

**PHOSPHORUS AND SEDIMENT – TWO OF THE MAJOR  
POLLUTANTS OF FRESHWATER STREAM ECOSYSTEMS –  
IMPACT UPON EPILITHON**

A thesis submitted for the degree of

**Doctor of Philosophy**

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By

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## **Declaration**

I hereby declare that no part of this thesis has been previously submitted to this or any other university as part of the requirements for a higher degree. The content of this thesis is the result of my own work unless otherwise acknowledged in the text or by reference.

The work was conducted in the field at Leicestershire, East Midlands of England and College of Medicine, Biological Sciences and Psychology, University of Leicester, UK during the period January 2013 to December 2016.

**Bassam Al-Yaseen**

## **Dedication**

This thesis is dedicated to my wife Saja who inspires, supports and loves. I could not have done it without you.

**Phosphorus and sediment – two of the major pollutants of freshwater stream  
ecosystems – impact upon epilithon**

**Bassam Mousa Abdulameer Al-Yaseen**

**Abstract**

Ecological degradation of rivers and streams resulting from multiple stressors is a big concern in the UK and other countries all over the world. The two largest stressors introduced by agriculture are phosphorus and fine sediment. The combined impacts of the multiple stressor and relative strength of each individual stressor needs to be understand.

A Number of ecological response variables were tested through a field mesocosm experiment, including algal and ecosystem variables: (1) The subsidy-stress for phosphorus and sediment (where at first, an ecological variable increases positively with the increased level of phosphorus and sediment until very high levels are reached, when negative effects would be expected); (2) Whether the stressors work individually or as multiple stressors and whether they interact; (2.a) Three ecological guilds of algae ('low profile' growth form, 'high profile' growth form, 'motile' growth form) were used in order to test whether the high profile growth form decreases and motile growth form increases with increase of sediment deposition, or whether (2.b) Both high profile growth form and motile growth form increase with increase concentration of phosphorus.

Most species showed subsidy stress responses for the gradient of phosphorus, but for the gradient of sediment the response was negative. Phosphorus and sediment together generally acted as multiple stressors and usually in a simple additive way, but complex interactions were also found. The algal community was impacted synergetically by phosphorus and sediment, as shown by the field study. The combined results from the field study and the mesocosm experiment indicate that phosphorus and sediment should be managed together in view of their acting most of the time as multiple stressors in their impacts on epilithic algae. Finally, in order to have a better evaluation for the possible reasons of a stream health decline, it is strongly recommended to measure routinely both fine sediment and phosphorus in the future.

## Summary

- 1 Multiple stressors introduction to streams, presenting a challenge where the relative strengths of each individual stressor and their combined multiple-stressor impacts must be understood. To investigate the patterns of the ecological response variables across different levels of two major stressors, increased levels of fine sediments and phosphorus.
- 2 A streamside mesocosm experiment was designed with eight phosphorus concentrations (using  $\text{KH}_2\text{PO}_4$ ) were used with eight levels of fine sediment in two replicate mesocosms of each treatment combination in early autumn 2015, and conducted a field preliminary study between May to September 2014 in streams in two regions of Leicestershire with different phosphorus gradients reported by a previous PhD student, but with previously unknown sediment gradients, namely as Eyebrook sampling sites and Upper Welland sampling sites.
- 3 The tested hypotheses were:
  - The subsidy-stress for phosphorus and sediment - where at first, an ecological variable increases positively with the increased level of phosphorus and sediment until very high levels are reached, which then have negative effects.
  - Whether the both stressors work alone or as multiple stressors and whether they interacted. Three ecological guilds of algae ('low profile' growth form, 'high profile' growth form, 'motile' growth form) were used in order to test that -
    - The high profile growth form decreases and motile algae growth form increases with increase of sediment deposition, and
    - Both high profile growth form and motile algae growth form increase with increased concentration of phosphorus.
- 4 In the 40-day long experiment (a 20-day colonization period and 20-day manipulative period), subsidy-stress patterns across the phosphorus gradient were frequently found for algal taxa and communities, but negative response were more widespread across the levels of sediment. Overall, fine sediment and phosphorus acted mostly as multiple stressors and sometimes in complex interactive ways.
- 5 The relative strengths of phosphorus and fine sediment impacts were similar for algal response variables, a finding that was also supported by the preliminary field data. My preliminary field data further suggested that sediment and phosphorus usually interact

in synergistic ways to influence algae, with sediment overwhelming any subsidy impact that phosphorus may have in isolation.

- 6 The combined field data and experimental results indicate that increased phosphorus concentrations and levels of sediment need to be measured together because they mostly act as multiple stressors in their impacts on algal response variables.

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## **List of abbreviations**

DIN	Dissolved Inorganic Nitrogen
N	Nitrogen
P	Phosphorus
SRP	Soluble Reactive Phosphorus
TP	Total Phosphorus
TRP	Total Reactive Phosphorus
TSZ	Transient Storage Zone
WFD	Water Framework Directive
Lux	lx

# CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

## 1.1 General Introduction

Algae are the most predominant primary producers in freshwater systems and are also responsible for the sequestration of nutrients such as Phosphorus (P). As a result they are very important components of both carbon and nutrient cycles (Vadeboncoeur and Steinman, 2002). Algal assemblages reflect their immediate water conditions, in these assemblages the main taxonomic group is often diatoms (Rouf, *et al.*, 2010), and diatoms have been used worldwide as a water quality biological indicators (Battarbee *et al.*, 2014).

Benthic (bottom-living) diatoms can respond quickly to both natural and anthropogenic environmental changes. For instance, the distribution and composition of benthic diatom species has been shown to be affected by a number of environmental factors, such as substrate type, current speed, light and temperature (Reavie and Smol, 2001; Saunders, 2011; Stewart and Lamoureux, 2012; Yang and Flower, 2012), in addition to sediment and P enrichment. Understanding the relationship between the composition of diatom community and different environmental conditions therefore has important applications for the conservation of biodiversity and the management of water quality. This is especially important in view of eutrophication as a world-wide problem, as a result of global increase in agricultural and domestic effluents (Liu *et al.*, 2012; Vonlanthen *et al.*, 2012).

Communities attached to a hard surface are the major producers within streams, particularly those with fast flow and are known as epilithon. The epilithon are mixed with fungi, protozoa and bacteria, making a biofilm (Battin *et al.*, 2003). Epilithon are the main primary producers up to mid-sized lotic ecosystems (third to sixth order) (Vannote *et al.*, 1980).

In all water systems, eutrophication and associated rapid increase of the epilithon have caused many problems, for instance depletion of oxygen, treatment problem for drinking water, toxicity, increased fish kills and decrease of recreational value (Quinn and Hickey, 1990; Biggs, 2000a; Smith and Schindler, 2009). The ecological cost of freshwater eutrophication in England and Wales is estimated to be between £75 million and £114 million per year (Pretty *et al.*, 2003). The European Water Framework Directive (WFD) stated that all water bodies should achieve “good ecological status” by 2015, this means that the biological community in water body should be as expected in the absence of anthropogenic impact, with only slight changes (WFD, 2000). For this ecological assessment, epilithon and macrophytes, are two of the required biological elements (Council of the European Commission, 1992).

High nutrient loading often results in epilithic blooms. Biggs (2000a) suggested that nutrient management should decrease both the duration and frequency of epilithic blooms. Depending on the river type, pragmatic management targets were developed by Mainstone and Parr, (2002) for the UK, which range from 0.02 mg L<sup>-1</sup> and 0.1 mg L<sup>-1</sup> Soluble Reactive Phosphorus (SRP), but the (Environment Agency, 2010) reported that more than half of the England's rivers have a SRP concentrations higher than 0.1 mg L<sup>-1</sup>. A P standard was generated by the "UK Water Framework Directive Technical Advisory Group" in 2005 for protecting the objective of ecological status in different categories of lakes and rivers, 0.05 mg L<sup>-1</sup> to 0.12 mg L<sup>-1</sup> Total Reactive Phosphorus (TRP) for good ecological status and from 0.03 mg L<sup>-1</sup> to 0.05 mg L<sup>-1</sup> TRP for high ecological status (Mainstone, Dils and Withers, 2008).

Nutrient loading to streams does not come alone, as indicated above; other factors such as light, temperature, substrate and current speed. There has been much less research into the combined effects of two or more factors affecting the epilithic – so-called 'stressors'. This thesis therefore, is an improvement in our knowledge of these combined effects as current knowledge of the effects of multiple stressors on ecosystem function is limited

## **1.2 Research Aims and Objectives**

The aims of this PhD study are to test the multiple stressor effects (individual and combined) on stream ecological response variables, and to investigate the interactions of two stressors (antagonistic or synergetic). My aim is to investigate biological response variables along gradients of inorganic nutrient (P) and fine sediment in order to test if they can act at low level as a subsidy and at high levels as stressors.

This research was completed in three phases: First phase, field study was carried out in two sub-catchments of the Welland to identify gradients in the environment and their effect on natural communities. Second phase, a mesocosm was constructed next to one stream and the two stressors added in order to test the effects of gradients upon communities. Third phase, the results of both earlier phases were analysed independently and jointly.

My research questions were:

- a) How does algal taxonomic composition in epilithic communities change with increasing P and with increasing sediment?
- b) How do the diatom contributions to the epilithic community change with these impacts?

c) How do the two factors interact with each other?

The literature review assesses current understanding of epilithon in terms of stream function, its sensitivity to the numerous stressors in that system, both individually and combined, and biomonitoring.

### **1.3 What Is The Role Of Epilithon In Streams?**

Epilithon provide a number of physical, chemical and biological functions within the stream ecosystem. Each depends on the epilithic biomass, so that as this biomass increases it becomes more important (Mulholland *et al.*, 1994). The epilithic' energy contribution within a stream can fluctuate significantly along the stream length and even in a short reach depending on the input of heterotrophs. For instance, input of detritus in mid reach streams is often low and the energy contribution amount of heterotrophs can be up to 60% (although seasonally). This is interpreted by the River Continuum Concept of Vannote *et al.*, (1980), which explains the relationships between the size of the stream and the functional and structural characteristics of the living communities in there. Epilithon' nutritional quality varies with water quality (Ledger and Hildrew, 2001), season (Ledger and Hildrew, 1998) and taxa (Lamberti, 1996). Epilithon could be considered as a "chemical modulator" which converts nutrients from inorganic form to organic form (Peterson and Grimm, 1992). Diurnal patterns in nutrient concentrations was observed by Triska *et al.*, (1989) who found that nitrate biotic uptake was higher in daylight. The epilithon also can help in purifying the water as it is capable of absorbing other materials, such as metal ions and serves as a breakdown site by bacteria for this and other organic matter contamination (Biggs, 2000b).

The boundary layer between the stream bed and the current, known as the transient storage zone (TSZ), is created by the biofilm is the interface for nutrient cycling to take place in streams (Webster and Patten, 1979; Paul *et al.*, 1991). The epilithon can either recycle nutrients already existing within the TSZ or extract the nutrients from the water column (Mulholland *et al.*, 1994), depending upon the epilithic mat's developmental stage. The downstream nutrient supply is influenced by inorganic nutrient uptake from the water column; however, once remineralisation or assimilation through successive trophic levels has occurred, these nutrients are transported further downstream where additional spiralling may happen (Paul *et al.*, 1991).

The stream near-bed hydraulic features can be changed by the epilithic accumulations, particularly the TSZ volume and dispersion coefficients, by altering the stream-bed roughness profile (Dodds and Biggs, 2002). The gaps between substrate in a rough stream bed can be

filled by the epilithic and subsequently smooth the profile, or the rougher biota can cover a smooth channel bed. As a result, even though epilithic growth may be influenced by hydrologic factors, the epilithon itself is capable of changing the stream bed roughness (Nikora *et al.*, 1997). The habitat and available food for higher trophic communities (such as fish and macro-invertebrates) also influenced by these changes. In lotic conditions, some taxa are capable of supplying habitat for epiphytes and meiofauna because they can offer a substratum that is attached securely against flow turbulence, provide protection from predators, supply food, for example the macro-alga *Cladophora* spp (Dodds and Gudder, 1992).

## **1.4 Factors Affecting Epilithic Growth In Streams**

Epilithic growth in streams is shaped to variable degrees by many different factors, such as light, temperature, stream flow, nutrient availability substrata properties and sediment.

### **1.4.1 Light**

Light is an essential requirement for phototrophic existence and photosynthesis responds to changes in light quantitatively (Hill, 1996). Light, for that reason, can limit the growth of epilithon even when other resources are available in greater quantities (Greenwood and Rosemond, 2005). Light maybe correlated with other factors, such as temperature, therefore it is difficult to establish its effects alone (Hill, 1996), nutrient concentrations can have an influence as well (Mosisch *et al.*, 1999)

Up to 95 % of incoming light, in small streams, can be obstructed by riparian vegetation (Hill *et al.*, 1995). This means that riparian vegetation often correlates with epilithic biomass (Hill and Harvey, 1990; Davies-Colley and Quinn, 1998; Hill and Dimick, 2002; Schiller *et al.*, 2007) but not if the vegetation is grazed heavily (Steinman *et al.*, 1992) An experiment conducted by Sabater *et al.*, (2000), where P and N were added to stream reaches, showed that nutrient retention and biomass doubled, but only when the surrounding riparian vegetation was removed, they concluded that light was the limiting resource. Some research has demonstrated that light and nutrients can co-limit stream systems (Rosemond, 1993; Larned and Santos, 2000; Hill and Fanta, 2008; Hill *et al.*, 2009; Liess *et al.*, 2009). Hill & Fanta (2008) indicated that 67% of epilithic biomass variation was explained by light alone, but light and P together explained 81% of the biomass increase. A few studies have shown that the light is less influential on epilithic growth than nutrient concentration (Rosemond *et al.*, 2000; Fanta *et al.*, 2010).

When light reaches the surface of the stream it is then attenuated by water molecules, suspended inorganic matter such as clay and silt and dissolved organic matter (Hill, 1996; Davies-Colley and Smith, 2001). Human actions often increase this turbidity (Quinn *et al.*, 1992). As soon as light does reach the epilithic mat it will be scattered and absorbed by the overstory cells (Hill, 1996). High-profile diatom species were observed by Lange *et al.*, (2011) to be prevalent at high light levels whereas low-profile species dominated at low light levels. Diatom taxa have been classified by Passy (2007) into low profile, high profile, or motile which are three special growth morphologies, selected to reflect their tolerance of differential potentials to physical disturbance and/or P limitation. Passy's study across the P gradient showed that under low P supply the community was dominated by the 'low profile' guild, the species of which did not develop thick algal mat, but as P supply increased the guild declined, and shading occurred, within algal multi-layered mats which were developed by members of the 'high profile' guild. Motile cells however, could escape physically from microhabitats with depleted resources, and with P augmentation the abundance of this guild increased.

#### **1.4.2 Temperature**

Most lotic species are poikilotherms (organisms whose temperature changes with their ambient) thus, physiological processes, productivity and growth rates are dependent on temperature (Giller and Malmqvist, 1998). The stream epilithon is usually significantly affected by water temperature, as other environmental like nutrient concentration or light usually tie in with temperature, making its impact not easy to distinguish (Larned, 2010). 10 to 30 °C is the optimal range of temperature for epilithon, with higher temperatures growth is reduced and heat stress induced (Larned, 2010). This temperature range suggests that in cold climates, thermal energy might be a limiting factor. Experiments elucidate that over a range of 5 to 25°C, photosynthesis increases and indicate the epilithic production in streams is linked strongly with temperature more than in oceans or lakes (Morin *et al.*, 1999).

Within the epilithon, different taxonomic groups dominate in different ranges of temperature: blue-green bacteria above 30 °C, yellow-brown and green algae between 15 and 30 °C, and diatoms between 5 and 20 °C (DeNicola and Hoagland, 1996). This tolerance indicates that in temperate rivers, the spatial patterns of epilithic communities are influenced by the thermal regime (Allan and Castillo, 2007).

Low flows, especially during summer, cause increases in water temperature as water depth decreases. According to Davies-Colley and Quinn (1998), wider streams (over 10 m width) are

exposed more to warming as they have less riparian shading. These factors in low flows increase the growth of epilithon (Biggs, 2000a). In contrast, low flows during winter, can lead to cooling and freezing, which damages epilithon (Angradi and Kubly, 1993).

### **1.4.3 Hydraulic pressure**

Many researchers have suggested that hydraulic pressure is the most important that determines the growth of epilithon. Other factors are influenced either indirectly or directly by hydraulics (Tett *et al.*, 1978; Biggs, 1996b; Elósegui and Pozo, 1998; Biggs *et al.*, 1999). Epilithon should stay attached to the substrate without being swept away by the current and must be capable as well of extracting the required nutrition from the current (Lampert *et al.*, 2007), this is called “subsidy stress” because flow has both harmful and beneficial effects (Biggs *et al.*, 1998). The beneficial effects include continual supply of gases and nutrients, which can increase the rate of metabolism and possibly also reproduction (Whitford and Schumacher, 1964; Stevenson *et al.*, 1996). The harmful effects are those related to increased pull, such as abrasion, shear stress and eventually sloughing. It also has been suggested that extracellular nutrients might be rinsed from the biofilm by increased current, which is a harmful as rendering nutrients in shorter supply (Humphrey and Stevenson, 1992).

In streams, the peak biomass occurs with the moderate or intermediate velocities of  $0.1 \text{ m s}^{-1}$  to  $0.2 \text{ m s}^{-1}$  (Biggs and Gerbeaux, 1993; Stevenson *et al.*, 1996). Peak biomass relies on light availability and river’s original nutrient status (Biggs and Close, 1989). Light intensity is lower in pools than in riffles as a result of deeper depths (Stevenson *et al.*, 1996). In moderate current, nutrients net gain (as the reload of the rinsed out nutrients happens quickly) plus the strong mechanical attachment are, in combination, reasons for greater biomass (Humphrey and Stevenson, 1992). In high nutrient streams, the benthic mat is thicker than in low nutrient streams, and to completely mix the overlying water through the thick mat, requires a greater current velocity (Horner *et al.*, 1990; Stevenson *et al.*, 1996). At  $0.3 \text{ m s}^{-1}$  to  $0.6 \text{ m s}^{-1}$  current velocities, and if the resources of light and nutrient are plentiful, increased biomass of filamentous green algae occurs (Stevenson *et al.*, 1996). Hondzo and Wang (2002) noticed that in stagnant water, filamentous green algal growth was minimal, emphasizing the importance of mixing to ensure nutrient influx to epilithon.

Greater biomass is provided by intermediate velocities over longer timescales, but over a short timescale, the picture is complicated. In low current velocity areas, the growing communities

develop faster and the biomass is greater than those communities growing in areas of high current velocities. They are packed loosely however, many cells are un-attached and many colonial or stalked forms are present (Keithan and Lowe, 1985). In medium to high current velocity areas, the communities are attached more strongly and will develop more slowly. In order to help the attachment against drag from current, the mucilage content may also be greater in these communities (Hoagland *et al.*, 1993; Biggs and Hickey, 1994; Peterson *et al.*, 1994). When floods occur, the more resistance communities are the ones growing in areas of medium to high current velocities (Peterson and Stevenson, 1992; Biggs and Thomsen, 1995).

Epilithic communities' architecture and growth form reflects their response to increased flow. For instance, diatoms, as colonising small organisms, use their mucilage in order to attach to the substrate along their length, so they can resist drag and benefit from increased flow to help nutrient diffusion (Allan and Castillo, 2007). Taking this position may also enable diatoms to withstand to some grazers (Peterson and Stevenson, 1992). Stalked or filamentous organisms, which are adapted more competitively to capture light and retrieve nutrients, will lose their nutrient uptake competitive advantage and face greater drag (Biggs *et al.*, 1998). Floods or extreme high water flow have the most effect upon the biomass of epilithic; Biggs, (1996a) explained that the accumulated biomass over long physical stability periods can be removed quickly by these disturbance forces.

Sediment size and type can have a considerable effect on the survival of epilithon, but this is itself affected by flow. When flow increases and causes scouring, the entrained sediments are abrasive to epilithon (Tett *et al.*, 1978; Francoeur *et al.*, 1998; Biggs *et al.*, 1999). High gravel and sand streams will have more epilithic abrasion (which is attached to immobile stratum) as a result of suspended sediments (Peterson, 1996). This gravel and sand can damage or dislodge attached epilithon (Blenkinsopp and Lock, 1994). If the small species are within recessed areas or crevices of the surface of substratum, they will be able to resist entrained the abrasive impact (Bergey, 1999). The entire epilithon will not be held in these crevices, but will does allow the survivors of flood disturbance to re-colonise (Bergey, 1999).

The community of epilithon will be an outcome of the cumulative impacts of previous flow events and current velocities (Tett *et al.*, 1978). Flood frequency also affects the diversity and the size of the epilithon (Biggs and Close, 1989; Fayolle *et al.*, 1998). Stable flow for a period of four to six weeks between floods is adequate for biomass accumulation (Biggs and Close, 1989; Biggs, 1996b; Biggs, 1996a).



#### **1.4.4 Substrata**

Submerged surfaces in natural aquatic environments are colonized quickly by epilithon. In the photic zones of aquatic systems, the epilithon attaches to diverse substrata (Wetzel, 1983). The epilithon comprises many habitat types, such as epilithos, epilithon and epipelon (Lane *et al.*, 2003), are of both industrial and ecological significance (Ford *et al.*, 1989).

Many types of substrate have been used in epilithon studies including hard substrates and living organisms. Some of these substrates contribute to the cycle of nutrient within the communities of epilithon attached on these substrates, living organism for example (Kahlert and Pettersson, 2002; Vadeboncoeur *et al.*, 2006). The hard substratum, on the other hand, works as inert adherent surface, and no evidence has been shown of chemical effects (Vadeboncoeur and Lodge, 2000). Several studies have found significant differences in periphytic characteristics between types of hard substrate (Sinsabaugh *et al.*, 1991; Sabater *et al.*, 1998; Àcs *et al.*, 2008). Epilithon characteristics also are affected by shape of substrate (Tuchman and Stevenson, 1980), microtopography (Murdock and Dodds, 2007) and the colonisation time (Cattaneo and Amireault, 1992; Liboriussen and Jeppesen, 2009).

#### **1.4.5 Fine sediment**

Water column turbidity increase created by suspended fine sediment will make light less available and consequently reduce diatom biomass and photosynthesis (Diehl, 2002). Biomass was reduced in experimental streams when clay was added (Parkhill and Gulliver, 2002). In reaches impacted by continuous fine sediment loads, gross primary production is lower because light is restricted by turbidity (Lloyd *et al.*, 1987).

It is not easy to separate the impacts of fine sediment other effects from reduced light availability. Once fine sediment has settled, IT has a direct shading impact on benthic algae, more so than when in suspension (Sand-Jensen, 1990; Vermaat and Hootsmans, 1994).

Shading from fine sediment deposition, for motile diatoms, may not cause as many problems as they can move to higher light intensities (Harper, 1976; Hay *et al.*, 1993; Yamada *et al.*, 2002; Dickman *et al.*, 2005). The initial response to 3-days (short term) sediment addition in indoor controlled experiment was a reduction in photosynthetic efficiency and chlorophyll a for the benthos, then 30-days after the deposition event, nearly a full compensation recovery happened but with different assemblage composition (Izagirre *et al.*, 2009).

Burial is another result, which changes the biological activity and flow patterns; concentrations of potentially and reactive reduced ions in sediments can also be toxic (Wetzel, 1983); Ivorra *et al.*, 2000; Ivorra *et al.*, 2002).

Smothering the substrata is the most profound impact of fine sediment deposition on diatoms. Non-motile species and especially chain-forming diatoms, cannot establish easily, changing the assemblages toward dominance by motile and single celled taxa (Dickman, Peart and Yim, 2005). The instability in patches results in lower biomass and taxon richness compared with patches that are more stable (Biggs *et al.*, 1998; Biggs and Smith, 2002; Matthaei *et al.*, 2003). Streams with unstable bed sediment support a lower biomass than those with stable beds (Iversen *et al.*, 1991; Biggs, 1995; Jowett and Biggs, 1997; Biggs *et al.*, 1999; Biggs and Smith, 2002).

River beds are a mosaic of depositing and eroding patches, with a history of disturbance that have both short term and long term impacts on benthic diatoms. As a result, within a river the assemblage of benthic diatoms may not be simple. Matthaei *et al.* (2003) found three months after a flood in the Isar river in Germany, where diatom chains were found buried in substratum, diatom taxon richness, total diatom density and algal biomass were highest in depositional patches; immediately after a second flood (six days) the biomass was highest in stable patches, and four weeks later, the diatoms were the most abundant in the erosive patches. They concluded that the history of disturbance and the succession of assemblage interacted together to determine patch development (Matthaei *et al.*, 2003). The development of assemblages will depend on the deposition rate and the frequency of fine sediment with which these deposits are remobilized after being disturbed by high flow.

Not all the fine sediment impacts are harmful to diatoms. Fine sediment deposition are generally nutrient rich, and nutrients will remobilise as a result of anoxic conditions that can develop (especially where there is high organic content). Benthic diatoms are in the perfect position to take advantage of these nutrients as they sit on the interface between the water and the sediment. Fine sediment deposits are rich in nutrients, can cause increased diatom growths and a shift towards nutrient-rich condition species as a consequence. The outcome depends on the balance of positive and negative impacts of fine sediment deposition on diatom growth, influenced by the stability and the rate of deposition. Where multiple stressors interact, complex effects may occur (Matthaei *et al.*, 2010). The fine sediment enrichment impact is particularly visible in permeable gravels, where the nutrients from fine sediment are dragged within the river bed in down-welling regions, whilst in up-welling regions, when the diatoms

attached to hard substrate return to the surface they can take advantages of these recycled nutrients (Dent *et al.*, 2001; Hildrew *et al.*, 2006).

Where fine sediment particles are both infertile and unstable, inorganic sands for example, increased growth presents small compensation for the negative impacts. Nutrient-rich fine sediment, on the other hand, can be colonized by the filamentous green algae, if relatively stable, such as *Ulvaintestinalis* and *Cladophora glomerata*.; in spite of chances for the epiphytic diatoms, these large species promote additional fine sediment deposition and decrease the assemblage of the benthic diatom that develops underneath them by burial and shading (Sand-Jensen *et al.*, 1989; Dodds, 1991).

#### **1.4.6 Nutrients**

Many micro and macro nutrients are required for protein synthesis and enzyme activity, although P and N are the primary nutrients in streams usually limiting the growth of epilithon. Limitation can also be caused by silicate which is required for diatom frustules (Haack and McFeters, 1982) even though this is unusual in streams (Allan and Castillo, 2007). In pristine river systems, the natural availability of P and N dissolved inorganic form from atmospheric inputs, upstream, surface runoff and ground water is much lower than their demand (Biggs and Close, 1989; Mainstone and Parr, 2002; Allan and Castillo, 2007). In many areas, the availability of P and N to fresh waters has increased by more than twenty times higher than background concentrations as a result of anthropogenic activities (Heathwaite *et al.*, 1996) for instance, 86% of SRP in inland waters is from human sources, while 70% of total nitrogen is from diffuse sources (Parr and Mason, 2003). The sources are surface water runoff from industrial pollution, sewage from animal and humans, agricultural fertilization and cultivation (Howarth *et al.*, 1996; Vitousek *et al.*, 1997; Galloway and Cowling, 2002), which all cause socioeconomic problems and eutrophication in water bodies (Pretty *et al.*, 2003).

In a pristine aquatic system, even though within catchments the P natural levels may vary, it is broadly agreed that UK upland rivers' SRP concentrations are below (Mainstone and Parr, 2002), however, due to the anthropogenic activities lowland rivers' natural levels are unknown (Dodds and Welch, 2000) but suspected to be below  $0.01 \text{ mg L}^{-1}$  (Demars and Harper 2005).

Anthropogenic impact in rivers can exceed Total P levels of  $1.5 \text{ mg L}^{-1}$  (Dodds *et al.*, 1998). The proposed SRP concentrations for lowland near-pristine upper limit is  $0.03 \text{ mg L}^{-1}$  (Mainstone and Parr, 2002), and worldwide average is  $0.025 \text{ mg L}^{-1}$  in natural systems for total

dissolved P concentration (Meybeck, 1982). Natural concentrations of both P and N geographically vary – with land cover, for instance TN and SRP are lower in wetlands than grazed pasture (Brion *et al.*, 2011), and with bedrock type, for instance, P levels are lower in crystalline bedrock rather than draining sedimentary areas (Dillon and Kirchner, 1975). It was initially believed that, due to the unidirectional flow, stream epilithon would not be nutrient limited (Grimm and Fisher, 1986). Nowadays this is known not to be the case as explained in studies of nutrient enrichment where, for instance, in a streamside mesocosm controlled nutrient gradient experiment, growth rates have been demonstrated to be N limited at 0.016 mg L<sup>-1</sup> SRP and at 0.086 mg L<sup>-1</sup> of dissolved inorganic nitrogen DIN (Stevenson *et al.*, 2006). The assessment of P and N ratio in the environment is a way to estimate if the epilithon is limited by P or N (Redfield, 1958).

Biggs (1990) shown in her study that nutrients are not always limiting in streams, as she found that below and above a P discharge the algal growth rates showed no differentiation, concluding that the concentration of nutrient upstream was already high enough to saturate the growth-rates of cells. Other environmental factors such as hydraulic pressure and temperature proved to be reliable additional indicators that growth rates were not nutrient-limited in the upstream communities. The relationship between biomass of epilithon in streams and uptake of nutrient has become a research and management issue, with the aim of decreasing the excess growth of epilithon (Biggs, 2000a). Usually the uptake rates increase with nutrient concentration till supply exceeds demand (Gregory *et al.*, 2002; Simon *et al.*, 2005). The uptake rates are related to the thickness of biofilm as it takes longer within thicker mats (Horner *et al.*, 1990), and also are related to the boundary layers (Mulholland *et al.*, 1994). Based on the hypothesis that diffusion through steady waters surrounding the algal cells controls nutrient mass transfer into the cells, Mulholland *et al.*, (1994) suggested the cycling of nutrient is related directly to the size of the TSZ.

The impacts of nutrients and hydraulics combined on the growth of epilithic community are highly correlated. Many laboratory and field studies have shown that during floods many nutrients forms are elevated, especially in enriched systems (Biggs and Close, 1989; Grimm and Fisher, 1989; Mulholland *et al.*, 1991; Humphrey and Stevenson, 1992; Peterson *et al.*, 1994; Biggs *et al.*, 1999; Biggs and Smith, 2002; Riseng *et al.*, 2004). In floods, SRP concentrations of SRP are more diluted, because at high flows the relative concentrations from point sources decrease (Jarvie *et al.*, 2006; Jarvie *et al.*, 2008). The impact of nutrients and velocity on the epilithon might be considered as a subsidy stress response as the chance of

nutrient depletion at the cell surface reduced with increasing velocity and the shear stress increasing with increasing velocity, which can cause sloughing (Biggs *et al.*, 1998). Streams could be considered to be in a retention mode at low velocities, and at high discharge they could be considered in a rinsing mode when epilithon have little chance to interact with nutrients input (Meyer and Likens, 1971; Royer *et al.*, 2006).

The optimal velocities for growth differ with the concentration of nutrient (Borchardt, 1994); the delivery benefit associated with high velocities are reduced by higher original nutrient concentration, even though in this case thicker biofilm mats might need to high velocities in order to deliver the nutrients to its base (Horner *et al.*, 1990). Humphery and Stevenson (1992) indicated that in nutrient rich streams, epilithic growth was stimulated, whereas in nutrient poor stream the growth was inhibited. This is because a nutrient net flux regardless of rinsing was still happening in nutrient rich stream, whereas in nutrient poor streams the nutrients washed off of the benthic mat and not reload again.

Individual species respond with varying efficiency to differences in nutrient concentrations (Paul *et al.*, 1991). This is because individual physiological characters, such as nutrient storage and uptake, in addition to different efficiency of usage (Borchardt, 1996). The varying storage and uptake abilities of species cause a co-limitation for nutrient in the community of a multi species environment (Tate, 1990; Francoeur, 2001; Tank and Dodds, 2003).

Epilithon are usually more effective at nutrient recycling in low nutrient concentration areas (Paul *et al.*, 1991), which generates a nutrient buffer so the diversity of the community does not necessarily change with the nutrient concentration changes (Mulholland *et al.*, 1991; Mulholland *et al.*, 1994; Greenwood and Rosemond, 2005).

(Rier and Stevenson, 2006) observed that diatoms still accumulated in their recirculating mesocosms even under exceptionally reduced nutrient conditions, and diatom growth could not be prevented, possibly due to heterotrophic diatom activity.. It is generally agreed that, in enriched streams, the most competitive species are elongate with high surface area and increased length, and these characteristics are most efficient in nutrient diffusion (Biggs *et al.*, 1998; Larned *et al.*, 2004). Pan and Lowe (1994) proved this also, when they found species succession from adnate to erect diatoms with increasing enrichment.

#### **1.4.7 Colonisation, drift and competition**

Initial colonisers of a bare substrate will be adnate algae, mainly diatoms. In order to reduce grazing and shear stress, these grow in a flat position on the substrate. These species, however, are poorly adapted for nutrient and light absorption and so are overgrown easily (McCormick and Stevenson, 1989). The first species to overgrow adnate algae are apically attached species due to their quick growth. In low current velocities these species stand erect on the substratum, and they consist of species such as *Synedra*. Eventually, slower growing filamentous species, stalked diatoms and sometimes motile species out-compete the apically attached and adnate species because of their better adaptations for light and nutrient absorption (Biggs, 1996a). This succession happens if the physical conditions are favourable within the stream, over time (Stevenson, 1996). Biggs (2006b) suggests as a rough guide that the incubation period before establishment of a mature community is four weeks.

Within streams, the drift is made up of benthic species and drift biomass is linked to benthic biomass (Butcher, 1932; Swanson and Bachmann, 1976). The emigration and immigration rates of epilithon vary and, depending on reproductive capacity, time of day and species. The daily turnover can be up to 5% in epilithic abundance (Stevenson, 1990). Autogenic factors can cause emigration, these factors include oxygen production and increased buoyancy post disturbance. Emigration may be due to allogenic factors such as grazer dislodgement, passing through the guts of grazers whilst staying alive, or disturbance caused by current velocity increase (Stevenson & Peterson, 1991). Drift abundances are positively related to immigration, a factor of emigration upstream. Current velocity is negatively related to immigration, as the rates of emigration exceed the rates of immigration with speeds greater than  $0.1 \text{ m s}^{-1}$ . Areas with slower flowing the rates of immigration exceeds the rates of emigration as these areas perhaps sinks for drift species (Stevenson & Peterson, 1991).

#### **1.5 Monitoring and Measuring Epilithon**

Water quality chemical measures, which include inorganic and organic pollutants, nutrients, salinity and acidity, all provide useful information, but they have cost and associated time constraints. Equipment, for instance, for continuous measurements should be left out in the field with the risk of destruction or flood damage., Biological measures however can indicate all water quality aspects over a number of years and provide a continuous measure of the impact of the environmental parameters . Biomonitoring gives an effective and affordable way to report a number of site conditions (Edward and David, 2010). Epilithon have some features

which make them better than other biota for biomonitoring (Lowe and Pan, 1996), such as they are smaller in size than other biota and so potentially more sensitive to pollution at lower concentrations and their communities are species-rich and each species has its own tolerances, so the assemblage represents an information-rich system;

Epilithic assemblage structure is widely measured by indices based on dominance, diversity, similarity and evenness (Ziglio, *et al.*, 2006). When comparing communities using these indices, differing site ranking may be produced according to the method and the weighting used (Nagendra, 2002). Another criticism of diversity indices, is that the lower and upper limits do not illustrate realistic ecological states. For instance, an unrealistic scenario is produced by the Shannon index, as zero lower limit meaning a community composed of a single species (Passy and Bode, 2004).

Bioindicator indices can either be numerical indices, depending on key indicator species, or a community evaluation involving multivariate analysis (Edward and David, 2010). The most common species can indicate the community type evaluation in relation to the water quality variables. For instance, (Round, 1993) based on results from different British rivers, suggested five increasing pollution zones and listed the main species of diatom recorded within those zones. The results from other studies, particularly the ones from different river sizes and from different geographical regions do not always agree with this type of result (Edward and David, 2010). Most bioindicator indices do not include filamentous algae but try to concentrate on diatom as epilithic representatives. Eventhough, diatoms in Europe are not generally responsible directly for the undesirable blooms of epilithon in rivers (Kelly *et al.*, 2009).

## **1.6 Freshwater Management and The Effects Of Multiple Stressors**

One of the main ecological degradations that rivers and streams experience is eutrophication as a result of the intensification of agricultural land use, which is threatening their biodiversity (Malmqvist and Rundle, 2002; Strayer and Dudgeon, 2010; Hamilton *et al.*, 2015). Dealing with multiple stressors resulting from human activities, for instance land use practices, is a big challenge in freshwater management (Sutherland *et al.*, 2006; Ormerod *et al.*, 2010). It is essential to understand the individual stressor effects in addition to the multiple stressors combined effects, in order to avoid ecological surprises that have resulted from the interactions of multiple stressors (Ormerod *et al.*, 2010).

A stressor is described as “a pollutant”, “pollution” or “pressure” in the policy and management context (Friberg, 2010); a variable that has exceeded its normal variation range as a result of human activities, and affects the individual taxa, ecosystem functioning or community composition. There could be positive or negative impacts on the biological response variables – the species (Townsend *et al.*, 2008).

The consequences of stressor loads on a stream ecosystems depend upon the catchment land use intensity (Allan, 2004). This increases with farming intensity as well as with the percentage land cover in the catchment under agriculture. There has been, in combination, an increase in fine sediment and P inputs to streams (Dolédéc *et al.*, 2006; Matthaei *et al.*, 2006) that mainly enter the stream via sub surface or surface runoff (Carpenter *et al.*, 1998; Cover *et al.*, 2008). These two types of disturbance are among the largest critical stressors in rivers and streams worldwide from agriculture (Allan, 2004; Paulsen *et al.*, 2008; Vörösmarty *et al.*, 2010)

Dissolved inorganic nutrients and deposited fine sediment are variables influenced by the geology of the catchment area (Richards *et al.*, 1996; Holloway *et al.*, 1998; Naden *et al.*, 2016). Researchers are trying to connect stressors directly from land use catchment changes, to the ecological endpoint changes, for example the composition of benthic algae that is usually used as an ecological condition indicator (Douterelo *et al.*, 2004; Kelly *et al.*, 2008; Delgado *et al.*, 2010).

The common theoretical framework for understanding the impacts of multiple stressor is that several potential outcomes will be produced for the ecological response variables: where the multiple stressor impact is additive, the outcome will be simple, where the multiple stressor combined impacts are either smaller or larger than impact of the additive single stressor, the outcome will be complex as stressors interact antagonistically or synergistically, respectively (Folt *et al.*, 1999; Vinebrooke *et al.*, 2004; Townsend *et al.*, 2008). The relationships of stressor response have been initially defined by using statistical approaches (parametric or nonparametric) for observational data across gradients of single stressor, (Yuan and Norton, 2003; Yuan, 2010) and extrapolation from these.

The multiple stressor impacts classification is one significant step in the research of multiple stressors. Understanding the response pattern's underlying mechanisms is another. It is important to test the hypotheses of multiple stressors that link the mode of action with expected outcome. Vinebrooke *et al.*, (2004) suggested that the effect of a second stressor (in case of two) on the biodiversity is set by whether the two stressors are tolerated by the species, which



is linked to the species' traits. No interaction outcome (simple additive) may be interpreted by two different theories: 1) "independent action" when the mode of action for chemicals is different, or 2) "concentration addition" when the modes of actions for different chemicals are equivalent (Greco *et al.*, 1995; Altenburger *et al.*, 2003). A possible consequence of different modes of action affecting each other is a departure from additive outcomes (antagonism or synergism). If different stressor combinations and each one results in individual multiple stressor outcomes (Crain *et al.*, 2008), and if natural stressors interact with anthropogenic stressors (Relyea and Hoverman, 2006) further complexity may emerge.

### **1.7 The Subsidy Stress Responses Theory**

"Perturbation theory" shows two ecological variables' response shapes across gradients of human perturbation (Odum *et al.*, 1979) – a unimodal shape, which is expected for the usable inputs, and a negative shape for the toxic inputs. The response across a gradient of subsidy stress describes a unimodal shape, where an ecological variable at low levels of perturbation is a subsidy, until reaching the maximum response at the perturbation point then the subsidy turns into stress. A stress response can show patterns of subsidy but both the negative (stress) and positive (subsidy) impacts are considered as a stressor impact outcome, as they cause changes from the reference conditions (Townsend *et al.*, 2008). The inflection point could be indicative of an ecosystem reduced stability (Odum *et al.*, 1979) and therefore it is a natural breakpoint that possibly is defined as a threshold of harm. Allan (2004) proposed another definition differentiating between these responses that indicate a biological condition's sudden decline at higher end and at lower end of the perturbation gradient.

Aquatic ecosystems are susceptible to various perturbation types from agricultural activities; the conceptual models of Odum *et al.* (1979) were applied by Quinn (2000) to benthic macro invertebrates, expecting P and light as usable inputs to conform to the responses of the subsidy stress, while sediment and pesticides to have negative effects only. Positive responses were shown by some invertebrates to the increasing amount of deposited fine sediment. Therefore, it could be considered as a usable input providing a habitat for some taxa in streams (Matthaei *et al.*, 2006; Townsend *et al.*, 2008).

Sedimentation was reported by most studies to have negative impacts on most invertebrate variables in streams (Rabení, 2005; Matthaei *et al.*, 2006; Larsen *et al.*, 2009), in agreement with the expectation of Quinn, (2000). On the other hand, macroinvertebrates' positive or

subsidy stress responses to the higher concentrations of P were more frequently reported (Heino *et al.*, 2007; Niyogi *et al.*, 2007).

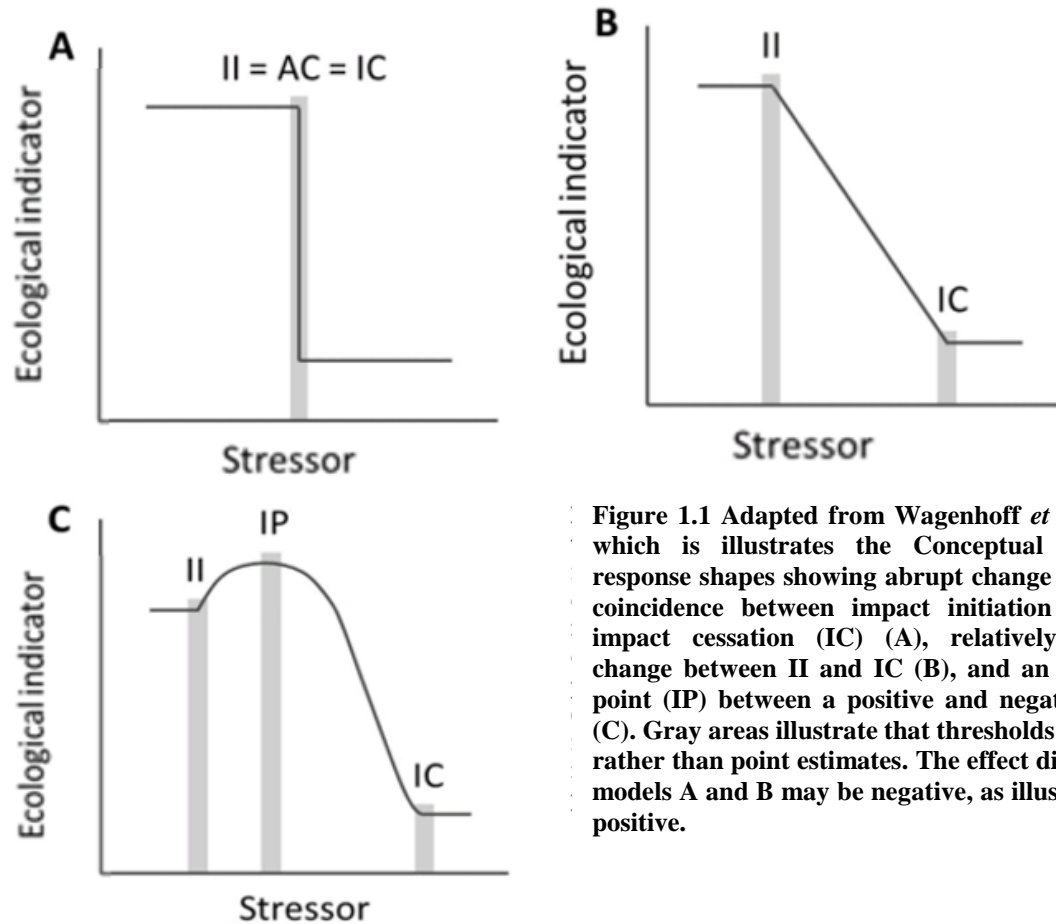


Figure 1.1 Adapted from Wagenhoff *et al.* (2017) which illustrates the Conceptual stressor–response shapes showing abrupt change (AC) and coincidence between impact initiation (II) and impact cessation (IC) (A), relatively gradual change between II and IC (B), and an inflection point (IP) between a positive and negative effect (C). Gray areas illustrate that thresholds are zones rather than point estimates. The effect direction of models A and B may be negative, as illustrated, or positive.

Wagenhoff *et al.* (2017) (Fig. 1.1.A–C) illustrated the conceptual stressor–response shapes for a wide single-stressor gradient that show thresholds of interest: 1) impact initiation (1st change of response rate away from zero, 2) impact cessation (last change of a positive or negative response rate to zero, 3) inflection (change of a positive to a negative response rate), and 4) abrupt change (i.e., the special case when the initiation of impact equals the cessation of impact). These thresholds have different ecological significance, which depends on the ecological indicator that is examined. For example, the initiation of impact thresholds at stressor values higher than background conditions can signify the resilience of an ecosystem, i.e., the capacity of ecosystem to absorb change in a driver variable without dramatic state change (Holling, 1973), whereas effect cessation can signify saturation (for example, with nutrients), exhaustion (for example, of habitat or capacity to assimilate nutrients), or severe

change of life sustaining attributes (for example, O<sub>2</sub> levels). Inflection can signify a subsidy stress gradient where the initial increase of sediment or nutrients has a positive impact on an ecological attribute (Odum *et al.*, 1979), such as a boost in macroinvertebrate diversity and production (Wagenhoff *et al.*, 2012). Last, abrupt change in the ecosystem functioning or structure of biotic community could signify the loss of one or several key species (Covich *et al.*, 1999).

The hypothesis of subsidy stress has not been studied specifically on the composition of the epilithic community or its biomass, even though theory suggests a unimodal response shape should be followed by epilithic diversity along disturbance and P gradients with the highest diversity at low to moderate levels (Biggs *et al.*, 1998). Additionally, functional variables of subsidy stress responses have been investigated in a very few studies. Along a gradient of land use stress, the metabolism of the ecosystem has followed a unimodal shape (Young and Collier, 2009) and stream tussock grass breakdown was correlated positively with elevated P concentration (Niyogi *et al.*, 2003). The observed effects of these two studies are probably multiple stressors operating product, which prevents any possible cause-effect relationships predations.

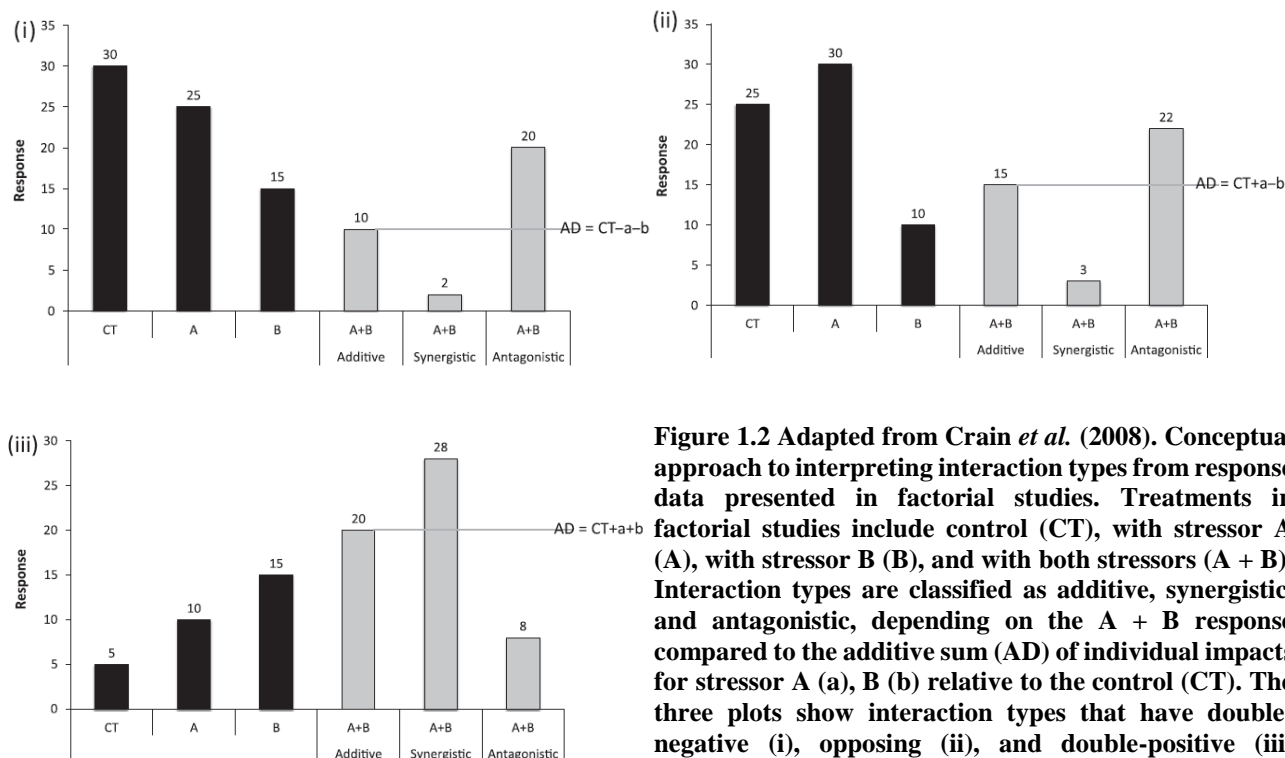
### **1.8 Multiple Stressor, Antagonism and Synergism**

The potential for complex antagonistic or synergistic interactions between multiple stressors shows one of the largest uncertainties when predicting ecological change (Sala *et al.*, 2000; Mothersill *et al.*, 2007; Darling and Côté, 2008). A consensus on synergism's operational definition is still lacking when classifying interactive effects, despite its common use in the scientific literature (Berenbaum, 1989; Folt *et al.*, 1999; Chou, 2010; Dunne, 2010; Vanhoudt *et al.*, 2012). In the context of ecological multiple stressors, synergism is used to define the cumulative impacts of multiple stressors that are greater than the additive sum of impacts produced by the stressors acting in isolation. This contrasts with the term "antagonism", which is used to define a cumulative impact that is less than additive (Hay *et al.*, 1994; Hay, 1996; Folt *et al.*, 1999). In an ecosystem, what is stressful or detrimental to one species could be beneficial to another, either directly or *via* species interactions. Stressor responses might follow a subsidy-stress gradient, e.g., for stream taxa in relation to the concentration of dissolved nutrient (Niyogi *et al.*, 2007). A stressor is therefore defined as a variable that, as a result of anthropogenic activity, exceeds its range of normal variation and impacts (whether positively

or negatively) individual taxa, composition of community, or ecosystem functioning (Breitburg *et al.*, 1999; Crain *et al.*, 2008; Townsend *et al.*, 2008).

The multiple stressor (additive, antagonistic and synergistic) responses for 171 coastal and marine system experimental studies, that manipulated two or more stressors, were assessed by Crain *et al.* (2008) who found both negative and positive single stressor responses. 38% of combined effects were antagonistic in individual studies, 36% were synergistic and 26% were additive, and an overall synergetic interaction effect was revealed across all studies. They concluded that the combined effects could be worse than expected on the basis of individual stressor basic knowledge. The three potential outcomes (antagonistic, synergistic and additive) can be applied when defining the stressor response surfaces along the gradients of two stressors but it is not always direct and simple because depending of the stressors levels, and across the stressors gradients, the two stressors might react differentially (Cottingham *et al.*, 2005; Piggott *et al.*, 2015).

Crain *et al.* (2008) analysed three interaction categories type based on the directions of individual stressor impacts: The two individual stressors operate positively (double positive), negatively (double negative), or with opposing (one negative and one positive) individual impacts relative to control conditions (Fig. 1.2). While the identification of an antagonism or synergism is usually straightforward when both stressors operate in the same direction (namely, double negative or double positive) (Folt *et al.*, 1999; Dunne 2010), for opposing individual impacts, the synergism definition could be contradictory because what is antagonistic to one stressor's impact direction is synergistic to the other stressor's impact direction and vice versa. Given the insufficiency of general agreement regarding these terms, Crain *et al.* (2008) believed that in the case where two individual stressors oppose each other, synergy only happens when the cumulative impact is more negative than the additive sum of the opposing individual impacts (see Fig. 1.2.ii). This could be appropriate in situations where the impact direction is implicitly negative (for example decreased survival rate), a definition like this is problematic from an ecological perspective because impact direction is completely context dependent. For example a data set for leaf matter decomposition where nutrient increase alone speeds up decay while sediment addition alone delays decay, but both stressors in combination cause a decay rate even faster than with nutrient increasing alone. This interactive pattern of leaf decay could be presented either negatively (as leaf mass remaining) or positively (as rate of leaf mass loss).



**Figure 1.2 Adapted from Crain *et al.* (2008). Conceptual approach to interpreting interaction types from response data presented in factorial studies. Treatments in factorial studies include control (CT), with stressor A (A), with stressor B (B), and with both stressors (A + B). Interaction types are classified as additive, synergistic, and antagonistic, depending on the A + B response compared to the additive sum (AD) of individual impacts for stressor A (a), B (b) relative to the control (CT). The three plots show interaction types that have double-negative (i), opposing (ii), and double-positive (iii) individual stressor impacts on the response variable of interest.**

## 1.9 What We Know and What Are The Gaps In Our Knowledge About These Impacts?

P is a vital nutrient for plant growth, and the concentration of P in surface water can be directly connected with the degradation of water quality through eutrophication (Sharpley *et al.*, 2001; Cordell and White, 2015). This is the term that describes the biological effects of elevated levels of plant nutrients concentrations (which are often P and nitrogen, but sometimes others such as potassium, silicon, calcium, manganese or iron) on the water ecosystem (Harper, 1992).

The loss of natural habitats with land conversion to farms and agricultural areas are major human impacts. The functioning and biodiversity of aquatic ecosystem are affected by these land use changes (Sala *et al.*, 2000; Bauer *et al.*, 2002). Researches on the impacts of human disturbance on biological assemblages have concentrated on responses to a single stressor, although most ecosystems under multiple stressors (Paine *et al.*, 1998). Anthropogenic impacts enhance biotic communities' changes and consequently ecosystem functioning (Pascoal *et al.*, 2003; Goudie, 2013; Loreau and Mazancourt, 2013).

The relationship between biomass of benthic algae and increasing levels of P concentrations have been studied extensively (Dodds *et al.*, 2002; Dodds, 2006; Smith and Schindler, 2009; Gudmundsdottir *et al.*, 2013; Sabater *et al.*, 2011). Growth assessment of benthic algae has

been done by nutrient concentration manipulation in field experiments (Bothwell, 1989; Walton *et al.*, 1995; Rier and Stevenson, 2006; Stevenson *et al.*, 2006), whilst links between algal biomass and P enrichment have been based on large scale surveys (Welch *et al.*, 1992; Dodds *et al.*, 1997; Chetelat *et al.*, 1999). In general, there is strong evidence that biomass increase of benthic algae and changing community composition are firmly related to the availability of P (Stevenson *et al.*, 1996; Wyatt *et al.*, 2010).

Diatom communities react to anthropogenic impact by changes in the ratio of tolerant: intolerant species to eutrophication (Fore and Grafe, 2002). Different field manipulative experiments have shown changes as an increase of growth variation and motile forms (Pringle, 1990; Kelly, 2003; Bellinger *et al.*, 2006; Wyat *et al.*, 2010; Gudmundsdottir *et al.*, 2013). Diatom community composition and relative abundance can quickly change and show adaptation to new nutrient conditions. This capability makes diatoms widely used to predict and understand the impacts of increasing levels of P on biological structure of river ecosystems (Kelly and Whitton, 1995; Kelly and Whitton, 1998; Kelly *et al.*, 1998; Kelly, 2003; Böhm *et al.*, 2013).

Benthic diatoms are thus frequently used for environmental condition assessment, such as P enrichment, habitat condition and water quality in rivers and streams (Kelly *et al.*, 1995; Pan *et al.*, 1999; Soininen *et al.*, 2004). Field studies can rarely link biotic patterns directly to a single variable (Oppenheim, 1991), although different species of diatom have shown different tolerance levels to different stressors in a laboratory experiment (Licursi and Gómez, 2013). A few studies such as those (Rier and Stevenson, 2002; Lange *et al.*, 2011) have been conducted in laboratory settings that analyze the diatom assemblages' responses to combined effects of multiple variables.

Establishment of causal linkages between ecological responses of stream and multiple stressors needs different research strategies (Culp and Baird, 2006). Integration of experiments and field surveys has been proposed by Cash *et al.* (2003) and Culp and Baird, (2006) because each approach has a different limitation and strength. A realistic study environment can be provided by field surveys but the possible interaction and coexistence of other influences on natural environmental gradients prevents the relationship of cause and effect being established when using the path only. A Controlled environment can be provided by experiments in stream mesocosms, but this lacks realism, especially regarding to a temporal or spatial scale. The gradients of multiple stressor can influence the ecological response variables in different ways. It is advisable to combine ecosystem, community and population level variables (Odum *et al.*,

1979; Culp *et al.*, 2000; Crain *et al.*, 2008, Sandin and Solimini, 2009) in addition to multiple organism or trophic levels (Biggs *et al.*, 2000). That would help to give a clearer understanding of the multiple stressor effect on the stream and to identify useful ecological indicators and indicator taxa.

Knowledge of the relative strengths of individual stressor effects and the combined effects of multiple stressors are crucial to make effective management decisions. Therefore, my thesis aims to investigate the individual and combined effects of multiple stressors on ecological response variables in order to inform resource management about potentially complex multiple-stressor interactions, the ecological response shapes to individual stressor gradients, the relative strengths of the individual stressors when both are operating

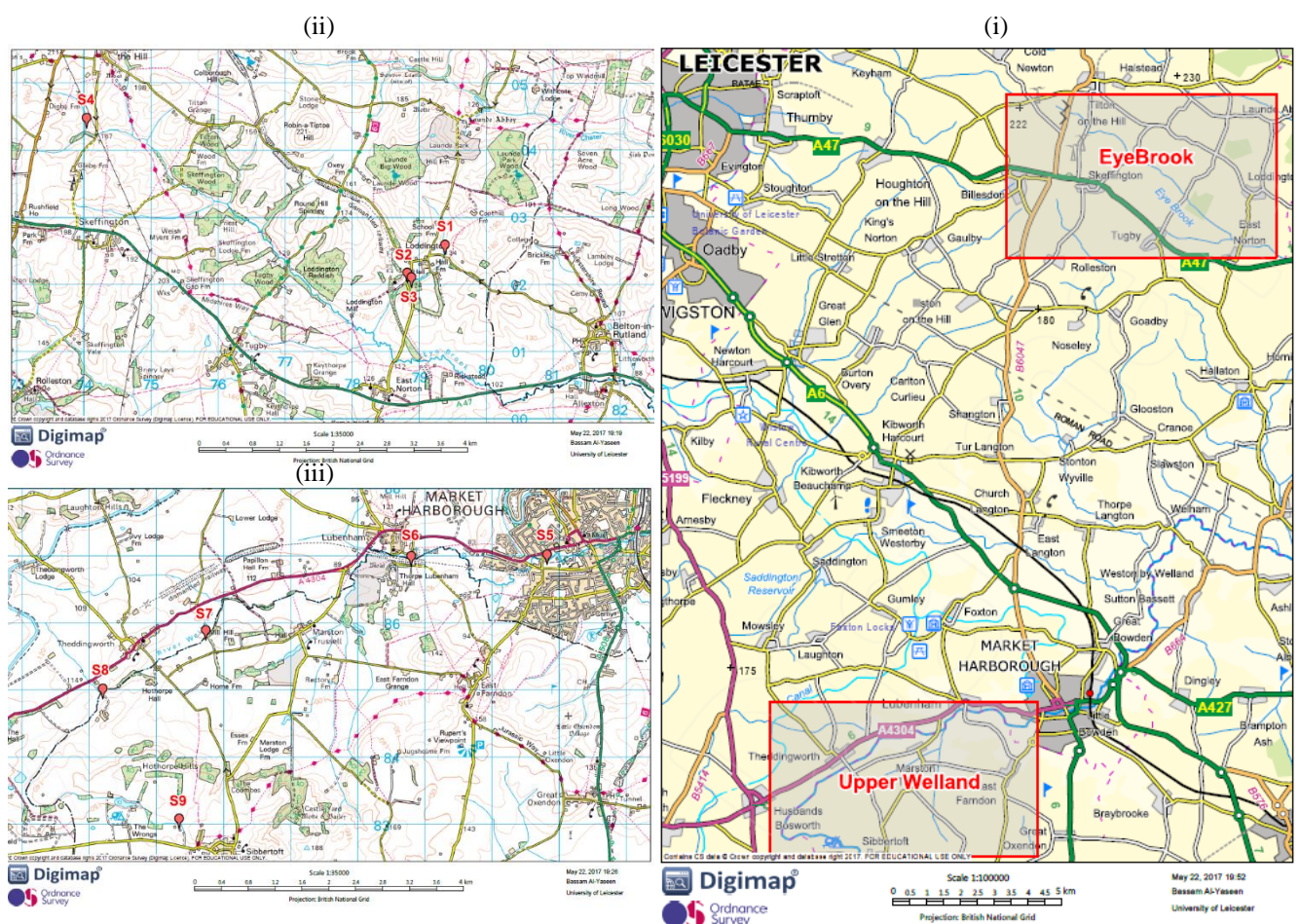
I used both a field survey and an experimental approach to draw conclusions about multiple-stressor effects. In my field preliminary observations (Chapter four), I tested three methodological objectives enabled me to design the mesocosm experiment of chapter five, where the epilithic algae responses to broad gradients of both phosphorus and fine sediment have been experimentally tested.



## CHAPTER TWO: STUDY SITES

### 2.1 Study Sites

Streams in two regions of Leicestershire with different P gradients (as (Wasiak, 2010), but with unknown sediment gradients were chosen as the main study sites and named as Eyebrook sampling sites and Upper Welland sampling sites (Fig. 2.1).



**Figure 2.1** Maps showing the location study sites (i) the EyeBrook and Welland Sampling sites (ii) four sites were chosen in the Eyebrook: S1) Loddington School Farm, S2) Loddington White Horse, S3) Loddington Lone Pine, S4) Tilton Digby Farm; and (iii) Five sites were chosen in Upper Welland: S5) Market Harborough, S6) Lubbenham; S7) Papillon Ford; S8) Hothorpe and S9) Sibbertoft.



The EyeBrook catchment lies in the heart of England, straddling the county boundaries of Leicestershire and Rutland. The Eye Brook is a tributary of the River Welland, which delivers its water into the Wash, the United Kingdom's largest estuary, its most important shellfish producing area, and a key site for migratory wading birds. If the Eye Brook catchment is 'isolated', it is only in the sense that it is rural. Most of the 67km<sup>2</sup> catchment is farmed, but the area also includes several large ancient semi-natural woods, and Eyebrook Reservoir, towards the bottom of the catchment has been an additional feature since 1940. Crops such as wheat and oilseed rape are produced, and livestock farms provide lamb and beef, as well as some milk (Stoate, 2010)

The Upper Welland catchment is predominately rural with mixed arable and livestock farming. The main Welland has a broad floodplain with steeply sloping bluff lines. The headwaters of the Welland and tributaries are more steeply sloping. This operational catchment supplies Rutland Water, an important wildlife and amenity site, but primarily a major source of drinking water. This main rivers have been heavily engineered to improve land drainage in the late sixties and early seventies. It used to be a popular fishery but is less important now (Environment Agency, 2016)

Digby farm as lowest impact due to pasture catchment, Schoool farm and White horse as affected by septic tank effluent; Sibbertoft as being at outlet of village sewage treatment works as well as effective source of the Welland in dry seasons

## 2.2 Mesocosm Experiment Study Site

The study was conducted at School Farm site (Fig. 2.2) from the 21<sup>st</sup> of September to the 30<sup>th</sup> of October 2015 (British early autumn). The School Farm stream, a tributary of the Eye Brook in Leicestershire, England ( $52^{\circ} 36' 45''$  N  $0^{\circ} 49' 47''$  W).



**Figure 2.2** Map showing the location of the mesocosm experiment site in Loddington School Farm, in Leicestershire where experiments investigating the responses of epilithon across wide gradients of sediment and P was conducted for six week (September – October, 2015).

## **CHAPTER THREE: MATERIALS AND METHODS**

### **3.1 Artificial Substrates**

Artificial substrates are used frequently to evaluate the assemblages of benthic algae in stream (Aloi, 1990; Cattaneo and Amireault, 1992). The artificial substrata I used were made of unglazed ceramic tiles, as recommended by Kelly and Whitton (1998) and APHA (1998) for monitoring and research programmes.

They consisted of unglazed ceramic tiles (4cm x 5cm) from Homebase, UK with tile thickness 0.65 cm approximately, cemented to a heavy base in sets of three. Substrate sets were placed in the river beds, two sets (i.e. six tiles) for each replicate. For testing whether the tiles are valid artificial substrates for epilithic growth or not, and to quantify the most effective current speed, tiles were placed at five different current speeds, in locations that contain similar sized natural substrates (stone) in each stream, and sampled up to four weeks (Cattaneo and Amireault, 1992; Hürlimann and Schanz, 1993). The ceramic tiles for quantifying the most appropriate length of time for tile to be exposed were placed next to each other in each stream and sampled on a weekly basis.

Two tiles per set (one from each base) were used for algal identification, one for biomass measurement and one for sediment measurement.

#### **3.1.1 Field collection**

Samples from natural (stone) and/or artificial substrate (tile) and water for P analyses were collected. Each substrate (stone or tile) was placed inside a plastic bucket containing stream water and labelled with the collection date, stream's name, temperature and current speed. The lids were attached to the buckets and transported back to the laboratory, then stored in a cold room overnight.

Algal colonisation on the tiles was measured after 1, 2, 3 4 5 and 6 weeks of exposure in order to find out the most appropriate length of time for exposure.

Water temperature was measured using a glass thermometer (Fisher Scientific Ltd, UK).

### **3.2 Algal Identification**

#### **3.2.1 Preparing samples**

Algae were removed by vigorously scrubbing the upper surface of the tile, and the same area of upper side (4cm x 5cm, the side most exposed to flowing water) of a stone with a clean

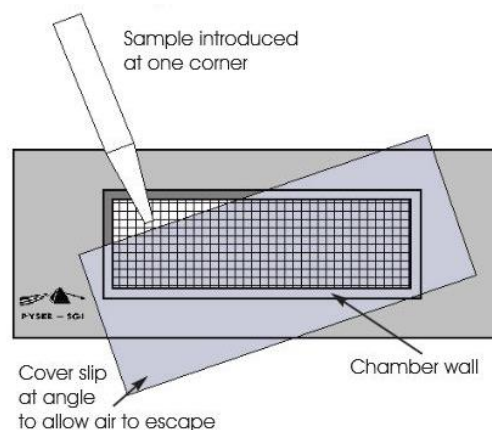
toothbrush to dislodge the algal community (Sharma *et al.*, 1990; (Sharma, Bhosle and Wagh, 1990; Bhosle *et al.*, 2005) into a petri dish with 20 ml of deionized (DI) water. The resulting suspension was then poured into a labelled Falcon tube of 50ml capacity using a funnel and made up to 50ml with DI. Care was taken to avoid equipment contamination between samples by rinsing both the toothbrush and the plastic petri dish before and after every single sample preparation (Kelly and Whitton, 1998; Kelly *et al.*, 1998).

### **3.3.2 Preservation of samples**

The prepared samples were fixed with Lugol's iodine to reach a final concentration of 1% by volume (Taylor *et al.*, 2005).

### **3.2.3 Counting using a Sedgewick-Rafter chamber**

The Sedgewick-Rafter (LeGresley and McDermott, 2010) was used. One chamber was placed on a clean paper towel to avoid scratching the bottom surface, with the cover glass placed at an angle across the chamber top (Fig. 3.1). One ml of the sample was then taken by using a pipette and clean tip and then carefully transferred to the chamber and the cover slip carefully slid into place. This allowed the air bubbles to escape during the filling procedure. Care was taken to prevent overfilling, so the cover glass did not float free and the volume of the sample in the chamber was known exactly. The Sedgewick-Rafter chamber was allowed to stand for 15 minutes before the cell count was made to allow the cells to settle to the bottom.



**Figure 3 1 Filling the Sedgewick-Rafter chamber which is constructed as a flat slide (76mm x 40mm) onto which is cemented a 'wall' to form a chamber or cell in the middle. This chamber is 50mm long x 20mm wide and 1mm deep and its base is marked with a grid of 100 x 1mm squares. (adopted from Pyser-SGI, 2010)**

### 3.2.4 Identification

Algal were identified in the laboratory, using Kelly and Council (2000) and John *et al.*, (2011) with a light CETI microscope, equipped with a mechanical stage and x 100 oil-immersion objective lens, total magnification was x 1000. Green algae and blue-green bacteria were identified to genus level whereas diatoms to species level.

## 3.3 Chlorophyll a Measurement

Chlorophyll *a* was measured spectrophotometrically following the procedure after APHA (1998).

### 3.3.1 Laboratory protocol

The algae were brushed off the upper surface of a tile by using a hard bristled toothbrush, and the brushed material were dislodged into a petri dish. The toothbrush and tile were continuously washed by using a squirt bottle filled with 50 ml distilled water. The resulting slurry was filtered onto a glass microfiber filter (47 mm, Fisher Scientific ) using a Whatman standard filtration apparatus; after filtration, the filter was placed into a grinding mortar, then 2-3 ml of 90% aqueous acetone solution was add and the slurry ground with a pestle. After grinding was completed, the contents were transferred into a labelled centrifuge tube, and final volume brought up to exactly 10.1 ml with more acetone. The stoppered centrifuge tubes were placed in the dark at 4 C° to steep for 14-18 hours. Next day the tubes were placed in centrifuge and

spun at 500 g for 5 minutes in order to clarify the samples. The Beckman Coulter spectrophotometer DU 730 turned on to begin running the samples. 3 ml of 90% aqueous acetone solution was transferred into the cuvette blank. The blank used to zero the spectrophotometer at all the selected wavelengths. Then 3 ml of extract sample was transferred into the cuvette. The absorbance of the sample was read at 750 and 664 nm (before acidification). The samples were analysed in the order in which they were extracted so that all samples had been steeped for approximately the same amount of time. Samples were then acidified with 0.1 ml of 0.1N HCl added to the sample cuvette after reading; gently inverted for 90 seconds in order to mix then the acidified extract volumes were again read at 750 and 665 nm. The cuvette was rinsed with 90% aqueous acetone solution and dried prior to measurement of the next sample.

### 3.3.2 Calculation

The following formula was used from Hauer and Lamberti (2011) to calculate the Chlorophyll *a* concentrations on each tile:

$$\text{Chlorophyll } a (\mu\text{g}/\text{cm}^2) = \frac{26.7(E_{664b} - E_{665a}) \times V_{ext}}{\text{area of substrate } (\text{cm}^2) \times L}$$

Where:

$E_{664b}$  = [{Absorbance of sample at 664nm – Absorbance of blank at 664nm} - {Absorbance of sample at 750nm – Absorbance of blank at 750nm}] before acidification

$E_{665a}$  = [{Absorbance of sample at 665nm – Absorbance of blank at 665nm} - {Absorbance of sample at 750nm – Absorbance of blank at 750nm}] after acidification

$V_{ext}$  = Volume of 90% acetone used in the extraction (ml)

$L$  = length of path light through cuvette (cm)

26.7 = absorbance correction (derived from absorbance coefficient for chlorophyll *a* at 664nm [11.0] × correction for acidification [2.43])

1.7 = maximum ratio of  $E_{664b}$ :  $E_{665a}$  in the absence of pheopigments.

### 3.4 Sediment Measurement

#### 3.4.1 Sediment sampling

Sediments deposited on the tiles were collected with minimum disturbance by using a sample container of exactly the same size as the tile, with its bottom removed. It was placed over the tile in the stream and then lifted out in order not to lose the fine materials. The sample container (Fig. 3.2) placed over tiles before they were removed from streams, which retained the sediment deposited on them with a watertight seal. Deposited sediment was poured into bottles. Bottles were labelled by sampling site and sample number and then transported to the laboratory for processing.



**Figure 3.2** Sample container for sediment collection which was cutting at the University workshop in order to fits the tiles used to measure the deposited sediments in the field

#### 3.4.2 Calculations

The following formula used by Hauer and Lamberti (2011) to calculate the weight of the sediment samples which were expressed as expressed as  $\text{mg cm}^{-2}$

$$\text{Sediment } (\text{mg cm}^{-2}) = \frac{(A - B)}{C}$$

Where:

A = the weight of the filter + sediment residue (expressed in mg),

B = the weight of the filter (expressed in mg),

C = the area (expressed in  $\text{cm}^2$ ) of the tile.

### 3.5 Methodology for Total Phosphorus (TP) In Kjeldahl Digests

#### 3.5.1 Summary of method

The sample was subjected to Kjeldahl digestion by heating a digestion block (SEAL Analytical) in the presence of digestion reagent (potassium sulphate, sulphuric acid and copper (II) sulphate). All P is converted to ortho-phosphate in Kjeldahl digestion. The residue was cooled, diluted and placed on the AQ2 discrete analyser for colorimetric determination followed the AQ2 method NO: EPA-135-A Rev. 2

Detection limit is  $0.009 \text{ mg L}^{-1} \text{ P}$

#### 3.5.2 Reagents and standards

##### 3.5.2.1 Preparation of reagents

- 10% (w/v) Sodium dodecylsulphate (SDS). 50 g SDS was added to a 500 mL volumetric flask, then about 400 mL deionized water was added and swirled to dissolve. The volumetric flask was filled to the mark with deionized water and mixed gently.
- Digestion reagent. 134 g potassium sulphate ( $\text{K}_2\text{SO}_4$ ) was dissolved in 700 mL deionized water in a 1 L volumetric flask, 134 mL concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) was carefully added. 11.4 g copper (II) sulphate pentahydrate was added and stirred to dissolve, then diluted to the mark with deionized water and inverted to mix.
- Alkaline EDTA rinse (1% w/v Disodium EDTA, 2% w/v NaOH). 10 g sodium hydroxide and 5 g disodium ethylene diamine tetra acetic acid were dissolved in a 500 mL flask containing about 400 mL deionised water. Then it was stirred to dissolve then diluted to 500 mL (the solution stored in a plastic bottle).
- Sulphuric acid, 5 N. 70 mL of concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) was very slowly added to approximately 400 mL of deionized water. The flask was cool to room temperature and diluted to 500 mL with deionized water and inverted to mix.
- Stock ammonium molybdate reagent, 4% w/v. 4g ammonium molybdate tetrahydrate was dissolved in 100 mL deionized water by stirring for 2 hours. It was Stored in a plastic bottle at  $4^\circ\text{C}$ .
- Antimony potassium tartrate,  $3 \text{ g L}^{-1}$ . 1.5 g antimony potassium tartrate was dissolved in 500 mL deionized water and stored  $^\circ\text{C}$  in a dark bottle at  $4^\circ\text{C}$ .



- Working colour reagent ( $10 \text{ g L}^{-1}$  ammonium molybdate,  $0.3 \text{ g L}^{-1}$  Antimony potassium tartrate). 20 mL of stock antimony potassium tartrate ( $3 \text{ g L}^{-1}$ ) and 50 mL of stock ammonium molybdate solution (4% w/v) were added to a 200 mL volumetric flask. Then it was diluted to 200 mL and inverted to mix. It was stored in a plastic bottle at  $4^\circ\text{C}$ .
- Ascorbic acid,  $60 \text{ g L}^{-1}$ . 6 g ascorbic acid, fine granular was dissolved in 100 mL of deionized water. The solution was stored at  $4^\circ\text{C}$ .
- Working acid (with o-phosphate spike). 80 mL of 5 normal sulphuric acid solution was added to a 200 mL volumetric flask containing about 50 mL deionized water, then 2 mL 10% SDS stock solution. This reagent was spiked with 3 mL of o-phosphate spike then diluted to 200 mL and mixed gently.

#### *3.5.2.2 Preparation of standards*

- Stock standard solution ( $800 \text{ mg P L}^{-1}$ ). 3.515 g potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ ) was dried at  $105^\circ\text{C}$ , weighed cooled in a desiccator then put into a 1000 mL volumetric flask. Deionized water was added, swirled to dissolve, diluted to the mark, and then stored at  $4^\circ\text{C}$ .
- Synthetic Kjeldahl blank matrix. 100 mL of digestion reagent was added to a 500 mL volumetric flask, then diluted to the mark and inverted to mix.
- Alkaline EDTA Rinse (to wash the cuvette post run). 5 g disodium EDTA dihydrate and 10 g sodium hydroxide was added to a 500 mL volumetric flask dissolved in deionized water and diluted to the volume and stirred to dissolve.

### **3.5.3 Procedure**

#### *3.5.3.1 Kjeldahl Digestion*

25 mL of sample and 5 mL of digestion reagent were added to each pre-cleaned digestion tubes, and mixed by using a vortex mixer. 3-4 boiling stones (suitable for Kjeldahl digestion) were added to prevent boil-over of digest; tear-drop stoppers were placed on the tubes. The digester tubes were placed in the block, and the block heated to  $160^\circ\text{C}$  for about two hours. When the tear-drop stoppers had stopped shaking, the temperature of the block was raised to  $380^\circ\text{C}$  and left for 30 minutes. The tubes were lifted with a rack from the block after digestion was

completed and left to cool for 15 minutes. 25 mL of distilled water was added to each tube and mixed with a vortex mixer.

#### *3.5.3.2 Analysis*

Standards and reagents were prepared as described above. After phosphate analyses were finished for the day, the cuvette rinsed with alkaline EDTA rinse solution to remove any reagent deposits.

Results were reported in  $\text{mg L}^{-1}$ .

### **3.6 Methodology of O-Phosphate by Discrete Automated Colorimetry**

#### **3.6.1 Summary of method**

Reaction with acidic molybdate in the presence of antimony formed an antimony phosphomolybdate complex which was reduced by ascorbic acid to an intensely blue complex: phosphomolybdenum blue. The absorbance of this complex was measured spectrophotometrically at 660 nm following the AQ2 method NO: EPA-128-A Rev. 5. This method conforms to USEPA method 351.2, version 2 (1993)

Detection limit is  $0.005 \text{ mg P}^{-1}$

#### **3.6.2 Reagents and standards**

##### *3.6.2.1 Preparation of Reagents*

- Ammonium molybdate, 4% (w/v). As for total P.
- 10% (w/v) Sodium dodecylsulphate (SDS). As for total P
- Sulphuric acid, 5 normal. 70 mL of concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) was slowly added to approximately 400 mL of deionized water. The flask becomes very warm. It was cool to room temperature and diluted to 500 mL with deionized water. Inverted to mix.
- As for total P.
- Working ascorbic acid,  $10 \text{ g L}^{-1}$  with 0.05% SDS. 1.0 g of ascorbic acid, fine granular was dissolved in 100 mL deionized water and stored at  $4^\circ\text{C}$ .
- Working colour reagent. 65 mL of 5 normal sulphuric acid followed by 7.5 mL of antimony potassium tartrate stock was added to a 100 mL volumetric flask and swirled to mix. Then

22 mL of 4% ammonium molybdate stock followed by 2 mL of 10% SDS stock solution was added. The contents swirled and the flask filled to the mark with deionized water and mixed. It was stored in a plastic container.

- Alkaline EDTA Rinse (to wash the cuvette post run). As for total P.

#### *3.6.2.2 Preparation of Standards*

- Stock standard solution ( $1000 \text{ mg P L}^{-1}$ ). 4.394 g potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ ), previously dried at  $105^\circ\text{C}$  and cooled in a desiccator was dissolved in deionized water and diluted to 1000 mL in a volumetric flask. It was stored at  $4^\circ\text{C}$ .
- Standard solution ( $20 \text{ mg P L}^{-1}$ ). 5 mL of stock standard solution ( $1000 \text{ mg P L}^{-1}$ ) was added to a 250 mL volumetric flask then diluted to the mark with deionized water and inverted to mix.

#### **3.6.3 Procedure**

Standards and reagents were prepared as described above. The samples were filtered through Whatman 45 mm pore diameter membrane filter. After o-phosphate analyses were finished for the day, the cuvette was rinsed with the alkaline EDTA solution to remove any reagent deposits. A reagent wedge (Fig. 3.3) was then filled with the alkaline EDTA solution and placed it in Position 1 of the reagent rack.



**Figure 3 3 Reagent wedge with on-board cooling, built-in level sensor to verify reagent volume**

### 3.7 Data Analyses

For methodology object (A) in Chapter 4 which is to show whether the tiles are valid artificial substrates for epilithic growth or not, an independent T-test was conducted to compare algal species grown on tile (artificial substrate) and on stone (natural substrate). ANOVA two-factor with replication was also conducted to compare the differences of species diversity (Shannon Wiener Diversity Index and Simpson Diversity index) on tiles and stones.

For methodology objective (B) in Chapter 4 which is to quantify the most appropriate length of time for tile to be exposed, ANOVA two-factor with replication statistical analysis was conducted to compare the differences of new and extinct species in different colonization time in four selected sites.

An independent T-test was conducted to compare the benthic algal species grown at high current speed and at low current speed for methodology objective (C) in Chapter 4 which is to quantify the most effective current speed.

Redundancy analyses (RDA) were performed using the R programme in order to produce diagrams show a simultaneous ordination of environmental variables, sites and species. This sort of constrained ordination presumes a linear response of the tested species along the environmental gradients. Ordinations were carried out on two sets of data. The first included the algal species and the second included the larger algal taxa (Cyanobacteria, Chlorophyceae and Bacillariophyceae) for all streams.

A Non-Metric Multidimensional Scaling (NMDS) with R package Vegan was performed using the Bray-Curtis dissimilarity matrix in order to determine the effects of the two manipulated stressors (P and deposited fine sediment) upon the composition of algal community, the abundances of all identified algal taxa (data not transformed) were used for this calculation.

A set of RDA models for each biological response variable was used to examine the relationships between the two stressors (P and fine sediment) and the biological response variables and to examine the study hypotheses. Johnson and Omland's (2004) protocol to perform the model selection was followed.

First step of the protocol was the biological hypotheses generation to create three competing models called single stressor model, simple-multiple model and complex multiple model. In each model, the predictor terms were P, sediment, and interaction of the two. The single stressor model predictor terms included only P or fine sediment. The simple-multiple model included

both P and fine sediments but no interaction. The complex multiple model included the P and fine sediments interaction.

The second step was fitting these models to the collected data.

The final step was conducting the RDA for each model to test the study hypotheses. For this, the species abundance data were standardised, because without standardisations, the analysis would be dominated by those species with the highest variation.

The environmental data were checked for collinearity, and the Variance Inflation Factors (VIF) were measured, so that VIFs above 10 could be avoided, as Borcard *et al.* (2011) recommended

To check if the data were really linear, the gradient length was checked with detrended correspondence analysis. The output gave the standard deviations of the axis lengths, and for linear analyses the standard deviations should not be much longer than between 2 and 3, preferably lower than that. For the sake of the completeness, the Hellinger transformation was performed to decrease gradient length for the ones with standard deviation more than 3.

Three community variables – algal species richness and the evenness index and relative abundances of ecological guilds, were determined, as they had been reported in previous diatom communities' studies by Passy (2007) to respond to P conditions. All taxa were assigned to one of three growth forms (high profile, low profile or motile) after Passy (2007), as shown in appendix 3.1.

### 3.8 Experimental Design

A total of 128 mesocosms (plastic containers) held tiles, with water running through them (Fig. 3.4.A). Eight P concentrations (using  $\text{KH}_2\text{PO}_4$ ) were used with eight levels of fine sediment in two replicate mesocosms of each treatment combination (Fig. 3.5).



**Figure 3.4 (a) Photo of the mesocosm experimental set-up consisting of two levels scaffolding and 128 tile-sets in Plastic Storage boxes. (b) Photo of a Plastic Storage box containing a tile-set taken at the end of the 20-day pre-colonisation period.**

P1 S1	P2 S1	P3 S1	P4 S1	P5 S1	P6 S1	P7 S1	P8 S1
P1 S2	P2 S2	P3 S2	P4 S2	P5 S2	P6 S2	P7 S2	P8 S2
P1 S3	P2 S3	P3 S3	P4 S3	P5 S3	P6 S3	P7 S3	P8 S3
P1 S4	P2 S4	P3 S4	P4 S4	P5 S4	P6 S4	P7 S4	P8 S4
P1 S5	P2 S5	P3 S5	P4 S5	P5 S5	P6 S5	P7 S5	P8 S5
P1 S6	P2 S6	P3 S6	P4 S6	P5 S6	P6 S6	P7 S6	P8 S6
P1 S7	P2 S7	P3 S7	P4 S7	P5 S7	P6 S7	P7 S7	P8 S7
P1 S8	P2 S8	P3 S8	P4 S8	P5 S8	P6 S8	P7 S8	P8 S8

Where:

P1= nothing to be added, P2= 0.462 mgL<sup>-1</sup>, P3= 0.832 mgL<sup>-1</sup>, P4= 1.492 mgL<sup>-1</sup>, P5= 2.686 mgL<sup>-1</sup>, P6= 4.835 mgL<sup>-1</sup>, P7= 8.703 mgL<sup>-1</sup>, P8= 15.665 mgL<sup>-1</sup>

S1= nothing to be added, S2= 2.243 mg.cm<sup>-1</sup> fine sediment, S3= 4.037 mg.cm<sup>-1</sup> fine sediment, S4= 7.267 mg.cm<sup>-1</sup> fine sediment, S5= 13.081 mg.cm<sup>-1</sup> fine sediment, S6= 23.546 mg.cm<sup>-1</sup> fine sediment, S7= 42.389 mg.cm<sup>-1</sup> fine sediment, S8= 76.301 mg.cm<sup>-1</sup> fine sediment.

**Figure 3 5 Experiment treatment combinations used during the of the 20-day manipulative period**

Eight blocks of plastic storage boxes, each block consisting of sixteen ceramic tiles-set were arranged for the P treatments. Within each P block, eight levels of sediment with two replicates were assigned to the sixteen ceramic tiles-set. The experiment ran for 40-days - a 20-day colonization period and 20-day manipulative period.

On day one of the manipulative period both stressors were introduced. P was continuously added for the whole period whereas sediment was only added once and remained on all sediment-added tiles until the end of the experiment. Gradients from the lowest level recorded in the site (as shown in Chapter 4) recorded earlier, to extremely high levels of each stressor treatment were chosen in order to simulate increasing anthropogenic stress levels.

During the colonisation period (first 20-day) all tiles showed rapid growth on the substratum surface (F. 2b). After this, a highly concentrated  $\text{KH}_2\text{PO}_4 \geq 99.0\%$  solution was dripped continuously into each water container in order to achieve the required P concentrations.

The School Farm stream water was the lowest level of P. The concentrations target for P and fine sediment from level two to level eight were set on a logarithmic scale, evenly spaced, to get the best use of statistical power (Smith, Bode and Kleppel, 2007; Friberg *et al.*, 2010; Yuan, 2010). P and sediment levels were set at 1.9 times higher than the previous level; level 1 was the actual concentrations of the School Farm stream. For ( $\text{KH}_2\text{PO}_4$ ), the target levels were ambient, 0.46, 0.83, 1.49, 2.69, 4.84, 8.70 and 15.67  $\text{mgL}^{-1}$  of ortho phosphate. The P containers had to be continuously refilled during the experiment manipulative period. Each mesocosm's content of dissolved P was monitored weekly to determine the total P using the AQ2 method NO: EPA-135-A Rev. 2 and Ortho-Phosphate using the AQ2 method NO: EPA-128-A Rev. 5.

The tiles were supplied with water pumped at a constant rate from the School Farm stream. Four pumps with capacity of 8  $\text{L. min}^{-1}$  each (Whale Lightweight Water Pump, Whale WP-WSF-UV0814; Jones Boatyard, United Kingdom) delivered water through a PVC hose (Homebase, United Kingdom) to a Y-Connector (Hozelock, UK), which split flow equally leading into two Slim Water Containers (Caravan Accessory Shop, United Kingdom) sitting on the first level of a scaffold. By gravity, each water container fed 16 individual plastic boxes with water through PVC hoses (Fig. 5.2.a).

Fine sediment (average grain size of 0.2 mm) was sourced from the School Farm stream floodplain and weighed out in advance. For the lowest treatment level, no sediment was added onto of that naturally provided in suspended sediment by the stream, then an evenly logarithmic scale was set from level two to level eight. The added amount of sediment was 0, 44.86, 80.74, 145.34, 261.62, 470.92, 847.78 and 1526.02  $\text{mg.cm}^{-1}$ , respectively on first day of the 20-day manipulative period and stayed on the tiles. Fine sediment was added directly on the tiles aiming for an even distribution across the surface whilst stopping the water flow for five minutes for sediment to settle. By the time when the water flow was restarted, all sediment was deposited on the tile surface where it stayed, with minimal loss (personal eye observation), for the 20-days of experiment time.



## CHAPTER FOUR: EFFECT OF PHOSPHORUS AND SEDIMENT GRADIENT UPON EPILITHIC ALGAE - FIELD STUDY

### 4.1 Abstract

This chapter presents the investigations of the epilithic communities on natural (stones) and artificial substrates at nine sites of two regions of Leicestershire situated in the East Midlands of England, UK. The artificial substrates were ceramic tiles 4cm x 5cm cemented to a heavy base, placed in the river bed.

Algal biomass is generally related to the concentrations of nutrient. In our studied stream, this was the situation for the epilithic algae. At Sibbertoft and Lone Pine, the recorded Chlorophyll a shows these sites had highest chlorophyll a in our study sites.

Algal biomass and densities increased across the gradients of sediment and nutrients concentrations. Quinn *et al.* (1997) have also reported streams with higher nutrients concentrations recorded greater biomass and densities compared to pristine streams. Gray and Ward (1982) suggested that the increased algal in streams with rising the levels of sediment might be resulted from the increased levels of nutrients included in that sediment.

The changes from community dominated by a very sensitive species to pollution such as *Brachysira vitrea* to a community dominated by a tolerant to pollution species such as *Rhoicosphenia abbreviate* and *Navicula cryptotenella* suggests that along the P gradients there is a biofilm functional changes which can provide an ecological justification for the ecological status of the stream.

*Brachysira vitrea* favoured by low nutrient concentrations where it's occurred in high relative abundances (Kelly *et al.*, 2007). *Rhoicosphenia abbreviate* recorded in oversaturated streams with high P (Rott *et al.*, 1998) and it is one of the most prolific diatom in under enriched streams conditions (Kelly *et al.*, 2007).

The group of tolerant taxa is largely dominated by *Navicula* and *Nitzschia* while sensitive category is dominated by *Achnantheidium* and *Fragilaria* (Kelly *et al.*, 2007). *Achnantheidium minutissimum* as relatively intolerant of eutrophication (Kelly *et al.*, 2007). The low abundance of these species as P concentration increase proves changes in the sites ecologically.

Both fine sediment and nutrient concentrations, were correlated with each other. This affects our ability to differentiate the effects of sediment and nutrients on the measured biotic responses in the studied streams. Definitive research of these differential impacts requires

experimental design with combine and separate manipulation of sediment and nutrients (see chapter 5 experiment).

When assessing a stream ecological condition or predicting the stream future condition, the knowledge of the multiple stressors impacts is very important (Paine *et al.*, 1998). With the few recent researches by Matthaei *et al.* (2010) and Ferreria and Chauvet (2011) of the multiple stressor impact indicating synergetic interactions, the current knowledge is still limited.

**Keywords:** artificial substrata (tile), natural substrata (stone), algal community, species composition, incubation time, new species, extinct species, stream, current speed.

## 4.2 Introduction

Diatoms are the species-rich group of benthic algae which easily affected by stresses, habitat biological physical, and chemical disturbances (Stevenson and Bahls, 1999; O'Driscoll *et al.*, 2012; Gray and Vis, 2013). The diatom community has been successfully used as a biological indicator to describe the present day status of both rivers and lakes (Watanabe *et al.*, 1986; Round, 1991) and to indicate the streams and rivers water quality in Europe and United States (Adams *et al.*, 2013) as a result of their role in the food web and their reproduction rapid rate (Stevenson and Bahls, 1999; O'Driscoll *et al.*, 2012; Gray and Vis, 2013). One of the habitats occupied by diatom is the epilithon, where they are attached to the surface of stones, rocks or pebbles (Paul *et al.*, 2016)

In streams, algae grow on substrates that vary in composition, origin, orientation, and size. These heterogeneities have always obstructed algal quantitative studies (Cattaneo and Amireault, 1992), because of the difficulties of quantitative removal of samples. Artificial substrata have been used for many years as substitutes for stones, as they are easier to sample (Hoagland *et al.*, 1982; Barbiero, 2000). The first glass slide was suspended in a lake by (Hentschel, 1916); since then algal researchers have investigated many and varied anchoring devices and materials. These techniques have been reassessed many times (Austin *et al.*, 1981; Aloï, 1990). Many researchers have thus chosen to study the assemblages of algae that grow on introduced artificial substrata, which simplify the natural complexity by providing consistent material, colonization time, size, and texture. They can be simply manipulated and they make both processes of the assemblage detachment and the sample area determination easy (Cattaneo and Amireault, 1992).

The substratum's physical nature is one of the components determining the abundance and distribution of stream organisms (Hynes and Hynes, 1970). There are many factors in addition to the artificial substratum type, such as incubation time, season, and current speed that affect the algal community development. It takes time for new introduced artificial substratum to reach the same extent of colonization (Korte and Blinn, 1983), so they need to be left in the stream water for enough time to allow representative communities of algal to develop on the artificial substratum surface (Reid *et al.*, 1995).

In most studies, artificial substrates are left in the stream water between two and four weeks before sampling. This time is logistically convenient for colonization and it avoids long exposures and the chance of loss to spates or vandalism. Moreover, such a time period is

suggested to be ideal because it avoids subsequent sloughing, and it allows the algal community to develop its biomass to the maximum (Federation and American Public Health Association, 2005). Kelly *et al.* (1998) recommended an incubation period of four weeks.

In river ecosystems, current speed differs temporally and spatially over a range of scales and affects the algal biomass by various mechanisms (Hart and Finelli, 1999). Current speed can have both negative and positive effect on the composition and the biomass of algal communities in stream (Horner *et al.*, 1990; Stevenson, 1996a). Increasing current speed can either negatively controls biomass production by increasing the shear stress on algae or positively by increasing the availability of the nutrient (Larned, Nikora and Biggs, 2004), thus both low and high speeds have the potential to change the ecosystem function and structure completely through accrual and scour of epilithon (Francoeur, 2001).

Comparative studies of the artificial and natural substrata assemblages are not lacking, the issue of whether artificial substrates adequately imitate natural ones is still unsolved, because; because there is a disagreement about these comparative results (Cattaneo and Amireault, 1992). These disagreements have two possible sources: 1) the substrata performance may depend on the used methodologies and the studied environment 2) the results evaluated differently by the researchers depending on the studies goals.

### **4.3 Objectives**

The objectives of this chapter are:

- 1) To show the differences between epilithic algae growing in a stream P gradient and;
- 2) The differences between epilithic algae growing in a sediment gradient (for example, species diversity, density and biomass);
- 3) In order to understand what the biological effects of a P and sediment gradient were in real life (in the field) and to help in design a field experiments gradients (chapter five), which will test whether there is an additive effect of the two (P and sediment) in combination, or whether they acted independently. In order to find the optimum field conditions in which to conduct the experiments, three preliminary observations was made to test three methodological objectives:  
[A] Whether the tiles are valid artificial substrates for epilithic growth or not.  
[B] To quantify the most appropriate length of time for tile to be exposed.  
[C] To quantify the most effective current speed.

So this chapter is to describe and analyse the differences in biology caused by two gradients. These three preliminary observations together will help in designing an experimental approach to examine the effects of P and sediment in isolation and together, in an effective way, guided by these results.

## 4.4 Materials and Methods and Study Sites

See chapter two and chapter three

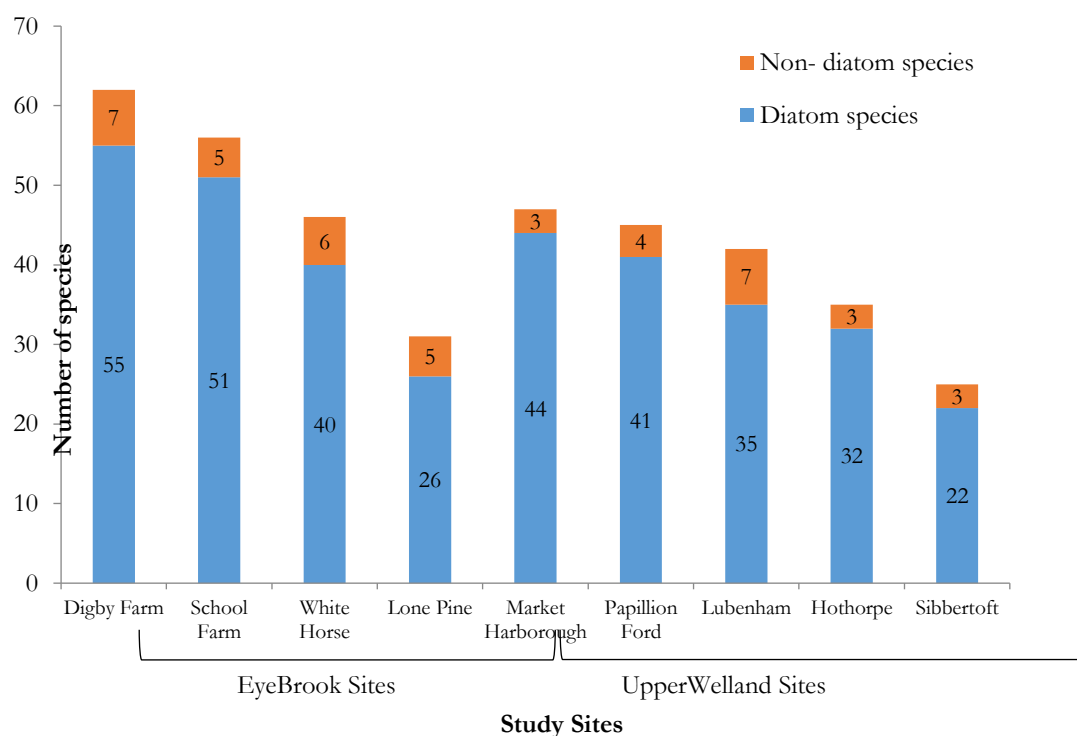
## 4.5 Results

### 4.5.1 Physical-chemical parameters

Results of the chemical and physical measurements of the study sites are shown in Table 4.1.

### 4.5.2 Algal community

The algal biomass consisted mainly of diatoms throughout the whole observations. Algal species at Eye Brook sites was maximum as 62 (55 diatom species and 7 non-diatom species) at Digby Farm, the lowest recorded species was 31 (26 diatom species and 5 non-diatom species) at Lone Pine. Compared to the Upper Welland sites, was maximum as 47 (44 diatom species and 3 non-diatom species) at Market Harborough, the lowest recorded species was 25 (22 diatom species and 3 non-diatom species) at Sibbertoft as shown in Fig. (4.1) and appendix (4.1).



**Figure 4.1** Algal species recorded on the artificial substrates (tiles) at nine different study sites for the preliminary field observation for the period from May to September 2014

**Table 4.1 Chemical and physical parameter of water at study sites. min= minimum value, avg= average value, max= maximum value , SD= standard deviation**

	Loddington School Farm (S1)	Loddington Lone Pine (S2)	Loddington White Horse (S3)	Tilton Digby Farm (S4)
	mean $\pm$ SD (min – max)	mean $\pm$ SD (min – max)	mean $\pm$ SD (min – max)	mean $\pm$ SD (min – max)
Total P (mgL <sup>-1</sup> )	0.37 $\pm$ 0.19 (0.21 - 0.66)	2.06 $\pm$ 1.29 (0.57 - 3.80)	0.54 $\pm$ 0.09 (0.44 - 0.66)	0.17 $\pm$ 0.04 (0.11 - 0.20)
Sediment (mg.cm <sup>-2</sup> )	2.06 $\pm$ 0.52 (1.20 - 2.80)	0.42 $\pm$ 0.09 (0.25 - 0.54)	0.40 $\pm$ 0.18 (0.11 - 0.80)	0.21 $\pm$ 0.12 (0.10 - 0.97)
Temperature (°C)	14.93 $\pm$ 1.96 (11.90 - 17.30)	14.31 $\pm$ 1.73 (11.30 - 16.20)	14.53 $\pm$ 1.63 (12.10 - 16.60)	14.01 $\pm$ 1.74 (11.50 - 16.00)
Light intensity (Lx)	212.17 $\pm$ 9.04 (200 – 224)	124.67 $\pm$ 3.20 (120 – 130)	207.33 $\pm$ 11.38 (196 – 225)	157.33 $\pm$ 31.41 (110 – 190)
Low current speed (m s <sup>-1</sup> )	0.21 $\pm$ 0.06 (0.03 - 0.10)	0.19 $\pm$ 0.05 (0.02 - 0.09)	0.23 $\pm$ 0.07 (0.01 - 0.10)	0.54 $\pm$ 0.13 (0.06 - 0.16)
High current speed (m s <sup>-1</sup> )	0.53 $\pm$ 0.10 (0.20 - 0.37)	0.49 $\pm$ 0.09 (0.21 - 0.35)	0.60 $\pm$ 0.12 (0.20 - 0.37)	0.49 $\pm$ 0.10 (0.21 - 0.35)
Chlorophyll a (mg.cm <sup>-2</sup> )	2.28 $\pm$ 0.33 (1.30 - 2.79)	2.48 $\pm$ 0.27 (1.99 - 2.85)	2.33 $\pm$ 0.35 (1.85 - 2.82)	1.86 $\pm$ 0.71 (1.10 - 2.93)

	Market Harborough (S5)	Lubenham (S6)	Papillon Ford (S7)	Hothorpe (S8)	Sibbertoft (S9)
	mean $\pm$ SD (min – max)	mean $\pm$ SD (min – max)	mean $\pm$ SD (min – max)	mean $\pm$ SD (min – max)	mean $\pm$ SD (min – max)
Total P (mgL <sup>-1</sup> )	0.41 $\pm$ 0.04 (0.33 - 0.50)	0.31 $\pm$ 0.09 (0.25 - 0.43)	0.96 $\pm$ 0.07 (0.80 - 1.13)	0.44 $\pm$ 0.03 (0.40 - 0.50)	0.59 $\pm$ 0.15 (0.21 - 0.82)
Sediment (mg.cm <sup>-2</sup> )	0.46 $\pm$ 0.04 (0.41 - 0.51)	0.44 $\pm$ 0.04 (0.41 - 0.49)	0.45 $\pm$ 0.04 (0.41 - 0.50)	1.50 $\pm$ 0.94 (0.59 - 2.82)	2.06 $\pm$ 1.29 (0.57 - 3.80)
Temperature (°C)	13.72 $\pm$ 1.55 (11.90 - 15.40)	13.72 $\pm$ 1.55 (11.90 - 15.40)	13.93 $\pm$ 1.12 (12.50 - 15.10)	14.05 $\pm$ 1.61 (11.60 - 16.00)	13.65 $\pm$ 1.54 (11.50 - 15.50)
Light intensity (Lx)	212.17 $\pm$ 9.04 (200 – 224)	178.50 $\pm$ 5.80 (173 – 186)	162.20 $\pm$ 11.52 (145 – 173)	158.00 $\pm$ 9.30 (145 – 170)	113.40 $\pm$ 6.54 (110 – 125)
Low current speed (m s <sup>-1</sup> )	0.41 $\pm$ 0.12 (0.04 - 0.13)	0.16 $\pm$ 0.05 (0.04 - 0.11)	0.19 $\pm$ 0.06 (0.02 - 0.10)	0.27 $\pm$ 0.07 (0.01 - 0.12)	0.07 $\pm$ 0.02 (0.01 - 0.03)
High current speed (m s <sup>-1</sup> )	0.50 $\pm$ 0.10 (0.20 - 0.35)	0.53 $\pm$ 0.10 (0.21 - 0.37)	0.53 $\pm$ 0.10 (0.21 - 0.35)	0.56 $\pm$ 0.10 (0.21 - 0.37)	0.24 $\pm$ 0.05 (0.07 - 0.15)
Chlorophyll a (mg.cm <sup>-2</sup> )	2.48 $\pm$ 0.27 (1.99 - 2.85)	2.40 $\pm$ 0.40 (1.79 - 2.95)	2.33 $\pm$ 0.37 (1.20 - 2.89)	2.43 $\pm$ 0.36 (1.79 - 3.01)	2.32 $\pm$ 0.29 (1.57 - 2.79)

The T-test analyses for methodological objective (A) to test whether the tiles are valid artificial substrates for epilithic growth or not. For samples collected on 13/08/2014 and 10/09/2014 from Market Harborough, and on 21/05/2014 and 18/06/2014 from Lubenham, Papillon Ford, Hothorpe, and Sibbertoft shown there were no significant difference across all studied sites between the species grown on tile and stone (Appendix 4.2). ANOVA two-factor with replication shown there was no significant effect of substrata type used (tile) on species diversity across the studied sites at the  $p > 0.05$  level (Appendix 4.3). Taken together, these

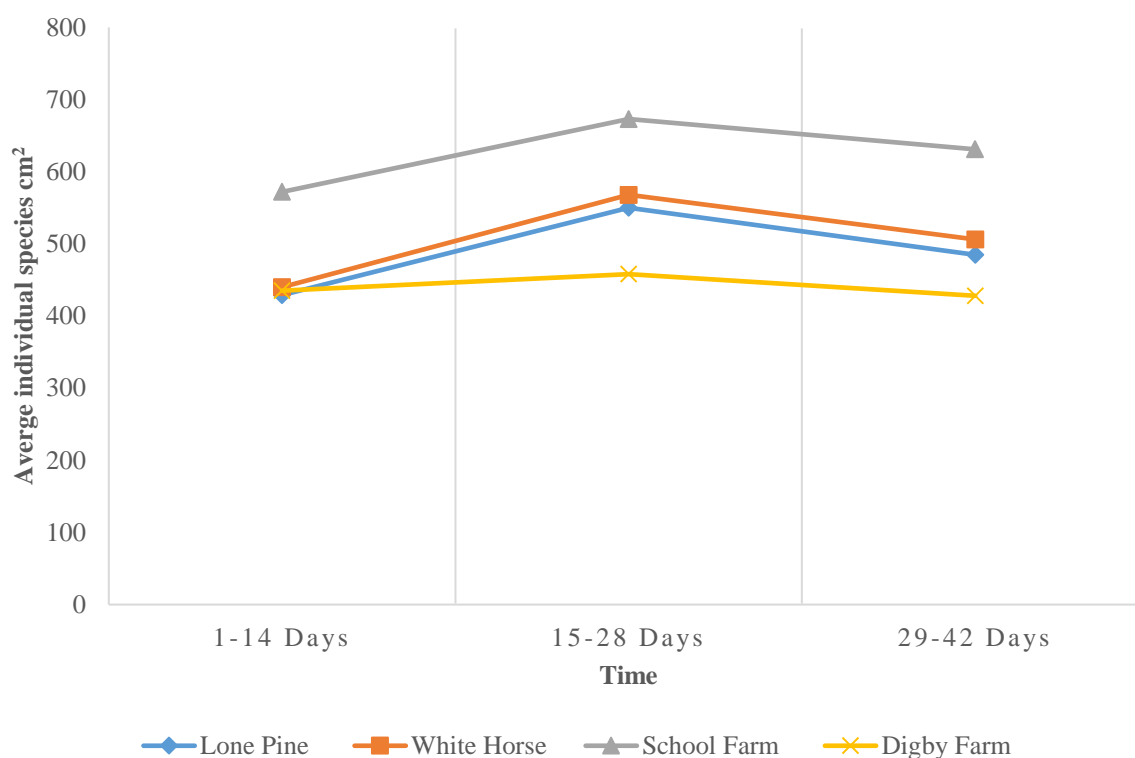
results prove that tile is a valid artificial substrate for epilithic growth, specifically, our results show no effect on the actual species and species diversity when compare the algal growth on both substrates

The species analysis of the tiles collected on weekly basis from the four selected sites for methodological objective (B) to quantify the most appropriate length of time for tile to be exposed in water showed that the majority of species were recorded in the first two weeks of exposure, and no new species recorded during week five or six, but some species started to go extinct<sup>1</sup> from the tiles after four weeks (table 4.2). There was a significant effect of colonization time across the studied sites at  $p > 0.05$  (Appendix 4.4). Figure (4.2) illustrates that maximum number of species was recorded in the period of the three to four weeks.

**Table 4.2 Number of new species recorded on the artificial substrates (tiles) at four study sites for the period of 42 days to quantify the most appropriate length of time for tile to be exposed**

Site	Time	New species	Extinct species
Lone Pine	1-14 Days	44	0
	15-28 Days	2	6
	29-42 Days	0	3
White Horse	1-14 Days	41	0
	15-28 Days	5	5
	29-42 Days	0	4
School Farm	1-14 Days	27	0
	15-28 Days	4	4
	29-42 Days	0	4
Digby Farm	1-14 Days	48	0
	15-28 Days	4	9
	29-42 Days	0	4

<sup>1</sup> Extinction, in biology, the dying out or termination of a species. Extinction occurs when species are diminished because of environmental forces (habitat fragmentation, global change, overexploitation of species for human use) or because of evolutionary changes in their members (genetic inbreeding, poor reproduction, decline in population numbers).



**Figure 4.2 Four sites average individual species recorded on the artificial substrates (tiles) at four study sites for the period of 42 days**

Both the statistical analysis and graph illustration show that the most appropriate length of time for tiles to be exposed is three to four weeks.

Throughout the entire study time for methodological objective (C) to quantify the most effective current speed the diversity as well as the abundance of the algal community was higher at low current speed ranged from  $0.01 - 0.29 \text{ m s}^{-1}$  than high current speed ranged from  $0.3 - 0.6 \text{ m s}^{-1}$  (there was a significant difference in the t test scores for high current and low current,  $p < 0.05$ ), the number of species colonizing the ceramic tiles at Eyebrook sites the maximum number of species colonization was 62 (average 47) at low current speed at Tilton Digby Farm, the maximum was 52 (average 43) at high current speed at Tilton Digby Farm. Compared to the Upper Welland sites, was maximum as 47 (average 41) at low current speed at Market Harborough, the maximum was 39 (average 35) at high current speed at Lubenham. The lowest current speed had the highest species richness and higher current speed the lowest. The recorded species richness seen is table 4.3.

The analysis for the samples collected on 13/08/2014 and 10/09/2014 from Market Harborough, and on 21/05/2014, 18/06/2014, 16/07/2014, 13/08/2014, and 11/09/2014 from each site in Lubenham, Papillion Ford, Hothorpe, and Sibbertoft, and on 07/05/2014,



04/06/2014, 02/07/2014, 30/07/2014, 27/08/2014, and 24/09/2014 from each site in Loddington Lone Pine, Loddington White Horse, Loddington School Farm, and Tilton Digby Farm shown there were significant differences across all studied sites at different current speeds for methodological objective C to quantify the most effective current speed (Appendix 4.5). The present study shows that lower current speed during the stages of colonisation leads to a greater biomass accumulation on the tiles. The general trend for the species abundance showing an inverse relationship with the current speed, where the minimum abundance was recorded at high current speed

**Table 4.3 Species richness recorded at different current speeds at the study sites for the time where low current speed ranged from 0.01 – 0.29 m s<sup>-1</sup> and high current speed ranged from 0.3 – 0.6 m s<sup>-1</sup>**

Site	Current Speed	Time	Species Richness			
			Tile1	Tile2	Tile3	Tile4
Lone Pine	Low	May	28	27	27	27
		June	28	26	28	29
		July A	29	27	29	27
		July B	30	30	30	28
		August	31	31	28	28
		September	27	28	27	29
	High	May	25	25	26	27
		June	23	28	26	27
		July A	27	27	26	27
		July B	28	29	28	30
		August	26	27	27	27
		September	26	26	27	24
White Horse	Low	May	37	37	40	36
		June	39	39	37	42
		July A	42	43	40	41
		July B	46	41	39	43
		August	42	41	42	38
		September	39	36	34	38

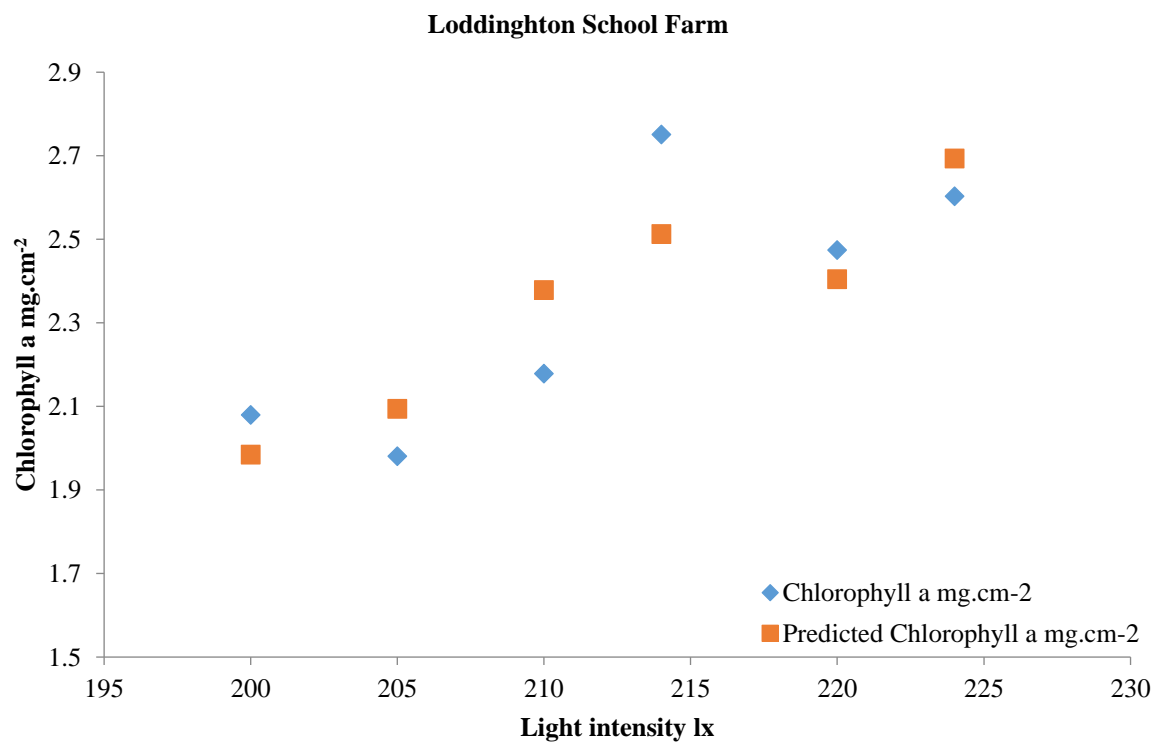
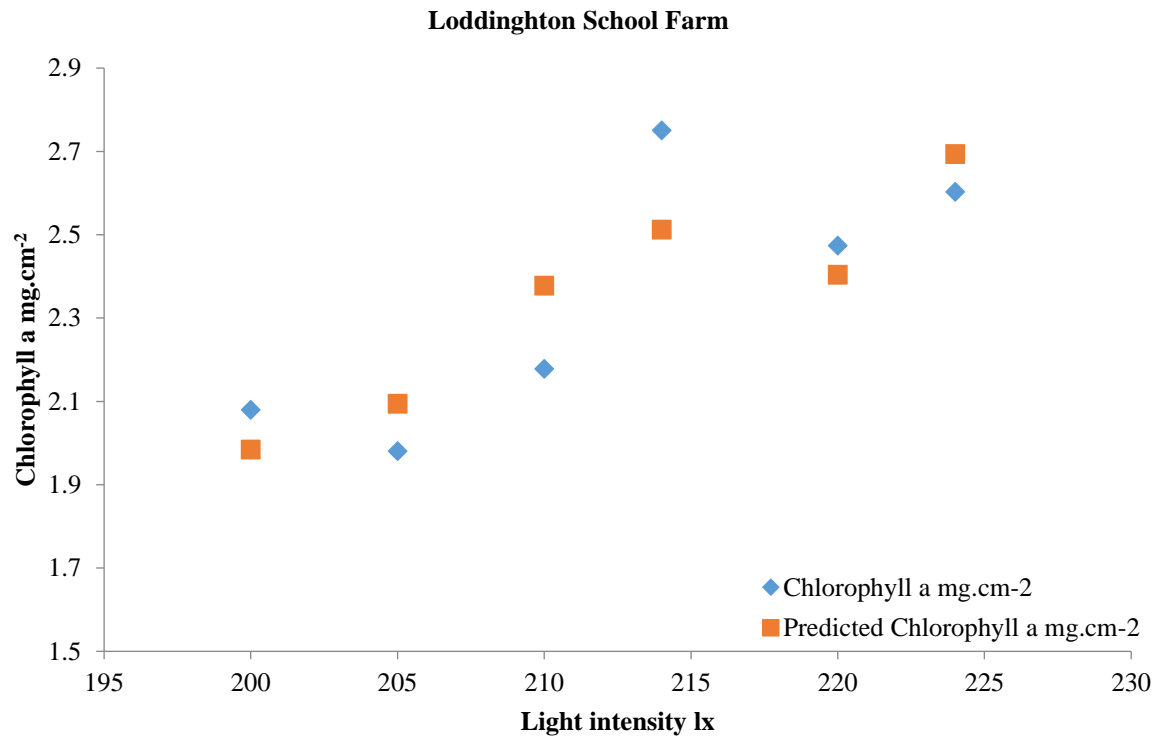
Site	Current Speed	Time	Species Richness			
			Tile1	Tile2	Tile3	Tile4
School Farm	High	May	32	37	34	35
		June	36	37	36	40
		July A	36	37	33	38
		July B	39	40	38	38
		August	37	40	37	39
		September	33	35	33	36
	Low	May	29	29	29	29
		June	32	36	32	39
		July A	48	49	46	47
		July B	46	52	52	52
		August	37	37	34	39
		September	32	36	33	38
Digby Farm	High	May	29	29	29	29
		June	35	38	33	30
		July A	34	43	33	35
		July B	41	42	40	37
		August	36	38	34	37
		September	30	36	32	33
	Low	May	42	39	39	40
		June	44	44	46	45
		July A	53	56	55	55
		July B	62	59	56	56
		August	43	42	42	44
		September	42	42	43	43
	High	May	37	37	34	32
		June	48	45	51	49
		July A	51	49	50	52
		July B	43	45	45	46
		August	37	40	41	40
		September	40	42	39	39

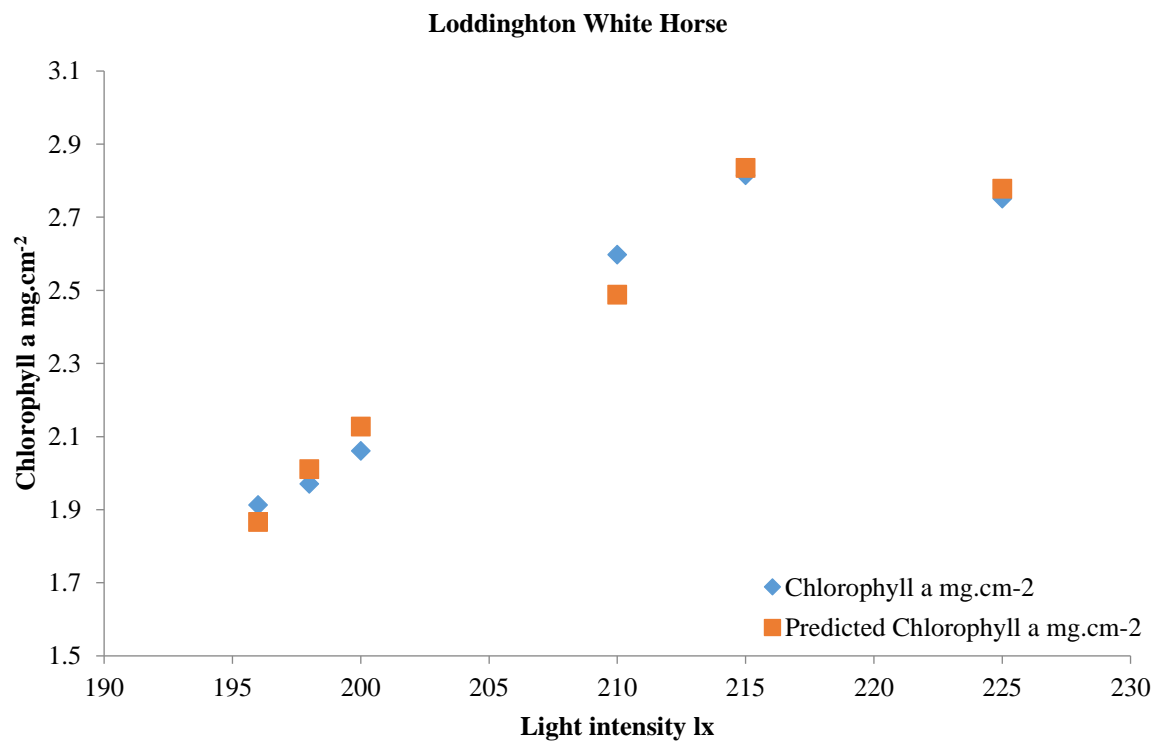
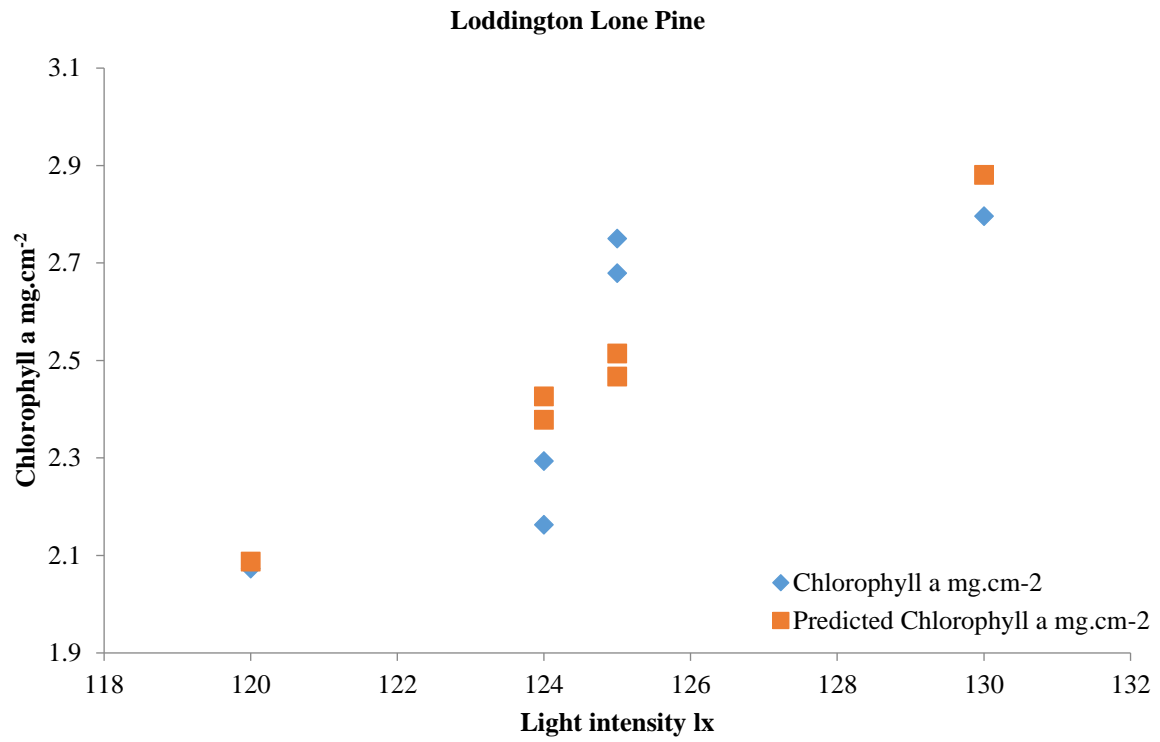
Site	Current Speed	Time	Species Richness			
			Tile1	Tile2	Tile3	Tile4
Lubenham	Low	May	37	37	35	35
		June	40	40	35	37
		July	42	42	39	41
		August	40	42	41	39
		September	37	38	37	40
	High	May	34	32	34	32
		June	35	35	32	34
		July	37	37	39	39
		August	38	39	38	38
		September	31	37	34	33
Papillon Ford	Low	May	36	36	34	36
		June	37	37	35	37
		July	42	38	40	45
		August	44	41	45	41
		September	36	37	35	34
	High	May	35	31	33	33
		June	35	35	30	35
		July	37	37	35	36
		August	36	36	32	33
		September	29	31	33	34
Hothorpe	Low	May	31	32	30	31
		June	33	30	31	33
		July	32	35	34	33
		August	31	34	32	32
		September	28	29	29	28
	High	May	29	29	30	30
		June	30	29	28	29
		July	32	30	30	29
		August	30	29	30	30
		September	28	28	29	29

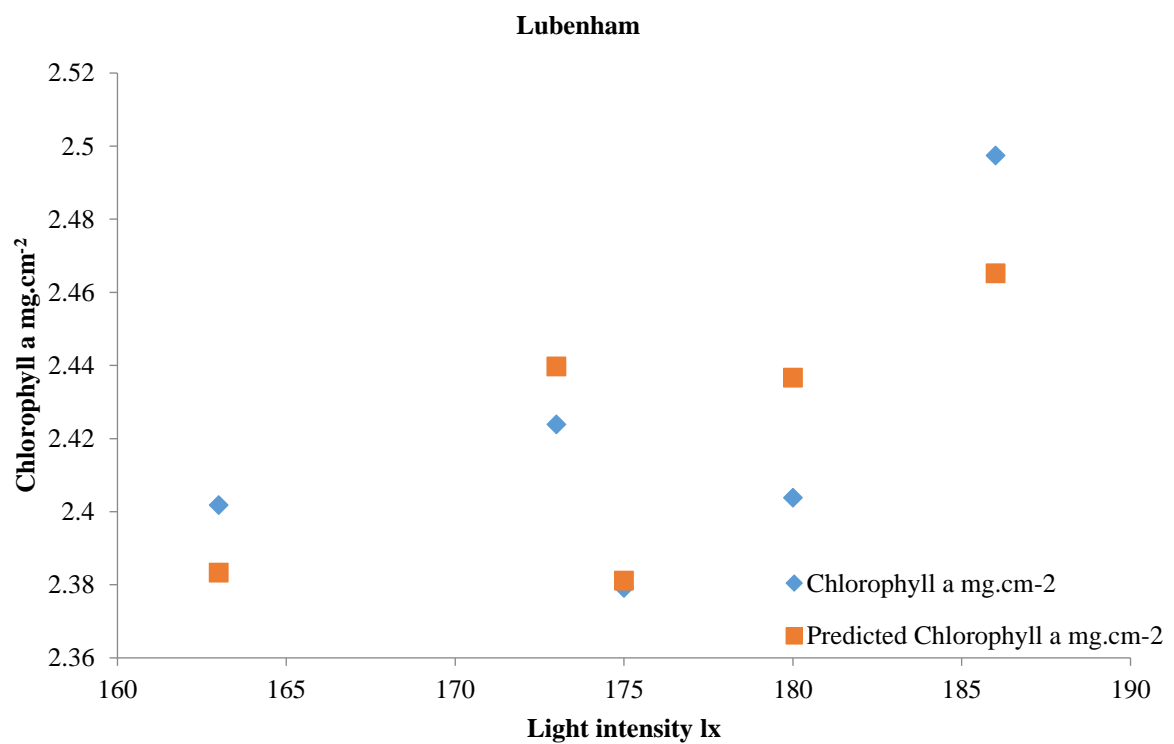
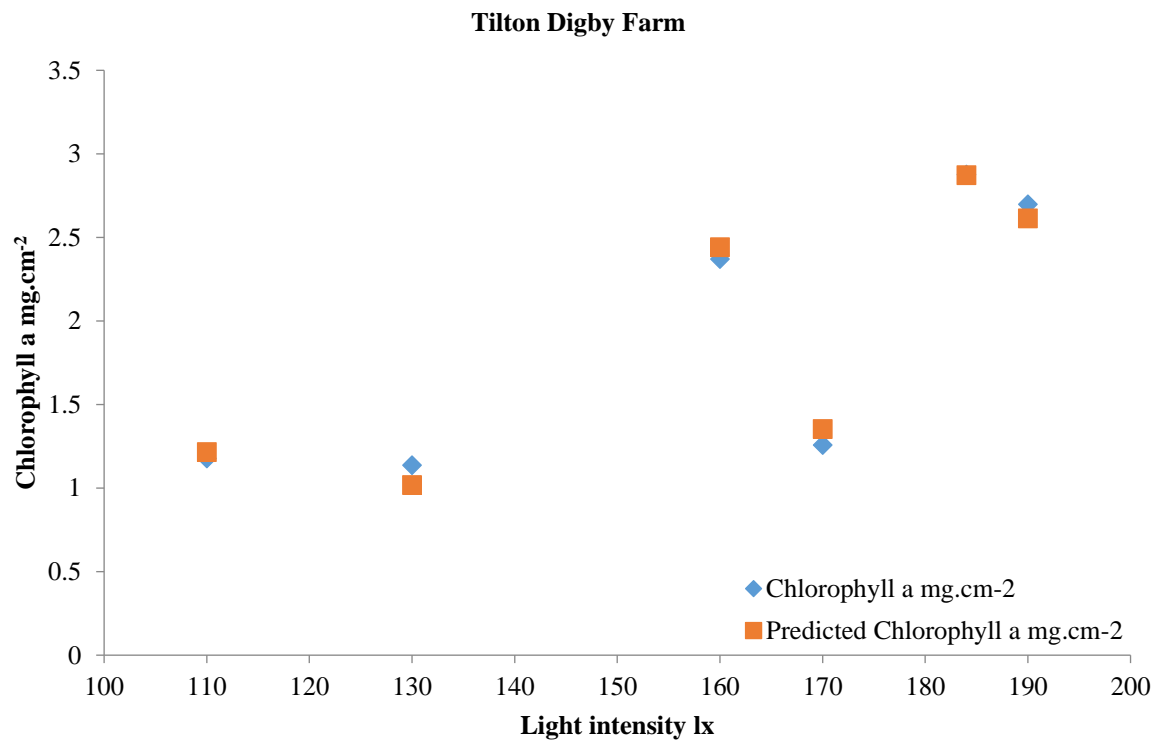
Site	Current Speed	Time	Species Richness			
			Tile1	Tile2	Tile3	Tile4
Sibbertoft	Low	May	24	24	23	24
		June	23	23	25	25
		July	25	25	22	25
		August	25	25	25	25
		September	25	25	25	24
	High	May	22	24	23	22
		June	24	25	24	23
		July	24	22	22	24
		August	25	24	25	24
		September	21	22	23	24

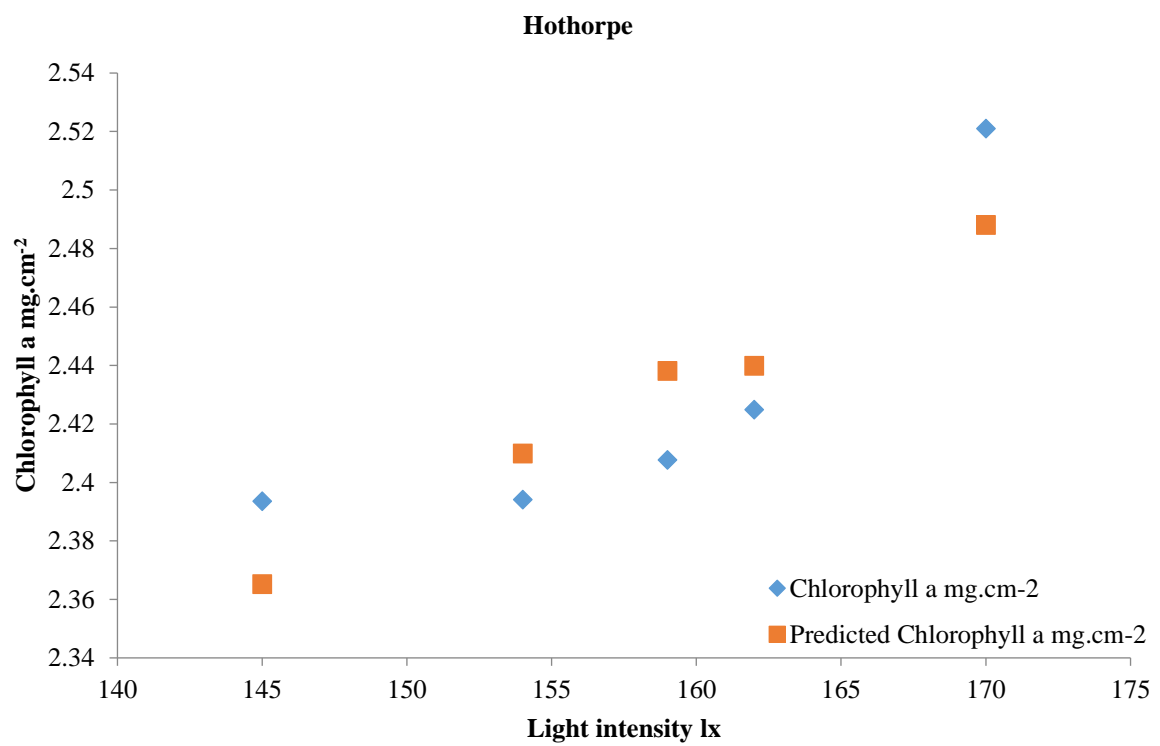
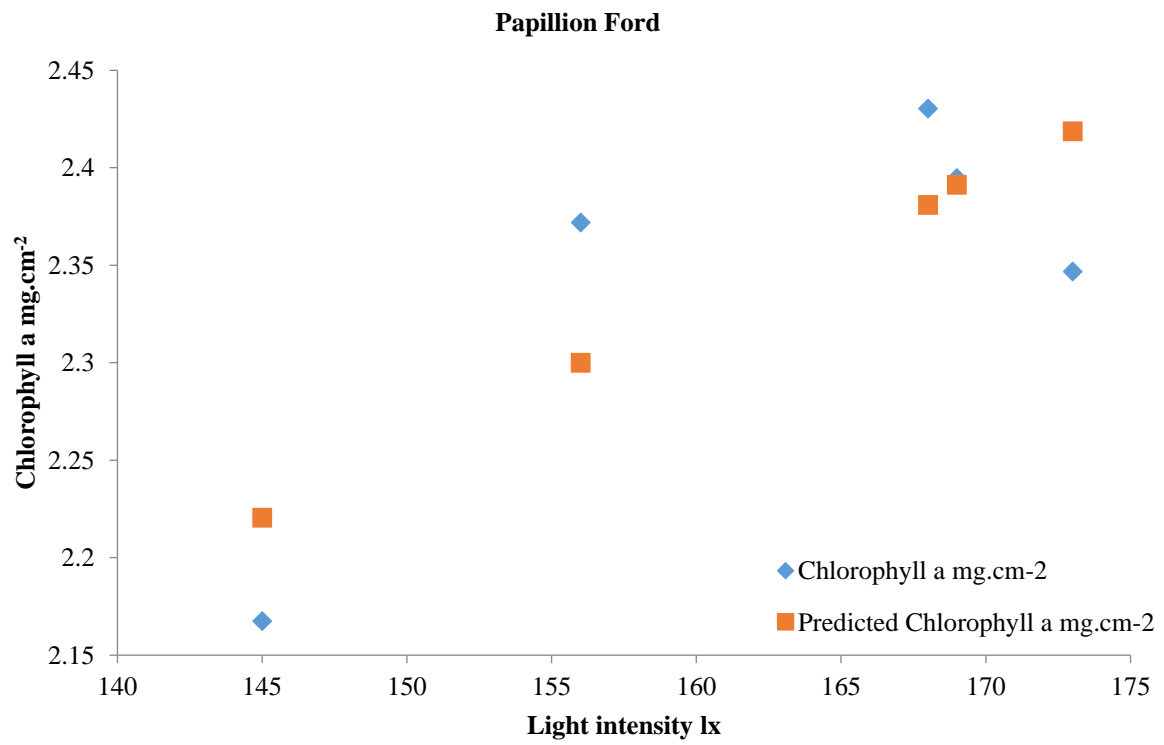
The statistical analysis for the seasonal differences across the study site showed that there is a significant change ( $P=0.033$ ) during the months of the study, namely that number of individuals and chlorophyll a concentration depended both on seasonal change (Appendix 4.6). In summer season (July) both the number of individual and chlorophyll a concentration significantly increased and reached their optimum recorded value.

The analyses for the recorded light and temperature illustrate that all sites are environmentally similar (Fig. 4.3 and Fig. 4.4), so the study sites could be compared by water quality alone. I accept that the light measurements, measuring Lux but not using a PAR meter and not measuring at equal times of day, were not adequate, but these were the best I had available to me during the year of field experimentation.

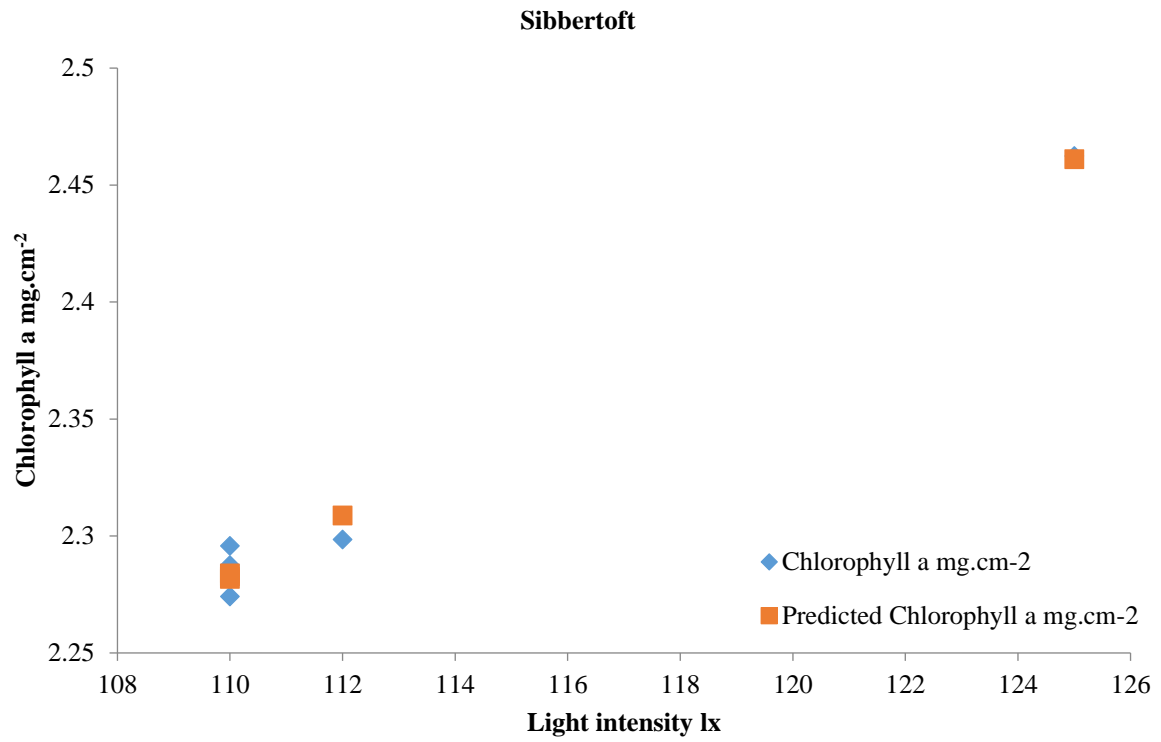




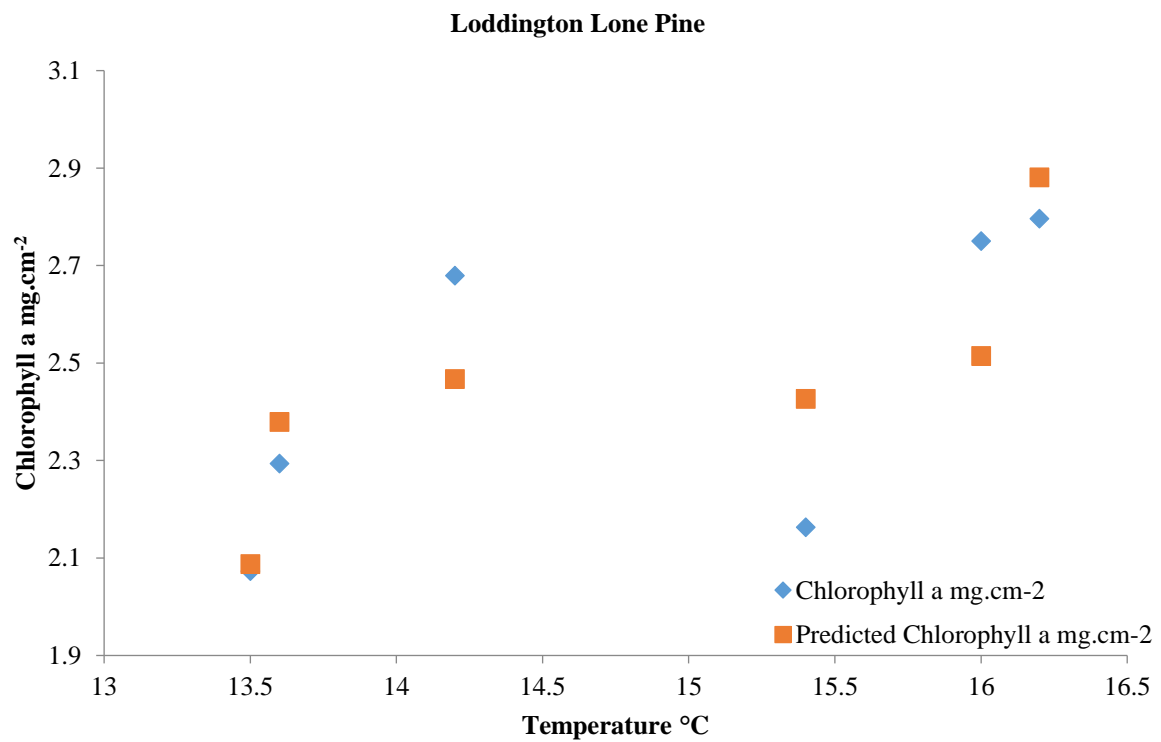
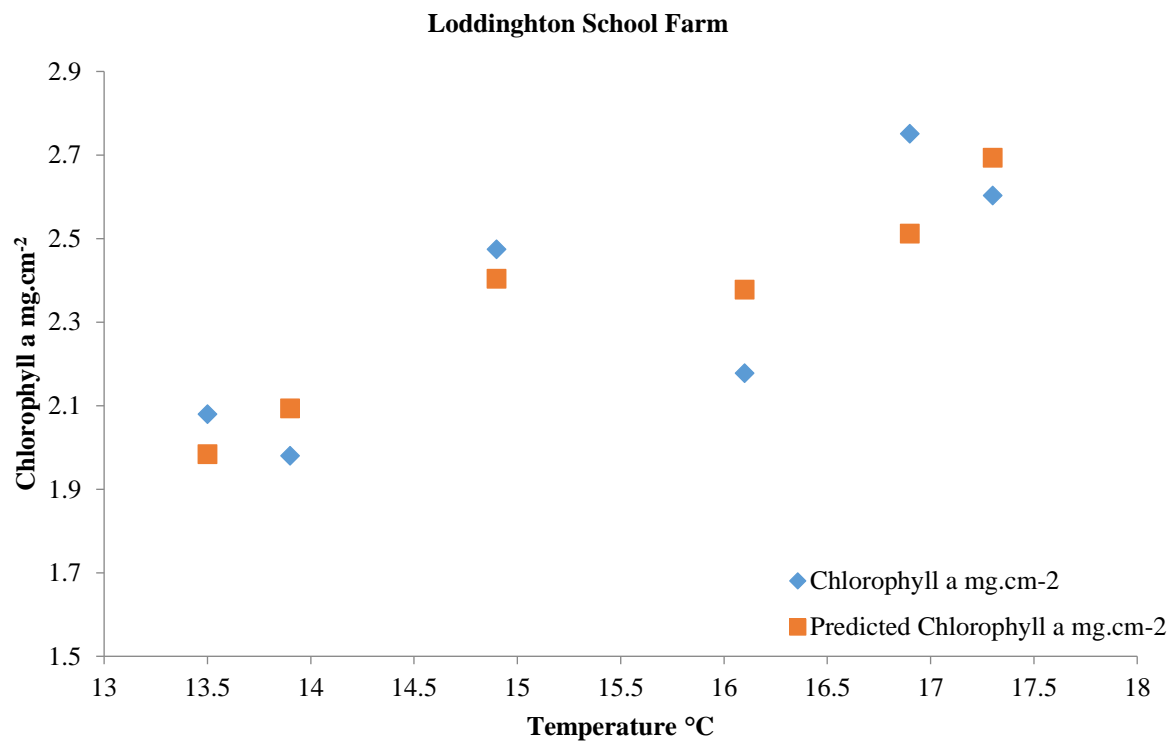


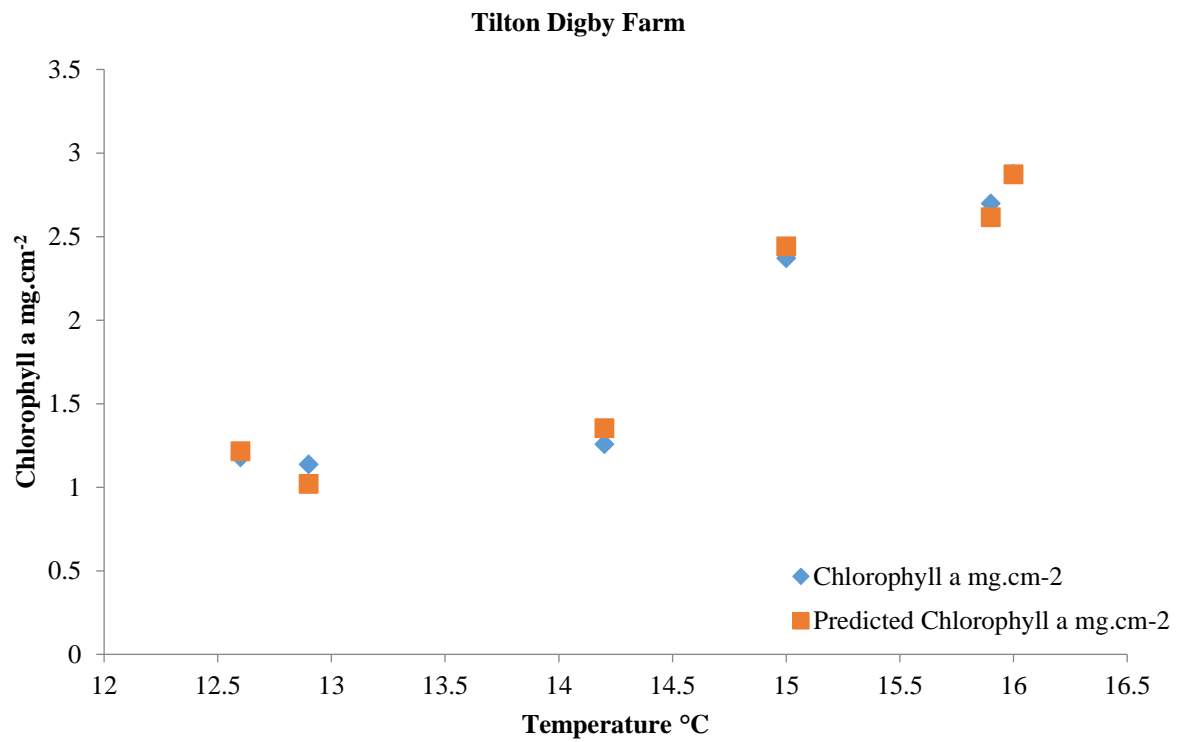
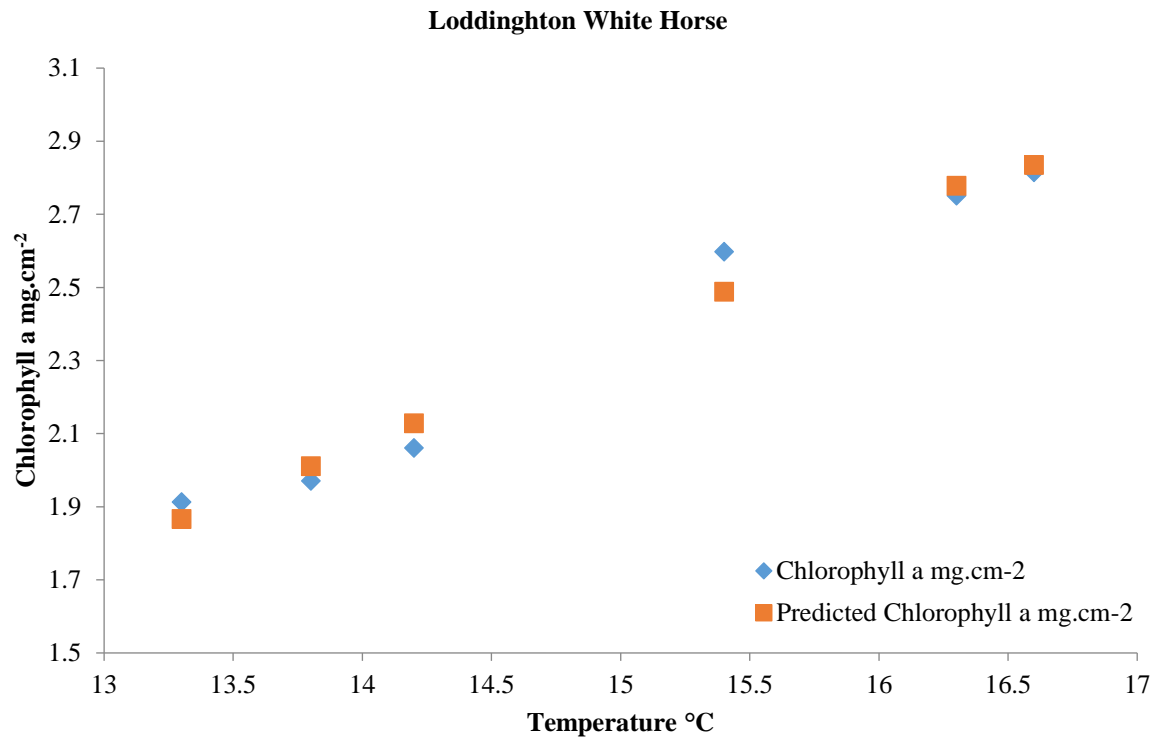


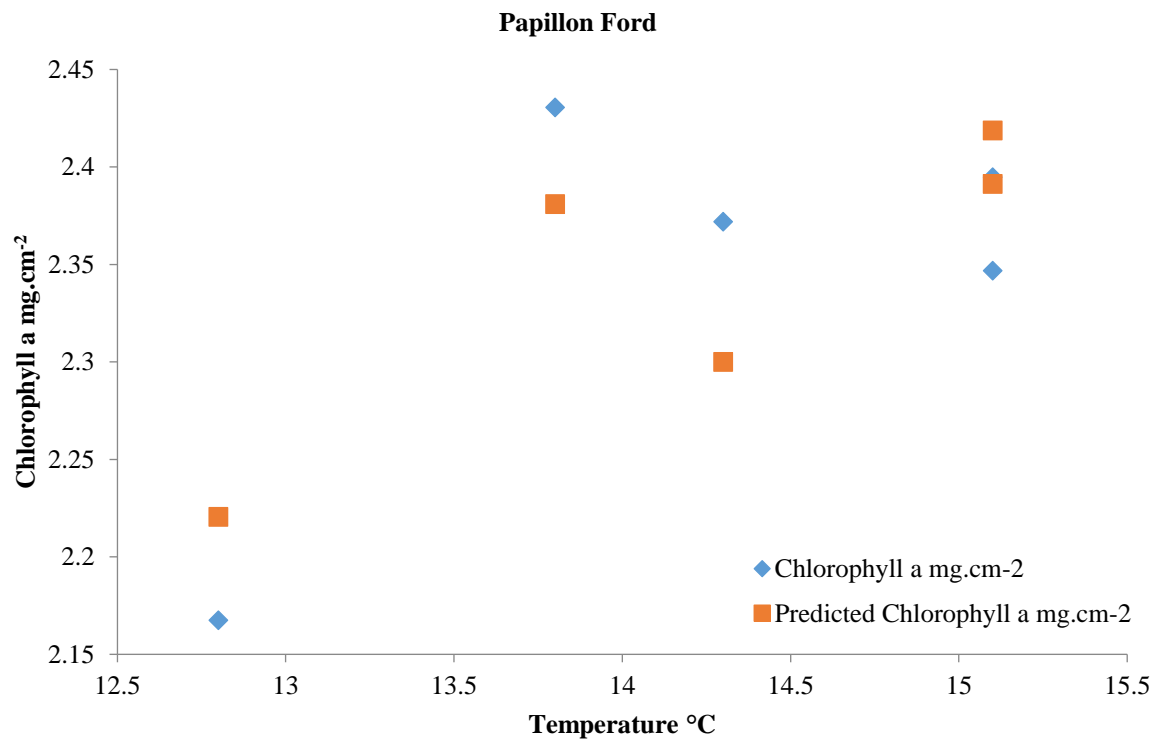
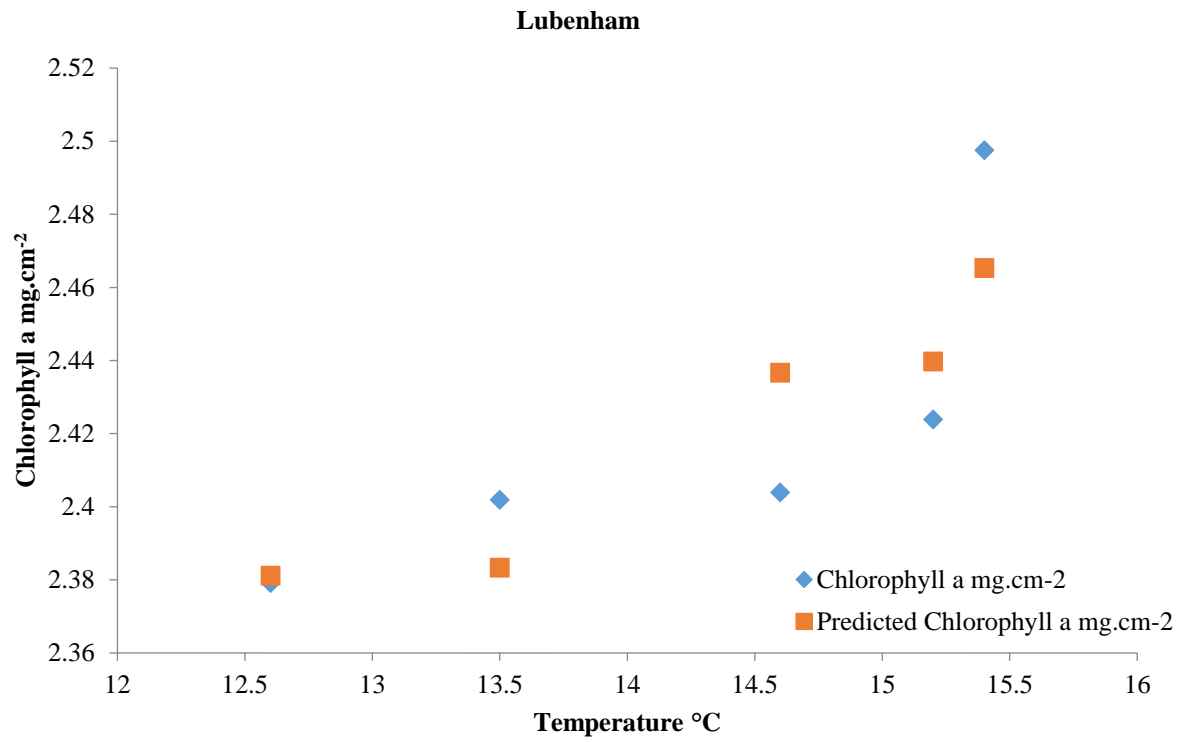


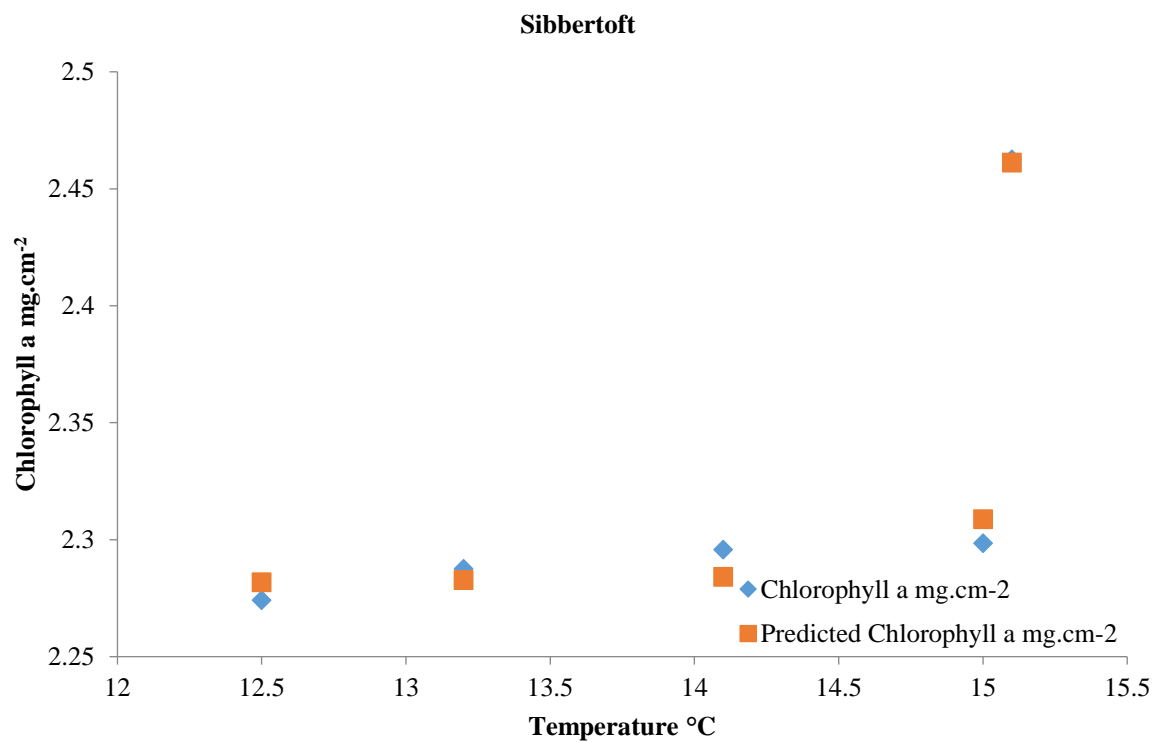
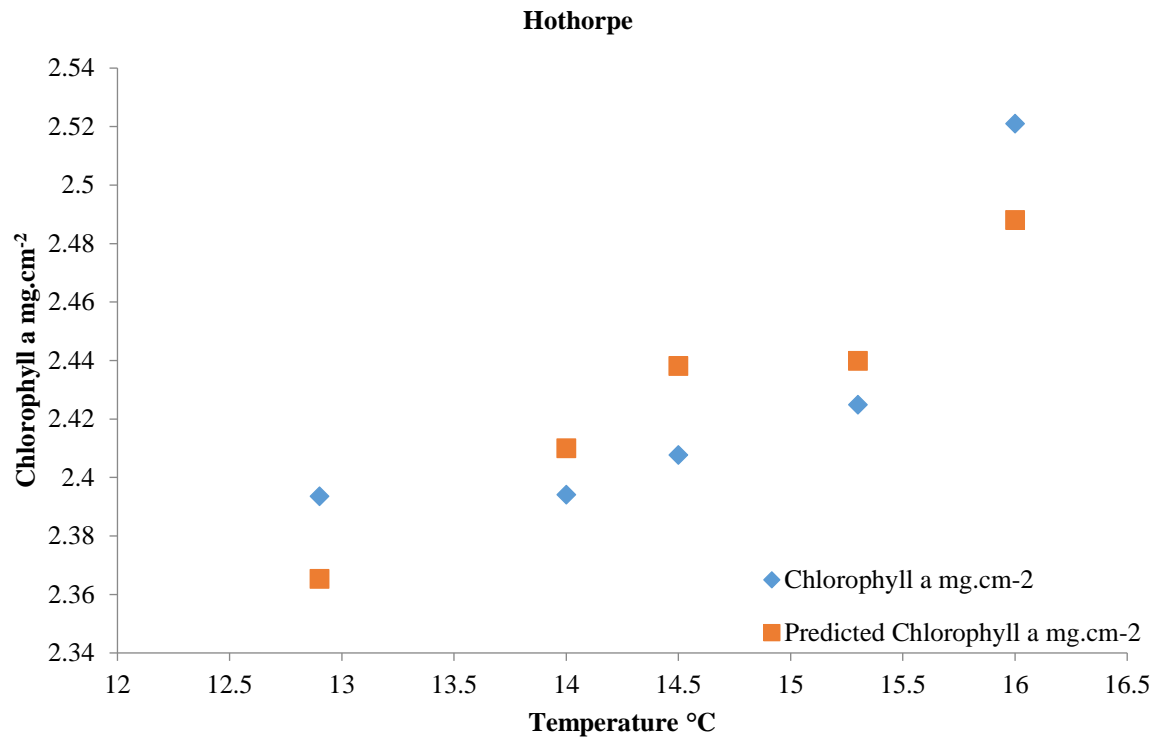


**Figure 4.3 Light analyses that showed that all sites are environmentally similar**









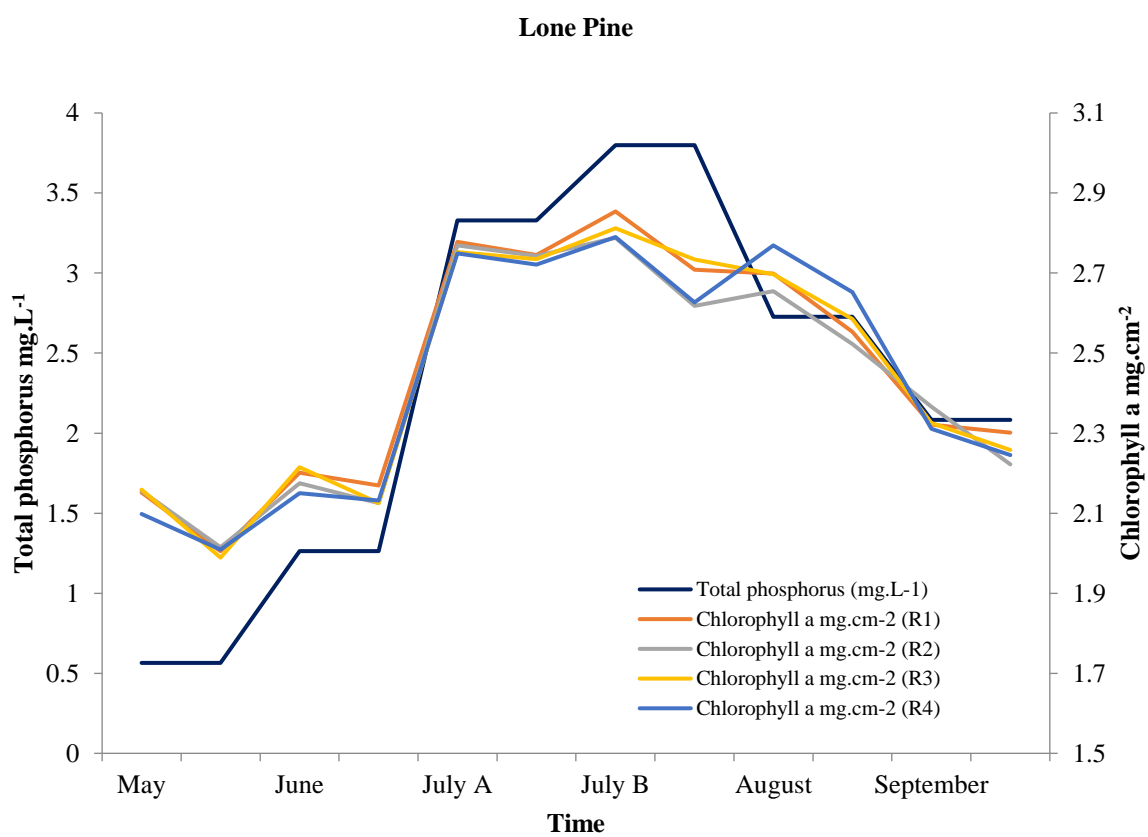
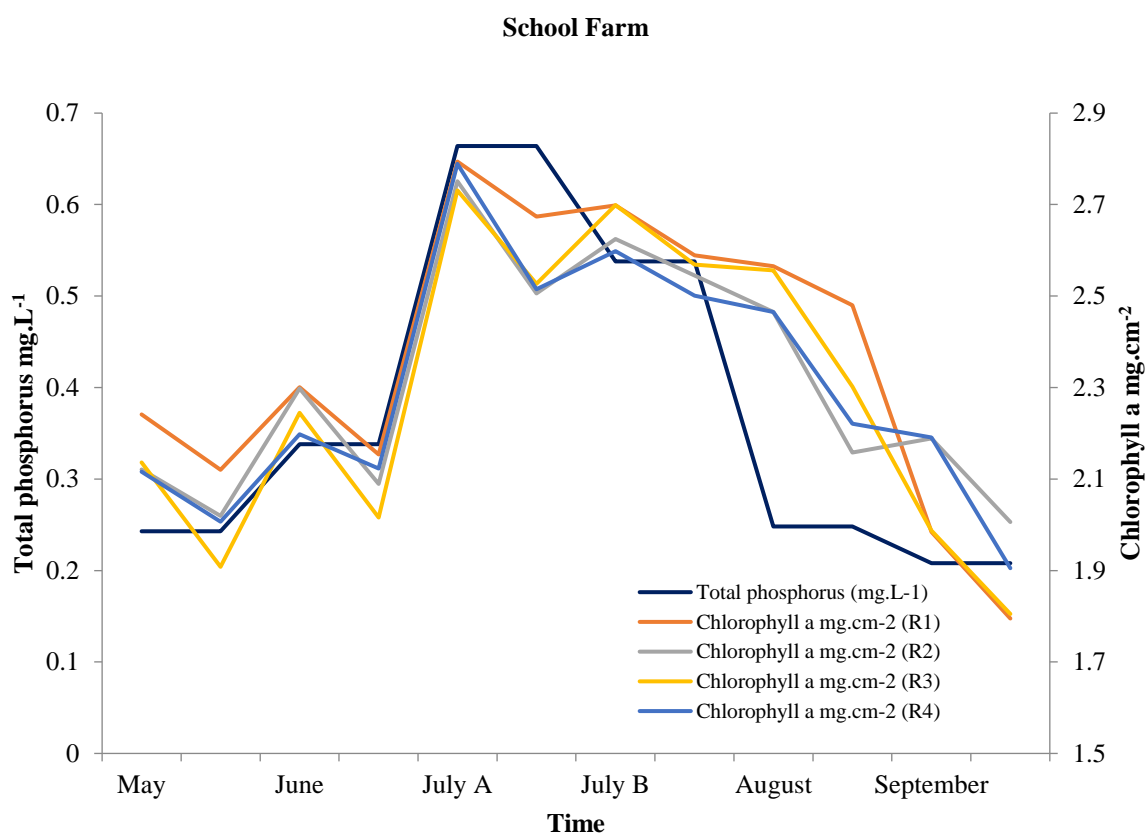
**Figure 4.4** Temperture analyses that showed that all sites are environmentally similar

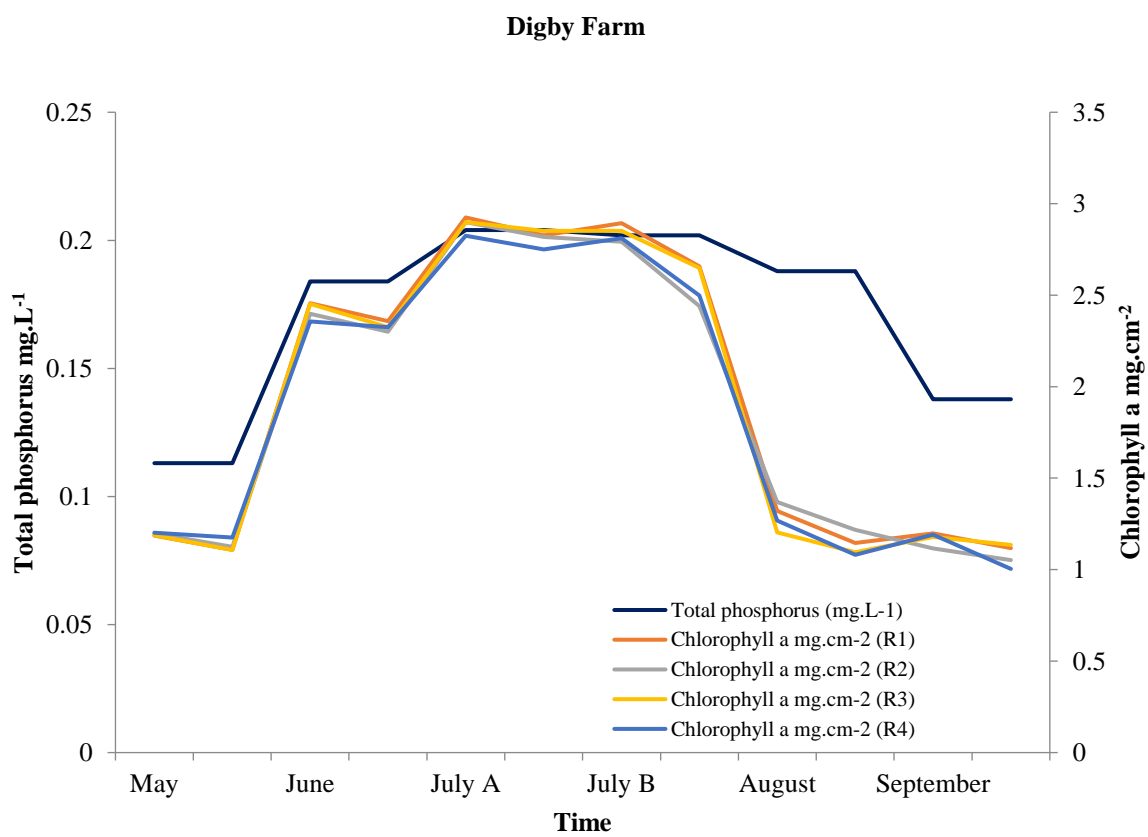
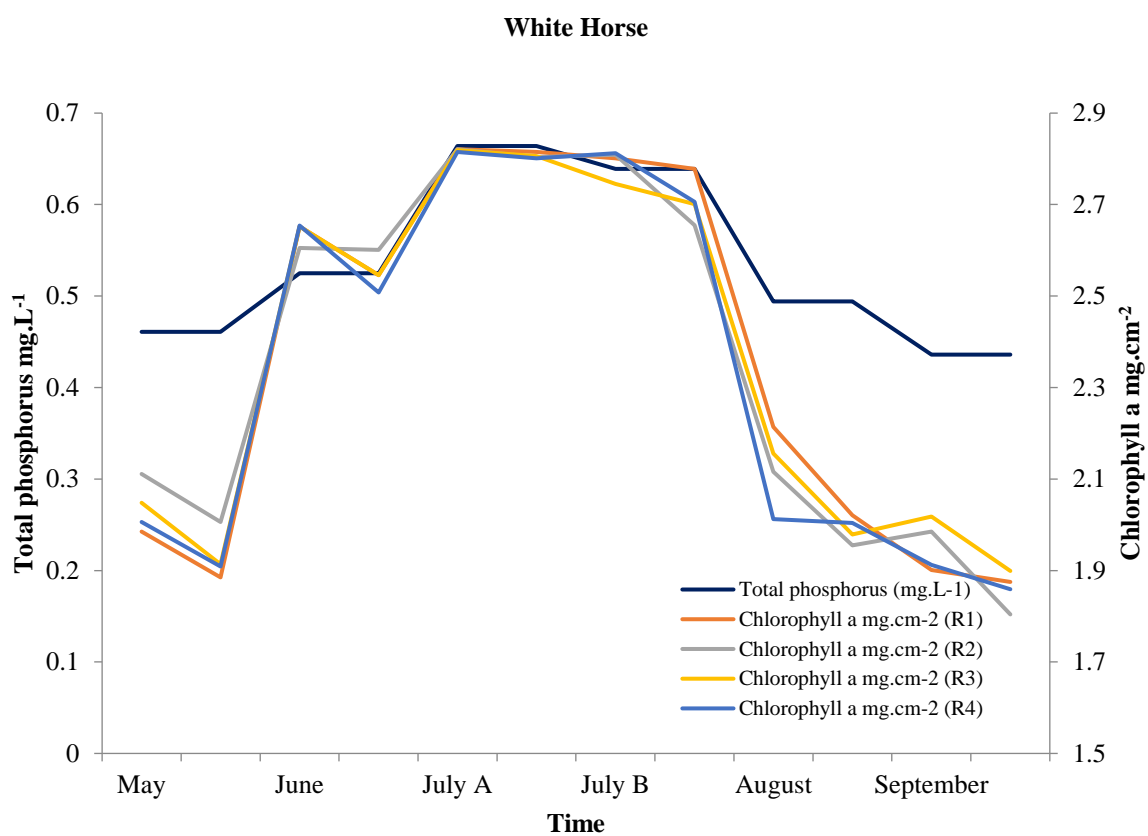
The illustrations for the total P and sediment gradients across sites over the study time show that the trends in total P are reflected by the biological trend in positive way. The results show chlorophyll a increased as the total P gradient increased (Fig. 4.5). The effect of sediment upon the biological activity (chlorophyll a) was negative, as chlorophyll a decreased as the sediment gradient increased (Fig. 4.6).

*Brachysira vitrea* was abundant across the whole current speeds as Digby Farm site within the EyeBrook where the lowest total P was recorded with range from 0.113 mgL<sup>-1</sup> to 0.204 mgL<sup>-1</sup>. Samples in Lubenham in Upper Welland area where P ranged between 0.245 mgL<sup>-1</sup> and 0.428 mgL<sup>-1</sup> were dominated by *Achnanthyidium minutissimum* with other nutrient sensitive species.

In the EyeBrook Lone Pine site, where the recorded P was within the range of 0.565 mgL<sup>-1</sup> to 3.799 mgL<sup>-1</sup> the dominant species was *Rhoicosphenia abbreviate* as this species is tolerant of moderate to heavy organic pollution. *Navicula cryptotenella* were recorded to be particularly dominant in Sibbertoft as there was a gradual increase with the increase of P from 0.587 mgL<sup>-1</sup> to 2.82 mgL<sup>-1</sup> which the highest recorded in the Upper Welland sites. *Brachysira vitrea* and *Achnanthyidium minutissimum* showed a linear decline with increasing levels of P, while the *Rhoicosphenia abbreviata* and *Navicula cryptotenella* showed a linear increase with increasing levels of P.

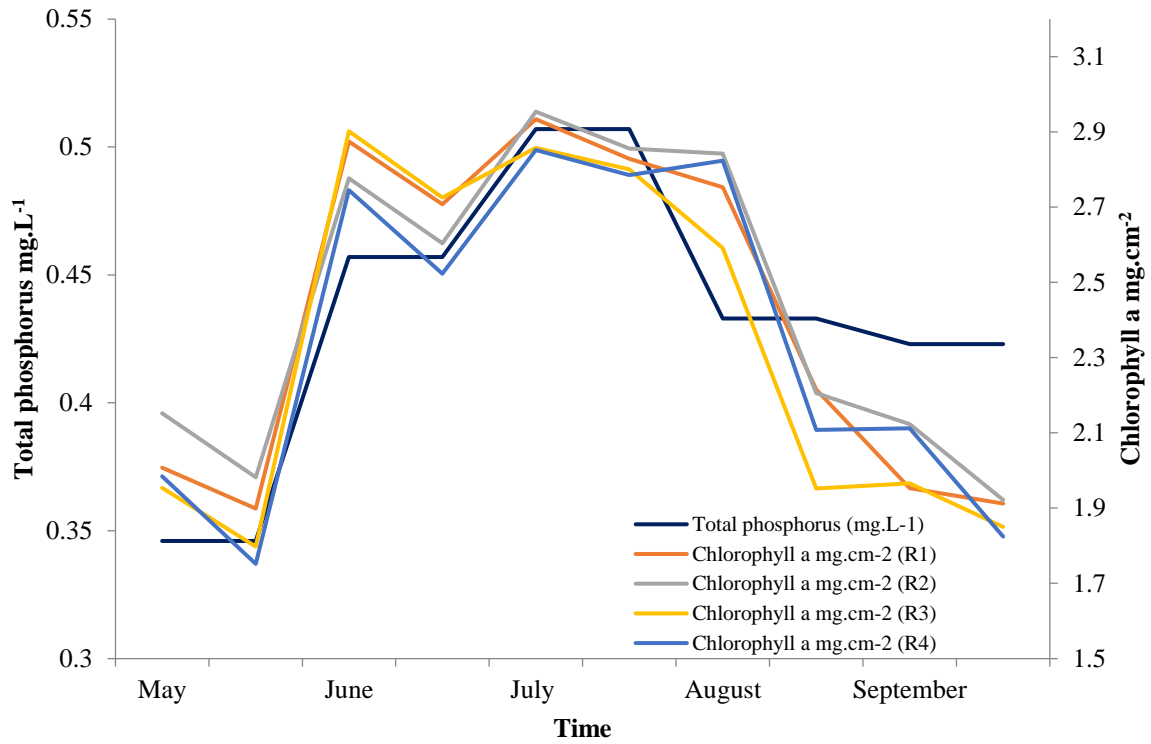
The locations of the study sites are shown in Fig. (3.1, 3.1 a, 3.1 b) and the environmental characteristics of each of the study sites are summarised in Table 4.1. Each study site has its own environmental characteristics that differentiate it from the other site. For example, the high current speed and low light intensity site with generally low P level has the lowest Chlorophyll a as it was recorded at Digby Farm. At the other end, the low current speed sites with high light intensity has the highest P concentration and the highest chlorophyll a, at Sibbertoft as example.



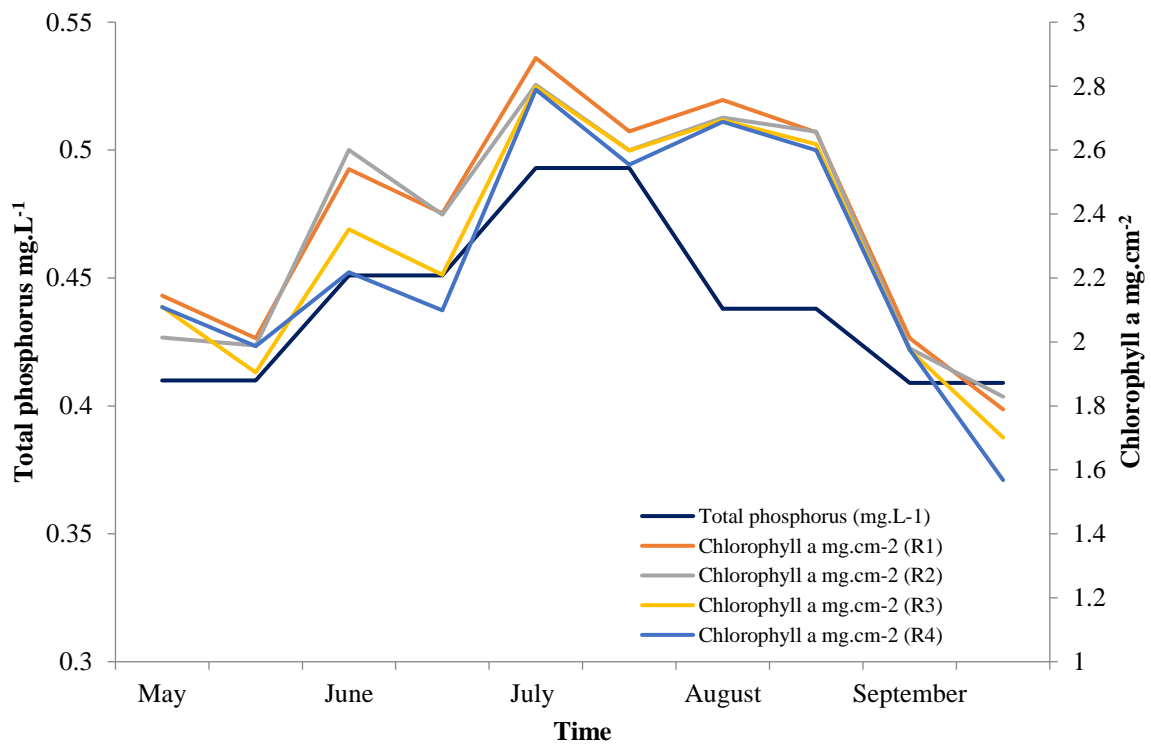


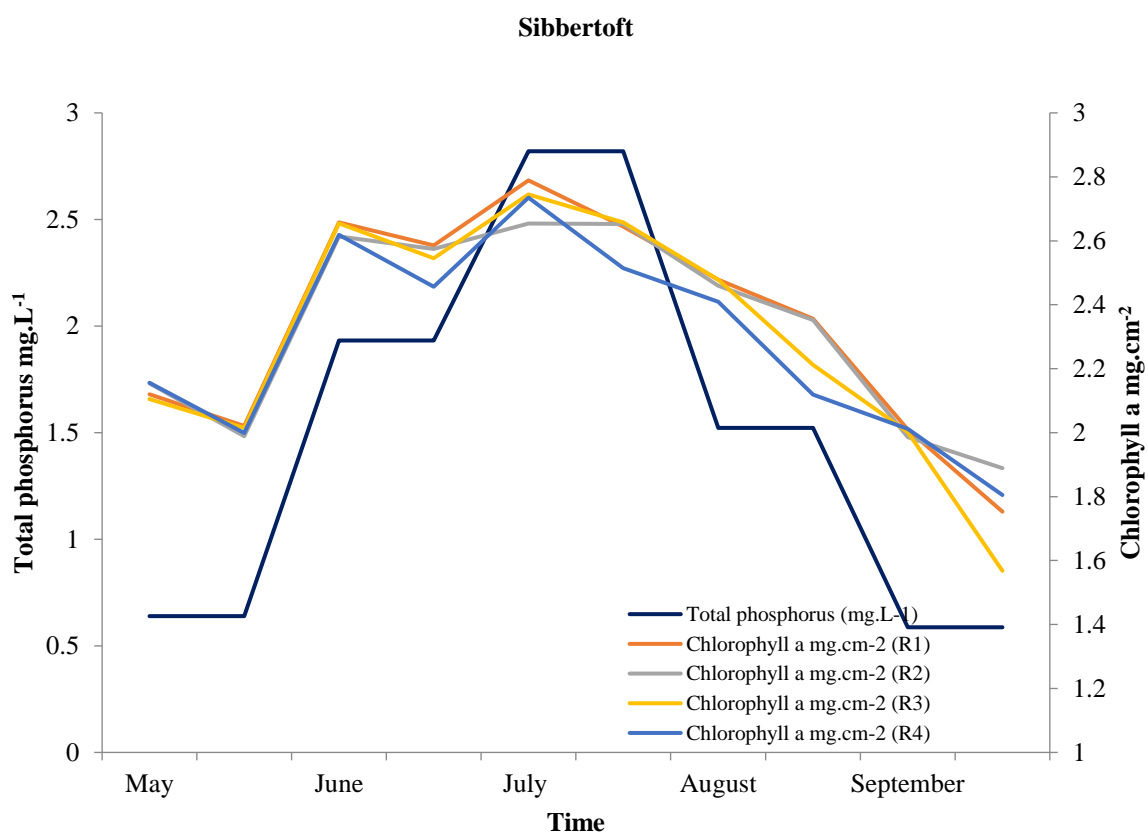
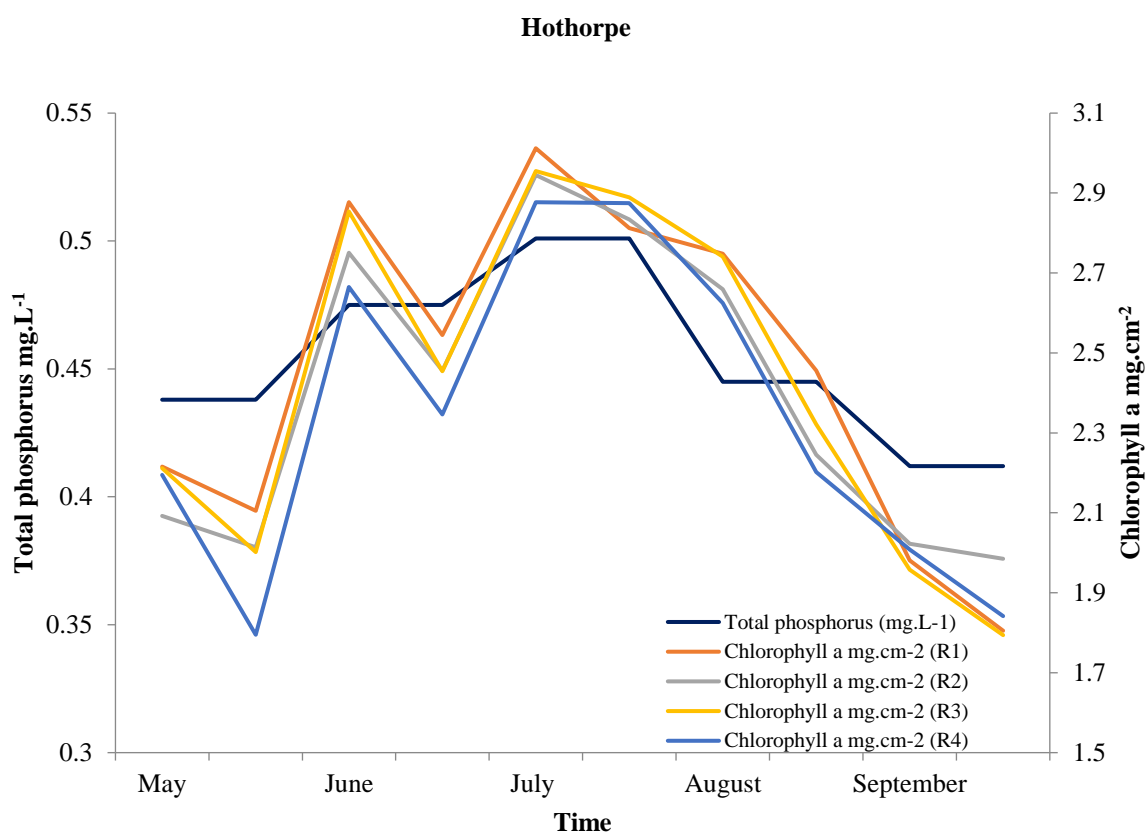


### Lubenhams

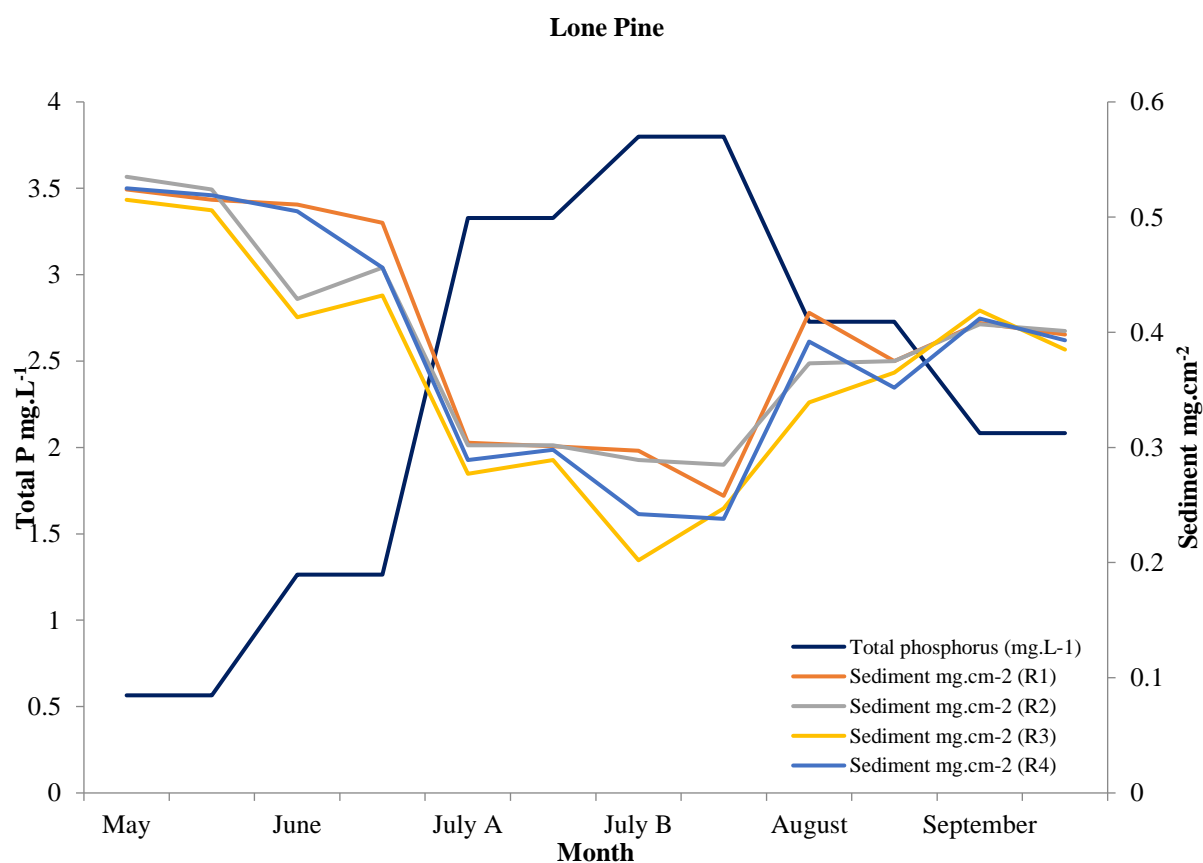
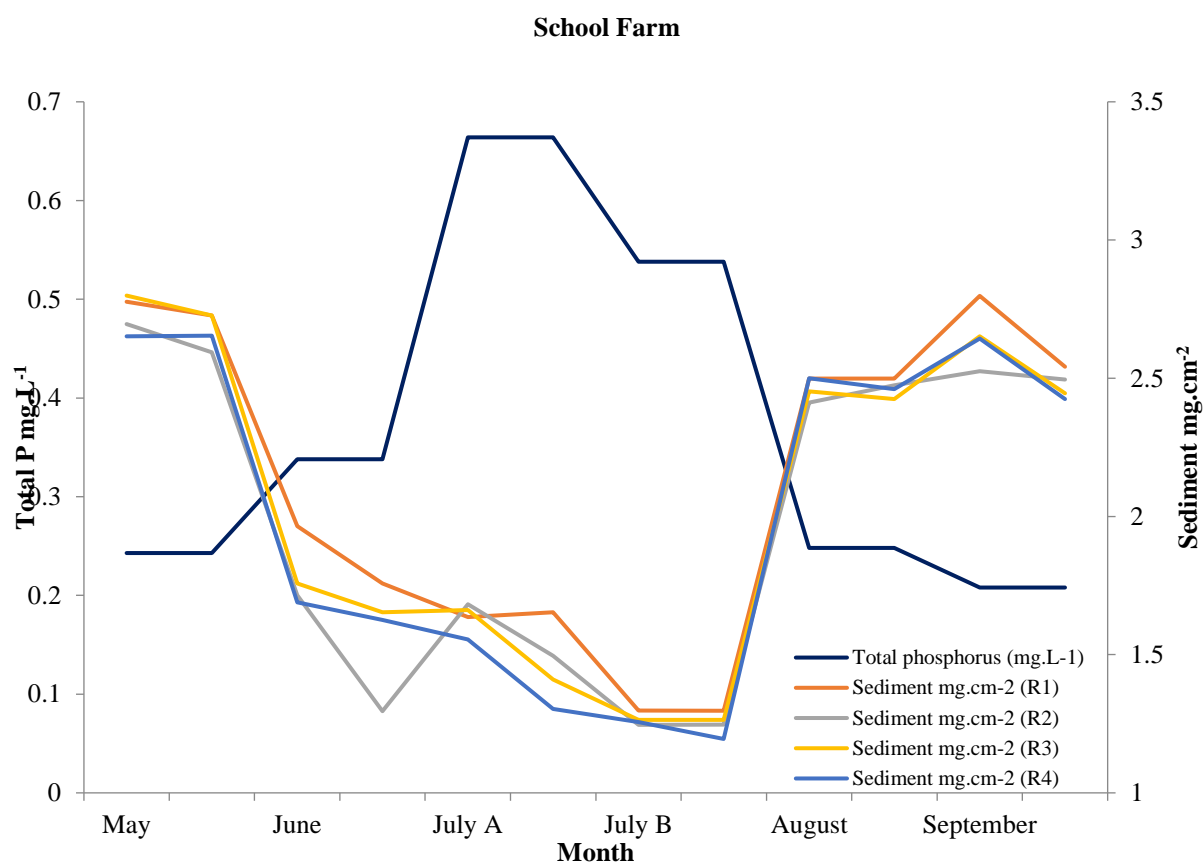


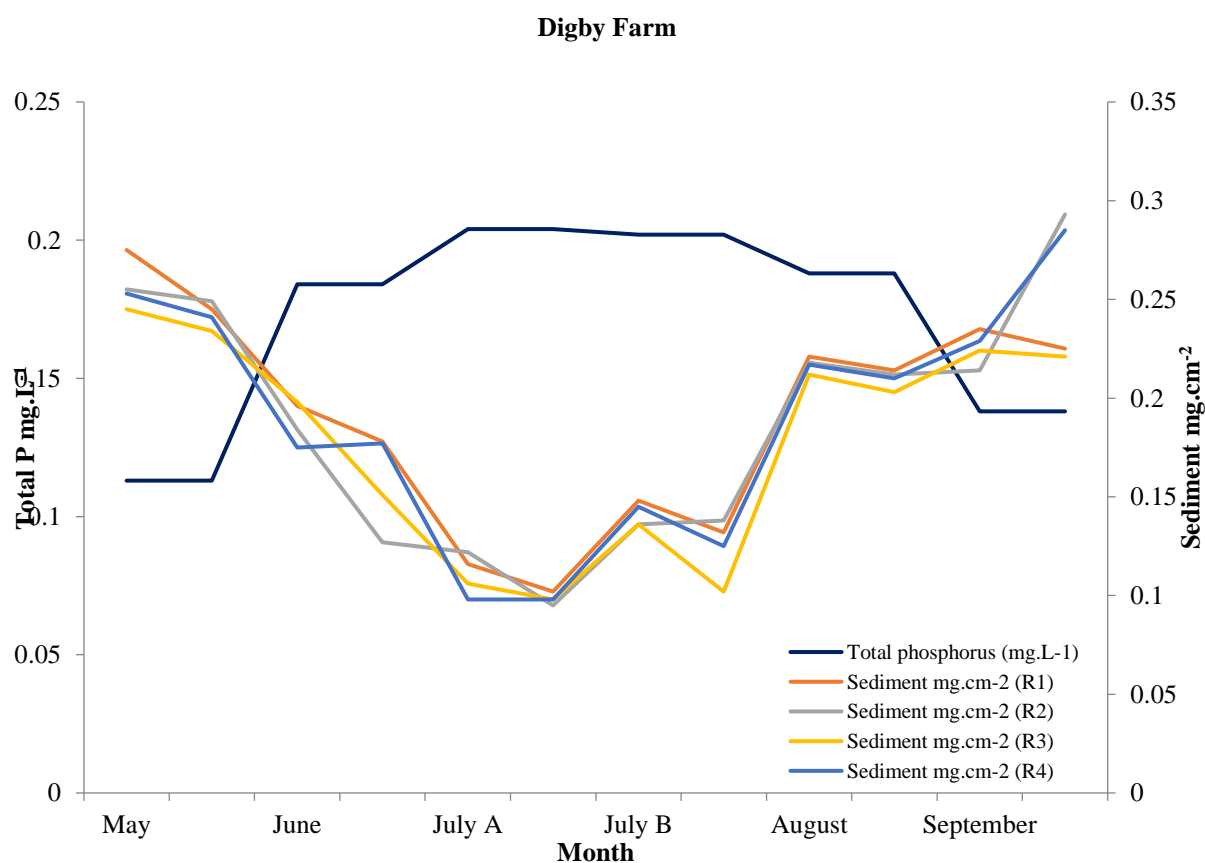
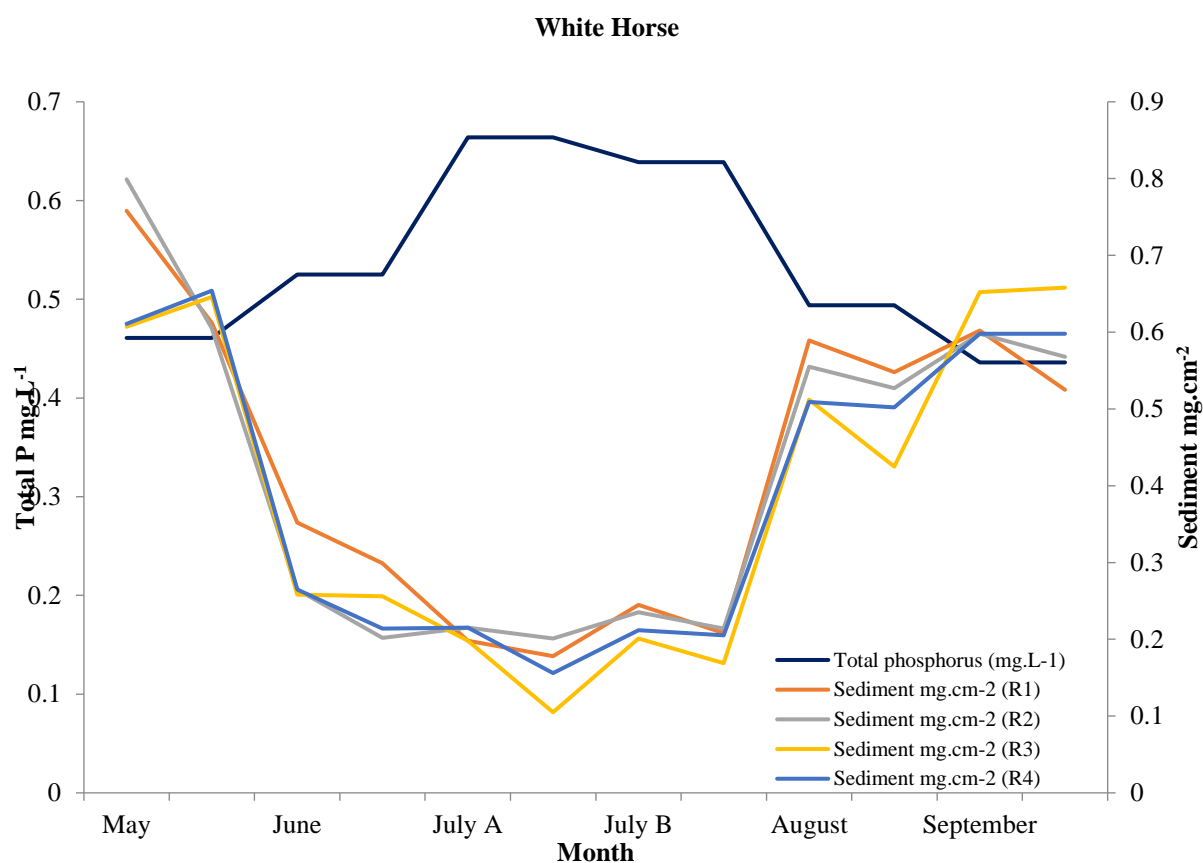
### Pappilon Ford

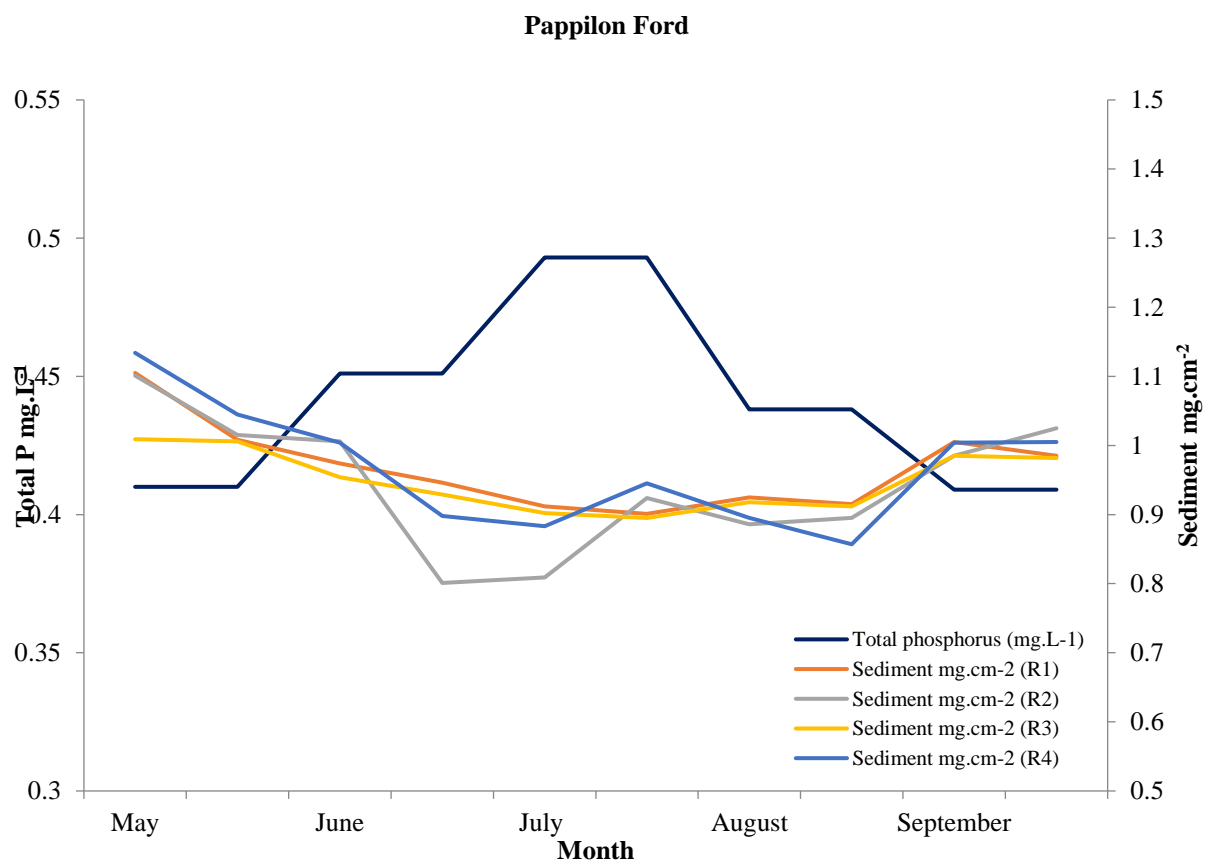
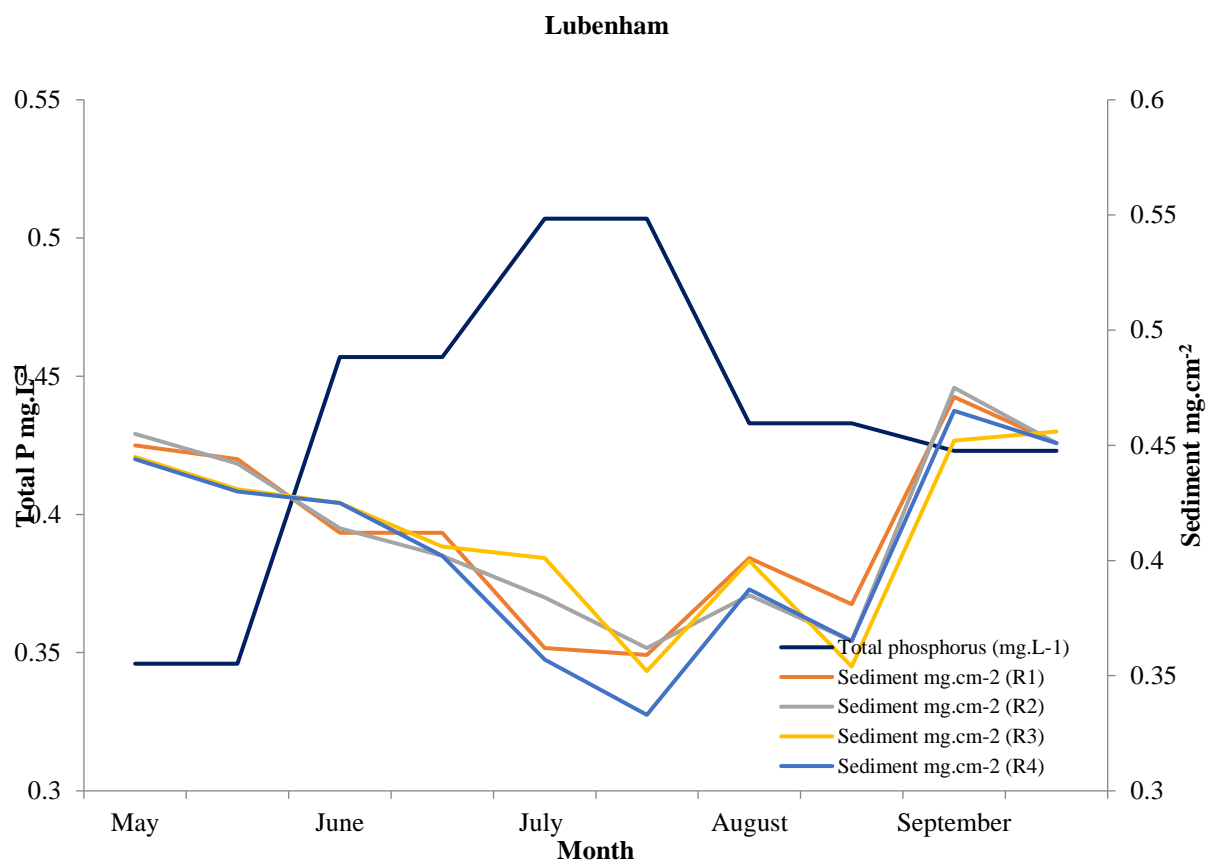


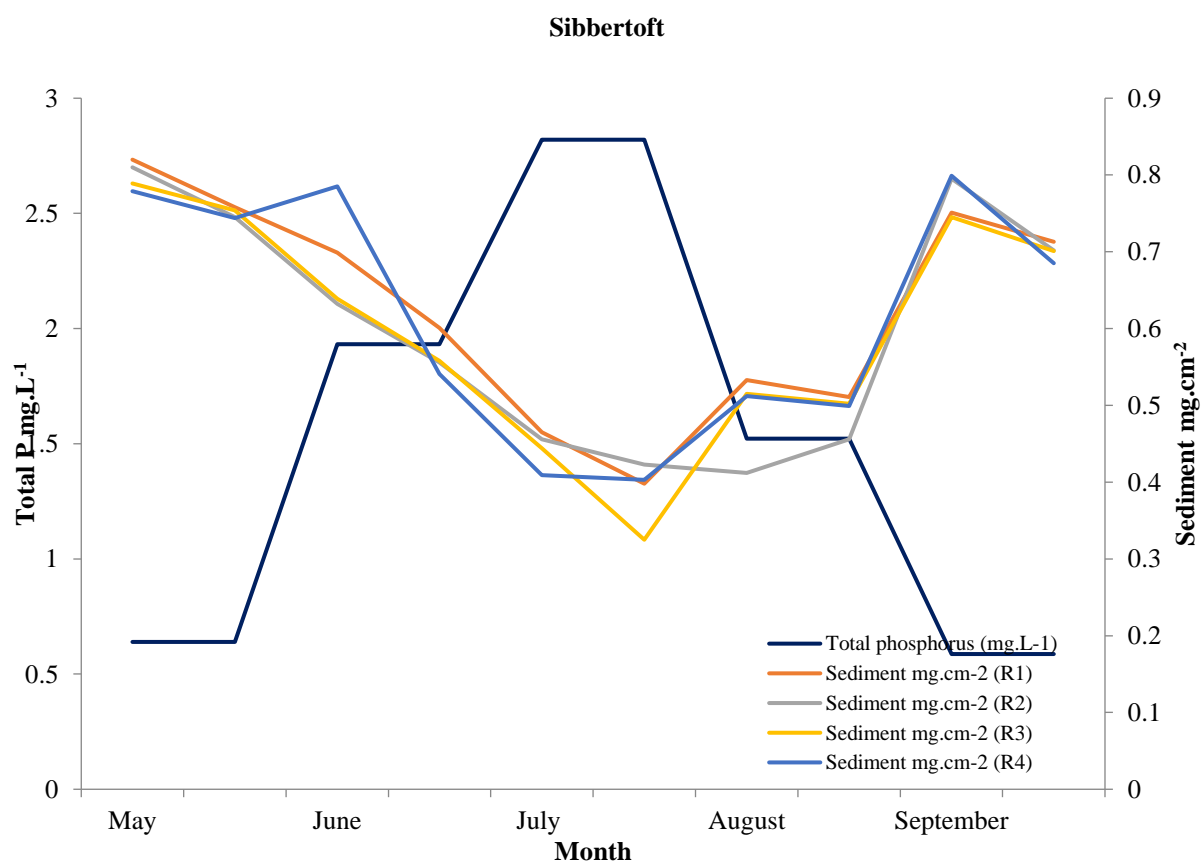
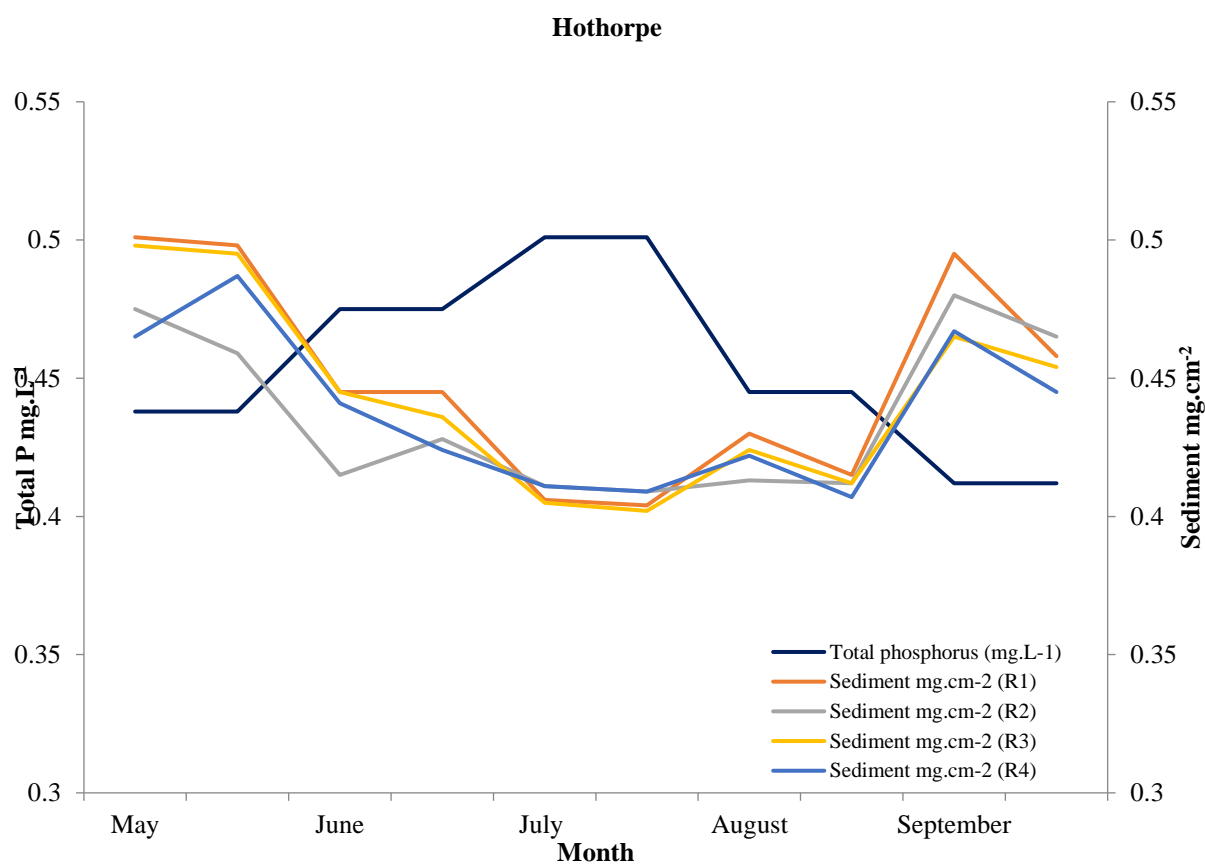


**Figure 4.5 Total P gradients effect on chlorophyll a across the study sites**





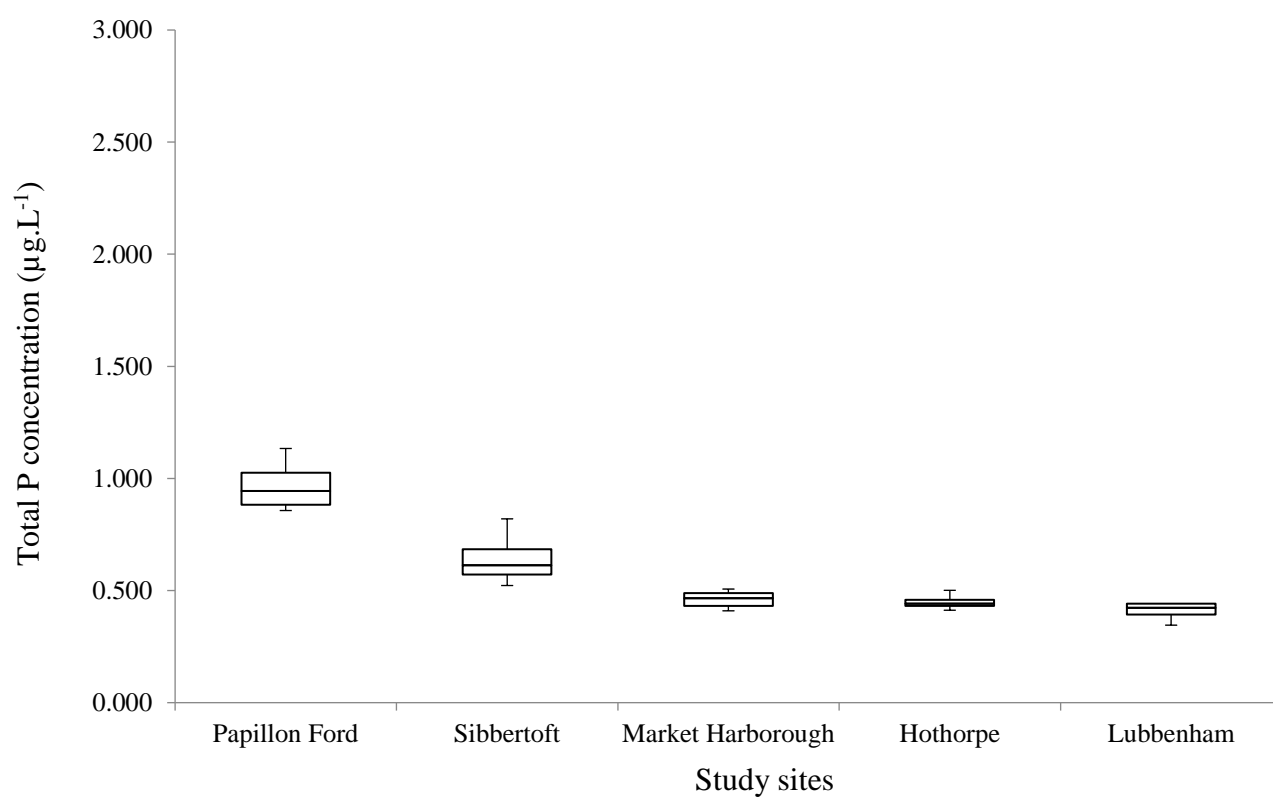
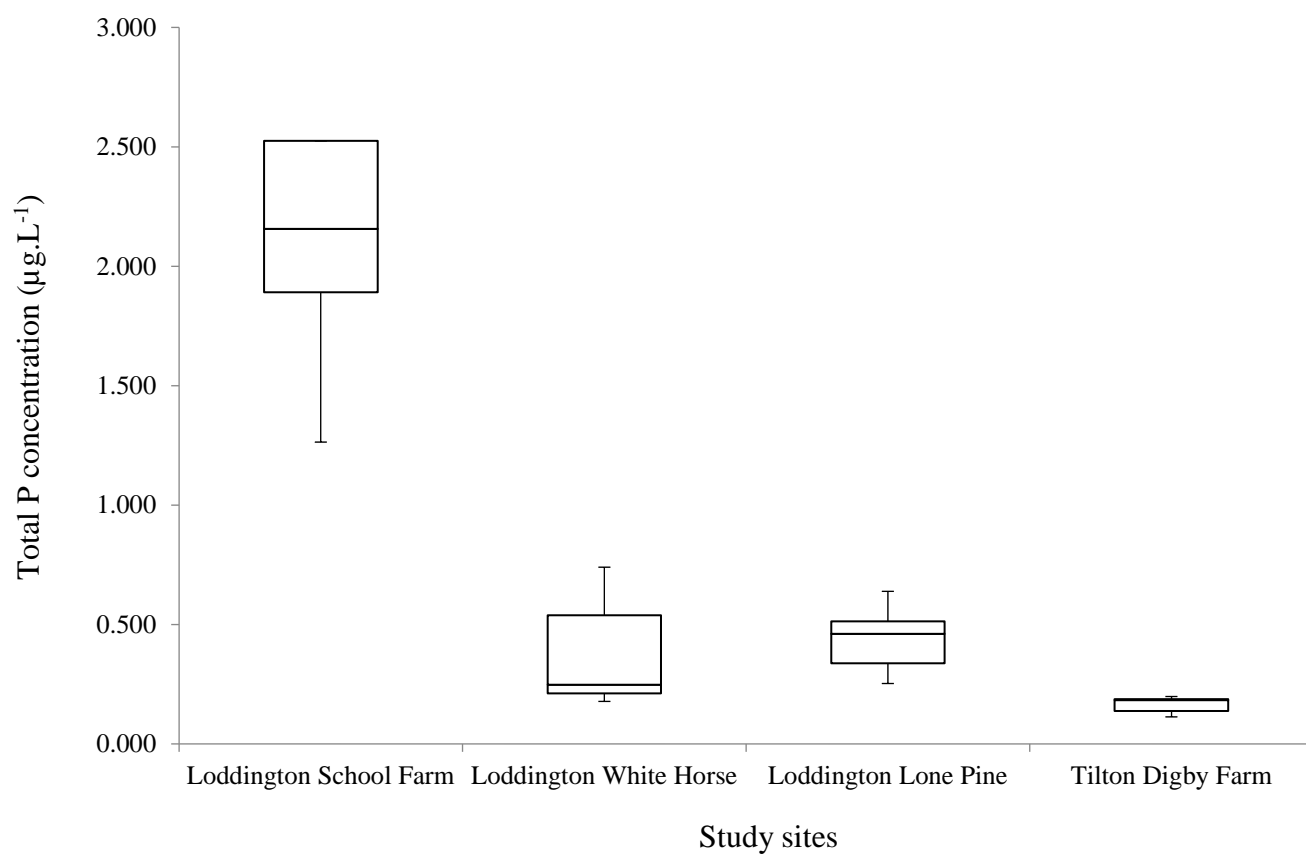




**Figure 4.6** Sediment gradients effect on chlorophyll a across the study sites

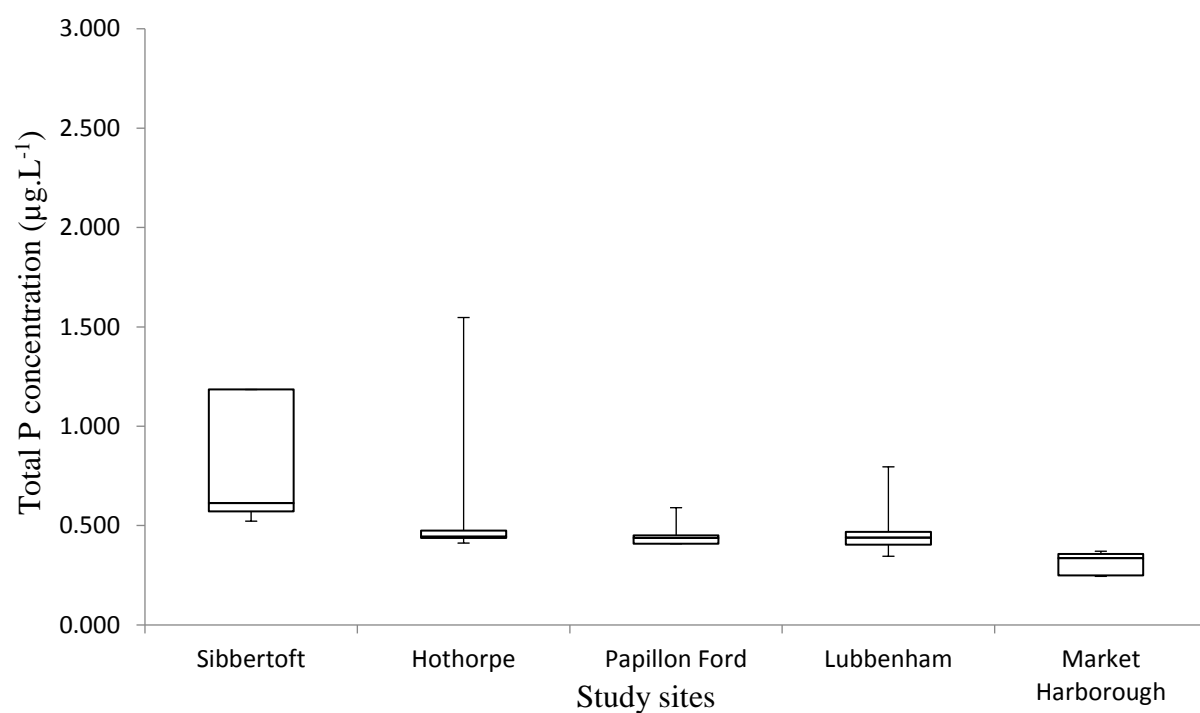
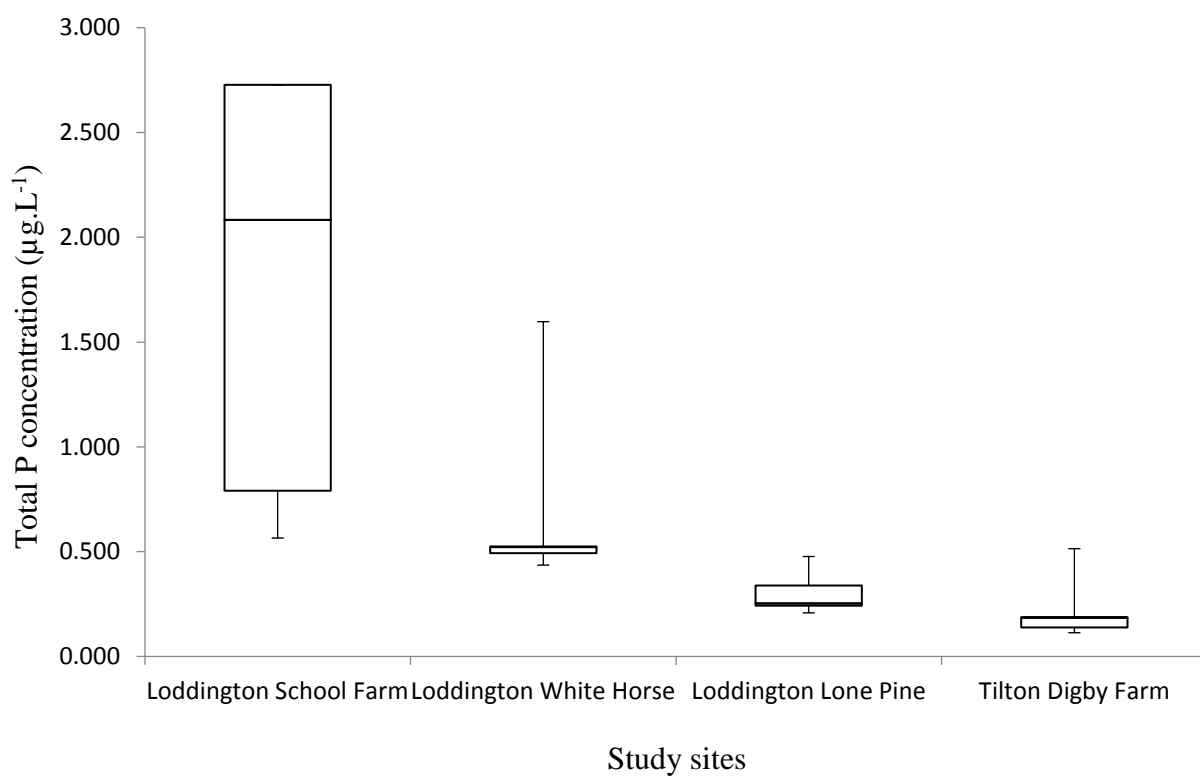
Sediment deposition at the EyeBrook sites was maximum as  $2.703 \text{ mg.cm}^{-2}$  at Loddington School Farm, the lowest was  $0.104 \text{ mg.cm}^{-2}$  at Tilton Digby Farm. Compared to the upper Welland sites, the maximum sediment deposition was  $1.053 \text{ mg.cm}^{-2}.\text{day}^{-1}$  at Pappilon Ford, the lowest was  $0.368 \text{ mg.cm}^{-2}$  at Lubenham (Fig 4.7).

Different total P concentration were recorded between the study sites, in which highest concentration for the Eyebrook sites was recorded at Loddington Lone Pine with  $3.328 \text{ mgL}^{-1}$ , minimum concentration was recorded at Tilton Digby Farm  $0.113 \text{ mgL}^{-1}$ . The highest concentration for the Upper Welland sites was recorded at Sibbertoft with  $2.82 \text{ mgL}^{-1}$ , minimum concentration was recorded at Lubenham with  $0.245 \text{ mgL}^{-1}$  with an average of  $0.314 \text{ mgL}^{-1}$  (Fig. 4.8).



**Figure 4.7 Sediment deposited rates for the study sites from May to September 2014**





**Figure 4.8 Total P gradients at the study sites from May to September 2014**

The RDA outcome for the individual species shows that all the environmental variables (total P and sediment, as explanatory variables. Total variance is 58.00 and constrained variables (the variables explained by the RDA axes) is 45.81, with unconstrained variables 12.19. This means that 79% of the variation can be explained by the explanatory variables. The eigenvalue axis shows that the first two axes of the RDA explains 42 out of the 58 total variances, and this means the first two axes is efficient to explain 72% of the environmental variables (Appendix 4.7).

Fig. (4.9) displays the first axis positively correlated with sediment content, light intensity and water temperature as listed in table 4.5, and separated sites with high concentrations of nutrient (Sibbertoft and Lone Pine) and high content of sediment (School Farm). In between these extreme sites, the others sites were recorded. The triplot reveals the individual species ordination. The relationship strength between the abundance of a particular species and the environmental variables is shown by the scores spatial arrangement, displayed by its position along the arrow representing the increase direction of a continuous variable. *Navicula tripunctata*, *Navicula capitatoradiata*, *Caloneis bacillum* and *Nitzschia palea* were the species most closely associated with high sediment content, on the other hand for the high P concentration the species were *Cosmarium*, *Gyrosigma attenuatum*, *Spirogyra* and *Gomphonema minutum*. Total P concentration was negatively correlated with both axes (axis one and axis two).

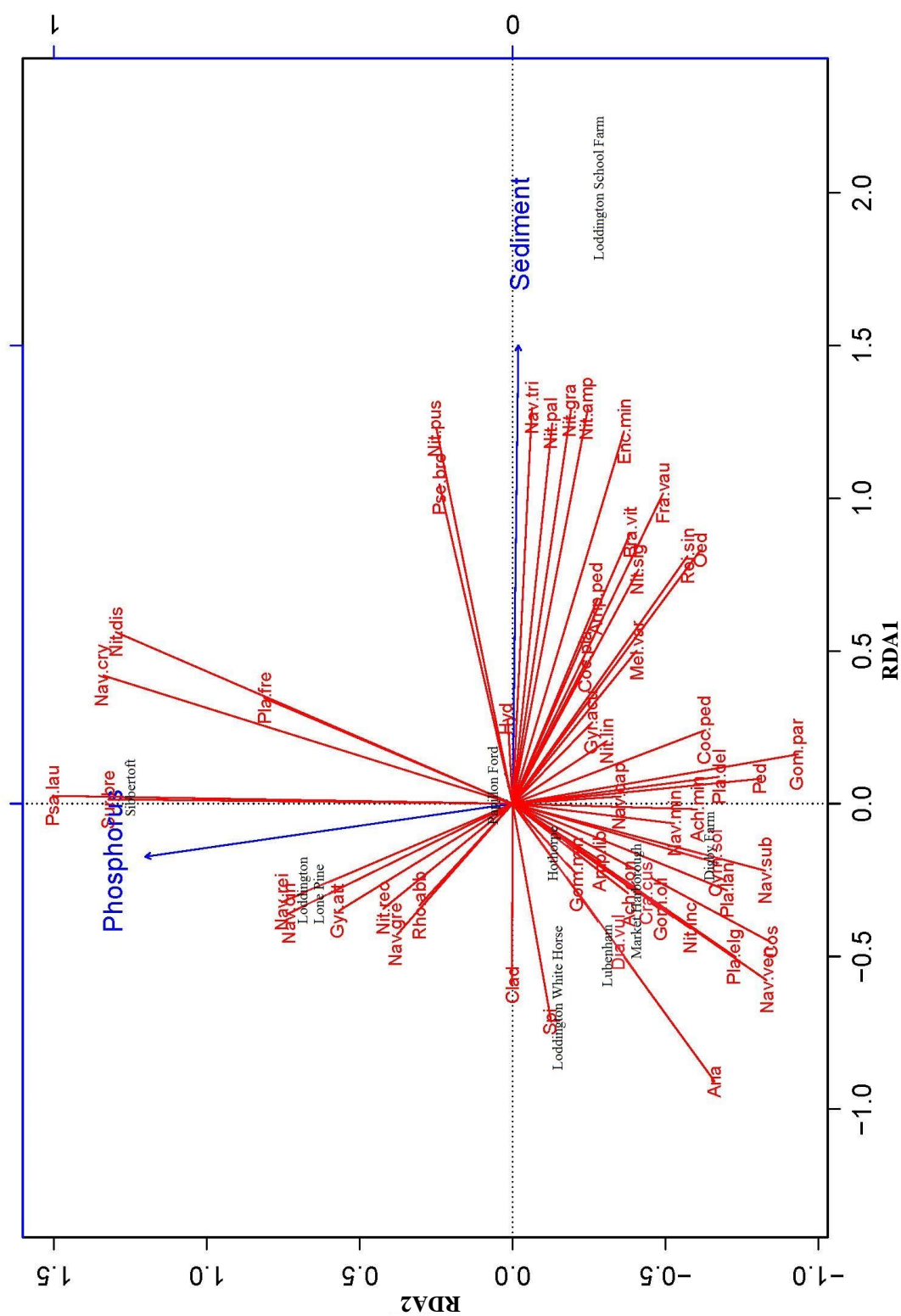


Figure 4.9 Triplot based on redundancy analysis (RDA) of the individual species in the 9 study sites

**Table 4.4 Inter set correlations of environmental variables with the first two axes for RDA of the individual species in the 9 study sites**

	Individual species		Algal groups	
	RDA1	RDA2	RDA1	RDA2
Total P	-0.16	-0.25	-0.29	-0.29
Sediment	0.91	0.02	-0.64	0.12

The algal groups' (Cyanobacteria, Chlorophyceae and Bacillariophyceae) relative abundance were used in another RDA (Table 4.4). First axis was negatively correlated with total P concentration and sediment content (Table 4.5, Fig. 4.10 and Appendix 4.8).

**Table 4.5 Percentage of relative abundance for the identified groups of algal which included in the RDA analyses in the 9 study sites.**

site	Algal groups (%)		
	Cyanobacteria	Chlorophyceae	Bacillariophyceae
Market Harborough	0.58	1.92	97.51
Lubenham	1.70	5.51	92.80
Papillon Ford	0.35	1.73	97.93
Hothorpe	1.23	0.88	97.90
Sibbertoft	0.77	1.34	97.89
Loddington Lone Pine	0.40	6.47	93.13
Loddington White Horse	0.70	7.01	92.29
Loddington School Farm	0.48	1.28	98.24
Tlton Digby Farm	2.69	7.850	89.46

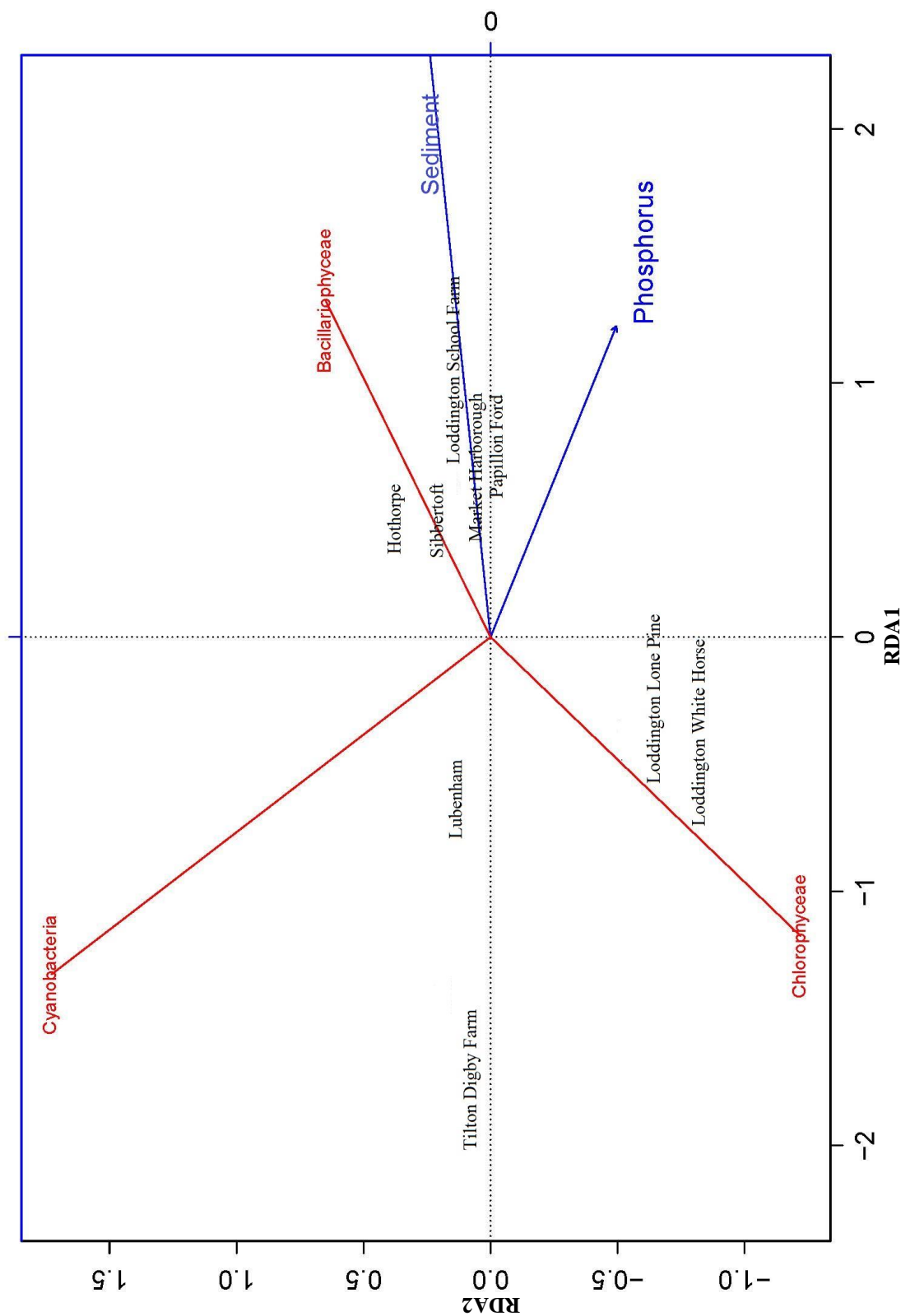
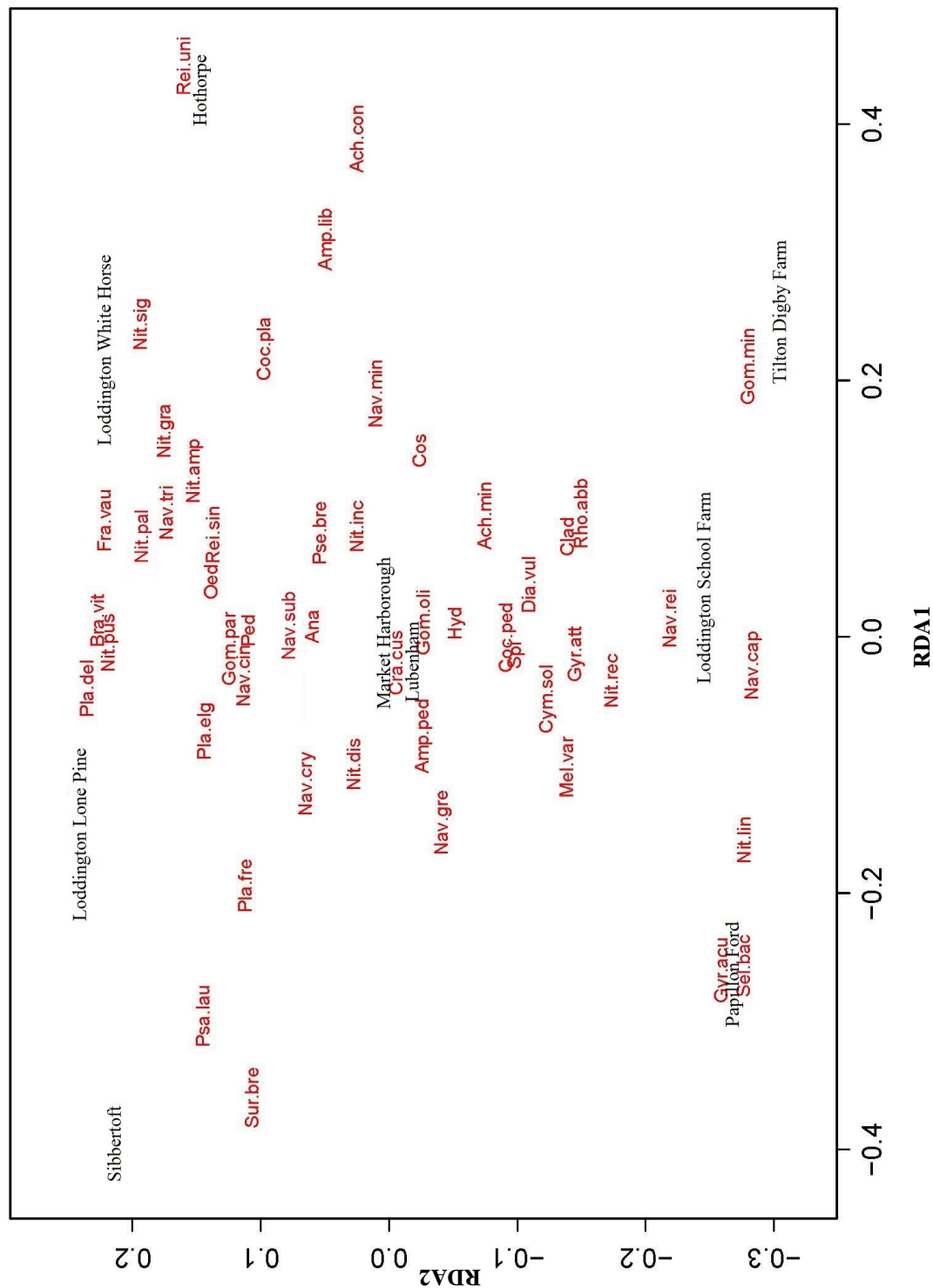
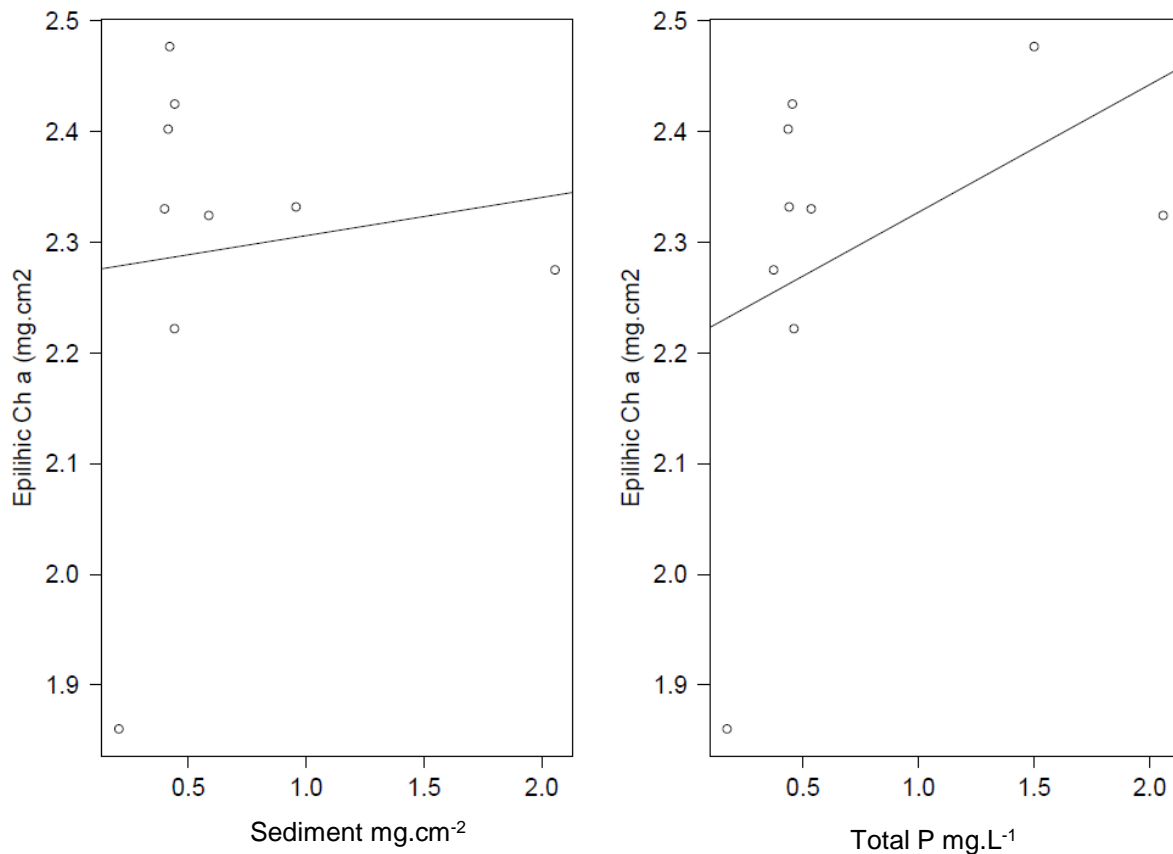


Figure 4.10 Triplot based on redundancy analysis (RDA) of algal groups (Cyanobacteria, Chlorophyceae and Bacillariophyceae) in the 9 study sites where Chlorophyceae was negatively correlated with total P concentration and sediment content

Axis one was positively related to the abundance of some genera *Sellaphora*, *Stenopterobia* and *Tabularia*) and negatively related to several others (i.e., *Achnanthes*, *Encyonema*, *Fragilaria*, *Staurosira*, *Arthrospira*, *Amphipleura* and *Luticola*). Axis two was positively related to *Craticula* and negatively related to *Fallacia* and *Thalassiosira* (Fig. 4.11).



Chlorophyll a increased with both sediment and nutrient concentration (Fig. 4.12). Streams with moderate sediment or nutrients concentration had slightly higher chlorophyll a amount than streams with high sediment and nutrients concentrations, indicating a non-linear response. Spearman rank correlation test was used to test the relationship and the result was 0.88 which shows very strong relationship



**Figure 4.12** Chlorophyll a in relation to sediment and nutrients (total P) concentration in the sampling sites.

#### 4.6. Discussion

The results of this study show that tiles were suitable artificial substrata for providing representative samples of natural diatom community composition in the streams studied. These tiles provided results to the range that replicates samples at each stream generally supported similar compositions of algal community, and there were no significant differences between natural and artificial substratum. The artificial substrata in our experiment reproduced diatom well. Similar results were seen in the work of (Castenholz, 1960) although the artificial substrates show less species diversity than natural substrates, but could be as a result of the colonization on the artificial substrates last for shorter time than the natural substrates which last for unknown. Furthermore, green algae are minimal on many artificial substrata, this could be a result of the diatoms fast growth and tend to be the first colonizers, while the latter forms need more time to grow (Stock and Ward, 1989).

Artificial substrata have been used progressively, as the artificial substrata was recommended by (Wahl and Mark, 1999; Albay and Akcaalan, 2003; Liboriussen, 2003) who considered the natural substrates less favourable than artificial substrates for experimental growth. Artificial substrata are easy handling, which makes them valuable in the researches where such processes as growth rates, immigration, and colonization are measured (Stevenson, 1984; Ács and Kiss, 1993; Barbiero, 2000). Artificial substrata are usually help in increasing the accuracy of the measurements by providing a uniform substratum for algal growth (Tuchman and Stevenson, 1980) and eliminating measurement problems associated of irregular natural substrata (Lamberti and Resh, 1985)

The sufficient incubation period resulted from this study was 28 days, which is considered to be enough time to establish a representative algal communities (Kelly *et al.*, 1998). The 28 day incubation period was during the summer season, which is the warmest of the sampling periods, and resulted in a representative assemblage of the algal communities, which shows that 4 weeks was a sufficient incubation time. The curve of immigration species is descending because the most successful distributed species would colonize at the beginning as the increased diversity in early days can be related to the fact that new taxa start to colonize (Hillebrand and Sommer, 2000), then followed by a significant decrease in the immigration overall rate. In contrast, the extinction curve is an ascending curve because as more species colonize on the tile through time, more species, exponentially, would become extinct where that could be related to dying, emigration and grazing effects (Biggs, 1996b).



The present study suggests that current speed plays a significant role in the colonizing pattern of algae in fresh water system. This was compatible to the results reported by (Steinaman and McIntire, 1986) where maximum colonization of algae was observed on tiles kept in low current speed indicating that low flow was an enhancing factor for algal colonization in rivers and stream. In slow current speed, greater cell abundance was recorded by (Lamb and Lowe, 1987). The dominance of diatoms in this study was similar to the findings of (Oemke and Burton, 1986). Under high current speeds algae could be subjected to various removal conditions from the substratum. It might be through scrape or due to bed disturbances which will be leading to a decline in the algal biomass (Stevenson, 1990; Bergey and Resh, 2006). This could be the reason behind reducing chlorophyll a production, cell abundance, and richness in high current speeds.

A significant impact of high current speed on changing the community composition as well as reducing the abundance of algal mats in stream was shown by Stanish *et al.* (2011). A negative relationship between algal cell numbers or biomass and current speed rate in fresh water ecosystems were recorded by (Heiskary and Markus, 2001; Ahearn *et al.*, 2006), and that is similar to my results which show an inverse relationship between algae colonization and high current speed.

Both fine sediment and nutrient concentrations, as expected, were correlated with each other in our study sites. This impacts our ability to differentiate the effects of sediment and nutrients on the measured biotic responses in the studied streams. Definitive research of these differential impacts requires experimental design with combine and separate manipulation of sediment and nutrients (see chapter 5 experiment). In this chapter discussion, an overall impacts assessment of algal development will be focused on. A particular attention is paid to whether the ecological responses to algal development in studied streams are linear or non-linear.

Algal biomass is generally related to the concentrations of nutrient. In our studied stream, this was the situation for the epilithic algae, even though, there are other factors can have impacts on algal biomass in streams such as hydrology, light, temperature, and invertebrate grazing (Biggs and Close, 1989; Biggs, 1996b; Suren and Duncan, 1999). At Sibbertoft and Lone Pine, the recorded Chlorophyll a shows these sites had highest chlorophyll a in our study sites. During floods, fine sediment maybe dragged within the high flows and scrub the algal biomass (Schofield *et al.*, 2004).

Algal metrics, including biomass, species richness and density had significant relationships with sediment and nutrients concentrations. In some situations, those relationships were fit with

a non-linear function. This subsidy stress relationships were recorded between algal biomass and nutrients concentrations.

Algal biomass and densities increased across the gradients of sediment and nutrients concentrations. Quinn *et al.* (1997) have also reported streams with nutrients concentrations recorded greater biomass and densities compared to pristine streams. The sediment finding harmonise with the results of Izagirre *et al.* (2009) who recorded algal biomass positive response in addition to photosynthetic efficiency when fine sediment was added experimentally. Gray and Ward (1982) suggested that the increased algal in streams with rising the levels of sediment might be resulted from the increased levels of nutrients included in that sediment.

The changes from community dominated by a very sensitive species to pollution such as *Brachysira vitrea* to a community dominated by a tolerant to pollution species such as *Rhoicosphenia abbreviate* and *Navicula cryptotenella* suggests that along the P gradients there is a biofilm functional changes which can provide an ecological justification for the ecological status of the stream.

*Brachysira vitrea* favoured by low nutrient concentrations where it's occurred in high relative abundances (Kelly *et al.*, 2007). *Rhoicosphenia abbreviate* recorded in oversaturated streams with high P (Rott *et al.*, 1998) and it is one of the most prolific diatom in under enriched streams conditions (Kelly *et al.*, 2007).

The group of tolerant taxa in both rivers and lakes is largely dominated by *Navicula* and *Nitzschia* while sensitive category is dominated by *Achnantheidium* and *Fragilaria* (Kelly *et al.*, 2007). *Achnantheidium minutissimum* as relatively intolerant of eutrophication (Kelly *et al.*, 2007). The low abundance of these species as P concentration increase proves changes in the sites ecologically.

When assessing a stream ecological condition or predicting the stream future condition, the knowledge of the multiple stressors impacts is very important (Paine *et al.*, 1998). With the few recent researches by Matthaei *et al.* (2010) and Ferreria and Chauvet (2011) of the multiple stressor impact indicating synergetic interactions, the current knowledge is still limited.

A subsidy stress hypothesis (at low levels of stressor, an ecological variable responds positively [increase] until the inflection point where the effect becomes negative [decrease]) has not been studied tested relating to the deposited sediment.

## CHAPTER FIVE: MULTIPLE STRESSOR IMPACTS ON EPILITHON COMMUNITIES

### 5.1 Abstract

The impacts of multiple stressors<sup>2</sup> on the epiphytic algal community are currently underexplored in comparison to macroinvertebrates, even though macroinvertebrates are likely to be less directly impacted by abiotic stressors than epilithon. In this study the shapes<sup>3</sup> of algal responses<sup>4</sup> were determined across two stressor (phosphorus and sediment) gradients after twenty days of exposure. Four hypotheses were tested:

(1) The subsidy-stress for phosphorus and sediment - where at first, an ecological variable increases positively with the increased level of phosphorus and sediment until very high levels are reached, which then have negative effects.

(2) Whether the both stressors work alone or as multiple stressors and whether they interacted. Three ecological guilds of algae ('low profile' growth form, 'high profile' growth form, 'motile' growth form) were used in order to test that -

(a) The high profile growth form decreases and motile algae growth form increases with increase of sediment deposition, and

(b) Both high profile growth form and motile algae growth form increase with increased concentration of phosphorus.

The subsidy stress was strongly supported along the phosphorus gradients and found frequently at both community and individual taxon levels. The subsidy stress patterns along the sediment gradient by contrast, were found in the *Nitzschia* species whereas remaining variables showed

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<sup>2</sup> A stressor is described as "pollutant", "pollution" or "pressure" in the policy and management context (Friberg, 2010); a variable that has exceeded its normal variation range as a result of human activities, and affects the individual taxa, ecosystem functioning or community composition. Effects could have positive or negative impacts on the biological response variables (Townsend, Uhlmann and Matthaei, 2008).

<sup>3</sup> The three response shapes of the ecological variables across sediment or/and phosphorus gradients are: (1) strictly positive (increase), 2) strictly negative (decrease) and (3) subsidy-stress shapes where it starts to increase then at the inflection points it decreases.

<sup>4</sup> The algal response variables include algal densities, taxonomic groups and ecological guilds and the community response variables include Chlorophyll a, species richness, species evenness, and total cell density and these are mentioned in table 5.1

either positive or negative responses. Overall, fewer epilithon variables responded to sediment than to phosphorus.

The common responses by the epilithon were single stressor responses, but phosphorus and sediment together generally acted as multiple stressors; usually in a simple additive way, perhaps as a result of the differences in the epilithon underlying mechanisms from one another. The interactions of complex multiple stressors were also found. Increasing phosphorus or sediment along their wide gradients had no large impact on cyanobacteria proportion, with a percentage increase from 3.16% to 4.03% and 3.16% to 4.42% respectively when analysed in isolation, but when phosphorus and sediment were delivered in concert the cyanobacteria proportion increased significantly from 4.14 % to 17.13 % and from 4.76 % to 16.89% respectively.

The algal growth forms' representation along the gradient of phosphorus hypothesis (hypothesis 2b) was partially supported, while the gradient of sediment hypothesis (hypothesis 2a) was fully supported. As predicted, the motile guild growth form increased and became widespread with the phosphorus increase over the high profile guild near the intermediate levels of phosphorus. The patterns were then overturned, where the high profile guild representation increased, and the motile guild decreased with further phosphorus increase. The motile guild representation and, due to this, the pattern of subsidy stress, could be considered as a useful detector to indicate the phosphorus enrichment early signs whilst for more severe enrichment cases this will be less useful.

**Keywords:** phosphorus, sediment, stressor, subsidy stress, single stressor, simple multiple stressor, complex multiple stressor, high profile guild, low profile guild, motile guild.

## 5.2 Introduction

Many rivers and streams in agricultural landscapes are degraded and in poor ecological condition as a result of multiple stressors. Two major stressors are fine sediment and phosphorus, which are known to have affected macroinvertebrate communities both individually and by their complex way of interaction (Townsend *et al.*, 2008; Matthaei *et al.*, 2010). Their effects on epilithic algal communities have received less attention, even though phosphorus is likely to impact the epilithic communities more directly than macroinvertebrates (Kelly & Whitton, 1998; Dodds, 2007). These two stressors were thus investigated in the lowland English stream environment.

Epilithic algae can be considered as stress indicators of anthropogenic pollution of aquatic ecosystems, so knowledge of multiple stressor interaction is important. Algal communities are routinely used as eutrophication and inorganic phosphorus pollution indicators (Kelly & Whitton, 1995; Whitton, 1999; Biggs, 2000; Dodds, 2007) by the regulatory authorities, especially the Environment Agency in the UK, but the range within which increased fine sediment composition can interact with phosphorus and cause enhanced responses is not known. One of the main determinants of algal functioning and community structure is the frequency of physical disturbance, which is usually high in substrata with moving and unstable fine particles (Biggs *et al.*, 1998). In this research I focus on the fine sediment levels as a stressor, however, the algal communities receive similar consequences from periodic movement and deposition of fine sediment as natural physical disturbance (Peterson, 1996). The range of deposition rate was indicated by He and Walling, (1996) as between 0.07 and 0.59 g.L<sup>-1</sup> in lowland English rivers. The suspended sediment is not meaningful in this study because it passes algae by; what is important is deposited sediment, and there is a little knowledge of that.

Different traits, or morphological adaptations, have been acquired by algal species in their evolution, reflecting the trade-offs between resource supply and constraints of disturbance (McCormick, 1996; Biggs *et al.*, 1998). Diatom taxa, reflecting this, have been classified by Passy (2007) into low profile, high profile, or motile which are three special growth morphologies, selected to reflect their tolerance of differential potentials to physical disturbance and/or phosphorus limitation. Passy's study across the phosphorus gradient showed that under low phosphorus supply the community was dominated by the 'low profile' guild, the species of which did not develop a thick algal mat, but as phosphorus supply increased the guild declined, and shading occurred, within algal multi-layered mats which were

developed by members of the 'high profile' guild. Motile cells however, could escape physically from microhabitats with depleted resources, and with phosphorus augmentation the abundance of this guild increased. Most motile taxa are more competitive (Pringle, 1990; McCormick, 1996). The guilds' behaviours led Passy (2007) to conclude that they might be good indicators of anthropogenic pollution.

Both fine sediment and inorganic phosphorus could be considered as usable inputs to aquatic ecosystems Odum *et al.* (1979), each with the possibility to create a subsidy stress response if in excess. An inorganic phosphorus increasing from low levels to intermediate levels might produce a subsidy effect for certain taxa (Biggs *et al.*, 1998; Chetelat *et al.*, 1999), the whole community (Liess & Hillebrand, 2006; Liess *et al.*, 2009) and increase primary production (Biggs, 2000; Dodds *et al.*, 2002). Field surveys and experiments have also showed increases in diatom community evenness and species richness with increasing phosphorus availability (Pringle, 1990; Stevenson *et al.*, 2008; Liess *et al.*, 2009). Eutrophic species will dominate the community and algal growth may become saturated at higher concentrations of phosphorus; no further subsidy will be produced as a result of further phosphorus increase, but potentially the algal response variables will be negative. The subsidy stress response, therefore, will be produced.

Deposited fine sediment can modify the response to augmented phosphorus. For instance, Pringle (1990) found that the immotile taxa response to experimentally added phosphorus depended on the type of substratum. Immotile taxa responded negatively to phosphorus when grown on sand-agar slides, but positively on glass slides. This could be as a result of the cells on the fine substratum understory being prevented from proliferation, by the upperstory dense community of motile cells. Benthic algae growing on hard substrata were more affected by the addition of phosphorus than those growing on fine sediment because in the habitat of fine sediment the physical conditions are less favourable or the access to the water column nutrient is reduced (Hillebrand & Kahlert, 2002). Fine sediment has been reported by Burkholder (1996) to sustain lower algal biomass where the movement of fine sediment particles buries or crushes algal cells. Conversely, habitat heterogeneity could increase when small amounts of fine sediment are deposited on coarse substrata and accordingly the algal species richness increases. Therefore, stressor-response relationships between epilithic community and fine sediment may take negative, positive or subsidy-stress shapes.

The phosphorus gradient subsidy-stress hypothesis was based on the assumption that the algal community is stimulated by increasing phosphorus, with subsidy effects on the whole

community as well as individual taxa, and negative effects caused by very high concentrations. The fine sediment gradient subsidy-stress hypothesis was based on the assumption that surface heterogeneity can be increased initially by the augmentation of sediment and species suppressed by stronger competitors are provided with additional microhabitats. The habitat heterogeneity decreases however with further sediment augmentation, as a result of substratum smothering and consequently the habitat for sediment sensitive taxa will be eliminated or reduced.

Recorded levels in the preliminary field observation were chosen to be a guide for the highest level used in this experiment. Jarvie *et al.*, (2008) and Friberg *et al.*, (2010) recorded that 247 mgL<sup>-1</sup> in the Wye river was the average total phosphorus concentration, with individual sites median concentrations ranging from 403 mgL<sup>-1</sup> in the Frome to 30 mgL<sup>-1</sup> in upper Wyne. The average Total Phosphorus for the Avon River was 194 mgL<sup>-1</sup>, with individual sites median concentrations ranging from 361 mgL<sup>-1</sup> in the West Avon to 21 mgL<sup>-1</sup> in Chitterne.

The aim of this study is to investigate the individual effect and the combined effects of increased fine sediment and soluble phosphorus on epilithic response variables in a field experiment. It follows from the field data gathered, which are analysed in Chapter 4 where both fine sediment and nutrient concentrations were correlated with each other in our study sites. The results of the preliminary observation show that the algal community was impacted in a synergetic way by phosphorus and sediment..

The following hypotheses were tested by using wide range of the two stressor levels along both gradients:

(1) The subsidy-stress for phosphorus and sediment - where at first, an ecological variable increases positively with the increased level of phosphorus and sediment until very high levels are reached, which then have negative effects.

(2) Whether the both stressors work alone or as multiple stressors and whether they interacted. Three ecological guilds of algae ('low profile' growth form, 'high profile' growth form, 'motile' growth form) were used in order to test that -

(a) The high profile growth form decreases and motile algae growth form increases with increase of sediment deposition, and

(b) Both high profile growth form and motile algae growth form increase with increased concentration of phosphorus.

### 5.3 Materials and Methods

See Chapter three

### 5.4 Results

Overall, diatoms (Bacillariophyceae) were dominant, with a representation of 85.55%, green algae (Chlorophyceae) followed with 8.86% and least abundant were blue-green bacteria (Cyanobacteria) with 5.60% of all counted cells (Appendix 5.2 shows all species found). The high profile guild was dominant at 21% (21 species), motile guild had 17% (17 species), and low profile guild was least abundant with 12% (21 species) of cells counted (Table 5.1).

**Table 5.1 Algal response variables statistics summary**

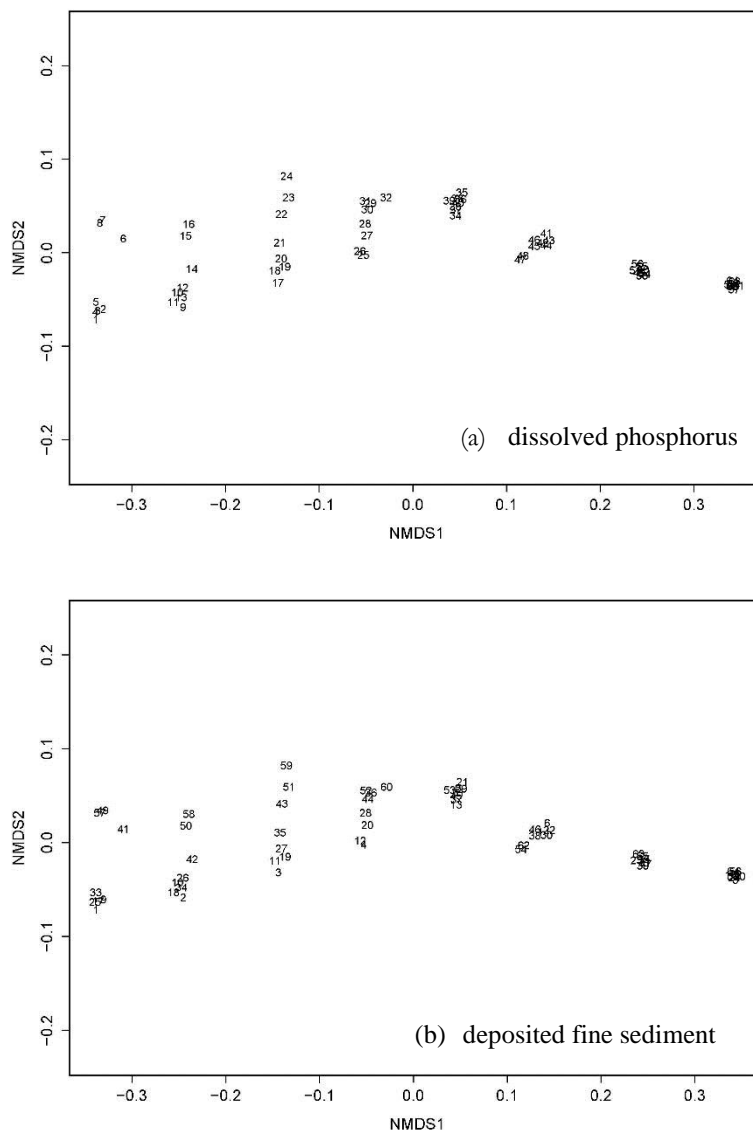
<b>Variables</b>	<b>Mean</b>	<b>St dev<sup>*</sup></b>
<b>Taxonomic group</b>		
Diatoms %	85.01	7.37
Cyanobacteria%	5.27	5.48
Green Algae	3.62	4.73
<b>Ecological guilds</b>		
High profile guilds %	42.26	9.88
Low profile guilds %	18.44	5.20
Motile%	37.09	9.03
<b>Community level variables</b>		
Chlorophyll a (mg.cm <sup>-2</sup> )	3.79	1.64
Species richness	52	6.09
Species evenness	0.43	0.11
Total cell density	2471	1461.81

\* Stdev= Standard Deviation



### 5.4.1 Algal response variables

The algal community composition NMDS plots (3D stress = 0.021) shown in Fig (5.1) illustrate that the communities associated with lower phosphorus and sediment levels (level 1 to level 4) are different from those with higher phosphorus and sediment levels (level 5 to level 8) by their positions on the opposite side of the NMDS plot. The increasing dissimilarity in gradient pattern with increasing phosphorus levels (Fig 5.1.a) was clearer than that with increasing fine sediment levels (Fig. 5.1.b).



**Figure 5.1** NMDS plots based on algal community composition dissimilarities in the tile-sets grouped by 8 treatment levels (a) dissolved phosphorus and (b) deposited fine sediment. The number are the levels of treatment where 1-8 (level 1), 9-16 (level 2), 17-24 (level 3), 25-32 (level 4), 33-40 (level 5), 41-48 (level 6), 49-56 (level 7), 57-62 (level 8).

#### 5.4.2 Single stressor

*Pediastrum* and *Cosmarium* (green algae) increased with increasing phosphorus levels (Fig. 5.2) while *Cymbella*, *Fragilaria* and *Synedra* (diatoms), excluding *Fragilaria vaucheriae* decreased (Fig. 5.3). Other algal species showed similar subsidy responses. The species subsidy response with maximum cell densities was recorded most of the time at level 4 and 5 of treatment,  $1.49 \text{ mgL}^{-1}$  and  $2.69 \text{ mgL}^{-1}$  respectively. The high profile guilds revealed a positive relationship with the increase phosphorus, contrary to the low profile guilds whereas the motile guilds showed an increase followed by a decrease (Fig. 5.4). The community variables showed various responses, whereby a positive response was recorded by chlorophyll a, a negative response by species richness, while the species evenness and total cell density showed a subsidy response (Fig. 5.5). The community was dominated by eutrophic taxa as the phosphorus concentrations increased further

Most diatom species reacted negatively to increasing fine sediment, with the exception *Brachysira vitrea*, *Navicula gregaria* and *Navicula minima* which reacted positively and *Nitzschia dissipata* and *Nitzschia pusilla* which showed a subsidy response with increasing levels of fine sediments (Fig. 5.5). Fig. 5.7 shows that Cyanobacteria were positively impacted and Bacillariophyceae negatively impacted. High and low profile guilds decreased with increase in fine sediment, while the motile guilds increased (Fig. 5.8). Chlorophyll and total density showed a negative response, decreasing with increasing sediment while species richness and species evenness showed a subsidy response (Fig. 5.9).

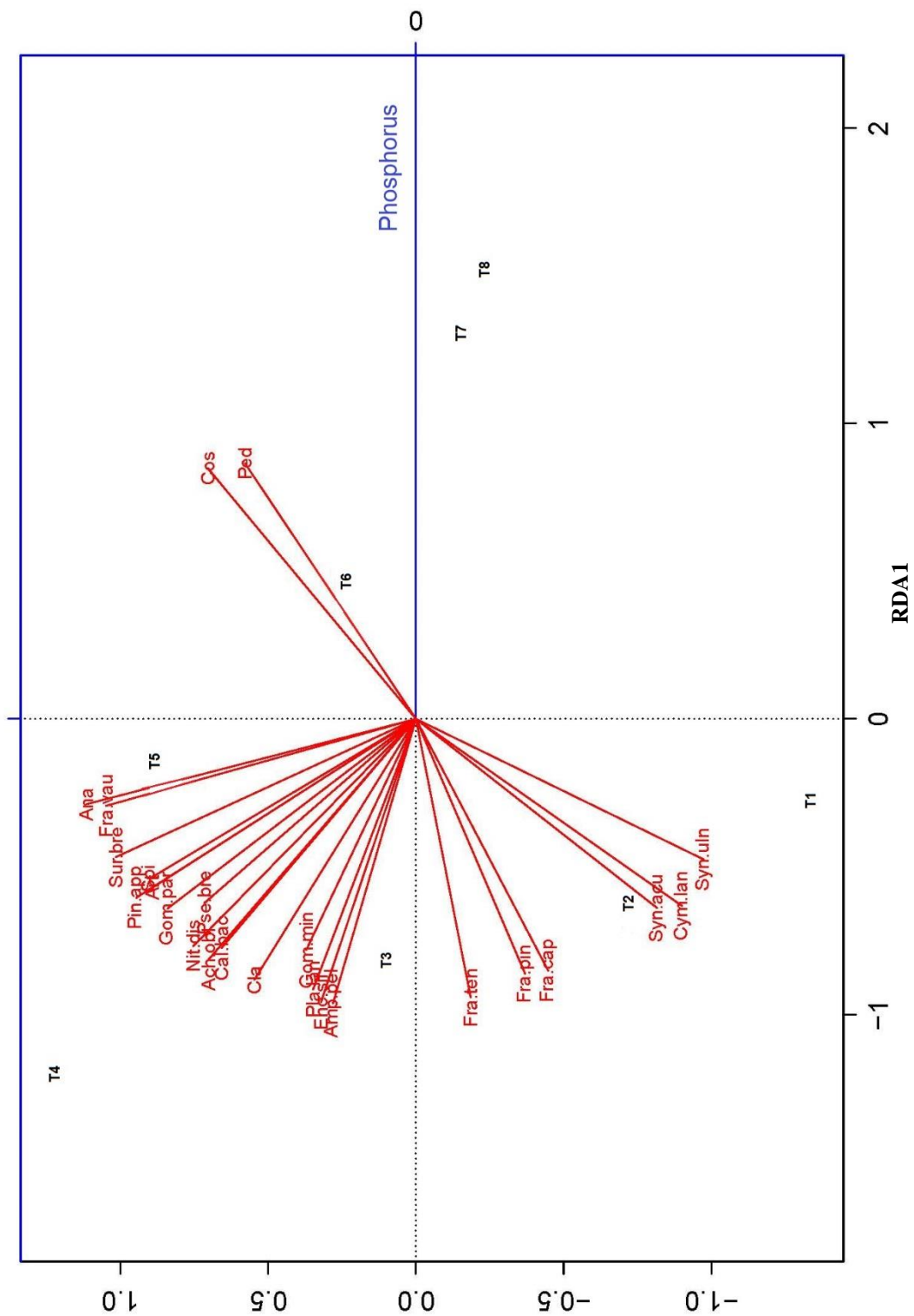


Figure 5.2 Triplot RDA Single Factor (Nutrient) where the blue line is the phosphorus, and the T is concentration where T1 is the lowest and the T8 is the highest level for phosphorus (treatment concentrations are listed in appendix 5.2).

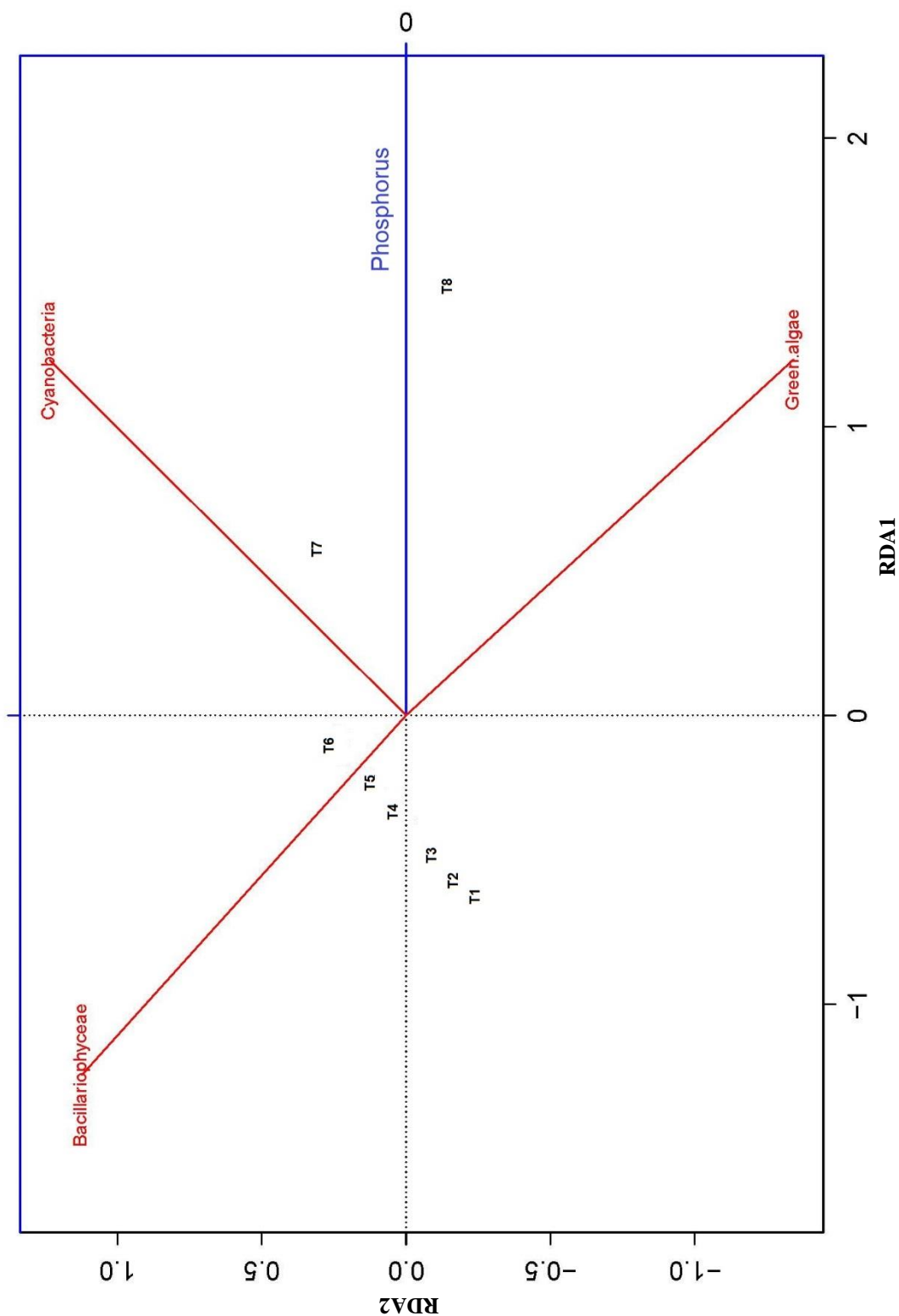


Figure 5.3 Triplot RDA Single Factor (Taxonomic group, the red lines) where the blue line is the phosphorus, and the T is concentration where T1 is the lowest and the T8 is the highest level for phosphorus (treatment concentrations are listed in appendix 5.2).



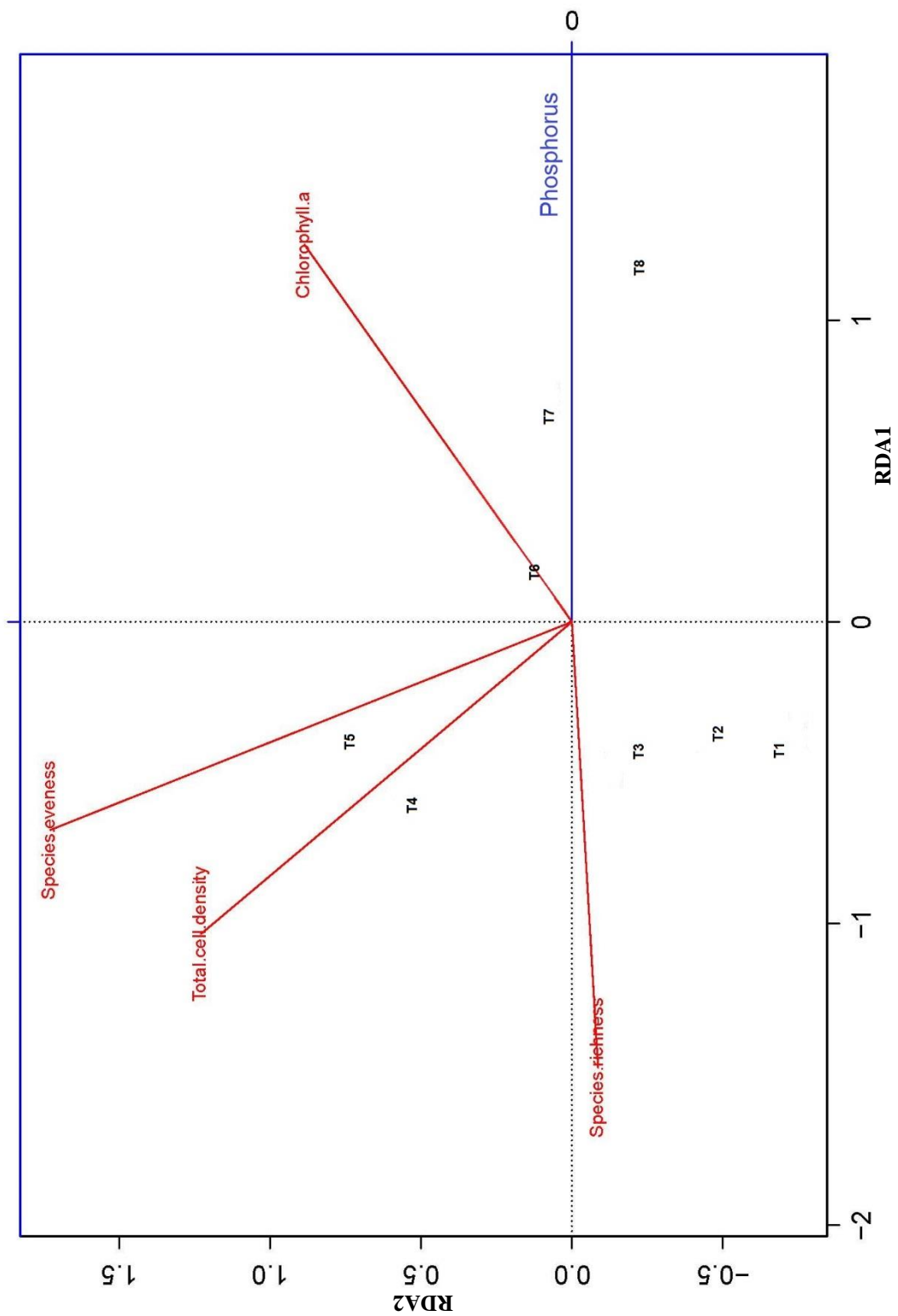


Figure 5.5 Triplot RDA Single Factor (Community Variables) where the blue line is the phosphorus, and the T is concentration where T1 is the lowest and the T8 is the highest level for phosphorus (treatment concentrations are listed in appendix 5.2).

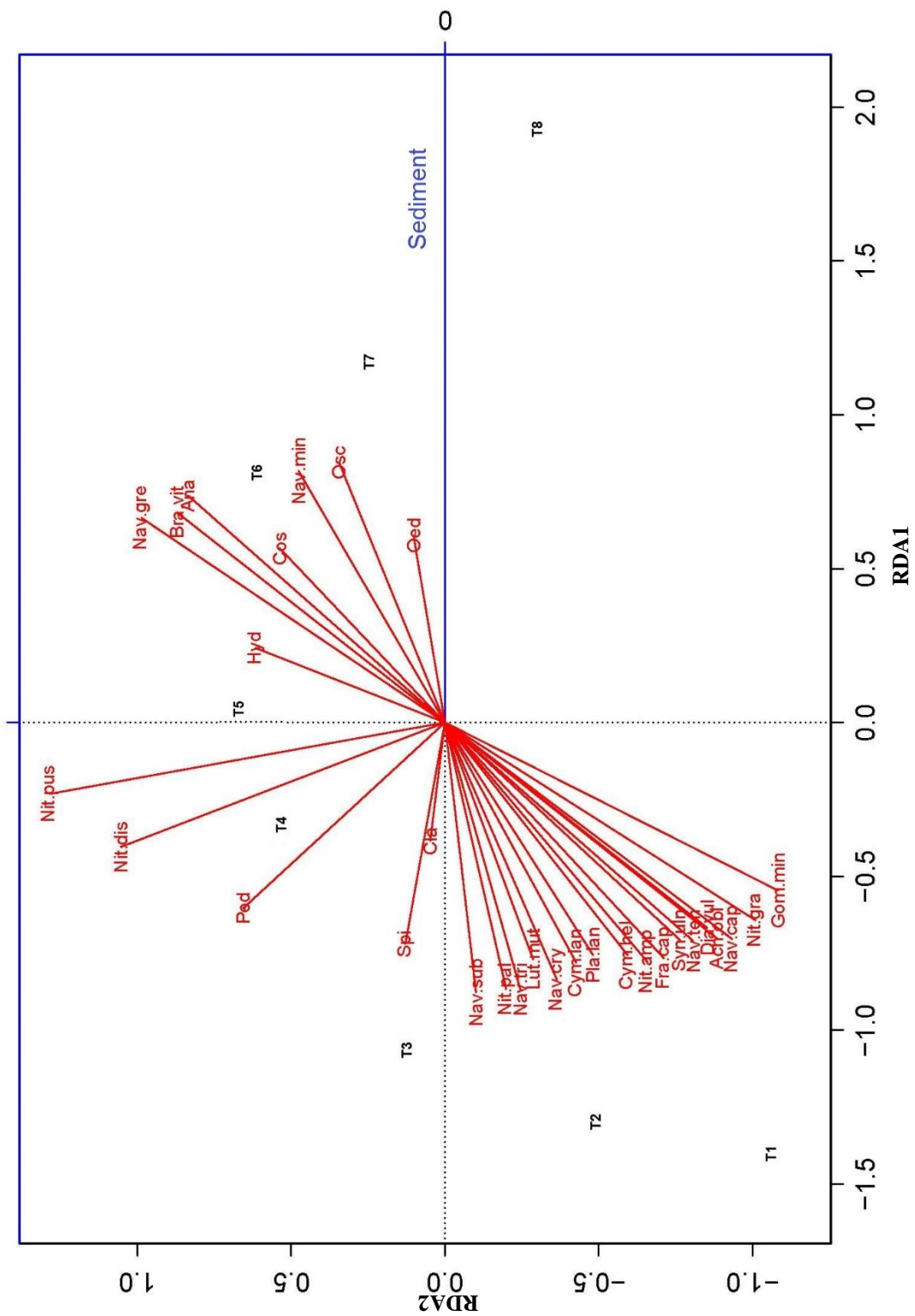


Figure 5.6 Triplot RDA Single Factor (Sediment) where the blue line is the sediment, and the T is concentration where T1 is the lowest and the T8 is the highest level for sediment (treatment concentrations are listed in appendix 5.2).

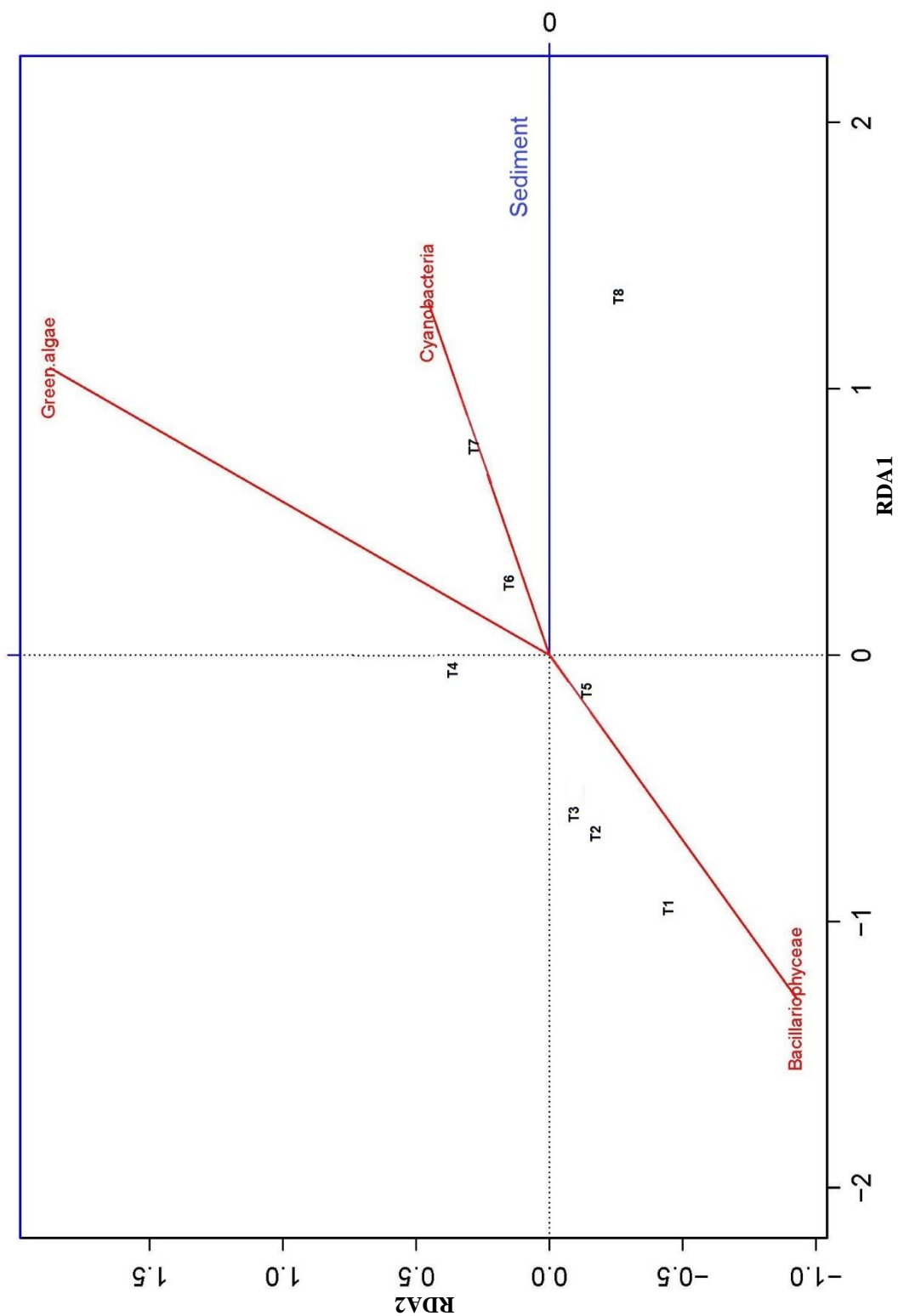


Figure 5.7 Triplot RDA Single Factor (Taxonomic group) where the blue line is the sediment, and T is concentration where T1 is the lowest and the T8 is the highest level for sediment (treatment concentrations are listed in appendix 5.2).





Figure 5.8 Triplot RDA Single Factor (Ecological guild) where the blue line is the sediment, and the T is concentration where T1 is the lowest and the T8 is the highest level for sediment (treatment concentrations are listed in appendix 5.2).

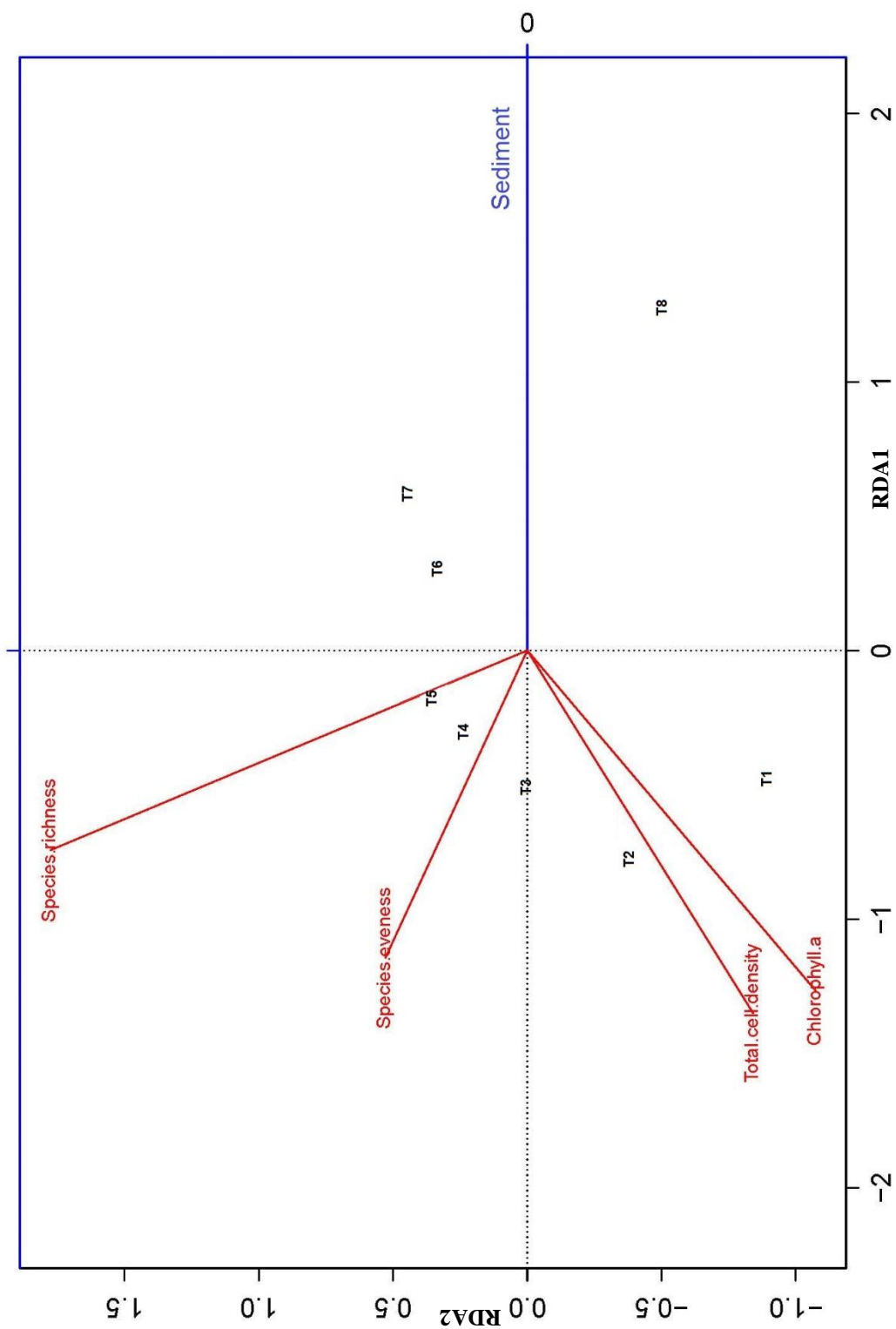


Figure 5.9 Triplot RDA Single Factor (Community Variables) where the blue line is the sediment, and T is concentration where T1 is the lowest and the T8 is the highest level for sediment (treatment concentrations are listed in appendix 5.2).

### 5.4.3 Simple multiple stressor

Only the green algae percentage showed a positive response to phosphorus where it increased from 1.29% to 22.26%. where both phosphorus and sediment impacted the response variables but without interactive impact (Fig. 5.10 to 5.13) Most variables showed subsidy stress responses for the phosphorus including algal evenness, *Encyonema minutum*, *Navicula capitatoradiata*, *Nitzschia dissipata*, *Nitzschia sigmoidea*, and three negative responses – the mean of species  $\text{cm}^{-2}$  (*Synedra acus* decreased from 41 to 0, and *Cymbella lanceolate* from 43 to 5), the percentage of high profile guild from 51.82% to 24.52%.

Negative impacts TO sediment, species were recorded for *Synedra ulna* from 56 to 0 mean  $\text{cm}^{-2}$ , *Cymbella lanceolate* from 43 to 0, *Nitzschia amphibia* from 212 to 2, *Encyonema minutum* from 149 to 1, *Encyonema silesiacum* from 82 to 12, algal cell density from 5989 to 882 and percentage of high profile guild from 51.82% to 33.12%). Ten positive responses were increased species mean  $\text{cm}^{-2}$  for *Navicula capitatoradiata* from 63 to 600, *N. cryptotenella* from 3 to 325, *N. gregaria* from 0 to 31, *N. lanceolate* from 12 to 377, *N. minima* from 0 to 16, *N. subminuscula* from 34 to 388, *N. tenelloides* 1 to 463, *Navicula tripunctata* from 19 to 582, algal evenness from 0.23 to 0.73, green algae from 1.29% to 2.49%). Other algal species showed subsidy stress responses.



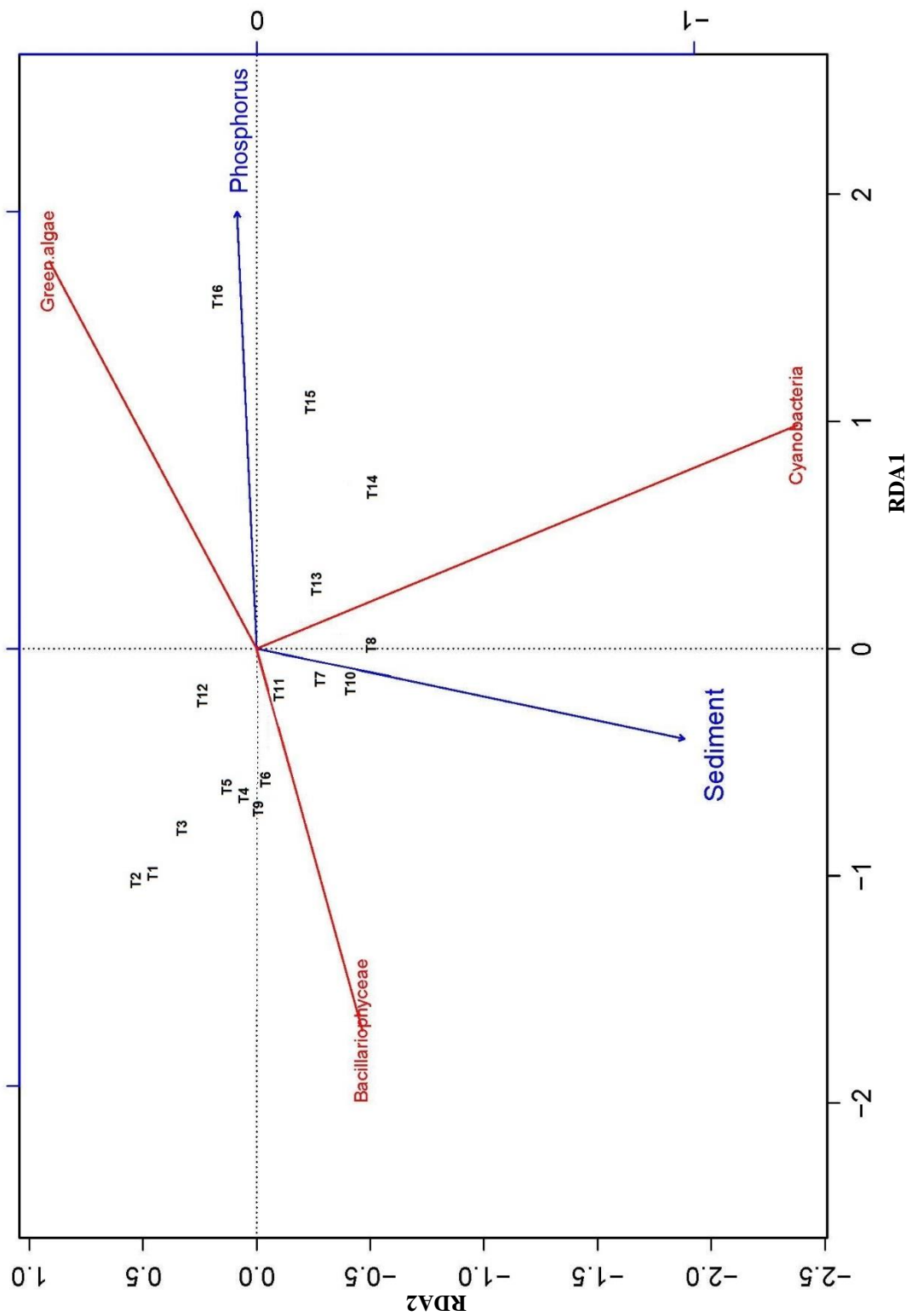


Figure 5.11 Triplot RDA Simple Multiple Response (Taxonomic groups) where T is concentration where T1 is the lowest and the T16 is the highest level for phosphorus and sediment (treatment concentrations are listed in appendix 5.2).

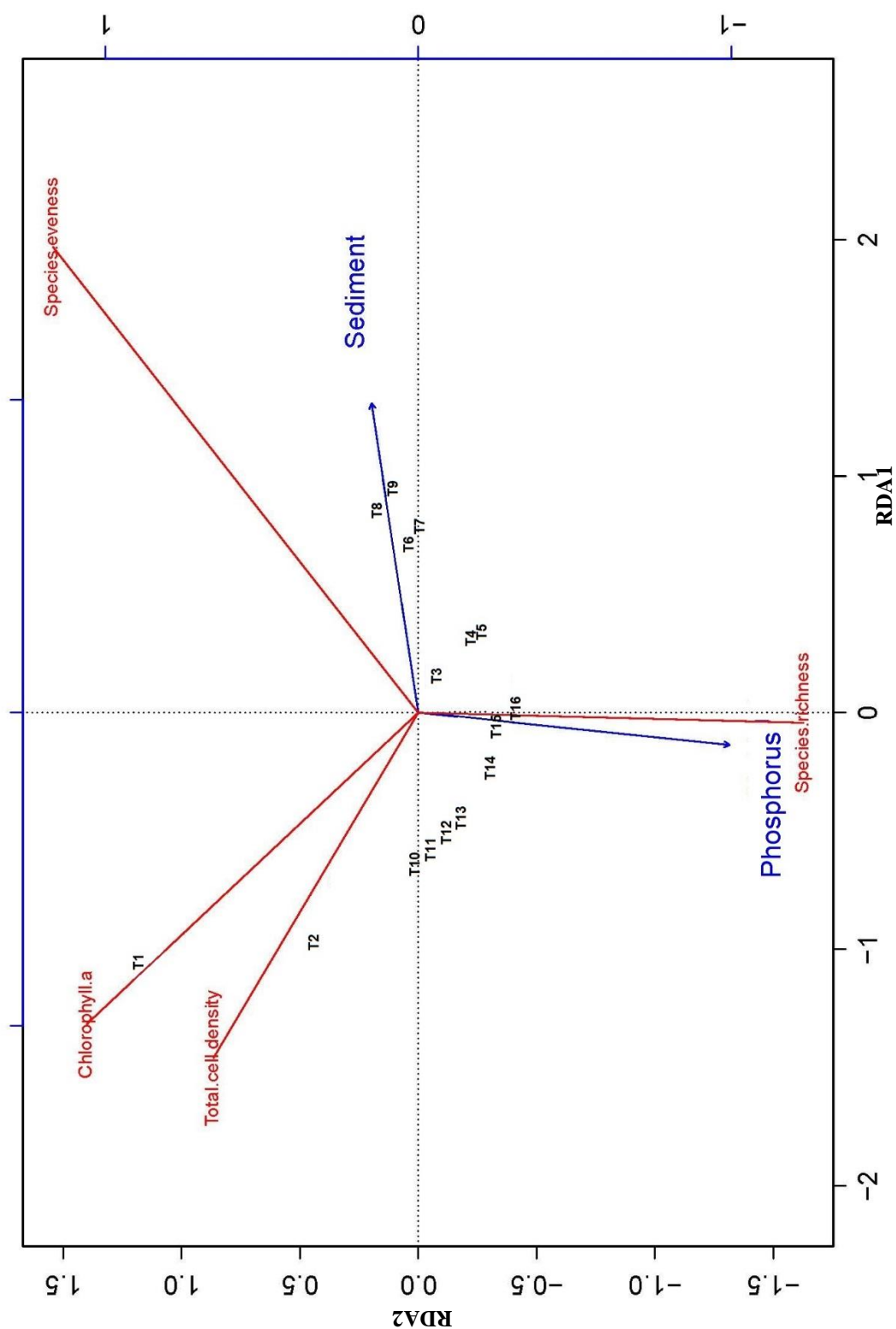


Figure 5.12 Triplot RDA Simple Multiple Response (Community variables) where the T is concentration where T1 is the lowest and the T16 is the highest level for phosphorus and sediment (treatment concentrations are listed in appendix 5.2).

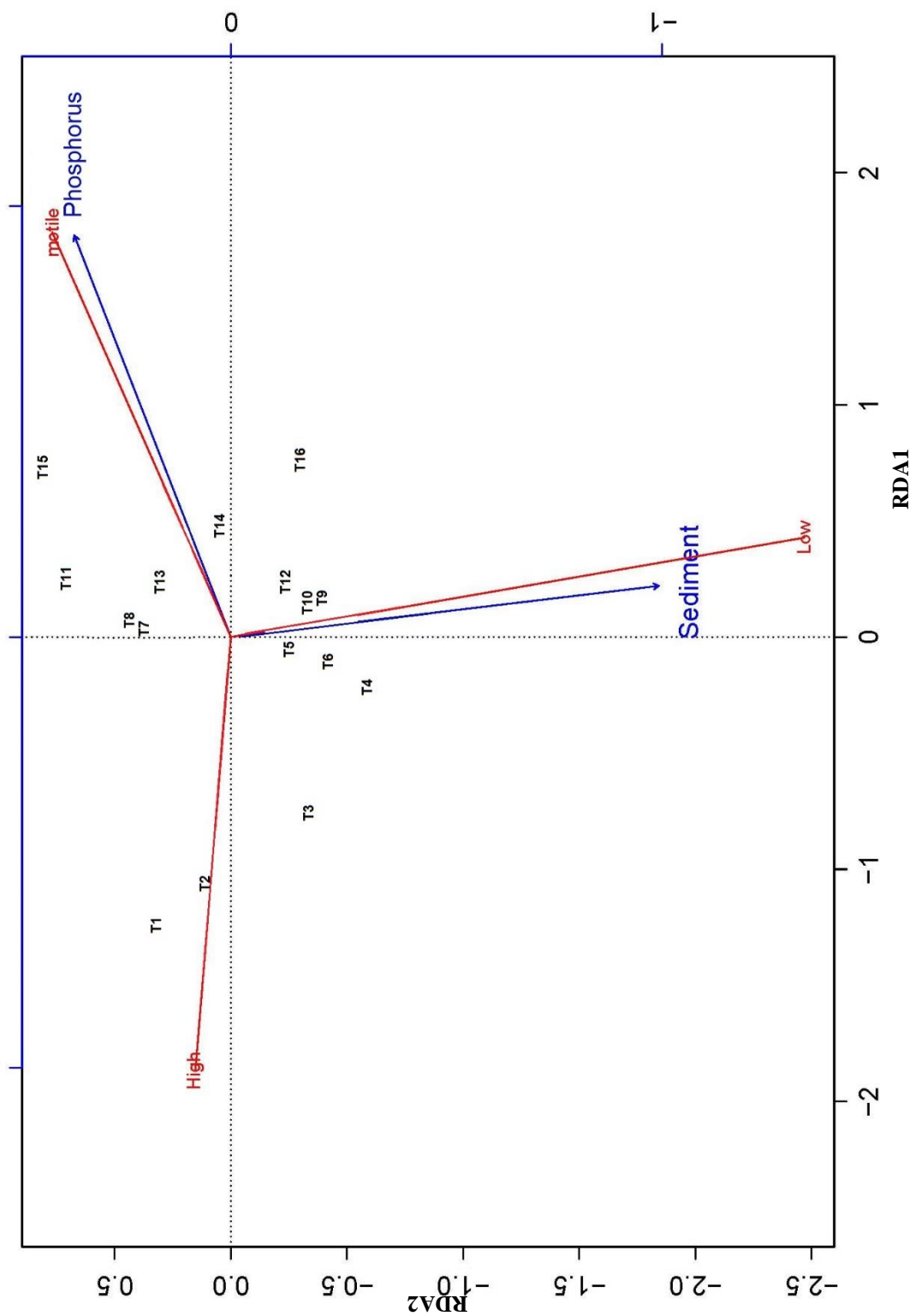


Figure 5.13 Triplot RDA Simple Multiple Response (Ecological guilds) where T is concentration where T1 is the lowest and the T16 is the highest level for phosphorus and sediment (treatment concentrations are listed in appendix 5.2).

#### 5.4.4 Complex multiple stressor

The percentage of motile guild and the percentage of cyanobacteria showed responses for the complex multiple stressors, where the relationship across phosphorus levels depended on the sediment gradient and the relationship across the sediment depended on the phosphorus (Fig. 5.14 to 5.17). At low levels of phosphorus and at low sediment there was a little impact on the cyanobacteria percentage. IT increased with rising levels of phosphorus at high levels of sediment and with rising levels of sediment at high levels of phosphorus. The phosphorus and fine sediment interactive effects on the motile guild percentage were more complex. A subsidy stress response was produced by the phosphorus and the top value was recorded at lower phosphorus levels with increasing sediment levels. Sediment had a positive impact on the motile guild, stronger at lower levels of phosphorus. This was an antagonistic interaction.

Cyanobacteria responded to complex multiple stressors. The individual effects to phosphorus and sediment at low levels of the other stressor were almost not noticeable, with a percentage increase from 3.16% to 4.03% and 3.16% to 4.42% respectively. At high gradient levels of other stressor, the positive impacts became stronger and cyanobacteria increased from 4.14 % to 17.13 % and from 4.76 % to 16.89% respectively. In the sediment free mesocosm, the algal community included cyanobacteria as a small percentage of the community, and as long as only a single stressor was employed, the relative abundance of the cyanobacteria still barely impacted if an increase in either phosphorus or sediments to high levels happened. The cyanobacteria percentage was nearly four times higher when both phosphorus and sediment levels were high, demonstrating that less suitable environmental conditions for diatom and green algae benefitted the cyanobacteria. In high phosphorus streams, cyanobacteria are more widespread (Douterelo *et al.*, 2004) even though the results of my study suggest that the proliferation of cyanobacteria is not caused by augmented in isolation.

Most of the epilithon variables showed either single stressor response or simple multiple stressor response, while the complex multiple response was followed least.





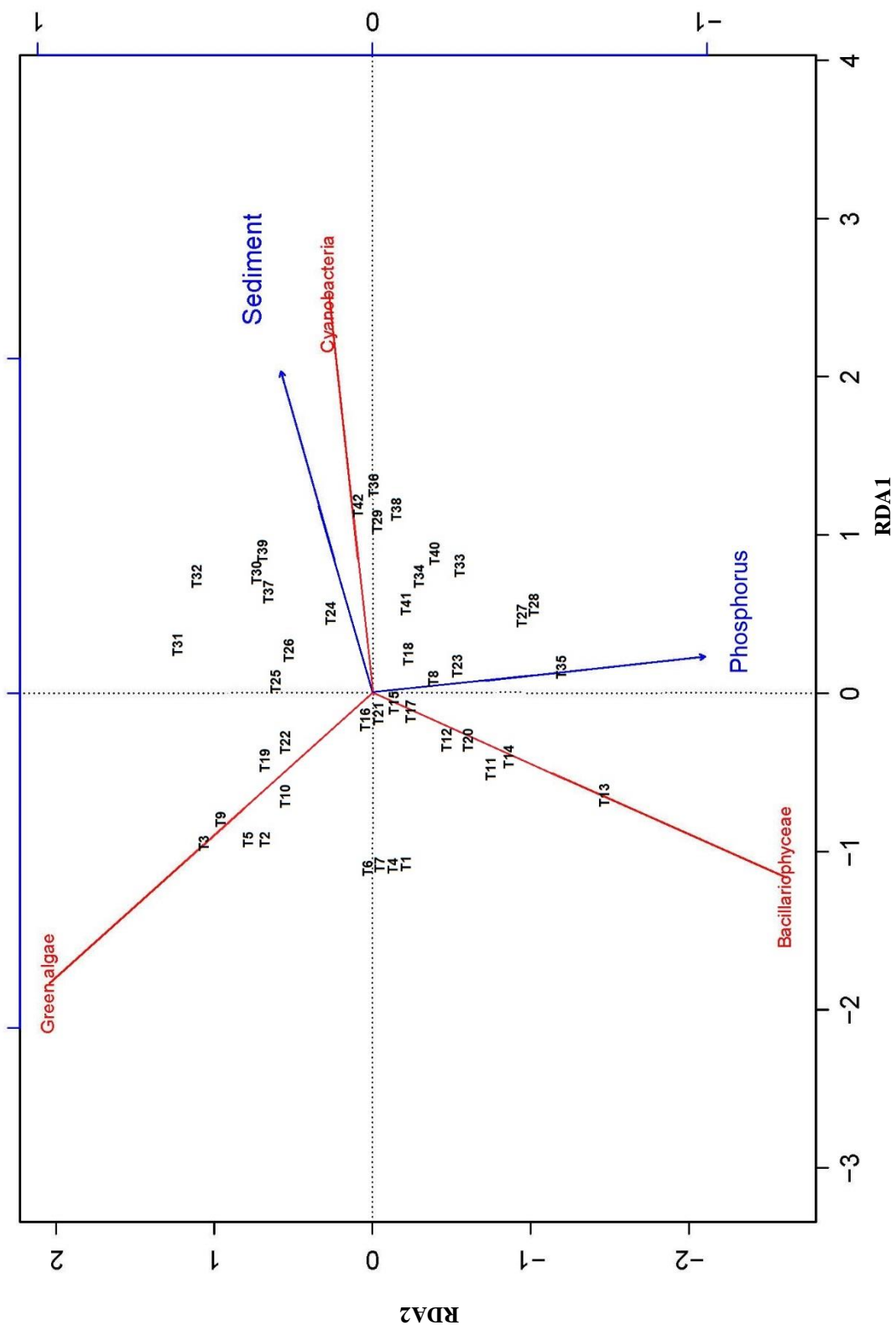


Figure 5.15 Triplot RDA Complex responses (Taxonomic Group) where T is concentration where T1 is the lowest and the T42 is the highest level for phosphorus and sediment (treatment concentrations are listed in appendix 5.2).

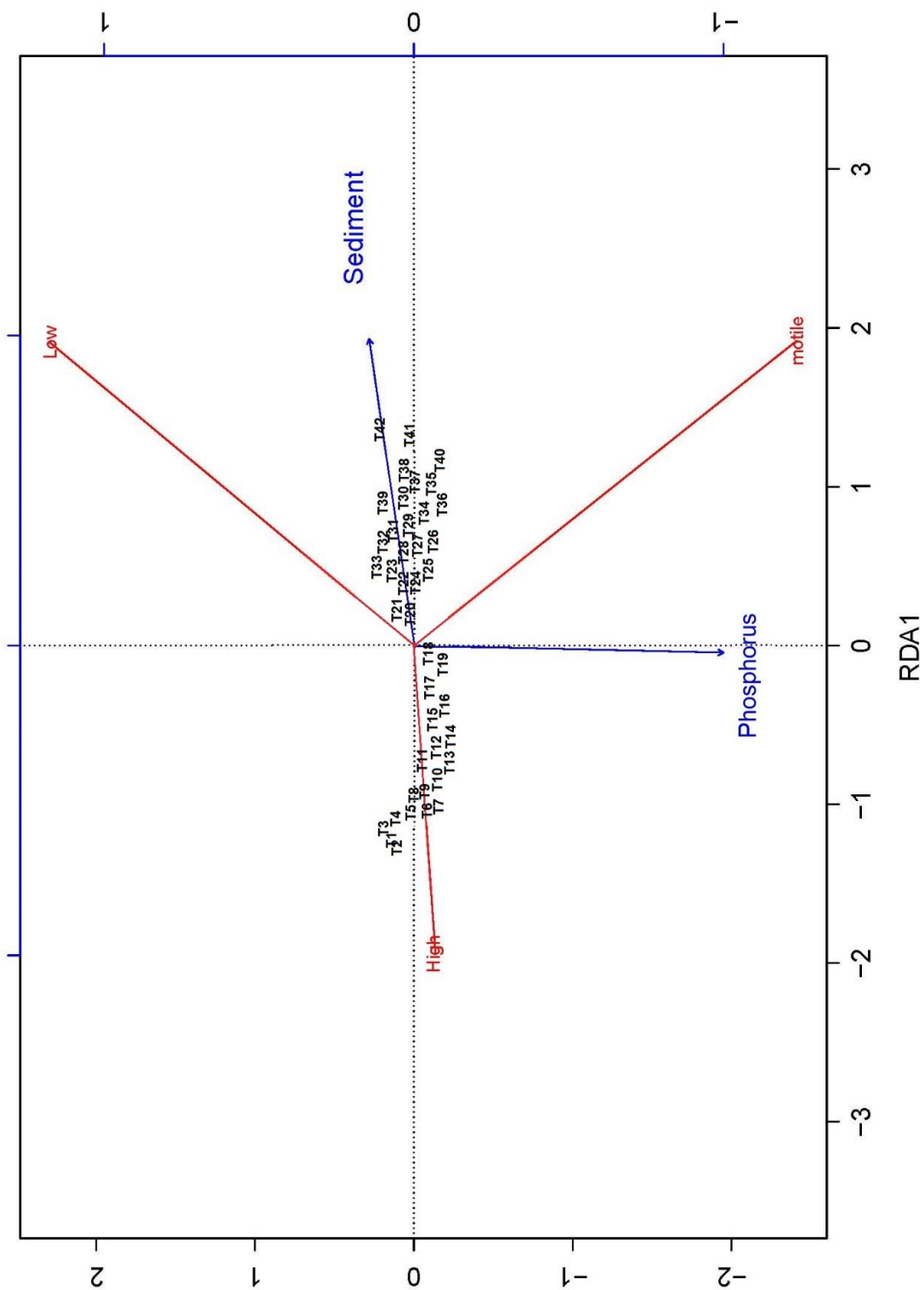


Figure 5.16 Triplot RDA Complex responses (Ecological Guilds) where T is concentration where T1 is the lowest and the T42 is the highest level for phosphorus and sediment (treatment concentrations are listed in appendix 5.2).

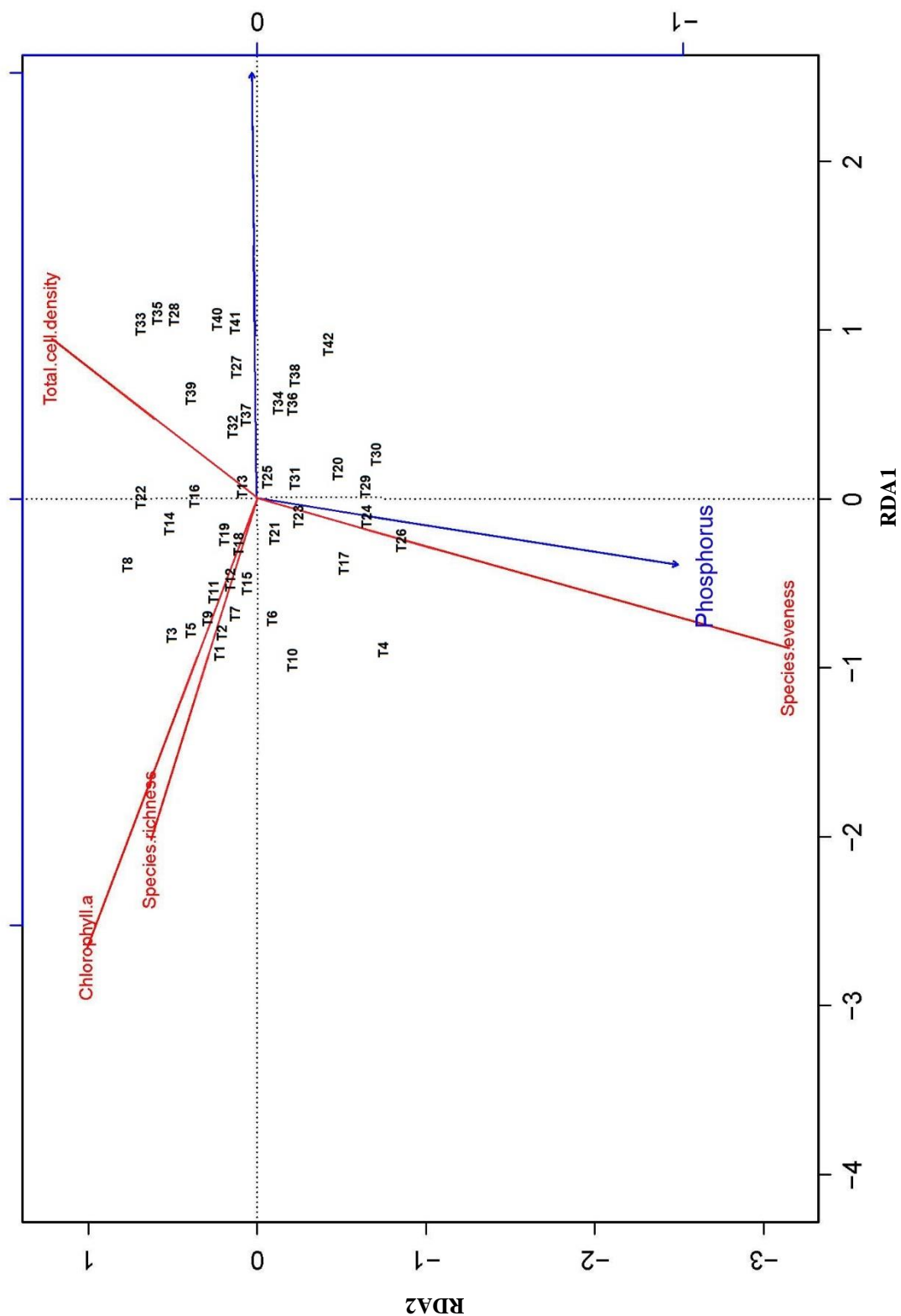


Figure 5.17 Triplot RDA Complex responses (Community variables) where T is concentration where T1 is the lowest and the T42 is the highest level for phosphorus and sediment (treatment concentrations are listed in appendix 5.2).

## 5.5 Discussion

### 5.5.1 Response shapes and subsidy stress hypothesis

The phosphorus gradient subsidy-stress hypothesis was supported by most of the species. The motile *Nitzschia* and *Navicula* species for instance showed a subsidy effect, as Kelly & Whitton, (1995) and Kelly *et al.*, (2008) state these two species are phosphorus tolerant from data collected in the United Kingdom. *Encyonema minutum* and *Fragilaria vaucheriae* on the other hand have been designated by Kelly *et al.* (2008) as water quality indicators because they are nutrient sensitive species. *Fragilaria vaucheriae* increase with phosphorus increase was found in some other researches (Biggs *et al.*, 1998 and Lange *et al.*, 2011). A decrease in the cell densities occurred after additional increase in the concentrations of added phosphorus, In spite of the high phosphorus concentration tolerance that seen by the most of the species that demonstrate a subsidy response. The taxon level subsidy stress hypothesis as a result was well supported.

The subsidy-stress pattern was followed by the total algal cell density at the algal community level. The taxonomic and community structure was affected by the phosphorus augmentation. Cyanobacteria and green algae increased in abundance. Biggs & Price (1987); Biggs (1995); Chetelat *et al.*, (1999) found that enriched phosphorus caused green algae proliferation, their abundance increase in this experiment was very clear. The phosphorus augmentation also impacted algal taxon richness, confirming Stevensson's *et al.*, (2008) field survey results and also results of Liess *et al.*, (2009). The phosphorus NMDS plot showed an obvious gradient pattern for the composition of the algal community indicating that phosphorus sensitive taxa such as *Cymbella lanceolate* and *Fragilaria pinnata* were replaced by tolerant ones such as *Navicula subminuscula* and *Nitzschia paleacea*.

*Nitzschia dissipata* and *Nitzschia pusilla* species showed a subsidy response to fine sediment. These two species' motile growth form could be the reason behind their toleration for the augmented sediment. Their decrease at very high levels of fine sediment could be as a result of a motile taxa vulnerability increasing to higher shear stress that happens when the high profile taxa decline (Dodds & Biggs, 2002).

At the community level, the hypothesis of sediment subsidy stress was not supported. Diatom percentage decreased from 98.43% to 91.28% with increasing fine sediment, and that could be because the extensive growth was prevented by the sediment particles' unstable nature.

Cyanobacteria percentage increased from 0.35% to 5.51% because the filaments of both *Oscillatoria* and *Anabaena* can grow, fasten and settle on fine sediment particles (Biggs, 1996). Species richness was also impacted by the fine sediment, where species that withstand fine sediment replaced the sensitive ones. For instance, the increased sediment produced negative responses from *Melosira varians* and *Nitzschia amphibia* which seem to be particularly sensitive to sediment, while only *Brachysira vitrea* and two *Navicula* species positively responded to sediment.

The *Navicula* species, which were increased with the fine sediment augmentation and the *Nitzschia* species, which showed a subsidy response both belong to the motile guild. It seems, as a result of that, that growth form is a valuable indicator of whether a species is affected negatively or positively by sediment. The total cell density, Chlorophyll a biomass and total algal cell densities followed the expectation and declined with the fine sediment augmentation, because as, Peterson (1996) mentioned, of the substrate instability as a result of increasing sediment, the community will be set back to the stage of early succession. Another potential reason for the sediment negative effect on algal growth is that the particles of deposited sediment could cause shading.

### **5.5.2 Single-stressor and multiple-stressor responses**

In general, the single stressor response of epilithon variables was more impacted by phosphorus than sediment. *Melosira varians* did not respond to phosphorus augmentation but responded to fine sediment, which was unexpected, as Kelly & Whitton, (1995) and Kelly *et al.*, (2008) described this species as eutrophication tolerant and normally abundant in high phosphorus rich streams. The chlorophyll a at the community level was impacted by sediment only. This is also an unexpected result as the phosphorus had no impact on the chlorophyll a, but it is not uncommon (Pan & Lowe, 1994) and reflects the fact that every concentration used in this experiment was high.

Most of the algal variables followed the simple multiple responses pattern, where both augmented phosphorus and fine sediment concentration impacted the epilithon variables but without interaction (simple additive way). All possible responses were recorded (subsidy, positive and negative along the phosphorus and sediment gradients). No evident coupling of the same response was recorded along these two stressor gradients, showing that augmented

phosphorus and fine sediment impacts mechanisms are very dissimilar from each other. This could explain the rarity of the complex multiple stressor impacts on epilithon that were recorded in this experiment.

Increasing sediment had a positive impact on the motile guild, for the complex multiple stressor patterns where the phosphorus interacted with fine sediment. This is a result of motile guild's tolerance to fine sediment across all the levels of phosphorus. The fine sediment impact was stronger when the levels of phosphorus were lower. That indicated the antagonistic interaction. The interactive impacts, in addition, modulated the motile guild response to the increasing concentrations of phosphorus. The inflection point, across all the fine sediment levels for the subsidy stress pattern, was AT phosphorus lower levels. The underlying mechanism of the complex interactive impacts is not very clear, as a result of the possibility of more than one pathway could be involved in the individual stressor response of motile guilds to the phosphorus.

### 5.5.3 Algal growth form and stressor gradients

Across sediment gradient, the representation of algal growth form hypotheses was fully supported. The dominant growth form was the high profile guild, even though it decreased from an average of 49.97% to 38.62%. ON THE other hand there was an increase from 32.57% to 37.73% and from 17.46% to 23.65% in the motile guild and low guild RESPECTIVELY with increasing sediment. These patterns agreed with Passy'S (2007) previous research where HE found that the distribution of the guildS can be habitat specific.

The hypothesis regarding increasing phosphorus should show an equivalent increase in both high profile algae and motile algae was partially supported. At all phosphorus gradients, the dominant group was the high profile guild but the three guild responded with their relative abundance to the increased phosphorus. The high profile and motile guilds showed reverse patterns of response as they are correlated in a negative way. As predicted, with increasing phosphorus the motile guild increased, but only up to 33%. The pattern reversed as predicted with further rise of phosphorus levels, as increase in high profile guild. Across the phosphorus levels, the low profile guild showed a significant increase with a relative abundance of 23.65% at the highest where it was 17.46% at the lowest phosphorus condition.

The high profile and motile guilds hypothesis was based on the outcomes of Passy (2007); Lange *et al.*, (2011) previous researches that involved diatom only species. These studies suggested high profile guilds should be favoured over low profile guilds with increasing levels of phosphorus, because high profile guilds can take up phosphorus more competitively. Many of the motile taxa are recorded as eutrophic, therefore motile guilds have increased. The inconsistency between my results and these researches could be partially that they included only diatoms, while my experiment included all algal taxa. Another possibility is the classification of the three guilds is an over simplification. For instance, some of the low profile guild species can grow epiphytically and result in escaping the limitation of resources under the high profile guild species canopy. Under enriched conditions, this form of life becomes more common (Kelly *et al.*, 2008; Veraart *et al.*, 2008).



## CHAPTER SIX: DISCUSSION

### 6.1. Introduction

The results of the field study showed that the algal community in School Farm, Loddington, was already impacted in synergy by both phosphorus and sediment, although this site was the only practical location for the experimental mesocosms. This affected my ability to differentiate the impacts of sediment from phosphorus on the measured biotic responses in the studied streams. Definitive research of these differential impacts required a mesocosm experimental design with combined and separate manipulation of sediment and nutrients (Culp and Baird, 2006). Integration of experiments and field surveys had been proposed by Cash *et al.*, (2003) and Culp and Baird (2006) because each approach has a different limitation and strength. A realistic study environment cannot be provided by field surveys because the possible interaction and coexistence of other influences on natural environmental gradients prevents the relationship of cause and effect being established. A controlled environment was therefore necessary in my stream mesocosm.

In both field and mesocosm experiment *Navicula tripunctata* and *Navicula capitatoradiata* were the species most closely associated with high sediment content, on the other hand for phosphorus concentration *Brachysira vitrea* showed a linear decline with increasing levels of P, while the *Rhoicosphenia abbreviata* and *Navicula cryptotenella* showed a linear increase with increasing levels of P.

Increasing phosphorus or sediment along their gradients had no large impact on the cyanobacteria's proportion with a percentage increase from 3.16% to 4.03% and 3.16% to 4.42% respectively when analysed in isolation, but when phosphorus and sediment were delivered in concert the cyanobacteria proportion increased significantly from 4.14 % to 17.13 % and from 4.76 % to 16.89% respectively.

In running waters, despite the usual co-occurrence of multiple stressors, most observational studies only quantified the relationships of stressor response for single stressors (Heino *et al.*, 2007; Niyogi *et al.*, 2007; Friberg *et al.*, 2010). The failure to consider the potential interactions of multiple stressors explains why researchers have found conflicting results sometimes, especially in regard to the impacts of increased concentrations of nutrient (Yuan, 2010). Inconsistencies could also appear in comparisons of researches that investigated gradients of different lengths, especially given the knowledge that increased nutrients could produce responses of subsidy-stress. In addition, a common field survey drawback is that natural

environmental factors variation could confound the impacts of anthropogenic stressors so that causal conclusion has to be done with extraordinary care. This is relevant even if the levels of multiple-stressors are under investigation, first of all as the stressors of interest could co-vary making it not possible to recognise their individual impacts (Niyogi *et al.*, 2007). Also, one of the stressors may co-vary with an un-measured naturally accompanied variable, which could have generated the impact (Miltner, 2010). As a consequence of this, concentrations of nutrients may be a proxy for a suite of other pollutants (Miltner & Rankin, 1998). It is achievable to reduce these limitation by designing a field mesocosm to investigate multiple environmental stressor.

The mesocosms I used were not ideal, Less eutrophic water would have given a more diverse epiphytic community, but the mesocosms were supplied with eutrophic stream water, which resulted in a smaller diversity. Nutrient enrichment by both phosphorus and nitrogen would have been better, in order to achieve a more realistic results as both elements typically co limit the primary production in running waters (Francoeur, 2001; Elser *et al.*, 2007). Many micro and macronutrients are required for protein synthesis and enzyme activity, although P and N are the primary nutrients limiting growth for phytobenthic in streams. In addition, growth limit can be caused by silica which is required for the frustules of diatoms (Haack & McFeters, 1982) even though this is rarer in streams (Allan & Castillo, 2007). In pristine aquatic ecosystems, demand for the dissolved inorganic forms of P and N is much more than their natural availability from surface run off, atmospheric inputs and upstream (Biggs & Close, 1989; Mainstone & Parr, 2002; Allan & Castillo, 2007). The activities of human beings have increased the P and N availability to fresh waters by more than twenty times background concentrations in many areas (Heathwaite *et al.*, 1996).

## **6.2. Links between field and experimental data**

The results for the phosphorus and sediment gradients across sites over the study time and the mesocosm experiment showed that the trends in P are positively reflected by the biological trends. Chlorophyll a increased as the total P gradient increased, indicating that primary production was stimulated. The effect of sediment upon the chlorophyll a was negative. Shade effects could help to explain this as shading experiments in streamside channels have shown low chlorophyll a in algae under high shade compared with unshaded conditions (Quinn *et al.*, 1997).

Field results showed increases in *Melosira varians* with increasing phosphorus availability, while in mesocosm experiments *Melosira varians* did not respond positively to phosphorus augmentation but responded to fine sediment, which was unexpected as Kelly & Whitton (1995) and Kelly *et al.* (2008) described this species as eutrophication tolerant, normally abundant in phosphorus-rich streams (Biggs *et al.*, 1998). This is probably because the species is more sensitive to sediment than nutrient.

Algal densities increased across the gradients of sediment and nutrients concentrations and the algal biomass decreased with the sediment increases Quinn *et al.* (1997) had reported streams with high nutrient concentrations recorded greater biomass and densities compared to pristine streams. The sediment findings harmonise with the results of Izaguirre *et al.* (2009) who recorded algal biomass negatively response in addition to photosynthetic efficiency when fine sediment was added experimentally. Gray and Ward (1982) suggested that the increased algal in streams with rising the levels of sediment have resulted from the increased levels of nutrients included in that sediment.

The changes from community dominated by a species sensitive to pollution, such as *Brachysira vitrea*, to a community dominated by species tolerant to pollution, such as *Rhoicosphenia abbreviate* and *Navicula cryptotenella* suggest that along the P gradients there are biofilm functional changes. *Navicula cryptotenella* favoured by high nutrient concentrations where it occurs in high relative abundances (Kelly *et al.*, 2007). *Rhoicosphenia abbreviate* has been recorded in oversaturated streams with high P (Rott *et al.*, 1998) and it is one of the most prolific diatom in under enriched streams conditions (Kelly *et al.*, 2007).

### **6.3. Hypotheses revisited**

#### **6.3.1. Hypothesis 1**

My results support the subsidy stress hypothesis for phosphorus and sediment (where at first, an ecological variable increases positively with the increased level of phosphorus and sediment until very high levels are reached, which have negative effects). Along the fine sediment gradient subsidy-stress patterns were found only for densities of *Nitzschia* species, whereas the remaining algal variables responded either negatively or positively. By contrast, subsidy-stress patterns along the nutrient gradient occurred frequently both at the algal taxon and community levels, strongly supporting the subsidy-stress hypothesis for nutrient enrichment. Overall, fewer epilithon variables responded sediment than to phosphorus. The hypothesis of sediment

subsidy stress at the community level was not supported. Diatom percentage decreased from 98.43% to 91.28% with increasing fine sediment, because growth was prevented by the sediment particles' unstable nature. Cyanobacteria percentage increased from 0.35% to 5.51% because the filaments of both *Oscillatoria* sp. and *Anabaena* sp. can grow, fasten and settle on fine sediment particles (Biggs, 1996).

The subsidy-stress pattern was followed by the total algal cell density at the algal community level. The taxonomic and community structure was affected by the phosphorus augmentation. Cyanobacteria and green algae increased in abundance. Biggs & Price (1987); Biggs (1995); Chetelat *et al.*, (1999) found that enriched phosphorus caused green algae proliferation, their abundance increase in this experiment was very clear. The phosphorus augmentation also impacted algal taxon richness, confirming Stevenson's *et al.*, (2008) field survey results and also results of Liess *et al.*, (2009). The phosphorus NMDS plot showed an obvious gradient pattern for the composition of the algal community indicating that phosphorus sensitive taxa such as *Cymbella lanceolata* and *Fragilaria pinnata* were replaced by tolerant ones such as *Navicula subminuscula* and *Nitzschia paleacea*.

### **6.3.2. Hypothesis 2**

The common responses by the epilithon were the single stressor responses, but phosphorus and sediment together generally acted as multiple stressors and usually in a simple additive way, perhaps as a result of the differences in the epilithon underlying mechanisms from one another. The interactions of complex multiple stressors were also found. Increasing phosphorus or sediment along their wide gradients had no large impact on the cyanobacteria's proportion with a percentage increase from 3.16% to 4.03% and 3.16% to 4.42% respectively when analysed in isolation, but when phosphorus and sediment were delivered in concert the cyanobacteria proportion increased significantly from 4.14 % to 17.13 % and from 4.76 % to 16.89% respectively.

Most of the algal variables followed the simple multiple responses pattern, where both augmented phosphorus and fine sediment concentration impacted the epilithon variables but without interaction (simple additive way). All possible responses were recorded (subsidy, positive and negative along the phosphorus and sediment gradients). No evident coupling of the same response was recorded along these two stressor gradients. This could explain the rarity of the complex multiple stressor impacts on epilithon that were recorded in this experiment.

### **6.3.3. Hypothesis 2 (a)**

Across sediment gradients, the representation of algal growth form hypotheses was fully supported. The dominant growth form was the high profile guild, even though it decreased from an average of 49.97% to 38.62%. On the other hand, there was an increase from 32.57% to 37.73% and from 17.46% to 23.65% in the motile guild and low guild respectively, with increasing sediment. These patterns agreed with Passy's (2007) research findings.

As predicted, the results showed motile guild growth form was increased and became widespread with the phosphorus increase over the high profile guild near the intermediate levels of phosphorus. The patterns were then overturned, where the high profile guild representation increased, and the motile guild decreased with further phosphorus increase. The motile guild representation and, due to this, the pattern of subsidy stress, could be considered as a useful detector to indicate the phosphorus enrichment early signs whilst for more severe enrichment cases this will be less useful.

### **6.3.4. Hypothesis 2 (b)**

The hypothesis that increasing phosphorus should show an equivalent increase in both high profile algae and motile algae, was partially supported. At all phosphorus gradients, the dominant group was the high profile. The motile guild, as expected, increased with increasing phosphorus, but only up to 33% and at the expense of the high profile guild. With further increase of phosphorus levels, the pattern reversed as high profile guild increased again. The low profile guild showed a significant increase across the phosphorus gradients with a relative abundance of 23.65% at the highest where it was 17.46% at the lowest phosphorus condition.

Overall, sediment and phosphorus acted mainly as multiple stressors and sometimes in complex interactive ways. The relative strengths of phosphorus and sediment impacts were similar for algal response variables, a finding that was also supported by my field results. My field results further suggested that sediment and phosphorus interacted in synergistic ways in sometimes, as recorded in School Farm for instance, with sediment overwhelming any subsidy impacts that phosphorus may have in isolation. The combined field results and mesocosm results revealed that increased of phosphorus concentrations and sediment levels need to be measured together because they mostly act as multiple stressors in their impacts on algal response variables.

#### 6.4. Relevance to other studies

*Achnantheidium minutissimum* was the dominant diatom in the low P gradients and is present in lower numbers in high P gradients. *A. minutissimum* is reported in the literature as an epiphytic taxon that can tolerate a wide range of nutrient levels, from oligotrophic to eutrophic (Whitmore, 1989; Van Dam, 1994; Ehrlich, 1995).

*Fragilaria* and *Synedra* increased in the study of Schelske and Stoermer (1972) to become major components of the final assemblage as P treatment concentration was increased. In my mesocosm experiment *Fragilaria capucina*, *Fragilariapinnata*, *Fragilaria tenera*, *Synedra acus* and *Synedra ulna* decreased with increasing P levels. Schelske and Stoermer's (1972) mesocosm experiment was enriched with various nutrients including P, Nitrogen and Silica, while in my mesocosm experiment only P was added, suggesting that P was the limiting nutrient, but silica was limiting for diatoms.

Species richness increased with sediment increase in my field study, possibly by reducing the competitive edge of dominant taxa in low-sediment, but adequate or high P, sites. On the other hand, the species richness decreased with increased soluble-P, as eutrophic species were dominant in the community and algal growth became saturated at higher concentrations of soluble-P. Where at the presence of saturating concentrations of P and CO<sub>2</sub> and constant temperature, algae undergoing steady state growth and may be termed "nutrient saturated". In the mesocosm, increased soluble-P had no effect on the taxon richness, this had been reported before by Pan and Lowe (1994). The species richness was not connected to either soluble-P or sediment, leading one to think that species richness is less useful indicator. The mesocosm result contrasts with the results of the Stevenson *et al* (2008) and Liess *et al* (2009) experiments, perhaps because they focused on diatoms only, while in my experiment I considered diatoms and algae. The lack of a subsidy pattern was related to the absence of P limitation stress across all soluble-P levels in my experiment; this mechanism was suggested for an increase in species richness with rising P levels (Biggs & Smith, 2002). On the other hand, the trophic water source for the mesocosms lacked algal species colonists that could exploit the small patches conditions provided by the treatments, limiting the biomass response to P often seen in enriched rivers (Biggs, 2000). However, a distinct gradient pattern of algal community composition was apparent in the NMDS plot for my P addition (Figure 5.1), indicating that tolerant taxa replaced those that were sensitive to P concentrations at higher P levels.

## 6.5. Indicators of stressor

The motile *Nitzschia* and *Navicula* species showed an increase first then a decrease as expected, as Kelly & Whitton, (1995) and Kelly *et al.*, (2008) state these two species are phosphorus tolerant from data collected in the United Kingdom. As explained in Chapter five (5.5.1 Response shapes and subsidy stress hypothesis) section, *Encyonema minutum* and *Fragilaria vaucheriae* on the other hand have been designated by Kelly *et al.* (2008) as water quality indicators because they are nutrient sensitive species. *Fragilaria vaucheriae* increase with phosphorus increase was found in some other researches (Biggs *et al.*, 1998 and Lange *et al.*, 2011). A decrease in the cell densities occurred after additional increase in the concentrations of added phosphorus, despite the well-known tolerance to high phosphorus concentration of most of the species that showed in my experiment a subsidy response.

All diatom species reacted negatively to increasing fine sediment, excluding *Brachysira vitrea*, *Navicula gregaria* and *Navicula minima* which reacted positively which suggest these species can adapt to increased sediment conditions. *Nitzschia dissipata* and *Nitzschia pusilla* showed a subsidy response with increasing levels of fine sediments, probably because these two are motile growth form tolerant of increased sediment. Their decrease at very high levels of fine sediment was probably a result of motile taxa's vulnerability to higher shear stress that happens (Dodds & Biggs, 2002). High and low profile guilds chlorophyll and total density decreased with increase in fine sediment, probably because of substrate instability as a result of increasing sediment, setting the community back to the stage of early succession (Peterson 1996). Another potential reason for the sediment negative effect on algal growth is that the particles of deposited sediment could cause shading.

Only the green algal percentage showed a positive response to phosphorus. Subsidy stress responses for the phosphorus were recorded by algal evenness, *Encyonema minutum*, *Navicula capitatoradiata*, *Nitzschia dissipata*, *Nitzschia sigmoidea*. Three negative responses were recorded by *Synedra acus*, *Cymbella lanceolate* and the percentage of high profile guild.

Negative impacts for sediment were recorded for *Synedra ulna*, *Cymbella lanceolate*, *Nitzschia amphibia*, *Encyonema minutum*, *Encyonema silesiacum*, algal cell density and percentage of high profile guild. Ten positive responses for *Navicula capitatoradiata*, *Navicula cryptotenella*, *Navicula gregaria*, *Navicula lanceolate*, *Navicula minima*, *Navicula*

*subminuscula*, *Navicula tenelloides*, *Navicula tripunctata*, algal evenness, green algae. Other algal species showed subsidy stress responses.

The percentage of motile guild and the percentage of cyanobacteria showed responses for the complex multiple stressor. Cyanobacteria have been recorded to be more widespread in high phosphorus streams (Douterelo *et al.*, 2004), even though the results of my study suggest that increased phosphorus in isolation is not responsible for the proliferation of cyanobacteria.

The richness of algal taxa was not connected in my research to phosphorus or sediment, therefore, this variable is less useful as an indicator. The motile taxa proportion, on the contrary, which has been recommended by Passy (2007) as a potential useful indicator to identify the stream phosphorus enrichment early signs, did show increases.

My high profile and motile guilds hypothesis was based on the outcomes of (Passy, 2007; Lange *et al.*, 2011); previous researches that involved diatom species only. In these studies, the authors stated that high profile guilds should be favoured over low profile guilds with increasing levels of phosphorus because high profile guilds can take up phosphorus more competitively. Many of the motile taxa are recorded as eutrophic, therefore, the motile guilds were supposed to increase. My result partially differed, and the inconsistency between my results and these researches were partially as a result that they included only diatoms while in my experiment all the algal taxa was included. The undesirable consequences of phosphorus enrichment in stream primarily connected with the emerging abundance of Cyanobacteria, with subsequent die off to their excessive biomass, with toxin production and oxygen consumption. As a result, the motile taxa might profit from the supply mode of limiting nutrients generated by cyanobacteria. On the other hand, the point along the P gradient where motility seemed to stop providing a competitive advantage, is an indication of P saturation for algal growth. As a result of that, other elements of community structure might come to the fore, such as tolerance of high P concentrations. Another reason is the classification of the three guilds is an over simplification. For instance, some of the low profile guild species can grow epiphytically and result in escaping the limitation of resources under the high profile guild species canopy. Under enriched conditions this form of life becomes more common (Kelly *et al.*, 2008; Veraart *et al.*, 2008).



## 6.6. Concluding remarks

The relationship between biomass of benthic algae and increasing levels of P concentrations have been studied extensively (Dodds *et al.*, 2002; Dodds, 2006; Smith and Schindler, 2009; Gudmundsdottir *et al.*, 2013; Sabater *et al.*, 2011). Growth assessment of benthic algae has been done by nutrient concentration manipulation in field experiments (Bothwell, 1989; Walton *et al.*, 1995; Rier and Stevenson, 2006; Stevenson *et al.*, 2006), whilst links between algal biomass and P enrichment have been based on large scale surveys (Welch *et al.*, 1992; Dodds *et al.*, 1997; Chetelat *et al.*, 1999). There is strong evidence that biomass increase of benthic algae and changing community composition are firmly related to the availability of P (Stevenson *et al.*, 1996; Wyatt *et al.*, 2010). Different field manipulative experiments have shown changes in diatoms as an increase of growth variation and motile forms (Pringle, 1990; Kelly, 2003; Bellinger *et al.*, 2006; Wyatt *et al.*, 2010; Gudmundsdottir *et al.*, 2013).

Benthic diatoms are frequently used for environmental condition assessment, such as P enrichment, habitat condition and water quality in rivers and streams (Kelly *et al.*, 1995; Pan *et al.*, 1999; Soininen *et al.*, 2004). Field studies can rarely link biotic patterns directly to a single variable (Oppenheim, 1991), although different species of diatom have shown different tolerance levels to different stressors in a laboratory experiment (Licursi and Gómez, 2013). A few studies such as those (Rier and Stevenson, 2002; Lange *et al.*, 2011) have been conducted in laboratory settings that analyse the diatom assemblages' responses to combined effects of multiple variables.

In this research, I integrated experiments and field surveys together (as was proposed by Cash *et al.* (2003) and Culp and Baird, (2006)) because each approach has a different limitation and strength. My research aimed to investigate the individual and combined effects of multiple stressors on ecological response variables in order to understand their potentially complex multiple-stressor interactions, the ecological response shapes to individual stressor gradients, and the relative strengths of the individual stressors when both are operating

I used both a field survey and an experimental approach to draw conclusions about multiple-stressor effects. Understanding the results of my field preliminary observations (Chapter Four) enabled me to design the mesocosm experiment of chapter five, where the epilithic algal responses to broad gradients of both phosphorus and fine sediment have been experimentally tested.

Sediment augmentation was demonstrated to be an extensive stressor that changed the algal communities' structure and taxonomy. Most taxa respond to the increasing sediment levels in a negative way, the inflection points for the species along the fine sediment levels where subsidy became stressor could be indicating the threshold for potential stressor. Consequently, when the fine sediment covered about 75% of the tile surface a distinct changes happened in the algal community. Similarly, the increased concentration of phosphorus where subsidy became stressor and caused dramatic alteration could indicate the phosphorus potential stressor threshold.

The richness of algal taxa was not connected to phosphorus or sediment, therefore, this variable is less useful indicator. The motile taxa proportion, on the contrary, has been recommended by Passy (2007) as a potential useful indicator to identify the stream phosphorus enrichment early signs.

Augmented phosphorus and fine sediment can impact the epilithon responses in opposing or similar ways, providing different results than what could be resulted in case of only just single stressor effects. The complex multiple stressor effects could happen (as revealed in this experiment) for part of the Cyanobacteria in the algal community. The proportion of Cyanobacteria was unaffected by augmented nutrient of sediment when operating in isolation across their wide stressors levels, but when both of these two stressors acted in concert, the Cyanobacteria proportion markedly increased.

This new knowledge needs to be taken into account where sediment is not measured together with nutrient in current routine monitoring of stream condition. Advantages of gathering this information in the future will help in identify the most effective mitigation measures to improve stream condition.

## APPENDICES

**Appendix 3.1** List of all diatom species, each species was assigned to one of the ecological guilds defined by Passy (2007); high profile, low profile and motile.

Species	Ecological guild
<i>Achnanthes oblongella</i>	Low profile
<i>Achnanthidium minutissimum</i>	Low profile
<i>Amphipleura pellucida</i>	Low profile
<i>Amphora pediculus</i>	Low profile
<i>Brachysira vitrea</i>	Low profile
<i>Caloneis bacillum</i>	Low profile
<i>Cocconeis placentula</i>	Low profile
<i>Cymbella ehrenbergii</i>	High profile
<i>Cymbella helvetica</i>	High profile
<i>Cymbella lanceolata</i>	High profile
<i>Diatoma vulgare</i>	High profile
<i>Encyonema minutum</i>	High profile
<i>Encyonema silesiacum</i>	High profile
<i>Fragilaria capucina</i>	High profile
<i>Fragilaria pinnata</i>	High profile
<i>Fragilaria tenera</i>	High profile
<i>Fragilaria vaucheriae</i>	High profile
<i>Gomphonema angustatum</i>	High profile
<i>Gomphonema minutum</i>	Low profile
<i>Gomphonema olivaceum</i>	High profile
<i>Gomphonema parvulum</i>	Low profile
<i>Karayevia clevei</i>	Low profile
<i>Luticola mutica</i>	Low profile
<i>Melosira varians</i>	High profile
<i>Navicula capitatoradiata</i>	Motile
<i>Navicula cryptotenella</i>	Motile
<i>Navicula gregaria</i>	Motile
<i>Navicula lanceolata</i>	Motile

Species	Ecological guild
<i>Navicula minima</i>	Motile
<i>Navicula subminuscula</i>	Motile
<i>Navicula tenelloides</i>	Motile
<i>Navicula tripunctata</i>	Motile
<i>Nitzschia amphibia</i>	Motile
<i>Nitzschia dissipata</i>	Motile
<i>Nitzschia gracilis</i>	Motile
<i>Nitzschia palea</i>	Motile
<i>Nitzschia paleacea</i>	Motile
<i>Nitzschia pusilla</i>	Motile
<i>Nitzschia sigmoidea</i>	Motile
<i>Pinnularia appendiculata</i>	Motile
<i>Planothidium delicatulum</i>	High profile
<i>Planothidium rostratum</i>	High profile
<i>Psammothidium</i>	High profile
<i>lauenburgianum</i>	
<i>Pseudostaurosira brevistriata</i>	High profile
<i>Reimeria sinuata</i>	Low profile
<i>Rhoicosphenia abbreviata</i>	High profile
<i>Staurosira elliptica</i>	High profile
<i>Surirella brebissonii</i>	Motile
<i>Synedra acus</i>	High profile
<i>Synedra ulna</i>	High profile

**Appendix 4.1 The complete species list for the preliminary field observation (May – September 2014) in the study sites**

**Lone Pine 07/05/2014**

Taxa	Tile 1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Amphipleura pellucida</i>	142	117	130	116	130	114	126	120
<i>Brachysira vitrea</i>	86	83	77	76	80	75	78	77
<i>Navicula cryptotenella</i>	42	35	38	34	39	33	38	35
<i>Gyrosigma acuminatum</i>	29	21	30	24	28	23	26	24
<i>Navicula decussis</i>	29	21	27	22	27	22	27	23
<i>Tabularia fasciculata</i>	28	22	26	22	26	22	25	23
<i>Navicula gregaria</i>	29	20	27	20	27	20	26	21
<i>Navicula lanceolata</i>	24	18	22	18	22	17	21	18
<i>Rhoicosphenia abbreviata</i>	20	18	18	15	19	16	18	17
<i>Nitzschia dissipata</i>	15	10	14	10	14	10	14	11
<i>Fragilaria</i>	14	10	12	10	13	10	13	10
<i>Cyclotella</i>	12	5	10	6	11	6	11	6
<i>Navicula tripunctata</i>	4	10	4	7	4	9	4	9
<i>Amphora pediculus</i>	7	3	7	3	6	3	6	3
<i>Navicula reichardtiana</i>	6	2	5	2	6	3	5	3
<i>Planothidium frequentissimum</i>	5	5	5	5	5	5	5	5
<i>Navicula cincta</i>	5	3	5	3	5	3	5	3
<i>Nitzschia sigmoidea</i>	5	3	5	3	5	3	5	3
<i>Nitzschia pusilla</i>	4	5	4	6	3	5	3	5
<i>Cocconeis placentula</i>	4	1	4	3	4	2	4	3
<i>Gomphonema minutum</i>	2	2	3	3	2	3	3	3
<i>Nitzschia palea</i>	3	2	3	3	2	3	2	3
<i>Navicula radiosa</i>	2	3	3	3	2	3	2	3
<i>Kolbesia ploenensis</i>	1	3	2	4	2	3	2	4

**Lone Pine 04/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma attentuatum</i>	127	125	124	124	121	119	122	115
<i>Rhoicosphenia abbreviata</i>	90	92	87	85	84	77	37	31
<i>Pseudostaurosira brevistriata</i>	44	42	41	42	41	39	40	39
<i>Stauroneis phoenicteron</i>	31	33	29	21	25	31	26	25
<i>Amphora pediculus</i>	31	32	29	25	21	30	23	21
<i>Navicula cryptotenella</i>	27	29	26	24	22	25	23	20
<i>Navicula gregaria</i>	27	25	25	26	21	14	20	15
<i>Achnantheidium minutissimum</i>	22	21	15	19	14	15	12	14
<i>Cocconeis placentula</i>	16	20	15	11	17	11	11	10
<i>Navicula reichardtiana</i>	21	23	19	12	17	13	5	6
<i>Nitzschia dissipata</i>	15	15	13	15	12	10	11	9
<i>Amphora inariensis</i>	9	11	9	8	5	6	3	6
<i>Diatoma vulgare</i>	7	12	7	5	7	5	2	3
<i>Surirella brebissonii</i>	11	13	8	5	2	2	1	2
<i>Gomphonema olivaceum</i>	8	5	5	6	3	4	3	5
<i>Planothidium lanceolatum</i>	8	6	5	5	1	2	2	8
<i>Cocconeis pediculus</i>	6	7	6	2	2	1	1	4
<i>Navicula meniscus</i>	5	4	4	2	2	2	4	5
<i>Gomphonema minutum</i>	5	4	2	6	3	0	2	5
<i>Tryblionella apiculata</i>	5	4	5	1	2	1	2	1
<i>Caloneis bacillum</i>	3	4	3	5	2	1	1	1
<i>Amphora montana</i>	4	5	4	2	1	2	0	3
<i>Gomphonema parvulum</i>	3	2	3	1	1	5	2	2
<i>Navicula lanceolata</i>	4	1	0	1	2	2	1	5
<i>Nitzschia lanceolata</i>	3	5	3	0	2	1	3	2
<i>Nitzschia sociabilis</i>	3	0	0	1	3	2	1	1
<i>Nitzschia recta</i>	3	0	0	1	2	1	3	2
<i>Amphora libyca</i>	3	1	2	0	1	0	0	2
<i>Diploneis marginestriata</i>	1	1	1	2	0	1	1	4
<i>Reimeria sinuata</i>	2	1	0	1	2	1	2	1
<i>Nitzschia paleacea</i>	0	1	1	1	0	2	1	4
<i>Navicula decussis</i>	2	2	0	2	1	2	0	0
<i>Navicula atomus</i>	1	0	0	1	1	0	0	5
<i>Nitzschia heufleriana</i>	0	2	2	1	0	1	0	0
<i>Cymatopleura solea</i>	0	0	1	2	0	1	0	0
<i>Nitzschia amphibia</i>	0	1	0	1	0	1	0	0

**Lone Pine 02/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma attentuatum</i>	133	135	132	122	129	130	127	122
<i>Rhoicosphenia abbreviata</i>	92	89	91	89	85	86	84	78
<i>Pseudostaurosira brevistriata</i>	42	51	42	39	39	35	40	22
<i>Stauroneis phoenicteron</i>	37	32	35	31	31	34	32	29
<i>Navicula gregaria</i>	33	35	31	29	30	30	30	28
<i>Amphora pediculus</i>	25	31	24	22	21	18	20	21
<i>Navicula cryptotenella</i>	28	25	25	24	21	13	12	18
<i>Achnanthyidium minutissimum</i>	20	29	19	15	14	13	13	11
<i>Navicula reichardtiana</i>	17	21	18	17	12	15	11	9
<i>Nitzschia dissipata</i>	14	18	13	11	10	11	10	8
<i>Surirella brebissonii</i>	11	19	11	9	11	9	10	9
<i>Cocconeis placentula</i>	7	12	9	8	5	9	5	9
<i>Gomphonema olivaceum</i>	8	11	6	5	5	8	4	8
<i>Amphora inariensis</i>	4	9	5	4	4	5	2	5
<i>Planothidium lanceolatum</i>	5	9	5	4	2	8	1	0
<i>Cymatopleura solea</i>	0	1	1	8	1	6	1	1
<i>Navicula menisculus</i>	4	6	3	3	2	5	1	1
<i>Reimeria sinuata</i>	0	1	3	2	3	6	3	1
<i>Navicula atomus</i>	2	0	4	3	3	8	1	0
<i>Cocconeis pediculus</i>	3	1	3	5	2	6	1	0
<i>Diploneis marginestriata</i>	1	1	2	4	1	5	1	1
<i>Gomphonema parvulum</i>	1	1	2	3	1	4	1	1
<i>Nitzschia amphibia</i>	1	1	2	1	1	4	1	2
<i>Tryblionella apiculata</i>	3	1	0	1	2	6	1	1
<i>Diatoma vulgare</i>	4	1	0	1	3	4	1	1
<i>Gomphonema minutum</i>	2	0	0	1	1	9	0	0
<i>Amphora montana</i>	2	2	1	3	0	5	0	0
<i>Encyonema silesiacum</i>	0	1	1	2	0	1	1	1
<i>Cocconeis placentula</i>	1	1	0	1	1	1	1	1
<i>Planothidium frequentissimum</i>	0	1	0	1	1	0	0	1
<i>Nitzschia paleacea</i>	0	1	0	1	1	2	0	0
<i>Amphora libyca</i>	1	1	1	1	0	1	0	0
<i>Nitzschia lanceolata</i>	0	1	1	1	0	1	0	0
<i>Navicula decussis</i>	0	0	0	1	1	1	1	0

**Lone Pine 30/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma attentuatum</i>	135	136	133	129	130	122	129	119
<i>Rhoicosphenia abbreviata</i>	92	99	92	99	90	99	96	100
<i>Pseudostaurosira brevistriata</i>	44	41	42	39	41	43	39	35
<i>Stauroneis phoenicnteron</i>	27	21	31	29	24	21	21	25
<i>Navicula gregaria</i>	30	24	29	19	21	19	19	15
<i>Amphora pediculus</i>	31	29	18	9	25	15	23	21
<i>Achnanthidium minutissimum</i>	20	24	21	22	22	16	21	20
<i>Navicula reichardtiana</i>	23	19	19	24	19	18	20	18
<i>Navicula cryptotenella</i>	25	20	22	24	19	15	14	12
<i>Surirella brebissonii</i>	15	18	13	15	8	5	5	2
<i>Cocconeis placentula</i>	17	12	14	12	8	9	4	3
<i>Amphora inariensis</i>	7	11	11	9	6	8	2	2
<i>Nitzschia dissipata</i>	12	12	8	11	6	5	5	2
<i>Planothidium lanceolatum</i>	7	10	6	9	5	5	5	4
<i>Gomphonema olivaceum</i>	8	6	6	8	5	1	1	0
<i>Cocconeis pediculus</i>	6	2	6	8	3	1	1	1
<i>Amphora montana</i>	4	0	4	6	3	1	2	0
<i>Gomphonema minutum</i>	3	0	7	5	2	1	1	1
<i>Diatoma vulgare</i>	4	1	3	8	1	1	0	1
<i>Caloneis bacillum</i>	3	1	3	4	0	2	2	0
<i>Diploneis marginestriata</i>	0	2	0	5	3	2	1	1
<i>Gomphonema parvulum</i>	3	6	2	1	0	6	0	0
<i>Amphora libyca</i>	1	1	2	2	1	6	1	1
<i>Encyonema silesiacum</i>	4	1	2	2	0	1	1	1
<i>Nitzschia recta</i>	2	5	2	0	1	2	0	1
<i>Nitzschia heufleriana</i>	0	1	1	1	3	2	1	0
<i>Nitzschia sociabilis</i>	1	1	2	1	0	2	2	1
<i>Tryblionella apiculata</i>	2	4	1	0	1	1	1	1
<i>Navicula menisculus</i>	2	2	0	0	1	3	2	0
<i>Synedra ulna</i>	2	0	2	1	0	3	0	1
<i>Gomphonema clavatum</i>	3	1	1	1	0	2	0	0
<i>Reimeria sinuata</i>	0	2	3	0	0	1	0	0
<i>Navicula atomus</i>	0	1	0	1	4	1	0	1
<i>Navicula lanceolata</i>	1	1	0	1	1	1	0	1
<i>Nitzschia lanceolata</i>	1	1	0	0	1	1	2	1
<i>Navicula decussis</i>	2	0	0	0	1	1	1	1
<i>Planothidium frequentissimum</i>	1	1	0	1	0	1	0	1
<i>Nitzschia paleacea</i>	0	1	1	1	0	1	0	1



**Lone Pine 27/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma attentuatum</i>	132	133	131	129	125	124	120	112
<i>Rhoicosphenia abbreviata</i>	91	89	87	85	82	84	81	92
<i>Pseudostaurosira brevistriata</i>	41	42	40	38	33	32	31	35
<i>Navicula cryptotenella</i>	27	25	19	21	22	24	21	16
<i>Stauroneis phoenicnteron</i>	30	29	29	26	24	21	21	14
<i>Amphora pediculus</i>	28	27	26	25	25	24	21	16
<i>Navicula gregaria</i>	28	24	28	29	21	23	20	21
<i>Navicula reichardtiana</i>	22	21	22	24	19	20	12	20
<i>Achnantheidium minutissimum</i>	19	20	19	21	23	20	12	15
<i>Cocconeis placentula</i>	15	16	14	15	12	13	11	6
<i>Nitzschia dissipata</i>	13	18	13	11	11	9	9	8
<i>Surirella brebissonii</i>	10	15	11	10	5	8	2	6
<i>Caloneis bacillum</i>	4	2	4	6	22	12	2	5
<i>Cocconeis pediculus</i>	5	6	6	6	2	5	1	0
<i>Planothidium lanceolatum</i>	6	8	4	6	4	6	2	0
<i>Amphora inariensis</i>	7	1	7	5	5	2	1	6
<i>Nitzschia recta</i>	4	2	5	6	3	6	2	0
<i>Diatoma vulgare</i>	5	5	4	6	3	5	2	2
<i>Amphora libyca</i>	2	2	2	6	2	2	9	6
<i>Gomphonema olivaceum</i>	7	1	5	2	2	5	1	0
<i>Gomphonema minutum</i>	4	1	4	2	2	5	1	6
<i>Encyonema silesiacum</i>	2	2	2	4	4	6	1	2
<i>Amphora montana</i>	3	1	3	2	3	4	3	5
<i>Navicula menisculus</i>	4	0	3	0	2	5	1	5
<i>Gomphonema parvulum</i>	2	3	3	5	2	3	1	2
<i>Tryblionella apiculata</i>	3	1	2	0	1	4	5	4
<i>Navicula decussis</i>	1	2	2	4	3	5	3	2
<i>Navicula lanceolata</i>	2	1	5	0	2	4	1	2
<i>Gomphonema clavatum</i>	2	1	2	5	1	2	2	0
<i>Reimeria sinuata</i>	2	2	2	3	1	0	0	0
<i>Nitzschia lanceolata</i>	2	1	2	3	1	0	1	0
<i>Nitzschia amphibia</i>	1	1	2	1	0	1	1	1
<i>Navicula atomus</i>	1	2	0	2	0	1	1	1
<i>Nitzschia sociabilis</i>	2	1	1	1	1	1	1	0
<i>Synedra ulna</i>	2	1	2	1	1	0	0	1
<i>Cocconeis placentula</i>	2	1	0	2	1	0	2	0
<i>Planothidium frequentissimum</i>	2	1	0	1	1	1	0	1
<i>Diploneis marginestriata</i>	1	1	1	0	0	2	0	2
<i>Nitzschia paleacea</i>	1	0	0	2	1	2	0	1
<i>Nitzschia heufleriana</i>	1	0	1	1	0	1	1	1
<i>Cymatopleura solea</i>	1	0	1	1	0	1	0	1

**Lone Pine 24/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma attentuatum</i>	123	124	122	115	108	103	100	99
<i>Rhoicosphenia abbreviata</i>	87	88	44	42	58	54	52	51
<i>Pseudostaurosira brevistriata</i>	43	33	42	41	35	34	32	31
<i>Navicula gregaria</i>	26	21	24	23	25	35	24	21
<i>Amphora pediculus</i>	27	25	25	21	12	28	21	19
<i>Navicula cryptotenella</i>	28	26	24	21	9	11	20	15
<i>Achnantheidium minutissimum</i>	22	21	21	16	20	12	18	14
<i>Navicula reichardtiana</i>	23	21	19	15	15	14	15	11
<i>Stauroneis phoenicteron</i>	22	22	18	14	14	13	11	10
<i>Nitzschia dissipata</i>	12	10	23	16	15	11	12	6
<i>Cocconeis placentula</i>	13	11	14	15	10	9	5	8
<i>Surirella brebissonii</i>	9	8	8	9	18	15	1	9
<i>Gomphonema minutum</i>	11	9	9	8	2	6	1	5
<i>Amphora inariensis</i>	8	6	5	6	4	5	2	2
<i>Nitzschia lanceolata</i>	3	9	5	6	3	2	2	2
<i>Gomphonema olivaceum</i>	8	6	5	5	1	2	1	0
<i>Cocconeis pediculus</i>	6	8	4	5	3	1	1	0
<i>Nitzschia amphibia</i>	4	5	4	5	0	2	2	2
<i>Diatoma vulgare</i>	4	4	4	5	0	3	3	5
<i>Navicula menisculus</i>	3	2	3	2	1	2	1	5
<i>Diploneis marginestriata</i>	1	2	2	2	1	5	0	1
<i>Planothidium frequentissimum</i>	1	2	0	5	0	5	2	0
<i>Nitzschia paleacea</i>	0	2	3	6	1	2	0	1
<i>Planothidium lanceolatum</i>	3	1	2	6	0	1	1	0
<i>Navicula decussis</i>	1	1	2	1	0	2	2	1
<i>Synedra ulna</i>	0	1	3	2	2	2	0	1
<i>Encyonema silesiacum</i>	2	2	0	2	1	3	1	1
<i>Nitzschia sociabilis</i>	2	0	4	1	1	1	1	1
<i>Caloneis bacillum</i>	3	1	3	1	0	1	1	1
<i>Navicula atomus</i>	0	1	2	1	0	2	2	0
<i>Gomphonema parvulum</i>	0	1	0	1	1	1	0	1
<i>Reimeria sinuata</i>	0	1	0	1	0	0	0	1
<i>Cymatopleura solea</i>	0	0	0	1	1	0	0	1

**White Horse 07/05/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	133	130	127	130	130	131	124	122
<i>Gomphonema minutum</i>	92	85	84	86	80	84	85	37
<i>Rhoicosphenia abbreviata</i>	42	39	40	41	39	40	42	40
<i>Kolbesia kolbei</i>	37	32	32	33	28	27	21	26
<i>Navicula gregaria</i>	33	31	30	32	27	28	25	23
<i>Navicula reichardtiana</i>	25	20	20	21	26	27	24	23
<i>Nitzschia vermicularis</i>	28	23	12	24	27	26	26	20
<i>Gomphonema olivaceum</i>	20	17	13	15	22	22	19	12
<i>Diatoma vulgare</i>	17	16	11	15	19	19	11	11
<i>Cocconeis pediculus</i>	14	9	10	11	14	15	12	5
<i>Navicula cryptotenella</i>	11	8	10	10	13	14	15	11
<i>Nitzschia palea</i>	7	9	5	7	11	12	8	3
<i>Planothidium lanceolatum</i>	8	6	4	6	4	4	5	2
<i>Tryblionella apiculata</i>	4	6	2	4	6	6	5	1
<i>Synedrella parasitica</i>	5	6	1	4	6	6	6	3
<i>Gyrosigma</i>	0	5	1	10	5	4	5	2
<i>Amphora inariensis</i>	4	2	1	3	5	4	2	1
<i>Navicula capitata</i>	0	5	3	2	5	5	2	4
<i>Encyonema prostratum</i>	2	2	1	3	3	3	6	2
<i>Nitzschia amphibia</i>	3	3	1	2	4	4	1	2
<i>Amphora libyca</i>	1	2	1	2	2	2	5	1
<i>Nitzschia gracilis</i>	1	3	1	2	2	2	2	0
<i>Achnantheidium biasoletiana</i>	1	3	1	2	2	2	1	2
<i>Achnanthes curtissima</i>	3	3	1	0	2	2	1	1
<i>Diploneis petersenii</i>	2	1	1	1	0	2	0	2
<i>Nitzschia dissipata</i>	0	2	1	1	1	0	0	3
<i>Gomphonema parvulum</i>	2	0	2	0	0	1	0	2
<i>Melosira varians</i>	2	1	2	1	0	1	0	2
<i>Navicula menisculus</i>	3	0	1	2	1	1	0	0
<i>Nitzschia archibaldii</i>	2	0	2	0	0	1	1	1
<i>Achnanthes conspicua</i>	1	1	0	0	1	1	1	1
<i>Navicula minima</i>	1	1	1	1	0	1	0	2
<i>Diploneis petersenii</i>	2	1	1	1	0	2	0	2
<i>Nitzschia dissipata</i>	0	2	1	1	1	0	0	3
<i>Gomphonema parvulum</i>	2	0	2	0	0	1	0	2

**White Horse 04/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	155	145	154	144	149	139	148	134
<i>Gomphonema minutum</i>	147	144	144	146	138	135	132	132
<i>Rhoicosphenia abbreviata</i>	61	55	61	56	58	51	53	51
<i>Kolbesia kolbei</i>	30	28	28	32	28	29	29	31
<i>Navicula gregaria</i>	22	21	21	24	19	21	24	26
<i>Nitzschia vermicularis</i>	29	28	21	19	15	16	18	21
<i>Navicula reichardtiana</i>	25	24	24	25	22	21	3	17
<i>Gomphonema olivaceum</i>	15	12	13	15	9	11	8	9
<i>Diatoma vulgare</i>	11	11	9	11	10	15	9	6
<i>Cocconeis pediculus</i>	11	15	12	11	9	11	4	5
<i>Nitzschia palea</i>	5	6	6	9	6	8	4	5
<i>Navicula cryptotenella</i>	10	5	8	9	5	6	5	6
<i>Planothidium lanceolatum</i>	8	5	8	8	4	8	3	2
<i>Tryblionella apiculata</i>	5	4	6	5	4	6	7	4
<i>Synedrella parasitica</i>	5	5	4	5	3	6	3	4
<i>Gyrosigma</i>	4	5	2	5	4	5	2	5
<i>Amphora inariensis</i>	7	2	5	6	3	6	2	2
<i>Navicula capitata</i>	4	2	4	6	3	5	3	4
<i>Nitzschia amphibia</i>	4	5	3	5	3	4	2	4
<i>Nitzschia gracilis</i>	3	2	3	6	2	1	1	12
<i>Encyonema prostratum</i>	7	2	5	4	3	2	1	5
<i>Amphora libyca</i>	4	6	3	4	2	5	2	1
<i>Nitzschia littoralis</i>	3	5	3	4	2	1	1	1
<i>Achnanthes curtissima</i>	3	2	3	5	1	1	1	5
<i>Achnantheidium biasoletiana</i>	3	2	3	5	0	2	1	2
<i>Cymatopleura solea</i>	1	2	1	0	1	2	0	6
<i>Navicula lanceolata</i>	2	2	2	0	1	2	1	1
<i>Nitzschia agnita</i>	2	1	1	1	1	2	0	1
<i>Nitzschia recta</i>	2	4	0	1	0	1	1	0
<i>Nitzschia linearis</i>	0	4	2	0	0	1	1	1
<i>Diploneis petersenii</i>	2	1	1	1	0	2	0	2
<i>Nitzschia dissipata</i>	0	2	1	1	1	0	0	3
<i>Gomphonema parvulum</i>	2	0	2	0	0	1	0	2
<i>Melosira varians</i>	2	1	2	1	0	1	0	2
<i>Navicula menisculus</i>	3	0	1	2	1	1	0	0
<i>Nitzschia archibaldii</i>	2	0	2	0	0	1	1	1
<i>Achnanthes conspicua</i>	1	1	0	0	1	1	1	1
<i>Navicula minima</i>	1	1	1	1	0	1	0	2

**White Horse 02/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	157	155	155	149	155	147	153	151
<i>Gomphonema minutum</i>	146	149	143	142	139	135	120	119
<i>Rhoicosphenia abbreviata</i>	58	61	66	68	66	61	67	65
<i>Nitzschia vermicularis</i>	29	29	29	32	27	29	25	21
<i>Kolbesia kolbei</i>	30	29	29	21	20	25	21	19
<i>Navicula gregaria</i>	22	25	21	25	23	27	19	15
<i>Navicula reichardtiana</i>	20	27	20	20	17	21	17	14
<i>Gomphonema olivaceum</i>	15	21	14	19	13	16	14	14
<i>Amphora inariensis</i>	11	16	9	15	9	11	6	1
<i>Cocconeis pediculus</i>	9	15	6	8	6	14	5	8
<i>Diatoma vulgare</i>	10	11	10	9	9	11	7	8
<i>Navicula cryptotenella</i>	10	14	9	5	7	9	6	6
<i>Nitzschia palea</i>	8	11	6	6	5	4	3	6
<i>Planothidium lanceolatum</i>	7	8	6	5	4	7	3	5
<i>Gomphonema parvulum</i>	7	9	6	2	0	8	6	5
<i>Achnantheidium biasoletiana</i>	6	5	4	5	4	7	2	4
<i>Achnanthes curtissima</i>	5	2	5	4	3	0	3	4
<i>Nitzschia archibaldii</i>	4	9	3	1	2	1	1	1
<i>Navicula lanceolata</i>	3	5	2	2	4	1	1	1
<i>Tryblionella apiculata</i>	4	2	4	5	2	1	3	0
<i>Navicula menisculus</i>	8	4	2	1	2	1	0	1
<i>Nitzschia recta</i>	3	5	2	1	3	0	1	6
<i>Amphora libyca</i>	5	1	0	1	2	2	4	2
<i>Synedrella parasitica</i>	2	2	2	5	2	4	1	1
<i>Surirella brebissonii</i>	3	1	3	6	2	1	0	2
<i>Navicula capitata</i>	3	2	2	3	2	0	1	3
<i>Gyrosigma</i>	3	0	0	5	0	1	2	5
<i>Nitzschia dissipata</i>	0	2	1	6	1	2	0	2
<i>Nitzschia capitellata</i>	1	1	2	2	2	1	1	4
<i>Navicula minima</i>	2	0	1	6	2	1	0	4
<i>Nitzschia littoralis</i>	2	2	0	1	5	1	1	0
<i>Nitzschia amphibia</i>	4	1	3	2	1	0	0	1
<i>Melosira varians</i>	1	0	1	5	1	0	1	0
<i>Encyonema prostratum</i>	1	1	0	2	1	1	1	1
<i>Achnanthes conspicua</i>	0	1	0	2	1	2	0	0
<i>Cymatopleura solea</i>	0	2	0	1	0	2	1	0

**White Horse 30/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	160	157	155	149	155	151	149	142
<i>Gomphonema minutum</i>	130	127	149	142	147	145	147	145
<i>Rhoicosphenia abbreviata</i>	80	78	70	68	69	66	61	60
<i>Nitzschia vermicularis</i>	27	27	25	24	25	32	24	25
<i>Kolbesia kolbei</i>	37	31	32	31	28	25	5	18
<i>Navicula gregaria</i>	22	31	20	29	18	19	17	12
<i>Navicula reichardtiana</i>	19	21	17	21	10	11	16	14
<i>Cocconeis pediculus</i>	13	21	11	14	10	8	9	11
<i>Gomphonema olivaceum</i>	16	21	11	9	8	4	5	6
<i>Navicula cryptotenella</i>	8	15	8	9	18	9	6	2
<i>Nitzschia linearis</i>	4	5	2	8	2	1	2	1
<i>Diatoma vulgare</i>	7	6	7	5	9	5	7	4
<i>Achnantheidium biasoletiana</i>	7	9	9	2	6	6	3	4
<i>Planothidium lanceolatum</i>	6	8	5	1	4	2	9	5
<i>Achnanthes conspicua</i>	1	2	1	5	7	6	8	7
<i>Gyrosigma</i>	5	8	3	2	12	5	1	5
<i>Synedrella parasitica</i>	9	5	7	6	3	4	2	2
<i>Navicula menisculus</i>	6	3	5	2	3	5	3	8
<i>Amphora inariensis</i>	6	3	5	2	3	4	2	5
<i>Nitzschia recta</i>	3	2	3	2	2	4	7	2
<i>Nitzschia gracilis</i>	5	4	4	1	2	2	3	0
<i>Navicula capitata</i>	4	0	4	1	3	2	2	1
<i>Nitzschia archibaldii</i>	3	1	7	4	1	2	1	1
<i>Tryblionella apiculata</i>	4	2	4	2	2	2	2	1
<i>Melosira varians</i>	4	2	4	1	3	0	2	2
<i>Diploneis petersenii</i>	3	2	5	0	2	1	1	1
<i>Gomphonema parvulum</i>	4	2	3	0	0	0	2	2
<i>Achnanthes curtissima</i>	4	1	3	0	2	0	1	1
<i>Nitzschia littoralis</i>	4	0	2	1	1	1	1	1
<i>Nitzschia capitellata</i>	2	1	2	2	0	1	1	1
<i>Nitzschia palea</i>	0	2	2	1	2	1	0	2
<i>Cymatopleura solea</i>	0	0	2	1	3	0	1	2
<i>Navicula minima</i>	0	5	0	1	1	0	1	1
<i>Nitzschia dissipata</i>	2	1	0	1	0	1	2	1
<i>Amphora libyca</i>	1	2	0	1	1	0	1	2
<i>Nitzschia agnita</i>	1	4	0	1	1	0	2	1
<i>Nitzschia amphibia</i>	0	0	0	1	1	0	1	4
<i>Surirella brebissonii</i>	0	1	0	1	0	0	0	2

**White Horse 27/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	157	154	155	157	152	142	150	149
<i>Gomphonema minutum</i>	146	146	144	141	142	145	137	135
<i>Rhoicosphenia abbreviata</i>	60	55	26	23	57	25	56	51
<i>Nitzschia vermicularis</i>	27	38	26	24	25	34	25	21
<i>Kolbesia kolbei</i>	28	21	21	18	20	25	19	21
<i>Navicula gregaria</i>	24	25	22	21	12	24	18	17
<i>Navicula reichardtiana</i>	18	21	14	15	20	21	11	12
<i>Gomphonema olivaceum</i>	14	21	34	21	13	12	7	9
<i>Diatoma vulgare</i>	12	15	7	9	11	24	9	9
<i>Cocconeis pediculus</i>	10	8	9	11	3	0	8	8
<i>Navicula cryptotenella</i>	9	8	7	12	5	1	2	2
<i>Planothidium lanceolatum</i>	6	6	4	4	5	9	3	5
<i>Amphora inariensis</i>	8	6	0	2	10	2	4	5
<i>Achnantheidium biasoletiana</i>	5	4	4	2	9	1	3	5
<i>Melosira varians</i>	1	0	5	2	4	6	5	5
<i>Nitzschia amphibia</i>	2	2	7	4	2	6	2	0
<i>Tryblionella apiculata</i>	3	2	4	6	1	3	1	1
<i>Nitzschia archibaldii</i>	3	2	2	5	1	6	0	1
<i>Amphora libyca</i>	3	2	2	2	2	2	5	1
<i>Gyrosigma</i>	3	2	3	4	3	1	1	2
<i>Synedrella parasitica</i>	2	2	1	1	0	1	1	12
<i>Nitzschia palea</i>	4	2	2	6	0	0	1	1
<i>Nitzschia capitellata</i>	1	2	2	6	2	2	1	1
<i>Nitzschia gracilis</i>	2	1	2	2	1	6	0	2
<i>Nitzschia agnita</i>	1	2	2	1	2	2	1	5
<i>Navicula lanceolata</i>	2	1	1	2	1	1	2	2
<i>Navicula menisculus</i>	4	0	0	2	3	2	0	0
<i>Navicula capitata</i>	3	1	2	2	1	1	0	2
<i>Nitzschia littoralis</i>	2	0	1	2	1	2	1	2
<i>Achnanthes curtissima</i>	2	1	0	2	0	1	1	2
<i>Cymatopleura solea</i>	1	2	0	3	0	2	1	0
<i>Achnanthes conspicua</i>	1	2	1	2	1	0	1	1
<i>Nitzschia recta</i>	2	0	1	0	0	2	0	1
<i>Encyonema prostratum</i>	1	2	1	1	0	1	0	1
<i>Nitzschia linearis</i>	1	2	0	2	0	0	1	1
<i>Nitzschia dissipata</i>	1	0	1	1	1	0	1	1
<i>Navicula minima</i>	2	1	1	0	1	1	0	2
<i>Diploneis petersenii</i>	1	2	1	1	1	1	0	0
<i>Gomphonema parvulum</i>	2	1	0	1	0	0	1	0
<i>Surirella brebissonii</i>	2	2	0	0	1	0	0	0

**White Horse 24/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	159	161	155	154	153	155	152	154
<i>Gomphonema minutum</i>	148	151	146	145	146	142	144	142
<i>Rhoicosphenia abbreviata</i>	66	67	69	59	63	62	60	56
<i>Kolbesia kolbei</i>	30	42	27	24	25	25	25	21
<i>Nitzschia vermicularis</i>	29	21	29	25	27	21	21	21
<i>Navicula gregaria</i>	26	11	21	26	19	21	17	15
<i>Navicula reichardtiana</i>	16	25	14	14	9	11	8	9
<i>Navicula cryptotenella</i>	11	15	12	15	10	16	7	5
<i>Cocconeis pediculus</i>	9	11	10	9	9	15	9	5
<i>Gomphonema olivaceum</i>	12	6	11	10	9	14	5	5
<i>Diatoma vulgare</i>	11	15	7	8	5	3	3	4
<i>Planothidium lanceolatum</i>	9	11	8	5	5	2	5	5
<i>Amphora inariensis</i>	7	12	6	6	5	2	4	4
<i>Nitzschia gracilis</i>	7	14	7	5	1	2	5	5
<i>Tryblionella apiculata</i>	5	3	6	6	4	0	1	2
<i>Achnantheidium biasolettiana</i>	6	3	6	5	0	1	2	1
<i>Nitzschia amphibia</i>	2	3	0	4	6	2	1	2
<i>Nitzschia palea</i>	3	5	3	5	2	1	1	1
<i>Nitzschia littoralis</i>	3	3	4	4	2	2	1	0
<i>Nitzschia recta</i>	3	2	1	0	3	1	0	1
<i>Achnanthes conspicua</i>	3	0	3	1	0	0	2	2
<i>Synedrella parasitica</i>	1	2	1	1	1	1	0	5
<i>Navicula menisculus</i>	4	1	0	2	0	0	3	1
<i>Melosira varians</i>	1	1	1	2	2	1	1	1
<i>Gomphonema parvulum</i>	1	1	0	2	2	2	0	2
<i>Nitzschia archibaldii</i>	4	1	3	2	0	0	0	1
<i>Navicula lanceolata</i>	3	2	2	0	0	1	1	1
<i>Encyonema prostratum</i>	2	2	0	1	1	1	1	1
<i>Navicula capitata</i>	2	0	1	2	0	2	0	2
<i>Navicula minima</i>	3	1	2	1	1	0	1	0
<i>Achnanthes curtissima</i>	2	1	2	1	1	1	0	1
<i>Nitzschia agnita</i>	1	0	1	1	1	1	0	5
<i>Amphora libyca</i>	1	0	0	2	0	2	1	2
<i>Cymatopleura solea</i>	0	1	0	1	1	1	0	2
<i>Diploneis petersenii</i>	0	0	0	0	0	1	1	1



**School Farm 07/05/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gomphonema parvulum</i>	133	135	132	122	129	130	127	122
<i>Nitzschia palea</i>	92	89	91	89	85	86	84	78
<i>Amphora pediculus</i>	42	51	42	39	39	35	40	22
<i>Psammothidium lauenburgianum</i>	37	32	35	31	31	34	32	29
<i>Nitzschia capitellata</i>	33	35	31	29	30	30	30	28
<i>Reimeria sinuata</i>	25	31	24	22	21	18	20	21
<i>Gomphonema olivaceum</i>	28	25	25	24	21	13	12	18
<i>Amphora inariensis</i>	20	29	19	15	14	13	13	11
<i>Planothidium lanceolatum</i>	17	21	18	17	12	15	11	9
<i>Planothidium delicatulum</i>	14	18	13	11	10	11	10	8
<i>Cocconeis placentula</i>	11	19	11	9	11	9	10	9
<i>Gomphonema olivaceoides</i>	7	12	9	8	5	9	5	9
<i>Navicula cryptotenella</i>	8	11	6	5	5	8	4	8
<i>Encyonema silesiacum</i>	4	9	5	4	4	5	2	5
<i>Staurosira elliptica</i>	5	9	5	4	2	8	1	0
<i>Rhoicosphenia abbreviata</i>	0	1	1	8	1	6	1	1
<i>Achnanthes oblongella</i>	4	6	3	3	2	5	1	1
<i>Navicula tripunctata</i>	0	1	3	2	3	6	3	1
<i>Cymbella helvetica</i>	2	0	4	3	3	8	1	0
<i>Fragilaria capucina</i>	3	1	3	5	2	6	1	0
<i>Amphipleura pellucida</i>	1	1	2	4	1	5	1	1
<i>Planothidium frequentissimum</i>	1	1	2	3	1	4	1	1
<i>Nitzschia paleacea</i>	1	1	2	1	1	4	1	2
<i>Fragilaria</i>	3	1	0	1	2	6	1	1
<i>Karayevia clevei</i>	4	1	0	1	3	4	1	1

**School Farm 04/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gomphonema parvulum</i>	115	116	113	121	101	99	97	89
<i>Nitzschia palea</i>	101	100	104	102	92	96	95	85
<i>Amphora pediculus</i>	59	56	51	42	44	42	39	31
<i>Psammothidium lauenburgianum</i>	53	58	48	48	33	32	42	39
<i>Nitzschia capitellata</i>	53	51	49	41	37	31	32	35
<i>Reimeria sinuata</i>	49	42	41	42	30	38	31	25
<i>Gomphonema olivaceum</i>	45	51	44	45	29	34	17	21
<i>Amphora inariensis</i>	25	25	21	21	21	25	19	20
<i>Planothidium lanceolatum</i>	28	26	26	23	17	21	11	16
<i>Planothidium delicatulum</i>	15	15	14	15	13	15	11	10
<i>Cocconeis placentula</i>	15	16	5	15	10	9	9	11
<i>Gomphonema olivaceoides</i>	11	17	12	13	10	8	2	11
<i>Navicula cryptotenella</i>	12	14	10	9	0	2	3	2
<i>Encyonema silesiacum</i>	9	8	7	9	6	2	6	2
<i>Staurosira elliptica</i>	7	6	12	12	5	2	1	2
<i>Rhoicosphenia abbreviata</i>	11	6	7	9	3	2	0	2
<i>Achnanthes oblongella</i>	6	8	5	6	6	2	0	0
<i>Navicula tripunctata</i>	3	6	3	9	1	2	0	2
<i>Cymbella helvetica</i>	4	5	4	6	3	6	1	0
<i>Fragilaria capucina</i>	5	5	5	5	0	2	0	1
<i>Amphipleura pellucida</i>	2	5	1	1	5	1	4	1
<i>Planothidium frequentissimum</i>	3	5	1	2	2	1	1	1
<i>Nitzschia paleacea</i>	4	0	1	0	0	1	1	0
<i>Fragilaria</i>	0	0	2	0	2	1	1	1
<i>Karayevia clevei</i>	1	0	0	0	1	0	1	2

**School Farm 02/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gomphonema parvulum</i>	107	104	101	102	97	99	91	88
<i>Nitzschia palea</i>	100	101	90	88	71	74	66	67
<i>Amphora pediculus</i>	87	81	66	65	57	51	52	42
<i>Psammothidium lauenburgianum</i>	66	59	51	60	50	51	49	51
<i>Nitzschia capitellata</i>	60	62	55	52	52	39	29	35
<i>Reimeria sinuata</i>	55	62	53	50	52	45	44	49
<i>Gomphonema olivaceum</i>	44	52	42	41	45	48	39	41
<i>Amphora inariensis</i>	28	32	25	30	26	35	21	23
<i>Planothidium lanceolatum</i>	28	31	26	31	19	25	17	21
<i>Cocconeis placentula</i>	18	20	16	21	4	2	10	11
<i>Planothidium delicatulum</i>	17	19	7	11	5	1	0	2
<i>Gomphonema olivaceoides</i>	2	5	9	12	5	3	5	6
<i>Rhoicosphenia abbreviata</i>	9	6	7	11	0	1	1	2
<i>Nitzschia paleacea</i>	7	5	5	6	2	2	1	3
<i>Amphipleura pellucida</i>	13	4	2	4	0	1	1	2
<i>Navicula cryptotenella</i>	5	4	4	4	0	2	0	1
<i>Achnanthes oblongella</i>	4	4	2	5	0	1	1	1
<i>Staurosira elliptica</i>	3	5	3	4	1	1	1	2
<i>Planothidium frequentissimum</i>	7	5	1	2	1	0	1	1
<i>Navicula tripunctata</i>	3	2	0	1	2	1	2	1
<i>Encyonema silesiacum</i>	1	0	0	1	2	1	1	2
<i>Cocconeis placentula</i>	0	0	1	1	2	2	1	1
<i>Fragilaria</i>	2	1	1	0	0	1	0	0
<i>Cymbella helvetica</i>	1	2	1	0	0	2	1	0

**School Farm 30/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gomphonema parvulum</i>	111	109	107	106	105	103	101	103
<i>Nitzschia palea</i>	101	100	100	99	91	88	92	95
<i>Amphora pediculus</i>	61	62	59	56	60	61	62	56
<i>Nitzschia capitellata</i>	59	54	49	45	41	34	39	41
<i>Psammothidium lauenburgianum</i>	57	57	26	35	31	32	27	11
<i>Reimeria sinuata</i>	47	45	43	41	41	39	40	45
<i>Gomphonema olivaceum</i>	44	48	42	15	31	26	21	21
<i>Planothidium lanceolatum</i>	28	26	27	34	26	24	25	24
<i>Amphora inariensis</i>	28	24	19	21	15	16	10	10
<i>Planothidium delicatulum</i>	15	19	15	21	11	15	10	9
<i>Cocconeis placentula</i>	19	21	12	21	10	11	11	9
<i>Rhoicosphenia abbreviata</i>	15	15	11	15	3	2	1	2
<i>Fragilaria capucina</i>	7	9	6	9	5	1	4	0
<i>Navicula tripunctata</i>	6	5	6	2	4	1	3	2
<i>Staurosira elliptica</i>	9	7	7	4	0	1	1	2
<i>Encyonema silesiacum</i>	6	2	6	6	4	1	1	2
<i>Navicula cryptotenella</i>	8	8	6	2	1	2	1	2
<i>Cymbella helvetica</i>	8	7	6	7	4	1	2	1
<i>Gomphonema olivaceoides</i>	7	6	9	5	0	0	7	0
<i>Achnanthes oblongella</i>	7	6	7	8	0	2	0	1
<i>Nitzschia paleacea</i>	3	5	2	1	1	3	0	2
<i>Karayevia clevei</i>	1	0	2	1	1	3	0	2
<i>Planothidium frequentissimum</i>	2	0	1	1	0	2	1	1
<i>Fragilaria</i>	0	0	0	1	1	3	1	1

**School Farm 27/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gomphonema parvulum</i>	110	111	109	111	107	103	102	99
<i>Nitzschia palea</i>	99	85	91	89	89	95	88	84
<i>Psammothidium lauenburgianum</i>	51	41	51	56	47	54	46	54
<i>Amphora pediculus</i>	57	57	52	54	12	21	50	45
<i>Reimeria sinuata</i>	45	46	43	35	39	41	31	34
<i>Gomphonema olivaceum</i>	43	41	35	36	40	35	38	41
<i>Nitzschia capitellata</i>	50	41	44	35	37	37	31	21
<i>Planothidium lanceolatum</i>	26	32	29	28	21	21	20	18
<i>Amphora inariensis</i>	23	31	21	29	17	21	16	24
<i>Cocconeis placentula</i>	13	12	11	14	10	15	9	11
<i>Planothidium delicatulum</i>	13	10	12	24	7	11	2	0
<i>Navicula cryptotenella</i>	8	9	7	11	6	5	6	6
<i>Gomphonema olivaceoides</i>	11	5	7	16	3	6	5	6
<i>Staurosira elliptica</i>	8	8	6	9	5	5	5	5
<i>Achnanthes oblongella</i>	8	5	7	9	0	2	0	6
<i>Rhoicosphenia abbreviata</i>	11	9	5	8	0	2	3	5
<i>Encyonema silesiacum</i>	5	8	4	5	3	1	1	1
<i>Planothidium frequentissimum</i>	5	7	2	6	4	2	3	2
<i>Cymbella helvetica</i>	5	8	4	5	2	1	1	1
<i>Fragilaria capucina</i>	5	5	7	4	0	1	1	2
<i>Navicula tripunctata</i>	5	4	4	5	0	1	1	1
<i>Fragilaria</i>	3	2	2	1	0	5	1	0
<i>Nitzschia paleacea</i>	5	1	0	1	2	3	1	1
<i>Cocconeis placentula</i>	3	1	2	0	1	2	0	0
<i>Karayevia clevei</i>	3	2	0	0	1	1	0	1
<i>Amphipleura pellucida</i>	3	1	0	0	1	0	0	0

**School Farm 24/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gomphonema parvulum</i>	112	110	110	104	101	95	91	95
<i>Nitzschia palea</i>	88	87	85	82	80	78	79	75
<i>Amphora pediculus</i>	75	75	70	71	71	65	56	84
<i>Psammothidium lauenburgianum</i>	53	65	51	42	50	41	49	41
<i>Nitzschia capitellata</i>	55	65	51	49	37	42	31	29
<i>Gomphonema olivaceum</i>	44	45	22	32	39	35	32	24
<i>Reimeria sinuata</i>	44	49	39	41	29	21	18	15
<i>Planothidium lanceolatum</i>	27	32	21	28	18	24	14	16
<i>Amphora inariensis</i>	25	31	21	16	11	12	7	2
<i>Cocconeis placentula</i>	15	21	9	2	0	2	5	4
<i>Rhoicosphenia abbreviata</i>	12	15	8	5	6	5	1	0
<i>Navicula tripunctata</i>	9	5	6	2	12	14	1	2
<i>Navicula cryptotenella</i>	7	5	7	2	5	5	2	0
<i>Staurosira elliptica</i>	8	4	4	6	4	2	2	0
<i>Planothidium delicatulum</i>	11	6	7	2	4	5	1	0
<i>Cymbella helvetica</i>	6	5	5	8	2	5	1	1
<i>Fragilaria capucina</i>	7	3	4	6	10	5	0	1
<i>Encyonema silesiacum</i>	7	3	5	5	0	2	1	1
<i>Achnanthes oblongella</i>	4	7	4	5	1	1	0	0
<i>Nitzschia paleacea</i>	0	1	1	4	4	0	1	1
<i>Amphipleura pellucida</i>	3	2	0	3	2	0	2	2
<i>Karayevia clevei</i>	3	1	0	2	1	1	1	3
<i>Fragilaria</i>	3	0	2	2	0	0	1	2
<i>Planothidium frequentissimum</i>	0	1	1	2	1	1	2	2

**Digby Farm 07/05/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	133	132	129	125	122	155	112	119
<i>Navicula lanceolata</i>	92	91	85	82	99	143	92	100
<i>Navicula gregaria</i>	42	42	38	33	43	66	35	35
<i>Planothidium lanceolatum</i>	37	35	21	22	21	29	16	25
<i>Nitzschia sigmoidea</i>	33	31	26	24	19	29	14	15
<i>Gomphonema angustatum</i>	25	24	25	25	15	21	16	21
<i>Amphora pediculus</i>	28	25	29	21	16	20	21	20
<i>Gomphonema parvulum</i>	20	19	24	19	18	14	20	18
<i>Achnantheidium minutissimum</i>	17	18	21	23	15	9	15	12
<i>Staurosira elliptica</i>	14	13	15	12	5	6	6	2
<i>Fragilaria vaucheriae</i>	11	11	11	11	9	10	8	3
<i>Rhoicosphenia abbreviata</i>	7	9	10	5	8	9	6	2
<i>Surirella brebissonii</i>	8	6	6	22	5	6	5	2
<i>Fragilaria pinnata</i>	4	5	6	2	5	6	0	4
<i>Cocconeis placentula</i> var. <i>lineata</i>	5	5	6	4	1	6	0	0
<i>Navicula subminuscule</i>	0	1	5	5	1	4	6	1
<i>Amphora</i>	4	3	6	3	1	5	0	0
<i>Pseudostaurosira brevistriata</i>	0	3	6	3	1	3	2	1
<i>Luticola mutica</i>	2	4	6	2	1	2	6	1
<i>Planothidium frequentissimum</i>	3	3	2	2	2	4	0	0
<i>Navicula tripunctata</i>	1	2	2	2	2	2	6	1
<i>Reimeria sinuata</i>	1	2	4	4	6	2	2	0
<i>Achnantheidium pyrenaicum</i>	1	2	2	3	6	0	5	1
<i>Cymbella helvetica</i>	3	0	0	2	1	2	5	1
<i>Encyonema minutum</i>	4	0	5	2	2	3	2	1
<i>Fragilaria capucina</i>	2	0	0	1	2	2	4	0
<i>Caloneis bacillum</i>	2	1	4	3	2	0	2	1
<i>Gomphonema olivaceum</i>	133	132	0	2	1	1	2	1
<i>Navicula minima</i>	92	91	5	1	3	2	0	0
<i>Pinnularia</i>	42	42	3	1	3	1	0	1
<i>Navicula tenelloides</i>	37	35	3	1	2	0	0	0
<i>Navicula cryptotenella</i>	33	31	1	0	1	3	1	0
<i>Nitzschia amphibia</i>	25	24	2	0	1	1	1	1
<i>Nitzschia pusilla</i>	28	27	1	1	1	0	0	0
<i>Diatoma vulgare</i>	20	22	1	1	1	0	1	0

**Digby Farm 04/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	83	88	78	77	70	68	63	61
<i>Navicula lanceolata</i>	59	54	55	51	49	51	21	23
<i>Navicula gregaria</i>	44	31	38	35	34	31	30	31
<i>Planothidium lanceolatum</i>	37	32	34	32	31	21	29	23
<i>Nitzschia sigmoidea</i>	28	31	25	24	21	24	19	21
<i>Gomphonema angustatum</i>	25	25	27	29	17	15	19	12
<i>Amphora pediculus</i>	23	25	21	21	7	9	17	15
<i>Gomphonema parvulum</i>	18	19	20	21	15	12	9	11
<i>Achnantheidium minutissimum</i>	12	11	12	11	14	15	11	10
<i>Staurosira elliptica</i>	13	15	11	10	7	9	10	12
<i>Fragilaria vaucheriae</i>	8	9	7	8	10	13	11	15
<i>Rhoicosphenia abbreviata</i>	14	13	8	9	7	8	2	2
<i>Surirella brebissonii</i>	9	11	2	0	7	8	7	2
<i>Fragilaria pinnata</i>	9	8	10	6	0	6	2	2
<i>Cocconeis placentula</i> var. <i>lineata</i>	9	10	9	10	0	4	7	9
<i>Navicula subminuscula</i>	5	8	6	5	2	5	7	3
<i>Amphora</i>	11	12	2	5	7	6	9	5
<i>Pseudostaurosira brevistriata</i>	7	9	6	4	5	6	5	6
<i>Luticola mutica</i>	5	6	12	11	4	5	1	2
<i>Planothidium frequentissimum</i>	6	8	5	6	3	5	7	9
<i>Navicula tripunctata</i>	3	5	15	13	1	4	2	0
<i>Reimeria sinuata</i>	8	8	3	5	2	0	7	1
<i>Achnantheidium pyrenaicum</i>	7	9	4	2	5	2	1	2
<i>Cymbella helvetica</i>	7	8	0	1	5	2	4	5
<i>Encyonema minutum</i>	3	5	1	3	1	1	3	5
<i>Fragilaria capucina</i>	5	6	3	0	1	0	3	5
<i>Caloneis bacillum</i>	4	5	4	1	3	0	1	3
<i>Gomphonema olivaceum</i>	4	5	3	0	0	2	0	3
<i>Navicula minima</i>	1	2	7	1	3	0	1	1
<i>Pinnularia</i>	1	2	4	2	2	1	1	1
<i>Navicula tenelloides</i>	2	0	0	1	3	1	1	2
<i>Navicula cryptotenella</i>	0	0	2	2	0	0	4	1
<i>Nitzschia amphibia</i>	0	1	1	1	1	2	1	4
<i>Nitzschia pusilla</i>	1	1	0	0	1	2	2	0
<i>Diatoma vulgare</i>	0	0	1	1	2	1	2	2
<i>Navicula veneta</i>	2	0	0	2	2	1	2	0
<i>Nitzschia dissipata</i>	1	0	0	2	2	1	1	1
<i>Navicula capitatoradiata</i>	0	1	0	0	1	1	0	4
<i>Cymbella microcephala</i>	2	0	0	2	1	1	2	0
<i>Cymbella prostrata</i>	1	1	1	1	1	1	1	1
<i>Navicula saprophila</i>	1	1	0	0	0	1	0	2
<i>Surirella roba</i>	0	0	0	1	0	1	1	1



**Digby Farm 02/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	73	77	72	70	66	64	61	56
<i>Navicula lanceolata</i>	55	51	55	51	51	51	49	51
<i>Navicula gregaria</i>	44	41	45	49	40	39	39	42
<i>Planothidium lanceolatum</i>	42	43	42	31	40	38	42	34
<i>Amphora pediculus</i>	33	35	29	32	22	21	17	21
<i>Gomphonema angustatum</i>	25	29	23	29	21	19	15	16
<i>Nitzschia sigmoidea</i>	22	25	21	21	15	19	19	15
<i>Gomphonema parvulum</i>	16	19	14	15	7	12	10	13
<i>Amphora</i>	11	15	9	11	8	5	7	12
<i>Surirella brebissonii</i>	13	18	9	12	7	6	5	6
<i>Achnantheidium minutissimum</i>	8	11	9	9	8	6	8	5
<i>Gomphonema olivaceum</i>	14	18	9	9	0	1	5	6
<i>Staurosira elliptica</i>	9	12	7	8	3	2	0	1
<i>Pseudostaurosira brevistriata</i>	7	12	7	5	3	3	3	3
<i>Nitzschia palea</i>	7	5	5	8	4	6	4	5
<i>Fragilaria pinnata</i>	9	5	3	9	5	4	0	2
<i>Planothidium frequentissimum</i>	8	8	7	8	3	5	4	2
<i>Nitzschia pusilla</i>	7	2	6	5	3	4	5	3
<i>Navicula subminuscula</i>	6	1	6	4	9	8	1	2
<i>Fragilaria vaucheriae</i>	8	5	5	6	4	3	1	4
<i>Fragilaria capucina</i>	5	5	3	4	2	3	1	1
<i>Cymbella helvetica</i>	8	4	6	5	3	3	1	5
<i>Achnantheidium pyrenaicum</i>	6	4	4	6	3	3	1	4
<i>Rhoicosphenia abbreviata</i>	6	4	7	5	0	1	1	2
<i>Gomphonema angustum/pumilum type</i>	4	4	4	5	2	2	1	1
<i>Nitzschia dissipata</i>	5	5	1	4	5	3	0	0
<i>Luticola mutica</i>	4	3	3	0	0	2	1	1
<i>Cocconeis placentula var. lineata</i>	6	5	0	2	1	1	0	1
<i>Pinnularia</i>	0	2	2	2	1	3	1	2
<i>Encyonema minutum</i>	2	2	3	2	0	1	2	1
<i>Navicula tenelloides</i>	3	2	1	1	1	3	1	1
<i>Navicula veneta</i>	2	1	2	1	2	2	1	1
<i>Cymbella prostrata</i>	0	2	2	1	1	1	1	0
<i>Reimeria sinuata</i>	2	1	2	2	1	2	1	0
<i>Caloneis bacillum</i>	2	0	0	1	1	2	2	2
<i>Navicula capitatoradiata</i>	1	1	1	2	0	1	0	2
<i>Gyrosigma acuminatum</i>	0	0	1	0	2	1	1	1
<i>Cymbella microcephala</i>	1	1	1	0	0	1	1	2
<i>Navicula minima</i>	0	1	1	2	0	0	1	1
<i>Diatoma vulgare</i>	1	0	0	0	1	0	3	2
<i>Surirella roba</i>	0	1	2	1	0	1	1	0
<i>Nitzschia amphibia</i>	0	1	0	1	0	0	0	1

**Digby Farm 30/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	77	78	71	69	69	68	63	69
<i>Navicula lanceolata</i>	52	52	50	45	46	45	43	41
<i>Planothidium lanceolatum</i>	40	41	40	38	36	32	31	32
<i>Navicula gregaria</i>	40	39	39	32	37	35	30	26
<i>Nitzschia sigmoidea</i>	28	24	27	24	21	19	20	24
<i>Amphora pediculus</i>	21	24	21	18	19	18	17	15
<i>Fragilaria vaucheriae</i>	18	21	15	13	9	11	7	16
<i>Cymbella helvetica</i>	15	19	13	15	9	12	5	5
<i>Planothidium frequentissimum</i>	16	19	16	15	8	7	4	4
<i>Gomphonema angustatum</i>	17	19	17	14	3	5	3	5
<i>Achnanthidium minutissimum</i>	11	15	10	9	7	6	0	5
<i>Caloneis bacillum</i>	12	15	9	11	6	4	4	4
<i>Gomphonema parvulum</i>	11	12	10	12	8	5	1	6
<i>Cocconeis placentula</i> var. <i>lineata</i>	14	12	9	5	0	3	4	6
<i>Amphora</i>	11	12	6	5	4	2	4	4
<i>Pseudostaurosira brevistriata</i>	13	10	11	8	3	2	1	5
<i>Rhoicosphenia abbreviata</i>	7	9	9	3	5	0	5	2
<i>Navicula saprophila</i>	11	13	7	2	2	1	1	4
<i>Fragilaria capucina</i>	8	9	6	5	0	4	2	1
<i>Nitzschia pusilla</i>	6	5	5	6	3	1	1	6
<i>Encyonema minutum</i>	6	5	6	5	1	4	0	5
<i>Fragilaria pinnata</i>	4	8	4	5	2	4	1	4
<i>Achnanthidium pyrenaicum</i>	6	5	5	0	0	5	1	5
<i>Staurosira elliptica</i>	12	7	2	2	0	1	1	2
<i>Gomphonema angustum/pumilum</i> type	4	2	3	4	0	4	1	2
<i>Gomphonema olivaceum</i>	4	3	2	5	1	5	1	0
<i>Cymbella microcephala</i>	4	3	3	1	1	5	1	1
<i>Nitzschia dissipata</i>	0	0	0	4	2	6	2	1
<i>Navicula veneta</i>	2	1	2	2	1	6	2	0
<i>Surirella roba</i>	1	1	2	1	2	5	0	1
<i>Nitzschia palea</i>	3	2	3	1	1	4	1	1
<i>Navicula minima</i>	0	1	0	1	2	3	1	1
<i>Navicula subminuscula</i>	2	1	1	2	0	4	1	1
<i>Navicula cryptotenella</i>	1	0	2	0	1	5	0	1
<i>Luticola mutica</i>	0	0	0	1	2	3	1	1
<i>Navicula tenelloides</i>	2	1	1	0	0	2	2	2
<i>Cymbella prostrata</i>	2	1	1	1	0	2	1	2
<i>Navicula tripunctata</i>	0	0	1	1	1		1	1
<i>Pinnularia</i>	0	1	1	0	0	1	0	0
<i>Gyrosigma acuminatum</i>	1	0	0	1	0	1	0	1

**Digby Farm 27/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	75	75	73	71	68	65	61	60
<i>Navicula lanceolata</i>	54	51	51	49	44	46	42	45
<i>Navicula gregaria</i>	38	23	39	35	32	32	30	35
<i>Planothidium lanceolatum</i>	39	29	33	31	29	31	20	24
<i>Amphora pediculus</i>	23	21	21	24	21	23	21	25
<i>Nitzschia sigmoidea</i>	26	29	24	21	17	24	21	26
<i>Gomphonema parvulum</i>	13	15	11	15	10	11	9	11
<i>Gomphonema angustatum</i>	15	14	11	11	9	14	7	4
<i>Staurosira elliptica</i>	10	11	9	12	7	12	2	5
<i>Achnantheidium minutissimum</i>	9	12	7	9	5	12	1	1
<i>Fragilaria vaucheriae</i>	6	8	4	6	1	2	0	6
<i>Rhoicosphenia abbreviata</i>	4	6	5	6	6	3	5	2
<i>Nitzschia palea</i>	5	6	15	8	1	1	2	3
<i>Cocconeis placentula</i> var. <i>lineata</i>	7	8	5	6	3	5	1	2
<i>Planothidium frequentissimum</i>	6	7	6	5	5	2	1	5
<i>Fragilaria pinnata</i>	6	9	4	6	3	5	1	2
<i>Pseudostaurosira brevistriata</i>	8	7	2	5	6	2	2	1
<i>Navicula tripunctata</i>	4	9	3	5	0	2	1	1
<i>Cymbella helvetica</i>	5	8	5	0	2	6	1	2
<i>Nitzschia dissipata</i>	4	4	4	2	2	6	1	4
<i>Achnantheidium pyrenaicum</i>	6	5	5	4	1	2	0	1
<i>Gomphonema olivaceum</i>	4	5	4	1	0	2	2	2
<i>Nitzschia pusilla</i>	2	6	2	1	1	1	2	4
<i>Navicula veneta</i>	1	3	2	1	1	3	1	2
<i>Gomphonema angustum/pumilum</i> type	2	5	2	2	1	3	0	2
<i>Navicula subminuscule</i>	4	6	1	1	0	1	2	2
<i>Surirella brebissonii</i>	3	3	2	0	0	1	1	5
<i>Cymbella microcephala</i>	2	5	2	1	1	2	1	0
<i>Luticola mutica</i>	2	2	2	2	1	2	1	1
<i>Fragilaria capucina</i>	2	0	0	1	1	2	2	2
<i>Pinnularia</i>	1	0	2	2	1	2	1	5
<i>Reimeria sinuata</i>	2	1	2	1	1	2	2	2
<i>Caloneis bacillum</i>	2	1	1	1	1	0	0	3
<i>Navicula cryptotenella</i>	1	2	2	1	1	2	1	1
<i>Nitzschia amphibia</i>	1	1	0	2	2	1	2	1
<i>Gyrosigma acuminatum</i>	1	1	1	0	1	2	1	1
<i>Navicula tenelloides</i>	1	0	1	1	0	1	2	2
<i>Navicula saprophila</i>	2	2	0	1	0	2	1	1
<i>Cymbella prostrata</i>	1	2	0	1	0	2	0	1
<i>Surirella roba</i>	1	1	0	1	1	2	0	2
<i>Encyonema minutum</i>	2	1	0	0	1	0	1	2
<i>Navicula minima</i>	1	2	0	1	1	2	1	0
<i>Navicula capitatoradiata</i>	1	1	1	0	0	0	0	1
<i>Diatoma vulgare</i>	1	2	0	0	0	0	1	1
<i>Amphora</i>	1	1	0	1	1	0	1	0

**Digby Farm 24/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	77	78	71	66	61	62	55	54
<i>Navicula lanceolata</i>	55	51	53	51	49	51	31	32
<i>Navicula gregaria</i>	48	51	42	41	29	32	31	29
<i>Planothidium lanceolatum</i>	40	42	38	32	31	29	29	28
<i>Amphora pediculus</i>	32	32	31	29	31	25	21	21
<i>Nitzschia sigmoidea</i>	35	38	31	25	26	24	20	19
<i>Gomphonema parvulum</i>	18	21	16	14	9	11	7	9
<i>Achnantheidium minutissimum</i>	12	15	9	8	8	9	6	11
<i>Amphora</i>	11	15	9	8	5	8	1	0
<i>Staurosira elliptica</i>	10	15	7	10	8	6	1	1
<i>Luticola mutica</i>	7	9	8	9	7	5	6	5
<i>Nitzschia palea</i>	7	9	7	12	1	6	3	2
<i>Navicula saprophila</i>	8	9	6	2	5	5	4	2
<i>Pseudostaurosira brevistriata</i>	11	10	8	3	1	6	3	2
<i>Gomphonema angustum/pumilum type</i>	6	9	6	3	4	0	5	6
<i>Rhoicosphenia abbreviata</i>	6	8	6	5	4	6	2	1
<i>Planothidium frequentissimum</i>	9	9	5	6	3	0	1	2
<i>Achnantheidium pyrenaicum</i>	9	8	0	3	7	5	5	2
<i>Surirella brebissonii</i>	6	5	5	5	3	6	1	1
<i>Gyrosigma acuminatum</i>	7	8	5	0	3	5	1	2
<i>Nitzschia dissipata</i>	5	8	3	2	1	3	2	1
<i>Cocconeis placentula</i> var. <i>lineata</i>	9	5	2	2	1	5	0	1
<i>Fragilaria pinnata</i>	5	8	5	1	3	2	0	0
<i>Diatoma vulgare</i>	5	3	5	1	2	2	0	1
<i>Cymbella microcephala</i>	2	5	1	6	0	5	1	3
<i>Navicula tripunctata</i>	9	5	1	1	0	1	1	0
<i>Cymbella prostrata</i>	4	4	3	3	0	1	0	1
<i>Gomphonema olivaceum</i>	5	3	3	2	2	1	0	2
<i>Reimeria sinuata</i>	2	4	1	8	0	2	1	1
<i>Navicula veneta</i>	2	5	1	3	2	2	2	2
<i>Navicula subminuscula</i>	4	3	3	2	0	1	3	0
<i>Caloneis bacillum</i>	2	4	2	3	0	4	1	2
<i>Nitzschia pusilla</i>	2	0	0	5	1	5	1	0
<i>Surirella roba</i>	0	1	0	5	2	4	1	2
<i>Navicula cryptotenella</i>	1	0	1	4	1	1	0	1
<i>Navicula tenelloides</i>	1	1	0	0	1	1	1	0
<i>Navicula capitatoradiata</i>	0	1	0	0	2	0	0	1
<i>Nitzschia amphibia</i>	1	0	0	1	1	1	1	1
<i>Navicula minima</i>	0	0	0	1	1	1	1	0
<i>Pinnularia</i>	0	0	1	1	0	0	0	0

**Lubenham 21/05/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	93	94	92	93	80	83	77	78
<i>Cymbella cistula</i>	88	87	88	87	75	73	71	69
<i>Gomphonema olivaceum</i>	54	55	55	55	45	44	46	41
<i>Gyrosigma attenuatum</i>	51	50	50	49	44	43	41	39
<i>Planothidium lanceolatum</i>	41	43	42	42	36	36	37	35
<i>Navicula cryptotenella</i>	32	30	31	33	26	29	21	20
<i>Navicula gregaria</i>	28	26	28	19	23	22	19	20
<i>Amphora pediculus</i>	21	22	15	20	15	12	14	15
<i>Cocconeis pediculus</i>	19	19	19	20	16	12	11	9
<i>Cyclotella meneghiniana</i>	15	14	9	12	11	8	5	4
<i>Nitzschia dissipata</i>	12	11	13	12	9	7	8	7
<i>Diatoma vulgare</i>	11	11	10	10	10	10	11	8
<i>Melosira varians</i>	12	11	10	9	7	8	9	7
<i>Cocconeis placentula</i>	11	11	11	9	8	8	5	3
<i>Reimeria sinuata</i>	9	11	8	9	9	8	6	2
<i>Planothidium frequentissimum</i>	6	4	7	8	5	6	1	0
<i>Nitzschia palea</i>	6	6	7	6	2	0	0	1
<i>Surirella brebissonii</i>	5	6	6	5	2	0	3	1
<i>Navicula cincta</i>	7	5	8	7	2	1	3	0
<i>Nitzschia</i>	3	1	4	6	2	1	2	0
<i>Tryblionella apiculata</i>	6	5	5	4	2	0	1	0
<i>Navicula capitatoradiata</i>	8	5	0	2	3	1	1	1
<i>Navicula subminuscula</i>	6	5	4	3	1	2	0	1
<i>Amphora</i>	3	2	3	1	0	1	2	1
<i>Achnanthes</i>	5	5	0	1	3	0	1	1
<i>Psammothidium lauenburgianum</i>	2	2	3	2	1	2	2	1
<i>Navicula tripunctata</i>	3	3	3	2	0	1	0	1
<i>Navicula minima</i>	1	2	3	3	0	1	2	1
<i>Navicula ignota</i>	1	1	1	1	1	1	1	0
<i>Pseudostaurosira brevistriata</i>	1	1	1	1	0	1	1	3
<i>Gomphonema parvulum</i>	2	1	0	0	2	0	1	1
<i>Placoneis elginensis</i>	2	1	2	0	0	0	0	1
<i>Craticula cuspidata</i>	1	2	2	0	1	0	0	0
<i>Nitzschia inconspicua</i>	1	1	0	1	2	1	1	0
<i>Cymatopleura solea</i>	1	1	0	1	2	0	1	2
<i>Meridion circulare</i>	1	1	1	1	0	1	0	0

**Lubenham 18/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	97	100	99	95	91	88	76	78
<i>Cymbella cistula</i>	88	85	80	83	79	80	77	75
<i>Gomphonema olivaceum</i>	55	53	51	52	51	55	52	55
<i>Gyrosigma attenuatum</i>	52	52	49	51	24	24	25	51
<i>planothidium lanceolatum</i>	44	46	43	35	22	21	42	24
<i>Navicula cryptotenella</i>	33	35	26	28	24	21	21	21
<i>Navicula gregaria</i>	23	25	25	26	22	20	20	19
<i>Amphora pediculus</i>	22	26	21	20	7	14	15	15
<i>Cocconeis pediculus</i>	18	19	12	12	13	15	11	12
<i>Cyclotella meneghiniana</i>	15	18	5	12	13	9	12	10
<i>Nitzschia dissipata</i>	13	15	11	10	10	9	2	6
<i>Melosira varians</i>	11	11	10	10	5	8	3	2
<i>Diatoma vulgare</i>	10	12	8	9	6	8	2	0
<i>Tryblionella apiculata</i>	5	7	5	9	4	7	3	0
<i>Nitzschia palea</i>	6	7	6	2	8	5	8	1
<i>Cocconeis placentula</i>	11	5	2	2	4	4	3	4
<i>Navicula tripunctata</i>	3	3	4	2	6	2	7	2
<i>Navicula capitatoradiata</i>	7	4	6	6	2	0	0	2
<i>Navicula subminuscula</i>	6	5	0	2	9	5	0	2
<i>Surirella brebissonii</i>	4	4	3	1	4	1	4	6
<i>Achnanthes</i>	4	5	3	1	5	2	1	3
<i>Diatoma mesodon</i>	0	2	0	2	2	0	1	2
<i>Nitzschia</i>	3	0	2	2	0	6	3	3
<i>Amphora</i>	2	3	6	1	3	1	3	3
<i>Reimeria sinuata</i>	9	2	0	0	0	1	1	0
<i>Cyclotella</i>	1	1	3	1	0	2	4	4
<i>Placoneis elginensis</i>	3	2	0	0	2	2	1	2
<i>Nitzschia inconspicua</i>	0	1	1	0	10	0	2	0
<i>Craticula cuspidata</i>	1	0	1	1	2	2	2	2
<i>Navicula ignota</i>	2	0	1	0	2	3	1	1
<i>Meridion circulare</i>	1	2	2	0	1	1	1	3
<i>Gomphonema parvulum</i>	2	1	2	2	1	0	1	2
<i>Encyonema silesiacum</i>	1	1	2	1	1	1	2	2
<i>Navicula minima</i>	2	0	0	2	2	2	0	1
<i>Caloneis bacillum</i>	0	2	3	1	2	0	1	1
<i>Navicula veneta</i>	1	0	1	3	2	1	1	0
<i>Gomphonema</i>	0	0	2	1	1	1	2	0
<i>Pseudostaurosira brevistriata</i>	1	2	2	1	0	1	1	1
<i>Gyrosigma acuminatum</i>	0	1	1	1	1	1	1	1
<i>Cymatopleura solea</i>	0	1	1	1	1	1	1	1

**Lubenham 16/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnanthydium minutissimum</i>	97	96	99	95	90	88	91	88
<i>Cymbella cistula</i>	95	94	60	89	81	80	79	77
<i>Gyrosigma attenuatum</i>	53	56	50	49	42	41	43	40
<i>Gomphonema olivaceum</i>	57	54	74	56	12	41	40	35
<i>planothidium lanceolatum</i>	47	47	44	45	42	29	36	32
<i>Navicula cryptotenella</i>	31	35	12	28	41	39	23	22
<i>Amphora pediculus</i>	26	23	24	18	21	12	13	15
<i>Navicula gregaria</i>	25	24	20	21	15	15	12	11
<i>Cocconeis pediculus</i>	21	22	17	18	11	9	2	0
<i>Cyclotella meneghiniana</i>	12	14	5	9	9	9	5	9
<i>Nitzschia dissipata</i>	9	10	11	8	5	7	0	2
<i>Placoneis elginensis</i>	2	4	2	2	4	5	25	16
<i>Cocconeis placentula</i>	10	10	8	8	5	7	2	2
<i>Reimeria sinuata</i>	8	9	0	2	4	5	10	8
<i>Navicula capitatoradiata</i>	6	1	5	6	5	5	2	5
<i>Achnanthes</i>	4	4	4	5	2	1	3	6
<i>Melosira varians</i>	8	2	0	0	5	5	2	2
<i>Planothidium frequentissimum</i>	6	7	0	5	6	2	0	1
<i>Nitzschia</i>	4	2	4	2	3	2	5	6
<i>Tryblionella apiculata</i>	7	5	2	1	2	1	2	2
<i>Encyonema silesiacum</i>	1	1	7	3	3	4	1	2
<i>Diatoma vulgare</i>	7	4	5	2	2	1	0	1
<i>Diatoma mesodon</i>	1	1	0	3	12	4	2	1
<i>Navicula cincta</i>	7	1	5	1	2	1	1	1
<i>Surirella brebissonii</i>	5	2	4	3	2	0	1	2
<i>Craticula cuspidata</i>	0	1	1	12	0	1	2	1
<i>Navicula tripunctata</i>	4	1	0	2	2	0	0	1
<i>Sellaphora seminulum</i>	0	0	1	2	1	1	1	0
<i>Navicula veneta</i>	0	1	1	2	2	1	2	2
<i>Gomphonema</i>	1	0	2	0	3	1	1	0
<i>Amphora</i>	3	0	2	0	1	2	1	1
<i>Cyclotella</i>	0	1	1	1	2	1	2	1
<i>Cymatopleura solea</i>	0	1	1	2	2	1	1	2
<i>Caloneis bacillum</i>	1	1	0	2	1	0	2	1
<i>Pseudostaurosira brevistriata</i>	0	1	1	1	2	1	1	2
<i>Navicula minima</i>	2	1	2	1	1	0	0	1
<i>Nitzschia inconspicua</i>	1	1	0	1	1	1	1	0
<i>Psammothidium lauenburgianum</i>	1	1	0	1	0	0	1	0
<i>Navicula ignota</i>	1	1	0	1	0	0	1	1
<i>Meridion circulare</i>	0	1	0	1	1	0	1	1

**Lubenham 13/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	112	109	107	108	90	87	84	85
<i>Cymbella cistula</i>	98	95	98	95	90	88	86	85
<i>Gomphonema olivaceum</i>	55	51	55	49	52	51	49	47
<i>Gyrosigma attenuatum</i>	50	49	49	45	41	39	45	44
<i>planothidium lanceolatum</i>	44	45	40	45	39	35	38	44
<i>Navicula cryptotenella</i>	36	39	29	27	31	21	26	25
<i>Navicula gregaria</i>	30	25	29	25	25	24	21	24
<i>Amphora pediculus</i>	27	28	25	24	19	14	15	18
<i>Cyclotella meneghiniana</i>	17	21	7	21	11	10	9	11
<i>Cocconeis placentula</i>	10	11	9	10	15	14	1	9
<i>Diatoma vulgare</i>	7	9	5	5	1	0	2	0
<i>Reimeria sinuata</i>	9	8	5	4	4	5	2	6
<i>Achnanthes</i>	4	5	2	4	1	6	1	2
<i>Nitzschia dissipata</i>	10	6	1	5	2	5	0	4
<i>Melosira varians</i>	6	2	5	0	4	2	4	3
<i>Tryblionella apiculata</i>	7	5	5	6	3	2	0	2
<i>Surirella brebissonii</i>	5	5	6	4	2	1	2	1
<i>Navicula minima</i>	1	2	11	2	0	1	4	2
<i>Navicula subminuscula</i>	6	9	2	2	2	0	1	1
<i>Meridion circulare</i>	1	0	5	2	2	0	2	1
<i>Nitzschia</i>	3	2	5	4	2	4	0	1
<i>Cyclotella</i>	0	2	5	2	2	1	1	2
<i>Nitzschia palea</i>	5	0	4	0	1	2	0	0
<i>Psammothidium lauenburgianum</i>	1	1	0	1	7	0	2	0
<i>Craticula cuspidata</i>	2	1	5	2	2	0	0	1
<i>Navicula veneta</i>	1	1	4	2	2	1	0	2
<i>Nitzschia inconspicua</i>	0	0	5	2	0	5	1	1
<i>Navicula cincta</i>	5	1	2	1	1	2	2	1
<i>Planothidium frequentissimum</i>	4	1	2	5	1	1	1	1
<i>Gomphonema parvulum</i>	3	1	2	2	0	2	1	1
<i>Placoneis elginensis</i>	2	1	0	2	2	1	1	1
<i>Amphora</i>	4	1	0	1	2	1	0	2
<i>Gomphonema</i>	0	1	2	0	0	2	1	0
<i>Pseudostaurosira brevistriata</i>	0	1	2	1	1	1	0	0
<i>Sellaphora seminulum</i>	0	1	0	0	2	2	1	0
<i>Gyrosigma acuminatum</i>	1	1	0	0	0	1	2	1



**Lubenham 11/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Achnantheidium minutissimum</i>	114	112	112	110	100	98	99	95
<i>Cymbella cistula</i>	90	88	78	75	80	82	76	81
<i>Gomphonema olivaceum</i>	53	55	55	54	49	45	42	41
<i>Gyrosigma attenuatum</i>	49	47	47	41	40	41	39	35
<i>planothidium lanceolatum</i>	42	44	17	21	39	35	29	31
<i>Navicula cryptotenella</i>	30	31	25	21	26	31	21	21
<i>Navicula gregaria</i>	29	27	27	25	22	26	20	21
<i>Amphora pediculus</i>	25	21	24	24	18	21	11	14
<i>Cocconeis pediculus</i>	24	21	11	10	18	20	15	13
<i>Nitzschia dissipata</i>	11	10	13	10	10	11	9	11
<i>Cyclotella meneghiniana</i>	13	13	11	10	9	11	5	7
<i>Diatoma vulgare</i>	9	7	15	12	20	9	0	2
<i>Tryblionella apiculata</i>	6	5	6	9	2	9	7	8
<i>Cocconeis placentula</i>	10	11	0	2	2	4	1	2
<i>Melosira varians</i>	9	5	7	5	0	2	2	2
<i>Reimeria sinuata</i>	8	5	5	4	4	5	1	3
<i>Surirella brebissonii</i>	5	4	4	2	0	2	9	5
<i>Achnanthes</i>	4	5	9	1	2	2	2	2
<i>Nitzschia</i>	4	5	3	5	1	2	10	2
<i>Navicula cincta</i>	6	2	6	2	6	0	0	2
<i>Navicula capitatoradiata</i>	6	0	1	0	5	0	3	2
<i>Amphora</i>	4	1	5	6	0	2	2	1
<i>Navicula subminuscula</i>	5	7	5	6	0	2	0	1
<i>Placoneis elginensis</i>	3	2	4	5	2	3	2	1
<i>Gomphonema parvulum</i>	3	4	3	5	2	2	2	2
<i>Navicula tripunctata</i>	4	2	4	2	0	1	2	1
<i>Navicula minima</i>	3	2	2	1	0	1	2	2
<i>Encyonema silesiacum</i>	1	2	2	2	2	1	0	5
<i>Meridion circulare</i>	0	2	2	1	1	1	2	1
<i>Cyclotella</i>	1	2	2	2	0	2	1	1
<i>Nitzschia inconspicua</i>	1	2	2	1	1	2	0	6
<i>Cymatopleura solea</i>	1	2	2	0	2	2	0	5
<i>Gomphonema</i>	0	1	2	0	1	1	1	1
<i>Navicula veneta</i>	1	1	1	1	0	2	2	1
<i>Caloneis bacillum</i>	0	0	0	1	1	1	1	1
<i>Craticula cuspidata</i>	1	1	0	1	0	2	1	2

**Papillion Ford 21/05/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma acuminatum</i>	93	95	91	88	88	87	84	88
<i>Stenopterobia sigmatella</i>	71	70	69	70	65	66	61	59
<i>Sellaphora bacillum</i>	63	65	62	65	77	69	58	54
<i>Cocconeis pediculus</i>	59	52	58	58	52	50	51	54
<i>Navicula gregaria</i>	58	55	35	41	54	51	55	49
<i>Melosira varians</i>	46	47	47	41	44	42	42	42
<i>Amphora pediculus</i>	45	45	41	44	40	41	39	41
<i>Rhoicosphenia abbreviata</i>	45	44	43	42	39	28	32	33
<i>Navicula cryptotenella</i>	13	21	15	19	12	15	11	15
<i>Achnantheidium minutissimum</i>	5	7	6	8	21	16	1	6
<i>Nitzschia linearis</i>	9	8	7	9	4	5	13	9
<i>Navicula lanceolata</i>	9	7	9	8	5	6	5	4
<i>Surirella brebissonii</i>	7	8	5	7	8	8	2	5
<i>Nitzschia dissipata</i>	7	8	8	9	2	5	2	4
<i>Navicula minima</i>	7	8	6	9	2	4	4	1
<i>Planothidium frequentissimum</i>	7	7	5	8	1	5	1	1
<i>Cocconeis placentula</i>	5	9	6	7	5	4	1	0
<i>Navicula tripunctata</i>	3	5	4	7	2	5	5	0
<i>Navicula capitata</i>	9	4	4	2	2	4	0	1
<i>Cymatopleura elliptica</i>	0	2	2	12	1	1	2	0
<i>Synedrella parasitica</i>	4	4	2	2	2	2	2	1
<i>Amphora libyca</i>	4	5	3	5	3	1	1	1
<i>Nitzschia sociabilis</i>	3	5	3	1	1	0	1	5
<i>Gomphonema parvulum</i>	1	4	5	2	2	1	3	2
<i>Gyrosigma attenuatum</i>	3	4	2	1	2	0	1	2
<i>Nitzschia amphibia</i>	1	5	3	4	2	1	1	1
<i>Caloneis silicula</i>	3	0	2	2	2	1	3	3
<i>Encyonema minutum</i>	3	1	3	1	2	0	0	3
<i>Cymatopleura solea</i>	3	4	2	1	2	1	1	2
<i>Amphora inariensis</i>	4	0	0	0	2	1	5	3
<i>Diatoma vulgare</i>	1	2	1	4	2	4	0	1
<i>Nitzschia recta</i>	1	2	2	2	2	5	1	0
<i>Gomphonema angustatum</i>	0	0	0	1	1	2	2	2
<i>Navicula laterostrata</i>	0	1	0	2	2	1	1	1
<i>Cyclotella meneghiniana</i>	1	1	0	1	1	2	2	1
<i>Caloneis amphisbaena</i>	1	0	0	1	0	1	2	2
<i>Planothidium lanceolatum</i>	0	1	0	1	0	1	1	2

**Papillion Ford 18/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma acuminatum</i>	92	99	93	92	90	90	88	87
<i>Stenopterobia sigmatella</i>	75	77	72	71	71	70	74	71
<i>Sellaphora bacillum</i>	65	62	66	65	60	59	60	61
<i>Cocconeis pediculus</i>	59	55	55	54	54	52	57	52
<i>Navicula gregaria</i>	57	59	55	52	42	41	33	32
<i>Melosira varians</i>	51	50	25	32	43	41	41	40
<i>Amphora pediculus</i>	46	45	40	35	25	21	32	31
<i>Rhoicosphenia abbreviata</i>	42	41	42	38	38	29	35	31
<i>Navicula cryptotenella</i>	14	24	16	14	11	10	18	16
<i>Cyclotella</i>	12	14	10	11	9	8	5	4
<i>Nitzschia linearis</i>	9	10	8	9	6	8	14	12
<i>Nitzschia levidensis</i>	7	8	5	6	17	8	1	2
<i>Navicula minima</i>	8	5	11	12	4	6	1	0
<i>Planothidium frequentissimum</i>	5	4	5	5	2	3	1	4
<i>Encyonema minutum</i>	3	6	5	6	2	3	3	0
<i>Navicula reichardtiana</i>	3	2	3	5	2	0	4	0
<i>Amphora inariensis</i>	5	5	4	6	2	4	1	0
<i>Nitzschia dissipata</i>	7	6	0	4	4	0	2	1
<i>Cocconeis placentula</i>	6	8	6	5	1	1	0	2
<i>Surirella brebissonii</i>	6	6	3	5	2	1	1	2
<i>Gyrosigma attenuatum</i>	3	7	2	5	2	0	1	3
<i>Synedrella parasitica</i>	3	2	3	5	0	1	0	2
<i>Gomphonema parvulum</i>	1	4	5	6	0	1	0	1
<i>Achnantheidium minutissimum</i>	5	6	0	1	2	0	1	1
<i>Cymatopleura solea</i>	2	2	2	0	2	1	1	1
<i>Navicula capitata</i>	3	4	3	1	1	0	0	1
<i>Nitzschia sociabilis</i>	2	1	2	0	1	1	2	2
<i>Nitzschia recta</i>	1	0	1	2	1	1	3	2
<i>Nitzschia amphibia</i>	0	2	2	1	1	1	1	2
<i>Caloneis silicula</i>	2	1	0	1	1	3	0	2
<i>Navicula laterostrata</i>	1	0	0	2	2	1	2	1
<i>Navicula tripunctata</i>	2	1	0	1	1	1	2	2
<i>Gomphonema angustatum</i>	2	1	0	2	1	2	1	1
<i>Planothidium lanceolatum</i>	0	1	0	1	0	0	1	0
<i>Meridion circulare</i>	1	1	0	1	0	1	0	0

**Papillion Ford 16/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma acuminatum</i>	91	92	92	91	91	92	90	88
<i>Stenopterobia sigmatella</i>	74	73	71	72	51	52	14	22
<i>Navicula gregaria</i>	55	51	58	55	55	48	51	41
<i>Cocconeis pediculus</i>	64	55	65	62	45	44	31	32
<i>Amphora pediculus</i>	53	51	55	51	42	39	43	49
<i>Rhoicosphenia abbreviata</i>	44	46	41	40	42	41	43	33
<i>Melosira varians</i>	44	42	46	42	41	39	24	35
<i>Sellaphora bacillum</i>	58	49	12	10	31	27	30	22
<i>Surirella brebissonii</i>	9	10	11	11	7	9	12	10
<i>Cyclotella</i>	16	15	11	10	5	9	4	3
<i>Nitzschia linearis</i>	10	11	8	9	5	3	4	2
<i>Navicula cryptotenella</i>	11	11	9	5	2	2	5	2
<i>Navicula lanceolata</i>	9	8	9	9	5	2	4	0
<i>Navicula tripunctata</i>	5	6	4	3	15	11	9	2
<i>Cocconeis placentula</i>	9	5	7	2	5	6	6	1
<i>Navicula minima</i>	6	6	5	5	5	6	1	0
<i>Gomphonema parvulum</i>	0	2	2	2	5	11	3	2
<i>Gomphonema angustatum</i>	0	2	1	2	10	9	5	0
<i>Nitzschia levidensis</i>	4	5	4	0	5	4	1	5
<i>Caloneis silicula</i>	5	4	4	2	2	5	5	4
<i>Nitzschia dissipata</i>	6	4	4	2	2	1	5	4
<i>Gyrosigma attenuatum</i>	6	2	2	0	3	6	4	1
<i>Planothidium frequentissimum</i>	5	4	0	2	4	2	5	2
<i>Amphora inariensis</i>	3	2	3	2	2	3	4	1
<i>Synedrella parasitica</i>	2	3	1	3	2	2	2	2
<i>Caloneis amphisbaena</i>	2	0	2	3	1	4	0	2
<i>Cymatopleura solea</i>	2	3	0	2	2	1	3	1
<i>Cymatopleura elliptica</i>	2	2	0	1	2	4	0	1
<i>Diatoma vulgare</i>	1	2	0	1	1	4	2	1
<i>Encyonema minutum</i>	1	1	0	2	2	1	1	2
<i>Cyclotella meneghiniana</i>	1	1	0	0	1	4	2	2
<i>Navicula laterostrata</i>	1	2	1	1	0	0	0	2
<i>Meridion circulare</i>	1	0	0	2	2	0	0	2
<i>Nitzschia amphibia</i>	0	0	0	2	0	0	2	1

**Papillion Ford 13/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma acuminatum</i>	97	91	89	99	92	91	96	91
<i>Stenopterobia sigmatella</i>	73	71	70	71	66	68	65	61
<i>Sellaphora bacillum</i>	69	66	65	62	62	39	61	59
<i>Cocconeis pediculus</i>	63	66	60	59	65	59	62	58
<i>Navicula gregaria</i>	61	59	62	54	62	61	60	62
<i>Amphora pediculus</i>	48	45	44	46	41	51	45	48
<i>Melosira varians</i>	49	48	42	41	35	29	32	32
<i>Rhoicosphenia abbreviata</i>	47	49	45	42	19	24	33	29
<i>Navicula cryptotenella</i>	17	20	15	16	14	15	18	16
<i>Cyclotella</i>	15	15	14	18	12	16	14	18
<i>Nitzschia linearis</i>	13	17	11	10	15	12	16	19
<i>Navicula lanceolata</i>	11	10	0	6	18	11	5	11
<i>Navicula minima</i>	11	12	9	7	7	9	1	8
<i>Nitzschia dissipata</i>	6	5	8	6	5	8	4	6
<i>Planothidium frequentissimum</i>	7	9	6	5	6	9	5	5
<i>Surirella brebissonii</i>	7	5	6	5	5	8	4	5
<i>Amphora libyca</i>	5	5	4	8	4	9	6	4
<i>Navicula reichardtiana</i>	3	1	2	5	10	12	7	4
<i>Synedrella parasitica</i>	3	1	6	6	5	4	2	4
<i>Nitzschia sociabilis</i>	2	2	5	5	5	2	0	5
<i>Cymatopleura solea</i>	2	1	0	7	6	1	2	4
<i>Navicula capitata</i>	3	2	2	4	0	2	1	1
<i>Navicula tripunctata</i>	2	1	1	4	1	1	1	1
<i>Nitzschia recta</i>	1	0	1	5	1	2	2	0
<i>Caloneis amphisbaena</i>	0	0	3	1	2	1	0	6
<i>Gyrosigma attenuatum</i>	2	1	0	0	1	0	1	6
<i>Cyclotella meneghiniana</i>	1	1	0	1	6	0	1	1
<i>Encyonema minutum</i>	2	0	1	1	1	2	1	1
<i>Cymatopleura elliptica</i>	1	1	1	1	0	0	1	2
<i>Caloneis silicula</i>	2	0	0	1	1	1	2	0
<i>Diatoma vulgare</i>	0	1	1	0	2	1	0	1
<i>Planothidium lanceolatum</i>	1	1	0	2	0	1	0	0
<i>Gomphonema angustatum</i>	1	1	1	1	0	2	0	1
<i>Navicula laterostrata</i>	1	1	0	1	1	2	0	1
<i>Nitzschia amphibia</i>	1	1	0	2	1	0	0	1
<i>Meridion circulare</i>	1	0	0	0	1	1	0	0
<i>Gomphonema parvulum</i>	0	1	2	1	0	0	0	0

**Papillion Ford 11/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Gyrosigma acuminatum</i>	95	99	94	95	78	75	84	85
<i>Stenopterobia sigmatella</i>	73	71	70	69	69	66	62	62
<i>Cocconeis pediculus</i>	61	58	60	59	54	52	51	55
<i>Sellaphora bacillum</i>	61	29	60	59	55	58	54	52
<i>Navicula gregaria</i>	57	58	52	51	46	45	42	41
<i>Melosira varians</i>	56	51	54	51	49	41	35	35
<i>Amphora pediculus</i>	46	52	44	45	38	32	32	36
<i>Rhoicosphenia abbreviata</i>	47	41	44	41	35	32	31	32
<i>Navicula cryptotenella</i>	19	45	15	24	12	10	12	25
<i>Cyclotella</i>	11	7	7	5	5	9	11	10
<i>Nitzschia linearis</i>	12	11	8	9	5	4	4	6
<i>Navicula lanceolata</i>	9	2	13	2	9	6	2	2
<i>Nitzschia dissipata</i>	9	10	7	5	5	4	3	3
<i>Planothidium frequentissimum</i>	8	9	5	9	2	5	4	2
<i>Surirella brebissonii</i>	7	9	5	9	2	3	1	1
<i>Achnantheidium minutissimum</i>	5	8	5	5	5	2	1	1
<i>Nitzschia levidensis</i>	6	5	5	7	0	1	1	1
<i>Cocconeis placentula</i>	7	5	3	7	2	1	0	1
<i>Synedrella parasitica</i>	5	1	4	8	3	1	0	1
<i>Amphora inariensis</i>	4	2	3	2	2	2	1	1
<i>Navicula tripunctata</i>	4	0	2	4	0	1	1	1
<i>Caloneis silicula</i>	3	1	2	2	0	1	1	0
<i>Amphora libyca</i>	4	0	0	2	2	2	2	0
<i>Gomphonema angustatum</i>	1	4	2	0	1	2	0	0
<i>Nitzschia amphibia</i>	1	3	1	1	0	1	1	2
<i>Nitzschia sociabilis</i>	2	5	1	0	0	0	1	0
<i>Caloneis amphisbaena</i>	2	1	0	1	1	1	0	2
<i>Nitzschia recta</i>	1	3	0	0	0	2	1	1
<i>Cymatopleura elliptica</i>	1	1	0	0	2	0	1	2
<i>Diatoma vulgare</i>	1	2	1	1	0	1	2	1
<i>Navicula reichardtiana</i>	2	2	0	1	0	1	1	1
<i>Meridion circulare</i>	1	2	0	1	0	2	0	2
<i>Cymatopleura solea</i>	0	1	0	1	2	0	1	0
<i>Navicula laterostrata</i>	0	2	0	1	0	1	2	1
<i>Gomphonema parvulum</i>	0	2	1	0	1	0	0	1

**Hothorpe 21/05/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Navicula radiosa</i>	138	133	127	125	120	118	119	117
<i>Nitzschia sigmoidea</i>	100	99	101	100	99	90	82	85
<i>Rhoicosphenia abbreviata</i>	71	70	72	68	70	68	65	65
<i>Cocconeis placentula</i>	63	60	60	56	59	55	50	51
<i>Fallacia subhamulata</i>	55	54	34	44	46	45	48	51
<i>Fallacia helensis</i>	29	24	29	31	32	32	30	36
<i>Amphora libyca</i>	23	22	23	25	20	21	21	20
<i>Cocconeis pediculus</i>	24	25	22	21	24	21	20	18
<i>Navicula minima</i>	19	16	17	14	15	14	12	13
<i>Caloneis silicula</i>	19	15	12	11	15	14	10	11
<i>Rhoicosphenia abbreviata</i>	14	13	13	11	15	13	11	11
<i>Achnanthes conspicua</i>	15	14	11	10	7	7	10	11
<i>Reimeria uniseriata</i>	10	11	11	10	9	8	7	11
<i>Navicula cryptotenella</i>	13	11	11	9	0	5	10	12
<i>Navicula tripunctata</i>	10	9	9	8	2	5	7	6
<i>Navicula subrotundata</i>	1	3	9	8	3	2	2	6
<i>Navicula lanceolata</i>	1	4	5	3	7	1	2	5
<i>Thalassiosira weissflogii</i>	9	7	4	3	0	4	1	0
<i>Achnantheidium</i>	5	4	4	3	2	4	1	0
<i>Amphora ovalis</i>	5	0	3	0	0	1	0	1
<i>Staurosira construens</i>	0	1	0	1	1	1	3	1
<i>Fragilaria vaucheriae</i>	3	1	2	1	0	1	0	2
<i>Reimeria sinuata</i>	0	1	2	1	1	0	2	1
<i>Nitzschia inconspicua</i>	1	2	0	1	5	0	0	1
<i>Navicula cincta</i>	2	0	2	1	1	0	1	0
<i>Achnanthes conspicua</i>	5	1	0	0	1	0	1	2
<i>Diploneis</i>	3	2	0	0	2	1	0	1
<i>Planorhynchium lanceolatum</i>	1	2	0	1	0	0	1	2
<i>Surirella brebissonii</i>	0	1	1	1	2	1	0	0
<i>Gomphonema pumilum</i>	0	0	1	0	0	1	1	0

**Hothorpe 18/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Navicula radiosa</i>	120	117	118	115	115	110	110	105
<i>Nitzschia sigmoidea</i>	118	115	107	101	102	99	100	89
<i>Rhoicosphenia abbreviata</i>	78	75	77	81	65	65	65	55
<i>Cocconeis placentula</i>	69	95	68	62	62	60	62	59
<i>Fallacia subhamulata</i>	59	58	55	52	52	51	49	48
<i>Fallacia helensis</i>	33	32	35	32	31	33	31	28
<i>Amphora libyca</i>	22	26	19	20	7	10	14	16
<i>Caloneis silicula</i>	17	18	17	15	12	10	11	15
<i>Navicula minima</i>	7	12	18	14	10	8	11	14
<i>Navicula cryptotenella</i>	12	14	14	13	10	8	9	11
<i>Cocconeis pediculus</i>	19	16	18	15	0	3	0	3
<i>Rhoicosphenia abbreviata</i>	15	15	7	9	11	8	5	8
<i>Navicula tripunctata</i>	14	16	10	11	7	6	5	8
<i>Achnanthes conspicua</i>	13	11	9	5	7	6	5	4
<i>Reimeria uniseriata</i>	11	10	6	5	5	6	0	1
<i>Melosira varians</i>	10	9	5	6	6	6	1	1
<i>Achnanthes conspicua</i>	7	8	6	5	0	2	5	1
<i>Thalassiosira weissflogii</i>	9	5	0	2	5	2	1	2
<i>Diploneis</i>	4	6	3	5	0	2	4	2
<i>Amphora ovalis</i>	5	8	5	4	2	1	0	2
<i>Navicula lanceolata</i>	1	0	7	6	5	1	0	2
<i>Navicula acceptata</i>	3	6	0	2	3	2	2	1
<i>Fragilaria vaucheriae</i>	3	0	4	6	0	1	2	2
<i>Achnanthidium</i>	6	1	0	2	1	2	1	1
<i>Planothidium frequentissimum</i>	0	3	3	1	1	0	0	1
<i>Reimeria sinuata</i>	1	2	1	2	1	1	1	0
<i>Nitzschia inconspicua</i>	1	2	2	1	1	0	0	1
<i>Staurosira construens</i>	1	0	1	1	1	0	1	1
<i>Navicula subrotundata</i>	1	0	1	0	1	2	1	0



**Hothorpe 16/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Navicula radiosa</i>	126	125	123	111	117	115	100	102
<i>Nitzschia sigmoidea</i>	105	100	85	95	89	88	88	89
<i>Rhoicosphenia abbreviata</i>	71	74	66	68	55	54	52	57
<i>Cocconeis placentula</i>	67	65	66	58	25	35	44	54
<i>Fallacia subhamulata</i>	49	49	19	20	33	31	30	41
<i>Cocconeis pediculus</i>	29	35	25	27	29	30	21	19
<i>Fallacia helensis</i>	31	32	30	28	25	31	22	18
<i>Amphora libyca</i>	26	25	7	18	21	25	19	15
<i>Navicula minima</i>	22	21	19	15	18	18	14	18
<i>Caloneis silicula</i>	21	20	15	16	8	11	4	9
<i>Achnanthes conspicua</i>	14	18	15	9	7	9	9	5
<i>Reimeria uniseriata</i>	14	14	11	10	10	11	9	5
<i>Navicula cryptotenella</i>	13	15	11	8	8	7	5	8
<i>Achnanthidium minutissimum</i>	18	19	7	9	7	5	2	3
<i>Navicula tripunctata</i>	12	11	10	10	6	5	5	4
<i>Melosira varians</i>	13	15	8	9	6	5	2	2
<i>Thalassiosira weissflogii</i>	9	11	2	5	6	5	2	2
<i>Fragilaria vaucheriae</i>	3	5	17	12	0	2	2	1
<i>Achnanthes conspicua</i>	7	9	0	2	2	2	1	1
<i>Navicula subrotundata</i>	4	6	3	0	2	5	2	1
<i>Achnanthidium</i>	4	5	1	2	7	1	3	0
<i>Amphora ovalis</i>	5	5	0	1	2	5	1	0
<i>Navicula lanceolata</i>	0	1	2	1	1	1	6	5
<i>Nitzschia inconspicua</i>	3	2	3	5	1	0	1	1
<i>Planothidium frequentissimum</i>	3	1	2	1	1	1	1	2
<i>Navicula cincta</i>	0	1	1	0	0	1	1	6
<i>Surirella brebissonii</i>	1	2	1	2	0	1	1	0
<i>Gomphonema pumilum</i>	1	1	2	1	0	1	0	1
<i>Reimeria sinuata</i>	0	0	1	1	0	2	0	1
<i>Planothidium lanceolatum</i>	0	1	0	0	1	0	0	2

**Hothorpe 13/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Navicula radiosa</i>	136	133	127	122	111	109	105	111
<i>Nitzschia sigmoidea</i>	116	118	126	128	101	98	102	99
<i>Rhoicosphenia abbreviata</i>	76	66	57	56	52	49	50	51
<i>Fallacia subhamulata</i>	61	62	55	55	59	51	42	53
<i>Cocconeis placentula</i>	68	29	18	44	60	55	60	51
<i>Fallacia helensis</i>	35	41	29	33	22	21	21	24
<i>Amphora libyca</i>	25	32	27	31	19	18	12	21
<i>Cocconeis pediculus</i>	28	25	18	20	20	19	15	21
<i>Caloneis silicula</i>	21	26	15	17	11	10	6	11
<i>Achnantheidium minutissimum</i>	16	14	0	9	12	10	12	15
<i>Reimeria uniseriata</i>	9	11	8	16	5	2	6	3
<i>Navicula cryptotenella</i>	15	12	15	12	0	2	2	5
<i>Achnanthes conspicua</i>	16	14	9	5	2	5	1	3
<i>Melosira varians</i>	10	9	11	8	5	2	5	2
<i>Navicula tripunctata</i>	11	14	9	6	0	5	1	0
<i>Thalassiosira weissflogii</i>	9	5	8	5	6	5	5	1
<i>Achnanthes conspicua</i>	5	6	5	5	4	5	4	0
<i>Fragilaria vaucheriae</i>	2	2	7	2	1	5	1	1
<i>Navicula subrotundata</i>	0	1	1	4	12	6	1	1
<i>Navicula acceptata</i>	2	2	3	0	2	2	1	1
<i>Amphora ovalis</i>	3	1	2	0	1	4	1	0
<i>Planothidium frequentissimum</i>	1	1	1	1	2	4	1	2
<i>Nitzschia inconspicua</i>	0	0	2	1	1	2	0	5
<i>Staurosira construens</i>	1	0	2	0	1	5	0	2
<i>Gomphonema pumilum</i>	0	1	1	1	0	0	1	3
<i>Navicula lanceolata</i>	0	1	0	1	2	0	2	2
<i>Reimeria sinuata</i>	1	0	0	0	0	1	0	2

**Hothorpe 11/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Nitzschia sigmoidea</i>	124	121	122	119	105	103	102	100
<i>Navicula radiosa</i>	127	121	120	118	88	89	85	84
<i>Rhoicosphenia abbreviata</i>	75	81	79	86	77	75	71	70
<i>Cocconeis placentula</i>	62	65	55	51	52	45	51	49
<i>Fallacia subhamulata</i>	51	45	44	41	40	39	36	32
<i>Fallacia helensis</i>	39	32	36	32	21	25	19	21
<i>Amphora libyca</i>	32	29	23	22	23	25	23	20
<i>Cocconeis pediculus</i>	31	31	27	24	20	24	19	18
<i>Caloneis silicula</i>	26	24	23	21	19	21	17	15
<i>Navicula minima</i>	19	15	17	15	12	10	0	2
<i>Rhoicosphenia abbreviata</i>	14	16	11	10	10	8	9	11
<i>Reimeria uniseriata</i>	13	15	11	14	10	5	9	5
<i>Navicula cryptotenella</i>	12	11	11	9	8	9	5	6
<i>Achnanthidium minutissimum</i>	15	16	9	2	8	5	4	5
<i>Melosira varians</i>	14	15	8	5	5	6	5	4
<i>Achnanthes conspicua</i>	14	15	7	4	5	4	1	1
<i>Achnanthes conspicua</i>	5	2	6	6	4	6	2	1
<i>Navicula cincta</i>	1	0	7	4	4	5	1	1
<i>Thalassiosira weissflogii</i>	9	5	2	0	1	4	1	1
<i>Nitzschia inconspicua</i>	0	0	2	1	2	2	2	2
<i>Planothidium frequentissimum</i>	0	1	0	1	5	1	2	0
<i>Navicula lanceolata</i>	1	0	1	2	1	1	2	0
<i>Gomphonema pumilum</i>	0	2	2	3	1	1	1	0
<i>Diploneis</i>	2	1	1	6	0	1	1	0
<i>Fragilaria vaucheriae</i>	2	1	2	3	0	1	0	1
<i>Amphora ovalis</i>	2	1	0	1	1	3	1	2
<i>Achnanthidium</i>	3	0	1	1	0	0	2	1
<i>Planothidium lanceolatum</i>	1	1	1	2	1	2	1	1
<i>Navicula acceptata</i>	1	1	1	0	0	2	1	0
<i>Reimeria sinuata</i>	1	1	1	0	1	2	1	0
<i>Surirella brebissonii</i>	0	1	0	2	1	2	1	0

**Sibbertoft 21/05/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	97	99	94	95	109	108	109	110
<i>Navicula cryptotenella</i>	71	72	71	62	89	91	91	92
<i>Gyrosigma acuminatum</i>	55	55	54	56	77	75	74	75
<i>Navicula decussis</i>	42	41	42	41	46	44	45	44
<i>Navicula gregaria</i>	27	24	25	24	33	36	34	35
<i>Navicula lanceolata</i>	19	20	21	19	28	28	27	26
<i>Tabularia fasciculata</i>	20	19	18	17	29	24	29	25
<i>Rhoicosphenia abbreviata</i>	18	18	18	15	23	23	24	25
<i>Nitzschia dissipata</i>	16	17	15	14	22	21	21	20
<i>Fragilaria</i>	13	13	12	13	16	15	14	16
<i>Cyclotella</i>	10	9	9	10	11	12	12	11
<i>Navicula reichardtiana</i>	9	9	9	5	11	6	10	8
<i>Planothidium frequentissimum</i>	8	9	8	7	8	8	4	9
<i>Amphora pediculus</i>	8	7	7	8	7	7	8	6
<i>Navicula cincta</i>	8	7	7	5	8	8	4	5
<i>Nitzschia sigmoidea</i>	9	8	8	4	6	6	4	5
<i>Navicula tripunctata</i>	6	5	5	6	5	5	7	4
<i>Nitzschia pusilla</i>	4	3	3	4	6	6	5	4
<i>Cocconeis placentula</i>	2	2	2	3	5	5	4	5
<i>Nitzschia palea</i>	0	2	2	1	0	0	2	0
<i>Kolbesia ploenensis</i>	1	2	0	1	0	1	0	0
<i>Surirella brebissonii</i>	1	0	0	1	0	1	0	0

**Sibbertoft 18/06/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	132	117	125	118	124	114	121	120
<i>Navicula cryptotenella</i>	115	101	103	96	105	96	101	101
<i>Gyrosigma acuminatum</i>	81	71	73	69	73	68	71	71
<i>Navicula decussis</i>	69	66	63	58	63	62	61	65
<i>Navicula gregaria</i>	54	50	52	48	52	48	49	50
<i>Navicula lanceolata</i>	35	31	33	29	32	29	31	30
<i>Tabularia fasciculata</i>	24	23	22	22	22	22	22	23
<i>Rhoicosphenia abbreviata</i>	18	17	17	18	18	18	18	19
<i>Nitzschia dissipata</i>	16	14	17	14	15	14	15	14
<i>Fragilaria</i>	15	13	16	13	15	13	14	14
<i>Cyclotella</i>	14	12	11	11	12	11	12	12
<i>Navicula reichardtiana</i>	12	10	11	10	11	11	11	11
<i>Planothidium frequentissimum</i>	12	11	10	10	11	11	11	11
<i>Amphora pediculus</i>	11	7	10	7	11	7	11	8
<i>Navicula cincta</i>	9	7	8	6	9	7	9	7
<i>Nitzschia sigmoidea</i>	8	7	4	5	6	6	6	6
<i>Navicula tripunctata</i>	5	5	4	4	4	5	4	5
<i>Nitzschia pusilla</i>	4	4	4	4	5	4	5	4
<i>Cocconeis placentula</i>	4	4	3	3	3	4	3	4
<i>Nitzschia palea</i>	4	3	4	2	4	3	4	3
<i>Kolbesia ploenensis</i>	4	2	4	2	3	2	3	2
<i>Surirella brebissonii</i>	3	2	2	2	2	2	2	2

**Sibbertoft 16/07/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	101	91	93	88	101	91	93	88
<i>Navicula cryptotenella</i>	86	80	78	78	86	80	78	78
<i>Gyrosigma acuminatum</i>	56	51	50	50	56	51	50	50
<i>Navicula decussis</i>	53	48	49	47	53	48	49	47
<i>Navicula gregaria</i>	45	40	41	38	45	40	41	38
<i>Navicula lanceolata</i>	31	30	28	28	31	30	28	28
<i>Tabularia fasciculata</i>	27	22	25	22	27	22	25	22
<i>Rhoicosphenia abbreviata</i>	21	21	20	20	21	21	20	20
<i>Nitzschia dissipata</i>	18	17	16	16	18	17	16	16
<i>Fragilaria</i>	11	14	11	13	11	14	11	13
<i>Cyclotella</i>	13	12	11	11	13	12	11	11
<i>Navicula reichardtiana</i>	11	10	10	9	11	10	10	9
<i>Planothidium frequentissimum</i>	10	10	10	10	10	10	10	10
<i>Amphora pediculus</i>	8	7	8	8	8	7	8	8
<i>Navicula cincta</i>	8	6	7	6	8	6	7	6
<i>Nitzschia sigmoidea</i>	7	6	5	5	7	6	5	5
<i>Navicula tripunctata</i>	6	5	5	5	6	5	5	5
<i>Nitzschia pusilla</i>	5	4	5	4	5	4	5	4
<i>Cocconeis placentula</i>	4	3	3	3	4	3	3	3
<i>Nitzschia palea</i>	5	3	5	3	5	3	5	3
<i>Kolbesia ploenensis</i>	4	4	4	3	4	4	4	3
<i>Surirella brebissonii</i>	4	2	4	2	4	2	4	2

**Sibbertoft 13/08/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	132	136	122	134	132	136	122	134
<i>Navicula cryptotenella</i>	79	78	71	78	79	78	71	78
<i>Gyrosigma acuminatum</i>	44	40	41	39	44	40	41	39
<i>Navicula decussis</i>	29	26	27	28	29	26	27	28
<i>Navicula gregaria</i>	27	26	25	27	27	26	25	27
<i>Navicula lanceolata</i>	26	23	24	24	26	23	24	24
<i>Tabularia fasciculata</i>	25	20	23	20	25	20	23	20
<i>Rhoicosphenia abbreviata</i>	14	14	15	14	14	14	15	14
<i>Nitzschia dissipata</i>	15	10	14	12	15	10	14	12
<i>Fragilaria</i>	11	9	13	11	11	9	13	11
<i>Cyclotella</i>	13	10	12	10	13	10	12	10
<i>Navicula reichardtiana</i>	7	7	6	7	7	7	6	7
<i>Planothidium frequentissimum</i>	6	6	5	7	6	6	5	7
<i>Amphora pediculus</i>	4	4	4	5	4	4	4	5
<i>Navicula cincta</i>	4	4	4	4	4	4	4	4
<i>Nitzschia sigmoidea</i>	3	4	3	4	3	4	3	4
<i>Navicula tripunctata</i>	4	2	3	3	4	2	3	3
<i>Nitzschia pusilla</i>	4	2	4	3	4	2	4	3
<i>Cocconeis placentula</i>	3	3	3	2	3	3	3	2
<i>Nitzschia palea</i>	4	1	3	2	4	1	3	2
<i>Kolbesia ploenensis</i>	2	2	2	2	2	2	2	2
<i>Surirella brebissonii</i>	2	0	1	0	2	0	1	0

**Sibbertoft 11/09/2014**

Taxa	Tile1	Tile 2	Tile 3	Tile 4	Tile 5	Tile 6	Tile 7	Tile 8
<i>Brachysira vitrea</i>	132	117	125	118	132	117	125	118
<i>Navicula cryptotenella</i>	115	101	103	96	115	101	103	96
<i>Gyrosigma acuminatum</i>	81	71	73	69	81	71	73	69
<i>Navicula decussis</i>	69	66	63	58	69	66	63	58
<i>Navicula gregaria</i>	54	50	52	48	54	50	52	48
<i>Navicula lanceolata</i>	35	31	33	29	35	31	33	29
<i>Tabularia fasciculata</i>	24	23	22	22	24	23	22	22
<i>Rhoicosphenia abbreviata</i>	18	17	17	18	18	17	17	18
<i>Nitzschia dissipata</i>	16	14	17	14	16	14	17	14
<i>Fragilaria</i>	15	13	16	13	15	13	16	13
<i>Cyclotella</i>	14	12	11	11	14	12	11	11
<i>Navicula reichardtiana</i>	12	10	11	10	12	10	11	10
<i>Planothidium frequentissimum</i>	12	11	10	10	12	11	10	10
<i>Amphora pediculus</i>	11	7	10	7	11	7	10	7
<i>Navicula cincta</i>	9	7	8	6	9	7	8	6
<i>Nitzschia sigmoidea</i>	8	7	4	5	8	7	4	5
<i>Navicula tripunctata</i>	5	5	4	4	5	5	4	4
<i>Nitzschia pusilla</i>	4	4	4	4	4	4	4	4
<i>Cocconeis placentula</i>	4	4	3	3	4	4	3	3
<i>Nitzschia palea</i>	4	3	4	2	4	3	4	2
<i>Kolbesia ploenensis</i>	4	2	4	2	4	2	4	2
<i>Surirella brebissonii</i>	3	2	2	2	3	2	2	2



**Appendix 4.2 T-test analyses across all studied sites between the species grown on tile and stone to test whether the tiles are valid artificial substrates for epilithic growth or not**

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	<i>Tile</i>	<i>Stone</i>		<i>Tile</i>	<i>Stone</i>
Mean	18.72	22.24	Mean	11.88	14.15
Variance	721.1	813.1	Variance	425.9	474.1
Observations	28	28	Observations	40	40
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	54		df	78	
t Stat	-0.48		t Stat	-0.48	
P(T<=t) one-tail	0.32		P(T<=t) one-tail	0.32	
t Critical one-tail	1.67		t Critical one-tail	1.67	
P(T<=t) two-tail	0.64		P(T<=t) two-tail	0.63	
t Critical two-tail	2.01		t Critical two-tail	1.99	
<b>Market Harborough 13/08/2014</b>			<b>Lubenham 18/06/2014</b>		
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	<i>Tile</i>	<i>Stone</i>		<i>Tile</i>	<i>Stone</i>
Mean	20.39	20.33	Mean	14.81	17.78
Variance	789.4	760.4	Variance	555.3	626.7
Observations	30	30	Observations	37	37
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	58		df	72	
t Stat	0.01		t Stat	-0.53	
P(T<=t) one-tail	0.50		P(T<=t) one-tail	0.3	
t Critical one-tail	1.67		t Critical one-tail	1.67	
P(T<=t) two-tail	0.99		P(T<=t) two-tail	0.6	
t Critical two-tail	2.00		t Critical two-tail	1.99	
<b>Market Harborough 10/09/2014</b>			<b>Papillion Ford 21/05/2014</b>		
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	<i>Tile</i>	<i>Stone</i>		<i>Tile</i>	<i>Stone</i>
Mean	14.79	14.91	Mean	15.08	17.34
Variance	486.8	484.3	Variance	576.2	650.4
Observations	36	36	Observations	35	35
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	70		df	68	
t Stat	-0.02		t Stat	-0.38	
P(T<=t) one-tail	0.49		P(T<=t) one-tail	0.35	
t Critical one-tail	1.67		t Critical one-tail	1.67	
P(T<=t) two-tail	0.98		P(T<=t) two-tail	0.70	
t Critical two-tail	1.99		t Critical two-tail	1.20	
<b>Lubenham 21/05/2014</b>			<b>Papillion Ford 18/06/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	<i>Tile</i>	<i>Stone</i>		<i>Tile</i>	<i>Stone</i>
Mean	19.18	22.54	Mean	18.63	22.67
Variance	943.2	992.4	Variance	437.2	505.8
Observations	30	30	Observations	29	29
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	58		df	56	
t Stat	-0.42		t Stat	-0.71	
P(T<=t) one-tail	0.34		P(T<=t) one-tail	0.24	
t Critical one-tail	1.67		t Critical one-tail	1.67	
P(T<=t) two-tail	0.68		P(T<=t) two-tail	0.48	
t Critical two-tail	2.00		t Critical two-tail	2.00	
<b>Hothorpe</b>	<b>21/05/2014</b>		<b>Sibbertoft</b>	<b>18/06/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances		
	<i>Tile</i>	<i>Stone</i>
Mean	22.57	22.52
Variance	1008	1032
Observations	29	29
Hypothesized Mean Difference	0	
df	56	
t Stat	0.01	
P(T<=t) one-tail	0.50	
t Critical one-tail	1.67	
P(T<=t) two-tail	0.99	
t Critical two-tail	2.00	
<b>Hothorpe</b>	<b>18/06/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances		
	<i>Tile</i>	<i>Stone</i>
Mean	20.65	20.83
Variance	441.7	442
Observations	29	29
Hypothesized Mean Difference	0	
df	56	
t Stat	-0.03	
P(T<=t) one-tail	0.49	
t Critical one-tail	1.67	
P(T<=t) two-tail	0.97	
t Critical two-tail	2.00	
<b>Sibbertoft</b>	<b>21/05/2014</b>	

### Appendix 4.3 Anova Two-Factor with replication to test the substra type effects on species diversity across the studied sites

Anova: Two-Factor With Replication					
SUMMARY	Shannon Wiener Diversity Index	Simpson Diversity index	Total		
Tile					
Count	10.00	10.00	20.00		
Sum	27.78	9.13	36.91		
Average	2.78	0.91	1.85		
Variance	0.00	0.00	0.92		
Stone					
Count	10.00	10.00	20.00		
Sum	28.01	9.14	37.16		
Average	2.80	0.91	1.86		
Variance	0.00	0.00	0.94		
Total					
Count	20.00	20.00			
Sum	55.79	18.27			
Average	2.79	0.91			
Variance	0.00	0.00			
ANOVA					
Source of Variation	SS	df	MS	F	P-value
Sample	0.00	1	0.00	0.89	0.35
Columns	35.19	1	35.19	20054.45	0.00
Interaction	0.00	1	0.00	0.68	0.41
Within	0.06	36	0.00		
Total	35.26	39			

Market Harborough 13/08/2014

Anova: Two-Factor With Replication						
SUMMARY	Shannon Wiener Diversity Index	Simpson Diversity index	Total			
Tile						
Count	10.00	10.00	20.00			
Sum	26.86	9.06	35.92			
Average	2.69	0.91	1.80			
Variance	0.00	0.00	0.84			
Stone						
Count	10.00	10.00	20.00			
Sum	27.21	9.08	36.29			
Average	2.72	0.91	1.81			
Variance	0.01	0.00	0.87			
Total						
Count	20.00	20.00				
Sum	54.07	18.14				
Average	2.70	0.91				
Variance	0.01	0.00				
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.00	1	0.00	1.41	0.24	4.11
Columns	32.28	1	32.28	12960.63	0.00	4.11
Interaction	0.00	1	0.00	1.09	0.30	4.11
Within	0.09	36	0.00			
Total	32.37	39				

Market Harborough 10/09/2014

Anova: Two-Factor With Replication							Anova: Two-Factor With Replication						
SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total		SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total	
Tile							Tile						
Count	10.00		10.00		20.00		Count	10.00		10.00		20.00	
Sum	27.78		9.13		36.91		Sum	27.47		9.02		36.49	
Average	2.78		0.91		1.85		Average	2.75		0.90		1.82	
Variance	0.00		0.00		0.92		Variance	0.00		0.00		0.90	
	10.00		10.00		20.00			10.00		10.00		20.00	
Stone	27.78		9.13		36.91		Stone	27.47		9.02		36.49	
Count	10.00		10.00		20.00		Count	10.00		10.00		20.00	
Sum	28.01		9.14		37.16		Sum	28.79		9.18		37.97	
Average	2.80		0.91		1.86		Average	2.88		0.92		1.90	
Variance	0.00		0.00		0.94		Variance	0.00		0.00		1.01	
	10.00		10.00		20.00			10.00		10.00		20.00	
Total	28.01		9.14		37.16		Total	28.79		9.18		37.97	
Count	20.00		20.00				Count	20.00		20.00			
Sum	55.79		18.27				Sum	56.26		18.20			
Average	2.79		0.91				Average	2.81		0.91			
Variance	0.00		0.00				Variance	0.01		0.00			
ANOVA							ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit	Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.00	1	0.00	0.89	0.35	4.11	Sample	0.054	1	0.05	48.92	0.00	4.11
Columns	35.19	1	35.19	20054.45	0.00	4.11	Columns	36.215	1	36.21	32525.71	0.00	4.11
Interaction	0.00	1	0.00	0.68	0.41	4.11	Interaction	0.033	1	0.03	29.99	0.00	4.11
Within	0.06	36	0.00				Within	0.040	36	0.00			
Total	32.37	39					Total	36.34	39				
Lubenham	21/05/2014						Lubenham	18/06/2014					

Anova: Two-Factor With Replication							Anova: Two-Factor With Replication						
SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total		SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total	
Tile							Tile						
Count	10.00		10.00		20.00		Count	10.00		10.00		20.00	
Sum	26.68		9.07		35.75		Sum	25.97		9.02		34.99	
Average	2.67		0.91		1.79		Average	2.60		0.90		1.75	
Variance	0.00		0.00		0.82		Variance	0.00		0.00		0.76	
	10.00		10.00		20.00			10.00		10.00		20.00	
Stone	26.68		9.07		35.75		Stone	25.97		9.02		34.99	
Count	10.00		10.00		20.00		Count	10.00		10.00		20.00	
Sum	28.22		9.21		37.43		Sum	27.21		9.12		36.34	
Average	2.82		0.92		1.87		Average	2.72		0.91		1.82	
Variance	0.00		0.00		0.95		Variance	0.00		0.00		0.86	
	10.00		10.00		20.00			10.00		10.00		20.00	
Total	28.22		9.21		37.43		Total	27.21		9.12		36.34	
Count	20.00		20.00				Count	20.00		20.00			
Sum	54.90		18.28				Sum	53.18		18.14			
Average	2.74		0.91				Average	2.66		0.91			
Variance	0.01		0.00				Variance	0.01		0.00			
ANOVA							ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit	Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.070379264	1	0.07	79.55	0.00	4.11	Sample	0.05	1	0.05	27.51	0.00	4.11
Columns	33.51636445	1	33.52	37882.14	0.00	4.11	Columns	30.70	1	30.70	18648.74	0.00	4.11
Interaction	0.049503256	1	0.05	55.95	0.00	4.11	Interaction	0.032	1	0.03	19.60	0.00	4.11
Within	0.031851131	36	0.00				Within	0.06	36	0.00			
Total	33.67	39					Total	30.84	39				
Papillion Ford 21/05/2014							Papillion Ford 18/06/2014						

Anova: Two-Factor With Replication							Anova: Two-Factor With Replication						
SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total		SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total	
Tile							Tile						
Count	10.00		10.00		20.00		Count	10.00		10.00		20.00	
Sum	25.03		8.85		33.88		Sum	25.80		8.97		34.77	
Average	2.50		0.89		1.69		Average	2.58		0.90		1.74	
Variance	0.00		0.00		0.69		Variance	0.01		0.00		0.75	
	10.00		10.00		20.00			10.00		10.00		20.00	
Stone	25.03		8.85		33.88		Stone	25.80		8.97		34.77	
Count	10.00		10.00		20.00		Count	10.00		10.00		20.00	
Sum	26.55		9.04		35.59		Sum	25.92		8.97		34.89	
Average	2.65		0.90		1.78		Average	2.59		0.90		1.74	
Variance	0.00		0.00		0.81		Variance	0.00		0.00		0.76	
	10.00		10.00		20.00			10.00		10.00		20.00	
Total	26.55		9.04		35.59		Total	25.92		8.97		34.89	
Count	20.00		20.00				Count	20.00		20.00			
Sum	51.57		17.89				Sum	51.72		17.95			
Average	2.58		0.89				Average	2.59		0.90			
Variance	0.01		0.00				Variance	0.01		0.00			
ANOVA							ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit	Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.07	1	0.07	61.04	0.00	4.11	Sample	0.00	1	0.00	0.09	0.77	4.11
Columns	28.	1	28.36	23568.72	0.00	4.11	Columns	28.51	1	28.51	7472.52	0.00	4.11
Interaction	0.04	1	0.04	36.72	0.00	4.11	Interaction	0.00	1	0.00	0.09	0.77	4.11
Within	0.04	36	0.00				Within	0.14	36	0.00			
Total	28.52	39					Total	28.65	39				
Hothorpe 21/05/2014							Hothorpe 18/06/2014						

Anova: Two-Factor With Replication							Anova: Two-Factor With Replication						
SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total		SUMMARY	Shannon Wiener Diversity Index		Simpson Diversity index		Total	
Tile							Tile						
Count	10.00		10.00		20.00		Count	10		10		20	
Sum	28.71		9.31		38.02		Sum	10.00		10.00		20.00	
Average	2.87		0.93		1.90		Average	27.81		9.25		37.06	
Variance	0.00		0.00		0.99		Variance	2.78		0.93		1.85	
	10.00		10.00		20.00			0.00		0.00		0.91	
Stone	28.71		9.31		38.02		Stone	10.00		10.00		20.00	
Count	10.00		10.00		20.00		Count	27.81		9.25		37.06	
Sum	28.86		9.33		38.19		Sum	10.00		10.00		20.00	
Average	2.89		0.93		1.91		Average	28.80		9.34		38.14	
Variance	0.00		0.00		1.00		Variance	2.88		0.93		1.91	
	10.00		10.00		20.00			0.00		0.00		1.00	
Total	28.86		9.33		38.19		Total	10.00		10.00		20.00	
Count	20.00		20.00				Count	28.80		9.34		38.14	
Sum	57.57		18.64				Sum	20.00		20.00			
Average	2.88		0.93				Average	56.61		18.59			
Variance	0.00		0.00				Variance	2.83		0.93			
ANOVA							ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit	Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.00	1	0.00	0.93	0.34	4.11	Sample	0.03	1	0.03	75.58	0.00	4.11
Columns	37.90	1	37.90	46051.71	0.00	4.11	Columns	36.14	1	36.14	93835.68	0.00	4.11
Interaction	0.00	1	0.00	0.55	0.46	4.11	Interaction	0.02	1	0.02	53.16	0.00	4.11
Within	0.03	36	0.00				Within	0.01	36	0.005			
Total	37.93	39					Total	36.21	39				
Sibbertoft	21/05/2014						Sibbertoft	18/06/2014					

**Appendix 4.4 Anova Two-Factor with replication for the selected sites to quantify the most appropriate length of time for tile to be exposed in water.**

SUMMARY	New species	Extinct species	Total
<hr/> 1-14 Days <hr/>			
Count	4	4	8
Sum	160	0	160
Average	40	0	20
Variance	83.33	0	492.86

<hr/> 15-28 Days <hr/>			
Count	4	4	8
Sum	15	24	39
Average	3.75	6	4.875
Variance	1.58	4.67	4.13

<hr/> 29-42 Days <hr/>			
Count	4	4	8
Sum	0	15	15
Average	0	3.75	1.88
Variance	0	0.25	4.13

<hr/> Total <hr/>			
Count	12	12	
Sum	175	39	
Average	14.58	3.25	
Variance	378.08	8.02	

<hr/> ANOVA <hr/>						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	1510.08	2	755.04	50.43	0.00	3.55
Columns	770.67	1	770.67	51.47	0.00	4.41
Interaction	2467.58	2	1233.79	82.41	0.00	3.55
Within	269.50	18	14.97			
Total	5017.83	23				



**Appendix 4.5 T-Test for the selected sites to quantify the most effective speed where low current ranged from 0.01–0.29 m s<sup>-1</sup> and high current ranged from 0.3–0.6 m s<sup>-1</sup>**

t-Test: Two-Sample Assuming Unequal Variances

	Low	High	t-Test: Two-Sample Assuming Unequal Variances		
				Low	High
Mean	2.87	2.76	Mean	2.82	2.64
Variance	0.00	0.00	Variance	0.01	0.01
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	6	
t Stat	3.89		t Stat	3.04	
P(T<=t) one-tail	0.00		P(T<=t) one-tail	0.01	
t Critical one-tail	1.94		t Critical one-tail	1.94	
P(T<=t) two-tail	0.01		P(T<=t) two-tail	0.02	
t Critical two-tail	2.45		t Critical two-tail	2.45	
<b>Market Harborough</b>	<b>13/08/2014</b>				

**Lubenhams 18/06/2014**

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.54	2.46	Mean	2.90	2.82
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5	
t Stat	2.92		t Stat	2.69	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.02	
t Critical one-tail	2.02		t Critical one-tail	2.02	
P(T<=t) two-tail	0.03		P(T<=t) two-tail	0.04	
t Critical two-tail	2.57		t Critical two-tail	2.57	
<b>Market Harborough</b>	<b>10/09/2014</b>				

**Lubenhams 16/07/2014**

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.02	1.86	Mean	2.75	2.12
Variance	0.01	0.01	Variance	0.01	0.01
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	6	
t Stat	2.47		t Stat	7.56	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.00	
t Critical one-tail	1.94		t Critical one-tail	1.94	
P(T<=t) two-tail	0.05		P(T<=t) two-tail	0.00	
t Critical two-tail	2.45		t Critical two-tail	2.45	
<b>Lubenhams</b>	<b>21/05/2014</b>				

**Lubenhams 13/08/2014**

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.04	1.88	Mean	2.82	2.60
Variance	0.01	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	6	
t Stat	3.13		t Stat	6.90	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.00	
t Critical one-tail	2.13		t Critical one-tail	1.94	
P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.00	
t Critical two-tail	2.78		t Critical two-tail	2.45	
<b>Lubenhams 11/09/2014</b>			<b>Papillion Ford 16/07/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.09	1.97	Mean	2.71	2.63
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	6	
t Stat	3.31		t Stat	3.63	
P(T<=t) one-tail	0.01		P(T<=t) one-tail	0.01	
t Critical one-tail	1.94		t Critical one-tail	1.94	
P(T<=t) two-tail	0.02		P(T<=t) two-tail	0.01	
t Critical two-tail	2.45		t Critical two-tail	2.45	
<b>Papillion Ford 21/05/2014</b>			<b>Papillion Ford 13/08/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.28	1.97	Mean	1.99	1.72
Variance	0.02	0.00	Variance	0.00	0.01
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	3	
t Stat	3.90		t Stat	4.51	
P(T<=t) one-tail	0.01		P(T<=t) one-tail	0.01	
t Critical one-tail	2.13		t Critical one-tail	2.35	
P(T<=t) two-tail	0.02		P(T<=t) two-tail	0.02	
t Critical two-tail	2.78		t Critical two-tail	3.18	
<b>Papillion Ford 18/06/2014</b>			<b>Papillion Ford 11/09/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.18	1.98	Mean	2.69	2.31
Variance	0.00	0.02	Variance	0.00	0.01
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	5	
t Stat	2.78		t Stat	6.10	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.00	
t Critical one-tail	2.13		t Critical one-tail	2.02	
P(T<=t) two-tail	0.05		P(T<=t) two-tail	0.00	
t Critical two-tail	2.78		t Critical two-tail	2.57	
<b>Hothorpe</b>	<b>21/05/2014</b>		<b>Hothorpe</b>	<b>13/08/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.79	2.45	Mean	1.99	1.86
Variance	0.01	0.01	Variance	0.00	0.01
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	4	
t Stat	5.28		t Stat	2.93	
P(T<=t) one-tail	0.00		P(T<=t) one-tail	0.02	
t Critical one-tail	1.94		t Critical one-tail	2.13	
P(T<=t) two-tail	0.00		P(T<=t) two-tail	0.04	
t Critical two-tail	2.45		t Critical two-tail	2.78	
<b>Hothorpe</b>	<b>18/06/2014</b>		<b>Hothorpe</b>	<b>11/09/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.95	2.85	Mean	2.13	2.01
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5	
t Stat	2.88		t Stat	8.74	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.00	
t Critical one-tail	2.02		t Critical one-tail	2.02	
P(T<=t) two-tail	0.03		P(T<=t) two-tail	0.00	
t Critical two-tail	2.57		t Critical two-tail	2.57	
<b>Hothorpe</b>	<b>16/07/2014</b>		<b>Sibbertoft</b>	<b>21/05/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.64	2.54	Mean	2.00	1.75
Variance	0.00	0.00	Variance	0.00	0.02
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	3	
t Stat	3.05		t Stat	3.65	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.02	
t Critical one-tail	2.13		t Critical one-tail	2.35	
P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.04	
t Critical two-tail	2.78		t Critical two-tail	3.18	
<b>Sibbertoft</b>	<b>18/06/2014</b>		<b>Sibbertoft</b>	<b>11/09/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.73	2.62	Mean	2.14	2.00
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	4	
t Stat	2.54		t Stat	8.81	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.00	
t Critical one-tail	1.94		t Critical one-tail	2.13	
P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.00	
t Critical two-tail	2.45		t Critical two-tail	2.78	
<b>Sibbertoft</b>	<b>16/07/2014</b>		<b>Loddington Lone Pine</b>	<b>07/05/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.46	2.26	Mean	2.19	2.14
Variance	0.00	0.01	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	3		df	6	
t Stat	3.28		t Stat	2.66	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.02	
t Critical one-tail	2.35		t Critical one-tail	1.94	
P(T<=t) two-tail	0.05		P(T<=t) two-tail	0.04	
t Critical two-tail	3.18		t Critical two-tail	2.45	
<b>Sibbertoft</b>	<b>13/08/2014</b>		<b>Loddington Lone Pine</b>	<b>04/06/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.76	2.73	Mean	2.33	2.26
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	3	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5	
t Stat	3.07		t Stat	3.61	
P(T<=t) one-tail	0.01		P(T<=t) one-tail	0.01	
t Critical one-tail	2.02		t Critical one-tail	2.02	
P(T<=t) two-tail	0.03		P(T<=t) two-tail	0.02	
t Critical two-tail	2.57		t Critical two-tail	2.57	
<b>Loddington Lone Pine</b>	<b>02/07/2014</b>		<b>Loddington Lone Pine</b>	<b>24/09/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.81	2.67	Mean	2.04	1.93
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	6	
t Stat	4.25		t Stat	2.83	
P(T<=t) one-tail	0.00		P(T<=t) one-tail	0.01	
t Critical one-tail	2.02		t Critical one-tail	1.94	
P(T<=t) two-tail	0.01		P(T<=t) two-tail	0.03	
t Critical two-tail	2.57		t Critical two-tail	2.45	
<b>Loddington Lone Pine</b>	<b>30/07/2014</b>		<b>Loddington White Horse</b>	<b>07/05/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.71	2.58	Mean	2.64	2.55
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	5	
t Stat	3.49		t Stat	4.01	
P(T<=t) one-tail	0.01		P(T<=t) one-tail	0.01	
t Critical one-tail	1.94		t Critical one-tail	2.02	
P(T<=t) two-tail	0.01		P(T<=t) two-tail	0.01	
t Critical two-tail	2.45		t Critical two-tail	2.57	
<b>Loddington Lone Pine</b>	<b>27/08/2014</b>		<b>Loddington White Horse</b>	<b>04/06/2014</b>	

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.82	2.81	Mean	1.95	1.86
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	5	
t Stat	3.75		t Stat	2.72	
P(T<=t) one-tail	0.01		P(T<=t) one-tail	0.02	
t Critical one-tail	2.13		t Critical one-tail	2.02	
P(T<=t) two-tail	0.02		P(T<=t) two-tail	0.04	
t Critical two-tail	2.78		t Critical two-tail	2.57	
<b>Loddington White Horse 02/07/2014</b>			<b>Loddington White Horse 24/09/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.79	2.71	Mean	2.15	2.01
Variance	0.00	0.00	Variance	0.00	0.01
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	5		df	5	
t Stat	2.73		t Stat	2.67	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.02	
t Critical one-tail	2.02		t Critical one-tail	2.02	
P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.04	
t Critical two-tail	2.57		t Critical two-tail	2.57	
<b>Loddington White Horse 30/07/2014</b>			<b>Loddington School Farm 07/05/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.12	1.99	Mean	2.26	2.10
Variance	0.01	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	6	
t Stat	3.00		t Stat	4.29	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.00	
t Critical one-tail	2.13		t Critical one-tail	1.94	
P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.01	
t Critical two-tail	2.78		t Critical two-tail	2.45	
<b>Loddington White Horse 27/08/2014</b>			<b>Loddington School Farm 04/06/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.77	2.56	Mean	2.09	1.88
Variance	0.00	0.01	Variance	0.01	0.01
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	6	
t Stat	4.96		t Stat	2.73	
P(T<=t) one-tail	0.00		P(T<=t) one-tail	0.02	
t Critical one-tail	2.13		t Critical one-tail	1.94	
P(T<=t) two-tail	0.01		P(T<=t) two-tail	0.03	
t Critical two-tail	2.78		t Critical two-tail	2.45	
<b>Loddington School Farm02/07/2014</b>			<b>Loddington School Farm24/09/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.66	2.55	Mean	1.19	1.13
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	3	
t Stat	3.26		t Stat	3.93	
P(T<=t) one-tail	0.01		P(T<=t) one-tail	0.01	
t Critical one-tail	1.94		t Critical one-tail	2.35	
P(T<=t) two-tail	0.02		P(T<=t) two-tail	0.03	
t Critical two-tail	2.45		t Critical two-tail	3.18	
<b>Loddington School Farm30/07/2014</b>			<b>Tilton Digby Farm 07/05/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.51	2.29	Mean	2.42	2.33
Variance	0.00	0.02	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	4	
t Stat	2.96		t Stat	3.30	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.01	
t Critical one-tail	2.13		t Critical one-tail	2.13	
P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.03	
t Critical two-tail	2.78		t Critical two-tail	2.78	
<b>Loddington School Farm27/08/2014</b>			<b>Tilton Digby Farm 04/06/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	Low	High		Low	High
Mean	2.89	2.81	Mean	1.17	1.08
Variance	0.00	0.00	Variance	0.00	0.00
Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	5	
t Stat	2.46		t Stat	2.65	
P(T<=t) one-tail	0.02		P(T<=t) one-tail	0.02	
t Critical one-tail	1.94		t Critical one-tail	2.02	
P(T<=t) two-tail	0.05		P(T<=t) two-tail	0.05	
t Critical two-tail	2.45		t Critical two-tail	2.57	
<b>Tilton Digby Farm</b>			<b>Tilton Digby Farm</b>		
<b>02/07/2014</b>			<b>24/09/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances		
	Low	High
Mean	2.84	2.56
Variance	0.00	0.01
Observations	4	4
Hypothesized Mean Difference	0	
df	4	
t Stat	4.69	
P(T<=t) one-tail	0.00	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.01	
t Critical two-tail	2.78	
<b>Tilton Digby Farm</b>		
<b>30/07/2014</b>		

t-Test: Two-Sample Assuming Unequal Variances		
	Low	High
Mean	1.29	1.13
Variance	0.01	0.00
Observations	4	4
Hypothesized Mean Difference	0	
df	6	
t Stat	3.32	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.94	
P(T<=t) two-tail	0.02	
t Critical two-tail	2.45	
<b>Tilton Digby Farm</b>		
<b>27/08/2014</b>		



**Appendix 4.6 Anova Two-Factor with replication for the seasonal differences across the study site during the months of the study,**

<i>May</i>			
Count	8	8	16
Sum	3829.75	16.73	3846.48
Average	478.72	2.09	240.41
Variance	4098.58	0.16	62492.35
<i>June</i>			
Count	8	8	16
Sum	4087.88	18.83	4106.70
Average	510.98	2.35	256.67
Variance	4049.82	0.02	70878.07
<i>July</i>			
Count	8	8	16
Sum	4381.38	20.94	4402.32
Average	547.67	2.62	275.14
Variance	2199.58	0.04	80248.94
<i>August</i>			
Count	8	8	16
Sum	4010.75	18.14	4028.89
Average	501.34	2.27	251.81
Variance	3633.29	0.20	68116.17
<i>September</i>			
Count	8	8	16
Sum	3645.63	16.54	3662.16
Average	455.70	2.07	228.89
Variance	3500.97	0.17	56509.97
<i>Total</i>			
Count	40	40	
Sum	19955.38	91.18	
Average	498.88	2.28	
Variance	4123.26	0.15	

<b>ANOVA</b>						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Sample	19459.08	4	4864.77	2.78	0.03	2.50
Columns	4932328.00	1	4932328.00	2821.24	0.00	3.98
Interaction	18974.13	4	4743.53	2.71	0.04	2.50
Within	122379.80	70	1748.28			
Total	5093141.00	79				

**Appendix 4.7 RDA for individual species outcome against the environmental variables (total P and sediment) for the preliminary field results**

Call:

```
rda(formula = Abu_fin ~ Total.phosphorus + Sediment, data = Env_fin, scale = TRUE)
```

Partitioning of correlations:

Inertia Proportion

Total 58.00 1.00

Constrained 45.81 0.79

Unconstrained 12.19 0.21

Eigenvalues, and their contribution to the correlations

Importance of components:

	RDA1	RDA2	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	15.62	8.99	7.82	6.178	3.94	3.25	6.86	5.33
Proportion Explained	0.27	0.16	0.13	0.11	0.07	0.06	0.12	0.10
Cumulative Proportion	0.27	0.42	0.56	0.67	0.73	0.79	0.91	1.00

Accumulated constrained eigenvalues

Importance of components:

	RDA1	RDA2
Eigenvalue	15.62	8.99
Proportion Explained	0.34	0.19
Cumulative Proportion	0.34	0.54

Scaling 2 for species and site scores

\* Species are scaled proportional to eigenvalues

\* Sites are unscaled: weighted dispersion equal on all dimensions

\* General scaling constant of scores: 4.641192

Species scores

	RDA1	RDA2	PC1	PC2	PC3	PC4
Achnanthidium.minutissimum	-0.07	-0.04	-0.25	0.29	-0.40	0.06
Amphora	-0.03	0.20	-0.36	-0.39	0.13	-0.08
Amphora.pediculus	0.23	0.15	0.06	0.18	0.41	-0.06
Anabaena	-0.25	0.13	-0.26	-0.39	0.07	0.20
Caloneis.bacillum	0.57	-0.05	-0.01	0.14	-0.04	0.01

Cladophora	-0.20	-0.50	-0.19	-0.09	-0.07	0.00
Cocconeis.pediculus	-0.05	0.18	0.01	0.36	0.28	0.17
Cocconeis.placentula	0.16	-0.07	-0.04	0.17	0.07	0.02
Cosmarium	-0.15	-0.17	-0.33	-0.0	-0.25	0.38
Cyclotella	-0.03	0.26	0.45	-0.08	0.03	0.06
Cyclotella.meneghiniana	-0.24	0.36	-0.08	0.26	-0.07	-0.07
Cymatopleura.solea	-0.24	0.30	-0.07	0.36	0.02	0.10
Diatoma.vulgare	-0.28	-0.08	-0.19	0.27	-0.21	-0.11
Encyonema.silesiacum	0.57	-0.03	-0.01	0.14	-0.05	0.01
Fragilaria.capucina	0.57	-0.03	-0.02	0.13	-0.05	0.02
Gomphonema.angustatum	0.58	-0.03	-0.04	0.10	-0.03	0.02
Gomphonema.olivaceum	-0.24	0.30	-0.10	0.26	-0.12	-0.07
Gomphonema.parvulum	0.27	0.11	-0.37	-0.30	0.09	0.07
Gyrosigma.acuminatum	0.02	0.13	0.10	0.17	0.34	0.15
Gyrosigma.attenuatum	-0.22	-0.26	0.12	0.14	0.20	-0.30
Hydrodictyon	0.10	-0.38	-0.01	0.06	0.09	0.16
Melosira.varians	0.10	0.14	0.07	0.25	0.32	0.16
Meridion.circulare	-0.19	0.41	-0.07	0.32	0.04	-0.16
Navicula.capitatoradiata	0.56	-0.01	-0.01	0.16	-0.06	0.04
Navicula.cincta	-0.20	0.28	0.29	-0.08	-0.22	-0.01
Navicula.cryptotenella	0.13	0.17	0.50	-0.07	-0.23	-0.07
Navicula.gregaria	-0.18	0.19	0.19	-0.18	0.20	0.02
Navicula.minima	-0.11	0.06	-0.07	0.18	0.16	0.15
Navicula.subminuscula	-0.07	0.38	-0.24	0.04	-0.01	-0.05
Navicula.tripunctata	0.56	0.02	0.04	0.12	-0.07	0.02
Navicula.veneta	-0.18	0.36	-0.28	-0.10	0.04	-0.05
Nitzschia.dissipata	0.20	0.03	0.46	-0.03	-0.02	-0.21
Nitzschia.inconspicua	-0.28	0.34	-0.11	0.26	-0.07	-0.03
Nitzschia.palea	0.58	-0.02	-0.02	0.05	-0.09	0.02
Oedogonium	0.43	0.22	-0.22	0.09	-0.06	-0.20
Pediastrum	0.20	0.02	-0.30	-0.29	0.09	0.20
Planothidium.frequentissimum	0.24	0.23	0.32	-0.35	-0.01	0.06
Planothidium.lanceolatum	0.03	0.25	-0.37	-0.13	-0.01	-0.37
Psammothidium.lauenburgianum	0.04	0.07	0.47	-0.30	-0.20	-0.10

Pseudostaurosira.brevistriata	0.46	-0.26	0.05	0.16	0.09	-0.15
Reimeria.sinuata	0.40	0.21	-0.22	0.13	-0.09	-0.27
Rhoicosphenia.abbreviata	-0.20	-0.50	0.01	0.11	0.12	-0.05
Spirogyra	-0.22	-0.29	-0.24	-0.14	-0.06	-0.13
Surirella.brebissonii	0.15	-0.26	-0.11	-0.06	0.18	-0.41
Tryblionella.apiculata	-0.10	0.22	-0.41	-0.13	-0.12	-0.30
Amphora.inariensis	-0.16	-0.50	-0.00	0.11	-0.01	-0.06
Amphora.libyca	-0.12	-0.06	-0.067	0.08	0.11	0.08
Encyonema.minutum	0.59	-0.01	-0.09	0.03	0.024	0.03
Navicula.lanceolata	0.17	0.10	-0.23	-0.50	0.16	0.03
Navicula.reichardtiana	-0.16	-0.52	0.10	0.03	-0.06	-0.13
Nitzschia.amphibia	0.58	-0.06	-0.04	0.13	-0.07	0.04
Nitzschia.recta	-0.13	-0.45	0.02	-0.06	0.27	-0.18
Fragilaria.vaucheriae	0.56	-0.00	-0.17	-0.09	0.04	0.03
Nitzschia.sigmoidea	0.34	-0.04	-0.11	0.05	0.02	0.07
Brachysira.vitrea	0.54	0.02	-0.15	-0.19	0.03	0.02
Nitzschia.pusilla	0.57	0.015	0.11	-0.02	-0.12	0.01
Gomphonema.minutum	-0.09	-0.30	-0.20	0.05	-0.38	0.14

Site scores (weighted sums of species scores)

	RDA1	RDA2	PC1	PC2	PC3	PC4
Market Harborough	-0.82	2.30	-1.17	1.41	-0.96	-3.16
Lubenham	-1.01	1.27	-0.57	0.99	-0.73	1.07
Papillon Ford	-0.51	0.53	1.40	1.07	3.35	1.70
Hothorpe	-0.48	-0.16	0.02	0.50	0.28	2.23
Sibbertoft	-0.07	0.84	3.49	-2.08	-1.51	0.06
Loddington Lone Pine	-0.66	-2.89	0.72	0.20	1.32	-2.62
Loddington White Horse	-1.09	-2.28	-1.11	0.25	-2.22	1.20
Loddington School Farm	4.31	-0.16	-0.33	1.06	-0.71	-0.18
Tilton Digby Farm	0.32	0.54	-2.44	-3.41	1.19	-0.31

Site constraints (linear combinations of constraining variables)

	RDA1	RDA2	PC1	PC2	PC3	PC4
Market Harborough	-0.80	2.30	-1.09	1.43	-0.95	-2.98
Lubenham	-1.49	0.99	0.35	0.92	0.11	2.44
Papillon Ford	0.13	0.86	0.74	1.24	2.43	1.04
Hothorpe	-0.75	-0.23	-0.65	0.32	0.31	0.54
Sibbertoft	0.01	0.89	3.30	-2.070	-1.66	-0.23
Loddington Lone Pine	-0.83	-2.99	1.10	0.18	1.64	-2.03
Loddington White Horse	-0.70	-2.09	-1.40	0.36	-2.73	1.05
Loddington School Farm	4.10	-0.27	-0.07	1.01	-0.39	0.14
Tilton Digby Farm	0.33	0.53	-2.28	-3.40	1.24	0.03

Triplot scores for constraining variables

	RDA1	RDA2	PC1	PC2	PC3	PC4
Total.phosphorus	-0.16	-0.24	0.84	-0.27	-0.19	-0.30
Sediment	0.91	0.02	0.18	0.37	0.02	0.09

**Appendix 4.8 RDA for Algal groups (Cyanobacteria, Chlorophyceae and Bacillariophyceae) relative abundance against the environmental variables (total P and sediment) for the preliminary field results**

Call:

```
rda(formula = algal_fin ~ Total.phosphorus + Sediment, data = Env_fin, scale = TRUE)
```

Partitioning of correlations:

Inertia Proportion

Total	3.00	1.00
Constrained	2.27	0.76
Unconstrained	0.73	0.24

Eigenvalues, and their contribution to the correlations

Importance of components:

	RDA1	RDA2	PC1	PC2	PC3
Eigenvalue	1.90	0.37	0.00	0.62	0.11
Proportion Explained	0.63	0.12	0.00	0.21	0.04
Cumulative Proportion	0.63	0.76	7.56	0.96	1.00

Accumulated constrained eigenvalues

Importance of components:

	RDA1	RDA2	PC1
Eigenvalue	1.90	0.37	0.00
Proportion Explained	0.84	0.16	0.00
Cumulative Proportion	0.84	1.00	1.00

Scaling 2 for species and site scores

\* Species are scaled proportional to eigenvalues

\* Sites are unscaled: weighted dispersion equal on all dimensions

\* General scaling constant of scores: 2.213364

Species scores

	RDA1	RDA2	PC1	PC2	PC3
Cyanobacteria	1.02	0.62	0.00	-0.19	-0.41
Chlorophyceae	0.96	-0.42	0.00	-0.73	0.11
Bacillariophyceae	-1.07	0.22	0.00	0.67	0.00

Site scores (weighted sums of species scores)

	RDA1	RDA2	PC1	PC2	PC3
Market Harborough	-0.61	0.15	0.09	-0.18	0.08
Lubenham	0.74	0.25	-0.64	-1.12	-0.89
Papillon Ford	-0.78	-0.07	-1.37	0.37	1.41
Hothorpe	-0.49	1.31	-0.34	1.74	-0.99
Sibbertoft	-0.63	0.59	1.58	0.25	0.14
Loddington Lone Pine	0.26	-1.66	0.20	-0.49	-0.29
Loddington White Horse	0.53	-1.48	0.01	-0.02	0.91
Loddington School Farm	-0.80	0.26	0.15	-0.21	-0.44
Tilton Digby Farm	1.77	0.65	0.33	-0.32	0.08

Site constraints (linear combinations of constraining variables)

	RDA1	RDA2	PC1	PC2	PC3
Market Harborough	-0.70	0.29	0.09	-0.18	0.08
Lubenham	0.07	0.42	-0.86	-1.12	-0.89
Papillon Ford	-0.44	0.44	-1.10	0.37	1.41
Hothorpe	0.32	-0.13	-0.44	1.74	-0.99
Sibbertoft	-0.49	0.52	1.61	0.25	0.14
Loddington Lone Pine	-0.03	-1.54	0.124	-0.50	-0.30
Loddington White Horse	0.61	-1.01	0.18	-0.02	0.91
Loddington School Farm	-0.95	0.15	0.06	-0.21	-0.44
Tilton Digby Farm	1.61	0.86	0.32	-0.32	0.08

Triplot scores for constraining variables

	RDA1	RDA2	PC1
Total.phosphorus	-0.29	-0.29	0.68
Sediment	-0.64	0.11	-0.09

# Appendix 5.1 All algal species found on the tiles in the mesocosm experiment

Phosphorus level	Sediment level	<i>Achnanthes oblongella</i>	<i>Achnanthidium minutissimum</i>	<i>Amphipleura pellucida</i>	<i>Amphora pediculus</i>	<i>Brachysira vitrea</i>	<i>Caloneis bacillum</i>	<i>Cocconeis placentula</i>	<i>Cymbella helvetica</i>	<i>Cymbella lanceolata</i>	<i>Diatoma vulgare</i>	<i>Encyonema minutum</i>	<i>Encyonema silesiacum</i>	<i>Fragilaria capucina</i>	<i>Fragilaria pinnata</i>	<i>Fragilaria tenera</i>	<i>Fragilaria vaucheriae</i>	<i>Gomphonema angustatum</i>	<i>Gomphonema minutum</i>	<i>Gomphonema olivaceum</i>	<i>Gomphonema parvulum</i>	<i>Karayevia clevei</i>	<i>Luticola mutica</i>	<i>Melosira varians</i>	<i>Navicula capitatoradiata</i>	<i>Navicula cryptotenella</i>	<i>Navicula gregaria</i>	<i>Navicula lanceolata</i>
1	1	50	70	50	44	2	37	32	14	42	12	41	55	125	111	75	0	23	44	9	15	9	26	7	86	38	5	62
2	1	57	76	63	50	13	77	35	0	38	14	69	65	113	100	85	0	25	54	11	25	11	5	12	345	102	89	170
3	1	60	81	64	52	54	75	47	0	23	22	82	68	102	41	88	5	30	83	19	30	19	0	15	505	110	103	186
4	1	98	102	74	72	23	117	48	0	0	34	126	82	90	80	104	76	40	102	23	46	23	0	23	600	221	174	377
5	1	86	104	43	78	35	95	60	0	0	27	149	48	47	42	39	68	67	26	35	51	35	0	35	432	325	93	298
6	1	91	67	48	74	12	23	29	0	0	17	33	52	25	32	25	35	27	22	54	43	44	0	27	150	55	84	110
7	1	37	60	35	32	7	10	25	0	0	3	11	23	0	0	0	0	18	7	3	4	3	0	2	70	25	0	54
8	1	14	45	12	25	0	1	2	0	0	2	1	12	0	0	0	0	3	0	1	2	1	0	0	63	3	0	12
1	2	49	66	48	38	3	13	25	13	43	6	38	48	123	110	69	0	21	38	8	13	7	25	7	82	33	8	60
2	2	63	77	20	58	15	18	26	0	39	8	39	38	101	108	95	73	29	8	10	11	10	5	7	87	40	79	81
3	2	66	79	22	61	33	40	28	0	2	18	42	39	91	97	86	66	31	18	22	24	22	0	24	94	92	71	73
4	2	34	90	25	32	41	48	31	0	0	22	52	42	81	87	76	1	27	22	26	30	26	0	30	84	81	63	65
5	2	36	98	26	34	69	81	34	0	0	37	88	44	42	45	40	0	30	37	44	51	44	0	51	44	42	33	2
6	2	41	123	33	39	85	96	41	0	0	45	105	52	2	0	4	0	68	46	54	61	53	0	63	2	2	2	0
7	2	55	145	39	52	131	148	47	0	0	69	162	55	0	0	0	0	48	71	83	94	82	0	97	0	0	0	0
8	2	100	264	78	95	202	228	78	0	0	106	249	85	0	0	0	0	80	109	102	145	126	0	140	0	0	0	0
1	3	40	65	48	30	3	13	24	12	38	6	34	44	122	110	66	0	17	37	7	12	6	24	4	76	33	19	54
2	3	93	5	31	60	18	18	26	0	30	7	25	40	119	104	92	78	29	10	8	6	8	5	10	100	82	104	76
3	3	98	5	34	63	40	40	28	0	35	16	26	41	107	94	83	70	31	22	18	13	18	0	4	90	62	94	68
4	3	51	5	38	33	49	48	31	0	0	19	66	44	95	83	74	1	27	26	22	15	22	0	26	80	66	83	61
5	3	54	5	40	35	83	81	34	0	0	32	111	46	49	43	38	0	30	44	37	25	37	0	44	42	34	43	2
6	3	54	6	50	44	102	96	41	0	0	39	132	54	1	1	4	0	36	54	46	30	45	0	54	1	0	0	0



Phosphorus level	Sediment level	<i>Achnanthes oblongella</i>	<i>Achnanthidium minutissimum</i>	<i>Amphipleura pellucida</i>	<i>Amphora pediculus</i>	<i>Brachysira vitrea</i>	<i>Caloneis bacillum</i>	<i>Cocconeis placentula</i>	<i>Cymbella helvetica</i>	<i>Cymbella lanceolata</i>	<i>Diatoma vulgare</i>	<i>Encyonema minutum</i>	<i>Encyonema silesiacum</i>	<i>Fragilaria capucina</i>	<i>Fragilaria pinnata</i>	<i>Fragilaria tenera</i>	<i>Fragilaria vaucheriae</i>	<i>Gomphonema angustatum</i>	<i>Gomphonema minutum</i>	<i>Gomphonema olivaceum</i>	<i>Gomphonema parvulum</i>	<i>Karayevia clevei</i>	<i>Luticola mutica</i>	<i>Melosira varians</i>	<i>Navicula capitatoradiata</i>	<i>Navicula cryptotenella</i>	<i>Navicula gregaria</i>	<i>Navicula lanceolata</i>
7	3	81	7	59	53	157	148	47	0	0	60	203	57	0	0	0	0	48	83	71	46	69	0	83	0	0	0	0
8	3	140	13	118	96	202	228	78	0	0	92	170	88	0	0	0	0	80	128	109	71	106	0	128	0	0	0	0
1	4	27	64	44	26	10	13	24	12	34	5	24	42	112	98	65	0	15	36	6	9	6	24	4	75	32	19	53
2	4	63	77	27	58	1	18	26	0	34	1	45	36	120	97	77	49	22	8	7	3	1	6	1	108	119	106	75
3	4	66	79	30	61	2	40	28	0	2	2	2	37	108	87	69	44	23	18	16	7	2	0	2	97	107	95	68
4	4	34	89	33	32	4	48	31	0	21	4	24	40	96	78	61	3	21	22	19	7	4	0	4	120	74	85	60
5	4	36	97	34	34	7	81	34	0	0	12	7	42	50	41	32	0	23	37	32	12	7	0	55	45	49	44	1
6	4	41	121	43	39	56	96	41	0	0	8	12	49	2	1	5	0	28	46	40	14	8	0	9	1	1	0	0
7	4	55	142	51	52	14	148	47	0	0	12	12	52	0	0	0	0	37	71	62	22	12	0	14	0	0	0	0
8	4	100	258	102	95	22	228	78	0	0	18	18	80	0	0	0	0	62	109	95	34	18	0	22	0	0	0	0
1	5	27	61	38	25	11	11	24	6	33	5	22	41	112	75	57	0	12	36	5	5	5	24	2	71	31	25	46
2	5	114	76	31	70	4	15	24	0	31	4	3	36	110	106	113	47	25	11	7	6	3	4	3	104	95	108	68
3	5	120	78	34	74	9	33	26	0	2	9	2	37	99	95	102	42	27	24	16	13	7	0	7	94	86	97	61
4	5	62	88	38	38	11	41	29	0	0	11	4	40	88	85	90	2	23	30	19	15	7	0	7	83	76	87	55
5	5	66	96	40	40	19	69	32	0	15	19	17	42	46	44	47	0	25	51	32	25	12	0	4	143	89	45	2
6	5	75	120	50	45	46	82	39	0	0	23	12	49	2	5	2	0	30	63	40	30	14	0	15	1	0	2	0
7	5	100	141	59	60	35	126	44	0	0	35	12	52	0	0	0	0	40	97	62	46	22	0	23	0	0	0	0
8	5	180	256	118	109	54	194	73	0	0	54	18	80	0	0	0	0	67	149	95	71	34	0	35	0	0	0	0
1	6	16	50	34	25	11	11	24	5	8	4	11	40	109	64	55	0	12	36	5	4	4	8	1	69	25	28	39
2	6	37	64	16	88	15	15	27	0	7	7	19	37	110	108	116	13	34	8	11	7	8	5	11	89	100	80	8
3	6	39	66	18	93	33	33	29	0	0	16	42	38	99	97	104	12	36	18	24	16	18	0	24	80	90	72	7
4	6	20	74	19	48	41	41	32	0	0	19	52	41	88	87	93	3	32	22	30	19	22	0	30	71	80	64	6
5	6	21	80	20	51	69	69	35	0	0	32	88	43	46	45	48	0	35	37	51	32	37	0	51	37	42	33	2

Phosphorus level	Sediment level	<i>Achnanthes oblongella</i>	<i>Achnanthidium minutissimum</i>	<i>Amphipleura pellucida</i>	<i>Amphora pediculus</i>	<i>Brachysira vitrea</i>	<i>Caloneis bacillum</i>	<i>Cocconeis placentula</i>	<i>Cymbella helvetica</i>	<i>Cymbella lanceolata</i>	<i>Diatoma vulgare</i>	<i>Encyonema minutum</i>	<i>Encyonema silesiacum</i>	<i>Fragilaria capucina</i>	<i>Fragilaria pinnata</i>	<i>Fragilaria tenera</i>	<i>Fragilaria vaucheriae</i>	<i>Gomphonema angustatum</i>	<i>Gomphonema minutum</i>	<i>Gomphonema olivaceum</i>	<i>Gomphonema parvulum</i>	<i>Karayevia clevei</i>	<i>Luticola mutica</i>	<i>Melosira varians</i>	<i>Navicula capitatoradiata</i>	<i>Navicula cryptotenella</i>	<i>Navicula gregaria</i>	<i>Navicula lanceolata</i>
6	6	24	100	25	58	85	82	42	0	0	39	15	51	5	4	2	0	42	46	63	39	45	0	3	152	91	1	0
7	6	32	118	29	77	131	126	48	0	0	60	162	54	0	0	0	0	56	71	97	60	69	0	97	0	0	0	0
8	6	58	215	58	140	170	194	80	0	0	92	249	83	0	0	0	0	93	109	149	92	106	0	149	0	0	0	0
1	7	14	44	29	21	13	6	22	2	5	3	1	40	105	59	52	0	8	35	4	2	2	5	1	68	19	30	26
2	7	28	40	10	49	14	8	26	0	2	6	15	28	95	103	105	9	42	6	6	3	6	3	6	86	102	80	3
3	7	29	41	11	52	31	18	28	0	1	13	33	29	86	93	95	8	45	13	13	7	13	0	13	77	92	72	3
4	7	15	46	12	27	37	22	31	0	0	15	41	31	76	82	84	2	40	15	15	7	15	0	15	68	81	64	2
5	7	16	50	13	29	63	37	34	0	0	25	69	33	40	43	44	0	44	32	25	12	25	0	25	35	42	33	0
6	7	18	63	16	33	78	44	41	0	0	30	82	39	2	1	2	0	53	31	31	14	30	0	31	1	1	0	0
7	7	24	74	19	44	120	68	47	0	0	46	12	41	0	0	0	0	71	48	48	22	46	0	1	160	95	0	0
8	7	44	135	38	80	185	105	78	0	0	71	194	63	0	0	0	0	118	74	74	34	71	0	74	0	0	0	0
1	8	12	44	23	21	14	8	18	2	2	1	1	31	101	48	45	0	6	34	4	2	1	5	1	65	16	31	23
2	8	33	45	3	49	19	11	19	0	5	8	17	43	91	98	113	12	37	7	6	3	7	5	6	88	89	109	5
3	8	35	46	3	52	42	24	20	0	0	18	38	44	82	88	102	11	39	16	13	7	16	0	13	79	80	98	5
4	8	18	52	4	27	52	30	23	0	0	22	44	48	73	79	91	1	35	19	15	7	19	0	15	71	71	87	4
5	8	19	57	11	29	88	51	25	0	0	37	74	50	38	41	47	0	38	32	25	12	32	0	25	37	37	45	4
6	8	22	71	11	33	109	61	30	0	0	45	88	59	1	0	2	0	46	40	31	14	39	0	31	1	0	1	0
7	8	29	84	6	44	168	94	34	0	0	69	135	62	0	0	0	0	61	62	48	22	60	0	48	0	0	0	0
8	8	53	153	12	80	202	145	57	0	0	106	8	95	0	0	0	0	102	95	74	34	92	0	0	165	102	0	0

P level	Sediment level	<i>Navicula</i> <i>minima</i>	<i>Navicula</i> <i>subminicula</i>	<i>Navicula</i> <i>tenellula</i>	<i>Navicula</i> <i>teinocostata</i>	<i>Nitzschia</i> <i>amabilis</i>	<i>Nitzschia</i> <i>decussata</i>	<i>Nitzschia</i> <i>ovacilis</i>	<i>Nitzschia palea</i>	<i>Nitzschia</i> <i>palaeosa</i>	<i>Nitzschia</i> <i>nucilla</i>	<i>Nitzschia</i> <i>sinuotecta</i>	<i>Pinnularia</i> <i>capandiculata</i>	<i>Planolithidium</i> <i>delicatulum</i>	<i>Planolithidium</i> <i>foveolatum</i>	<i>Planolithidium</i> <i>lanceolatum</i>	<i>Psammolithidium</i> <i>lanceolatum</i>	<i>Pseudostauroneis</i> <i>brachystriata</i>	<i>Reimera</i> <i>cinnamomea</i>	<i>Rhoicosphenia</i> <i>abbeutata</i>	<i>Stauroneis</i> <i>elliptica</i>	<i>Surirella</i> <i>brachicostis</i>	<i>Synedra acus</i>	<i>Synedra ulna</i>	<i>Anabaena</i>	<i>Oscillatoria</i>	<i>Cladophora</i>	<i>Cosmarium</i>
1	1	0	71	75	93	26	14	38	31	90	0	71	26	19	15	22	7	41	22	11	70	22	41	56	2	5	3	2
2	1	0	205	320	157	67	16	42	34	330	0	215	29	32	25	37	12	69	37	19	78	46	37	50	5	14	5	10
3	1	7	298	352	377	113	18	151	214	350	10	445	35	37	30	38	15	80	43	33	95	82	24	0	13	24	9	18
4	1	0	388	463	582	131	62	158	247	480	17	512	47	85	46	58	23	123	66	35	97	85	0	0	40	24	11	22
5	1	0	210	337	442	202	52	197	178	312	5	410	78	88	71	89	35	77	72	54	36	91	0	0	36	22	10	24
6	1	0	225	331	337	212	42	129	124	100	0	119	20	7	13	18	3	15	8	4	7	18	0	0	26	11	8	25
7	1	0	100	40	122	18	12	33	28	55	0	82	10	7	6	8	2	11	6	1	0	6	0	0	12	7	6	28
8	1	0	40	1	19	5	5	12	6	0	0	19	2	5	0	1	2	2	0	0	0	1	0	0	3	6	5	34
1	2	0	71	72	92	20	16	38	31	82	5	65	24	20	15	22	12	41	24	11	65	19	31	45	5	7	5	5
2	2	0	100	17	30	27	18	63	23	70	1	21	32	1	4	3	11	20	6	1	95	13	19	32	3	10	6	2
3	2	0	90	38	32	28	26	24	24	63	2	22	34	1	9	7	2	21	6	2	86	29	0	0	0	0	0	0
4	2	0	80	44	78	71	29	27	27	56	2	56	30	4	11	7	4	52	15	4	76	34	0	0	0	0	0	0
5	2	0	5	74	132	120	32	30	30	1	0	95	33	7	19	34	7	88	25	7	40	57	0	0	0	0	0	0
6	2	0	0	88	153	140	39	36	36	0	0	110	40	8	23	54	9	102	37	9	2	70	0	0	0	0	0	0
7	2	0	0	135	235	215	44	41	41	0	0	169	53	22	35	18	14	157	45	14	0	108	0	0	0	0	0	0
8	2	0	0	208	251	210	73	68	68	0	0	260	88	18	54	28	22	242	69	22	0	166	0	0	0	0	0	0

P level	Sediment level	<i>Navicula</i> <i>minima</i>	<i>Navicula</i> <i>subminicula</i>	<i>Navicula</i> <i>tenelloides</i>	<i>Navicula</i> <i>teuanostrata</i>	<i>Nitzschia</i> <i>amabilis</i>	<i>Nitzschia</i> <i>dissecta</i>	<i>Nitzschia</i> <i>ovacilis</i>	<i>Nitzschia palea</i>	<i>Nitzschia</i> <i>palaeosa</i>	<i>Nitzschia</i> <i>pusilla</i>	<i>Nitzschia</i> <i>sinuatiloba</i>	<i>Pinnularia</i> <i>capandiculata</i>	<i>Planolithidium</i> <i>delicatulum</i>	<i>Planolithidium</i> <i>foveolaticornis</i>	<i>Planolithidium</i> <i>lanceolatum</i>	<i>Psammolithidium</i> <i>lanceolatum</i>	<i>Pseudostauroneis</i> <i>brachystriata</i>	<i>Reimera</i> <i>cinnamomea</i>	<i>Rhoicosphenia</i> <i>abbeutata</i>	<i>Stauroneis</i> <i>elliptica</i>	<i>Surirella</i> <i>brachicornis</i>	<i>Synedra acus</i>	<i>Synedra ulna</i>	<i>Anabaena</i>	<i>Oscillatoria</i>	<i>Cladophora</i>	<i>Cosmarium</i>
1	3	1	70	62	91	19	39	32	29	80	16	55	23	20	14	20	10	41	21	12	64	8	24	42	11	9	5	1
2	3	0	92	17	31	28	17	27	28	91	2	21	32	0	3	1	7	20	1	3	112	11	22	38	0	0	0	0
3	3	0	83	38	33	29	19	67	30	82	4	22	34	0	7	2	16	21	1	7	101	24	7	24	4	15	8	5
4	3	0	74	44	82	75	21	32	34	73	1	56	30	0	7	4	19	52	4	7	89	30	0	0	0	0	0	0
5	3	0	1	74	139	127	23	35	37	2	0	95	33	0	12	34	32	88	7	12	46	51	0	0	0	0	0	0
6	3	0	0	88	162	148	28	42	45	0	0	110	40	10	36	54	40	102	41	23	2	63	0	0	0	0	0	0
7	3	0	0	135	249	228	32	48	51	0	0	169	53	13	22	11	62	157	12	23	0	97	0	0	0	0	0	0
8	3	0	0	208	245	142	53	80	85	0	0	260	88	0	34	24	95	242	18	35	0	149	0	0	0	0	0	0
1	4	5	69	62	90	15	41	27	29	73	18	45	23	15	11	19	9	31	15	11	61	12	25	35	14	15	7	4
2	4	0	88	17	28	20	16	23	12	86	5	21	29	1	1	3	0	15	7	1	109	3	23	32	0	0	0	0
3	4	0	79	38	29	21	17	24	13	77	1	22	31	1	2	7	0	16	7	2	98	7	1	2	0	0	0	0
4	4	0	71	44	75	52	133	87	14	68	1	56	27	4	4	7	0	41	19	4	87	7	0	12	8	16	12	4
5	4	0	1	74	127	88	21	30	15	2	0	95	30	7	7	30	0	69	32	7	45	55	0	0	0	0	0	0
6	4	0	0	88	148	102	25	36	18	0	0	110	36	11	25	45	19	80	37	45	1	15	0	0	0	0	0	0
7	4	0	0	135	228	157	28	41	20	0	0	169	48	54	12	18	0	123	57	14	0	23	0	0	0	0	0	0
8	4	0	0	208	252	242	47	68	33	0	0	260	80	18	18	28	75	189	88	22	0	35	0	0	0	0	0	0

P level	Sediment level	<i>Navicula</i> <i>minima</i>	<i>Navicula</i> <i>subminicula</i>	<i>Navicula</i> <i>tenellulae</i>	<i>Navicula</i> <i>teinocutata</i>	<i>Nitzschia</i> <i>amabilis</i>	<i>Nitzschia</i> <i>dissecta</i>	<i>Nitzschia</i> <i>ovacilis</i>	<i>Nitzschia palea</i>	<i>Nitzschia</i> <i>palaeosa</i>	<i>Nitzschia</i> <i>pusilla</i>	<i>Nitzschia</i> <i>sinuotecta</i>	<i>Pinnularia</i> <i>capandicula</i>	<i>Planolithidium</i> <i>delicatulum</i>	<i>Planolithidium</i> <i>foveolaticornis</i>	<i>Planolithidium</i> <i>lanceolatum</i>	<i>Psammolithidium</i> <i>lanceolatum</i>	<i>Pseudostauroneis</i> <i>braconistata</i>	<i>Reimera</i> <i>cinnata</i>	<i>Rhoicosphenia</i> <i>abbeutata</i>	<i>Stauroneis</i> <i>collata</i>	<i>Surirella</i> <i>bebbianii</i>	<i>Synedra acus</i>	<i>Synedra ulna</i>	<i>Anabaena</i>	<i>Osillatoria</i>	<i>Cladophora</i>	<i>Cosmarium</i>
1	5	11	69	62	85	14	51	25	26	71	18	25	22	8	3	15	6	27	11	2	59	11	21	32	19	14	2	5
2	5	0	91	15	20	21	12	23	22	108	5	21	33	11	3	7	8	24	15	3	116	1	19	29	0	0	0	0
3	5	0	82	33	21	22	13	24	23	97	0	22	35	12	7	16	18	25	16	7	104	2	1	2	0	0	0	0
4	5	0	73	41	52	56	14	27	26	87	0	56	31	30	7	18	22	63	41	7	93	4	0	0	0	0	0	0
5	5	0	1	69	88	95	100	30	29	1	0	95	34	51	12	51	37	106	69	12	48	74	0	0	9	19	15	6
6	5	0	0	82	102	110	18	36	35	0	0	110	41	59	14	54	46	123	80	15	1	46	0	0	0	0	0	0
7	5	0	0	126	157	169	20	41	40	0	0	169	55	91	22	48	71	189	123	23	0	14	0	0	0	0	0	0
8	5	0	0	194	242	260	33	68	67	0	0	260	92	140	34	74	109	208	189	35	0	22	0	0	0	0	0	0
1	6	13	68	61	84	9	22	21	21	71	9	13	21	1	2	2	5	25	13	7	62	9	11	11	22	16	1	6
2	6	0	91	13	11	13	42	42	44	110	5	18	30	45	35	35	28	21	46	24	101	13	10	10	0	0	0	0
3	6	0	82	29	12	14	45	45	47	99	0	19	32	47	78	78	62	22	48	53	91	29	0	0	0	0	0	0
4	6	0	73	33	30	34	51	51	53	88	1	49	29	119	92	92	75	56	123	63	81	34	0	0	0	0	0	0
5	6	0	1	56	51	57	56	56	58	1	0	83	32	201	155	155	127	95	208	106	42	57	0	0	0	0	0	0
6	6	0	0	67	59	66	67	29	70	0	0	97	39	234	185	158	157	110	242	131	1	70	0	0	11	25	20	7
7	6	0	0	103	91	102	76	76	80	0	0	149	52	360	285	243	242	169	372	202	0	108	0	0	0	0	0	0
8	6	0	0	158	140	157	127	127	133	0	0	229	87	554	352	374	104	172	572	295	0	166	0	0	0	0	0	0

P level	Sediment level	<i>Navicula minima</i>	<i>Navicula subminicula</i>	<i>Navicula tenuiloides</i>	<i>Navicula tenuicostata</i>	<i>Nitzschia amabilis</i>	<i>Nitzschia delicatula</i>	<i>Nitzschia gracilis</i>	<i>Nitzschia palea</i>	<i>Nitzschia paleacea</i>	<i>Nitzschia pusilla</i>	<i>Nitzschia sinuotuberosa</i>	<i>Pinnularia conradii</i>	<i>Planolithidium delicatulum</i>	<i>Planolithidium fenestraticum</i>	<i>Planolithidium lanceolatum</i>	<i>Psammolithidium lanceolatum</i>	<i>Pseudostauroneis brevisetacea</i>	<i>Reimera cinnamomea</i>	<i>Rhoicosphenia abbreviata</i>	<i>Stauroneis elliptica</i>	<i>Surirella brevisetacea</i>	<i>Synedra acus</i>	<i>Synedra ulna</i>	<i>Anabaena</i>	<i>Oscillatoria</i>	<i>Cladophora</i>	<i>Cosmarium</i>
1	7	14	66	59	78	2	15	21	20	65	7	12	21	1	2	2	1	12	12	5	59	7	7	6	21	19	4	7
2	7	0	86	3	21	3	55	34	42	93	0	11	29	42	29	33	21	3	45	21	80	10	6	5	0	0	0	0
3	7	0	77	7	22	3	59	36	45	84	0	12	31	44	64	73	47	3	47	47	72	22	1	1	0	0	0	0
4	7	0	69	7	56	7	66	42	51	74	1	30	27	112	78	89	56	7	119	56	64	26	0	0	0	0	0	0
5	7	0	1	12	95	12	73	46	56	2	0	51	30	189	132	150	95	12	201	95	33	69	0	0	0	0	0	0
6	7	0	0	14	110	14	88	55	67	0	0	59	36	220	157	153	117	14	234	117	1	54	0	0	0	0	0	0
7	7	0	0	22	169	34	24	27	76	0	0	91	48	338	242	235	180	22	360	180	0	83	0	0	15	26	21	8
8	7	0	0	34	152	34	167	105	127	0	0	140	80	520	256	362	242	34	554	150	0	128	0	0	0	0	0	0
1	8	22	61	52	71	2	11	21	11	58	5	8	21	0	1	1	0	9	6	5	54	2	4	11	28	32	2	6
2	8	0	82	17	1	3	44	29	31	91	2	17	29	35	33	36	31	13	37	15	49	17	4	10	0	0	0	0
3	8	0	74	38	1	3	47	31	33	82	1	18	31	37	73	80	69	14	39	33	44	38	1	0	0	0	0	0
4	8	0	66	44	4	7	53	35	38	73	1	45	27	93	89	96	82	34	97	41	39	45	0	0	0	0	0	0
5	8	0	1	74	7	12	58	38	42	2	0	76	30	157	150	162	139	57	164	69	20	76	0	0	0	0	0	0
6	8	0	0	88	8	14	70	46	51	0	0	88	36	183	179	165	172	66	191	85	1	94	0	0	0	0	0	0
7	8	0	0	135	12	22	80	52	58	0	0	135	48	282	275	254	265	102	294	131	0	145	0	0	0	0	0	0
8	8	0	0	208	18	22	15	23	97	0	0	208	80	434	423	391	198	157	362	202	0	223	0	0	18	32	25	14

## Appendix 5.2 The treatment explanation for the triplot RDA for the mesocosm experiment

### Treatment explanation for the simple responses Phosphorus (Fig. 5.2, 5.3, 5.4, 5.5)

T	Phosphorus mgL <sup>-1</sup> P	Sediment mg.cm <sup>-1</sup>
T 1	0.257	1.246
T 2	0.462	1.246
T 3	0.832	1.246
T 4	1.492	1.246
T 5	2.686	1.246
T 6	4.835	1.246
T 7	8.703	1.246
T 8	15.665	1.246

### T explanation for the Complex responses (Fig. 5.6, 5.7, 5.8, 5.9)

T	Phosphorus mgL <sup>-1</sup> P	Sediment mg.cm <sup>-1</sup>
T 1	0.257	1.246
T 2	0.257	2.243
T 3	0.257	4.037
T 4	0.257	7.267
T 5	0.257	13.081
T 6	0.257	23.546
T 7	0.257	42.389
T 8	0.257	76.301

### T explanation for the Complex responses (Fig. 5.10, 5.11, 5.12, 5.13)

T	Phosphorus mgL <sup>-1</sup> P	Sediment mg.cm <sup>-1</sup>
T 1	0.257	1.246
T 2	0.257	1.246
T 3	0.257	2.243
T 4	0.257	4.037
T 5	0.257	7.267
T 6	0.257	13.081
T 7	0.257	23.546
T 8	0.257	42.389
T 9	0.257	76.301
T 10	0.462	1.246
T 11	0.832	1.246
T 12	1.492	1.246
T 13	2.686	1.246
T 14	4.835	1.246
T 15	8.703	1.246
T 16	15.665	1.246

**T explanation for the Complex responses (Fig. 5.14, 5.15, 5.16, 5.17)**

T	Phosphorus mgL-1 P	Sediment mg.cm <sup>-1</sup>
T 1	0.462	4.037
T 2	0.462	7.267
T 3	0.462	13.081
T 4	0.462	23.546
T 5	0.462	42.389
T 6	0.462	76.301
T 7	0.832	2.243
T 8	0.832	7.267
T 9	0.832	13.081
T 10	0.832	23.546
T 11	0.832	42.389
T 12	0.832	76.301
T 13	1.492	2.243
T 14	1.492	4.037
T 15	1.492	13.081
T 16	1.492	23.546
T 17	1.492	42.389
T 18	1.492	76.301
T 19	2.686	2.243
T 20	2.686	4.037
T 21	2.686	7.267
T 22	2.686	23.546
T 23	2.686	42.389
T 24	2.686	76.301
T 25	4.835	2.243
T 26	4.835	4.037
T 27	4.835	7.267
T 28	4.835	13.081
T 29	4.835	42.389
T 30	4.835	76.301
T 31	8.703	2.243
T 32	8.703	4.037
T 33	8.703	7.267
T 34	8.703	13.081
T 35	8.703	23.546
T 36	8.703	76.301
T 37	15.665	2.243
T 38	15.665	4.037
T 39	15.665	7.267
T 40	15.665	13.081
T 41	15.665	23.546
T 42	15.665	42.389



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