Ecology and biogeochemistry of fluvial microphytobenthic communities on soft sediments

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To Shannon,

Thank you for everything.

SUMMARY

Freshwater ecosystems are the link between the terrestrial and the marine biomes. Terrestrially-derived nutrients travel through, and are transformed within these ecosystems, outflowing eventually to the sea. A major player in small rivers are microphytobenthic (MPB) communities - groups of algae, bacteria, fungi and other protists existing within a polysaccharide matrix. These biofilm communities exist on a transitional zone (the hyporheic zone) between surface water and underground water. This thesis explores the ecology and biogeochemistry of microphytobenthic communities over multiple spatial scales within the Hampshire Avon river catchment, south England. Across three contrasting sub-catchment geology types - clay, greensand and chalk - algal abundance and composition, algal extracellular organic carbon, bacterial abundance and physical characteristics including organic carbon and nutrient concentrations were examined. Results from this study showed that the contrasting habitats indeed carried communities under different physical and ecological pressures. Variability between samples highlighted the heterogeneity of MPB communities and confirmed through comparisons of multiple rivers within the same sub-catchment. Diatom community composition showed a distinct gradient, where the greensand rivers were found to form a transitional zone between the two extremes of clay and chalk rivers. Interactions found between algal extracellular carbon and pore- and surface water chemistry hinted at a change in interactions between autotrophs and heterotrophs, and indicated the first known example of shifting facilitation/competition found in MPB communities on natural sediment. Potential nutrient flux was also shown to vary between geology and light levels, with differences being found between habitats within the same river. An attempt to remove variability in these systems using simulated natural sediment was not fully successful, although supported previous findings regarding the impact of light on biogeochemical cycles through oxygen flux. This work shows that further investigation is needed on natural MPB communities, with greater sampling effort required to overcome heterogeneity.

Acknowledgements

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List of abbreviations

AoA Ammonia oxidising archaea

AoB Ammonia oxidising bacteria

BFI Baseflow index

C Carbon

CHO Carbohydrate

CHO*HW* Hot water extracted carbohydrate

CHO*D* Dissolved carbohydrate

DOC Dissolved organic carbon

DOM Dissolved organic matter

EOC Extracellular organic carbon

EPS Extracellular polymeric substances

EPS*70* Extracellular polymeric substances precipitated with 70% ethanol

HAC Hampshire Avon catchment

MPB Microphytobentos

N Nitrogen

NGS Next-generation sequencing

NPOC Non-purgeable organic carbon

P Phosphorus

POM Particulate organic matter

SA:V Surface area to volume ratio

Sw Nutrient spiral length

TC [flask] Tissue culture flask

TDI Trophic diatom index

TIC Total inorganic carbon

TOC Total organic carbon

WFD European Union Water Framework Directive

Contents

1 INTRODUCTION

1.1 Thesis rationale

Freshwater systems are at the forefront of impact from many anthropogenic activities. Their location and connection to terrestrial environments modified for agricultural and urban purposes, combined with their relatively small size, results in an environment which is incredibly susceptible to the indirect effects of human activity. Small rivers are intrinsically linked to the terrestrial environment of the surrounding catchment, due to water's movement over (run-off) and through (underground flow) a catchment on route to the river channel. The composition of a catchment is vital in understanding the flow of water above and below ground. The long-term ratio of above to underground water flow (known as the base-flow index) is primarily dictated by the permeability of the catchment's soil and bedrock, while anthropogenic factors such as agriculture, deforestation and urbanisation can also impact the base-flow index (BFI). Smaller rivers are more likely to be influenced by their surroundings than larger rivers and lakes, due to their large sediment surface area to water volume ratios (SA:V). Primary and secondary production in smaller rivers is dominated by a community of microbes inhabiting the majority of illuminated surfaces such as sediment – microphytobenthic communities. Microphytobenthic communities are typically an assemblage of microalgae, bacteria, fungi and protists that exist primarily within an extracellular matrix that can be found on plants (epiphytic), rocks (epilithic) and on soft sediments (epipelic). This matrix, as well as housing microphytobenthic communities, also plays roles in sediment stability, retention of nutrients and as a source of fixed carbon for heterotrophic microorganisms. By inhabiting the sediment

of rivers, microphytobenthic communities experience a unique combination of conditions.

It has been well established that species composition is influenced by many factors such as substratum structure and resource availability. Hydrochemical conditions have been shown to be contrasting between ground and surface waters due to differential biotic and abiotic processes, and the meeting point of these two environments (termed the hyporheic zone) is a transitional zone of many factors such as oxygen. Therefore, biogeochemical processes, such as the cycling of the macronutrients C, N and P, are mutually linked while being differentially driven by proportions of surface and underground flow.

This thesis aims to examine the biogeochemistry and ecology of microphytobenthic communities across a natural gradient of catchment geology types within lowland rivers in England.

It will focus on how shifts in substratum type and water chemistry - driven primarily by differences in underlying catchment geology - contribute to overall system production, biogeochemical processing and interactions between components of the microphytobenthos. The river system which was studied in this thesis is the Hampshire Avon Catchment, in South England. This catchment will be studied across multiple landscape scales; ranging from sediment and fine organic matter within the river to the catchment as a whole. Rivers and tributaries within a catchment are classified in a traditional order system (Strahler, 1952), with low order rivers being smaller and

typically headwaters (Figure 1.1) - as two water courses of the same order meet, the water channel after the confluence gains an order (e.g. two 2nd order rivers meeting would create a 3rd order river). In this river classification, headwaters are considered to be orders 1-3, medium rivers orders 4-6 and large rivers assigned an order of more than 6 (Vannote *et al*., 1980). The river continuum concept (Vannote *et al*., 1980) also suggests that the biological components of a river are linked to this physical classification (Figure 1.2).

The interactions between catchment and biogeochemical cycling are not fully understood, and this thesis aims to investigate catchment interactions with benthic biogeochemical activity within a single river system. This approach of using contrasting catchments over a relatively small area provides an opportunity to study catchment biogeochemistry while minimising variance in factors such as temperature, climate, biogeography and anthropogenic activity.

1.2 Inland freshwaters

In established large scale nutrient turnover models, lotic ecosystems (rivers and streams) are considered simply as a transport route, interconnecting the terrestrial and marine environments (Aufdenkampe *et al*., 2011; Battin, 2009; Luyssaert *et al*., 2009; Cole *et al*., 2007). This low-key role has also been echoed within the hydrological cycle, where rivers have been described as the "veins of the landscape, concentrating run-off and transporting it to the base of erosion" (Pusch *et al*., 1998; Williamson *et al*., 2008). These interpretations suggest that rivers and streams are of little

importance to biogeochemical cycling. However, river morphology can influence the biogeochemical contribution to a whole system. Smaller rivers with narrow channels for example would be subject to a higher magnitude of riparian shading, thereby limiting the growth of higher photoautotrophic organisms such as macrophytes. In larger rivers, riparian vegetation would not fully shade the river channel, allowing macrophytes to grow without experiencing shading.

Other factors, such as higher river flow (and the coarser sediment structure that typically accompanies it) and higher responsiveness to rainfall also inhibit macrophyte growth in low-order rivers. Because of these physical constraints, it is often MPB communities which dominate headwater rivers. As MPB communities are positioned on the sediment-water interface and are considered to be the dominant primary producers in headwater rivers, they play a significant role in biogeochemical processes at these sites. Due to a river's unique position in linking terrestrial, groundwater and surface aquatic systems, the characteristics of the catchment can dramatically impact the chemistry of the water flowing through the river (Clark *et al.*, 2018). Examples from other studies have indicated that calcium and nitrate concentrations in surface waters are correlated to inputs of these compounds from groundwater sources (Jarvie *et al*., 2005) .

1.3 Catchment influences on water movement

As water reaches solid surfaces following precipitation, it starts to leach organic material (Pusch *et al*., 1998). This process of leaching continues to occur throughout

the catchment, leading water to be enriched with dissolved organic matter (DOM) as it enters the river system (Pusch *et al*., 1998). While surface water run-off can carry nutrients, it also erodes and transports soil and particulate organic matter (POM, such as leaf litter and organic material in soil) to a river system. Because hydrological action plays a large role in transporting dissolved and particulate nutrients from vegetation and sediments, the route that it takes through the catchment will be a factor in determining the overall flux of these nutrients from land to aquatic systems. Geological porosity, often assessed on the base flow index (BFI), is one factor which

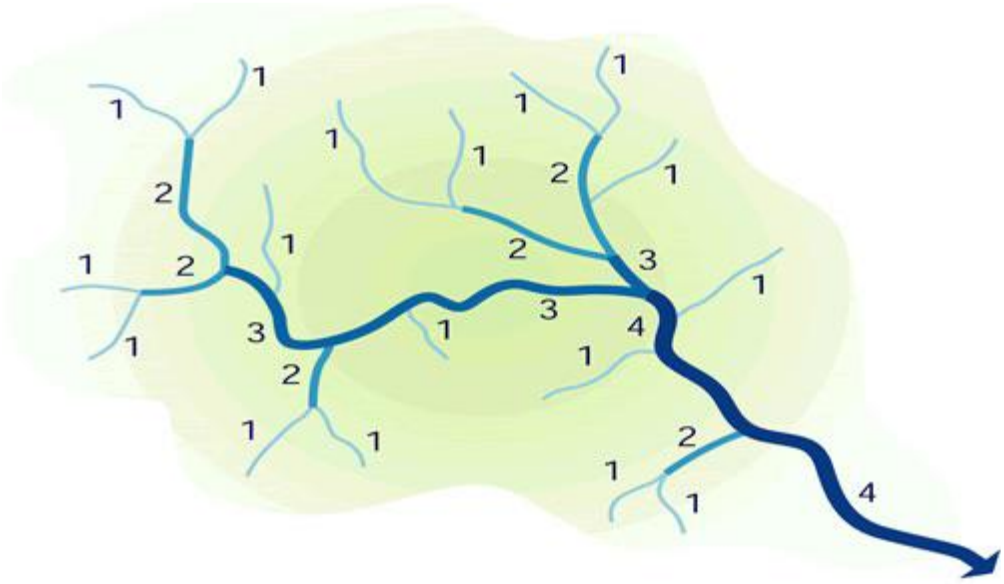

Figure 1.1 River order classification. Each unique tributary is given a river order of 1. The river resulting from the confluence of 2 first order rivers is assigned as order 2. This is cumulative where rivers of the same classification meet. If a second order river were to combine with a third order river however, the river order would not increase downstream of the confluence. Image credit http://www.fgmorph.com/fg_4_7.php, last accessed 07/09/2015.

needs to be considered along with soil moisture, vegetation, and evapotranspiration in assessing groundwater flow (Szilagyi *et al*., 2005). BFI is a measure of the proportion of base (underground) flow to total water run-off within a catchment (Bloomfield *et al*., 2009). Lower BFI catchments promote surface runoff as a transfer of water from land to rivers. Rivers fed by surface run-off tend to experience higher turbidity from soil erosion, and a flashier hydrology - defined by a small lag time and high variability in water levels after storm events (Figure 1.3a). The flashy hydrology is created as water is unable to percolate through and be stored within an aquifer, ultimately making the river the main reservoir for water in these catchments. This leads to large amounts of overland flow, which through abrasion can lead to high levels of soil erosion. With higher soil erosion should also come high levels of particulate organic matter (POM), potentially influencing biotic and abiotic nutrient cycling. When examined over a long period of time, the result of flashy hydrology can be identified as a sharp rise in a flow-duration curve (Figure 1.3c) at lower percentile flow rates. Groundwater-fed rivers are found in areas with a high BFI, and have a typically less flashy hydrology as water is more easily stored in the porous soils and bedrock (Figure 1.3b), and the resulting flow-duration curve from long-term data sets will represent a steady flow (Figure 1.3d). The aquifer that this creates acts to feed the river with water even during periods of low above-ground flow. Differences in hydrology caused by the geological characteristics will therefore likely influence the transfer of nutrients from the terrestrial environment to the aquatic.

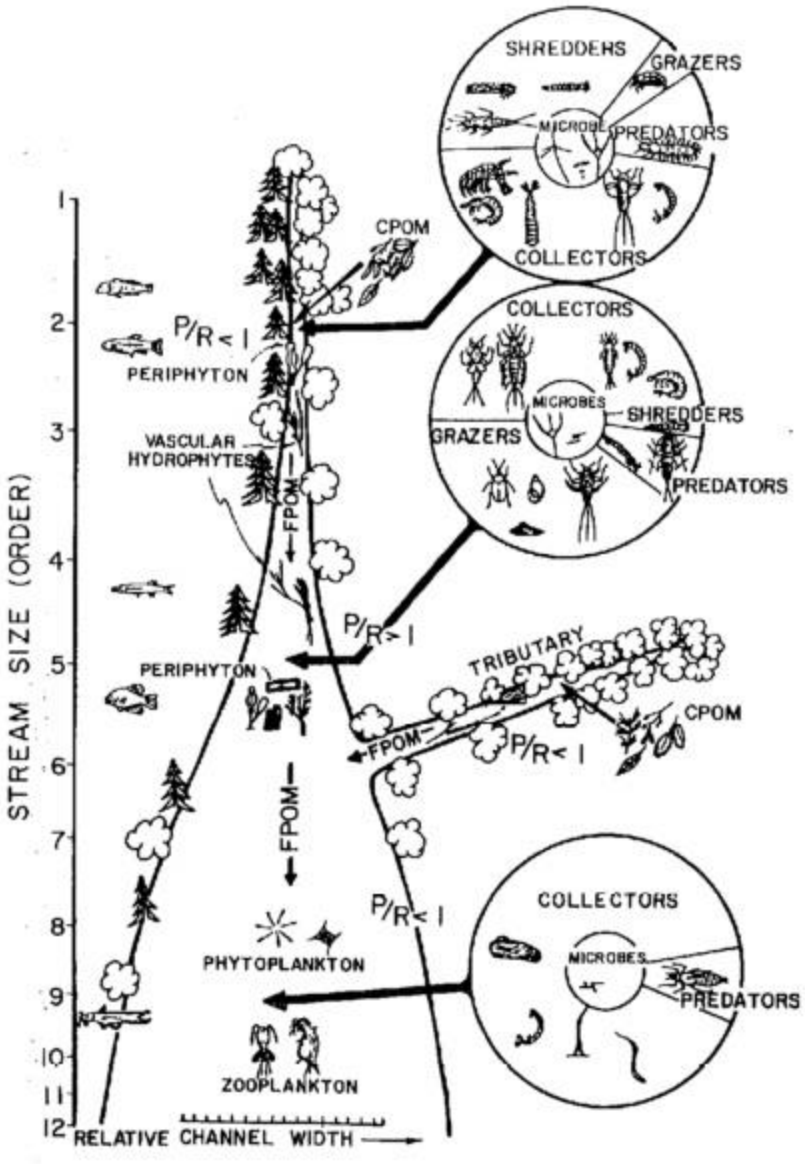

Figure 1.2. River continuum concept, from Vannote *et al*. (1980). The river continuum concept suggests that there will be a shift in community composition, and therefore function, with the size of the river. In low order rivers, channel shading by riparian vegetation limits growth of macrophytes, allowing microphytobenthic communities to dominate.

The groundwater-surface interface - also termed the hyporheic zone - is described as a 'dynamic ecotone' (Trimmer *et al*., 2009). The physical region of the hyporheic zone in river systems is a challenge to determine, due to temporal and spatial variation caused by water movement through rivers and groundwater (Brunke *et al*., 1997), as well as terrestrial inputs. Because they are transitional zones, they represent a wide range of redox conditions and as such are important in biogeochemical cycling of multiple elements (Bengtsson *et al*., 2014; Briggs *et al*., 2015; Thomas *et al*., 2001). Bacterial production has been shown to be supported by hyporheic flow, with increases in bacterial activity correlated to subsurface water flow (Battin, 2000). In this case, water and dissolved nutrient retention time in sediments is considered an important factor explaining increased bacterial activity (Battin, 2000). The importance of the hyporheic zone is increased in low order and headwater rivers, where there is likely to be greater groundwater flow relative to surface water and large sediment surface area-to-volume ratio.

1.4 Sediment characteristics

As rivers are formed by terrestrial erosion, river sediments are typically representative of wider catchment conditions. Catchment soils can be transported to rivers through hydrological and biophysical means. Run-off can cause large amounts of soil to be washed into rivers, and this can contribute to a large proportion of the sediment load in a river. For instance, work undertaken on chalk rivers in south England suggests that up to 97 % of the fine sediment in a river reach is derived from terrestrial run-off

(Sanders *et al*., 2007). This can not only change the physical characteristics of the sediment environment, but also the concentration of nutrients. Jones *et al*. (2001, as cited in Bechtold *et al*., 2012) reported that up to 50 % of nitrogen and phosphorus loadings in rivers could be predicted when land use is primarily agricultural where soil transport is generally high, resulting in transport from terrestrial environments making up a significant factor in river chemistry. As sediment can be retained in freshwater systems such as rivers and lakes, these waterbodies can also contribute to nutrient sinks; around 33 Tg C yr^{-1} is estimated to be stored in these sediments in Europe (Ciais *et al*., 2008). It has been estimated that lateral carbon transport - the movement of carbon away from where it is sequestered as CO_2 - could be as high as 165 Tg C yr^{-1} in Europe alone (Ciais *et al*., 2008). Terrestrially derived (allochthonous) carbon is also a major contributor to sediment metabolic processes, with CO_2 out-gassing estimated to be 90 Tg C yr^{-1} (Ciais *et al*., 2008), suggesting that while the majority of carbon in rivers is transported, a significant amount is also used in secondary production.

Water requires a high level of kinetic energy to be able to break down, suspend and transport particulate matter. Pools and riffles - common attributes of rivers - are a typically used example of how hydrology can shape the river sediment. In riffles, the channel is constricted in depth by the presence of large, difficult to move stones and pebbles. These areas are typically fast flowing, and as such are less likely to contain fine sediments. Pools on the other hand are an expansion of the river channel. In pools, river flow reduces as the area for flow is greatly increased, leading to deposition of

finer particles. Pools are characteristically dominated by softer sediments because of the lower river velocity found in these environments. Biotic factors, such as macrophyte growth, are also able to influence hydrology and therefore sediment structure. Sanders *et al*. (2007) was able to show the impact of *Ranunculus* spp. in both fine sediment accumulation and to biogeochemical cycling. Growth of macrophytes was able to significantly slow river velocity by around 0.4 m s^{-1}, to close to 0.1 m $s^{-1.}$ This led to a peak deposition of fine sediments around 20 cm above the chalk river bed - a large deposition event in rivers with a mean depth of 22 cm. Catchment characteristics, such as changes in BFI and interactions between surface/groundwaters, are therefore important factors to consider when assessing river biogeochemical processes, particularly when examining benthic systems such as MPB communities.

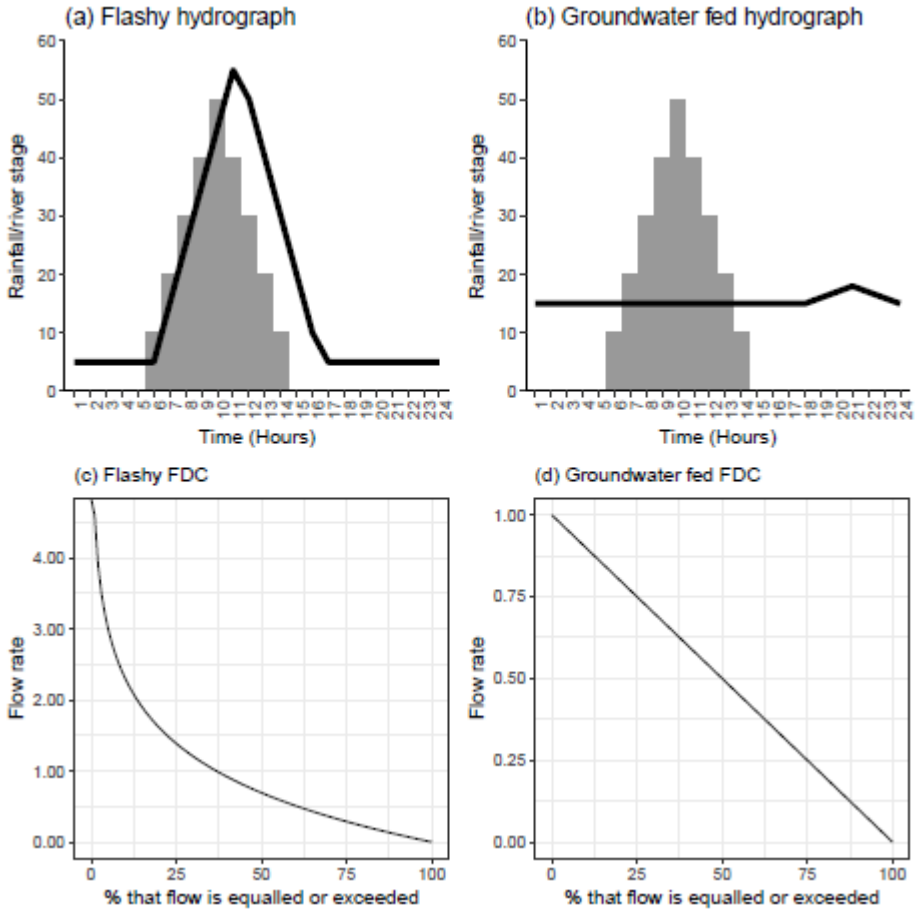

Figure 1.1 Hypothetical hydrographs and flow-duration curves (FDC) representing differences in river response to the same rainfall event under contrasting sub-catchment base flow conditions. The low BFI catchment (a) shows an initially low river stage (line), and a rapid increase in stage response to rainfall (bars), along with a rapid decline in stage shortly after the rainfall event. The high BFI catchment (b) is categorised by a higher initial stage throughout the rainfall event, with a small fluctuation spread over a longer time frame. Flow duration curves calculate a river's response to rainfall over a longer period of time. In flashy rivers (c), FDCs show low flow is not equalled or exceeded for the majority of the sampling period, while higher flows are rare occurrences. In groundwater fed rivers, flow rates are evenly distributed over the sampling period, resulting in a more linear response.

1.5 The biogeochemical role of small rivers in the landscape

Although the concept of rivers being biogeochemically inactive has now been refined with further research (for a review see Battin *et al*., 2009), a proportion of nutrients is indeed transported downstream. Nutrient cycle conceptual models are commonly used to define the biogeochemical transformation of chemicals. This model works well as nutrient cycles can be carried out in a relatively static location (such as a terrestrial environment), and because they are adapted to relatively straight-forward visualisation. The flowing nature of a river however removes the static concept of a nutrient cycle. Instead, it has been proposed that nutrients spiral, with the cyclic transformation of elements being carried out over a large longitudinal distance. The spiralling distance (S_W) is defined as the distance a molecule must travel in order to complete a full biogeochemical cycle (Bouwman *et al*., 2013), and is determined both by abiotic (flow rate, depth, river bed topography) and biotic (abundance, resource requirement) factors. Due to their position in a catchment, headwater and small rivers play a large role in the processing of non-point pollution (such as agricultural run-off) due to proximity to the sources, and the high SA:V ratio of sediments (Lyon & Ziegler, 2009). More clearly understanding the role of freshwater environments in the processing of nutrients is important, not only due to ecological issues but also legislative ones. As of writing, implementing the European Union's Water Framework Directive (WFD, European Parliament, (2000) in the UK is currently a priority of the government and UK Environment Agency. It has been proposed that catchment management plans overlook nutrient interactions within a river, and simple plans

which aim to reduce initial loading may not be effective where the biological and ecological interactions with nutrients are not fully understood (Withers & Jarvie, 2008). Therefore, it is important to gain a deeper understanding of the processes that are involved in biogeochemical cycling within riverine sediments, and how increased knowledge can contribute to more effective management of catchment systems.

1.6 The microphytobenthos

Microphytobenthic communities, as suggested by the river continuum concept (Vannote *et al.*, 1980, Figure 1.2), are typically the dominant primary producers in smaller rivers. MPB are mixed microbial communities consisting of many algal components (Underwood *et al*., 2005), as well as heterotrophic bacteria and other microbial groups such as protozoa and fungi (Bruckner *et al*., 2008). MPB exist within a carbon-rich polysaccharide matrix consisting of extracellular polymeric substances (EPS), carbohydrates and other secreted compounds. MPB communities can be found on most wet, illuminated substrata. A high diversity of organisms is found within an MPB community, leading authors to compare biofilm communities to rainforests (Hansen *et al*., 2007, as cited in Lyon, 2007). High levels of habitat heterogeneity, coupled with evolutionary and ecological processes such as niche differentiation, cooperation and facilitation have led to the development of a wide range of biogeochemical activity within a relatively small area (Arnon *et al*., 2007).

The EPS matrix in which MPB communities exists can play many roles, such as habitat modification by stabilising sediment structure (Gerbersdorf *et al*., 2005; Gerbersdorf

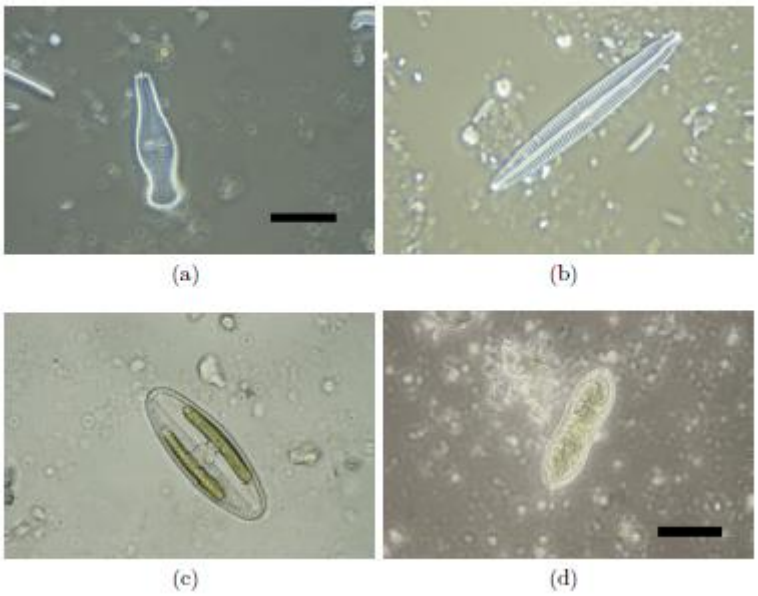

Figure 1.4. A range of permanently mounted (Figures 1.4a, 1.4b) and glutaraldehyde fixed diatoms (Figures 1.4c, 1.4d). Figure 1.4a,b,c images were taken under 1000 x magnification, while Figure 1.4d was taken under 400 x magnification. Figure 1.4a, *Gomphonema truncatum*, Figure 1.4b, *Navicula lanceolata*, Figure 1.4c *Navicula* sp. showing chloroplast structures, Figure 1.4d *Cymatopleura solea* showing chloroplast structures. Figure 1.4a shows a scale bar of 20 um for Figures 1.4b and 1.4c. Figure 1.4d shows a scale bar of 40 m. Full authorities are listed in the Appendix.

et al. 2008; Gerbersdorf & Wieprecht, 2015; Passarelli *et al*. 2013; Smith & Underwood, 1998; Stal & de Brouwer, 2003). Stability of sediments is increased through EPS acting as a "glue", in which particle cohesion is improved. In simulated fine sediments (particle size < 63 µm diameter), increasing EPS and extracellular carbohydrates was shown to increase the resistance of sediments to physical action by up to two times that of uncolonised sediment (Gerbersdorf *et al*., 2009).

1.7 Diatoms as major components of the MPB

Diatoms (Figure 1.4, Bacillariophyceae) are typically abundant in many MPB communities. Diatoms are single celled algae, with some species able to form colonial chains. They are considered to account for around 20 % of global photosynthesis (Mann, 1999 as cited in Jones *et al*., 2012), with most of this being dominated by marine planktonic species. Diatoms can be suspended as phytoplankton, forming a large component of global oceanic primary productivity, and when sessile they are found in illuminated benthic environments. One of the group's defining features is the silica cell wall, also known as a frustule, which is used extensively in taxonomic studies. Although approaches have been made to identify live diatoms (Cox, 1996), these are typically only effective down to genus level for most investigators - while chemical preparation of diatoms leaves only frustules which are essential for fine-taxonomic studies. The trade-off with this method comes with the lack of distinction between dead and live cells, with the accumulation of dead diatoms being found in areas of sediment deposition, such as soft sediment rivers (Cox, 1990). Morphology of diatom

frustules is also likely to influence retention in the sediment, potentially leading to a bias for certain taxa to exist within this accumulation of dead diatoms. Recent attempts have integrated morphological taxonomy with molecular studies, although this has proved difficult as the knowledge gap between the two disciplines is vast; for example in a study to establish sequencing techniques, only 28 species were able to be positively identified using next-generation sequencing (NGS) out of 242 operational taxonomic units (OTU, Visco *et al*., 2015) - an eight per cent identification rate. However, it is expected that with time, molecular approaches will become more useful as the methodology continues to be explored due to lowering costs and increasing accessibility (Clark *et al*., 2018).

While diatoms are primarily photoautotrophic, interactions with endosymbiotic bacteria has led to some diatom genera (such as *Denticula* and *Epithemia*) to also be classified as nitrogen fixers (Soininen *et al*., 2016). Other studies have suggested that diatoms can utilise heterotrophy (Tuchman *et al*., 2006), although experimental design leads into question the role of bacterial contaminants in heterotrophic utilisation of the compounds tested.

Like all microbes, the small size and short generation time of diatoms result in communities that are incredibly sensitive to environmental conditions. It is for this reason that diatom community structure is used in assessing waterbody condition through many methods such as the Trophic Diatom Index (TDI, Kelly, 2000). Such indices are deemed to be effective enough to contribute to the management of rivers

through legislation such as the European Union's Water Framework Directive (European Parliament, 2000), despite nutrient-independent factors (such as substratum properties) also influencing community composition and biomass (Cox, 1990, Cox, 1994; Murdock & Dodds, 2007; Potapova & Charles, 2005). Interestingly, the role of substratum properties is often overlooked in diatom community structure. Typical analysis of MPB communities are carried out on epilithic communities, with sediment dwelling communities deemed unnecessary to study (Cox *et al*., 2011) - when in reality these can have large roles in community composition and biogeochemical cycles due to their proximity to permeable sediments and the microbial communities contained within.

1.8 Heterotrophic bacteria in MPB communities

Bacteria can be a major component of the MPB assemblage and the importance of bacterial biofilms in general has re-emerged as a focus for research in many different scientific fields (Battin *et al*., 2009). As bacteria are a broad group with wide functional diversity, it is important to understand species' distributions and composition and the factors which drive these. It has been suggested from correlative work that temperature and dissolved organic carbon (DOC) influence the dominant bacterial group (Hullar *et al*., 2006), which may introduce, remove or change the contribution and/or presence of individual biogeochemical fluctuations into river systems. This work also found terrestrial species within benthic biofilms, which the authors suggested entered the river with run-off from the land. How land type and land use

impact the input of terrestrial species into rivers is yet unknown. The function that these organisms play in the biofilms was unknown (Hullar et al., 2006), however non-aquatic bacteria (e.g. laboratory strains of *E. coli*) have been shown to have a positive effect on initial biofilm formation in other studies (Bruckner *et al*., 2011). These results highlight the role that terrestrial communities can play in the biogeochemical function of the MPB community, as differences in terrestrial diversity also influence species which are introduced into the aquatic environment.

1.9 Ecology of diatoms

Diatoms show complex relationships with other diatoms in a biofilm, and there are examples of both cooperation and competition. As with many other environments, increased biodiversity has been found to increase primary productivity (Vanelslander *et al*., 2009), and in intertidal environments, vertical migration within soft sediments allows diatoms within biofilms to express behavioural photo acclimation - which reduces negative light effects such as photoinhibition (Underwood, 2005). This adaptation works in a mutually beneficial manner for diatoms across the community, as they are able to stratify in relation to light conditions - effectively maximising photoefficiency. This changing stratification allows diatoms to exploit a niche in the light environment, preventing issues such as photoinhibition light conditions are not optimal (Underwood, 2005). The role of biodiversity improving productivity in diatoms was also assessed using a number of species composition experiments (Vanelslander *et al*., 2009). This work showed that increased species diversity allowed communities

to express higher overall production rates due to increased facilitation. Interestingly, results suggested that at least one species tested responded physiologically to increased diversity, by reducing chlorophyll-*a* content. This hints that the diatom is potentially mixotrophic, as there is less requirement on photosynthesis for carbon acquisition. Later research by another group showed evidence of diatom mixotrophy through incorporation of a labelled polysaccharide (13C) to diatom chloroplast RNA under light limitation (Taylor *et al*., 2013).

Diatoms can also have competitive interactions. Light has been shown to influence these competitive interactions - the marine diatom *Nitzschia* c.f. *pellucida* has been shown to release the chemical cyanogen bromide (BrCN) at sunrise (Vanelslander *et al.*, 2012). This chemical is a strong algaecide, resulting in chloroplast damage and ultimately cell death in competing diatoms. In some cases, complete cell death was witnessed after only 120 minutes exposure to the chemical (Vanelslander *et al*., 2012). As the release of the algaecide is stimulated by increasing light levels, it will coincide with light-stimulated migratory patterns in this environment, proving this is a strong competitive weapon.

Niche differentiation - a process whereby organisms are at their most competitive in specific conditions - has also been found in estuarine diatoms, where salinity, ammonium and nitrate preferences were compared (Underwood & Provot, 2000). This experiment demonstrated that, not only did species have differences in environmental preference, but they also had different ranges of tolerance to the factors investigated.

The authors stress that while they only tested salinity, ammonium and nitrate, other factors could play a role in defining tolerances. This issue is highlighted in research by (Cox, 1994), who showed that despite laboratory studies to determine a niche, diatom taxa were often found outside expected conditions.

Diatoms have also been shown to have niche preferences to other abiotic factors such as light and substratum structure. A study on a lake in Germany found that certain diatoms were selective of their habitats, for example *Amphora ovalis* was found to preferentially dominate sandy (epipsammic) habitats, while another diatom *Navicula rhynchocephala* was found to only be abundant in high light conditions (Cox, 1984). Similar results were found in a river study where community composition was impacted by sediment type, and also highlighted differences in epiphytic assemblages growing on angiosperms and bryophytes (Cox, 1990). Work similar to this has been repeated across large spatial scales, and has supported this work by finding contrasting diatom community composition on different substrata (Potapova & Charles, 2005). One way of understanding the impact of taxa on a system is to carry out a trait-based analysis. Traits - for instance mode of attachment and size - add a functional aspect to how communities are examined (Lange *et al*., 2015). Many species can carry out the same function, such as secretion of EPS and extracellular organic carbon (EOC). The abundance of these functional groups - rather than the individuals contributing - may result in two communities being unique at a species level, but at a system level they can have the same impact. Trait-based approaches for diatoms were included within

an algal community study (Lange *et al*., 2015). Traits examined included cell size, attachment and motility, providing evidence that using a range of traits to assess communities, as opposed to solely relying on species provided information on controls of algal community composition (Lange *et al*., 2015). Another study of species and traits showed that environmental gradients were observed using trait data, while species data was more varied at each site (Soininen *et al.*, 2016). This study however focused on motility, morphological profile and tolerance to acidic conditions. As this is an emerging approach for use with diatoms and algal communities, it is likely that it can be optimised further.

1.10 Ecology of Bacteria

Bacteria-bacteria interactions are gaining increasingly intensive research attention, and this is typically focused on bacterial communication (Battin *et al*., 2007). As it is now accepted that bacteria thrive in close proximity to others, the interest in communication such as quorum sensing has gained momentum. Quorum sensing describes a process whereby gene expression is controlled by population density (Keller and Surette, 2006). In these situations, signals are released by all bacteria and upon reaching a certain concentration a response is triggered. The signalling molecules - auto-inducers (AI) - bind to response regulators in the bacterial cell, facilitating these changes (Amin *et al*., 2012). One of the first instances of quorum sensing was discovered in the marine bacterium *Vibrio fischeri* - which exhibits bioluminescence at high cell densities (Amin *et al*., 2012, and references therein).

The bacterial community composition in microbial communities has been shown to be important for ecosystem processes such as nutrient cycling. In a pelagic system, it was shown that external bacterial enzyme production can be limited and specific to a bacterial species. For instance, it was found that only 80 % of taxa in a sample produced the enzyme β-glucosidase, production ranged from 0 - 35 µmol MUF produced $cell^{-1}$ h^{-1}, suggesting that the functional ecology for breaking down recalcitrant carbon sources is dependent on diversity (Martinez *et al*., 1996). The collective production of these enzymes has been shown to increase under experimental glucose additions in freshwater biofilms, suggesting that labile carbon sources can be obtained in a supply-demand type relationship (Ylla *et al*., 2009). However, it can be argued that this may not be a representative experiment as glucose is not readily available or common as a carbon source in many natural systems.

1.11 Inter-kingdom interactions in the MPB

Diatoms have been well documented to produce high quantities of extracellular polymeric substances and carbohydrates. The production quantity and composition of carbohydrates has been linked to limitation of nutrients other than C (Underwood *et al.*, 2004). For instance, when carbon is fixed by photosynthesis, it can only be utilised by growth where other nutrients are not limiting (Danger *et al.*, 2013; Scott *et al*., 2008). Due to the abundance of C available, this limitation results in excess carbon being assimilated via photosynthesis, which is excreted from the cell. While the ecosystem engineering properties of extracellular organic carbon have been discussed

earlier, this extremely labile carbon can also fuel the metabolic activity of heterotrophic bacteria.

Living within a biofilm creates inevitable interactions between the individuals within the MPB community. Instead of the individual organisms that are studied, microbes form functional communities (Davey & O'Toole, 2000), creating competition and cooperation between community inhabitants. However, these interactions have proved to be difficult to observe, as systems need to be manipulated into breaking down these relationships to understand interactions between components (Amin *et al*., 2012).

Ecological interactions between members of the MPB community can be driven by niche partitioning and co-metabolism mechanisms. Another potential driver of interactions between different organisms can be the exchange of secondary metabolites - chemicals produced by the cell that are not essential for maintenance of cellular growth or normal function of the organism (Keller and Surette, 2006). In this respect, secondary metabolites can be considered as the currency of microbial interactions (Amin *et al*., 2012), with both sides of a mutualistic interaction exchanging goods to receive others. As in larger ecological systems, MPB communities experience interactions between primary producers and heterotrophic organisms. Because MPB communities are the dominant primary producer group in low-order rivers, the majority of autochthonous carbon (carbon produced within the system) is produced by microalgae such as diatoms. Similarly to intra-kingdom groups, the trophic

interactions between the components of the MPB communities can be facilitative, competitive or cooperative (Amin *et al*., 2012; Fitter & Hillebrand, 2009).

Algal activity can influence bacterial activity through metabolic processes such as photosynthesis. Photosynthesis, through removal of inorganic carbon, can raise the pH of a biofilm community to around pH 9 (Francoeur & Wetzel, 2003). It has been shown that photosynthesis-derived changes in pH within a biofilm can stimulate bacterial excretion of leucine-aminopeptidase (LAMP) - an extracellular enzyme which is known to degrade amino acids (Francoeur & Wetzel, 2003). In this series of experiments, it was shown that while other factors (such as labile organic carbon, dissolved inorganic carbon) also played a role in LAMP regulation, pH exhibited the most significant effect. Bacteria have been demonstrated to influence the carbon allocation of a benthic diatom. In an experiment diatom cultures were paired with bacteria and bacterial spent media, both of which influenced growth and EPS production rates in diatoms (Bruckner *et al*., 2011). Although bacterial cells do produce EPS, it has been deemed less significant (in quantity) than diatom-derived EPS (Bruckner *et al*., 2008). The exact products which are transferred between the two parties are unknown, although one of the major hypotheses for bacterial supplementing diatom production is the exchange of vitamins, primarily vitamin B12 – cobalamin (Amin *et al.*, 2012). Recent studies of planktonic systems have suggested that vitamin B12 is indeed shared between algal and bacterial cells (Grant *et al*., 2014),

although these conclusions were based on modelling rather than experimental manipulation.

Autotrophs and heterotrophs in MPB communities are likely to be coupled, whereby they facilitate growth of each other through the exchange of nutrients and other metabolic products which would limiting in the natural environment. Evidence for this was found by (Rier & Stevenson, 2001), who demonstrated that algal biomass can correlate with bacterial cell density, suggesting that bacterial and algal growth are linked. This was also shown in another study examining the impact of light on these interactions (Rier *et al*., 2007), whereby bacterial abundance was correlated to algal biomass on certain substrata. Trophic coupling interactions however can be suppressed in high nutrient conditions, where the need to facilitate growth is non-existent. Here, the autotrophic and heterotrophic constituents of the community can become decoupled (Scott *et al*., 2008). This decoupling of components could change the taxonomic composition of MPB communities as species which rely on the interactions will be outcompeted, potentially leading to changes in function by the system.

A potential driver of these interactions is likely to be heterotrophic consumption of EPS and EOC as a carbon source. Work has shown that colloidal (water soluble) extracellular carbon (CHO_D) is a preferential C source for bacteria in intertidal mudflats, despite high levels of OC being found within the same sediment (Taylor *et al*., 2013). This uptake was shown to be significant, with the authors reporting that

although EPS constituted to ~ 5 % of total organic carbon in experimental slurries, 100 % of the addition was utilised in 30 hours. This high rate consumption of a relatively scarce carbon source highlights its importance to these systems. However, the incorporation of multiple fractions of colloidal carbon has been shown to influence bacterial community structure (Haynes *et al.*, 2007), due to differential bioavailability. Changes in the relative abundance of these fractions could have add selection pressure to a bacterial community, which may also select for contrasting biogeochemical functionality.

A model example of the interactions between auto- and heterotrophic organisms was recently established using culturing, transcriptomic and metabolite analysis of bacteria and the planktonic diatom *Pseudonitzschia multiseries* (Amin *et al*., 2015). The experiments showed that axenic (bacteria free) cultures show significantly lower growth rates, while those with a consortium of associated bacteria outperformed diatoms grown with a single bacterial species. The bacteria seemed to benefit from co-culture too; although this was highly specific as a strain of *Sulfitobacter* used only showed increased growth with *P. multiseries*, and not with other marine diatoms (Amin *et al*., 2015). The study concludes that interaction between the diatoms and the bacteria are driven by exchange of the metabolite taurine (alga to bacteria, a carbon source) and this is used to transform NO_3^- to NH_4^+ by the bacterial cell. Diatoms then exploit this easily-assimilated N source.

This series of experiments shows that auto- and heterotrophs can facilitate and even have a positive impact on the growth of a co-habiting partner. These interactions are also able to have an impact on biogeochemical processing. Algal biomass was shown to correlate not only to bacterial abundance, but also extracellular enzyme activity in experimental communities (Rier *et al*., 2007), suggesting that primary producers influence heterotrophic activities.

Microbial priming describes an interaction whereby the breakdown of allochthonous carbon is facilitated by autochthonous carbon. While this process has been well studied in terrestrial systems (Kuehn *et al*., 2014) it is a relatively new area of study in aquatic systems. By seeding heterotrophs, primary producers are able to stimulate the breakdown of recalcitrant organic matter, allowing heterotrophs and autotrophs to benefit from newly available sources of macronutrients (Danger *et al.*, 2013; Kuehn *et al*., 2014). This relationship has been demonstrated in benthic and peatland communities (Danger *et al*., 2013; Kuehn *et al*., 2014; Wyatt & Turetsky, 2015). In peatland systems, recalcitrant carbon was deemed likely to be behind increases of bacterial activity in the presence of algae (Wyatt & Turetsky, 2015). In this example, much like many others, algal derived EOC was hypothesised to fuel the production of complex extracellular enzymes by heterotrophic bacteria. This allowed energy to be used as part of niche differentiation on the bacterial component in order to gain access to carbon which would not otherwise be bioavailable. Priming has been estimated to

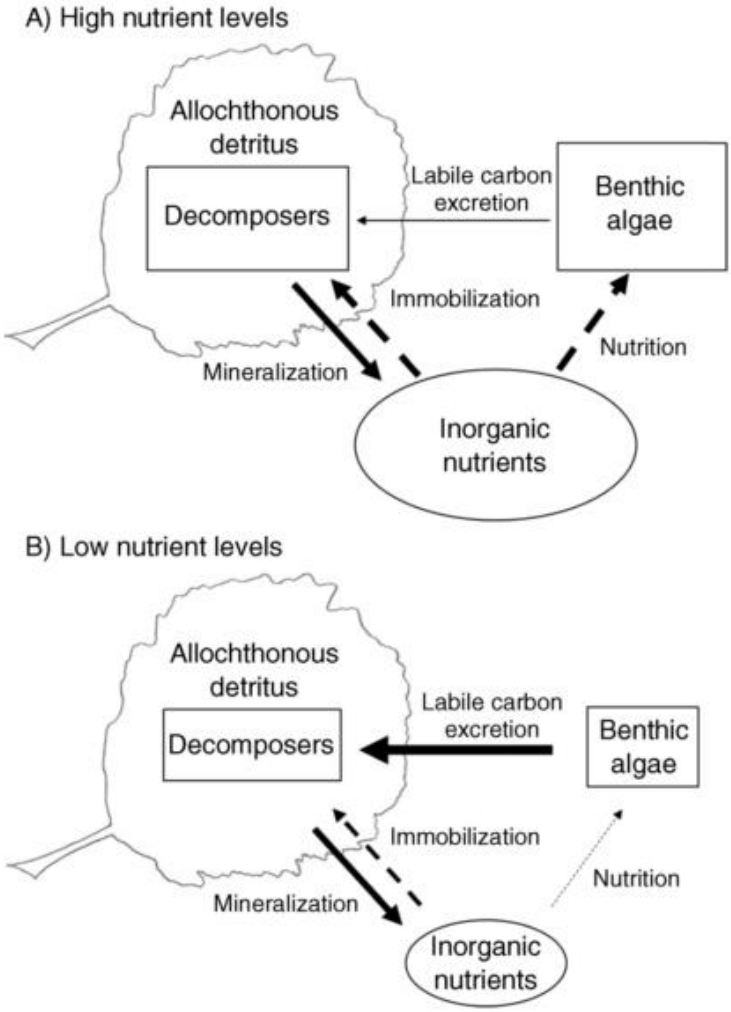

Figure 1.1. Conceptual model of the interactions between photoautotrophs and heterotrophs in headwater rivers (Danger *et al*., 2013). Arrows represent matter transfers and width is proportional to the magnitude of transfers. Dotted and continuous lines represent competitive interactions and mutualistic pathways respectively. (A) Under high nutrient conditions, competition is reduced, and C exudates released by nutrient-unlimited algae represent a minimal fraction of primary production. (B) Under low-nutrient conditions, competition for inorganic nutrients is high, resulting in reduced algal growth, since decomposers are superior to plants in competition for nutrients. Nutrient limitation also stimulates the release of algal C exudates. The use of this labile C by decomposers should, in turn, increase the mineralisation of recalcitrant organic matter through a "priming effect" (Figure and legend modified from Danger *et al*., 2013).

unlock a large quantity of C; for instance, in a review by Guenet *et al*. (2010), a study quoted found a 10-fold increase in allochthonous C resulted in a 50 - 500 % increase in turnover of a recalcitrant carbon source.

Interactions such as priming and trophic coupling however are likely to be sensitive to the background nutrient concentrations. Work by (Danger *et al*., 2013) suggested that the priming effect of trophic coupling only occurred during low nutrient conditions (Figure 1.5). Here, due to the low inorganic nutrient pool, algal photosynthesis exceeds the supply of nutrients used to capitalise on newly fixed carbon as growth. As fixed carbon is now in excess relative to other nutrient requirements, it is excreted from the cell as it cannot utilised in growth.

In the model system described by (Danger *et al*., 2013), this excess labile carbon stimulates the breakdown of detrital material by stimulating the energetic creation of extracellular enzymes. Similarly to results found by Scott *et al*. (2008), Danger *et al*. (2013) found that the facilitative relationship between auto- and heterotrophs was broken at high nutrient concentrations, where facilitative interactions became competitive over nutrients. Both of these studies contrast with an investigation into a priming effect in the hyporheic zone, which found no evidence to suggest priming is occurring (Bengtsson *et al*., 2014). The location of the hyporheic zone however may limit the input of EOC required for a priming effect as acknowledged by the authors (Bengtsson *et al*., 2014), and this may result in the experimental mesocosms utilised during this study being unprepared to deal with artificial C additions. The diversity of

results found indicate the complexity of inter-kingdom ecology is still yet to be understood.

Where work has shown that nutrients impact trophic coupling and/or microbial priming, there are implications for our understanding of how MPB systems respond to nutrient addition. It seems that at high nutrient concentrations, the relationship governing taxonomic composition - and by proxy ecological function - is broken down as individuals can obtain all necessary resources independently. This could lead to changes in functionality of a system, as the organisms that exist during steady trophic coupling will likely be out-competed in a non-facilitative system.

1.12 Thesis aims

The aim of this thesis is to examine the influence of catchment geology on microphytobenthic communities, and their associated ecological processes. This will be achieved by first characterising microphytobenthic communities and water chemistry across a seasonal cycle in a catchment made up of multiple, contrasting geology types – focusing on recording sediment chemical composition, abundance and community composition of microalgae (diatoms) and the fractionation of extracellular organic carbon (EOC, EPS). Secondly, incubation experiments will be carried out, recording potential for primary production/respiration, nutrient exchange between sediment and water, and impacts of light levels on these processes. Finally, directly manipulated microphytobenthic communities grown under high DOC and multiple

light conditions will be tested for potential nutrient flux between the sediment and water under adapted light conditions.

Chapter 3 presents a characterisation of MPB communities across a seasonal cycle in three contrasting catchment geology types within the Hampshire Avon Catchment (HAC), south England. This chapter describes the only know microphytobenthic study to incorporate a range of sediment types, seasonal analysis and a wide range of MPB community traits. Chapter 4 presents a comparison of biogeochemical fluxes within a selection of the sites that were first explored in Chapter 3, along with contrasting habitats within each site. Here, *in-situ* light-controlled incubations provide evidence of differences between the role that MPB communities play in the cycling of C, N & P using natural MPB communities. Based on results gained from Chapter 3 4, Chapter 5 examines the role of light and carbon in the coupled production of algae and bacteria. Here, experimental communities were created *in-situ*, allowing the colonisation of chemically disinfected, homogeneous sediment patches. Both light and DOC controls were in place for the 5-week colonisation period, after which flux measurements similar to those in Chapter 4 were taken to examine the activities of fully established communities.

Overall findings, novel findings and implications from this thesis are discussed in Chapter 6. This thesis is supported by data collected as part of a NERC Macronutrient Cycles consortium: 'The role of lateral exchange in modulating the seaward flux of C, N & P'. The contributions of others' data are indicated in each Chapter.

2 GENERAL METHODOLOGY AND MATERIALS

2.1 Sampling rational and site selection

2.1.1 The Hampshire Avon Catchment

The Hampshire Avon catchment lies in the south of England and covers an area of around 1,750 km^2 (Nadolski, 2012), while the population is around 230,000. The underlying geology is primarily chalk (Figure 2.1), with ribbons of other bedrock such as greensand and clay. The area is home to typical English chalk streams – characterised by groundwater dominated, gravel bed streams with exceptional water clarity - which are commercially valuable for salmonid fisheries (Sanders *et al*., 2007) and watercress production. Other dominant geology types found within this catchment include greensand, and clay – in turn creating a natural gradient of geological permeability. Individual study sites are given in Table 2.1. In two of the sites (GA2 and CW2), two dominant habitats of 'river bed' and 'macrophyte stands' were sampled, denoted by habitat type MC and MP respectively. The selection of these sites was based on three major factors: 1. Three of the sites were also paired to terrestrial sites, which were sampled alongside the rivers as part of the NERC Macronutrients programme, 2. These sites were also in use as part of the DEFRA Test Catchment (DTC) programme, and 3. The range of geologies provides a conceptual gradient of base-flow conditions, with clay catchments being considered to be less permeable, while chalk catchments are considered to be highly permeable.

2.1.2 Sampling design

This work was embedded within the NERC Macronutrient Cycles programme, and as such was an effort to take a holistic approach to answering the overall goals of the project. As such, site selection and temporal sampling regime were set as part of the wider project. However, sampling within each aspect of the project was optimised to the relative aims and objectives. Other work in this project has resulted in the following publications: Heppell *et al*. (2017), Lansdown *et al*. (2016) and Lansdown *et al*. (2012)**.**

Table 2.1. Names, locations and background information for sites used in this study. Coordinates give the start of the reach sampled (main channel only).

Site code	River Name	Geology	Location	Coordinates
AS1	Sem	Clay	Prior's Farm	51.055166N, 2.156794W
AS2	Sem	Clay	Share Farm	51.045813N, 2.111229W
AS3	Sem	Clay	Cool Cottage	51.066300N, 2.143318W
GN1	Nadder	Greensand	Share Farm	51.045151N, 2.110883W
GA2	Avon (W)	Greensand	Puckshipton House	51.319403N, 1.861784W
GA3	Avon (W)	Greensand	Rushall	51.306540N, 1.824670W
CE1	Ebble	Chalk	Stoke Farm	51.028296N, 1.925272W
CW2	Wylye	Chalk	Whitecliff Farm	51.143090N, 2.207420W
CA3	Avon (E)	Chalk	Rushall Farm	51.304841N, 1.809903W

2.2 Water sample collection

2.2.1 Water Chemistry and Hydrology

Water chemistry was monitored both continuously (starting April 2013) via autosampler equipment and during site sampling visits in the HAC. Water samples acquired during sampling were taken upstream of any disturbance using a sterile syringe and filtered using MINISART® 0.2 µm syringe filters (Sigma, UK) into sterile universal containers (25 ml). Water samples (total volume of 40 ml) were stored chilled until return to the laboratory, where samples were then frozen until required. Chemical analysis of the water samples was carried out on a Dionex® (California, USA) ICS3000 with UHP water and KOH eluent, and an AS18 column, measuring major anion and cation concentrations.

Continuous water chemistry was sampled through automatic bank-side samplers as part of the DEFRA test-catchment programme and are now available via the Environmental Information Data Centre (Hants Avon DTC consortium, 2016a, 2016b). Sampled liquids were stored untreated in a covered container, which was sampled every 48 hours.

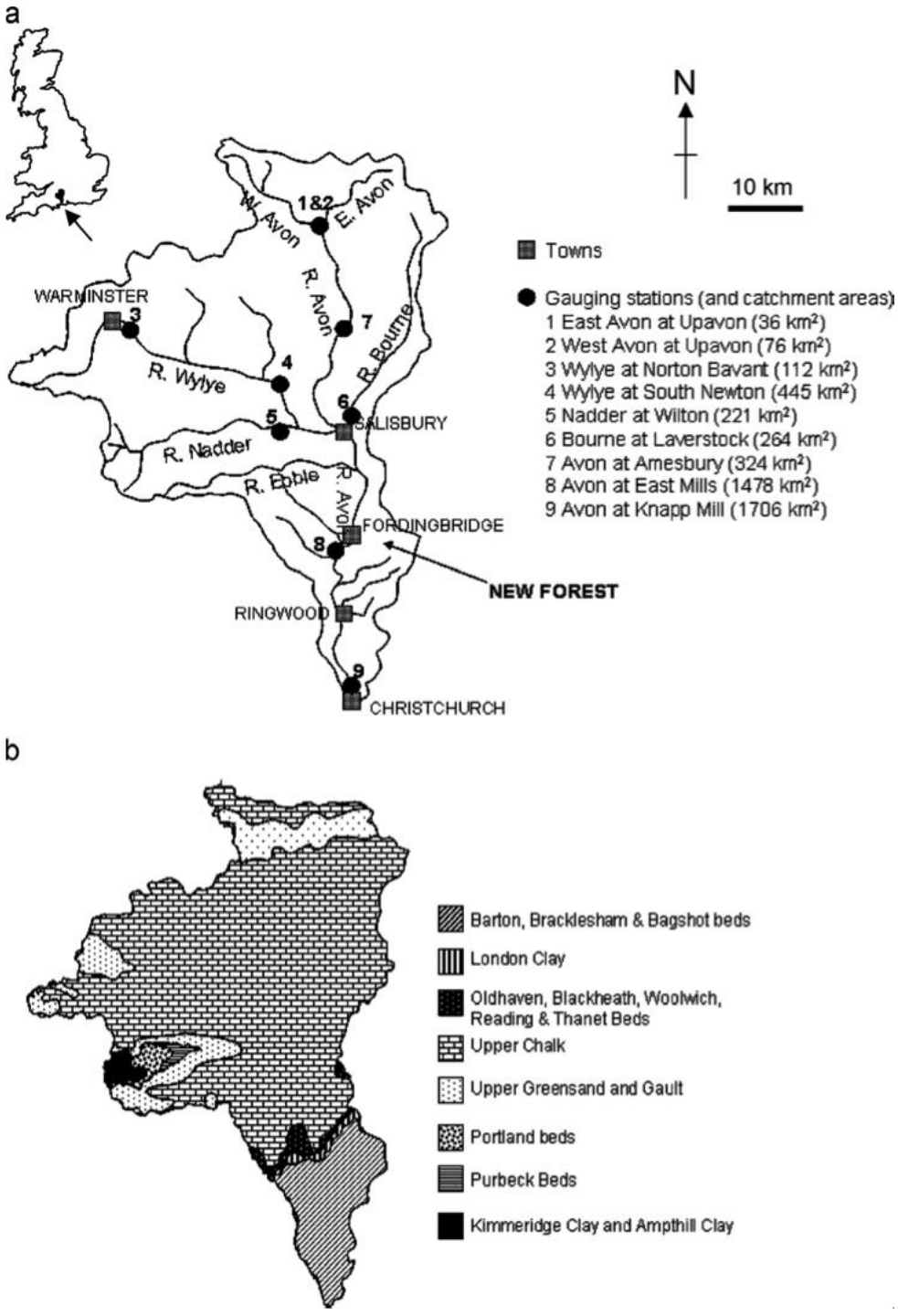

Figure 2.1. The Hampshire Avon Catchment (HAC) Panel (a) shows the catchment with major towns and gauging stations. Panel (b) shows the underlying geology of the catchment.

2.3 Sediment sample collection

Sediment samples were collected using a system of corers to create paired samples for a multitude of analyses. Replicates within each river reach were taken, with all but molecular samples being replicated as n = 5, with more expensive molecular analysis and diatom community composition being replicated as n = 3. Previous work has suggested, based on models produced for both hard and soft sediment, that a single sample would be sufficient to examine the community for environmental monitoring purposes (Potapova & Charles, 2005). This advice however was developed in a study which used pooled samples, effectively removing any aspect of within-site variation and fine resolution which this study aimed to achieve. Therefore, in this study multiple independent samples as described above were chosen in order to account for microspatial variability of river sediments within each reach studied. Soft sediment was targeted and collected using a plastic core (internal diameter of core = 8 cm) 5 times at each reach, along a 5 m transect. This sediment core - typically around 5 cm deep - was then placed into a plastic tray in order to sub-core. Four sub-cores were taken from each of the larger replicates to store the samples in the most appropriate way for each given analysis. Sub-cores were extracted from this initial core using cut-off 20 ml syringes (internal diameter 2 cm), and then decanted into sterile universal containers (Figure 2.2). Samples were stored according to their final analysis (see each analysis section for storage conditions).

2.4 Sample analysis

2.4.1 Pigment analysis

Sediment cores taken for pigment analysis were stored chilled (< 4 °c) and in darkness immediately following collection. Samples were analysed for the photosynthetic pigment chlorophyll-*a* and its degradation products, phaeopigments. Chlorophyll-*a* and phaeopigment concentrations were measured using the cold methanol extraction method (Stal *et al*., 1984). This method includes correction for phaeopigments – a breakdown product of chlorophyll-*a* that can be used as a proxy for degradation (such as through grazing) of algal cells. Sediment samples were freeze-dried and homogenised using a pestle and mortar. A small quantity (around 500 mg) was then weighed, and 4 ml of Methanol buffered with $MgCO_3$ was added. These samples were then vortex mixed for 10 seconds and stored in the dark at 4 °C for 24 hours.

Following chilling, samples were vortex mixed once more, and then centrifuged at 2300 g for 15 minutes. Supernatant was decanted into cuvettes and measured spectrophotometrically at 665 nm and 750 nm. Samples were then acidified using 10 % HCl to determine phaeopigment concentrations. Final chlorophyll-*a* and phaeopigment concentrations were calculated using the equations 2.1 & 2.2 respectively against a methanol blank processed alongside each sample batch.

2.4.2 Sediment carbon

Sediment collected for carbon analysis was stored in darkness at < 4 °c after collection. Upon returning to the laboratory, samples were frozen at - 20 °c. At the point of

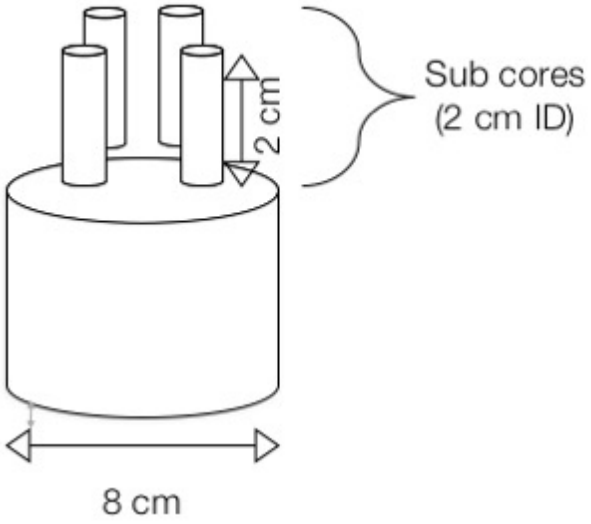

Figure 2.2 Schematic of sediment sample design used throughout this thesis. A large core (internal diameter 8 cm, depth ~ 5 cm) was extracted from the river bed, which was then sub-cored (internal diameter 2 cm, depth 2 cm) to produce paired samples. These paired samples were treated according to the analysis to be carried out in the laboratory. During the incubation experiments (Chapters 4 and 5) sub-cores were used to replicate each individual light treatment.

Equation 2.1 and 2.2. Calculations required to determine chlorophyll-*a* and phaeopigment concentrations from cold-ethanol extracted samples (Stal *et al*., 1984).

$$\frac{K \times (((Abs665_{b} - Abs750_{b}) - (Abs665_{a} - Abs750_{a})) \times Vs \times 1000)}{(AC \times (g^{-1}\ sediment\ DW))} \quad (2.1)$$

$$\frac{K \times F \times ((4.14 \times (Abs665_{a} - Abs750_{a}) - (Abs665_{b} - Abs750_{b})) \times Vs \times 1000)}{(AC \times (g^{-1}\ sediment\ DW))} \quad (2.2)$$

*Abs*665$_b$ = absorbance at 665 nm **before** acidification,

*Abs*750$_b$ = absobance at 750 nm **before** acidification.

*Abs*665$_a$ = absorbance at 665 nm **after** acidification.

*Abs*750$_a$ = absorbance at 750 nm **after** acidification.

K = 1.34.

AC = 74.5.

F = 0.947.

Vs = volume of solvent used (ml).

analysis, samples were thawed and dried in a drying oven at 55 °c. Total sediment carbon (TC) and organic carbon (OC) were measured using a Shimazdu (Kyoto, Japan) TOC-V® with attached Solid-State Module (SSM). TC samples were not treated before being measured. OC samples were purged of inorganic carbon by the addition of 1 ml of 2M HCl, then dried overnight at 60 °c to remove moisture before measurement.

2.4.3 Extracellular organic carbon

Samples for carbohydrate and EPS analyses were extracted from the sediment cores used for sediment carbon analysis (section 2.4.2), and as such were stored in the same way following sampling (darkness at < 4 °c). Carbohydrate methods were adapted from (Hanlon *et al*., 2006). After freeze-drying, multiple extractions were made on a known mass of sediment. Carbohydrates and EPS were measured using phenol-sulphuric acid assay (PSA, Hanlon *et al*., 2006), with the samples being measured spectrophotometrically at 485 nm.

Dissolved carbohydrates (CHO_D) and EPS were extracted from a known quantity of sediment (approximately 500 mg) which was placed into a centrifuge tube, along with 4 ml of UHP water. This was then vortex mixed for 10 s and incubated at 25 °C for 30 minutes. Samples were then centrifuged at 4000 g for 30 minutes, and triplicates of supernatant (0.4 ml) were decanted into glass boiling tubes for CHO_D analysis through PSA, which was carried out immediately to minimise degradation of the CHO. A further sample of supernatant (1.5 ml) was also extracted and added to 3.5 ml absolute

ethanol (EtOH), giving a final concentration of 70 % EtOH for EPS extraction. The dried sediment pellet was then retained for further analysis.

EPS_{70} samples were stored overnight in darkness at 4 °c, then centrifuged at 4000 g for 30 minutes to form an EPS pellet. Excess EtOH was then removed, and the pellet was allowed to air dry. Once dry, the EPS pellet was then resuspended in 1.5 ml UHP water, decanted (0.4 ml) into triplicate glass boiling tubes for PSA.

The original sediment pellet was then subsequently used to extract hot water soluble carbohydrates (CHO_{HW}). Any remaining water was decanted from the centrifuge tube, following which 4 ml UHP water was added. This was then heated to 100 °C in a water bath for 1 hour, and then the sample was cooled and centrifuged at 4000 g for 30 minutes, then split into triplicate 0.4 ml volumes in boiling tubes for PSA.

2.4.4 MPB Community Analysis

MPB community analysis cores were fixed on site using 20 ml universals prefilled with 0.5 % glutaraldehyde in order to fix samples. Before preparation in the laboratory, sediment samples were vortex mixed briefly (5 s) before being sonicated for 10 minutes to suspend any attached algae. Diatom community composition was assessed in a quantitative method, using a method adapted Trophic Diatom Index (Kelly *et al*., 2008). After sonification, samples were vortex mixed for 10 seconds. Standard 1000 µL pipette tips were cut to provide a wide mouthed tip (internal diameter ~ 0.5 cm), which allowed for the uptake of larger particles of sediment. Using these large pipette tips, 2 ml of mixed solution was decanted into a centrifuge tube. These samples were

then centrifuged at 2300 g for 15 minutes, forming a pellet of material. Excess glutaraldehyde above the pellet was removed and replaced with 0.5 ml of ultra-high purity (UHP) water, and 2 ml of saturated $KMnO_4$. After 24 hours, 5 ml of 32 % HCl was added to the tubes, which were then heated in a water bath at 80 °C for 1 hour. Samples were centrifuged again (2300 g for 15 minutes) and supernatant removed. The pellet was resuspended in 2 ml of UHP water and centrifuged as above. This step was repeated 5 times in order to remove HCl from the samples. The clean samples were then dried on gently heated coverslips and mounted on microscope slides using the diatom mountant Naphrax®. Finished slides were analysed using a light microscope (1000 x magnification), with individual diatom frustules being identified to species level, or where not possible to the highest taxonomic level. Diatoms were identified using a combination of Hartley (1996), Kelly (2000) and Kelly *et al*., (2005).

2.4.5 Light manipulation slurry incubations

Light manipulation slurry experiments were carried out independently of primary sampling to minimise the impact of sampling disturbance on results. Sediment cores were taken from each patch type (main channel or sediment from under a macrophyte stand, Figure 2.2). Surface sediment from the top 2 cm was added to unvented Nunc (ThermoFisher, UK) tissue culture (TC) flasks and river water added to the flasks eliminating any headspace gas. Samples for a t0 measurement of O_2 and water chemistry were taken in triplicate as flasks were filled. Filled tissue culture flasks were secured to a plastic board (Figure 2.3). Light levels (ambient, 50 % ambient, 25 %

ambient and 0 %) were controlled using neutral density filters and aluminium foil. Ambient light levels were measured using an Onset (MA, USA) HOBO® logger attached to the plastic boards (Figure 2.3) which sampled light levels (LUX) and temperature (°c) at a 30 second resolution.

2.4.6 Dissolved oxygen

Dissolved oxygen (DO) measurements were carried out using a modified Winkler titration Figure 2.3, Winkler, 1888). Modern methods of measuring dissolved oxygen - such as oxygen electrodes - can result in more accurate measurements immediately in the field; however due to the experimental design aimed at replicating the environment within multiple, simultaneous isolated microcosm of small volumes, Winkler titrations were selected. A number different of factors were taken into consideration, such as the small volume of water sample (25 ml overall), and the need to fix the sample as quickly as possible. While the issue of fixing the sample in the field would have been mitigated by using electrodes, stabilising the system would have added to sample recovery time, potentially leading to nutrient and/or oxygen saturation/depletion. The fragility of oxygen electrodes in a field environment, where equilibration of atmospheric oxygen is a major concern was also taken into consideration. Also due to replication efforts required in what was assumed to be a higher heterogenous environment, cost was also a factor in the decision to use Winkler titrations for the recording of oxygen concentrations.

Liquid samples were collected directly from the incubation flasks in the field using a syringe with attached silicon tubing, and water samples were drawn from the tube slowly to minimise formation of air bubbles and therefore minimise atmospheric equalisation. Samples were decanted to overflowing into air-tight Labco (Lampeter, UK) Exetainers® (12 ml) with glass beads to aid mixing in the absence of any airspace. DO was fixed with the addition of 0.1 ml $MnSO_4$, followed by a NaOH and KI solution (0.1 ml). The addition of these solutions generates a $Mn(OH)_2$ to form, which is rapidly oxidised – fixing the O_2 and preventing any biological utilisation. Although at this point oxygen is mad unavailable to any biological activity, there is still a small risk of iodine in the sample being oxidised or reduced, providing incorrect results.

On return to the laboratory, samples were titrated to determine dissolved oxygen concentration. Calibration preceded each batch processed in the lab. In order to calibrate each of the solutions, an F-factor was calculated according to Equation 2.3. 12 ml UHP was added to a conical flask along with 0.1 ml concentrated H_2SO_4 which acts to liberate iodine, which is equimolar to $MnO(OH)_2$. 0.1 ml of both alkaline iodide solution and $MnSO_4$. 1 ml of 0.01 M KIO_3 was then added, and then titrated as below. For each of the calibrations and samples, 0.1 ml of concentrated H_2SO_4 was added to the vials and mixed. The contents of the vial were then placed in a conical flask, and a sodium thiosulphate solution was slowly added until the sample turned a pale-yellow colour. At this point, 0.2 ml of starch indicator was added which turned the solution a

dark blue/black. The titration was then carried out until the solution became clear using sodium thiosulphate.

2.4.7 Molecular analysis

Sediment samples for molecular analysis were cored as per Section 2.3 using sterile syringes. Sediment cores were immediately frozen in a cryogenic vessel at -80°C until return to the laboratory. Upon return, samples were stored at -80 °C until extraction.

Molecular material was extracted from the samples using a Mo-Bio Powersoil® DNA Isolation Kit (QIAGEN, Germany). The forward primer Bakt_341F 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG3', and reverse primer Bakt_805R 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACT ACHVGGGTATCTAATCC3' (Herlemann et al., 2011) were used to target the 16S rRNA gene, specifically focusing on the V3 and V4 regions. Equal volumes of index and PCR reagent were used for each of the samples. 12.5 µL of RedTaq® DNA polymerase (Sigma-Aldrich®, UK) were added to a PCR tube along with 5.0 µL of both the forward and reverse primers. 2.5 µL of sample index was added to the tubes, with exception to a negative control to detect possible contamination. PCR was optimised using a random combination of samples each run to the following conditions; a first step of 5 minutes at 94 °C, followed by step two which consisted of 28 cycles of denaturation (30 seconds at 94 °C), annealing (30 seconds at 56 °C) and extension (30 seconds at 73 °C). A final step of elongation was carried out for 10 minutes at 72 °C, followed by

chilling at 4 °C. In order to examine reaction quality, samples were run on 1.5 % agarose gel using electrophoresis against a molecular ladder.

2.4.8 Data Analysis

All data analysis was carried out using R (version 3.6.2 - "Dark and Stormy Night", (R Development Core Team, 2011). Specific packages used are listed in Table 2.2. For comparing sediment samples, a nested-ANOVA approach was used. A nested design was considered the best solution as it fit within the multiple landscape level approach utilised in the sampling design. Each landscape level was treated as a level in the ANOVA, which allowed for simultaneous comparisons of samples within a sub-catchment geology group and overall sub-catchments. A confidence interval of 95 % was used for all analysis in order to reject null hypotheses.

Where direct comparison of multiple sites was required, such as in Chapters 4 and 5, a standard ANOVA was used. This allowed for comparison of the different variable

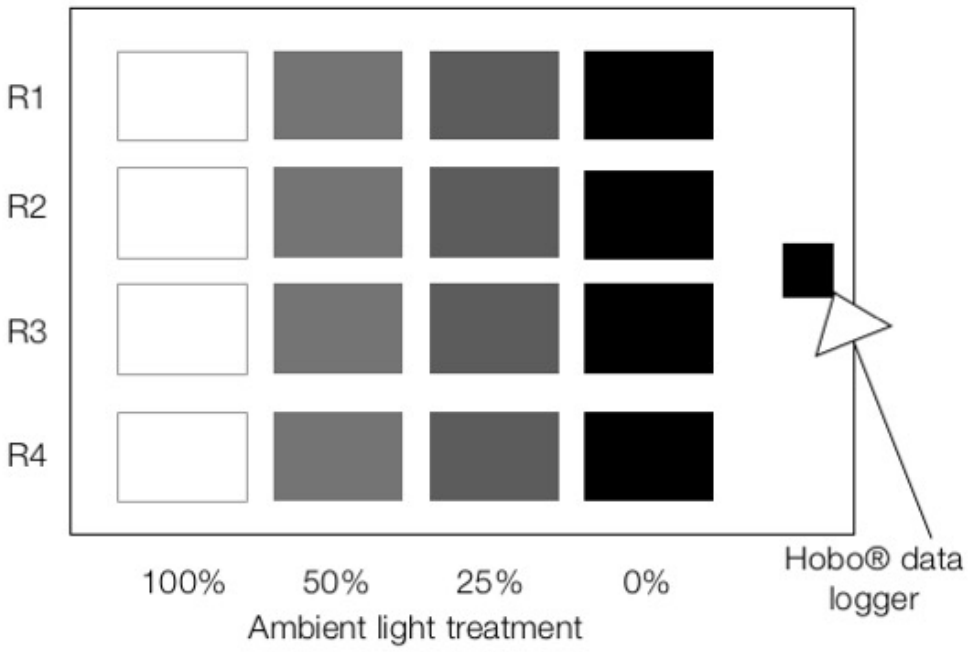

Figure 2.3 Example locations on plastic board (60 cm x 40 cm) for light manipulation slurry incubations. Each sediment core was divided into 4 sub-cores as per Figure 2.2. Light was controlled by using neutral density filter (25 % ambient light and 50 % ambient light) and aluminium foil (0 % light). A HOBO® light and temperature pendant was secured to a non-shaded area on the board to record conditions during the incubation period.

treatment levels following light manipulated incubation experiments at each of the different sites.

Diatom community composition was analysed using multiple methods. First, the trophic diatom index (TDI) was calculated for each of the replicates. Although the TDI is intended for use on rock scrap/hard substratum samples, the method was applied with the intention investigating its suitability on a range of substratum. Indicator species analysis (ISA) was also carried out for diatom community composition data. Inclusion of ISA allowed for a similar comparison to that generated by the TDI by using species presence/absence as indicators of conditions.

Species turnover allowed comparison of unique sites (at all sampling levels) to be compared seasonally. Species turnover was calculated by assessing the species present at time period 1 and time period at 2, the number of species gained and lost at each of the time periods, with the result presented as percentage change as shown presented in (Soininen & Eloranta, 2004).

Person's correlation analysis was carried out to investigate links between variables. Multiple, logical interactions were investigated to examine potential interactions during exploratory data analysis. Due to the nature of correlations and natural systems, these relationships where then investigated in more detail, to ascertain any responses in more confidence.

All graphic output was produced using the **ggplot2** package, with packages such as **grid** and **gridExtra** being used for layout purposes.

Table 2.2 List of R packages, examples of use case and supporting information used during data analysis presented throughout this thesis.

R package name	Purpose	Supporting information
compute.es	Linear modelling	(Re, 2013)
knitr	Compiling of document, data visualisation	(Xie, 2015)
Ggally	Data visualisation	(Schloerke et al., 2014)
ggplot2	Data visualisation	(Wickham, 2009)
gridExtra	Data visualisation	(Auguie, 2015)
Hmisc	Data visualisation	(Jr, with contributions from Charles Dupont, & many others., 2015)
indicspecies	Diatom community analysis	(De Cáceres, 2013)
lme4	Linear modelling	(Bates, Mächler, Bolker, & Walker, 2015)
lmerTest	Linear modelling	(Kuznetsova, Bruun Brockhoff, & Haubo Bojesen Christensen, 2016)
lubridate	Time series data management	(Grolemund & Wickham, 2011)
lsmeans	Linear modelling	(Lenth, 2016)
multcomp	Linear modelling	(Hothorn, Bretz, & Westfall, 2008)
multcompView	Linear modelling	(Graves, *et al*., 2015)
pastecs	Time series data analysis	(Grosjean & Ibanez, 2014)
plotrix	Data visualisation	(Lemon, 2006)
reshape	Data manipulation	(Wickham, 2007)
scales	Data visualisation	(Wickham, 2015)
vegan	Diatom community analysis	(Oksanen et al., 2015)

2.4.9 Data Deposition

In accordance with funding body requirements, raw data produced in this project was archived with a national data centre (NERC Environmental Information Data Centre, Centre for Ecology & Hydrology, (McKew, Leung, Underwood, Warren, & Whitby, 2016), doi:10.5285/976602b3-a58d-460c-a52d-088d0bb09989 & (Warren & Underwood, 2016), doi:10.5285/aec7f752-6be6-4626-bbdb-921bf8d7e3ce). These datasets are also presented in this thesis as Appendix II and Appendix III.

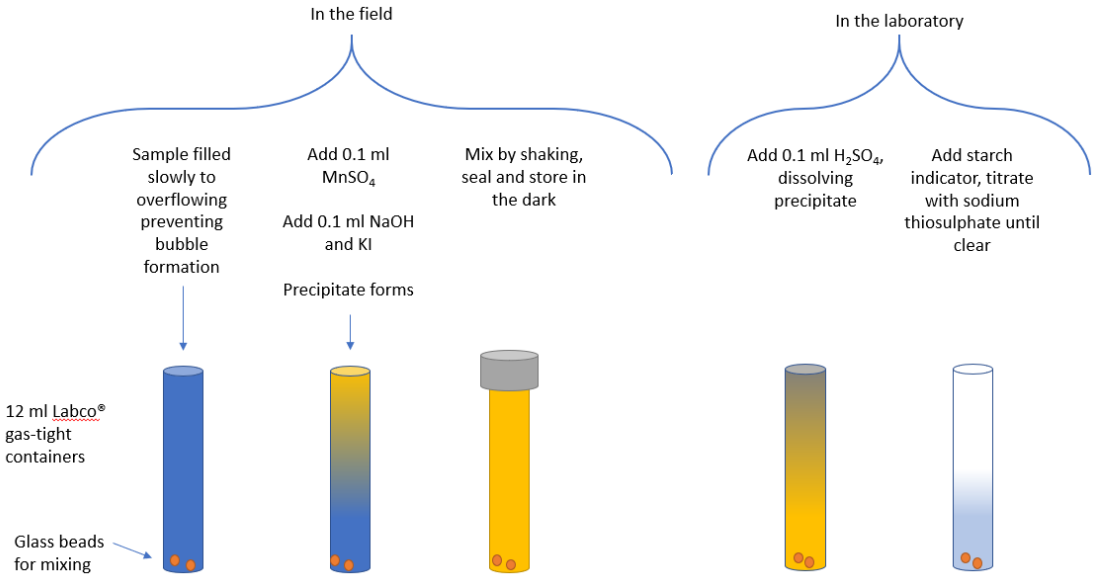

Figure 2.4. Schematic of Winkler titration, indicating chemical additions and visual representation of the solution at each stage of the procedure. Sample water is added to overflowing – while ensuring no bubble formation – to 12 ml Labco® gas-tight containers preloaded with 2 glass beads. 0.1 ml of $MnSO_4$ followed by 0.1 ml of a solution of NaOH and KI fix the oxygen in the same, creating a precipitate. This is then mixed and stored in the dark. On return to the laboratory, 0.1 ml of H_2SO_4 is added to dissolve the precipitate. Starch indicator is then added, and the sample is titrated with a known volume of sodium thiosulphate until clear.

3 MICROPHYTOBENTHIC COMMUNITIES ON CONTRASTING SOFT SEDIMENTS IN THE HAMPSHIRE AVON CATCHMENT, SOUTH ENGLAND

3.1 Introduction

Freshwaters make up a small amount of liquid water on earth, yet significantly contribute to the global biogeochemistry of nutrients such as carbon, nitrogen and phosphorus (Aufdenkampe *et al*., 2011; Battin *et al*., 2009; Luyssaert, *et al*., 2009; Cole *et al*., 2007). A river catchment - defined as the area in which water drains to the river channel from to a single outflow - can be largely heterogeneous at many different scales. Underlying geology can play a major role in determining characteristics such as hydrology within a catchment or sub-catchment, as bedrock and soil composition directly influence underground water flow. Underground flow, simplified by the base-flow index (BFI), is a long-term ratio of surface to underground flow of water as described in (Bloomfield *et al*., 2009). Much of the work on the heterogeneity of catchments has focused on land use, which while also controlling hydrology, are more likely to be point-sources of knowledge rather than reflecting the wider catchment area (Bechtold *et al*., 2012).

Catchment hydrology has been shown to impact many factors that control biological production in river systems. The path of water through a catchment can influence concentrations of dissolved and particulate materials transported into a water body, thus influencing input of dissolved nutrients and organic matter, and particulate organic matter. Work on an alpine stream system has suggested that the underground flow supports bacterial production in the sediment through delivery of materials such

as macronutrients, with the sediment itself acting to retain and accumulate nutrients (Battin, 2000).

In small rivers and streams, the microbiome is a major contributor to primary and secondary production. Microphytobenthic (MPB) communities - groups of algae, bacteria, fungi, and protists living within an extracellular polysaccharide (EPS) matrix - are able to grow without competition from higher plants and their associated fauna, as bank vegetation shades the channel bed. As such, higher plants are typically light limited, allowing the proliferation of MPB on a wide range of surfaces. There have been shown to be intrinsic links between the autotrophic and the heterotrophic components of MPB communities. Many studies have shown that there are positive correlations between algae and bacteria (Carr *et al*., 2005; Kalscheur *et al*., 2012; Rier & Stevenson, 2001, Rier & Stevenson, 2002; Scott *et al*., 2008), although some authors have questioned whether this is a trophic link (Scott *et al*., 2008) or simply heterotrophs exploiting autotrophic cells as a colonisation surface (Rier & Stevenson, 2002).

Diatoms - a major component of the MPB - are incredibly sensitive to environmental conditions due to their small size, surface-area to volume ratio and short generation time. It is because of this sensitivity that diatoms are used in multiple biological indices, one example being the trophic diatom index (TDI, Kelly, 2001). Like many of the diatom and alga-based indices, the TDI relies on the niche of a particular diatom species along the broadness of this niche - and in this example, this is primarily driven

by phosphorous concentrations. This allows the grading of rivers based on the biological reactions to nutrient conditions and is one of many vital tools used in monitoring water bodies according to legislative directives (e.g. the Water Framework Directive, European Parliament, 2000).

Because of the specific applications of metrics such as the TDI, they are often utilised incorrectly in ecological studies. For instance, the TDI utilises a reference community of MPB colonised on a solid surface (such as rocks and pebbles), excluding all other potential habitats within a stream or river on which MPB can be successful. Although this limitation can be understood in the case of the TDI due to its intended usage, many studies on the ecology and biogeochemistry of MPB also focus on the use of standardised - and sometimes artificial - substratum. Work by (Murdock & Dodds, 2007) examined many of the substratum used in studies of MPB under SEM, demonstrating the varied topography. It was found that glazed tiles - often used in experiments - had a very homogeneous topography, with peak vertical distance no larger than 50 µm. In comparison, house bricks showed a varied topography, with peaks of up to 400 µm (Murdock & Dodds, 2007). As most benthic diatoms range between 2 µm and 200 µm, elevations of 200 µm over a distance of <50 µm are likely to create microhabitats with differences in light levels, nutrient retention and nutrient replenishment. A large study carried out across the United States comparing microalgal communities growing on hard and soft substrata also found differences in the diversity and richness of diatoms, along with differences in the proportion of the

sample represented by diatoms, and the most common diatom species (Potapova & Charles, 2005). Using hard substrata to analyse predominantly soft sediment rivers also means that samples are not representative of the water body itself, while also excluding within-sediment processes which have been shown to have great importance. The wider community has therefore overlooked a more functionally and biologically diverse system in soft sediments (Cox, 1988), which in some rivers are the predominant bed form. Therefore, understanding how soft sediments select for diatoms and the wider MPB community. is important to understand. Soft sediments, which are heavily studied in estuarine systems, present a range of conditions which can be different to the standard 'rock scrape' method utilised in many freshwater studies. Interactions between surface and groundwater for example create the potential of delivery of nutrients and abiotic conditions that may not be present in the opposing environment. For example, due to rates of respiration exceeding primary productivity, groundwater can become anoxic. Through percolation from surface waters, anoxic groundwaters can be supplemented by oxygen, facilitating the development of oxic microsites in an otherwise anoxic environment. In a study of surface waters influencing benthic denitrification, the mass transport of chemicals was found to be influenced by water velocity (Arnon *et al*., 2007). In this study, the sediment pore water concentration of oxygen was shown to correlate with water velocity, although due to increased removal of other substrates denitrification peaked during intermediate flow. The sediment and hyporheic zone are also considered to be a source of other factors relevant to the current study such as allochthonous dissolved

organic carbon (DOC, Bengtsson *et al*., 2014), influencing transfer of chemicals, nutrients and metabolites which are already known to control microphytobenthic activity.

Our understanding of freshwater MPB communities is primarily driven through examining those living on hard surfaces. This study will expand our knowledge of soft sediment microphytobenthic communities across multiple examples of soft sediment found in the Hampshire Avon river catchment (HAC), assessing these communities at different spatial scales.

3.1.1 Aims, objectives and hypotheses

The aim of the study presented in this chapter is to describe physiochemical characteristics and benthic microbial communities in the Hampshire Avon catchment over a seasonal cycle spanning 1 year. To achieve this aim, the hydrological, hydrochemical and biological samples will be taken over a multitude of river reaches within the Hampshire Avon at multiple points throughout the year. Data collected will cover many aspects of the river benthos, including algal abundance, carbon concentrations (of multiple labilities and forms), and water chemistry of each of the catchments. By utilising data collected alongside this project – such as long term river flow and nutrient concentrations – these objectives are able to cover a large number of variables which are likely to influence microphytobenthic communities across different catchment geology types.

Specifically, I hypothesise that:

1. Clay river water will exhibit greater concentrations of dissolved macronutrients (C, N and P) than chalk rivers, with greensand river water showing intermediate concentrations.
2. River level response times to catchment rainfall will decrease within increasing base-flow (defined by BFI).
3. Algal biomass will be at its greatest during April sampling (spring in the northern hemisphere), while February sampling will show the lowest concentrations.
4. Phaeopigment concentrations will be greatest in chalk rivers due to high biomass and diversity of grazers.
5. Algal community composition will be linked to environmental conditions, with flashier rivers containing more 'attached' diatoms, and more stable rivers having an increased abundance of non-attached and stalk diatoms.

3.2 Methods

3.2.1 Site Selection

The Hampshire Avon catchment (HAC) covers around 1,750 km^2 in the south of England, and has a population of around 230,000 (Nadolski, 2012). The catchment is primarily composed of a chalk underlying geology, although significant other bedrock types are present. During the study period (2013-2015), the Hampshire Avon was included in the DEFRA Test Catchment (DTC) programme, and as such was highly

instrumented for water quality and river hydrology measurements. Work in the HAC was carried out in nine distinct sites within seven rivers, which were based on rivers draining from three catchment types; clay, greensand and chalk. These three sites represent a gradient of base flow conditions, ranging from low BFI (clay rivers) to high BFI (chalk rivers). In order to assess differences between rivers grouped within a geology type, triplicate rivers from each type were sampled (Table 2.1).

3.2.2 River characteristics

A total of 7 rivers were sampled within the Hampshire Avon (details in Chapter 2, Table 2.1), presenting a gradient of baseflow from relatively non-permeable clay to highly permeable chalk geology types. The River Sem was the sole river sampled for analysis of clay rivers. As no other clay rivers are found within the catchment, the River Sem was sampled at three distinct reaches in order to provide replication at multiple landscape scales. Greensand rivers were more abundant, with the River Nadder and the River Avon (east, sampled at two locations) being sampled. Three distinct rivers were also sampled to define Chalk rivers, with these being the River Ebble, River Wylye and River Avon (west, downstream of confluence with other tributaries).

Table 3.1 Trait classification and criteria for diatoms. Classifications are based on size, motility, attachment method (if any) and two classifications used for analysis in the Trophic Diatom Index (Kelly, 2001). Other trait classifications are adapted from Lange *et al.*(2015).

Attribute	Classification	Criteria
Cell size	Nano	5 ≤ 100 µm^2
	Micro	100 ≤ 300 µm^2
	Meso	300 ≤ 600 µm^2
	Macro	600 ≤ 1500 µm^2
	Large	> 1500 µm^2
Motility	Attached	Non-motile
	Constrained	Constrained motility within stalks/EPS channel
	Motile	Able to move through gliding
Attachment	N/A	Not attached to substratum
	Pad	Attached with a pad like appendage
	Stalk	Attached to/within a mucilage stalk
TDIs (Sensitivity)	1	Species favoured by very low nutrient concentrations
	2	Species favoured by low nutrient concentrations
	3	Species favoured by intermediate nutrient concentrations
	4	Species favoured by high nutrient concentrations
	5	Species favoured by very high nutrient concentrations
TDIv (Indicator)	1	Low environmental specificity
	2	Intermediate environmental specificity
	3	High environmental specificity

3.2.3 Water chemistry

Water samples (25 ml) were taken upstream of any activity, and before any sediment coring activity was carried out. Water chemistry was assessed using ion chromatography on a Dionex® ICS3000 (with UHP water and KOH eluent, and an AS18 column). Ions assessed were nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}).

Although the instrument used is used ubiquitously for water quality measurements, there are limits in detection. The manufacturer claims that for this specific instrument and column, there are detection limits for nitrate (0.5000 mg L^{-1}), nitrite (0.0125 mg L^{-1}) and phosphate (0.1500 mg L^{-1}). Where samples were below these detection levels, the instrument would record a value of 0 – however because of the limitations it is not possible to determine if concentrations are truly 0. Where this occurred, samples were given an arbitrary value of 50 % of the lowest recorded.

3.2.4 Primary sampling

Sediment cores (internal diameter = 8 cm, n = 5) were taken longitudinally within a river reach at 5 m intervals. Sediment was recovered and subsequently sub-cored using cut-off 20 ml syringes (internal diameter = 2 cm, n = 4) and samples were stored depending on the required analysis detailed in sections 3.2.5 - 3.2.10.

3.2.5 Chlorophyll-*a*

Sediment cores for chlorophyll-*a* and phaeopigment analyses were stored chilled (0-5 °c) until return to the laboratory. Upon return, samples were immediately frozen at 20 °c. After freezing, samples were freeze-dried, and homogenised using a pestle and

mortar. Pigments were extracted using the cold methanol method (Stal *et al*., 1984). A known amount (typically 500 mg) of sediment was added to 4 ml methanol, and samples were vortex mixed, then chilled in darkness at 4 °c for 24 hours. After the extraction period, samples were centrifuged at 2300 G for 15 minutes, with the supernatant measured spectrophotometrically at 665 nm and 750 nm. Samples were acidified with 1 M HCl and reanalysed at 665 nm and 750 nm for correction for phaeopigments (Equation 2.1 and Equation 2.2.).

3.2.6 Sediment carbon

Sediment total organic carbon (TOC) was analysed using a Shimazdu® TOCV with attached solid-state module (SSM). Sediment was frozen at -20 °c upon return to the laboratory, and later thawed at 60 °c. After thawing, around 500 mg of sample was loaded into pre-combusted SSM ceramic boats for organic carbon (OC) measurement, and incinerated at 900 °c. A further sample, also around 500 mg, was loaded into a separate pre-combusted SSM ceramic boat and acidified overnight with the addition of 1 ml of 2 M HCl. This was then incinerated at 900 °c. Samples were standardised using a 5-point calibration from 0-1000 mg TOC in the form of desiccated glucose, as per manufacturer recommendations.

3.2.7 Pore water Extracellular organic carbon (EOC), dissolved organic carbon (DOC), and carbohydrate fractions

Carbohydrates were analysed in sediment frozen upon return to the laboratory (-20 °c) which was later freeze-dried and homogenised using a pestle and mortar.

Carbohydrates and extracellular polymeric substances (EPS) were then extracted sequentially on the same sediment (around 300 mg). The method is based on that of (Hanlon et al., 2006), although this has been adapted in order to analyse coarser sediment (Section 2.4.3 for further details).

4 ml of UHP water (Milli-Q®) was added to around 300 mg of sediment in a centrifuge tube. Samples were vortex mixed, and incubated at 30 °c for 30 minutes. After the incubation period, samples were fractionated. DOC samples were decanted and frozen for later analysis using a Shimazdu® TOC-V liquid sample module. EPS was extracted by decanting samples with a final volume of 70 % EtOH, and as such all results presented represent EPS_{70}. EPS was precipitated chilled (4 °c) for 24 hours. After this precipitation period, samples were centrifuged at 4000 G for 30 minutes. EtOH was then decanted, and the samples dried before being reconstituted in UHP water. This sample was then used for measurement of EPS through the PSA assay (described below). CHOD was decanted directly into glass tubes for PSA analysis.

In order to extract hot-water soluble carbohydrates (CHO_{HW}), 5 ml of UHP water was added to the sediment used in the previous extractions of DOC, EPS and CHO_D. Samples were incubated at 100 °c for 1 hour, after which they were centrifuged at 4000 G for 30 minutes. Supernatant was then decanted into glass tubes for PSA analysis.

To quantify concentrations of EPS_{70}, CHO_D and CHO_{HW}, a phenol sulphuric acid (PSA) assay was carried out. 1 ml of 0.5 % v/v phenol was added to each sample, and to a

series of phenol-free blanks, 1 ml of UHP water. 5 ml of conc. H_2SO_4 was then added rapidly to both the samples and the blanks. After this, samples were left to cool at room temperature for 30 minutes, and then centrifuged at 4000 G for 30 minutes. Samples were then measured spectrophotometrically at 485 nm.

3.2.8 Diatom community analysis

A sediment core was fixed on site using 0.5 % glutaraldehyde. On return to the laboratory, samples were sonicated for 10 minutes (>20 kHz). After vortex mixing the samples at a slow speed for 10 seconds, 2 ml of the sample was extracted into a centrifuge tube using a wide mouth pipette (internal diameter 5 mm). After cleaning the samples in order to remove excess glutaraldehyde, organic material in the samples was then digested using 2 ml saturated $KMnO_4$. After a 24-hour period, concentrated HCl was added to the samples, and heated to 80 °c for 1 hour. Once cleaned, samples were mounted on glass slides using the diatom mountant Naphrax®. 300 frustules were counted following the methods of the Trophic Diatom Index (TDI; Kelly *et al*., 2008). Diatoms were identified using a combination of Hartley (1996), Kelly (2000) and Kelly *et al*., (2005). Diatom traits were classified according to Table 3.1. Species-specific information was gathered from multiple sources, namely Spaulding *et al*. (2010) and Kelly *et al*. (2005). Trait classes were adapted from those derived by (Lange *et al*. 2015).

In order to make the data comparable between sites, rare species - diatom taxa contributing less than 2.5 % of any one sample - were excluded. 2.5 % was chosen as a cut off as preliminary analysis using higher (5 %) and lower (1 %) exclusion points

returned too few and too many diatoms, respectively, to cover the spectrum of environments that were sampled, as some areas are predicted to have higher biodiversity than others. Indicator species analysis was carried out following methods suggested by Bakker (2008). Groups were selected based first on river geology type and sampling period, in order to determine changes in characteristic species across the year. In order to follow the nested design of the study, each site within a geology was also analysed with sampling period, to examine the relationship between individual sites.

3.2.9 Bacterial abundance

Samples for molecular analysis were immediately flash frozen in liquid nitrogen and stored in a cryogenic container before being stored at -80 °c on return to the laboratory. Bacterial abundance was measured using qPCR. The V3 and V4 regions of the 16S rRNA gene were targeted using the primers Bakt_341F and Bakt_850R, detailed in Chapter 2 (Herlemann *et al*., 2011). Sample concentration was measured using fluorescence from a multi-plate DNA quantification spectrophotometer.

3.2.10 Statistical analysis

All statistical analysis was carried out using the R language and environment for statistical computing (version 3.6.2 - "Dark and Stormy Night", R Development Core Team, 2011). A full list of R packages used, their function and citation are given in Chapter 2. Sampling date, river geology and individual river site comparisons were analysed using a nested ANOVA approach, with Tukey *post-hoc* analysis being carried

out to closer determine differences. Multiple analyses were carried out on diatom community data.

In order to streamline diatom community composition analysis, species which accounted for more than 2.5 % of any sample were retained for further analysis. Species turnover was calculated as per (Soininen & Eloranta, 2004), and given in equation 3.1. Specific packages utilised for the creation of figures and tables are listed in Chapter 2 (Table 2.2). Mean results are presented ± standard error.

Equation 3.1.

$$T = \frac{specG + specL}{taxaS1 + taxaS2} \times 100$$

Where: T = species turnover, *specG* = species gained (present at sampling period 2, not at sampling period 1), *specL* = species lost (present at sampling period 1, not at sampling period 2), *taxaS1* = number of taxa at sampling period 1, *taxaS2* = number of taxa at sampling period 2.

3.2.11 Background data

Background monitoring data were collected for some of the sites (AS1, AS3, and CE1) by the DEFRA test catchment (DTC) research program. Datasets for multiple variables were collected, summarised in Table 3.2. These datasets were accessed from http://www.environmentdata.org/ (last accessed 15/11/2016, with citations detailed in References).

Flow data was used to construct flow-duration curves (FDC) as a representation of river's response to rainfall. In order to do this, flow data was converted to percentiles, and plots were created to show the proportion of time at which each flow percentile is equalled or exceed.

Table 1.2. Datasets available online from the DEFRA test catchment (DTC) project conducted at the same time as data collected for this thesis. Table shows the availability of datasets which cover the sampling periods presented in this chapter.

		Site code			
Variable	Unit	AS1	AS3	CE1	CW2
Ammonium	mg L^{-1}	Yes	Yes	Yes	Yes
Nitrate	mg L^{-1}	-	-	-	Yes
Non-purgeable organic carbon (NPOC)	mg L^{-1}	Yes	Yes	Yes	Yes
pH	N/A	Yes	Yes	Yes	Yes
Total dissolved nitrogen (TDN)	mg L^{-1}	Yes	Yes	Yes	Yes
Total dissolved phosphorous (TDP)	mg L^{-1}	Yes	Yes	Yes	Yes
Total nitrogen (TN)	mg L^{-1}	Yes	Yes	Yes	Yes
Total phosphorous (TP)	mg L^{-1}	Yes	Yes	Yes	Yes

3.3 Results

Water chemistry

Point water samples were taken and analysed for nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}, Table 3.3). Nitrate (NO_2^-) concentrations ranged from 1.65 mg L^{-1} to 40.55 mg L^{-1}, at the clay river AS2 in August and at chalk river CW2 in April respectively. Nitrate was above instrument detection levels at all sites, and was recorded at lowest concentrations during August for all sites (clay, 3.88 ± 1.150 SE mg L^{-1}; greensand, 7.88 ± 0.741 SE mg L^{-1} and chalk, 14.09 ± 0.505 SE mg L^{-1}), while peak concentrations within catchment types were experienced at varied sampling periods (clay 12.58 ± 4.866 SE mg L^{-1} recorded April 2013; greensand 24.417 ± 1.486 SE mg L^{-1} recorded in February 2013 and chalk 39.82 ± 0.452 SE mg L^{-1} recorded in April 2013). At each individual sampling point, clay rivers had the lowest recorded concentrations of nitrate, while chalk rivers had the highest nitrate concentrations (Table 3.3).

ANOVA analysis revealed that overall nitrate concentrations were significantly different for both sampling period ($F_{3,23}$ = 23.53, p = < 0.001) and catchment geology ($F_{2,23}$ = 37.56, p = < 0.001). A significant difference in nitrate concentrations was also seen between individual geology types over the four sampling periods, although this was not as strong a difference as those seen for sampling period and catchment geology individually ($F_{3,\ 23}$ = 3.21, p = 0.020). Tukey *post-hoc* analysis revealed that nitrate concentrations were lowest in clay rivers in February and April (7.20 ± 2.343 mg L^{-1}, p = 0.002 and 12.58 ± 4.486 mg L^{-1}, p = 0.002 respectively). Greensand and chalk

rivers had similarly high mean nitrate concentrations in February, April and November (Table 3.3). Nitrate in chalk rivers in April was significantly higher than both greensand and clay rivers (p = < 0.001 for both comparisons). During the November sampling period, all three geology types had significantly similar nitrate concentrations. Throughout the whole sampling period, clay rivers exhibited similar nitrate concentrations. Both greensand and chalk rivers showed significantly higher concentrations of nitrate in February and April compared to August and November (p = < 0.05 for all comparisons).

Table 3.3 Point measurement concentrations of nitrate (mg L^{-1}), nitrite (mg L^{-1}) and phosphate (mg L^{-1}) of water samples measured on a Dionex® Ion Chromatogram. No samples were taken from GA3 in February due to site inaccessibility. Samples labelled with an asterisk were below detection levels of the given analysis and given a holding value of 50 % of the lowest recorded value.

Date	Geology	Site	Nitrate	Nitrite	Phosphate
February	Clay	AS1	2.58	0.01*	0.48
		AS2	8.80	0.01*	0.22
		AS3	10.21	0.01*	0.20
	Greensand	GN1	22.93	0.01*	0.41
		GA2	25.90	0.01*	0.45
		GA3	ND	ND	ND
	Chalk	CE1	36.13	0.01*	0.18
		CW2	29.80	0.19	0.18
		CA3	27.87	0.01*	0.69
April	Clay	AS1	5.83	0.32	0.03
		AS2	10.82	0.10	0.10
		AS3	21.07	0.03	0.06
	Greensand	GN1	8.93	0.06	0.10
		GA2	31.93	0.03	0.32
		GA3	31.21	0.04	0.23
	Chalk	CE1	39.31	0.04	0.06
		CW2	40.55	0.01*	0.11
		CA3	38.99	0.11	0.22
August	Clay	AS1	5.47	0.02	0.02*
		AS2	1.65	0.03	0.02*
		AS3	4.53	0.02	0.02*
	Greensand	GN1	9.35	0.03	0.02*
		GA2	6.97	0.04	0.02*
		GA3	7.33	0.01	0.02*
	Chalk	CE1	14.48	0.06	0.22
		CW2	13.08	0.03	0.02*
		CA3	14.69	0.17	0.02*
November	Clay	AS1	8.33	0.19	0.02*
		AS2	7.33	0.04	0.02*
		AS3	4.85	0.01	0.02*
	Greensand	GN1	9.97	0.03	0.02*
		GA2	10.30	0.06	0.02*
		GA3	9.94	0.04	0.02*
	Chalk	CE1	16.09	0.03	0.02*
		CW2	14.50	0.05	0.02*
		CA3	14.80	0.10	0.02*

Nitrite (NO_2^-) concentrations were recorded below detection level at all sites within a geology type on two occasions, while concentrations overall only increased above a mean of 0.1 mg L^{-1} on a single occasion. Below detection level (BDL) concentrations were recorded in clay and greensand rivers in February (Table 3.3). Conversely, the highest concentration of NO_2^- were recorded in clay rivers in April (0.14 ± 0.088 SE mg L^{-1}) – 68 % higher concentration than found at the next highest nitrite recording – chalk rivers in August (0.09 ± 0.042 SE mg L^{-1}). In clay and greensand rivers, peak nitrite concentrations were reached in April (0.14 ± 0.088 SE mg L^{-1} and 0.05 ± 0.009 SE mg L^{-1} respectively), while in Chalk rivers nitrate was relatively consistent over the all sampling periods, with the highest concentrations found in August (0.09 ± 0.417 SE mg L^{-1}), and the lowest concentrations found in April (0.05 ± 0.032 SE mg L^{-1}), representing a 58.5 % difference of nitrite concentrations between the extreme conditions. Due to concentrations being below detection level in clay and greensand rivers, such a comparison is not possible, as the data does may or may not represent true null values.

Overall nitrite concentrations were not statistically different between sampling points, geology type or between sampling periods within each geology type. Tukey *post-hoc* analysis also revealed that at the majority of sampling dates, all three geology types had similar concentrations of nitrite – one exception to be noted is during April 2013 sampling (p = 0.0153, Tukey). Within geology types, there were no differences between nitrite concentrations at each of the sampling points. Statistical analysis of nitrite data were limited due to many recordings being below instrument detection

level, with holding values for BDL recordings not providing true concentrations (Table 3.3).

As with nitrite, phosphate was also found to be in low concentrations with some samples, and many concentrations were below instrument detection level (marked in Table 3.4 with asterisks). Seasonal patterns were similar across all geology types, with highest phosphate levels found in February 2013 for clay (0.29 ± 0.091 SE mg L^{-1}, greensand (0.43 ± 0.022 SE mg L^{-1}) and chalk rivers (0.35 ± 0.171 SE mg L^{-1}). August and November recorded the lowest concentrations of phosphate in all geology types, being below detection level for all but chalk rivers in August (0.07 ± 0.071 SE mg L^{-1}, with a single recording at CE1, Table 3.3).

Phosphate concentrations across all sites were significantly different between sampling date ($F_{3, 23}$ = 16.641, p = > 0.001), although these results do include holding results for a high number of samples which recorded as BDL (Table 3.3). During February 2013 sampling, within each sampling period there were significant differences in phosphate concentration between all of the geology types (Tukey, p = > 0.001 for each geology type). In April, clay and chalk rivers had similar phosphate concentrations, with greensand rivers exhibiting a significantly higher concentration of phosphate (Tukey, p = > 0.01).

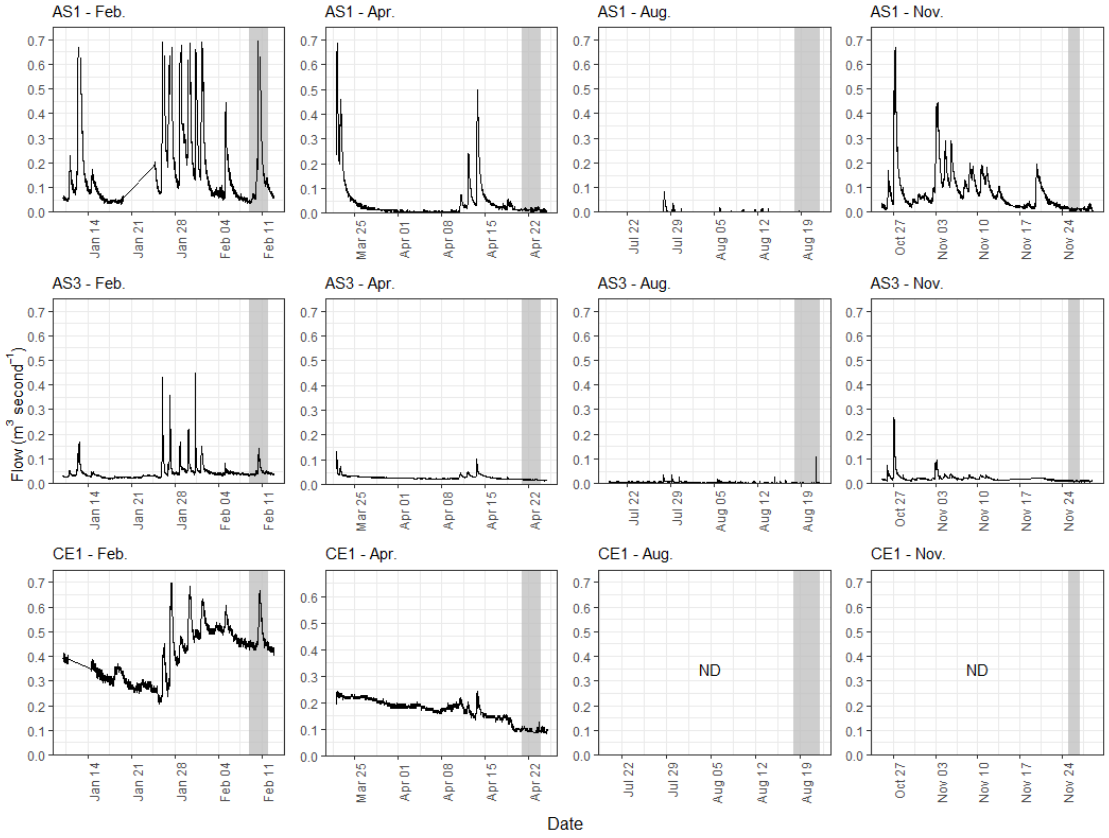

Figure 3.1. High resolution (every 15 minutes) flow rates gathered from DTC data for each of the sampling periods at sites AS1 (top row), AS3 (middle row) and CE1 (bottom row). The grey shaded area on each plot denotes the sampling period for original data presented throughout this thesis.

DTC continuous monitoring data

As continuous monitoring data were collected as part of an external project, the rivers spanned, and the periods covered do not completely coincide with other measurements presented in this chapter. However, data are presented where there are overlaps of sampling period (Figure 3.1). Notably, the two clay rivers (AS1 and AS3) experienced the sharpest increases in flow rate, particularly in January/February. Similar permutations in the river flow rate can be seen at similar dates for each of the rivers. During February, the chalk river CE1 did experience a high flow period which occurred at the same time as peaks in flow for AS1 and AS3 – however there was no return to previous flow rates recorded before the sharp increase in flow rates (Figure 3.1).

To compare flashiness – or rainfall response - of a river, flow duration curves were created. Flow duration curves (Figure 3.2) present the percentage of time that flow equalled or exceeded an indicated flow level. Both clay rivers (AS1 and AS3) exhibit low flow rates for a large proportion of the time. For instance, AS3 in February exceeds a flow rate of 0.10 m3 s^{-1} only 4 % of the time. However, within these lowest 4 percentiles, the rate of flow increases dramatically (~ 500 % to the highest recorded flow of 0.6 m^3 s^{-1}). A flow of 0.20 m^3 s^{-1} is only equalled or exceeded 1 % of the time (Figure 3.2).

Similar results are seen throughout the data for AS1 and AS3. CE1 flow duration curves in February and April (ND for August and November) show that high flows are more

common. For instance, the low flow measured for CE1 in April is 0.08 $m^3 s^{-1}$, and this increases by only 0.1 $m^3 s^{-1}$ for 50 % of the time to a flow of 0.18 $m^3 s^{-1}$. This more gradual change is seen at both sampling periods. These results cannot be tested statistically due to the nature of the data - particularly regarding serial correlation (Gordon, 2004) - but clearly show differences in flashiness of the rivers in response to rainfall in the catchment over the gradient of base-flow index.

As with flow data collected by the DTC project, accompanying nutrient data were also patchy over sites and periods covered. Recorded values of NPOC, TN and TP - where available (Figure 3.3) - show that nutrient concentrations track the permutations in river flow rates shown in Figure 3.1. This can be best seen at AS1 in the period before April sampling, where an increase in NPOC, TN and to an extent TP are shown around 06/04/2013. A similar increase in NPOC and TN are also shown at the same sampling period in CE1, although the increase is slightly later, peaking around a week later (15/04/2013). Nutrient concentrations recorded by the DTC project (Figure 3.3) were consistently lower than those recorded in point measurements (Table 3.1), although the methods and nitrogen states being sampled were different between the two data sets.

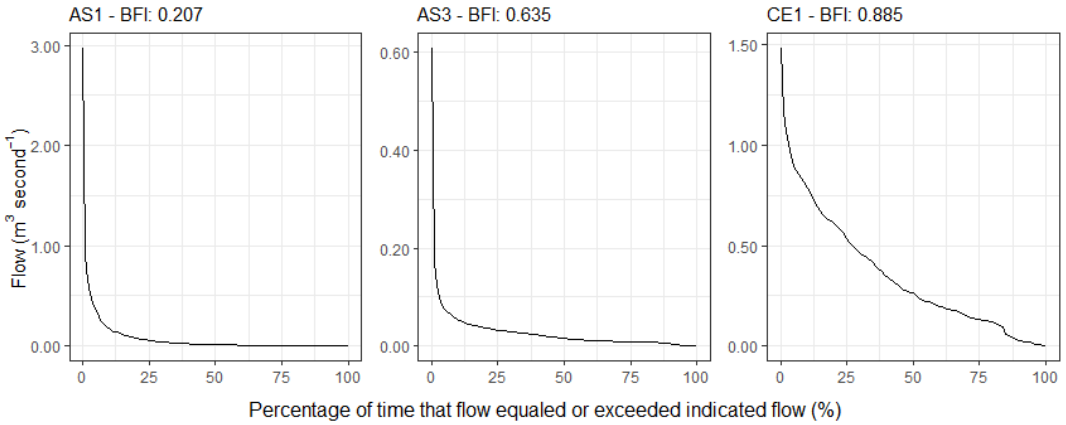

Figure 3.2 Flow duration curves for AS1, AS3 and CE1. Data shown is based on the percentiles of the flow data for all available data, and the flow rate that is equalled or exceeded. Due to differences of peak flow rates at each site, each Y axis is individual to each plot. BFI indicates measured base-flow index at each site.

Algal biomass and phaeopigments

Chlorophyll-*a* was used as a proxy for algal biomass (Figure 3.4). There was a wide range of chlorophyll-*a* concentrations across the sites, geology types and seasons. Figure 3.4 shows chlorophyll-*a* concentrations grouped by both geology type and by individual sites. Overall, individual chlorophyll-*a* concentrations ranged from 0.09 µg chlorophyll-*a* g^{-1} sed. DW (AS1, February) to 29.86 µg chlorophyll-*a* g^{-1} sed. DW (CE1, November).

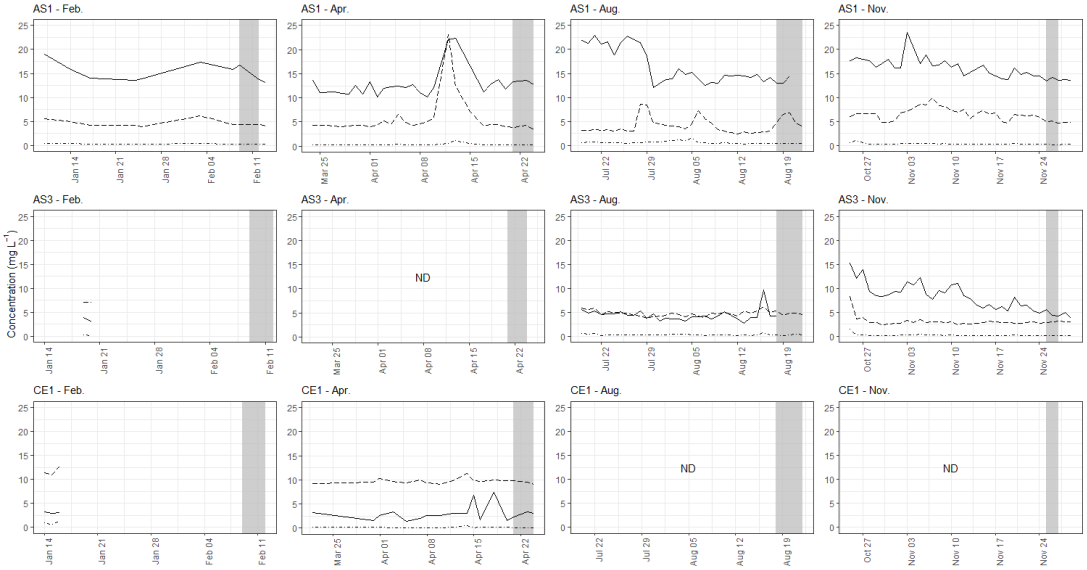

Figure 2.3 Concentrations of major nutrients (NPOC, TN, TP) gathered from DTC data for each of the sampling periods at sites AS1 (top row), AS3 (middle row), and CE1 (bottom row). Solid lines denote NPOC, dashed lines TN and dot-dashed lines TP. Resolution of samples is every 2 days. The grey shaded area on each plot denotes the sampling period for original data presented throughout this chapter.

Chlorophyll-*a* concentrations when grouped by geology type showed a smaller range (Figure 3.4b), although some common themes are shared when looking at individual sites. For instance, chalk rivers had consistently high chlorophyll-*a* abundance – ranging from 6.31 ± 0.638 µg g^{-1} sed. DW in February to 20.65 ± 2.178 µg g^{-1} sed. DW in November.

Both greensand and chalk rivers showed similar seasonality with chlorophyll-*a* abundance (Figure 3.4), with peak concentrations recorded in April and November. Clay rivers did not follow this pattern of highs in April and November, instead showing a peak of chlorophyll-*a* abundance in August (7.84 ± 1.702 µg g^{-1} sed. DW).

February 2013 chlorophyll-*a* concentrations found within each sample site were found to be statistically similar to that of others within a geology group (Figure 3.4). However - and for chalk rivers in particular - during the later sampling periods of April, August and November 2013 there are significant differences within each geology level.

Overall ANOVA results showed that chlorophyll-*a* concentrations differed between sampling date ($F_{3, 129}$ = 9.20, p = < 0.001), catchment geology type ($F_{2, 129}$ = 50.19, p = < 0.001), catchment geology type crossed with sampling date ($F_{6, 129}$ = 3.62, p = 0.002) and at individual sites nested within catchment geology types crossed with sampling date ($F_{23, 129}$ = 8.81, p = < 0.001, Figure 3.4). A gradual increase in chlorophyll-*a* was seen in clay rivers from the February sampling period throughout the year, with significantly higher chlorophyll-*a* being found in the later sampling periods of August (p = 0.047 compared with February) and November (p = < 0.001 compared with

February). A development of this increase is shown in April, although biomass was not significantly different from any of the other time periods.

Greensand rivers had only two statistically distinct groups, where chlorophyll-*a* was found to be more abundant in April than in February (p = < 0.001) and August (p = 0.013). At each of the sampling periods, chlorophyll-*a* was significantly higher in chalk rivers than in greensand rivers (p = < 0.001 for all periods). Similar differences were found between chalk and clay rivers, with the exception of August, where chlorophyll-*a* concentrations were similar. During this sampling period, mean algal biomass was 7.84 ± 1.702 µg Chl-*a* g^{-1} sed. DW and 7.81 ± 1.664 µg Chl-*a* g^{-1} sed. DW for clay and chalk rivers respectively. Concentrations of chlorophyll-*a* were similar between clay and greensand rivers at all sampling points except for August (Figure 3.4).

Sediment in the clay rivers (AS1, AS2 and AS3) exhibited relatively similar chlorophyll-*a* concentrations throughout the different sampling periods (Figure 3.4). The only sampling period that there were significant differences between chlorophyll-*a* in clay rivers was August, where significantly higher concentrations were found in AS1 compared to AS2 and AS3 (p = <0.001 for both sites). Greensand rivers were also relatively homogeneous in chlorophyll-*a* concentration. As in clay rivers, only a single significant difference was found; GN1 having higher recorded levels of algal biomass than GA2 during August (p = 0.015). Chlorophyll-*a* concentrations in GA3 were similar to both of the other greensand sites during this time period. Chalk rivers show within-geology differences during the April, August and November sampling periods. During

April and November, CE1 had significantly higher chlorophyll-*a* than both CW2 and CA3 (p = < 0.001 for both rivers and both periods), however during August, chlorophyll-*a* concentrations in CE1 were significantly lower than both CW2 and CA3 (p = < 0.001). AS1 and AS3 also show differences in seasonality, as chlorophyll-*a* concentrations are similar between the two sites with the exception of the August sampling point. Here, there is a sharp increase in biomass at AS1, with this river showing some of the highest biomass measured in this period (15.71 ± 2.11 µg Chl-*a* g^{-1} sed. DW).

Phaeopigments - a group of pigments indicative of chlorophyll-*a* degradation - were typically recorded at a higher concentration than chlorophyll-*a* concentrations (Figure 3.5). Mean concentrations at each geology type, particularly in chalk rivers in the later sampling periods of August 2013 and November 2013, were at the highest concentrations, recording 18.18 ± 5.99 and 20.81 ± 5.91 µg phaeopigments g^{-1} sed. DW. respectively. In clay and greensand rivers, the increase in phaeopigment concentration was also seen in August and November; rising from < 2.5 µg g^{-1} sed. DW at both geology types to 9.51 ± 1.782 (November) and 7.21 ± 1.289 (August) µg g^{-1} sed. DW respectively (Figure 3.5).

Overall, phaeopigment concentrations were shown to be significantly different between sampling date ($F_{3, 130}$ = 19.72, p = < 0.001) and sub-catchment geology ($F_{2, 130}$ = 17.22, p = < 0.001). There was also a significant difference measured within geology types at the different sampling points ($F_{6, 130}$ = 2.61, p = 0.020), although the difference was not as clear as the overall models. Phaeopigment concentrations at individual

sites were also found to be significantly different at between sampling periods ($F_{23, 130}$ = 5.69, p = < 0.001), with August and November having higher concentrations than February and April (Figure 3.5).

Sediment carbon dynamics

Total organic carbon (TOC) showed a large range of results over the different geology types and sampling periods (Figure 3.6), ranging from 7.35 ± 0.807 mg TOC g^{-1} sed. DW (greensand rivers, November) to 57.23 ± 11.488 mg TOC g^{-1} sed. DW (clay rivers in August). In clay and greensand rivers, August samples contained the highest concentration of TOC, whereas in chalk rivers, samples taken in April had the highest concentration (10.93 ± 2.451 mg TOC g^{-1} sed. DW).

Total organic carbon (TOC) differed significantly by sampling date ($F_{3, 117}$ = 6.40, p = < 0.001), geology type ($F_{2, 117}$ = 49.60, p = < 0.001), within geology types at different sampling dates ($F_{5, 117}$ = 2.87, p = 0.018) and between individual rivers nested in catchment geology types at different sampling dates ($F_{20, 117}$ = 7.10, p = < 0.001).

Total organic carbon (TOC) was found to be highest in clay rivers throughout the year when compared by broad geology type and by individual sites (Figure 3.6, p = < 0.01 for all time periods). Seasonally, there were little differences between TOC measured at each of the individual geology types, with clay rivers always having the highest concentrations of sediment TOC, and greensand and chalk rivers being similarly low throughout the year, with the exception of August, when TOC in greensand was significantly higher than in clay (p = 0.002). Within one site (GA3) there was a large

variation in TOC concentration during August, with a mean value of 31.84 ± 20.52 mg TOC g^{-1} sed. DW - the mean TOC being significantly higher than that found in the other greensand sites GN1 and GA2 (p = < 0.001).

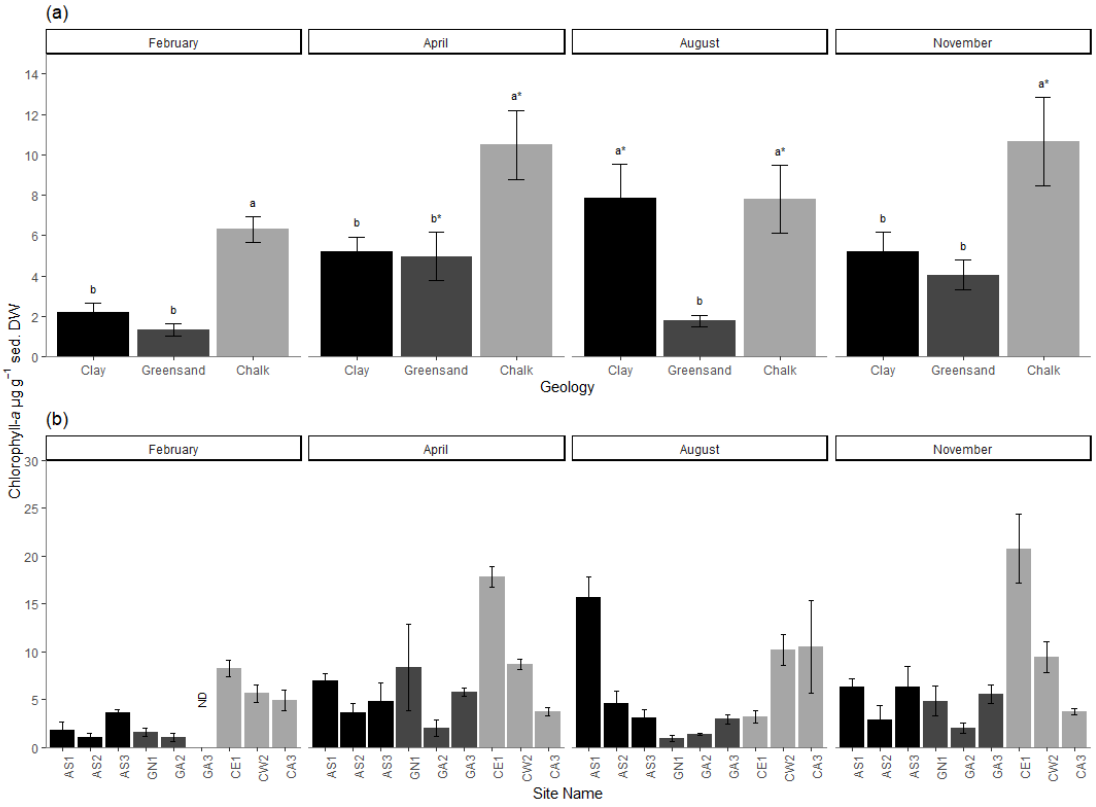

Figure 3.4 Mean (±SE) chlorophyll-*a* concentrations grouped by (a) river sub-catchment geology, and (b) river site. Letters shown above geology level bars represent significant grouping based on least-mean squares (Tukey) *post-hoc* analysis. Those followed by an asterisk denote geology types where significant differences of chlorophyll-*a* were found at different within-group river sites. ND indicates that no data were collected.

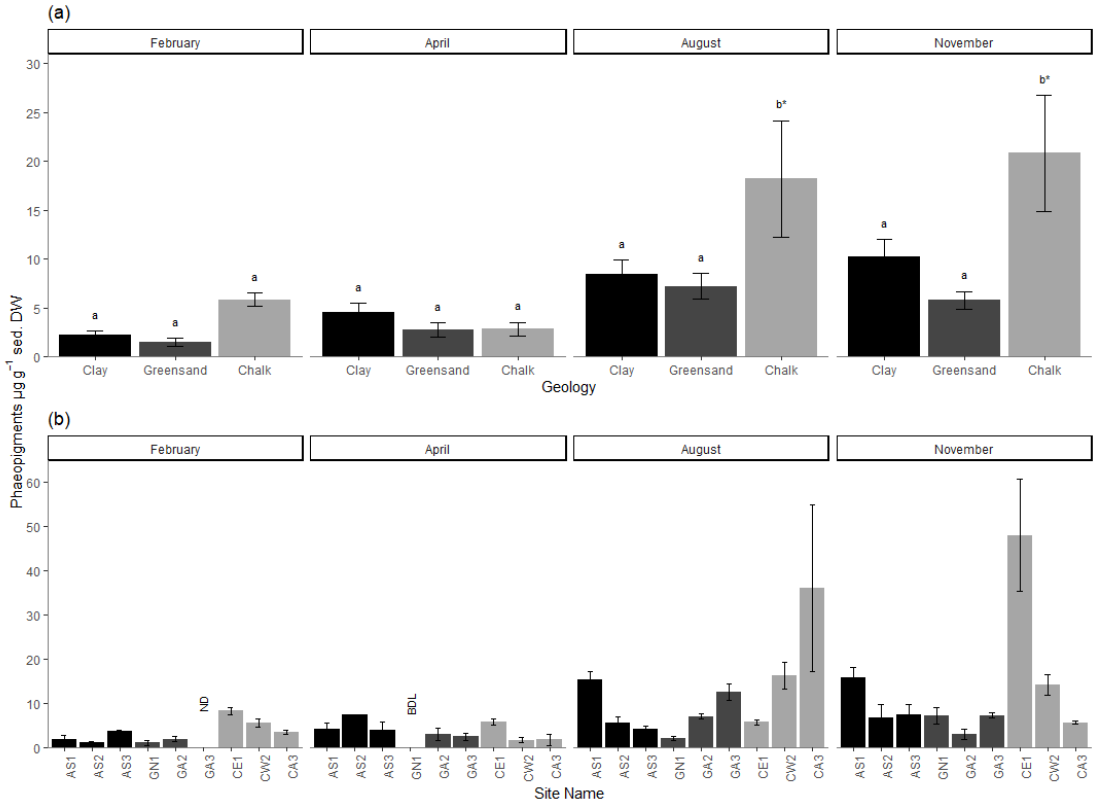

Figure 3.4 Mean (±SE) Phaeopigment concentrations grouped by (a) river sub-catchment geology, and (b) river site. Letters shown above geology level bars represent significant grouping based on least-mean squares (Tukey) *post-hoc* analysis. Those followed by an asterisk denote geology types where significant differences of phaeopigment concentrations were found at different within-group river sites. ND indicates that no data were collected.

Carbohydrates and pore water DOC

Dissolved carbohydrates (CHO_D) concentrations did not show great differences between sampling period within each geology (Figure 3.7), with the range of concentrations in greensand ranging from 29.56 ± 8.826 µg g^{-1} sed. DW (February) to 48.78 ± 9.429 µg g^{-1} sed. DW (August), while the largest range was found in clay rivers – between 115.60 ± 18.652 µg g^{-1} sed. DW (February) to 203.04 ± 42.453 µg g^{-1} sed. DW (April). There was no pattern of maximum and minimum measured CHO_D between the geologies, and each recorded greatest and smallest concentrations at different sampling periods (Figure 3.7).

CHO_D concentrations were significantly different during each sampling period ($F_{3, 128}$ = 2.81, p = 0.042), catchment geology type ($F_{2, 128}$ = 44.84, p = < 0.001) and between sites nested within geology types over the sampling period ($F_{22, 128}$ = 3.02, p = < 0.001, Figure 3.7). There was no significant difference overall in CHO_D in sampling period at each of the catchment geology types ($F_{6, 128}$ = 2.01, p = 0.069). During all sampling periods, clay rivers had significantly more CHO_D than greensand and chalk rivers (p = < 0.005 for all comparisons). Variation within catchment geology types was widespread in clay rivers, occurring in February, April and August. February and April sampling showed that AS1 had significantly higher CHO_D than AS2 (p = 0.006), although neither site was statistically different from AS3. In August, CHO_D concentrations in AS1 were significantly higher than both AS2 and AS3 (p = < 0.001). Greensand rivers all exhibited

similar CHOD concentrations at each sampling point, while CE1 and CW2 were found to be significantly different in November (p = 0.027).

Hot-water extracted carbohydrates (CHO_{HW}) showed a similar trend to concentrations of CHO_D – for instance, there were a small range of results within geology types (greensand ranged from 29.56 ± 8.826 µg g^{-1} sed. DW in February to 43.43 ± 9.515 µg g^{-1} sed. DW in August), and there were no consistent seasonal responses between the different sub-catchments (Figure 3.8). Over the majority of the dataset, concentrations of CHO_{HW} were slightly lower than those recorded for CHOD, with chalk rivers being the exception over most of the year. During February, August and November sampling periods in chalk rivers, CHO_{HW} was at a higher concentration than CHO_D (see Figures 3.7 and 3.8), the greatest difference being seen in August, where concentrations of CHO_{HW} reached 57.45 ± 25.452 µg g^{-1} sed. DW compared to CHO_D concentrations of 34.25 ± 3.599 µg g^{-1} sed. DW, although variability within these two samples was greatly reduced in CHO_D compared to CHO_{HW}.

CHO_{HW} were significantly different between sampling periods ($F_{3, 114}$ = 3.54, p = < 0.017), sub-catchment geology ($F_{2, 114}$ = 41.42, p = < 0.001), and at different sites nested within geology ($F_{20, 114}$ = 2.90, p = < 0.001, Figure 3.8). No significant overall effect was found within geology types at different sampling periods.

CHO_{HW} was significantly higher in clay rivers in April compared to greensand and chalk rivers (p = < 0.001 for both comparisons). No other comparisons of geology type at each sampling period showed any significant differences. Results for each river within

a geology type showed that there were relatively few differences, and where differences were found, they occurred in clay rivers. In February, AS1 was found to be significantly higher than AS2 (p = 0.049), while AS3 showed similar concentrations of CHO_{HW} to AS1 and AS2. Differences between clay rivers were also found in August and November, with AS1 once again having significantly higher concentrations of CHO_{HW} than AS2, and AS3 (p = < 0.02 for all comparisons).

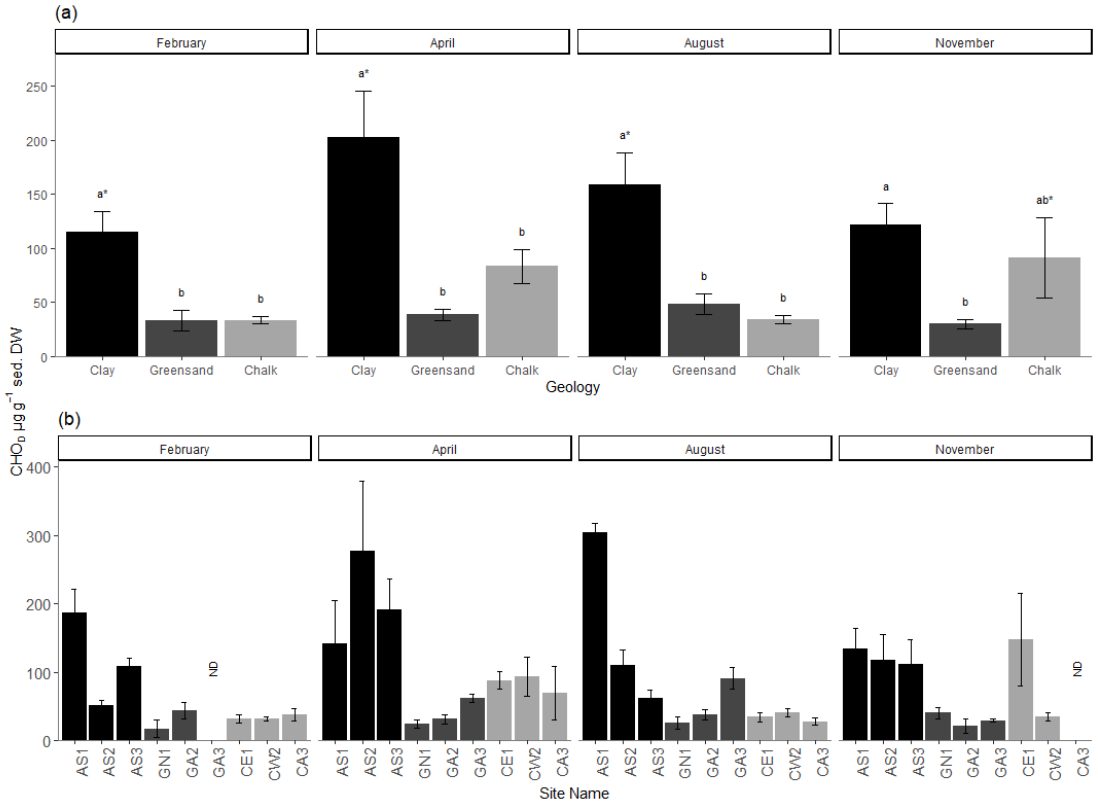

Figure 3.7. Mean (± SE) dissolved carbohydrates (CHO_D) grouped by (a) river sub-catchment and (b) river site. Letters shown above geology level bars represent significant groupings based on least-mean squares (Tukey) *post-hoc* analysis. Those followed by an asterisk denote geology types where significant differences of CHO_D were found at different within-group river sites. ND indicates that no data were collected.

EPS_{70} was recorded in lower concentrations than other carbohydrate fractions (Figure 3.9), ranging from 11.74 ± 4.047 µg g^{-1} sed. DW (August, greensand) to 46.31 ± 9.418 µg g^{-1} sed. DW (April, clay). Throughout the sampling period, there were minimal changes in greensand rivers, results showing that there were only a 31 % increase in mean concentrations between the smallest and greatest points (February and April), whereas in clay rivers the percentage difference between maximum and minimum EPS_{70} concentrations was ~ 49 % between February and November. As with CHO_{HW}, EPS extracted with 70 % ethanol (EPS_{70}) in the overall model was found to be different between sampling period ($F_{3, 84}$ = 3.86, p = 0.012), catchment geology ($F_{2, 84}$ = 12.91, p = < 0.001) and between individual sites within geology type over the sampling period ($F_{15, 84}$ = 3.81, p = < 0.001). Similarly to CHO_{HW} concentrations (Figure 3.8), when comparing EPS_{70} concentrations between geology types at each sampling period, there were few differences (Figure 3.9). The only significant result was found in April, where clay rivers had significantly higher EPS_{70} compared to greensand and chalk rivers (p = ≤ 0.01). Missing data in August and November does not allow us to see if the pattern in the later periods for EPS_{70} also matches that found in CHO_{HW}. Individual rivers within each geology type were largely similar, with differences only being seen in Chalk rivers in February ($CW2_{CH}$ significantly higher than CA3, p = 0.046), clay rivers in April (AS3 significantly higher than both AS1 and AS2, p = ≤ 0.02) and again in clay rivers during August (AS1 significantly higher than AS2 and AS3, p < 0.001). No data were collected for chalk rivers due to laboratory processing issues.

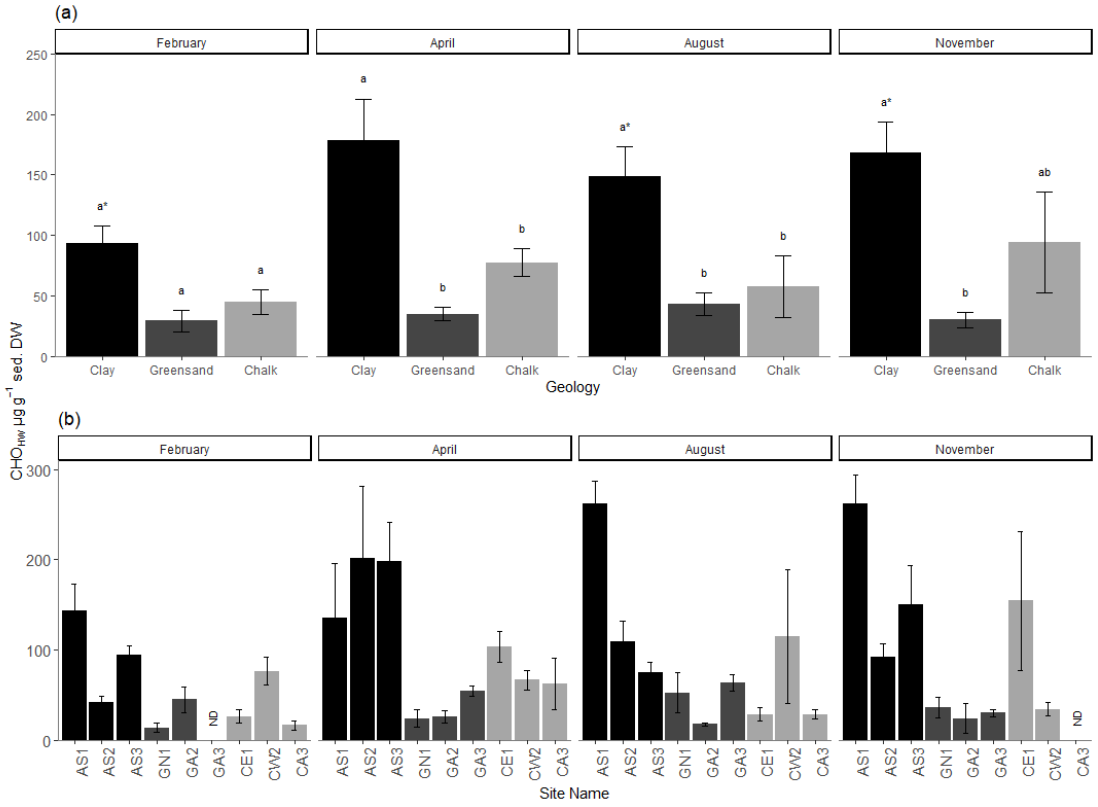

Figure 3.8. Mean (± SE) hot water extracted carbohydrates (CHO_{HW}) grouped by (a) river sub-catchment geology, and (b) river site. Letters shown above geology level bars represent significant groupings base on least-mean squares (Tukey) *post-hoc* analysis. Those followed by an asterisk denote geology types where significant differences of CHO_{HW} were found at different within-group river sites. ND indicates that no data were collected.

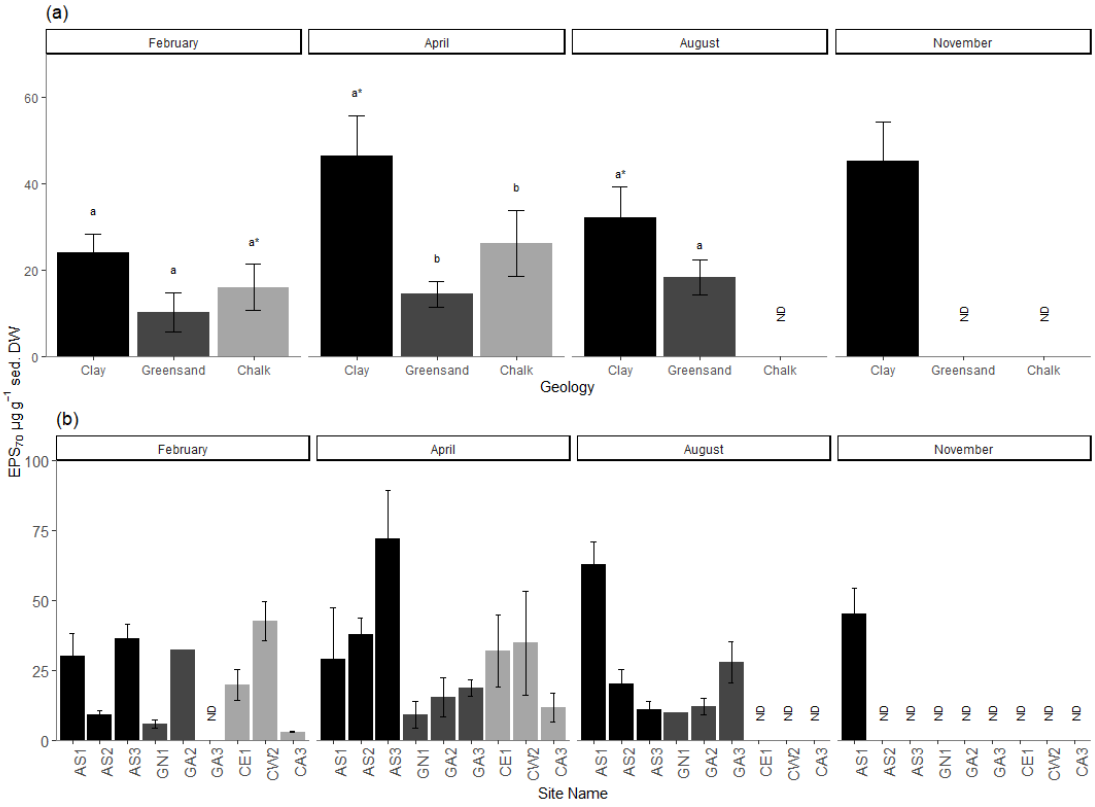

Figure 3.9. Mean (± SE) EPS_{70} grouped by (a) river sub-catchment geology, and (b) river site. Letters shown above geology level bars represent significant groupings based on least-mean squares (Tukey) *post-hoc* analysis. Those followed by an asterisk denote geology types where significant differences of EPS_{70} were found at different within-group river sites. ND indicates that no data were collected.

Carbohydrate fractions (CHO_D, CHO_{HW}, EPS_{70}) were compared with sediment DOC concentrations (Figure 3.10) and inorganic nutrients (nitrate, nitrite and phosphate, Figure 3.11). All three fractions of carbohydrates were shown to be positively correlated with sediment DOC concentrations (Pearson's correlation, p = 0.001 for all comparisons), with r^2 values ranging from 0.325 (EPS_{70}) to 0.808 (CHO_{HW}). When compared with nitrate, CHO_D and CHO_{HW} were both found to have a negative relationship (p = 0.009 and 0.008 respectively). No interaction was found between nitrate and EPS_{70} (Figure 3.11). When compared against nitrite concentrations, only CHO_{HW} showed a (negative) correlation (p = 0.044). While significant, the regression fit was low, with an r^2 of 0.025. Neither CHO_D nor EPS_{70} showed any significant trend.

Phosphate concentrations were the only inorganic nutrient to show a relationship with EPS_{70} concentrations. Negative correlations were found between phosphate concentrations and both EPS_{70} and CHO_{HW} concentrations (p = < 0.01 for both comparisons, Figure 3.11).

Diatom community composition

In total 220 species were recorded over during the study (Appendix 1), encompassing 46 genera. Following initial processing, taxa which were not found represent over 2.5 % of any sample were removed, resulting in 58 species that were deemed to be sufficiently common amongst the samples for comparison. Remaining diatoms proportionally represented between 87.71 to 100.00 % of individual samples, with a mean representation of 94.96 ± 0.32 % across the whole dataset, and as such still

accounted for a large proportion of diatoms recorded. The species which were kept belonged to 27 genera, with the majority of species belonging to the Genus *Navicula*. Other high-abundance genera included *Achnanthes*, *Cocconeis*, *Nitzschia*, *Planothidium*, *Staurosirella* and *Surirella* – a full list of taxa used in the analysis, and raw counts, are available in Appendix 1.

Indicator species analysis (ISA) was carried out at multiple levels, following the nested experimental design used throughout this Chapter (Table 3.4). Groupings were manually assigned according to geology and sampling date. As the most common diatoms were used for analysis, 58 taxa were included in the analysis. Using geology as the sole grouping variable (excluding individual sites and sampling periods), 24 taxa were significant indicators of geology types (Table 3.4). However, only 7 of these were unique to a single geology type, whereas the remaining 17 were shared across two groups. Clay and greensand rivers shared a total of 4 species, while greensand and chalk rivers shared the highest number of indicating taxa at 13 (Table 3.4).

Greensand rivers had no significant indicator species, while clay and chalk rivers identified 6 and 1 indicator taxa respectively. A list of taxa which are in each of these groups is shown in Table 3.4.

Differences in sampling periods were described within each geology level, resulting in 3 separate analyses (clay, greensand and chalk). Clay rivers showed differences in significant indicator species throughout the range of sampling periods. Of the 10 species shown to be significant indicators, no species was indicative of February or

November alone, while 1 species (*Navicula reichardtiana*) was found to significantly indicate the April sampling period (p = 0.004). August had 3 significant species (*Gyrosigma attentuatum*, *Navicula capitata* & *Staurosirella pinnata*). None of these species were found to be significant in the overall analysis (Table 3.4). The only species found to be a significant indicator within the clay rivers which was also found in the overall analysis was *Gyrosigma acuminatum*, which represented April and August sampling periods in clay rivers (p = 0.016), and Clay and greensand rivers overall (p = 0.019). For greensand rivers, 20 species were found to have significant indicator values. In total, seven species showed a strong indicator value for February, April and August sampling periods. Once again, none of the taxa with high indicator values from this group matched those representing the geology throughout the whole year. Two common taxa were shared between the whole year analysis (Table 3.4); *Achnanthes conspicua* - indicator of greensand rivers in February April, and *Navicula atomus* - a significant indicator for greensand in April and August. No significant indicators were found for November in greensand rivers. Chalk rivers had no significant indicators - all diatom taxa input into the analysis were not significant indicators of any sampling period.

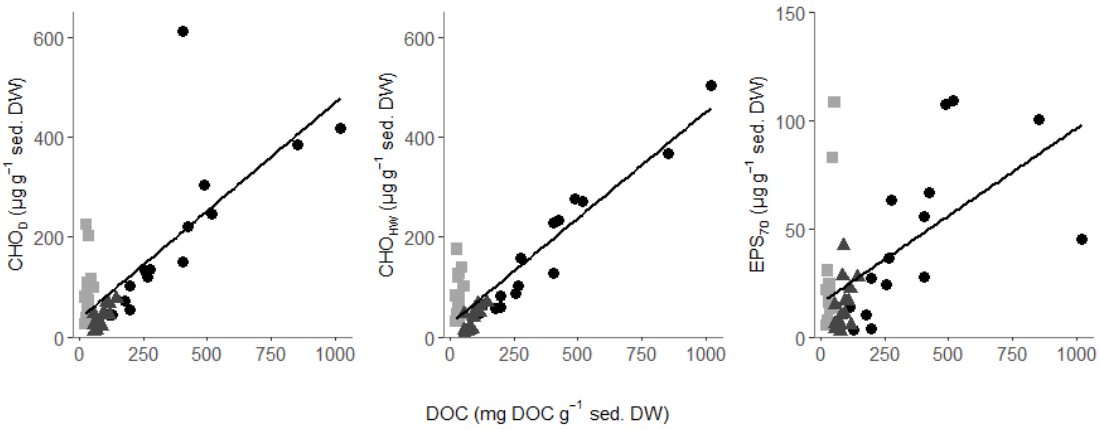

Figure 3.10. CHO_D, CHO_{HW} and EPS_{70} concentrations plotted against DOC concentration results from sediment pore water. Note changes in Y axis scale. Circles represent clay rivers, triangles greensand rivers and squares chalk rivers.

Within-geology indicator taxa were also compared to follow the nested design of this study. In this case, the 3 rivers which were sampled within the geology type were used as a grouping factor, along with date. Similar to the geology level analysis, chalk rivers showed no indicator species for any of the sampling periods. In the three clay rivers (AS1, AS2 and AS3), no species was identified as an indicator species across all sampling periods. As with the other analyses, there were few shared indicator taxa with the overall dataset. *Navicula lanceolata* is a significant indicator of AS3 in April and August (p = 0.036), as well as clay and greensand rivers across the whole year. Other notable results include *Cocconeis pediculus* being significant in AS3 in April and August (p = 0.044), while also representing greensand and chalk rivers in the whole dataset analysis. Greensand rivers GN1 and GA2 had a low number of significant indicators (4 and 3 respectively). GN1 included *Surirella brebissonii* as an indicator species for February and April (p = 0.038) which also was a significant indicator for clay rivers in the whole year analysis (Table 3.4). *Staurosirella leptostauron*, a species found to represent greensand rivers in the whole year analysis, was also present as an indicator for GN1 during August and November (p = 0.046). Of the identified indicator species for GA2, none were also found in the whole year analysis. Significant indicators were only found for February (*Gyrosigma sp.*, p = 0.032) and August (*Gyrosigma attentuatum*, p = 0.042 & *Navicula angusta*, p = .0.042). In GA3, there were more indicator species (11), of which many related to species found to be indicator across the whole year analysis.

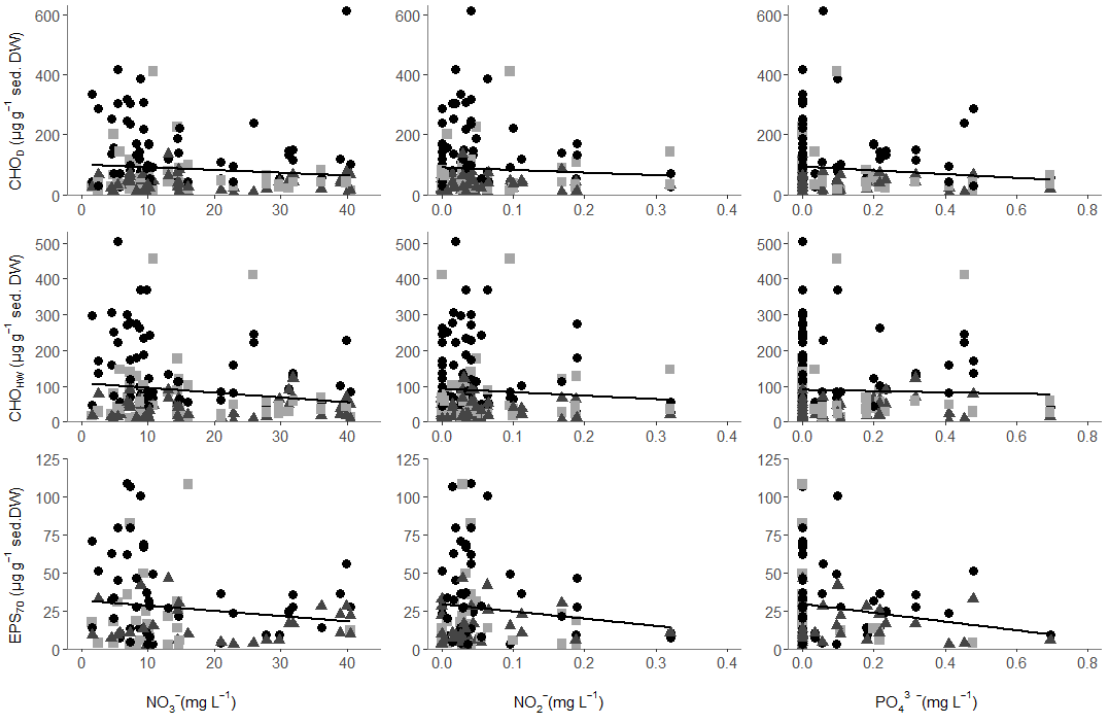

Figure 3.11. CHO_D, CHO_{HW} and EPS_{70} concentrations plotted against nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) concentrations in water. Circles represent clay rivers, triangles greensand rivers and squares chalk rivers.

Species turnover (Table 3.5) ranged from 28.79 % (AS1, February to April) to 58.54 % (GA2, April to August). Where species turnover values were available for between multiple sampling periods, the greatest change in all sites except CE1 and CW2 was found between April and August. At CE1 and CW2, the largest species turnovers were February to April (32.33 %) and August to November (50.54 %) respectively. Species turnover between month was patchy for some sites (Table 3.5). Where turnover was assessed between all sampling periods, the largest change was seen in GA2, which had 45-50 % species change (Table 3.5). The chalk river CE1 showed low species turnover between February and April and between April to August, with 26.05 % and 33.33 % change respectfully. However, between August to November, CE1 saw a turnover of 50.54 %, showing a large increase in turnover during the later part of the year. Overall, more change was seen between April to August, where turnover values ranged from 31.75 % to 58.54 % (which was also the highest value recorded), whereas values between February and April ranged from 26.05 to 38.32 %. Diatom size class traits were compared against chlorophyll-*a* concentrations measured from matched sediment cores (Figure 3.12). Diatoms belonging to the 'nano' size class (Table 3.1) were found to be highest in abundance at lower chlorophyll-*a* concentrations (Pearson's correlation, $p = 0.001$), while relative abundance of 'meso' diatoms showed a positive correlation to chlorophyll-*a* concentrations ($p = 0.008$). Fit for this correlation however was low, with an r^2 value of 0.093.

Table 3.4. List of diatom taxa which are significant indicators for each geology type across the whole year. Asterisks denote level significant calculated by indicator species analysis (ISA, *p* value, *** = 0.001, ** = 0.01, * 0.05). Subscripted numbers indicate each species TDI_v classification, as described in Table 3.1. Taxonomic authorities are given in the Appendix. No species were deemed to be indicators of greensand rivers alone.

Clay rivers	Clay and greensand rivers	Greensand and chalk rivers	Chalk rivers
Diploneis oblongella$_1$**	*Gyrosigma acuminatum*$_2$**	*Achnanthes conspicua*$_2$*	*Cocconeis placentula* var. *euglypta*$_1$*
Frustulia vulgaris$_2$***	*Navicula lanceolata*$_2$**	*Cocconeis pediculus*$_2$**	
Navicula gregaria$_1$***	*Navicula molesiformis*$_1$***	*Diatoma vulgare*$_3$*	
Navicula telloides$_3$**	*Navicual slevicensis*$_1$*	*Kolbesia kolbei*$_2$*	
Pinnularia appendiculata$_3$*		*Navicula atomus*$_1$*	
Surirella brebissonii$_1$***		*Pseudostaurosira brevistriata*$_2$**	
		Staurosirella lapponica$_1$**	
		Staurosirella leptostauron$_1$**	
		Staurosirella pinnata$_1$**	
		Staurosira construens$_1$**	
		Staurosira elliptica$_1$**	
		Synedra ulna$_1$**	

Diatom attachment method (not attached, with a mucilage pad or with a mucilage stalk) was compared with base-flow index (BFI, Figure 3.13). Significant correlations were seen between pad attached diatoms and increasing BFI (Pearson's correlation, $p = 0.001$), while no other attachment method was significantly correlated with BFI.

Bacterial abundance

Throughout the whole sampling period, 16S copy abundance was highest in clay rivers, while chalk and clay rivers had the lowest concentrations at different sampling points (Figure 3 .16). Highest abundance of 16S was recorded in clay rivers in August ($7.29 \times 10^9 \pm 5.53 \times 10^8$) while the lowest abundance was seen in greensand rivers in February ($1.20 \times 10^9 \pm 6.91 \times 10^7$). Variability of samples was high across the dataset, as seen on the error bars in Figure 3.16. This was expected when grouped at the geology level, due to the variation seen in individual rivers.

Overall ANOVA results showed that 16S copy abundance (Figure 3.16) was different between sampling period ($F_{3, 71} = 3.98$, $p = 0.011$), catchment geology type ($F_{2, 71} = 16.78$, $p = < 0.001$), between individual geology types at different sampling periods ($F_{6, 71} = 2.28$, $p = 0.045$) and at sites within geology types at each date ($F_{23,71} = 3.16$, $p = < 0.001$). Tukey *post-hoc* analysis revealed that during the February, April and August sampling periods, clay rivers exhibited significantly higher 16S copy numbers in comparison to greensand and chalk rivers. In April, chalk rivers were not significantly different to either clay or greensand rivers, and results in November showed that there were no differences between 16S abundance.

Table 3.5 Species turnover at each site between the sampling months of February to April, April to August, and August to November. Species turnover (percentage change of species between sampling periods) was calculated according to methods used in Soininen and Eloranta (2004). Entries without figures could not be calculated due to an incomplete dataset.

Site	February to April	April to August	August to November
AS1	28.79	39.09	-
AS2	-	-	-
AS3	-	34.31	52.00
GN1	38.32	-	-
GA2/A	43.94	51.09	46.48
GA3	37.93	58.54	-
CE1	26.05	33.33	50.54
CW2/A	32.33	31.75	-
CA3	-	51.19	-

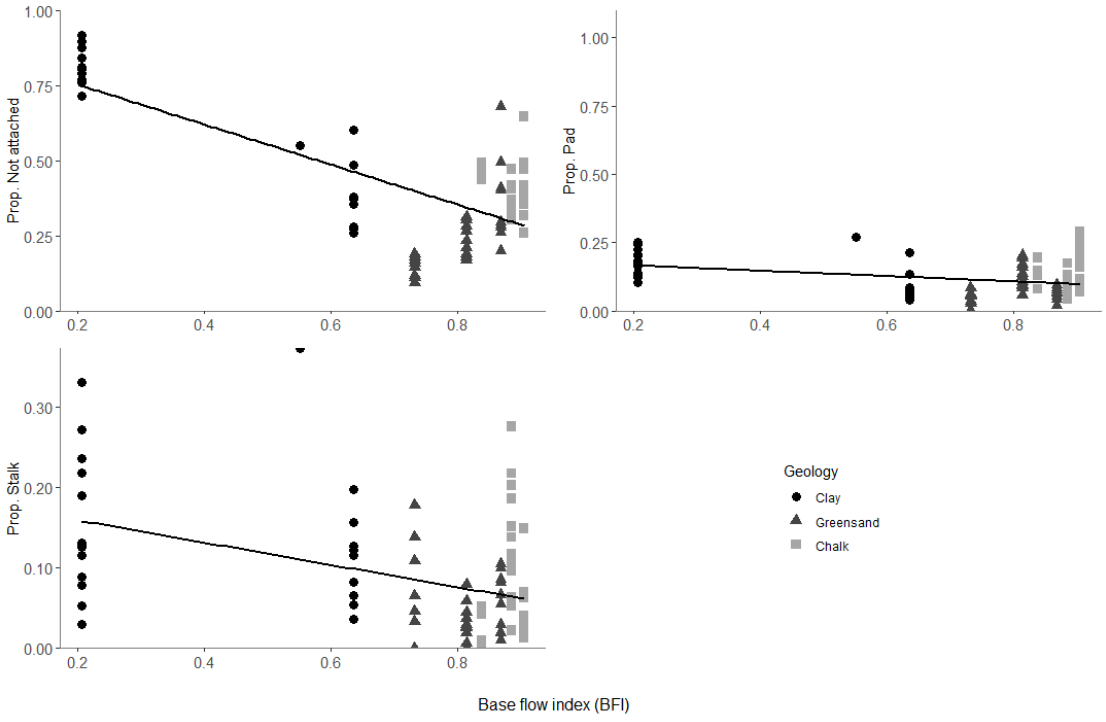

Figure 3.133. Base flow index (BFI) plotted against the proportion of each diatom attachment class (not attached, mucilage pad or mucilage stalk) found within a sample.

The only sampling period where there were significant differences between all of the groups was August, where chalk rivers had significantly lower concentrations of 16S than both greensand (p = 0.003) and clay rivers (p = < 0.001).

16S copy numbers were mostly consistent within the geology types at each sampling period, with the exception of August (Figure 3.16). Here, one site from clay rivers (AS1, p = < 0.007) and one site from greensand rivers (GA3, p = < 0.001) were found to have higher 16S abundance than the other rivers in their respective geology type. At all other sampling periods, and at all other river geology types, there were no significant differences between individual rivers.

3.4 Discussion

3.4.1 Water chemistry and hydrology

Concentrations of the macronutrients nitrate, nitrite and phosphate obtained through point measurements were found to be different across sampling period and geology type. Consistently throughout the analysis, clay rivers were lower in nitrate concentration compared to greensand and chalk rivers, which were similar at all sampling periods with the exception of August. Low concentrations of nitrite and phosphate resulted in only a small insight into the dynamics of these nutrients across the year and geology, but where data were available only phosphate was deemed to change between sampling periods. Neither of these two ions were found to be

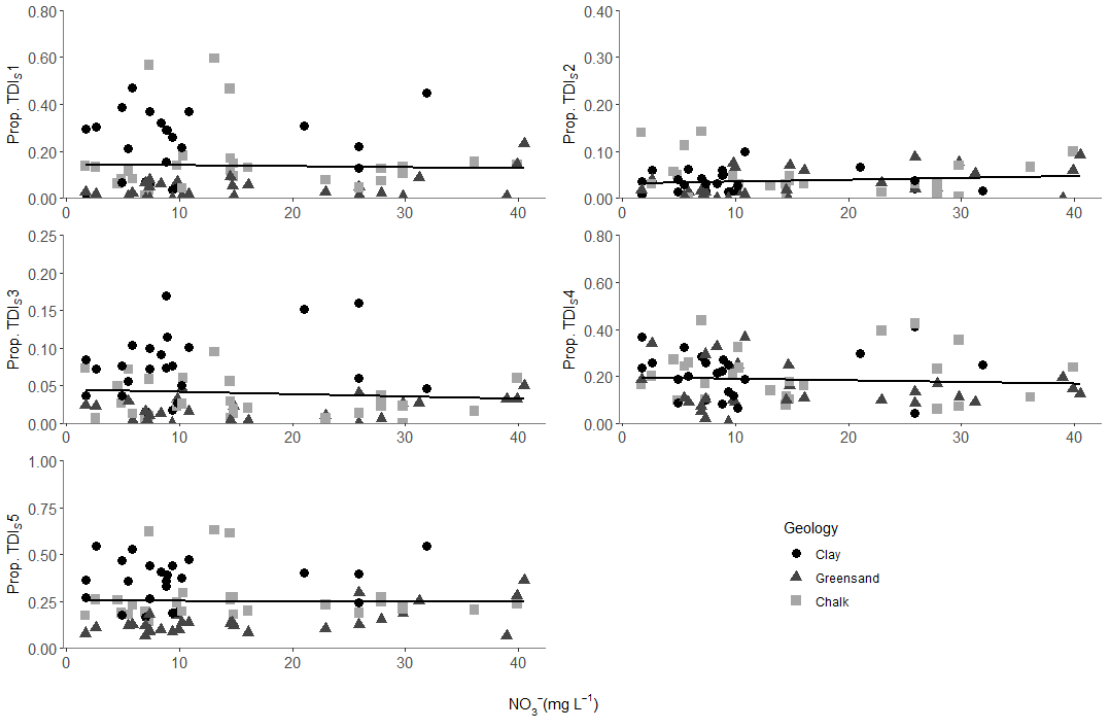

Figure 3.14. Proportional abundance of diatoms bellowing to TDI_S groups 1-5 plotted against nitrate concentrations. *p* values quoted refer to Pearson's correlation coefficient.

different between geology types, with *post-hoc* analysis revealing that phosphate increased significantly for all sites in the later half of the year (August and November). Although nitrite concentrations were not statistically different, it is important to note the relationship between nitrite concentrations and river flow; for instance, where river flow was highest in February clay rivers, nitrite levels were below the instrument's detection level. However, when flow was low in April, clay rivers recorded the highest concentration of nitrite over the whole dataset. The Hampshire Avon Catchment has been subject to many studies in hydrology and nutrient dynamics - most notably (Jarvie *et al*., 2005) - who were able to show influence of catchment geology on nutrient conditions. The annual mean concentrations of N and P presented by Jarvie *et al*., (2005), when compared with the results of this study, show that nutrient concentrations are similar or lower than expected for most of the year, with exception to results obtained in February 2013, which were found to be similar in some rivers but much higher than expected in others.

Results suggested that base-flow was not correlated with increases of nutrients above expected means in February, and instead it can be suggested that local catchment conditions at (and upstream of) the sampling sites are likely to be contributing to these results. Another likely reason for a particular site - GN1 - exhibiting much higher concentrations of P than expected could be due to increased P concentrations in groundwater from greensand catchments, as reported by (Jarvie *et al*., 2005). Interestingly, two chalk rivers in February also showed higher than expected

concentrations of P (CE1 and CW2). Continuous chemistry data obtained from the DTC data (Hants Avon DTC consortium, *2016a,e,f,h*) were typically lower than that measured in the point measurements, which concentrations of nitrate peaking at 25 mg L^{-1}, compared to highs of > 50 mg L^{-1} being measured at the point samples. While the DTC dataset does not account for all of the sites profiled in the point measurements, there is still a discrepancy between data collected by DTC autosamplers and point measurements recorded during the present study when matching sites are compared, with point measurements being around 15 mg L^{-1} higher than that of the continuous monitoring data. Due to the nature of the continuous data collection, there is a potential that biological activity had reduced nutrient concentrations between sampling periods, which could account for this difference. Point measurements were immediately passed through a 0.2 µm filter, which would have effectively removed suspended microbes from the sample, minimising the biological activity. When comparing the DTC results with those of (Jarvie *et al*., 2005), DTC samples also show lower concentrations of N compounds and P, supporting this hypothesis.

As expected, clay rivers were showed a flashier response to chalk rivers. Due to missing data from the DTC project, no greensand rivers were sampled at a similar time to the rest of this study. Flow duration curves revealed that for the majority of clay rivers sampled, there was a sharp increase in flow during the lower 10 percentiles. In contrast, the data available for the chalk river CW2 showed that there was an almost

linear flow duration curve, where around 50 % of the flow was reached 50 % of the time. These results support given base-flow index figures for each of the environments that flow data was obtained for regarding sub-catchment hydrology, and show that river responses to rainfall in a relatively small area can be vastly different.

There were matched permutations in nutrient concentrations and flow rates measured by the DTC project (Figures 3.1, 3.2, 3.3). This is particularly clear in AS1 in April, where sharp increases in flow rate and nutrient conditions occurs around April 08, persisting to around April 15. Results such as these offer insights into terrestrial-aquatic linkages, and supports work which suggests a large proportion of organic and inorganic nutrient enrichment can be terrestrially derived (Jarvie *et al.*, 2005). Interestingly, nutrient concentrations did not experience fluctuations to the same extend in the chalk river CW2. Levels of TN from the DTC data, along with high levels of NO3 - measured during the point measurements suggest that influences from groundwater are likely to sustain high concentrations of N. Jarvie *et al*. (2005), who measured concentrations of N in the groundwater of the Hampshire Avon catchment, suggest that the long residence time of this aquifer, along with high nutrient inputs from agricultural activity result in groundwater being a significant input of N to surface waters. Given the differences in BFI shown via the flow duration curves, this is likely to have more an impact in chalk rivers than clay rivers, which consistently had low concentrations of both NO_3^- in point measurements and TN in DTC continuous data, supporting the hypothesis that BFI will have an impact on surface water chemistry.

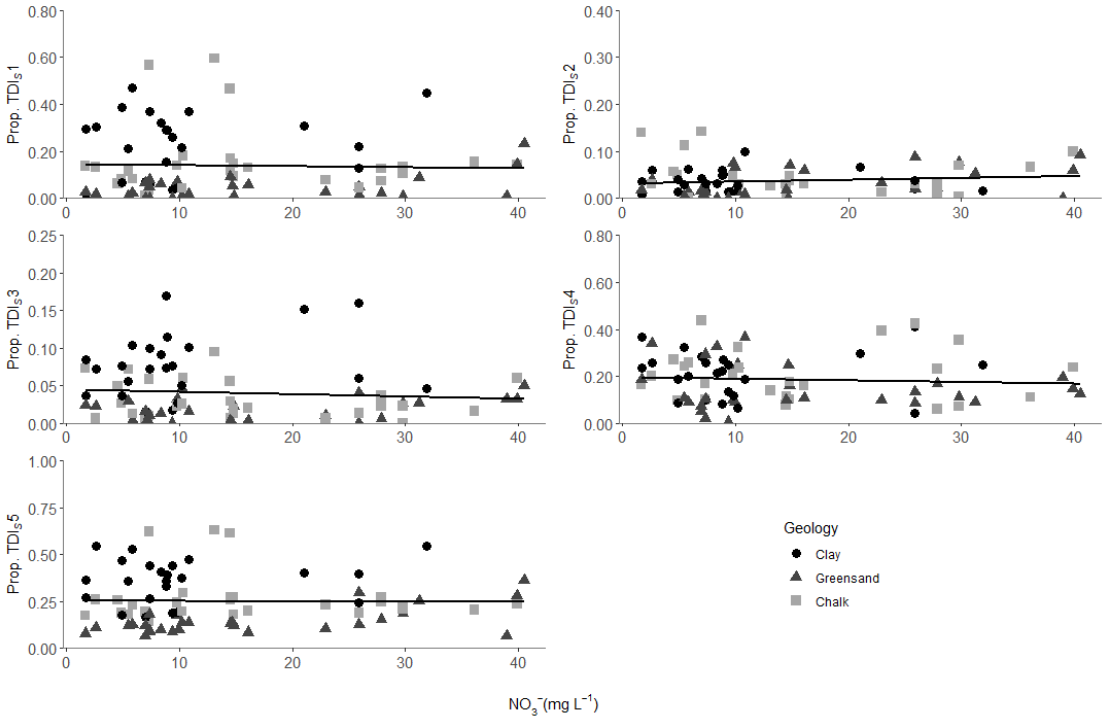

Figure 3.15. Proportional abundance of diatoms bellowing to TDI_S groups 1-5 plotted against nitrate concentrations.

3.4.2 Chlorophyll-*a*

Algal biomass, which was estimated through chlorophyll-*a* analysis, largely followed hypothesised patterns. Characteristically clear water chalk streams were always highest in biomass, and apart from the August sampling period chalk streams were significantly higher than both clay and greensand rivers. Results also showed that chlorophyll-*a* was lowest in clay chalk streams during February, which is to be expected given the low quality and quantity of solar radiation during the northern hemisphere's winter. Peaks in chalk and greensand rivers were found during the April and November sampling period, with a light but significant decrease in biomass being recorded in August. This corresponds with summer in the northern hemisphere, and as such it can be speculated that this is driven by competition with higher plants such as macrophytes and riparian vegetation. The increase in biomass in naturally turbid clay rivers at this time suggests that although competition is occurring between the MPB community and macrophytes for light, the light limitation driven by water turbidity is likely to limit growth at a time where MPB can benefit from lower competition with macrophytes and higher plants.

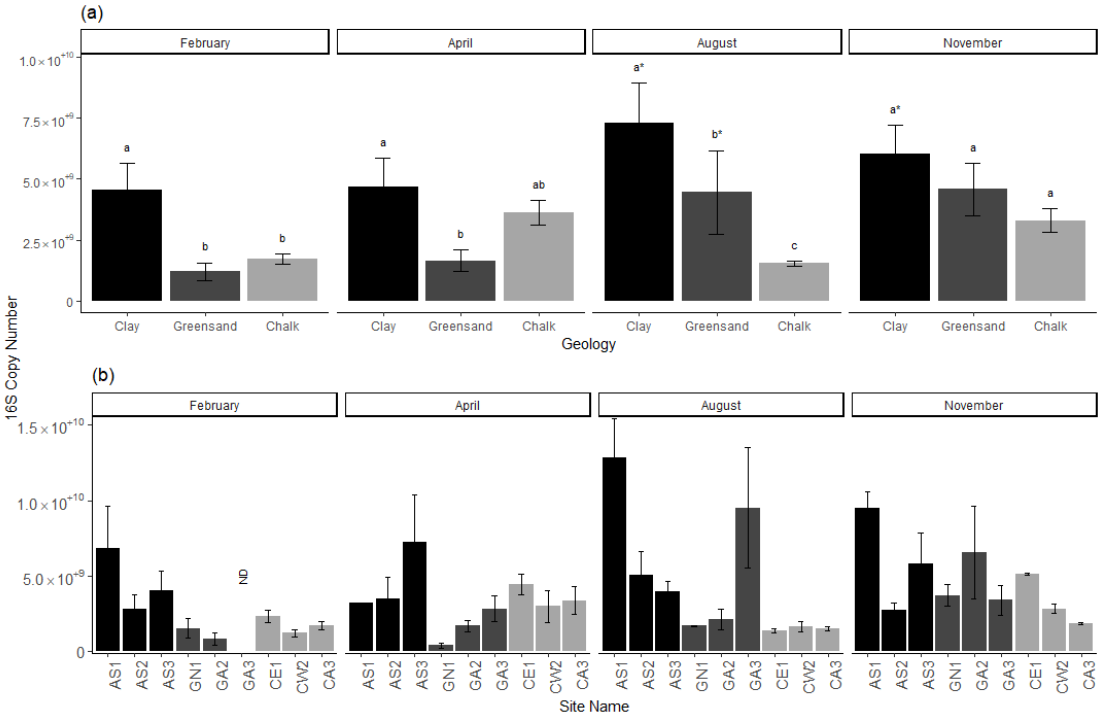

Figure 3.16. Mean (SE) 16S abundances grouped by (a) river sub-catchment geology, and (b) river site. Letters shown above geology level bars represent significant groupings based on least-mean squares (Tukey) *post-hoc* analysis. Those followed by an asterisk denote geology types where significant differences of 16S were found at different within-group river sites. ND indicates that no data were collected.

3.4.3 Sediment carbon

Clay rivers experienced the highest levels of TOC at all sampling periods, although on two occasions there was a high level of variability between the three clay rivers sampled. Greensand and chalk rivers were equally low throughout the year with the exception of August, where a large variation of TOC concentrations in a greensand river lead to greensand rivers overall having a higher concentration of TOC than chalk rivers.

Clay rivers were expected to have high concentrations of TOC due to the characteristics of fine sediment, where deposition leads to the entrainment of both allochthonous and autochthonous organic material. Greensand and chalk sediments however have larger sediment particle sizes, which is likely to reduce retention of materials and therefore contribute to increased transport of organic material downstream. The fine nature of clay sediments also leads to a larger surface-area:volume ratio for colonisation by microorganisms, which can be seen in 16S rRNA concentrations when compared between the sites (Figure 3.16). The lack of algal biomass, along with the high concentrations of TOC and high abundance of 16S rRNA suggest that clay rivers are likely to be net heterotrophic. Relative heterotrophy is important to consider in global biogeochemical cycles, as this would contribute to an increased liberation of fixed carbon as CO_2.

3.4.4 Carbohydrates and pore water DOC

Strong positive correlations were seen between all recorded carbohydrate fractions and pore water DOC extracted from the sediments. Conversely, certain fractions were found to be negatively correlated to inorganic nutrients; in particular CHO_D and CHO_{HW} when compared with NO_3^-. These results echo those found in studies of Scott *et al*. (2008) and Danger *et al*. (2013), who suggested that algal EOC excretion was linked to competition for inorganic nutrients with heterotrophic microorganisms. Competition for nutrients can be inferred from EOC concentrations, as algal cells are known to fix carbon at the highest rate possible. However, where there are limitations of inorganic nutrients this carbon cannot be utilised, and so is excreted from the cell (Rier *et al*., 2006; Underwood, 2005). As diatoms in particular excrete large amounts of carbon for attachment and motility, these systems provide an interesting insight into how resources are allocated under differing nutrient conditions.

3.4.5 Diatom community composition and trait-based analysis

Diatom community composition allowed insight to many aspects of the microphytobenthic community. Large numbers of taxa were recorded (220), which demonstrates the wide reaching nature of this study; for instance in another large analysis covering 52 sub-catchments in boreal areas recorded 217 taxa (Teittinen *et al*., 2015). This large number of taxa recorded could be due to the large changes in community composition at each site and sampling period - turnover analysis showed that the most significant change in species composition between sites was seen from

April to August (for all sites except CW2/A), while turnover was more limited from February to April.

Similar analysis was carried out by Potapova & Charles (2005), and common species were also found to be indicators. The authors of this particular study grouped communities base on “soft” and “hard” sediment. Two species of diatom which were found to be indicators of clay rivers were also found in the “soft substrate” class of indicators (*Diatoma vulgaris*, *Navicula gregaria*, Potapova & Charles (2005). Two species of indicators of greensand and chalk rivers (*Cocconeis pedi*culus*, *Nitzschia dissipata*) were also shown to be indicators of ’hard substrate". Interestingly, at one study site (Ridge and valley, Potapova & Charles, 2005) *Nitzschia dissipata* was found to be indicative of soft substrates, despite showing a significant indicator value for hard substrates at a different site as well as being indicative of greensand and chalk rivers.

Diatom traits were used as an alternate method to taxonomic identification to assess trends from a functional perspective (Lange *et al*., 2015). Due to the specificity of diatom species to each river geology, traits provide an insight into drivers of diversity across this wide range of communities. In order to assess communities, the proportion of each trait class was compared. The strongest relationships found were a negative correlation between “nano” diatoms and chlorophyll-*a* concentration, and an increase of “pad” attached diatoms with increasing BFI. With size class and chlorophyll-*a*, concentrations of chlorophyll-*a* were only shown to increase with the proportion of

"meso" diatoms, indicating that the abundance of this intermediate size class is the largest contributor to overall algal biomass.

Conversely, nano diatoms are only competitive at low algal concentrations, suggesting that although smaller cells are known to be faster growing and more competitive for dissolved nutrients (Lange *et al.*, 2015), competition for light through shading likely to be a stronger interaction.

Pad attached diatoms being more abundant in high BFI rivers suggests that river flashiness is an important consideration for diatom competition. While stalk diatoms showed no overall trend, their proportion of the community peaks at intermediate BFI levels. Due to the wide range of base-flow indexes sampled in this study, there is a lack of rivers which had intermediate BFI, and as such there is a potential that any relationship between attachment type and BFI is missed. Community proportion of TDI_S values compared against nutrient concentrations showed only a single interaction, where TDI_S 1 diatoms were found to be significantly negatively correlated with concentration of NO_3^-. No other interactions were found when comparing water chemistry and TDI_S scores, which contrasts with the widely used methods of the TDI. The TDI is designed around a diatom's response to dissolved phosphate concentrations (Kelly, 2001), and as such where no correlations between TDI_S score and phosphate concentrations were found were surprising. Suspected reasons for this include the differences in sampling design; whereby the TDI uses hard substrata such as tiles while rivers sampled were composed of finer, soft sediment. These results

potentially show that substratum type can influence diatom community composition and/or local nutrient conditions to an extent where soft sediment communities cannot be employed for biomonitoring.

3.4.6 Bacterial abundance

Bacterial abundance was measured by 16S rRNA analysis using qPCR. Results from this analysis showed similar trends to sediment TOC. Clay rivers consistently exhibited the highest abundance in 16S rRNA, with high abundances only being matched by greensand rivers in April and August, and similar results being found across all three geology types in November. Results within geology types were fairly consistent, with differences in 16S rRNA abundance only being seen in clay and greensand rivers. As with TOC, variances in the greensand rivers in August is likely to be driven by the wide range of 16S rRNA recorded at GA3, where a single observation causes a large error of the mean. High abundance of 16S rRNA in clay rivers is likely to be a product of high TOC concentrations within the sediment (Figure 3.6) and the high SA:V provided by the fine sediment characteristics of clay rivers.

Results presented in this chapter highlight the variability of microphytobenthic communities on varying soft sediments within a relatively small geographical area. Results showed that despite rivers being in the same overall catchment, there were significant differences in algal and bacterial biomass, total organic carbon and algal EOC concentrations both between synthetically defined geology types and within them. Within the diatom community, there were distinct species that were shown to

be indicative of a particular geology type, although no single species was shown to be an indicator of solely greensand rivers. Diatom trait based analysis presented an overall view of the diatom communities in this rivers from a functional aspect, and results complimented those of (Murdock & Dodds, 2007), stressing the importance of substratum on diatom community composition. These results also suggest that physical interactions of nutrients with the sediment itself (such as retention in porous sediment) may also play a role in influencing the ecology of microphytobenthic communities.

Although correlative, relationships shown between carbohydrate fractions, sediment DOC and inorganic nutrients strongly support the theory of trophic coupling (Scott *et al*., 2008). The extent to which DOC plays a role in increasing competition between these groups is unknown because of the large number of factors, but the strong relationships found in clay and greensand rivers between EOC and DOC suggest that high nutrient concentrations are likely to lead to algal/bacterial competition for inorganic nutrients. This evidence is further supported by the negative correlation between EOC and inorganic nutrients, which suggests at high nutrient concentrations assimilated C through photosynthesis is being utilised for cell function.

The aim of this study was to describe physiochemical characteristics and benthic microbial communities within the Hampshire Avon catchment over a seasonal cycle. The extensive sampling effort and dataset presented within this thesis provides an insight into the catchment and it's varying geology types, along with the variability of

microphytobenthic communities over landscape scales ranging from within the same reach to over the whole catchment.

In studying these variables, this work has presented a snapshot of the conditions within these rivers over an annual cycle. However, as seen with the continuous data for nutrients and river flow, even the scales used in this Chapter may not be sufficient to explain all interactions between the wider environment and microphytobenthic communities. Despite these limitations, there were interactions seen – such as with DOC concentrations and EOC abundance – which support existing theories within the literature. The present study raises questions to how these communities act functionally in each of the seasonal states recorded.

4 SEDIMENT OXYGEN AND NUTRIENT FLUX IN THE HAMPSHIRE AVON RIVER CATCHMENT

4.1 Introduction

Habitat characteristics are a major factor in determining community form and function throughout natural history, as well as providing opportunities for organisms to adapt to specific niche environments. At a larger landscape scale, river habitats can be classified into groups such as underlying geology and the spatial position of a river (Chapter 3). However, at a smaller scale, river habitats can be classified within a single reach, each with abiotic characteristics that impact biological and biogeochemical activity. Typically, these habitat features are formed by erosion and deposition of sediment by the surface water. For instance, erosive forces will suspend and transport smaller, lower mass sediment particles, leaving behind larger sediment. However, in slow-flowing water, suspended particles are likely to be deposited as the forces exerted by the water column are insufficient to maintain sediment suspension. As such hydrology can have a dramatic impact on the sediment structure of a river bed. Surface water, and therefore rates of erosion and deposition, can also be influenced by biotic components of a river channel, for instance macrophytes. In chalk streams found in England brook water crowfoot (*Ranunculus* spp.) is a characteristic macrophyte, and this species has been shown to significantly reduce river velocity which can modify sediment structure (dominated by fine silts), channel depth (channel depth reduced by ~ 90 % compared to open sections), water movement (significantly lower than in the open channel) and light quality (decreased by shading from macrophyte canopies) (Sanders *et al*., 2007). The significance of these factors is important to consider at a landscape scale, where macrophyte stand and open sections of river can exist within

a small distance of around 5 m, thus creating contrasting habitats - and therefore potentially contrasting communities and processes - within a single river reach. Differences in sediment topography have been shown to impact water movement (Arnon *et al*., 2007), resulting in habitat types having contrasting biogeochemical delivery, retention and removal over a small spatial scale.

Benthic habitat heterogeneity in streams and rivers has been assessed extensively in macroinvertebrate and fish, with many studies highlighting that diversity of habitats over a small spatial scale can have large impacts. For instance, work has shown Chironomids adapt their attachment preferences to the sediment structure, leading to decreased predation by fish in gravel areas, and therefore higher levels of algal grazing (Power, 1992). Invertebrates have also been shown to expand their temporal ranges in systems, due to diversity of habitats creating multiple refuges which may usually be removed due to seasonal effects such as storms (Brown, 2003). However, few studies focus on microbial communities over different habitat types.

As well as impacting the larger components of small river systems, local changes in sediment structure can also influence benthic metabolism, due to some sediments – such as fine sands – not being stable enough to produce mature microbial communities (Atkinson *et al*., 2008). Further work has shown that while underlying geology can influence bacterial communities in the Arctic, habitat type (sediment and larger stones) are more likely to be the driver of diversity in similar rivers (Larouche *et al*., 2012).

As well as shaping the river channel, hydraulic flow is also a factor considering delivery and transport of suspended and dissolved molecules require for biogeochemical cycling (Arnon *et al*., 2007). This is particularly true in permeable sediments, where the surface-water interface and associated hyporheic zone is known to be a biogeochemical hotspot (Bengtsson *et al.*, 2014; Briggs *et al*., 2015; Thomas *et al*., 2001). The combination of surface-water velocity and permeable sediment was shown to impact vital biogeochemical processes such as denitrification (DN) through the combined delivery of nitrate (required for DN) and oxygen, which inhibits the DN process (Arnon *et al*., 2007). Flow velocity has also been shown to influence biofilm uptake of dissolved carbohydrate fractions from the water differentially – for instance it has been shown that heterotrophic bacteria may switch to carbohydrate fractions which are less labile when delivery of sources easier to uptake are limited by flow (Singer *et al*., 2011). As a result, there may be trophic shifts found at local scales, such as in open channel and sediment populated my macrophytes in the heterotrophic community. Where the trophic change does occur, there may be interactions between autotrophic production of EOC and nutrient dynamics between auto- and heterotrophs as potentially seen in Chapter 3. Hydraulic flow has also been shown to influence diatom communities directly, but the effect was found to be dependent on water turbidity (Soininen, 2004). Water flow rates could therefore also influence the species composition – and therefore production of EOC over a relatively small spatial area.

Light quality is important to any community dependent on photoautotrophy, not only for primary production but also for production of oxygen. Reduced light from shading has been shown to influence bacterial abundance and composition (Lehmann et al., 2015), potentially an effect of light altering the algal community which act as substrate within the biofilm for bacteria. Interactions between light levels and biogeochemical cycles are well established, and as well as carbon, interactions can be driven by to the photosynthetic production of oxygen. For instance, biogeochemical processing of nitrogen is split between oxic (nitrification) and anoxic processes (denitrification). While oxygen concentrations are not solely driven by light, it is an important factor to consider when studying microphytobenthic communities dominated by photoautotrophs. Experimental work has also shown that light can impact community limitations in MPB - for instance communities that were exposed to high light levels were found to be nutrient limited while those in low-light were not - described as serial co-limitation (Warren *et al*., 2017).

Labile carbon is also a factor driven by light as well as nutrients. The excretion of extracellular organic carbon (EOC) in diatoms is a process which is influenced by attachment type (pad, stalk or motile) as well as a method to remove excess carbon fixed during photosynthesis relative to other nutrients. EOC availability is an important factor to consider when investigating biogeochemical cycles in rivers, as it acts within a feedback loop paired to other macronutrients such as N and P. Evidence for EOC and nutrient interaction has also been found in the natural environment (Chapter 3),

complimenting experimental manipulations discussed above. Freshwater sediments are important centres of biogeochemical cycles, with respiration alone estimated to contribute around 90 Tg C yr-1 to the atmosphere (Ciais *et al*., 2008). However, work on a wide spectrum of nutrients has shown that catchments, and the sediment within them, can play a large role in determining both surface and ground water chemistry (Jarvie *et al*., 2005).

Substratum topography is often overlooked in MPB studies, yet has been shown to impact the communities on multiple levels, including through retention, delivery and removal of particulate and soluble materials, and selection of components of the MPB such as diatoms (Cox *et al*., 2011; Murdock & Dodds, 2007; Potapova & Charles, 2005). Sediment structure has also been shown to change the relative impact of other environmental conditions; whereby community responses to variables such as nutrients and pH were not similar for hard substratum compared to soft substratum (Potapova & Charles, 2005). Land use can also influence MPB communities, as different land uses create contrasting impacts of POM delivery, run-off, nutrient leaching and other factors similar to those experienced in different catchment geologies. Algal communities in forested areas have been shown to be impacted by bottom-up processes – such as nutrient limitation and habitat availability (Cibils-Martina *et al*., 2019). However, in grassland streams, grazing by macroinvertebrates was shown to have a greater impact, with the authors suggesting that differences in hydrology was the cause for these changes (Cibils-Martina *et al*., 2019).

The mass transport of dissolved molecules required for biogeochemical cycling has been shown to be influenced by both water velocity and sediment topography (Arnon *et al*., 2007). Because of the nature of depositary sediments in relation to erosive sediment (from the main channel), these influences are likely to be differentiated between the two sediment types.

Influences from underground flow are also likely to be differentiated between main channel sediment and depository sediments. (Jarvie *et al.,* 2005) demonstrated that pore water hydrochemistry was vastly different between geology types, which is a trend that could also apply at the smaller sediment scale. Differences between the sediment could also influence rates of nutrient retention, further complicating our understanding of biogeochemical cycles between these closely situated contrasting habitats.

The aim of this study was to influence of habitat choice on the MPB community and biogeochemical cycles. The Hampshire Avon catchment provides ideal habitats to investigate habitat choice there are drastic changes in habitat conditions over a small spatial scale (~ 5 m). By using a similar approach as taken in Chapter 3 to characterise the different habitats, I aim to eliminate effects of water source, and use novel methods for measuring potential flux of macronutrients and gasses allowed investigation into biogeochemical processes within the MPB community.

I hypothesise that:

1. Algal biomass and carbohydrate concentrations will decrease under macrophyte stands.

2. However, due to higher surface area and retention of organic matter, sediment TOC will increase under macrophyte stands compared to the main channel.

3. I expect both of these to be different seasonally due to changes in the extent of the macrophyte stands.

4. I predict that primary productivity will be higher in the main, unshaded channel, and as a result of this;

5. Oxic processes (such as nitrification) will be linked to this higher production of oxygen.

4.2 Methods

4.2.1 Site selection

Two of the sites from those sampled in Chapter 3 – the greensand River Avon (GA2) and the chalk River Wylye (CW2) were sampled (Table 2.1) within the Hampshire Avon Catchment (Figure 2.1), with two habitat types being targeted (Main channel; MC and Macrophyte sediment; MP). These specific rivers were sampled because a) they were used previously in Chapter 3 to allow for direct comparisons, and b) because they were the only rivers within the Hampshire Avon Catchment which had established and extensive areas of macrophyte growth. While the size of the macrophyte stands changed throughout the sampling cycle, where present there was sufficient habitat to

correctly replicate samples. On some occasions, macrophyte stands were not present – and therefore sampling was unable to be carried out. Clay rivers and other rivers within the greensand and chalk sub-catchments did present macrophyte growth, but at these sites there were not enough examples to fully provide replication.

4.2.2 Primary sampling

Sediment cores were extracted from the main channel and macrophyte sediment at 5 m intervals using a plastic sediment corer (internal diameter = 8 cm, n = 5, Figure 2.2). Sediment was sub-cored for further analysis using cut off 20 ml syringes (internal diameter = 2 cm, n = 4). Sub-cores from which algal pigments and carbohydrates were stored on ice until return to the laboratory. These samples were then frozen at - 20 °c. For algal pigment analysis, Chlorophyll-*a* was extracted from freeze-dried sediment using the cold methanol method, with a step to correct for phaeopigments (Stal *et al*., 1984). Sediment carbohydrates were also extracted from freeze dried sediments, following methods adapted from Hanlon *et al.* (2006). Extractions of CHO_D, CHO_{HW} and EPS_{70} were extracted using methods detailed in Chapter 2 and Chapter 3.

4.2.3 In river incubation experiments

In river incubations were carried out at two separate occasions in August 2013 (GA2 – MC, GA2 - MP and CW2 - MC) and July 2014 (CW2 - MC and CW2 - MP). Sediment cores were extracted in a similar way to those collected during primary sampling. At 5 m intervals, a large sediment core of each sediment type was taken (internal diameter

= 8 cm, n = 4) which was then divided into 4 smaller sediment cores using cut-off 20 ml syringes (internal diameter = 2 cm, n = 4). Each of these sub-cores was then placed into individual tissue culture flasks (TC flasks, 50 ml volume, unvented) and secured to a plastic board (Figure 2.3). Each of the sub-cores was then covered according to the 4 light treatments used in the incubation - 100 % ambient (uncovered), 50 % ambient (50 % neutral density (ND) film), 25 % (75 % ND film) and 0 % ambient (aluminium foil). Attached to each plastic board was a HOBO® logger, recording light and temperature at a resolution of 10 s.

As each TC flask was filled with stream water (removing any air head space) samples were taken for t0 measurement of dissolved oxygen via Winkler titration (detailed below) and a 0.2 µm filtered sample for nutrient analysis. Incubations were carried out for a known duration of around 2 hours, after which samples were removed minimising sediment disturbance and processed.

TC flasks were first sampled for dissolved oxygen upon opening of the TC flask to minimise diffusion of oxygen from the air. Upon completing collection of the oxygen samples, a further sample was taken and filtered (using sterile 0.2 µm filters) for later nutrient analysis. Dissolved oxygen was calculated using a modified Winkler titration. Samples were extracted minimising surface disturbance and therefore gas transfer. Samples were then decanted to overflowing in 7 ml air tight glass exotainers (Labco®, UK), preloaded with glass beads to enhance mixing. Dissolved oxygen was fixed on site by adding 10 µl $MnSO_4$ and 10 µl of a solution of NaOH and KI, which reacted with the

dissolved oxygen in the sample to create an equimolar precipitate of $Mn(OH)_3$, minimising biological activity. After transport to the laboratory in darkness, DO concentration was established by acidifying the sample, leading to the liberation of iodine. Sample concentrations where then determined using titration of a starch indicator with KIO_3.

4.3 Results

4.3.1 Within-reach differences

Abundance of chlorophyll-*a* peaked at a mean of 4.28 ± 0.408 µg g^{-1} sediment DW within the macrophyte stand, compared to 2.05 ± 0.500 µg g^{-1} sediment DW recorded in the main channel (Figure 4.1). Chlorophyll-*a* concentrations in the greensand river (Figure 4.1 a) were shown to be significantly different across both date ($F_{3, 27}$ = 6.05, p = 0.002) and across sampling date crossed with habitat types ($F_{2, 27}$ = 6.41, p = 0.005). In the overall model, there were not found to be significant differences between chlorophyll-*a* between the two habitat types. At individual sampling periods, differences in chlorophyll-*a* concentrations between the two habitats were found only during April (Tukey, p = 0.006). No data was collected at the greensand macrophyte stands in February due to lack of suitable habitat, most likely caused by the high river flow conditions at the time of sampling.

Chlorophyll-*a* abundance in chalk rivers was typically greater than those found in the greensand rivers, with results ranging from 5.67 ± 0.939 µg g^{-1} sed. DW (main channel, February 2013) to a peak of 27.55 ± 9.022 µg g^{-1} sed. DW (macrophyte sediment,

August). Similarly to results found from greensand rivers, the abundance of chlorophyll-*a* in chalks river was also shown to be significantly different between sampling period ($F_{3, 30}$ = 4.12, p = 0.015), and between habitat types ($F_{1, 30}$ = 1.98, p = 0.008). As found between the greensand habitats, there was only a single sampling period where chlorophyll-*a* concentration was found to be different between the habitat types - although this was found in August (Tukey, p = 0.003) where, like GA2, macrophyte sediment had a higher abundance of 27.56 ± 4.035 µg g^{-1} sed. DW compared to 10.18 ± 0.726 µg g^{-1} sed. DW in the main channel.

Phaeopigments – a product of chlorophyll-*a* degradation, showed a large variation of abundance at each of the different sites and sampling periods (Figure 4.2). Over the whole dataset, concentrations ranged from a minimum of 0.11 ± 0.361 µg g-^{1} sed. DW to 123.83 ± 56.458 µg g^{-1} sed. DW at greensand macrophyte sediment in April and chalk macrophyte sediment in August respectively. The high abundance of phaeopigments and with the high variability of the samples within the chalk macrophyte sediments was driven by a large variation in each of the replicates. However, 3 of the 5 replicates recorded phaeopigment abundances above 90 µg g-1 sed. DW, showing that the greater figure was unlikely to be drawn from a single anomalous measurement.

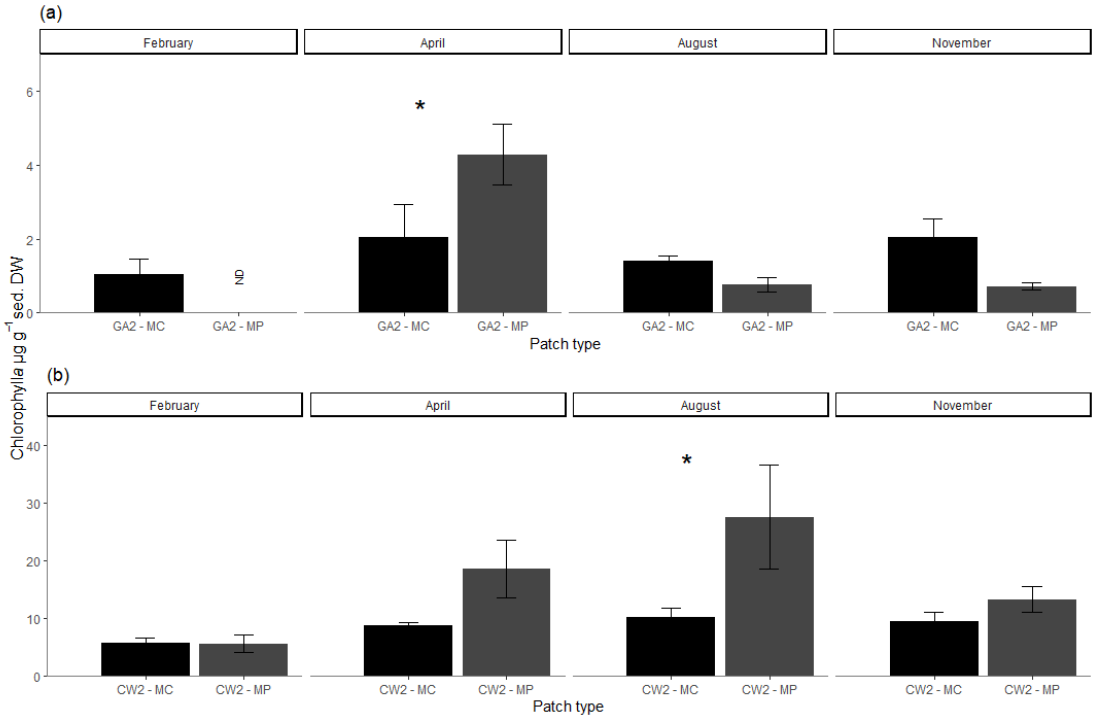

Figure 4.1. Chlorophyll-*a* abundance compared between the main channel (dark bars) and macrophyte sediment (light bars) in GA2 (a) and CW2 (b). Asterisks denote significant differences in chlorophyll-*a* abundance between the two sediment types within each site.

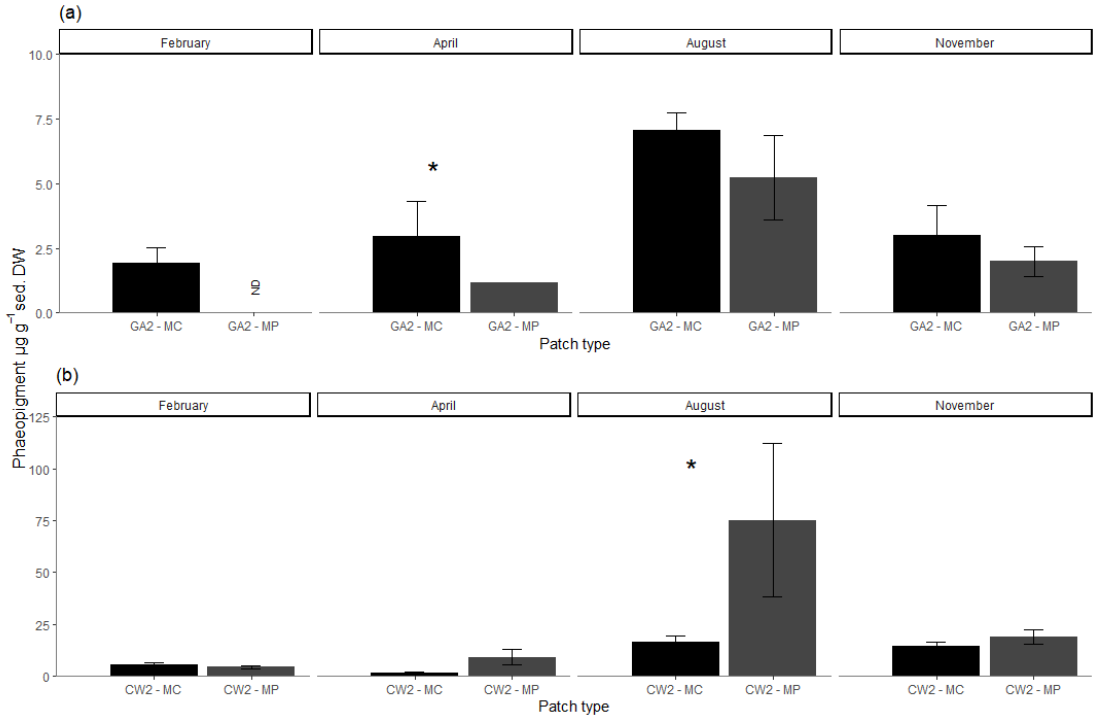

Figure 4.2. Phaeopigment abundance compared between the main channel (dark bars) and macrophyte sediment (light bars) in GA2 (a) and CW2 (b). Asterisks denote significant differences in phaeopigment abundance between the two sediment types within each site.

When statistically compared using an ANOVA, phaeopigment abundances within the chalk river were found to be significantly different between the sampling periods ($F_{3, 30} = 4.45$, $p = 0.01$), between the habitat types ($F_{1, 30}$, $p = 0.04$) and also between each of the habitat types at each of the sampling periods ($F_{3, 30} = 4.21$, $p = 0.04$). Within the greensand river, phaeopigments were not found to be significantly different between the different habitat types at each sampling period – a significant difference was found at a single sampling point – chalk rivers in August (Tukey, $p = > 0.001$). There was a significant difference between phaeopigment abundance at each of the sampling periods overall ($F_{3,27}$, p -= 0.03) and between the habitat types throughout the whole year ($F_{1, 27}$, $p = > 0.001$).

Dissolved carbohydrate (CHO_D) concentrations in the greensand river showed a relatively narrow range of results (Figure 4.3), with only a 67 % difference between the highest concentration (62.36 ± 4.905 µg g^{-1} sed. DW – macrophyte sediments in April) and the lowest concentration (20.55 ± 10.298 µg g^{-1} sed. DW – main channel, November).

Statistical analysis showed that differences between CHO_D concentrations in GA2 were not significant in the overall ANOVA model during neither sampling period, habitat type or during the different habitat types at each sampling period. Results from individual sampling periods showed that there was only a significant difference between CHO_D concentration habitat types in April (Tukey, $p = 0.024$), while there were no other differences found.

Chalk river sediment exhibited a large range of CHO_D concentrations, particularly within macrophyte sediments where concentrations were recorded, from 4.28 ± 0.7359 µg g^{-1} sed. DW in February to a peak of 220.17 ± 31.471 µg g^{-1} sed. DW in April. This large variability in results was not seen in the main channel of the chalk river, with results showing CHO_D concentrations between 31.96 ± 2.691 µg g^{-1} sed. DW (February) and 93.42 ± 29.053 µg g^{-1} sed. DW in April. Although April results were the highest for CHO_D in the main channel of the chalk river, variance was high at 68.9 %. The chalk river CW2 showed significant differences to all of the variables in the overall model, including sampling period ($F_{3, 27}$ = 13.86, p = < 0.001), habitat type ($F_{1, 27}$ = 13.92, p = < 0.001) and sampling period crossed with habitat type ($F_{2,27}$ = 8.15, p = 0.002). Significant differences in CHO_D were seen between habitat types in April and August (Tukey, p = 0.001 and p = < 0.001 respectively).

The hot water extracted carbohydrate fraction (CHO_{HW}) was found to be of similar concentrations to CHO_D, and also followed similar trends (Figure 4.4). For instance, concentrations of CHO_{HW} were typically higher in the chalk river (maximum of 278.68 ± 47.258 µg g^{-1} sed. DW; April, chalk macrophyte sediments compared to 50.69 ± 5.362 µg g^{-1} sed. DW; greensand macrophyte sediment in April). One noticeable difference was seen in the comparison of CHO_{HW} and CHO_D – in greensand rivers during November sampling, mean concentration of CHO_{HW} was higher in the main channel than the macrophyte sediment, whereas CHO_D recorded the opposite result.

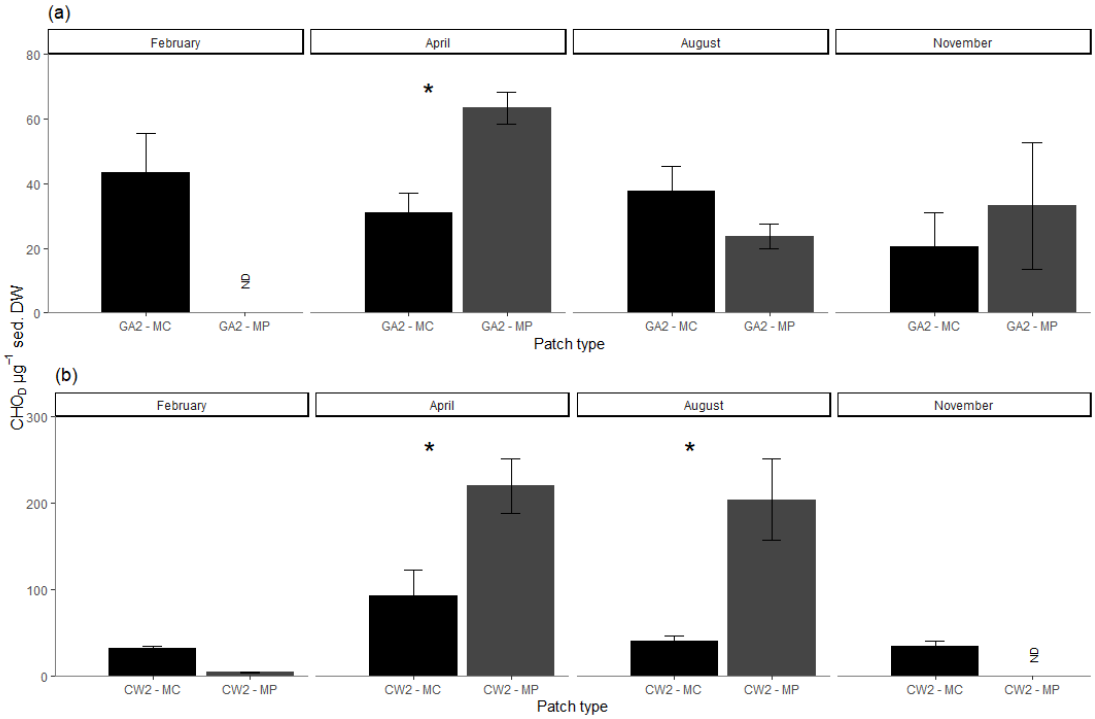

Figure 4.3. Dissolved carbohydrates (CHO_D) concentration between the main channel (dark bars) and macrophyte sediment (light bars) in GA2 (a) and CW2 (b). Asterisks denote significant differences in CHO_D concentrations between the two sediment types within each site.

Hot water extracted carbohydrates (CHO_{HW}) within GA2 showed no overall difference in concentration between patch type (Figure 4.4). The overall ANOVA model showed that the only factor to impact concentration in the greensand river was sampling period ($F_{3, 27} = 3.61$, $p = 0.026$). Differences were seen overall in CW2 when CHO_{HW} was compared with sampling period ($F_{3, 26} = 4.93$, $p = 0.008$), habitat type ($F_{1, 26} = 8.64$, $p = 0.007$) and sampling period crossed with habitat type ($F_{2, 26} = 4.91$, $p = 0.016$). The rivers sampled in this Chapter were sampled simultaneously with samples collected for Chapter 3, a comprehensive dataset for concentrations of major nutrients (NO_3^-, NO_2^- and PO_4^{3-}) can be found in Table 3.4.. These measurements, while not featuring replication, were closer to previously published values compared to continuous data, which was also analysed in Chapter 3. The potential causes for this were discussed in Chapter 3 (section 3.4).

Diatom species composition in the macrophyte stands was dominated by the diatoms *Navicula lanceolata*, *Planothidium lanceolata* and *Cocconeis placentula*. Species turnover between each month was low (a maximum of 26.84 %, found between April and August at GA2 - MP), particularly when compared to the main channel where turnover was 51.09 % (Table 3.6).

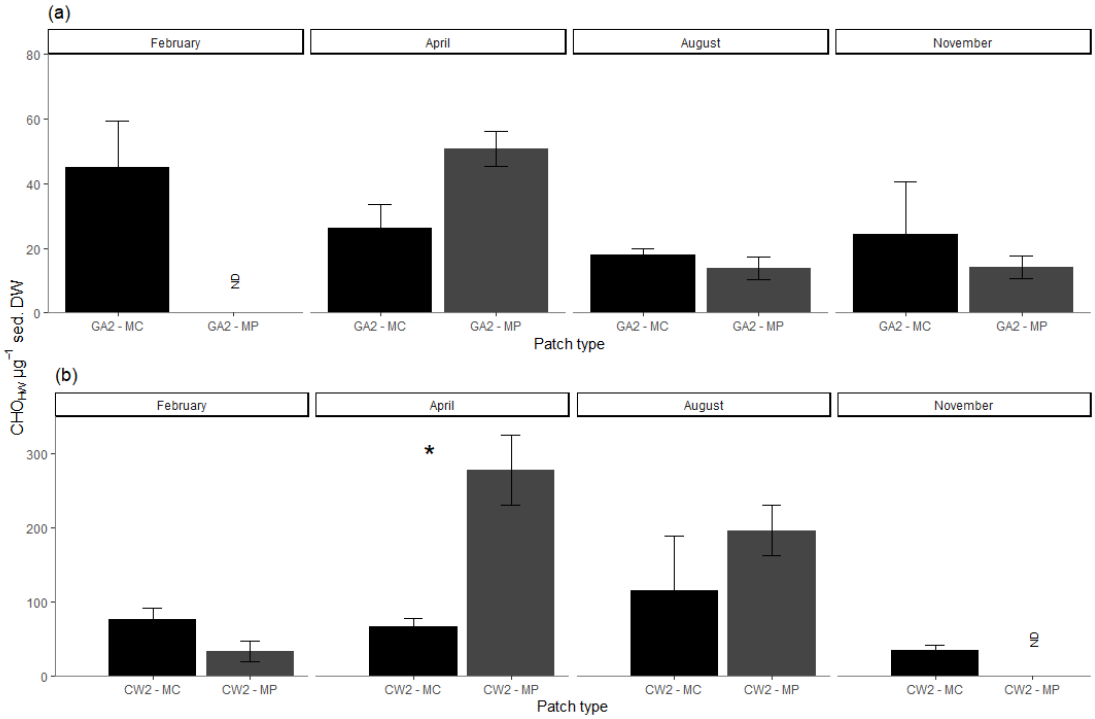

Figure 4.4. Hot water extracted carbohydrates (CHO_{HW}) concentrations between the main channel (dark bars) and macrophyte sediment (light bars) in GA2 (a) and CW2 (b). Asterisks denote significant differences in CHO_{HW} concentrations between the two sediment types within each site.

4.3.2 Incubation nutrient flux

During the incubation period, light and temperature were recorded at a resolution of 10 s (Figure 4.7). As expected, the characteristically clear water found in chalk streams allowed the chalk river to have the highest light levels (an average over the incubation period of 88.67 ± 1.008 µmol PAR m^2 s^{-1}, whereas the main channel of the greensand river had a mean light level of 53.38 ± 0.496 µmol PAR m^2 s^{-1}. Light concentrations in the macrophyte stands were further reduced – with a mean light level of 16.85 ± 0.243 µmol PAR m^2 s^{-1} – a reduction of 68.4 % compared to the main channel - and maximum light levels recorded in under the macrophyte stands was lower than the mean of the main channel (44 µmol PAR m^2 s^{-1} and 53.38 ± 0.496 µmol PAR m^2 s^{-1} respectively). Light conditions were found to be significantly different between the chalk river, the

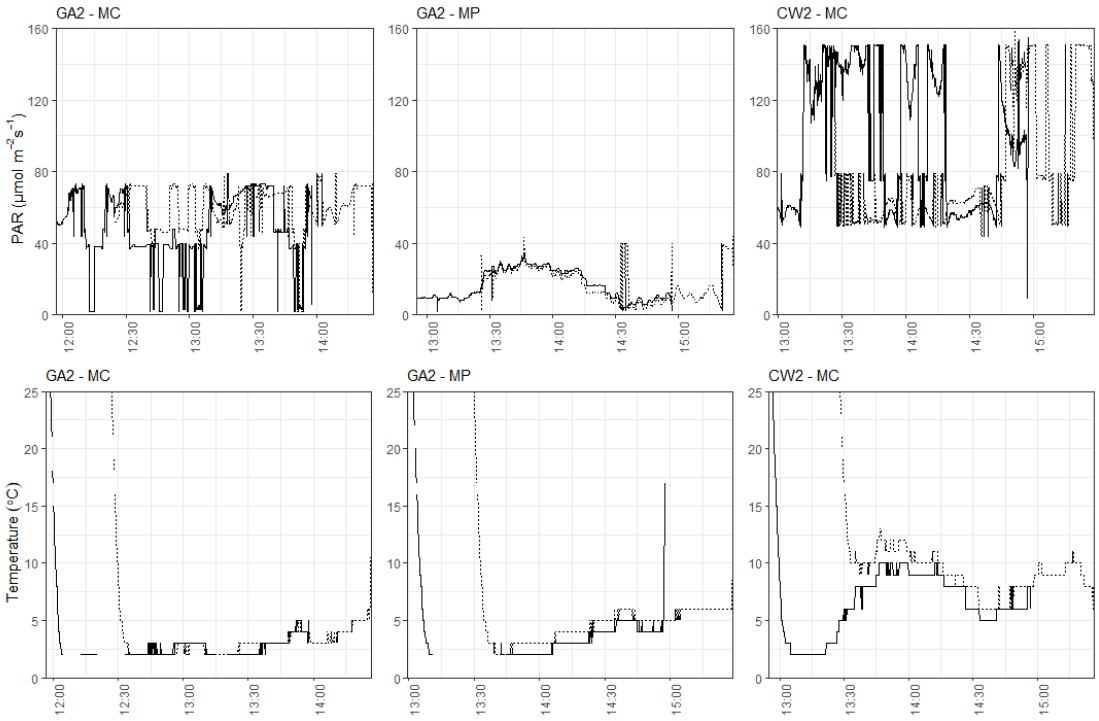

Figure 4.5. Recorded light (PAR, µmol L^{-1} s^{-2}) and temperature (°C) recorded during incubation experiments in 2013. Each column shows data collected from a different site/patch, while lines distinguish results from each individual incubation board.

greensand main channel, and also the greensand macrophyte sediment ($F_{2, 4392}$ = 2925, p = < 0.001 overall, and p = < 0.001 for each individual Tukey comparison).

Temperature was also found to be different between the sites, with a mean temperature of 7.94 ± 0.073 °c recorded in the chalk river (Figure 4.5). In comparison, the greensand river recorded temperatures of 3.15 ± 0.065 °c on the main channel, and 4.42 ± 0.073 °c in the macrophyte stands. ANOVA analysis showed that these temperatures were significantly different ($F_{2, 3900}$ = 1213, p = < 0.001), and that each of the three incubation treatments experienced significantly different temperatures (Tukey, p = < 0.001 for all comparisons).

Dissolved nutrient concentrations were recorded in order to establish a starting concentration (t0) of the water used in for the timed incubation. Replicate samples from 2013 showed that starting concentrations of Nitrate (NO_2^-) were higher in the chalk river (19.31 ± 0.065 mg L^{-1}) than in the greensand river (12.84 ± 0.173 mg L^{-1}). Nitrite concentrations showed an inverse result to that of nitrate, with chalk rivers showing lower concentrations compared to greensand (0.03 ± 0.001 mg L^{-1} and 0.06 ± 0.001 mg L^{-1} respectively), with phosphate concentrations showing a similar pattern (8.69 ± 0.131 mg L^{-1} in chalk and 10.61 ± 0.621 mg L^{-1} in greensand). Each of the t0 nutrient concentrations showed minimal variability throughout replication, ensuring that starting conditions were as similar as the natural nutrient regime as possible.

Nitrate (NO_3^-) flux during each of the 2013 incubations showed uptake of nitrate by sediments for at all light treatments at all sites (Figure 4.6). The range of results was

relatively low (minimum - 0.15 ± 0.045 mg L^{-1} hr^{-2} in the main channel of the greensand river at 100 % ambient light, to maximum of - 0.87 ± 0.105 mg L^{-1} hr^{-2} in the chalk main channel at 50 % ambient light). There were no significant differences in nitrate flux between the two habitat types in the greensand river – however the sediment uptake of NO_3^- was significantly higher in the chalk river ($F_{2,34}$ = 30.38, p = < 0.001), with no differences being found between the greensand habitat types. When comparing each individual treatment across the habitat types, there were no differences at 0 % ambient light. However, all of the illuminated treatments (25 %, 50 %, 100 %) all showed that sediment uptake of NO_3^- was significantly different in the two rivers (Tukey, p = < 0.05 for all comparisons, Figure 4.6). There were no differences between the habitat types in the greensand river. When examining each habitat type, there were no significant differences found between any of the light treatments.

Nitrite (NO_2^-) fluxes indicated that sediments are a source of NO_2^- to the water column (Figure 4.6). Fluxes were smaller than those of nitrate, with a maximum change of 0.54 ± 0.079 mg L^{-1} (chalk main channel, 50 % ambient light) and a minimum of 0.05 ± 0.023 mg L^{-1} (greensand macrophyte sediments at 25 % ambient light). Flux in all individual incubations was positive from sediment to water, with the lowest overall flux being recorded at 0.015 mg L^{-1} hr^{-2}, and low variance from the mean indicate that samples were relatively consistent with regards to nitrite flux.

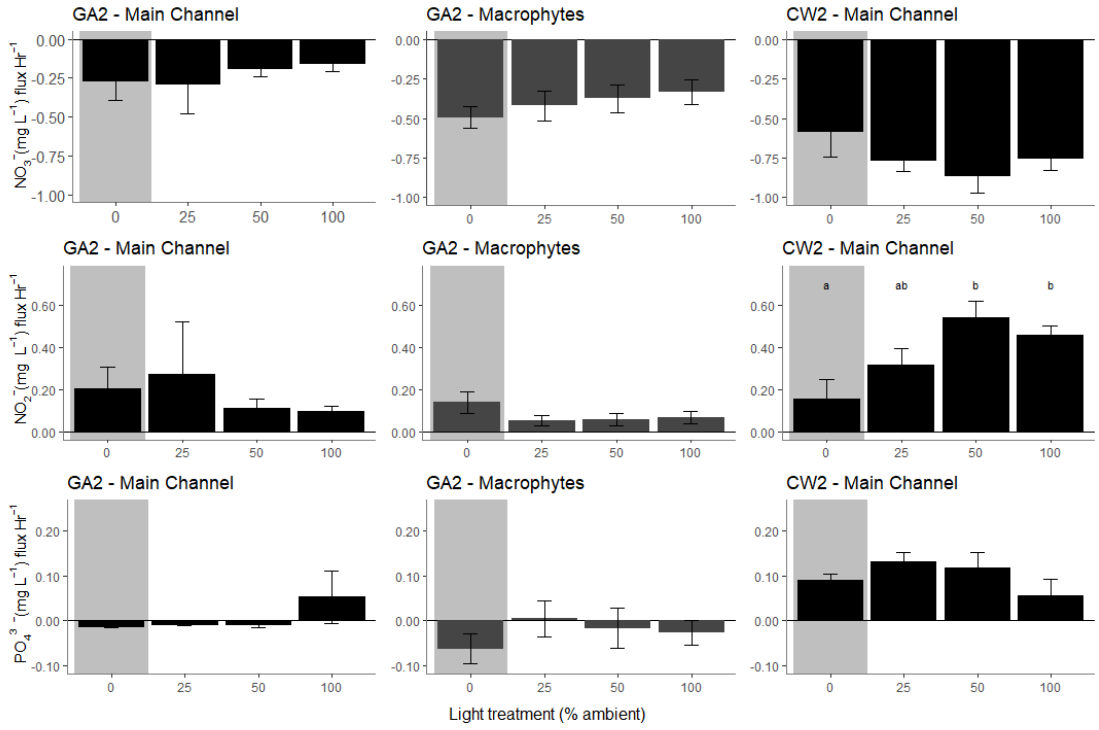

Figure 4.6. Potential flux of NO_3^-, NO_2^- and PO_4^{3-} per hour recorded during incubations carried out during 2013.

Nitrite was shown to be the only nutrient that showed significant differences between flux measurements in each treatment at an individual habitat type. At CW2, the main channel flux in NO_2^- was shown to be different between the treatments ($F2$, 12 = 19.62, p = < 0.001), with Tukey *post-hoc* tests showing that this was driven by the highly illuminated (50 % and 100 % ambient) light treatments releasing a significantly higher rate of NO_2^- compared to the 0 % light treatment.

Phosphate (PO_4^{3-}) flux was varied across the individual samples, with mean values being calculated from - 0.62 ± 0.033 mg L^{-1} hr^{-2} (sediment uptake of PO_4^{3-}, greensand macrophyte sediment at 0 % ambient light) to a 0.12 ± 0.034 mg L^{-1} hr^{-2} release of phosphate from the sediment in the chalk main channel at 25 % ambient light (Figure 4.7). Both the main channel and the macrophyte sediment in showed a wide variation of phosphate flux rates with release and uptake of phosphate from sediments. The main channel of the chalk river showed that the sediment was a source of P for each light treatment, although release of P was lowest at 0 % and 100 % light (0.09 ± 0.012 and 0.06 ± 0.037 mg L^{-1} hr^{-1} respectively).

Overall, no significant difference was found in phosphate flux in the different light treatments. Similarly to the other macronutrients analysed in this experiment, there was a significant difference in sediment P uptake/release at each of the different habitat types ($F_{2,34}$ = 15.00, p = < 0.001), with this difference being attributed to the chalk river showing significantly higher positive flux of P from the sediment in all but 100 % ambient light treatments (Tukey, p = < 0.05).

Oxygen flux within the incubations showed mostly a net reduction in oxygen (Figure 4.7). There were different ranges of oxygen consumption for the greensand habitats, with the main channel ranging from - 0.54 ± 0.191 µmol O_2 L^{-1} hr^{-1} to 0.32 ± 0.046 µmol O_2 L^{-1} hr^{-1}, and macrophyte sediment showing a range between -1.06 ± 0.248 µmol O_2 L^{-1} hr^{-1} and -1.66 ± 0.358 µmol O_2 L^{-1} hr^{-1}. The small range of results from the greensand sediments was in contrast to the greater range of results found in the main channel of the chalk river, which shifted from net heterotrophy (-0.77 ± -0.151 µmol O_2 L^{-1} hr^{-2}) at 0 % ambient light to net primary production (0.15 ± 0.870 µmol O_2 L^{-1} hr^{-2}) at just 25 % ambient light (Figure 4.7). Incubations carried out in 100 % ambient light in the chalk river indicated that each replicate experienced net primary production, with the mean oxygen flux being 0.68 ± 0.231 µmol O_2 L^{-1} hr^{-2}.

Statistically, O_2 fluxes were not found to be different between treatment types in both the main channel and the macrophytes of GA2. A significant difference in O_2 flux was seen in CW2 (main channel, $F_{3, 12} = 14.70$, $p = < 0.001$), which as discussed above was the only river sediment to experience an increase in O_2 over the incubation period. After calculating the average light levels experienced by each of the replicates during the incubations, these were plotted against the flux in O_2 (Figure 4.8). A polynomial curve was plotted in order to fit the light-curve nature of this data. In 2013, there was a significant correlation between average light levels and oxygen production (Pearson's correlation, $p = < 0.001$). Confidence intervals

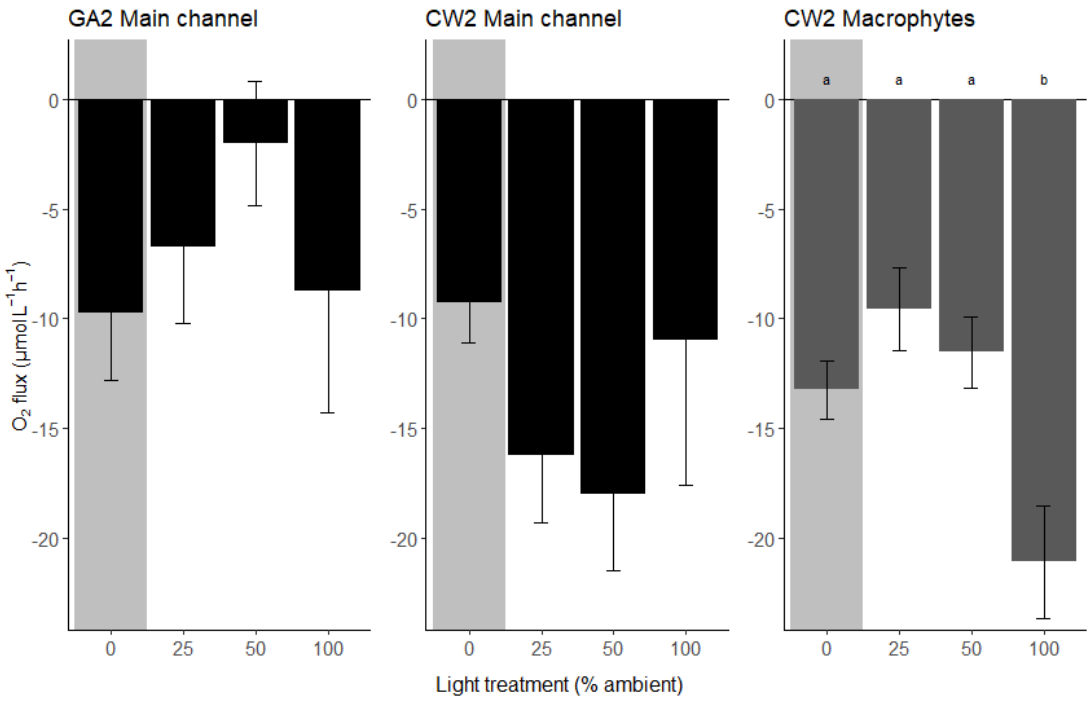

Figure 4.7. Mean O_2 flux (±SE) recorded during incubations carried out during 2013. Letter above bars indicate significant groupings.

applied to the polynomial line suggested that net primary production (O_2 flux > 0) is reached at around 100 µmol PAR m^2 s^{-1}. Some sites however extend well beyond this (CW2 - MC for example at > 150 µmol PAR m^2 s^{-1}).

The same experiment was repeated during July 2014. Similar to the incubations carried out in 2013, there were limitations based on availability of macrophyte sediment required to compare habitat types within a single site. On this occasion, there was insufficient macrophyte growth in the greensand river to be able to carry out incubations, whereas macrophyte growth was greater in the chalk river compared to 2013, allowing chalk macrophyte to be included in the incubation experiments. Light levels were much higher in the 2014 incubations, with the highest average light levels being found in the chalk river main channel (282.52 ± 3.933 µmol PAR m^2 s^{-1}), and even the lowest light levels – recorded under macrophyte stands in the chalk river – matching the highest light levels recorded in the 2013 incubations (88.85 ± 3.191 µmol PAR m^2 s^{-1}, Figure 4.11). There was a significant difference between the light levels experienced in each of the habitats throughout the incubation period ($F_{2, 7644}$ = 766.3, p = < 0.001).

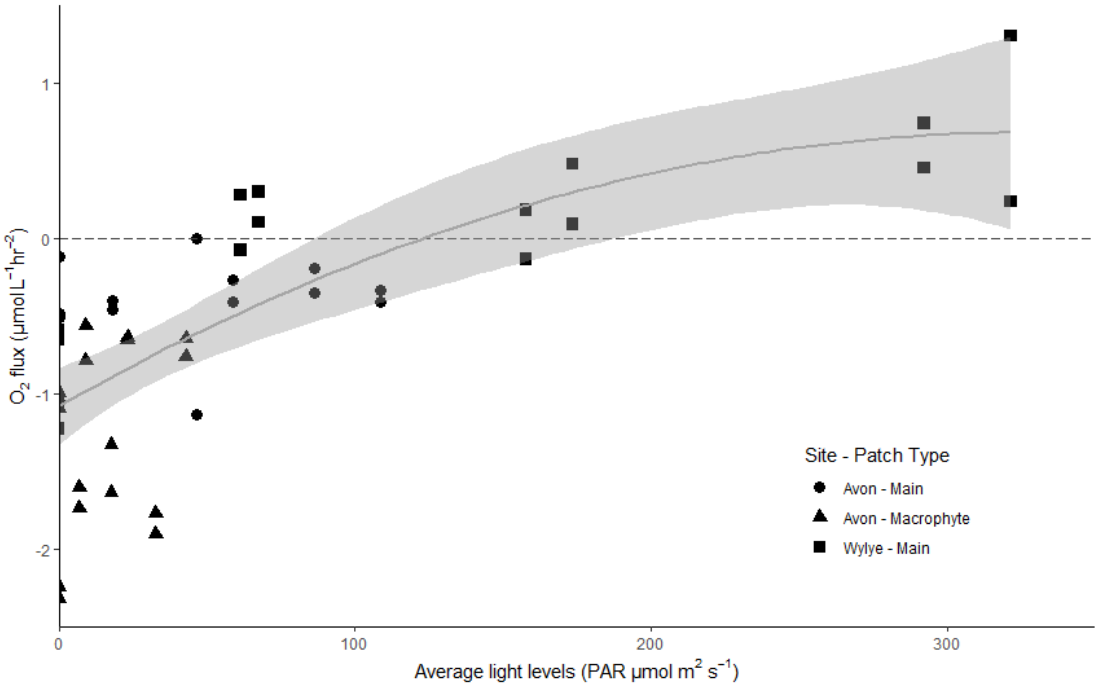

Figure 4.8. Change in oxygen concentration plotted against the average light levels experienced throughout the 2013 incubation period. Shaded area indicates confidence intervals for polynomial curve.

Water temperature was also higher during the 2014 incubations than experienced in the 2013 period (Figure 4.9). The main channel of the chalk river had the highest temperature, with a mean of 19.05 ± 0.049 °c, while the chalk macrophyte stands and the greensand river experienced temperatures of 17.82 ± 0.0537 °c.

During this time, no significant differences were seen between any of the light treatments in either of the habitat types for any of the nutrients recorded. Nitrate flux was variable in each of the habitat types, with the main channel ranging from 0.32 ± 0.243 mg L^{-1} hr^{-2} at 0 % ambient light to 0.53 ± 0.199 mg L^{-1} hr^{-1} at 50 % ambient light. However, each individual incubation showed a high level of variation, and for the 0 % ambient and 25 % ambient light treatments replicates exhibiting sediment uptake and release of NO_3^-. The macrophyte sediment also experienced a large range between replicates, and also had the only example of an overall sediment uptake of NO_3^- - with the mean flux of nitrate in 25 % ambient light being recorded at -1.19 ± 1.441 mg L^{-1} hr^{-1}.

Nitrite fluxes also showed a large variation between the multiple replicates in 2014 (Figure 4.10). While in the main channel the sediment acted as a source of NO_2^- for each of the light treatments, the range of results was very small (0.01 ± 0.007 mg NO_2^- L^{-1} hr^{-1} - 0 % ambient light to 0.03 ± 0.021 mg NO_2^- L^{-1} hr^{-1} – 25 % ambient light), and there

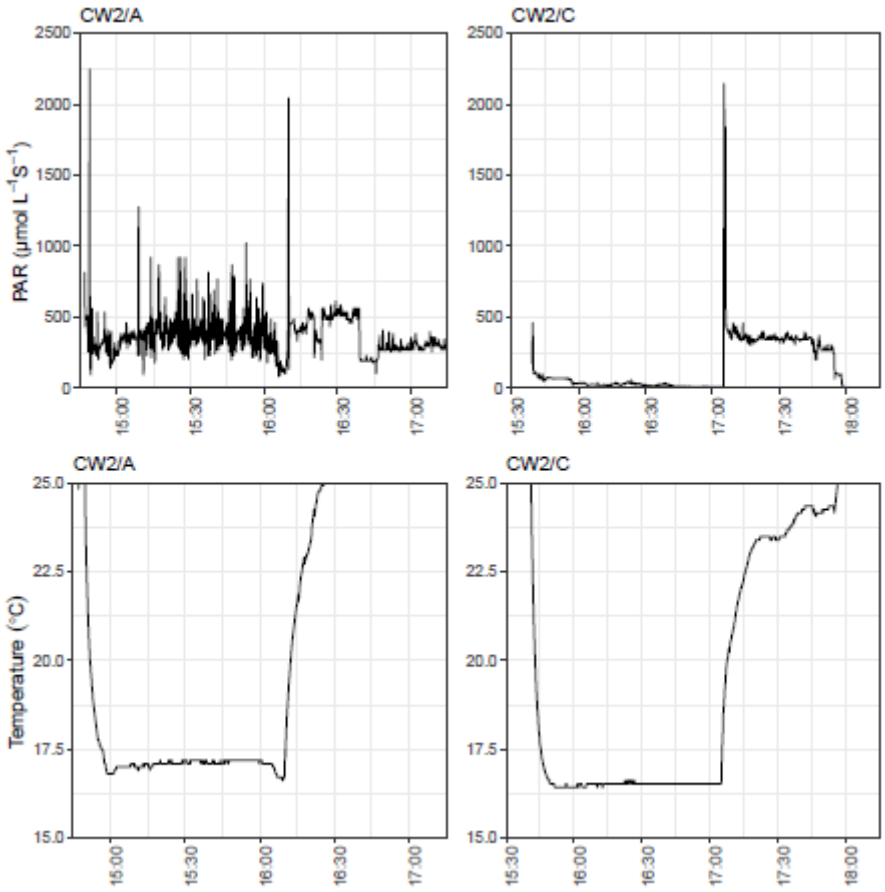

Figure 4.9 Light (PAR, µmol L^{-1} S^{-2}) and temperature recorded during incubation experiments in 2014. Each column shows data collected from a different site/patch, while line types distinguish results from each individual incubation board. Data has a resolution of 10 minutes.

were no trends over the differing treatments. Nitrate showed high variation of means and individual incubation NO_2^- flux – with raw results ranging from -0.008 to 0.037 mg NO_2^- L^{-1} hr^{-1} – and a maximum mean of 0.01 ± 0.007 mg NO_2^- L^{-1} hr^{-1} recorded at 25 % ambient light. Interestingly, one of the treatments – 100 % ambient light – showed an almost unchanged flux of NO_2^-, with a mean change of > 0.001 ± 0.005 mg NO_2^- L^{-1} hr^{-1} change recorded over the incubation period.

O_2 fluxes in 2014 incubations were always negative (Figure 4.10). There were no significant differences in oxygen flux found in CW2 - MC, but a significant reduction of oxygen was seen in CW2 - MP ($F_{3, 14}$ = 6.92, p = 0.004). This interaction was driven by a significant loss of oxygen in the 100 % light treatment (Tukey, p = < 0.05 for all comparisons) in relation to the other light treatment. Phosphate was recorded as below detection levels for all samples, and so no analysis was carried out.

Where O_2 flux was always negative in 2014 (Figure 4.11), plotting average light levels against individual recordings resulted in no light level threshold where sediments were net autotrophic (Figure 4.12). The polynomial curve produced by the data suggests that net-autotrophy would be achieved in higher light levels – averaging over 600 µmol PAR m^2 s^{-1} – which were not experienced during the incubations (Figure 4.12).

4.4 Discussion

The aim of this chapter was to examine differences between contrasting habitats within the same sub-catchment of the Hampshire Avon. This was driven by finding

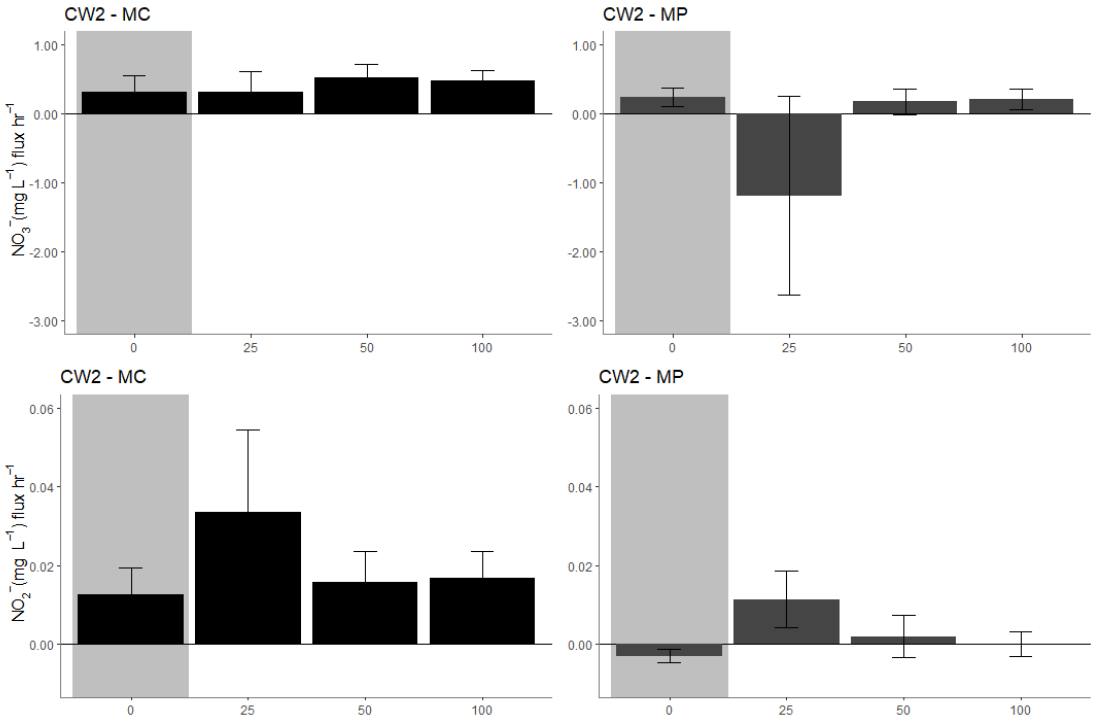

Figure 4.10 Potential nutrient flux of NO_2^- and NO_3^- per hour recorded during incubations carried out during 2014. PO_4^{3-}. Data was collected, but all concentrations were below detection level.

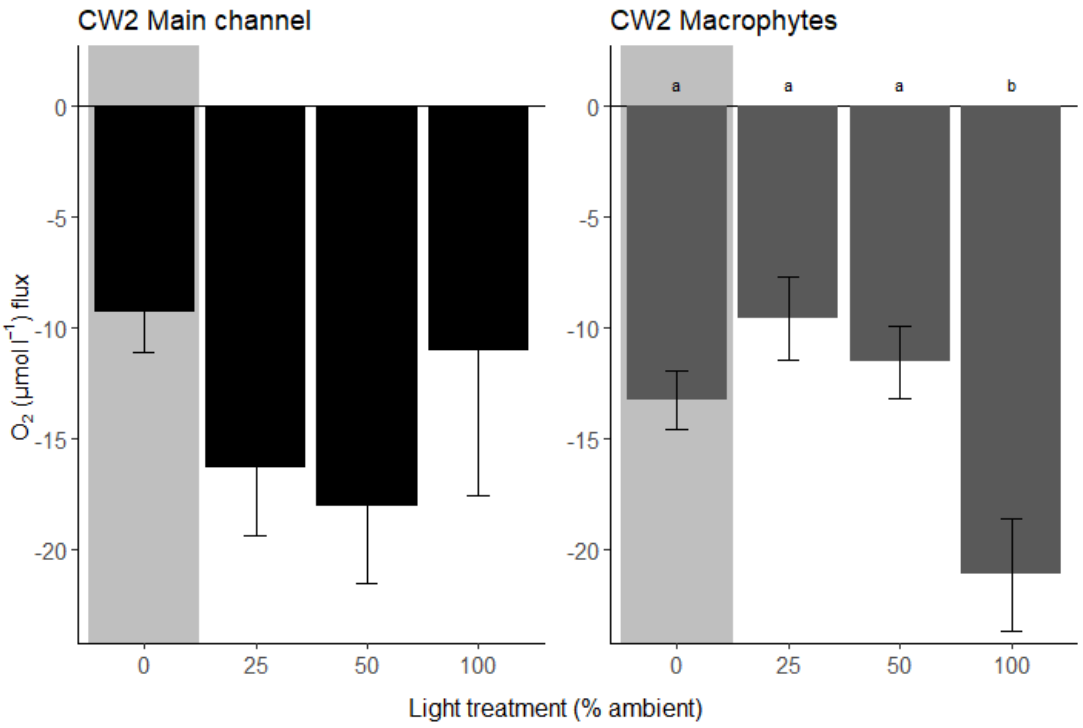

Figure 4.11. Mean O_2 flux (±SE) recorded during incubations carried out during 2013. Letter above bars indicate significant groupings.

differences between rivers belonging to similar geology types in Chapter 3. In this chapter, it has been shown that the two habitats within a river can diverge, particularly at levels of peak productivity. For instance, chlorophyll-*a* was recorded in significantly higher abundance in macrophyte stands in both greensand the chalk rivers, with these differences being found during April and August respectively. Similar responses were seen for CHO_D, and to an extent in CHO_{HW}. In these limited models, even where only a single difference was found, the overall ANOVA results suggested that significant differences in these factors could be seen between the habitat types in each river.

Macrophyte stands exhibiting higher levels of chlorophyll-*a* was not expected. The shaded environment, along with finer sediments and high levels of sediment deposition were expected to select for mostly motile diatoms (which are able to deal with constant deposition, Jones *et al*., 2012) and thus limit diversity. Despite this, and particularly in the greensand rivers, an abundant diatom at all times was *Cocconeis placentula* - a diatom which has a stalk attachment method. *Cocconeis spp.* are well established to be an epiphyte (growing on higher plants, Cox *et al*., 2011), so it could be hypothesised that the sediment retains cells originally growing on the macrophytes themselves. However, due to the nature of the methods used in this study, it was impossible to determine whether these cells were alive or merely dead cells from the above macrophytes (Cox, 1996). Increased concentrations of carbohydrates of all forms suggests that diatoms may play a large role in stabilising macrophyte sediment (Gerbersdorf *et al*., 2008), and also correlates with high abundance of motile diatoms.

Motile diatoms are likely to thrive in this area due to sediment deposition rates, as a method of dealing with covering is to move through the sediment to reach the surface-water interface (Jones *et al*., 2012). This method could also account for the high levels of chlorophyll-*a* in the sediments, as the increased proportion of fine sediments would increase the habitat carrying capacity in these areas.

Another benefit of EOC stabilised sediments is increased community stability for all inhabitants of the MPB. Fine sediment, when in a new environment, can be easily disturbed, leading to communities never reaching their successional climax. The established communities that are found in more stable sediment – for instance those sampled in this Chapter – may be more consistent regarding processes such as biogeochemical cycling. During the nutrient flux incubations that were carried out in 2013, only nitrite flux was found to differ between any of the light treatments. Sediment collected from CW2 – MC showed a large release of nitrite which was found to be significantly stimulated by higher light levels of 50 % and 100 % ambient light. This interaction was only seen in the well illuminated chalk stream - and results closely mirror those found with nitrate uptake at the same site, which was significantly higher than that measured in either the main channel or macrophyte stands of the greensand site. The chalk sediment was the only site to also show net primary production at high light levels, which were significantly different to the 0 % treatment but no differences in O_2 production were seen between the different light treatments. Light and temperature data recorded during the 2013 incubations was found to be of poor

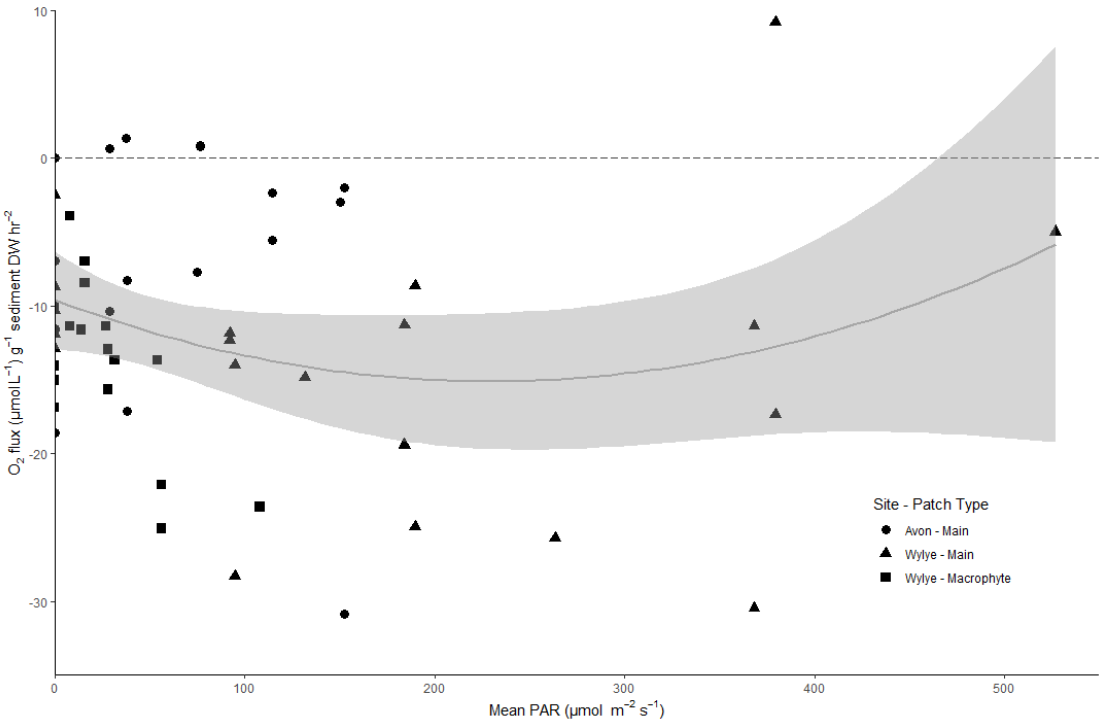

Figure 4.12. Change in oxygen concentration plotted against the average light levels experienced throughout the 2014 incubation period. Shaded area indicates confidence interval for polynomial curve.

quality (for example a peak of over 100 °c was recorded within one of the rivers). This was likely due to instrument malfunction. Although data were not completely reliable, light levels were averaged during the incubation period and plotted against the change in oxygen. A classical light response curve could be seen, indicating that the lack of net primary productivity seen in greensand rivers is likely due to light limitation. Chlorophyll-*a* concentration was also significantly lower that greensand rivers compared to chalk rivers (also seen in Chapter 3), supporting this hypothesis. Light levels are also likely to be behind increased nitrite release from chalk sediments, as nitrification is an oxic process which would be inhibited in areas of low oxygen production/high oxygen consumption (greensand rivers). Interestingly there was a slight decrease of mean nitrate flux at the highest light levels. It has been hypothesised that increased primary productivity also increases community respiration (Romaní *et al*., 2004) - and so at high light levels slightly lower oxygen concentrations within microhabitats may reduce nitrification. This was not seen in the oxygen flux data collected, however this measurement was recorded in the water column, whereas the biogeochemical activity is going to be present in the sediment pore water itself.

During 2014, the nutrient and oxygen flux was found to be very different compared to measurements taken during 2013. Despite only carrying out incubations in chalk river sediments, no net primary productivity was measured. Light treatments were also found to be significantly different, with the greatest oxygen consumption being found

at 100 % ambient light in macrophyte sediments. At the time of the experiment, sediments were covered with filamentous algae mats. This is likely to have outcompeted any MPB communities for light, and therefore primary production in sediments was limited. However, there were signs of biogeochemical activity, particularly respiration, suggesting that the primary production of the filamentous algae was fuelling sediment microbial metabolism.

The results presented in this chapter highlight the high variability in microphytobenthic communities (as shown in Chapter 3) and the biogeochemical cycles in which they play such a large role. Unlike work carried previously in this thesis, these measurements provide a potential of each measured variable, whereas the static measurements carried out in Chapter 3 provide a snapshot of the conditions of each variable at that moment in time. While both approaches are useful in understanding ecological systems, they both have advantages and disadvantages. For instance, although the characterisation of the two rivers studied in this chapter were featured previously, without directly recording factors such as the diatom community composition, it is not possible to be confident that the sediment sampled for incubation is representative of the overall river reach. This is further highlighted by the variability shown in Chapter 3, which used a large nested sample design to define river reaches at multiple landscape scales.

Another variable which was not addressed in the series of incubations was temporal changes to the environment. Although samples were taken over two consecutive

years, and both in July of each year (summer in the northern hemisphere) the results from each year presented contrasting results, particularly when considering oxygen flux. This suggests that between the sampling periods, the local environment changed enough to shift from net autotrophy to net heterotrophy, despite the experiments being carried out at similar times of the year. Other work has also shown similar interannual divergences, where diatom community composition was thought to be impacted by factors such as rainfall and anthropogenic factors such as water extraction in Mediterranean springs (Lai et al., 2018).

The results obtained in this Chapter compliment those from Chapter 3 – here however the inclusion of incubations to measure biogeochemical potential has allowed insight into functional differences between and within rivers at multiple landscape scales. In order to fully utilise the results of this chapter, modelling approaches could be used in the future. The short incubation time combined with multiple light levels could provide some insight into daily changes in ecosystem function. Each light treatment was constantly shaded, and so the light curve produced (Figures 4.10 & 4.11) could be used to model daily overall production. This approach would provide an opportunity to extrapolate this data to cover the whole reach, as multiple habitat types were sampled. Data presented in this Chapter however would not provide enough detail for studies of this nature – as there would be no reference information for seasonal conditions which have shown to be highly variable (Chapter 3). For instance, macrophyte growth is more likely to shade sediments during the summer months at

which this study was carried out, leading to a greater difference between MP sediments and those in the main channel. Seasonal succession within the microphytobenthos is also important to consider, as algal biomass peaks during the spring season (April, Figure 4.3). Seasonal changes in community composition are also not taken into consideration, and as such the results from the experimental data presented here only provides a narrow outlook of benthic biogeochemical processes.

Algal community structure has been shown to influence biogeochemical activity (Ishida *et al*., 2008). In a study of potential denitrification across multiple algal communities, it was shown that communities with a high proportional abundance of diatoms were most effective. While the composition of other algal groups was not considered in this study, future work should include other groups as a factor.

Many articles in the literature have highlighted the need to understand microspatial differences in freshwater sediments (e.g. Scott *et al*., 2008), and results from this chapter support this idea. Perhaps most interesting is the difference in the same habitat between 12 months of sampling. The main channel of CW2 was sampled in both the 2013 and the 2014 incubation campaigns, however they presented almost opposite results, particularly in regards to oxygen flux. This indicates that other factors that were not assessed in this study are at play, and in order to fully understand how MPB communities drive biogeochemical cycles a more intensive survey over multiple years with multiple factors may be necessary.

5 EFFECTS OF LIGHT AND DISSOLVED ORGANIC CARBON ON INTERACTIONS WITHIN MICROPHYTOBENTHIC COMMUNITIES IN RIVERS

5.1 Introduction

Ecological interactions are drivers of many functions in natural history, including evolution, ecosystem services and biogeochemical processes. While these ecological interactions can be both facilitative and competitive, many benefits to ecosystem function have been seen due to increased diversity - including in microphytobenthic communities - where niche differentiation to light conditions was shown to increase overall community productivity through cell stratification (Vanelslander *et al*., 2009). Microbial communities are also known to be able to complete many biogeochemical functions. The proximity of primary producers and heterotrophs in MPB communities provides the opportunity to directly trade beneficial substances - for example carbon, oxygen, and other nutrients - between different functional groups. The impact of the proximity to other functional groups in MPB communities is further compounded by strong redox gradients (the hyporheic zone) which increases functional diversity within these areas. Complex internal biogeochemical processes within the MPB can therefore be overlooked when examining systems at a large scale, as downstream concentrations of nutrients are typically the target of landscape scale studies.

Investigations into the interactions between different microbial groups within the MPB have yielded interesting results and further questions. As discussed previously in this thesis a primary carbon source for heterotrophic bacteria is autochthonous carbon - which is primarily extracellular organic carbon (EOC) produced by algae such as diatoms.

Light has been shown to impact many factors within MPB communities; including allocation of algal EOC (Moerdijk-Poortvliet *et al*., 2018), oxygen production (Chapter 4), community respiration (Romaní *et al*., 2004), and nitrification (Chapter 4). Light can also impact the stoichiometry of algae, which also has implications for the wider ecosystem through bottom-up stoichiometry (Guo, *et al*., 2016). Interactions between some of these factors has also been examined. It was suggested that under low-nutrient conditions that competition for inorganic nutrients between algae and bacteria limited algal growth, in turn increasing EOC production due to algal nutrient limitation (Scott *et al*., 2008). Other work has expanded on this, suggesting that this increase of labile carbon (relative to allochthonous sources) results in a feedback loop in which overall system productivity increases (Kuehn *et al*., 2014). The above examples were concluded from experimental data, and little work exists in natural MPB communities. Results from Chapter 3 show increases in extracellular carbon in response to dissolved organic carbon, which suggests that DOC concentrations within the sediment matrix may also promote these interactions.

These findings were also interesting because there were no manipulations of carbon conditions. In many studies, nutrient diffusing substratum (NDS) are often used to feed MPB communities grown directly on the surface. These methods - while cost effective and easily manipulatable, remove aspects of MPB found in soft sediment environments such as the hyporheic zone. These studies also favour use of glucose as

a carbon source - This carbon may not be the natural DOC source found within rivers, so therefore the community response may not represent typical DOC inputs.

This chapter aimed to investigate the impacts of light and carbon on MPB communities in two rivers. By using novel methods, the NDS principle was adapted to generate a DOC source within the sediment itself. Communities were also colonised over a 5 week period under varying light conditions. Previous work on these rivers (Chapter 3) also allowed for identification of the dominant carbon compound found within the DOC to be used to enrich DOC concentrations.

5.2 Methods

DOC and light manipulated experimental sediment was incubated in two rivers - GA2 and CW2 - both of which were previously studied in this thesis (see chapter 3). The colonisation period commenced 7 July 2015 at the two sites.

DOC was manipulated using 15 cm^2 discs of 2 % bacteriological agar (Oxoid, Thermo scientific, UK). DOC was added with additions of Sodium Acetate conforming to 100 % (DOC + 1) and 1000 % (DOC + 2) of DOC measured in pour water during August 2013. A control disc was made containing only 2 % agar. Discs were placed at around 3 cm depth of the final sediment set up. A sand and gravel aggregate was used as an artificial substratum. Sediment was sterilised in 3 % H_2O_2 for 1 hour, and rinsed using distilled water. Sediment was placed into plastic plant pots (maximum diameter 9 cm, height 6 cm) along with DOC amendments.

Light levels were controlled as per chapters 4. 100 % ambient light was left uncovered, while 50 % and 25 % ambient light was covered with a corresponding neutral density filter. 0 % light treatments were covered with aluminium foil, although in order to allow flow of water to the sediment filters were placed above the surface. Therefore light levels were controlled to estimated light levels, although all replicates were treated equally.

Light and temperature was measured continuously using a HOBO light/temperature logger (ONSET®, USA) set at a 30 minute resolution. After a period of 5 weeks, samples were removed from the river and sub-cored according to methods in Chapter 4. Each sample was placed into a TC flask and incubated according to the conditions under which it was colonised for 2 hours. t0 and resulting nutrient concentrations and dissolved oxygen was measured in line with methods from Chapter 4.

5.3 Results

5.3.1 Colonisation period conditions

Light levels between the two sites were found to be, at times, an order of magnitude greater in the chalk river compared to the greensand river, although for the majority of the time light levels were relatively similar (Figure 5.1). The average light levels recorded in during daylight hours in the chalk river was 113.20 ± 8.84 µmol PAR m^2 s^{-1}, while the greensand river experienced mean light levels of 42.31 ± 1.741 µmol PAR m^2 s^{-1} during daylight hours. There were large variation in the light levels throughout the colonisation period, but both rivers – due to their relatively close proximity –

showed higher levels of light in the later part of the experiment; particularly from August onwards (Figure 5.1).

Temperature was relatively consistent in the chalk river, showing diurnal fluctuation of around 3 °c, and mean temperature throughout the colonisation period was found to be 12.88 ± 0.08 °c in the chalk river, 14.64 ± 0.06 °c in the greensand river – both factors being typical of an aquifer fed river (Figure 5.1). Temperature in the greensand river, while on average greater than that of the chalk river, was more variable – diurnal fluctuations were less pronounced than in the chalk river (around 1 °c), but the temperature decreased by around 2.5 °c consistently during the last week of July. Temperature then increased sharply by around 2 °C over a two-day period, where it stayed at an intermediate temperature between 13 and 15 °C.

Incubation of colonised material begun on the 10th and 11th of August at the chalk river CW2 and the greensand river GA2 respectively. Due to instrument failure, the resolution of light and temperature was unable to be reduced from 30 minutes. For this reason, data during the incubation period for light and temperature were set from the mean data recorded in the 5 previous days for each site, providing a short term dataset over the whole period. This resulted in mean light levels of 95.84 ± 9.556 µmol PAR m^{-2} s^{-1} and temperatures of 12.50 ± 0.137 °C recorded in the chalk river, and mean

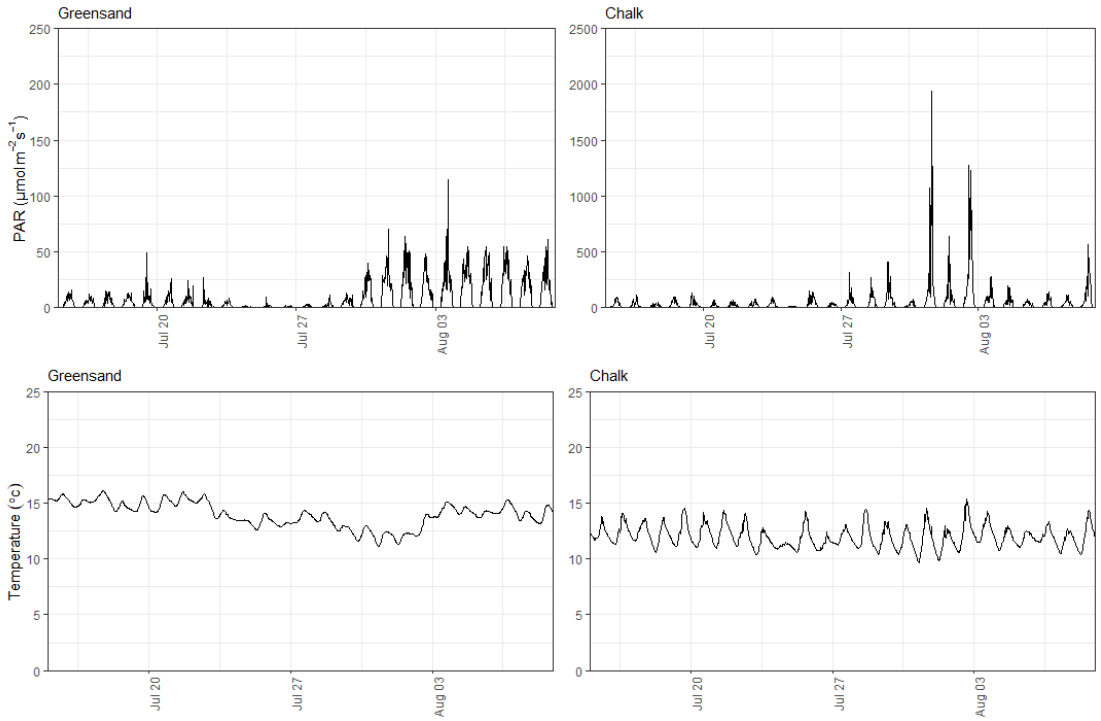

Figure 5.14. Data for light and temperature recorded by Hobo data loggers during the 5 week colonisation period in Greensand and Chalk rivers. Note the difference in order of magnitude experienced for PAR between the two test sites.

light levels of 37.18 ± 1.133 µmol PAR m^2 s^{-1} and temperatures of 14.24 ± 0.034 °c in the greensand river. These figures are representative of the overall dataset, and so were used in confidence.

Starting concentrations of oxygen in each of the rivers was relatively consistent, with chalk rivers having higher concentrations of dissolved oxygen than greensand rivers (68.76 ± 0.257 µmol O_2 L^{-1} and 59.19 ± 0.352 µmol O_2 L^{-1} respectively). Relative changes in oxygen concentration over the incubation period for greensand rivers were always negative, ranging from -20.17 ± 12.723 % to -1.57 ± 1.235 %. Despite this large range of oxygen fluxes experienced in the replicates, no significant differences were seen in O_2 concentrations by either light or DOC treatments in greensand rivers.

5.3.2 Post-colonisation incubation experiments

Oxygen flux over the whole experiment showed a wide range of results (Figure 5.2), ranging from -20.17 ± 12.724 µmol O_2 L^{-1} hr^{-1} recorded in greensand rivers (0 % ambient light, +1 DOC) to a maximum of 29.80 ± 12.868 µmol O_2 L^{-1} observed in the chalk river

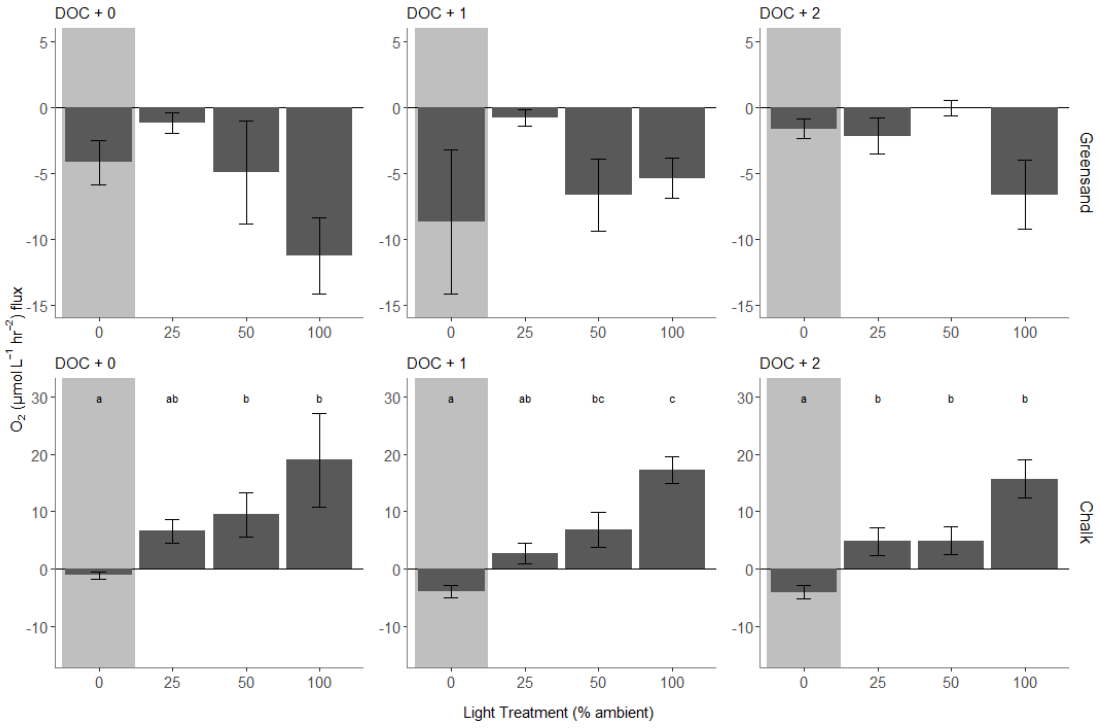

Figure 5.2. Changes in O_2 concentration during incubations for each river, light treatment and DOC treatment.

(100 % ambient light, +0 DOC). Flux of O_2 was always negative in greensand rivers (Figure 5.2), and individual replicates only showed positive values in 41 % of the conditions, the highest being found at 50 % ambient light and +0 DOC treatments (3.67 µmol O_2 L^{-1} hr^{-1}). In contrast, the greatest negative flux of O_2 recorded over the dataset was – 58.20 µmol O_2 L^{-1} hr^{-1}, indicating extremely high levels of respiration in the greensand river at 0 % ambient light and +1 DOC. Mean values of O_2 flux in the chalk river were mostly positive, with only three treatments seeing net heterotrophy (0 % ambient light and all 3 DOC conditions, Figure 5.2). The greatest negative O_2 flux recorded in the chalk river (-12.92 ± 3.481 µmol O_2 L^{-1} hr^{-1}) was second highest mean value across the dataset.

Oxygen flux was found to be different between sites ($F_{1, 72}$ = 69.59, p = < 0.001), light treatment ($F_{3, 72}$ = 13.03, p = < 0.001) and light treatments at each individual site ($F_{3, 72}$ = 11.70, p = < 0.001, Figure 5.2). Tukey *post-hoc* analysis revealed that differences in oxygen flux between light treatments in chalk rivers were the drivers for this overall effect, with all three DOC treatments experiencing increased O_2 in illuminated treatments over dark treatments. A light gradient was seen in + 100 % DOC treatment, with 100 % ambient light treatment being distinct from both 0 % and 25 % light treatments (Tukey, p = < 0.01 for both comparisons).

Significant differences in NO_3^- were seen in across light treatments in the overall model ($F_{3,72}$ = 7.18, p = < 0.001), which was determined to only be applicable to chalk rivers under the 1000 % increase in DOC treatment (DOC + 2, Tukey, p = < 0.01 for both

0 % - 50 % and 0 % - 100 %). There were no other significant differences between NO_3^- flux in any other treatment. Nitrite and phosphate concentrations were only found to be above detection levels in the greensand site. Fluxes of NO_2^- and PO_4^{3-} were not found to be affected by neither light nor DOC treatments (Figure 5.2).

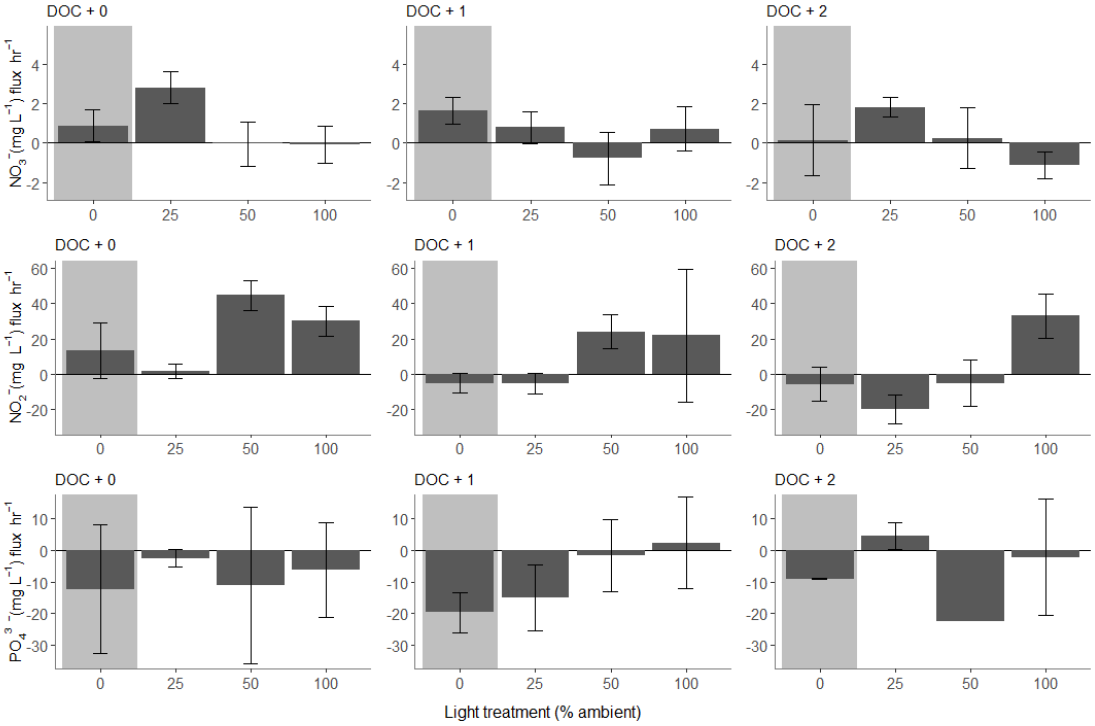

Figure 5.3. Nutrient flux during from greensand sediment incubation experiments.

5.4 Discussion

This chapter aimed to manipulate light and dissolved organic carbon in order to assess the response in biogeochemical cycles and to identify potential trophic interactions between auto- and heterotrophs. Two distinct rivers were used in order to generate two populations from distinct seeding populations. Light was shown to influence oxygen flux, but only in well illuminated chalk rivers. No response was found to DOC treatment for any biogeochemical interaction. Due to data constraints, it was also not possible to attempt to quantify any trophic interactions. The response to light is similar to that which was seen in Chapter 4. The change in O_2 was found to be greatest being dark and light treatments in the 100 % DOC treatment (DOC + 1), which could have been driven by trophic interactions. This may be the tipping point between where bacteria can utilise DOC in order to transform nutrients which are then liberated for algal growth, but not completely dominate a system. This recycling is similar to that seen in Danger *et al.* (2013), who showed that increased heterotroph activity was able to increase the pool of inorganic nutrients available for growth. If this is the case, then the reduction of an effect seen in the high DOC treatment (DOC + 2) would suggest at this point that in chalk streams bacteria are able to solely rely on allochthonous sources of carbon for growth (Scott *et al.*, 2008), and therefore begin out compete algal cells.

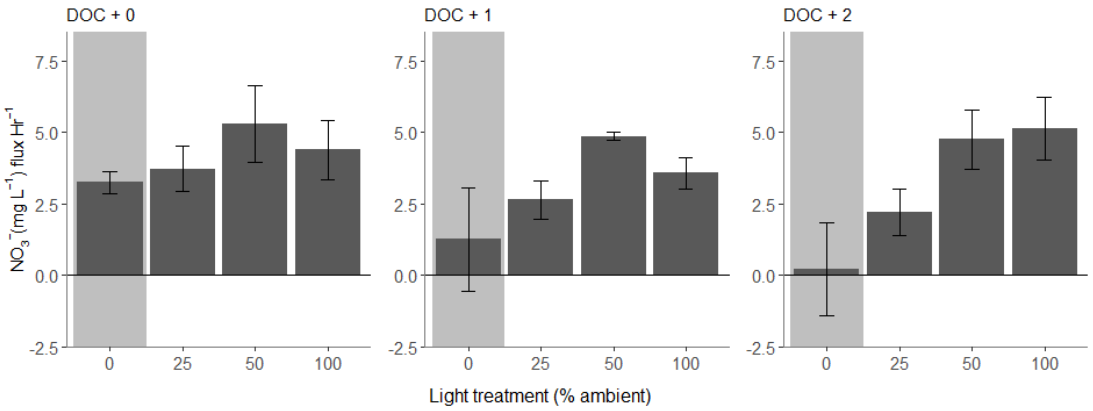

Figure 5.4. Nutrient flux during from chalk sediment incubation experiments.

This experiment utilised standardised sediment in an effort to reduce variability between samples, unlike work in Chapter 3 and Chapter 4 which instead used naturally occurring sediment. The purpose of this was to provide a similar colonisation surface, which had benefits and weaknesses. For instance, this allows for a more standardised approach to understanding each system – similar to deploying methods such as glass tiles used in many of the studies previously listed. Conversely, the standardisation of sediment may reduce the similarity to natural communities (Murdock & Dodds, 2007), leading to results not being fully representative of the intended outcome. Using a standardised approach for sediment would also remove sediment surface-area as a factor, standardising biomass to the best extent and removing any influence of sediment surface area on retention of dissolved nutrients (Bottacin-Busolin *et al*., 2009).

Direct interaction between algae and bacteria through priming is under heavy debate for its overall importance. Literature available is split between these interactions having a significant impact (Danger *et al*., 2013; Halvorson, Scott *et al*., 2016; Kuehn *et al*., 2014; Scott *et al*., 2008; Soares, *et al*., 2017) whereas others have either failed to find an effect or it has been minimal (Bengtsson *et al*., 2014; Catalán *et al*., 2015). The data presented in this chapter supports work where no priming effect has been found, although the presence of priming in some environments indicates that it is a complex process that may take a large amount of effort to fully understand.

The overall outcomes from the experiment presented in this chapter were inconclusive. One limitation of the work may be due to the nutrient diffusion discs not providing consistent and constant additions of DOC to the whole community. Because of the unknown flow of water over and through each sample, DOC may not have had an impact because it was not directly available to biofilm communities on the sediment surface. Many studies that utilise nutrient diffusion as a method for manipulating chemical delivery to systems use nutrient diffusing substrata – a well established experimental design which relies on communities growing directly on an artificial membrane surface (Bechtold *et al*., 2012; Gu & Wyatt, 2016; Warren *et al*., 2017) This experiment attempted to replicate this while also introducing a sediment overlay for colonisation, over a large temporal scale from colonisation to incubation, which may have exhausted C supplies.

Another potential reason for not finding evidence of interactions between DOC and light may be due to the timing of the experiment. Chapter 3 showed that concentrations of chlorophyll-*a* – indicative of algal biomass – were significantly lower in greensand rivers in August compared to April and November samples. While not statistically different, chalk rivers also showed a similar response, with biomass peaking in April and November. These peaks in algal biomass were also echoed in 16S abundance. Thus, despite the colonisation period covering a 5-week period, the communities may not have been at maximum potential for nutrient flux. With sub-optimal conditions, the small scale (spatially and temporally with the microcosm style

design) may have been insufficient to document consistent results. While the timing of the experiment to non-peak activity may have resulted in inconclusive results, it does provide an example of responses at a time of low activity – something which is rarely reported in scientific studies. Results such as these solidify concerns for sampling intensity discussed in Chapters 3 and 4.

Loss of connection to neighbouring ecosystems may have also resulted in modifying the manipulated microbial communities relative to their natural state. The primary linked system which could potentially influence MPB is the hyporheic zone. The removal of a closely linked site of high biogeochemical activity may have changed microscale delivery and retention of nutrients in sediment pore water, which were not assessed in the context of this study.

In order to gain a clearer understanding of these systems and how they act, future studies should utilise larger areas and shade natural sediment which could then be sampled for incubations. It would also be of benefit to repeat any nutrient flux experiments throughout the year to capture the variability which was highlighted in Chapter 3.

6 GENERAL DISCUSSION

Freshwater microphytobenthic communities on soft sediments are vital components within the realm of global nutrient cycles, despite only covering a small percentage of the Earth's surface. As 'veins of the landscape', rivers and their associated ecosystems can be influenced by many different factors, and understanding how and why rivers – particularly headwaters – transform terrestrial inputs is increasingly important.

Headwater rivers, such as those featured in this thesis, are subject to influences from terrestrial run-off and underground hydrological flow, the relative importance of each reliant on factors such as land use, geological features and sediment permeability. The dominant microbial groups in these systems – typically microphytobenthic communities of algae, bacteria and protists – are at the centre of biogeochemical cycles, but are not well studied in freshwater lotic environments.

The aim of this thesis was to examine the influence of catchment geology on microphytobenthic communities, and their associated ecological processes.

Chapter 3 aimed to examine river sediment communities over multiple catchment geologies - and in doing so developed a comprehensive, seasonal dataset which allowed for interesting insights into the variability of MPB communities in rivers which were classified as similar. Studies on rivers are typically focused on a single stream or reach. Where larger landscape scales are used (such as catchments), work is typically focused around land-use changes. While the variables which are focused upon in land-use studies are similar to those explored in the thesis, the impact of land-use would be contrasting against different underlying geology types. Therefore a more integrated

approach to the two would be beneficial in understanding the linkages between the terrestrial environment and microphytobenthic communities.

Chapter 3 was unique as the variables measured covered such a great span of the river. The mosaic of geologies that exist within the Hampshire Avon catchment (Figure 2.1) allowed for interesting comparisons across a gradient of base-flow; the proportion of water which enters a river through underground flow. It was able to show that rivers which existed within the same catchment were highly variable, and the nested design allowed for insight into rivers within the same sub-catchment. Chapter 4 expanded upon this spatial variability by examining within-reach differences, and combined these findings indicate the need to incorporate variability at all spatial scales into future experimental and survey design. Although fully understanding the finer drivers of each of these factors would be beneficial, it is unrealistic to assume that every future endeavour would cover such a wide range of influencing factors.

Diatom community composition allowed insights into the ecology of microphytobenthic communities, particularly how site-specific sediment and hydrological conditions can impact community development. Diatoms, which are used extensively as bioindicators, were shown to be heavily site dependent. Clay and chalk rivers – each at opposite ends of the BFI spectrum, had species which were indicative of the geology type. Greensand rivers had no defining diatom taxa which could be used as indicator species, but shared many species with both clay and chalk rivers. These findings suggest that diatom taxa within the Hampshire Avon Catchment are

distributed across the BFI gradient, and that some species which are valuable as nutrient bioindicators may change in abundance independently of water chemistry. Factors such as these are important to consider when assessing a group for tools such as bioindicators – and some of the current tools, such as the Trophic Diatom Index (TDI) do not local biogeography into account. The issue of substratum type is somewhat negated by TDI and other diatom indices, largely because of the use of standardised substratum. However, if the standardised substratum used for multi-river comparisons are not available or added artificially, then some significant species may not show expected abundances.

One of the thesis aims was to explore MPB communities across a range of natural environments relevant to understanding nutrient cycles within these diverse communities. Chapter 3 focused on characterising microphytobenthic communities across 3 distinct geology types within the Hampshire Avon catchment. Results presented showed differing seasonal cycles between the catchment sub-geologies for abundance of photosynthetic pigment, algal EOC and diversity, nutrient concentrations and bacterial abundances. Correlative work also suggested the presence of microbial trophic interactions under nutrient stress, supporting hypotheses from work based in other similar systems.

The complexity of river systems was explored further in Chapter 4, where two predominant habitat types – the main, uncovered channel and macrophyte sediment – were compared not only through static measures but also by through examining

sediment/water nutrient and oxygen flux. The findings of this study highlight some important points. Firstly, the differentiation between the habitat types over such a small spatial scale (< 15 m reach) shows how heterogeneity within a river channel. The significance of the heterogeneity spanned light levels, community composition and nutrient flux from sediment to water. Many studies on MPB biogeochemical fluxes in rivers occur without considering these factors – which depending on relative cover can have significant influences on overall system function.

To a lesser extent than in Chapter 3, Chapter 4 also investigated temporal differences between nutrient flux potential in river sediment. Light manipulated incubations carried out in the same reach in 2013 and 2014 showed contrastingly different results. The main channel of CW2 (chalk) showed net autotrophy in 2013 – with significant increases in dissolved oxygen being found in illuminated incubations – whereas in 2014 DO decreased at all light levels. The cause of the shift from net-autotrophy to net-heterotrophy was unable to be determined, but results such as these show that in order to truly understand river systems, a multi-year approach or long term monitoring is required. Therefore, results from Chapter 3, while spanning an annual cycle, can still only be seen as a snapshot of results rather than a comprehensive characterisation of the HAC for future predictions.

Chapter 3 presented the first account of negative correlations between production of EOC and dissolved organic carbon in a natural system (as opposed to experimentally manipulated). These results are consistent with the theory of trophic coupling, or

priming, which suggests that the metabolic processes of autotrophs and heterotrophs is linked. Many manipulative experiments have been conducted – with mixed results – which show that under high DOC concentrations, bacteria and other heterotrophs preferentially uptake this as opposed to algal EOC. This can lead to competition for nutrients, and therefore DOC is an important factor to consider when considering other biogeochemical cycles.

Chapter 4 presented the first functional recordings in the thesis. Nutrient and oxygen flux was shown to be active in sediment microbial communities, although varied across geology types and also habitat types within the same river. The variability of both community structure and sediment properties & biogeochemical processes such as oxygen flux supported each other, it can be concluded that differences in the biogeochemical potential of sediments is driven by changes to the microbial community and its interactions with the wider environment.

In an effort to assess the impact of additional, labile carbon on microbial processes, Chapter 5 experimented with creating MPB communities adapted to multiple light levels and DOC concentrations. It was a novel experimental design in that it used diffusion discs contained within standardised sediment, but the novelty of the design may have been a factor which lead to the experiment being inconclusive.

The incubation phase of the study was also carried out during a period of lower biomass, meaning that smaller incremental changes may have been masked by insufficient levels of activity to be detected using the methods utilised.

Fluxes of some nutrients – namely nitrate and phosphate – were too low to be detected by the methods used on many occasions. While this indicates that any existing changes may only be small, there may be significant shifts in nutrient concentrations which are undetectable. There is also the possibility that nutrients are tightly recycled, implying that as soon as they are freely available they are quickly assimilated.

The landscape scales used over this thesis ranged from the whole catchment of 1,750 m^2 to river reaches of around 5 m in length. The purpose of the use of such a wide range of landscape scales was to try to understand the ecology of microphytobenthic communities at multiple different levels, which are likely to be employed for legislative purposes by different organisations. For instance, local legislation such as land planning may take into consideration terrestrial nutrient transfer as well as nationwide legislation on fertiliser usage. While many landscape scales were covered in this thesis, the variability at each level and also each variable shows that more sampling effort would be required to make more comprehensive decisions.

Variation was shown to be large over all aspects of the project, particularly diatom communities. This has implications for future scientific work regarding biomonitoring, where standard approaches use sample pooling to replicate sampling at individual sites (Potapova & Charles, 2002). While many biomonitoring studies focus on hard substratum over the soft sediments which were sampled extensively in this thesis, variability of such environmentally sensitive microbial communities is likely to occur.

The study site of this thesis – the Hampshire Avon – represents a model catchment made up of multiple geology types. While some of the catchment geology types and combinations will be unique to the HAC, broad conclusions can be drawn which may be applied to other river systems within the UK. The significant variable shared within each of these geology types was base-flow index – the proportion of surface water to underground water through a catchment. The work presented in this thesis represents a gradient of base-flow conditions, along with associated sediment structure. These two variables are most clearly linked when examining the indicator species analysis in Chapter 3, where greensand rivers were shown as a transitional zone in preference between clay and chalk sub-catchments – sharing a total of 15 species which could be indicators for each underlying geology. While not directly applicable to other catchments within the UK, the work in this thesis may influence further research into BFI as a driver for MPB communities. The importance of BFI and catchment heterogeneity would need to be considered before using any similar approach, though this work demonstrates that variability can occur over a relatively small spatial scale.

Different influences, such as BFI, water chemistry from natural and anthropogenic sources, along with local variance of seasons and pressures makes the application of this work in the broad sense unsuitable to be applied elsewhere. This research highlights the importance of quantifying river heterogeneity when assessing other river systems, and the fine details of interactions between nutrient cycles and light, and oxygen production can be used as a base to build research in other areas upon.

The complexity of MPB communities does not allow every factor to be investigated, although some trends were easier to confer. There were large variations in static factors (algal biomass, TOC, algal EOC) not only between geology type, but also within geology types. While the definition of geology types was synthetic, diatom community analysis carried out in Chapter 3 showed that these were likely to overlap, and on the presented range of permeability (BFI), the intermediate geology type - greensand - was found to share significantly abundant diatom species with both clay and chalk rivers, while having no independent indicators. These results have influence on efforts to understand MPB communities and the rivers in which they lie, such as biomonitoring. For instance, the diatom trait based analysis carried out in Chapter 3 found no correlation on soft sediments between nutrient concentration and scores defined by the trophic diatom index (TDI; Kelly, 2001). These results mirror those found by Murdock & Dodds (2007) and Potapova & Charles (2005), both of whom showed the significance of colonisation type on selection of diatom communities. The TDI itself is vindicated as a biomonitoring method because of the standardised approach to targeting “rock” based communities, and standard sampling methods. However, the concern of rivers being dominated by soft sediments causing false results (Murdock & Dodds, 2007) due to adaptations of communities to their environments is supported by this work. Issues such as these are further amplified when examining differences between habitats of the same river. Chapter 4 found differences in community composition between MPB colonising the main channel, and MPB in macrophyte stand sediment. A comparable analysis was undertaken by

Potapova & Charles (2005), who examined the differences between hard and soft sediments in rivers. Their findings also showed that habitat type can drive MPB community structure, which implies that the themes seen here are common throughout rivers world-wide. Differences in biomass of both algae (through chlorophyll-*a*) and bacteria (through 16S rRNA quantification) were also shown to be different at multiple spatial scales. This has potential impacts for predicting biogeochemical cycling, which was shown to be true in Chapter 4 at both the geology/river scale explored in Chapter 3, and also within rivers across different habitat types. As with the seasonal data recorded in Chapter 3, this supports arguments to explore the level of heterogeneity in freshwater ecosystems when extrapolating data in application such as large scale modelling (Bechtold et al., 2012; Scott et al., 2008), where proportional contributions to major functions (such as nitrification, denitrification) could be scaled to habitat type found within a river. Modelling the wider implications of habitat type in headwater rivers is beyond the scope of this thesis, however utilising methods such as these will be beneficial for nutrient models in agricultural and urban areas.

This work, namely Chapter 4, presented evidence that oxygen produced during photosynthesis was able to stimulate the oxic process of nitrification, leading to increases in the release of NO_2^- into the surrounding water column. This was paired, although not significantly, with uptake of NO_3^-, suggesting the large algal influence on these systems. This also suggests that there is differentiation of uptake by algae and

bacteria in a facilitative interaction, as nitrification liberates nitrite from NH3 and NH4+. However, as seen throughout the thesis, these interactions are subject to change. Despite covering both the main channel and macrophyte sediment during the 2014 sampling campaign, results from this particular time period showed very different responses to nutrient and oxygen flux. During this time there was net respiration throughout the different light treatments, suggesting high levels of respiration at this moment in time.

Interactions within the microphytobenthic communities were not able to be determined in Chapter 5. There were however indications in all chapters that this was occurring, from the production of algal EOC (EPS and carbohydrates) in relation to water nutrient and sediment DOC concentrations, and through changes in oxygen flux in relation to DOC treatments. Further work would aim to allude these interactions by examining factors such as extracellular enzyme production, and radio-labelled tracer experiments. Many researchers have examined both of these methods in MPB communities (McKew *et al*., 2013; Taylor et al., 2013; van Oevelen *et al*.,2006).

Many of the methods used in Chapter 5 were novel. As such, this acted similar to a pilot study, which could contribute to the largely inconclusive results. Allowing the MPB to develop under varying light conditions was intended to account for light variances not seen in Chapter 4, where fully illuminated MPB communities were tested under temporary darkness. Many studies which examine light conditions on MPB communities do allow for development under these conditions Although this thesis

was composed from a relatively large dataset, the variability of MPB communities leads to the requirement for a higher sampling effort in any future studies. Some work has suggested that only a single, pooled sample would be sufficient to study MPB in freshwaters (Potapova & Charles, 2005). However approaches such as these are limiting in first avoiding the natural heterogeneity of these microbial systems, and also misses an opportunity to assess the variability between rivers.

The differences in sediment types present many conditions which were not assessed in this thesis. Water flow through sediment has an impact on microbial production (Battin, 2000), and, although this was inferred throughout the study of the BFI gradient in the Hampshire Avon, it was not directly measured. The overlap between these arbitrary catchment geology types is also apparent when looking at diatom community composition, and so clearer definitions would allow for the production of a model in order to better identity MPB communities and their biogeochemical contributions in relation to river conditions.

Further work would aim to tie down interactions by repeating experiments carried out in Chapter 5 in a more concise way. More samples, and a greater characterisation of nutrient profiles after addition will allow insight into underlying processes that are found in MPB systems. Stable isotope labelling would also benefit work in Chapters 4 and 5, describing the fate of nutrients in the system and monitoring their progression through the ecosystem.

This work was among the first to examine MPB communities on a range of soft sediments at multiple spatial scales. It has shown that these environments and communities can be significantly different in physical and biological composition, seasonal response and biogeochemical processing. It also highlighted the inherit heterogeneity and challenges of sampling natural, soft sediment in rivers, pushing the requirement for more intensive study in the future.

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APPENDIX 1: FULL LIST OF TAXA IDENTIFIED IN THIS THESIS AND AUTHORITIES

Note: Bold diatom taxa were included in all analysis.
***Achnanthes conspicua* Mayer**
Achnanthes spp. Bory
Achnanthidium microcephalum Kützing
***Achnanthidium minutissimum* (Kützing) Czarnecki**
Achnanthidium spp. Kützing
Achnanthidium subatomus Lange-Bert
Achnanthes oblongella Østrup
Achnanthes rupestris Krasske
***Amphora libyca* Ehrenberg**
Amphora normanii Rabenhorst
Amphora ovalis Kützing
***Amphora pediculus* (Kützing) Grunow**
***Amphora* spp. (Kützing) Ehrenberg**
***Amphora veneta* (Kützing)**
Asterionella spp. Hassall
Cavinula variostriata (Krasske) D.G. Mann ex Round
***Cocconeis pediculus* Ehrenberg**
***Cocconeis placentula* Ehrenberg**
Cocconeis placentular var. *lineata* (Ehrenberg) Van Heurck
***Cocconeis placentular* var. *euglypta* (Ehrenberg) Grunow**
Cocconeis spp. Ehrenberg
Craticula halophila (Grunow ex Van Heurck) D. G. Mann ex Round
Craticula cuspidata (Kützing) D. G. Mann ex Round
Cymatopleura elliptica (Brébisson ex Kützing) W. Smith
Cymatopleura solea (Brébission & Godey) W. Smith
Cymbella lanceolata (Ehrenberg) Kirchner
Cymbella ovalis (Kützing) Brébission & Godey
Cymbella spp. Agardh
Diadesmis contenta (Grunow ex Van Heurck) D. G. Mann ex Round
Diatoma ehrenbergii Kützing
Diatoma mesodon (Ehrenberg) Kützing
Diatoma spp. J. B. M. Bory de St-Vincent
Diatoma tenue Agardh
***Diatoma vulgare* Bory**
***Diploneis oblongella* (Naegeli) Cleve-Euler**
Ecyonema reichardtii (Krammer) D. G. Mann
***Encyonema silesiacum* (Bleisch in Rabenhorst) D. G. Mann**

Encyonema spp. Kützing
Epithemia turgida (Ehrenberg) Kützing
Eucocconeis flexella (Kützing) P. T. Cleve
Eunotia bilunaris (Ehrenberg) Mills
Eunotia exigua (Brébison ex Kützing) Rabenhorst
Eunotia minor (Kützing) Grunow in Van Heurck
Eunotia spp. Ehrenberg
Eunotia subarcuatoides Alles, Norpel & Lange-Bertalot
Fallacia pygmaea (Kützing) Stickle & Mann ex Round
Fallacia spp. Stickle & Mann ex Round
Fragilaria bidens Heiberg
***Fragilaria* capucina Desmaziére**
Fragilaria capucina var. mesolepta (Rabenhorst) Rabenhorst
Fragilariforma constricta (Ehrenberg) D. M. Williams & Round
Fragilaria perminuta Lange-Bertalot
Fragilaria vaucheriae (Kützing) Petersen
Fragilariforma virescens (Ralfs) D. M. Williams & Round
Fragilaria spp. H. C. Lyngbye
Fragilariforma spp. (Ralfs) Williams & Round
Frustulia rhomboides (Ehrenberg) de Toni
Frustulia spp. Rabenhorst
***Frustulia vulgaris* (Thwaites) de Toni**
***Gomphonema angustatum* (Kützing) Rabenhorst**
Gomphonema angustum Agardh
Gomphonema clavatum Ehrenberg
Gomphonema gracile Ehrenberg
Gomphonema minutum (Agardh) Agardh
Gomphonema olivaceum (Hornemann) Brébisson
Gomphonema parvulum (Kützing) Kützing
***Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot**
***Gomphonema* spp. Ehrenberg**
Gomphonema truncatum Ehrenberg
***Gyrosigma acuminatum* (Kützing) Rabenhorst**
***Gyrosigma attentuatum* (Kützing) Rabenhorst**
***Gyrosigma* spp. Hassall**
Hantzschia (Ehrenberg) Grunow
Karayevia clevei (Grunow in Cleve & Grunow) Round & L. Bukhtiyarova
***Kolbesia kolbei* Hust.**
Kolbesia ploenensis (Hust.) Round & L. Bukhtiyarova
Luticola goeppertiana (Bleisch in Rabenhorst) D. G. Mann ex Round et al.
Luticola mutica (Kützing) D. G. Mann ex Round et al.
Luticola ventricosa (Kützing) D. G. Mann ex Round et al.
Martyana martyii Round in Round et al.

Martyana spp. Round in Round et al.
***Meridion circulare* (Grev.) C. Agardh**
***Navicula angusta* Grunow**
***Navicula atomus* (Kützing) Grunow**
***Navicula capitata* Ehrenberg**
Navicula capitatoradiata Germain
Navicula cari Ehrenberg
***Navicula cincta* (Ehrenberg) Ralfs in Pritchard**
Navicula cryptocephala Kützing
Navicula cryptotenella Lange-Bertalot
Navicula decussis Østrup
Navicula digitoradiata (Gregory) Ralfs in Pritchard
***Navicula gregaria* Donkin**
***Navicula ignota* Krasske**
***Navicula lanceolata* (Agardh) Ehrenberg**
Navicula menisculus Schumann
***Navicula minima* Grunow in Van Heurck**
Navicula minusculoides Hustedt
***Navicula molestiformis* Hustedt**
Navicula protracta (Grunow in Cleve & Grunow) Cleve
Navicula radiosa Kützing
***Navicula reichardtiana* Lange-Bertalot in Krammer & Lange-Bertalot**
***Navicula reinhardtii* (Grunow) Grunow in Cleave & Möller**
Navicula rhynchocephala Kützing
Navicula saprophila Lange-Bertalot & Bonik
***Navicula slesvicensis* Grunow in Van Heurck**
***Navicula* spp. Bory de St. Vincent**
Navicula subminuscula Manguin
Navicula subrotundata Hustedt
Navicula tenelloides Hustedt
Navicula tripunctata O. F. Müller
Navicula trivialis Lange-Bertalot
Navicula veneta Kützing
Navicula virdula (Kützing) Ehrenberg
***Nitzschia amphibia* Grunow**
Nitzschia angustata (W. Smith) Grunow in Cleave & Grunow
***Nitzschia capitella* agg. Hustedt in A. Schmidt**
Nitzschia cf. *liebetruthii* Rabenhorst
Nitzchia clausii Hantzsch
Nitzschia denticula Grunow in Cleave & Grunow
***Nitzschia dissipata* (Kützing) Grunow**
Nitzschia epithemoides Grunow in Cleave & Grunow
Nitzschia filiformis (W. Smith) Van Heurck

Nitzschia flexa Schumann
Nitzschia fonticola (Grunow in Cleave & Grunow) Grunow in Van Heurck
Nitzschia frustulum (Kützing) Grunow in Cleave & Grunow
Nitzschia gracilis Hantzsch
Nitzschia heufleriana Grunow
Nitzschia inconspicua Grunow
Nitzschia lacuum Lange-Bertalot
***Nitzschia linearis* W. Smith**
Nitzschia microcephala Grunow in Cleave & Grunow
Nitzschia palea (Kützing) W. Smith
Nitzschia perminuta (Grunow in Cleave & Grunow) M. Peragallo
Nitzschia pusilla Grunow
Nitzschia recta Hantzsch ex Rabenhorst
Nitzschia sigma (Kützing) W. Smith
Nitzschia sigmoidea (Nitzsch) W. Smith
***Nitzschia* spp. Hassall**
Nitzschia subacicularis Hustedt
Nitzschia tubicola Grunow in Cleave & Grunow
Nitzschia vermicularis (Kützing) Hantzsch in Rabenhorst
Pinnularia abaujensis (Pantoscek) Ross
***Pinnularia appendiculata* (Agardh) Cleave**
Pinnularia borealis Ehrenberg
***Pinnularia* spp. Ehrenberg**
Pinnularia viridis (Nitzsch) Ehrenberg
Placoneis clementis (Grunow) E. J. Cox
Placoneis elginesis (Gregory) E. J. Cox
Placoneis spp. Mereschkowsky
Planothidium daui (Foged) Lange-Bertalot
Planothidium delicatulum (Kützing) Round et L. Bukhtiyarova
***Planothidium frequentissium* (Lange-Bertalot) Round et L. Bukhtiyarova**
Planothidium granum (Hohn & Hellerman) Lange-Bertalot
***Planothidium lanceolata* (Brébisson) Lange-Bertalot**
Planothidium rostratum (Østrup) Round et L. Bukhtiyarova
Planothidium spp. Round et L. Bukhtiyarova
Psammothidium lauenburgianum (Hust.) L. Bukhtiyarova et Round
***Pseudostaurosira brevistriata* (Grunow in Van Heurck) D. M. Williams et Round**
***Reimeria sinuata* (Gregory) Kociolek & Stoermer**
***Rhoicosphenia abbreviata* (Agardg) Lange-Bertalot**
Sellaphora bacillum (Ehrenberg) D. G. Mann
***Sellaphora pupula* agg. (Kützing) Mereschkowsky**
Sellaphora seminulum (Grunow) D. G. Mann
***Sellaphora* spp. Mereschkowsky**
Semiorbis spp. R. M. Patrick in R .M. Patrick et Reimer

***Stauroneis anceps* Ehrenberg**
Stauroneis kriegeri Patrick
Stauroneis phoenicenteron (Nitzsch) Ehrenberg
Stauroneis smithii Grunow
Stauroneis spp. Ehrenberg
***Staurosira construens* Ehrenberg**
***Staurosira elliptica* (Schumann) D. M. Williams et Round**
Staurosira spp. Ehrenberg
***Staurosirella lapponica* (Grunow in Van Heurck) D. M. Williams et Round**
***Staurosirella leptostauron* (Ehrenberg) D. M. Williams et Round**
***Staurosirella pinnata* (Ehrenburg) D. M. Williams et Round**
Staurosirella spp. D. M. Williams et Round
Surirella amphioxys Turpin
Surirella angusta Kützing
***Surirella brebissonii* Krammer & Lange-Bertalot**
Surirella crumena Brébisson ex Kützing
Surirella linearis W. Smith
Surirella minuta Brébisson ex Kützing
Surirella ovalis Brébisson
Surirella roba Leclercq
Surirella spp. Turpin
***Synedra acus* Kützing**
Synedra capitata Ehrenberg
Synedra parasitica (W. Smith) Hust.
Synedra parasitica var. *subconstricta* (Grunow in Van Heurck) Hust.
Synedra rumpens Kútzing
Synedra spp. Ehrenberg
***Synedra ulna* (Nitzsch) Ehrenberg**
Tabellaria spp. Ehrenberg
Trybilonella apiculata Gregory
Trybilonella hungarica (Grunow) Frenguelli

Appendix 2: Raw Data

date	geology	site.code	site.name	chl.a	phaeo	colloidal.ch	hw.cho	eps.30
February	Clay	1	AS1	3.572835	3.572835	288.707	170.771	
February	Clay	1	AS1	0.570298	0.570298	144.618	98.003	
February	Clay	1	AS1	3.126892	3.126892	166.566	121.014	
February	Clay	1	AS1	0.094683	0.094683	96.783	82.405	
February	Clay	1	AS1			238.099	244.393	
February	Clay	2	AS2	1.908053	1.908053	63.793	61.233	
February	Clay	2	AS2	2.248322	2.248322	58.59	55.566	
February	Clay	2	AS2	0.202742	0.202742	54.474	29.374	
February	Clay	2	AS2	0.7608	0.7608	56.261	39.932	
February	Clay	2	AS2	0.267127	0.267127	25.575	26.303	
February	Clay	3	AS3	4.164478	4.164478	90.951	83.834	
February	Clay	3	AS3	2.516881	2.516881	107.768	85.081	
February	Clay	3	AS3	3.632429	3.632429	79.468	74.124	
February	Clay	3	AS3	4.326064	4.326064	114.821	136.243	
February	Clay	3	AS3	3.576312	3.576312	147.575	92.858	
February	Chalk	1	CE1	6.262081	6.262081	39.301	36.351	
February	Chalk	1	CE1	8.509321	8.509321	15.156	11.684	
February	Chalk	1	CE1	11.43798	11.43798			
February	Chalk	1	CE1	7.541758	7.541758	41.994	41.711	
February	Chalk	1	CE1	7.804502	7.804502	27.627	16.278	
February	Chalk	2	CW2/A	8.116451	8.116451	28.784	20.71	
February	Chalk	2	CW2/A	4.970104	4.970104	40.821	103.158	
February	Chalk	2	CW2/A	7.363653	7.363653	31.772	67.441	
February	Chalk	2	CW2/A	4.997098	4.997098	24.671	101.566	
February	Chalk	2	CW2/A	2.869025	2.869025	33.739	89.006	
February	Chalk	3	CA3	7.132224	4.641321	41.92		
February	Chalk	3	CA3	4.601217	3.513971	42.376	27.197	
February	Chalk	3	CA3	2.871373	2.607401	16.167	12.874	
February	Chalk	3	CA3	2.570301	2.314501	20.382	9.525	
February	Chalk	3	CA3	7.578614	4.538611	66.696		
February	Greensand	1	GN1	1.50422	0.579533		6.004	
February	Greensand	1	GN1	1.235248	0.056146		8.347	
February	Greensand	1	GN1	3.150802	3.263536	42.741	30.61	
February	Greensand	1	GN1	1.539996	0.331657	6.284	21.109	
February	Greensand	1	GN1	0.551924	1.150407	2.51	4.54	
February	Greensand	2	GA2/A	0.568496	1.764505	23.403	20.235	
February	Greensand	2	GA2/A	0.972088	1.485637	66.086	76.353	
February	Greensand	2	GA2/A	2.693677	3.637416	53.882	61.719	
February	Greensand	2	GA2/A	0.742565	0.790763	67.205	64.693	
February	Greensand	2	GA2/A	0.218195	-0.21252	6.331	1.947	
February	Greensand	3	GA3					
February	Greensand	3	GA3					
February	Greensand	3	GA3					
February	Greensand	3	GA3					
February	Greensand	3	GA3					
April	Clay	1	AS1	6.189786	-0.05077	71.252	56.376	1.74
April	Clay	1	AS1	4.90543	1.22314	44.157	65.582	
April	Clay	1	AS1	8.058921	1.795121	54.614	60.209	
April	Clay	1	AS1	9.257361	7.861842	385.309	368.439	0.876

Appendix 3: Raw Diatom Count

	AS1-1	AS1-2	AS1-3	AS1-4	AS1-5	
Achnanthes_conspicua		8	1	0	0	2
Achnanthes_sp.		0	0	0	0	0
Achnanthidium_microcephalum		0	0	0	0	0
Achnanthidium_minatissium		3	1	0	0	1
Achnanthidium_sp.		0	0	0	0	0
Achnanthidium_subatomus		0	0	0	0	0
Achnanthes_oblongella		0	0	0	0	0
Achnanthes_rupestris		1	0	0	0	0
Amphora_lybica		2	3	0	0	2
Amphora_normanii		0	0	0	0	0
Amphora_ovalis		0	0	0	0	0
Amphora_pediculus		24	17	0	0	19
Amphora_sp.		2	0	0	0	0
Amphora_ventra		0	0	0	0	0
Asterionella_sp.		0	0	0	0	0
Cavinula_variostraiata		0	0	0	0	0
Cocconeis_pediculus		2	3	0	0	6
Cocconeis_placentula		5	9	0	0	0
Cocconeis_placentula_var_lineata		0	0	0	0	0
Cocconeis_placentular_var._euglycpta		0	0	0	0	0
Cocconeis_sp.		0	0	0	0	0
Craticular_halophilia		0	0	0	0	0
Cruticule_cuspidata		0	0	0	0	0
Cymatopleura_elliptica		0	0	0	0	1
Cymatopleura_solea		0	3	0	0	0
Cymbella_lanceolata		0	0	0	0	0
Cymbella_ovalis		0	0	0	0	0
Cymbella_sp.		0	0	0	0	0
Diadesmis_contenta		0	0	0	0	0
Diatoma_ehrenbergii		0	0	0	0	0
Diatoma_mesodom		0	0	0	0	0
Diatoma_sp.		0	1	0	0	0
Diatoma_tenue		0	0	0	0	0
Diatoma_vulgare		0	0	0	0	0
Diploneis_oblongata		8	5	0	0	20
Ecyonema_reichardtii		0	0	0	0	0
Encyonema_silesiacum		0	0	0	0	0
Encyonema_sp.		0	0	0	0	0
Epithemia_thrgdia		0	0	0	0	0
Eucocconeis_flexella		0	0	0	0	0
Eunotia_bilunaris		1	0	0	0	0
Eunotia_minor		0	0	0	0	0
Eunotia_sp.		0	1	0	0	1
Eunotia_subarcuatoides		0	0	0	0	0
Facilla_pymaea		0	0	0	0	0
Facilla_sp.		0	0	0	0	0
Fragilaria_bidens		0	0	0	0	0
Fragilaria_capucina		0	4	0	0	0
Fragilaria_capucina_var._mesolepta		0	0	0	0	0