Contrasts in stream water chemistry impact aquatic ecosystem functioning in an agricultural landscape

Moragh Wallis Stirling

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Department of Geography and Environmental Science

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Signed

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Abstract

Given the importance of freshwater ecosystems to human health and wellbeing, the resilience of aquatic communities to pollution from a variety of sources is of increasing concern. Current indices concentrate on structural measures to define stream health. These include community assemblages, in-stream water chemistry, and hydrological impairment through physical modifications. However, ecosystem services provided by freshwater communities rely on the underlying biogeochemical cycles that are a function of metabolic processes. At present, these are not routinely used in assessments of ecological status.

A paired sub-catchment approach was used to study the effects of different land management practices on in-stream water chemistry, and their consequences to aquatic functional integrity in an agricultural landscape. The study provided an opportunity to assess the potential for ecosystem functional measures to complement the structural measures that are used to define impact on aquatic communities.

High resolution analysis of the nutrient chemistry within two study reaches underpinned comparisons of community aerobic respiration, greenhouse gas transfer across the sediment-water interface and macro-invertebrate mediated processing of organic matter. This programme of measures identified clear differences between the study sub-catchments. It revealed that the management of animal waste, and control on the delivery of fine sediments to a watercourse, were key influences on in-stream functional integrity. The delivery of inorganic nutrients as a result of fertiliser application was also evident. However, the significance of this signal was masked by the overriding effect of high loads of organically loaded fine sediments and low flow in the Priors Farm reach.

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List of sampling sites and associated abbreviations.

These abbreviations are used throughout the thesis and refer to the sites described in chapter 4 and illustrated on page 69.

Daily sampling sites:

Cool's Cottage downstream	CCDS
Priors farm downstream	PFDS

Weekly sampling sites:

Cool's Cottage headwater	CCHW
Cool's Cottage upstream	CCUS
Cool's Cottage piped spring	CCSP
Priors Farm, tributary 1	PF1
Priors Farm, tributary 2	PF2
Priors Farm, tributary 3	PF3
Priors Farm upstream	PFUS

Occasional sites:

Cool's Cottage headspring	CC1
Cool's Cottage Ruddlemoor	CCRU
Cool's Cottage sinkhole	
Cool's Cottage woodland edge	
Cool's Cottage culvert (road drain)	
Priors Farm confluence	PF conf

Abbreviations.

List of abbreviations, commonly used throughout the thesis:

Chl-a	Chlorophyll-a
Defra	Department of the environment, food and rural affairs
DWPA	Diffuse Water Pollution from Agriculture
DOC	Dissolved Oxygen
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
DTC	Demonstration Test Catchments
EA	Environment Agency
ER	Community Aerobic Respiration
EU	European Union
ESS	Environmental Stewardship Schemes
GPP	Gross Primary Production
NEP	Net Ecosystem Production
NH_4-N	Total ammonium Nitrogen
PON	Particulate Organic Nitrogen
PP	Particulate Phosphorus
SRP	Soluble Reactive Phosphorus
SUP	Soluble Unreactive Phosphorus
TDN	Total Dissolved Nitrogen
TDP	Total Dissolved Phosphorus
TN	Total Nitrogen
TON	Total Oxidised Nitrogen
WFD	Water Framework Directive

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Chapter 1. **Introduction.**

Surface and groundwater sources represent an essential natural resource to humans and deliver a range of benefits. These include: the provision of clean water for drinking, and irrigation; creation of habitats that support food production and biodiversity; processing organic matter and other nutrients such as nitrogen (N) and phosphorus (P); climate regulation by acting as both a source and sink for greenhouse gases; facilitating the development of large communities, through the transport of materials and the removal of waste products; and promoting wellbeing and good health through recreation (The Ramsar Convention, 1971). These benefits have been formally recognised in modern welfare economics and are termed 'ecosystem services'. Ecosystem services are driven by underlying biogeochemical processes and are dependent on a healthy, functioning ecology. Globally, pressures from population growth and increasing economic development have led to the degradation and loss of inland waters and it is predicted that the need for the ecosystem services provided by them, such as freshwater, denitrification and protection against floods and storms, will increase, while their capacity to provide them declines (The Millennium Ecosystem Assessment, 2005). In the second half of the 20th century, many surface waters in the UK were polluted resulting in habitat degradation and a decline in the provision of ecosystem services (Durand et al., 2011). Considerable resources were spent on licencing, monitoring and controlling point sources of pollution such as industrial effluents and sewage treatment work outfalls. Despite a noticeable reduction in the concentrations of some chemical pollutants, river ecosystems are still impacted with higher than expected concentrations of nutrients and sediments (Walling et al., 2003; 2008; Ballantine et al., 2009; Collins et al., 2009). With increasing controls on point source pollution from wastewater treatment works and industrial effluents, attention has turned to sources of diffuse pollution. Key sources include domestic sewage systems that are unconnected to the mains network (Bowes et al., 2005; Neal et al., 2000; May et al., 2011), runoff from roads or track ways (Collins et al., 2007; Collins et al., 2010b), and agriculture (Heathwaite and Johnes, 1996; Walling et al., 2003; Walling et al., 2008; Johnes et al., 2007; Stromqvist et al., 2008). Increasing demand for new housing, the rise in alternative land use such as solar farms and the drive towards ever cheaper food that financially impacts producers, result in mounting pressure to increase the intensity of production on the remaining agricultural land. Increases in the delivery of fine sediments, as well as increases in the flux of N, P and organic matter to watercourses, are some of the consequences arising from this increased intensification (Neal et al., 2002; Prior and Johnes, 2002; Evans et al., 2004; Worrall and Burt, 2007a; Worrall and Burt, 2007b; Jarvie et al., 2008; Collins et al., 2009) While it is straightforward to measure the concentrations of pollutants being discharged from a point source, it is more difficult to quantify inputs from diffuse sources. A variety of modelling approaches can be used to estimate inputs from a range of land use types. For example, The National Export Coefficient uses a range of data including land use, population density, livestock numbers, fertiliser application, and atmospheric inputs to predict N and P delivery to watercourses, on a sub-catchment to regional and national scale (Johnes and Hodgkinson, 1998; Johnes et al., 2007). However, the most frequently used measure of diffuse pollution remains its effects on the environment. The consequences of increased fertiliser application following the Second World War are now well recognised, and nutrient enrichment is a key metric of impacted streams. For many years, P was seen as the limiting nutrient in freshwater aquatic systems. A framework for classifying stream status, ranging from low nutrient (oligotrophic) to enriched (eutrophic or hyper eutrophic), was developed, based on total phosphorus (TP) concentrations (Vollenweider and Kerekes, 1980) and legislation was put in place to control phosphorus in water courses. Limits on the concentration of nitrate in drinking water were introduced as a response to health concerns and led to controls on the use of N fertilisers in vulnerable zones. The Nitrate Directive in 1991 (91/676/EEC) and Groundwater Directive in 2006 (2006/118/EC) limited nitrate concentrations in groundwaters to 50 mgl^{-1} – well above the suggested maximum concentration for TN of 2 mg l^{-1} proposed as the target for surface waters (Durand et al., 2011). However, there is, as yet, no environmental legislation to control N in surface waters. An additional problem arises from the dependence of many legislative and managerial policies on the monitoring of dissolved inorganic nutrients, missing the importance of other, potentially biologically active forms such as dissolved organic N and P (DON, DOP), and those associated with particulate matter (PON, PP), either suspended in the water column or deposited on the stream bed. The impact of fine sediments is of increasing concern, not only due to the transport of associated nutrients, but the extensive smothering of benthic sediments reduces oxygen penetration into the stream bed and has severe consequences on stream ecology (Nogaro et al., 2010) (Jones et al., 2009). Cementation of gravels, for example, causes a lack of oxygen reaching fish spawning grounds (redds), including those of commercially important species such as salmon and trout (Collins et al., 2013; Pattison et al., 2014; Sear et al., 2014). Another, less explored consequence of fine sediment deposition may be an increasing dependence on anaerobic pathways to process organic matter in the stream bed, accompanied by an increase in the production of greenhouse gases (Pina-Ochoa and Alvarez-Cobelas, 2006; Pretty et al., 2006; Sanders et al., 2007; Jones et al., 2008; Trimmer et al., 2010).

The European Union Water Framework Directive (WFD) (2000/60/EC) brought together many existing regulatory frameworks under one umbrella. Indicators of status adopted by the WFD are based on structural measures (Jones et al., 2010) These include chemical water quality; the community composition of macro-invertebrates, diatoms, macrophytes and fish; and hydrological impairment as a result of physical modifications. The extent to which the functional integrity of surface waters is impacted by multiple stressors is, so far, not included in standard assessments of ecological status. However, two key ecosystem processes, leaf litter degradation and aerobic respiration have been used to study the effects of broad land use change (e.g. deforestation, urbanisation, agricultural intensity) on ecosystem health (Lecerf et al., 2006; Baldy et al., 2007; McKie and Malmqvist, 2009; Young and Collier, 2009; Clapcott et al., 2010; Imberger et al., 2010) and frameworks for categorising impact based on these functional indicators have been proposed (Gessner and Chauvet, 2002; Young et al., 2008). The extent to which these measures of ecosystem function are sensitive to the effects of different land management practices within a land use category is less clear (Magbanua et al., 2010) but they represent a potentially powerful tool to monitor changes brought about by increased anthropogenic impact, or by targeted mitigation measures designed to counteract its effects.

This research programme used a paired catchment approach to compare measures of ecosystem function in two headwater streams flowing through agricultural catchments with similar land use and surface geology, but different land management practices.

The research was designed to address the following research questions:

1.1. Research questions

RQ1. Are there differences in water quality between two headwater streams flowing through agricultural catchments with similar land use and surface geology, but different land management practices?

RQ2. Can differences in water chemistry or sediment characteristics be linked to agricultural management practices?

RQ3. Are there differences in short term metabolic processes between two headwater streams flowing through agricultural catchments with similar land use and surface geology, but different land management practices?

RQ4. Are there differences in time integrated ecosystem processes between two headwater streams flowing through agricultural catchments with similar land use and surface geology, but different land management practices?

RQ5. Do the process measurements used to compare the study reaches represent potential methods to monitor changes to functional integrity, following modifications to land management practices?

1.2. Thesis structure

The research programme was embedded within the Demonstration Test Catchment (DTC) research platform funded by Defra, and focussed on the River Sem sub-catchment of the Hampshire Avon. The River Sem is a tributary of the River Nadder and is atypical of the Hampshire Avon being mainly underlain by low permeability Gault clay rather than the Chalk that is characteristic of the majority of the catchment. The two study areas, Priors

Farm and Cool’s Cottage, are small agricultural sub-catchments that are predominantly used for stock grazing. Their proximity (the outlets to the sub-catchments were within 1 km of each other) ensured a high degree of similarity in meteorological conditions throughout the study period, October 2011 to June 2014.

Chapter 2 presents an overview of current understanding on the functioning of aquatic systems and the pressures on them resulting from human activities. Considerations of the importance of nutrient balance (stoichiometry) and the effects of disrupting natural cycles are linked to the research questions outlined in section 1.2.

Chapter 3 provides a detailed comparison of the two study sub-catchments, providing evidence for both similarities and differences resulting from intrinsic characteristics and from differences in land management practices. These are discussed in relation to their impact on the hydrology, in-stream nutrient chemistry and sediment characteristics of the study reaches. **Chapter 4** describes the approach used to address the research questions. It details the methodologies employed for sample collection, processing and chemical analyses, and the protocols employed during the incubations designed to measure ecosystem processes.

The results are presented in the following three chapters. **Chapter 5** provides a detailed, high resolution description of the in-stream nutrient chemistry of the two study reaches. A combination of spatial and temporal variation in nutrient chemistry defined differences in the sources of nutrients between the sub-catchments and, together with chapter 3, addresses the first research questions, RQ1 and 2.

Chapter 6 addresses RQ3. Short term metabolic processes were measured to determine whether the differences in in-stream environments, identified in chapters 3 and 5, impacted on rates of community aerobic respiration, or on the preferential utilisation of different nutrient fractions during incubations. Photosynthetic primary production and the relationship between it and aerobic respiration were also investigated as a possible indicator of contrasting trophic status. Finally, in chapter 6, considerations on the potential implications of land management for greenhouse gas production by anaerobic processes were explored, by measuring *in situ* accumulation of methane (CH_4) nitrous oxide (N_2O) and carbon dioxide (CO_2) across the sediment-water interface during closed incubations.

Chapter 7 addresses RQ4. In order to establish the ecosystem functional response to environmental conditions integrated over time, the rates of macro-invertebrate mediated processes, namely leaf litter degradation and herbivory, were measured and compared between the two study reaches. The association between process rates and observed macro-invertebrate community structure was explored, and the relationship between macro-invertebrate structure and function and water quality integrated over a variety of time scales are discussed. The final chapter, **Chapter 8**, is a synopsis of the research project and presents an overview of the impact of different management practices on key ecosystem processes. The thesis concludes with an evaluation of the applicability of each of the functional measures adopted in the project to routine assessments of stream health.

Chapter 2. **Pressures and controls on stream health.**

2.1. Agricultural sources of diffuse pollution.

Diffuse Water Pollution from Agriculture is considered to be a major contributor of non-point source contaminants to surface waters in rural landscapes. Harmful anthropogenic inputs associated with agriculture include high levels of nutrients, organic carbon in the form of animal wastes, pesticide and herbicide applications and disproportionate sediment input. Recognition of the impact that changes in agricultural practice after the 2nd world war have had on groundwater stores has focussed attention on ‘fertiliser’ chemicals, nitrogen and phosphorus in particular. More recently, the impacts of high loads of fine sediments are being recognised as having serious ecological consequences through reducing available oxygen and cementing the loose gravel substrate required for successful fish egg survival. Runoff from fields and stock trackways that ends up on roads are a major source of sediments, as roads provide an efficient conduit between fields and watercourses, (Collins and Walling, 2007; Walling et al., 2008; Ballantine et al., 2009; Collins et al., 2010b).

2.2. Legislative and economic drivers, Demonstration Test Catchments and some existing mitigation measures.

The legislative framework behind water quality improvements in the 20th century have been well documented in many publications. Much of the early legislation was driven by concerns for human health eg. the EU Nitrates Directive (91/676/EEC) and EU Drinking Water Directive (98/83/EC). Environmental priorities were included in the EU Habitats Directive (92/43/EEC) and protected sites legislation. The cost to Water Companies of meeting EU drinking water standards and strict regulations on mitigation of environmental damage has encouraged their participation in research and implementation of strategies designed to improve the quality and sustainability of water resources.

Alongside the economic cost to water companies, the major driving force behind the continued clean-up of surface waters is the EU Water Framework Directive (WFD) that incorporates many of the previous directives and is both ambitious and proactive. One of its great strengths has been to provide not only clear targets for improvement but also a specific time frame for their implementation. The WFD has prompted a large scale re-evaluation of the anthropogenic consequences to the ecosystem services provided by surface waters and increasingly, the necessarily less well documented influence of diffuse pollution is being recognised as a major factor in the changing character of rivers and groundwaters.

Common Agricultural Policy (CAP) grants have changed their emphasis from supporting wholly food security driven measures, following the Second World War, to a balance between production and environmental priorities. In the UK, Environmental Stewardship Schemes (ESS) recognise the contribution the farming community makes to maintaining the countryside and aim to encourage environmentally sympathetic farming practices, particularly in environmentally sensitive areas. To this end, a team of Catchment Sensitive Farming officers provide advice on practical issues such as matching crop selection to soil and climate types and on the financial incentives available to implement these mitigation measures.

In the light of recent pressure to reduce CAP grants to farmers and the resurgence of concerns over food security, the need to justify ESS grants has become increasingly urgent. The first step to assigning a cost benefit analysis to these payments is to define the specific benefits expected from the interventions designed to reduce DWPA and to determine their efficacy. In the UK, the Department of Environment Food and Rural affairs (Defra) have commissioned a comprehensive research programme in three representative catchments to study the responses of the aquatic environment to the on farm mitigation measures supported by the ESS payments. The Demonstration Test Catchments (DTC) have been chosen to represent a highland catchment, the River Eden in Cumbria; a lowland catchment, the River Wensum in East Anglia and an intermediate, chalk catchment, the Hampshire Avon in Wiltshire and Hampshire.

The majority of mitigation measures concentrate on reducing the delivery of DWPA to water bodies in a variety of ways:

- In cultivated fields, the timing of agrochemical application to avoid excessive loss due to heavy rainfall immediately after application is a key and cost effective measure, benefitting both the farmer who avoids loss of expensive chemicals through leaching (particularly nitrogen) or association with soil particles, (particularly phosphorus and pesticides).
- Careful choice of crops that optimise the farm soil type and reduce the time bare soil is exposed to erosive weather also benefits both farmer and the environment through a reduction in erosion of productive topsoil, although some compensation may be required to offset loss of production of high yield cash crops such as maize and rape.
- Ploughing in a direction that increases downslope overland flow, or that leads directly onto a track or roadway can significantly increase soil inputs to water bodies, as roads often provide a direct and uninterrupted conduit for sediment transport

(Collins et al., 1997; Collins et al., 2007; Collins et al., 2010a). Changing the direction of cultivation can make a significant contribution to reducing the loss of soil to watercourses.

- Minimal cultivation methods such as reduced surface tillage and timing the use of heavy machinery to avoid compaction are all mutually beneficial measures.

For dairy and livestock farmers, measures that aim to reduce soil compaction through a reduction in stocking levels are examples of strategies that are unlikely to benefit the farmer and therefore unlikely to have significant voluntary uptake, although work by Walling et al. (2003; 2008) has shown that soil losses caused by compaction through overstocking is a significant proportion of total soil loss within a catchment (Collins et al., 2009; Collins et al., 2010a).

The provision of riverbank fencing and in-field drinking troughs to reduce bankside erosion caused by stock drinking directly from the river are examples of currently funded mitigation schemes. These also reduce the direct input of organic carbon and nitrogen in the form of animal wastes. Interruption of the pathway for waste slurries from farmyards and track ways by drainage and treatment systems or by roofing open yards to reduce surface runoff are also examples of targeted ESS grants.

The provision of ponds to collect runoff and ‘buffer strips’ alongside water courses are further examples of the concentration of effort dedicated to reducing the delivery of contaminants, particularly phosphate and pesticides that are associated with soil particles eroded from cultivated land. Methods aimed at enhancing the receptor ecosystem’s ability to process DWPA are less common. The development of wetland areas and wet meadows has primarily been seen as a flood defence mechanism. However, by increasing retention time, these areas allow further local processing of allocthonous material, potentially reducing the input of organic matter and agrochemicals to downstream reaches. Recent work highlights the need for appropriate monitoring and management of these schemes to account for ‘saturation’ and subsequent leaching to watercourses. Resistance in take up of these options may be based on both cost of implementation and the loss of apparently productive riparian land.

2.3. Physical controls on nutrients.

In healthy aquatic systems, the ecosystem balance is maintained through a limited supply of the nutrients essential for growth, predominantly carbon, nitrogen and phosphorus. ‘Natural’ concentrations are determined by catchment and aquifer geology and hydrology. Understanding the mechanisms that control the bioavailability of limiting nutrients underpins efforts to improve water quality in impacted systems (Ensign and Doyle, 2006)

The uptake and processing of nutrients and their fate as recycled or stored are controlled by both biological and geophysical processes, (Battin et al., 2003; Alexander et al., 2007; Battin et al., 2008; Alexander et al., 2009) Physical processes include the photodegradation of organic matter (Amado et al., 2006; Alexander et al., 2009) and temperature. Geomorphology influences the flow regimes that in turn dictate physical states such as shear stress, retention time and substrate characteristics. Turbulence affects the availability of oxygen for chemical reactions. Redox potential, and the presence of chemicals such as iron and carbonate control the equilibrium point of available nutrients (Evans and Johnes, 2004; Evans et al., 2004; Withers and Jarvie, 2008; Durand et al., 2011). A more detailed discussion of the nitrogen and phosphorus cycles is given below, section 1.1.8.

Biological processes exert strong control on the fate of nutrients and their delivery to downstream reaches. This biological ecosystem service is highly influenced by the community structure that develops in response to the physical and chemical conditions in the river, (Battin et al., 2008; Singer et al., 2010; Clapcott and Barmuta, 2010; Nogaro et al., 2010).

2.4. Biological controls on nutrients.

The factors influencing community development track the dominant physical controls: light, temperature, nutrient status, oxygenation, flow and shear stress, retention time, substrate material, sediment quantity and particle size and the delivery of allocthonous organic matter. The link between biological efficiency and physicochemical conditions result in both positive and negative feedback controls on nutrient cycling and availability (Findlay and Sinsabaugh, 2006; Fukuda et al., 2006; Clapcott and Barmuta, 2010; Covino et al., 2010). Distinct ecosystems within the stream are the seston and the benthic communities. For nutrient transformations, one critical distinction is the proximity of individual components to each other. Biofilms, on substrate (epilithic), woody debris (epixylic) and on macrophytes (epiphytic), provide a level of commensalism unavailable to free living organisms. The three dimensional arrangement of organisms within the film optimises the availability of nutrients to community members (Fukuda et al., 2006; Besemer et al., 2009; Bouletreau et al., 2010). Benthic, interstitial and epixylic films are the most likely to provide medium to long term storage of macronutrients, being a captive food source for grazing macroinvertebrates and fish. Aggregates within the water column provide an additional framework for biofilm development. Their retention within the reach is dependent on filter feeders. Free living organisms have the advantage of immediate availability of labile nutrients, whereas the biofilm communities may be limited by the rate of diffusion of key nutrients through the slime itself (Flemming and Wingender, 2010).

2.4.1. **Stoichiometry.**

In 1934, a key observation was made when Redfield (1934), found that the balance of major nutrients in the oceans stayed remarkably constant. This ratio, named after Redfield, has strong implications for the regulation of biogeochemistry and prompted further research into the principles behind nutrient balance and its repercussions. While the Redfield ratio has been found to be less than universal, the principle of co-dependence stands. The processing of carbon, nitrogen and phosphorus are intimately linked, making it essential to consider their delivery, transport and fate together.

In the majority of heterotrophs, the ratios of carbon (C), nitrogen (N), and phosphorus (P) are strictly maintained as a consequence of their body structure (Schade et al., 2011). The requirement for both energy and materials must be met from their food source and are, therefore, coupled. Excess nutrients within a food source, which cannot be organically bound with carbon, are excreted and recycled into the environment, potentially driving alternative pathways of resource utilisation.

In contrast, the ratios of C, N and P in autotrophs are highly variable. Because they fix their own organic carbon from inorganic sources through photosynthesis, their mechanisms for energy and nutrient capture are decoupled. Excess, non-limiting nutrients in their growth medium can be stored within the cell vacuole to be used when concentrations of a limiting nutrient increase. This ‘luxury consumption’ is a strong survival strategy where the concentration of nutrients fluctuates (Sterner and Elser, 2002). A good example of such variation is in rivers and streams that are subject to pulses of high nutrient runoff from agricultural land, particularly after inappropriate fertiliser application or soil erosion.

A consequence of these differences is that the carbon:nutrient (C:nut) ratio of a food source, limits heterotrophic ability to assimilate nutrients; while in autotrophs, not limited by light, it is the N:P ratio in the medium that controls assimilation and growth. Studies by (Elser et al., 1990; Downing and McCauley, 1992), cited in Sterner and Elser (2002), found a N:P ratio of around 31 (14 by mass) to be the threshold that determines whether N or P concentrations limit the growth of algae in freshwater lakes. This ratio was remarkably similar to the thresholds described for terrestrial systems (Verhoeven et al., 1996) and has been proposed as a significant threshold for the switch between N or P limitation. The N:P ratio of the environment is likely to vary over an annual cycle resulting in communities that experience alternating N and P limitation at different times.

Some communities, notably bacteria and fungi, are able to utilise dissolved N and P, in both organic and inorganic forms. These ‘saprophytes’ select a required nutrient by controlling the production of extracellular enzymes. This allows them to utilise energy sources with a high C:N ratio such as leaf litter. However, bacteria in particular are nutrient rich and provide a high quality diet to consumers such as protists and ciliates.

The transfer of microbial carbon into the higher trophic levels is increased through the production of biofilm. Production of biofilms allow the development of communities with complementary metabolic ‘skills’, facilitating the breakdown of refractory compounds. Benthic, epilithic, epixylic (on woody debris) and epiphytic films provide a food source for grazing protists and macro-invertebrates. ‘Sticky’ biofilm increases the effective particle size of free living microbial communities through aggregation. This makes them easier prey for filter feeding organisms such as foraminifera and macroinvertebrates.

Analyses by (Dodds et al., 2004; Dodds, 2006) strengthen the premise that variation in the relationship between community metabolism and nutrient uptake is controlled by the C:N and C:P ratio of the food source, particularly in heterotrophic streams where a substantial input of allocthonous C fuels heterotrophic production. The speciation of inorganic N and P may affect the ease of their uptake. However, as the exchange between forms is dynamic, their ratio may be less important than the quality of the carbon source. Since both autotrophs and microbial heterotrophs can utilise both organic and inorganic N and P, light limitation of autotrophy in streams may be less important for the microbial community, particularly in detritus based headwaters where carbon is not limiting. Investigations examining the relationship between community metabolism and nutrients showed that the higher C:N ratio of allocthonous vs autochthonous material stimulates heterotrophic nitrate uptake in detritus based streams (Fellows et al., 2006b; Chung and Suberkropp, 2008). This study demonstrates the adaptability of microbial heterotrophs to variable quality in food sources.

2.4.2. **Organic carbon.**

Input of fixed carbon to surface waters is traditionally partitioned into primary production, transfer from upstream reaches and allocthonous material from the catchment such as leaf detritus, particulate organic matter carried on soil particles, dead insects and other terrestrial fauna and dissolved organic matter. New research has demonstrated the occurrence of significant methane fixation by chemoautotrophs in chalk streams with strong connections to groundwater sources (Trimmer et al., 2010), although it is not yet clear whether this previously unrecognised source makes a significant contribution to carbon budgets.

In much of the literature, a distinction exists between the transient storage of macronutrients in biomass and their more permanent storage or removal through burial and outgassing, (Billett et al., 2006). Recent improvements in understanding the complex mechanisms of nutrient cycling through biogeochemical processes blur this line. The metabolic processes of the microbial community are central to these transformations. Coupled cycles of aerobic and anaerobic metabolism alter the redox potential in sediments, the hyporheic zone, and in aggregates within the water column. Redox state strongly influences the soluble phase of nutrient stores within streams.

Examples of transient storage are incorporation into benthic or epixylic and epiphytic biofilms, uptake by macrophytes and meiofauna, such as macro-invertebrates and juvenile fish, and burial in shallow sediments subject to seasonal flushing. Community respiration and the production of soluble exudates that are utilised within the reach are examples of recycling.

2.5. Nitrogen and phosphorus.

Understanding the transformation and cycling of N and P is key to understanding their bioavailability and the consequences of increasing concentrations in freshwater ecosystems. The concentrations and cycling of inorganic N and P dominate early discussions, chemical availability being thought to mirror bioavailability. More recently, comparisons of the upper limits of phytoplankton biomass, as measured by chlorophyll a (Chl-a), with total N (TN) and total P (TP) showed strong correlation in lakes. An intercept of the upper limit of biomass with the measured nutrient, 0 mg/l for P and 0.7mg/l for N suggests all P is available for plant growth in these ecosystems but that some N remains unavailable to metabolic processes (Scheffer, 1998).

Further insights were made possible by the development of techniques capable of distinguishing the dominant fractions or speciation of N and P (Johnes and Heathwaite, 1992). This allowed the differentiation of inorganic, soluble organic and particulate fractions of the total nutrient concentrations to be assayed. A further step change was the semi-automation of these analyses making higher time resolution of their interactions achievable. This is particularly important when dealing with biological transformations. The diurnal patterns associated with primary production are long established and their effects on the nutrient chemistry in freshwaters are known to have significant effects.

The different forms and availability of nutrients make their timing and delivery to a watercourse a major factor determining their processing. These differences often result in nitrogen and phosphorus cycling being discussed separately, although their biological processing is intimately linked through stoichiometric controls, see section 2.4.1.

2.5.1. **Nitrogen:**

Nitrogen is the major constituent of the atmosphere. In its molecular form as N_2 gas, it has limited bioavailability. Transformation of nitrogen is predominantly mediated by biological systems. Fixation by microbes, either free living, or in association with some plants, the legumes, converts molecular nitrogen, first into ammonia (NH_3), then nitrite ions, (NO_2^-), and ultimately nitrate ions, (NO_3^-), in a process known as nitrification. Plants and some microbes can incorporate nitrate directly into their biomass to forms such as amino acids and proteins, yielding organic nitrogen. The cycle is completed when metabolic processes reduce the organic matter to ammonia and then re-oxidise it to nitrate, known as nitrification. Depending on oxygen availability, nitrate can either be re assimilated into organic matter or reduced to nitrite and denitrified back to molecular nitrogen releasing N_2O, a powerful greenhouse gas, as a by-product.

Due to its small size and low negative charge, nitrate is rarely bound to soil constituents and leaches freely through soils when in excess of growth requirements.

Many organic nitrogen compounds such as urea, are highly soluble and nitrogen is often delivered to rivers in dissolved organic forms, readily available to photosynthetic organisms where its uptake will be strongly influenced by light availability. The processes involved in nitrogen cycling and its control in freshwater systems has been comprehensively reviewed by Durand et al. (2011) but some key factors are presented here. In low nutrient, open canopy streams in the summer, NO_3^- demand by photoautotrophs alone can exceed measured uptake of NO_3^- as measured by solute injection studies (Fellows et al., 2006b). These findings demonstrate that primary production is highly dependent on the recycling role of primary heterotrophs, and provide a plausible mechanism for the strong coupling between heterotrophic activity and primary production reported by (Scott et al., 2008) and others, that is lost in high nutrient streams. In the same study, Fellows et al. found higher than expected rates of night time NO_3^- uptake into autotrophs, suggesting it can be a subsidy for their growth, using excess carbon fixed during daylight hours.

In-stream processing of this N in headwater streams is an important ecosystem service that reduces its transport to downstream reaches, where high N availability is linked to an enhancement of primary production and resultant eutrophication (Johnson et al., 2009; Hilton et al., 2006). In these low order streams, the high ratio of bed substrate to water volume provides excellent habitat, both for benthic autotrophs and for the microbial communities responsible for denitrification that can exceed 40% of total nitrate uptake (Mulholland et al., 2008)

2.5.2. **Phosphorus:**

In contrast to the delivery of predominantly soluble nitrogen, large pulses of particulate P are often associated with storm events, where increased flow rates reduce the retention time within the reach and, therefore, the potential to process it locally. On the other hand, hydromorphological elements, such as sinuosity and impoundment by natural woody debris dams or man-made structures, increase retention time and may aid its incorporation into benthic biomass and up the food chain. In a positive feedback loop, decreasing redox levels, resulting from bacterial respiration, favour the dissolution of P by switching the equilibrium of metal bound forms to soluble phosphate, fuelling benthic processes and further reducing redox. Transformation between the forms of inorganic phosphorus is strongly influenced by the prevailing physicochemical conditions. Phosphate is bound or adsorbed to soil particles, where its strong negative charge is attracted to metallic ions such as iron and aluminium, constituents of clays. Changes in ambient redox and pH result in a change in the strength of this adsorption and can trigger its retention or release as orthophosphate (soluble reactive phosphate, SRP). In areas high in calcium such as chalk catchments, low pH can result in the stable formation of an insoluble mineral. This can result in export of phosphorus from the aquatic system through burial.

2.6. Effects of diffuse pollution.

2.6.1. High sediment load.

Sediment has multiple effects on river ecosystems. Chemically, agricultural soils are associated with bound phosphorus which can be transformed to available phosphorus once it reaches the microbial communities of the river. In low flow conditions and in fine sediments, a ‘cap’ can inhibit transformations across the interface and can have a strong influence on the retention / release of sediment stored P (Jarvie et al., 2008). Physical effects of fine sediments are equally harmful to the aquatic ecosystem. Compaction and the smothering of gravel beds damages fish spawning grounds. Increased turbidity reduces the available light available to the algal and macrophyte communities and changes its spectrum. This alters the community structure, favouring faster growing (and dying) algal forms that decompose on senescence, leading to oxygen depletion. Epiphytic algae increase, further shading macrophytes and reducing their ability to fix and process nutrient inputs (Jarvie et al., 2008; Hilton et al., 2006). These epiphytic communities process a smaller proportion of the available nutrients and are prone to removal by high flows and sediment ‘poisoning’ making them a more transient store of received nutrients.

Microbial communities respond to habitat changes including sediment quantity and dimension. Bacterial growth rates and whole community metabolism are highest in fine sediments when compared to rates in gravel or cobble substrates, (Clapcott et al., 2010; Clapcott and Barmuta, 2010). Fine sediments, however, cause problems for many invertebrates, so transfer of biomass from bacteria to higher trophic groups may be limited and their increased activity can lead to increased mineralisation of nutrients that are then exported downstream. Problems associated with excessive sediment also include significant losses of macroinvertebrate functional groups. Filter feeders are unable colonise the unstable substrate, reducing the ability to retain the increased levels of fine particulate organic matter (FPOM) within the reach (Benstead et al., 2009). For these reasons the deposition of fine sediment associated with runoff from agricultural catchments is a significant problem that attracts funding for mitigation measures aimed at attaining good ecological status under the EU Water Framework Directive.

2.6.2. **Eutrophication**

Eutrophication is most apparent in slow moving or still water bodies where high phytoplankton numbers, driven by high nutrient concentrations, cause visible changes to water colour and clarity, and through senescence and dieback at the end of the growing season, alter the nature of the substrate, lead to reduction of available oxygen in the water column though microbial decomposition and result in loss of biodiversity and increased purification costs in water supply. In faster flowing water, flushing prevents the development of high phytoplankton biomass and new definitions of eutrophy have been developed (Hilton et al., 2006; Dodds, 2006). In particular, the development of excessive, short lived epiphytic and benthic algae that restrict macrophyte metabolism through competition and shading, is cited as an indicator of poor water quality.

As a result of these easily recognised and harmful effects of eutrophication, the story of inorganic nutrients and their transport has dominated investigations. High nitrate concentrations in streams have been shown to inhibit both nitrification and total nitrate uptake (Alexander et al., 2009; Fellows et al., 2006b). In their model of nitrate uptake, biogeochemical factors such as nutrient concentrations, temperature and residence time were all shown to be important predictors of nitrate uptake rate. In streams with high ambient NO_3^-, even in summer with an open canopy, photoautotrophic demand could not keep up with NO_3^- supply. In streams with a closed canopy and light restricted primary production, nitrification in sediments released NO_3^- that exceeded demand by photoautotrophs resulting in net export to downstream reaches, (Mulholland et al., 2006).

Much of this work concentrates on inorganic fractions of N and P, missing the pool of nutrients in dissolved organic and particulate form. The relative proportions of nitrogen species and phosphorus fractions do not remain constant, either between or within water bodies throughout the year, making it impossible to describe ecologically significant chemical water quality by studying the inorganic fractions alone (Durand et al., 2011; Heathwaite and Johnes, 1996; Johnes and Burt, 1993; Heathwaite et al., 1996). While nitrate, ammonium and phosphates are known to be readily available for biological uptake, many organic forms can also be used directly and, as communities adapt to utilise any available resource, almost all nutrient delivered to a watercourse will be processed, albeit at different time scales (Maberly et al., 2002). For example, exo-enzymes produced by microbial populations can release readily available nutrients from complex organic matter and seasonal increases in macrophyte biomass will die off in the winter and decompose or be transported to downstream reaches. Recalcitrant particulate matter may settle as sediment but will be vulnerable to re-suspension and transport during high flow events, or may be transformed as a result of changing temperature and redox conditions throughout the year. It is likely that only a small proportion of nutrient is truly removed from the watercourse through natural in stream processing such as terrestrial insect development from their macroinvertebrate juvenile stages.

2.6.3. **Microbial/ algal interactions.**

A recent review stresses the importance of the microbial community for both storage and transfer of nutrients and for their release and recycling (Findlay, 2010). Sterner and Elser consider saprophytes to be responsible for the 2nd largest flux of macronutrients in the biosphere, second only to photosynthesis (Sterner and Elser, 2002). Large scale comparisons between land use and functional metrics showed measures of gross primary production and the activity of primary heterotrophs were the most responsive indicators of broad land use categories, (Clapcott et al., 2010). Understanding how microbial populations and processes respond to DWPA is, therefore, a crucial component in linking ecosystem structure and function.

One of the most consistent observations from recent research is the decoupling of bacterial and algal production in streams with high nutrient concentrations. In high light and low nutrient conditions, bacterial production correlates closely with primary production (Rusanov et al., 2009; Scott et al., 2008). This relationship breaks down with increasing nutrient concentrations, supporting the theory that heterotrophic bacteria can use high C:N food sources in nutrient rich streams. Scott et al. suggest their data support the premise that algae rely on bacterial remineralisation and recycling of nutrients in oligotrophic streams, while Rusanov et al. focus on the competition for high quality (low C:N) food that exists between bacterial and algal populations in low nutrient conditions. In biofilms, a similar relationship has been linked with the release of DOC from the film into the surrounding water that can range from 2 – 45% of net primary production (NPP), (Ziegler and Lyon, 2010). In experimental incubations at a range of nutrient concentrations, the source of released (excess) DOC in low nutrient conditions, is mainly as carbon from older biofilm constituents, while that released from biofilms in nutrient rich conditions is dominated by carbon fixed during the incubation period. Bacterial incorporation of this newly fixed carbon does not mimic the increase in new carbon fixed by algae in nutrient rich conditions, allowing the release of labile carbon to downstream reaches (Lyon and Ziegler, 2009; Ziegler et al., 2009; Ziegler and Lyon, 2010). These observations are linked with land use in a study by Williams et al. (2010) who found that riparian land use altered both the quantity and quality of exported DOM. Streams in agricultural catchments exported more labile DOM than wetland or forest streams. The source of DOM has been shown to have a strong effect on bacterial activity and community composition (Judd et al., 2006; Judd et al., 2007). The addition of DOM from soil water had a greater effect on stream bacteria, with production increasing by 3 to 7 fold, much more than the increase stimulated by the addition of DOM from a stream source. This strong microbial response to inputs of C, N and P is further demonstrated by the work of Benstead et al. (2009) in field investigations that show a 15 fold increase in the export of FDOM in enriched streams compared with a control reach. In addition, the export of acclimatised microbial populations to downstream reaches may increase the ability of higher order streams to process nutrients (Battin et al., 2008).

2.6.4. **Microbial /macro-heterotroph interactions.**

The previous section deals with the interactions between microbial communities and primary producers. Fixation of macronutrients within a reach will be dependent on burial within sediments or their transfer to higher trophic levels. Few studies quantify bacteriophagy in freshwater streams but see (Konigs and Cleven, 2007), who found no evidence for a significant pathway through ciliate grazing of bacteria in a hyporheic zone. Determining the contribution heterotrophic microbiota make to the nutritive value of biofilm to grazers is difficult (Withers and Jarvie, 2008). Fungi, on the other hand, have been shown to contribute to the nutrition of macroinvertebrate shredders, although their contribution to dietary intake, which can be up to 100% of growth requirements, depends on the growth stage (instar) of the macroinvertebrate (Chung and Suberkropp, 2009). Predominantly, the impact of microbial transformations is thought to be as remineralisers and a synergistic relationship exists both up and down trophic levels. We have seen how the remineralisation of limiting nutrients is crucial to primary producers. This in turn affects the herbivorous macroinvertebrates. Detritivores, too, are reliant on the release of macronutrients to subsidise high C:N ratio carbon sources such as terrestrial leaf litter. In high nutrient streams, microbial degradation of leaf litter can equal that of combined microbial and macroinvertebrate grazing in low nutrient streams, (Chung and Suberkropp, 2008). The metabolic response of the microbial community to changes in nutrient status was greater in their study than that of the macroinvertebrates. In another study, macro-invertebrate community structure was significantly affected by nutrient status (Baldy et al., 2007). Bacterial biomass increased throughout a wide range of enrichment (defined by phosphate concentrations) while shredder taxa of macroinvertebrates were intolerant of high P and high ammonia (NH_3 concentrations. Fungal requirements for N and P are thought to be lower (Duarte et al., 2009). In 2007, Baldy et al. also found that increasing P concentrations had a positive effect on fungal biomass but for a limited range. At their defined range of eutrophic and hypereutrophic P concentrations, fungal biomass levelled off and ultimately decreased. The strong feedback links and high speed responses available to microbial communities through physiological changes of their exo-enzyme production, (Sinsabaugh and Shah, 2010) and short generation times allowing rapid colonisation following introduction, leading to changes in community structure (Judd et al., 2007), facilitate whole community response to variations in nutrient status.

Extrapolating short term, experimental responses into longer term management strategies is a challenge. For example, in a five year nutrient enrichment study, responses to nutrient enrichment were shown to vary between mass specific, physiological responses and area specific, whole system, responses (Suberkropp et al., 2010). In the short term, nutrient subsidies increased primary production, microbial and macroinvertebrate biomass and production. Over the five year period, however, the reduction in leaf litter substrate resulted in reduced areal microbial biomass and, therefore, nutrient uptake potential.

2.7. The ecosystem service / function/ process model

The ecosystem service, function and process model has evolved from a recognition of the vital contribution a functioning ecosystem makes to human survival, health and wellbeing (The Ramsar Convention, 1971; The Millennium Ecosystem Assessment, 2005). In this model, each ecosystem service, for example ‘provisioning’, ‘regulating’ or ‘cultural’, is represented by a suite of ecosystem functions, each of which are reliant on a complex group of processes. One illustration of the nested nature of this model is demonstrated in Figure 2:1 (McInnes, 2008; McInnes et al., 2008).

In their seminal paper Gessner and Chauvet (2002) advanced the case for measurements of ecosystem function to complement structural indices. The ability of low order streams to process the key nutrients, N, P, and organic carbon, is of considerable interest and the extent to which anthropogenic inputs disrupt this ecosystem service has stimulated research into the mechanisms that control nutrient cycling and to quantify their assimilation, retention, sequestration and release.

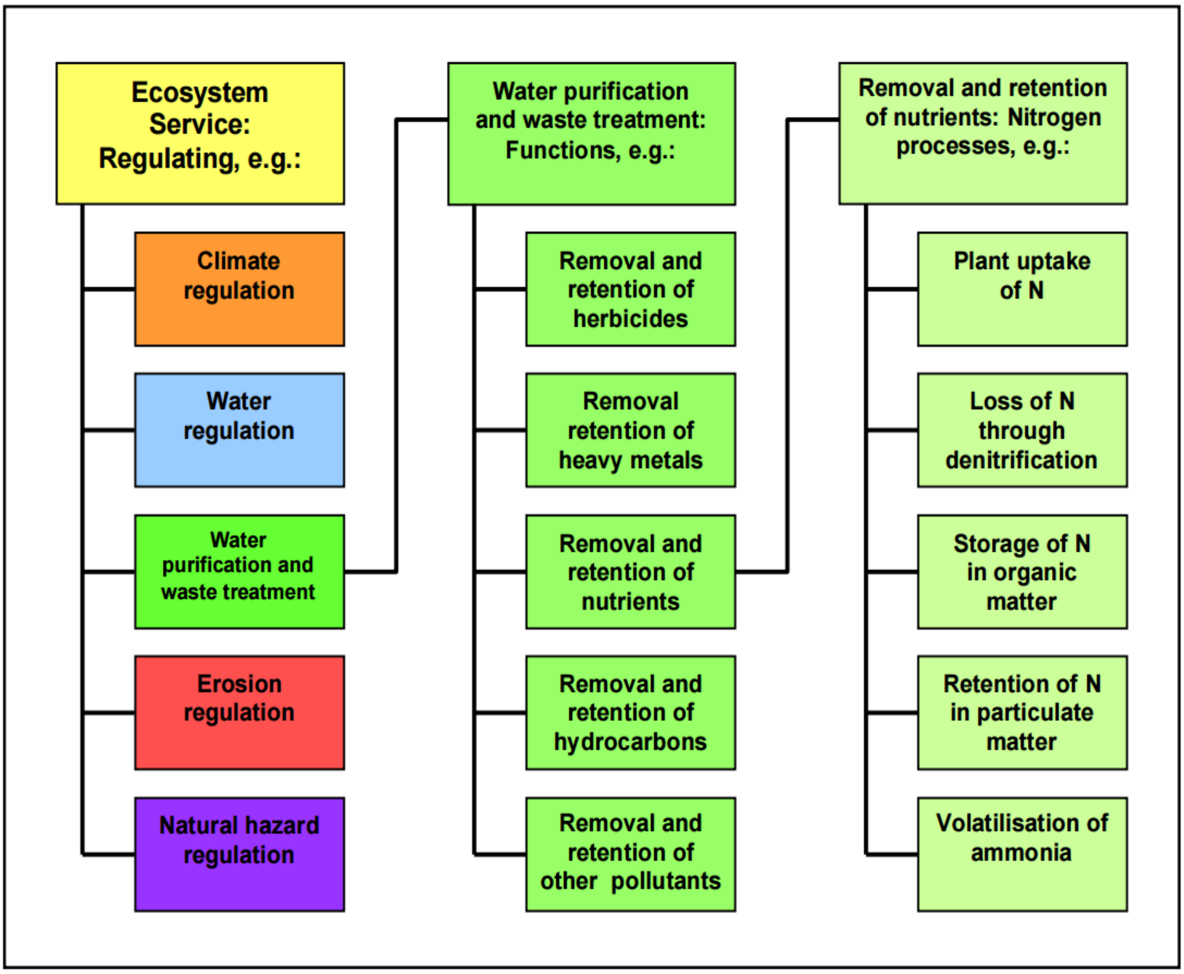

Figure 2:1 Relationships among ecosystem services, functions and processes: example for removal and retention of nitrogen as a component of the regulating ecosystem service (McInness et al., 2008).
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Studies by Nelson (2000)and Bunn and Davies (2000) cited in Gessner and Chauvet (2002), describe observations of the decoupling of functional and structural changes in response to environmental stress. In a further example, the reduction of phosphate in a lowland river, where sewage treatment works had improved phosphate stripping techniques, resulted in a significant reduction the concentration of SRP. Photosynthetic production (a functional metric), however, did not decline (Neal et al., 2010), confirming the complex nature of cause and effect in aquatic ecosystem functional response to a single parameter change.

Linking biodiversity and ecosystem functioning with reference to food webs, Woodward (2010) described the critical control that vertical interactions (consumer: resource) in a food web can exert - termed trophic cascades - in contrast to horizontal interactions that often exhibit less sensitivity to the loss of taxa (functional redundancy).

2.7.1. **Leaf Litter degradation.**

Gessner and Chauvet (2002) champion leaf litter breakdown rates as a good candidate for a standard assay for ecosystem function. It has the advantage of being fairly straightforward while providing a measurement, integrated over time that examines ecosystem response to a variety of stressors at different trophic and organisational levels. (McKie and Malmqvist, 2009) used the technique to compare in-stream responses to light availability in forested and clear felled streams of different trophic status. Short term 'physiological' responses and longer term 'system' change can be distinguished using leaf degradation studies as a model. In their five year study, Suberkropp et al. (2010), describe the short term physiological subsidy provided by enhanced nutrient concentrations, driving increased detrital degradation rates and contrast that with the resultant, long term reduction in detrital organic carbon, driving a reduction in nutrient uptake (through stoichiometric controls) and their increased availability for downstream export.

Leaf litter breakdown dynamics are also used in the carbon budget studies of Benstead et al. (2009) and in determining the relative contributions of microbial and invertebrate activity to bioavailability. In 2008, Chung and Suberkropp examined fungal / invertebrate interactions and established the key role played by microbial transformations in bioavailability of key nutrients. Disadvantages in the use of leaf litter degradation as a standard assay include the variability of leaf litter from different tree species and differing reactions of communities pre-adapted to ambient leaf species (Kominoski et al., 2007). Discussions on the applicability of single or mixed LL packs can depend on logistics (frequency of sampling vs sufficient degradation) and the aim of the study – carbon budgets or comparison between stream reaches or nutrient status. The use of cellulose strips has been suggested as an artificial substrate to eliminate some of the variability inherent in the leaf litter methodology (Imberger et al., 2010), although these may have limited relevance to calculations of nutrient dynamics and budgets in natural systems.

2.8. The Demonstration Test Catchment Programme:

The DTC consortium uses a suite of measures to assess ecological response in small sub-catchments. Defining environmental responses, following changes in land management practice can be a long term process. The criteria for evaluating status take into account the physical, chemical and biological descriptions of a water body. These can be defined as ‘structural’ measures and each has associated difficulties and limitations, for example:

- Without high resolution, long term monitoring (which is both expensive and / or labour intensive), chemical measures provide a snapshot of chemical water quality.
- Sediment stress is difficult to measure and highly subject to short term weather conditions, again lending itself to a snapshot measure.
- Assessing changes in traditional ‘short term’, time-integrated biological indicators, such as macro-invertebrate and diatom assemblages, is labour intensive and requires a high degree of expert knowledge. Populations are highly seasonally variable giving a time lag in identifying real change.
- Other structural changes, for example fish populations, may take years or decades to confirm a stable change.

The processes that underlie how these elements interact with each other and with the wider environment both respond to and drive changes in the characteristics that define ecological status. These processes are described here as ‘functional’ measures. One of the central research questions of this studentship is to assess whether integrated ecosystem functional response may prove to be a faster, more sensitive indicator of changes to a combination of sediment and nutrient stressors within a reach. Many of these processes are mediated by microbial populations that are able to respond rapidly to changes in available resources; either through changing community structure as a result of short generation times, or by manipulating intra or extracellular enzyme activity. In low nutrient, open canopy streams, primary production and community respiration are tightly coupled. Carbon fixed by photosynthesis becomes available for heterotrophs and in a feedback loop, microbial recycling makes essential nutrients available for primary producers. This coupling is disrupted by the high nutrient input associated with diffuse pollution from agriculture. In the heavily shaded headwater streams of the Prior’s Farm and Cool’s Cottage sub-catchments, primary production may be less of a control for bacterial populations than available allocthonous carbon sources. The High DOC concentrations in the Prior’s Farm reach may be reflected in bacterial numbers and activity. Extracellular phosphatase activity from bacterial or fungal sources may correlate negatively with SRP concentrations but positively with less immediately reactive fractions. Other ecosystem processes that have been linked to ecological stressors include macroinvertebrate herbivory and processing of leaf litter detritus (detritivory).

Chapter 3. **Site Description:**

3.1. The Hampshire Avon:

The Hampshire Avon is situated in the South of England and flows from Upavon, Wiltshire, in the North to Christchurch, Dorset on the South coast (Figure 3.1). It has a predominantly chalk catchment with an area of ~1700km^2. The Upper Avon is well connected to its underlying chalk aquifer while the Nadder valley, a western tributary lying south of the Mere Fault, is formed from a deep incision through the dominant chalk geology. Here the up-warped Kimmeridge Clay, that constitutes its bedrock geology, restricts permeability (Allen et al., 2014).

Throughout the catchment, land use is largely agricultural, although with large conurbations including the outskirts of Shaftesbury, that together with Warminster, Salisbury, Ringwood and Christchurch, comprise the majority of the population in the catchment. It hosts both SAC and SSSI conservation sites, largely due to its chalk streams that support fisheries, tourism and watercress production, which are economically important for the region. The upper reaches of the Rivers Wylye and Nadder have been important for both salmon and brown trout spawning grounds. However, increasing sedimentation of their spawning gravels (redds) has been blamed for decreasing populations throughout the catchment (The Environment Agency, 2009; The Environment Agency, 2005).

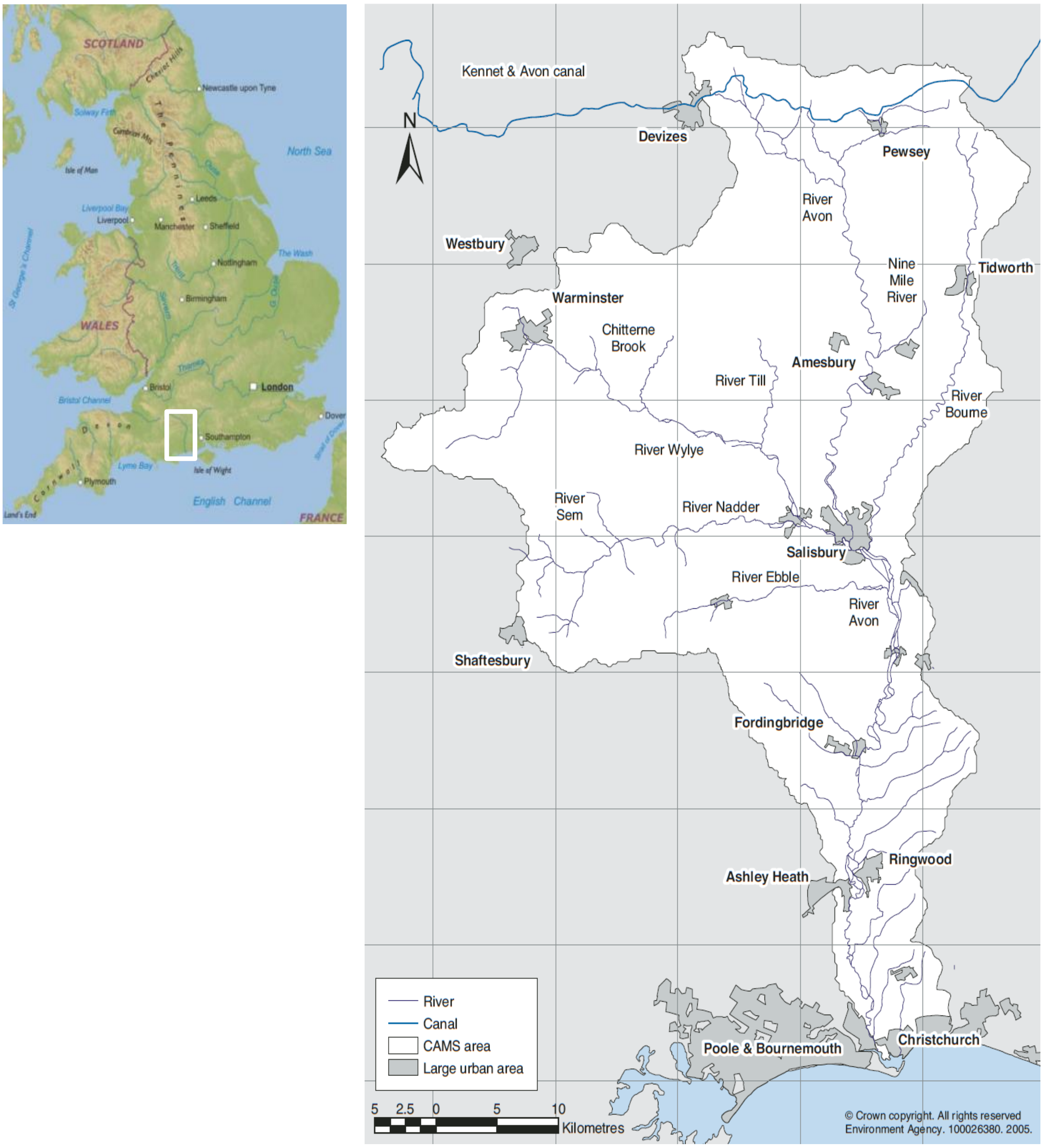

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Figure 3:1 Map of the Hampshire Avon Catchment showing major waterbodies and urban areas. Reproduced from the Environment Agency Catchment Abstraction Management Plan, 2005.

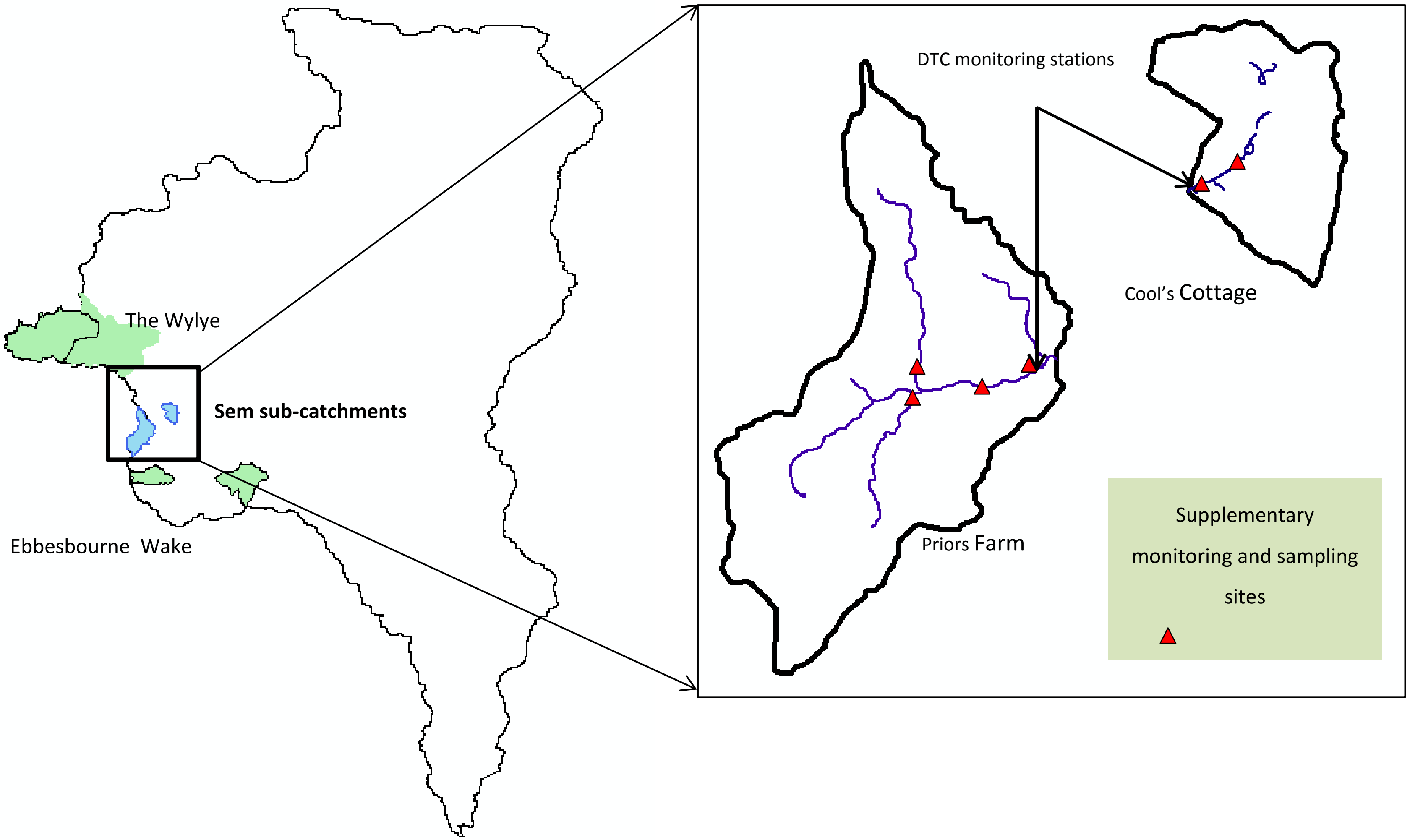

Figure 3:2 The Hampshire Avon catchment showing the DTC sub-catchments. The Study area for this project concentrates on the River Sem sub-catchments: Priors Farm and Cools Cottage.

3.1.1. **DTC sub-catchments, site selection and meteorology**

Four sub-catchments in the Hampshire Avon were chosen for intensive study as part of the Hampshire Avon DTC research platform (Figure 3:2). The Wylye and the Ebble are chalk sub-catchments and represent predominantly arable (River Wylye) and pasture (Ebble) agricultural types. The River Sem is a tributary of the River Nadder and rises in the West of the Hampshire Avon catchment near Shaftesbury. The River Sem sub-catchment was chosen for this project. The low permeability of the soils and underlying geology in the sub-catchments were considered likely to exhibit a more rapid response to changes in land management practice (within the time frame of the research project), than would a permeable, chalk catchment. In addition, the impermeable nature of the underlying geology makes the River Nadder and its tributaries particularly vulnerable to the delivery of inputs from the catchment via surface flow. The findings reported here are based on data collected during a 32 month period from October 2011 to June 2014, a time frame that experienced some weather extremes (Figure 3:3).

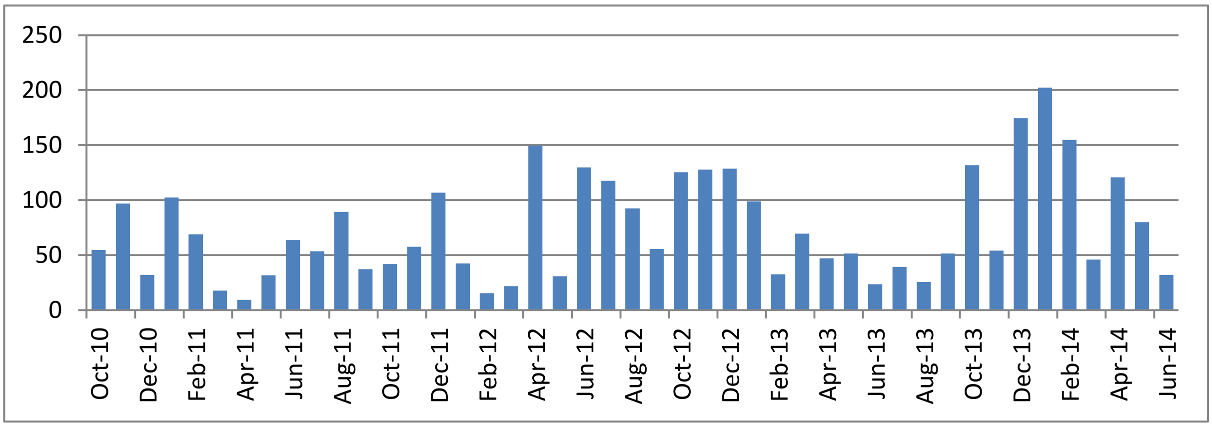

Figure 3:3 Monthly rainfall totals (mm) at Tisbury weather station - Data provided by E.A. SW region

Year 1 (WY 2011) followed the drought conditions of summer 2011. Persistent rain during the winter of 2011/2012 was followed by heavier rain through the summer of 2012 (April – Sept). In year 2 (WY 2012) the high rainfall persisted until April 2013, but the summer was dry. These conditions were followed by the extreme wet winter of 2013/2014 that caused extensive flooding in the UK and, most noticeably, in neighbouring Somerset, over the Christmas period and early spring. For both sub-catchments, the persistent rain during 2012 resulted in saturated soils, leading to stock being housed throughout the summer. Combined with periods of drought, these conditions resulted in secondary pressures being exerted on the in-stream communities (Section 3.3.2.).

In addition to the importance of the local hydrology, biological activity - and therefore ecosystem function, responds to temperature. Year 2 was characterised by a late spring, with water temperatures not rising above 10^{o}C until mid-April, in comparison to years 1 and 3 where temperatures reached 10^{o}C by early March (Figure 3:4). In Year 3 however, signs of spring were also late to develop, evidenced by the late emergence of leaf cover and low in-stream primary production (chapters 6 and 7).

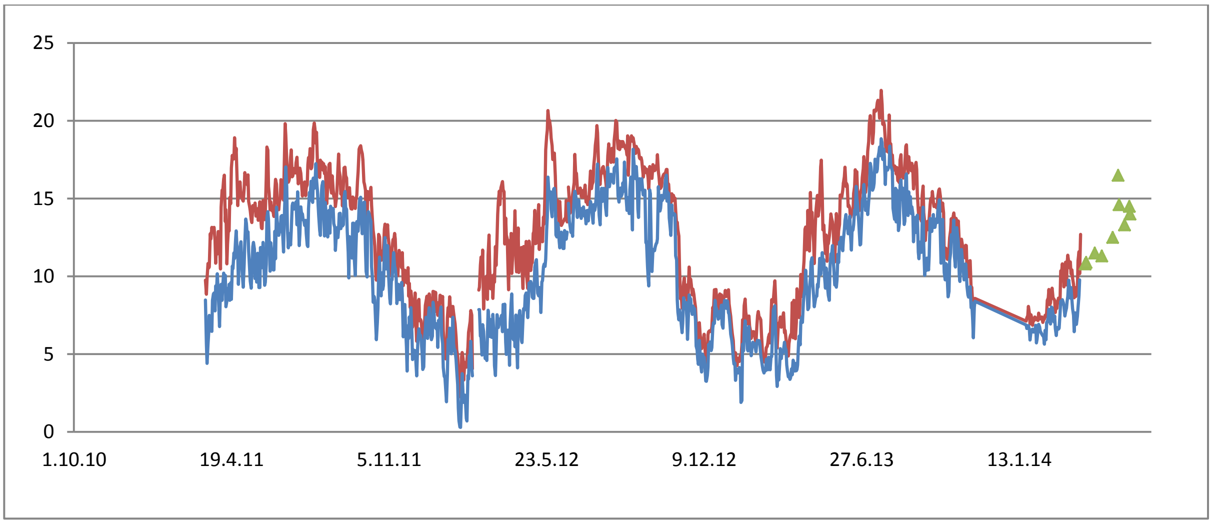

Figure 3:4 Daily minimum and maximum river temperatures at Cools Cottage during the study period: (▲) weekly spot samples.

3.1.1.1. Study sites

Within the River Sem catchment, two neighbouring, headwater streams provided the basis for a paired catchment study. The monitoring stations at Priors Farm and Cool's Cottage are sited at the outlets of small sub-catchments, approximately 5km^2 and 2km^2 respectively, with similar dominant soil types and land-use (Figure 3:5 and Figure 3:6). Their proximity to each other (1.6 km apart at the monitoring stations), means they experience the same meteorological conditions. The two sites experience differing levels of impact from agriculture, however, and provide a natural laboratory that has enabled observations of ecosystem functional responses to different stress levels, and allowed a distinction to be made between system responses to anthropogenic stressors, and to those arising from the effects of natural meteorological variability. Initial assessment of the DTC sub-catchments suggested similar geology and hydrology would provide a strong basis for a paired catchment approach. The following, detailed sub-catchment descriptions identify key similarities and differences between the study sites.

3.2. The Cool's Cottage sub-catchment

The Cool's Cottage sub-catchment covers an area of 1.75 km^2. It is sparsely populated with only a few dwellings: not currently serviced by mains drainage (Figure 3:7 and Figure 3:8). The study reach comprises a first and second order stream, flowing NE to SW with a combined total length of approximately 1.7 km (from two sources) to the outlet where the DTC monitoring cabinet is sited. The absolute length is seasonally affected by the recession of the ephemeral head of the secondary tributary during dry weather. It is a shallow, fairly fast flowing stream with a channel width of approximately 1m throughout the study reach.

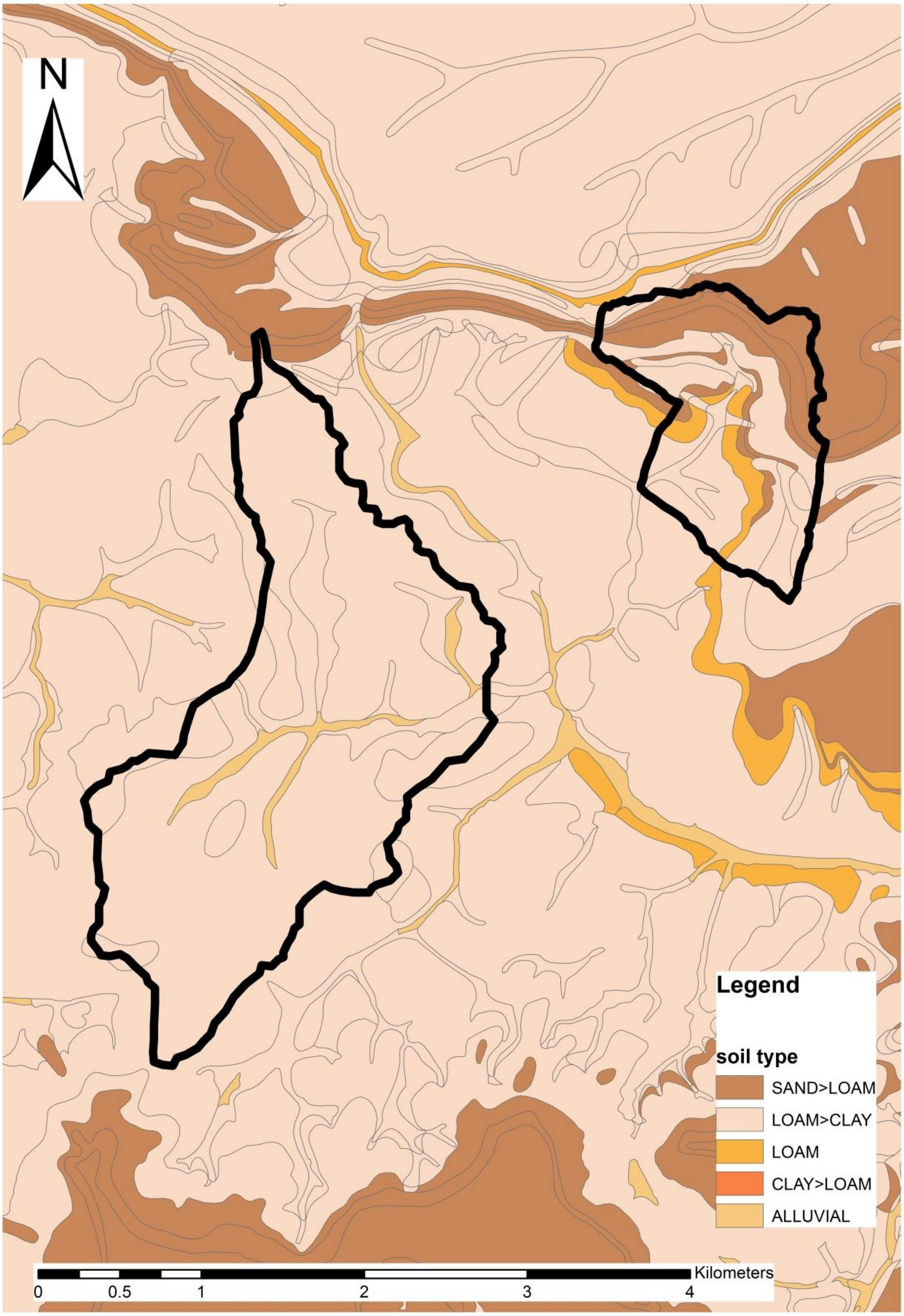

Figure 3:5 Soils of the Sem sub-catchments.

Figure 3:6 The Sem sub-catchments showing dominant land use.

Figure 3:7 The view from Cools Farm towards the DTC monitoring station

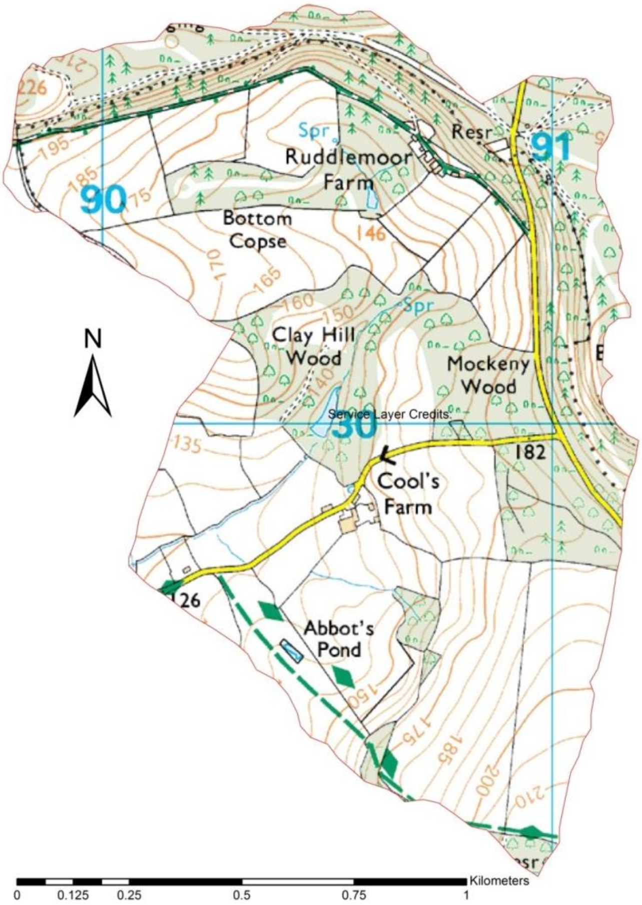

Figure 3:8 The Cools Cottage sub-catchment

Mean daily discharge over the two full water years (WY) 2011 and 2012 were 19 l s-1 (range 2.3 – 148 l s-1) and 26 l s-1 (range 3 – 144 l s-1) respectively. The sub-catchment has an elevation of 120 m from its highest point (240 m A.O.D.) to the outlet (120 m A.O.D); with an average slope of 100.5 m km-1. The Cool's stream flows through a mixed, broadleaf woodland; a man-made lake; improved pasture with riparian fencing; a domestic pond (now silted up and forming a small wetland) and into another, smaller wet woodland area, before reaching the monitoring station at Cool's Cottage. The secondary tributary also acts as a drainage ditch carrying surface runoff from a rural road into the main channel. The road is narrow, with steep banks that are prone to damage by large vehicles. Several springs drain into the road that, consequently, acts as an efficient conduit of water from land to stream. The ditch contributes a visible input of fine sediment just upstream of the pond that has silted up within the last 30 years. During WY 2011, construction work at Cool's Farm, combined with the high rainfall, is likely to have increased this fine sediment load.

3.2.1. **Bedrock geology and hydrology.**

For much of the lower sub-catchment the underlying geology is Kimmeridge Clay. This formation has very low permeability, restricting connectivity between surface and groundwater. However, although the lower sub-catchment is underlain by clay, the headwaters rise in a compound series of geological profiles including Lower Greensand, Upper Wardour and Portland Limestone, Gault Mudstone and Chert Sandstone (Figure 3:**10**). The complex geology results in the upper catchment being punctuated with an abundance of springs, issues and sinkholes, (Figure 3:**9** and Figure 3:11 (d)). In order to determine the likely geological source of the springs feeding the Cool's Cottage study reach, a series of samples were analysed by ICP-OES (Chapter 4) and their Mg:Ca ratio was determined after Allen et al. 2014: (Figure 3:12). This analysis illustrates the contrasting nature of the groundwater sources in the Cool's Cottage reach with two distinct signatures.

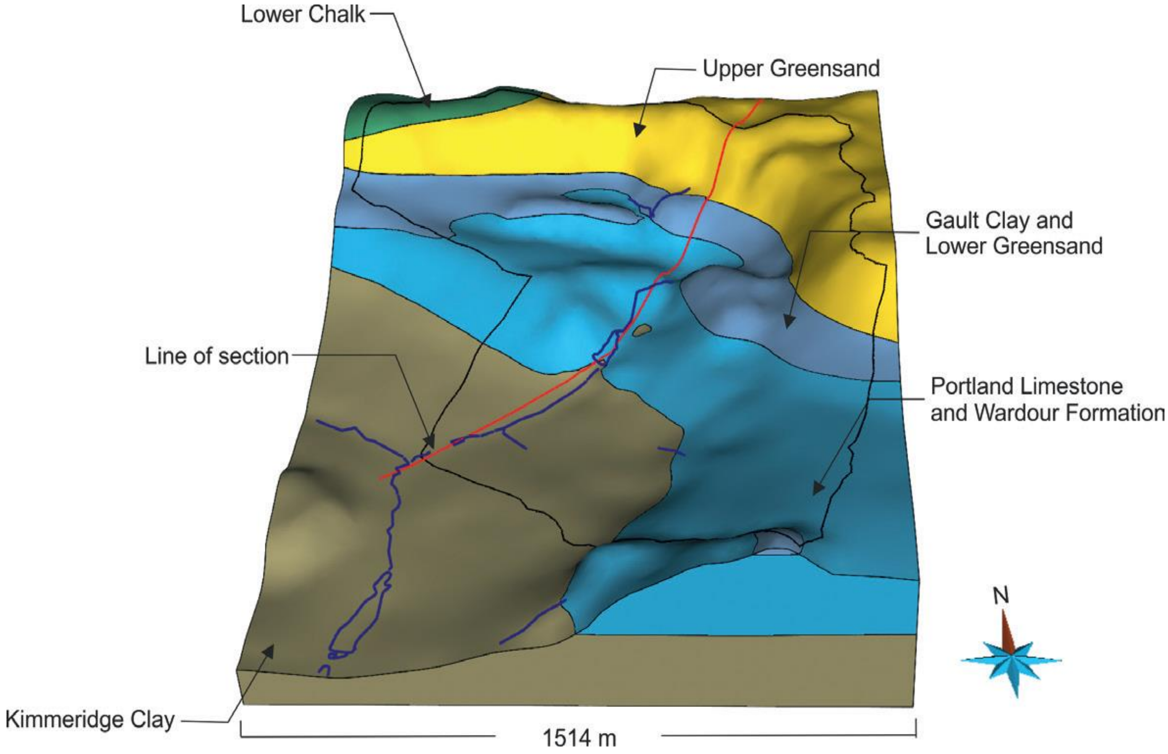

Figure 3:10 Bedrock geology of the Cools Cottage sub-catchment. Reproduced from Quarterly Journal of Engineering Geology and Hydrogeology 2014, v.47; p65-80. D. J. Allen, W. G. Darling, J. Davies, A. J. Newell, D. C. Gooddy and A. L. Collins : doi: 10.1144/qjegh2013-043: NORA.
Geological features, BGS, ©NERC. NEXTMap Britain elevation data from Intermap Technologies.

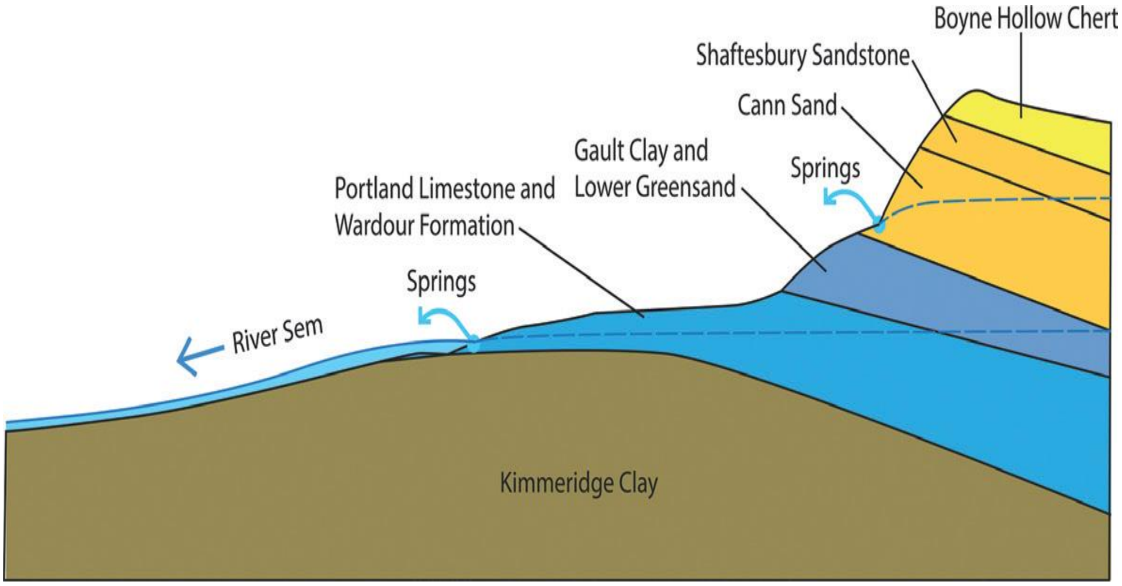

Figure 3:9 Schematic diagram showing the principal hydrogeological functioning of the Sem Cools Cottage sub-catchment. Reproduced from Quarterly Journal of Engineering Geology and Hydrogeology 2014, v.47; p65-80. D. J. Allen, W. G. Darling, J. Davies, A. J. Newell, D. C. Gooddy and A. L. Collins : doi: 10.1144/qjegh2013-043: NORA.
Geological features, BGS, ©NERC. NEXTMap Britain elevation data from Intermap Technologies.

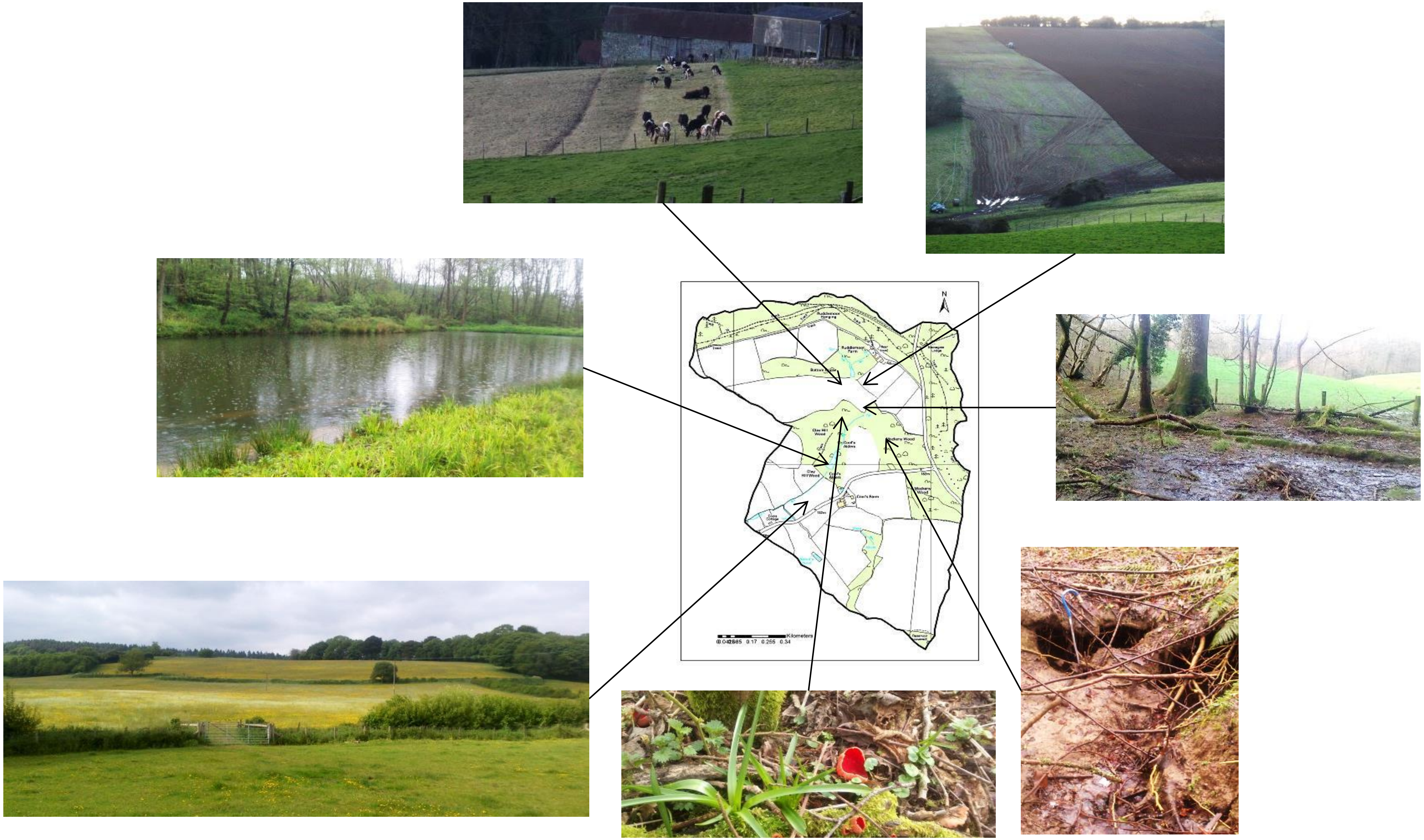

Figure 3:11 Key characteristics of the Cools Cottage sub-catchment. Clockwise from the top: a, all year strip grazing; b, parallel ploughing; c, accumulation of sediments above the Cools Cottage reach headwaters; d, sinkhole formed in the permeable upper catchment; e, forest flora diversity, away from the sediment accumulation; f, improved pasture adjacent to the Cools Cottage reach; g, the lake in Clay Hill Wood.

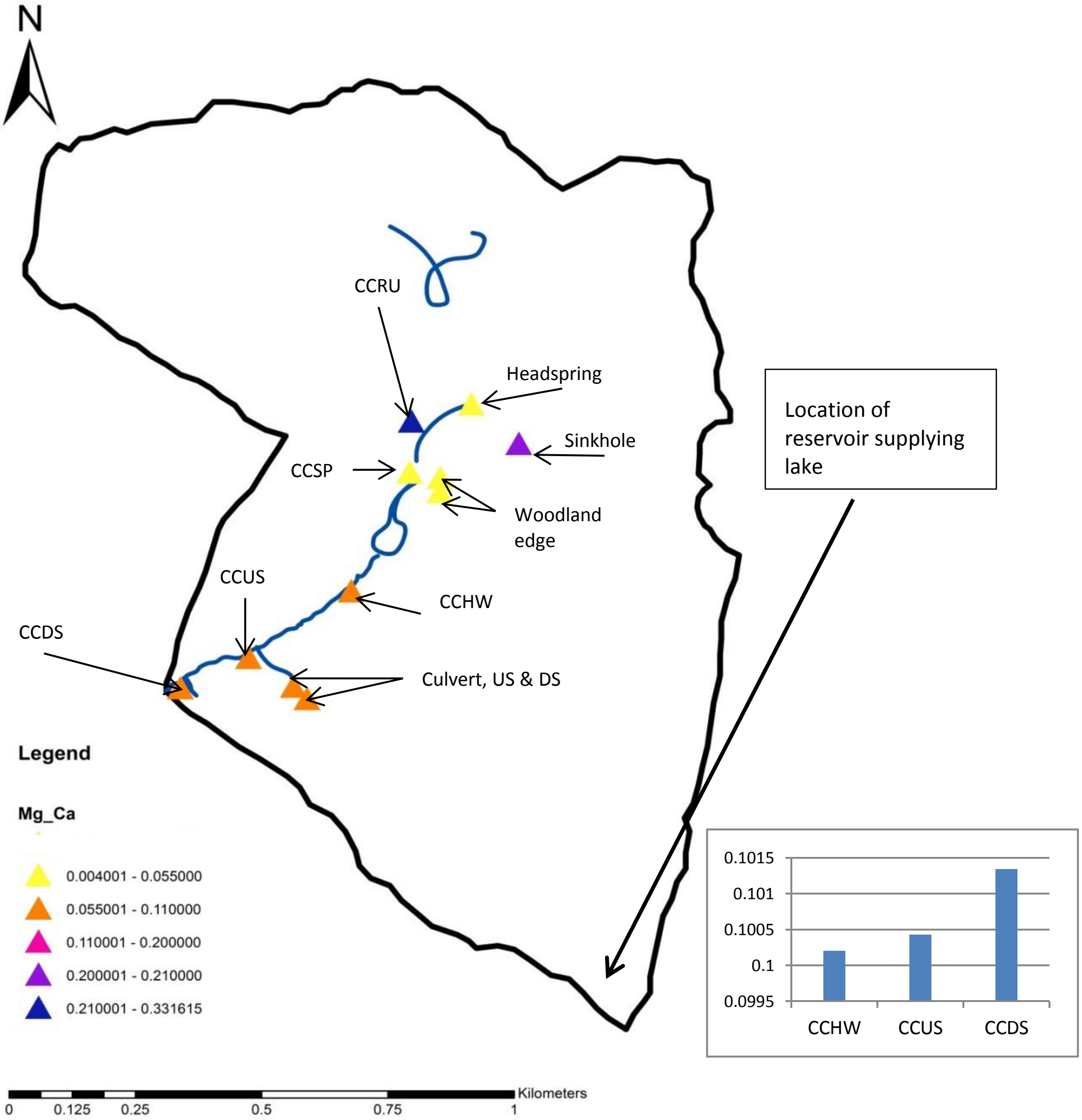

Figure 3:12 The Cools Cottage sub-catchment showing main drainage channels, sampling sites and respective Mg:Ca ratios. (inset: increasing Mg: Ca ratio with distance downstream).

Those with a very low Mg: Ca ratio are likely to originate from the Portland Limestone aquifer, while the higher Mg: Ca ratios, exhibited by the spring downstream of Ruddlemoor Farm (CCRU) and at the sinkhole, are indicative of a Greensand source. Despite the incursion of the north-eastern most corner of the sub-catchment into the West Melbury chalk formation, there is little evidence for this aquifer providing significant input to the Cool's Cottage reach (Allen et al., 2014). As part of a system for stock watering, previous landowners at Cool's Farm installed a network of pipes from the reservoir in the southernmost corner of the sub-catchment. They provide a constant supply that feeds the man-made lake in Clay Hill Wood, immediately downstream from the stream's source. Discharge from the most substantial of these piped sources (CCSP) was measured on two occasions during the dry season in September 2013. Discharge from the pipe on 9.9.13 & 16.9.13 was 0.17 and 0.22 l s^{-1} respectively, a contribution of approximately 5% of those days' discharge (4.82 & 4.88 l s^{-1}) and 10% of the lowest measured discharge (2.35 l s^{-1}) at the sub-catchment outflow, from this single source alone. The reservoir is also situated in the Wardour Formation and Portland Limestone, and the high calcium carbonate content of limestone provides a likely explanation for the low Mg:Ca ratios of these samples.

The lake in Clay Hill Wood (Figure 3:11(g)) provides a mixing pot for the uppermost water sources within the sub-catchment, and the heightened Mg:Ca ratio at CCHW (Figure 3:12), the first regular sampling point downstream of the lake, indicates the result of this mixing. Some local 'in-stream' processing is likely to arise from the increased residence time the lake affords. It hosts a large population of *Elodea canadensis* that is subject to periodic clearance and removal by the landowner, providing a possible sink for some nutrients. Below this lake, the increasing Mg:Ca ratio with distance downstream (inset Figure 3:12), suggests a decreasing influence from groundwater sources in the lower part of the sub-catchment. This is consistent with the progression from permeable to impermeable geology and with the resulting increase in contribution from surface flow from the surrounding pasture. The Base Flow Index (BFI) for the sub-catchment is 0.49 (data supplied by BGS); midway between those of groundwater dominated chalk streams (BFI >0.7) and rivers with low permeability and a higher proportion of surface flow (BFI <0.35), reflecting this mixture of sources.

3.2.2. Soils, land use and management.

The soils in the sub-catchment are classed as deep to intermediate by the UK Soil Observatory and are a mixture of predominantly sandy and clayey loams in the upper sub-catchment and loam to clay in the lower reaches (Figure 3:5). The clay and clayey loams are subject to waterlogging in wet weather, exacerbated when underlain by low permeability Gault and Kimmeridge Clay. This makes them difficult to manage, being prone to poaching and panning and rendering them unsuitable for arable tillage or grazing in wet weather. Prior to this study, land was used for maize production but widespread problems of runoff and sediment loss resulted in local farmers reverting high risk maize fields to pasture (Collins, A.C., pers. comm.). Woodland covers an area of 70 ha, approximately 40% of the sub-catchment. The land immediately adjoining the DTC cabinet is used as pasture with some woodland, currently used for grazing a small beef cattle herd during the summer. Deteriorated fencing allows the cattle direct access to the stream, and field observations indicate that this was a common occurrence. The remaining land adjacent to the stream (~45 ha, approximately 27% of the sub-catchment) is predominantly organically managed, improved pasture that is used for beef cattle grazing, limited to the summer months (Figure 3:11(f)). It is also used periodically for sheep grazing at other times. Some fodder crops are harvested in the late summer. At the start of the study period, new fencing was installed between Cool's Cottage and the stream's emergence from Clay Hill Wood. Additional drinking troughs were also installed at some distance from the stream to reduce damage caused from overuse of the existing, streamside troughs. Waste management for this herd is as solid manure, stored at the farm complex and applied to the land in dry weather. Upstream of Clay Hill Wood and to the north of the sub-catchment there is a substantial risk of soil loss and the delivery of fine sediment to the reach from the steep slopes in the upper sub-catchment. During year 1 of this study, New Zealand style strip grazing (high impact- high stock density) in this part of the catchment caused extensive soil erosion, aggravated by the wet spring and early summer of 2012. This was followed by ploughing parallel to the slope (Figure 3:11 (a) & (b)), actions likely to have contributed to the build-up of fine sediment above the spring sources in Clay Hill Wood (Figure 3:11 (c)). The remainder of the habitat in Clay Hill Wood supports a diverse flora (Figure 3:11 (e)). The study reach is provided some protection by trapping of mobilised fine sediment in the wood itself, and by the lake that acts as a settling pond for suspended sediment. Further upslope still, the upper perimeter of the sub-catchment is dominated by broad-leafed woodland. The southernmost corner of the sub-catchment (the location of the covered reservoir that supplies the lake) is used for arable and horticultural crop production. Inorganic fertilisers are applied to approximately 25% of the sub-catchment on the land furthest from the stream, in the northwest and south of the sub-catchment.

3.2.3. **River habitat and sediment characteristics**

Typical of many headwater streams in an agricultural setting, the stream drains the surrounding pasture and has been modified by dredging and straightening. It is broadly a trapezoidal channel with high banks and lined with hedges for much of its length. In the summer dense riparian growth, including brambles (*Rubus fruticosus*), dog roses (*Rosa* *canina*), Hemlock Water-dropwort (*Oenanthe crocata*) and fool's watercress (*Apium nodiflorum*) line the banks, some of which are also hedged with hawthorn (*Cretaegus monogyna*) and alder (*Alnus glutinosa*). These result in poor light penetration to the stream bed (Figure 3:13.a). For much of the reach the river bed is predominantly gravel and sand, with pebbles and some cobbles. (Figure 3:13.b). The interstices are clogged with finer material resulting in a cemented bed. Where flow is obstructed, either by artificial channel modification or fallen trees, finer materials (silt and clays) have been deposited and in places these have accumulated to depths of over 10 cm. Where these sediments are protected from flushing, the anoxic zone, indicated by black colouration and sulphurous odour, is present at a sediment depth of only a few mm.

Figure 3:13 (a) dense vegetation along the cools cottage reach that restricts light reaching the stream bed and (b) bed sediments at Cools Cottage.

Determination of fine sediment load was carried out by another member of the Hampshire Avon DTC consortium, the Queen Mary University of London - Rivers Communities Group (QMUL.RCG), using the re-suspension cylinder technique (Collins and Walling, 2007; Duerdoth et al., 2015). This gives the fine sediment load in kg m^{-2} of 'surface' (easily re-suspended sediment mobilised by stirring of the water column alone) and 'total' fine sediment (requiring significant disturbance of the overlying water column and the upper 5-10 cm of the river bed to re-suspend). During the first year of this study (October 2011 – October 2012) the reach average total mass of fine sediment was 2.6 kg m^{-2}. Of this, 12% (0.312 kg m^{-2}) was organic matter, determined as ash free dry mass (AFDM: 500^{o}C). On average, 30% of the total was 'surface' fine sediment, with the remainder being incorporated in the interstices to a depth of approximately 10 cm.

The overall sediment size distribution in the stream bed was determined from cores collected from the experimental site at the DTC monitoring cabinet. Here, 81% of the sediment was made up of particles that passed through a 2 mm sieve and of those, 86% passed through the 0.06 mm sieve. Silt and clay fractions, therefore, constitute around 70% of the total bed sediment sampled from these cores. Analysis of organic matter content, by Loss On Ignition by mass (L.O.I. 550^{o}C) returned an average of 0.677kg m^{-2}. Organic matter constituted 5.1% of the < 2mm fraction and 4.35% of total sediment.

3.2.4. **Biological indicators of ecological status**

Surveys of macrophytes, macro-invertebrate assemblages and diatoms were carried out by QMUL. RCG, and analysed to obtain scores for a series of biological indices that, collectively, contribute to the classification of WFD ecological status. The River Invertebrate Classification Tool (RICT) comprises a group of scoring systems, designed to identify impacts from a variety of stressors. In the UK, two indices form the basis of the WFD classification based on macro-invertebrate assemblages: NTAXA (Number of scoring TAXA) and ASPT (Average Score Per Taxon); both derived from a scoring system developed for the National River Quality Survey in 1980 and known as the BMWP (Biological Monitoring Working Party) and standardised in a WFD report the SNIFFER report, (Clarke et al., 2011; Davey-Bowker et al., 2008). These tests are used to gauge general degradation (NTAXA) and stress due to organic pollution (ASPT). The ratio of ‘observed’ to ‘expected’ scores are used to produce the Ecological Quality Index (EQI) of a test site and predetermined bands produce a classification of ‘High’, ‘Good’, ‘Moderate’, ‘Poor’ or ‘Bad’ status. Two further tests; PSI (Proportion of Sediment-sensitive Invertebrates) and LIFE (Lotic-invertebrate Index for Flow Evaluation), that indicate stress due to sediment load and impaired flow respectively, are presented. An additional assessment of the overall habitat is provided by indices calculated from the results of macrophyte (LEAFPACS) and diatom (DARES) surveys.

Results from the ASPT (average EQI throughout the study period: 1.06) and NTAXA (average EQI throughout the study period: 1.41) indicate high status - derived from macro-invertebrate assemblages. The LIFE index (average EQI throughout the study period: 1.025) shows no evidence of habitat degradation as a result of impaired flow. However, there is evidence of moderate stress as a result of sediment load from the PSI_{sp} index (average score throughout the study period: 0.8).

Results from the LEAFPACS index return moderate to poor status for the Cool’s Cottage study reach. This result is matched by the DARES index of diatom community assemblage. Together, these are likely to arise from the lack of light due to the pronounced riparian shading described above.

3.3. Priors Farm sub-catchment

The Priors Farm sub-catchment covers an area of 5 km^2. It is largely agricultural but has a greater population than the Cool's Cottage sub-catchment, with communities at East Knoyle, Sedgehill and Kings Settle (Figure 3:14). The majority of dwellings, including a residential Park that houses 60 residents and staff, are not connected to mains drainage and rely on septic tanks. There is a small industrial estate at Kings Settle which is served by a small sewage treatment works (STW) that discharges within the sub-catchment (at Semley Common). The STW serving East Knoyle discharges into another tributary of the River Sem that joins the Priors Farm reach, downstream of the Priors Farm sub-catchment, at Kinghay and does not, therefore, impact on the measurements made within this study. The western perimeter of the sub-catchment is fringed by farms and bounded by the A350, a major trunk road that is likely to contribute fine sediments and road runoff with associated pollutants. The study reach comprises a first to third order stream, flowing west to east with a combined length of approximately 4 km; the absolute length is seasonally affected. It is a shallow, fairly fast flowing stream, although subject to drying out during long periods of dry weather. It demonstrates a riffle and pool configuration with a width of 1 – 2m (except at the DTC cabinet where the stream channel is approximately 3m width). Over the annual cycle, mean daily discharge for the two full WY 2011 and 2012 were 57.4 $l\ s^{-1}$ (range 0 – 1346 $l\ s^{-1}$) and 71.8 $l\ s^{-1}$ (range 0 – 945 $l\ s^{-1}$) respectively. There is little topographic variation within the sub-catchment. Although its highest point is 200m A.O.D., the majority is below 150m A.O.D.

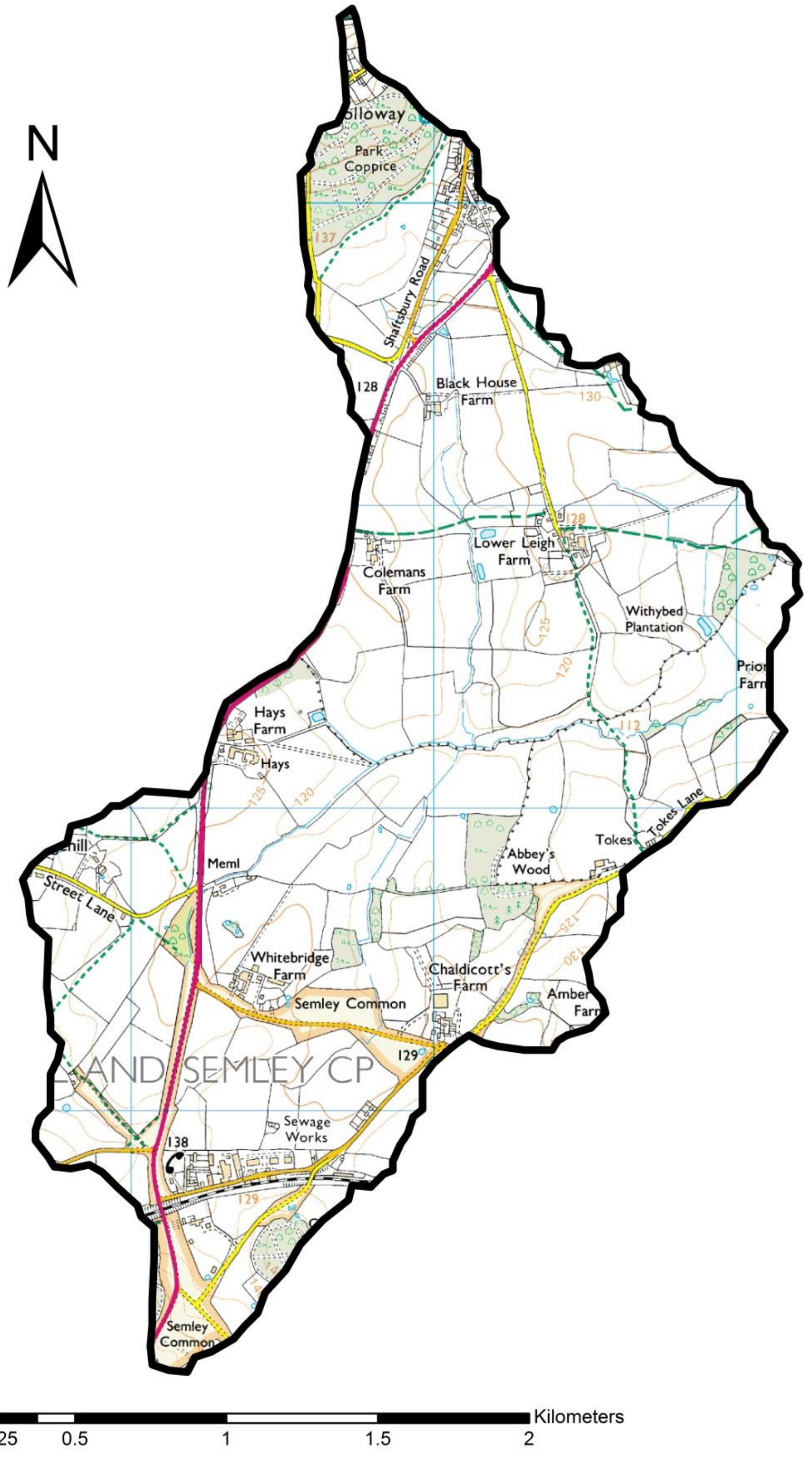

Figure 3:14 The Priors Farm sub-catchment

Three tributaries converge approximately 1 km upstream of the monitoring station (Figure 3:19). Two, PF1 and PF2, flow through improved pasture for dairy herds, where improvements to slurry containment and discharge practices form part of the mitigation works planned for the DTC project. The third, PF3, rises in Semley Common and flows through unimproved, marshy grassland and a small wood before reaching improved pasture, approximately 0.5km upstream of its confluence with PF1 and PF2. From the confluence to the outlet, the river mainly runs through improved pasture before reaching the monitoring station at Priors Farm (PFDS).

3.3.1. **Bedrock geology and hydrology.**

The sub-catchment is almost entirely underlain by impermeable Kimmeridge Clay, with small outcroppings of Gault Clay and Upper Greensand in the south and a larger one in the north at East Knoyle (Figure 3:15)

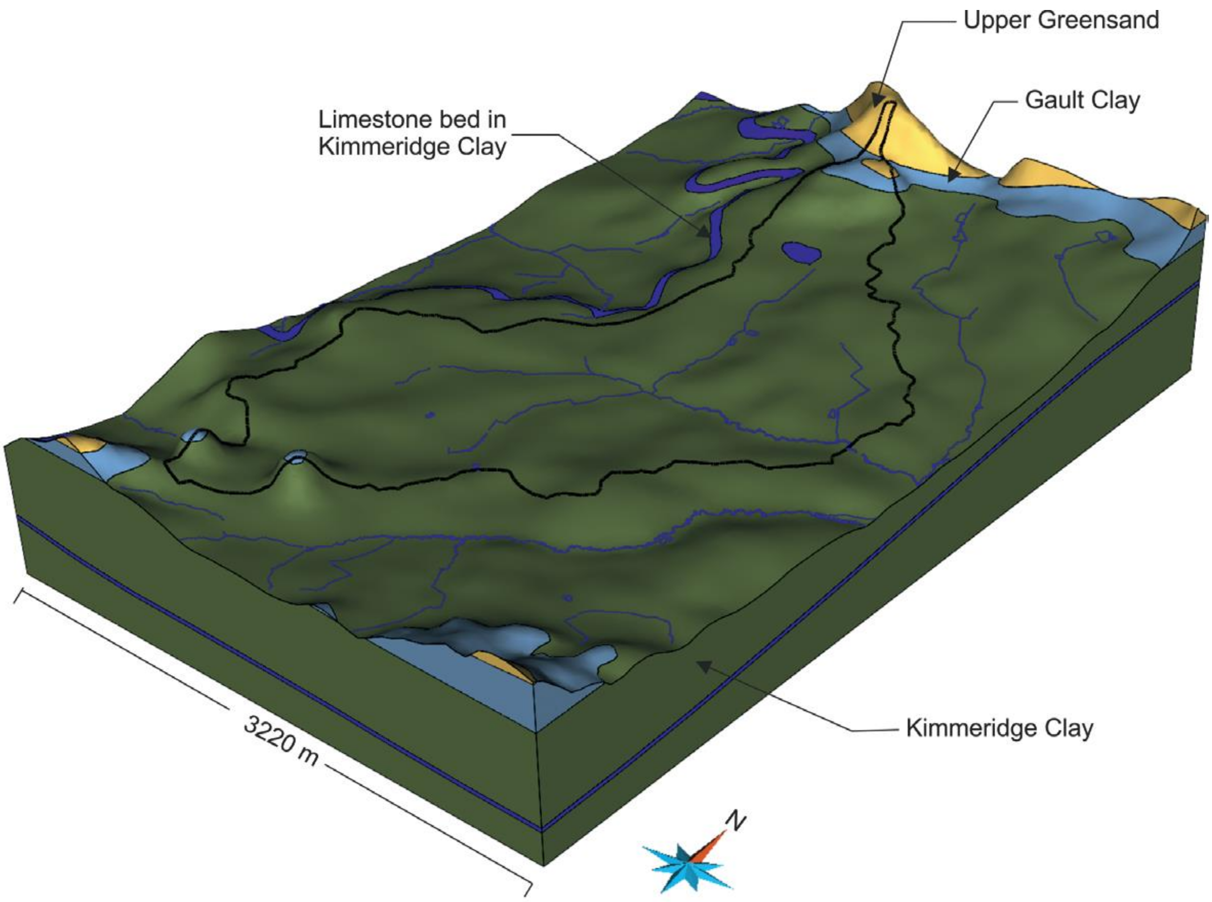

Figure 3:15 Bedrock geology of the Priors farm sub-catchment. Reproduced from Quarterly Journal of Engineering Geology and Hydrogeology 2014, v.47; p65-80. D. J. Allen, W. G. Darling, J. Davies, A. J. Newell, D. C. Gooddy and A. L. Collins : doi: 10.1144/qjegh2013-043: ©NORA. Geological features, BGS, ©NERC. NEXTMap Britain elevation data from Intermap Technologies.

As a consequence, the river is dominated by surface runoff (BFI 0.23; data supplied by BGS), and exhibits a characteristically flashy hydrograph (Figure 3:16). It experiences periods of no flow in dry weather, resulting in isolated pools that can become stagnant in the summer months. Conversely, during heavy rainfall the channel is overtopped. The resulting overbank flow causes erosion of fertile soil and contributes substantially to the delivery of sediment to the river (Figure 3:18(c)). There are limestone beds within the Kimmeridge Clay that could possibly provide a source of groundwater to the stream (Allen et al., 2014). Analysis of the Mg:Ca ratio, however, demonstrates a uniformity of source, with little variation throughout the sub-catchment and suggests that, if present, these sources contribute little to the overall discharge (Figure 3:19)

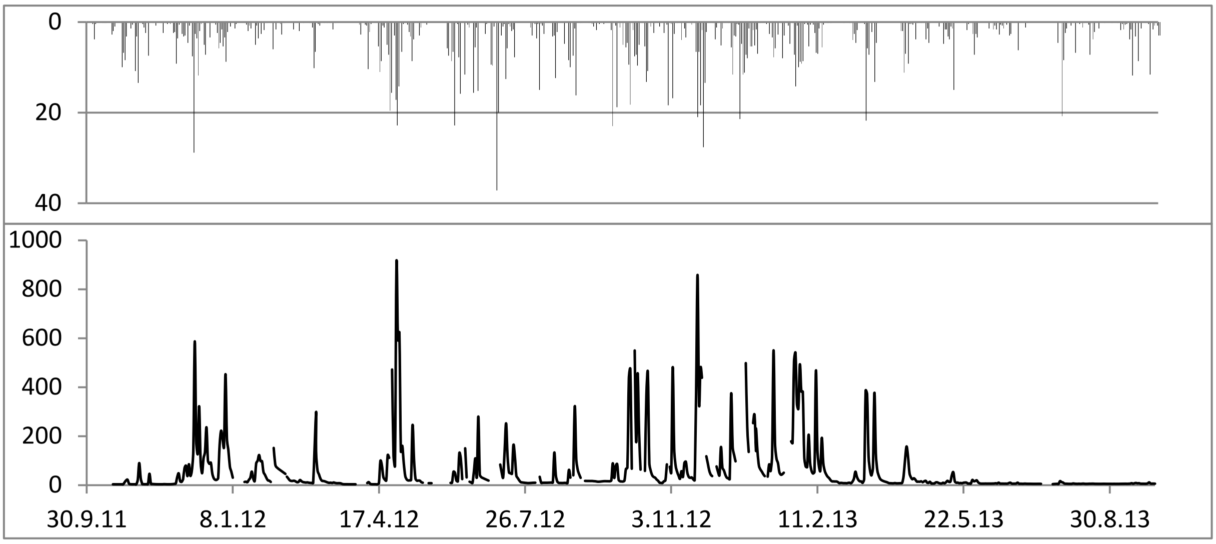

Figure 3:17 Daily rainfall (mm) at Tisbury (Wilts) and discharge (l s^{-1}) at the outlet from the Priors Farm sub-catchment

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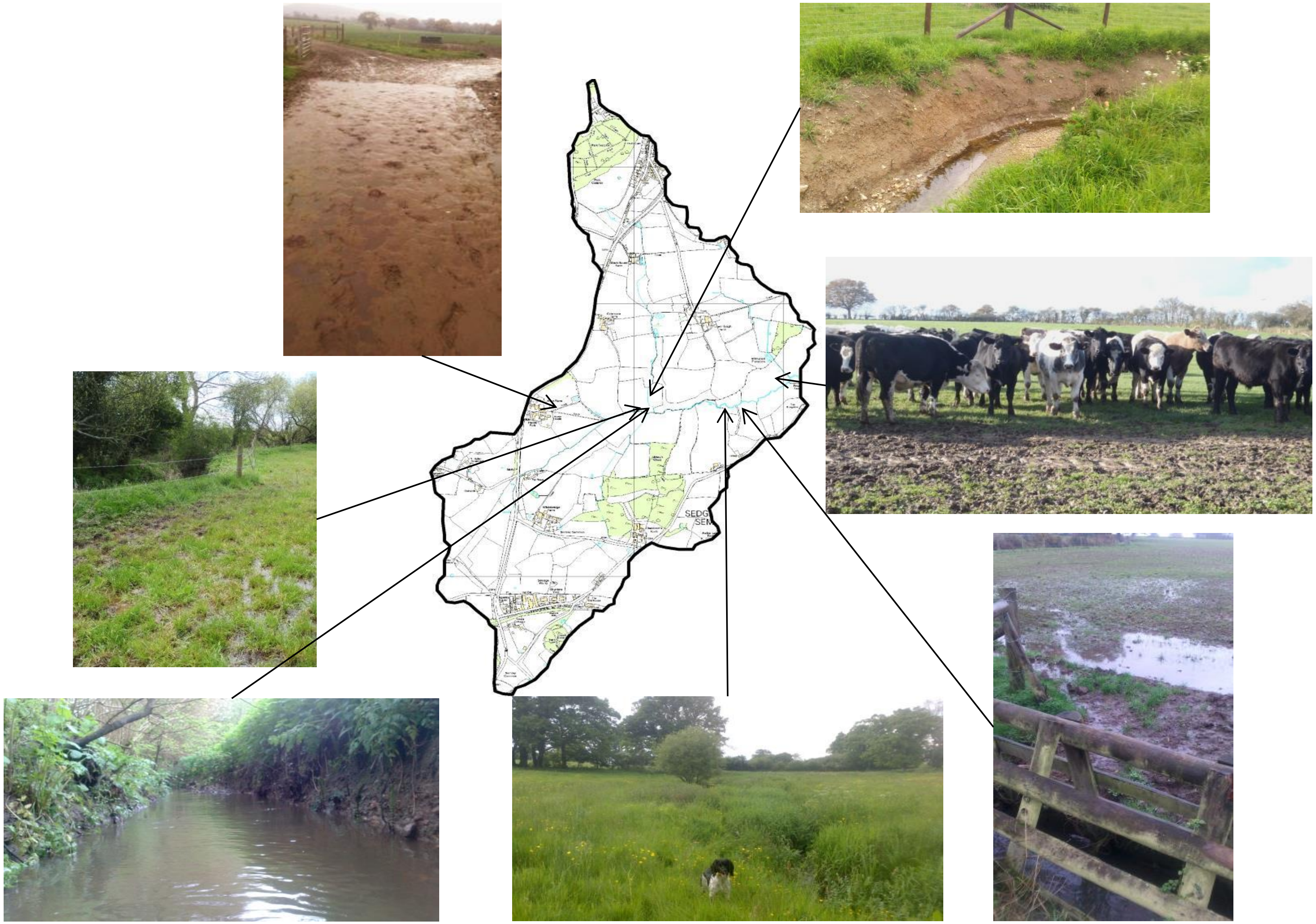


Figure 3:18 Key characteristics of the Priors farm sub-catchment: a, dredged channel at PF 1; b, soil damage caused by grazing in wet weather; c, overland flow delivering fine sediment to the river channel; d, unfenced stream channel; e, 'u' shaped, shaded and dredged channel; f, waterlogged pasture adjacent to the Priors Farm stream; g, footsteps in organically loaded fine sediment at Priors Farm headwater.

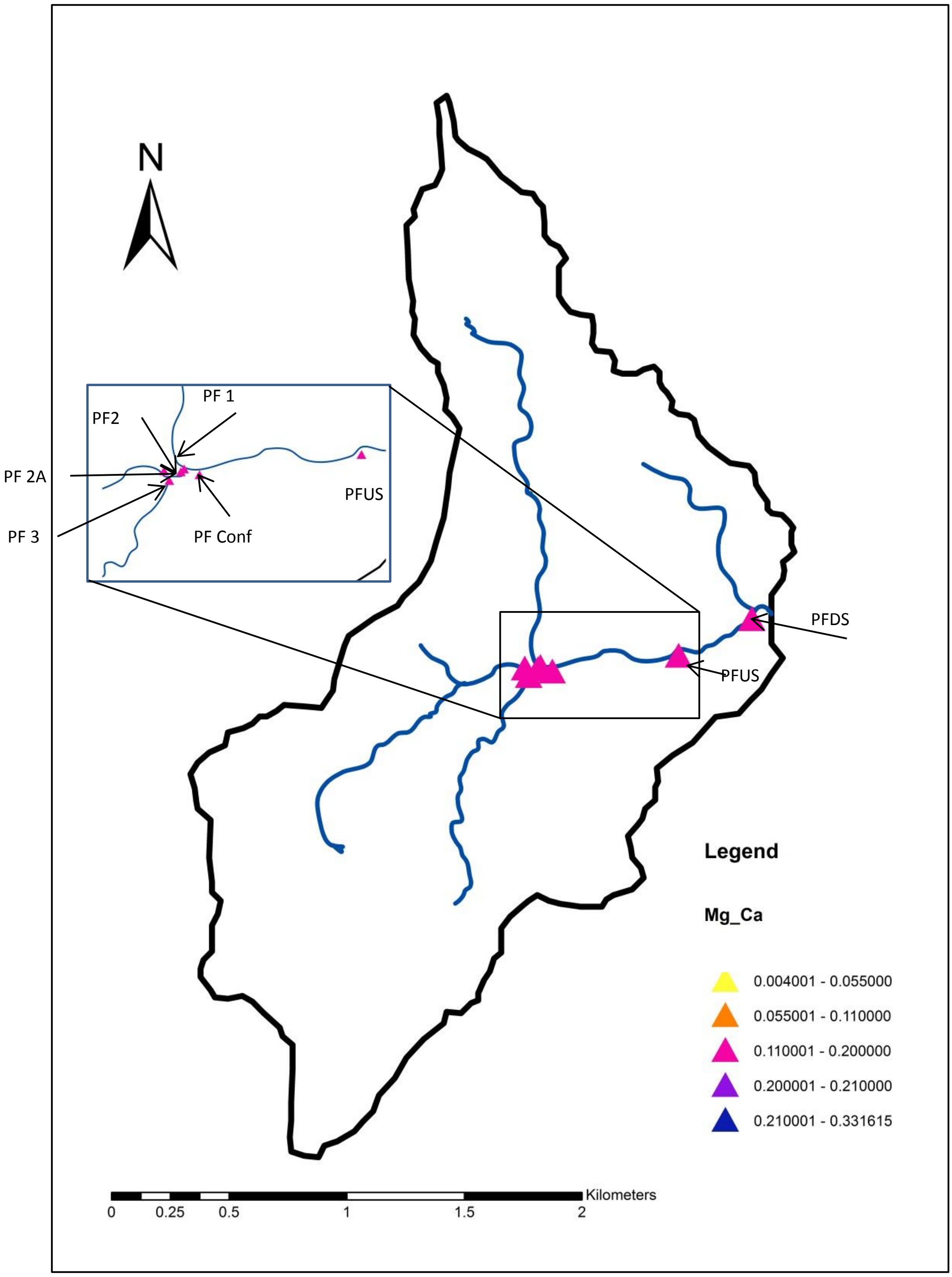


Figure 3:19 The Priors farm sub-catchment showing main drainage channels, sampling sites and respective Mg:Ca ratios (inset: Priors Farm tributaries)

3.3.1. **Soils, land use and management.**

The soils in the sub-catchment are classed as deep to intermediate by the UK Soil Observatory and are predominantly derived from claystone and mudstone, yielding a soil texture of loam to clay. Along the stream valleys, soils derived from riverine clay, sand and gravels result in textures ranging from clay to sandy loams (Figure 3:5). Combined with the underlying geology, these soils make the sub-catchment prone to waterlogging, and pastures deteriorate rapidly when grazed during wet weather (Figure 3:18 (b) & (f)). There is little established woodland, approximately 40 ha in total, constituting 8% of the sub-catchment. There has, however, been recent planting along the lower reaches close to Priors Farm, both along the river banks and in the adjoining fields. The unimproved grassland of Semley Common occupies a further 40 ha and the settlements of East Knoyle and Kings Settle constitute approximately 35 ha. Elsewhere land use is dominated by improved grassland (~ 320 ha, 63% of the sub-catchment), some of which has recently reverted from maize and other arable crops, and is used for stock grazing, predominantly for dairy production, with some sheep grazing in the winter. Along the study reach a combination of fencing and channel topography prevents stock access to the river, with the exception of a short reach (approximately 200 m) where field observations indicated that cattle had been in the river on several occasions (Figure 3:18 (d)). Manure management throughout the sub-catchment is predominantly slurry storage with umbilical and spray plate surface application to the fields primarily, during dry weather. The high rainfall experienced during 2012 and 2013, and in the winter of 2013/2014, exerted extreme pressure on slurry storage capacity and there were periods when it proved essential to apply slurry to the field in suboptimal conditions in order to prevent storage lagoons from over-topping. Despite this, slurry storage capacity was exceeded on a number of occasions throughout the study period, resulting in several incidents where farmyard manure or slurry entered the watercourse. This was indicated by field observations of colour and odour; and confirmed by peaks in the concentrations of NH_4-N and DOC- and steep troughs in DO concentration (Figure 3:20 & chapter 5). There was some export of slurry from the sub-catchment to a third party in 2012 and 2013, relieving the storage crisis for a time. The extreme weather, however, meant the storage capacity of the recipient was also reached, precluding further export for a period. As part of the DTC programme of mitigation works, some yard roofing was undertaken. Without the finance to effectively separate clean and dirty water, however, this provided little alleviation to the slurry and manure storage crisis. Clean and dirty water separation remains a target for future DTC mitigation works in this sub-catchment. The poor weather caused additional problems on another farm, where a degraded track-way resulted in a mixture of sediment and manure being delivered to the stream during heavy rainfall (Figure 3:18(g)). During the second year of this study, the DTC programme funded improvements to the track-way, and the provision of a swale and series of settling ponds to trap sediment. These improvements are expected to alleviate pollutant delivery from this source in the future. In addition to slurry application, inorganic fertilisers are applied within the sub-catchment, including to some fields adjacent to the watercourse.

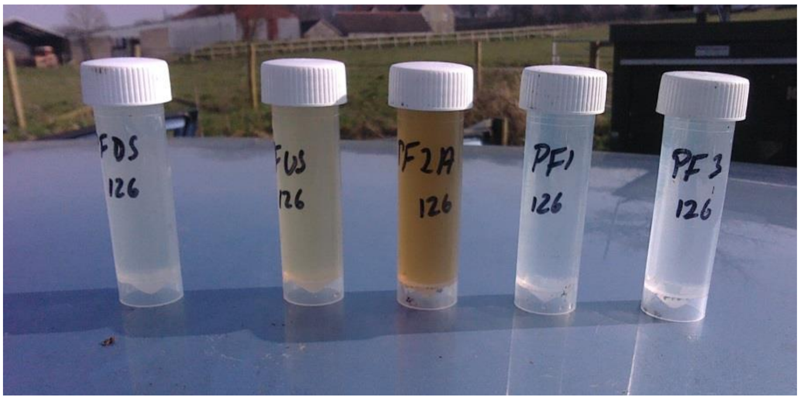


Figure 3:20 Visible signs of a 'slurry' event: spatial samples from the Priors Farm reach

3.3.2. **River habitat and sediment characteristics**

The gradient throughout the Priors Farm sub-catchment is low and much of the reach has been modified in the past to increase drainage of the adjacent fields. Towards the end of year 1 of the present study, where the tributaries (PF1 and 3) flow through improved grassland, they were dredged and fenced as part of the DTC project. These sections of the reach have trapezoidal, straight channels and the recent clearance of vegetation associated with the dredging works, allows good light penetration to the stream bed, but destabilises bankside soil until vegetation is re-established (Figure 3:18 (a)).

Elsewhere, the river exhibits greater sinuosity. The channel morphology is U shaped, with steep, clay banks (Figure 3:18 (e)). These are mostly lined with hedges of blackthorn (*Prunus spinosa*), alder (*Alnus glutinosa*) and some oak (*Quercus spp*), mixed with brambles (*Rubus fruticosa*) and nettles (*Urtica dioica*); allowing little light to reach the stream bed, particularly in the summer. Where light reaches the stream, fool's watercress (*Apium nodiflorum*) is common. However, riparian woodland is present along much of the reach, some established and some newly planted, further reducing light availability. The river bed is a mixture of gravel, sand and clay.

At the confluence of the three tributaries, and where flow is obstructed (mainly by fallen trees), fine sediment accumulates to depths of up to 40 cm. The combination of fine sediment load and periodic pulses of high organic content slurry, resulted in low dissolved oxygen concentrations (Chapter 5) and the sediments become anoxic within 1 mm of the surface, exhibiting characteristic black colouration and sulphurous odour. In WY 2011 (Oct 2011 – Oct 2012), the reach average total mass of fine sediment was 4.2 kg m^{-2}. Of this, 16% (0.672 kg m^{-2}) was organic matter determined as ash free dry mass (AFDM: 500^{o}C). On average, only 18% of the total was ‘surface’ fine sediment with the remainder being incorporated into the interstices to a depth of approximately 10 cm (QMUL.RCG,).

The overall sediment size distribution in the stream was determined from cores at the upstream experimental site, PFUS. Here, 61.5% of the sediment was made up of particles that passed through a 2 mm sieve and of those, 80% passed through the 0.06 mm sieve. Silt and clay fractions, therefore, constitute around 49% of the total sediment sampled from these cores. Analysis of organic matter content, by L.O.I ($550^{o}C$) returned an average of 1.245 kg m^{-2}. Organic matter constituted 8.5% of the < 2mm fraction and 5.95% of total sediment.

3.3.3. Biological indicators of ecological status

One of the most obvious visual signs of nutrient enrichment to surface waters is the development of fast growing algal communities. In the Priors Farm study reach, wherever there is sufficient light, dense populations of filamentous algae develop, smothering macrophytes and stream bed sediments when they die and decay (Figure 3:21).

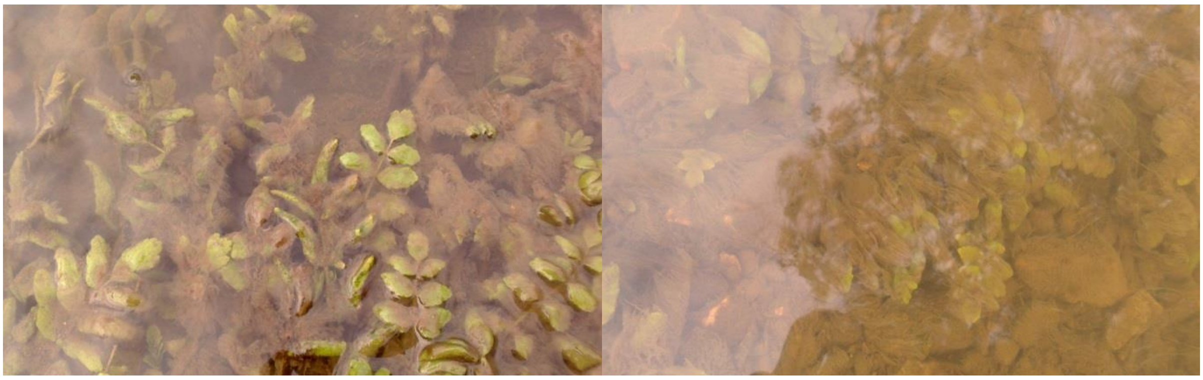


Figure 3:21 Filamentous algae smothering macrophytes and benthic sediments at Priors Farm

The scores obtained from the macro-invertebrate assemblages collected from the Priors Farm study reach indicated moderate stress from organic pollution (ASPT 0.85) and from impaired flow (LIFE 0.88). Stress as a result of sediment load was severe, (PSI_{sp} 0.35). Despite these low scores, NTAXA, the index of general degradation, returned a score that would denote high status if used in isolation (NTAXA 1.10), emphasising the need for using a wide range of classification tools to assess stream ecological status. Results from the LEAFPACS and DARES indices returned moderate to good status for the Priors Farm reach.

3.4. Overview of key similarities and differences in the study sites

The detailed study of the two sub-catchments revealed both similarities and differences. These can be briefly summarised as follows:

3.4.1. Intrinsic sub-catchment characteristics

Both of the sub-catchments studied in this research programme are small ($5km^2$ and $2km^2$ for Priors Farm and Cool's Cottage, respectively); with similar soil types; a mixture of clay, clayey loam and sandy loam. The underlying geology through which the streams flow is similar, predominantly low permeability, Kimmeridge clay, although the Cool's Cottage headwaters are fed by springs arising from more permeable geology in the upper sub-catchment. Meteorological conditions were the same for both sub-catchments and both exhibited a lag in flow response to the onset of rain following the dry summer of 2011. Subsequently, both exhibited the flashy response to rainfall that is typical of clay soils. Peak flows at the two sites are closely aligned, both to each other and to rainfall (Figure 3:22), demonstrating that peak flow is largely dominated by surface runoff or interstitial through-flow. In contrast, the baseflow response differs in the two sub-catchments. The flow at

Priors Farm rapidly declines to minimum discharge following intense rain events, while the return to baseflow at Cool's Cottage shows some damping (Figure 3:22), reflecting the contribution of groundwater spring sources in the headwaters of the Cool's Cottage reach and the buffering effect of the lake in Clay Hill Wood. During the dry summer of 2013 for example, the river at Priors Farm dried up leaving isolated pools along the stream bed while at Cool's Cottage, flow continued throughout the summer (Figure 3:22).

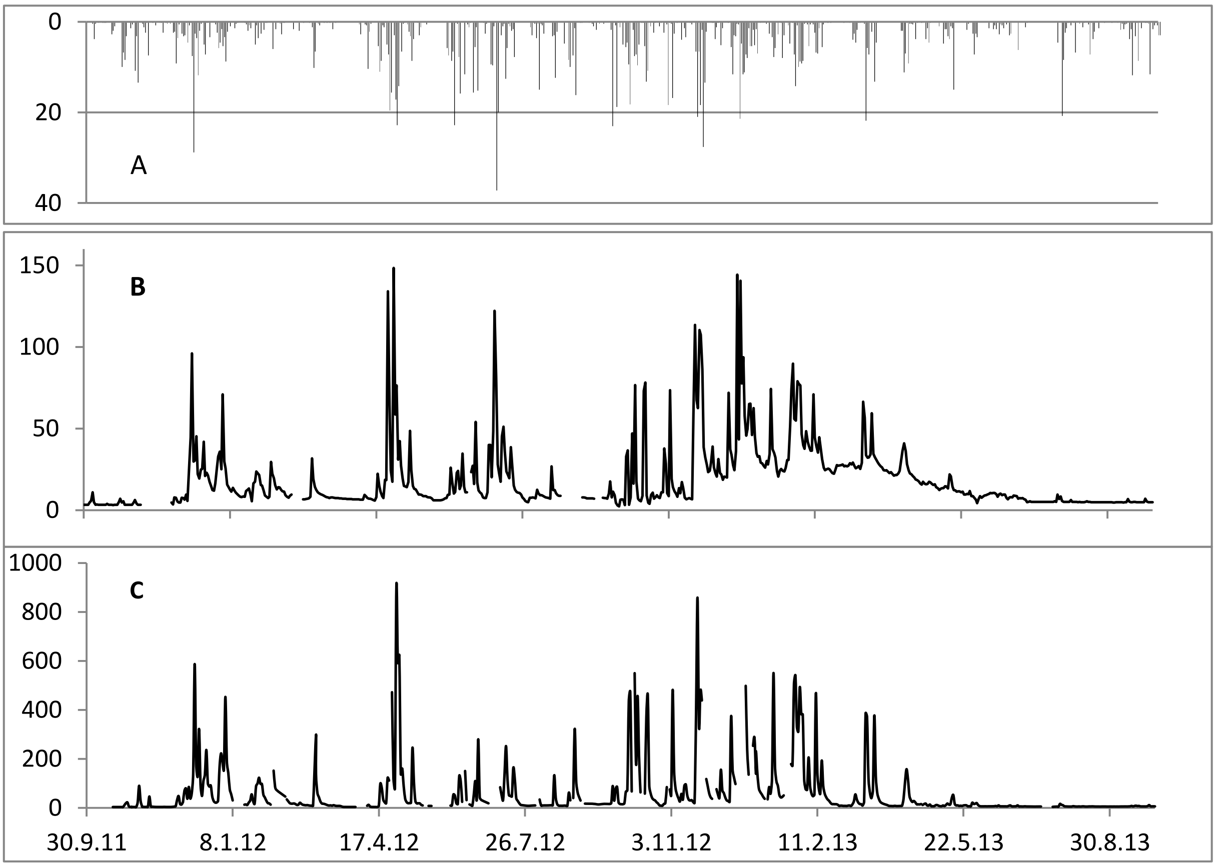


Figure 3:22 (A) Daily rainfall (mm) at Tisbury (Wilts) and discharge (l $^{s-1}$) at the outlets from the (B) Cools Cottage and (C) Priors Farm sub-catchments

3.4.2. **Management driven characteristics**

Both sub-catchments were predominantly used for stock grazing. However, the intensity of land use, and differences in management practices associated with dairy and beef production resulted in contrasts in the quantity and quality of inputs from the sub-catchments reaching the streams. In the Priors Farm sub-catchment, there was some cultivation of fields adjacent to the stream that contributed to the input of sediments and associated nutrients during wet weather (Figure 3:11 (c)). In addition, both slurry and inorganic fertilisers were applied to pasture immediately adjoining the stream, whereas in the Cool’s Cottage sub-catchment, the pasture surrounding the stream was managed organically, and waste applied as solid manure during dry weather. This pasture appeared to act as a buffer between the Cool’s Cottage study reach and the areas in the sub-catchment that were managed more intensively. Frequent movements of dairy herds from pasture to farmyard (for milking for example) resulted in accelerated damage to soil structure in the Priors Farm sub-catchment and, together with run-off from degraded farm track-ways, contributed substantial inputs of organically rich, fine sediments to the watercourse (Figure 3:11(g)). The storage capacity for slurry in the Priors Farm sub-catchment proved insufficient to deal with the weather conditions experienced during the study period, and the failure of stores to contain it resulted in periodic pulses of high organic content input being delivered to the watercourse on several occasions (see chapter 5).

3.5. Conclusions

The detailed site descriptions presented above identified differences in catchment geology that influenced the relative proportion of surface flow versus base-flow in the study reaches, and differences in the management and intensity of land use between the two sub-catchments. Both study sub-catchments exhibited characteristics that could be linked to land management. For example, a combination of management practices and wet weather resulted in high rates of sediment and organic-rich nutrient being delivered to the Priors Farm reach, that was not observed in the Cool’s Cottage reach.

A key question arising from chapter 3 is, therefore, whether these differences in catchment characteristics will have a measureable impact on water quality, thus providing an opportunity to study the impact of these fluxes on stream ecosystem function?

This question will be addressed in chapter 5 where a detailed description of nutrient chemistry in the two study reaches is presented. The approach and methods employed to address this question are presented in chapter 4.

Chapter 4. **Sampling strategy and methods**

4.1. Programme design

Chapter 2 detailed a variety of process measurements that have been proposed as suitable for describing in-stream ecosystem function and for use as indicators of stream health (Rosenfeld and Mackay, 1987; Meyer, 1989; Gessner and Chauvet, 2002; McTammany et al., 2003; Young et al., 2008; Young and Collier, 2009; Clapcott et al., 2010; Imberger et al., 2010). In this chapter, the sampling strategy and programme of process measurements designed to investigate their sensitivity to variations in in-stream water chemistry and sediment characteristics at differing temporal scales is described. To this end, short term measurements (24 hrs) of key metabolic processes (photosynthetic gross primary production (GPP), aerobic community respiration (ER) and the transfer of greenhouse gasses across the sediment-water interface) were conducted in conjunction with longer term (30 days) studies of leaf litter degradation and macro-invertebrate grazing. These were set in the context of an intensive monitoring programme of in-stream nutrient chemistry, designed to establish whether it reflects the dissimilarities in the sub-catchments identified in chapter 3.

4.2. Hydrochemistry

4.2.1. **Sampling programme:**

In order to interpret the results of the measurements of in-stream processes, it was essential to have a strong understanding of the hydrochemical environment and the variability in water chemistry over the study period within the two sub-catchments. Because the process measurements employed in this study respond at different time scales

(Snell et al., 2014), it was important to characterise the hydrochemical environment at appropriate spatial and temporal resolution. Therefore, the sampling programme was conducted at a range of temporal frequencies (Figure 4:1). These can be categorised as:

- ‘high resolution’ (daily and sub-daily storm) samples to describe the temporal variability in the ambient, in-stream nutrient concentrations experienced by the biota and to identify specific events. All samples were analysed to determine:
    - dissolved organic carbon (DOC),
    - nitrogen (N) species: total ammonium nitrogen (NH_4-N) measured as the sum of ammonium (NH_4^+-N) and ammonia (NH_3-N); total oxidised nitrogen (TON) measured as the sum of nitrite (NO_2-N) and nitrate (NO_3^--N), dissolved organic nitrogen (DON) and particulate organic nitrogen (PON).
    - phosphorus (P) fractions: soluble reactive phosphorus (SRP) measured as orthophosphate (PO_4-P), soluble unreactive phosphorus (SUP, primarily in the form of dissolved organic P (DOP)) and particulate phosphorus (PP).
- ‘medium resolution’ (weekly) grab samples to characterise spatial variability in nutrient concentrations along each stream from the source to the high resolution sampling site at each sub-catchment outlet. Samples were analysed to determine DOC, N species and P fraction concentrations.
- ‘occasional’ grab samples. The ratio of calcium (Ca) and magnesium (Mg) has been shown to be a good means of distinguishing between groundwater sources (Allen et al., 2014). These occasional samples were analysed to determine a range of cations, notably Ca and Mg to distinguish between possible sources of water in the sub-catchments (see chapter 3.)

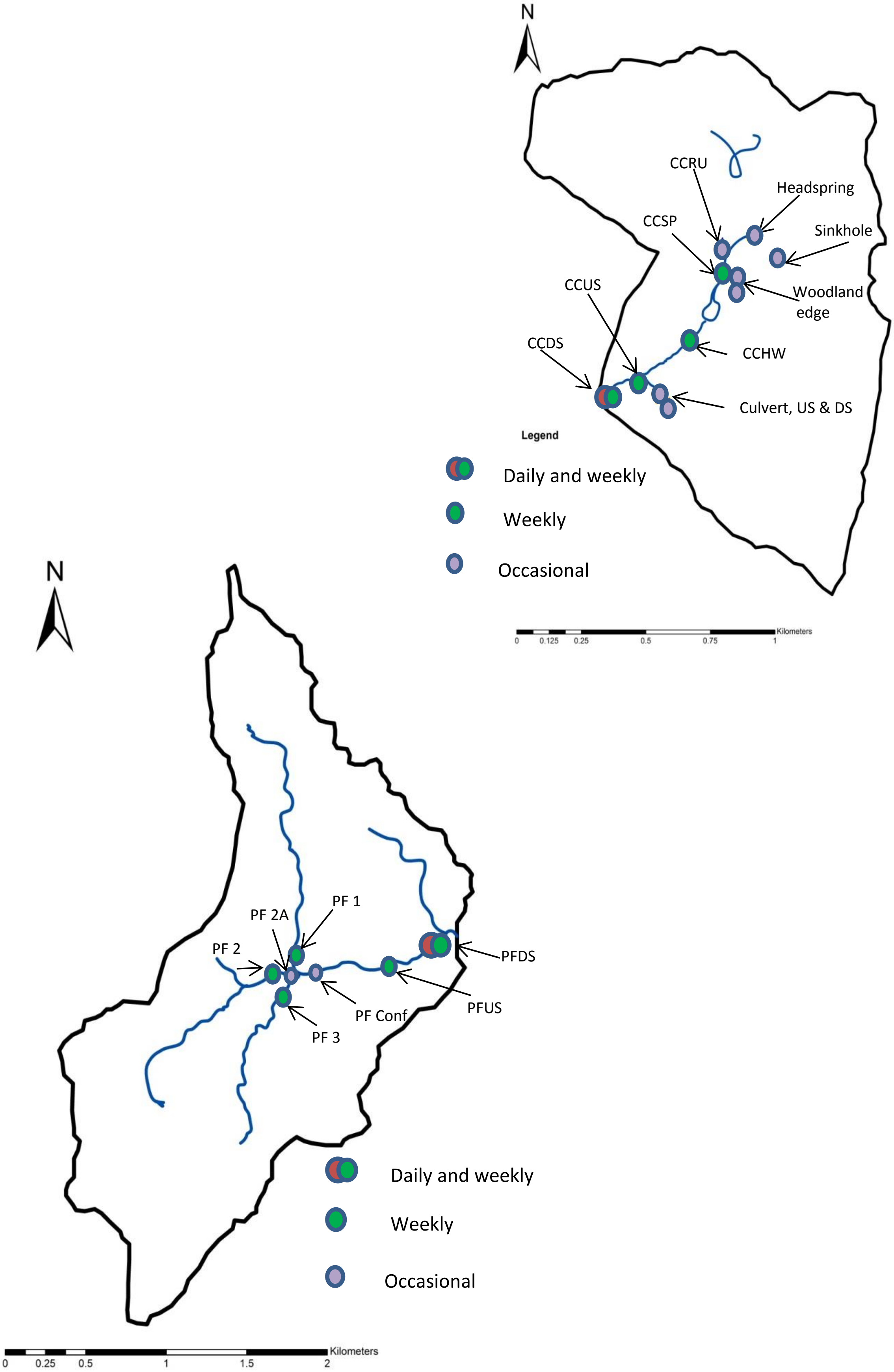


Figure 4:1 The locations and frequecy of the water chemistry sampling programme A, Cool's Cottage; B, Priors Farm.

In addition, continuous observations (15 minute intervals) were made of a range of physico-chemical variables likely to influence or respond to the in-stream processing of nutrients within each study reach. These included: dissolved oxygen concentrations (DO) and temperature (measured using an Aanderaa Oxygen–Optode -2), turbidity (measured using a YSI sonde series 6), water velocity and stage height (measured using a Mace FloPro XCi). Stream discharge ($m^3 s^{-1}$) was calculated from water velocity ($m s^{-1}$), and the cross sectional area (m^2) given by the stage height at a fixed point with a known channel width at the catchment outlet sampling station. Where necessary (at the Priors Farm downstream site), discharge was corrected for out-of-bank flow using stage height and a weir equation based on (Brater and King, 1976), Lloyd. C, pers comm).

4.2.2. **Nutrient chemistry**

High resolution water samples were collected from the sub-catchment outlets by streamside auto-samplers (ISCO 3700) that were housed in the DTC monitoring cabinets and linked to the Mace Flo-Pro acoustic Doppler velocity meters that controlled the storm sampling programme at each site. The regular, daily samples were supplemented by additional, flow proportional sampling, triggered by cumulative discharge with regularly updated threshold values. These threshold values were determined by the ADAS field team supporting the instrumentation platform on the DTC programme. Samples were stored within the auto-sampler units and collected weekly by ADAS staff (Figure 4:2). On collection, one aliquot of each sample was filtered through 0.45µm pore size cellulose nitrate filter (Whatman). Filtered and unfiltered aliquots were transferred to Nalgene HDPE storage bottles and stored at $4^{\circ}C$ in cool-boxes. These were shipped by overnight courier to the University of Reading (UOR) laboratory.

Figure 4:2 One of the weekly sample collections from the Priors Farm monitoring station

This sampling regime resulted in samples being stored in the streamside cabinets for periods of up to one week. Refrigerated storage in the dark is routinely used to preserve water samples for nutrient analysis over short periods (Kotlash and Chessman, 1998; Gardolinski et al., 2001). Lack of mains power in the monitoring cabinets, however, gave rise to the samples being stored at ambient temperature, albeit in the dark. Some degradation of samples as a result of both biological and physicochemical processes was, therefore, an inevitable consequence of the sampling programme. Both NH_4-N and SRP are particularly sensitive to degradation during storage. The partitioning of NH_4-N into NH_4^+ and NH_3 is determined by pH and temperature, with the proportion of NH_3 increasing with increasing temperature and pH (Figure 4:3). NH_3 is volatile, so higher temperatures can lead to the loss of N from stored samples (samples stored in the cabinets remained open to the atmosphere until collected). Higher temperatures will also increase any degradation of samples due to biological activity, and changes to both N speciation and P fractionation are likely to occur.

In addition, fluctuations in temperature and pH are likely to result in changes to the adsorption or desorption of SRP on particulate matter within the samples (Kotlash and Chessman, 1998; Jarvie et al., 2002; Evans and Johnes, 2004). With these limitations in mind, uncertainties in the extent to which the analyses of stored samples are a true representation of the original sample must be considered.

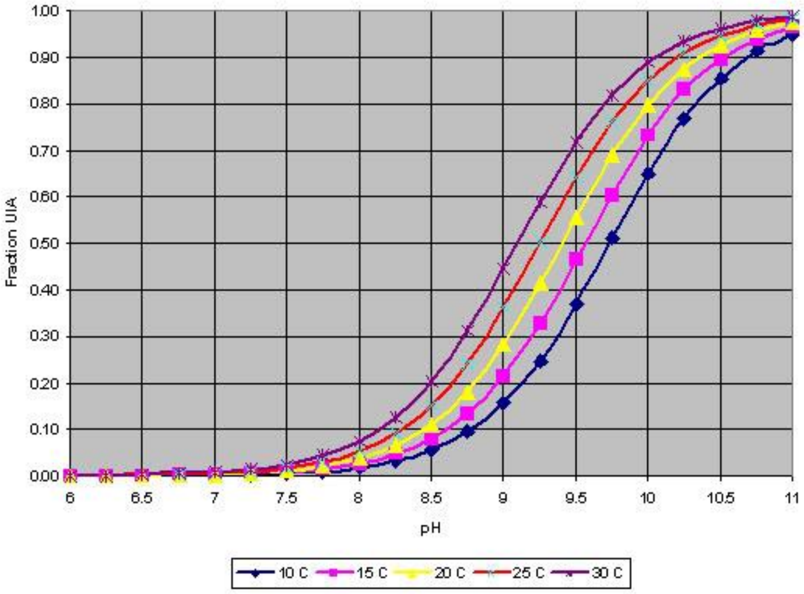


Figure 4:3 The relationship between pH and temperature and the proportion of NH4-N as un-ionised ammonia, NH3.

A grab sample was collected at the same time as the auto-samplers were emptied and processed using the same procedures. Where the weekly grab samples were taken before that day's triggered samples, comparisons between fresh samples and those stored for a week, were possible. These identified differences in the effect of storage between sites. The most substantial changes in water chemistry related to storage identified by this method were the loss of NH_4-N at concentrations higher than 0.3 mg l^{-1} at the Priors Farm downstream site, loss of SRP at the Cool's Cottage downstream site, increases in both particulate N and P at both sites with a small, concomitant loss of DON and SUP at the Priors

Farm downstream site. A trend analysis conducted over multiple sampling sites and using storage time as the variable, indicated a systematic loss of NH_4-N alone, with other determinands seemingly unaffected (Lloyd C., pers comm).

To increase the spatial coverage of data collection within the sub-catchments, and to identify possible origins of water pollution, additional weekly samples were collected in HPDE bottles, at sites along the study reaches, upstream of the sub-catchment outlet (Figure 4:1). Water samples were also collected from incubation chambers as part of the study of metabolic processes (see section 4.5.1 and Chapter 6). These samples were stored overnight, in the dark at 4°C. On arrival at the laboratory, one aliquot of each sample was filtered through 0.45 µm pore size cellulose nitrate (Whatman) filters. Filtered and unfiltered aliquots were processed and analysed with the routine daily samples using the protocols detailed below.

4.2.3. Sample processing and analysis

4.2.3.1. Dissolved Organic Carbon

As the Hampshire Avon flows through a predominantly chalk catchment, the standard procedure used for determining DOC was the ‘non purgeable organic carbon’ (NPOC) method, chosen to minimise interference from the high concentration of inorganic carbon that is characteristic of chalk streams. This procedure was adopted throughout the Hampshire Avon DTC to facilitate the processing of the regular samples and maintain consistency between them. On arrival at the laboratory, 10 ml of the filtered samples were acidified with 100 µL of 15% hydrochloric acid (HCl) to a pH of between 2 and 3, to convert inorganic carbon to CO_2 which was then driven off (sparged) using CO_2 free air. Remaining (non-purgeable) carbon was measured using a Shimadzu TOC analyser, T5000 in which, 100 µL of sparged sample was injected into the combustion column, where high temperature (680°C) catalytic oxidation of the sample resulted in the production of CO_2. The concentration of CO_2 was calculated from peak areas generated by a non-dispursive infrared gas detector, calibrated against 0, 10 and 20mg L^{-1} standard solutions.

4.2.3.2. Nitrogen and Phosphorus

Dissolved inorganic nitrogen and phosphorus were analysed using a Skalar San ++ multi-channel, continuous flow auto-analyser. The auto-analyser was configured for the simultaneous measurement of total NH_4-N, TON and SRP. These soluble inorganic reactive forms were analysed using standard colourimetric techniques adapted for use on the auto-analyser:

- NH_4-N was measured using a modified Berthelot reaction (Crooke and Simpson, 1971). In an acid solution (pH 5.2) NH_4-N is chlorinated to monochloramine. This reacts with salicylate to form 5-aminosalicylate that forms a green complex on oxidation . It was measured at 660 nm.
- NO_3^- is reduced to NO_2^- by hydrazinium sulphate. NO_2^- produces an azo dye when reacted with sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride. This complex was measured at 540 nm (Henriksen and Selmer-Olsen, 1970).
- Phosphate reacts with ammonium heptamolybdate and potassium antimony (III) oxide tartrate to form a complex that turns blue when reduced by ascorbic acid. It was measured at 880 nm (Murphy and Riley, 1962).

Concentrations were calculated from peak heights, calibrated against freshly prepared standard solutions and corrected for drift by the addition of a further standard, repeatedly checked at an interval of 10 samples.

For the determination of the unreactive soluble and insoluble or particulate nutrients, 10 mL of filtered and unfiltered aliquots were subject to alkaline persulphate digestion, catalysed by high temperature and pressure in a microwave unit (CEM Mars Xpress) after Johnes and Heathwaite (1992) to produce soluble reactive forms. These were then analysed using the same procedures as above. Dissolved organic nitrogen (DON), particulate nitrogen (PON), soluble unreactive phosphorus (SUP) and particulate phosphorus (PP) were determined by difference (Figure 4:**4**).

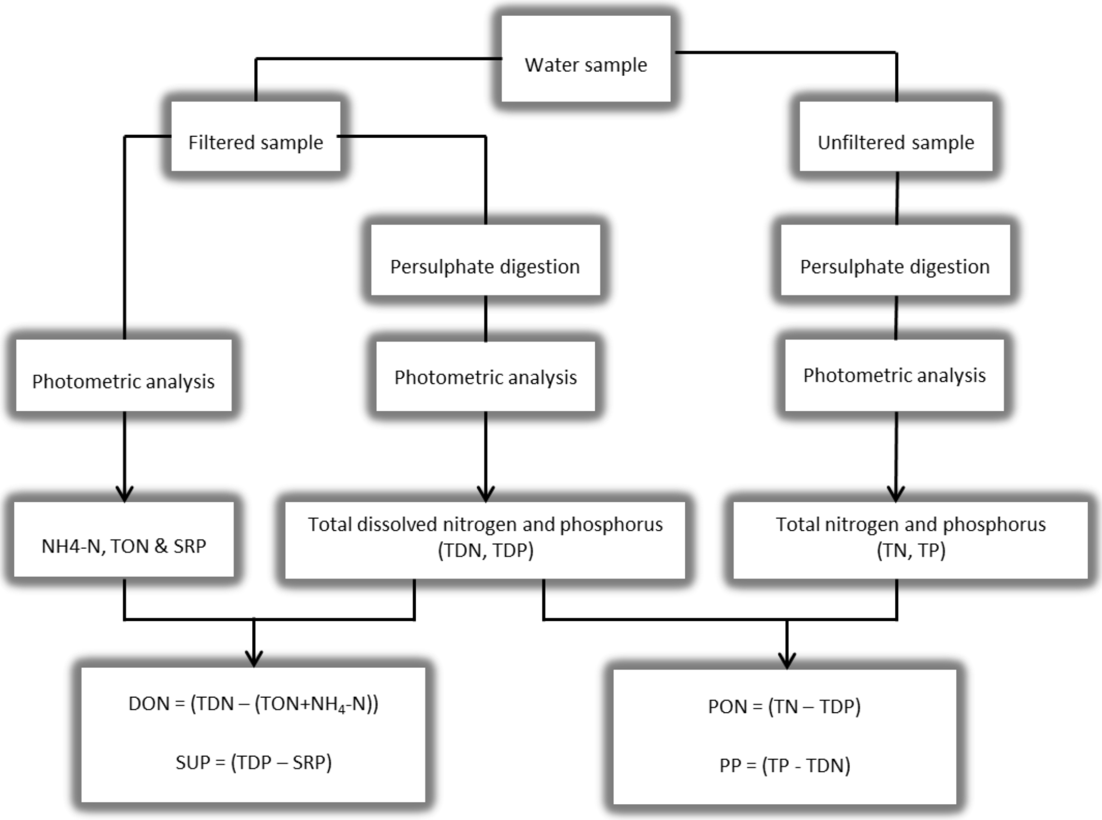


Figure 4:4 Determination of nitrogen species and phosphorus fractions by persulphate digestion after Johnes and Heathwaite 1992

4.2.4. **Nutrient load**

While instantaneous measures of nutrient concentrations are likely to be most relevant to microbial and algal processes in the water column, much of the biological activity in streams is concentrated at the sediment-water interface or in the benthos itself. Knowledge of the input of nutrients, integrated over time, may be a more effective descriptor of impact on the whole community than instantaneous concentrations in the water column. Nutrient load, defined as the mass of that nutrient passing a given cross section of the stream per unit time may, therefore, be a more appropriate measure. Nutrient load at the sub-catchment outlets was compared over the two full water years covered by the period of study, and was calculated from the instantaneous concentration at the time of sampling and daily mean discharge data, as discussed in Johnes, (2007) using equation 4.1

$$\text{Load} = K \sum_{i=1}^{n} (Ci \, Qpi)$$

Equation 4.1

Where:

- K = conversion factor to take account of period of record
- C_i = instantaneous concentration associated with individual samples (mg L^{-1})
- Q_{pi} mean discharge for interval between samples (L^3 s^{-1})
- n = number of samples

4.3. Bed sediment characteristics

On completion of the benthic incubations, the channel bed sediment cores within the chambers were recovered and frozen for analysis of grain size distribution and organic matter content.

4.3.1. **Grain size**

Once thawed, a subsample of each sediment core was washed through a stacked series of sieves with mesh sizes 2 mm, 500 µm, 250 µm, and 63 µm to separate the gravel (>2 mm), coarse and fine sand (2 mm – 500 µm ; 500 µm- 63 µm) and silt and clay (<63 µm) fractions. Sediment particles that passed through the 63 µm sieve were collected in large foil trays to facilitate rapid evaporation of the large volume of water generated. The contents of the sieves were transferred to foil trays, dried to a constant weight at 60 °C and weighed to give the proportion of each grain size in the subsample.

4.3.2. **Organic matter content.**

Oven dried samples were transferred to ceramic crucibles, weighed and combusted overnight in a muffle furnace at 550 °C (Rowell, 1994). At this temperature, organic carbon is driven off as CO_2 so weight loss is attributed to organic carbon. A second subsample was dried and weighed without fractioning to give total organic content. Organic matter is reported as loss on ignition (LOI.).

4.4. Calcium and Magnesium

Concentrated nitric acid was added to filtered samples to a final concentration of 5%. The samples were analysed by inductively coupled plasma atomic emission spectroscopy on a Perkin Elmer 7300 Dual View ICP-OES.

4.5. Short term metabolic processes

4.5.1. **Approach**

A series of incubations were performed during a twelve month period from June 20th, 2013 to June 3rd, 2014, to explore variations in key metabolic processes between the two sub-catchments. Four measures of ecosystem metabolism were made, namely:

Aerobic respiration;

- Whole stream and water column aerobic respiration were calculated from the change in oxygen concentration (Δ O_2) in dark benthic chambers and water bottles over 24 or 48 hours, respectively.

Primary production:

- Short term (24 or 48 hr) photosynthetic primary production was measured by comparing the change in dissolved oxygen concentration (Δ O_2) in light and dark chambers.

Nutrient processing:

- Changes in the concentration of N species, P fractions and DOC were measured in benthic chambers during 24 hour incubations.

Greenhouse gas flux

- The transfer of methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) across the sediment-water interface was measured during 24 hour incubations.

Initial incubations (June – September 2013) were carried out at two sites in the study area; Cool's Cottage and the Priors farm downstream site. In September 2013 a further site was added upstream in the Priors Farm reach (PFUS) to more closely mirror the ambient light conditions prevalent throughout the Cool’s Cottage sub-catchment and representing approximately 60% of the Priors Farm study reach (Figure 4:**5**).

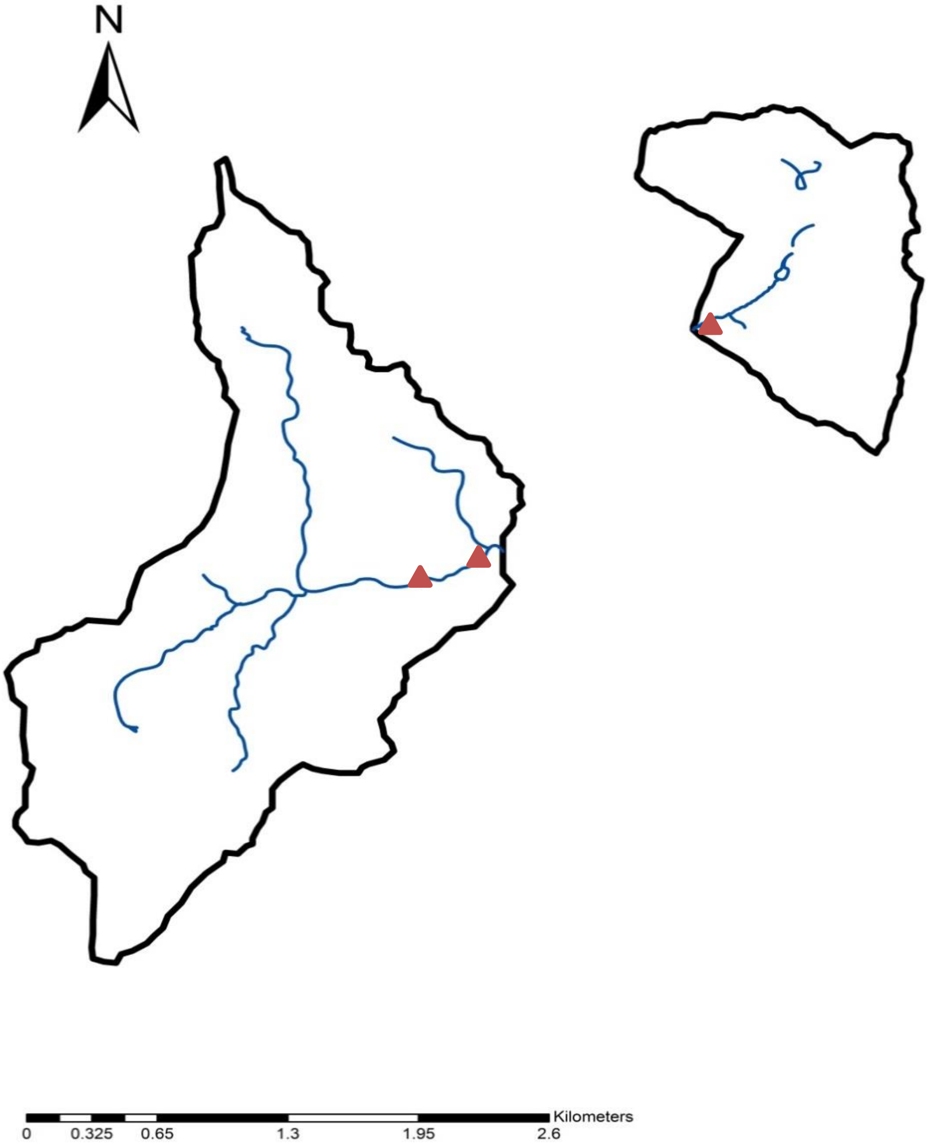


Figure 4:5 The location of the incubations measuring short term metabolic processes

The sites were chosen using the following criteria; depth, substrate and aspect:

1. **Depth:** There needed to be sufficient water depth to fully accommodate the incubation chambers and to ensure an adequate volume of water in the chamber, both for the incubation and for subsequent water sampling.
2. **Substrate:** The sites were chosen to ensure that, as far as possible, the substrate was comparable between sites. The chambers had to be driven into the river bed on each occasion, and there were few sites where this was possible without significant physical disturbance of the sediment structure.
3. **Aspect:** At closed canopy sites, incubation chambers were installed where watercourse direction and the height of the banks allowed maximum light penetration to the water surface. This provided conditions that were typical of the study reach and facilitated comparison of metabolic processes in both light and dark chambers.

The timetable of short term, community metabolism measurements was determined by the meteorological and hydrological conditions at the study sites. Metabolic rates are strongly influenced by temperature, so it was important to conduct incubations over the range of ambient water temperatures occurring at each site. Measurements were scheduled to include spring and summer, to capture seasonal variations that are potentially influenced by temperature, daylight hours and shading from riparian vegetation. Both high and low water levels precluded installation of the incubation chambers used in the project, thereby restricting measurements to summer / autumn 2013 and spring /early summer 2014.

The measures were grouped into **‘**dedicated’ incubations: (20.6.13, 12.7.13, 12.9.13, 24.9.13, 6.3.14 and 13.3.14)**,** where only aerobic respiration and photosynthetic primary production were calculated, and ‘combined’ incubations: (22.7.13, 1.4.14, 20.5.14 and 3.6.14), where a subsample of water was removed from the chamber for headspace gas analysis at the end of the incubation, but prior to the final DO readings being taken (Table 4:1).

Table 4:1 Timetable of short term community metabolism measurements

Date	Dedicated aerobic measures in benthic chambers	Combined measures	Gas transfer only	Aerobic measures in water bottles
20.6.13	CCDS, PFDS			CCDS, PFDS
12.7.13	CCDS, PFDS			CCDS, PFDS
22.7.13		CCDS, PFDS		
12.9.13	CCDS, PFDS, PFUS			CCDS, PFDS, PFUS
24.9.13	CCDS, PFDS, PFUS			CCDS, PFDS, PFUS
6.3.14	CCDS, PFDS, PFUS			CCDS, PFDS, PFUS
13.3.14	CCDS, PFDS, PFUS			CCDS, PFDS, PFUS
1.4.14		CCDS, PFDS, PFUS		
8.4.14			CCDS, PFDS, PFUS	
20.5.14		CCDS, PFDS, PFUS		
3.6.14		CCDS, PFDS, PFUS		PFDS

A water sample was also taken from the combined incubation chambers, after the final DO reading, to assess changes in the concentrations of N species, P fractions and DOC. On one occasion, (8.4.14), low sample volumes precluded the measurement of O_2 after removal of the subsample required for headspace analysis. Only greenhouse gas transfer across the sediment-water interface was recorded on this date. The contribution of the water column to community aerobic respiration (ER) and photosynthetic gross primary production (GPP) was calculated from changes in DO concentration in light and dark water bottles incubated alongside the benthic incubation chambers.

4.5.1.1. Detailed methodology

Short term metabolism was characterised at each site in incubation chambers, small enough to be pushed or driven into the bed sediments while minimising disturbance, and in bottles tethered alongside them. For both the benthic chambers and water bottle incubations, light and dark versions were installed. Materials used in the construction of the incubation vessels were chosen for their low permeability to water, O_2 and CO_2. The bottles used were made of polyethylene terephthalate (PET) from a proprietary supplier of carbonated water (Buxton). PET has a very low permeability constant for both oxygen (O_2) and CO_2 (Bhadha, 1999). Pilot studies revealed that using black plastic or tape to produce the dark bottles resulted in water temperature increases, and aluminium foil was therefore used to exclude light (Figure 4:6). The light benthic chambers were constructed of 80 mm diameter, ‘Perspex’ (poly methyl methacrylate) pipe and the dark chambers, 66 mm diameter PVC (poly vinyl chloride) soil pipe (FloPlast). The seal for both was a double sheet of 180 gauge polyethylene (Pro-Loc) fixed in place with triple elastic bands. The dark chambers were then topped with aluminium foil, held in place with elastic bands, to exclude light (Figure 4:7).

Figure 4:6 The bottles used to measure water column aerobic metabolic processes in the light and dark.

Figure 4:7 The benthic incubation chambers used to measure community aerobic metabolic processes in the light and dark.

The benthic incubation chambers were pushed into the stream bed to a minimum depth of 10 cm. At low water levels during low flow periods, this depth increased to ensure the chamber was completely submerged and no air was trapped in the chamber. Where the interstices were clogged by fine sediment resulting in a cemented stream bed, a metal former, with the same diameter as the chambers, was hammered into the sediment and removed, facilitating installation of the chambers. Care was taken to minimise disturbance to the bed sediment as much as possible during installation. The chambers were then allowed to equilibrate with the overlying water, following which, the chambers were sealed. Extreme care was taken to exclude any air bubbles from the chambers and bottles prior to sealing. Equilibration was assumed to have occurred when the DO concentration of the chamber water and stream water were the same. The time for equilibration varied with flow, generally within a few minutes, but up to 15 minutes during low flow periods at Priors Farm. Initial DO readings and water samples for the measurement of nutrient concentrations (t=0) were taken from the river, except during September 2013 when there was no flow in the Priors Farm study reach and the chambers did not reach equilibrium with the overlying water, even after 40 minutes. On these two dates, initial DO readings and water samples for the measurement of nutrient concentrations (t=0) were taken from individual chambers.

Figure 4:8 Benthic incubations installed on site: A and B, PFDS; C CCDS.

4.5.1.2. Aerobic respiration and primary production – dedicated incubations

Benthic chambers were incubated *in situ* for 24 hours (Figure 4:8) and water bottles for either 24 or 48 hours (Figure 4:9), after which a final DO reading was taken. For the benthic chambers, a small incision was made in the polythene seal and the DO probe inserted immediately to take a final DO reading. This procedure was also followed for the readings in the PET bottles.

Figure 4:9 Benthic incubation chambers and water bottles installed at the Priors Farm upstream site

Volumetric ecosystem aerobic respiration (ER) was calculated from ΔO_2 in the dark incubation chambers using Equation 4.2

$$ER_{(24)} = \Delta O_2D_{(24)} = -1x\ (O_2D_{(t)} - O_2D_{(t=0)}\ x\ (24/t))$$

Equation 4.2

Where:

- $ER_{(24)}$ = ecosystem aerobic respiration over 24 hrs
- t= incubation time in hrs
- $O_2D_{(t)}$ = DO concentration in dark chambers at time t
- $O_2D_{(t=0)}$ = DO concentration in dark chambers at the start of the incubation

Volumetric net ecosystem production (NEP) was calculated from ΔO_2 in the light incubation chambers using Equation 4.3

$$NEP_{(24)} = \Delta O_2 L_{(24)} = O_{2\ (t)} - O_{2\ (t=0)}\ x\ (24/t)$$

Equation 4.3

Where:

- O_2L (t) = DO concentration in light chambers at time t
- O_2L (t=0) = DO concentration in light chambers at the start of the incubation

Volumetric GPP was calculated using Equation 4.4

$$GPP_{(24)} = NEP_{(24)} - ER_{(24)}$$

Equation 4.4

After the final DO reading was taken, the internal height of the chamber to bed sediment was measured in four places, giving the volume of the incubation chamber and allowing daily process rates to be quoted on an aerial basis as $mgO_2\ cm^{-1}d^{-1}$ (Equation 4.5).

$$\text{Aerial ER} = ER_{(24)/}1000\ x\ H.$$

Equation 4.5

Where: H = chamber height (cm)

Results were reported as the mean of 3 ‘pseudo’ replicates (1 sample each from 6 chambers, 3 light and 3 dark at each site).

4.5.1.3. Greenhouse Gas transfer – combined incubations

Benthic chambers for the combined incubations were installed following the procedure described above. For the combined experiments, at the end of the incubation, 60 ml of sample was removed from the chamber, using a luer lock syringe equipped with a 19G (1.1 mm) hypodermic needle. The polythene seal on the chambers was allowed to deform to a concave shape to compensate for fluid loss. Nevertheless, removal of this subsample resulted in a systematic error that was compensated for (see chapter 6). The 60 mL sample was transferred using a 21G (0.8mm) hypodermic needle, to a pre-prepared, helium filled Duran bottle (Schott) whose lid was fitted with an air tight seal and equipped with a septum (Supelco), housed in a brass 8 mm straight coupling (B&Q plumbing supplies), or 8 mm gland (RS supplies; Figure 4:10).

Figure 4:10 Duran bottles modified for headspace analysis and fitted with housings for the silicon septa.

The bottles were pre-dosed with 3 mL of 50%w/v zinc chloride (ZnCl) to stop further biological processes (Elkins, 1980; Pretty et al., 2006; Walker et al., 2010; Hinshaw and Dahlgren, 2013). Pressure equilibration was achieved by inserting a second hypodermic needle, free to vent to the atmosphere. Once equilibrated to atmospheric pressure, the septa were covered with insulation tape (as an extra precaution) and the bottles shaken vigorously for 60 seconds. Samples were stored overnight to optimise headspace equilibration, aided by two further sessions of vigorous mixing by shaking. On the following day, after a final shaking, 60 mL of headspace gas was sampled using water displacement (Figure 4:11) into a second luer lock syringe and transferred to a pre-evacuated 22 mL, gas tight vial, fitted with a silicon septum. The 60 mL volume allowed for flushing of the gas tight vials with 2 x their volume of sample before being completely filled.

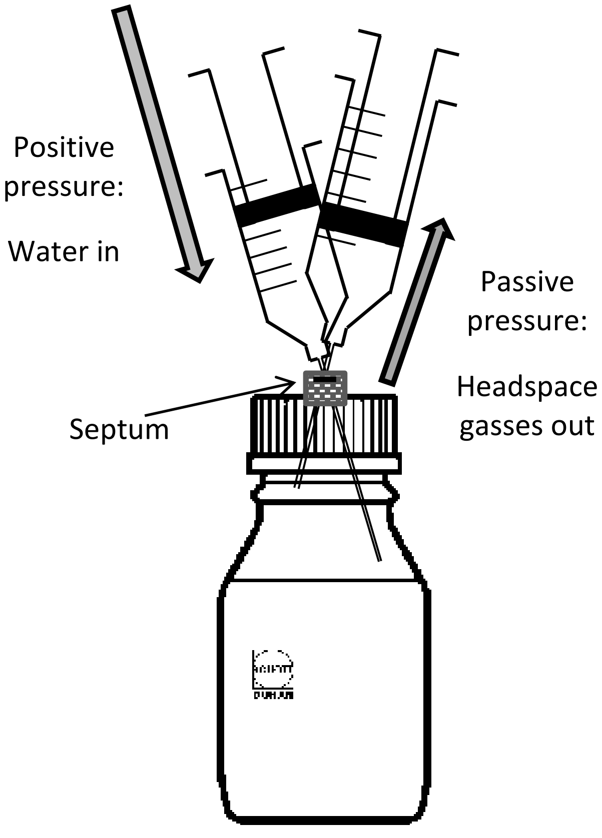


Figure 4:11 The method used to extract the headspace gasses after equilibration using positive pressure displacement.

Pressure equilibration was achieved by the insertion of a second hypodermic needle, free to vent to the atmosphere. The gas vials were delivered to the Forestry Commission's Research facility at Alice Holt, Surrey, UK, where they were stored in an atmospherically controlled store until analysis.

4.5.1.4. Greenhouse gas analysis

Headspace analysis was carried out by Forest Research. Headspace gases were analysed simultaneously (Hall and Dowdell, 1981) on a Perkin Elmer Clarus 500 Gas Chromatograph, equipped with a TurboMatrix 110 automatic headspace sampler. The sample was split and component gases were separated at 50°C, using N_2 as the carrier gas, by passing through parallel, 30 m megabore (0.53 mm I.D.) capillary 'Elite-Plot Q' columns (fused silica lined with 'Chromosorb 101', 'Porapak Q' and 'Haysep Q'). CO_2 was reduced to CH_4 by a 'methanizing catalytic converter' and the resulting two peaks of CH_4 were measured using a Flame Ionisation Detector (FID) heated at 350°C and supplied with combustion gases, H_2 and air, with flow rates of 45 and

450 mL min^{-1} respectively. N_2O was measured using an Electron Capture Detector (ECD) heated at 375°C. Headspace concentrations were calculated from peak areas, calibrated and corrected for drift using three calibrations standards (AirProducts UK Ltd):

1. 0.2ppm N_2O + 1.2 ppm CH_4 + 300 ppm CO_2
2. 1.0 ppm N_2O + 6 ppm CH_4 + 1500 ppm CO_2
3. 5.0 ppm N_2O + 30 ppm CH_4 + 7500 ppm CO_2

Sample concentrations of CO_2 were outside the range of the calibration standards and a second analysis was conducted on samples that had been diluted by a factor of 3, and corrected for dilution and loss of sample.

4.5.1.5. Calculation of greenhouse gas concentrations

Data supplied by Forest Research returned values for greenhouse gas headspace concentrations in ppm. GHG concentrations in the original water samples were calculated using the ideal gas law and the solubility coefficients (K_o) calculated by Weiss (1974) for CO_2 and Weiss and Price (1980) for N_2O. For CH_4, K_o was derived from the Bunsen coefficient (β) calculated by Yamamoto et al. (1976) using Equation 4.6.

Atmospheric pressure was not recorded and is given the value of 1hPa throughout.

$$K_o = \beta / V_{(T)}.$$

Equation 4.6

where $V_{(T)}$ is the volume of the gas at a measured temperature expressed in °K.

The steps used in back calculating the original concentration in the water sample were as follows:

$$C_{(ws)}(nM) = (nmoles_{(hs)} + nmoles_{(wp))/}V_{(ws)}$$

Equation 4.7

$$nmoles_{(hs)} = (P_{(v)}/RT) \times V_{(hs)}$$

Equation 4.8

$$nmoles(wp) = P_{(V)} \times K_{o(T)} \times V_{(wp)}$$

Equation 4.9

Where

- $C_{(ws)}$ = calculated Molar concentration of original sample
- hs = headspace in Duran bottle
- wp = water phase in Duran bottle
- ws = original water sample
- V = volume in litres (L)
- $P_{(V)}$ = measured concentration of headspace gas (ppb)
- R = universal gas constant, 0.082058
- T = equilibrium temperature (°K)
- $K_{o(T)}$ = solubility coefficient at equilibrium temperature T

To allow comparisons between sites, GHG transfer across the sediment-water interface was calculated on an aerial basis as above (4.5.1.2,). As found in other studies (Pretty et al., 2006; Sanders et al., 2007; Trimmer et al., 2010), a high degree of spatial heterogeneity was observed. For this reason, data are reported both on an individual basis and as the mean of 3 pseudo replicates, light and dark at 3 sites (1 sample each from 6 chambers, 3 light and 3 dark at each site).

4.6. Time integrated ecosystem processes

4.6.1. Leaf litter degradation

The in-stream processing of leaf litter was investigated using leaf packs (Petersen and Cummins, 1974; Gessner and Chauvet, 2002; Hladyz et al., 2011b). Oak leaves, collected immediately after abscission, were dried at 80°C. 5 g (+/- 0.05 g) of leaves were weighed and packed into mesh bags before being installed in the study sites. Leaf packs were tethered to 0.6 m reinforced steel bars driven into the stream bed. Two mesh sizes (4 mm and 0.5 mm) were chosen to allow or exclude macro-invertebrates (Figure 4:12). Temperature was recorded during the incubation on waterproof loggers (Hobo UA 002 64), tethered to the steel bars, but with sufficient clearance from the leaf packs to prevent interference from them.

The leaf packs were incubated for 30 days. At the end of the incubation period, the bags were removed from the stream by lifting into a plankton net (1 mm mesh) to prevent loss of leaf fragments from the coarse mesh bags. The bags were sealed in individual plastic bags and stored in a freezer until analysis. Contents of the bags were washed through a 500 µm mesh sieve to remove fine sediment particles and emptied into a sorting tray. Sticks and non-oak leaf fragments were removed from the coarse mesh bags. Macro-invertebrates were removed, dominant taxa noted and stored in 80% ethanol for later examination. Leaf fragments were washed clean of sediment and transferred to a foil tray, dried at 80°C and weighed. The loss of leaf litter mass was calculated from the change in dry mass of leaf litter over the incubation period. Temperature compensated rate coefficients ($-k\ dd^{-1}$) were derived from an exponential model of decay (Petersen and Cummins, 1974; Gessner and Chauvet, 2002; Barlocher, 2005b), using Equation 4.10.

$$-k\ dd^{-1} = \ln (M_f/M_i) / dd$$

Equation 4.10

Where:

- $k\ dd^{-1}$= rate of leaf loss per degree day
- M_f = leaf mass (g) after incubation
- M_i = leaf mass (g) at the start of the incubation
- dd = degree days

Leaf loss in the fine mesh bags ($-k_{(mic)}.dd^{-1}$) was attributed to microbial action and physical and chemical processes such as leaching and flow related damage (Barlocher, 2005a). Leaf loss in the coarse mesh bags ($-k_{(tot)}.dd^{-1}$) was attributed to the combination of these processes and macro-invertebrate grazing. The proportion of leaf loss due to macro-invertebrate action ($-k_{(invert)}.dd^{-1}$) was calculated from the difference in leaf loss from the coarse and fine mesh bags.

4.6.2. **Epilithic primary production and macro-invertebrate herbivory**

Unglazed ceramic tiles were anchored to the stream bed by fixing to engineering bricks that were secured by the reinforced steel bars tethering the leaf packs (Figure 4:12). At each location two tiles were installed (Figure 4:13). One tile was unaltered (‘grazed’ tile) and the other had its vertical edges covered in petroleum jelly (Figure 4:13) to exclude crawling macro-invertebrates (McAuliffe, 1984b). Epilithic primary production was calculated from the chlorophyll a (Chl-a) concentration extracted from the biofilm on the ‘un-grazed’ tile and herbivory was calculated from the difference in Chl-a between the ‘grazed’ and ‘un-grazed’ tiles.

Figure 4:12 Coarse and fine leaf litter bags, used to measure leaf litter degradation and the ceramic tiles, used to measure epilithic primary production and macro-invertebrate herbivory, prior to installation.

Figure 4:13 The ceramic tiles installed on the stream bed. The petroleum jelly coating the vertical sides of the right hand tile can just be seen.

4.6.2.1. Measurement of chlorophyll a:

At the end of the 30 day incubations, each pair of tiles was gently lifted from the water, the accrued periphyton on the tile surface removed by vigorous scrubbing with a toothbrush, and the resultant suspension washed into Nalgene HDPE bottles which was frozen for later analysis. Once thawed, the resultant slurry was made up to 300 mL and divided into 100 mL aliquots. The concentration of Chl-a was measured in one 100 mL aliquot after filtering through a GFC filter (Whatman), using a 24 hour, 90% acetone extraction (Talling and Driver, 1961). The filter was macerated for several minutes using a broken glass rod prior to extraction. After 24 hours extraction in the dark at 4°C, the tubes were agitated and centrifuged at 3500 rpm for 20 minutes. The supernatant was transferred to a disposable cuvette and absorbance was measured on a Cecil 1012 UV/Vis Spectrophotometer at a wavelength of 665 nm (corrected for background at 750 nm) before and after acidification. The concentration of Chl-a was calculated after Lorenzen (1967), and is reported as mg Chl-a m^{-2}. Macro-invertebrate herbivory was calculated from the difference in Chl a concentration on the grazed and un-grazed tiles and the temperature corrected grazing rate given by (Equation 4.11 and Equation 4.12)

$$\text{Herbivory } (p_{(herb)} dd^{-1}) = p_{(gross)}\, dd^{-1} - p_{(net)}\, dd^{-1}$$

Equation 4.11

And

$$\textit{Periphyton accrual } (p\ dd^{-1}) = \ln \text{periphyton accumulation} / dd$$

Equation 4.12

Where:

- Periphyton accumulation = Chl $a_{(f)}$ = chlorophyll a, mg m^{-2} after 30 days
- dd = degree days
- $p_{(gross)}\, dd^{-1}$ = rate of chlorophyll accrual on ungrazed tiles
- $p_{(net)}\, dd^{-1}$ = rate of chlorophyll accrual on grazed tiles

4.7. Statistical Analysis

Statistical tests such as Pearson’s correlation coefficients and ANOVA were carried out using MINITAB 16 statistical software. For testing statistical differences, analysis of variance was followed by Tukey’s test for *post hoc* pair-wise comparisons. Differences were considered significant when $P < 0.05$.

The following chapters present the findings from this suite of measures, designed to address the research questions posed in chapter 1. Chapter 5 is the first of these results chapters and provides a detailed comparison of the in-stream chemistry between the two study reaches.

Chapter 5. **Characterising nutrient spatial and temporal variability in two headwater sub-catchments**

5.1. Overview

In this chapter, samples collected along the study reach upstream of the sub-catchment outlets were analysed to identify and compare spatial variability in nutrient concentration, speciation and fractionation in the two sub-catchments. Temporal changes in nutrient fraction concentrations at the sub-catchment outlets are linked to observations of rainfall and stream discharge within the study reaches. Collectively, these data help to identify sources of nutrient enrichment within each sub-catchment. This information helps with understanding the potential differences in their biological availability and subsequent effects on ecosystem function. Annual load estimates for the two full water years covered by the study (WY 2011 and 2012) are compared for the two sub-catchments. The monitoring period extended beyond WY2012, however, and provides detailed background characterisation of the chemical environment during the process rate measurements conducted throughout (October 2011 to June 2014).

5.2. Variation in nutrient chemistry in the source waters of the Cool's Cottage sub-catchment

5.2.1. **Nitrogen**

Guided by the Mg:Ca ratios described in chapter 3, the occasional sampling points were designated as 'limestone dominated sources' [piped source (CCSP), spring source (CC3) and the woodland edge], predominantly from the south of the sub-catchment and exhibiting low

Mg:Ca ratios; and 'greensand dominated sources' [spring source (CC1), Ruddlemoor and the sinkhole], from the north and east of the sub-catchment and demonstrating high Mg:Ca ratios (figure 3.12). At least one of the limestone dominated sources (CCSP) continued to flow throughout the study period while observable surface flow from the greensand sources ceased in dry periods. The limestone dominated sources have relatively high concentrations of TN (range 4.7 – 11.9 mg l^{-1}; (Table 5:1 a) and are characterised by high TON (mean concentrations of 7.03 – 9.13mgl^{-1}) constituting 88 – 94% of TN. This proportion is high, even when compared with chalk streams, generally considered to be nitrate rich. The greensand dominated sources have much lower concentrations of TN (range 0.9 – 2.5 mg l^{-1}; (Table 5:1 b) and correspondingly low concentrations of TON (0.94 – 3.45 mg l^{-1}), contributing 46 – 89% to TN. NH_4-N concentrations are consistently low for the limestone sources (range, 0.01 – 0.2 mg l^{-1}, 0.3 – 1% of TN). NH_4-N concentrations are similar from the greensand sources but constitute a higher proportion of TN (range, 0.02 – 0.07 mg l^{-1}, 2 – 7% of TN, Table 5:1, b). A similar pattern is observed for DON (range 0.0 – 2.0 mg l^{-1}, 4-11% of TN in the limestone sources and 0.0 – 0.76 mg l^{-1}, 6 – 47% of TN in the greensand sources) and PON which only contributes 1% to TN (0.0 – 0.12 mg l^{-}1) in the limestone sources and 3 – 14% in the greensand sources (0.04 – 0.26 mg l^{-1}).

5.2.2. **Phosphorus**

The distinction between the limestone and greensand dominated sources is less clear for the phosphorus fractions. SRP ranges from 0.01 to 0.123 mg l^{-1} across all sites, with both the highest and lowest concentrations occurring in the greensand sources (Table 5:2 a and b). Overall, SRP contributes between 34 and 48% of total P. A similar pattern is observed for SUP with mean concentrations ranging between 0.04 and 0.091 mg l^{-1} across all sites (37 –

61% of TP), again with both the highest and lowest mean concentrations occurring in the greensand sources. Concentrations of PP range from 0.0 – 0.083 mg l^{-1}. The contribution of PP to TP varies from 5 – 17% (0.0 – 0.039 mg l^{-1}) for most sites with only the sinkhole exhibiting a high proportion of PP at 54% (0.083 mg l^{-1}).

5.2.3. **Carbon**

Mean concentrations of DOC from all sampling points upstream of the lake in Clay Hill wood were low (1.58 – 2.06 mg l^{-1}; Table 5:2b) with the exception of the sinkhole site which had a moderately high DOC concentration of 9.5 mg l^{-1}, (Table 5:2)

Together, these data demonstrated that the headwater springs, upstream of the lake in Clay Hill Wood, were free from the influence of surface sources of organic matter. Only the sinkhole exhibited higher concentrations of DOC. Land cover above the sinkhole was predominantly broad-leaf woodland and the low NH_4-N concentration of this source indicated that the DOC is unlikely to have originated from manures or sewage effluent, but was more likely to be derived from plant material. SRP concentrations were also low compared with the Priors farm reach and suggested there was little input from contemporary inorganic fertilisers. High TON concentration in the limestone dominated sources, on the other hand, may indicate the effects of 'historic' fertiliser application having infiltrated the Portland Limestone and Wardour aquifer.

Table 5:1 A comparison of nitrogen species in the groundwater sources of the Cool's Cottage reach; (a), limestone dominated sources; (b), greensand dominated sources

Limestone Sources (a)	TN (mg N l^{-1})	NH_4-N (mg N l^{-1})		TON (mg N l^{-1})		DON (mg N l^{-1})		PON (mg N l^{-1})	
Spring CC3	**10.39**	**0.030**	**(0.3%)**	**9.13**	**(88%)**	**1.12**	**(11%)**	**0.12**	**(1%)**
	8.90 - 11.88	0.012 - 0.05		8.44 - 9.82		0.22 - 2.01		0.23 - 0.00	
Woodland Edge	**7.83**	**0.047**	**(1%)**	**7.03**	**(90%)**	**0.67**	**(9%)**	**0.08**	**(1%)**
	4.70– 10.33	0.02 – 0.11		4.60 – 9.33		0.05 – 1.35		0.00 – 0.34	
Piped Source	**8.13**	**0.058**	**(1%)**	**7.22**	**(89%)**	**0.77**	**(9%)**	**0.09**	**(1%)**
	5.35-12.24	0.018-0.196		4.71-10.55		0.00-2.64		0.00-0.51	

Greensand Sources (b)	TN (mg N l^{-1})	NH_4-N (mg N l^{-1})		TON (mg N l^{-1})		DON (mg N l^{-1})		PON (mg N l^{-1})	
Sink Hole	**1.59**	**0.065**	**(4%)**	**0.734**	**(46%)**	**0.76**	**(47%)**	**0.04**	**(3%)**
Ruddlemore	**1.05**	**0.047**	**(4%)**	**0.57**	**(54%)**	**0.16**	**(15%)**	**0.28**	**(26%)**
	0.94– 1.16	0.026 – 0.068		0.55 – 0.59		0.15 – 1.16		0.13 – 0.42	
Spring CC1	**2.50**	**0.039**	**(2%)**	**2.23**	**(89%)**	**0.14**	**(6%)**	**0.09**	**(4%)**
	1.83-3.45	0.022-0.079		1.37-3.42		0.00-0.26		0.00-0.26	

Table 5:2 A comparison of phosphorus species in the groundwater sources of the Cool's Cottage reach; (a), limestone dominated sources; (b), greensand dominated sources

Limestone (a) Sources	TP (mg P l^{-1})	SRP (mg P l^{-1})		SUP (mg P l^{-1})		PP (mg P l^{-1})		DOC (mg P l^{-1})
Spring CC 3	**0.171**	**0.059**	**(34%)**	**0.084**	**(49%)**	**0.029**	**(17%)**	
	0.162 – 0.180	0.059 - 0.058		0.066 – 0.103		0.020 – 0.038		
Woodland Edge	**0.162**	**0.071**	**(44%)**	**0.079**	**(49%)**	**0.011**	**(7%)**	**1.784**
	0.127– 0.188	0.058 – 0.103		0.046 – 0.103		0.00 – 0.033		1.08 – 2.63
Piped Source	**0.131**	**0.045**	**(35%)**	**0.080**	**(61%)**	**0.006**	**(5%)**	**2.06**
	0.100-0.165	0.021-0.062		0.045-0.115		0.00-20.020		1.06-6.91

Greensand (b) Sources	TP (mg P l^{-1})	SRP (mg P l^{-1})		SUP (mg P l^{-1})		PP (mg P l^{-1})		DOC (mg C l^{-1})
Sink Hole	**0.151**	**0.010**	**(7%)**	**0.059**	**(39%)**	**0.083**	**(54%)**	**9.51**
Ruddlemore	**0.208**	**0.123**	**(59%)**	**0.065**	**(31%)**	**0.019**	**(9%)**	**2.02**
	0.167– 0.249	0.119 – 0.127		0.040 – 0.091		0.00 – 0.039		1.89 – 2.15
Spring CC1	**0.131**	**0.048**	**(36%)**	**0.068**	**(52%)**	**0.015**	**(11%)**	**1.58**
	0.103-0.151	0.035-0.061		0.049-0.102		0.003-0.029		0.74-2.50

Table 5:3 Longitudinal variation in nutrient chemistry along the Cool's Cottage study reach throughout the study period. The piped source is included for comparison.

Cool's Cottage	NH4-N (mg N l^{-1})	TON (mg N l^{-1})	DON (mg N l^{-1})	PON (mg N l^{-1})	SRP (mg P l^{-1})	SUP (mg N l^{-1})	PP (mg N l^{-1})	DOC (mg C l^{-1})
Piped Source	**0.058**	**7.18**	**0.52**	**0.13**	**0.045**	**0.080**	**0.006**	**1.48**
	0.018 – 0.196	4.71 – 10.55	0.00 – 1.79	0.00 – 0.51	0.021 – 0.062	0.045 – 0.116	0.00 – 0.017	1.06 – 2.22
Headwater	**0.062**	**3.90**	**0.74**	**0.25**	**0.026**	**0.079**	**0.030**	**3.25**
	0.011 – 0.337	1.59 – 7.96	0.00 – 3.28	0.00 – 0.89	0.00 – 0.087	0.00 – 0.359	0.00 – 0.165	1.41 – 7.42
Upstream site	**0.063**	**3.36**	**0.70**	**0.38**	**0.051**	**0.072**	**0.071**	**4.08**
	0.009 - 0.311	1.34 - 7.63	0.00 - 2.71	0.00 -1.69	0.002 - 0.200	0.00 - 0.388	0.00 - 0.363	1.70 - 9.23
Downstream site	**0.063**	**3.20**	**0.75**	**0.38**	**0.049**	**0.072**	**0.083**	**4.46**
	0.003 - 0.172	1.18 -7.80	0.00 - 2.6	0.00 -1.90	0.00 - 0.110	0.008 - 0.421	0.00 - 0.611	1.81 - 11.38

5.3. Longitudinal variation in nutrient chemistry in the Cool's Cottage study reach

For the purposes of this investigation, the headwater of the study reach was taken to be the point at which the stream emerges from Clay Hill Wood, where the various sources described in section 5.2 have been mixed to a more uniform condition in the lake. This is a short reach of 450m from the headwater to the sub-catchment outlet at the downstream site. Between the headwater site and the intermediate sampling point at the upstream site, 200m from the sub-catchment outlet, there is an input from a tributary that carries runoff from the road into the stream (figure 3.12).

5.3.1. **Nitrogen**

Mean concentrations of TON decreased with distance downstream from 3.90 to 3.20 mg l^{-1} (Table 5:**3** and Figure 5:1) and supported the idea that high TON concentrations in the limestone dominated source waters were a result of historic, rather than contemporary land use. NH_4-N and DON concentrations showed little change along the study reach. The most noticeable change was in PON concentrations that increased from 0.25 to 0.38 mg l^{-1} between the headwater and the upstream site but remained constant between the upstream and downstream sites, suggesting the major input was from the road drain.

5.3.2. **Phosphorus**

The influence of the road drain is more marked in the P fractions, with substantial increases in both SRP and PP concentrations between the headwater and upstream sites (Table 5:**3**, Figure 5:1). There was little change in SUP concentrations; a small decrease between the headwater and upstream sites suggested that lower concentrations of SUP in the road drain may have diluted the source water. Mean SUP concentrations remained constant between the upstream and downstream sites.

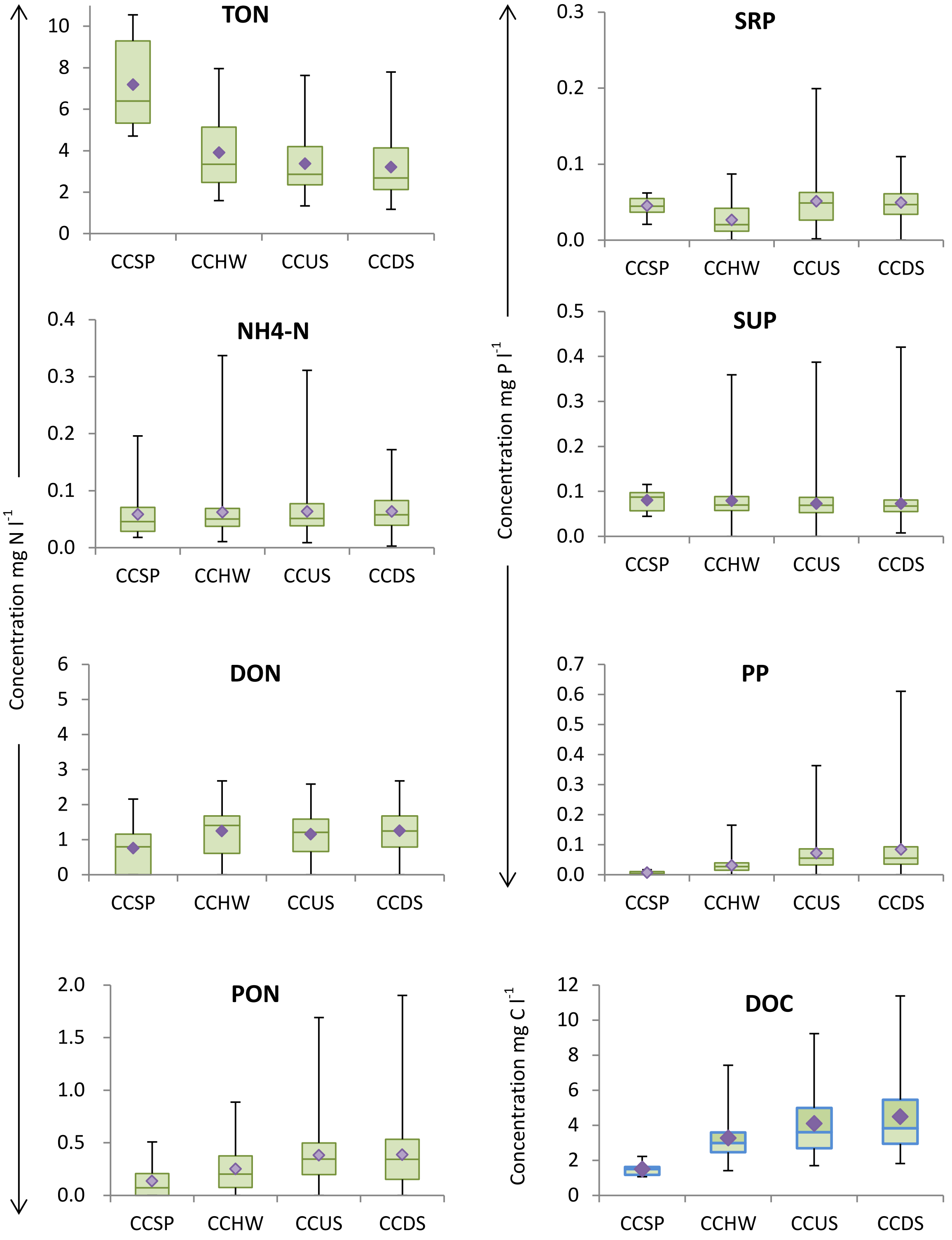


Figure 5:1 Longitudinal variation in nutrient chemistry along the Cool's Cottage study reach. Locations are abbreviated: CCSP, piped source; CCHW, headwater; CCUS, upstream site and CCDS, downstream site at the sub-catchment outlet

5.3.3. **Carbon**

DOC concentrations increased with distance downstream indicating a contribution from surface sources (Figure 5:1). The increase in concentration per metre was more marked between the headwater and upstream sites than between the upstream and downstream sites reflecting the contribution from the road drain and the efficiency of the road as a conduit for delivering surface pollution to the watercourse.

5.4. Speciation and fractionation of nitrogen and phosphorus in the Cool's Cottage sub-catchment

Daily and sub-daily samples were analysed for all nutrient species and fractions at the sub-catchment outlet. Because of the influence of sample storage on NH_4 –N and SRP it is the convention to report full speciation and fractionation for samples analysed within 24 hours of collection only (chapter 4). The following analyses relate to grab samples collected weekly and analysed within 24 hours of collection. Higher resolution temporal dynamics are discussed in section 5.7 in order to capture key transport events that may have been missed by the weekly sampling regime.

5.4.1. **Nitrogen**

Over the full study period, TON dominated nitrogen concentrations at Cool's Cottage, constituting 73.6% of TN at the sub-catchment outlet. DON was the next highest constituent, contributing 16.7% to TN with PON contributing 8.5%. NH_4-N was present in low concentration and contributes only 1.4% to TN.

5.4.2. **Patterns in nitrogen speciation in the Cool’s Cottage study reach**

There was a pronounced annual pattern in concentrations of TON downstream of the lake in Clay Hill Wood that was not apparent in the piped source from the reservoir in the south of the sub-catchment (Figure 5:2).This indicated either substantial TON uptake within the lake during the summer, when there was a large population of *Elodea canadensis*, or a considerable shift in the dominance of different sources during the annual cycle. If variation in source dominance was the sole driver of the annual cycle, however, observations on site, that showed a higher contribution of flow from the piped source to total flow during dry weather (chapter 3), would predict higher TON concentrations during the summer, rather than the observed decline.

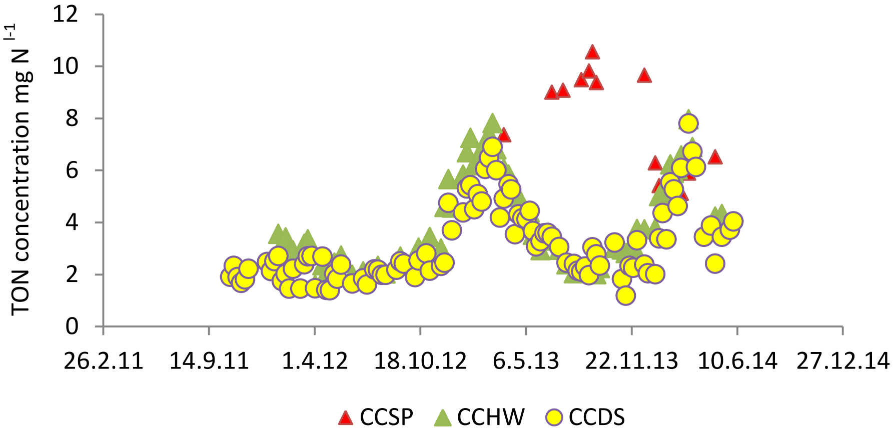


Figure 5:2 Differential variation in TON concentrations in the Cool's Cottage sub-catchment, above and below the lake in Clay Hill Wood.

This strong annual cycle was not so marked in the other nitrogen species and resulted in a change in the relative importance of each species over the annual cycle. However TON remained dominant throughout the study period (Figure 5:3).

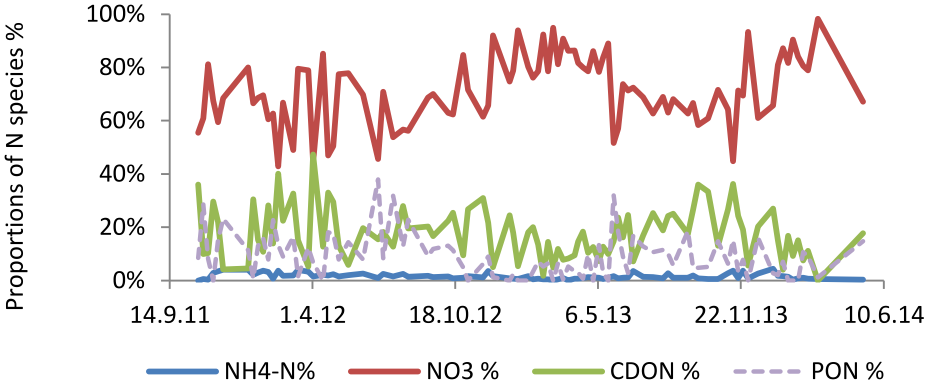


Figure 5:3 Temporal variation in the proportion of nitrogen species at the outlet from the Cool's Cottage sub-catchment

5.4.3. **Phosphorus**

The balance between phosphorus fractions was more equal. PP contributed the highest proportion at 40.4%, with SUP constituting 34.7%. Over the full study period, SRP contributed 24.8% to TP.

5.4.4. **Patterns in phosphorus fractionation in the Cool’s Cottage study reach**

There was a marked annual pattern in SRP concentrations in the Cool’s Cottage study reach with annual minima occurring during the spring (Figure 5:4). This pattern is most pronounced at the headwater site, immediately downstream of Clay Hill wood, but was also observed at the sub-catchment outlet. This observation reinforced the idea that photosynthetic primary production in the lake, which was not shaded, resulted in substantial uptake of inorganic nutrients during the spring and summer. As with the comparison among the nitrogen species, the strong annual pattern in SRP was not observed in the SUP and PP fractions, and resulted in different fractions dominating the phosphorus pool throughout the study period (Figure 5:5).

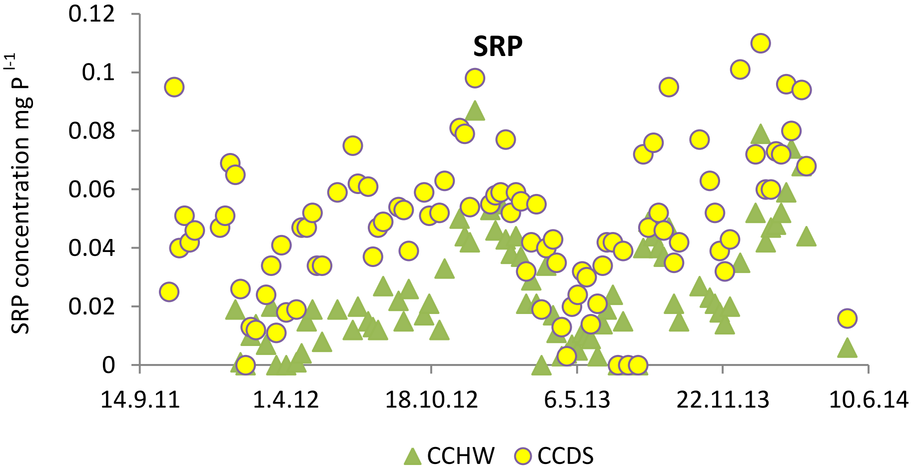


Figure 5:4 Variation in SRP concentrations in the Cool's Cottage sub-catchment below the lake in Clay Hill Wood.

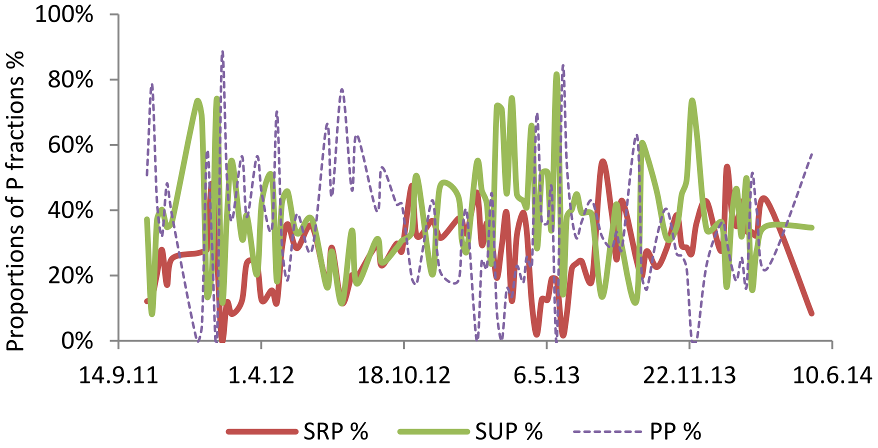


Figure 5:5 Temporal variation in the proportions of phosphorus fractions at the outlet from the Cool's Cottage sub-catchment

5.5. Temporal variation in stoichiometry at Cool's Cottage

The seasonal patterns observed in the speciation and fractionation of nitrogen and phosphorus result in changing N:P ratios throughout the annual cycle. This ratio exerts an important control on photosynthetic primary production. Although the threshold that determines which nutrient limits growth is likely to vary between biotic groups, a molar ratio of 31 (14 by mass) was proposed by Sterner and Elser (2002) based on earlier work in both terrestrial and freshwater ecosystems e.g. Elser et al., (1990); Downing and McCauley, (1992); Verhoeven et al., (1996), and is included here as a guide. This ratio was exceeded at the sub-catchment outlet throughout the study period for both dissolved inorganic species (NH_4-N+TON:SRP) and total dissolved species (TDN:TDP, Figure 5:6a – c) suggesting available phosphorus may limit metabolic processes in this reach. The higher proportion of phosphorus existing as PP than nitrogen as PON results in lower TN:TP ratios providing a store of phosphorus that may be re-mineralised as an additional resource (see chapter 6)

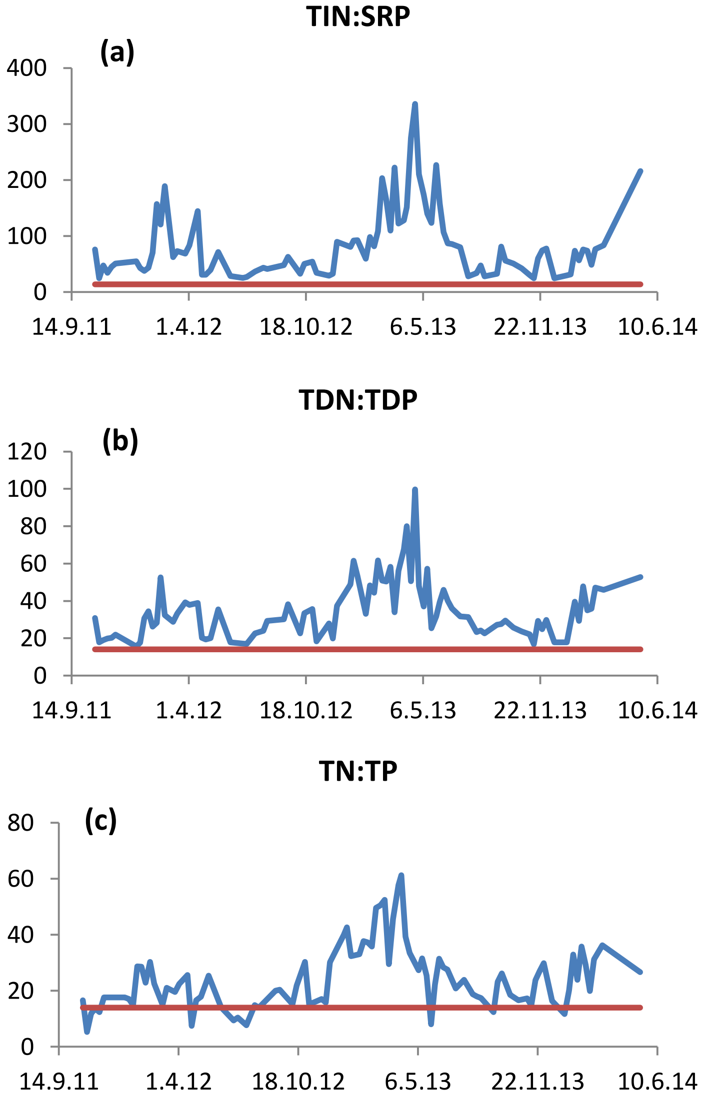


Figure 5:6 Temporal variation in the N:P ratio (by mass) at the Cool's Cottage sub-catchment outlet; (a) inorganic species, (SRP + TON):SRP, (b) dissolved species (TDN:TDP) and (c) TN:TP. The red line represents a N:P ratio (by mass) of 14, one estimate of the point at which limitation of photosynthesis in lakes switches from N to P (see text).

5.6. Nutrient load at the outlet from the Cool's Cottage sub-catchment.

The two study years exhibited very different meteorological conditions, although the total rainfall for both years was similar (rainfall totals were 861 mm in year 1 compared with 820mm in year 2; source EA Tisbury weather station). Year 1, however, had a dry winter (285mm October to March) that followed a dry year (676 mm total rainfall in year 0, WY 2010 with only 284 mm between April and September). Heavy rain fell in the summer of year 1 (576 mm between April and September) and in year 2, heavy winter rain (582 mm between October 2012 and March 2013) fell on already saturated soils. The summer of 2013 was dry (238 mm between April and September) and continued dry until late summer. Further heavy rain fell in the winter of year 3 (762 mm between October 2013 and March 2014;(figure 3.3). Total discharge was higher in year 2 (714 ML a^{-1} compared with 521 ML a^{-1} in year 1) and while some of this difference will be due to seasonal vegetative growth and transpiration in the wet summer of 2012, accounting for a greater proportion of water uptake than the heavy winter rain in year 2, these patterns also reflect the effects of soil saturation on flow and illustrate the importance of considering antecedent conditions when interpreting nutrient fluxes.

Partly as a result of the increased discharge, the annual load of TN in year 2 was substantially higher than in year 1 (3.7 t a^{-1} compared to 1.7 t a^{-1}). However, higher concentrations of TON also contributed to the increase mean concentration 3.37 mg l^{-1} compared with 1.84 mg l^{-1} in year 1 (Table 5:4). Mean concentrations of DON were also slightly higher in year2 (0.86 mg l^{-1} and 1.06 mg l^{-1} in year 1 and year 2, respectively) while other nitrogen species remained constant (NH_4-N, 0.060 mg l^{-1} and 0.059 mg l^{-1} year 1 and year 2 respectively and PON, 0.678 mg l^{-1} and 0.662 mg l^{-1} year 1 and year 2 respectively).

Table 5:4 A comparison of the mean concentrations of nitrogen species over two years at the outlet to the Cool's Cottage sub-catchment, contributing to the difference in load (figure 5.7)

Year	NH4 (mg N l^{-1})		TON (mg N l^{-1})		DON (mg N l^{-1})		PON (mg P N^{-1})	
1	**0.060**	±0.039	**1.841**	±0.436	**0.860**	±0.412	**0.678**	±0.708
2	**0.059**	±0.034	**3.375**	±1.320	**1.058**	±0.416	**0.662**	±0.541

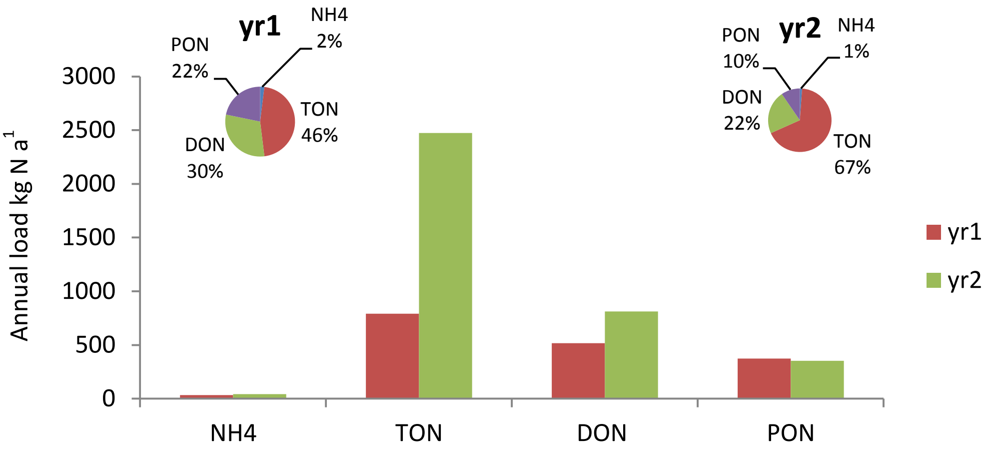


Figure 5:7 A comparison of the contribution to total nitrogen load by nitrogen species over two water years at the outlet to the Cool's Cottage sub-catchment. Total nitrogen load was 1.7 tonnes per annum (t a^{-1}) in year 1 and 3.7 (t a^{-1}) in year 2.

A similar trend was observed for the annual load of TP (0.162 t a^{-1} in year 1 compared to 0.220 t a^{-1} in year 2,). As with DON, the mean concentration of SUP was slightly higher in year 2 (0.065 mg l^{-1} in year 1 compared with 0.072 mg l^{-1} in year 2, (Table 5:4, Table 5:7), while PP was slightly lower in year 2; 0.194 mg l^{-1} compared with 0.210 mg l^{-1} in year 1. The mean concentration of SRP was similar in both years, 0.039 mg l^{-1} and 0.040 mg l^{-1} for year 1 and year 2, respectively. The annual load of DOC was constant over the two years (4.74 t a^{-1}

in year 1 and 4.72 t a^{-1} in year 2), although the mean concentration was lower in year 2 (5.19 mg l^{-1}) than in year 1, (7.07 mg l^{-1}).

Table 5:5 A comparison of the mean concentrations of phosphorus fractions and DOC over two years at the outlet to the Cool's Cottage sub-catchment, contributing to the difference in load (figure 5.8). DOC load was 4.7 t a^{-1} in both years.

Year	SRP (mg P l^{-1})		SUP (mg P l^{-1})		PP (mg P l^{-1})		DOC (mg C l^{-1})	
1	**0.042**	±0.026	**0.060**	±0.026	**0.210**	±0.196	**7.07**	±3.578
2	**0.040**	±0.019	**0.072**	±0.023	**0.194**	±0.139	**5.19**	±2.238

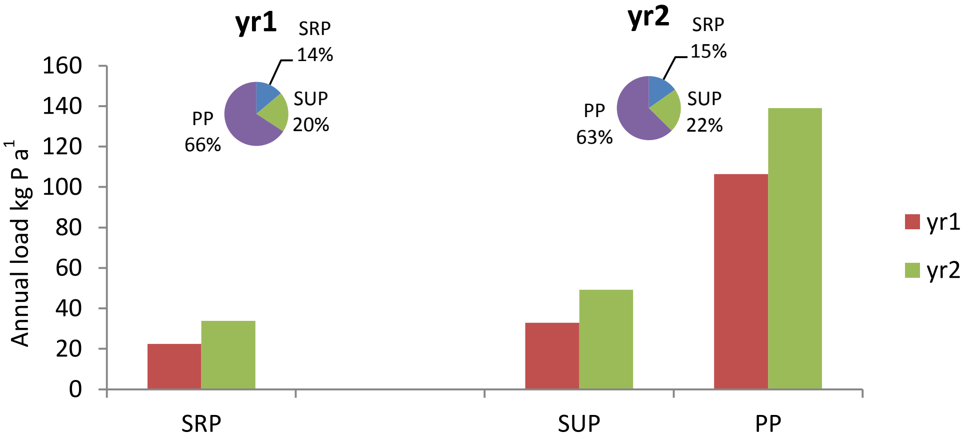


Figure 5:8 A comparison of the contribution to total phosphorus load by phosphorus fractions over two water years at the outlet to the Cool's Cottage sub-catchment. Total phosphorus load was 0.16 tonnes per annum (t a-1) in year 1 and 0.22(t a-1) in year 2.

5.7. High resolution temporal variation in nutrient concentrations at the outlet from the Cool's Cottage sub -catchment

While recognising the influence of sample storage on the speciation and fractionation of nitrogen and phosphorus, important additional information on in-stream nutrient chemistry and its response to hydrological conditions can be obtained from the higher resolution that the daily samples provide. Short term changes are likely to be common in such flashy streams and may well be missed by limiting the sampling frequency to weekly collections.

5.7.1. Nitrogen dynamics

Peak TON concentrations lag behind the peak flows in the winters of 2012 and 2013 by two months (Figure 5:9).Together with the lack of a corresponding peak following the onset of flow following the dry summer of 2011, and after the higher flows of the spring and summer of 2012,this supports the contention that the control of TON is not dominated by surface sources but, more likely, influenced by contributions from groundwater sources and the reservoir, that take longer to respond to rainfall. This pattern of low concentrations of TON in the spring and early summer reflects the pattern seen in section 5.4.2 and reinforces the interpretation that biological processing in the lake in Clay Hill wood exerts significant control on in-stream nutrient chemistry for some distance downstream within this sub-catchment. Biological processing through photosynthetic primary production in the lake was likely to exert a stronger influence than local in-stream processing at the sub-catchment outlet, bearing in mind the heavy shading at the sampling station at Cool's Cottage. Low photosynthetic primary production at the sub-catchment outlet was reflected in the patterns in dissolved oxygen concentrations that did not exhibit the peaks in summer daytime concentrations that would be expected if there was a high degree of photosynthetic primary production at the site.

Concentrations of PON were highly variable but showed weak minima following high flows in the summer of 2012, winter 2012 / 2013 and winter 2013 / 2014 suggesting there may have been some flushing of the stream bed during these events, although there was little evidence of peaks in concentration associated with high flow events (peaks in load, however, would have resulted from the combination of higher discharge and consistent concentrations). Both PON and NH_4-N showed small peaks in concentration during the dry weather and low flows of summer 2013. DON showed little variation throughout the study period, again suggesting little input from surface sources.

5.7.2. **Phosphorus dynamics**

As seen in section 5.2.2., the source waters had a lesser effect on phosphorus fractions than on nitrogen species. SRP concentrations rose with the onset of winter rains after the dry summer of 2011). The wet year of 2012 resulted in higher concentrations throughout the year, and the high rainfall in winter 2013/2014 was also accompanied by an increase in SRP concentrations. This suggested a link with surface sources, exacerbated by low phosphorus uptake in the sub-catchment, due to limited growth during the winter. SRP concentrations reached minima in the spring of all three years suggesting that uptake in the lake during photosynthetic primary production influenced nutrient chemistry for some distance downstream. As with DON, SUP concentrations did not exhibit clear seasonal or annual patterns. PP concentrations, however, mimicked those of PON and SRP. This may reflect increased delivery from the sub-catchment during periods of wet weather – or perhaps reinforces the proposal that PP provided a 'backup' resource for metabolic processes when more readily biologically available sources of P were depleted (Chapter 6).

5.7.3. **Dynamics of dissolved organic carbon**

DOC concentrations responded to peaks in flow. This observation tallies with patterns in longitudinal concentrations that suggested delivery of DOC from surface sources in the sub-catchment. (Figure 5:10). Concentrations of DOC increased with the onset of the winter rain after the dry summer in 2011, remained high (for this catchment) through the wet season, but decreased during the winter of 2012, despite continuing rain and increased discharge. This was likely to be influenced by the removal of grazing stock from the surrounding fields to protect waterlogged pasture from erosion and compaction. DOC concentrations increased with the onset of autumn rain in 2013 suggesting some input from the surrounding pasture following the summer grazing of stock.

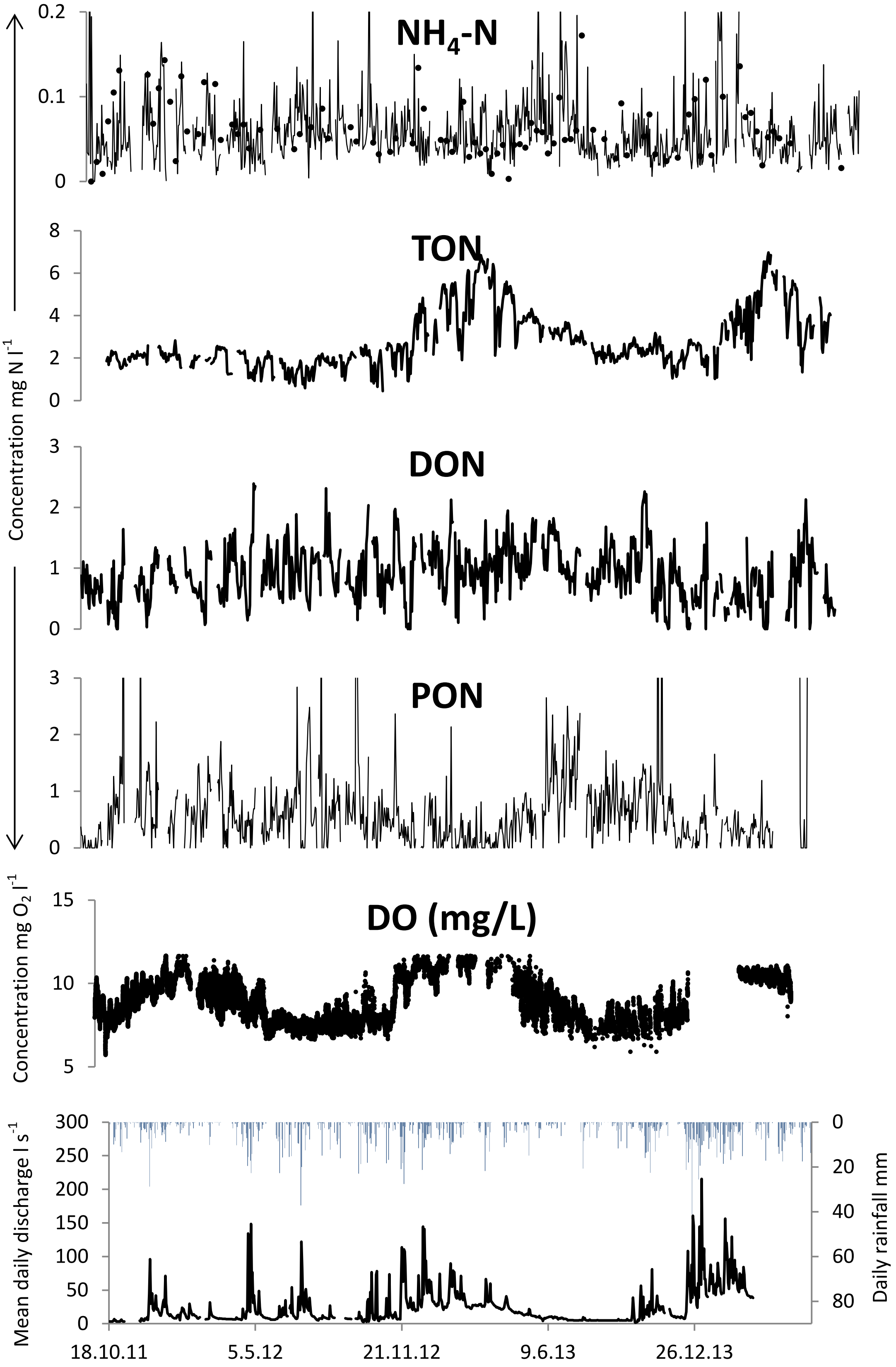


Figure 5:9 Temporal variation in nitrogen species and dissolved oxygen at the outlet to the Cool's Cottage sub-catchment. Weekly grab samples of NH4-N are represented as points, while daily samples that may have degraded during storage are represented as a line plot.

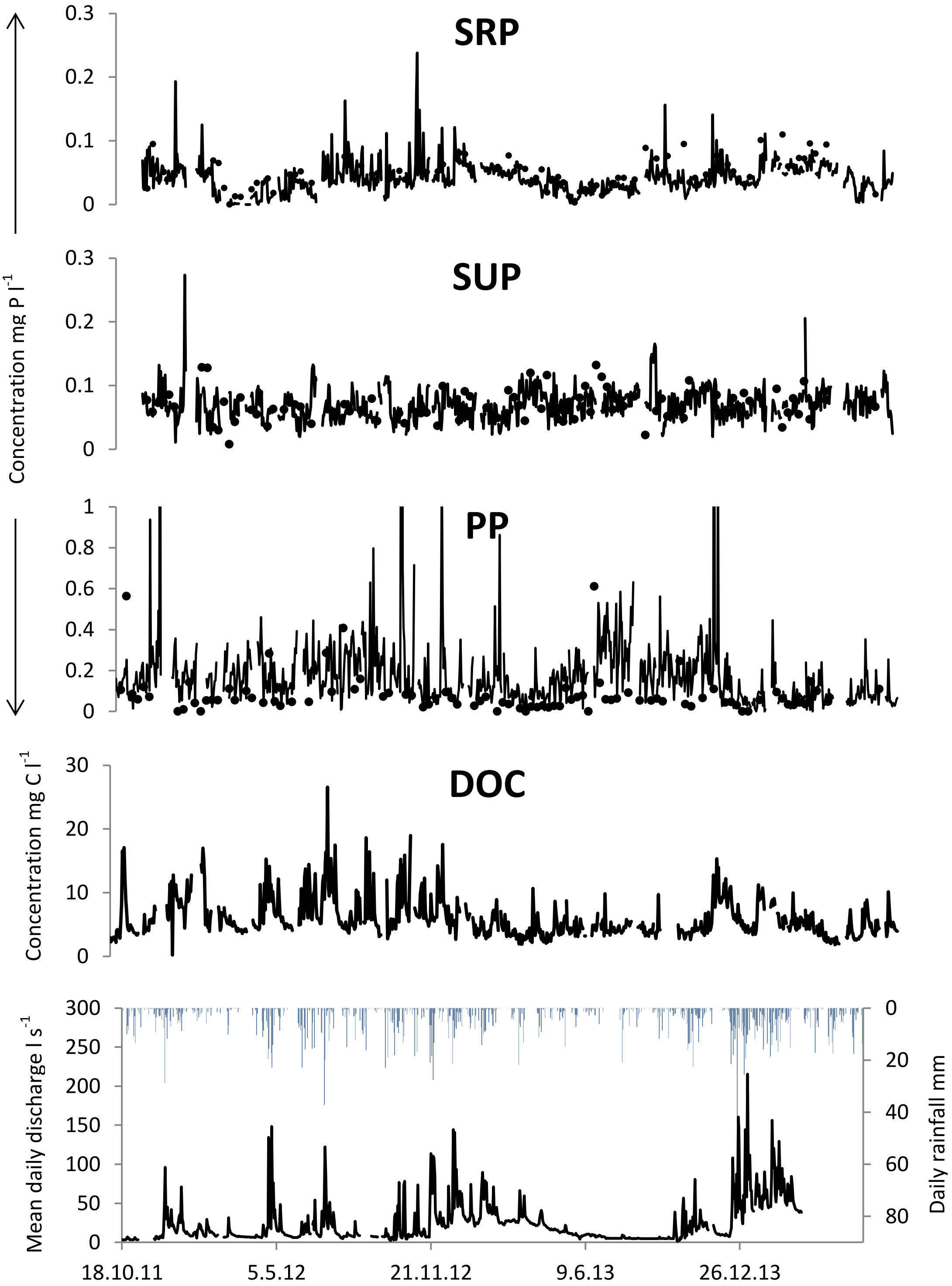


Figure 5:10 Temporal variation in phosphorus fractions and dissolved organic carbon at the outlet to the Cool's Cottage sub-catchment. Weekly grab samples of phosphorus fractions are represented as points, while daily samples that may have degraded during storage are represented as a line plot.

5.8. Variation in the nutrient chemistry of the tributaries of the Priors Farm sub-catchment

As discussed in chapter 3, the Mg:Ca ratios throughout the Priors Farm sub-catchment show little variation, suggesting a greater uniformity of source than experienced in the Cool's Cottage sub-catchment. Nevertheless, the nutrient chemistry of the three tributaries PF1, PF2 and PF3 does vary. PF1 and PF2 rise close to the steadings of Hays Farm and Coleman's Farm in the West of the sub-catchment, while PF3 rises in the South, in Semley common, through which it flows before reaching grazing pasture shortly before it joins PF1 and PF2 (figure 3.18.). This contrast in provenance is reflected in the tributaries' nutrient concentrations, speciation and fractionation, and is discussed in the following section. Furthermore, within the study period, two occasions on which pulses of exceptionally high organic matter input coincided with field sampling days are discussed in greater detail in section 5.9. This provides additional background information on the conditions experienced by the in-stream community in this study reach and their possible implications to ecosystem function. Data from these extreme events are omitted from this overview to aid the interpretation of underlying trends in nutrient concentration, speciation and fractionation.

5.8.1. Nutrient speciation and fractionation in the tributaries of the Priors Farm sub-catchment

The following analyses relate to grab samples collected weekly and analysed within 24 hours of collection. Higher resolution temporal dynamics are discussed in section 5.14 in order to capture key transport events that may have been missed by the weekly sampling regime.

The nutrient chemistries of PF1 and PF2 exhibited a strong influence from organic fractions that was absent from the tributary PF3. Both PF1 and PF3 dried out in dry weather and the peak concentrations of TON and SRP in PF3 occurred in the summer of 2013, coinciding with lowest flow (based on field observations and recorded flow at the sub-catchment outlet; no flow was recorded for the tributaries). In contrast, the highest concentrations of TON and SRP in PF1 and PF2 occurred during periods of increased discharge, and suggested that the transport of surface nutrients was a key mechanism for nutrient enrichment in these tributaries.

5.8.2. **Nitrogen**

All three tributaries had similar mean concentrations of TN (range, 4.4 – 5.6 mg l^{-1}, Table 5:6). PF3 is characterised by high TON during the dry season, with concentrations reaching 12 - 14 mgl^{-1} in the summer of 2013, and a mean concentration of 3.23 mgl^{-1} over the study period, constituting 59.7% of TN. Both PF1 and PF2 had lower mean concentrations of TON, 1.38 and 2.81 mgl^{-1}, constituting 31.1 and 50.0% of TN respectively. NH_4-N concentrations were consistently low at PF3 (mean 0.05 mg l^{-1}, 1.1% of TN), as is DON (mean 1.82 mg l^{-1}, 34.9% of TN) while at PF1 and PF2, mean NH_4-N concentrations are higher (0.317 and 0.257 mg l^{-1}, contributing 7.1 and 4.6% of TN, for PF1 and PF2, respectively). A similar pattern is observed for DON (2.29 and 2.11 mg l^{-1}, 51.8% and 37.5% of TN, for PF1 and PF2, respectively) and PON (0.44 mg l^{-1} at both sites; 10.0% and 7.9% of TN, for PF1 and PF2, respectively) with PF3 exhibiting the lowest mean concentration of PON (0.23 mg l^{-1}, 4.3% of TN).

Table 5:6 A comparison of nitrogen species in the tributaries of the Priors farm reach, throughout the study period; For PF2, summary data in black exclude the extreme event of 4.3.13, to aid understanding the underlying trends. Figures in red (PF2E) include this extreme event and illustrate its influence on the overall contributions (see text).

Priors farm	TN (mg N l^{-1})	NH_4-N (mg N l^{-1})		TON (mg N l^{-1})		DON (mg N l^{-1})		PON (mg N l^{-1})	
PF1	**4.44** 1.97 – 12.23	**0.317** 0.016 – 4.231	**(7%)**	**1.38** 0.06 – 6.22	**(31%)**	**2.30** 0.94 – 5.9	**(52%)**	**0.44** 0.00 – 1.89	**(10%)**
PF2	**5.62** 3.02 – 12.96	**0.257** 0.043 – 0.864	**(5%)**	**2.81** 0.62 – 10.14	**(50%)**	**2.11** 0.30 – 3.36	**(37%)**	**0.44** 0.00 – 2.26	**(8%)**
PF2 E	6.57 3.02 – 68.68	0.956 0.043 – 46.40	(15%)	2.79 0.62 – 10.14	(40%)	2.12 0.30 – 3.36	(32%)	0.712 0.00 – 18.29	(11)
PF3	**5.42** 2.48 – 17.99	**0.058** 0.017 – 0.182	**(1%)**	**3.23** 0.34 – 14.88	**(48%)**	**1.89** 0.53 – 3.23	**(35%)**	**0.23** 0.00 – 0.85	**(4%)**

Table 5:7 A comparison of phosphorus fractions and dissolved organic carbon in the tributaries of the Priors farm reach, throughout the study period; For PF2, summary data in black exclude the extreme event of 4.3.13, to aid understanding the underlying trends. Figures in red (PF2E) include this extreme event and illustrate its influence on the overall contributions (see text).

Priors farm	TP (mg P l^{-1})	SRP (mg P l^{-1})		SUP (mg P l^{-1})		PP (mg P l^{-1})		DOC (mg P l^{-1})
PF1	**0.45**	**0.155**	**(34%)**	0.116	(26%)	0.190	(42%)	15.75
	0.064 – **2.013**	0.035 - 0.797		0.029 – 0.679		0.00 – 1.154		9.68 - 27.56
PF2	**0.504**	**0.203**	**(40%)**	0.084	(17%)	0.226	(45%)	13.78
	0.121– 1.007	0.041 – 0.541		0.00 – 0.195		0.080 – 0.707		9.16 – 22.83
PF2	**0.580**	**0.230**	**(40%)**	0.094	(16%)	0.265	(46%)	14.77
	0.121– 5.592	0.041 – 2.00		0.00 – 0.753		0.080 – 2.840		9.16 – 67.46
PF3	**0.338**	**0.162**	**(48%)**	0.088	(26%)	0.088	(26%)	13.73
	0.180 – 1.347	0.025 – 1.196		0.019 – 0.207		0.00 – 0.456		9.48 – 19.45

Table 5:8 Longitudinal variation in nutrient chemistry between the Priors Farm upstream and downstream sites throughout the study period.

Priors Farm	NH_4-N (mg N l^{-1})	TON (mg N l^{-1})	DON (mg N l^{-1})	PON (mg N l^{-1})	SRP (mg P l^{-1})	SUP (mg N l^{-1})	PP (mg N l^{-1})	DOC (mg C l^{-1})
Upstream site	**0.437**	**2.25**	**1.62**	**0.59**	**0.171**	**0.095**	**0.215**	**15.28**
	0.00 – 10.468	0.08 – 7.49	0.35 – 5.67	0.00 – 6.20	0.029 – 0.943	0.00 – 0.664	0.00 – 1.832	9.15 – 53.30
Downstream site	**0.321**	**2.20**	**1.59**	**0.50**	**0.161**	**0.090**	**0.174**	**15.02**
	0.00 - 4.998	0.07 - 7.55	0.49 - 4.43	0.00 - 4.09	0.034 - 0.595	0.003 - 0.356	0.00 - 1.641	9.45 – 39.11

5.8.3. **Phosphorus**

In contrast to TN, TP was lower at PF3 than at the other two sites (Table 5:7), 0.388 mg l^{-1} at PF3 compared with 0.454 and 0.504 mg l^{-1} at PF 2 and PF3, respectively. The mean concentration of SRP was highest at PF2 (0.203 mg l^{-1} (40.2% of TP), compared with 0.155 mg l^{-1} (34.1% of TP) and 0.162 mg l^{-1} (47.9% of TP) at PF1 and PF3, respectively. SUP was highest at PF1 (0.116 mg l^{-1}, 25.5% of TP) while PF2 and PF3 exhibited similar mean concentrations 0.084 mg l^{-1} and 0.088 mg l^{-1} (16.6 and 26.1% of TP), respectively. Mean concentration of PP was highest at PF2 (0.226 mg l^{-1}, 44.7% of TP) and lowest at PF3 (0.088 mg l^{-1}, 26% of TP). The mean concentration of PP at PF1 was 0.190 mg l^{-1}, 41.9% of TP.

5.8.4. **Carbon**

Concentrations of DOC from the three tributaries were similar at all sites, 15.7 mg l^{-1} at PF1, 13.8mg l^{-1} at PF2 and 13.7 mg l^{-1} at PF3 (Table 5:7). The contrast in the balance of nitrogen species and phosphorus fractions at PF1 and 2 with those at PF3 is consistent with their proximity to dairy farms that manage their waste as slurry. Several incidents were witnessed when high concentrations of nutrients, notably NH_4-N and DOC, were accompanied by a green colouration to the water and the characteristic odour of farmyard manure. These are discussed further in sections 5.5 and 5.10. The combination of high DOC and high NH_4-N is characteristic of animal or human waste. On at least two of these occasions, after high rainfall, the containment of slurry is known to have failed suggesting a probable source for these observations.

5.9. Transport and fate of high organic matter pulses.

One of the events described in section 5.5 occurred on 7th January 2013. Following intense rainfall, the tributary at PF1 was observed to be dark green and odorous. An additional sample was collected further upstream, at the ditch leading from the breached slurry store, to supplement the weekly sampling points in the study reach. Changes in the concentrations of different species and fractions varied with distance downstream suggesting that abiotic processes, such as dilution, were not solely responsible for the processing of this organic matter pulse (Table 5:9).

Between PF1 and the Priors farm upstream site, there are inputs from PF2 and PF3; therefore, the contributions from these streams will affect the changes to in-stream nutrient chemistry. Between the upstream site and the sub-catchment outlet at the downstream site, however, there were no observed additional surface water inputs. At both stages, the highest loss (expressed as percent) was in the NH_4-N concentration. Between the upstream and downstream sites, NH_4-N reduced by 17% compared with 14% for DON and 10% for SUP. Other fractions showed an increase, with DOC increasing by 3%, TON by 8% and SRP by 13%. While this disproportionate loss of NH_4-N may result from preferential biological processing, it may also indicate that the $NH_4^+ \rightleftarrows NH_3$ pair may not have reached equilibrium at sampling points close to the source, resulting in a higher proportion of NH_4-N being present as NH_3, and leading to increased loss due to volatilisation. This has implications on the effect of these high organic matter pulses to ecosystem function as NH_3 is harmful to many organisms, including some macro-invertebrates. This is further discussed in chapter 7.

Table 5:9 Concentrations of organic pollutants following a breached slurry store and their dispersal downstream.

Location	**date**	**NH$_4$-N (mg N l^{-1})**	**TON (mg N l^{-1})**	**SRP (mg N l^{-1})**	**DON (mg N l^{-1})**	**SUP (mg P l^{-1})**	**PON (mg P l^{-1})**	**PP (mg P l^{-1})**	**DOC (mg C l^{-1})**
Coleman's Farm outlet	**7.1.13**	**216**	**6.6**	**28.8**	**154**	**5.25**	**81.75**	**14.62**	**864**
PF1		2.52	1.172	0.246	3.94	0.131	1.54	0.56	27.5
PFUS		0.24	1.192	0.090	2.93	0.066	0.09	0.13	14.2
PFDS		0.21	1.288	0.102	2.51	0.060	0.09	0.12	14.7
PF2	**4.3.13**	**46.4**	**1.30**	**2.00**	**2.39**	**0.399**	**18.1**	**4.20**	**134**
PFUS		10.4	1.69	0.32	1.38	0.074	6.20	1.83	53.3
PFDS		1.35	2.05	0.06	2.66	0.184	0.89	0.27	16.2

A second incidence of high organic matter input coincided with a field sampling day on the 4th of March 2013 and facilitated a further investigation into the transport of matter downstream. Analysis of the samples downstream from PF2, where the characteristic colour and odour were again observed, revealed a different pattern of loss among the different fractions. On this occasion there was a much larger reduction in the concentrations of NH_4-N, SRP and DOC between the upstream and downstream sites, but the concentrations of TON, DON and SUP were higher at the downstream site. It is possible that the timing of this pulse of organic matter meant that it had not reached the downstream site at the time of sampling. These observations emphasise the value of high resolution (real-time or sub daily) sampling in interpreting such discrete events and their implications to biological function.

5.10. Longitudinal variation in nutrient chemistry in the Priors Farm study reach

The distance between the confluence of the three tributaries (PF1, PF2 and PF3) and the regular sampling and incubation sites at the upstream site and sub-catchment outlet, acts in a similar fashion to the lake in Clay Hill Wood in the Cool's Cottage sub-catchment, and ensures the disparate contributions from the three tributaries are well mixed. The study reach stretches for 1000m from the upstream sampling site to the sub-catchment outlet and, although there is a surface hedge-line ditch midway between the two sites, surface water flow was only observed here in the extreme weather of winter 2013 / 2014.

5.10.1. **Nitrogen**

Over the full study period, there was a small reduction in the mean concentrations of all nitrogen species from upstream to downstream suggesting greater contributions from point sources in the headwaters than from diffuse sources, despite the application of both inorganic fertiliser and organic slurry within the sub-catchment. This reduction was proportionately greater in the NH_4-N and PON concentrations than the other nitrogen species (**Error! Reference source not found.** and Figure 5:11(a). While these changes in mean concentration are small, maximum concentrations were highly variable and always greater at the upstream site. For those days when measurements were taken at all sites, the maximum recorded value of NH_4-N at the upstream site was 10.5 mg l^{-1} and 5.0 mg l^{-1} at the downstream site. (The maximum concentration of NH_4-N, recorded over the full study period at the downstream site was higher at 15 mg l^{-1} (Figure 5:19); but there was no corresponding value for the upstream site on that occasion). The contrast between the two sites is much less extreme for DON (maximum concentrations of 5.87 mg l^{-1} and 4.63 mg l^{-1} for the upstream and downstream sites, respectively) and PON (maximum concentrations of 6.20 mg l^{-1} and 4.09 mg l^{-1} for the upstream and downstream sites, respectively), while TON maxima are comparable for the two sites (7.49 mg l^{-1} and 7.55 mg l^{-1} for the upstream and downstream sites, respectively).

5.10.2. **Phosphorus**

The mean concentrations of phosphorus fractions also decrease with distance downstream (Table 5:**8** and Figure 5:11); SRP from 0.171 mg l^{-1} to 0.161 mg l^{-1}, SUP from 0.095 mg l^{-1} to 0.09 mg l^{-1} and PP from 0.215 mg l^{-1} to 0.174 mg l^{-1}. As with the nitrogen species, maximum concentrations are also lower at the downstream site. The maximum recorded concentration of SRP at the upstream site was 0.94 mg l^{-1} compared with 0.59 mg l^{-1} at the downstream site. Maximum concentrations of SUP show a greater difference between sites than their corresponding mean concentrations: 0.66mg l^{-1} at the upstream site compared with 0.35 mg l^{-1} at the downstream site. The maximum PP concentration at the upstream site was 1.83 mg l^{-1} compared with 1.64 mg l^{-1} at the downstream site.

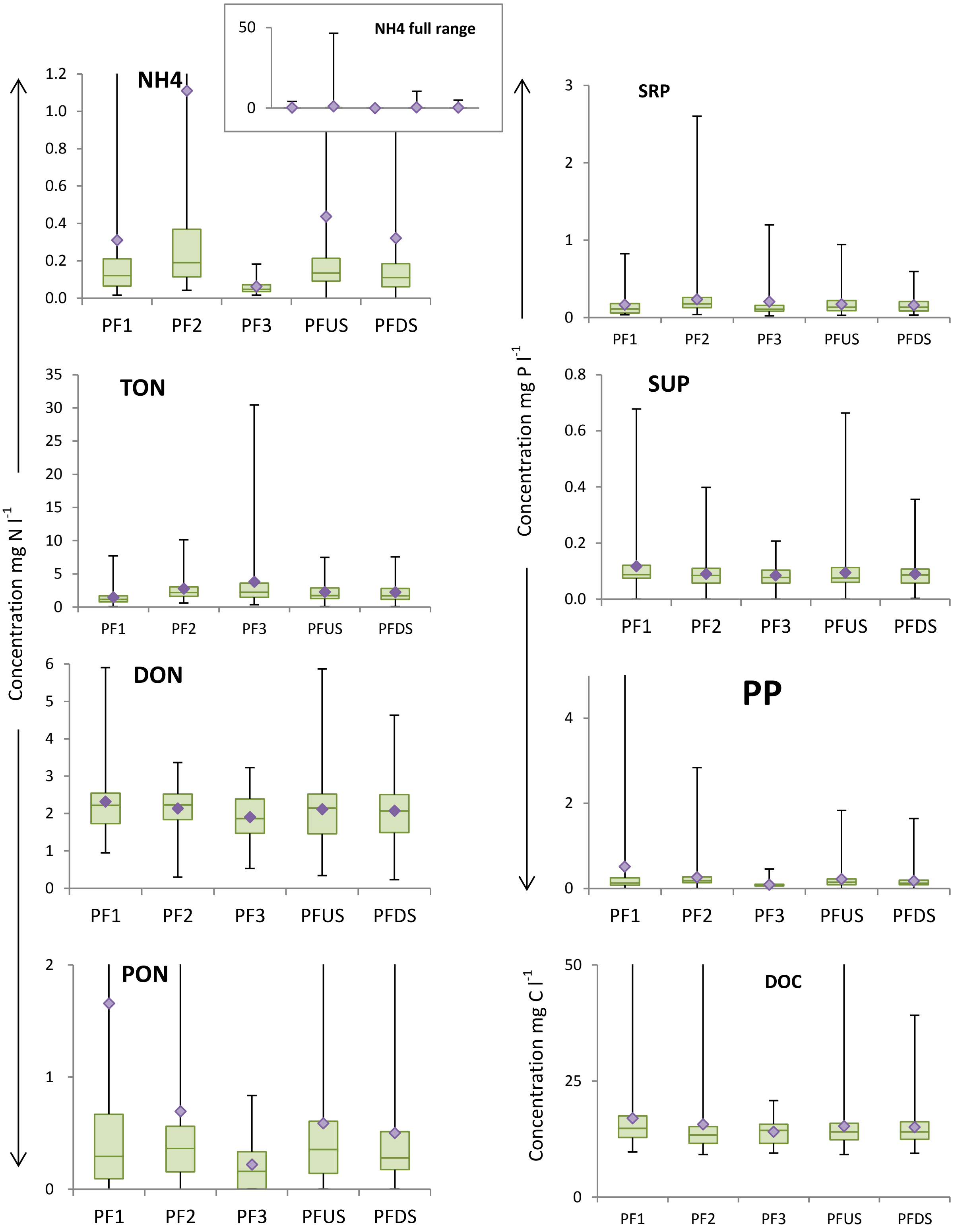


Figure 5:11 Spatial variation in nutrient chemistry in the Priors Farm sub-catchment. Locations are abbreviated: PFUS, upstream site and PFDS, downstream site at the sub-catchment outlet. The influence of extreme events is illustrated by the high variability and disparity between median and mean concentrations at all sites except PF3. Concentrations of TON at PF3 respond to the tributary drying out in the summer.

5.10.3. **Carbon**

A high degree of variability is also seen in the DOC concentrations (Table 5:**8 a**nd Figure 5:11). Taken over the full study period, mean concentrations are similar at the two sites (15.3 mg l^{-1} and 15.0 mg l^{-1} for the upstream and downstream sites, respectively). Maximum concentrations, however, are highly variable with a maximum recorded concentration of 53.3 mg l^{-1} at the upstream site compared to 39.1 mg l^{-1} at the downstream site. As reported for NH_4-N, higher DOC concentrations (maximum concentration of 94.9 mg l^{-1}) were recorded at the sub-catchment outlet but without upstream data available for comparison.

5.11. Patterns in nutrient speciation and fractionation at the Priors Farm sub-catchment outlet

The following analyses relate to grab samples collected weekly and analysed within 24 hours of collection. Higher resolution temporal dynamics are discussed in section 5.14.

5.11.1. **Nitrogen**

Unlike the Cool's Cottage sub-catchment, over the full study period, there is no overriding dominant species at Priors Farm. On average, TON constitutes 48.6% of TN at the sub-catchment outlet (2.20 mg l^{-1},Table 5:**8**); however, the concentration and its proportion of TN is affected seasonally (Figure 5:12), and drops to as low as 11% in the summer and as high as 86% during the winter. The dominance of different nitrogen species alternates between TON and DON (Figure 5:13). DON contributes 39.9% to TN overall (1.55 mg l^{-1}), with a maximum contribution of 65.7% during the summer and a minimum contribution of 10.1% in the winter. The contribution of NH_4-N throughout the period is 6.7% (0.31 mg l^{-1}), but can rise to 30.8% during discrete events as discussed in section 5.9. PON contributes 10.7% (0.48 mg l^{-1}), with a contribution that ranges from 0 – 36.4%.

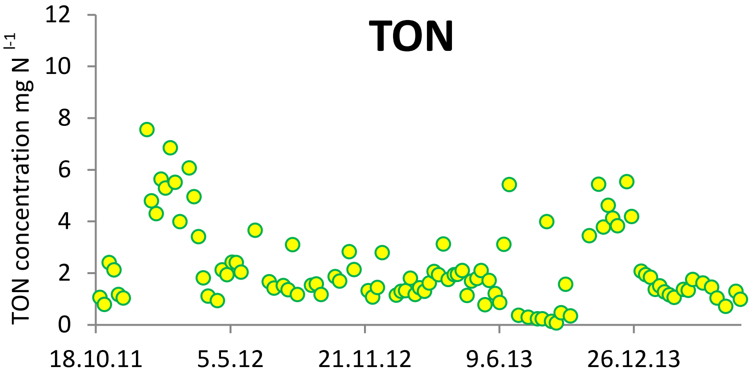


Figure 5:12 Temporal variation in TON concentrations at Priors Farm.

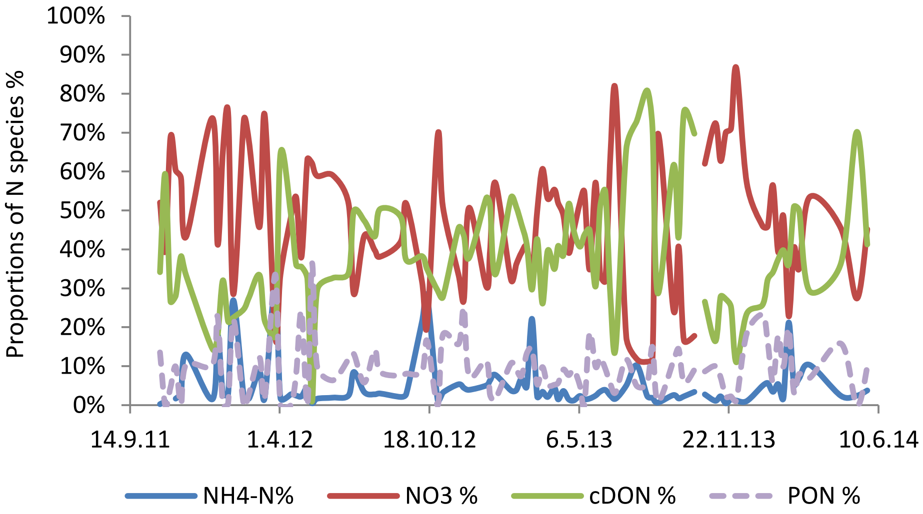


Figure 5:13 Temporal variation in the proportion of nitrogen species at Priors Farm

5.11.2. **Phosphorus**

As at Cool's Cottage, the balance between phosphorus fractions is more equal. Over the full study period SRP and PP contribute 39.9% and 38.4% respectively (0.17 mg l^{-1} and 0.16 mg l^{-1}, Table 5:11), while SUP constitutes the lowest proportion of TP at 21.7% (0.091 mg l^{-1}). There is a clear seasonal pattern in SRP concentrations (Figure 5:14), and a marked dominance of SRP in the summer of 2013 when TON concentrations are very low (Figure 5:15) suggesting a possible limitation caused by low available nitrogen at this time.

5.12. Temporal variation in stoichiometry at Priors farm

The lack of riparian shading at the monitoring station at Priors Farm promotes high light levels in the summer giving the potential for high primary production (further discussed in chapter 6). Therefore, seasonal variation in the N:P ratio can provide information on possible limiting factors for photosynthesis at this site. For both dissolved inorganic species (NH_4-N+TON : SRP) and total dissolved species (TDN : TDP), this ratio fluctuates around the threshold of 14 (by mass), being substantially lower during the summer and early autumn for all three study years (Figure 5:16). During the winter, spring and early summer, however, the ratio exceeds the threshold value and may drive remineralisation of less biologically available phosphorus fractions, by the activation of phosphatase, an exo-enzyme to maintain productivity in the spring (Chapter 6).

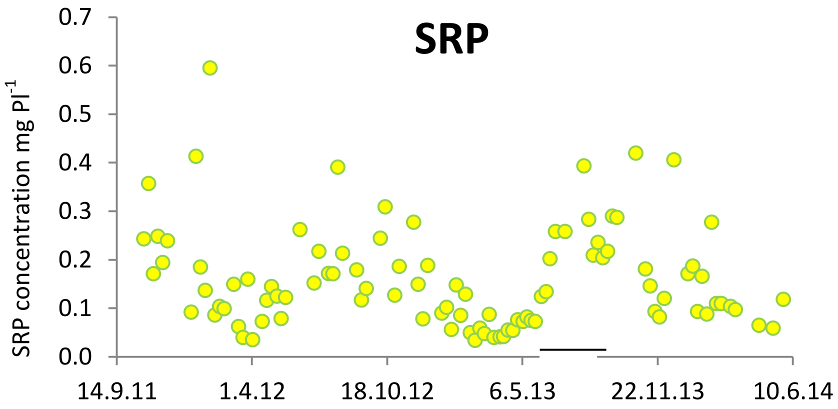


Figure 5:14 Temporal variation in SRP concentrations at Priors Farm.

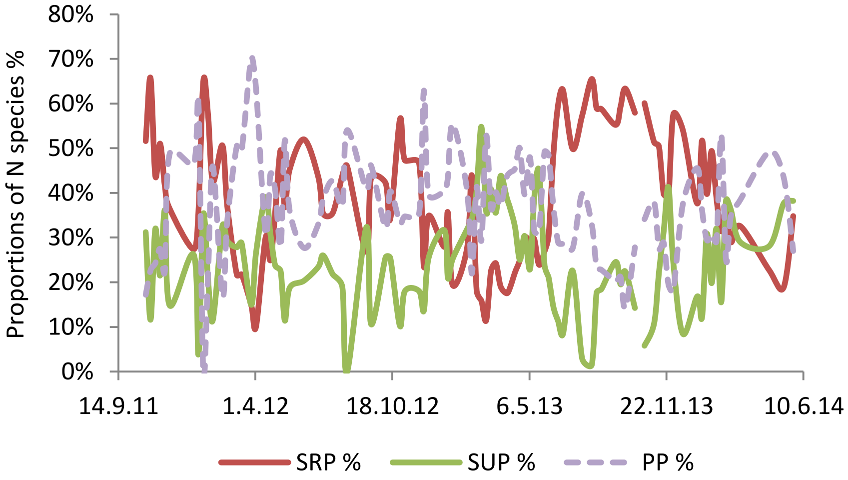


Figure 5:15 Temporal variation in the proportion of phosphorus fractions at Priors Farm

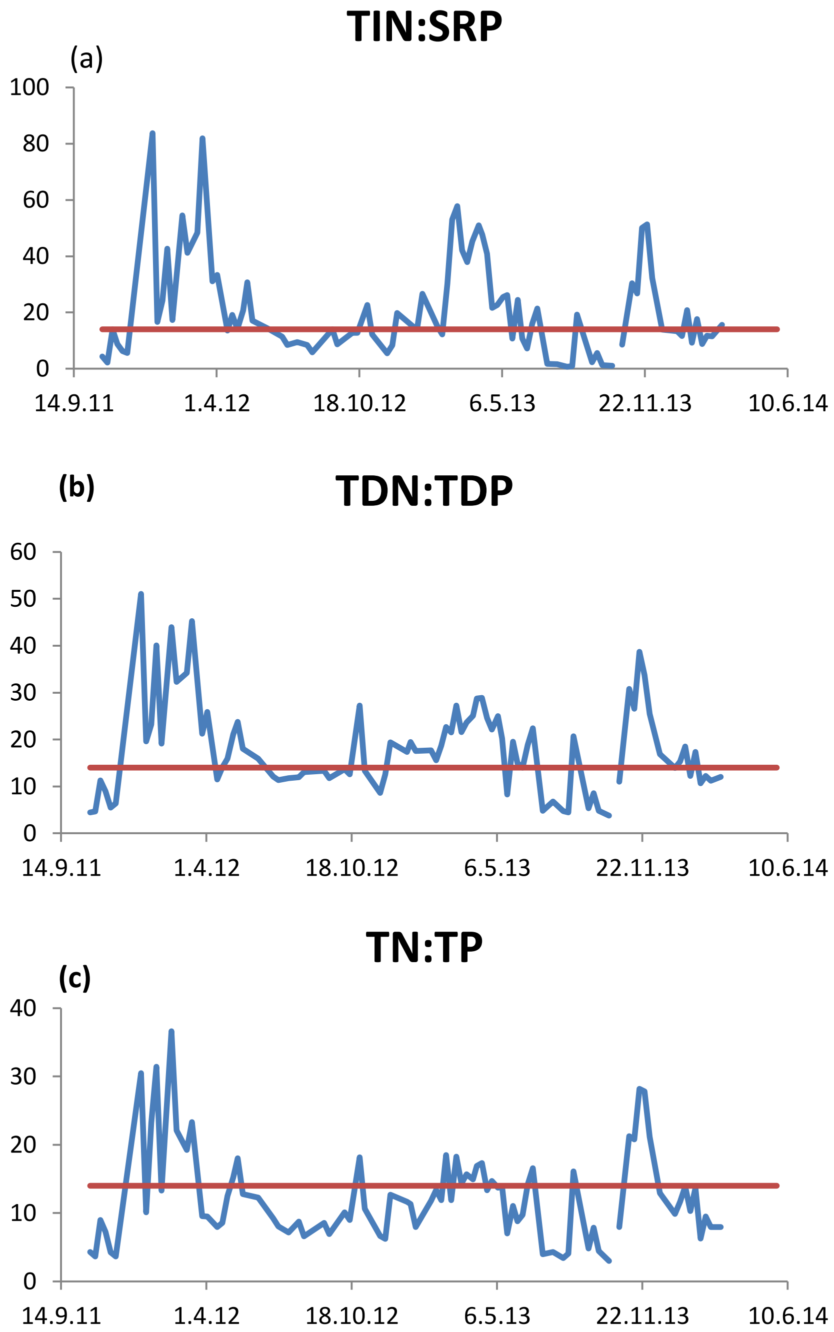


Figure 5:16 Temporal variation in the N:P ratio (by mass) at the Priors Farm sub-catchment outlet; (a) inorganic species, (SRP + TON):SRP, (b) dissolved species (TDN:TDP) and (c) TN:TP. The red line represents a N:P ratio (by mass) of 14, the point at which at which limitation of photosynthesis in lakes switches from N to P.

5.13. Nutrient load at the outlet from the Priors Farm sub-catchment.

As at Cool's Cottage, total discharge at the Priors Farm sub-catchment outlet was higher in year 2 (2259 ML a^{-1} compared with 1812 ML a^{-1} in year 1). Mainly as a result of the increased discharge, the annual load of TN in year 2 was higher than in year 1 (10.3 t a^{-1} compared to 9.3 t a^{-1}, a rise of 11%; Figure 5:17). The mean concentration of TON was slightly lower in year 2, with a mean concentration of 1.57 mg l^{-1} compared with 2.69 mg l^{-1}. However, mean concentrations of DON and NH_4-N remained the same at ~ 1.9 mg l^{-1} and 0.21 mg l^{-1} respectively (Table 5:9). PON was substantially lower in year 2 than in year 1, with a mean concentration of 0.29 mg l^{-1} in year 2 compared with 0.86 mg l^{-1} in year 1. A smaller rise was observed for the annual load of TP (1.2 t a^{-1} in year 1 compared to 1.3 t a^{-1} in year 2, a rise of 8%; (Figure 5:18). The mean concentration of SUP was lower in year 2: 0.102 mg l^{-1} in year 1 compared with 0.089 mg l^{-1} in year 2 (Table 5:11). PP was also substantially lower in year 2; 0.21 mg l^{-1} compared with 0.43 mg l^{-1} in year 1. The mean concentration of SRP was higher in year 1: 0.17 mg l^{-1} compared with 0.15 mg l^{-1} for year 2. Although the mean concentration of DOC was slightly higher in year 1 (16.97 mg l^{-1}in year 1 and 15.62 mg l^{-1} in year 2; (Figure 5:10), the lower discharge in year 1 resulted in an annual load of 28.9 t a^{-1} compared to 40.9 t a^{-1} in year 2, a rise of 41%.

Table 5:10 A comparison of the mean concentrations of nitrogen species over two years at the outlet to the Priors Farm sub-catchment, contributing to the difference in load (figure 1.17)

Year	NH4 – N (mg N l^{-1})		TON (mg N l^{-1})		DON (mg N l^{-1})		PON (mg P N^{-1})	
1	**0.217**	±1.047	**2.69**	±1.68	**1.88**	±1.39	**0.86**	±1.23
2	**0.212**	±0.478	**1.57**	±1.08	**1.96**	±0.65	**0.29**	±0.43

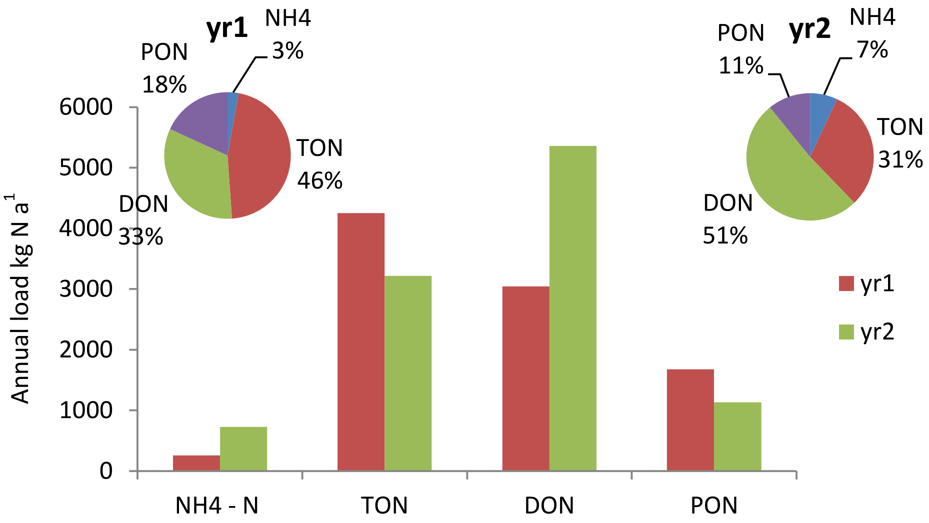


Figure 5:17 A comparison of the contribution to total nitrogen load by nitrogen species over two water years at the outlet to the Priors farm sub-catchment. Total nitrogen load was 9.3 tonnes per annum (t a-1) in year 1 and 10.3 (t a-1) in year 2.

Table 5:11 A comparison of the mean concentrations of phosphorus fractions and DOC over two years at the outlet to the Priors farm sub-catchment, contributing to the difference in load (figure 1.18). DOC load was 28.9 t a-1 in year 1 and 40.9 t a^{-1} in year 2.

Year	SRP (mg P l^{-1})		SUP (mg P l^{-1})		PP (mg P l^{-1})		DOC (mg C l^{-1})	
1	**0.169**	±0.114	**0.102**	±0.148	**0.429**	±0.391	**16.97**	±7.33
2	**0.151**	±0.094	**0.089**	±0.036	**0.212**	±0.160	**15.62**	±3.86

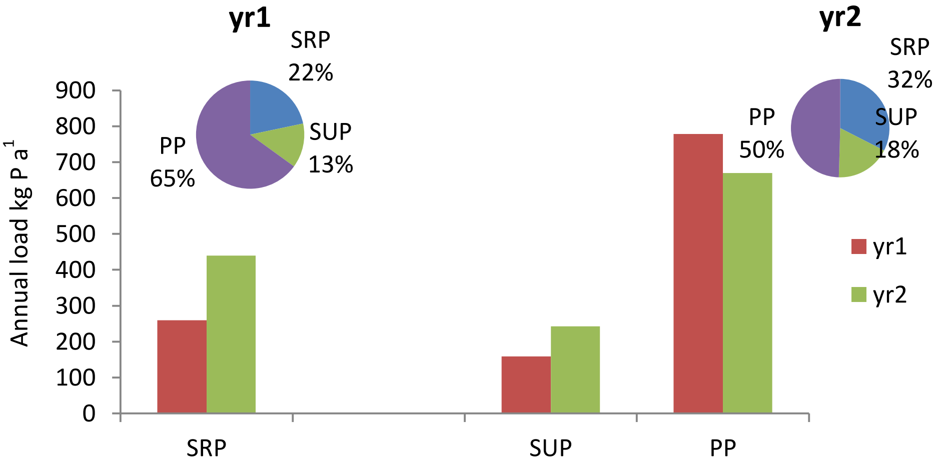


Figure 5:18 A comparison of the contribution to total phosphorus load by phosphorus fractions over two water years at the outlet to the Priors Farm sub-catchment. Total phosphorus load was 1.3 tonnes per annum (t a-1) in year 1 and 1.2(t a-1) in year 2.

5.14. High resolution temporal variation in nutrient concentrations at the outlet from the Priors Farm sub -catchment

The extreme variability in nutrient inputs in the Priors Farm sub-catchment, as described in the previous sections, emphasises the importance of high resolution analyses of in-stream chemistry for interpreting both nutrient fluxes and their implications for ecosystem function. While the weekly observations were able to capture a few of the extreme events that are likely to influence in-stream community structure and function, the high resolution sampling, albeit with intrinsic limitations on absolute accuracy with regard to in-stream speciation and fractionation, provide a better overview of the frequency and severity of these events. The consequences of high and intense rainfall, leading to problems of slurry containment, are illustrated by the timing of these events.

5.14.1. **Nitrogen dynamics**

Unlike at Cool's Cottage, the concentration of TON responded rapidly to the onset of rain following the dry summer of 2011, but fell in the spring of 2012 despite continuing rains. This may have been due to the reservoir of TON in the soils of the sub-catchment becoming depleted following the wet weather and biological uptake from the soil, as spring productivity began (Figure 5:19). The two minima in TON concentrations during this phase, 19.1.12 and 3.2.12 (0.41 and 0.34 mg l^{-1} NO_3-N), coincide with two pulses of high NH_4-N and DOC (6.0 and 14.4 mg l^{-1} NH_4-N and 48.2 and 94.9 mg l^{-1} DOC, respectively), with a similar, but less severe incident occurring on 25.2.12 (1.83 mg l^{-1} NO_3-N, 8.06 mg l^{-1} NH_4-N and 43.1 mg l^{-1} DOC). These incidents are also indicated by intense peaks in DON and PON and sharp troughs in dissolved oxygen (section5.14.4, Figure 5:21). Further intense rain in the spring of

2012 result in a smaller rise in TON concentration in the early summer, but despite continuing rain in the summer and winter, the concentration of TON remains relatively low (~1.2 mg l^{-1}) for the rest of 2012 and through to the spring of 2013. During this period, slurry from both Coleman's Farm and Hays Farm was being exported from the sub-catchment until the capacity of the receiver was reached in the spring of 2013 and the export stopped (see chapter 3). Concentrations of TON rose in the spring of 2013 but declined during the dry summer and remained low until the onset of rain in late October 2013, when they rose in response to increased surface and interstitial flow. This peak in TON concentration declined rapidly, despite continuing rain, again suggesting depleted TON stores in the soils of the sub-catchment, following the removal of grazing stock from the sub-catchment to winter housing. NH_4-N, DON and PON all exhibit discrete spikes in concentrations following the onset of intense rain in the winter of 2012, and periodically thereafter. These indicators of animal waste were absent for the period during which slurry was being exported from the sub-catchment.

5.14.2. **Phosphorus dynamics**

SRP concentrations rise with the onset of winter rain after the dry summer of 2011 following which, they exhibit a strong annual cycle with lows in the spring of each of the study years (Figure 5:20). Maximum concentrations of SRP occur during the late summers of 2012 and 2013. This reversal of the pattern observed for TON supports the proposal of a switch in the factors limiting primary production at this site. Concentrations of SUP exhibit a weaker annual cycle than SRP. Concentrations of PP are highly variable with sharp peaks coinciding with intense rainfall events. This is consistent with sediment inputs being driven by overland flow.

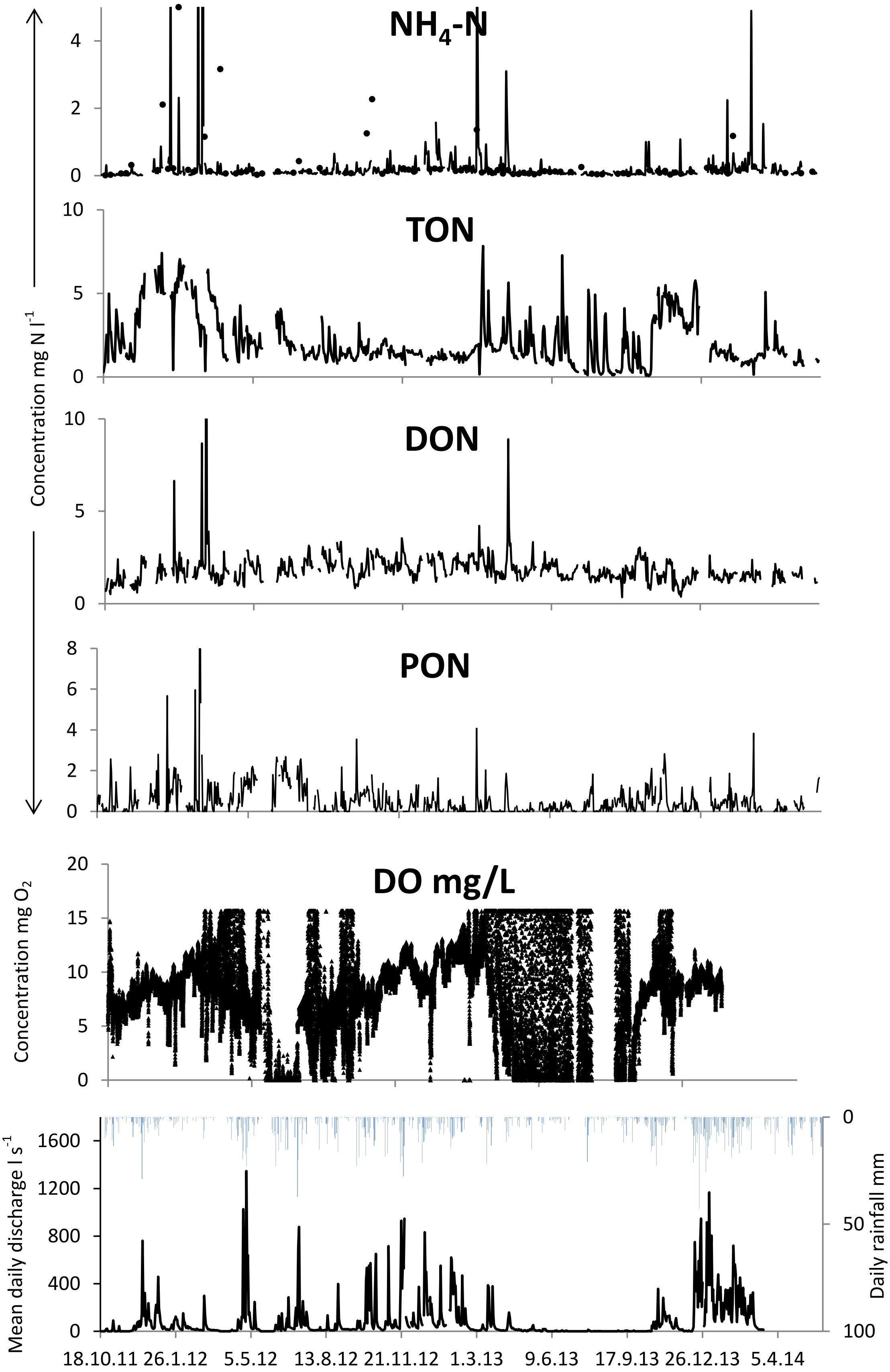


Figure 5:19 Temporal variation in nitrogen species and dissolved oxygen at the outlet to the Priors Farm sub-catchment. Weekly grab samples of NH$_4$-N are represented as points, while daily samples that may have degraded during storage are represented as a line plot.

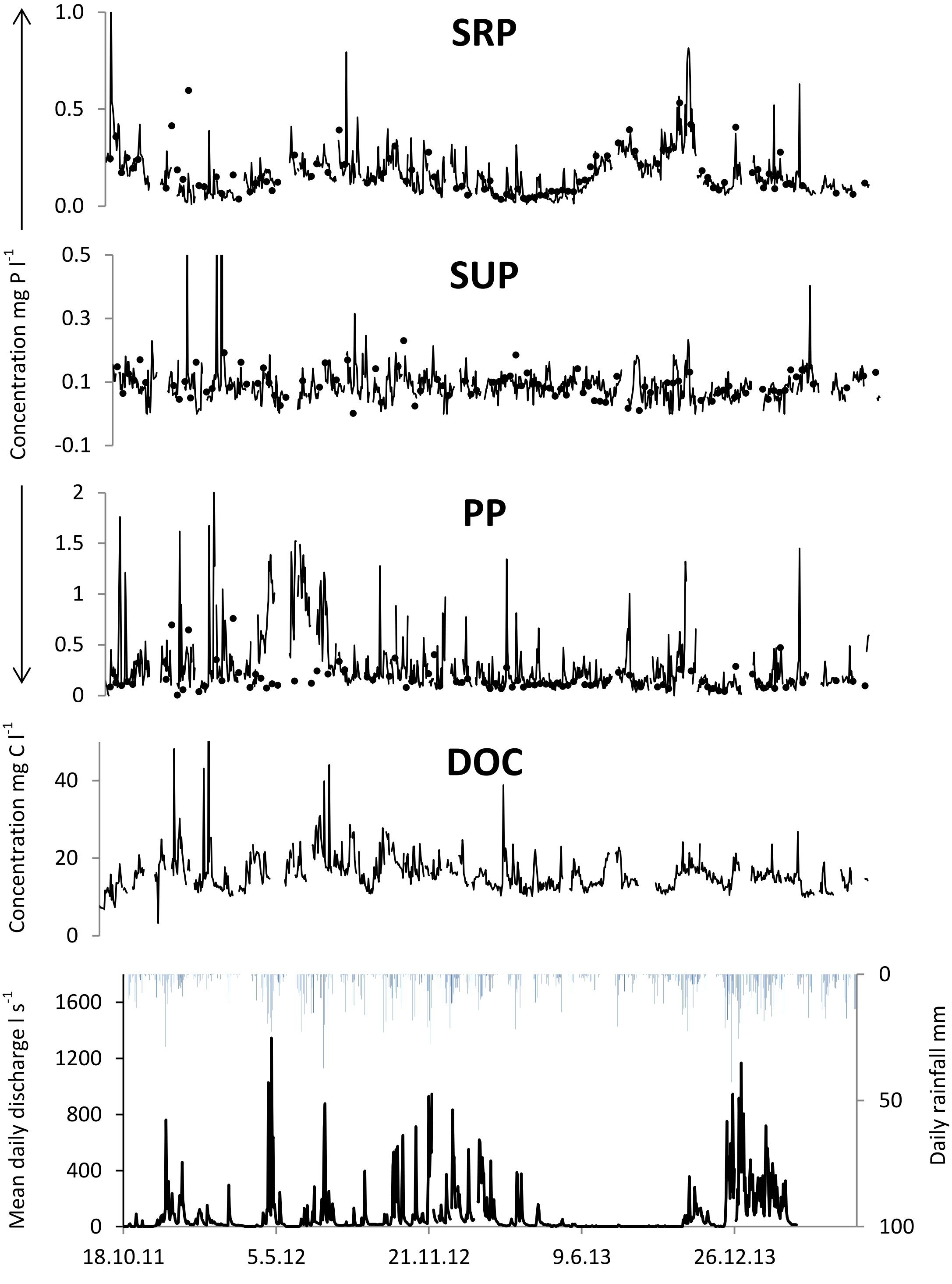


Figure 5:20 Temporal variation in phosphorus fractions and dissolved organic carbon at the outlet to the Priors Farm sub-catchment. Weekly grab samples of phosphorus fractions are represented as points, while daily samples that may have degraded during storage are represented as a line plot.

5.14.3. **Dynamics of dissolved organic carbon**

As at the outlet from Cool's Cottage, DOC concentrations respond to peaks in flow, rising after the onset of rain in October 2011 and again following the summer rain in 2012. There is a further increase in DOC concentration after the dry summer of 2013, with the onset of rain in October. In this sub-catchment, however, DOC concentration is also a powerful indicator of pollution events as discussed in the following section (and see Figure 5:20).

5.14.4. **Dissolved Oxygen**

The conditions at the Priors Farm monitoring station caused some problems for the dissolved oxygen sensor. The combination of high nutrient concentrations and light availability resulted in substantial biofouling of the sensor that significantly reduced its sensitivity (Figure 5:21), particularly during the spring and summer when extensive algal growth occurred at this site (see chapter3). There are, however, periods when the sensor performed well and illustrate the consequences of the pulses of organic matter, for example those that occurred in early 2012 (e.g. 19.1.12, 30.1.12, 25.2.12 and 2.3.12) and on the occasions described in section 1.9 (7.1.13, and 3.3.13). The magnitude of the diurnal variation in dissolved oxygen is also apparent with extremes of both supersaturation and hypoxic conditions being experienced by the in-stream communities during the summer months- especially during the period of no flow in summer 2013.

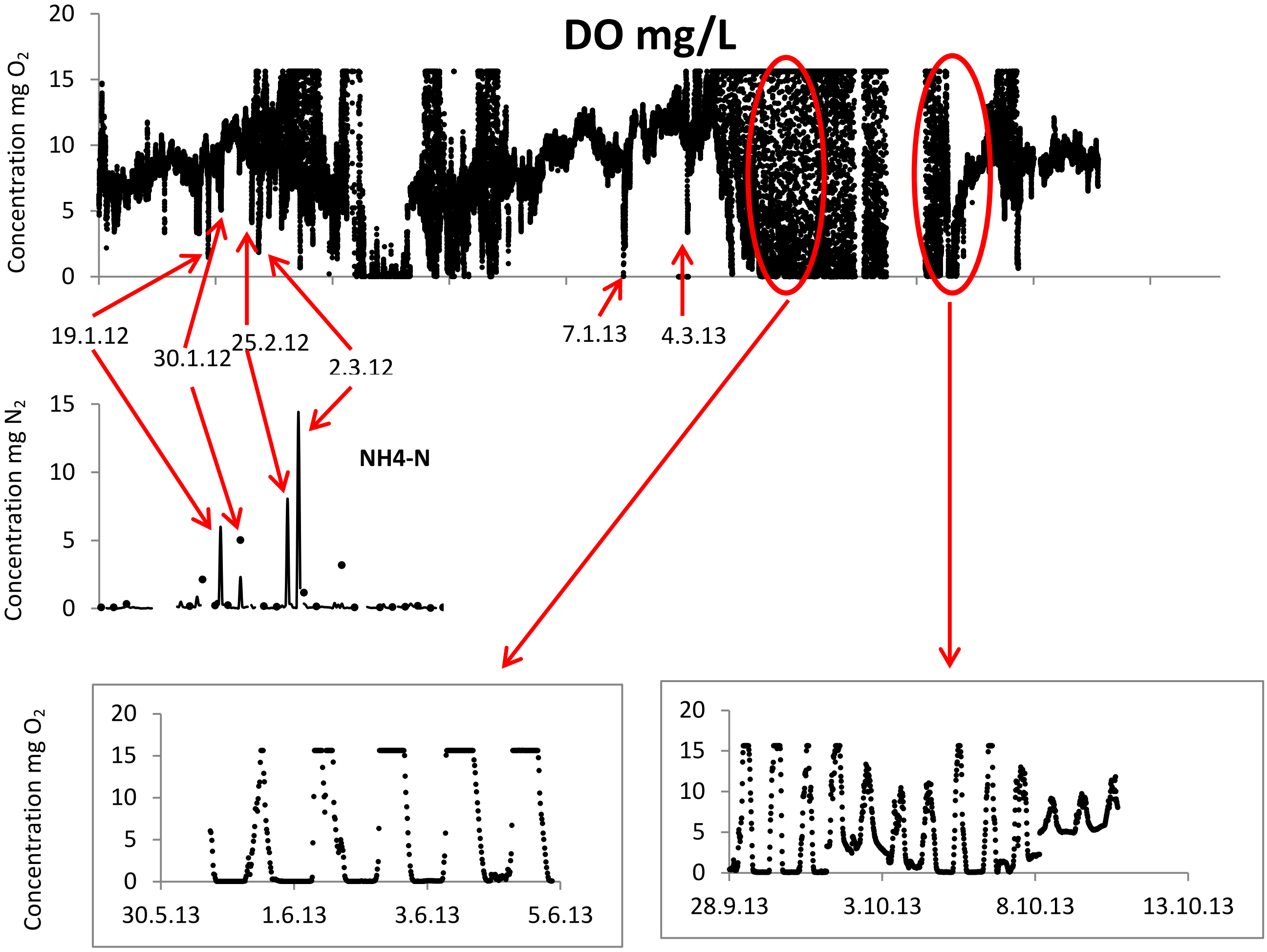


Figure 5:21 Expanded sections of the dissolved oxygen record at Priors Farm showing the response to organic matter pulses (indicated by NH4-N concentrations) and reduced sensitivity during algal blooms (Note rise in DO of 10 mgl-1 in 15 minutes at 0630 for several days in June 2013.

5.15. A comparison of the hydrochemistry in the Cool's Cottage and Priors Farm sub-catchments

The data presented here demonstrate clear differences between the study sub-catchments in relation to water chemistry. Annual loads for all nutrient species and fractions are higher at Priors Farm than at Cool's Cottage. Only TON has higher mean concentrations at Cool's Cottage than at Priors Farm and the reduction in concentration with distance downstream suggested that TON was not delivered to the reach from surface sources. Rather, it appeared to be a legacy of historic farming practice, or of activities outside the sub-catchment affecting the groundwater source feeding the headwater springs. Concentrations of SRP, DOC and NH_4-N were all substantially lower than in the Priors Farm reach. However, there was some evidence to support delivery of these nutrients from surface sources, with a marked contribution from the road drain. Cool's Cottage nutrient chemistry was strongly influenced by annual cycles driven by biological processes in the lake in Clay Hill Wood.

In contrast the Priors Farm reach exhibited high concentrations of SRP, NH_4-N and DOC. Concentration of other fractions were also higher than in the Cool's Cottage reach, but the disparity was smaller. With no apparent groundwater sources, these nutrients must have been delivered to the watercourse from surface sources. In addition to high background concentrations, Priors Farm experienced pulses of very high DOC and NH_4-N, linked with episodes of low DO. These appeared to originate from insufficient slurry containment during prolonged periods of wet weather. The consequences of high nutrient concentrations were exacerbated by periods of very low or no flow in the Priors Farm reach while at Cool's Cottage, flow was maintained throughout the study period.

These contrasts in nutrient concentrations and in their speciation and fractionation between the two sub-catchments resulted from both the intrinsic differences in the sub-catchments, and the differences in land management described in chapter 3. The paired catchment approach adopted for this study provided an opportunity to assess their effects on in-stream ecosystem function. The consequences to both short term metabolic function and the longer term functional measures of macro-invertebrate herbivory and detritivory are explored in chapters 6 and 7.

Chapter 6. **Characterising short term ecosystem function in two headwater sub-catchments.**

At its most fundamental, ecosystem function is itself a function of metabolism: the acquisition of energy through respiration (aerobic and anaerobic); the assimilation of materials into organic matter, and the transformation of organic and inorganic compounds resulting from these processes that both respond to, and influence the environment around them.

In this chapter, community aerobic respiration, primary production and the relationship between them are compared in the two study reaches. To a lesser extent, anaerobic respiration using NO_3^- and CO_2 as electron acceptors is compared by estimating the flux of the greenhouse gases, CO_2, CH_4 and N_2O across the sediment-water interface.

6.1. Overview

A series of incubations were performed during a twelve month period from June 20th, 2013 to June 3rd, 2014, to explore variations in key metabolic processes between the two sub-catchments namely: community aerobic respiration, community photosynthetic primary production and the accumulation of greenhouse gases across the sediment-water interface. Incubations at the Priors Farm downstream site and at Cool's Cottage were conducted within 3 m of the DTC cabinets. The Priors Farm downstream site was unshaded and from 12th September 2013 a second, shaded site was adopted in the Priors Farm reach to more closely mirror the ambient light conditions prevalent throughout the Cool's Cottage sub-catchment and to represent the shaded reaches (approximately 70%) of the Priors Farm study reach (Figure 6.1). For full details of methods and incubation timetable see chapter 4.

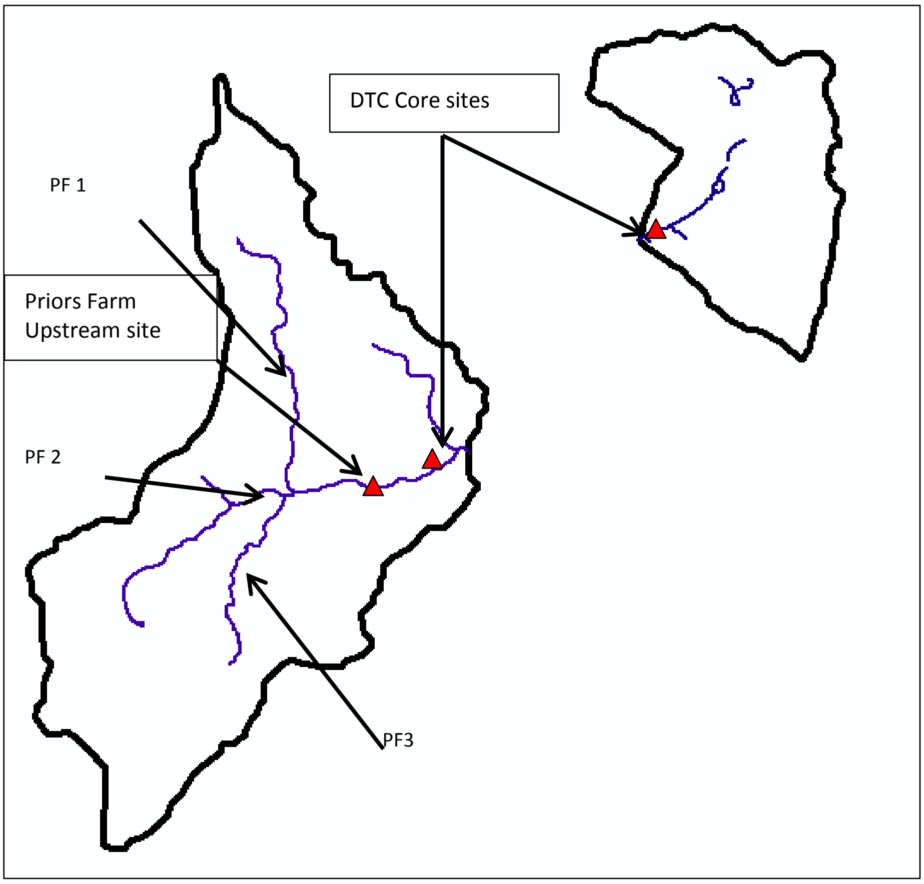


Figure 6:1 Location of experimental sites and tributaries.

6.2. Aerobic respiration and nutrient transformations.

The renewed search for reliable and transferable indicators of stream health has led to recent work revisiting measurements of aerobic respiration as a useful measure of ecosystem function (McTammany et al., 2003; Uehlinger, 2006; Young et al., 2008). Another approach is to explore the consistent relationships between aerobic respiration and temperature, and between carbon respired and nitrogen produced, to help make predictions on a global scale (AcuÑA et al., 2008; Demars et al., 2011; Yvon-Durocher et al., 2010; Yvon-Durocher et al., 2011; Trimmer et al., 2012).

6.2.1. **Key characteristics of respiration and nutrient dynamics:**

There was a strong relationship between temperature and aerobic respiration measured in benthic chambers at the Cool's Cottage and Priors Farm downstream sites (Figure 6:2).

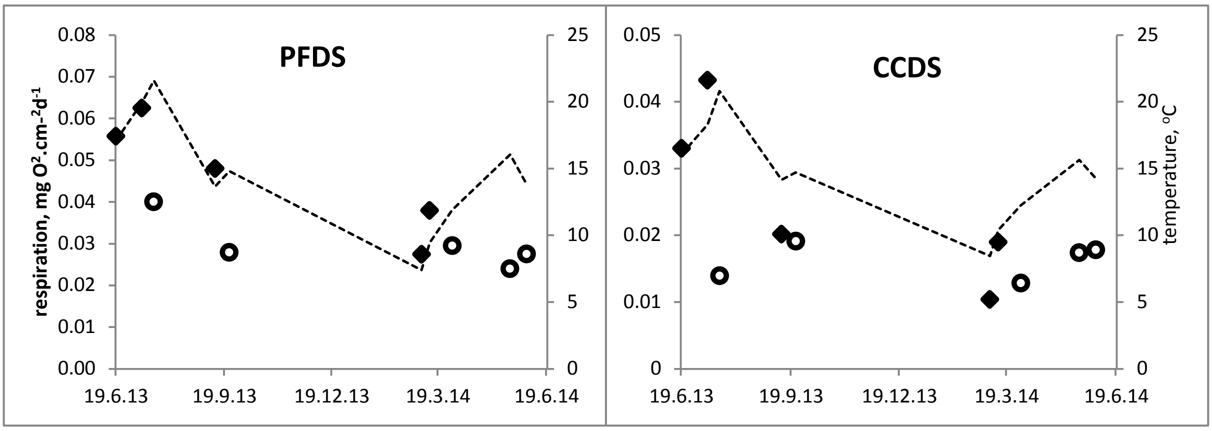


Figure 6:2 Temporal variation in ecosystem respiration (ER) - A, Priors Farm Downstream. B, Cool's Cottage. Closed symbols, dedicated measures of aerobic respiration: Open symbols, combined measures of greenhouse gas release and aerobic respiration. Dotted line, temperature

However, a systematic error was introduced in the combined incubations, where a subsample was removed for headspace analysis of greenhouse gases (Chapter 4). Using the dedicated respiration incubations, the relationship between aerobic respiration and temperature was used to correct for this systematic error in the combined incubations. (Figure 6:3). The corrected values are used in all future discussions.

There was a significant difference ($F = 26$, $P < 0.0001$) between aerobic respiration at Cool's Cottage and in the Priors Farm reach. There was no significant difference between aerobic respiration at the Priors Farm upstream and downstream sites ($F=1.4$, $P > 0.2$). Ecosystem respiration at Cool's Cottage and at the Priors Farm sites ranged from 0.010-0.043 and 0.027 -0.071 mg O_2 cm^{-2} day^{-1} respectively, equating to 0.1 – 0.7 g O_2 m^{-2} day^{-1}, with a mean value of 90% of community aerobic respiration being attributable to benthic respiration (88% and

91% in the Priors Farm and Cool’s Cottage reaches, respectively;(Table 6:1). There was significant seasonal variation at all three sites (F=6.3 P < 0.0001).

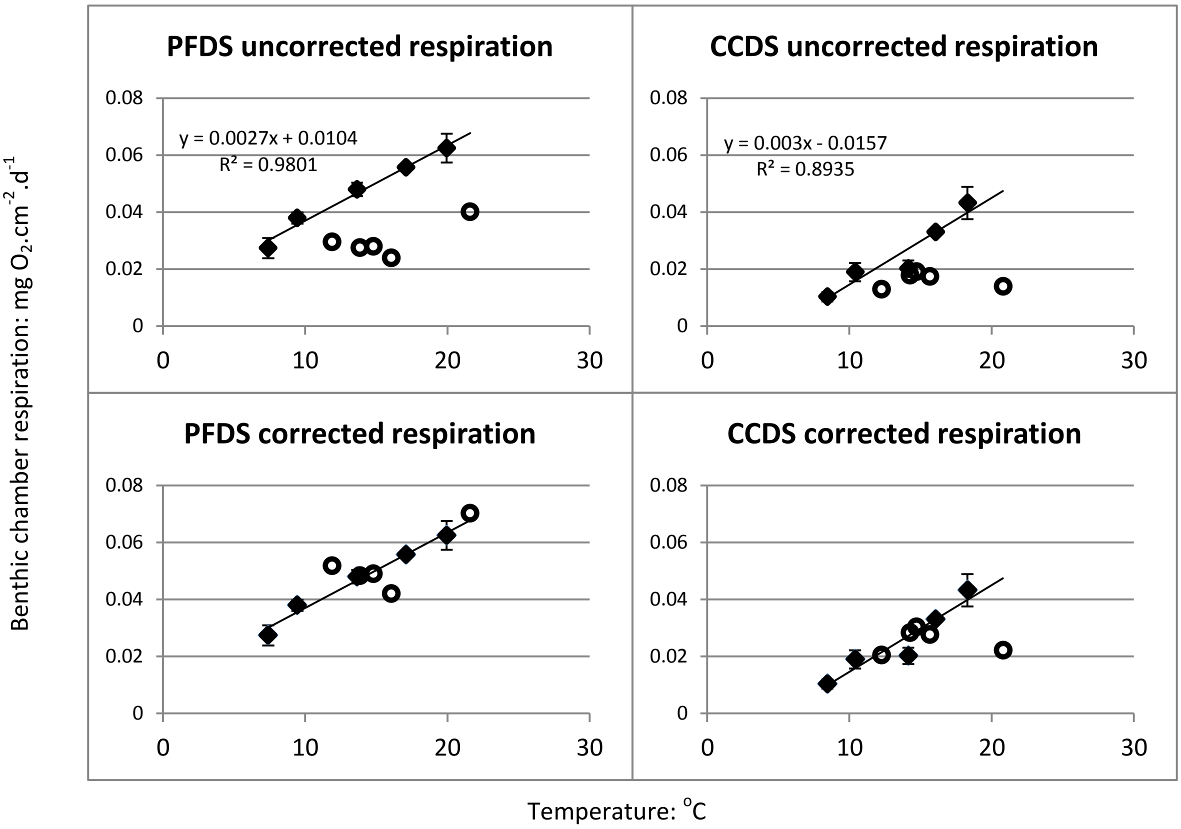


Figure 6:3 Relationship between temperature and benthic respiration before and after correction for systematic error. A and C Priors Farm downstream site; B and D Cool’s Cottage: Closed symbols, dedicated measures of aerobic respiration; open circles, combined measures.

Recent work by Trimmer et al. (2012) and Yvon-Durocher et al. (2010 & 2011) have reported the predictive power of temperature in modelling changing respiration in aquatic environments on a global scale. The following section explores the value of this approach to understanding the drivers of key metabolic processes at a local level.

Table 6:1 Rates of aerobic respiration in dark benthic chambers and water bottle incubations at Cool’s Cottage and the Priors Farm downstream site.

	Cools Cottage				**Priors Farm**			
date	areal benthic respiration (mg O^2 cm^{-1} d^{-1})	volumetric benthic respiration (mg O^2 cm^{-1} d^{-1})	water column respiration (mg O^2 l^{-1} d^{-1})	**wc %**	areal benthic respiration (mg O^2 cm^{-1} d^{-1})	volumetric benthic respiration (mg O^2 l^{-1} d^{-1})	water column respiration (mg O^2 l^{-1} d^{-1})	**wc %**
19.6.13	0.0330	4.13	0.56	13%	0.0557	5.07	0.71	14%
11.7.13	0.0432	5.50	0.12	2%	0.0624	7.68	1.24	16%
21.7.13	0.0223	5.44			0.0702	9.62		
11.9.13	0.0201	2.80	0.28	10%	0.0479	6.48	0.25	4%
23.9.13	0.0306	5.64	0.33	6%	0.0489	7.78	0.37	5%
5.3.14	0.0103	1.44			0.0274	4.09	1.00	24%
12.3.14	0.0189	3.59	0.42	12%	0.0379	6.34	0.60	9%
31.3.14	0.0205	3.78			0.0517	10.18		
19.5.14	0.0278	4.44			0.0420	9.76		
2.6.14	0.0286	4.54			0.0483	8.51		
mean				**9%**				**12%**

6.2.1.1. Kinetics of aerobic respiration

The activation energy of a system is defined by the key transformations required to support community metabolism. It stems from the energy required to prime essential enzymatic processes and can be determined from further analysis of the relationship between respiration and temperature, in particular by expressing this relationship as a standardised rate (Equation 13).

Equation 13: $\ln R_S(T)=\ln [R(T)/R(T_C)]=E_a(1/kT-1/kT_C)$.

Where:

ln RS(T) = standardised respiration rate, ln [R(T)/R(TC)]

R(T) = respiration rate at a measured temperature, in °K

$R(T_C)$ = respiration rate at a fixed, reference temperature T_C, in °K

k = the Boltzman constant ($8.62\text{x}10^{-5}$ eV k^{-1})

E_a = activation energy in eV (electron volts)

For the current analysis, the reference temperature was chosen as 288 °K (15 °C) representing the median temperature recorded during the incubations.

In a heterotrophic system, where respiration is not constrained by photosynthesis, the theoretical activation energy, E_a, approaches that of the respiration complex, 0.62 eV. This value is derived from the two molecules of ATP (E_a = 0.31eV mol^{-1}) required to 'prime' glycolysis (Brown et al., 2004). A large scale modelling study using data from the literature (Trimmer et al. 2012) demonstrated widespread adherence to this prediction. At the local level, deviation from this theoretical value may indicate that another factor is limiting metabolism, and E_a is likely to approach that exhibited by the processes required to overcome that limitation (Sinsabaugh and Shah, 2010).

Using equation 1, E_a can be calculated from the relationship between the standardised respiration rate (ln R_S) and standardised temperature ($1/kT_C$-$1/kT$). To calculate E_a, only the dedicated respiration incubations from the downstream sites were used. E_a at the Priors Farm and Cool's Cottage downstream sites were estimated as 0.43 and 0.92eV respectively (Figure 6:4). At Priors Farm, E_a was close to the theoretical value for the global scale E_a (i.e. 0.5eV), demonstrated by Trimmer et al. (2012) and Yvon-Durocher (2010). However, at Cool's Cottage, despite a strong relationship with temperature, the estimate of activation energy was substantially higher, around 0.9eV.

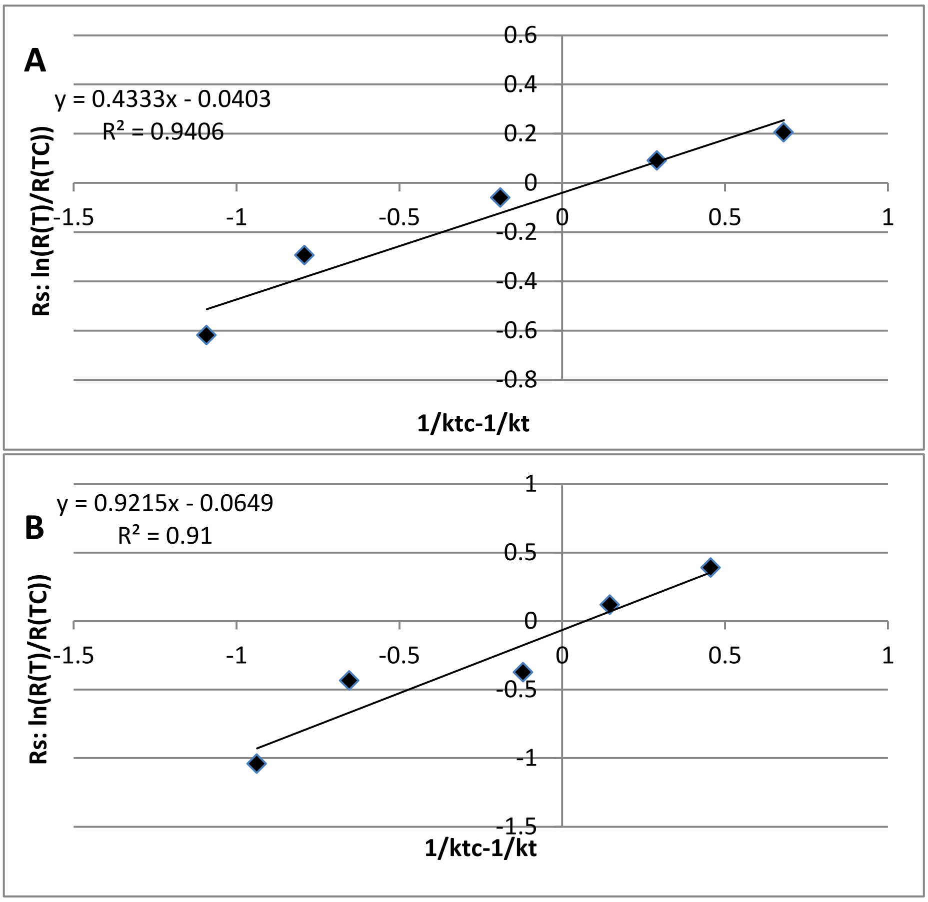


Figure 6:4 Calculation of activation energy for Ecosystem Respiration at A, Priors Farm downstream site, B Cool's Cottage

This value is closer to that demonstrated by Sinsabaugh and Shah (2006), 0.86eV, which occurred as a result of resource limitation. To explore the possible causes of these differences, the relationships between respiration and in-stream nutrient fractions were examined at these two sites.

6.2.1.2. Aerobic respiration and nutrient chemistry

The response of community aerobic respiration to in-stream water chemistry has been explored widely in the literature, most commonly evaluating the role of DOC and the inorganic species NO_3^- and PO_4^{3-} (Young and Huryn, 1999; Mulholland et al., 2001; Mulholland et al., 2006; Fellows et al., 2006a; Fellows et al., 2006b; Uehlinger, 2006; Roberts et al., 2007; Valett et al., 2008). In this study, the correlation between aerobic respiration and DOC, and with all nitrogen species and phosphorus fractions, were explored.

The contribution of the water column to community respiration was calculated from the relative values of volumetric respiration (mg O_2 l^{-1}) observed in the benthic chambers and in dark water bottles incubated alongside them (Table 6:1). The average contribution of the water column was 9% and 12% at Cool's Cottage and Priors Farm, respectively. The uncharacteristically high contribution of the water column at Priors Farm in March 2014 coincided with the start of one of the periodic 'pollution events' where slurry entered the watercourse upstream of this site (see chapter 5). Under 'background' conditions, the average contribution of the water column to ER at PFDS was 10%.

Respiration in the dark water bottles was predicted to respond more sensitively to in-stream water chemistry than respiration in the benthic chambers: ~ 90% of the respiration in the chambers was attributable to benthic processes, which were expected to be more strongly influenced by sediment characteristics than water chemistry. Table 6:2 describes the relationships between water chemistry and the observed aerobic respiration in both the dark benthic chamber and water bottle incubations. However, as established above (section 6.2.1.1), temperature exerts a significant control on respiration. Studies of another ecosystem functional metric, leaf litter degradation, confronted this issue by normalising rates to temperature and presenting data as rates per degree day, (ln R_T dd^{-1}). Including temperature in the analysis of the relationship between water chemistry and aerobic respiration distinguished between metabolic responses to nutrient concentrations and coincidental covariance with temperature (Table 6:3). Overall, the inclusion of temperature in this analysis had the effect of increasing the number of significant relationships between nutrients and respiration at Cool's Cottage and reducing them at Priors Farm. However, at both sites, the statistical significance of the relationships identified between respiration and key nutrients increased (Table 6:3).

In general, and contrary to expectations, respiration in the water column appeared to be less sensitive to variations in nutrient chemistry than did respiration in the whole stream incubations. This is likely to be a consequence of both fewer incubations being carried out (i.e. smaller $\underline{n}$ value, giving a weaker statistical significance), and much lower respiration being recorded in the water bottles than in the benthic chambers. The values for change in dissolved oxygen concentration over the incubation period (Δ O_2) in the water bottles ranged from 0.11 to 1.2 mg O_2 l^{-1}, approaching the limit of detection given the experimental protocol. These factors resulted in a higher degree of uncertainty surrounding the measures of water column respiration. Hence, the exploration of the relationships between in-stream nutrients and respiration is concentrated on results from the benthic chamber incubations.

Table 6:2 Pearson correlation matrix describing the relationship between observed rates of aerobic respiration and in-stream water chemistry at Cool's Cottage and the Priors Farm downstream site. Significance is indicated as *P* values. DOC*, correlation with 'slurry' point removed, (see text).

	Cools Cottage			**Priors Farm**		
date	areal benthic respiration mg O^2 cm^{-1} d^{-1}	volumetric benthic respiration mg O^2 l^{-1} d^{-1}	Significance		areal benthic respiration mg O^2 cm^{-1} d^{-1}	volumetric benthic respiration mg O^2 l^{-1} d^{-1}
NH_4-N	0.21	0.89 **	**** $P < 0.001$	NH_4-N	-0.71 **	0.39
TON	-0.71 **	0.36	*** $P < 0.005$	TON	-0.52 ~	-0.11
DON	0.61 ~	-0.19	** $P < 0.05$	DON	0.71 **	0.69 ~
PON	0.82 **	-0.57	* $P < 0.1$	PON	-0.07	-0.79 ~
SRP	-0.54 ~	0.29		SRP	0.48 ~	0.14
SUP	-0.10	-0.66		SUP	0.22	0.8 ~
PP	0.87 ***	-0.41	~ weak trend	PP	0.31	-0.42
DOC	0.77 **	0.10	0.1 <) < 0.2	DOC	0.69 **	0.72 ~
				DOC*	0.85 **	0.77 ~

Table 6:3 Pearson correlation matrix describing the relationship between temperature-corrected aerobic respiration and in-stream water chemistry at Cool's Cottage and the Priors Farm downstream site. Significance is indicated as *P* values. . DOC*, correlation with 'slurry' point removed, (see text).

	Cools Cottage			**Priors Farm**		
date	areal benthic respiration (ln RT dd^{-1})	volumetric benthic respiration (ln R(wc) dd^{-1})	Significance		areal benthic respiration (ln RT dd^{-1})	volumetric benthic respiration (ln R(wc) dd^{-1})
NH_4-N	0.18	0.72 ~	**** P < 0.001	NH_4-N	-0.9 ***	0.43
TON	-0.83 ***	0.03	*** P < 0.005	TON	-0.43	0.15
DON	0.67 **	0.12	** P < 0.05	DON	0.67 **	0.69 ~
PON	0.73 *	-0.46	* P < 0.1	PON	0.04	-0.8 ~
SRP	-0.73 **	-0.04		SRP	0.35	0.08
SUP	-0.29	-0.69 ~		SUP	-0.14	0.83 *
PP	0.76 **	-0.28	~ weak trend	PP	0.32	-0.39
DOC	0.7 **	0.34	0.1 <) < 0.2	DOC	0.45 ~	0.60
				DOC *	0.72 **	0.55

Aerobic respiration at Cool’s Cottage appeared to be more responsive to in-stream water chemistry than at Priors Farm (Figure 6:5 and Figure 6:6), with significant correlations being returned for most of the fractions. Aerobic respiration at Cool’s Cottage exhibited a strong negative relationship with both TON and SRP. Other fractions; DON, PON, PP and DOC returned positive trends (Figure 6:5).

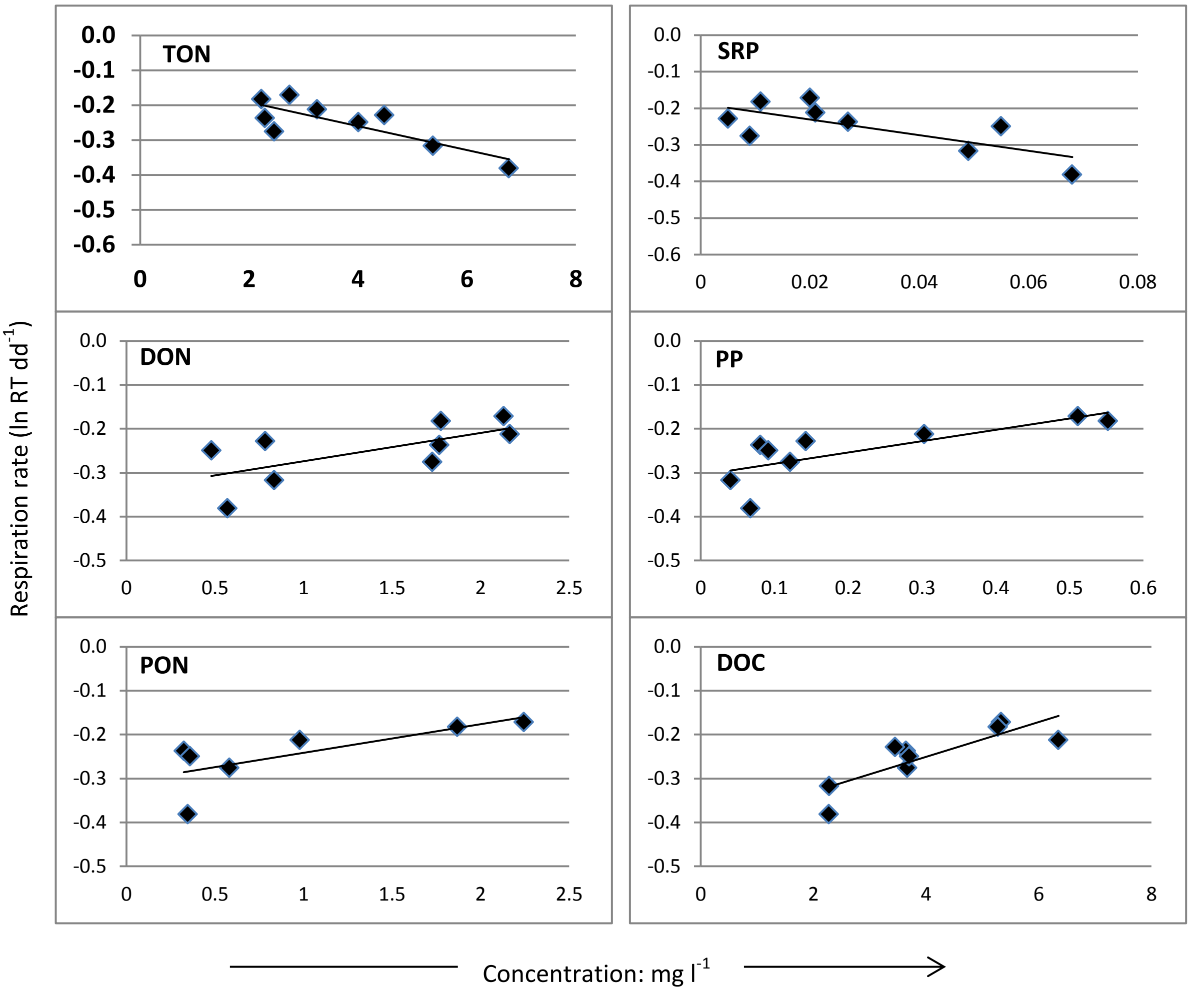


Figure 6:5 Relationships between ER and nutrient fractions at CCDS (only those fractions where relationships were statistically significant are shown)

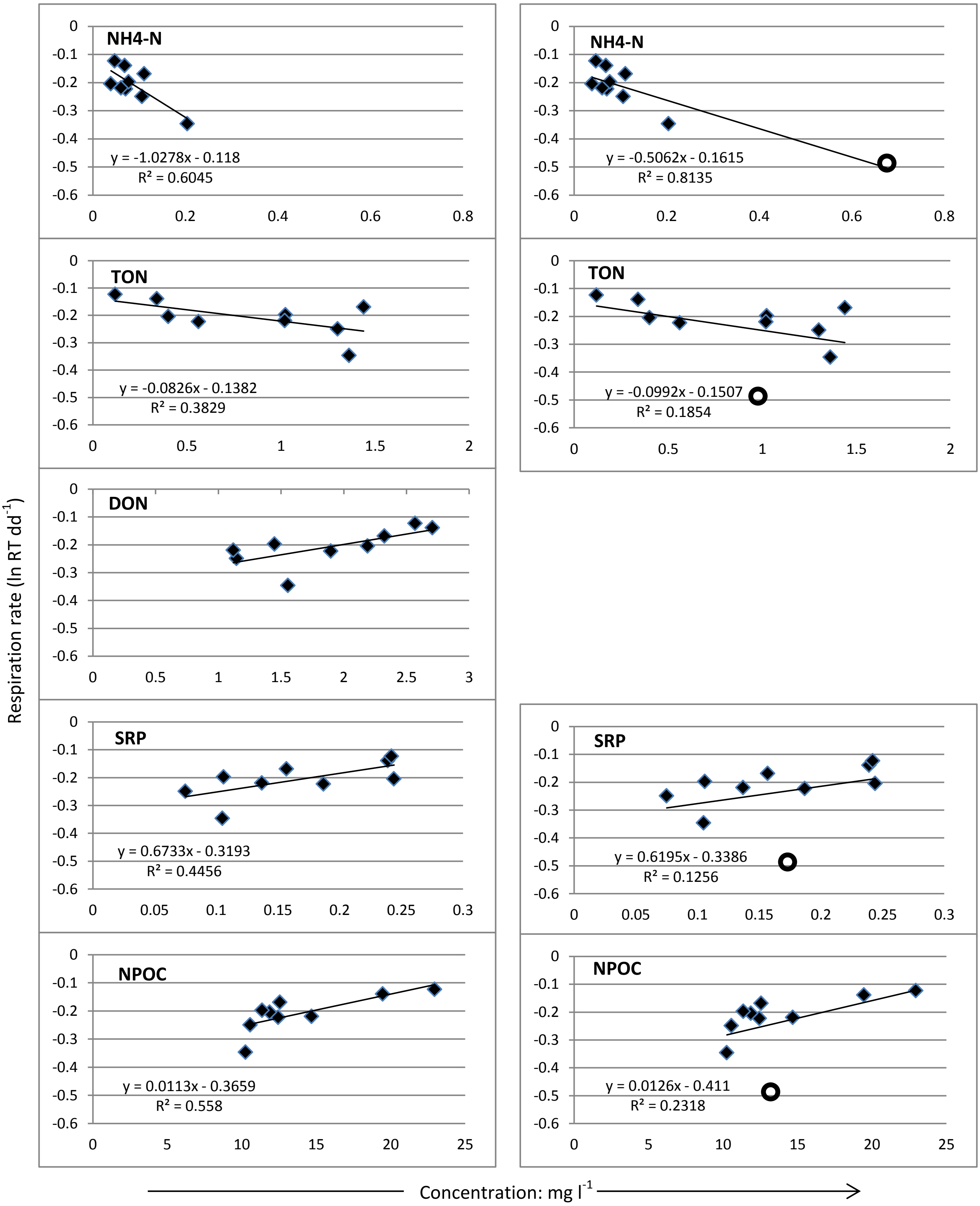


Figure 6:6 Significant relationships between ER and nutrient fractions at PFDS. Open circles denote 'slurry day' Left-hand panel shows the relationships during 'background conditions': Right hand panel shows the effect of including data from days where a pulse of high organic matter enters the reach.

At Priors Farm, the most dominant relationship was a strong and highly significant ($P<0.001$) negative correlation between aerobic respiration and NH_4-N. Positive correlations with aerobic respiration were returned for both DON and DOC. In order to examine the relationships between nutrients and aerobic respiration at Priors Farm in the absence of a plug of slurry, relationships were established with and without the values returned during this event. With the exception of NH_4-N, removal of this outlier did not change the nature of the relationships (given by the slope), but did increase their significance (Figure 6:6).

The high significance attached to the influence of DOC to aerobic respiration was surprising as there were substantial reserves of organic matter (3 – 10% w/w) stored in the sediments at both sites (chapter 3). Although the sediment community was responsible for ~90% of aerobic respiration, the relationship with DOC suggested that, most of this activity is at the sediment-water interface, fuelled by inputs of DOC. Organic carbon (OC) stored in the sediment may have been unavailable for aerobic respiration, with the oxidised zone restricted to a thin layer near the sediment-water interface. An increase in DOC may lead to increased activity in the water column if there is a community present that is able to exploit this resource. An examination of the influence of DOC on the relative contribution of the water column to ER (Figure 6:7) showed that at Priors Farm, even without the slurry input, the percentage of respiration attributable to the water column was influenced by DOC. This suggested that a microbial population able to respond rapidly to inputs of DOC was present in the seston in the Priors Farm reach. A much smaller range of DOC concentration was observed at Cool's Cottage, and there was no relationship between DOC and the contribution of the water column to aerobic respiration (Figure 6:7b). Observations using fluorescence microscopy revealed the presence of colloidal matter in samples from the

Priors Farm reach that was not seen in samples from the Cool's Cottage reach (data not presented). This flocculated matter provides an ideal substrate for water column microbial communities. The increase in the proportion of aerobic respiration contributed by the water column in the Priors Farm reach appears to have been exaggerated by inputs of slurry.

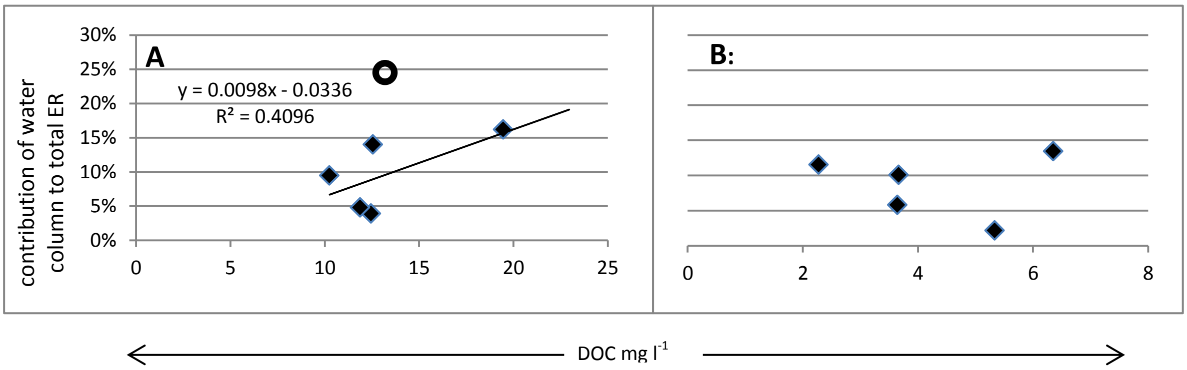


Figure 6:7 The influence of DOC on the contribution of the water column to whole stream respiration.
A: Priors Farm, B: Cool's Cottage

A further illustration of the effect of high DOC on aerobic respiration could be seen from the changes in daytime water column DO concentrations at different sites along the study reach (Figure 6:8). The concentration of DOC in the Priors Farm reach was highly variable with several pulses of high organic matter being recorded throughout the study period (chapter 5). A pulse of high DOC (98.89 mg l^{-1}) was recorded in the tributary PF1 in October 2013 (location shown in Figure 6:1). Dilution by the other tributaries, PF1 and PF2 reduced the concentration of DOC at PFUS (the next sampling point downstream) to 39.12 mgl^{-1}. Despite this substantially lower concentration of organic carbon at PFUS, anoxic conditions were observed at both sites. Under these conditions, the system appeared to be saturated with DOC that cannot be fully processed. Aerobic respiration appeared unable to increase further in response to such an elevated DOC concentration due to the lack of oxygen, with the concentration of DOC further downstream at PFDS unchanged at 39.11 mg l^{-1}. The delay in recovery at PF1 over the following week compared to that at PFUS (Figure 6:8) suggested significant 'fallout' of organic matter continued to swamp the microbial community at this site. A less severe incident occurred in February 2014 (DOC concentration at PF1 22.1 mg l^{-1}) which resulted in much smaller reduction in DO concentration and a faster recovery (Figure 6:8). In this incident, the reduced drop in DO at PFUS, relative to that at PF1, suggested that the system was not saturated with DOC at this level and could respond with higher respiration rates in response to more moderate incidents. In the neighbouring tributary, PF3, no such extreme and discrete pulses were recorded and the DO concentration remained high throughout. These data support the hypothesis of a direct relationship between input of extreme DOC concentration and increased water column contribution to whole stream respiration.

At Cool's Cottage, the response of aerobic respiration to DOC was more sensitive than that at the Priors Farm downstream site (Figure 6:9) possibly indicating a higher reliance on DOC throughout the study period. The dependence of aerobic respiration on DOC may be linked to the quality, rather than quantity, of sediment organic matter. Observations made on site suggest that, at Cool's Cottage, organic matter in the sediment was predominantly of vegetative origin (sticks, bark and leaves). Such organic matter tends to be more recalcitrant than the animal wastes that dominate the sediment organic matter in the Priors farm reach and it is likely that the particulate organic matter in the Cool's Cottage reach would require additional, extracellular, enzymatic breakdown before being available for respiration.

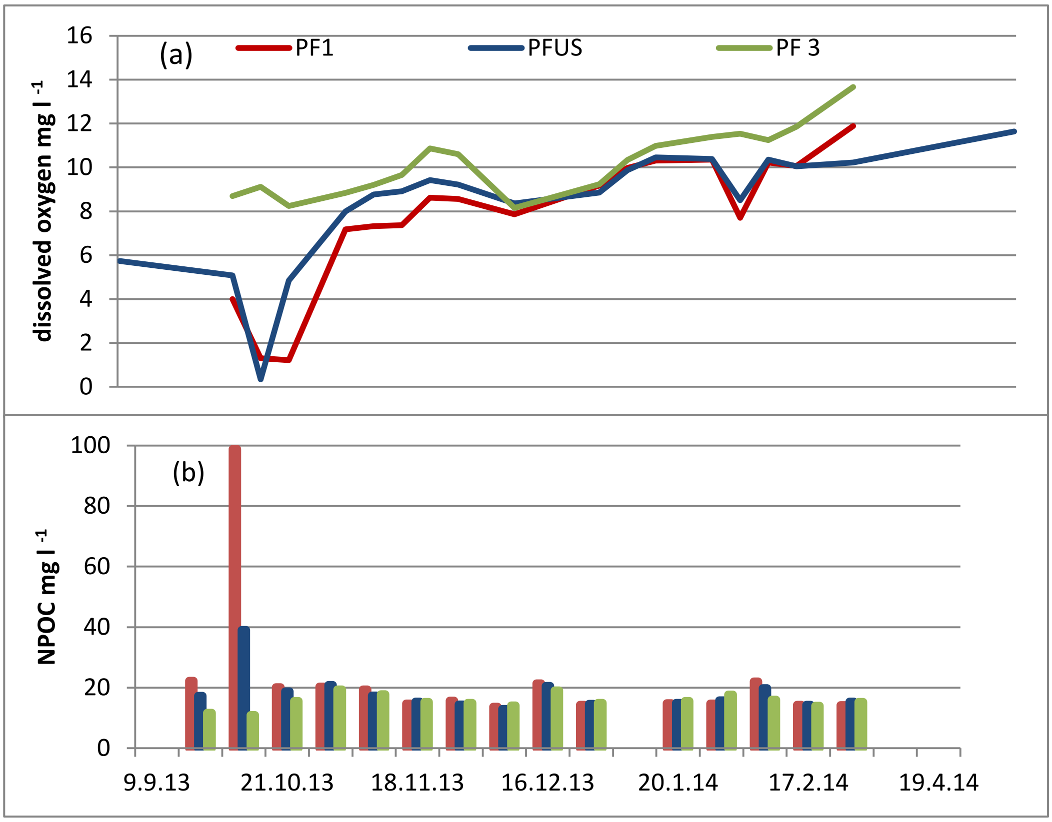


Figure 6:8 The relationship between DOC and dissolved oxygen during a pollution incident: (a) dissolved oxygen concentrations at three sites in the Priors Farm sub-catchment (See Figure 6.1. for locations);
(b) corresponding DOC concentrations.

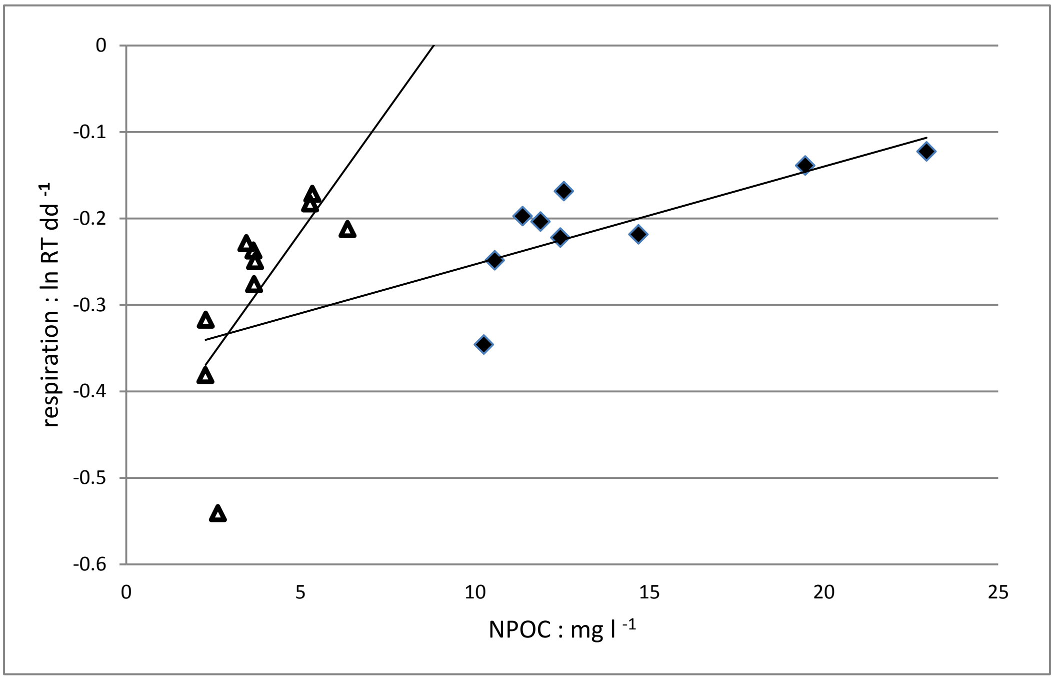


Figure 6:9 A comparison of the relationship between respiration and DOC at Cool's Cottage (open symbols) and Priors Farm (closed symbols).

6.2.1.3. Nutrient Transformations in closed benthic incubation chambers

Studying correlations between aerobic respiration and nutrient fractions can reveal significant relationships, but does not distinguish cause and effect. To investigate these further, transformations between nutrient fractions during incubations were observed in summer 2013 and spring and early summer 2014. These dates did not coincide with the slurry pulse and are, therefore, more representative of background conditions.

Nitrogen:

A striking observation was the similarity of the nitrogen transformations between the sites. At all sites TON was consumed during all the incubations (Figure 1.10). Similarly NH_4-N increased at all sites and during all incubations. The magnitude of change was always greatest in the Priors Farm reach. (Figure 6:10). At both downstream sites, DON was produced in early spring (April 2014), but was either consumed or showed no change during the late spring and early summer (May & June 2014). At the Priors Farm upstream site, DON increased in all incubations. Initial concentrations of both TON and DON were close to or above 1 mg l^{-1} (Table 6:4), suggesting that neither species was limiting.

Phosphorus:

In contrast to the behaviour of the nitrogen species, the sites exhibited differences in phosphorus fraction dynamics. The increase in SRP concentration in the spring and early summer at the Priors Farm upstream site and at Cool’s Cottage aligned with the increase in DOC concentrations, suggested that the remineralisation of organic matter was a key process. Such mobilisation of stored phosphorus may limit, or delay, the success of any mitigation of phosphate inputs from the catchment. A similar effect may be the cause of the limited success of phosphate reduction in achieving reduced primary production, or the resumption of ‘normal’ ecosystem function, following SRP stripping at waste water treatment works (WWTW) (Neal et al., 2000; Neal et al., 2010; Comber et al., 2012; Yates and Johnes, 2013). At the Priors Farm downstream site the opposite pattern was seen, with net SRP consumption occurring during all incubations.

DOC:

DOC concentrations rose consistently over the course of the incubations, with the single exception of the Priors Farm downstream site in June 2014. This finding supports the hypothesis that remineralisation of sediment organic matter contributed to community respiration.

Overall, combining information on nutrient transformations with initial nutrient concentrations aided the interpretation of the relationships between aerobic respiration and nutrient concentrations reported in Table 6:3. Where the initial concentration was high (Table 6:4), any relationship was unlikely to be a response to limitation. Rather, increased aerobic respiration was revealed as a possible cause (not effect) of such changes in concentration (for example, the consumption of TON at all sites, Figure 6:10). At Cool’s Cottage, on the other hand, the increase in SRP and DOC suggested that remineralisation supported community metabolism at this site.

Table 6:4 In-stream nutrient concentrations at the start of incubations used to study nutrient dynamics.

date	respiration	Cools Cottage						respiration	Priors Farm					
	(mg O^2 cm^{-1} d^{-1})	NH4 - N	TON	DON	PO4 - P	SUP	DOC	(mg O^2 cm^{-1} d^{-1})	NH4 - N	TON	DON	PO4 - P	SUP	DOC
21.7.13	0.0223	0.01	2.22	1.78	0.011	0.064	5.3	0.0702	0.047	0.12	2.57	0.242	0.081	22.9
31.3.14	0.0203	0.039	5.38	0.83	0.049	0.136	2.3	0.0517	0.106	1.30	1.14	0.075	0.143	10.6
19.5.14	0.0276	0.08	4.48	0.78	0.005	0.046	3.5	0.0420	0.077	1.02	1.45	0.106	0.056	11.4
2.6.14	0.0283	0.069	4.01	0.48	0.055	0.023	3.7	0.0483	0.061	1.02	1.12	0.137	0.021	14.7

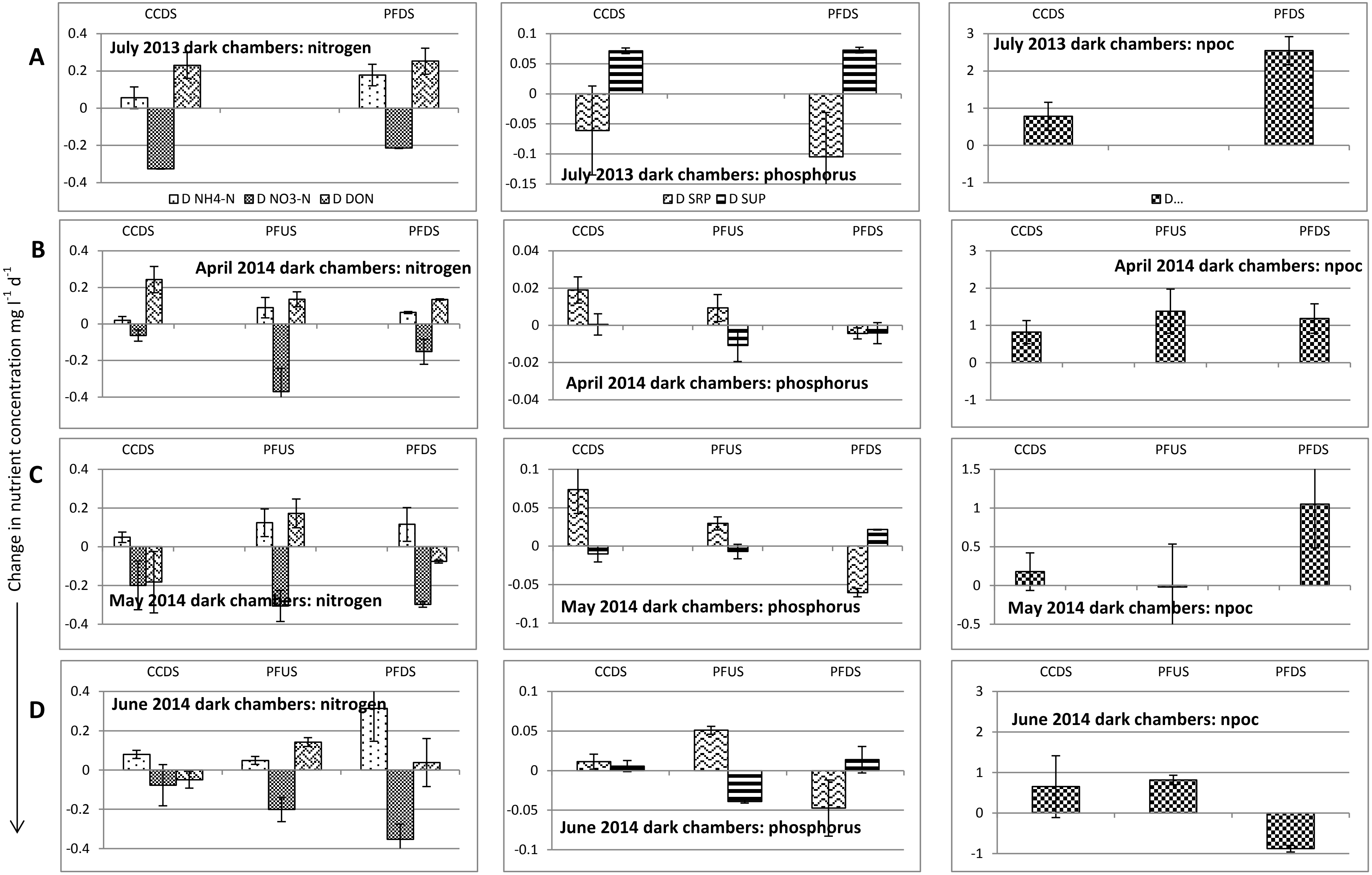


Figure 6:10 Dissolved nutrient transformations during 24 hr dark benthic chamber incubations, A: July 2013, B: April 2014, C: May 2014, D: June 2014

6.2.1.4. Available phosphorus as the limiting factor in the Cool's Cottage reach

Temporal variation in SRP concentration in the Cool's Cottage reach during the spring, before the development of a closed canopy (see chapter 5), suggested a demand for SRP that outstripped supply. Nutrient transformations within the incubation chambers indicated that this may have stimulated the remineralisation of SRP from other phosphorus fractions. SRP concentrations in the light incubation chambers (data not shown) did not increase in line with those of the dark incubations, indicating the additional demand for this resource from autotrophs. The study of system kinetics provided further evidence of possible phosphorus limitation.

A wide range of values for the E_a of phosphatases have been reported in the literature, but a recent review by (Hui et al., 2013) suggested an average value of around 0.34eV mol^{-1} for both acid and alkaline phosphatases. Unlike the respiration complex, however, phosphatases act outside the protective environment of the cell and may not, therefore, be acting at optimum pH (Antibus et al., 1986). The optimum pH for acid phosphatase is ~ 4 and for the alkaline phosphatase, ~ 9 (Hui et al., 2013; Bae and Barton, 1989). The pH at Cool's Cottage was consistently around 7.8, which is sub-optimal for both enzyme groups, and likely to cause an increase in apparent E_a. Nevertheless, the sum of the average E_a for phosphatases given by Hui (2013) and the E_a of the respiration complex approached that observed at the Cool's Cottage downstream site.

The evaluation of E_a, has been shown to be a useful tool in the study of ecosystem dynamics. Sinsabaugh and Shah (2010) used a similar approach in a more sophisticated and detailed set of experiments. They studied the E_a for a range of extracellular enzymes and were able to identify changes in dominant resources throughout the annual cycle. Their results supported the findings of (Allen and Gillooly, 2009) that the balance of resources (i.e. stoichiometry) can be as important as temperature in determining rates of metabolic processes.

6.2.2. **Aerobic respiration as an indicator of stream health.**

Values for in-stream community aerobic respiration reported in the literature range from 0.0 to 37.4 g O^2 m^{-2} day^{-1}. This wide range reflects the variety of methodologies employed (in situ benthic chambers and disturbed sediment samples; one and two point, open channel diel modelling; laboratory assays), stream order (1 – 7), temperature (4 – 25^{o}C), catchment area (2- 15000 ha) and land use (urban to forest).

The majority of reported values for in-stream community aerobic respiration using open channel methods lie within the range 0.0 – 16 g O^2 cm^{-2} day^{-1} (McTammany et al., 2003; Fellows et al., 2006a; Fellows et al., 2006b; Uehlinger, 2006; Roberts et al., 2007; Young and Collier, 2009; Young et al., 2008) and others) while values as low as 0.6 – 2.1 g O^2 m^{-2} day^{-1} (Bott et al., 1985) and 0.1 – 0.8 g O^2 m^{-2} day^{-1} (Hedin, 1990b; Hedin, 1990a) have been reported by researchers using closed chamber incubation methods. Higher values of up to 37 g O_2 m^{-2} day^{-1} have been reported by Clapcott et al. (2010) although the methods of determining ER were not reported.

For *in situ* measurements of in-stream metabolism, the chamber incubation method used in this study has been more recently superseded by variations of the open channel oxygen concentration method based on the techniques developed by Odum (1956) and Kosinski (1984). Direct comparisons of ecosystem respiration measured using these two techniques report discrepancies (Bott et al., 1978; Kosinski, 1984; Marzolf and Mulholland, 1994;

Marzolf et al., 1994; Webster et al., 1995; Chen et al., 1998a; Chen et al., 1998b; Hopkinson and Smith, 2005; Fellows et al., 2006b), with the chamber method consistently returning values of around 0.3 times the values derived from open channel methods. Both methods have their sources of uncertainty. The closed chamber method is likely to lead to an underestimate through a combination of potential resource limitation and the removal of laminar flow that tends to increase the width of the 'boundary layer' at the sediment-water interface and restricts the rate of diffusion of oxygen and nutrients across this layer. Marzolf et al. (1994) calculated that community respiration estimated using open channel methods can be up to 300% greater than that estimated using chamber incubations.

The open channel method is also subject to considerable uncertainties. Small errors in the measurement of temperature, DO and atmospheric pressure can significantly alter the calculation of the oxygen deficit and re-aeration rate, which are key to determining respiration. A combination of low ΔO_2 (less than 5 mg l^{-1} d^{-1}) and medium to high re-aeration rates (determined by temperature, atmospheric pressure, flow velocity and turbulence), can result in overestimates of up to 300% (Chen et al., 1998a; Chen et al., 1998b). Such conditions are typical of those found in the Cool's Cottage reach, although ΔO_2 in the Priors Farm reach was considerably higher. Discrepancies of a similar order of magnitude have also been reported in studies of estuarine community respiration where the use of open water diel changes in O_2 to measure respiration has been established over a longer time period (Hopkinson and Smith, 2005). A further, significant source of error has been identified by Demars et al. (2011): one of the central assumptions of open channel methods (one and two point diel measurements) is in-stream homogeneity, a condition unlikely to be true for many streams and demonstrably not so in the Priors Farm reach (chapter 5).

Other measurements of ER have relied on *ex situ* measurements eg. (AcuÑA et al., 2008; Hill et al., 1998) that are more akin to measurements of potential ER. These techniques necessarily disrupt the sediment structure that is shown to be an important determinant for the depth of development of anoxic conditions. In their comparison of methods, Wilson and Dodds (2009) demonstrated that measurements made using sediments where structure was artificially altered returned higher estimates of ΔO_2 than *in situ*, measurements on minimally disturbed sediment.

The validity of these discrepancies between methods is called into question by the relatively few examples of direct comparison of community respiration using the contrasting methodologies (but see Bott, 1978; Fellows et al. 2006). Both Webster et al., (1995) and Hopkinson and Smith (2005) acknowledge that their comparisons represent composite data, collated from different sites using the differing methodologies. Indeed, Webster et al. (1995) stress that the higher estimates of community respiration recorded using the open channel method are concentrated on higher order streams. Nevertheless, overall, the weight of evidence indicates that closed chamber incubations return values of community respiration approximately 30% of those measured using the open channel methods.

With this in mind, and in order to place the results from the present study in context with other research, 'method corrected' aerobic respiration in the Cool's Cottage and Priors Farm sub-catchments was estimated (at three times the observed values) as 0.3 – 1.2 and 0.6 to 2.1 g O_2 m^{-2} d^{-1} respectively. These are at the low end of the range reported in the literature and. Using the framework for assessing stream health using aerobic respiration suggested by Young et al. (2008), these values indicated mild to severe impacts on both sub-catchments. In addition (as noted above), the incubation dates did not coincide with the periodic pulses of high organic matter that were a feature of the Priors Farm reach. Thus these estimates are likely to represent minimum rates for this river. Crucially, the same methodology was used throughout the study and for all sites, giving confidence in the validity of comparisons between the two study sub-catchments, but see further discussions in Chapter 7.

The results presented here provide evidence that studies of aerobic respiration can be a powerful tool for monitoring variability in stream health. When combined with measurements of temperature, in-stream chemistry and nutrient dynamics, this study shows that estimates of aerobic respiration can be sufficiently sensitive to distinguish differences between streams in paired sub-catchments.

The measurement of aerobic respiration, analysis of the kinetics of respiration and observations of nutrient transformations during incubations provided strong evidence of a clear distinction between the ecosystem functioning of the two streams. The apparently low values of community aerobic respiration in both sub-catchments are likely to be influenced by the fine sediment load resulting in poor aeration of the stream bed. In the Priors Farm reach this is further compounded by high concentrations of organic carbon and low or restricted flows. Further processing of organic carbon in the sediment, therefore, is likely to be a function of anaerobic processes. The transfer of greenhouse gases across the sediment-water-interface in the two study reaches was compared, to investigate if this additional metric provides a further key to assessing ecosystem function (section 6.4).

6.3. Primary production.

Excessive algal growth is one of the most visible consequences of nutrient enrichment. In many freshwater and coastal ecosystems, however, heterotrophic production is not limited by *in situ* primary production resulting from organic subsidies due to the inputs of organic matter from external (allochthonous) sources, both natural and anthropogenic. The extent of this decoupling of primary production and respiration has been proposed as an index of stream health, with the ratio of primary production to respiration used as a measure of functional integrity (Young et al., 2008).

To characterise the study reaches in these terms, whole stream and water column net ecosystem production (NEP) were measured in clear incubation chambers and bottles installed alongside the respiration incubations. Gross primary production (GPP) was calculated from the difference between NEP and aerobic respiration measured in the dark incubations (Chapter 4).

6.3.1. **Comparing the drivers of primary production**

6.3.1.1. Contribution of the water column

Despite extending the water bottle incubations to 48 hrs, water column GPP was too low to measure on most dates at the Priors Farm upstream site and at Cool's Cottage, returning small positive or negative values for the majority of incubations (Figure 6:11). At the Priors Farm downstream site the water column contributed only 4% to whole stream GPP. The following discussions focus on the measurement of whole stream GPP, measured in the benthic incubation chambers.

6.3.1.2. Whole Stream ecosystem and primary production

Temporal changes in GPP followed a similar pattern to those of respiration (Figure 6:11). As predicted, GPP at the Priors Farm downstream site was consistently higher than at the other two sites, emphasising the effect of riparian shading on this core metabolic function. At the Priors Farm downstream site, NEP was positive and GPP/R > 1 during the late spring and summer seasons, indicating a system supported by autotrophy, dropping only in March. Incubations were not carried out during the winter. By contrast, at the two shaded sites, NEP was always negative and GPP/R always < 1.(Figure 6:11).

6.3.2. Primary production as an indicator of stream health.

Observations of the rate of photosynthesis alone do not provide a useful measure of the influence of biogeochemical pressures on stream health as a consequence of its dependence on light availability. Variation in GPP within the Priors Farm reach was much greater than that between the two shaded sites in different sub-catchments (Cool's Cottage and the Priors Farm upstream site). However, GPP/R did give an indication that the Priors farm upstream site had greater access to alternative resources, in that GPP/R at this site was lower than that at Cool's Cottage (median 0.244 and 0.377 respectively; Table 6:5). In general, while some important information regarding the metabolism of the streams could be gathered from measuring GPP and studying its relationship to respiration, its usefulness as an index of ecosystem health was limited. The constraints of stream topography and riparian vegetation, and thus light availability, on GPP prevented a direct comparison between reaches and sub-catchments in the context of contrasting water quality.

Table 6:5 Comparison of summary data for the indicators of ecosystem function, GPP and P/R, between July 2013 and June 2014 in the two study reaches. GPP mg O_2 m^{-2} d^{-1}.

Cool's Cottage					**Priors Farm upstream**				**Priors Farm downstream**			
	med	**mean**	**min**	**max**	**med**	**mean**	**min**	**max**	**med**	**mean**	**min**	**max**
GPP	0.013	0.014	-0.002	0.030	0.006	0.009	0.000	0.026	0.064	0.057	0.002	0.125
GPP/R	0.378	0.360	-0.075	0.761	0.244	0.292	0.015	0.753	1.153	1.066	0.066	2.009

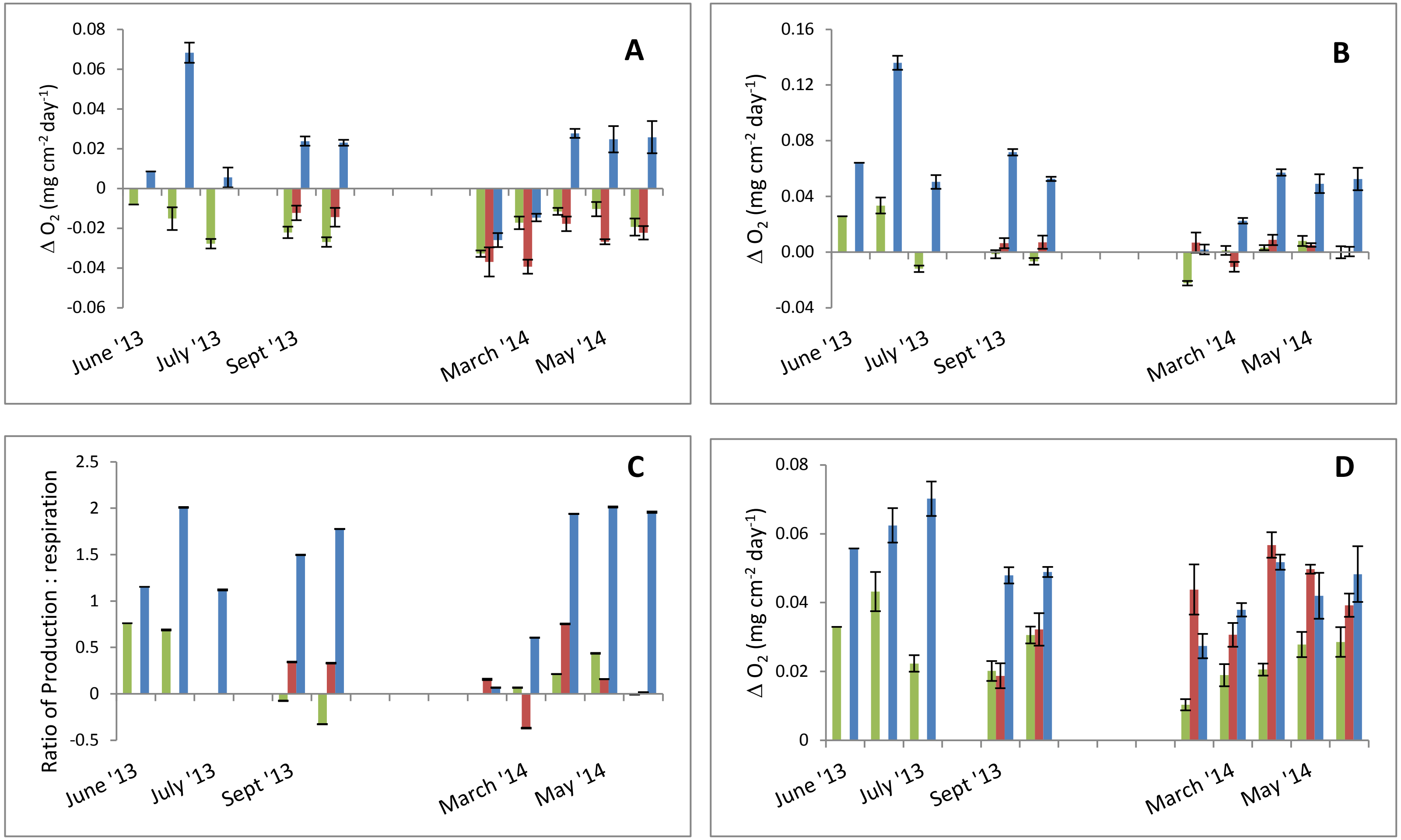


Figure 6:11 Temporal patterns in key aerobic metabolic processes: A, Net ecosystem production, (ΔO_2, mg cm^{-2} day^{-1}); B, Photosynthetic primary production , (ΔO_2, mg cm^{-2} day^{-1}); ; C, P/R; D, Community aerobic respiration (ΔO_2, mg cm^{-2} day^{-1}).
Green, Cool's Cottage Red Priors Farm upstream, Blue, Priors Farm downstream,

6.4. Anaerobic respiration and the production of greenhouse gases.

The deposition of large amounts of fine sediment can restrict the availability of dissolved oxygen and nutrients from the water column to the benthic community, significantly reducing aerobic processes within the river bed (Collins et al., 2013) and leading to the development of a shallow anoxic zone. In anoxic zones, energy is acquired by the means of alternative metabolic pathways using a wide range of terminal electron acceptors. Anaerobic processes are notoriously difficult to unravel, as they consist of the intricate and intertwined cycling of components in differing redox states. The emission of greenhouse gases as a result of these processes is of increasing concern Both methane (CH_4) and nitrous oxide (N_2O) have high greenhouse warming potential (GWP) when compared to carbon dioxide ($CH_4 = 28 \times CO_2$ and; $N_2O = 265 \times CO_2$) and their production is highly sensitive to temperature (Yvon-Durocher et al., 2011), resulting in a detrimental positive feedback loop.

Determination of the rates of true denitrification, methanogenesis, fermentation or indeed anaerobic respiration by alternative pathways, was outside the scope of this study and were not measured. Rather, the focus of this work was to measure the transfer of greenhouse gases across the sediment-water interface, and the potential to use this metric as a means of monitoring changes in ecosystem health. To this end, three sites were chosen to investigate whether the sub-catchments exhibited differences in the production of greenhouse gases and whether in-stream chemistry or physical factors were the dominant drivers of any dissimilarities observed.

6.4.1. **Nitrous Oxide**

Of the alternative modes of respiration, the reduction of nitrate by denitrification is the next most efficient to the reduction of oxygen (although with a poorer energy yield than would be expected from the redox potential of the NO_3^-/N_2 redox pair (King, 2005). Denitrification is an important pathway in the processing and removal of nitrogen from aquatic systems. Global rates of denitrification are estimated to represent a significant proportion of respiration with a ratio of 0.07 – 0.09 moles of N_2-N produced per mole of O_2 consumed (Pina-Ochoa and Alvarez-Cobelas, 2006; Trimmer et al., 2012).

In-stream denitrification is carried out by facultative anaerobic bacteria, accentuating the adaptability of the microbial community and its potential to utilise any available resource. Only in anoxic conditions and in the presence of NO_3^- do the membrane bound sites of denitrification become active, reducing non-essential energy consumption and optimising growth efficiency. NO_3^- is first reduced to NO_2 followed by NO, N_2O and finally, in some cases, N_2.

As a result, some attention has been paid to the potential for enhanced denitrification as a means of reducing the delivery of NO_3^- to watercourses via runoff from agricultural land (Ernfors et al., 2012; Ledgard et al., 2012; Ruser et al., 2012). The denitrification process however, is sometimes ‘incomplete’ with an, as yet, unpredictable proportion of NO_3^- being converted to N_2O, a potent greenhouse gas with a warming potential of 265 times that of CO_2 Thus the proposed use of accelerated denitrification as a mitigation strategy is an example of pollution swapping, with attendant potential negative consequences. Additionally, denitrification has been shown to be highly sensitive to temperature (Bonnett et al., 2013), providing a strong positive feedback for the increase in warming potential from this source.

6.4.1.1. Nitrous oxide accumulation in benthic chambers

The atmospheric concentration of CH_4, N_2O and CO_2 were obtained from the global data-set presented for 2014 on the NOAA and AGAGE websites accessed 19.1.15 (Blasing, 2014). Across all sites, the background in-stream concentration of N_2O was between 100 and 250nM, i.e. supersaturated by 700 to 1800% with respect to the atmosphere. The surface water concentrations were high in comparison to other published data, [e.g. ~40nM, (Pretty et al., 2006), 7 – 36 nM (Hinshaw and Dahlgren, 2013)], and suggested that significant denitrification and N_2O transfer across the sediment water interface was occurring in both the Cool's Cottage and Priors Farm reaches.

There was no significant difference in N_2O accumulation across the sediment-water interface between light and dark chambers ($F = 7$, $P > 0.4$). Therefore, data from dark and light chambers were combined for further statistical analyses. There was no significant difference between rates of N_2O accumulation at the Priors Farm upstream and downstream sites ($F= 1.4$, $P > 0.2$). Rates of N_2O accumulation were significantly higher in the Cools Cottage reach than the Priors Farm reach, $F = 10$, $P < 0.005$) (Table 6:6). The interaction between site and date showed a significant temporal variation for both sites, $P < 0.001$, but there was no relationship with the overlying water temperature (Figure 6:12 and Figure 6:13).

Table 6:6 Summary data for rates of N_2O accumulation (µmol N_2O m^{-2} h^{-1}) in benthic chambers between July 2013 and June 2014 in the two study sub-catchments.

	Cool's Cottage				**Priors Farm upstream**				**Priors Farm downstream**			
µmol m^{-2} h^{-1}	med	mean	min	max	med	mean	min	max	med	mean	min	max
July	0.1292	0.1687	-0.0012	0.4175					-0.0326	-0.0206	-0.0822	0.0649
March	-0.0133	0.0701	-0.0885	0.5598	0.0431	0.0369	-0.0299	0.1046	0.0104	0.0006	-0.0771	0.0593
April	0.0995	0.1565	-0.0750	0.6156	-0.0900	-0.0512	-0.1516	0.0680	-0.0001	-0.0070	-0.0675	0.0459
May	0.1988	0.2072	0.1487	0.2806	0.0681	0.0570	-0.0323	0.1070	0.0641	0.0628	-0.0365	0.1461
June	0.0102	0.1055	-0.0394	0.6322	0.0538	0.0539	-0.0203	0.1117	0.0244	0.0261	-0.0303	0.1107
annual	0.0552	0.1397	-0.0885	0.6322	0.0507	0.0261	-0.1516	0.1117	0.0197	0.0147	-0.0822	0.1461

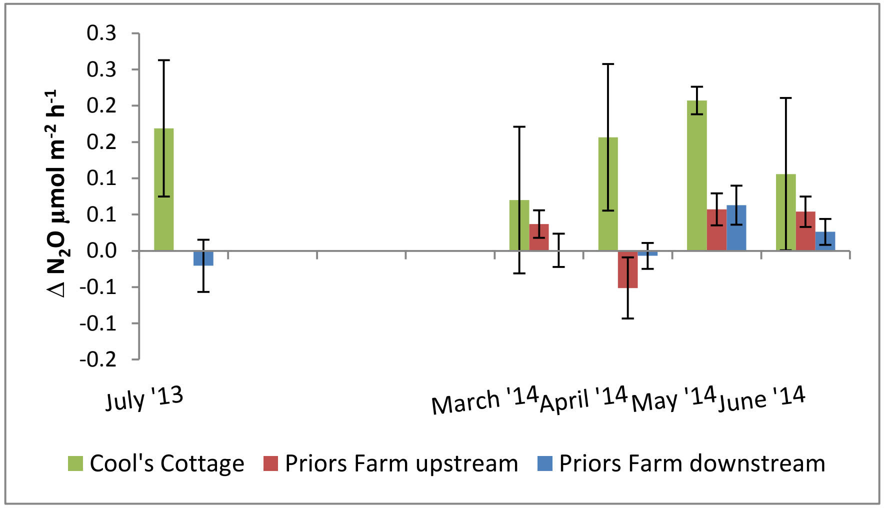


Figure 6:12 Temporal patterns in N_2O accumulation (µmol m^{-1} h^{-1}) at the three study sites

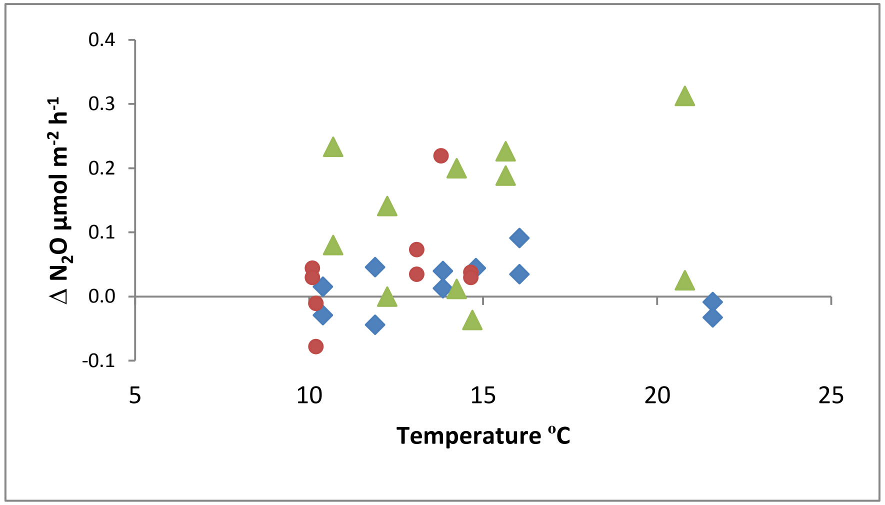


Figure 6:13 Relationship between N_2O accumulation and temperature at the three study sites; no significant relationship was observed

6.4.2. **Methane**

Methane production is one of the least efficient of the anaerobic pathways for respiration The electrode potential (a measure of the potential for energy production) of the CO_2 / CH_4 redox pair is -0.24V, compared with +0.82V and +0.75V for the O_2/H_2O and NO_3^-/N_2 redox pairs respectively (King, 2005). Methanogens are obligate anaerobes resulting in their spatial separation from sites of aerobic CO_2 production. Despite being the least thermodynamically favourable of the anaerobic respiratory pathways, methanogenesis benefits from a ready supply of raw materials (CO_2 and H) produced *in situ*, from other metabolic processes such as denitrification, fermentation and sulphate reduction. As a result, methanogenesis is not as limited by the diffusion of substrates through the compacted sediment from the overlying water as are aerobic respiration and denitrification. Thus methanogens are able to exploit reserves of benthic organic matter that are unavailable to other anaerobes after the exhaustion of more efficient alternative electron acceptors.

6.4.2.1. Methane accumulation in benthic chambers

The atmospheric concentration of CH_4, was obtained from the global data- set presented for 2014 on the NOAA and AGAGE websites accessed 19.1.15 (Blasing, 2014). The background in-stream concentration of CH_4 varied between the three sites. Concentrations ranged from 85nM at Cool's Cottage in March/April 2014 (approximately in equilibrium with the atmosphere) to 3µM at the Priors Farm downstream site in July 2013 (supersaturated in relation to the atmosphere) when there was low flow and the daytime temperature reached 21.6°C. Concentrations were always lowest at Cool's Cottage.

There was no significant difference in CH_4 accumulation across the sediment-water interface between light and dark chambers ($F = 0.2$, $P > 0.6$). Therefore, data from dark and light chambers were combined for further statistical analyses. Average rates of CH_4 accumulation in the benthic chambers were significantly higher at the Priors Farm downstream site ($F = 33$, $P < 0.0001$), outstripping the other two sites by between one and two orders of magnitude (Table 6:7). There was no significant difference between rates of CH_4 accumulation at the Priors Farm upstream site and at Cool's Cottage ($F= 0.98$, $P > 0.3$). The interaction between site and date showed a significant temporal variation for the Priors farm downstream site, $F=5.08$, $P < 0.001$, but not at Cool's Cottage or the Priors farm upstream site($F= 1.96$, $P > 0.1$) (Figure 6:14). There was no relationship with the overlying water temperature (Figure 6:15).

The maximum recorded rate of accumulation in a single incubation chamber, 150 $\mu g\ m^{-2}\ h^{-1}$, was recorded at the Priors Farm downstream site in June 2014 This figure approached that emitted from organically rich histosols (Bonnett et al., 2013) and far exceeds that found escaping from the sediments in a chalk stream (Trimmer et al., 2010). At the other two sites CH_4 emissions were closer to those observed from other river sediments and lower than observed from flooded intact soil cores.

6.4.2.2. Key drivers of methane transfer.

Anaerobic fermentation of organic material produces partially reduced, small organic compounds that are further reduced to methane by methanogens. The high photosynthetic activity and productivity at PFDS resulted in a ready supply of fresh organic substrate, in the form of decaying algal biomass, over and above that which was imported from the catchment. This ready source of carbon, along with high amounts of fine sediments reducing oxygen penetration into the river bed, is likely to have resulted in the high rate of methane accumulation observed in the incubation chambers at this site. The lower *in situ* productivity at Cool's Cottage and the Priors Farm upstream site, together with the lower DOC concentration at Cool's Cottage, may have contributed to the lower rates of CH_4 accumulation at these sites. Nevertheless, there are other factors that may have influenced the transfer of CH_4 across the sediment/water interface. Daytime water temperatures during the summer were approximately 2 °C warmer at the Priors Farm downstream site when compared to the upstream site. However, while CH_4 production has been shown to be temperature dependent (Bonnett et al., 2013), the difference in temperature cannot fully explain the large differences in CH_4 accumulation observed between these sites. Other likely contributory factors include sediment structure and substrate availability. Although, as far as possible, sites were chosen to maximise the similarity in sediment characteristics, size fractionation of the sediment samples collected from the incubation chambers following deployment revealed a lower proportion of fine sediments in the cores from the Priors Farm downstream site when compared to the upstream site (Chapter 3). The resultant difference in hydraulic conductivity may explain some of the difference between the two sites in the Priors farm reach. Additionally, the higher light levels at the downstream site may have promoted macrophyte growth, which was absent at the other two sites. Macrophytes have been demonstrated to increase CH_4 emission by up to two orders of magnitude (Trimmer et al., 2010) by facilitating transport through their stems. While care was taken to avoid incorporating macrophytes in the incubation chambers, poor visibility (<2cm) and prolonged immersion in cold water reducing manual sensitivity made it possible that some subsurface roots were cut and incorporated within the incubation chambers at the Priors Farm downstream site.

Table 6:7 Summary data for rates of CH_4 accumulation (µmol CH_4 m^{-2} h^{-1}) in benthic chambers between July 2013 and June 2014 in the two study sub-catchments.

	Cool's Cottage				Priors Farm upstream				Priors farm downstream			
µmol m^{-2} h^{-1}	median	mean	min	max	median	mean	min	max	median	mean	min	max
July	0.2142	0.6786	0.0068	2.2791					43.5639	47.6127	35.2427	68.0803
March	0.1929	0.1754	0.0731	0.2550	0.0708	0.0839	-0.1381	0.3695	14.1086	22.2359	8.3798	57.6162
April	0.0459	0.5518	0.0098	3.1321	-0.0134	0.2480	-0.0733	1.0275	19.9008	29.6114	3.5969	68.6633
May	0.3409	1.4992	0.0506	7.0881	-0.0806	-0.0270	-0.1855	0.1727	27.9182	31.1110	9.2890	66.7544
June	2.2397	1.9681	0.3108	3.5860	0.0062	1.7183	-0.3956	9.6083	66.2207	83.4641	13.0442	149.7691
annual	0.2208	0.9957	0.0068	7.0881	-0.0120	0.5417	-0.3956	9.6083	37.4590	43.7671	3.5969	149.7691

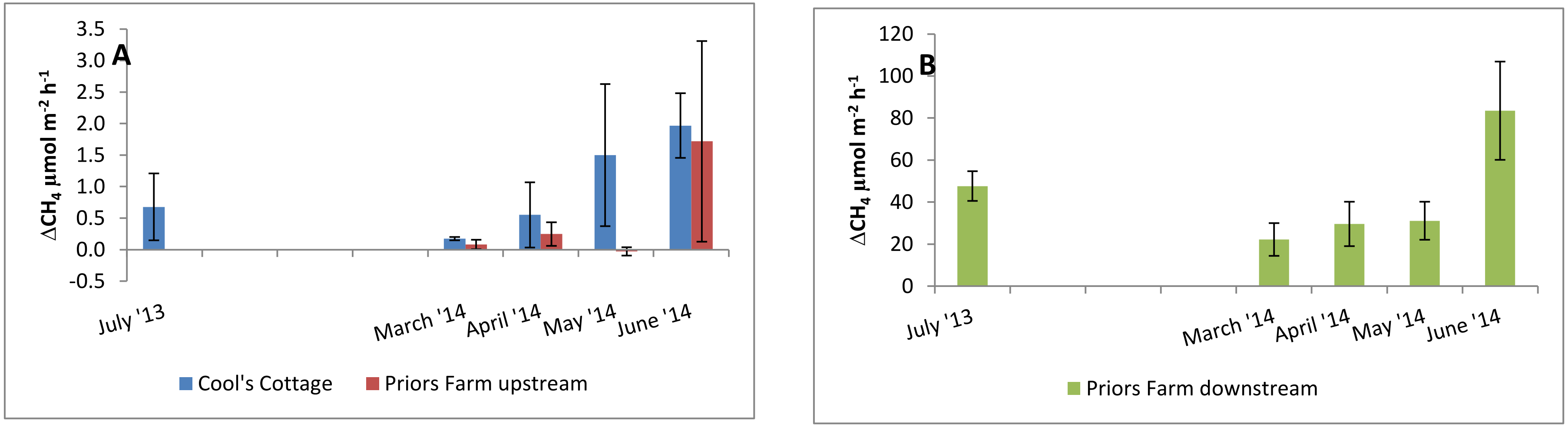


Figure 6:14 Figure 6:12 Temporal patterns in CH_4 accumulation (µmol m-1 h-1) at the three study sites, A, Cool's Cottage and Priors Farm upstream; B, Priors farm downstream.

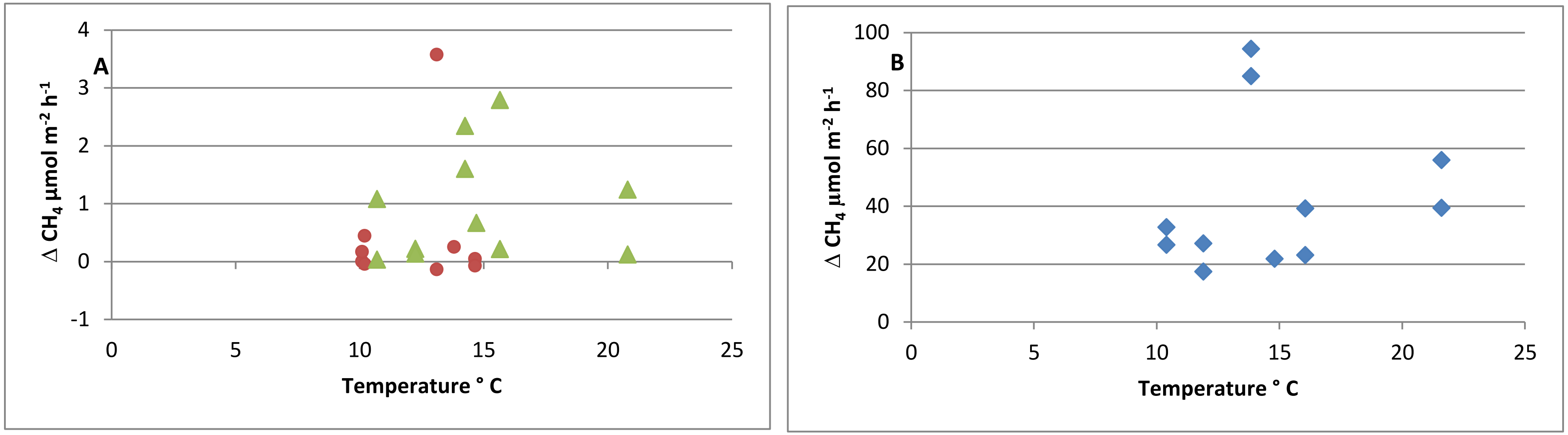


Figure 6:15 Relationship between rate of CH$_4$ (µmol m^{-2} h^{-1})accumulation and temperature, A: temperature relationship between CH$_4$ accumulation at Cool's Cottage and the Priors Farm upstream site; B: temperature relationship between CH4 accumulation and the Priors Farm downstream site: no significant relationship was observed

Table 6:8 Summary data for rates of CO_2 accumulation (µmol CO_2 m-2 h-1) in benthic chambers between July 2013 and June 2014 in the two study sub-catchments

	Cool's Cottage				Priors Farm upstream				Priors Farm downstream			
µmol m^{-2} h^{-1}	med	mean	min	max	med	mean	min	max	med	mean	min	max
July	245.91	243.46	211.01	271.01					-279.29	-286.53	-505.40	-82.14
March	299.75	330.93	142.79	734.54	108.41	156.15	-34.99	444.13	43.27	76.43	-8.17	205.28
April	113.18	87.89	-75.03	214.89	84.58	80.23	22.74	126.90	33.14	40.90	-20.91	115.26
May	32.54	-1.83	-339.18	245.38	29.99	26.90	-97.84	191.40	88.13	93.75	-137.87	368.50
June	29.29	11.87	-104.40	126.11	251.13	241.37	113.17	390.55	359.11	385.57	236.37	552.80
annual	134.45	126.68	-339.18	734.54	101.75	132.76	-97.84	444.13	68.95	88.52	-505.40	552.80

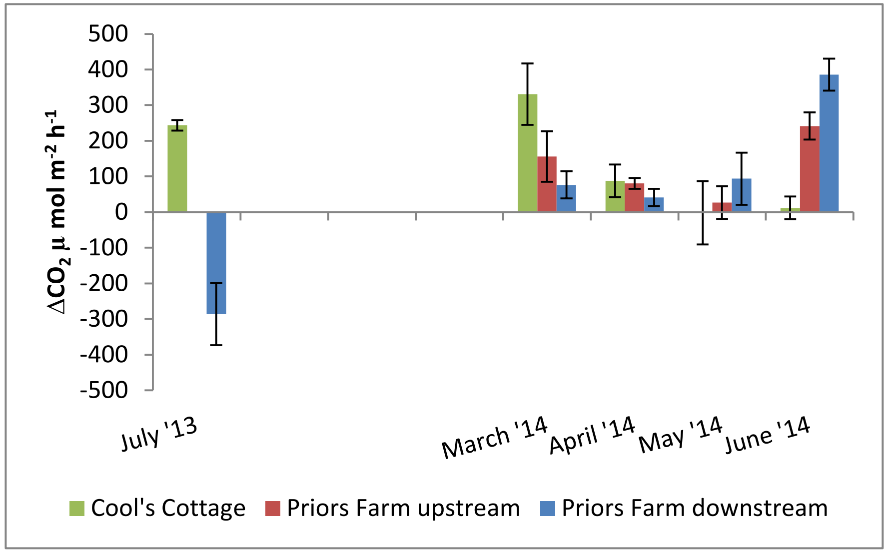


Figure 6:17 Temporal patterns in CO_2 accumulation (µmol m-1 h-1) at the three study sites

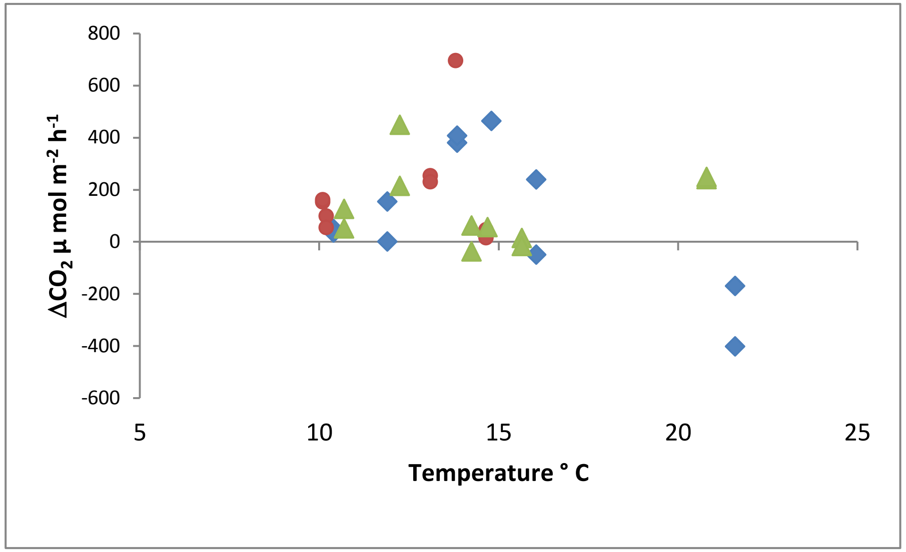


Figure 6:16 Relationship between CO_2 accumulation and temperature at the three study sites; no significant relationship was observed

6.4.3. **Carbon dioxide**

Carbon dioxide is the most ubiquitous of the greenhouse gases, in terms of its role in many of the anaerobic (and aerobic) respiratory pathways. It is produced by sulphate reducing bacteria, through fermentation, anaerobic and aerobic methane oxidation, photorespiration, and of course, by aerobic respiration. It is consumed during photosynthesis and by methanogens. Of these processes, only aerobic respiration and primary production have been truly quantified in this study. However, measurement of the accumulation of CO_2 in the incubation chambers may help in the interpretation of other processes. Additionally, the flux of CO_2 across the sediment water interface has a consequence for the generation of greenhouse gas and, therefore, was studied here in its own right, in an approach comparable to that used for both N_2O and CH_4 flux.

6.4.3.1. Carbon dioxide accumulation in benthic chambers

The atmospheric concentration of CO_2 was obtained from the global data- set presented for 2014 on the NOAA and AGAGE websites accessed 19.1.15 (Blasing, 2014). The background in-stream concentration of CO_2 varied little between the three sites the majority of measurements ranged from 300 to 900 µM (with the exception of the Priors Farm downstream site in July, 1300 µM). In-stream concentrations at all sites were super-saturated with respect to atmospheric concentrations by 1800% - 7500%.

Contrary to expectations, there was no significant difference in CO_2 accumulation across the sediment-water interface between light and dark chambers ($F = 1.3$, $P > 0.2$). Therefore, data from dark and light chambers were combined for further statistical analyses. There was no statistical difference in CO_2 accumulation between sites ($F = 0.4$, $P > 00.6$ (Table 6:**8**), echoing the results of Bonnet et al. (2013) who found no statistical difference in CO_2 accumulation between the soil types in their flooded cores.

Significant net CO_2 uptake was only observed in the benthic chambers at the Priors Farm downstream site in July (Figure 6:17). The maximum rate of transfer across the sediment water interface was 20 mg m^{-2} h^{-1}, lower, but in the same order of magnitude as that emitted from undisturbed wetland soil cores 70 – 150 mg m^{-2} h^{-1} (Bonnet et al., 2013). As a comparison, heterotrophic respiration as measured by O_2 uptake in the rivers, equated to the production of 11 – 45 mg m^{-2} h^{-1} CO_2. Combined light and dark rates of CO_2 accumulation exhibited no relationship with the overlying water temperature (Figure 6:16).

The respiratory quotient (RQ) is the ratio of moles CO_2 produced per mole O_2 consumed. It ranges from 1.3 for glycolic acid; through 1.0 for sugars, to 0.67 for fatty acids (Williams and del Giorgio, 2005). Using RQ values of 1.0 and 0.67 to cover a range of likely substrates, the accumulation of CO_2 (measured using headspace gas analysis) was compared to the calculated CO_2 produced from respiration (measured by O_2 consumption in dark chambers (Figure 6:18). This analysis revealed a CO_2 'deficit' for all incubations (apart from at Cool's Cottage in March) that could not be accounted for by the accumulation of CH_4 in the chambers.

6.4.3.2. The CO_2 'deficit': implications for ecosystem production.

The CO_2 'deficit' described above suggests a drawdown of CO_2 into the sediment that was not used for photosynthesis (oxygen uptake data for these calculations were from dark incubations only), and that may have been utilised for anaerobic reactions such as methanogenesis. As the data were corrected for the methane transferred across the sediment water interface, the deficit may represent transient storage of carbon as methane in compacted sediments (see section 6.4.1), or possibly, as longer term carbon sequestration via chemosynthetic pathways (e.g. methanotrophy) as has been recently described in chalk streams (Jones et al., 2008; Jones and Grey, 2011; Trimmer et al., 2009). Oxidation of methane, both aerobic and anaerobic is known to be significant in estuaries, lakes and the ocean floor. In 2006, Kankaala et al. demonstrated that, on an annual basis, around 80% of methane transported across the sediment-water interface of a stratified lake was removed by methane oxidation in the water column, with a maximum rate of 18mmol m^{-3} d^{-1} at the oxic/anoxic boundary. Further anaerobic oxidation of methane, either by sulphate reducing bacteria (at the expense of CO_2) or by nitrifiers (at the expense of NO_3^-) was likely to have been occurring in the anoxic zones of the sediments, with the potential for organic products being incorporated into biomass. Furthermore, methane derived carbon has been shown to make an important, albeit small, contribution to the food-chain, even in exposed, well lit, clear streams with high rates of primary productivity (Jones et al., 2008; Trimmer et al., 2009; Jones and Grey, 2011). In the shaded sites at Cool's Cottage and the Priors Farm upstream site, it is possible that this pathway was a more significant source of fixed carbon. The lower deficit at Cool's Cottage may be simply have been due to a combination of limited methanogenesis and *transient* storage, as a result of lower organic carbon resources. On the other hand, low rates of photosynthetic productivity, coupled with low dissolved organic carbon input from the catchment, may have increased the likelihood of methanotrophy contributing to the productivity of the reach. Methane oxidation at the expense of NO_3^- has the added benefit of removing excess NO_3^- where N:P ratios are unbalanced (Sterner and Elser, 2002; Baker et al., 2000; King, 2005). The NO_2 thus produced can be further reduced to N_2 via a synotrophic relationship with anaerobic ammonia oxidising bacteria (Haroon et al., 2013). Since methane oxidation produces CO_2, significant methane oxidation may be one explanation for the lower deficit at Cool's Cottage. This form of nitrate reduction does not contribute to the N_2O pool, and indeed may compete with that process, providing a possible, additional explanation for the apparently low rates of N_2O transfer across the sediment water interface at Cool's Cottage.

6.5. Metabolic metrics as indicators of change.

Indices designed for using metabolic processes as functional indicators of stream health have so far concentrated on aerobic processes (Webster and Meyer, 1997; Young and Collier, 2009; Young et al., 2008; Clapcott et al., 2010; Fellows et al., 2006a; Magbanua et al., 2010), despite an increasing awareness of the importance of the benthic and hyporheic zones for overall stream metabolism (Pina-Ochoa and Alvarez-Cobelas, 2006; Pretty et al., 2006; Sanders et al., 2007). The interest in anaerobic processes in rivers is accelerating (Pina-Ochoa and Alvarez-Cobelas, 2006; Sanders et al., 2007; Jones et al., 2008; Trimmer et al., 2010) as it is realised that environments previously thought of as oxic are impacted by inputs of fine sediment and organic matter (Collins et al., 2013; Pattison et al., 2014) making them anoxic zones.

The data presented here emphasise the importance of these alternative metabolic pathways and the contribution they make to the productivity of headwater streams. The results highlight the need to incorporate measures of these processes to fully describe ecological status. Given the long understood importance of benthic sediments for community respiration, and an increasing awareness of the widespread development of anoxic conditions, a greater knowledge of these anaerobic processes would represent a substantial step forward in our understanding of in-stream ecosystem function and the development of functional indices of stream health. The development of techniques to measure anaerobic processes using stable isotopes and whole-stream determination of nitrogen metabolism (analogous to the diel oxygen curves for photosynthesis and aerobic respiration) is gaining pace (Trimmer et al., 2012). More widespread use of these methods could greatly enhance the assessment of the impairment of stream functional integrity in impacted streams.

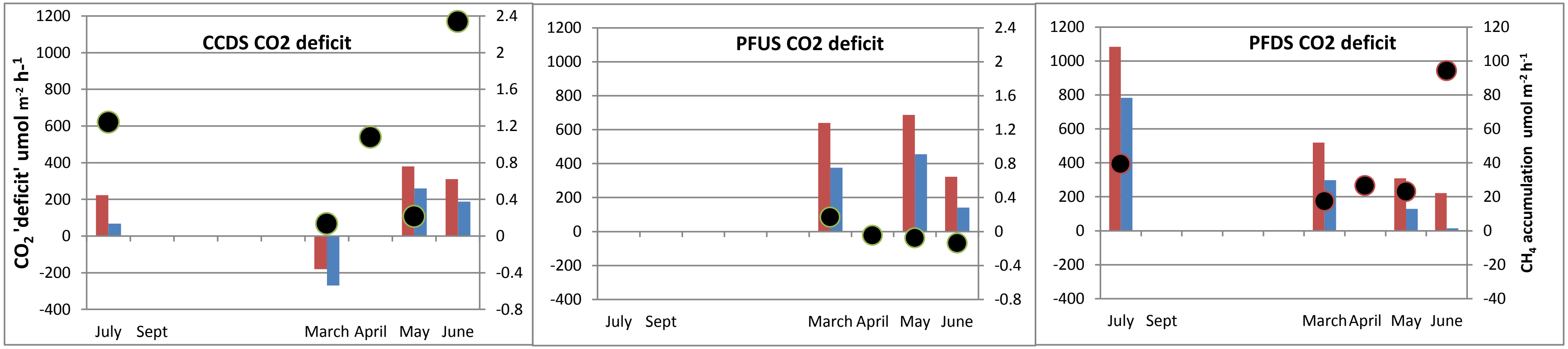


Figure 6:18 Variation in CO_2 'deficit' over time, calculated from O_2 consumption - CO_2 accumulation in dark chambers using RQ = 1 (red) and RQ = 0.67 (blue). The mean rate of CH_4 accumulation in corresponding chambers is also shown (circles: secondary axis).

Chapter 7. **Macro-invertebrate processes**

The metabolic processes discussed in chapter 6 are characterised by their potential to react quickly to changes in environmental parameters. Ecosystem processes that respond over a longer time period can provide an indication of in-stream conditions, integrated over the medium term. Macro-invertebrate community composition is routinely used in assessments of ecological status (Jones et al., 2010). Ecosystem functional measures that rely on macro-invertebrate processes represent a complementary tool that can provide additional information on in-stream functional integrity. In reference streams, leaf litter is an important food source for heterotrophic communities living in shaded headwater reaches. In un-shaded reaches, photosynthetic primary productivity, often in the form of epilithic algal (periphyton) accumulation, provides additional support for heterotrophic production. The degree to which these processes are de-coupled can provide a measure of impact in altered streams (Battin et al., 2008; Findlay, 2010). Leaf litter processing has been used in a variety of studies to determine the degree to which ecosystem function has been compromised by environmental stressors such as nutrient enrichment, pH, metal toxicity, broad land use change and food-web disruption e.g. (Petersen and Cummins, 1974; Gessner and Chauvet, 2002; Gulis and Suberkropp, 2003; Woodcock and Huryn, 2005; Ferreira et al., 2006; Gulis et al., 2006; Lecerf et al., 2006; Baldy et al., 2007; McKie and Malmqvist, 2009; Magbanua et al., 2010; Hladyz et al., 2011a; Woodward et al., 2012). In this chapter, the medium term ecosystem process rates associated with leaf litter degradation, periphyton accrual and macro-invertebrate herbivory measured at the study sites are compared in the light of the macro-invertebrate community assemblages present. The degree to which these processes may have been influenced by the differences in sub-catchment characteristics described in chapters 3 and 5 is discussed further in chapter 8.

The rate of leaf litter degradation was determined on seven occasions throughout the study period between October 2011 and June 2014. Measurements of the rate of periphyton accumulation and macro-invertebrate herbivory were made on six of these occasions between May 2012 and June 2014 (Table 7:1). In order to determine the contribution made by macro-invertebrate detritivory to leaf litter loss, leaf litter bags with coarse and fine mesh were deployed to respectively allow or exclude access to macro-invertebrates. For full details of the method and calculations see Chapter 4. Briefly, the rate of leaf loss attributable to macro-invertebrate processes was calculated from the differences between the rates of loss in each coarse and fine mesh bag pair. Loss of leaf material from the fine mesh bags was attributed to a combination of microbial degradation and abiotic processes such as abrasion and leaching, hereafter termed microbial breakdown (see section 7.5). Five replicate pairs of bags (coarse and fine mesh) and tiles (grazed and un-grazed) were installed at two sites (upstream and downstream) in each of the two study reaches. In May 2013, only the downstream sites were studied and problems with access meant that only four replicate pairs were installed at the upstream site in the Cool's Cottage reach from August 2012 to April 2014. Nevertheless, a total of 250 litter bags and 210 ceramic tiles were installed on site and incubated for 30 days. Recovery of samples from both litter bags and ceramic tiles was good, 94% for the litter bags and 96% for the tiles. Samples were lost due to displacement and burial by gravel (caused by high flows during deployment), or by damage to containers during storage. Additional losses from the litterbags were caused by poor fine-mesh bag construction in the first deployment resulting in loss of integrity that allowed macro-invertebrate access. These bags were discarded and the problem was corrected in all subsequent deployments. Temperatures were recorded at fifteen minute intervals during the incubations (chapter 4). Mean daily in-stream temperatures ranged from 4.3°C at the Priors Farm downstream site in February 2013 to 17°C at the Cool's Cottage upstream site in August 2012. The maximum difference in mean daily temperatures between streams was 2°C in February 2013 (Table 7:2). Water temperatures were higher in the Cool's Cottage reach for all deployments, with an average difference between streams of 0.8°C.

7.1. Leaf litter degradation

7.1.1. Key findings:

There were clear, substantial and significant differences in the rates and patterns of leaf litter decomposition between the two study reaches.

- Rates of leaf litter degradation were greater in the Cool's cottage reach than in the Priors Farm reach.
- Rates of leaf litter degradation in the Cool's Cottage reach were dominated by macro-invertebrate processes, while those in the Priors Farm reach were more evenly distributed between macro-invertebrate and microbial processes.
- Temporal patterns in the rates of leaf litter degradation were significantly different between the two study reaches

7.1.2. Patterns in leaf litter degradation in the Cool's Cottage reach

The following sections describe patterns in leaf litter processing. 'Absolute' loss refers to the mass in grams of litter lost over the course of a 30 day incubation. Temperature compensated rate coefficients ($-k\ dd^{-1}$) were derived from an exponential model of decay (Petersen and Cummins, 1974; Gessner and Chauvet, 2002; Barlocher, 2005b), and used to explore patterns of leaf litter degradation within sites and to compare leaf litter degradation dynamics between the two study reaches (Chapter 4).

7.1.2.1. Patterns in absolute leaf litter degradation

There was substantial leaf litter degradation in the Cool's Cottage reach. The highest mean absolute loss from the coarse bags was at the upstream site, with 85% (by mass) of material lost over the 30 day incubation. In the fine bags, the maximum absolute loss was 41%, also at the upstream site. (Table 7:2). The maximum absolute loss for both fine and coarse bags occurred in August 2012, coinciding with the highest in-stream water temperature. There was a strong seasonal variation in absolute loss in both coarse and fine bags (Figure 7:1).

7.1.2.2. Patterns in temperature compensated rates of leaf litter degradation

Rates of leaf litter degradation were comparable to degradation rates for oak litter recorded by other studies (Petersen and Cummins, 1974; Mackie and Malmqvist, 2009; Hladyz et al., 2011; Woodward et al., 2012). There was a small but significant difference between the upstream and downstream sites in both the coarse and fine mesh bags $P < 0.02$. Macro-invertebrate mediated degradation rates showed no significant difference between the upstream and downstream sites $P = 0.7$, indicating that the observed difference between sites in the coarse mesh bags was driven by microbial processes . Data from the two sites were combined to provide reach averaged rates for further analyses.

Table 7:1 Schedule of incubations undertaken to determine leaf litter degradation and herbivory

		Cool's Cottage				**Priors Farm**			
Season	**Date**	**Upstream**		**Downstream**		**Upstream**		**Downstream**	
		Leaf Litter	**Herbivory**	**Leaf Litter**	**Herbivory**	**Leaf Litter**	**Herbivory**	**Leaf Litter**	**Herbivory**
Autumn '11	15.10.11 - 29.11.11	**x**		**x**		**x**		**x**	
Spring '12	14.05.12 - 14.06.12			**x**	**x**			**x**	**x**
Summer '12	23.07.12 - 23.08.12	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Winter '12-'13	02.02.13 - 25.02.13	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Spring '13	20.05.13 - 20.06.13	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Autumn '13	02.09.13 - 02.10.13	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Spring '14	19.03.14 - 19.04.14	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**

Table 7:2 Absolute leaf loss, as a percentage of original mass, in coarse and fine mesh bags.

		Cool's Cottage						**Priors Farm**					
Season	**Date**	**Upstream**			**Downstream**			**Upstream**			**Downstream**		
		Coarse	**Fine**	***Temp***	**Coarse**	**Fine**	***Temp***	**Coarse**	**Fine**	***Temp***	**Coarse**	**Fine**	***Temp***
Autumn '11	15.10.11 - 29.11.11	26%	11%	*10.3*	19%	6%	*10.3*	15%	14%	*10.3*	13%	8%	*10.3*
Spring '12	14.05.12 - 14.06.12				74%	19%	*14.1*				39%	16%	*13.8*
Summer '12	23.07.12 - 23.08.12	85%	41%	*17.0*	78%	30%	*17.0*	33%	27%	*15.8*	23%	20%	*15.8*
Winter '12-'13	02.02.13 - 25.02.13	10%	6%	*6.3*	14%	5%	*5.9*	13%	0%	*4.6*	12%	0%	*4.3*
Spring '13	20.05.13 - 20.06.13	82%	28%	*14.1*	75%	22%	*13.5*	35%	14%	*12.5*	31%	9%	*12.6*
Autumn '13	02.09.13 - 02.10.13	45%	22%	*14.0*	46%	19%	*13.6*	17%	12%	*13.0*	14%	14%	*13.0*
Spring '14	19.03.14 - 19.04.14	83%	15%	*10.3*	68%	13%	*10.3*	26%	8%	*8.7*	21%	8%	*9.1*

Table 7:3 Rate coefficients of leaf litter degradation in the Cool's Cottage and Priors Farm study reaches. Rate of total degradation in the coarse bags (-$k_{(tot)}$dd^{-1}); rate of microbial degradation in the fine bags (-$k_{(mic)}$dd^{-1}); and rate of macro-invertebrate mediated degradation (-$k_{(invert)}$dd^{-1}).

	Cool's Cottage					Priors Farm				
Season	**-$k_{(tot)}$ dd^{-1}**	***s.d***	**-$k_{(mic)}$ dd^{-1}**	***s.d***	**-$k_{(invert)}$ dd^{-1}**	**-$k_{(tot)}$ dd^{-1}**	***s.d.***	**-$k_{(mic)}$ dd^{-1}**	***s.d***	**-$k_{(invert)}$ dd^{-1}**
Autumn '11	0.0008	*0.0004*	0.0003	*0.0001*	0.0005	0.0005	*0.0005*	0.0004	*0.0004*	0.0002
Spring '12	0.0029	*0.0005*	0.0005	*0.0001*	0.0024	0.0012	*0.0012*	0.0004	*0.0003*	0.0008
Summer '12	0.0036	*0.0014*	0.0008	*0.0002*	0.0027	0.0007	*0.0002*	0.0006	*0.0001*	0.0001
Winter '12-'13	0.0009	*0.0004*	0.0003	*0.0002*	0.0005	0.0012	*0.0006*	0.0001	*0.0002*	0.0010
Spring '13	0.0038	*0.0014*	0.0007	*0.0001*	0.0032	0.0011	*0.0003*	0.0003	*0.0001*	0.0007
Autumn '13	0.0015	*0.0004*	0.0006	*0.0001*	0.0009	0.0004	*0.0001*	0.0004	*0.0001*	0.0001
Spring '14	0.0046	*0.0016*	0.0005	*0.0001*	0.0041	0.0010	*0.0005*	0.0003	*0.0001*	0.0007

Mean rates of total leaf litter degradation (coarse mesh bags: $-k_{(tot)}.dd^{-1}$) ranged from 0.0008 to a maximum rate of 0.0046 (Table 7:3) and exhibited pronounced seasonal variation; F = 34, P <0.0001. Despite correction for temperature, leaf litter degradation rates were lowest in late autumn and winter and highest in late spring and summer (Table 7:3). The magnitude of the variation in total leaf litter degredation was dominated by the component attributable to macro-invertebrates: there was less variation between deployments in the fine mesh than coarse mesh bags (F = 33.8 and F= 9.6 for coarse and fine bags respectively). The proportion of leaf litter degradation attributable to macro-invertebrates was always greater than that attributable to microbial processes: mean 74%, range 59% in February 2013 to 90% in April 2014 (Table 7:4, Figure 7:2). Both processes exhibited significant seasonal variation, P < 0.0001 (Figure 7:3, a and b). Mean macro-invertebrate degradation rates ($-k_{(invert)}\ dd^{-1}$) varied from a minimum of 0.0005 in winter (October 2011 and February 2013) to a maximum of 0.0041 in April 2014, whereas mean microbially mediated degradation rates ($-k_{(mic)}\ dd^{-1}$) ranged from 0.0003 in February 2013 to 0.0008 in August 2013 (Table 7:3).

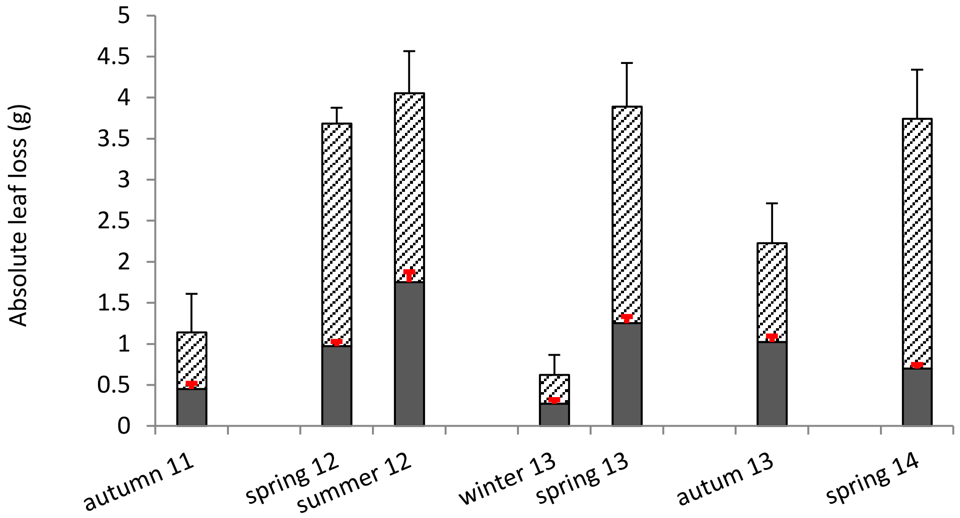


Figure 7:1 Seasonal variation in absolute leaf litter loss at Cool's Cottage. Hatched column, macroinvertebrate mediated loss; grey column, microbially mediated loss; bars are standard error.

Table 7:4 The relative contribution from microbial and macro-invertebrate processes to total leaf litter degradation in the Cool's Cottage and Priors Farm study reaches.

Relative contribution to total leaf litter degradation

	Cool's Cottage		**Priors Farm**	
	micro	**invert**	**micro**	**invert**
Autumn '11	36%	64%	66%	34%
Spring '12	17%	83%	34%	66%
Summer '12	23%	77%	80%	20%
Winter '12-'13	41%	59%	12%	88%
Spring '13	18%	82%	32%	68%
Autumn '13	37%	63%	84%	16%
Spring '14	90%	90%	30%	70%
mean	**26%**	**74%**	**48%**	**52%**

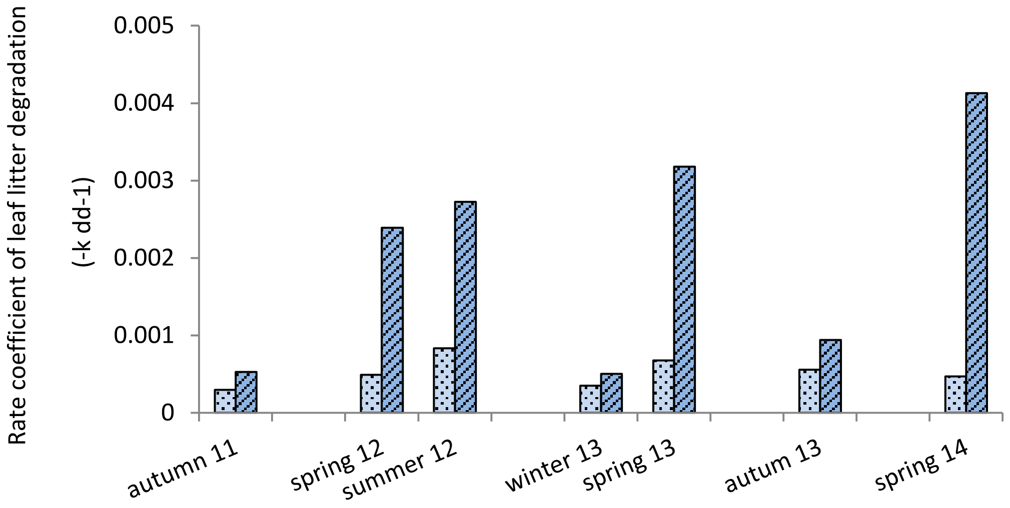


Figure 7:2 Seasonal variation in the relative contribution of microbial and macro-invertebrate processes to leaf litter degradation in the Cool's Cottage reach. Hatched column, macro-invertebrate rate coefficient ($-k_{(invert)}dd^{-1}$); stippled column, microbial rate coefficient ($-k_{(mic)}dd^{-1}$).

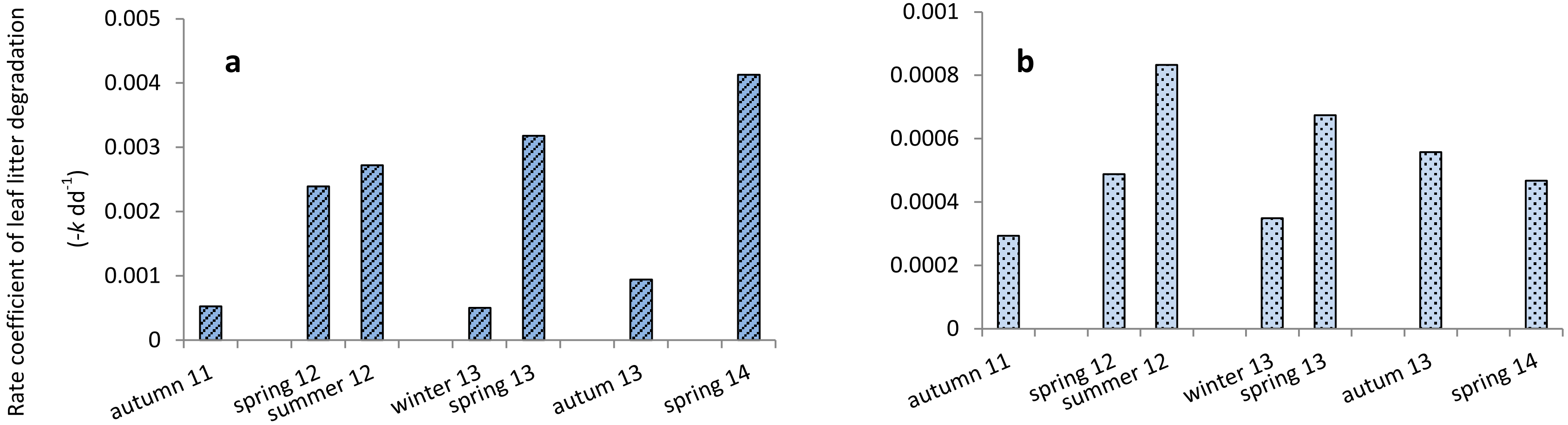


Figure 7:3 Seasonal variation in temperature compensated rate coefficients of leaf litter degradation in the Cool's Cottage reach: a, macro-invertebrate mediated rate coefficient (-$k_{(invert)}$dd^{-1}); b microbial rate coefficient (-$k_{(mic)}$dd^{-1}).

7.1.1. **Patterns in leaf litter degradation in the Priors Farm reach**

7.1.1.1. Patterns in absolute leaf litter degradation

Absolute leaf litter loss was lower in the Priors Farm reach than in the Cool's Cottage reach, with maximum loss of 45% (by mass) in the coarse bags in the two spring deployments, May 2012 and April 2014. In the fine bags, the maximum absolute loss was 32%, coinciding with the highest temperature in August 2012 (Table 7:2). Absolute loss in both coarse and fine bags exhibited strong seasonal variation (Figure 7:4), but the pattern was different to that in the Cool's Cottage reach. These relationships were described further using temperature compensated degradation rates ($k\ dd^{-1}$), as for the Cool's Cottage reach.

7.1.1.2. Patterns in temperature compensated rates of leaf litter degradation

There was no significant difference between the upstream and downstream sites in either the coarse or fine bags ($p > 0.1$). To provide an estimate for the sub-catchment, data from the two sites were combined for further analyses. Rates of leaf litter degradation were low when compared to values recorded for oak litter recorded by other studies (Petersen and Cummins, 1974; Mackie and Malmqvist, 2009; Hladyz et al., 2011; Woodward et al., 2012), and to those in the Cool's Cottage reach. Mean rates of total leaf litter degradation (coarse mesh bags: $-k_{(tot)}.dd^{-1}$) ranged from 0.0004 in September 2013 to a maximum of 0.0012 in February 2014. (Table 7:3).

Temporal variation in rates of total leaf litter degradation , although significant ($P < 0.0001$), was lower in the Priors Farm reach than in the Cool's Cottage reach (F=13 compared with F=34 for the Cool's Cottage reach) and followed a different temporal pattern: the maximum rate of total leaf litter degreadation occurred in winter. Mean macro-invertebrate degradation rates ($-k_{(invert)}\ dd^{-1}$) varied from a minimum of 0.00007 in September 2013 to a maximum of 0.001 in February 2014, compared with $-k_{(mic)}\ dd^{-1}$ that ranged from 0.0001 in February 2013 to 0.0006 in August 2012(Table 7:3). In contrast to the Cool's cottage reach, the rate of microbially mediated degradation exceeded that attributable to macro-invertebrate detritivory on three out of the seven deployments, with the maximum microbial contribution occuring in late summer and autumn (August 2012 and September 2013) coinciding with the highest temperatures (Figure 7:5). The proportion of degradation attributabe to macro-invertebrates in the Priors farm reach was consequently lower than in the Cool's Cottage reach with a minimum contribution of 16% in September 2013 and a maximum of 88% in February 2013, an average of 52% overall (Table 7:4). Both $-k_{(invert)}.dd^{-1}$ and $-k_{(mic)}.dd^{-1}$ exhibited significant seasonal variation, $P < 0.0001$, (Figure 7:6a and b). There was greater temporal variation in rates of total leaf litter degradation than in rates of microbial leaf litter degradation (F = 13.3 and F= 8, respectively) indicating that macro-invertebrate processes were responsible for more of the temporal variation than microbial processes.

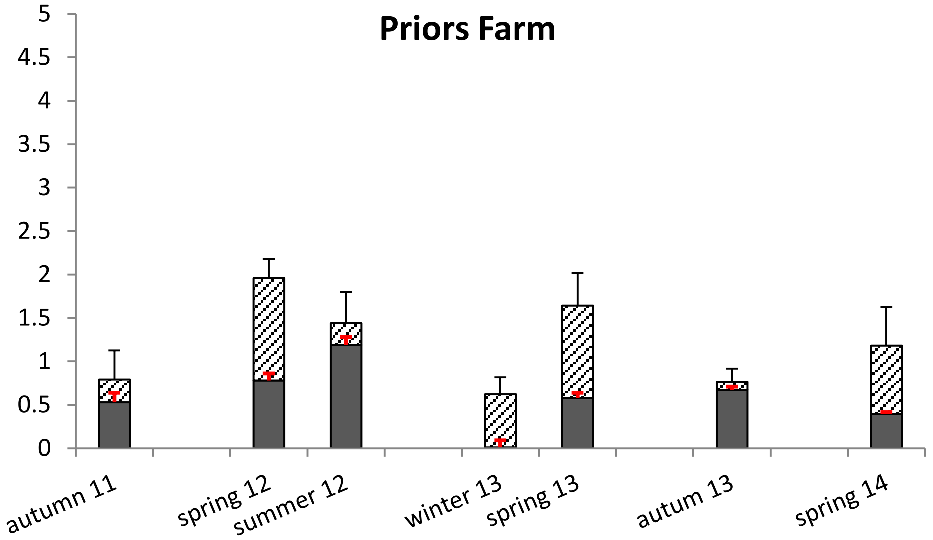


Figure 7:4 Seasonal variation in leaf litter loss at Priors Farm. Hatched column, macroinvertebrate mediated loss; grey column, microbially mediated loss; bars are standard error.

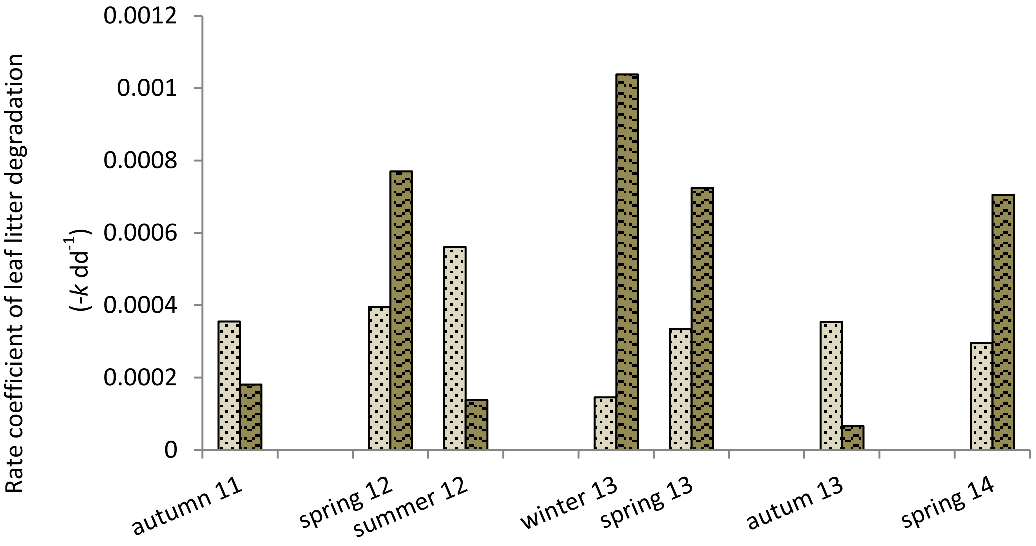


Figure 7:5 Seasonal variation in the relative contribution of microbial and macro-invertebrate processes to leaf litter degradation in the Priors Farm reach: hatched column, macro-invertebrate rate coefficient (-$k_{(invert)}dd^{-1}$); stippled column, microbial rate coefficient (-$k_{(mic)}dd^{-1}$).

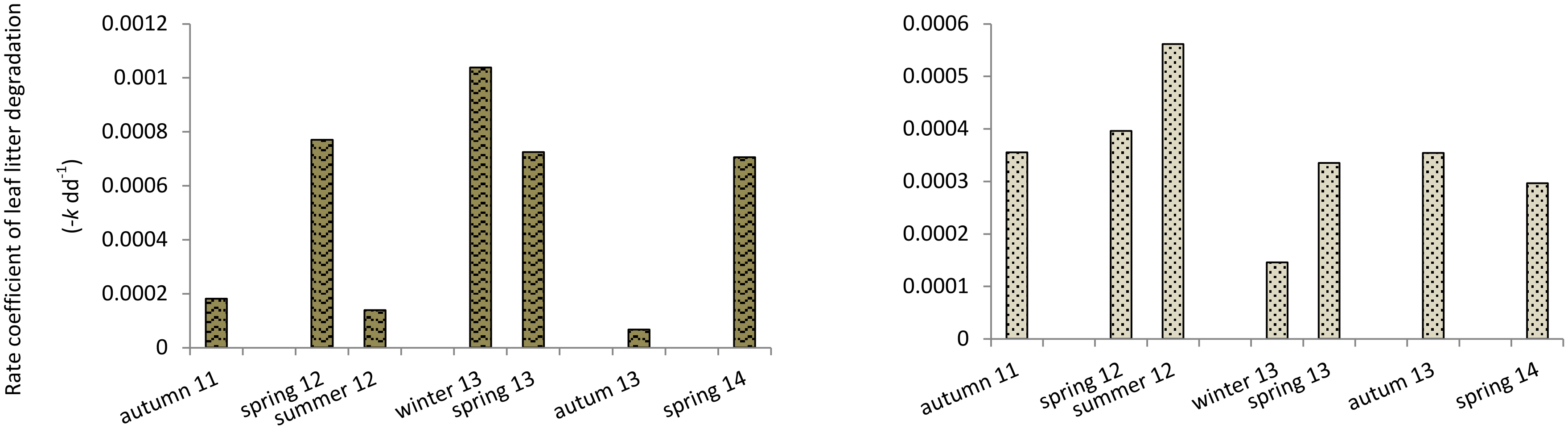


Figure 7:6 Seasonal variation in temperature compensated rate coefficients of leaf litter degradation in the Priors Farm reach: a, macro-invertebrate mediated rate coefficient ($-k_{(invert)}dd^{-1}$); b microbial rate coefficient ($-k_{(mic)}dd^{-1}$).

7.1.2. **Contrasts in leaf litter processing in the two study reaches**

Between stream differences in the rates of leaf litter degradation, were significant for both macro-invertebrate ($F=112$, $p < 0.0001$) and microbially mediated degradation ($F= 36$, $p < 0.0001$). Although the difference between the reaches was most pronounced for the rate of macro-invertebrate mediated degradation, both processes were suppressed in the Priors Farm reach when compared to the Cool's Cottage reach (Figure 7:7, a– c). Analysis of the patterns in leaf litter degradation revealed additional differences between the two study reaches, both in the proportion of degradation attributable to macro-invertebrate processes and in the patterns of seasonal variation: The interaction between reach and date was highly significant $P < 0001$. In the Cool's Cottage reach, minimum ($-k_{(invert)}\ dd^{-1}$) occurred in winter, whereas winter was the period of maximum macro-invertebrate contribution to ($-k_{(tot)}.dd^{-1}$) in the Priors Farm reach (Figure 7:8). These data suggest that factors other than background temperature and seasonal variation were influencing leaf litter dynamics, and are discussed in the context of macro-invertebrate community structure in section 7.4.

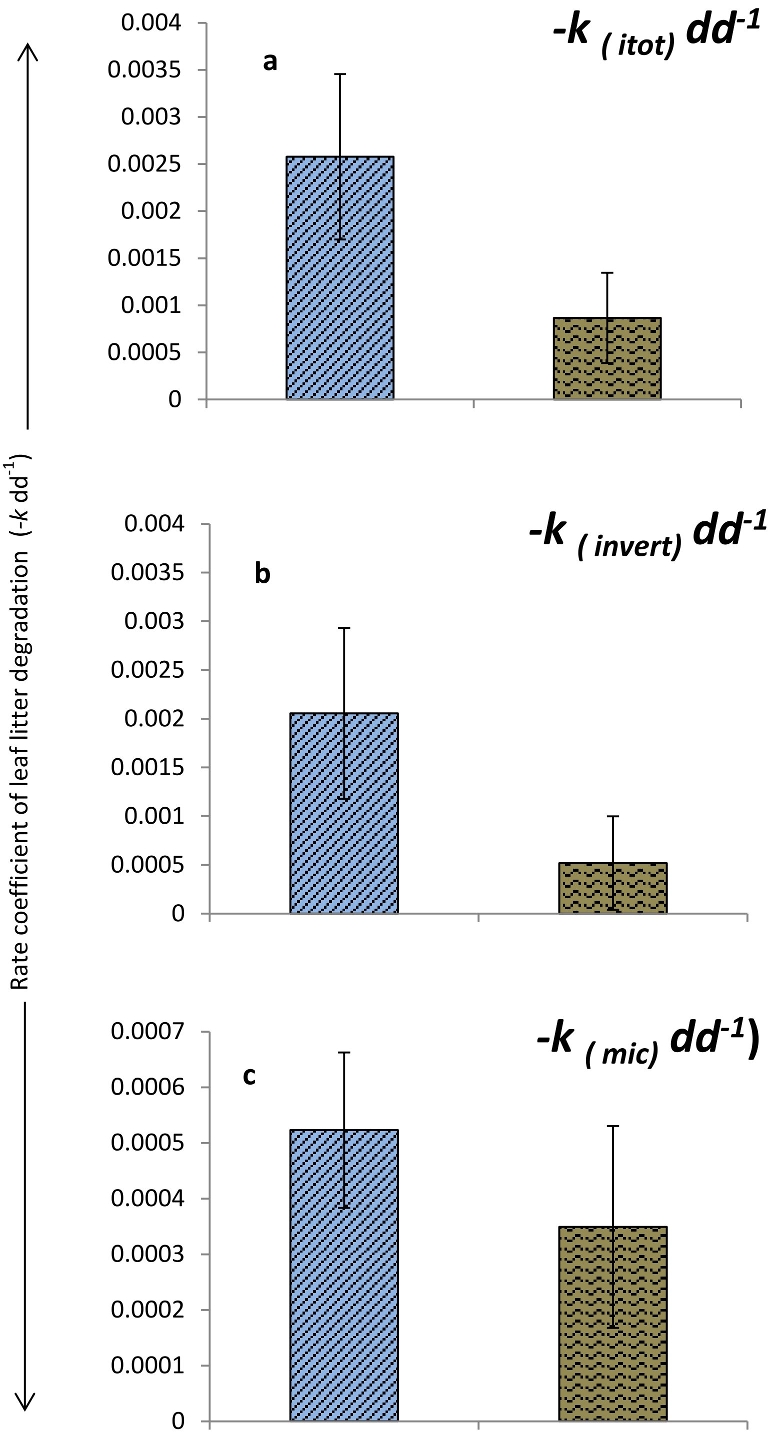


Figure 7:7 Comparison of leaf litter degradation rates in the two study sub-catchments; a, total degradation, b degradation attributable to macro-invertebrates; microbially mediated degradation: blue columns, Cool's Cottage, brown columns, Priors Farm, Error bars are standard error.

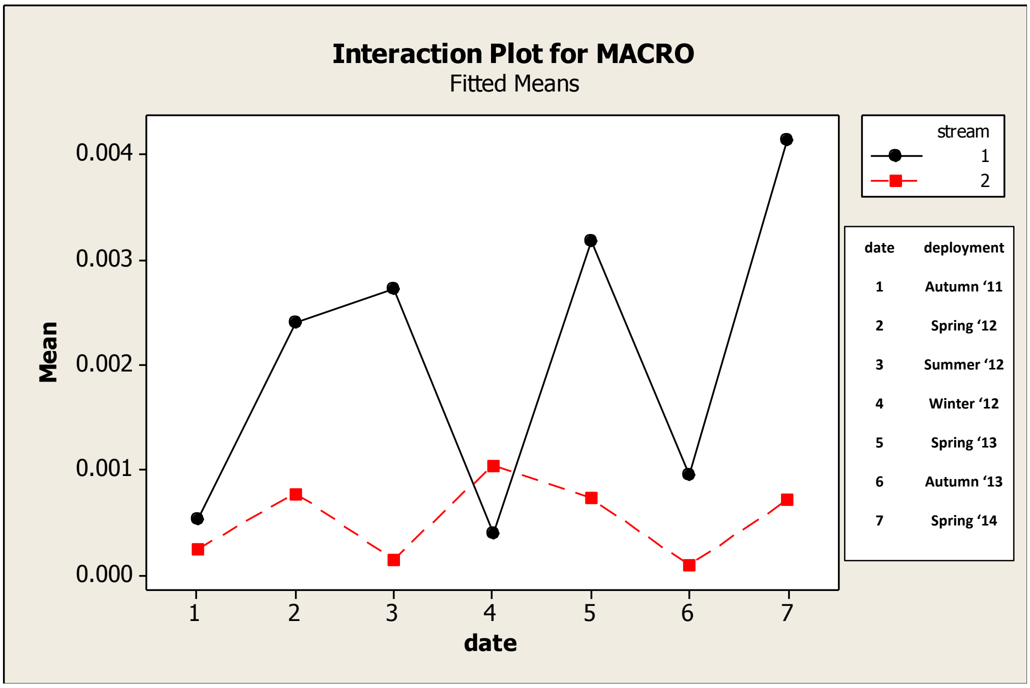


Figure 7:8 Graphical output from 'Minitab', illustrating contrasting seasonal variation in macro-invertebrate leaf processing the two study reaches: stream 1, Cool's Cottage, stream 2, Priors Farm

7.2. Epilithic primary production and macro-invertebrate herbivory

7.2.1. Key findings

There were significant differences in the rates of epilithic algal (periphyton) accrual between sites, and rates of herbivory between streams.

- Periphyton accrual was highest at the Priors farm downstream site.
- There was no significant difference in periphyton accrual on un-grazed tiles between the Cool's Cottage reach and the Priors Farm upstream site.
- Rates of herbivory were greater in the Cool's Cottage reach than in the Priors Farm reach.
- Temporal patterns were significantly different between the two study reaches.

Both light and nutrients are likely to limit photosynthetic primary production at different times throughout the seasons and at different study sites. Only the downstream site in the Priors Farm reach remained unshaded throughout the year. The accumulation of periphyton on un-grazed tiles reflected these conditions and was highly variable, both within and between sites.

7.2.2. Patterns in periphyton accumulation

As for leaf litter processing, data are reported both as absolute, measured values of periphyton 'accumulation' over the full incubation period, expressed as mg Chlorophyll-a (Chl-a) m^{-2}, and as temperature compensated rates of periphyton 'accrual' expressed as $p\ dd^{-1}$ (McAuliffe, 1984a; McAuliffe, 1984b; Mitchell and Wass, 1996).

7.2.2.1. Patterns in measured periphyton accumulation

In the Cool's Cottage reach there was no significant difference in periphyton accumulation between the upstream and downstream sites ($P = 0.25$). Over the full study period, mean accumulation was 1.9 mg Chl-a m^{-2} on the grazed tiles and 4.2 mg Chl m^{-2} on the un-grazed tiles (Table 7:6). Growth of periphyton on the un-grazed tiles demonstrated significant seasonal variation ($P < 0.0001$). The highest mean value of 10.6 mg Chl m^{-2} was recorded in April 2014 at the downstream site and the lowest, 0.4 mg Chl m^{-2} at both sites, in September 2013 (Figure 7:9a).

There was a significant difference between the Priors Farm upstream and downstream sites, $P < 0.0001$. As predicted because of the lack of shading, the accumulation of periphyton was highest at the Priors Farm downstream site. Over the full study period, mean accumulation on the un-grazed tiles was 57.3 mg Chl-a m^{-2} and 58.7 mg Chl-a on the grazed tiles

(Table 7:5). Growth of periphyton on the un-grazed tiles demonstrated significant seasonal variation ($P < 0.0001$). The highest mean value of 113 mg chl a m^{-2} was recorded in April 2014. This was the only site where minimum periphyton accumulation occurred in the winter, 6.1 mg Chl-a m^{-2} in February 2013 (Table 7:4 and Figure 7:9 b).

The Priors farm upstream site had the lowest periphyton accumulation overall; site mean was 3.9 mg Chl-a m^{-2} on the un-grazed tiles and 4.3 mg Chl-a m^{-2} on the grazed tiles. The maximum, but highly variable mean accumulation was 17.1 (range 1.1 – 41.3 mg Chl-a m^{-2}) occurred in April of 2014 before canopy development and minimum was 0.2 mg Chl-a m^{-2} in September 2013 (Table 7:4 and Figure 7:9 (a)).

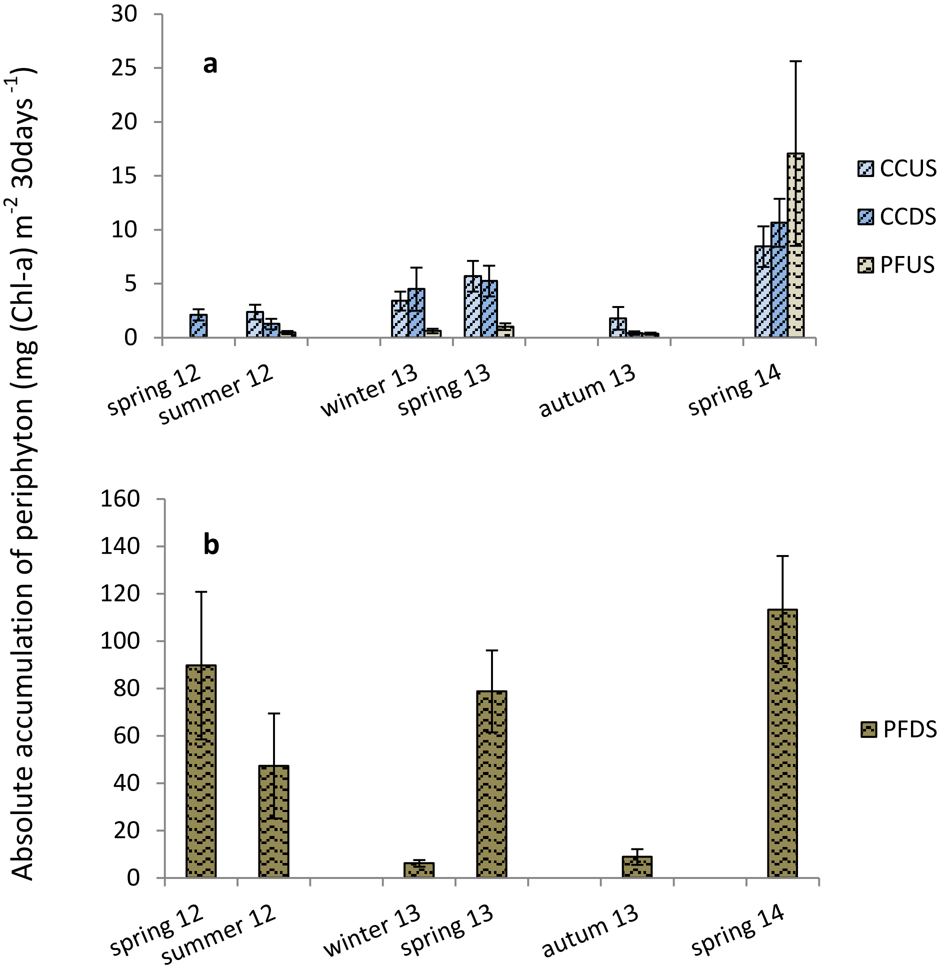


Figure 7:9 Seasonal variation in absolute accumulation of periphyton on un-grazed tiles over the 30 day incubations, measured as mg chlorophyll-a m^{-2} 30 $days^{-1}$:
a, shaded sites, blue columns, Cool's Cottage, brown column, Priors Farm upstream site;
b, unshaded site, Priors Farm downstream

Table 7:5 Absolute accumulation of periphyton on grazed and un-grazed tiles over the 30 day incubations, measured as mg chlorophyll-a m^{-2} 30 days^{-1}

	Cool’s Cottage								**Priors Farm**							
	Upstream				**Downstream**				**Upstream**				**Downstream**			
	grazed	***s.d***	**un-grazed**	***s.d***	**grazed**	***s.d***	**un-grazed**	***s.d***	**grazed**	***s.d***	**un-grazed**	***s.d***	**grazed**	***s.d***	**un-grazed**	***s.d***
Autumn ‘11																
Spring ‘12					1.9	*1.2*	2.1	*1.1*					77.9	*44.4*	89.6	*62.3*
Summer ‘12	1.6	*1.2*	2.4	*1.4*	0.8	*0.7*	1.3	*1.0*	0.6	*0.3*	0.5	*0.3*	47.0	*37.5*	47.2	*44.3*
Winter ’12-‘13	1.8	*1.2*	3.4	*1.7*	1.2	*0.7*	4.5	*4.0*	0.9	*0.4*	0.6	*0.4*	5.9	*4.9*	6.1	*2.8*
Spring ‘13	3.5	*2.6*	5.7	*2.8*	4.4	*3.6*	5.2	*2.8*	1.5	*0.6*	1.0	*0.6*	92.3	*48.7*	78.8	*34.6*
Autumn ‘13	0.4	*0.3*	1.8	*2.1*	0.2	*0.2*	0.4	*0.3*	0.5	*0.4*	0.4	*0.2*	9.6	*4.0*	8.8	*6.6*
Spring ‘14	2.0	*0.6*	8.4	*3.8*	2.9	*1.5*	10.6	*4.5*	18.0	*17.3*	17.1	*17.1*	119.8	*61.8*	113.2	*45.4*
mean	**1.9**	***1.2***	**4.3**	***2.4***	**1.9**	***1.3***	**4.0**	***2.3***	**4.3**	***3.8***	**3.9**	***3.7***	**58.7**	***33.6***	**57.3**	***32.7***

Table 7:6 Rate coefficients of periphyton accrual in the Cool's Cottage and Priors Farm study reaches. Rate of accrual on grazed tiles (p (net) dd-1); rate of accrual on the un-grazed tiless (p (gross) dd-1);

	Cool’s Cottage								**Priors Farm**							
	Upstream				**Downstream**				**Upstream**				**Downstream**			
	$p_{(net)}$	***s.d***	$p_{(gross)}$	***s.d***	$p_{(net)}$	***s.d***	$p_{(gross)}$	$p_{(net)}$	***s.d***	$p_{(net)}$	***s.d***	$p_{(gross)}$	***s.d***	$p_{(net)}$	***s.d***	$p_{(gross)}$
Autumn ‘11																
Spring ‘12					0.001	*0.002*	0.001	*0.001*					*0.010*	0.001	*0.010*	0.001
Summer ‘12	0.001	*0.001*	0.001	*0.001*	-0.001	*0.002*	0.000	*0.002*	*-0.001*	*0.001*	*-0.002*	0.002	*0.007*	0.002	*0.006*	0.003
Winter ’12-‘13	0.002	*0.005*	0.006	*0.003*	0.000	*0.004*	0.007	*0.005*	*-0.002*	*0.003*	*-0.006*	0.006	*0.013*	0.009	*0.015*	0.007
Spring ‘13	0.002	*0.002*	0.004	*0.001*	0.003	*0.002*	0.004	*0.001*	*0.001*	*0.001*	*0.000*	0.002	*0.012*	0.002	*0.011*	0.001
Autumn ‘13	-0.003	*0.002*	0.000	*0.002*	-0.004	*0.002*	-0.003	*0.002*	*-0.002*	*0.001*	*-0.003*	0.001	*0.006*	0.001	*0.005*	0.002
Spring ‘14	0.002	*0.001*	0.006	*0.001*	0.003	*0.002*	0.007	*0.002*	*0.008*	*0.006*	*0.008*	0.006	*0.017*	0.002	*0.017*	0.002
mean	0.001	*0.002*	0.004	*0.002*	0.000	*0.002*	0.003	*0.002*	*0.001*	*0.003*	*-0.001*	0.003	*0.011*	0.003	*0.011*	0.003

7.2.2.2. Patterns in temperature compensated rate of periphyton accrual

Rates of accrual on the grazed tiles ($p_{(net)}\ dd^{-1}$) at PFDS were significantly different to the other sites ($P < 0.0001$). For rates of accrual on the un-grazed tiles ($p_{(gross)}\ dd^{-1}$), in addition to a significant difference between PFDS and the other sites, there was a significant difference between PFUS and the two sites in the Cool's Cottage reach ($P < 0.0001$). There was no significant difference in either measure between the upstream and downstream sites in the Cool's Cottage reach (Table 7:6, Figure 7:10).

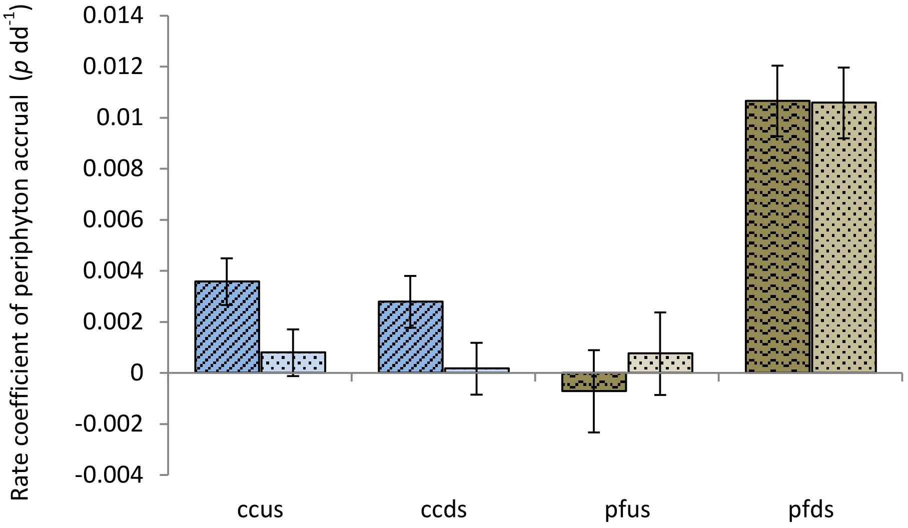


Figure 7:10 Mean rate coefficients of periphyton accrual over the full study period: hatched columns, un-grazed tiles ($p_{(gross)}dd^{-1}$), stippled columns, grazed tiles ($p_{(net)}dd^{-1}$); blue columns Cool's Cottage, brown columns, Priors Farm.

Once corrected for temperature, seasonal patterns in periphyton accrual on both grazed and un-grazed tiles were similar for the Cool's Cottage reach and at the Priors Farm downstream site, although different in magnitude. On the un-grazed tiles, maximum rates of accrual occurred in winter '12-'13 and spring 2014, and minimum rates occurred in

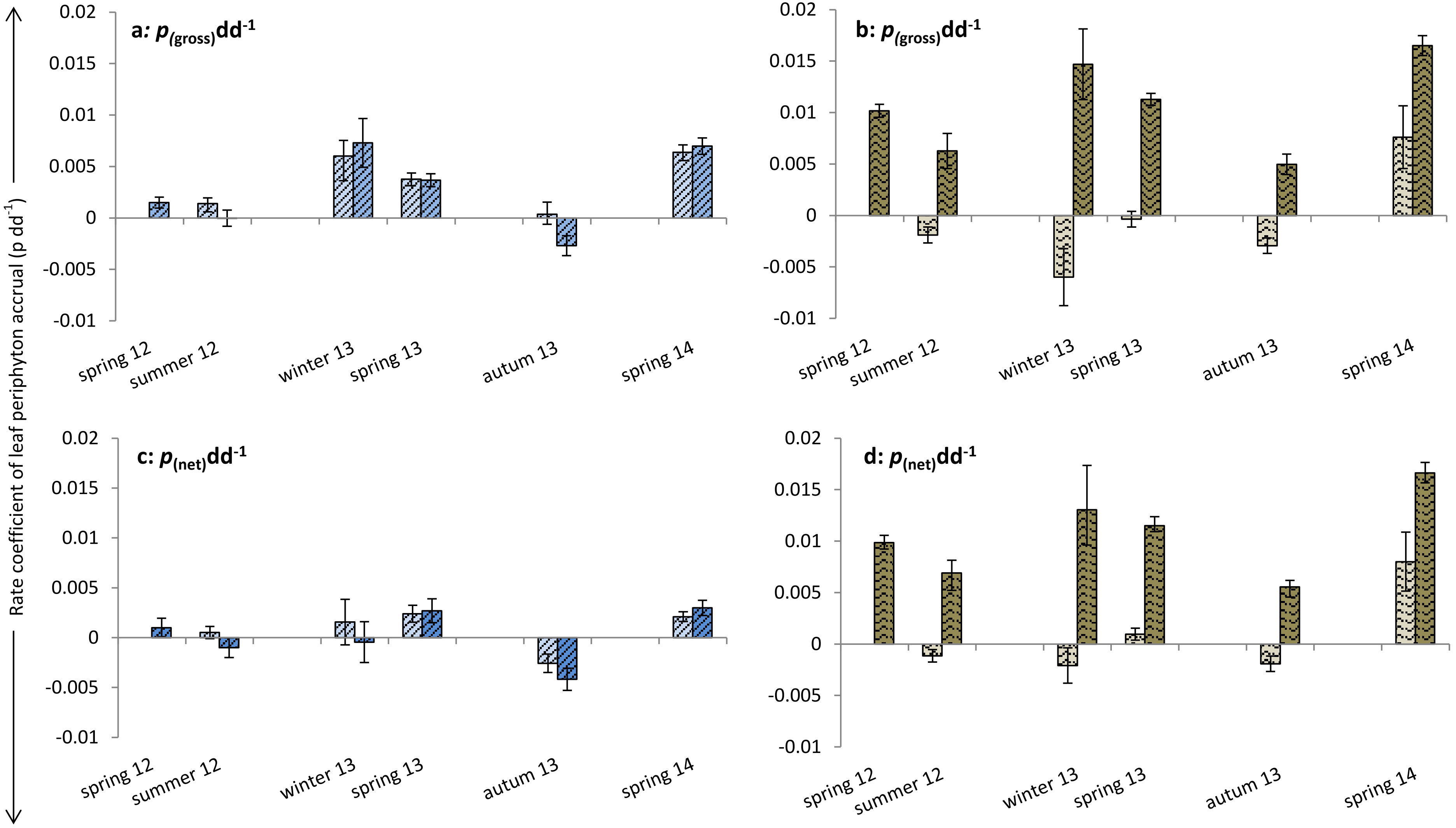
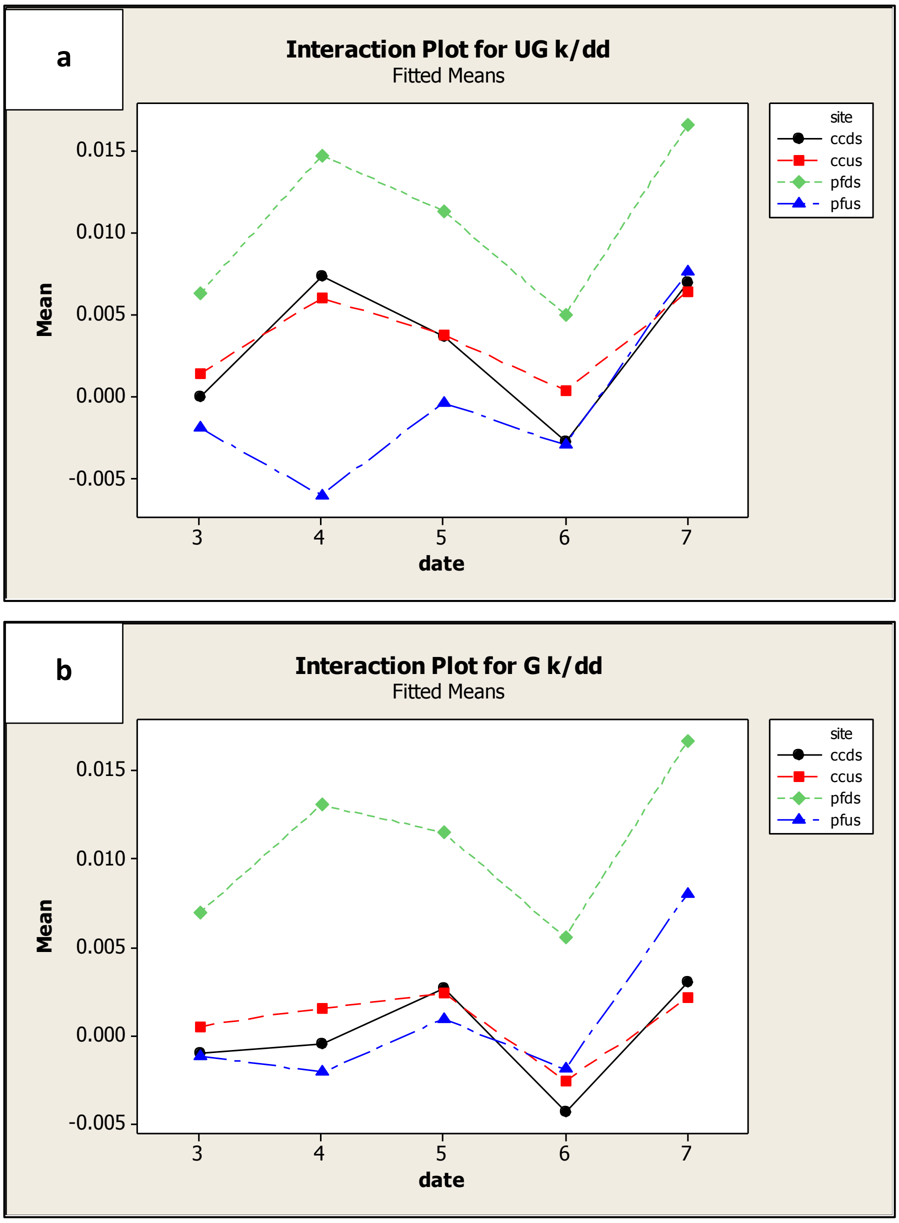


Figure 7:11 Seasonal variation in rate coefficients of periphyton accrual on grazed tiles (p $_{(net)}$ dd^{-1}); and un-grazed tiles (p $_{(gross)}$ dd^{-1}): a, Cool's Cottage un-grazed tiles; b, Priors Farm un-grazed tiles; c, Cool's Cottage grazed tiles; d, Priors Farm grazed tiles; pale columns, upstream sites, darker columns, downstream sites.

autumn ‘13. On the grazed tiles, the magnitude of the seasonal variation was lower than that observed on un-grazed tiles at both sites in the Cool’s Cottage reach. No such difference was observed between the two measures at the sites in the Priors Farm reach (Table 7:6 and Figure 7:11, a-d). The interaction between site and date was significant for both the grazed tiles ($P < 0.05$) and un-grazed tiles ($P < 0.0001$) confirming that temporal patterns of periphyton accrual were different between sites (Figure 7:12 a and b).


Interaction Plot for UG k/dd
Fitted Means
Interaction Plot for G k/dd
Fitted Means

date	deployment
1	Autumn ‘11
2	Spring ‘12
3	Summer ‘12
4	Winter ‘12
5	Spring ‘13
6	Autumn ‘13
7	Spring ‘14

Figure 7:12 Graphical output from 'Minitab', illustrating seasonal variation in periphyton accrual on a, un-grazed tiles (p $_{(gross)}$ dd^{-1}) and b, grazed tiles (p $_{(net)}$ dd^{-1}); and demonstrating the reduction on the grazed tiles in the Cool’s Cottage reach.

7.2.3. Macro-invertebrate herbivory

The rates of herbivory ($p_{(herb)}\, dd^{-1}$), calculated from the difference in rates of accrual between each pair of grazed and un-grazed tiles (chapter 4), were significantly different (F = 35, $P < 0.0001$) between streams .

Table 7:7 Rate coefficients for herbivory in the Cool's Cottage and Priors Farm study reaches ($p_{(herb)}dd^{-1}$)

	Cool's Cottage				**Priors Farm**			
	Upstream		**Downstream**		**Upstream**		**Downstream**	
	grazing	***s.d.***	**grazing**	***s.d.***	**grazing**	***s.d.***	**grazing**	***s.d.***
Autumn '11								
Spring '12			0.0005	*0.0017*			0.0003	*0.0011*
Summer '12	0.0009	*0.0013*	0.0010	*0.0016*	-0.0008	*0.0024*	-0.0006	*0.0013*
Winter '12-'13	0.0044	*0.0054*	0.0077	*0.0035*	-0.0039	*0.0035*	0.0017	*0.0056*
Spring '13	0.0014	*0.0014*	0.0010	*0.0012*	-0.0013	*0.0023*	-0.0002	*0.0009*
Autumn '13	0.0029	*0.0021*	0.0016	*0.0008*	-0.0005	*0.0004*	-0.0006	*0.0015*
Spring '14	0.0043	*0.0013*	0.0038	*0.0030*	-0.0004	*0.0027*	-0.0001	*0.0009*
mean	**0.0028**	***0.0023***	**0.0026**	***0.0020***	**-0.0014**	***0.0023***	**0.0001**	***0.0019***

There was no significant difference between the rates of herbivory at the upstream and downstream sites in the Cool's Cottage reach $P > 0.7$. More surprisingly, given the difference in algal production, there was no significant difference in rates of herbivory between the two sites in the Priors Farm reach, $P > 0.5$: there was no detectable herbivory at either site in the Priors Farm reach (Table 7:7, Figure 7:13).

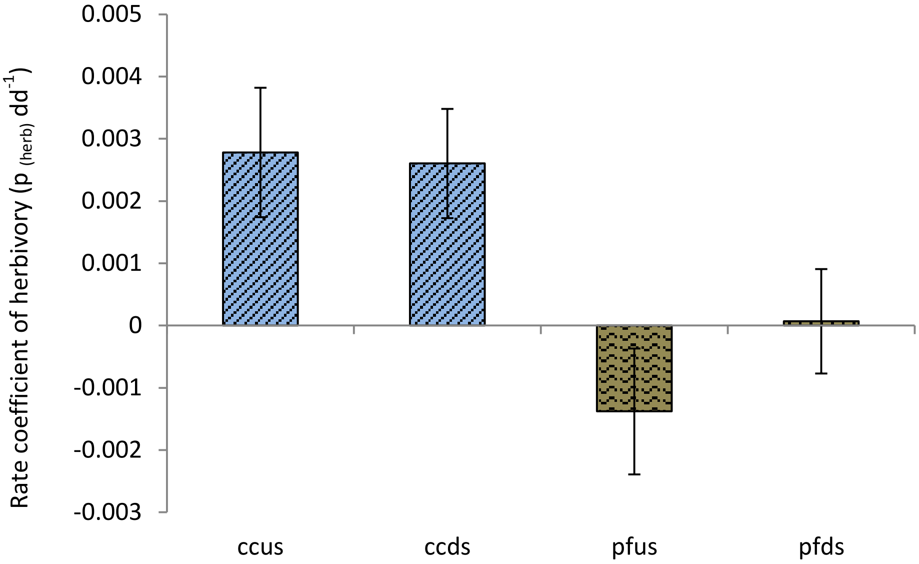


Figure 7:13 Mean grazing rate coefficients over the full study period ($p_{(herb)}dd^{-1}$): blue columns Cool's Cottage, brown columns, Priors Farm; bars are standard error.

Data from both sites in each reach were combined to provide a reach scale estimate for further analysis. Temporal patterns in herbivory, shown by the interaction between stream and date were significantly different between streams (*P* = 0.003,Figure 7:14).

Figure 7:14 Graphical output from 'Minitab', illustrating contrasting seasonal variation in grazing rate coefficients ($p_{(herb)}$ dd^{-1}) in the two study reaches: stream 1, Cool's Cottage, stream 2, Priors Farm

The maximum rate of herbivory in the Cool's Cottage reach was in winter '12-'13 (Table 7:7, Figure 7:15) and coincided with the minimum rate of macro-invertebrate detritivory (section 7.1.2). Rates of herbivory in the Cool's Cottage reach were also high in spring 2014, coinciding with maximum macro-invertebrate detritivory. Herbivory was undetectable throughout the year in the Priors Farm reach (Table 7:7, Figure 7:16). There are theoretical limitations to the methodology that can result in an underestimate of herbivory in highly productive sites (Mitchell and Wass, 1996), such as at the Priors Farm downstream site. However, further scrutiny of the data (see section 7.5) provided no evidence to reject the results. These findings show a significant reduction in the rate of another key ecosystem process in the Priors Farm reach compared with the Cool's Cottage reach, and demonstrate a severe impact on the macro-invertebrate community in the Priors Farm reach.

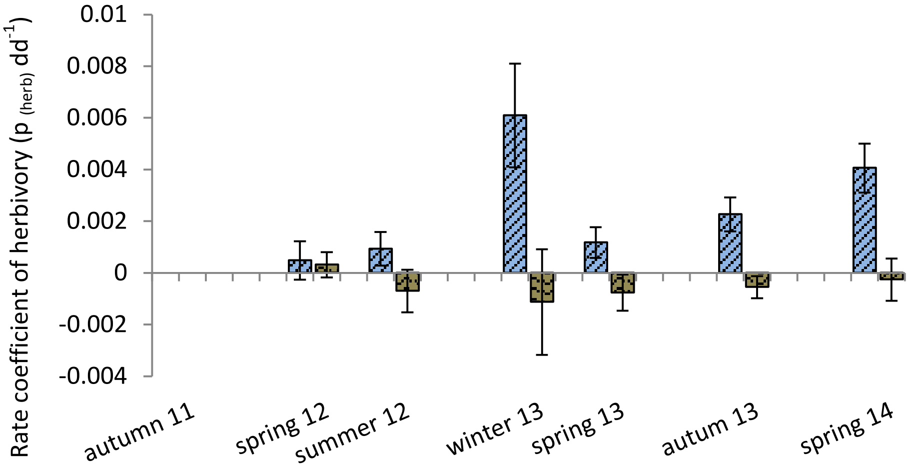


Figure 7:15 Seasonal variation in grazing rate coefficients: blue columns, Cool's Cottage; brown columns, Priors Farm; bars are standard error.

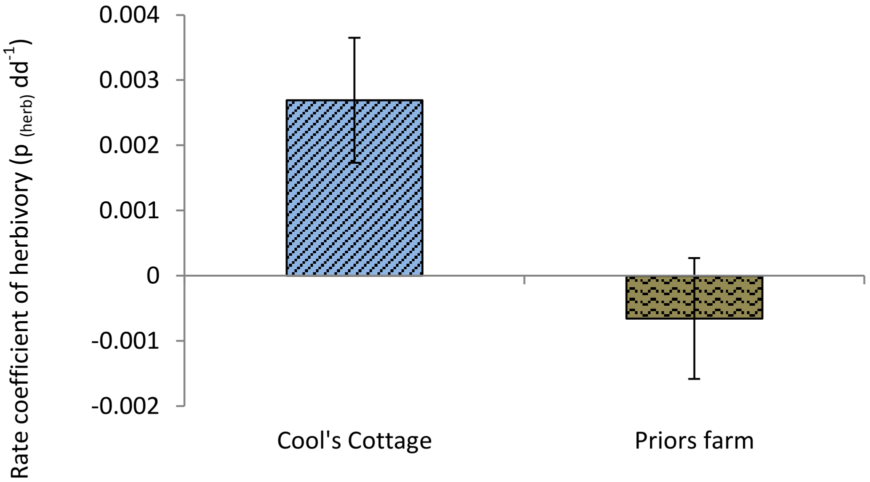


Figure 7:16 Comparison of herbivory in the two study reaches;: blue column, Cool's Cottage, brown column, Priors Farm, error bars, standard error.

7.3. Macro-invertebrate communities

Macro-invertebrate abundances, identification and trait data were provided by the QMUL River Communities Group: these data extended over most of the study period from October 2011 to November 2013. Two sites were sampled in each stream using the RIVPACS standardised semi-quantitative three minute kick sample (Clarke et al., 2011). However, due to the time required for sample processing, full data sets were only available for both sites per stream for the first three sampling dates that functional measures were collected, and one site per stream thereafter. Where these data were available, temporal variation in total macro-invertebrate abundance and in taxon richness were reported as the mean. Macro-invertebrate abundance was higher in the Cool's Cottage reach than in the Priors Farm reach, although there was high within-stream variability (Table 7:8). Where both replicates were available, site A was consistently and substantially higher than site B, introducing possible bias for the later samples where only site A data were available (inset, Figure 7:17).

Table 7:8 Macro-invertebrate abundance and taxon richness at Cool’s Cottage and Priors Farm.

	Cool’s Cottage					**Priors Farm**				
	Abundance				**Taxon count**	**Abundance**				**Taxon count**
	A	**B**	**mean**	***+/-***	**mean**	**A**	**B**	**mean**	***+/-***	**mean**
Spring ‘11	5174	4708	4941	*233*	62	3379	2151	2765	*614*	51
Summer ‘11	4466	1710	3088	*1378*	28	3485	1770	2627	*857*	42
Autumn ‘11	5473	2200	3836	*1636*	33	2527	3141	2834	*307*	57
Spring ‘12	8671	2343	5507	*3164*	38	2543	4818	3680	*1137*	50
Summer ‘12	9947	2289	6118	*3829*	37	6280	1908	4094	*1608*	46
Autumn ’12	4562				40	788				37
Spring ‘13	9630				49	894				29
Summer ‘13	5031				36	3185				35
Autumn ‘14	1408				42	2570				57
Total no individuals				**67612**		**39439**			**58%**	
Excluding dredged samples				**53420**		**37757**			**71%**	

The macro-invertebrate sampling site at Priors Farm was dredged in late summer 2012, following which there was a substantial drop in macro-invertebrate abundance (autumn 2012 and spring 2013), with abundance recovering by summer 2013. (Figure 7:17). The experimental sites, where the litter bags and tiles were deployed, were upstream of the dredged section and were not directly affected.

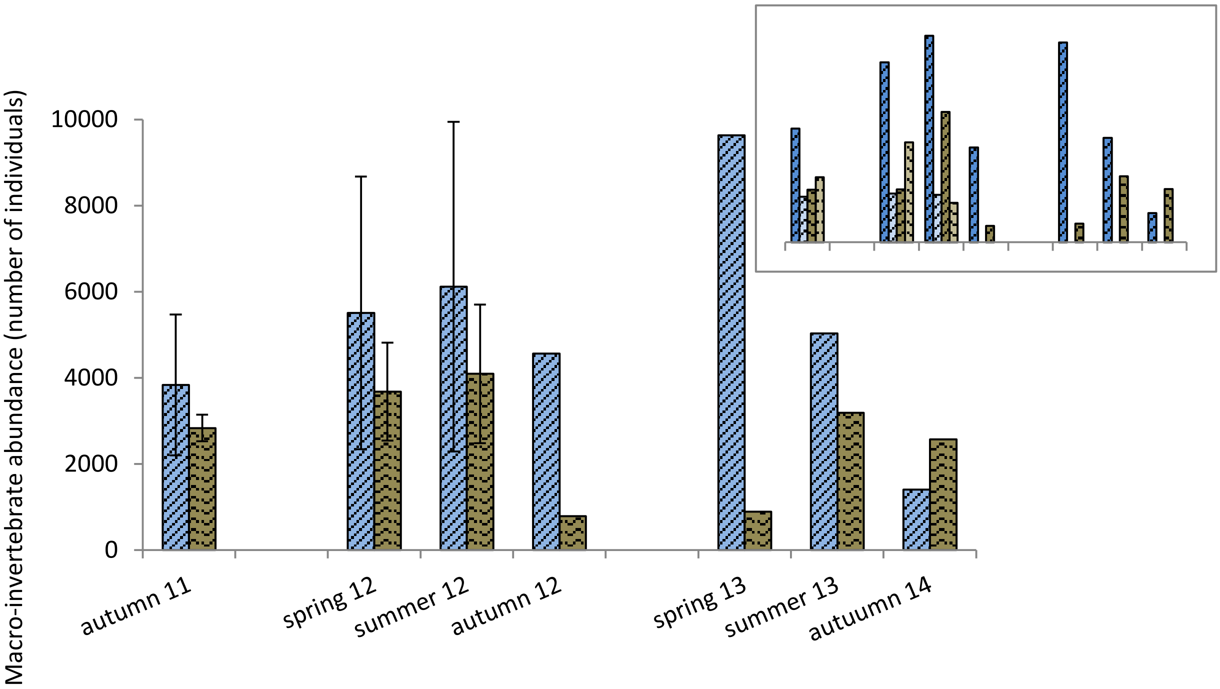


Figure 7:17 Seasonal variation in macro-invertebrate abundance at Cool's Cottage and Priors Farm: blue columns, Cool's Cottage; brown columns, Priors Farm. Inset: Illustration of the consistently higher macro-invertebrate abundance in site A at Cool's Cottage, thereby potentially conferring a bias in the samples for which there are no replicates.

When these sampling dates were removed from the calculation of relative abundance for the two reaches, the proportion of macro-invertebrate numbers at Priors Farm, relative to that at Cool's Cottage was 71% (Table 7:8). Taxon richness in the Priors Farm reach was slightly higher than in the Cool's Cottage reach (Table 7:8). This difference was largely made up of higher proportions of predators, absorbers and parasites in the Priors Farm reach, with the relative proportions of taxa assigned to scrapers, shredders, deposit feeders and filter feeders similar in both study reaches (Table 7:9, Figure 7:18).

Table 7:9 Contribution of functional feeding groups as a proportion of macro-invertebrate populations at Cools Cottage and Priors Farm. In this table and in figure 1.8, the assignation of many taxa to multiple feeding groups results in the percentages adding up to more than 100% of the number of individuals. In this context, 'observations' is the number of taxa, multiplied by the number of groups to which it was assigned.

Functional feeding group		**Cools' Cottage**		**Priors Farm**	
Scrapers	SCR	27646	41%	16055	41%
Shredders	SHR	27603	41%	17724	45%
Deposit feeders	DEP	23560	35%	14405	37%
Filter feeders	FF	17677	26%	11405	29%
Predators	PRE	2284	3%	7183	18%
Absorbers	ABS	1187	2%	4989	13%
Parasites	PAR	1073	2%	6883	17%
Piercers	PIE	90	0.1%	350	1%
Unassigned	UNA	747	1%	1151	3%
Total number of observations		**101867**		**80145**	
Total number of individuals		**67612**		**39439**	

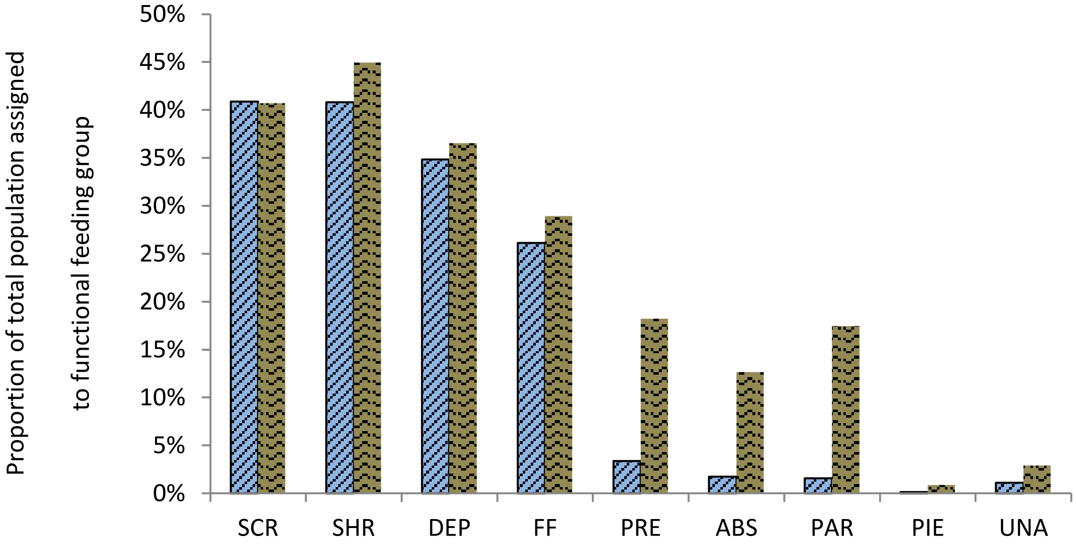


Figure 7:18 Distribution of functional feeding groups at Cool's Cottage and Priors Farm: blue columns, Cool's Cottage; brown columns, Priors Farm.
Abbreviations as for Table 7:9

The trait data of those taxa with a cumulative abundance of more than 100 individuals from all samples (Table 7:10) were studied in more detail. Many taxa are assigned to more than one functional feeding group, and the number of functional feeding groups to which they were assigned was used to infer their degree of specialism. There was a higher proportion of generalists (defined here as individuals assigned to multiple (>2) functional feeding groups) in the Priors Farm reach. This bias was also observed in functional feeding group specific comparisons for scrapers and shredders, the functional feeding groups expected to contribute most to the ecosystem processes of macro-invertebrate leaf litter degradation and herbivory measured in this study (Figure 7:19, a-c).

Of the taxa assigned to the shredder functional feeding group, *Gammarus pulex* was the most abundant taxon in the Cool's cottage reach, contributing 24% of the total number of individuals counted throughout the study period (Table 7:10) whereas in the Priors Farm reach, *Gammarus pulex* constituted only 4%, with *Assellus aquaticus* contributing 7%. *Chironomidae* (assigned to multiple functional feeding groups), were abundant in both reaches (16% and 25% at Cool's cottage and Priors Farm respectively). *Ptychoptera sp.*, (assigned to both shredder and deposit feeder functional feeding groups) contributed 2% to the total in the Cool's Cottage reach but were absent from the Priors Farm reach. Other taxa assigned to the shredder functional feeding group in the Priors Farm were the snails, *Anisus sp.*, *Potamopyrgus antipodarum* and *Gyraulus sp*. that together contributed 10% of all individuals counted. Both study reaches had large populations of *Pisidium sp*, a filter feeder (12% and 6% at Cool's cottage and Priors Farm respectively), and of *Tubificidae* (17% and 13 % at Cool's Cottage and Priors Farm respectively), a deposit feeding oligochaete, whose preferred substrate is mud and sand, reflecting the high proportion of fine sediments in the stream beds of both reaches (Chapter 3).

Table 7:10 Macro-invertebrate taxa with a combined total of more than 100 individuals from all samples within each site

Taxon	Cool's Cottage	Priors Farm
Agapetus sp.	137	
Anisus (Disculifer) vortex		1066
Asellus aquaticus		2620
Baetis rhodani	222	154
Ceratopogonidae	308	
Chironomini [tribe]	358	6065
Elmis aenea	112	180
Ephemera sp.	154	
Gammarus pulex	16424	1750
Gyraulus sp.		126
Habrophlebia fusca		169
Helophorus sp.		272
Limnius volckmari	825	
Limnephilidae	196	508
Orthocladiinae [sub-family]	715	811
Oulimnius sp.		271
Pisidium sp.	7830	2179
Potamopyrgus antipodarum		2807
Ptychoptera sp.	1351	
Simulium sp.	102	141
Tanypodinae [sub-family]	1107	790
Tanytarsini [tribe]	8427	2163
Tubificidae	11756	4937

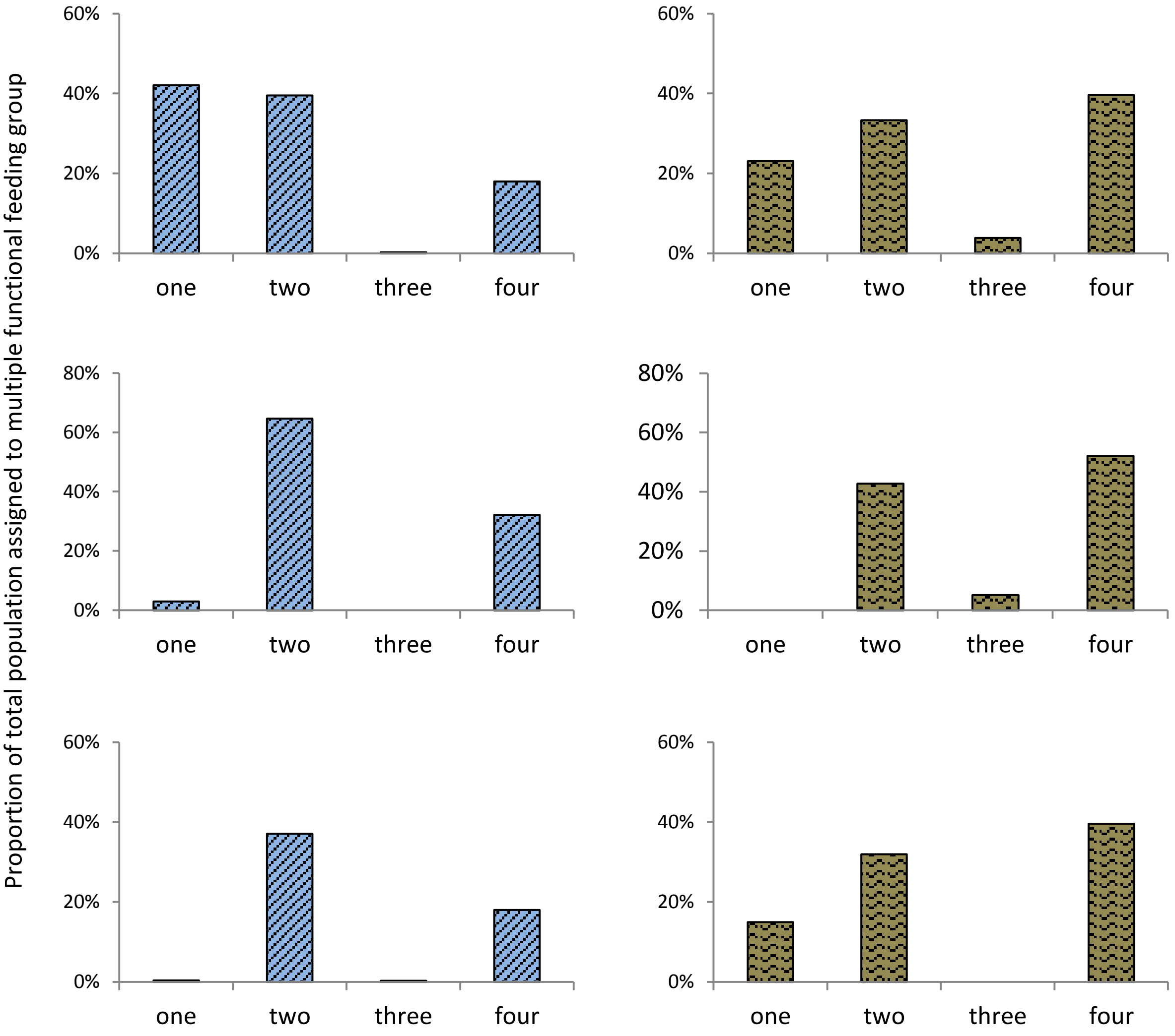


Figure 7:19 Proportion of taxa assigned to multiple functional feeding groups at Cool's Cottage and Priors Farm: a, proportion of all taxa with more than 100 individuals; b, proportion of scrapers and c, proportion of shredders. Blue columns, Cool's Cottage; brown columns, Priors Farm

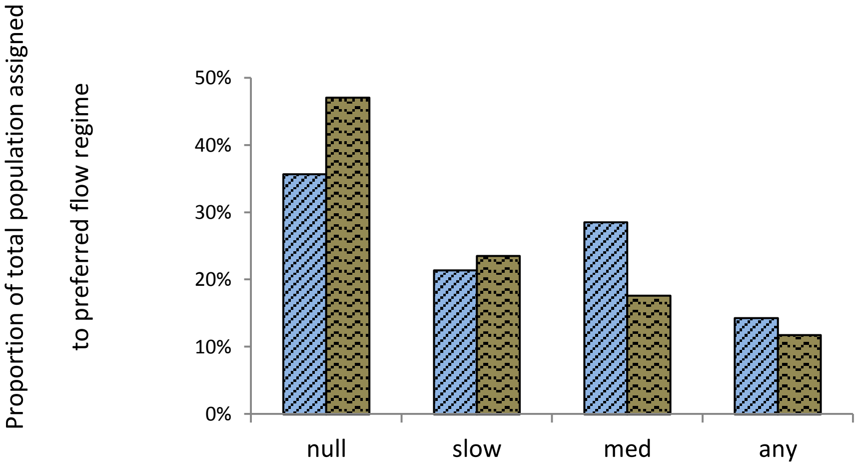


Figure 7:20 Flow regime preferences for those taxa with more than 100 individuals from all samples: blue columns, Cool's Cottage; brown columns Priors Farm.

Macro-invertebrate assemblages associated with the leaf litter packs were not quantified. However, visual assessments of the populations picked from the leaf packs, prior to drying and weighing, were broadly similar to the benthic communities. In the samples from the Priors farm reach, however, the contribution of *Assellus aquaticus* appeared to be greater than in the benthic samples, and *Limnephilidae* were commonly recovered from leaf litter packs from both streams. These apparent differences in density between benthic and litter pack assemblages suggested active feeding preferences in these two taxa.

The preference for flow regime was also examined and there was a slightly higher proportion of individuals in the Priors Farm reach that preferred, or tolerated zero or slow flow than in the Cool's Cottage reach (Figure 7:20).

7.4. Interactions between macroinvertebrate community structure and function

A range of factors, can affect both species richness and behaviour. In this section, process rates are discussed in the context of the macro-invertebrate community assemblages and possible errors arising from methodological bias. Further considerations of these findings, in the wider context of contrasting sub-catchment characteristics, are discussed in chapter 8.

The differing rates of macro-invertebrate detritivory and herbivory observed are likely to have been influenced by more than one factor. The lower macro-invertebrate abundance in the Priors Farm reach is unlikely to be the only cause of the observed low rates of ecosystem function, as not all species make the same contribution to process rates. Some species, although present in small numbers can have a disproportionate effect on ecosystem processes.

7.4.1. **Detritivory**

Macro-invertebrate abundance at Priors Farm averaged 71% of that at Cool’s Cottage (excluding the data immediately following dredging; Table 7:9), but rates of macro-invertebrate mediated detritivory ranged from 5% to 34% of that at Cool’s Cottage, except in February 2013 (Table 7:11).

Table 7:11 Macro-invertebrate detritivory at Priors Farm ($-k_{(invert)}\ dd^{-1}$); expressed as a percentage of that at Cool's Cottage

Season	**Priors Farm: Cools’ Cottage**
Autumn ‘11	34%
Spring ‘12	32%
Summer ‘12	5%
Winter ’12-‘13	**206%**
Spring ‘13	23%
Autumn ‘13	7%
Spring ‘14	17%

Research using mesocosms, aimed at untangling the drivers of leaf litter degradation, has found that macro-invertebrate numbers alone cannot explain rates of leaf litter degradation, and community diversity exerts a key influence (Woodward, 2009; Tolkkinen et al., 2013). Predictions based on the distribution of functional feeding groups can also be misleading as they are based on morphological characteristics and not necessarily related to expressed feeding preferences (MacNeil et al., 1997; Rawer-Jost et al., 2000; Baldy et al., 2007; Woodward, 2009; Lauridsen et al., 2014). For example, although *Asellus aquaticus* is assigned to the 'shredder' functional feeding group, its main feeding mode on leaves is to scrape rather than chew, and predominantly feeds on the fungal assemblages that colonise and condition them (Graca et al., 1993; Chung and Suberkropp, 2009). Examination of their gut contents (Lecerf et al., 2006; Baldy et al., 2007 Lauridsen et al. 2014) has also shown that *Asellus aquaticus* feed on fine particulate organic matter (FPOM). Species interactions, both within and between trophic levels, as well as species richness are important in influencing ecosystem function (MacNeil et al., 1997; Woodward, 2009; Tolkkinen et al., 2013). In this study, the shredder community at Cool's Cottage was dominated by *Gammarus pulex*, an efficient shredder. *Gammarus pulex* is less tolerant of organic pollution than *Asellus aquaticus* (Whitehurst and Lindsey, 1990; Maltby, 1995; MacNeil et al., 2002); the latter dominates the *Crustaceaea* at Priors Farm. However, where conditions are more favourable for *Gammarus pulex*, it out-competes *Asellus aquaticus* and may even feed on them (Fries and Tesch, 1965; Oseid and Smith, 1979). The contribution of macro-invertebrates to total leaf litter degradation ($-k_{(inv)}dd^{-1}/- k_{(tot)}dd^{-1}$) at Priors Farm was highest in February 2013, when the proportion of *Gammarus pulex* in the *Crustaceaea* was greatest (Table 7:12 and Figure 7:21). This ratio has previously been proposed as a good indicator of organic pollution (Whitehurst and Lindsey, 1990; MacNeil et al., 2002). Notwithstanding the uncertainties in the specificity of functional feeding groups, a higher proportion of individuals in the Priors Farm reach are assigned to multiple functional feeding group and are likely to feed preferentially on easily digestible and nutrient rich foods in preference to high C:N foods that require conditioning, such as oak leaf litter (Sterner and Elser, 2002; Woodward et al. 2009; Hladyz et al,. 2011; Lauridsen et al. 2014).

Table 7:12 The proportions of *Gammarus* in the Crustaceaea and macro-invertebrate mediated leaf litter degradation at Priors Farm.

Priors Farm

Season	**Proportion of Gammarus in the crustaceaea**	**Proportion of leaf processing by macro-invertebrates**
Autumn '11	24%	34%
Spring '12	8%	66%
Summer '12	26%	20%
Autumn/winter '12	70%	88%
Spring '13	95%	68%
Summer '13	15%	
Autumn '13	74%	16%
Spring '14		70%

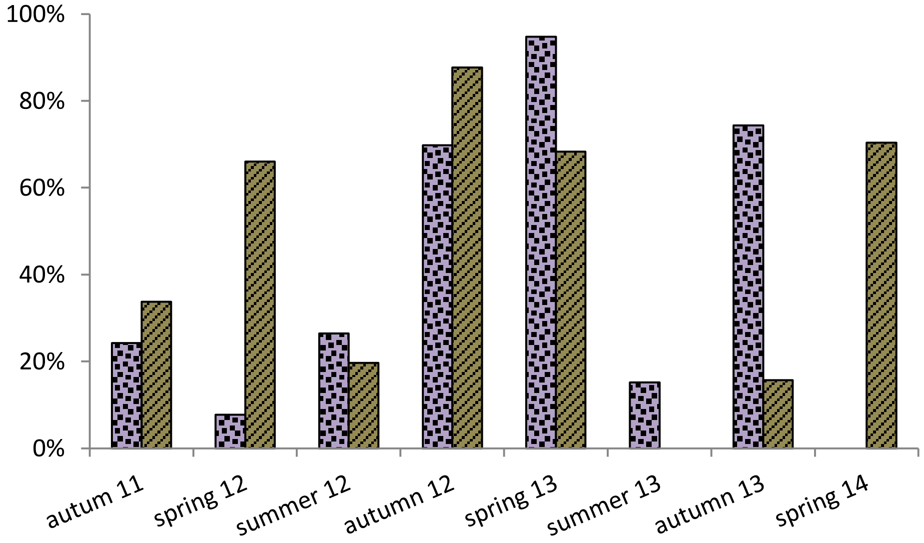


Figure 7:21 Relationship between *Gammarus pulex* and macro-invertebrate leaf litter processing at Priors Farm: stippled bars represent the proportion of *Gammarus* in the *Crustaceaea*, brown columns represent the proportion of total leaf degradation attributable to macro-invertebrates.

7.4.2. **Herbivory:**

Three of the four sites exhibited low photosynthetic primary production, echoing the findings in chapter 6 and indicating that shading was an important limiting factor, at least in the Priors Farm reach. In the Cool's Cottage reach, both sites were shaded in the summer and the extent to which light limited algal growth was, therefore, not assessed. In common with other eutrophic streams, filamentous algae were prolific in the Priors Farm downstream site and were observed at the upstream site before canopy closure. Filamentous algae were not observed in the Cool's Cottage reach, even in an un-shaded stretch of the stream downstream of the sub-catchment outlet. The low grazing rates in the Priors Farm reach suggest that filamentous algae may not be palatable to the crawling macro-invertebrates in this reach. As with the degradation of leaf litter, the ready availability of alternative food sources may have resulted in preferential feeding strategies for those taxa with the ability to exploit that resource (Lecerf et al., 2006; Baldy et al., 2007; Woodward et al., 2012; Lauridsen et al., 2014). In the Cool's Cottage reach, *Agapetus* sp. and Simuliidea were commonly found on the grazed tiles; although other potential grazers that were recorded in the benthic communities at Cool's Cottage, were not. *Agapetus* sp. have been shown to suppress populations of other grazing macro-invertebrates as a result of resource competition for periphyton biomass (McAuliffe, 1984b). Simuliidae are filter feeders and not constrained by this competitive pressure. Macro-invertebrates were not observed on the grazed tiles from the Priors Farm reach.

In addition to nutrient status and grazing pressure, physical factors impact on algal community structure and abundance (Law, 2011; Law et al., 2014b). The most obvious of these is light. However, stream velocity also plays an important role through the action of shear stress and, combined with sediment load, sloughing of attached algae through abrasion, known as scouring (Biggs and Close, 1989). Differences in stream velocity favour different algal morphologies due to variations in their attachment modes and strengths. Low growing communities that attach along their length, for example, tend to demonstrate stronger attachment than filamentous algae that attach via a stalk (Allan, 2007). On the other hand, stalked algae have a competitive advantage in slow flowing waters, particularly in low light or nutrient conditions, where the greater surface area in contact with the water promotes better nutrient uptake and access to light (Biggs et al., 1998).

During high flows, both average daily velocities and peak velocities during storm events (recorded at 15 minute intervals) were higher at the Priors Farm downstream site than at the Cool’s cottage downstream site (Figure 7:22).

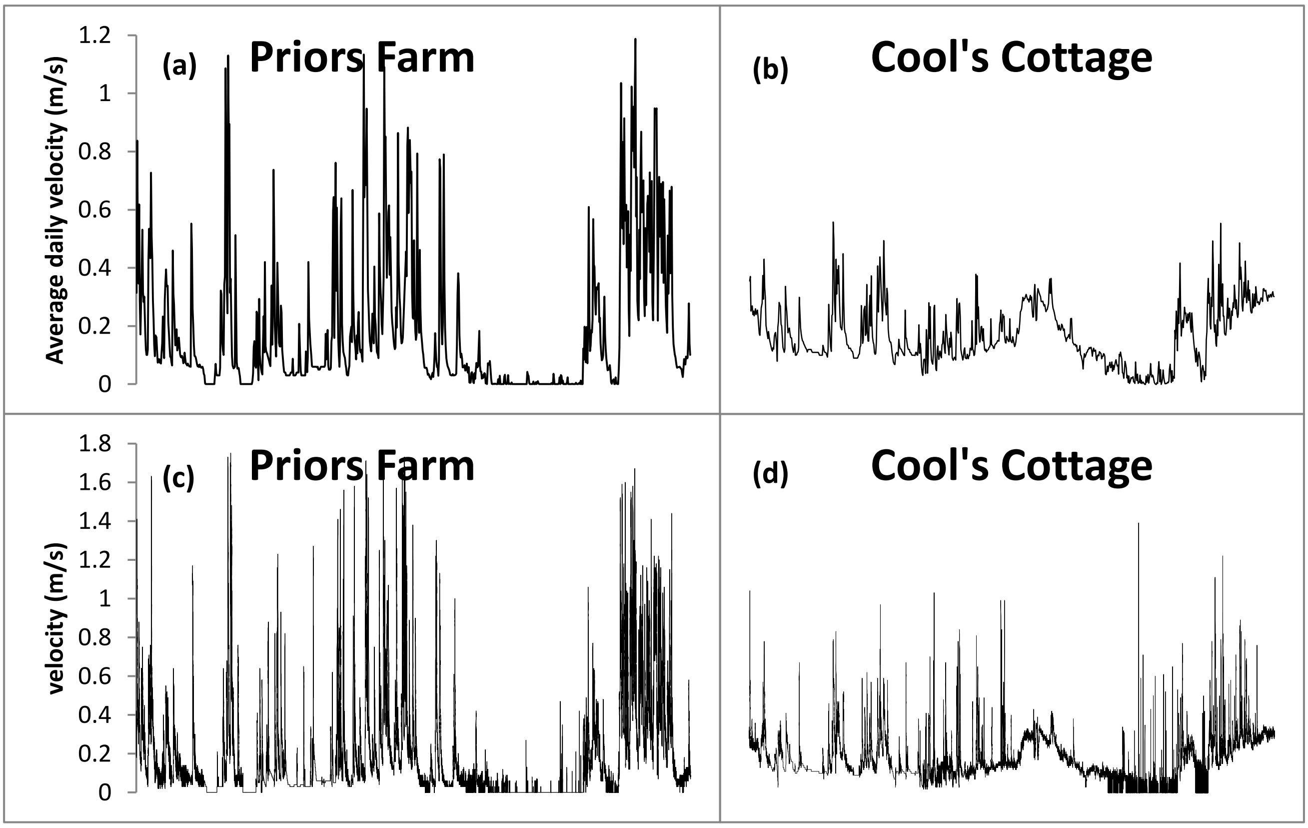


Figure 7:22 Stream velocity between December 2011 and April 2014 in the two study reaches: (a) and (b), average daily velocity; (c) and (d), high resolution velocity (15 minute intervals), showing short term response to rainfall events.

Nevertheless, filamentous algae were abundant in the Priors Farm reach, but were not observed in the Cool's Cottage reach during the study period. The seasonal pattern of accumulation at Priors Farm, taken together with velocity data, suggest that shear stress due to these higher flows did not constrain algal accumulation during this study, with the possible exception of February 2013 (Figure 7:23).

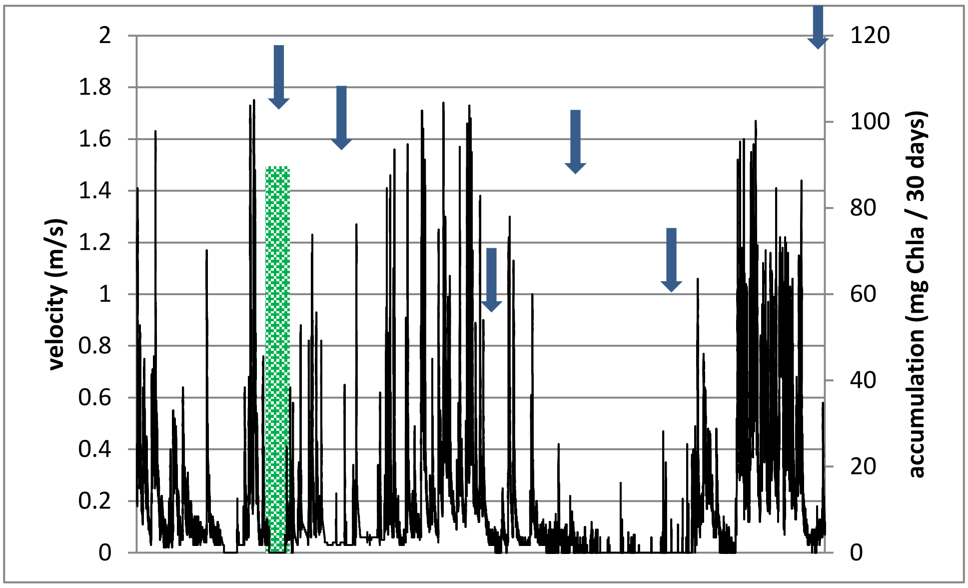


Figure 7:23 High resolution velocity (m/s) at the Priors Farm downstream site showing periphyton accumulation (mg Chla) during 30 day ceramic tile incubations.

Both study reaches were impacted by high loads of fine sediment (Chapter 3) giving similar conditions for scouring as a result of abrasion. High flows that occurred during deployments of the ceramic tiles used to measure phytoplankton accumulation and macro-invertebrate herbivory, may have resulted in some scouring and loss of algal standing stock. The experimental sites were chosen to represent similar deployment depths and substrate (Chapter 4), and the proximity of the study sites and simultaneous deployments ensured both study reaches experienced similar meteorological conditions, allowing a representative comparison between sites. In addition, for the calculation of herbivory, each tile in a paired experiment was subject to the same conditions of stream velocity, turbulence and shear stress, with the un-grazed tile acting as an internal control for the grazed tile. Thus, the experimental protocol allows for the measurement of realistic, *in situ*, accumulation and macro-invertebrate herbivory, within the constraints of methodological bias that are further explored in section 7.5.

7.4.3. **Top-down predator-prey interactions**

Fish were more abundant in the Priors Farm reach than in the Cool's Cottage reach. Bullhead (*Cottus gobio*), stone loach (*Barbatula barbatula*), minnows (*Phoxinus phoxinus*) and sticklebacks (*Gasterosteus aculeatus*) were all recorded at Priors Farm, and they may have had an effect, both on the abundance of macro-invertebrates and on their feeding behaviour. In order to avoid visual predators, macro-invertebrates avoid the exposed surface of the tiles and stick to more protected environments in the gravel. This effect has been reported following the introduction of predatory fish that reduced macro-invertebrate mediated herbivory and detritivory more than it reduced macro-invertebrate density (Jones pers. Comm.). The American signal crayfish (*Pacifastacus leniusculus)*, found at Cool's Cottage, but not at Priors Farm, is also an efficient predator, but it is unlikely that similar avoidance tactics would be as effective against it.

7.5. Methodological bias:

The model used to calculate macro-invertebrate detritivory and herbivory relies on the assumption that the two measured processes rates from which they are derived are still in an exponential phase (Mitchell and Wass, 1996). If the faster of the two processes has reached the lag or stationary phase, the variable calculated from the difference will be underestimated. Furthermore, if the process to be calculated somehow benefits the slower process measured, an apparent negative rate may occur in the calculated variable. For example, if periphyton growth on the un-grazed tile, $p_{(gross)}dd^{-1}$, has reached a density where it is subject to self-shading, growth on the grazed tile, $p_{(net)}dd^{-1}$ may catch up over the course of the incubation, leading to an underestimate of herbivory, $p_{(herb)}dd^{-1}$. It is also possible that counterintuitive positive effects of grazing can occur, for example if sediment builds up around the algae and restricts growth, grazing macro-invertebrates on the grazed tiles may displace this sediment, potentially increasing $p_{(net)}dd^{-1}$ and resulting in a negative value for $p_{(herb)}dd^{-1}$.

Periphyton accumulation at the Priors farm downstream site reached a maximum of 119 mg Chl-a 30 days^{-1} in spring 2014. It is possible that this represents a maximum threshold for periphyton growth at this site, thereby leading to underestimates of $p_{(herb)}dd^{-1}$. However, much denser periphyton growth developed on the tiles that remained in situ between deployments, suggesting that the stationary phase of growth had not been reached during the 30 day incubations and $p_{(herb)}dd^{-1}$ was not underestimated as a result of this potential methodological bias. Certainly, higher growth rates have been observed in other nutrient rich rivers. For example, Bowes et al. (2012) measured algal accumulation of 140 mg Chl-a m^{-2} over nine days in the Thames. In addition, calculation of the rate of herbivory at the Priors Farm downstream site during deployments with much lower rates of periphyton accumulation, yielded similarly low values. Taken together, these results do not provide evidence to reject the findings presented in section 7.2.3. Intermediate sampling of a subset of tiles part-way through the incubation would help to resolve whether these potential sources of error are significant in future deployments.

In the measurement of macro-invertebrate detritivory, rapid degradation in the coarse mesh bags, $-k_{(tot)}dd^{-1}$, may lead to resource limitation and an underestimate of $-k_{(invert)}dd^{-1}$. From the data presented here, rapid processing of leaf litter in the Cool's cottage reach may have led to resource limitation in the spring and summer deployments when up to 85% of the material was lost by the time the leaf bags were recovered (Table 7.2). This may have resulted in an underestimate of the absolute value of $-k_{(invert)}dd^{-1}$ on these dates. However, the patterns of both seasonal variability and overall litter processing in comparison to the Priors Farm reach remain unchanged.

Further errors in the estimation of $-k_{(invert)}dd^{-1}$ may arise from well recognised limitations in the litterbag methodology. Preferential loss of leaf litter from the coarse mesh bags due to abrasion during high flows is one such factor, as the fine mesh bags are likely to offer more protection from physical damage. It is possible that this could have contributed to the heightened rate of macro-invertebrate mediated leaf litter degradation in the Priors Farm reach in February 2013. However, this proportional increase was not seen in the Cool's Cottage reach that was subject to the same increase in flow regime, and is likely to have experienced similar rises in turbulence and suspended sediment during this deployment. Another factor that can increase the uncertainty in measuring rates of litter loss is the leaching of material, early in a deployment. The relative contribution of leaching to total degradation is highly variable and related to leaf species, velocity and water temperature (Barlocher, 2005a). In this assessment of the contrast in the dynamics of leaf litter degradation between two neighbouring reaches, such losses were assumed to be comparable between the study sites. The low rates of mass loss in the fine bags in the Priors

Farm reach suggested that losses due to leaching were low during this study. Consequently, calculations of litter degradation were not adjusted for leaching and abrasion.

7.6. Conclusions

The data presented in this chapter demonstrate a significant suppression of the key ecosystem processes, detritivory and herbivory in the Priors Farm reach when compared to the Cool's Cottage reach. The contrast in both overall process rates and their seasonal variability was very marked between the two study reaches. Seasonal sampling revealed strong minima in macro-invertebrate detritivory during the autumn at both sites, the season traditionally chosen for studies of leaf litter degradation. Differences in the macro-invertebrate assemblages were also recorded. However, biotic indices based on community structure were less emphatic in their distinction between the streams (chapter 3) and the scale of the difference in process rates between the two study reaches was greater than the differences in benthic macro-invertebrate communities might suggest. This echoes the finding of other research where community structure and function have been found to respond differently to environmental stressors (McKie and Malmqvist, 2009). They indicate that a change in macro-invertebrate behaviour, over and above changes to community structure, arise from the environmental stressors that impact on key ecosystem processes. This suggests that targeted on-farm mitigation works have the potential to promote rapid improvements in ecosystem function delivered by these processes, where a change in behaviour may be a faster response than a change in community structure. For example, a substantial reduction in the delivery of organic rich fine sediments may alter the feeding habits of macro-invertebrate detritivores and herbivores.

One unexpected outcome of this work was the independence of the measurement of herbivory to light at the Priors Farm downstream site, where the high productivity might have been expected to obscure any conclusive results. The data presented here show that these measures of ecosystem function provide a sensitive and straightforward, complementary tool to assess in-stream health and recovery. Further considerations of how the differences in sub-catchment characteristics have impacted on these processes are discussed in chapter 8.

Chapter 8. In-stream ecosystem functional response to variations in the aquatic environment

The main aims of the project were: to describe and compare the in-stream chemistry of two headwater streams flowing through agricultural land with similar land use and underlying geology; to identify the consequences of contrasting in-stream chemistry to ecosystem function, and to assess the potential for using ecosystem functional metrics as an aid to management, by providing complementary measures of stream health to augment currently used structural measures. Current standard methods are based on describing what is in the stream – not what it is doing. Characterising the community structure is time consuming and highly dependent on extensive expert knowledge. In addition, community structure does not take account of behavioural changes in the community, whereas functional changes give an integrated picture of both community structure and behaviour; and may be more readily understood as having a direct bearing on the provision of ecosystem services.

8.1. Key findings

8.1.1. Contrasts in water chemistry and in-stream environments

A detailed comparison of the study sub-catchments provided evidence that, despite broad similarities in land use and the geology through which the streams flow, a combination of differences in source geology and land management contributed to contrasting in-stream environments that impacted on communities and ecosystem function (Figure 8:1, A – F).

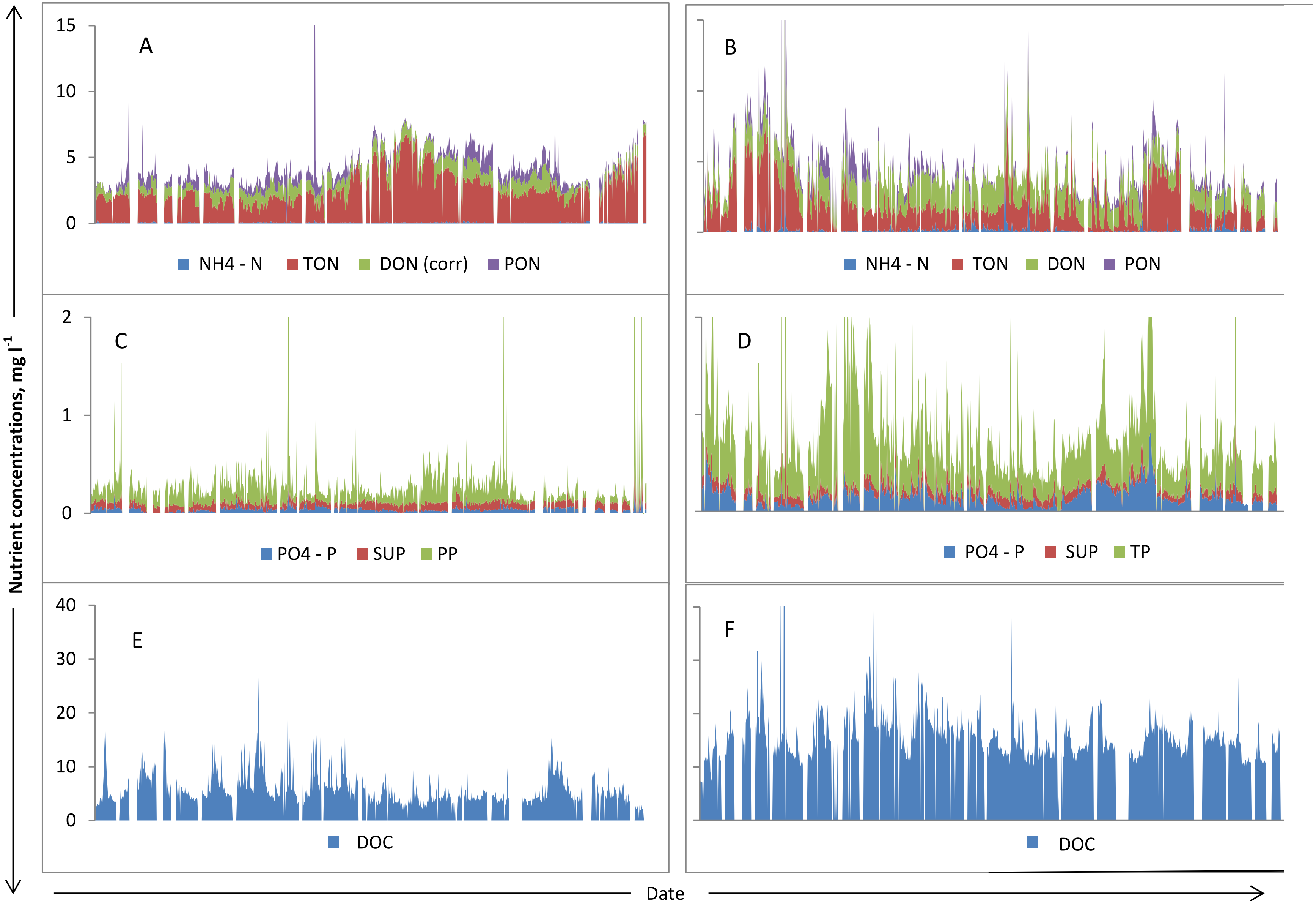


Figure 8:1 Illustration of the contrasting nutrient chemistries at Cool's Cottage and Priors farm; A, C, E; Cool's Cottage: B, D and F; Priors Farm

While the underlying geology of both streams is based on clay and subject to the flashy flow regime associated with impermeable catchments, the Cool's Cottage study reach was supplemented by groundwater sources and water supplied from a local reservoir that maintained flow throughout the study period. The residence time for these sources was increased by the presence of a lake that acts as a header tank for the reach, allowing substantial in-stream processing to occur and influencing the chemistry of the study reach below it. Land cover upstream of the lake is largely woodland, and sources of organic matter entering it are likely to be derived from leaf litter decomposition and characterised by high C:N ratios. Downstream of the lake, the land is farmed organically and used as pasture for a beef herd. Waste is managed as solid manure. Cattle are excluded from the pasture in the winter and stock movements are minimal throughout the year. The remaining areas of the upper catchment are farmed more intensively with strip grazing of dairy cattle and some arable land supplemented with inorganic fertilizers. The organically managed pasture, therefore, acts as a buffer zone and, despite visible inputs of particulates from a road drain upstream of the study reach, the stream is characterised by low concentrations of DOC, DON, SRP and PP when compared to the Priors Farm reach (Figure 8.1 A – F).

The Priors Farm study reach is supplied by surface water runoff and interstitial flow from the sub-catchment, with no apparent groundwater sources. As a result it was subject to periodic episodes of zero flow resulting in isolated pools along the study reach. The land was predominantly used as pasture for dairy herds. Cattle were excluded from the pasture during wet weather. However, while outdoors, stock were regularly moved from pasture to the farm for milking, resulting in the mobilisation of fine sediments with increased organic matter content, that were transported to the stream in wet weather. Additional inputs of fine sediments resulted from surface runoff from an arable field adjacent to the stream. Waste in this sub-catchment was largely managed as slurry, and slurry stores had insufficient capacity during the extreme wet weather experienced throughout 2012 and the winter of 2013 – 2014; this resulted in pulses of discharge characterised by low dissolved oxygen, high DOC and high NH_4-N concentrations reaching the stream (chapter 5).

While both streams were impacted by high loads of fine sediment, the Priors Farm reach was significantly enriched with organic matter when compared to the Cool's Cottage reach. Together with intermittent low or zero flow, this resulted in several periods when concentrations of dissolved oxygen in the Priors Farm reach were severely impaired, with implications for both short term metabolic processes and macro-invertebrate community structure and behaviour.

Reports of ecosystem functional response to environmental stressors often use concentrations of dissolved inorganic nutrients, usually SRP and DIN, as indicators of anthropogenic impact. In these livestock dominated catchments, dissolved inorganic nutrients often represent only a small fraction of the total nutrient load (chapter5). In the Cool's Cottage reach; only 15% of phosphorus is represented by SRP, and 27% in the Priors Farm reach. Phosphorus associated with particulate matter (PP) dominates the phosphorus load in both study reaches; 64% in the Cool's Cottage reach and 57% in the Priors Farm reach, with a significant proportion present as SUP; 21% in the Cool's Cottage reach and 16% in the Priors Farm reach. Although DIN represents a higher proportion of the nitrogen load in both study reaches (58% and 45% in the Cool's Cottage and Priors Farm, respectively), DON and PON constitute substantial additional resources to the in-stream communities.

8.1.2. **Short term metabolic functional metrics**

Examination of the relationships between aerobic respiration and in-stream nutrient concentrations emphasised the reciprocal nature of nutrient / ecosystem process interactions. At Cool's Cottage, aerobic respiration was negatively correlated with both SRP and TON while positively correlated with DON, PON, PP and DOC; and at Priors Farm, aerobic respiration was negatively correlated with NH_4-N while positively correlated with DON and DOC. These results highlight a key area missing from our ability to model the effects of nutrient enrichment on ecosystem function; essentially, there is a lack of information on how much of these potential resources are readily available to the in-stream community. More refractory dissolved nutrients, and those in particulate form, can be accessed through the action of exo-enzymes. However, where more labile nutrients are available, the production, or activation of these enzymes is unlikely to be energetically favourable, resulting in a hierarchy of nutrient uptake. In chapter 6, the changes in speciation and fractionation of nutrients during 24-hour dark incubations suggested that readily available phosphorus may have been limiting in the Cool's Cottage reach during spring and summer, stimulating the release of available phosphorus from more refractory sources. Counter-intuitive increases in DOC during dark incubations in both reaches seemed to indicate that particulate organic matter was also being utilised to supplement growth. Overall, in both study reaches, aerobic respiration was low and suggested medium to severe impact using the framework proposed by (Young et al., 2008). High concentrations of DOC, N and P in the Priors Farm reach weaken any suggestion of nutrient limitation and suggest that low diffusion of oxygen into the sediments is the most probable limiting factor in this reach. Poor oxygen availability in the sediments is also likely to impact the Cool's Cottage reach.

The increase in fine sediments clogging stream beds is a recurring feature of anthropogenic impact. Where this is combined with increasing organic matter, the prevalence of anoxic sediments are set to increase and a better understanding of anaerobic processes becomes a priority. Temperature exerts a strong influence on the production of greenhouse gasses (Bonnett et al., 2013), making increased greenhouse gas emissions from stream beds a likely consequence of predicted temperature rises. Measurements of greenhouse gas exchange across the sediment–water interface provided an indication that the suppression of aerobic respiration increased the resources available to anaerobes and amplified the warming potential of impacted streams through the production of CH_4 and N_2O. In the Cool's Cottage reach, TON was the dominant nitrogen species and N:P ratios did not fall below the threshold proposed by Sterner and Elser (2002) as the switch between nitrogen and phosphorus limitation (chapter 5). The transfer of N_2O across the sediment-water interface in the Cool's Cottage reach suggests this resource was exploited as an electron acceptor in denitrification, while at Priors Farm, where the N:P ratio was lower, N_2O production was also lower (chapter 6). A striking result was the substantial difference between methane transfer across the sediment-water interface at the two sites in the Priors Farm reach. Methanogens are obligate anaerobes and, therefore, unlikely to be present in the upper layers of sediment that are exposed to light and oxygen. Nonetheless, methane transfer across the sediment-water interface at the unshaded downstream site in the Priors Farm reach was up to two orders of magnitude greater than at the shaded upstream site which experienced similar in-stream water chemistry. These measurements of gas transfer across the sediment-water interface are examples of net ecosystem function – not processes per se. The underlying mechanisms controlling these functions need further investigation if management recommendations are to take account of these findings.

8.1.3. **Time integrated functional metrics**

For processes that are integrated over a longer time period (weeks rather than hours), environmental conditions were expected to act synergistically on the community within a reach. It was not expected, therefore, that relationships between process rates and single parameters would be as clear as those for the short term functional metrics described in chapter 6; this proved to be the case.

There were significant differences between the study reaches both in leaf litter degradation and macro-invertebrate herbivory. Macro-invertebrate mediated leaf litter degradation ($k_{(invert)}dd^{-1}$) was significantly correlated with macro-invertebrate abundance ($P < 0.05$) and relationships between nutrient chemistry and process rates echoed the differences in nutrient chemistry between reaches (chapter 7). Herbivory, on the other hand, showed no correlation with either macro-invertebrate abundance or periphyton accrual, but did exhibit a positive relationship with both discharge and velocity, and with nutrient concentrations that again reflected characteristic differences in nutrient chemistry between reaches. More detailed examination of the relationships between nutrient fractions and within-stream variability were inconclusive. However, there were suggestions of some possible nutrient limitation in the Cool's Cottage reach that impacted on algal accrual, and an increase in macro-invertebrate mediated process rates during higher flows at Priors Farm (chapter 7). Episodic discharges with high organic content and accompanied by flocculated material appeared to provide an alternative resource to both microbial and macro-invertebrate communities in the Priors Farm reach; this may have impacted on feeding choices (Lauridsen et al., 2014) and resulted in the reduced rates of detritivory and herbivory recorded in the Priors Farm reach when compared to the Cool's Cottage reach.

Macro-invertebrate processes are also affected by low DO concentrations. Uncertainties in the reliability of the data retrieved from the DO sensor at Priors Farm (chapter5) precluded statistical analysis of this potentially critical influence. However, the combination of low flow and high organic matter In the Priors Farm reach adversely affected dissolved oxygen concentrations, with extreme DO fluctuations at the unshaded downstream site and chronic low DO concentrations at the upstream, shaded site (chapters 3 and 5). Studies on the effects of low concentrations of DO on macro-invertebrates show limited lethal effects on prolonged (24 hr) exposure and increased deaths in survivors for up to 30 days thereafter (Maltby, 1995). Exposure to less extreme concentrations (particularly over prolonged periods as experienced at the upstream site in the Priors Farm reach) leads to behavioural effects that are accompanied by reduced feeding activity (Jones et al., 2009). *Gammarus pulex* is more sensitive to low DO concentrations than *Asellus aquaticus* (Maltby, 1995; Jones et al., 2009), leading to the dominance of *Asellus aquaticus* in the Priors Farm reach. The increase in the ratio of *Gammarus pulex* to *Asellus aquaticus* in the winter, when the reach experienced periods of high flow and increased DO concentrations, supports these observations.

8.2. Evaluating ecosystem functional metrics in assessments of stream health - and future directions

To be effective, a measure of stream health needs to be sensitive to the stressors of interest, and robust to other environmental factors. Three distinct groups of ecosystem function were assessed for their suitability as routine measures of functional integrity, thereby complementing current standard, structural measures. The results presented here indicate that all process measurements were sensitive to the pressures that arise from differing management practices. However, each has different limitations and implications both to logistics and resources.

8.2.1. **Community aerobic respiration**

Aerobic respiration is one of the most fundamental ecosystem processes and was proved sensitive to the different environments in the two study reaches (chapter 6). In its simplest form (measurement of changes in dissolved oxygen concentration in closed chambers as used in this study), it is straightforward, and relatively low cost. However, issues arising from low frequency of incubations were identified. Discrete measurements of community respiration failed to coincide with the episodic pulses of high organic matter that appeared to be a dominant factor in the loss of functional integrity in the Priors Farm study reach. Methods, such as one and two point, open-channel diel monitoring can be modified to provide continuous monitoring of community respiration (Uehlinger, 2006) that would overcome this limitation. Continuous monitoring of DO is a high cost, high maintenance option, especially in the conditions prevalent at the Priors Farm downstream site. However, reliable, continuous measurements of dissolved oxygen, combined with accurate measures of atmospheric pressure, would make possible the high frequency estimation of whole stream community respiration that is needed to capture the effects of these episodes on this key ecosystem process.

8.2.2. **Anaerobic respiration**

The net accumulation of greenhouse gasses across the sediment-water interface varied with contrasting in-stream chemistry. Although not individual process measurements, they represent a useful measure of ecosystem function in streams with a high proportion of fine sediments in the stream bed. Direct measurements of anaerobic processes present significant problems. Measurements using stable isotopes and narrow bore piezometers have demonstrated high within-site variability (Pretty et al., 2006; Sanders et al., 2007; Trimmer et al., 2009) and the methods are costly, making direct measurements unsuitable for routine use. However, the development of techniques to measure whole-stream nitrogen metabolism (analogous to the diel oxygen curves for photosynthesis and aerobic respiration) is gaining pace (Trimmer et al., 2012 and references therein), and future uptake of these methods would allow the inclusion of anaerobic processes in the improved assessment of aquatic ecological status.

8.2.3. **Time integrated functional metrics**

Rates of detritivory and herbivory were highly sensitive to the different environmental conditions experienced by the in-stream communities in the Priors farm and Cool’s Cottage study reaches. Process rates within this study fitted well into the proposed frameworks for assessing the degree of impact based on these key metrics. However, the bimodal response of these variables to dissolved inorganic N and P highlights the need for a better understanding of the availability of other nutrient fractions. The development of a rapid and reliable index of the availability of nutrients other than SRP and DIN, perhaps through a combination of biological assays and optical characterisation of DOM, would greatly assist in our understanding of the detrimental effects of high level pollution. In both processes, temporal variation in rates of detritivory and herbivory were much greater than within-stream variability. This seasonal variation emphasises the need for process measurements to be conducted throughout the year; for example, substantial minima in leaf litter degradation in the autumn (commonly the season chosen for studies of leaf litter processing) would result in underestimates of the differences between these two sub-catchments. Despite the strong influence of light on rates of algal productivity, rates of herbivory proved to be independent of light in the Priors Farm reach and highlighted the increased availability of alternative food sources in the Priors Farm reach. Methods for the measurements of leaf litter processing and macro-invertebrate herbivory are straightforward and inexpensive, making them suitable for routine assessments of in-stream ecosystem functional integrity.

Despite being one of the most obviously visible manifestations of nutrient enrichment, photosynthetic primary production proved the least useful variable in distinguishing between the study reaches. The overriding factor influencing this process was light, making it less suitable for many small headwater streams that are often shaded by bankside vegetation (unless clearance is the environmental stressor to be tested). It remains a useful measure of ecosystem function in unshaded reaches where the ratio of photosynthetic primary production to community respiration can be a powerful indicator of the extent to which these processes become de-coupled with increasing impact (Battin et al., 2008).

8.3. Conclusions

The outputs from this project confirm the value of measuring ecosystem function in assessments of aquatic ecological status. Extreme variation in environmental conditions, particularly in streams subject to episodic pulses of organically rich inputs (as in the Priors Farm reach), emphasises the importance of high frequency monitoring of all nutrient species and fractions as well as of key ecosystem processes. Future research into the availability of 'black box' nutrient fractions to the in-stream community is key to improving our understanding of the mechanisms behind detrimental human impact. Understanding the mechanisms behind deterioration of functional integrity in impacted streams is essential to effective targeting of management strategies.

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Appendix A – Index to data files on accompanying CD.

Data files for Chapter 3

1. Daily and monthly rainfall totals at Tisbury weather station: *(data provided by Environment Agency, SW region).*
2. Daily minimum and maximum river temperatures at the outlet to the Cool's Cottage sub-catchment: *(data provided by ADAS).*
3. Locations and Mg:Ca ratios for the spatial sampling sites in the Cool's Cottage and Priors Farm sub-catchments.

Data files for Chapter 5

1. Spatial variation in nutrient concentrations at weekly & occasional sampling sites in the Cool's Cottage and Priors Farm sub-catchments.
2. Nutrient fractions and NP ratios at the outlets to the Cool's Cottage and Priors Farm sub-catchments.
3. Daily discharge and nutrient concentrations at the outlets to the Cool's Cottage and Priors Farm sub-catchments: *(discharge data provided by ADAS).*
4. High resolution DO concentration at the outlets to the Cool's Cottage and Priors Farm sub-catchments: *(data provided by ADAS).*

Data files for Chapter 6

1. Summary of key short term aerobic metabolic indicators.
2. Aerobic metabolic rates during benthic incubations_master (for stats).
3. Nutrient transformations during benthic incubations.
4. Daytime dissolved oxygen readings at weekly sampling sites.
5. Greenhouse gas accumulation during benthic incubations_master (for stats).

Data files for Chapter 7

1. Absolute leaf loss by mass and degradation rates for individual deployments.
2. Leaf litter degradation rates_ master (for stats).
3. Absolute algal accumulation and herbivory; and process rates_master for stats).
4. Macro-invertebrate numbers and trait data *(data provided by QMUL_RCG).*
5. Stream velocity at Cool’s Cottage *(data provided by ADAS).*
6. Stream velocity at Priors Farm *(data provided by ADAS).*