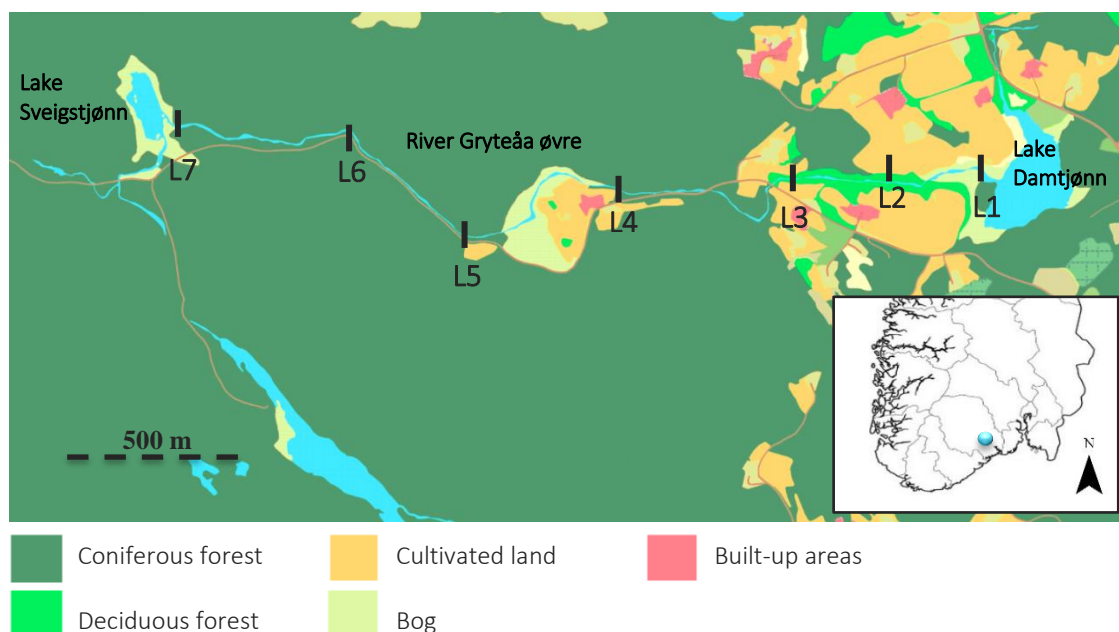
Figure 3. River Gryteåa øvre (Lona) in Southeast Norway and surrounding vegetation types. Straight lines indicate the seven locations (L1 – L7) where samples for water quality analyses were obtained. Modified map from http://kilden.skogoglandskap.no

2.2. Study design

2.2.1. Water quality analyses

This investigation is based on algal and environmental data collected at the study site during summer-autumn 2015. Water samples were collected in June, August and October from seven different locations along the river (Figure 3) in order to study possible mesoscale variations in water chemistry caused by local changes in surrounding environment and by human land use. Water quality analyses were conducted at the water laboratory of the University College of Southeast Norway (campus Bø). Water samples were kept in darkness and refrigerated until their analysis. pH analyses were carried out within 48 hours after sample collection, and the rest of parameters were analysed within five months after sample collection. An overview of the different chemical parameters, instruments and methods used in this study is given in Table 1.

Mean resulting values for calcium (Ca^{2+}), water colour and total organic carbon (TOC) were used to corroborate the national river type of the river Gryteåa øvre. Class limits given in the reviewed Norwegian guide for classifying the environmental status in water (Direktoratsgruppa 2013) were used for this purpose.

Table 1. Instruments and methodologies used for the analysis of water quality parameters in Gryteåa øvre 2015. NS = Norwegian Standard.

Chemical parameters	Unit	Instrument	Method
pH		PHM 219 pH METER	Electrochemical method – NS 4720
Total organic carbon (TOC)	mg/l	Aurora Model 1030	Heating sodium persulfate oxidation/ Non dispersive infrared detection –Intern method
Water colour	mg Pt/l	Perkin Elmer Lambda 25	Spectrophotometry – NS 4787
Total phosphorus (TP)	µg P /l	Perkin Elmer Lambda 25	Spectrophotometry – NS 4725
PO_4^{3-}	µg P/l	Perkin Elmer Lambda 25	Spectrophotometry – NS 4724
Total nitrogen (TN)	µg N/l	FIAlab-2500	Spectrophotometry – Intern method
NO_3^-	µg N/l	DIONEX ICS 1100	Ion chromatography – Intern method
NH_4^+	µg N/l	DIONEX ICS 1100	Ion chromatography – Intern method
Ca^{2+}	mg/l	DIONEX ICS 1100	Ion chromatography – Intern method
Mg^{2+}	mg/l	DIONEX ICS 1100	Ion chromatography – Intern method
K^+	mg/l	DIONEX ICS 1100	Ion chromatography – Intern method
Na^+	mg/l	DIONEX ICS 1100	Ion chromatography – Intern method
Cl^-	mg/l	DIONEX ICS 1100	Ion chromatography – Intern method
SO_4^{2-}	mg/l	DIONEX ICS 1100	Ion chromatography – Intern method

2.2.2. Benthic algae analyses

The periphyton samples were obtained between 13.8.2015 and 20.8.2015 in a period of similar weather conditions. 32 samples were collected along the whole length of the river and a selection of environmental variables was measured at each of the sampling points. The sampling points were chosen according to a range of environmental gradients (shading degree, flow type and depth) and –when possible- their different combinations. This approach was implemented in order to maximize sampling effort and to ensure that the samples reflected the heterogeneity of the different microenvironments present at the study site. The GPS co-ordinates for each of the sample locations were obtained and they are given in Appendix 7.

The procedures for sampling the periphytic material were based on the guidelines given in Biggs and Kilroy (2000) and the European Standard EN 15708:2009 (CEN 2009). The sampling methodologies depend on whether the substratum type is loose sediment, removable substratum or large boulders/bedrock. Since different sampling methodologies may lead to considerable differences in sampling effort, most of the samples in this study were obtained from removable substrata (pebbles and cobble) and by using the same sampling methodology. Anecdotal data from loose sediments (1 sample) and from large, non-removable substrata (2 samples) were also collected, but were not used in species richness analyses. An overview of the collection techniques implemented in this study is given in Table 2.

The percentage cover of the algal mat and/or filaments on the sampled substrata (removable substrata and large substrata) was estimated by using a grid/quadrant (Figure 4) and noted down on the field sheet. The type of algal mat and/or filaments was also identified according to the guidelines given in Biggs and Kilroy (2000) (Table 3). Each mat type was given a rank from 1 to 3 based on a qualitative estimation of the mat biovolume. Percentage cover and biovolume rank were merged together to form a new variable by multiplying both values. This was done in order to get an approximation of the relative biovolume among samples.

Table 2. Methodologies used in the present study for the sampling of soft-bodied benthic algae in running waters. Adapted after Biggs and Kilroy (2000) and the European standard EN 15708:2009.

Substratum type	Sampling method
Loose sediments: organic fine, clay, silt, sand, fine gravel.	An inverted petri dish lid was pressed into the top layer of substratum. Sediments and algal material were isolated in the lid by sliding a spatula blade under it. The sample was brought to the surface while holding the spatula under the lid. The petri dish was emptied into a tray and transferred into a labelled sample container (60 ml wide-mouth jar).
Removable substrata: gravel, pebbles, cobble and boulders.	The substrate was removed from water and placed into a tray. A representative area of 10x10 cm on the top of the rock was chosen by using a grid/quadrant. When a smaller substratum was used, the area to be sampled was noted down on the field sheet. Filamentous algae and thick growths were scraped with a scalpel and washed onto the tray by using a squirt bottle filled with stream water. Following, an unused toothbrush was used to scrub the area during approximately 30 seconds, and was rinsed off into the tray. The content was transferred into a labelled sample container (60 ml wide-mouth jar).
Large non-removable substrata: large boulders and bedrock.	A double syringe periphyton sampler –as described in Biggs & Kilroy (2000) - was used to obtain samples from non-removable substrata. The algal material collected into the syringe was transferred into a labelled sample container (60 ml wide-mouth jar).

Table 3. Classification of periphyton communities according to their visual macroscopic characteristics. Based on (Biggs and Kilroy 2000).

Type of algal mat/filaments	Colour	Code	Biovolume rank
Thin mat or film (<0,5 mm thick)	Green	1	1
	Light brown	2	
	Black/dark brown	3	
Medium mat (0,5-3 mm thick)	Green	4	2
	Light brown	5	
	Black/dark brown	6	
Thick mat (>3 mm thick)	Green	7	
	Light brown	8	
	Black/dark brown	9	
Short filaments (< 2cm long)	Green	10	
	Brown/reddish	11	
Long filaments (> 2 cm long)	Green	12	3
	Brown/reddish	13	

Figure 4. Photographs from field work in the river Gryteåa øvre (August 2015). a) Inspection of the river bed and its periphytic communities by using an aquascope. b) Quadrant used for estimating the percentage cover of the algal mat and the area to be sampled. c) Picture showing the scraping procedure during periphyton sampling in removable substrate. Photo: Miguel A. Segarra.

After sample collection, jars containing the samples were placed on ice and in darkness into a polystyrene box for transport to the laboratory. Once at the laboratory a representative subsample of ~ 2,8 ml was removed from each jar and placed into a 3,6 ml tube. This was done by mixing the content of each sample and taking three aliquots with a clean plastic pipette. The mouth of the plastic pipettes was cut in order to get a wider opening and allow the sampling of algal filaments and thicker algal masses. The subsamples were preserved with glutaraldehyde (~ 5 % final concentration) by adding 0,8 ml of a 25% stock solution of glutaraldehyde to the sub-sample tubes. The sub-samples were kept cool and in darkness, and they were used for the taxonomic

composition analysis. The original sample containers were stored frozen at -25 °C for possible future analyses.

The analyses of the taxonomic composition of the samples were based on specialized literature. Cyanobacterial taxa were identified according to Komárek and Anagnostidis (1999), Komárek and Anagnostidis (2005), and Komárek (2013). Eukaryotic algae were identified in line with John et al. (2002), Coesel and Meesters (2007) and Rueness et al. (2011). Filamentous taxa such as *Mougeotia*, *Oedogonium*, *Spirogyra*, and *Zygnema* cannot easily be identified at species level without culturing, and they were therefore classified into morphological groups according to Schneider and Lindstrøm (2011). Other sources such as algaebase.org (Guiry and Guiry 2016), Gutowski and Forster (2009), and help from specialised taxonomists were used for ratifying the taxonomic identifications.

The taxonomic analyses were carried out using optical microscopy at a magnification of 400-1000x. A microscope camera was used for taking photographic images of the different taxa. This was done in order to be able to assure the quality of the data and provide taxonomic consistency (Manoylov 2014). The photographic images were also used during taxonomic identification to conduct measurements using the image editing program GIMP©. In addition to taxonomic identification, taxa were ranked according to their relative contribution to the algal biovolume in the sample, and a simplified version of the ranking system proposed by Biggs and Kilroy (2000) was implemented for this purpose. Taxa having a major contribution to the sample biovolume were considered dominant and given the rank 3. Occasional and common taxa that could not be considered dominant in the sample were given the rank 2. Rare taxa that occurred just once or a few times and had a very low contribution to the algal biovolume in the sample were given the rank 1.

2.2.3. Microhabitat analyses

A selection of environmental variables were measured at each sampling point before collection of the algal material. River width and distance from the sampling point to the closest river bank were noted down. Flow type at the sampling point was ranked from 1 to 6 according to the descriptions given in Table 4. Total water column depth (m) and

mean water velocity (m s^{-1}, 2 cm above sampling point) were measured at the exact location of the algal growths with a water velocimeter type Marsh MacBirney electronic flow meter 2000. Water level and discharge data from three neighbouring water bodies were obtained from The Norwegian Water Resources and Energy Directorate (NVE 2016) in order to check for possible significant changes occurring during the sampling period. After this verification, it was considered that no modifications of depth and water velocity data were necessary.

Table 4. Flow type classification that was used in the present study. V = water velocity. Adapted after Haury et al. (1991).

Rank	Flow type description	Associated mesohabitats
1	Scarcely perceptible flow	Pools, lentic channels and marginal deadwater.
2	Very low current velocity and no surface turbulence. *V < 20 cm/s*	Stillwater channels and glides (flat, slick).
3	Intermediate current velocity with low turbulence. *20<V<40 cm/s*	Runs
4	Moderate current velocity and turbulence. *V > 40 cm/s*	Riffles
5	Current velocity and turbulence very important *V > 40 cm/s*	Rapids
6	Chute/Cascades with very high velocity	Chutes and vertical waterfalls.

Photosynthetically active radiation (PAR, in µmol of photons m^{-2} s^{-1}) was measured with a LI-COR Quantum Sensor just above the water surface at each sampling point. The samples and measurements were carried out under clear sky conditions in order to assure similar measurement conditions. Still, changes in light intensity and in the position of the shadows from surrounding vegetation during the day can lead to inaccurate measurements. To further exam this source of error, the amount of light was also estimated qualitatively by determining the degree in which surrounding vegetation blocked the sunlight at the sampling points. The shading degree originated by riparian vegetation was ranked from 1 to 5 according to the descriptions given in Table 5.

The substrate size from which the samples were obtained was registered according to Heggenes et al. (2002) (Table 6). Temperature, conductivity, and dissolved oxygen were also measured at each sampling point in order to identify possible local built-up physicochemical environments. A conductivity meter model WTW LF91 was used to

obtain the conductivity data, and the instrument EcoSense® DO200 provided temperature and dissolved oxygen data. These data did not provide enough variation to be used in the data analyses

Table 5. Classification of the degrees of shading originated by riparian canopy cover that was used in the present study.

Shading degree	Rank
Open Just low vegetation at both sides	1
Little covered Just some bushes that can produce little shading.	2
Partly covered Sparse trees at one or both banks.	3
Mostly covered Trees at both banks	4
Completely covered Dense trees at both banks blocking most of the direct light.	5

Table 6. Substrate-type classification that was used in the present study. After Heggenes et al. (2002).

Substrate type	Size in mm	Rank
Organic fine	<10	1
Organic coarse	>10	2
Clay or silt	0,004 – 0,06	3
Sand	0,061 – 2	4
Fine gravel	2,1 – 8	5
Gravel	8,1 – 16	6
Small pebble	16,1 – 32	7
Pebble	31,1 – 64	8
Small cobble	64,1 – 128	9
Cobble	128,1 – 256	10
Large cobble	256,1 – 384	11
Boulder	384,1 – 512	12
Large boulder	>512	13
Bedrock (smooth or rough)		14

2.3. Data analyses

2.3.1. Ecological indices

The ecological indices AIP (Acidification Index Periphyton) and PIT (Periphyton Index of Trophic status) were calculated according to Scheider and Lindstrøm (2009, 2011). Both indices are defined as the average of the indicator values associated with the indicator taxa present at the sampling location. However, in this study, AIP and PIT were calculated for each of the samples in order to study possible variations in sample values associated to microenvironmental factors. In accordance with Scheider and Lindstrøm (2009, 2011), AIP was calculated when at least three indicator taxa were present, while PIT could be calculated with a minimum of two indicator taxa:

$$AIP = \frac{\sum_{i=1}^{n} IV_i}{n_i}$$

$$PIT = \frac{\sum_{i=1}^{n} IV_i}{n_i}$$

AIP: Acidification Index Periphyton;
IV$_i$: indicator value of species "i" in AIP
(Schneider & Lindstrøm 2009);
n: number of indicator species.

PIT: Periphyton Index of Trophic status;
IV$_i$: indicator value of species "i" in PIT
(Schneider & Lindstrøm 2011);
n: number of indicator species.

2.3.2. Land use and water quality

Differences among the seven water sampling locations (Figure 3) and among sampling periods (June, August and October) were tested by carrying out two-way ANOVA tests without replicates. Significant differences (p < 0,05) among locations and/or sampling periods were inspected further by plotting graphs of the measured parameters per sampling location and month. Two sections of the river were considered for testing differences in TP, PIT and AIP based on the resulting differences in water parameters and presence of human activities along the river continuum. The division for conforming the sample groups was an intermediate point between sampling locations 4 and 5 (Figure 1). Two-sample t-tests were implemented for the comparison of TP, PIT and AIP mean values between the upstream and downstream groups of samples.

2.3.3. PCA, regression models and hypothesis testing

Principal Component Analysis (PCA) is an ordination method that examines linear relationships among variables. A PCA was conducted with the statistical software PAST© (Hammer et al. 2001) in order to analyse the relationships among the measured environmental factors. Algal richness, relative biovolume and resulting values from PIT and AIP of each sample were also included in the analysis in order to detect possible associations with the environmental factors.

Previous to this ordination analysis, the variables were checked for normality by carrying out Shapiro-Wilk tests. The histograms from non-normally distributed data were visually examined for skewness. When the frequency distribution of the data was skewed to the right (i.e. had a long tail on the right side), the data points were transformed by applying a log base-10 transformation according to the guidelines given in Whitlock and Schluter (2009). Following the same guidelines, the data were transformed by squaring each data point when the resulting frequency distribution was skewed left. An overview of the variables included in the principal component analysis and the different transformations that were carried out is given in Table 7.

Table 7. Environmental and biological variables included in the principal component analysis (PCA) and data transformations implemented in order to improve the data requirements.

Variable	Unit	Transformation required	Abbreviation in PCA
Elevation above sea level (GPS data)	m	Log base-10	LogAlt
Distance to the closest river bank	m	Log base-10	LogDistanceCB
Photosynthetically active radiation (PAR)	$\mu mol\ m^{-2}\ s^{-1}$	Log base-10	LogIrrad
Shading degree		None	ShaD
Depth	m	Log base-10	LogDepth
Mean water velocity	$m\ s^{-1}$	Log base-10	LogVel
Flow type		Log base-10	LogFlowT
Substrate type		Square transformation	SubsT^2
Relative biovolume		Log base-10	LogCov*V
Total taxonomic richness		None	NoAlg
Cyanobacterial richness		None	NoCyan
Green algal richness		None	NoGAlg
Red algal richness		None	NoRAlg
Periphyton index of trophic status (PIT)		Log base-10	LogPIT
Acidification index periphyton (AIP)		None	AIP

Since the data come from variables measured in different units, all variables were standardized to zero mean and unit variance before conducing the PCA. This was done automatically by the statistical program by choosing the option "Matrix > Correlation" in the PCA analysis (Hammer et al. 2001). The option "Iterative imputation" was chosen in PAST© for handling missing data points according to the guidelines given in Ilin and Raiko (2010).

After examining the relationships among the different variables denoted by the PCA biplot, correlations and regressions were implemented to confirm possible associations and dependence between pairs of variables. Polynomial models were used when they improved the fit and significance of the regression. Since PCA just assumes linear responses between variables, possible nonlinear relationships between variables that were not shown by the PCA were also explored. Shapiro-Wilk tests were conducted in order to test if the regressions met the assumption of normally distributed residuals and equal variance of residuals. F-tests were carried out to test the overall significance of the regression models (Whitlock and Schluter 2009). When a response variable was significantly predicted by two different explanatory variables, 3D wireframe plots and contour plots were obtained in Minitab 17 Statistical Software (2010) in order to examine the potential relationships between the three variables.

Differences of the mean values for PIT and AIP were tested in relation to the variable 'depth' as treatment. Two groups were considered ($\leq 0,2$ m and $0,2 - 1$ m) based on the inflection point given when analysing the relationship between depth and the mean water velocity or the flow type (Appendix 4). Normality and homoscedasticity were checked previously to the comparison of the groups. This was done by implementing Shapiro-Wilk tests and F-tests. The distribution of PIT ($\leq 0,2$m) could not be considered normal and the nonparametric Mann-Whitney U-test was therefore used to compare the distribution of PIT ($\leq 0,2$m) and PIT ($0,2$-1m). Both AIP groups –AIP ($\leq 0,2$m) and AIP (1-2m) - were normally distributed, but the assumption of equal variances could not be met. The Welch's approximate t-test was therefore chosen for comparing the means of the AIP groups (Whitlock and Schluter 2009).

2.3.4. DCA, TWINSPAN and CCA

Detrended Correspondence Analysis (DCA) is an indirect ordination method that gives information about sample similarity based only on taxa composition and abundances. This method was applied to the algal data by using R and default settings in the *vegan* package. The environmental variables (not transformed) were *post hoc* fitted into the DCA plot. Since taxa with very few records are not likely to be placed truthfully in their ecological space, taxa with fewer than 4 occurrences were not included in the analysis. The dataset included 62 algal taxa after the removal of rare taxa.

TWINSPAN classification (Hill et al. 1979) was used to explore sample similarity, and resulting groups of samples were compared with the DCA results. A modified TWINSPAN (Roleček et al. 2009) was conducted with the package TwinspanR in R using five pseudospecies cut-levels (0 - 2 - 5 - 10 - 20). Four sample clusters were selected, and the dominant taxa in each of the sample clusters were identified. Kruskal-Wallis tests were carried out to determine whether there were significant differences in resulting PIT and AIP values among the sample clusters obtained in the TWINSPAN analysis.

Canonical Correspondence Analysis (CCA) is a direct ordination method that was conducted in PAST© in order to test the significance of the different environmental variables for the different algal taxa. Environmental variables were transformed previous to the CCA analysis in the same way as for the PCA. Only taxa with more than three occurrences in the dataset were included, as it was done in the DCA analysis. Sample clusters obtained in the TWINSPAN classification were also compared with the position of the samples in the CCA biplot for environmental relationships and sample similarity.

3. Results

3.1. Water quality

The mean calcium concentration was 2,7 mg/l and indicated that the water body is a calcium-poor river (Ca^{2+} 1-4 mg/l) according to the Norwegian classification system for water bodies (Direktoratsgruppa 2013). Mean TOC concentration was 6,1 mg/l and mean water colour was 43 mg Pt/l (min. 32; max. 65). These values indicated that Gryteåa øvre is a humic river according to the same guidelines (TOC 5-15 mg/l; Water colour 30-90 mg Pt/l). In conformity with these characteristics (low-land, calcium-poor and humic) this is considered a Norwegian *river type 6*, and its northern river code is R-N3.

Water quality parameters from the seven sampling locations (Figure 3) were studied in order to identify possible variations along the river continuum and/or sampling periods. The resulting p-values from the tests for equal means (two-way ANOVA tests without replication) indicated that there are statistically significant differences in the measured values for TOC, TP and Ca^{2+} among sampling sites and among sampling periods (June-August-October) (Table 8). Water colour, pH, Mg^{2+}, Na^+, Cl^-, NO_3^- and SO_4^{2-} showed significant statistical differences among sampling periods but not among sampling sites (Table 8, Figure 5).

TOC and Ca^{2+} concentrations are important when it comes to explain the buffering capacity of natural waters (Schneider 2011). These parameters showed a very slight trend along the river continuum with decreasing TOC and increasing Ca^{2+} concentrations towards the downstream end of the river (Figure 6). pH showed statistically significant differences among sampling months (Figure 5), but all values fell within the class limits of very good acidification status for *river type 6* (pH 7,2-6,2).

Table 8. Mean values for water quality parameters at seven sampling locations in the river Gryteåa øvre in 2015. Grey cells (^) indicate parameters that present significant statistical differences among locations and sampling periods. Yellow cells (*) indicate parameters with significant statistical differences among sampling periods (two-way ANOVA without replication).

Location	Parameters - Mean values (June-August-October)													
	pH*	TOC (mg/l)^	Water colour (mg Pt/l)*	TP (µg P/l)^	PO_4^{3-} (µg P/l)	TN (µg N/l)	NO_3^- (µg N/l)*	NH_4^+ (µg N/l)	K^+ (mg/l)	Na^+ (mg/l)*	Ca^{2+} (mg/l)^	Mg^{2+} (mg/l)*	SO_4^{2-} (mg/l)*	Cl^- (mg/l)*
Loc. 1	6,6	6,0	41	5,1	<1	420	27	29	0,17	1,2	2,7	0,38	1,3	0,98
Loc. 2	6,7	6,0	42	5,1	<1	396	27	25	0,17	1,2	2,8	0,40	1,3	0,96
Loc. 3	6,7	6,0	46	5,1	<1	458	28	51	0,17	1,2	2,7	0,39	1,3	0,97
Loc. 4	6,6	6,1	41	4,4	<1	217	17	25	0,16	1,2	2,7	0,39	1,3	0,94
Loc. 5	6,7	6,1	43	3,5	<1	292	19	27	0,16	1,1	2,7	0,39	1,3	0,93
Loc. 6	6,7	6,3	44	4,0	<1	288	22	20	0,15	1,1	2,6	0,39	1,3	0,92
Loc. 7	6,6	6,3	42	3,9	<1	232	13	32	0,16	1,1	2,6	0,39	1,3	0,94
Total mean	6,7	6,1	43	4,4	<1	329	21	31	0,16	1,2	2,7	0,39	1,3	0,95
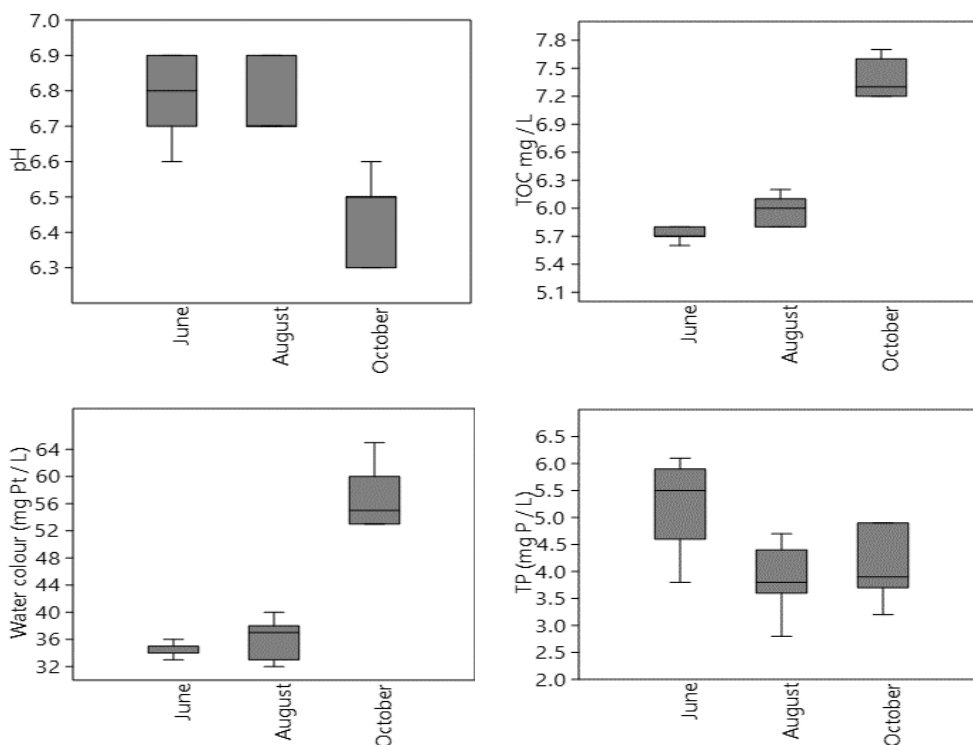
Figure 5. Box plots for selected water quality parameters that showed statistically significant differences (two-way ANOVA without replication; p < 0,05) among sampling periods (June, August and October 2015) in the river Gryteåa øvre.

Total phosphorus (TP) concentrations at the downstream sampling locations (1, 2, 3 and 4) were slightly higher than in the upstream half of the river (locations 5, 6 and 7) (Figure 6). A two-sample t-test for equal means indicated that there are statistically significant differences (p = 0,003) between the downstream group of samples ($\overline{TP}$ = 4,9 µg P/l; 95% CI [4,4 5,4]) and the upstream group of samples ($\overline{TP}$ = 3,8 µg P/l; 95% CI [3,3 4,2]). TP concentrations were in every case within the class limits of very good trophic status for *river type 6* (1-17 µg P/l). Total nitrogen (TN) concentrations were also within the class limits of very good trophic status (1-475 µg N/l) (Direktoratsgruppa 2013).
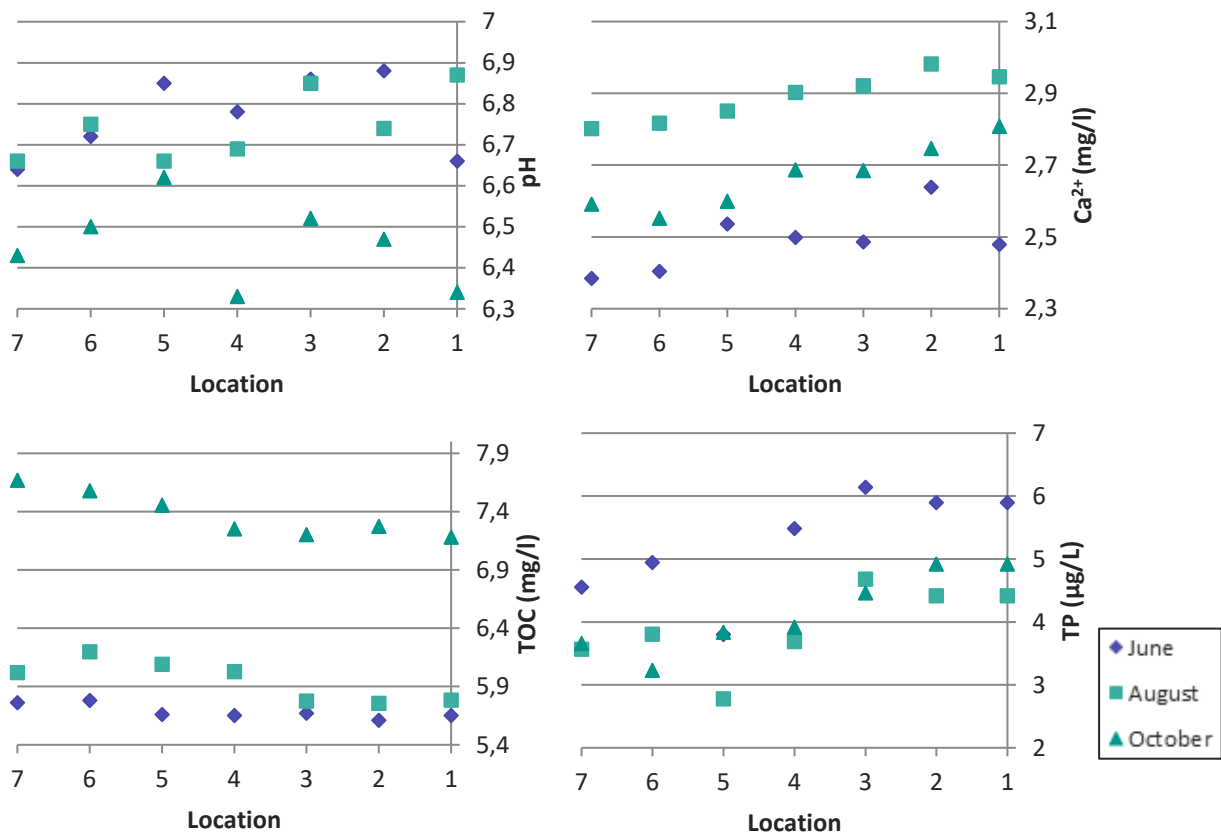
Figure 6. Selected water quality parameters from seven sampling locations along the river Gryteåa øvre in June, August and October 2015. Locations are placed in the upstream-downstream direction (7 = most upstream location; 1 = most downstream location).

3.2. Algal diversity and ecological indices

This study included 32 periphytic samples where 172 soft-bodied benthic algae were identified (see taxa list in Appendix 9). The largest portion of this number was formed by green algae (Chlorophyceae: 113 taxa) and cyanobacteria (Chyanophyceae: 48 taxa).

Five red algal taxa (Rhodophyceae), two chrysophytes (Chrysophyceae), one xanthophyte (Xanthophyceae), one dinoflagellate (Myzozoa) and one colonial ciliate were also registered. Of these taxa, 110 were registered in three or less periphytic samples (Figure 7). The most frequent species was the cyanobacterium *Stigonema mamillosum* with 24 occurrences. This taxon was followed in no. of occurrences (noc.) by the green algae *Closterium parvulum* (noc. = 23), *Oedogonium* b (noc. = 20), *Bulbochaete* sp. (noc. = 19), *Cylindrocystis* sp. (noc. = 18), *Zygnema* b (noc. = 18), the cyanobacteria *Calothrix* sp. (noc. = 18) and *Leptolyngbya* sp. (noc. = 18), and the red alga *Batrachospermum* sp. (noc. = 14). When taking into account their abundance in the periphytic samples, *Stigonema mamillosum, Oedogonium* b, *Zygnema* b, *Batrachospermum* sp. and *Mougeotia* b were among the most contributing taxa to the sample biovolumes. Mean soft-bodied algal richness per sample was 23 taxa (min. 3; max. 50).
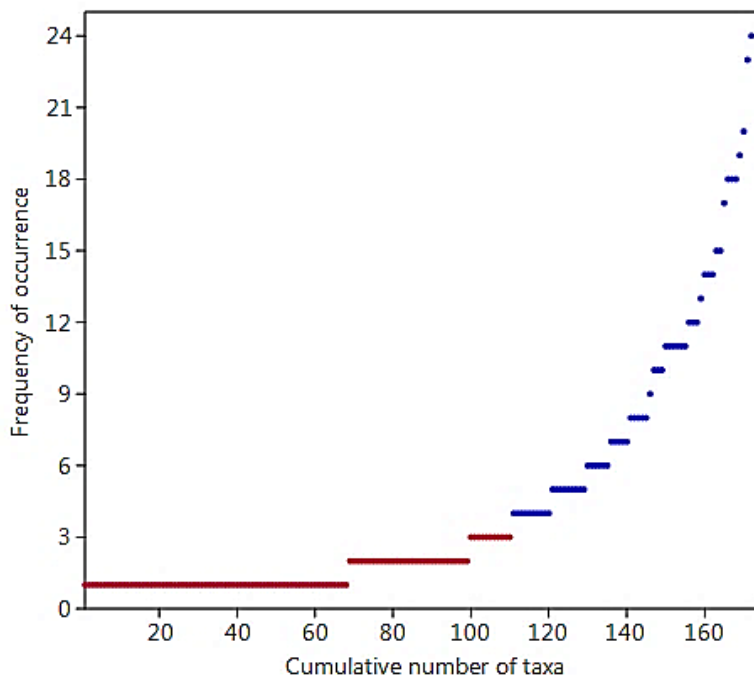
Figure 7. Cumulative distribution of taxa occurrence (soft-bodied benthic algae) in 32 periphytic samples from river Gryteåa øvre (2015). Red spots represent taxa occurring in three or less samples. Only taxa occurring in more than three samples (blues spots) were included in the Detrended Correspondence Analysis (DCA) and in the Canonical Correspondence Analysis (CCA).

PIT values from the different periphytic samples ranged from 4,80 to 10,56. All samples but one had a PIT value that fell in the class for very good condition with regard to trophic status for Norwegian *river type 6* (PIT < 9,50). The PIT value of the remaining sample fell in the class for good trophic status (PIT 9,5 - 16). Mean PIT of the samples was 6,60, a value that is comparable to the reference value for Norwegian *river type 6* (PIT=6,71) (Direktoratsgruppa 2013). AIP values obtained in this study ranged from 6,38 (bad acidification status) to 7,01 (very good acidification status). The values of most of the samples fell into the class for good acidification status (6,77-6,59). The mean AIP value for the water body was 6,69 and indicated an overall good status in respect to acidification (Direktoratsgruppa 2013).

PIT and AIP values between the upstream and downstream groups of periphyton samples were compared. Mean PIT values for the upstream (n=12) and downstream (n=20) groups of periphyton samples were 6,10 and 6,91 respectively. Mean AIP values for the same groups of periphyton samples were 6,69 and 6,68. Resulting p-values from two-sample t-tests were in both cases higher than the significance level (α = 0,05) and the null hypotheses of equal means were not rejected. There is therefore no statistical evidence for stating that PIT and AIP indices provided different results in the upstream and the downstream half of the river.

3.3. Microhabitat and biological responses

The linear relationships among environmental variables, taxa richness, relative biovolume and resulting values from ecological indices were explored by carrying out a PCA (Figure 8). A comparison between the resulting scree plot of eigenvalues and the eigenvalues expected under a random model (Broken Stick) (Appendix 3, Figure A3-1) indicated that the first and second component are the dimensions which are most desirable to explore in this PCA analysis (Hammer et al. 2001).

The first component explained 27,56 % of the variance in the data set, with a bootstrapped confidence interval (BCI) of 20,04 - 37,46 % (N = 10000). This component was highly correlated with the environmental variables depth (LogDepth), flow type (LogFlowT), mean near-bed water velocity (LogVel) and substrate type (SubsT^2). When inspecting this group of environmental variables, it was possible to identify a negative

relationship between depth and the rest of variables. The ecological indices PIT and AIP also appeared to have a connection with this group of environmental variables. The second component explained 21,32 % of the variance in the data set (BCI 15,51 - 32,10 %). Total algal richness (NoAlg) and the different richness subgroups were strongly correlated to this axis. Photosynthetically active radiation (LogIrrad) and shading degree (ShaD) had a strong negative relationship and were also important variables contributing to the second component. Red algal richness (NoRAlg) and relative biovolume (LogCov*V) varied along with the amount of light. The altitude (LogAlt) and the relative distance of the sample to the closest river bank (LogDistanceCB) had a low explanatory power.
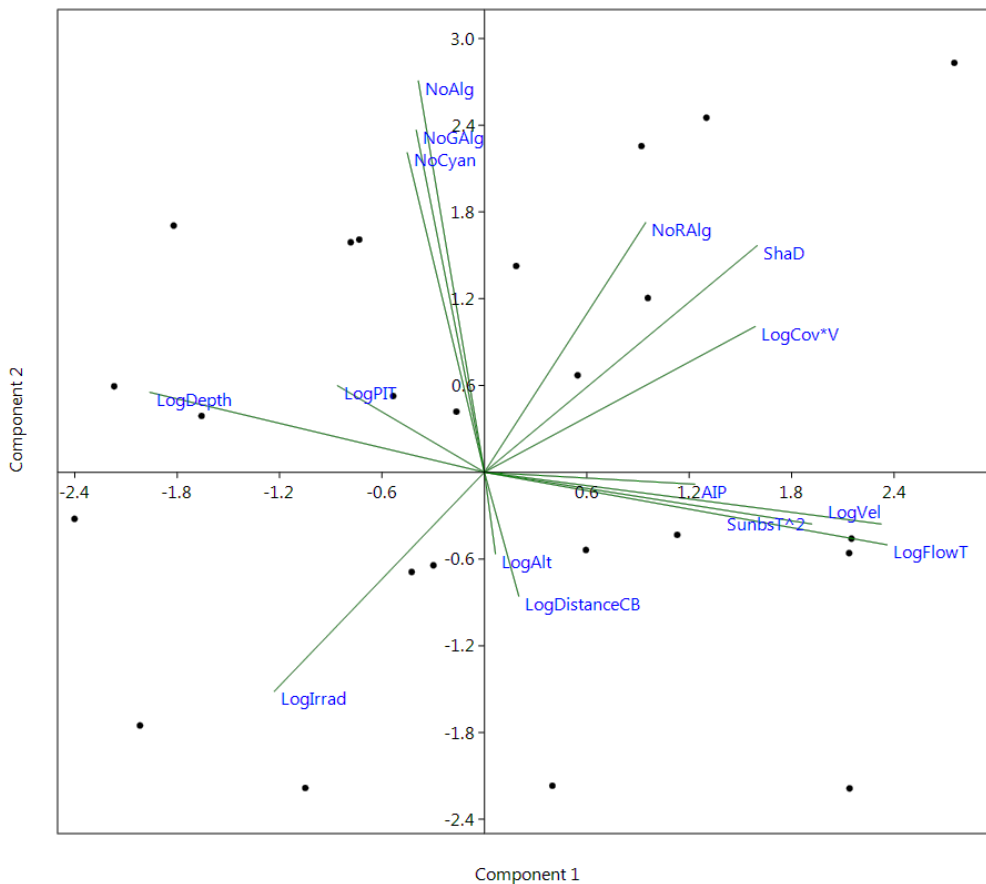
*Figure 8. PCA analysis showing relationships among environmental variables (LogAlt, LogDistanceCB, LogIrrad, ShaD, LogDepth, LogVel, LogFlowT and SubsT^2), taxa richness (NoAlg, NoCyan, NoGAlg and NoRAlg), relative biovolume (LogCov*V) and resulting values from ecological indices (LogPIT and AIP). Data from river Gryteåa øvre, August 2015.*

Further analyses with regression models gave a more detailed picture of the relationships between different variables (see Appendix 5). The environmental variables that best explained variations in relative biovolume (LogCov*V) where those related to light irradiance and water flow. The relationship between relative biovolume and PAR (log transformed) was best explained by a polynomial regression model with order 3 ($p < 0.05$) (Figure 9 a). The resulting curve showed a hump-shaped relationship between both variables, with higher algal biovolumes at intermediate light intensities. The degree of shading also showed a statistically significant relationship with relative biovolume, but it had a slightly lower explanatory power (Appendix 5).

The relationship between relative biovolume and mean water velocity (log transformed) was better explained by a polynomial regression model with order 2 ($p < 0.05$). The resulting trend was an increasing relative biovolume with increasing water velocity (Figure 9b). The resulting relationship when using the flow type as predictor variable was analogous, but the model had a lower explanatory power (Appendix 5).

When displaying relative biovolume together with PAR and mean water velocity in a 3D scatterplot it is possible to identify and explain a notable portion of the variability that is present in the models based on one predictor variable. A high variability in relative biovolume at low and high PAR (Figure 9 a) is explained by mean water velocity, with high biovolumes being associated to high water velocities (Figures 9 c and 9 d). Simultaneously, the great variability in relative biovolume that it is found at low water velocities (Figure 9 b) is well explained by the hump-shaped curve resulting from the interaction with light (Figures 9 c and 9 d).

PAR was the predictor variable that explained variations in total algal richness. This relationship between algal richness and PAR (log-transformed) followed a similar pattern as with biovolume, but in this case, it was best explained by a polynomial regression model with order 2 ($p < 0.05$) (Figure 10 a). Individual regression analyses for green algae and cyanobacteria also showed higher richness at intermediate light intensities, while red algal richness decreased with increasing light intensities (see Appendix 6). When using the degree of shading as the predictor variable (Table 5), the occurrence of red algal taxa appeared to be positively correlated to the shading by riparian canopy cover ($p < 0.05$) (Figure 10 b).
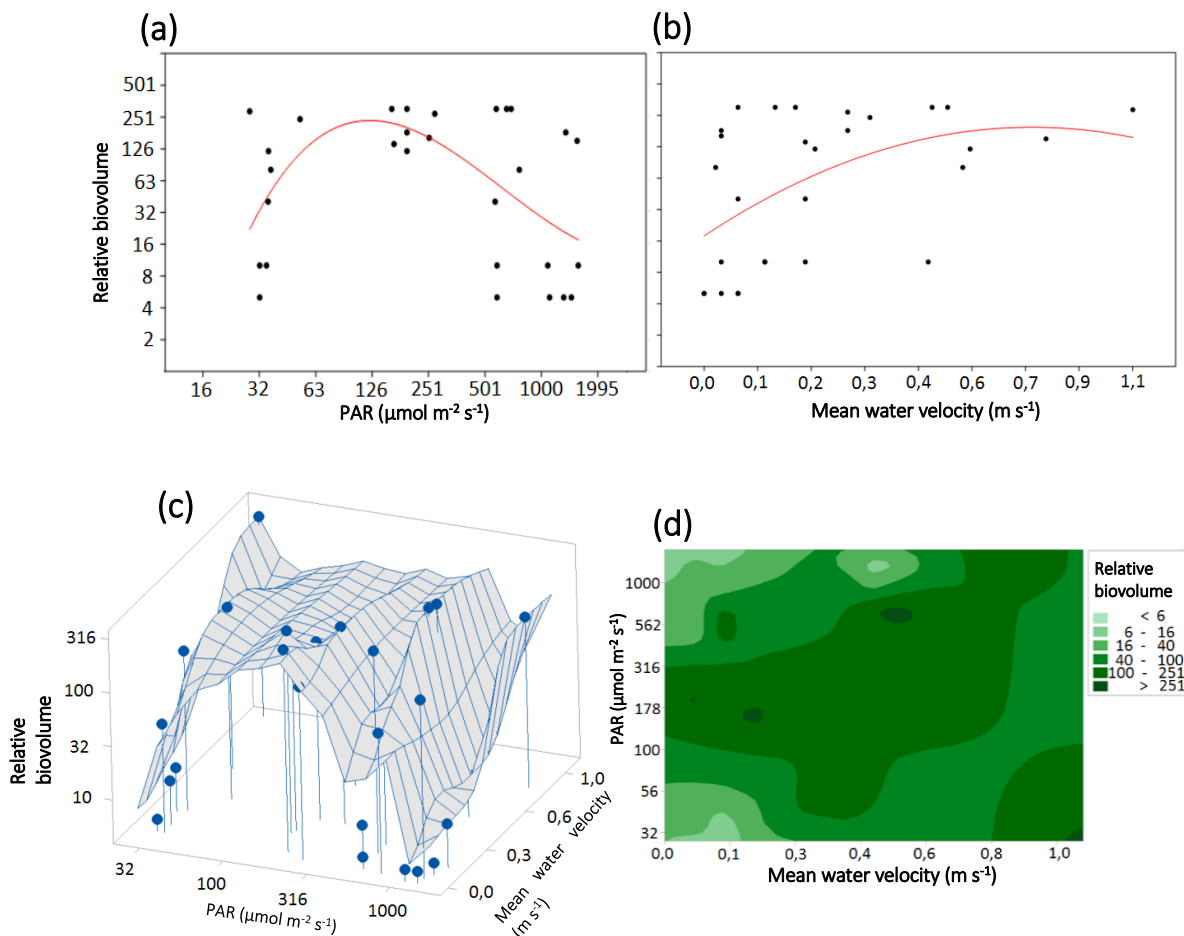
Figure 9. Patterns of relative biovolume in the river Gryteåa øvre (August 2015) in relation to photosynthetically active radiation (PAR; μmol m⁻² s⁻¹) and mean water velocity (m s⁻¹). (a) Polynomial regression model (order 3) showing the variation in relative biovolume as a function of measured PAR ($R^2 = 0,275$; $p = 0,042$). (b) Polynomial regression model (order 2) showing the variation in relative biovolume as a function of mean water velocity ($R^2 = 0,253$; $p = 0,022$). (c) Wireframe plot showing the relationships between relative biovolume as a function of PAR and mean water velocity. The same data are displayed as a contour plot in (d). All variables are represented in logarithmic scale and real values.
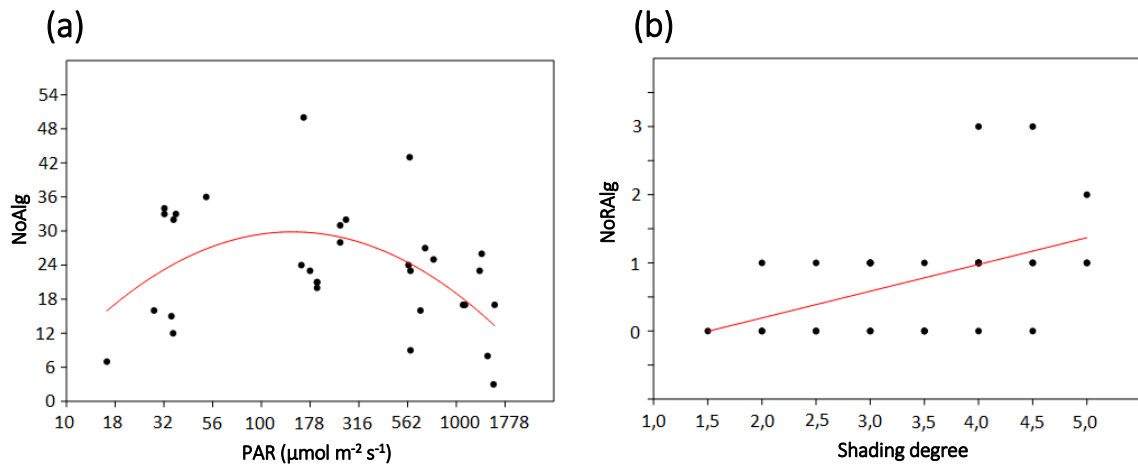
Figure 10. Patterns in algal richness in the river Gryteåa Øvre (August 2015) in relation to light irradiance. (a) Polynomial regression model (order 2) showing the variation in total richness of soft-bodied algae (NoAlg) as a function of photosynthetically active radiation (PAR; µmol m⁻² s⁻¹) (R2 = 0,256; p = 0,014). PAR is represented in logarithmic scale. (b) Red algal richness (NoRAlg) as a function of the degree of shading originated by riparian canopy cover (1 = Open; 5 = Completely covered) (R2 = 0,220; p = 0,007).

Depth was the environmental variable that best explained the variability of the ecological indices (PIT and AIP) among the group of environmental variables correlated with the first PCA component (Figure 8). A linear regression model showed a positive and significant relationship (p = 0,036) between the samples' PIT and depth (Figure 11). The regression line fell entirely in the class of very good trophic status (PIT < 9,5). Differences between two sample groups based on depth (≤ 0,2 m and 0,2 − 1 m) were also tested by conducting a Mann-Whitney U-test. Mean PIT for the group with samples from shallow waters (≤ 0,2 m; n = 18) was 6,23 (95% CI [5,63 6,82]) while the group from deeper waters (0,2 − 1 m; n = 14) presented a mean PIT of 7,08 (95% CI [6,03 8,13]). The resulting p-value from the Mann-Whitney U-test was higher than the significance level (α = 0,05) and indicated that there are no significant differences in PIT between the median values of both groups (Figure 12).
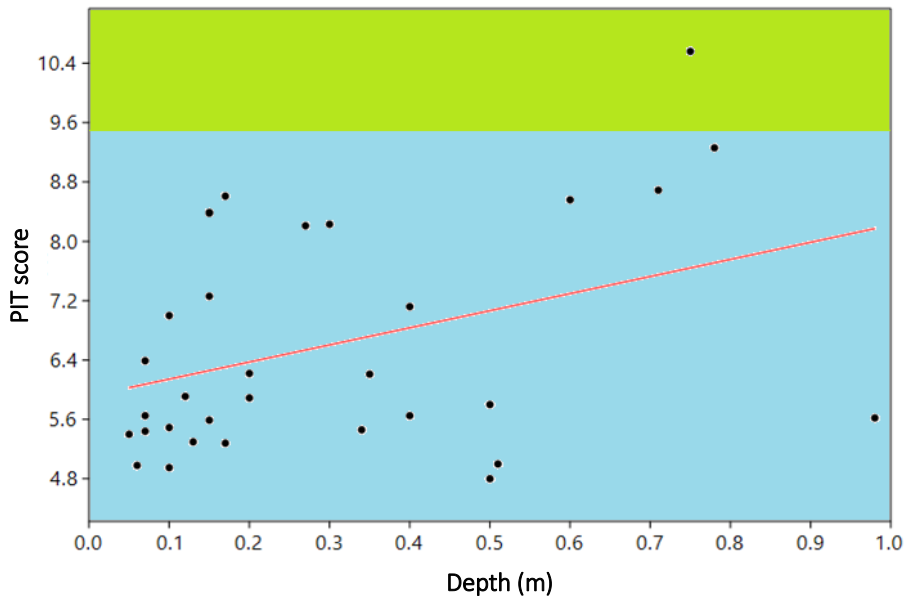
Figure 11. Linear regression model showing the variation in Periphyton index of trophic status (PIT) -calculated for individual samples- in relation to depth ($R2 = 0,139$; $p = 0,036$). Blue and green colours indicate the limit between the classes very good and good trophic status (Direktoratsgruppa 2013). Data from river Gryteåa øvre, August 2015.
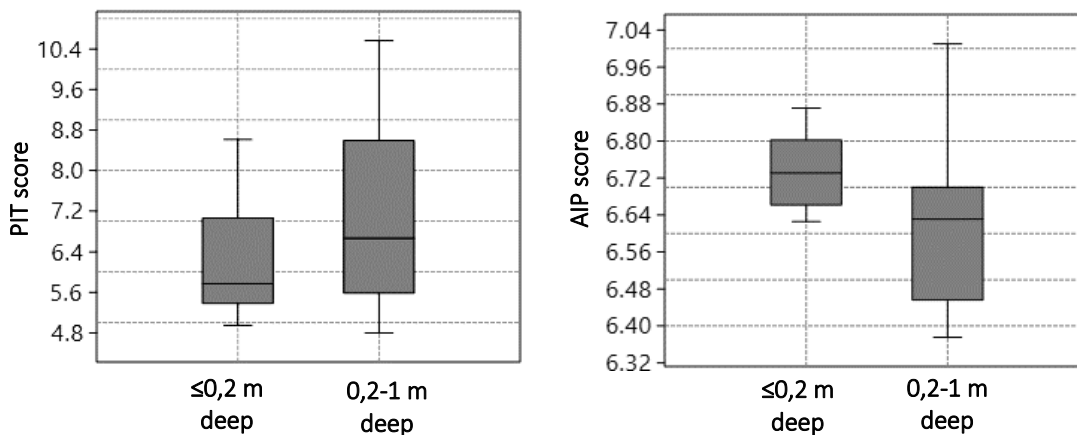
Figure 12. Box plots showing the median, 25-75 percent quartiles, and minimal and maximal values for the samples' PIT and AIP values in the depth groups $\leq 0,2$ m (n = 18) and $0,2 - 1$ m (n = 14). Data from river Gryteåa øvre, August 2015.

The AIP scores of the samples showed a negative and significant relationship with depth (p = 0,001). The regression model covered to a large extent the range of values of the classes good and moderate acidification status (Figure 13). Differences between depth groups ($\leq$ 0,2 m and $0,2 - 1$ m) were tested by carrying out a Welch's approximate t-

test. The group with samples from shallow waters had a mean AIP of 6,74 (95% CI [6,70 6,78]), and the group from deeper waters (n = 14) presented a mean AIP of 6,62 (95% CI [6,52 6,72]). The resulting p-value from the statistical test (p = 0,03) indicated that there are significant differences between the AIP means of both sample groups (Figure 12).
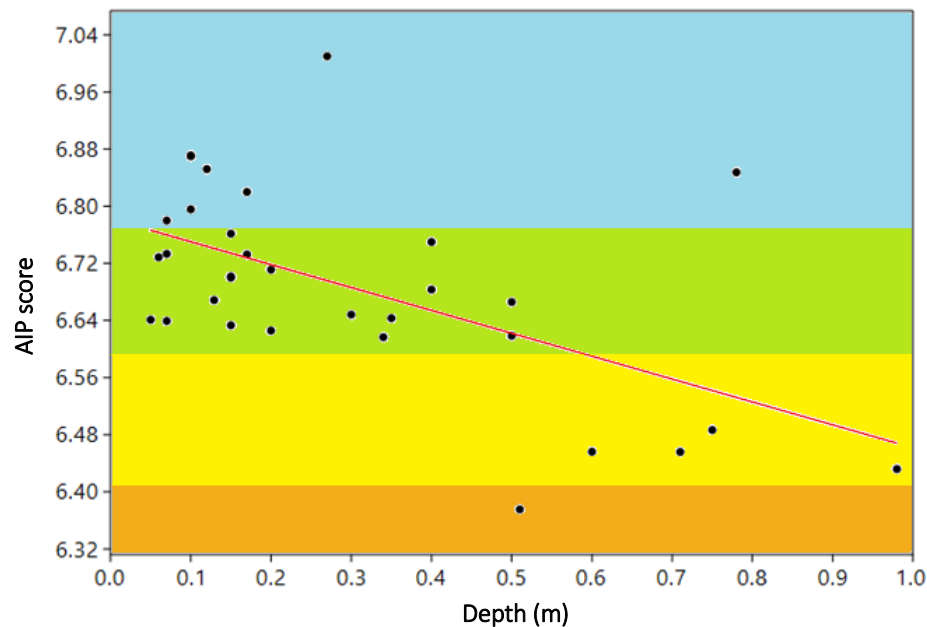
Figure 13. Linear regression model showing the variation in Acidification index periphyton (AIP) in relation to depth. Orange, yellow, green and blue colours indicate the ranges of the classes bad, moderate, good and very good acidification status according to Direktoratsgruppa (2013). R2 = 0,319; p = 0,001. Data from river Gryteåa øvre, August 2015.

3.4. Classification and ordination analyses

Four clusters of periphytic samples were separated by TWINSPAN and these are shown in the DCA plot (Figure 14). The TWINSPAN classification matched well with the relative position of the samples in the DCA ordination. The resulting clusters consisted of three groups of 15 (cluster 1), 9 (cluster 2) and 7 samples (cluster 4), and a fourth sample that remained alone. Algal richness, PIT, AIP and algal composition of the three groups of samples are given in Table 10. The sample that remained alone was characterized by having the highest mean water velocity measured the studied samples (0,8 m/s), and an algal richness of only three taxa: *Lemanea fluviatilis*, *Chamaesiphon rostafinskii* and *Leptolyngbya* sp., where *Lemanea* was the dominant taxon in the sample.
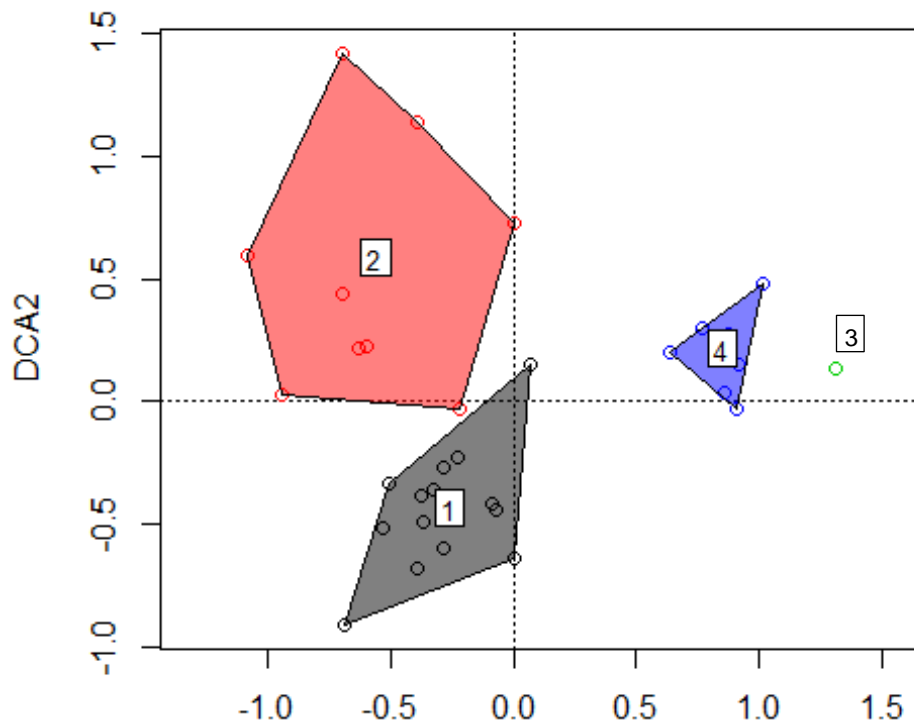
Figure 14. TWINSPAN classification clusters of periphytic samples in relation to sample positions given by the DCA ordination analysis. Data from river Gryteåa øvre, August 2015.

Clusters 1, 2 and 4 were tested for differences in PIT and AIP by implementing Kruskal-Wallis tests for equal medians. Resulting p-values indicated that there are statistically significant differences in PIT (p = 0,041) among clusters. The null hypothesis of equal medians was not rejected in the case of AIP (p = 0,420).

Figure 15 shows the relative position of a selection of taxa in relation to the DCA axes. *Post hoc* fitted environmental vectors for depth, water flow, substrate type and shading degree showed significant correlations (p < 0,05) with the species associations suggested by the DCA. Non-attached unicellular taxa such as the desmids *Closterium parvulum*, *Closterium diantum*, *Cosmarium subcostatum*, *Cylindrocystis* sp., *Euastrum ansatum* and *Haplotaenium minutum* occurred positively correlated with depth. The cyanobacteria *Cylindrospermum* sp., *Geitlerinema splendidumn* and *Oscillatoria tenuis* were also associated with deeper environments. On the other side, taxa such as *Tetraspora cylindrica*, *Cyanophanon mirabile*, *Calothrix* sp., *Zygnema* b (22-25 μm), *Oedogonium* d (29-32 μm), *Lemanea fluviatilis*, *Phormidium autumnale* and *Capsosira brebissonii* appeared to be associated with larger substrates in shallower and faster

flowing waters. Oedogonium b (13-18 µm), *Lemanea fluviatilis, Phormidium autumnale, Capsosira brebissonii, Cosmarium dickii, Cosmarium majae, Netrium* sp*., Closterium incurvum, Coleodesmium sagarmathae, Tolypothrix sp, Microspora amoena* and *Chamaesiphon confervicolus* were positively linked to the degree of shading. Measured photosynthetically active radiation (PAR; *Irrad* in Figure 15) did not have a significant explanatory power when *post hoc* added to the DCA (p = 0,250).

Table 10. Summary statistics for the sample clusters obtained in the TWINSPAN classification of the periphytic samples from the river Gryteåa øvre (August 2015).

	Cluster 1	Cluster 2	Cluster 4
No. of samples	15	9	7
Mean richness (min.; max.) SD	15,67 (5; 29) 5,81	19,89 (15; 27) 3,33	23,00 (7; 35) 9,59
Mean PIT (min.; max.) SD	5,88 (4,95; 8,69) 1,046	7,53 (4,8; 10,56) 2,054	6,99 (5,65; 8,61) 1,808
Mean AIP (min.; max.) SD	6,71 (6,43; 6,87) 0,133	6,64 (6,38; 7,01) 0,197	6,69 (6,63; 6,76) 0,059
Thirteen most dominant taxa in the cluster	*Stigonema mamillosum* *Zygnema* b (22-25 µm) *Mougeotia* b (15-21 µm, short cells) *Batrachospermum* sp. *Oedogonium b* (13-18 µm) *Bulbochaete* sp. *Mougeotia* e (30-40 µm) *Calothrix* sp. *Oedogonium* a (5-11 µm) *Oedogonium* a/b (19-21 µm) *Spirogyra* a (20 - 42 µm, 1 K. L) *Leptolyngbya* sp. *Scytonema mirabile*	*Batrachospermum* sp. *Closterium parvulum* *Closterium limneticum* *Leptolyngbya* sp. *Oedogonium* b (13-18 µm) *Leptolyngbya crassior* *Closterium dianale* *Mougeotia* a/b (10-18 µm) *Oedogonium* a (5-11 µm) *Calothrix* sp. *Phormidium autumnale* *Scytonema mirabile* *Bulbochaete* sp.	*Oedogonium* c (23-28 µm) *Microspora amoena* *Zygnema* b (22-25 µm) *Phormidium autumnale* *Stigonema mamillosum* *Oedogonium* d (29-32 µm) *Oedogonium* b (13-18 µm) *Calothrix* sp. *Capsosira brebissonii* Unidentified red alga *Lemanea fluviatilis* *Chamaesiph. confervicolus* *Tolypothrix* sp.
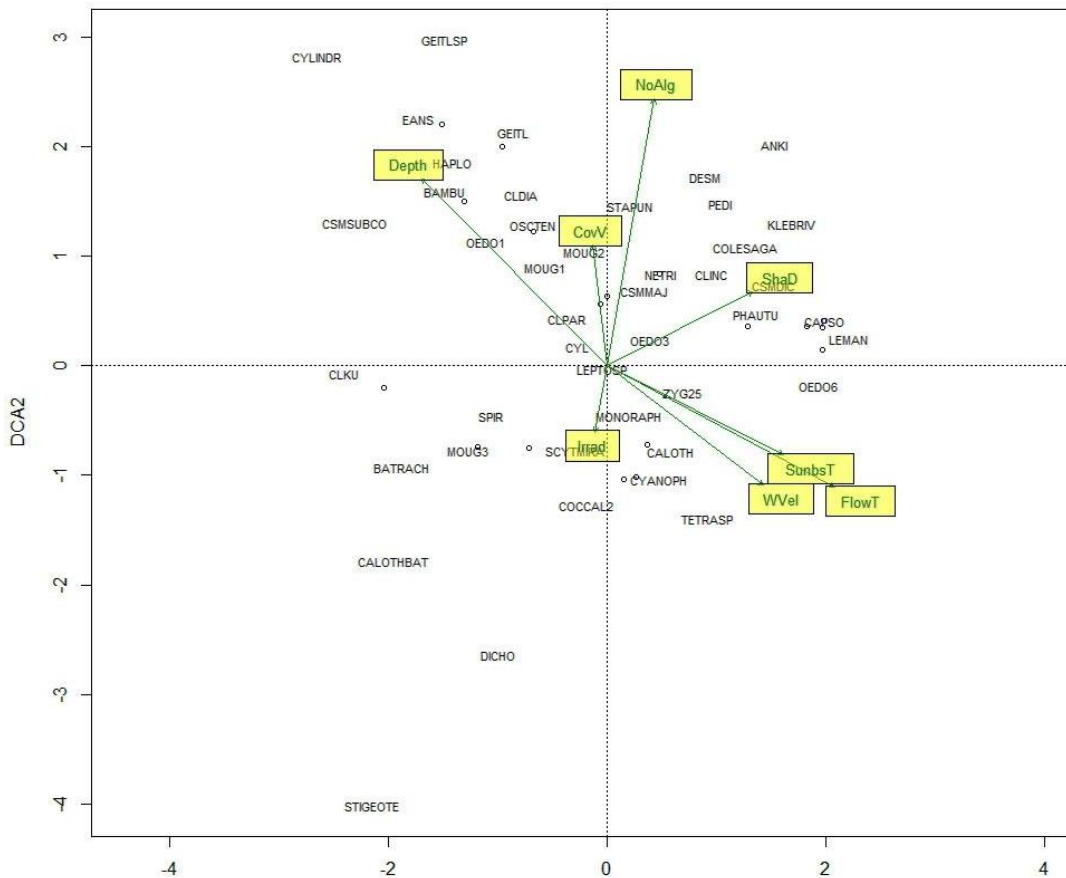
Figure 15. DCA scatterplot showing relative positions of selected soft-bodied benthic algae in relation to axes 1 and 2. Environmental variables were post hoc introduced after the DCA ordination. Data from river Gryteåa øvre, August 2015.

In contrast to DCA, Canonical Correspondence Analysis (CCA) is a direct ordination method where the construction of the axes is based on the environmental variables, and samples and taxa are subsequently correlated to them. In the resulting CCA ordination the first and the second axes explained, respectively, 28,52 % and 22,54 % of the variance in the data set. The relationships among environmental variables were similar to those indicated by the PCA, and the relative position of taxa and samples agreed with those obtained in the DCA. The sample clusters obtained in the TWINSPAN classification were considerably well grouped in the CCA biplot (Figure 16).
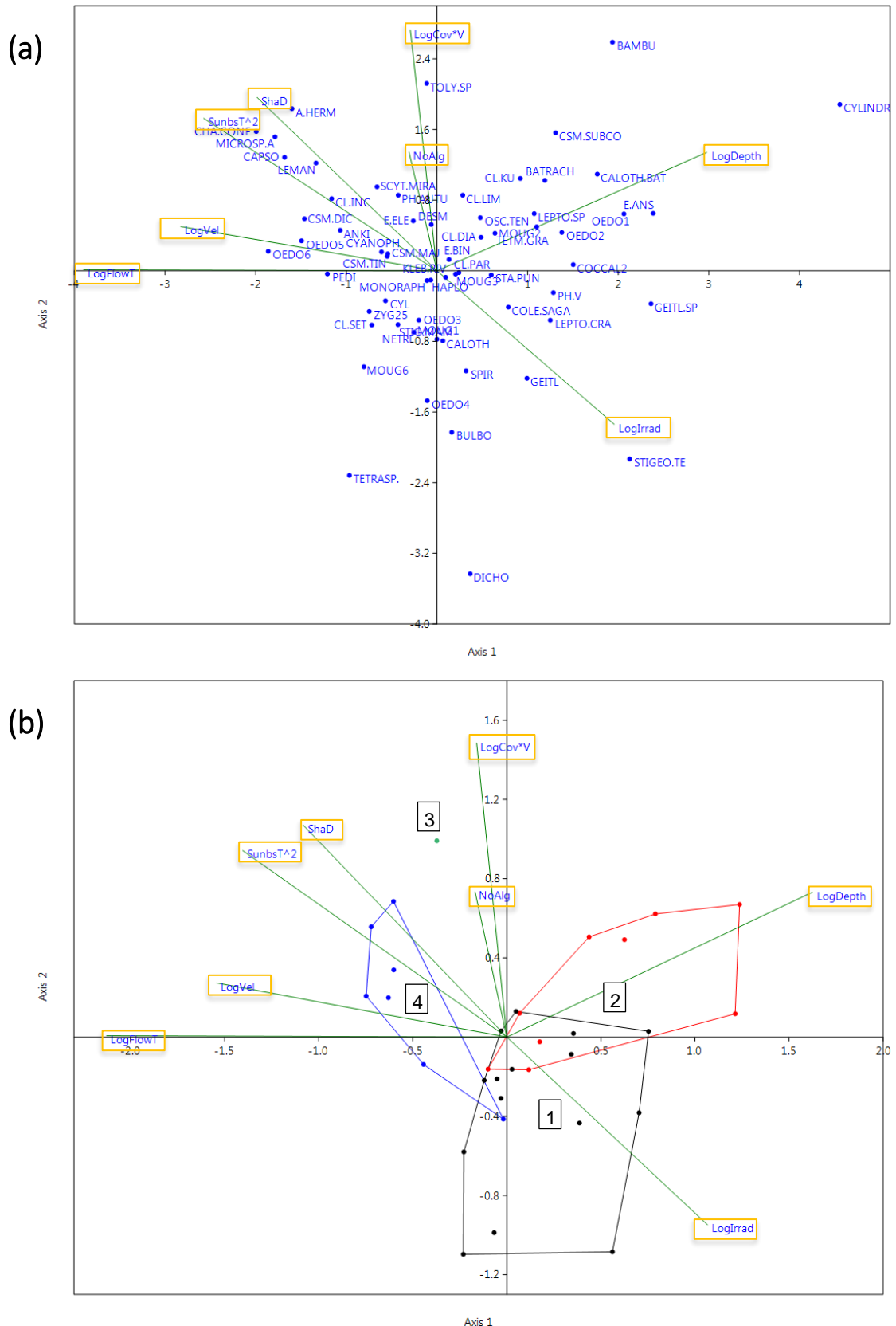
Figure 16. CCA biplots showing relationships among environmental variables and correlated positions of algal taxa (a) and samples (b). TWINSPAN classification clusters based on sample similarity are included in the samples' biplot (cluster 1= black; cluster 2 = red; cluster 3 [one single sample] = green; cluster 4 = blue). Data from river Gryteåa øvre, August 2015.

4. Discussion

4.1. River continuum gradients

Water quality analyses enabled to confirm that the river Gryteåa øvre is a calcium-poor ($[\overline{Ca}^{2+}]$ = 2,7 mg/l) and humic river (mean water colour = 43 mg Pt/l; $\overline{TOC}$ = 6,1 mg/l). Determining the water body type is essential in order to be able to implement the systems for evaluating the ecological status that are currently valid in Norway (Direktoratsgruppa 2013). TP is a parameter that can be used as proxy for trophic status of the water bodies (Schneider and Lindstrøm 2011) and in Norway this parameter is used as a supporting element for the classification of the ecological status of rivers and lakes (Direktoratsgruppa 2013). Mean TP of the samples obtained from the river ($\overline{TP}$ = 4,4 µg P/l) pointed out a very good ecological status with regard to eutrophication. TP concentration showed an increasing trend along the stream, and there where statistically significant differences between the mean TP of the samples obtained from the upstream locations 5, 6 and 7 and the samples from the downstream locations 1,2, 3 and 4 (Figure 3). This headwater-to-mouth increase in nutrients is a well-known pattern that occurs often along the river continuum due to the summative effect of input sources (Vannote et al. 1980). Nonetheless, concentrations indicated ultraoligotrophic conditions, and raised in a so small degree that changes were very unlikely to be significant in ecological terms. A two-sample t-test between mean PIT of the upstream and downstream algal samples did not indicate statistically significant differences between both groups. This result supports the presumption that the increasing phosphorus concentrations towards the downstream end of the river Gryteåa øvre were not sufficiently prominent to have measurable effects on the river's ecological processes.

In addition to TP, there were several other water quality parameters that showed statistically significant differences among sampling stations and/or months (Table 8). In the same vein as TP, measured variations were very small and they were not expected to have meaningful spatiotemporal influences on the river's biota.

4.2. Microenvironmental variables

Several environmental variables that were measured at each of the algal sampling points showed logical associations in the PCA analysis. The resulting PCA showed that flow type and water velocity were, as expected, highly positively correlated (Figure 8). It is therefore advisable, considering the importance of water velocity for benthic organisms, to register the flow type (Table 4) when conducting periphyton surveys in lotic systems when a water velocimeter is not available. Flow type and water velocity had a strong negative relationship with depth. As expected, substrate size was positively associated with water velocity, and negatively associated with depth. A general microhabitat gradient when considering these interrelated variables would span from deeper and quiet waters with small substrates, to shallower and faster waters with cobble and larger substrates. Taking into account the high interrelation among these variables in the river Gryteåa øvre, it is reasonable to think that having the information of one of them can give us a fair picture of the hydrologic environment at a given point of this or a similar river. Nevertheless, and as evidenced in this study, these factors have different explanation power when predicting biological variables such as algal biomass or species richness.

Photosynthetic active radiation (PAR) and shading degree where strongly negatively correlated. In the same way as with water velocity and flow type, it is therefore recommended to register the degree of shading (Table 5) when conducting periphyton surveys, since it can give valuable information about the light conditions when we do not have a photometer available. As with the hydrologic variables addressed before, the explanatory power of PAR and shading degree when predicting biological variables can vary.

An important point when examining a PCA is that it only displays linear relationships among variables. Using the iterative imputation method for handling missing data points during the analysis can lead to an overestimation of the strength of the PCA components (Ilin and Raiko 2010). This is however not expected to be the cause of concern in this study due to the usage of a very nearly complete data matrix.

4.3. Algal biomass and algal richness

4.3.1. Variations in algal biomass

Biomass is undoubtedly a variable of interest when studying photosynthetic organisms. Chlorophyll-a, ash-free dry mass (AFDM) and cell biovolume measurements are extensively used methods for estimating algal biomass, but each of them present its own strengths and weaknesses. Chlorophyll-a is the most common way to indirectly estimate biomass of photosynthetic organisms. However, variations in chlorophyll content among different taxa and fluctuations associated to changes in environmental conditions can lead to significant error (Baulch et al. 2009; Hill et al. 2009). AFDM measures the total amount of organic material in the sample, including both autotrophic and heterotrophic living and dead organisms, plus debris of diverse origins. Combining these two variables can provide complementary information and be combined to form a ratio (Biggs and Kilroy 2000). Measurements of biovolume are often considered as the most reliable algal biomass estimations, but they are time consuming and there are error sources associated to the geometric shape approximations, cell counts, and variation in cell densities in different taxa (Baulch et al. 2009).

Due to working capacity limitations none of the aforementioned methodologies could be implemented in this study. Variations in algal biomass were therefore estimated from the percentage cover and the type of algal mat or filaments registered in the field in a similar way as it is frequently done in terrestrial floristic investigations (Odland et al. 2014). The resulting variable was denominated *relative biovolume*, has no units and is unfortunately not easily comparable to any universal biomass measurement. Despite the inconvenience of not having conventional biomass measurements, *relative biovolume* allowed making comparisons among the periphyton samples of this study and could be regarded as a response variable. Further studies should however employ standard procedures for estimating algal biomass in order to make the results universally comparable.

Another consideration when studying biomass in streams is that the relative contribution of benthic algal biomass might vary in space throughout the accrual cycle

(Figure 1). Periphyton samples in this study were obtained in late summer from a lake-fed river. Lake-fed rivers are characterized by having a low flow variability (Biggs and Kilroy 2000), and by the end of summer periphyton communities were expected to have had enough time to develop throughout the growing season. Benthic algal communities in the river Gryteåa øvre were therefore assumed to have passed the biomass peak of the biomass cycle and occur in a mature state corresponding the carrying capacity level (Figure 1).

The present study's PCA analysis showed that the *relative biovolume* of the algal samples appeared highly associated to the variables *shading degree* and *PAR* (Figure 8). Further regression analyses showed that these variables had a unimodal relationship that was better explained by polynomial models -rather than linear models (Appendix 5). A 3rd order polynomial model between log-transformed relative biovolume and PAR (Figure 9 a) showed that the highest relative biovolumes occurred at PAR intensities of 100 to 300 μmol m^{-2} s^{-1}. Most short-term photosynthetic-irradiance studies suggest that photosynthesis in stream benthic algae is saturated at irradiances between 100 to 400 μmol m^{-2} s^{-1} (Hill 1996; Hill et al. 2001; Hill et al. 2009). Lower irradiances experienced in severely shaded streams can be powerful constraint on benthic algal photosynthesis (Hill et al. 2009). High light intensities in high-elevation mountain streams have been linked to low algal biomasses as a result of photoinhibition (Wellnitz and Rader 2003). At this point, we could argue that lower algal biovolumes at both extremes of the PAR gradient might be explained by light limitation at low PAR intensities (< 60 μmol m^{-2} s^{-1}) and photoinhibition at very high PAR intensities (> 600 μmol m^{-2} s^{-1}). Although not obvious in the PCA, further regression analyses showed that relative biovolume was positively correlated to water velocity (Figure 9 b). Gryteåa øvre is an oligotrophic river with $\overline{\text{TP}}$ concentrations of 4,4 μg P/l. It is therefore reasonable to register larger relative biovolumes at higher water velocities, where benthic algae can profit from a continuous input of nutrients and a greater mass transfer of metabolites (Biggs 1996; Stevenson 1996).

Relative biovolume variability was especially notable at both extremes of the PAR gradient and at low water velocities. A 3D wireframe plot representing relative biovolume as a function of PAR and water velocity helped understanding this variability

(Figure 9 c). Relative biovolume presented a hump-shaped relationship with PAR at low water velocities, with higher algal biovolumes at intermediate light intensities. On the other hand, algal biovolume remained high at all irradiances when samples were obtained from high water velocities. High biovolumes at low light irradiances and high water velocities might be explained by synergistic effects between nutrient and light availability. Hill et al. (2011) investigated the way in which the availability of one of these resources facilitated the utilization of the other. Results showed higher ratios of chlorophyll a : biomass in phosphorous-enriched streams that might be associated to a more efficient use of light. Considering the enhancement of nutrient availability as current velocities increase in oligotrophic rivers (Biggs 1996; Stevenson 1996), there might be an increased photosynthetic activity even at low irradiances explained by resource synergy. A similar reasoning may apply for the high biovolumes registered at high irradiances and high water velocities. Algae developing in these environments may have an increased nutrient-uptake capacity and be simultaneously less susceptible to photoinhibition (Hill et al. 2011).

An alternative explanation of the variation in biovolume along the water velocity gradient at high light intensities might be related to biological factors. Grazing by benthic invertebrates is known to be a determining factor associated to biomass losses (Steinman 1996) and increased heterogeneity of biomass distribution within streams (Hillebrand 2008). High light intensities have been associated with increased growth of benthic herbivores in oligotrophic streams (Hill et al. 2001; Hill et al. 2010) and greater algal biomass losses caused by grazers (Wellnitz and Rader 2003; Hillebrand 2005). At the same time, current velocity has been proved to reduce grazer effectiveness (Poff and Ward 1995). These relationships may therefore explain the low relative algal biovolumes in high light and low current conditions in the river Gryteåa øvre due to a possibly enhanced grazing pressure. Hydrologic stress on herbivores at more intense current velocities may be associated to a reduced grazing pressure and explain the occurrence of high algal biovolumes.

A final consideration when analysing biovolume patterns in this study is that algal samples obtained at low and intermediate current velocities were considerably better represented than samples from high velocities. An increased number of samples

obtained in fast waters (> 40 cm s^{-1}) would improve the strength of the results, and should therefore be considered in future investigations.

4.3.2. Variations in algal richness

Between-stream variation in algal taxonomic richness have been studied in relation to environmental factors such as flood disturbance and water quality (Biggs and Smith 2002; Cardinale et al. 2006; Schneider et al. 2013). However, less attention has been paid to changes in richness associated to microhabitat variations within streams. Although not obvious in the PCA that was conducted in this study (components 1 and 2), further regression analyses showed that total algal richness had a hump-shaped association with PAR (Figure 10). This unimodal response might be explained in a similar way as biovolume, appealing to light limitation for many species at low irradiances, and photoinhibition and/or richness decline by grazing at very high light intensities. Separated analyses for green algae and cyanobacteria showed a similar response, while red algae presented a significant negative relationship with PAR (Appendix 6). This association of red algal richness with light was also explained by a significant positive correlation with the degree of shading (Figure 10). Red algae have phycobilins as accessory pigments that allow them to harvest blue-green light and develop in deeper waters than other taxa (Lee 2008). In deep environments light is more limited and just shorter wavelengths are available (Brönmark and Hansson 2005). Forest canopy results also in an important light attenuation due to a selective transmission and reflection by leaves. Most part of the visible light spectrum is absorbed in severely covered areas, with exception of wavelengths between 520 and 620 nm (Théry 2001). These wavelengths match well with the absorbance ranges of phycobilins, which have absorption maxima at 575 nm (phycoerythrin) and 620 nm (phycocyanin) (Lee 2008). Freshwater red algae might consequently be able to use severely shaded stream environments more efficiently than other taxa, explaining the positive relationship between red algal richness and shading degree that is presented in this study.

4.4. Microhabitat and ecological indices

The periphyton index of trophic status (PIT) was developed by Schneider and Lindstrøm (2011) by calculating taxon's TP optima in Nordic rivers. The Acidification Index for

Periphyton (AIP) was developed by calculating pH-optima of soft-bodied benthic algae in Norwegian rivers (Schneider and Lindstrøm 2009). Taxon indicator values included in these ecological indices provide a very good foundation to place taxa in their right ecological niche with regard to river trophic and acidification status. The sampling methodology for implementing the PIT and AIP indices follows the guidelines of the European Committee for Standardization (CEN 2009) and consists in collecting all visible benthic algae from a 10 m length transect in the river. In addition, microscopic algae are collected by brushing about 8 X 8 cm from the upper side of 10 stones from the sampling transect. Sampling methodology is therefore designed to obtain all algal species occurring in a river segment without taking into account microenvironmental conditions within the stream. However, CEN (2009) does provide guidelines regarding the election of sampling locations. According to these guidelines it is desirable to choose sampling locations where water flows and mixes continuously in order to avoid eventual built-up local physicochemical environments. It is indeed the effect of microenvironmental conditions this investigation had its focus on, and therefore mean taxa optima for PIT and AIP were calculated for each of the single periphyton samples obtained in line with Table 2.

Resulting PIT and AIP values were included in the PCA analysis and showed a high correlation with the first component, together with the variables depth, water velocity, flow type and substrate size. Further regression analyses indicated that depth was the environmental factor that best predicted PIT and AIP scores of the samples. PIT had a significant positive correlation with depth, and the regression line fell entirely in the class of very good trophic status (PIT < 9,5) (Figure 11). Most samples obtained from shallow waters where mainly composed of algae with low PIT indicator values (IV). Higher PIT values were originated by the presence of three cyanobacteria that are associated to eutrophic conditions: *Oscillatoria tenuis* ($IV_{O.\ tenuis}$ = 44,24), *Oscillatoria limosa* ($IV_{O.\ limosa}$ = 39,10) and *Geitlerinema spendidum* ($IV_{G.\ splendidum}$ = 43,42) (Figure 17). These cyanobacteria occurred in both shallow and deep waters, and the positive slope of the regression model was the result of the scarcity of samples free for these cyanobacteria as deep increased. Deeper environments in Gryteåa øvre were usually associated to low water velocities and smaller substrates. Slow environments are characterized by increased sedimentation rates of sediment particles and organic

matter that hinder the growth capability of many algal taxa. The cyanobacteria *O. tenuis, O. limosa* and *G. splendidum* are motile taxa that have the capacity to glide and oscillate to overcome the effects of being covered by sediments (Komárek and Anagnostidis 2005). Furthermore, they have phycobilins as accessory pigments, allowing them to harvest short-wave light in deeper environments (Lee 2008). These taxa occur often associated to mud, sandy bottoms and decaying organic matter (Komárek and Anagnostidis 2005), having a privileged access to nutrients that are product of mineralization (Burkholder 1996). This combination of environmental and biological characteristics makes these taxa more likely to occur in deep and slow environments, even in oligotrophic streams. It is therefore essential to follow the sampling guidelines from CEN (2009) regarding the choice of sampling locations in order to ensure that sampled taxa represents the general trophic status of the stream and not local physicochemical environments. It is also important to conduct a sampling that includes both a careful visual inspection of macroscopic algae occurring in the sampling location as well as a systematic sampling of benthic algae along a transect. This will ensure a representative sampling of the algal diversity, and provide a resulting PIT value that reflects the overall trophic status of the water body.
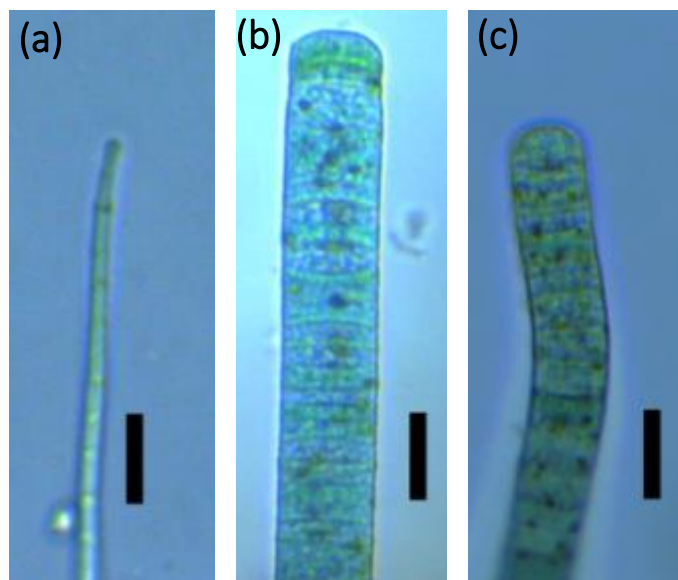
Figure 17. Cyanobacteria from the river Gryteåa øvre (2015) with highest PIT indicator values: (a) Geitlerinema splendidum, (b) Oscillatoria limosa (c) Oscillatoria tenuis. Black bar indicates 10 µm. Photo: Miguel A. Segarra.

Samples were grouped according to depth (≤ 0,2 m and 0,2 – 1 m) and a Mann-Whitney test for equal medians did not indicate statistically significant differences in PIT between groups (Figure 12). Visual inspection of the PIT distribution in relation to depth (Figure 11) shows that the threshold depth that appears to be related to changes in PIT is approximately 0,5 m. The number of samples obtained from deep environments (0,5-1 m deep) was however small (n = 8) compared to the number of samples from shallow waters (< 0,5 m; n = 24). In order to be able to compare these groups with an acceptable statistical power, future investigations should include a more balanced distribution of data points along the depth gradient.

AIP showed a significant negative relationship with depth, and the regression line crossed the threshold value between good and moderate acidification status (Figure 13). In this case, a comparison between depth groups (≤ 0,2 m and 0,2 – 1 m) did also show statistically significant differences in AIP, with deeper samples having a lower mean AIP than samples from shallower environments (Welch approximate t-test; p = 0,02) (Figure 12). Registered taxa with AIP indicator values less than 6,56 were *Closterium parvulum* ($IV_{C.parv.}$ = 6,55), *Chamaesiphon rostafinskii* ($IV_{Ch. rost.}$ = 6,45), *Bulbochaete sp.* ($IV_{Bulbochaete}$ = 6,43), *Coleodesmium* sagarmathae ($IV_{Co. sag.}$ = 6,26), Stigonema mamillosum ($IV_{S.mamillosum}$ = 6,25), *Scytonema (Myochrotes) mirabile* ($IV_{Sc.mirabile}$ = 5,65), *Mougeotia* a/b (10-18 µm) ($IV_{Moug. a/b}$ = 5,57), *Stigonema ocellatum* ($IV_{S.ocellatum}$ = 5,38), *Capsosira brebissonii* ($IV_{C.brebissonii}$ = 5,19) and *Stigonema hormoides* ($IV_{S.hormoides}$ = 5,19) (Figure 18). An inspection of the algal taxa occurring above and under 0,5 m depth revealed that there were no differences in the mean number of the aforementioned acidic taxa in shallow and deep stream environments (3,3 "acidic taxa" per sample). However, samples obtained from deeper environments (0,5-1 m) contained a significant lower number of algal taxa with AIP indicator values above the threshold for good acidification status (3,1 "non-acidic taxa" per sample) compared to samples obtained from shallower waters (depth < 0,5 m; 5,2 "non-acidic taxa" per sample) (Mann-Whitney test for equal medians; p = 0,017). This explains the prevailing low AIP values in samples obtained from deep environments and the negative relationship between AIP and depth.
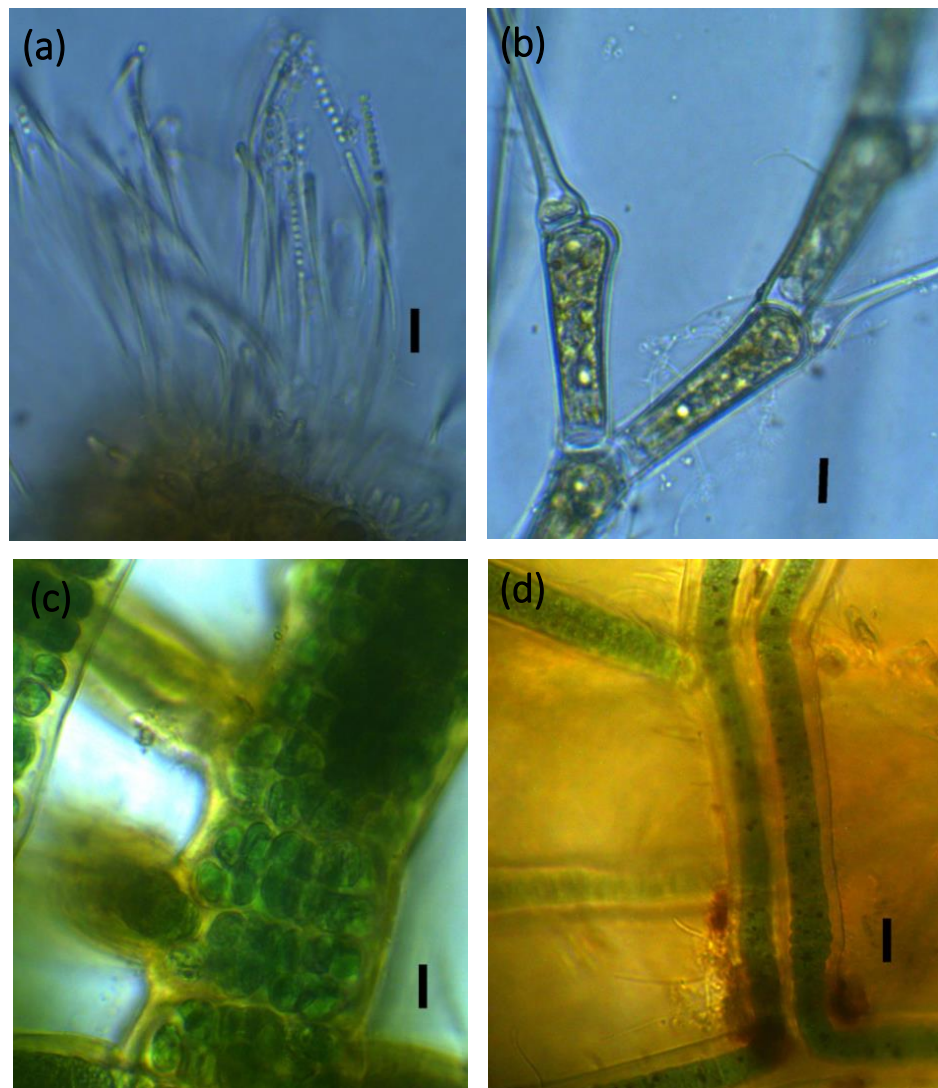
Figure 18. Selection of algal taxa from the river Gryteåa øver (2015) with AIP indicator values under the threshold between god and moderate acidification status for river type 6 (AIP = 6,56). (a) Chamaesiphon rostafinskii (b) Bulbochaete sp., (c) Stigonema mamillosum, (d) Scytonema (Myochrotes) mirabile. Black bar indicates 10 µm. Photo: Miguel A. Segarra.

Ecological differences between acidic and non-acidic taxa do not provide an obvious line of reasoning for explaining changes along the depth gradient as it has earlier attempted with PIT because there are no differences in motility. However, seven out of the ten "acidic taxa" present in this study are cyanobacteria and do have phycobilins as accessory pigments that might allow them to use light more efficiently than other taxa in deep environments. On the other hand, only 40% of the "non-acidic taxa" have phycobilins (i.e. cyanobacteria and red algae).

An alternative line of reasoning might be linked to possible microenvironmental differences in TOC concentrations. Schneider (2011) investigated the impact of calcium and TOC on the acidification index for periphyton (AIP) and found a negative correlation between AIP and TOC in very Ca-poor rivers (< 1 mg Ca/l). Despite the fact that this study was based on comparisons between rivers, local conditions within rivers may originate variations in TOC close to the sediments at a microhabitat scale (Ouyang et al. 2006). Deep and slow flowing waters are related to accumulation of organic matter and production of organic acids that might explain local pH reductions in Ca-poor rivers. These built-up microenvironmental conditions might explain changes in benthic algal composition, expressed as reductions in AIP in deeper waters of the river Gryteåa øvre.

4.5. Algal diversity and algal assemblages

With 172 algal taxa (including morphological forms), we can say that the river Gryteåa øvre presents a very high algal diversity. This is the result of an important sampling effort that included 32 periphyton samples distributed along the whole river length, and consciously taken from a considerable variety of microhabitats. In addition, many of the algal taxa found in the river might have their origin in the feeder lake (Lake Sveigstjønn, Figure 3), resulting in a significant higher richness due to the combination of algal taxa from lentic and lotic systems. This high algal richness made taxonomical identification very demanding, and constituted a notable portion of the total effort for the elaboration of the present study. Nevertheless, and despite numerous examinations of the taxonomical identifications and consultations with experts, inaccurate algal identifications are to be expected. According to Manoylov (2014) up to 80 % of the variation in the results of diatom indices may come from the taxonomist, while minor error sources may result from the sampling (10%), preparation of samples and diatom slides (5%) and from the replicates of each slide (5%). Assessing soft-bodied algae is according to Manoylov even more complex, because "the diversity of the groups precludes anyone having all required literature for species-level identification". Two additional complications associated with the identification of soft-bodied algae are the lack in specific literature for algal identification in Norway (identification keys are often developed for other specific regions), and the abundance of name synonyms that can lead to mismatches between identification keys and species lists from ecological

indices. Despite these difficulties, Manoylov (2014) argues that ecological indices based on algae work because random and unbiased errors in algal identifications should increase the variability of indicator values without altering the central tendency.

In addition to the calculation and analysis of ecological indices (see sections 2.3.1, 3.3, and 4.4), taxonomic information was used to examine floristic associations and gradients. Resulting sample clusters from the TWINSPAN analysis allowed to study which algae are more prone to occur together (Table 10). TWINSPAN classification and DCA ordination are exclusively based on floristic similarity, and TWINSPAN clusters matched well with the sample position suggested by the DCA analysis and there was no cluster overlapping (Figure 14). Even more interesting is the relationship between TWINSPAN clusters and the Canonical Correspondence Analysis (CCA). As a regression, CCA uses environmental factors as explanatory variables, and maximizes the correlation of sample points and taxa (that act as response variables). Surprisingly enough, TWINSPAN sample clusters -based exclusively on algal composition- had a clear correlation with different environmental vectors, giving weight to the importance of the environmental variables that were included in this study for explaining the taxonomic composition of the samples (Figure 16 b). Light intensity appeared to be the variable that best explained the position -and algal composition- of samples from cluster 1. Samples and taxa from cluster 2 were positively correlated with deep and slow flowing environments with small substrates. Samples and algal taxa from cluster 4 appeared to be associated to faster waters and shaded environments. The PIT values of the clusters were analysed and cluster 2 presented the highest mean PIT (Table 10). A Kruskal-Wallis test for equal medians showed statistical differences in PIT among clusters ($p < 0,05$). Further analyses with a Mann-Whitney pairwise tests indicated that PIT differences were detected between clusters 1 and 4 ($p = 0,019$), but not with cluster 2. This is attributable to the high variability of PIT values in samples from cluster 2 (Appendix 8). AIP did not show statistically significant differences among TWINSPAN clusters.

Floristic classification (or clustering) is very often too rigid and sets artificial borders where in fact there is a community continuum. This can result in clusters of samples that differ in some species, but that share many other taxa (see Table 10 and taxa occurring in several clusters: e.g. *Stigonema mamillosum*, *Batrachospermum* sp.,

Oedogonium b (13-18 µm) and *Calothrix* sp.). Ordination, on the other hand, allows to explore sample similarity and taxa relationships without the rigidity of clustering. The combination of both methodologies was however very informative in this study.

In addition to sample similarity (Figure 14), the Detrended Correspondence Analysis (DCA) provided information about species relationships and likelihood to occur together (Figure 15). *Post hoc* introduced environmental variables had logical associations with the position of benthic algal taxa. The association of depth with several desmid algae might be explained by an increased settlement of these unicellular taxa. Although most desmids exhibit a benthic lifestyle, they are easily detached from the substrate by the water current (Coesel and Meesters 2007). It is therefore reasonable to expect a higher sedimentation rate in environments with lower current velocities. Depth was also associated to the cyanobacteria *Geitlerinema splendidumn* and *Oscillatoria tenuis*. This relationship agrees with the reasoning presented in point 4.4 about their biological traits. Their high PIT indicator values also support the positive relationship between PIT and depth that has been discussed earlier. Large substrates and fast flowing waters were associated to current-demanding taxa such as *Lemanea* sp. (Whitford 1960; Rueness et al. 2011) and *Cyanophanon mirabile* (Komárek and Anagnostidis 1999). *Lemanea* sp. was also associated with the degree of shading, and this agrees with the efficient use of light in phycobilin-holding taxa.

Environmental vectors in the DCA were *post hoc* fitted in the species ordination diagram (Figure 15) and they gave information about the environmental conditions that correlated better with the species ordination. On the other hand, CCA analysis locates single species in relation to their connection with the existent environment. It is therefore expected to find a better relationship between single species and their optimal environment in the CCA than in the DCA. Resulting species-environment associations obtained in CCA analysis were generally very resembling to the ones shown in the DCA. This might indicate that environmental conditions are important not only for single species, but for also for algal species assemblages.

5. Conclusion

Statistically significant differences in water quality parameters were detected along the river continuum, but differences were not important enough to be considered of ecological relevance. This was confirmed by testing possible differences in PIT and AIP between the upstream and the downstream sections of the river, and the null hypothesis for equal means was not rejected ($p > 0,05$). Relationships among environmental variables at a microhabitat scale were investigated, and it was found a strong association between the variables depth, water velocity, flow type and substrate size. Depth was negatively correlated to water velocity and flow type; substrate size had a positive relationship with water velocity and was negatively associated to depth. This group of variables appeared to be associated to the ecological indices PIT and AIP in the PCA analysis, and further regression analyses indicated that depth was the factor that best explained variations in these indices within the river. PIT presented a significant positive relationship with depth, but the regression line did not cross the threshold value between very good and good trophic status. AIP was negatively correlated to depth, and the regression line crossed the threshold value between good and moderate acidification status. Further analyses with a better representation of samples from deeper environments are needed to corroborate these relationships.

Another important group of explanatory environmental variables were photosynthetically active radiation (PAR) and shading degree. These variables presented a negative relationship, and were associated with the richness of algal taxa and the relative biovolume of the samples. Total algal richness showed a hump-shaped relationship with PAR. Richness of Chlorophyceae (green algae) and Cyanophyceae (cyanobacteria) presented a similar relationship with PAR. Red algal richness (Rhodophyceae) had a negative relationship with PAR and a positive association with the degree of shading originated by riparian canopy cover. Relative biovolume presented also a unimodal relationship with PAR, but an important fraction of the model variability was solved when including water velocity as a predictor variable. A 3D wireframe plot showed that relative biovolume had a hump-shaped association with PAR at low water velocities, while biovolumes remained high at elevated PAR and water velocities. Explanations for these responses include variations in grazing activity, source

limitation and source synergy. A better distribution of data points along PAR and water velocity gradients, together with the conduction of standard biomass analyses (i.e. chlorophyll-a and AFDM) and the study of the communities of benthic invertebrates should be considered in future investigations.

TWINSPAN sample clusters based on algal composition were clearly associated to different environmental variables when visualized in the CCA ordination biplot. This corroborated the importance of measured microenvironmental variables for the algal composition of the periphyton samples. A DCA scatterplot with *post hoc* added environmental vectors showed significant correlations between species associations and the environmental factors depth, water flow, substrate type and shading degree. A CCA biplot with relationships among environmental variables and soft-bodied benthic algae indicated the most relevant factors explaining the presence and abundance of each single algal taxa.

Changes in spatial environmental conditions within streams are important for explaining benthic algal biomass, richness and species composition. Microhabitat might simultaneously influence the resulting values of the ecological indices based on soft-bodied algae that are currently used in Norway. This influence may be specially linked to the election of sampling location and sampling methodologies. Following current sampling standards is therefore important in order to assure a full sampling of the algae occurring in the waterbody, and getting index values that reflect the true ecological status. Registering microenvironmental variables during periphyton assessments is desirable in order to enhance our understanding of algal ecology in lotic systems. This may also contribute to a better resolution and resolving power of ecological indices in the future.

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Appendixes

Appendix 1 – Water quality analyses

Tables showing resulting values for the parameters that were included in the water quality analyses. P-values for the water quality parameters that showed statistically significant differences among sampling locations and/or sampling periods (test for equal means: two-way ANOVA without replications) are indicated in bold. Water samples were obtained in June, August and October 2015 from seven sampling points along the oligotrophic river *Gryteåa øvre* (southeast Norway).

Table A1-1. pH.

pH				
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	6,7	6,9	6,3	6,6
Location 2	6,9	6,7	6,5	6,7
Location 3	6,9	6,9	6,5	6,7
Location 4	6,8	6,7	6,3	6,6
Location 5	6,9	6,7	6,6	6,7
Location 6	6,7	6,8	6,5	6,7
Location 7	6,6	6,7	6,4	6,6
Mean	6,8	6,7	6,5	6,7

*Two-way ANOVA without rep.: p-value (locations) = 0,27; **p-value (months) = 5,31x10^{-5}***

Table A1-2. Total organic carbon.

TOC (mg/L)				
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	5,51	5,78	7,18	6,00
Location 2	5,44	5,76	7,28	5,98
Location 3	5,54	5,78	7,20	6,01
Location 4	5,61	6,03	7,25	6,12
Location 5	5,50	6,09	7,45	6,14
Location 6	5,77	6,20	7,58	6,33
Location 7	5,69	6,02	7,67	6,27
Mean	5,58	5,95	7,37	6,12

*Test for equal means: **p-value (locations) = 0,0062; p-value (months) = 8,51x10^{-13}***

Table A1-3. Water colour.

LONA	Water colour (mg Pt/L)			
	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	34	35	53	41
Location 2	33	32	60	42
Location 3	34	38	65	46
Location 4	34	33	55	41
Location 5	35	38	55	43
Location 6	36	40	57	44
Location 7	35	37	53	42
Mean	35	36	57	43

*Test for equal means: p-value (locations) = 0,36; **p-value (months) = 9,30x10^{-9}***

Table A1-4. Total phosphorous.

LONA	TP (µg P/L)			
	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	5,9	4,4	4,9	5,1
Location 2	5,9	4,4	4,9	5,1
Location 3	6,1	4,7	4,5	5,1
Location 4	5,5	3,7	3,9	4,4
Location 5	3,8	2,8	3,8	3,5
Location 6	4,9	3,8	3,2	4,0
Location 7	4,6	3,6	3,7	3,9
Mean	3,9	4,4	3,7	4,4

*Test for equal means: **p-value (locations) = 0,00029;** p-value (months) = **2,24x10^{-15}***

Table A1-5. Phosphate.

LONA	PO$_4^{-3}$ (µg P/L)			
	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	0,00	0,00	0,92	0,31
Location 2	0,00	0,00	1,94	0,65
Location 3	0,00	1,18	0,11	0,43
Location 4	0,00	0,00	0,00	0,00
Location 5	0,97	0,18	0,00	0,38
Location 6	0,00	0,09	0,00	0,03
Location 7	0,00	0,00	0,00	0,00
Mean	0,14	0,21	0,42	0,26

Test for equal means: p-value (locations) = 0,74; p-value (months) = 0,15

Table A1-6. Total nitrogen.

	TN (µg N/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	170	858	233	420
Location 2	183	771	233	396
Location 3	154	987	233	458
Location 4	185	233	233	217
Location 5	154	233	490	292
Location 6	232	233	401	288
Location 7	231	233	233	232
Mean	187	507	293	329

Test for equal means: p-value (locations) = 0,79; p-value (months) = 0,063

Table A1-7. Nitrate.

	NO_3^- (µg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	n.a	14,56	39,49	27,03
Location 2	n.a	18,78	34,16	26,47
Location 3	n.a	15,03	39,99	27,51
Location 4	2,37	13,91	35,54	17,27
Location 5	5,00	16,69	35,5	19,06
Location 6	n.a	9,53	33,95	21,74
Location 7	3,75	7,31	27,63	12,90
Mean	3,71	13,69	35,18	20,78

*Test for equal means: p-value (locations) = 0,105**; p-value (months) = 2,36x10^{-6}***

Table A1-8. Ammonium.

	NH_4^+ (µg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	46,37	11,14	n.a.	28,76
Location 2	8,13	41,98	n.a.	25,06
Location 3	101,15	11,49	40,99	51,21
Location 4	45,06	11,02	17,89	24,66
Location 5	27,26	n.a.	n.a.	27,26
Location 6	25,15	14,20	n.a.	19,68
Location 7	52,65	38,13	3,64	31,47
Mean	43,68	21,33	20,84	31,02

Test for equal means: p-value (locations) = 0,59; p-value (months) = 0,097

Table A1-9. Sulphate.

	SO_4^{-2} (mg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	1,41	1,18	1,32	1,30
Location 2	1,40	1,18	1,29	1,29
Location 3	1,51	1,20	1,32	1,34
Location 4	1,38	1,18	1,31	1,29
Location 5	1,37	1,14	1,31	1,27
Location 6	1,38	1,11	1,28	1,26
Location 7	1,37	1,18	1,25	1,27
Mean	1,40	1,17	1,30	1,29

Test for equal means: p-value (locations) = 0,28; **p-value (months) = 4,96x10^{-8}**

Table A1-10. Calcium.

	Ca^{+2} (mg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	2,48	2,95	2,81	2,7
Location 2	2,64	2,98	2,75	2,8
Location 3	2,49	2,92	2,69	2,7
Location 4	2,50	2,90	2,69	2,7
Location 5	2,54	2,85	2,60	2,7
Location 6	2,40	2,82	2,55	2,6
Location 7	2,38	2,80	2,59	2,6
Mean	2,5	2,9	2,7	2,7

Test for equal means: **p-value (locations) = 0,0015; p-value (months) = 1,25x10^{-9}**

Table A1-11. Magnesium.

	Mg^{+2} (mg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	0,33	0,40	0,43	0,38
Location 2	0,34	0,42	0,44	0,40
Location 3	0,35	0,39	0,43	0,39
Location 4	0,34	0,41	0,42	0,39
Location 5	0,35	0,40	0,42	0,39
Location 6	0,35	0,41	0,41	0,39
Location 7	0,35	0,40	0,41	0,39
Mean	0,34	0,41	0,42	0,39

Test for equal means: p-value (locations) = 0,76; **p-value (months) = 2,24x10^{-8}**

Table A1-12. Potassium.

	K$^+$ (mg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	0,17	0,16	0,19	0,17
Location 2	0,15	0,19	0,17	0,17
Location 3	0,17	0,16	0,17	0,17
Location 4	0,15	0,16	0,18	0,16
Location 5	0,16	0,16	0,16	0,16
Location 6	0,14	0,15	0,16	0,15
Location 7	0,15	0,16	0,16	0,16
Mean	0,16	0,16	0,17	0,16

Test for equal means: p-value (locations) = 0,14; p-value (months) = 0,074

Table A1-13. Sodium.

	Na$^+$ (mg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	1,18	1,20	1,14	1,17
Location 2	1,17	1,22	1,12	1,17
Location 3	1,17	1,19	1,14	1,17
Location 4	1,16	1,17	1,15	1,16
Location 5	1,16	1,14	1,09	1,13
Location 6	1,14	1,15	1,13	1,14
Location 7	1,15	1,16	1,09	1,13
Mean	1,16	1,17	1,12	1,15

Test for equal means: p-value (locations) = 0,68; **p-value (months) = 0,0025**

Table A1-14. Chloride.

	Cl$^-$ (mg/L)			
LONA	11.06.2015	13.08.2015	13.10.2015	Mean
Location 1	1,00	1,02	0,93	0,98
Location 2	1,00	0,98	0,89	0,96
Location 3	1,00	1,00	0,90	0,97
Location 4	0,97	0,95	0,90	0,94
Location 5	0,97	0,96	0,86	0,93
Location 6	0,97	0,95	0,84	0,92
Location 7	0,97	0,98	0,86	0,94
Mean	0,98	0,98	0,88	0,95

Test for equal means: p-value (locations) = 0,47; **p-value (months) = 3,97x10^{-7}**

Table A2-1. Field measurements. River Gryteåa Øvre. August 2015.

Sample number	Weather	Altitude (m)	Scratched surface (cm)	River width (m)	Distance closest bank (m)	Temperature °C	Conductivity	Oxygen (%)	PAR (μmol m⁻² s⁻²)	Shading degree	Depth (m)	Mean water velocity (m/s)	Flow type	Substrate type	Mat or filaments	Colour	Cover	Biovolume rank	Cover*Biov.rank (Relative biovol.)
1	Sunny	96	10x10	8	3	15,9	19,5	95,2	1315,3	2	0,71	0	1	10	Thin mat	Light brown	5	1	5
2	Sunny	96	10x15	3	3	16,1	19,6	104	1444,1	2,5	0,3	0,06	2	9	Thin mat	Green	5	1	5
3	Sunny	96	10x10	9	0,5	16,4	19,5	106,2	52,06	4	0,07	0,33	3	10	Long filaments	Green	80	3	240
4	Sunny	96	10x10	5	2,5	16,4	19,5	102,5	35,39	5	0,2	0,21	3	11	Short filaments	Green	60	2	120
5	Sunny	102	10x10	4	1	17	19,4	99,3	177,4	4	0,15	0,24	3	10	n.a.	Green	25	n.a.	n.a.
6	Sunny	102	5x5 Forceps + syringe	3	0,5	17	19,4	99	36,39	4,5	0,17	0,56	4	14	Short filaments	Green and brown	40	2	80
7	Sunny	104	10x10	3	1	17,3	19,3	95,3	164,5	4	0,4	0,19	2	10	Short filaments	Green and brown	70	2	140
8	Sunny	104	10x10	3	1	17,5	19,3	95,3	34,52	n.a.	0,35	0,11	2	11	Medium mat	Dark	5	2	10
9	Sunny	86	Petri dish	3	1,5	15,6	19,9	100	253,2	3,5	0,75	0,03	1	5	n.a.	n.a.	n.a.	n.a.	n.a.
10	Sunny	86	10x5	3	1,5	15,6	19,9	100	253,2	3,5	0,78	0,03	1	9	Short filaments	Brown/Reddish	80	2	160
12	Sunny	91	10x10	8	1	15,6	19,9	100	1347,8	2	0,5	0,03	1	12	Medium mat	Brown	90	2	180
13	Sunny	92	5x5 Forceps + syringe	2	1	16,4	17,7	100	1549,5	3	0,07	0,8	5	14	Short filaments	Brown/Reddish	75	2	150
14	Sunny	123	10x10	2,5	0,5	18	17,2	100	31,77	4,5	0,5	0,06	2	10	Thin mat, short fil.	Dark/Brown	5	1	5
15	Sunny	126	10X10	3,5	0,5	18	17,2	100	31,77	4,5	0,1	0,19	2	10	Thin-medium	Dark/brown	5	2	10
17	Sunny	127	10x10	4	1	18,3	17,2	98,6	271,2	4	0,15	0,28	3	11	Long filaments	Green	90	3	270
18	Cloudy	131	10x5	4	1	18,4	n.a.	98,6	160,14	3	0,27	0,17	2	9	Long filaments	Brown/Reddish	100	3	300
19	Sunny	111	10x10	4	2	15,8	20,62	100	192,78	4	0,51	0,06	2	11	Long filaments	Brown/Reddish	100	3	300
20	Sunny	141	10x10	4	2	15,8	18,4	100	192,78	4	0,13	0,58	3,5	12	Medium mat	Green	60	2	120
21	Sunny	141	10x10	4	2	15,8	18,4	100	192,78	4	0,07	0,28	3	13	Medium mat	Green	90	2	180
23	Sunny	135	10x10	11	2	16,6	18,4	99	763,5	3	0,4	0,02	1	9	Filaments	Brown/Reddish	40	2	80
26	Sunny	130	10x10	6	1	17	17,4	100	1570,7	1,5	0,06	0,11	2	10	Medium mat	Light brown	5	2	10
27	Sunny	125	10x10	1,5	0,3	17,1	17,4	95,1	1106,2	2	0,1	0	1	10	Brown thin mat + green colonies		5	1	5

Table A2-1 (continuation)

Sample number	Weather	Altitude (m)	Scratched surface (cm)	River width (m)	Distance closest bank (m)	Temperature °C	Conductivity	Oxygen (%)	PAR ($\mu mol\ m^{-2}\ s^{-2}$)	Shading degree	Depth (m)	Mean water velocity (m/s)	Flow type	Substrate type	Mat or filaments	Colour	Cover	Biovolume rank	Cover+Biov.rank (Relative biovol.)
28	Sunny	123	10x10	1	0,3	17,1	17,4	100	1081,6	2,5	0,17	0,47	4	10	Medium mat	Green	5	2	10
29	Sunny	111	10x10	3	0,5	17,3	19,5	100	28,1	5	0,05	1,09	5	11	Long filaments	Green	95	3	285
30	Sunny	110	10x10	3	1,5	17,3	19,5	100	16,1	5	0,12	0,42	4	12	Sparse bryophytes	n.a.	n.a.	n.a.	n.a.
31	Sunny	110	10x10	4	1	17,3	19,5	100	35,2	4,5	0,2	0,06	2	11	Short filaments	Brown/Reddish	20	2	40
32	Partly cloudy	131	10x10	2,5	1	17,1	19,4	100	653,6	3	0,1	0,48	4	12	Long filaments	Green	100	3	300
34	Partly cloudy	150	10x10	3	1,5	16,9	19,4	100	567,6	3	0,34	0,19	2	12	Short filaments	Brown/Reddish	20	2	40
35	Partly cloudy	157	10x7	10	1	17	19,5	100	581,1	3	0,98	0,03	2	9	Thin mat	Green?	5	1	5
36	Partly cloudy	157	10x10	8	1	17	19,5	100	581,1	2,5	0,6	0,03	2	10	Medium mat	Brown	5	2	10
38	Partly cloudy	166	10x10	1	0,5	18,6	19,7	100	690,7	3,5	0,15	0,52	4	11	Long filaments	Green and brown	100	3	300
39	Partly cloudy	166	10x10	4	1	18,6	19,7	100	575,5	3,5	0,15	0,13	2	10	Green glob. colonies + Brown fil.		100	3	300

Appendix 3 – Multivariate analyses

Table A3-1. PCA summary: principal components (PC), eigenvalues, explanatory power and confidence intervals.

PC	Eigenvalue	% variance	Eig 2.5%	Eig 97.5%
1	4.13428	27.562	20.038	37.463
2	3.19738	21.316	15.508	32.102
3	1.6026	10.684	5.8607	16.051
4	1.29837	8.6558	3.2345	13.951
5	1.10577	7.3718	2.2748	13.597
6	0.987968	6.5865	0.71799	11.286
7	0.678724	4.5248	0.55549	8.2553
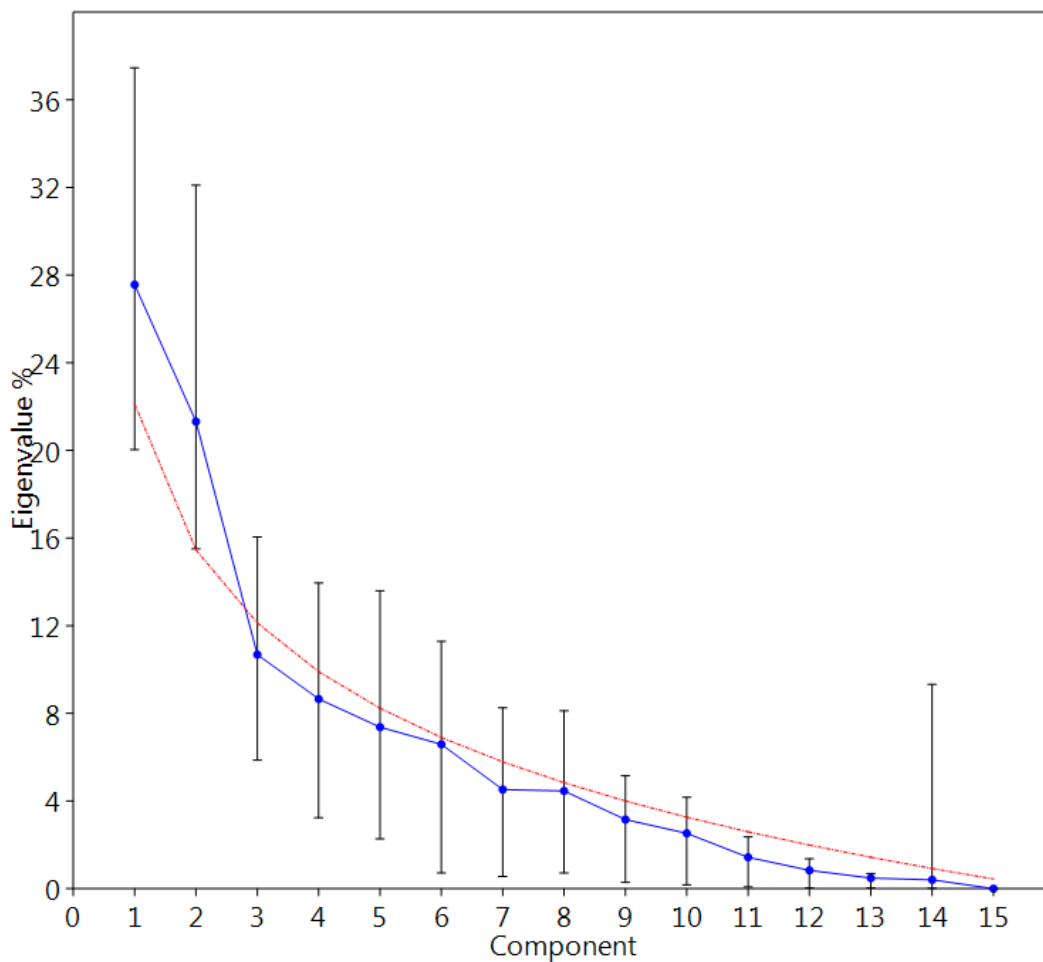
Figure A3-1. Scree plot and broken stick (red line) indicating the number the principal components that are likely to be most significant (in this case PC 1 and PC 2).

Table A3-2. PCA loadings (coefficients) for the first and the second principal component.

	PC 1	PC 2
LogAlt	0.008472	-0.099666
LogDistanceCB	0.037784	-0.16157
LogIrrad	-0.2298	-0.28168
LogDepth	-0.36497	0.10237
LogVel	0.43312	-0.066861
LogFlowT	0.43948	-0.093903
SunbsT^2	0.35746	-0.067805
LogCov*V	0.29603	0.18695
Shading degree	0.29834	0.29119
LogPIT	-0.16013	0.11252
AIP	0.22988	-0.013763
NoAlg	-0.071817	0.50576
NoRAlg	0.17656	0.32063
NoGAlg	-0.074294	0.44298
NoCyan	-0.083638	0.41089

Table A3-3. Fit and significance of the *post hoc* fitted environmental variables in the DCA scatterplot.

	r2	Pr (>r)
Irrad	0.1063	0.250
Depth	0.3111	0.005 **
WVel	0.1304	0.187
FlowT	0.2483	0.052 .
SunbsT	0.2211	0.049 *
NoAlg	0.3184	0.002 **
CovV	0.4641	0.453
ShaD	0.4706	0.021 *

Table A3-4. CCA summary: axes, eigenvalues, explanatory power and p values.

Axis	Eigenvalue	%	p (999 permutations)
1	0,22302	28,52	0,027
2	0,17625	22,54	0,001
3	0,14327	18,32	0,002
4	0,084412	10,79	0,084
5	0,065706	8,402	0,12
6	0,052066	6,658	0,127
7	0,037325	4,773	0,25
8	$5,0083 \times 10^{-6}$	0,0006404	0,965

Appendix 4 - Mean near-bed water velocity and depth
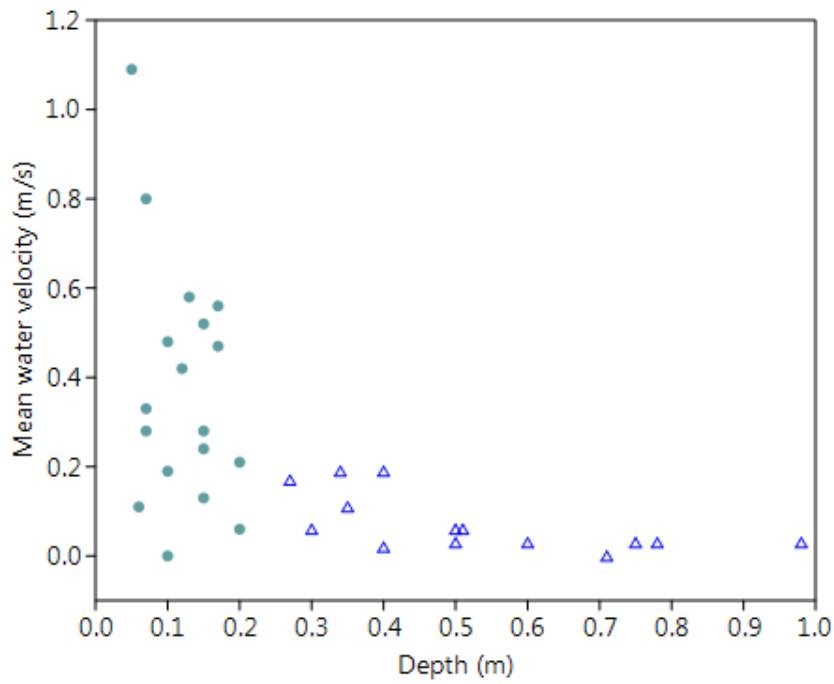
Figure A4-1. Relationship between depth and mean water velocity at the different sampling points in the river Gryteåa øvre (August 2015). Triangles and dots show the two groups (≤ 0,2 m and 0,2 − 1 m) that where considered for testing differences in mean values for PIT and AIP.

Appendix 5 – Regression models

Table A5-1. Regression models between measured environmental variables and the response variables "relative biovolume" and "algal richness" in the river Gryteåa øvre (August 2015). Model equation, coefficient of determination (R^2) and p-value (p) are printed in bold when the model is statistically significant ($p < 0,05$).

	Relative biovolume (LogCov*V)			Algal richness (NoAlg)		
	Equation	R^2	p	Equation	R^2	p
Altitude (LogAlt)	-0,1756x + 2,075	0,0004	0,9137	1,863 x + 19,53	0,0002	0,9359
Distance from the closest bank (LogDistanceCB)	4,481x + 1,277	0,1099	0,0848	-47,17 x + 28,99	0,0729	0,1350
Water velocity (LogVel)	**-17,33x² + 8,484x + 1,249**	**0,2533**	**0,0224**	-395,6x² + 80,1x + 21,88	0,1139	0,1731
Flow type (LogFlowT)	**1,841x + 0,7765**	**0,1409**	**0,0447**	-113,3x² + 101,7x + 3,443	0,1098	0,1848
Depth (LogDepth)	-0,6048x + 2,556	0,0906	0,1125	-20,6x² + 61,75x - 20,14	0,0724	0,3361
Photosynthetically active radiation (LogIrrad)	**0,9126x³ - 7,644x² + 19,99x - 14,34**	**0,2748**	**0,0423**	**-15,36x² + 66,3x - 41,71**	**0,2561**	**0,0136**
Shading degree (ShaD)	**-0,232x² + 1,846x - 1,628**	**0,2658**	**0,0210**	2,75x + 14	0,0633	0,1719
Substrate type (SubsT²)	0,8036x + 0,7972	0,1300	0,0546	-6,497x + 30,74	0,0459	0,2387

Table A5-2. Regression models between measured environmental variables and PIT/AIP scores in periphyton samples from the river Gryteåa øvre (August 2015). Model equation, coefficient of determination (R^2) and p-value (p) are printed in bold when the model is statistically significant ($p < 0,05$).

	Periphyton index of trophic status (PIT)			Acidification index periphyton (AIP)		
	Equation	R^2	p	Equation	R^2	p
Altitude (LogAlt)	-4,782x+16,55	0,0716	0,1454	-0,0819x+6,866	0,0029	0,7742
Distance from the closest bank (LogDistanceCB)	5,272x+5,969	0,0421	0,2595	0,006789x+6,685	8,34E⁻⁰⁶	0,9875
Water velocity (LogVel)	-3,734x+6,926	0,0390	0,2784	0,5648x+6,637	0,1065	0,0683
Flow type (LogFlowT)	-2,916x+8,127	0,0763	0,1259	0,2565x+6,552	0,0704	0,1421
Depth (m)	**2,307x + 5,912**	**0,1387**	**0,0357**	**-0.3203x + 6.782**	**0.3192**	**0.0008**
Photosynthetically active radiation (LogIrrad)	0,2509x+6,01	0,0105	0,2509	-0,02764x+6,752	0,0152	0,5007
Shading degree (ShaD)	-0,1412x+7,108	0,0076	0,6414	0,01138x+6,648	0,0059	0,6819
Substrate type (SubsT²)	**-1,639x+8,477**	**0,1354**	**0,0383**	0,08213x+6,592	0,0405	0,2691

Appendix 6 – Algal richness as a function of PAR (Log Irrad)
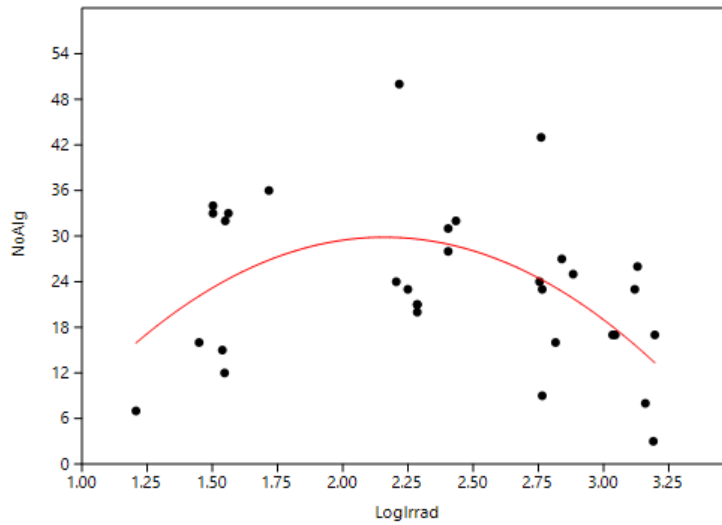
Figure A6-1. Total algal richness as a function of PAR (Log Irrad)
Polynomial regression, order 2
R2: 0,25615
p: **0,013695**
Equation: $-15,36x^2 + 66,3x - 41,71$
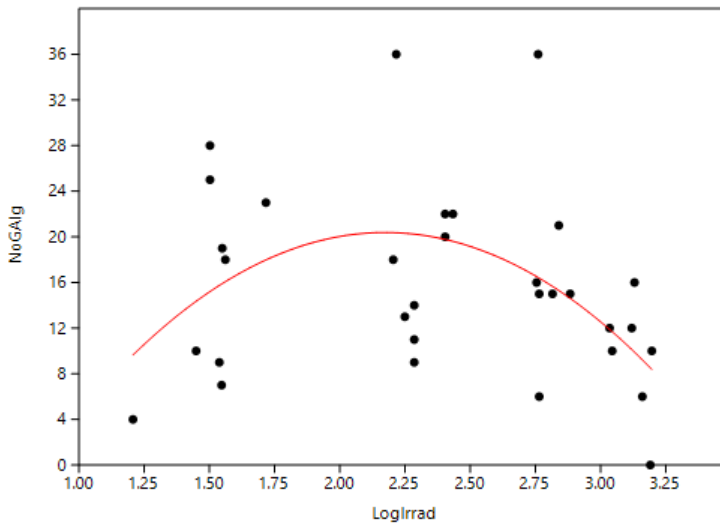
Figure A6-2. Richness of green algae as a function of PAR (Log Irrad)
Polynomial regression, order 2
R2: 0,21527
p: **0,029745**
Equation: $-11,48x^2 + 49,9x - 33,85$
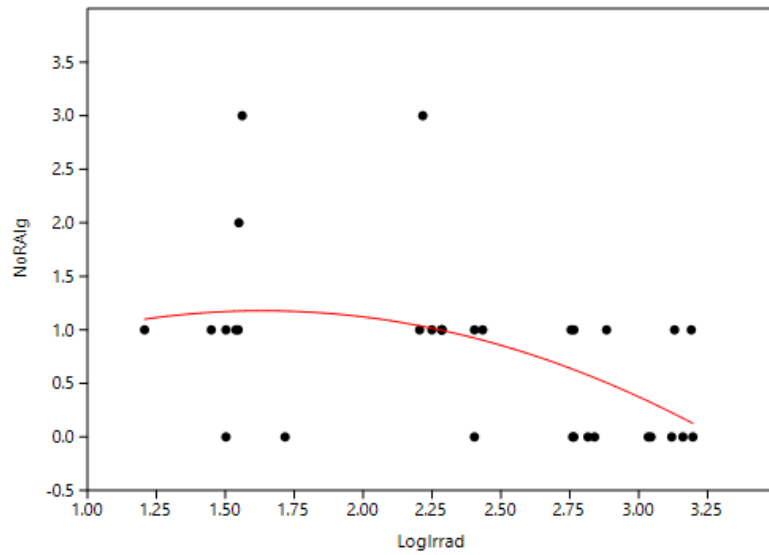
Figure A6-3. Richness of red algae as a function of PAR (Log Irrad)
Polynomial regression, order 2
R2: 0,21658
p: **0,029034**
Equation: $-0,4315x^2 + 1,411x + 0,02503$
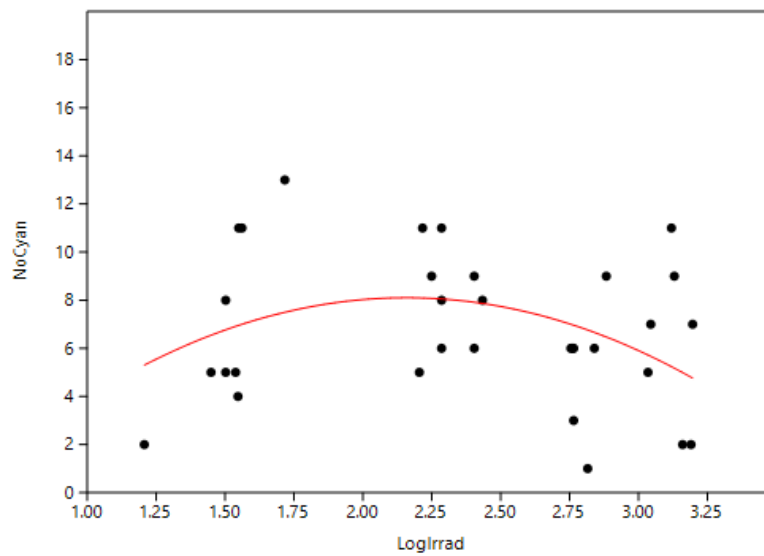
Figure A6-4. Cyanobacterial richness as a function of PAR (Log Irrad)
Polynomial regression, order 2
R2:   0,11977
p: 0,15727
Equation: $-3,098x^2 + 13,37x - 6,32$

Appendix 7 - GPS coordinates.

Table A7-1. Location of periphyton samples obtained from the river Gryteåa øvre in August 2015.

Sample name	Time	Latitude	Longitude	Altitude (m)
S1	2015-08-13 12:00:48	59.353690000	9.054471000	96.2
S2	2015-08-13 12:24:14	59.353769000	9.054630000	97.0
S3	2015-08-13 13:02:20	59.353693000	9.054151000	95.0
S4	2015-08-13 13:23:43	59.353714000	9.053926000	94.8
S5	2015-08-13 14:21:15	59.353563000	9.052157000	102.4
S6	2015-08-13 14:20:14	59.353566000	9.052200000	102.3
S7	2015-08-13 14:18:53	59.353439000	9.051782000	103.9
S8	2015-08-13 14:41:32	59.353262000	9.051435000	103.7
S9	2015-08-14 11:27:55	59.353996000	9.059809000	86.1
S10	2015-08-14 11:27:55	59.353996000	9.059809000	86.1
S11	2015-08-14 11:27:55	59.353996000	9.059809000	86.1
S12	2015-08-14 12:02:09	59.353915000	9.059724000	90.8
S13	2015-08-14 12:31:37	59.353733000	9.058818000	91.6
S14	2015-08-14 13:35:45	59.353139000	9.048898000	123.0
S15	2015-08-14 13:49:50	59.353124000	9.048865000	126.2
S17	2015-08-14 14:19:43	59.353111000	9.048310000	135.1
S18	2015-08-14 15:00:38	59.352983000	9.046058000	130.6
S19	2015-08-19 09:51:22	59.353030000	9.047311000	111.0
S20	2015-08-19 10:21:13	59.352963000	9.046940000	140.8
S21	2015-08-19 10:21:13	59.352963000	9.046940000	140.8
S23	2015-08-19 11:06:06	59.352868000	9.045728000	135.4
S26	2015-08-19 12:38:33	59.351978000	9.039967000	130.3
S27	2015-08-19 12:54:19	59.351967000	9.040287000	125.0
S28	2015-08-19 13:11:56	59.351947000	9.040196000	123.5
S29	2015-08-20 10:53:43	59.351515000	9.037090000	110.7
S30	2015-08-20 10:53:11	59.351508000	9.037004000	109.6

S31	2015-08-20 10:52:28	59.351497000	9.036992000	110.3
S32	2015-08-20 11:29:33	59.351628000	9.036512000	130.7
S34	2015-08-20 12:02:08	59.352639000	9.034307000	150.4
S35	2015-08-20 12:45:12	59.353779000	9.030961000	157.0
S36	2015-08-20 12:45:57	59.353716000	9.031143000	156.9
S38	2015-08-20 13:41:57	59.353666000	9.023471000	167.1
S39	2015-08-20 13:52:11	59.353625000	9.023240000	165.6

Appendix 8 – Ecological indices and TWINSPAN clusters
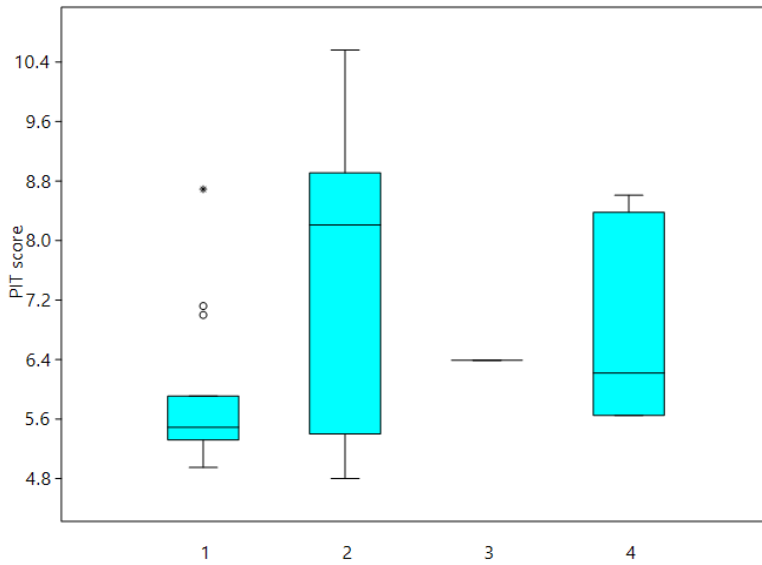
Figure A8-1. Box plots showing PIT values dispersal in TWINSPAN sample clusters from the river Gryteåa øvre (2015).
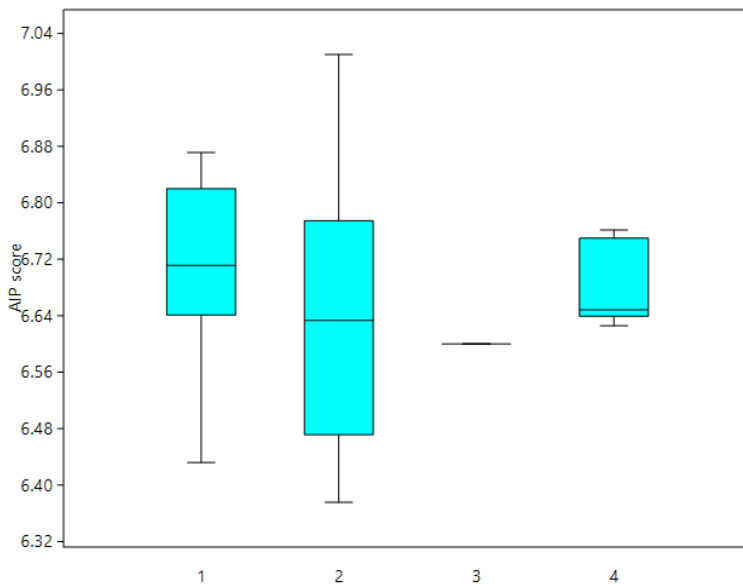
Figure A8-2. Box plots showing AIP values dispersal in TWINSPAN sample clusters from the river Gryteåa øvre (2015).

Appendix 9 – Algal taxa

Table A9-1. Soft-bodied benthic algae from the river Gryteåa øvre (August 2015), number of occurrences, and indicator values for PIT and AIP.

	Abbreviation	Number of occurrences	PIT indicator value	AIP indicator value
Cyanobacteria				
Aphanocapsa sp.	ACAPSA	1	7,24	
Aphanothece sp.	ACETE	1	7,83	
Calothrix sp. (Rivularia?)	CALOTH.RIV	1		
Calothrix sp.	CALOTH	17	5,21	
Calothrix sp. (on Batrachospermum)	CALOTH.BAT	5	5,21	
Capsosira brebissonii	CAPSO	4	3,98	5,19
Chamaecalyx swirenkoi	CHACALYX	1		
Chamaesiphon confervicolus	CHA.CONF	5	6,61	7,05
Chamaesiphon rostafinskii	CHA.ROST	3	4,37	6,45
Chroococcus sp.	CHRO	2	3,57	
Coccal cyanobacterium in mucilaginous colony	COCCAL1	1		
Coccal-colonial cyanobacterium (*Chlorogloea*?)	COCCAL2	8		
Cyanophanon mirabile	CYANOPH	11	4,39	6,71
Cylindrospermum sp.	CYLINDR	2		
Dichothrix gypsophila / orisiana	DICHO	7	4,55	
Geitlerinema sp.	GEITL	6		
Geitlerinema splendidum	GEITL.SP	5	43,42	
Heteroleibeinia (long cells)	HETERO1	1	7,98	
Heteroleibleinia pusilla?	HETERO2	1	7,98	
Heteroleibleinia sp.	HETERO.SP	2	7,98	
Leibleinia sp.	LEIB	1		
Leptolyngbya crassior	LEPTO.CRA	11	3,82	
Leptolyngbya sp.	LEPTO.SP	18	7,83	
Leptolyngbya sp1. (up to 4 µm)	LEPTO.4	4	7,83	
Limnothrix redekei (planktic)	LIMNO.RE	1		
Oscillatoria limosa	OSC.LIM	2	39,10	7,10
Oscillatoria princeps	OSC.PRIN	2		
Oscillatoria proboscidea	OSC.PROB	2		
Oscillatoria sp.(9 µm, constricted)	OSC.SP	1		
Oscillatoria tenuis	OSC.TEN	7	44,24	
Phormidium autumnale sensu lato (4-7 µm)	PH.AUTU	12		7,17
Phormidium heteropolare	PH.HETER	3	3,40	6,80
Phormidium sp. (tergestinum?)	PH.TERG	3		
Phormidium sp. group V	PH.V	5		
Phormidium sp.	PH.SP	2		
Phormidium subfuscum	PH.SUB	1		
Pseudanabaena batrachospermorum	PSEUD.BAT	2		
Pseudanabaena limnetica	PSEUD.LIM	1		
Rivularia sp?	RIV.CAL.DIC	1		
Schizothrix sp.	SCHIZ	1	4,71	
Scytonema (Myochrotes) mirabile	SCYT.MIRA	9	3,37	5,65

Spirulina sp.	SPIR	1		
Stigonema hormoides	STIG.HOR	3	*1,87*	5,19
Stigonema mamillosum	STIG.MAM	24	*3,88*	6,25
Stigonema ocellatum	STIG.OCE	1	*3,34*	5,38
Stigonema tomentosum	STIG.TOM	1	*4,43*	
Tolypothrix sp. (disorta?)	TOLY.SP	8	*5,72*	7,17
Coleodesmium sagarmathae	COLE.SAGA	6	*4,82*	6,26

Chlorophyceae

Unidentified alga (4 celled)	UND.ALG	1		
Ankistrodesmus sp.	ANKI	5		
Bambusina sp.	BAMBU	5		
Bulbochaete sp.	BULBO	19	*4,65*	6,43
Chaetophora elegans	STIGEO.TE	4	*5,91*	7,36
Chlamydocapsa sp	CHLAMY	1		
Closterium abruptum	CL.ABRU	1		
Closterium angustatum	CL.ANGU	1		
Closterium archerianum	CL.ARCH	1		
Closterium baillyanum	CL.BAI	1		
Closterium calosporum	CL.CALO	2		
Closterium closteroides	CL.CLOS	1		
Closterium closteroides var. Intermedium	CL.CLOS.IN	3		
Closterium costatum / regulare	CL.COST	2		
Closterium dianae var. Arcuatum	CL.DIA.A	2		
Closterium dianale	CL.DIA	10		
Closterium directum	CL.DIR	2		
Closterium gracile	CL.GRA	2		
Closterium incurvum	CL.INC	11		
Closterium intermedium	CL.INT	1		
Closterium kuetzingii	CL.KU	4		
Closterium limneticum	CL.LIM	11		
Closterium parvulum	CL.PAR	23		
Closterium ralfsii	CL.RAL	2		
Closterium rostratum	CL.ROS	1		
Closterium setaceum	CL.SET	4		
Closterium striolatum	CL.STR	1		
Closterium sublaterale	CL.SUB	1		
Cosmarium abbreviatum	CSM.ABB	2	*5,14*	
Cosmarium blyttii	CSM.BLY	1	*5,14*	
Cosmarium contractum	CSM.CON	3	*5,14*	
Cosmarium depressum	CSM.DEP	2	*5,14*	
Cosmarium dickii	CSM.DIC	8	*5,14*	
Cosmarium didymoprotupsum / obtusatum	DSM.DID	2	*5,14*	
Cosmarium difficile	CSM.DIF	2	*5,14*	
Cosmarium goniodes	CSM.GON	1	*5,14*	
Cosmarium impressulum	CSM.IMP	1	*5,14*	
Cosmarium majae	CSM.MAJ	8	*5,14*	
Cosmarium moniliforme	CSM.MON	1	*5,14*	
Cosmarium phaseolus	CSM.PHA	1	*5,14*	

Cosmarium pseudopyramidatum	CSM.PSE	2	5,14	
Cosmarium punctulatum	CSM.PUNC	1	5,14	
Cosmarium reniforme	CSM.REN	2	5,14	7,28
Cosmarium sp1	CSM.SP1	2	5,14	
Cosmarium subbroomei	CSM.SUBB	2	5,14	
Cosmarium subcostatum	CSM.SUBCO	6	5,14	
Cosmarium subcucumis	CSM.SUBCU	3	5,14	
Cosmarium subreinschii	CSM.SUBRE	2	5,14	
Cosmarium sutumidum	CSM.SUT	3	5,14	
Cosmarium tinctum	CSM.TIN	12	5,14	
Cosmarium undulatum	CSM.UND	2	5,14	
Cylindrocystis sp. / Penium sp.	CYL	18		
Desmodesmus sp.	DESM	7		
Dictyosphaerium pulchellum	DICTY	1		
Euastrum ansatum	E.ANS	6	5,47	
Euastrum bidentatum	E.BID	1	5,47	
Euastrum binale	E.BIN	11	5,47	
Euastrum denticulatum	E.DEN	1	5,47	
Euastrum elegans	E.ELE	10	5,47	
Euastrum lacustre	E.LAC	1	5,47	
Euastrum oblongum	E.OBL	1	5,47	
Euastrum pectinatum	E.PEC	1	5,47	
Euastrum sp	E.SP	1	5,47	
Gonatozygon brebissonii	GON.BRE	1		
Gonatozygon kinahanii	GON.KIN	2		
Haplotaenium minutum	HAPLO	6		
Hyalotheca sp.	HYALO	1		
Hyalotheca sp.? Ulothrix sp.?	HYALO.ULO	1		
Klebsormidium flaccidum	KLEB.FLA	3	4,87	
Klebsormidium rivulare	KLEB.RIV	4	4,00	
Micrasterias radiosa	MIC.RA	2		
Micrasterias truncata	MIC.TR	1		
Microspora amoena	MICROSP.A	6	11,58	
Monoraphidium sp	MONORAPH	14		
Mougeotia a/b (10-18 µm)	MOUG2	14	4,53	5,57
Mougeotia a2 (3-7 µm)	MOUG1	7	4,01	
Mougeotia b (15-21 µm, short cells) / Mougeotiopsi	MOUG3	15	5,55	
Mougeotia c (21-24 µm)	MOUG4	2	10,71	
Mougeotia d/e (25-30 µm)	MOUG5	3	5,87	6,98
Mougeotia e (30-40 µm)	MOUG6	15	4,53	7,16
Netrium sp.	NETRI	11	4,57	
Oedogonium a (5-11 µm)	OEDO2	13	5,84	
Oedogonium a 1 (3-4 µm)	OEDO1	4	4,59	
Oedogonium a/b (19-21 µm)	OEDO4	10	7,57	
Oedogonium b (13-18 µm)	OEDO3	20	7,73	6,92
Oedogonium c (23-28 µm)	OEDO5	8	9,09	7,09
Oedogonium d (29-32 µm)	OEDO6	5	10,87	7,27
Oedogonium e (35-44 µm)	OEDO7	2	16,05	7,27
Pediastrum tetras	PEDI	5		

Pleurotaenium ehrenbergii	PLEURO	2		
Schizochlamis gelatinosa	SCHIZ	1	4,61	
Spirogyra a (20 - 42 µm, 1 K. L)	SPIR	12	8,38	7,01
Staurastrum echinatum	ST.ECH	1	3,05	
Staurastrum furcatum/pseudopisciforme	ST.FUR	1	3,05	
Staurastrum lapponicum	ST.LAP	2	3,05	
Staurastrum paradoxum (4 arms)	ST.PAR	1	3,05	
Staurastrum punctulatum	STA.PUN	4	3,05	
Staurastrum sp. (3+3 arms small)	ST.1	2	3,05	
Staurastrum sp. (3+3 arms)	ST.2	1	3,05	
Staurastrum sp. (3+3? Long arms) planctonicum?	ST.3	3	3,05	
Staurastrum sp. (4+4 arms)	ST.4	1	3,05	
Staurastrum sp. (5+5 arms)	ST.5	1	3,05	
Staurastrum vestitum	ST.VES	1	3,05	
Staurodesmus lunatum	STAUROD.LUN	1	4,33	
Staurodesmus mucronatus	STAUROD.MUC	1	4,33	
Tetmemorus granulatus	TETM.GRA	7		
Tetmemorus laevis	TETM.LAE	1		
Tetraspora sp. (cylindrica?)	TETRASP.	4	5,34	7,38
Xanthidium sp.	XANTH.1	1		
Xanthidium sp. (acanthophorum)	XANTH.2	1		
Xanthidium sp. (armatum)	XANTH.3	1		
Zygnema a (19 µm)	ZYG19	1	4,45	
Zygnema b (25 µm)	ZYG25	18	4,76	6,99

Rodophyceae				
Batrachospermum gelatinosum	BATRACH	14	7,68	7,12
Unidentified red alga *(Chantransia* stage - *chalybaea*) L/B >3	CHAN1	1		
Unidentified red alga *(Chantrasia* stage - *pigmea*) L/B = 1-3	CHAN2	1		
Lemanea fluviatilis	LEMAN	4	6,98	7,11
Unidentified purple red alga (*A. hermannii*?)	A.HERM	5		

Chrysophyceae				
Dinobryon sociale	DIN.SO	1		
Dinobryon sertularia	DIN.SE	1		

Xanthophyceae				
Vaucheria hamata	VAUCH	1	5,84	

Dinophyceae				
Peridinium sp.	PERID.SP	1		

Others				
Ophrydium versatile (colonial ciliate)	ORPH.VER	1	5,36	

Table A9-2. Occurrence and abundance of soft-bodied algae in 32 periphytic samples from the river Gryteåa øvre, August 2015 (1=rare; 2=common; 3 =abundant).

NoAlg = total algal richness in the sample; NoRalg = richness of red algal taxa in the sample; NoGAlg = richness of green algal taxa in the sample; NoCyan = richness of cyanobacterial taxa in the sample; PIT = Periphyton Index of Trophic status (sample score); AIP = Acidification Index Periphyton (sample score). For abbreviations of taxa see Table 9A-1.

Sample nr	1	2	3	4	5	6	7	8	9	10	12	13	14	15	17	18	19	20	21	23	26	27	28	29	30	31	32	34	35	36	38	39
BATRACH										3	2		2		3	3	3	3	2	3				3	1	3		3		1		
CHAN1							3																									
CHAN2						2																										
LEMAN				3		3	3					3																				
A.HERM				2	1	2	3	1																								
UND.ALG							1																									
ANKI			1				1	1																								1	1
BAMBU						1			1		1	1				1																
BULBO		1	1	1	1				1				2	1	1				1		2	2	3	1			1	2	2	1		1
STIGEO.TE															1	1			1			3										
CHLAMY							1																									
CL.ABRU													1																			
CL.ANGU													1																			
CL.ARCH																			1													
CL.BAI	1																															
CL.CALO																		2	1													
CL.CLOS														2																		
CL.CLOS.IN									1							1															1	
CL.COST	1												1																			
CL.DIA.A							1																					1				
CL.DIA							1	1	1				1		1	1			1											1	1	1
CL.DIR									1								1															
CL.GRA							1			1																						
CL.INC			1	1		1	1	1					1		1	1											1			1		1
CL.INT												1																				
CL.KU										1					1	1														1		
CL.LIM						1			1			1	1		1	1	1		1											1	1	1
CL.PAR			1	1	1	1	2		1		1	1	1	1	1	1	1	1	1	1	1	1	1					1		1	1	1
CL.RAL																														1	1	
CL.ROS									1																							
CL.SET													1																	1	1	1
CL.STR																		1														
CL.SUB												1																				
CSM.ABB							1																				1					
CSM.BLY																			1													
CSM.CON							1						1											1								
CSM.DEP										1			1																			

CSM.DIC			1	1		1	2							1					1								1					1	
DSM.DID			1															1															
CSM.DIF										1																							1
CSM.GON			1																														
CSM.IMP							1																										
CSM.MAJ			1		1		1						1	1				1									1						1
CSM.MON			1																														
CSM.PHA							?																										1
CSM.PSE												1		1																			
CSM.PUNC																															1		
CSM.REN	1									1																							
CSM.SP1	1																																1
CSM.SUBB														1																	1		
CSM.SUBCO									1	1		1		1													1						1
CSM.SUBCU							1																	1			1						
CSM.SUBRE												1																					1
CSM.SUT							1												1	1													
CSM.TIN			1	1			1					1		1	1							1			1	1					1	1	
CSM.UND																			1														1
CYL			1	1	1	1						1	1	1	1	1	2	1		1		1					1	1		1	1		1
DESM			1				1	1	1					1				1															1
DICTY											1																						
E.ANS	1								1	1	1			1																			1
E.BID																																	1
E.BIN						1	1		1	1		1	1							1			1				1	1					1
E.DEN										1																							
E.ELE			1	1			1	1		1		1		1						1							1						1
E.LAC														1																			
E.OBL									1																								
E.PEC																																	1
E.SP						1																											
GON.BRE																		1															
GON.KIN						1							1																				
HAPLO									1	1		1	1																		1	1	1
HYALO										1																							
HYALO.ULO											1																						
KLEB.FLA	2										2		1																				
KLEB.RIV				1	2		1		1																								
MIC.RA										1			1																				
MIC.TR																			1														
MICROSP.A			1	2	2	3	3	1																									
MONORAPH	1		1	1		1	1						1	1	1			1			1		1				1				1	1	
MOUG2	1	1		1		1	1		1	1	1			1		1														1	1	1	1
MOUG1			1										1			1	1				1											1	1
MOUG3							1		1			2	2	3		1	3	3	1		1		2	2	2	2	2						
MOUG4	1					1																											
MOUG5									1					1					1						1							1	

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	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31
MOUG6			2	2	1		1					1	2	3			1	1	1	2			2	2			2			1	
NETRI			1	1	1		1		1			1	1		1					1	1										1
OEDO2	1			1				1	2			2	1		3			1		1		1			1	2		1			
OEDO1						1		1	1									1				1									
OEDO4	1	1					3						2				1		2		1	1			2		1				
OEDO3	1		2	2	2	2	2			2		3	2			1		2	2		3		1	2		2	1	1	3	1	
OEDO5		1	3	3	2	2	3	1				3																			
OEDO6		1	3	3	2	2																									
OEDO7		1		2																											
PEDI			1				1					1	1																		1
PLEURO							1	1																							
SCHIZ																											1				
SPIR						1		1	1				1				1			1	3	1		1	2	1			1		
ST.ECH						1																									
ST.FUR																															1
ST.LAP																										1					1
ST.PAR												1																			
STA.PUN						1						1					1														1
ST.1											1																				1
ST.2																															1
ST.3							1				1																		1		
ST.4											1																				
ST.5											1																				
ST.VES																															1
STAUROD.LUN												1																			
STAUROD.MUC																															1
TETM.GRA							1	1		1		1	1																1		1
TETM.LAE			1																												
TETRASP.					3								2						2					1							
XANTH.1																															1
XANTH.2							1																								
XANTH.3								1																							
ZYG19												1																			
ZYG25			2	2	2	2	2	2				3	3			2			2		2	3	2	2	3	2	1		3		
ACAPSA											1																				
ACETE																												1			
CALOTH.RIV																	1														
CALOTH			2	3		3	2		1		2	1		2	1		2	3	3	2				1		1	1	1			
CALOTH.BAT										2	2	2		2									2								
CAPSO			3	3		1	3																								
CHACALYX					1																										
CHA.CONF			1	2	1	2	2																								
CHA.ROST			2						3											3											
CHRO																1															1
COCCAL1						1																									
COCCAL2	1		1	2							1		2			3			1				1								
CYANOPH			2	2	1		1			1		2		1	2	2			1	1		1		1							

CYLINDR									2	2																						
DICHO	1			1											1						2	3	2							1		
GEITL							1		2					1							1	1									1	1
GEITL.SP									3					1						1											1	1
HETERO1																														1		
HETERO2	1																															
HETERO.SP							1							1																		
LEIB																			1													
LEPTO.CRA									2	1		1		1	1					1		1	2						1	1		1
LEPTO.SP			1	1	1	1		1	1	2	2	1	1	1	1		2	2		2						2		2	1			
LEPTO.4	1			1	1				1																							
LIMNO.RE									1																							
OSC.LIM								1	1																							
OSC.PRIN	1								1																							
OSC.PROB									1										1													
OSC.SP			1																													
OSC.TEN	1				1	1			1						1															1		1
PH.AUTU			2	2	2	2	3	1	2				1			2				1			1							2		
PH.HETER																					2	2	2									
PH.TERG	1					1	1																									
PH.V	1	1				1			1											2												
PH.SP			1						1																							
PH.SUB	1																															
PSEUD.BAT								3	1																							
PSEUD.LIM														1																		
RIV.CAL.DIC			1																													
SCHIZ																1																
SCYT.MIRA			2			3						2		3		3	3	3				2								1		
SPIR																										1						
STIG.HOR			1													3				1												
STIG.MAM	1		2	3	2	2	2	1				2	3	3		1	3	3	1	2	2	3	2	2	1	2	2	1	1			
STIG.OCE				1																												
STIG.TOM														1																		
TOLY.SP			2				2	3	1			2		1		2				1												
COLE.SAGA	1	1		3	3				2															3								
PERID.SP														1																		
DIN.SO																														1		
DIN.SE																													1			
VAUCH						2																										
ORPH.VER																																2
NoAlg	23	8	36	32	23	33	50	15	28	31	26	3	34	33	32	24	21	21	20	25	17	17	17	16	7	12	16	24	9	23	27	43
NoRAlg	0	0	0	2	1	3	3	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1	1	0	1	0	1	0	0
NoGAlg	12	6	23	19	13	18	36	9	22	20	16	0	28	25	22	18	9	14	11	15	10	10	12	10	4	7	15	16	6	15	21	36
NoCyan	11	2	13	11	9	11	11	5	6	9	9	2	5	8	8	5	11	6	8	9	7	7	5	5	2	4	1	6	3	6	6	6
PIT	8,7	8,2	5,7	6,2	8,4	8,6	5,7	6,2	10,6	9,3	5,8	6,4	4,8	7,0	5,6	8,2	5,0	5,3	5,4	7,1	5,0	5,0	5,3	5,4	5,9	5,9	5,5	5,5	5,6	8,6	8,4	7,3
AIP	6,5	6,6	6,6	6,6	6,8	6,7	6,7	6,6	6,5	6,8	6,7	6,8	6,6	6,9	6,7	7,0	6,4	6,7	6,7	6,7	6,7	6,8	6,8	6,6	6,9	6,7	6,9	6,6	6,4	6,5	6,7	6,6

Microspora amoena
Photo: Miguel A. Segarra