University of Reading

Dissolved organic matter in riparian wetlands: concentration dynamics, chemical composition and implications for water quality.

Doctor of Philosophy

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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.

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Abstract

Wetlands are ecosystems capable of improving or maintaining water quality in surface water bodies. River water quality issues include long-term dissolved organic matter (DOM) increases that can affect the ecology of aquatic ecosystems. As such, a more detailed understanding of DOM in wetlands and its association with adjoining water bodies are required.

This study investigates the concentration dynamics and chemical composition of DOM in riparian wetlands. Furthermore, an assessment is made of the impact the studied wetlands have on the adjacent streams with respect to DOM and inorganic nutrient concentrations as well as metal toxicity. Two riparian wetlands with contrasting characteristics, and their adjoining streams, were selected for the study. One of the wetlands was a peat-forming, groundwater-fed wetland, located at a hillslope within an undisturbed surrounding area. The other was an ephemeral valley bottom wetland, located within an arable catchment.

Results show that DOM varies in time and space, quantity and character, within and among different wetlands. The drivers of this variation included wetland type, land use, land elevation, soil depth and temperature. Both wetlands demonstrated a capacity to act as DOM sources. Acute toxicity tests with *Daphnia magna*, showed that DOM exported from the peat-forming wetland can reduce tungsten toxicity. The ability of the ephemeral wetland to reduce or buffer phosphorus reaching the adjacent stream was challenged. Findings of this study showed that wetlands should not be considered as an effective mitigation measure for targeting diffuse pollution. Extrapolation from studied to non-studied wetlands should therefore be treated with extreme caution.

"There is no royal road to science, and only those who do not dread the fatiguing climb of its steep paths have a chance of gaining its luminous summits".

Karl Marx

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Symbols and notations

DOM	Dissolved organic matter
DOC	Dissolved organic carbon
DIC	Dissolved inorganic carbon
TDC	Total dissolved carbon
DON	Dissolved organic nitrogen
TDN	Total dissolved nitrogen
NH_4	Total ammonium
SRP	Soluble reactive phosphorus
SUP	Soluble un-reactive phosphorus
TDP	Total dissolved phosphorus
UV	Ultra violet
DTC	Demonstration test catchment
Kow	Octanol-water partition coefficient

Chapter 1- Introduction

Wetlands are ecosystems capable of improving or maintaining water quality in surface water bodies. Thus, growing concerns over river water quality, provide incentive on the study of the link between riparian wetlands and the adjacent streams and rivers.

River water quality issues include long-term dissolved organic matter (DOM) increases in rivers (Evans et al., 2005, Freeman et al., 2001a). Such an increase can affect aquatic ecosystems in various ways. DOM serves as a nutrient supply and major source of carbon and energy for microorganisms (Docherty et al., 2006, Qualls and Richardson, 2003, Young et al., 2004) and affects acidity (Driscoll et al., 1989, Garcia-Gil et al., 2004). It also influences light attenuation in the water column and photochemistry that affects the highly important photic zone and primary production in aquatic ecosystems (Costa et al., 2013, Jones and Lennon, 2015), as well as the bioavailability and toxicity of metals (Aiken et al., 2011). Despite the role of DOM in aquatic systems, DOM is a neglected factor in land and stream status management (Stanley et al., 2012). Wetlands are considered to be major contributors to stream DOM (Mulholland, 2008). Hence, the study of DOM behaviour within wetlands and the effect of wetlands on stream DOM is crucial for surface water management and mitigation measures. Investigation of DOM quality characteristics can provide indications of the DOM ecological function in the aquatic ecosystems and the effect on metal toxicity.

Concerns on river water quality are also related to inorganic nutrients. The concentrations of nitrogen and phosphorus have increased severely, because of intensification of agriculture and livestock production (e.g.Addiscott et al., 1992, Arheimer and Liden, 2000, DEFRA, 2009). Legislation in place sets goals on inorganic nutrient levels in streams, so that aquatic ecology is protected (e.g. Water Framework Directive 2000/60/EC, Nitrate Directive 91/676/EC). Wetlands have the ability to store and transform nutrients, therefore reducing or buffering nutrients reaching surface waters (Blackwell et al., 2009). Therefore, evaluation of the role of wetlands as diffuse nutrient pollution mitigations features is vital in achieving the goals set.

Tungsten is one of the least regulated metals for which evidence of the adverse effects on animals and humans is increasing. Studies on tungsten toxicity are especially relevant for sites close to military grounds. Furthermore, filling the gap in knowledge on

the effect of DOM in tungsten toxicity can provide evidence on the role of wetlands in tungsten toxicity in rivers.

The aim and objective of this study was to investigate the concentration dynamics and chemical composition of DOM in riparian wetlands and to assess implications for the adjacent streams with respect to DOM and inorganic nutrients concentrations as well as metal toxicity.

The research questions asked were:

RQ1. What are the concentrations of DOM and inorganic nutrients within the study wetlands, and do they differ spatially? (Chapter 5)

RQ2. What are the main factors, among topography (elevation), seasonality, wetland type, sub-catchment land use and depth that influence DOM and inorganic nutrients concentrations in wetlands? (Chapter 5)

RQ3. What is the status of the streams in terms of DOM and inorganic nutrient levels and the related ecological status as defined by the existing legislation? (Chapter 7)

RQ4. How does the river upstream and downstream of the wetlands compare in terms of DOM and inorganic nutrient concentrations? (Chapter 7)

RQ5. What are the differences in DOM quality in the studied wetlands? (Chapter 6)

RQ6. Is the study of tungsten toxicity relevant to the study area? (Chapter 8)

RQ7. What effect do differences in the levels and quality of DOM, in hardness and pH, have on the toxicity of tungsten to *Daphnia magna*? (Chapter 8)

To answer the research questions, two riparian wetlands with contrasting characteristics, and their adjoining streams, were selected for the study. One of the wetlands was a peat-forming, groundwater-fed wetland, located at a hillslope within an undisturbed surrounding area. The other was an ephemeral valley bottom wetland, located within an arable catchment.

The structure of the thesis is presented in Figure 1.1.

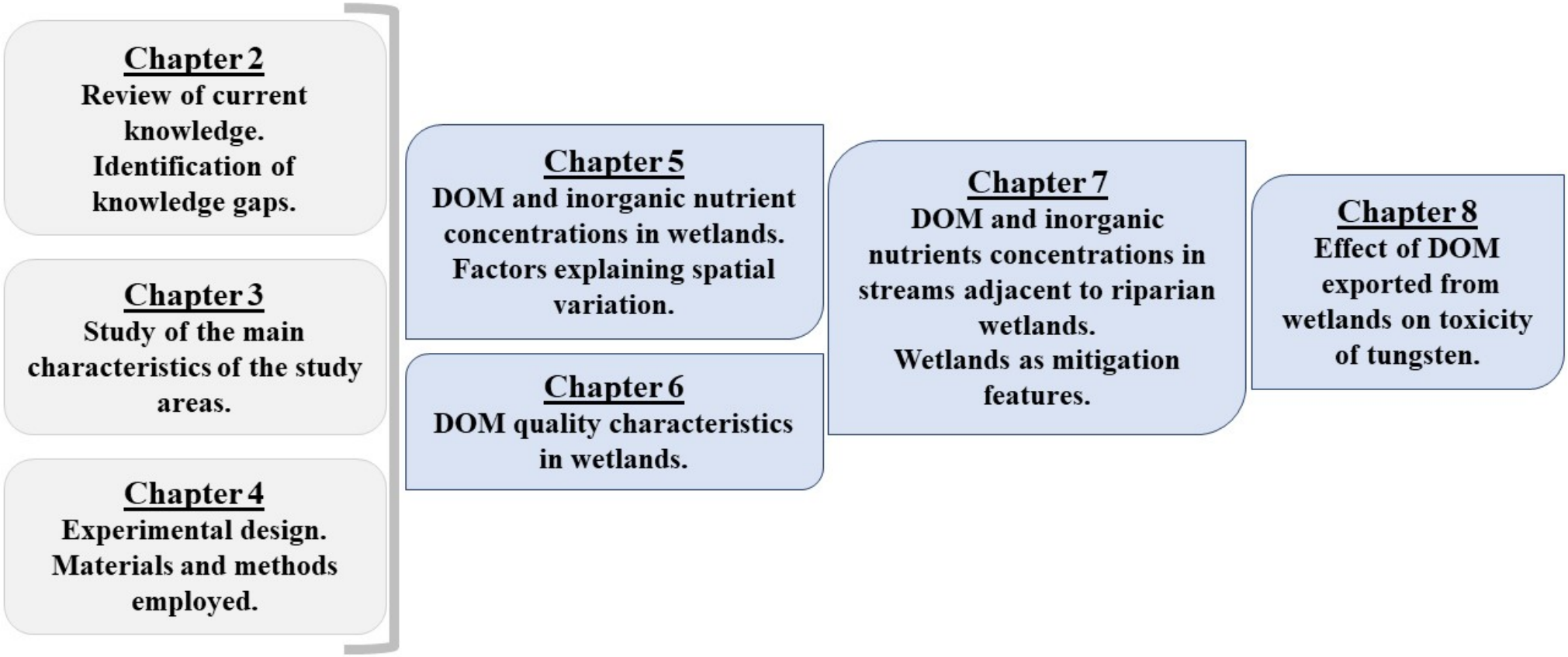


Figure 1.1 Thesis chapters diagram

Chapter 2- Overview of the role of dissolved organic matter in aquatic ecosystems. The distinct role of wetlands.

2.1 Overview

To meet the aims and objectives presented in Chapter 1, a detailed understanding is required of the significance of dissolved organic matter (DOM) in aquatic ecosystems, especially in relation to wetland biogeochemistry and contaminant toxicity. This chapter presents an overview of the current literature documenting the role of DOM in aquatic ecosystems, its interactions with metals, and the fluxes, transformations and transfers of DOM in wetlands.

2.2 DOM in aquatic ecosystems: Why it matters

2.2.1 Definition

DOM is a very broad term describing any organic component smaller than 0.45 μm. It is therefore very difficult to characterise its chemical composition, as it includes countless number of compounds. Some of these compounds have low molecular weight and are chemically recognisable; e.g. carbohydrate, amino sugars, peptides, proteins, lipids and lignin. Low molecular weight DOC is common in lowland and intensively farmed catchments and those with dense human populations. The remainder of the compounds do not belong to any discrete category in biochemistry; these exceedingly complex compounds are collectively known as humic substances, and comprise 80% of DOM (Thurman, 1985b).

Humic substances are naturally occurring, biogenic and heterogeneous, and can be characterised as being yellow to black in colour, of high molecular weight and refractory. Humic substances can be divided into three types based on water solubility at different pH values: humin (not soluble in water), humic acids (water soluble at pH > 2), and fulvic acids (water soluble under all pH conditions) (Aiken et al., 1985, Maccarthy, 2001, Stevenson, 1994). There are further differences between fulvic and humic acids. Compared to humic acids, fulvic acids have lower molecular weight, higher oxygen, lower carbon, and more acidic functional groups (Stevenson, 1982). The elemental composition of humic substances in natural water is shown in Table 2.1 (Thurman, 1985b).

Table 2.1 Elemental composition of humic substances in natural waters (Thurman, 1985b)

	%C	%O	%H	%N	%S
Humic acids and humin	50-60	30-35	4-6	2-4	0-2
Fulvic acids	40-50	50-55	>4	1-3	0-2

The origin of DOM can either be autochthonous (e.g. derived from algae and macrophytes) or of allochthonous origin (e.g. from anthropogenic and terrestrial sources). In aquatic solutions, DOM concentration, composition, and chemistry are highly influenced by the sources of organic matter, temperature, ionic strength, pH, major cation composition of the water, surface chemistry of sediment sorbents, and the presence of photolytic and microbiological degradation processes (Leenheer and Croue, 2003).

DOC is the most widely used parameter when referring to DOM. It was originally introduced by Malcolm and Leenheer (1973) and is the simplest measure of DOM. It is the main form of organic carbon in aquatic ecosystems and can reach concentrations of 50 mg/L (Thurman, 1985b, Wetzel, 2001). DOC mostly comprises humic substances, in percentages that are estimated to be 50% in rivers and 70-90% for wetlands. Hydrophilic acids and non-humic substances also form part of DOC (Aiken et al., 1985, Thurman, 1985b). Prairie (2008) characterised DOC as the "great modulator", pointing out that, unlike nutrients, it plays a distinctive role in modifying other variables in aquatic ecosystems. Stanley et al. (2012), in a review of the contemporary changes in DOC, argued that DOC should be an integral part of stream management. Hence this study focuses on DOC.

2.2.2 Role in aquatic ecosystems

DOM provides a major source of carbon and energy for microorganisms. Although the low-molecular weight, labile pool of DOM is more easily and rapidly degraded by bacteria, part of the high molecular compounds can also be broken down. Docherty et al. (2006) showed that DOM concentration and quality controls the structure and function of freshwater microbial communities. They found that the microbial community develops so that eventually the high molecular DOM can be metabolised. Biodegradability of humic and fulvic compounds was also confirmed by Young et al. (2004). However, biodegradation of humic substances is very slow and constrains

biodegradation of other fractions of the DOM (Qualls and Richardson, 2003). Low molecular weight DOM is utilised more rapidly i.e. is more biologically reactive compared to high molecular weight DOM (Khodse and Bhosle, 2011). Increase of lower molecular weight carbon results in elevated microbial abundance and activity (Wilcox et al., 2005).

DOM plays an important role in aquatic ecosystems. It serves as a nutrient supply as it transports significant amounts of carbon, nitrogen and phosphorus that are released by microbial metabolism and photo-degradation (Qualls and Richardson, 2003, Wiegner and Seitzinger, 2001). DOM influences light attenuation in the water column, due to its ability to absorb ultraviolet and visible available radiation. Costa et al. (2013) demonstrated that coloured DOM is the primary control on light attenuation in low suspended sediments rivers and the secondary control in high suspended sediment rivers. Terrestrial DOC correlates with light attenuation, affecting primary production and respiration (Jones and Lennon, 2015). DOM concentrations and chemistry have been linked to levels of acidity in wetland porewater, rivers and lakes globally (Driscoll et al., 1989, Eshleman and Hemond, 1985, Gorham et al., 1998, Kullberg et al., 1993). Humic acids have been proven to possess pH buffering capacity (Garcia-Gil et al., 2004). The effect of DOM, specifically DOC, on contaminant toxicity is discussed in section 2.3.

To summarise, DOM is very closely linked with the structure, function and productivity of aquatic ecosystems. There is evidence in the literature that DOM levels in rivers have increased. Freeman et al. (2001a) reported a long term rise of DOC levels in UK rivers. Evans et al. (2005) reviewed data from 22 UK upland rivers and showed a 91% average increase in DOC over the previous 15 years and reviewed similar trends in rivers of Northern Europe and America. DOM in rivers and streams is correlated with percentage of wetland area as described in section 2.4.

2.3 DOC effect on contaminant toxicity in aquatic organisms

First an introduction to basic terms of toxicology and ecotoxicology is needed. Paracelsus (1943 -1541) set the foundations of toxicology, noting that "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." In other words, toxicity is dose dependent. Toxicity can be defined as the relative (conditions dependent) ability of a substance to cause adverse effects in living organisms. Toxicity can be acute, effects caused over a short period of time, or chronic,

developed over longer periods (Waller and Allen, 2010). The fraction of the exposure dose that is available to biological response is defined as bioavailable (Rand et al., 2003). Bioavailability integrates complex processes such as contaminant interaction with the environment, transport to the organisms, passages across the biological membrane (Anderson and Hillwalker, 2010). Bioconcentration occurs when uptake of a contaminant into an organism is greater than elimination, whereas bioaccumulation is the total amount of the contaminant in the organism (Anderson and Hillwalker, 2010).

DOC affects the water solubility, bioavailability, bioconcentration and toxicity to aquatic organisms of several contaminants. DOC can bind to and affect the toxicity of hydrophobic chemicals (Chin et al., 1997, DePaolis and Kukkonen, 1997, Landrum et al., 1984, McCarthy and Jimenez, 1985a, McCarthy and Jimenez, 1985b, Servos and Muir, 1989). Bioavailability of chemicals is also controlled by DOC (Driscoll et al., 1995, Hassett and Anderson, 1982, Voice et al., 1983). Studies have shown that DOC in concentrations higher than 2 mg/l can reduce the uptake in organisms, bioconcentration and toxicity of hydrophobic chemicals with a log Kow higher than 6 (Qiao and Farrell, 2002). The effects of DOC on the bioconcentration of organic chemicals in aquatic animals have been summarised by Haitzer (1998), showing that bioconcentration generally decreases, but in concentrations lower than 10 mg/L the opposite effect has also been observed. DOC can increase the water solubility of chemicals, for example DDT (Hassett and Anderson, 1979, Matsuda and Schnitze, 1971, Ogner and Schnitze, 1970, Wershaw et al., 1969). Not only accumulation but uptake of organic micropollutants has been shown to be affected by different fractions of DOC (Kukkonen et al., 1990).

Metals comprise the largest group of identified chemical elements (Smith and Nordberg, 2015). Metals extensive use results in widespread exposure to living organisms that are unable to break them down (Nordberg et al., 2015). Metals can be toxic to living organisms including humans. The need to incorporate water quality parameters such as pH, hardness and organic carbon in environmental risk assessments of metals is widely acknowledged (Janssen et al., 2000). Metal toxicity standards are defined using models such as the biotic ligand model that integrate DOM complexation (Niyogi and Wood, 2004). Still further advances are needed in explaining and incorporating in models the trace metal complexation of DOM (Aiken et al., 2011).

Published studies presented in this section have proven that DOC decreases metal toxicity, thereby offering a protective role against metals in aquatic ecosystems. Wang (1987) presented a literature review to that date on factors affecting metal toxicity to aquatic organisms, showing that organic compounds are included in these factors. Since then more research has been published on the effect of DOC on the toxicity of metals to aquatic species. De Schamphelaere and Janssen (2004) used natural DOM from two lakes and a creek for toxicity tests and found that DOC concentration had a significant effect on copper toxicity regardless of source, presenting a linear decrease. A significant decrease in copper toxicity with increasing DOC concentration has been reported elsewhere; for example, Kramer et al. (2004); Park et al. (2009). Jo et al. (2010) showed that the toxicity of a copper and cadmium mixture declined with increasing DOC concentration and was not affected by the chemical properties of DOM. Although the literature is dominated by studies on copper, the same effect has been reported for other metals such as zinc (Heijerick et al., 2003), cadmium (Penttinen et al., 1998), cobalt-complexed cyanide (Little et al., 2007) and others. There is certainly scope for studying the effect of DOC in lesser studied metals.

2.3.1 DOC interaction with metals

Humic substances can decrease metal bioavailability by binding and consequently lowering toxicity. As described in 2.2.1, the chemical composition of humic substances is very heterogeneous. Therefore, the trace metal binding affinity of natural waters containing DOC of different composition varies (Benedetti et al., 1995). Toxicity of metal ions in natural waters is also affected by competition among ions for humic substances for binding sites (Kinniburgh et al., 1999).

There is an extensive range of studies on DOC complexation of metals. The most studied functional groups to which metal ions bind are carboxylic (-COOH) and phenolic (-OH) (Aiken et al., 1985, Dewit et al., 1993, Perdue et al., 1984). Other less abundant functional groups, such as thiols (-SH), and amines ($-NH_2$), can also bind trace metals (Herrin et al., 2001, Karlsson et al., 2006, Smith et al., 2002). Metal ion complexation by DOC modifies metal bioavailability as complexed metals are not bioavailable (Aiken et al., 2011). Aiken et al. (2011) showed that DOC also affect nanoparticles kinetics, clustering and aggregation that contribute to the dissolved metal pool.

2.4 DOM in wetlands

The word "wetland" was first defined by Shaw and Fredine (1956) as "lowlands covered with shallow and sometimes temporary or intermittent waters. They are referred to by such names as marshes, swamps, bogs, wet meadows, potholes, sloughs, and river-overflow lands. Shallow lakes and ponds, usually with emergent vegetation as a conspicuous feature, are included in the definition, but the permanent waters of streams, reservoirs, and deep lakes are not included. Neither are water areas that are so temporary as to have little or no effect on the development of moist-soil vegetation". Since Shaw and Fredine (1956), many definitions have been given. The Ramsar convention defines wetlands as "Areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh brackish, or salt including areas of marine water, the depth of which at low tide does not exceed 6 meters" (Navid, 1989).

The 2005 Millennium Ecosystem Assessment (MEA, 2005) estimated the global wetland ecosystem coverage to be more than 1280 million hectares, equal to 1.5 of Brazils' total area. While wetlands are continually under threat from destruction or degradation, they are ecosystems of paramount importance (Maltby, 2009). For centuries, many cultures have been dependent on wetlands, for example in rice growing regions (MEA, 2005). They benefit populations financially by providing support to fisheries, agriculture and tourism (Roggeri, 2009, Turner et al. 2009). Being an ecotone between terrestrial and aquatic systems, wetlands are unique ecosystems. They support biological diversity, have a great ecological significance and play an important role in the global water and carbon cycles (Maltby and Barker, 2009). The Ramsar convention has listed 1947 wetlands of international importance that cover 190,084,700 hectares. The services provided by wetlands are summarised in Figure 2.1, and include among others: provision of food, fresh water, fibre and fuel, climate regulation, groundwater recharge/discharge, erosion regulation and flood control.

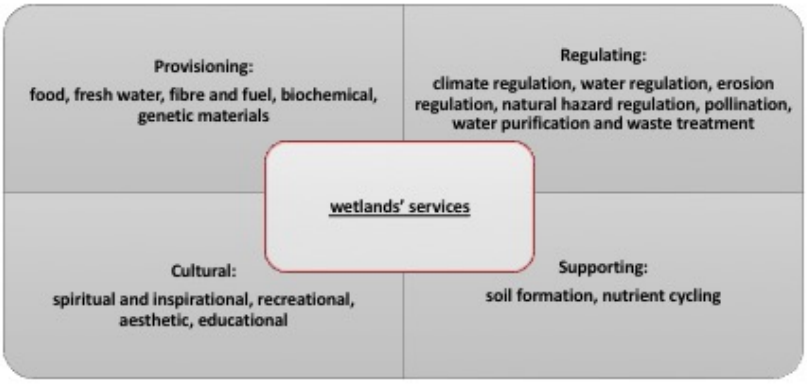


Figure 2.1 Wetland services (MEA, 2005).

Wetlands are known as nature's kidneys (Mitch and Gosselink, 2000). Because they improve the water quality of the passing water, they have been used as models for the engineering of treatment wetlands (Brix, 1994, Kadlec and Wallace, 2009, Vymazal and Březinová, 2015). However, the tendency is that wetlands are declining in size and at the same time larger amounts of contaminants are produced, which are often of increasing toxicity (Zedler and Kercher, 2005). Therefore, the dwindling wetland resource is not always able to control diffuse pollution (Catallo, 1993, Raisin and Mitchell, 1995). Many studies have dealt with the risks of contaminant inputs in wetlands to biota (Clark et al., 1993, Gross et al., 2004, Zillioux et al., 1993). The need to address the rise in agricultural pollution has led to an increase of constructed field wetlands (Ockenden et al., 2012). Still another aspect has been underestimated. Wetlands can play an important indirect role in toxicity and bioavailability of contaminants by exporting DOC.

Wetlands have been shown to be the main source of DOM to adjacent water bodies (Gorham et al., 1998, Hope et al., 1997, Mulholland, 1997). The increase of DOC in rivers and oceans has in many cases been associated with export from wetlands (Freeman et al., 2001b, Tranvik and Jansson, 2002). The export from wetlands supplies the aquatic ecosystems with humic substances (Stern et al., 2007) and labile forms of carbon (Schiff et al., 1998). However, wetland DOM export increases riverine DOM complexity (Wilson and Xenopoulos, 2009). DOM in wetland-affected streams is characterised as less labile and less microbially accessible compared to streams in agricultural catchments (Williams et al., 2010). DOM from wetlands can affect significantly the chemistry (Billett et al., 2006) and biology (Sun et al., 1997) of the receiving aquatic systems. The ecological significance of DOM and the link between

riverine DOM and wetlands signify that gaining a better understanding of DOC in wetlands can enhance our ability to manage catchments' ecological status.

2.4.1 DOM in wetlands

The transfers and transformations of DOC in wetlands have been described in many books; for example, Dise (2009), Mitch and Gosselink (2000), Reddy et al. (1999). The main processes involved in the aerobic zone is respiration, whereas in the anaerobic zone fermentation, methanogenesis, and sulphate, iron, and nitrate reduction dominate (Kayranli et al., 2010). In the aerobic zone, between the soil surface and water table (when wetlands are not completely waterlogged), DOC and particulate organic carbon (POC) originate from the senescence and decomposition of living material. The decomposition of organic material, such as leaves, is through the process of aerobic respiration, releasing carbon dioxide to the atmosphere. This process is mediated by detritivores and decomposer organisms (Dise, 2009).

As new organic material is deposited at the surface and older material is decomposed, at some depth below the surface oxygen becomes depleted and limits the rate of decomposition. As a result, POC becomes compressed in the anaerobic zone. With very low oxygen, or anaerobic conditions, fungi and microorganisms reduce complex DOC and POC compounds to simpler ones, such as lactic acid and ethanol, via fermentation (Dise, 2009). These simpler organic molecules in the anaerobic zone comprise an energy pool available for anaerobic organisms with different reduction efficiency. Denitrifying bacteria are more efficient compared to iron-reducing bacteria. Methanogenic archaea and sulfate reducing bacteria are the least efficient.

The organic fraction of nitrogen generally represents the majority of nitrogen in wetlands and is mostly not bioavailable, whereas the inorganic fraction makes up less than 5%. The organic nitrogen pool consists of amino acids, amines, proteins and humic substances. The inorganic pool in wetlands consists largely of ammonium (NH_4^+) plus nitrate (NO_3^-) and nitrite (NO_2^-) (White and Reddy, 2009). Organic nitrogen is converted biologically to ammonium by nitrogen mineralisation/ammonification in aerobic and anaerobic zones. Ammonium levels are positively correlated with nitrogen mineralisation rates (White and Reddy, 2000). Ammonification is dependent on soil microbial biomass (McLatchey and Reddy, 1998). Accumulation of ammonium in wetlands is also related to the anaerobic conditions leading to low nitrification rates.

Unlike nitrogen and carbon cycles that include gaseous products that escape the wetland ecosystem, the phosphorus cycle lacks such products and hence accumulates in wetlands. However, phosphorous is usually the primary limiting nutrient in wetlands and freshwater aquatic ecosystems. Organic phosphorus accounts for a substantial fraction of phosphorous in wetlands (Reddy et al., 1999, Richardson et al., 2007, Wetzel, 1983). Labile organic phosphorous can be enzymatically hydrolysed whereas complex forms of organic phosphorous such as fulvic and humic acids resist decomposition.

2.4.2 DOC fluxes in wetlands depend on external sources

DOC levels in wetlands depend on climatological and hydrological factors. Precipitation is a source of DOC in low concentration; but throughfall from tree canopies and stemflow can significantly increase the concentrations. It has also been shown that DOC from rainfall can be a significant source of low molecular weight carbon (Dalva and Moore, 1991, Likens et al., 1983, McDowell and Likens, 1988). Throughfall was found to increase DOC phenolics, carbohydrates, carboxylic acids, aldehydes, and amino acids and to alter the composition of precipitation DOC. This might be explained by leaf leaching and leaf washing (McDowell and Likens, 1988, Tukey, 1970).

Storms appear to have an important role in DOC fluxes. Hinton et al. (1998) found that due to leaching and DOC flushing during storms, the export from wetlands accounted for most of the stream DOC. Pastor et al. (2003) showed that the main factor controlling DOC export from peatlands, bogs and fens is discharge. This can be the decisive factor for either net export or net retention DOC. DOC in streams is correlated with storms and discharge, though flow-independent discharge of DOC from sewage treatment works can distort this relationship (Mann and Wetzel, 1995, McDowell and Likens, 1988).

Dissolved organic carbon concentration in wetlands can also show seasonality. This can be explained by thermal and hydrological regimes (Dalva and Moore, 1991), primary production and microbial activity (Bonnett et al., 2006, Mann and Wetzel, 1995) resulting in peak concentrations of DOC in summer. This trend might differ with the type of wetlands (Pinney et al., 2000). Bioavailability of DOC can also show seasonality, related to soil freezing and thawing (Wiegner and Seitzinger, 2004).

2.4.3 DOC levels and spatial distribution

DOC concentrations in soil pore water vary greatly in different wetland types. The values reported in the literature show a wide range. This range is further extended by the different methods and sampling depths studied by different researchers. Table 2.2 shows examples of values reported, ranging from 4.43 to 140 mg/L.

Subsurface pore-water chemistry depends on the hydrogeology of a wetland. By definition, wetlands can be ground water or rainwater fed and hence differ regarding the source, quality and levels of organic carbon. The position of the water table and duration of the water saturation determines the dominance of anaerobic processes over aerobic. Groundwater flow will also characterise discharge and recharge areas (Gilvear and Bradley, 2009). Hydrological flowpaths within the wetland also affect transfer and export of DOC. Geological characteristics, such as an impermeable clay layer, isolate the wetland system from the groundwater basin. The landscape location of the wetland also affects organic carbon levels e.g. hillslope wetlands. Elevation affects drainage and redox status causing carbon concentration to increase with distance down the hillslope profile, slope and elevation (Boothroyd et al., 2015, Creed et al., 2013, Hancock et al., 2010).

Variability in DOC concentration in soil pore waters occurs even at a small scale, affected by micro-topography in wetlands e.g. hummocks, hollows and lawns features. This phenomenon can be explained by fluctuation in the water table and hydrological flowpaths (Branfireun, 2004, Ulanowski and Branfireun, 2013). Local variations in soil type and profile characteristics also influence DOC levels in pore water. DOC concentrations in the topsoil are expected to be higher than the deeper horizons because of higher DOM input (Michalzik and Matzner, 1999, Qualls and Haines, 1991, Thurman, 1985b). However, Schiff et al. (1998) explained that although DOC in wetlands is higher closer to the surface, the maximum concentration occurs in the depth of highest accumulation coinciding with lowest hydraulic conductivity depth. Mann and Wetzel (1995) were inconclusive regarding DOC behaviour with depth. Similarly, Orem et al. (1997) reported a variety of DOC profiles with depth in the Florida Everglades. Some of the sites showed multiple DOC maximum values. More often the DOC concentration increased with depth reaching a maximum value and then decreased constantly. The fact that many researchers report average DOC across different soil layers presents additional difficulties in drawing conclusions on DOC behaviour with depth. Further research is

needed to study DOC profiles as a function of depth below the 30cm top soil; e.g. Olson and Al-Kaisi (2015); Hiederer (2009)). This need is boosted by studies suggesting that most DOC export is via the subsurface waters (Mann and Wetzel, 1995).

It is clear that DOC in wetlands is a complex system as it is affected by numerous factors that are not replicated in different wetlands. Research to date has focused on peatlands, especially in relation to carbon dioxide emissions. Other types of wetlands such as ephemeral wetlands have not received adequate attention (Boeckman and Bidwell, 2007). Many factors affecting DOC transfers and transformations such as hillslope position of wetlands have been overlooked (Boothroyd et al., 2015). Given the ecological significance of DOC, further research is needed to study the spatiotemporal behaviour of DOC in wetlands. Variation of DOC, even at the small (m x m) scale, presents the risk that under-sampling the wetlands could lead to invalid conclusions.

Table 2.2 DOC (mg/L) soil pore water levels reported in research publications.

DOC concentration (mg/L)	Wetland type	Measured at, using	Reference
27.1 in Bog, 32.1 in forested wetland, 14.6 in fen	bog, forested wetland, fen	piezometers in 25 cm depth	Fellman et al. (2008)
5-140 in wetland soil pore water	forested wetland and sloping bog	lysimeters at 10 and 20 cm depth	D'Amore et al. (2010)
63.7 ± 2.1 in pore water	poor fen peatland	piezometers at 25 cm depth	Kane et al. (2014)
22.23 ± 4.48 mean in July, mean 24.85 ± 1.45 mean in August	bog	pore-water sipper, 0 – 5 cm bellow water table	Ulanowski and Branfireun (2013)
15.05 ± 3.69 mean in July, 15.31 ± 1.51 mean in August	fen	pore-water sipper, 0 – 5 cm bellow water table	Ulanowski and Branfireun (2013)
32.5 ± 18.7 at 0 - 45 cm, 21.8 ± 10.1 at 50 - 100 cm and 21.4 ± 6.8 at surface	small headwater riverine wetland	dipwells	Bradley et al. (2007)
19 - 38	bog, fens	piezometers at 20, 50 and 90 cm depth	Moore (2003)
8.2 - 52.8 at 1 - 10 cm, 15.1 - 31.3 at 20 - 50 cm	peatland	suction soil water samplers	Clark et al. (2008)
59 overall average	forested swamp	perforated bottles at 30, 60 and 90 cm depth	Dalva and Moore (1991)
4.43 -9.70 at 0 cm, 17.80 - 33.99 at 20 cm, 6.32-21.46 at 30 cm, 7.10 - 48.17 at 60 cm, 7.49 - 30.90 at 90 cm, 5.97 - 31.14 at 120 cm	riverine wtland	porous ceramic cup samplers at 20, 30, 60, 90 and 120 cm	Mann and Wetzel (1995)

Chapter 3- Site description and study area

3.1 Overview

The selection of the two study areas was based on two main considerations. The first being the fact that the study is part of the Hampshire Avon Demonstration Test Catchment (DTC) project, jointly supported by the Department for Environment Food & Rural Affairs (DEFRA) and Environment Agency (EA). The project aims to reduce the impact of agricultural diffuse water pollution on ecological function, provide important findings to improve water quality status in UK waters, and to meet the EU Water Framework Directive targets. So site selection took into account location within the Hampshire Avon river catchment, accessibility of the wetlands and permission to install equipment and sample on a regular basis.

The second consideration was the need to address the research questions of the current thesis as described in Chapter 1; this required a paired study approach, using two wetlands with contrasting characteristics. The wetlands differ in hydrogeology and seasonality. Millersford is located in the New Forest National Park. This wetland is permanently wet, located along a shallow foot slope and alluvial toe slope, perpendicular to the flow of Millersford brook. The area is exposed to extensive grazing and is characterised by peaty soils and impermeable bedrock. The vegetation is uniform, consisting of mosses and grasses. Ebbesbourne is essentially a flat site at the bottom of an arable catchment. It is characterised by alluvial soils over gravel beds and chalk. It is seasonally dry and protected from grazing for most of the year. During summer the wetland is dominated by reed, canary grass and hemlock water dropwort vegetation. It is a bottom valley wetland that acts as a collection pool and protects the adjacent area from flooding. This gives it the potential to collect contaminants from the surrounding land use.

The contrasting characteristics of the wetlands give the potential for the comparison of quantitative and qualitative analysis of DOM between them to deliver valuable outcomes. The differences detected can provide links with geomorphology and hydrology, as well as land use. Association of DOM with wetland characteristics will be investigated in the next sections.

3.2 The Hampshire Avon River catchment

The Hampshire Avon River catchment (Figure 3.1) is situated in southern England. Covering an area of approximately 1,750 km^2, it has a human population of 230,000. The catchment is mainly rural with only 2% of the catchment urbanised (Environment Agency, 2012). The River Avon rises in the Vale of Pewsey and meets the English Channel at Christchurch. The river flows through the counties of Wiltshire, Hampshire and Dorset. Major commercial and residential areas include Salisbury, Fordingbridge, Ringwood and Christchurch. The upper catchment contains the major tributaries Ebble, Nadder, Wylye and Bourne. The geology is dominated by Cretaceous Chalk and is generally characterised by high bedrock permeability. The land is mostly rural, with intensive arable farming in the Chalk-lands (Marsh and Hannaford, 2008). The south of Salisbury part of the catchment is composed of small streams. It covers part of the New Forest national park and is dominated by heather moorland and woodland.

As evidenced by the Base Flow Index (BFI) ranging from 0.88 in Ringwood to 0.91 in Amesbury and East Mills (Table 3.1), the River Avon is groundwater fed. There is an indication that this might not be true for the small streams draining off the New Forest plateau. The BFI value for Dockens Water at Moyles Court (number 12 on the map) is 0.39. BFI is a unit of the fraction of river runoff originated from stored sources. Impervious clay catchments typically have BFI values of 0.15 to 0.35 whereas most Chalk streams have greater than 0.9 because of the dominance of the groundwater component in the river discharge (Marsh and Hannaford, 2008). The main hydrometric characteristics of the catchment are presented in Table 3.1.

Being one of the most biodiverse river systems in lowland England, the River Avon flows over 71 Sites of Special Scientific Interest (SSSI), 9 Special Areas of Conservation, and several Special Protection Areas and Ramsar designations. The New Forest National Park and the World Heritage Site of Stonehenge are important environmental sites within the catchment. Areas of Outstanding Natural Beauty (AONB) include Wessex Downs, Cranbourne Chase, West Wiltshire Downs. River Avon and its tributaries are habitat for internationally rare or vulnerable species such as flowing water vegetation (*Ranunculus* species), Atlantic salmon, bullhead, brook and sea lamprey, Desmoulin's whorl snail (Wheeldon, 2003) .

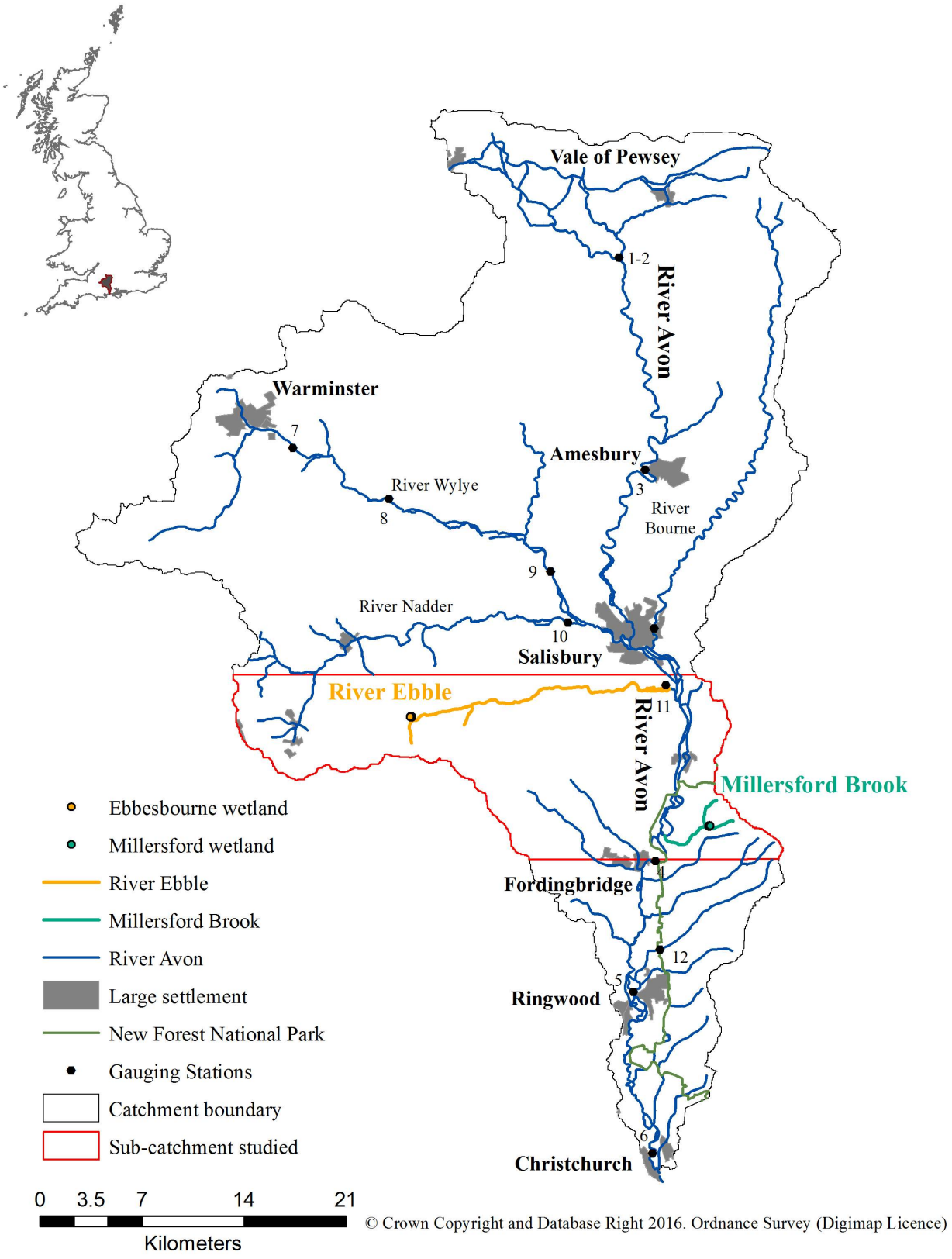


Figure 3.1 Map of the Hampshire Avon catchment (developed using ArcGIS 10.1). The river Avon is shown in blue, river Ebble in yellow and Millersford Brook in green. The locations of the studied wetlands are shown with a yellow (Ebble) and green (Millersford) dot. Numbers 1-12 indicate the gauging stations numbered after Table 3.1.

Table 3.1 Hampshire Avon river catchment hydrometric characteristics observed at the Centre of Ecology and Hydrology gauging stations (2008). In the land use column, the letter H indicates that heath dominates. In the bedrock permeability column, the formations of mixed permeability are not tabulated.

	Gauging station	Base Flow Index	Mean annual rain (mm)	Mean annual runoff (mm)	Mean flow (m^3s^{-1})	Factors affecting runoff	Proportion of time soils are wet	Mean drainage path slope	Bedrock permeability (%)			Land use				Urban extend (%)
									High	Moderate	Very low	Woodland	Arable/ horticultural	Grassland	Mountain/ heath/bog	
1	**East Avon at Upavon**	0.89	793	304	0.82	Natural	32	55	40	60	0	11	51	32	0	1
2	**West Avon at Upavon**	0.71	781	258	0.69	Groundwater abstraction	34	43	59	41	0	11	55	30	0	1
3	**Avon at Amesbury**	0.91	779	350	3.55	Groundwater abstraction	34	51	74	26	0	9	33	49	0	1
4	**Avon at East Mills**	0.91	843	333	15.4	Natural	34	64	80	16	3	10	39	42	<1	2
5	**Avon at Ringwood**	0.88	813	340	20.0	Public water supplies	34	62	75	14	3	12	38	40	1H	2
6	**Avon at Knap Mill**	0.90	842	363	19.5	Public water supplies	34	61	73	13	3	13	37	40	2H	2
7	**Wylye at Norton Bavant**	0.87	949	314	1.11	Groundwater abstraction Effluent return	35	74	64	34	2	13	43	34	<1	3
8	**Wylye at Stockton**	0.89	953	277	2.20	-	35	76	82	17	<1	12	30	48	<1	1
9	**Wylye at South**	0.89	860	290	4.07	Natural	35	70	90	10	<1	9	31	51	<1	1
10	**Nadder at Wilton**	0.82	913	417	2.90	Natural	35	79	46	41	13	16	49	30	<1	1
11	**Ebble at Bodenham**	0.85	900	246	0.76	Natural	35	95	97	3	0	6	55	31	<1	1
12	**Dockens Water at Moyles Court**	0.39	818	413	0.22	-	33	57	0	0	0	48	6	23	21H	1

3.2.1 Sub-catchment studied

The sub-catchment, in which both study sites are located, is magnified in in 3.2.

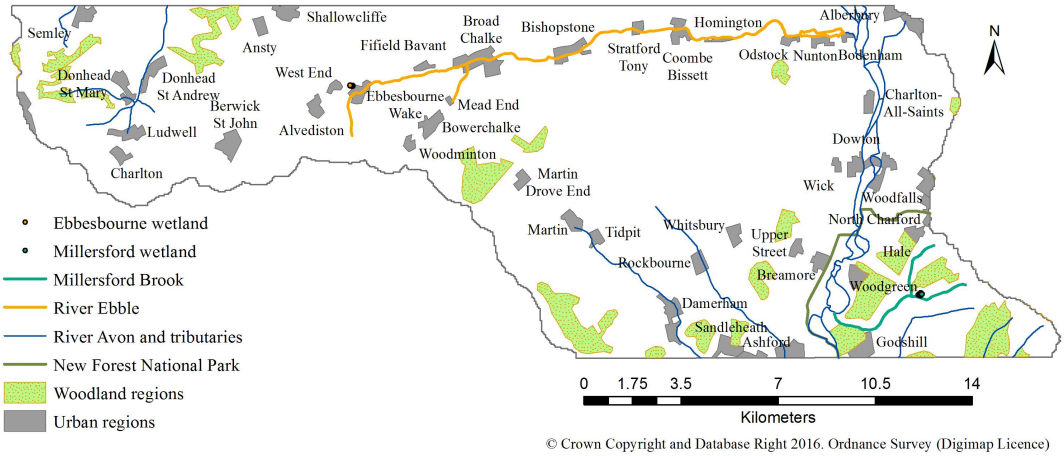


Figure 3.2 Map of the sub-catchment studied (developed using ArcGIS 10.1). The river Ebble is shown in yellow and Millersford brook in green. The studied wetlands are shown in the same colours as the rivers. Urban and woodland regions are indicated with grey and green colours respectively.

The River Ebble (Figure 3.3) is one of the main tributaries of the river Avon. The river rises at Alvediston and meets the Avon south of Salisbury, at Bodenham (Figure 3.2), covering a distance of approximately 20 km. The river flows over chalk underlain by Greensand in an arable area with urban areas across it (Figure 3.5). Bedrock permeability in the Ebble at Bodengam is the highest in the River Avon catchment, with high permeability bedrock comprising 97% of the bedrock. At the same location, arable exploitation reaches 55% of the land use.

Figure 3.3 Photograph of the river Ebble off Ebbesbourne Wake village (high flow).

The Millersford brook (Figure 3.4) is located within the New Forest National Park, a very important site for nature conservation. It rises in the New Forest plateau, in an area classified as a combination of heather grassland and woodland (Figure 3.5). The stream flows over clay, silt, sand and gravel (Figure 3.5). It meets the River Avon, 6 km to the west, north of Fordingbridge.

Figure 3.4 Photographs of Millersford Brook adjacent to the wetland (during low flow).

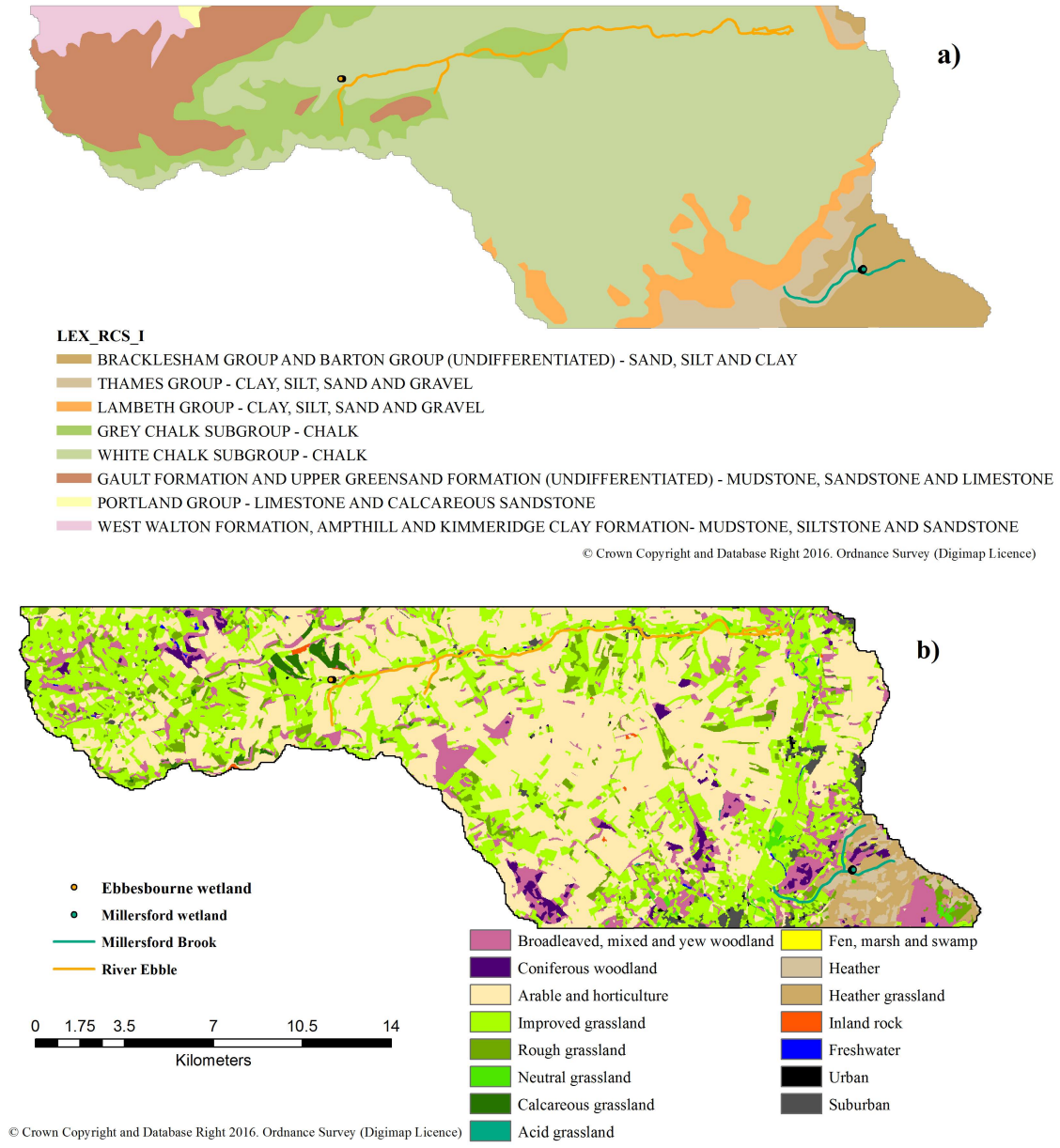


Figure 3.5 Geology (a) and land use (b) map of the sub catchment studied. The Ebble river and wetland are shown in yellow and the Millersford Brook and wetland in green.

3.3 Characteristics of the wetlands

The soil properties of the two wetlands were investigated. Model estimates for topsoil properties and soil group characterisation were applied. Based on a modelling approach, the Centre for Ecology & Hydrology the Countryside Survey used topsoil maps (0-15 cm) maps, generated by Countryside Surveys of 1978, 1998 and 2007 to provide detailed soil data (Emmett et al., 2010). A Soil Parent Material Model was developed by the British Geological Survey to map the upper 2–3 m of soil geology. The derived soil properties are summarised in Table 3.2. As expected, the soil in Millersford has higher

organic content (e.g. loss on ignition >50%, compared to 0-10 in Ebbesbourne), higher water content, is less compacted (see bulk density values, Table 3.2) and strongly acidic.

Table 3.2 Soil properties of the 2 wetland study sites. Soil group refers to soil texture groupings (%clay, %silt and %sand) that are categorised as light soils (i.e. sand/silt rich), medium soils (i.e. loams) and heavy soils (i.e. clay-rich). Highly variably textured soils or the presence of peat is classified as "mixed or organic". Model estimates of topsoil properties [Countryside Survey] © Database Right/Copyright NERC — Centre for Ecology & Hydrology. All rights reserved. Contains British Geological Survey materials © NERC 2014.

	Ebbesbourne wetland	Millersford wetland
Topsoil properties		
Soil pH	>8	<5
Soil moisture (%)	<25	55-60
Bulk density (g cm^{-3})	1-1.2	0.4-0.6
C:N ratio	<11	>25
Carbon concentration (g kg^{-1})	0-25	100-500
Carbon density (t ha^{-1})	50-60	>90
Loss on ignition (%)	0-10	>50
Olsen P (mg kg^{-1})	35-40	20-25
Soil properties		
Soil Group	Medium and/to light soils	Mixed or organic soils
Soil layer thickness	Shallow	Deep

3.3.1 Millersford

The location of the Millersford wetland study site is detailed in Figure 3.6.

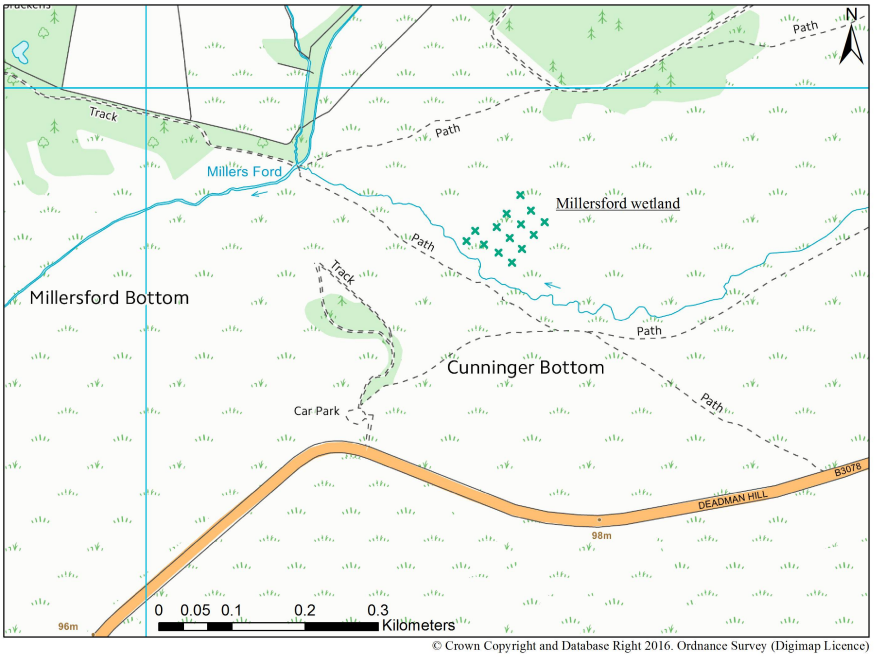


Figure 3.6 Location of wetland study site in Millersford (green crosses).

The wetland is surrounded by scenic landscapes (Figure 3.7) that are used for recreation. Mainly cattle, horses and ponies graze the area. No pesticides are used but feed material is provided to the animals.

Figure 3.7 Photographs of the valley (top left), Millersford wetland is distinct in light brown (bottom left), photos of the wetland (top and bottom right).

The area studied is not flat. Millesford Brook flows in a valley surrounded by hills as shown in Figure 3.8. The riparian wetland spreads across a hill slope. The closest to the stream part of the wetland is at 69 m height above the sea level, whereas the highest at 74 m height above the sea level.

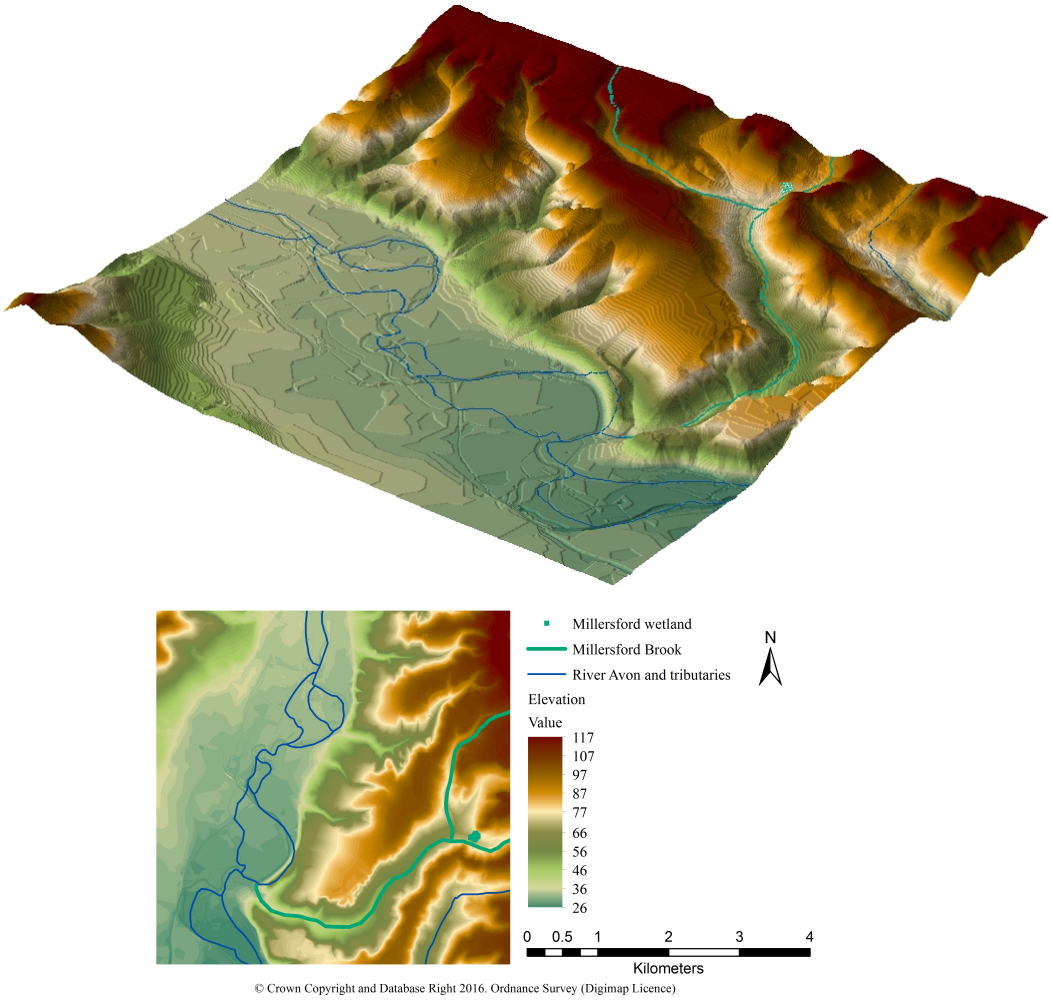


Figure 3.8 Visualisation of surface elevation using elevation model (top) and top view (bottom) of the Millersford site (developed using ArcGIS 10.1).

The Millersford wetland is located in a hillslope/ valley bottom site, and its substrate categorises it as a groundwater fen (Gilvear and Bradley, 2000). It is thus expected to be minerotrophic and groundwater fed, showing low seasonality in water table behaviour. Its acidic pH would classify it as bog. However, observations of the water level during the study using dipwells, confirmed that the wetland was waterlogged for the entire duration of the study. Hence it is colcluded that it is mainly groundwater and not rainwater fed.

3.3.1.1 Subsurface soil structure

The soil profile was studied using Ground Penetrating Radar (GPR) (Sir20, Geophysical Survey Systems, Inc., processed using Radan SIR20 software and GSSI hardware). GPR is a non-destructive method, that can be used for subsurface characterisation using transmission and reflection of electromagnetic waves. The basic

way that GPR works is illustrated in Figure 3.9. An overview of the GPR principles of operation can be found in many studies (e.g. Jol, 2009, Rubin and Hubbard, 2006). GPR results give an indication of the nature and location of the subsurface features based on the amplitudes of the received echoes and the corresponding arrival times. Soil characteristics such as electrical conductivity will influence the propagation velocity, attenuation and penetration depth of electromagnetic energy of the GPR system resulting in different output. Hence, GPR has been used for stratigraphy and hydrogeology studies of various soils including organic soils and peatlands.

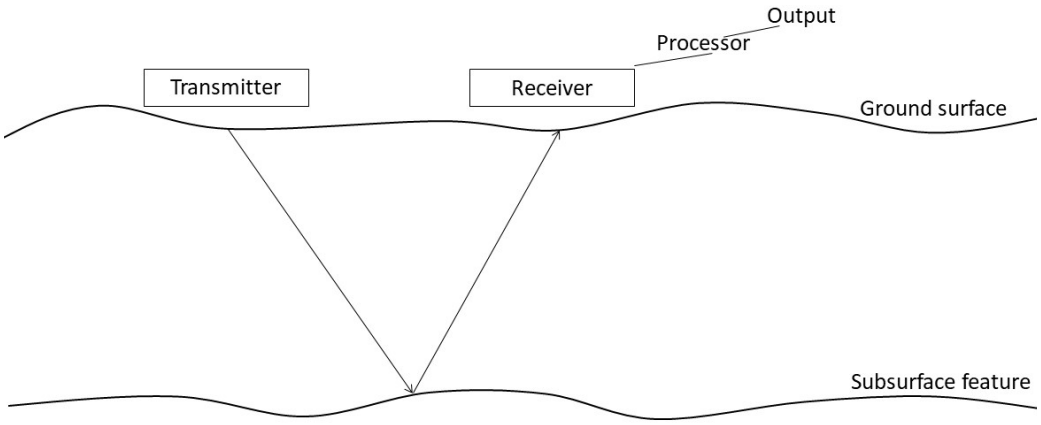


Figure 3.9 GPR principle way of function (adapted from Dong and Ansari (2011).

GPR can be very useful for subsoil investigations as typically soil horizons, soil moisture contents, physical and chemical soil properties correspond to the radar reflections (Jol, 2009). Indeed, strong radar reflections were found to correspond to the impermeable clay layer in Millersford as confirmed by field observations using an auger. The transects studied are shown in Figure 3.10**.**

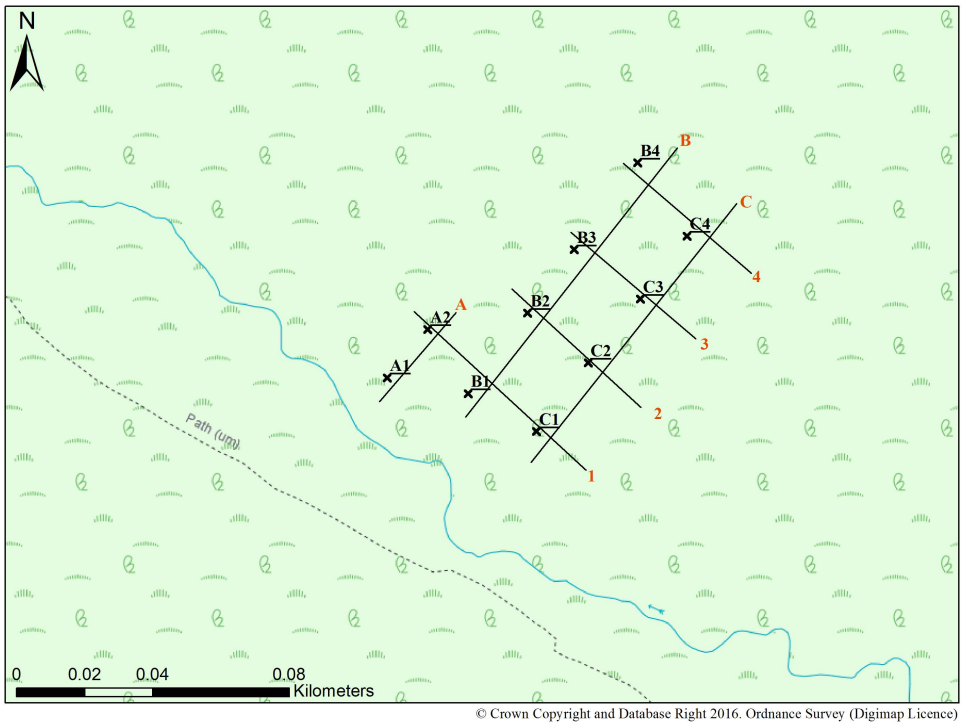


Figure 3.10 Map of the sampling grid in the Millersford wetlands, showing the 7 transects studied and the marked locations across them.

Soil samples using an auger were investigated in all marked locations and in points in between. The depth of the impermeable clay layer was recorded and observations on the soil characteristics were documented. GPR was then used for a more detailed spatial investigation of the subsoil.

The profile showed variability mainly across the hill slope (from 1 to 4) and less so across the horizontal transects (A to C) of the wetland (Figure 3.10). It is noteworthy that differences occurred within a range of just a few centimetres.

The top peat layer covered up to 30 cm in the locations closer to the river, with much less in the points located up the hillslope (e.g. B4, C4). The depth of the impermeable clay layer varied across the wetland. This layer was often mixed with gravel, although bits of gravel was observed in shallower depths in many locations. The quality of the clay layer also differed across the wetland. The most compacted, resistant, impermeable clay was observed from the middle of the wetland, horizontal transect 2 upwards.

Ground penetrating radar images are shown in Figure 3.11, Figure 3.12 and Figure 3.13. The estimated location of the impermeable clay layer is marked in blue colour. At

location A2 impermeable clay is located at 60 cm depth and at A1 shallower at less than 40 cm depth. In between the samplers the same layer is located at 40 cm depth. In transect B, impermeable clay layer is deepest at B4 (90 cm), shallowest at B2 and B3 (45 cm) and at 80 cm depth in B1. A ditch can be observed between B2 and B3. The differences in the clay layer depth show possible higher water retention times in the ditch and in B1. Transect C appeared less variable in impermeable layer depth (65 cm at C4, 60 cm at C1 and C3, 40 cm at C2), but indicate possible water retention at C3. In the horizontal transects, differences in impermeable layer depth are more pronounced in transect 4. The layer is found at greater depth at B4 compared to C4 (90 cm and 65 cm respectively). The rest of the transects show relatively little variation.

Based on the soil profiles, preferential flow is expected to occur with increasing distance down slope, with the part of the wetland closest to the riverbed acting as a collection point.

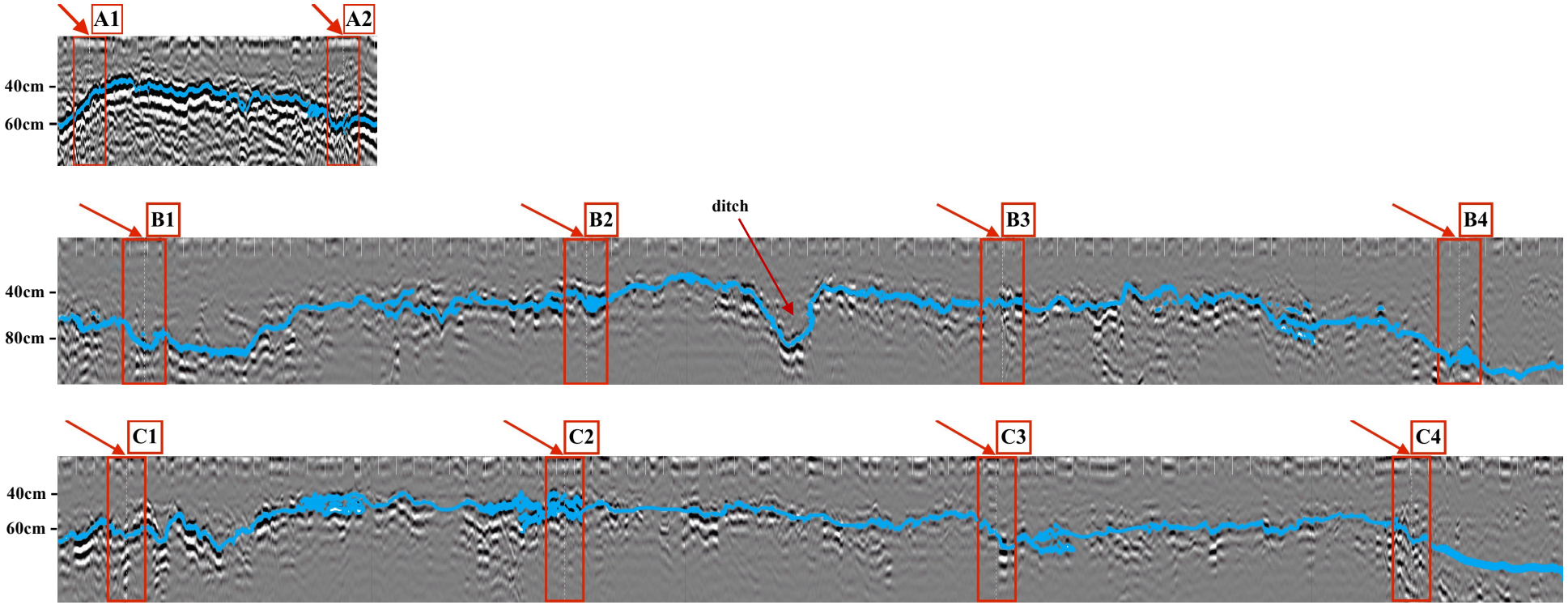


Figure 3.11. Ground penetrating radar image of transects A, B and C. Depth (cm) is shown on the left side. Locations of the samplers are shown in white dotted lines and red frames. Impermeable clay layer is marked in blue.

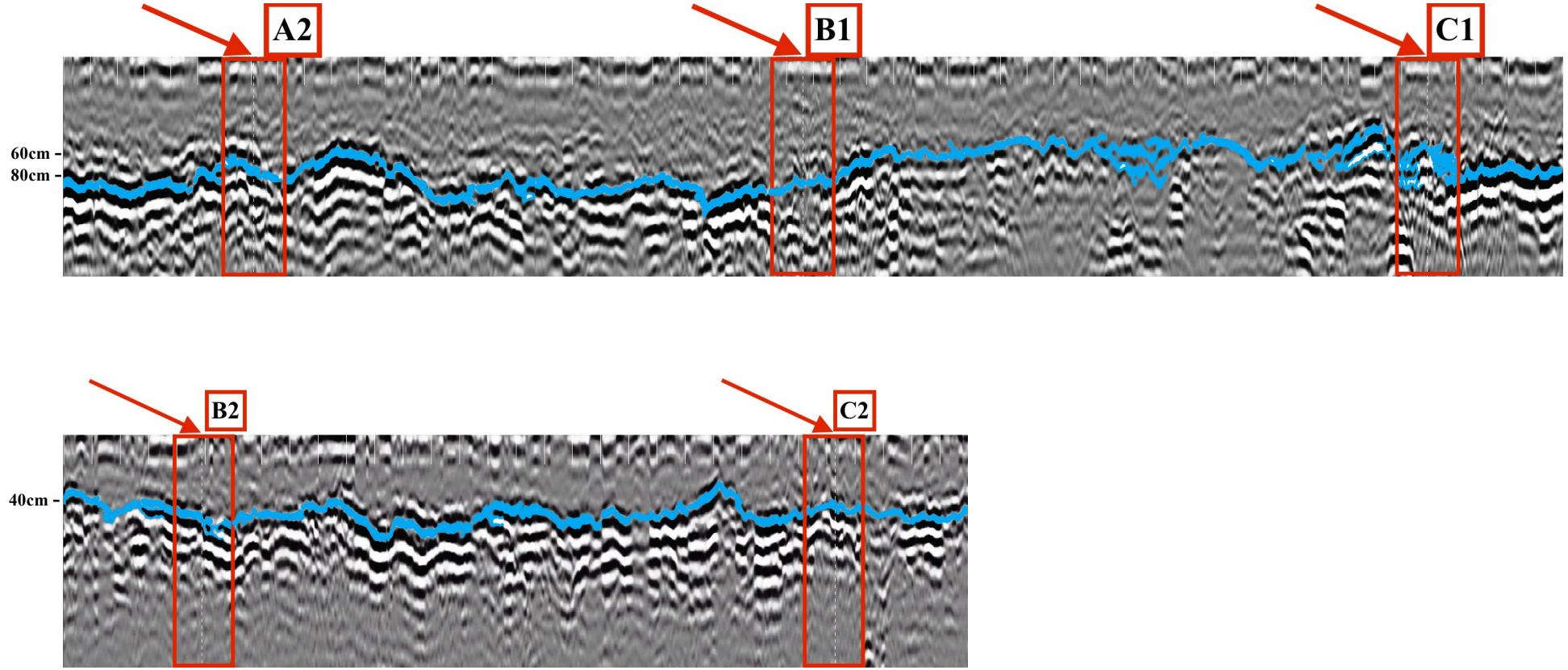


Figure 3.12 Ground penetrating radar image of transects 1 and 2. Depth (cm) is shown on the left side. Locations of the samplers are shown in white dotted lines and red frames. Impermeable clay layer is marked in blue.

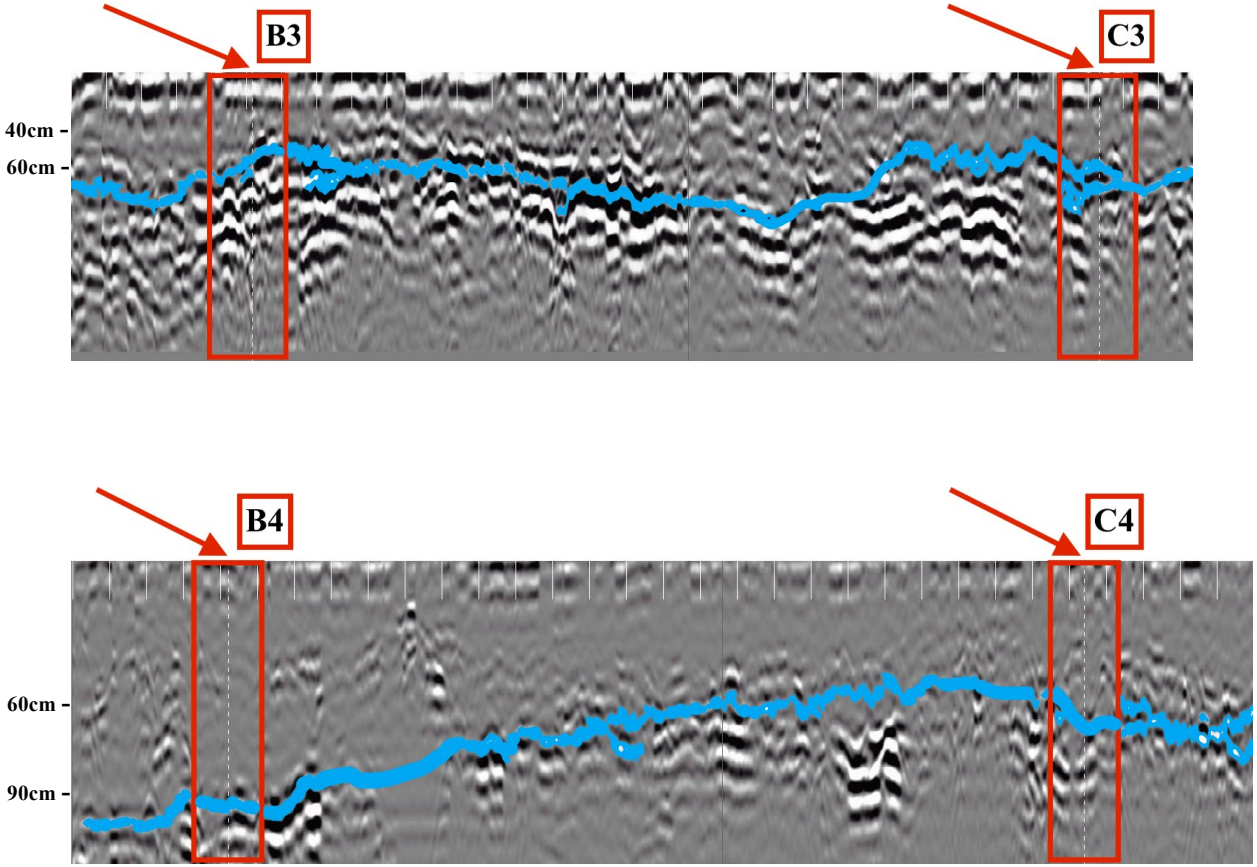


Figure 3.13. Ground penetrating radar image of transects 3 and 4. Depth (cm) is shown on the left side. Locations of the samplers are shown in white dotted lines and red frames. Impermeable clay layer is marked in blue.

3.3.2 Ebbesbourne

The wetland was partly constructed by the residents of Ebbesbourne Wake for flood defence. The river Ebble flows adjacent to the wetland before reaching a pond and then the village.

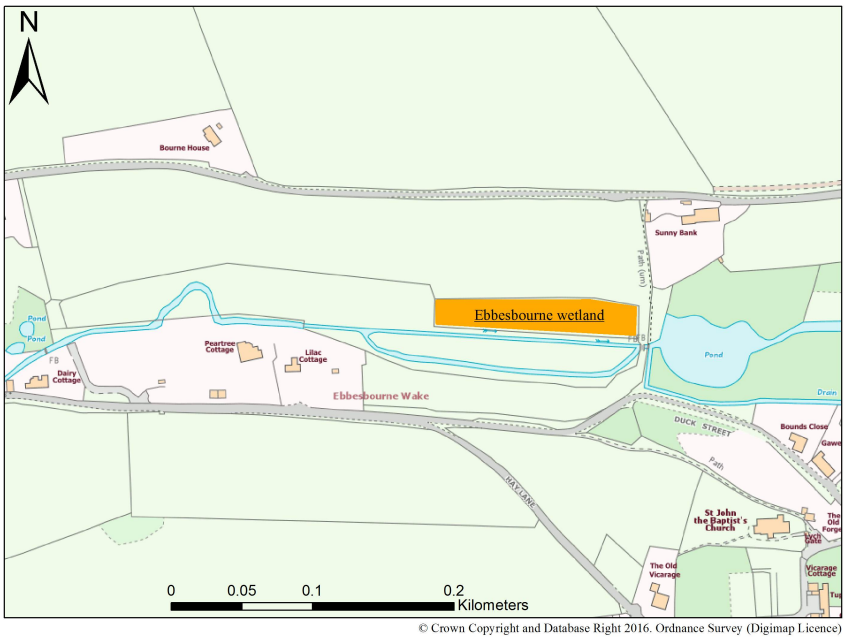


Figure 3.14 Location of wetland study site in Ebbesbourne (highlighted with yellow).

The wetland is fenced and was used for grazing in the past but not during the study. Cattle grazed the area outside the fence. All the area, wetland included, was mowed once a year.

Figure 3.15 Photographs of the Ebbesbourne wetland surrounded by the fence (top), River Ebble adjacent to the wetland (bottom).

The wetland is situated in a flat plain site (Figure 3.16), with no significant variation in elevation. It resembles the characteristics of a backswamp floodplain, for which seasonal flooding is the dominant process (Gilvear and Bradley, 2000). As such, it is expected to show high seasonality.

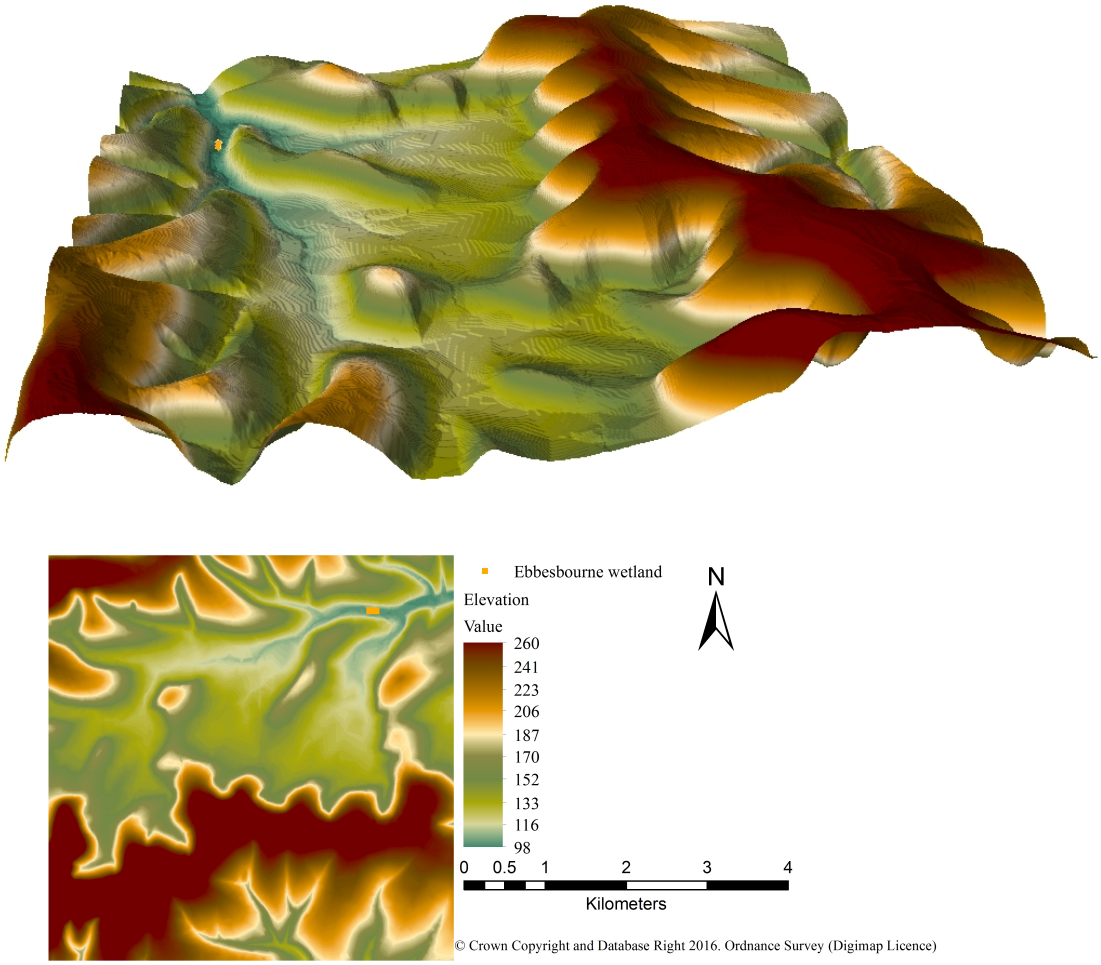


Figure 3.16 Visualisation of surface elevation using elevation model (top) and top view (bottom) of the Ebbesbourne site (developed using ArcGIS 10.1).

3.3.2.1 Subsurface soil structure

Unlike Millersford, the soil profile of the wetland is uniform. Impermeable clay is met at around 60 cm depth. A typical example is the GPR image after scanning 18 m across the wetland, shown in Figure 3.17 . This was confirmed with field observations. The presence of calcium carbonate in shallower depths was also observed.

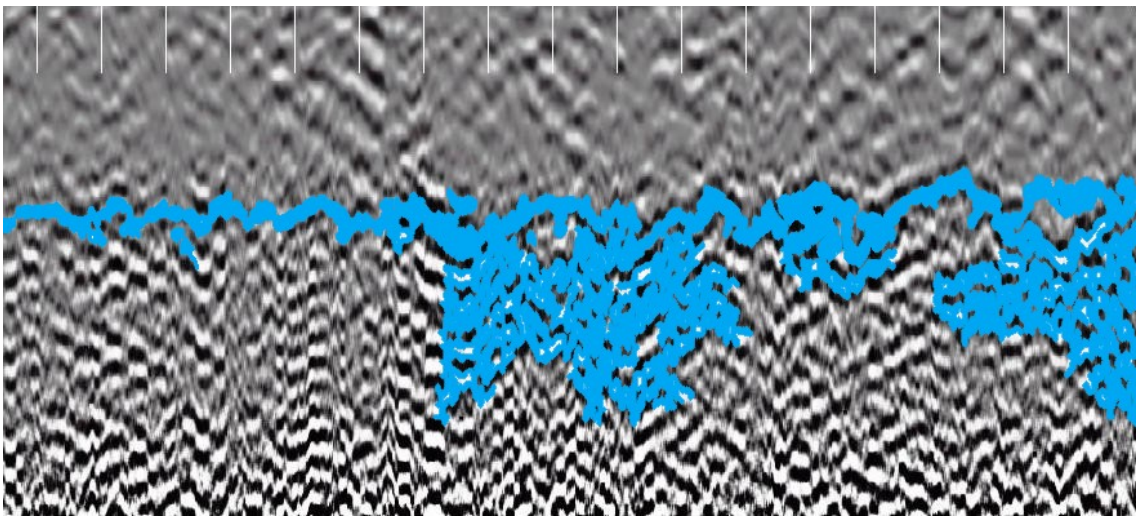


Figure 3.17 Ground penetrating radar image of typical Ebbesbourne wetland soil profile. Locations of the samplers are shown in white dotted lines and red frames. Impermeable clay layer is marked in blue.

Given the permeability of the soils in Ebbesbourne and the uniformity of the soil profile, no particular subsurface flow patterns are expected within the wetland.

Chapter 4- Materials and methods

4.1 Overview

This chapter describes the sampling strategy, along with the materials and methods employed to collect and analyse samples. Based on the study description in Chapter 3, and the aims and objectives in Chapter 1, the sampling strategy had to meet the need for spatial DOM analysis of the wetlands soil pore water and the streams. This was addressed by installing ceramic cup samplers in an extended sampling grid in each wetland. Also, the study of the vertical distribution of DOM was performed by installing the samplers at 20, 40, 60cm depth and at 80cm in some sampling points in Millersford. The depths were defined after the study of the soil profiles in Chapter 3. All the samples were analysed for the concentrations of dissolved carbon, nitrogen, phosphorus fractions for the quantitative analysis of DOM; the absorbance and fluorescence spectra were evaluated for the qualitative analysis of DOM. Both the quantitative and qualitative evaluations of DOM aimed to describe the spatial and vertical distribution within the wetland (Chapters 5 and 6 respectively) but also to investigate the role of the wetlands in determining the levels and composition of DOM in the adjacent stream (Chapter 7). For the latter purpose, data from the Avon Demonstration test catchment project for Ebble River have been incorporated.

4.2 Experimental design

The rationale for the study of DOM in riparian wetlands and the adjacent streams has already been discussed in Chapters 1 and 2. In order to meet the objectives of the study, described in Chapter 1, the experimental work was designed as illustrated in Figure 4.1. To correlate DOM levels or quality characteristics with the variables of interest, correlation coefficients are estimated and significant difference using statistical analysis were tested. As shown, differences in DOM levels and quality characteristics in different sampling locations, depths and seasons are studied to conclude on spatial, vertical and temporal patterns of DOM. Selection of the study area and the contrasting characteristics of the two wetlands have already been discussed in Chapter 3. Data collected from the two sites are compared to draw conclusions on the effect of wetland types and land use on DOM concentration and quality in wetlands and the adjacent streams. The effect of

each wetland to the adjacent stream DOM is evaluated by comparing data upstream and downstream the wetland.

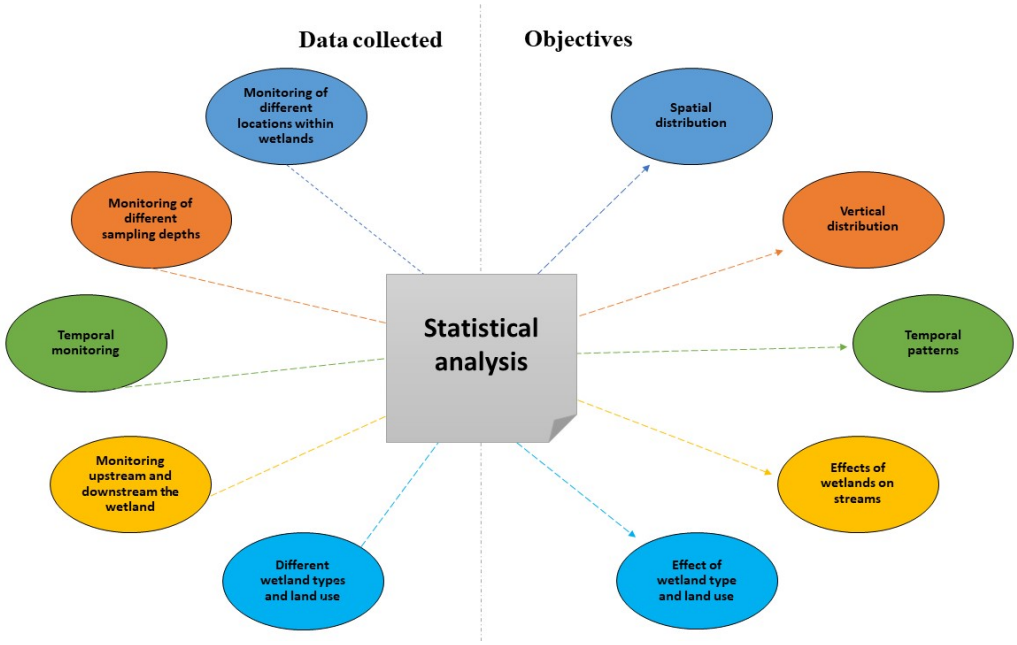


Figure 4.1 Experimental design diagram.

The objective to study the quantitative and qualitative spatial distribution of DOM in riparian wetlands was met by distributing the sampling points across the whole area of each wetland. This ensured that samples were collected close to the streams, from the edges and centre of the wetlands and in the case of Millersford from different locations across the hillslope. The maximum number of sampling points was reasonably restricted by the landowner of the Ebbesbourne wetland (Mr Robin Long) and the managers of the Millersford wetland (Verderers of the New Forest). Therefore, samplers in 15 sampling points in Millersford wetland and 12 sampling points in Ebbesbourne could be installed.

The area covered by the Millersford wetland is more than 4000 m^2. The grid of the sampling points is shown in Figure 4.2. Most of the wetland has a length of approximately 75 m and a width of approximately 50 m. To obtain a representative grid of samplers across the wetlands, 12 sampling points were distributed across the 1-4 and B-D transects. Within each transect, the distance between adjacent samplers was 25 m. The part of the wetland outside of this grid was covered with the transects 0 and A. The distance between transects 0 and 1 was 17 m. Distance in between sampling points of transect 0 was 25 m. Unfortunately, equipment at the edge of the wetland, closer to a

walking path, was vandalised. Therefore, results from transects D and sampler B0 are not presented.

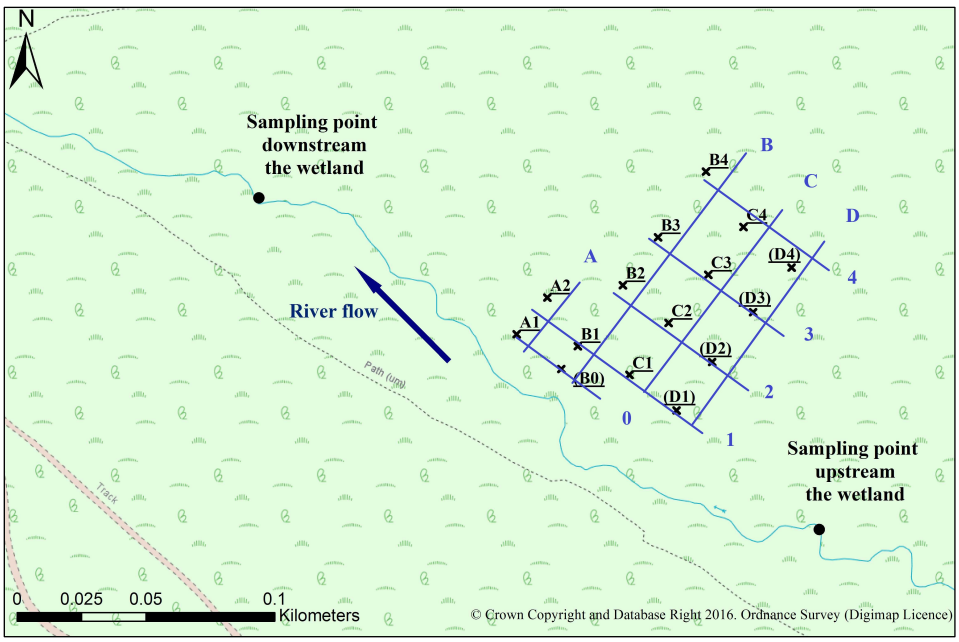


Figure 4.2 Map of the sampling locations at Millersford, as estimated by high precision GPS. Wetland sampling points are indicated with a cross, river sampling points indicated with points. Retired sampling points are in parenthesis. Transects are shown in blue.

The area covered by the Ebbesbourne wetland is almost 1000 m^2, with a length of approximately 90 m and width 11 m. A sampling grid of 4x3 transects was created, as presented in Figure 4.2. Within each long transect, adjacent samplers had a distance of 30 m and within each short transect the adjacent samplers were 5.5 m away.

The decision on the depths sampled was justified on the basis of a lack of knowledge on wetland samples collected bellow 30 cm depth. The vast majority of published research in soil organic carbon and soil pore water DOM, including wetlands, focuses on the upper 30 cm of the soil profile. However, the literature explains that a large amount of organic carbon is present in the 30-100 cm layer, often equal to that found in the 0-30 cm layer (Batjes, 1996, Hiederer, 2009). The objective of this study is to capture the flux of DOM quantity and quality throughout the soil profile. The need to study DOM behaviour in deeper soil layers is especially important at Millersford, as it is a groundwater fed wetland and situated on a hillslope (§3.3.1). These wetland features indicate that soil pore water movement is complex and could affect DOM pools in subsurface layers.

Maximum sampling depth for each sampling point was decided after the study of the soil profiles (§3.3.1.1 and §3.3.3). The depth of the impermeable clay layer in Millersford varied from 40 cm to 90 cm, with the surface peat layer up to 30 cm deep. Accordingly, the sampling equipment was installed at 20, 40 and where possible (because of the impermeable layer depth) 60 and 80 cm. However, sample collection from 20 and 80 cm depth was problematic and results are not presented in the current study. The collection of soil water at 20 and 80 cm depth would be possible using different sampling equipment (e.g. different vacuum pump). The sampling points where sampling equipment was installed at 60 cm depth are B2, B3, B4, C2 and C4.

Two additional sampling points, in the Millersford brook, were included; one was upstream and the other downstream of the wetland, in order to investigate the effect of the exported DOM from the wetland to the brook. The location of all sampling points is shown in Figure 4.1.

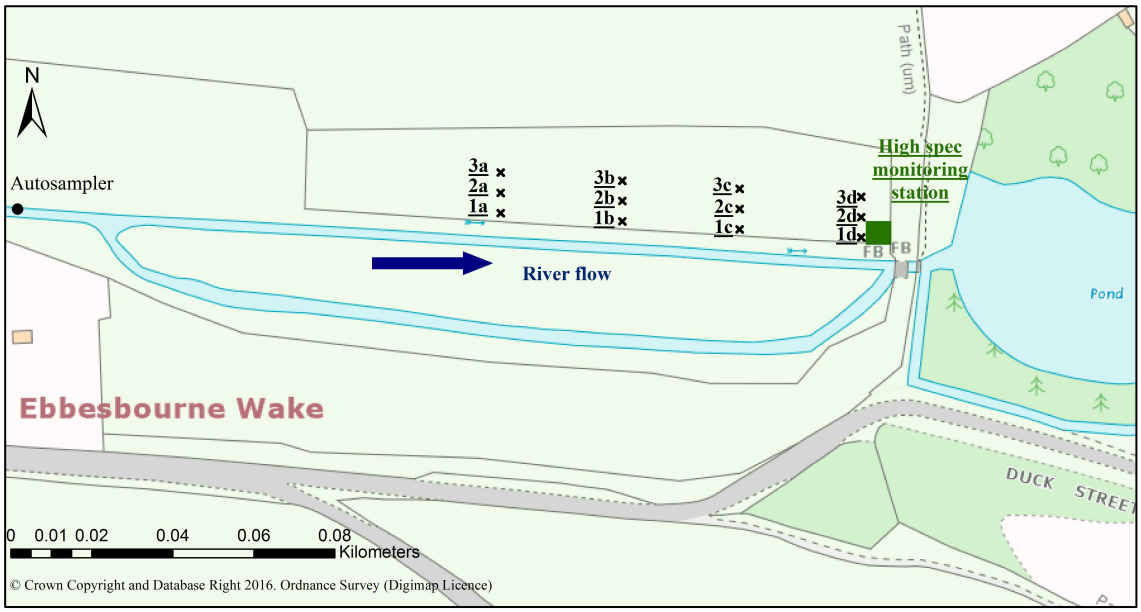


Figure 4.3 Map of the sampling locations at Ebbesbourne. Wetland sampling points are indicated with a cross, the high spec station location indicated with a green box and the autosampler location with a point.

In the Ebbesbourne wetland, the saturated clay layer is found at around 60 cm depth. Three depths, 20, 40 and 60 cm were studied. As part of the DTC project, the Ebbesbourne site was equipped with a high-specification monitoring station that collected water samples for analysis and monitored dissolved oxygen, pH, temperature, conductivity, turbidity, chlorophyll a, water level and flow rate. The station was

positioned to collect water and data downstream of the wetland. Additionally, an autosampler was installed upstream of the site, to collect water samples before entering the wetland. The location of the autosampler and monitoring station are shown in Figure 4.3.

Temporal variation was assessed by collecting samples during different months and seasons of the year. The Millersford wetland and Millersford Brook were monitored from October 2011 to December 2012 on a monthly basis. The Ebbesbourne wetland and river remained dry at the beginning of the study due to the 2011-2012 drought in England. This was followed by a sudden change in April 2012, and the wetland was monitored monthly from July 2012 until May 2013 when it dried up again. The Ebble River was monitored from May 2012 to May 2013 daily.

4.3 Instrumentation

Both sites were instrumented with suction cups, for soil water sampling at the different soil depths. This is by far the most commonly used technique for *in situ* water extraction, and offers potential for inter-site comparisons (Weihermuller et al., 2007). The porous suction samplers, made of porous PTFE (teflon) mixed with silica flour (Prenart Equipment ApS), accumulate the water from the adjacent soil. Each sampler is connected to a PVC pipe long enough to be accessible on the ground surface; as such, water in the samplers can be removed with the aid of a vacuum pump connected via a sampling tube, and collected in 1L polypropylene bottles. For each depth, the samplers were installed in the ground at 45^{o}, so that the sample collected was representative of that depth. Silica flour was introduced into the borehole before installation to achieve optimal contact with surrounding soil. In order to protect animals from choking on the sampling tubes and prevent equipment damage, the bottles were placed in light-proof insulated cylinders buried in the ground and all tubing was covered with concrete slabs as shown in Figure 4.4. Soil pore water samples were transferred to acid washed (5%HCl) polyethylene and glass sample bottles immediately after extraction from the soil.

Figure 4.4 Photograph of the materials used to protect the soil water sampling equipment and animals.

Dipwells were installed in the Millersford wetland to measure water table levels. They were made of perforated 5cm diameter PVC pipes wrapped in shade cloth. One dipwell per sampling nest was installed in proximity of less than a meter. The installation depth was close to the impermeable layer surface.

4.4 Sample analysis

Upon arrival at the laboratory, all samples were filtered through Whatman 0.45µm cellulose nitrate filters and stored in the dark at 4°C.

4.4.1 Quantitative nutrient analysis

Inorganic fraction analysis for N and P and microwave digestion were done within 24h. A sub-sample was acidified, as described in 4.4.1.1. All other analysis was completed within a week. All the sample bottles were acid washed (5% HCl).

4.4.1.1 Dissolved Carbon

Dissolved carbon and its fractions were determined by high temperature catalytic oxidation (Peltzer and Brewer, 1993, Sugimura and Suzuki, 1988, Vidal et al., 2014), using a Shimadzu TOC 5000 carbon analyser. Total dissolved carbon (TDC) was oxidised to carbon dioxide in an oxygen rich environment, in a combustion furnace that contained a platinum catalyst, at 680°C. The carbon dioxide was then detected by an

infrared gas analyser. Dissolved inorganic carbon (DIC) was measured by detecting (again with the infrared gas analyser) the carbon dioxide isolated after sparging the sample with acid (25% phosphoric acid). Dissolved organic C (DOC) was calculated by subtracting the DIC concentration from the TDC concentration.

The samples collected at Ebbesbourne (both soil pore waters and river water samples) were analysed using the non-purgeable organic carbon measurement employing the same piece of equipment. This method is more suited for samples in which TDC consists largely of DIC. This is because calculation of DOC by subtracting DIC from TDC could result in significant errors. Non- purgeable DOC concentration was determined after the sample was acidified to pH 2.5 with HCl. The sample was then sparged (for 5 min) with carbon dioxide purified air, thus converting the DIC to carbon dioxide that was then removed. After this procedure DOC was measured following the method described above for TDC.

For both methods, the sample was injected 5 times and the resulting mean concentration was accepted if the coefficient of variance was <3%. All fractions were calculated using a five point calibration curve (using potassium hydrogen phthalate for TDC and DOC, sodium hydrogen carbonate and sodium carbonate for DIC). For every set of samples, solutions of 20 mg/L TDC and DOC and 5 mg/L DIC (for the first method) were also analysed to check for drift (5% deviation of the real value). Drift was not detected for any of the batches.

4.4.1.2Dissolved nitrogen and phosphorus

Nitrogen and phosphorus fraction analysis is illustrated in Figure 4.5. All fractions were determined using a Skalar San^{++} multi-channel continuous flow autoanalyser.

All inorganic fractions were measured automatically in filtered untreated samples. Total oxidised nitrogen (TON) was measured as the sum of nitrates and nitrites with the hydrazine reduction method (Kamphake et al., 1967, Kempers and Luft, 1988). After reducing the nitrate to nitrite, a highly coloured azo dye is formed that was then colourimetrically measured at 540 nm(Henriksen and Selmer-Olsen, 1970). Total ammonium (NH_3-N and NH_4-N) determination was based on a modified Berthelot reaction producing a green coloured complex measured colourimetrically at 660 nm (Krom, 1980). Soluble reactive phosphorus (SRP) determination was performed after

reactions with ammonium heptamolybdate and potassium antimony (III) oxide tartrate in an acidic medium. The antimony-phospho-molybdate complex formed was then reduced by ascorbic acid and the intensely blue coloured complex was measured colourimetrically at 880 nm (Boltz and Mellon, 1948, Murphy and Riley, 1962).

Total dissolved nitrogen and phosphorus determination (TDN and TDP) was based on the persulfate microwave digestion as described by Johnes and Heathwaite (1992) using a CEM Mars Express microwave digestion unit. High acidity by persulfate oxidation, high temperature and pressure by microwave were used to convert phosphates to orthophosphates and oxidise all nitrogen to nitrate (Delia et al., 1977, Johnes and Heathwaite, 1992, Koroleff, 1972, Koroleff, 1977). The sample digests were then analysed as described above for the inorganic species.

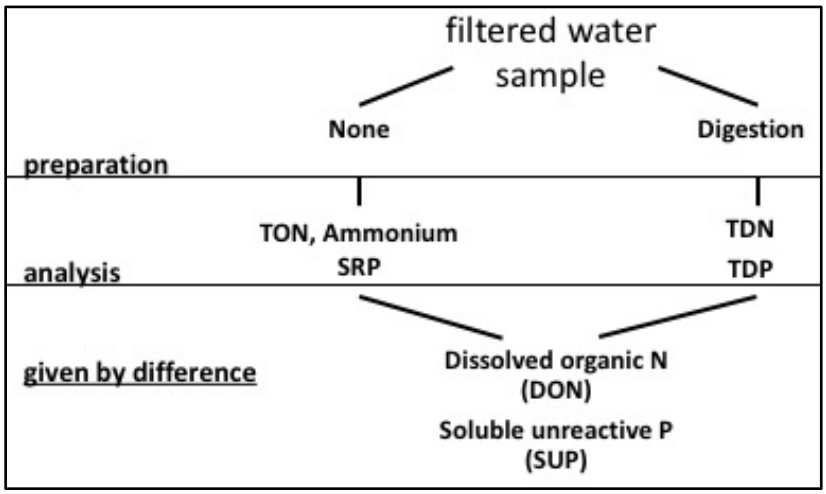


Figure 4.5 Sample analysis for dissolved N and P fractions, modified from Johnes and Heathwaite (1992).

Dissolved organic nitrogen (DON) was calculated by subtracting the inorganic N species from TDN. Soluble unreactive phosphorus (SUP) was calculated as the difference between TDP and SRP. Phosphorus fractions are described in the literature by a variety of terms. This has partially attributed to there being no clear or consistently explained difference between the organic and inorganic fractions. For example, SRP could contain organic compounds and SUP condensed inorganic compounds (Denison et al., 1998, McKelvie, 2005). Moreover, the filtered samples could contain colloidal fractions (Whitton and Neal, 2011). Nomenclature includes filterable reactive phosphate (FRP) and dissolved reactive phosphorus (DRP), in place of SRP; also, the terms filterable

hydrolyzable phosphate (FHP), filterable organic phosphate(FOP), dissolved unreactive (DUP) and dissolved hydrolyzable phosphate (DHP) exist alongside SUP (Ellwood and Whitton, 2007, Jarvie et al., 1998, Whitton and Neal, 2011, Withers et al., 2007). In this study, SRP and SUP are used as surrogates for dissolved inorganic and organic phosphates, respectively, taking into consideration the above remarks.

4.4.1.3 Detection limits

The detection limits of the analytical instruments used, were examined. According to IUPAC (Freiser et al., 1987):

"The limit of detection, expressed as the concentration, c_L, or the quantity, q_L, is derived from the smallest measure, x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is given by the equation:

$x_L = x_{bi} + k s_{bi}$

where x_{bi} is the mean of the blank measures, s_{bi} is the standard deviation of the blank measures, and k is a numerical factor chosen according to the confidence level desired".

4.4.1.3.1 Skalar

The estimated limits of detection for the Skalar San^{++} multi-channel continuous flow autoanalyser are presented in Table 4.1.

Table 4.1 Skalar detection limits (mg/L)

Nitrate	SRP	NH_4-N
0.17 mg/L	0.01 mg/L	0.18 mg/L

The values were calculated, using the results of analysis of blank samples that can be seen in Table 4.2.

Table 4.2 Blank samples analysis results (mg/L)

Replicate	Nitrate	SRP	NH_4-N
1	0.059	0.007	0.007
2	0.046	0.009	-0.008
3	0.071	0.007	0.066
4	0.041	0.005	0.005
5	0.103	0.004	0.045
6	0.010	0.003	0.024

7	0.005	0.007	0.007
8	-0.012	0.005	0.024
9	0.044	0.011	0.019
10	-0.018	0.002	0.007
11	0.012	0.001	0.026
12	0.076	0.002	0.236
13	0.021	0.001	0.026
14	0.102	0.007	0.046
15	0.054	0.007	0.024
16	0.048	0.001	0.047
17	0.066	0.006	0.041
18	0.168	0.001	0.034
19	0.020	0.001	0.038
20	0.025	0.001	0.102
21	0.045	0.001	0.036
22	0.059	0.002	0.040
23	0.104	0.006	0.044
24	0.046	0.003	0.086
25	0.097	0.001	0.030
26	0.037	0.002	0.044
27	0.009	0.007	0.098
28	0.020	0.002	0.057
29	0.033	0.005	0.037
30	0.073	0.006	0.030
Mean	**0.049**	**0.004**	**0.045**
St. deviation	**0.040**	**0.003**	**0.045**

4.4.1.4 Shidmazu TOC-L series analyser

Two methods were used, and they were both checked for limit of detection of the carbon analyser. For the first method used for the Millersford samples, the detection limits are shown in Table 4.3.

Table 4.3 Carbon analyser detection limits (mg/L) using the TOC method

DOC	TDC	DIC
0.05 mg/L	0.09 mg/L	0.05 mg/L

Detection limits were estimated based on the results presented in Table 4.4.

Table 4.4 Blank samples analysis using the TOC method (mg/L)

Replicate	DOC	TDC	DIC
1	0.030	0.062	0.032
2	0.031	0.075	0.044

3	0.014	0.028	0.014
4	0.033	0.098	0.065
5	0.031	0.042	0.011
6	0.013	0.031	0.018
7	0.012	0.021	0.009
8	0.025	0.036	0.011
9	0.037	0.061	0.024
10	0.015	0.026	0.011
11	0.013	0.021	0.008
12	0.013	0.021	0.009
13	0.021	0.030	0.009
14	0.024	0.037	0.013
15	0.014	0.025	0.011
16	0.018	0.034	0.016
17	0.014	0.026	0.012
18	0.021	0.036	0.015
19	0.019	0.037	0.018
20	0.023	0.035	0.012
21	0.014	0.031	0.017
22	0.022	0.033	0.011
23	0.015	0.025	0.011
24	0.030	0.041	0.011
25	0.010	0.027	0.017
26	0.001	0.015	0.014
27	0.055	0.067	0.013
28	0.030	0.040	0.009
29	0.036	0.047	0.011
30	0.020	0.029	0.009
mean	**0.022**	**0.038**	**0.016**
st.dev	**0.011**	**0.018**	**0.012**

Limits of detection were also checked for the NPOC method used for the Ebbesbourne samples. Based on the data presented in Table 4.5, the limit of detection of the carbon analyser was estimated to be 0.05 mg/L.

Table 4.5 Shidmazu carbon analyser blank readings using the NPOC method (mg/L)

Replicate	Conc.
1	0.029
2	0.009
3	0.043
4	0.028
5	0.032
6	0.019
7	0.014
8	0.023
9	0.017

10	0.021
11	0.009
12	0.007
13	0.026
14	0.030
15	0.000
16	0.022
17	0.009
18	0.020
19	0.002
20	0.019
21	0.001
22	0.022
23	0.007
24	0.031
25	0.009
26	0.001
27	0.011
28	0.010
29	0.025
30	0.029
Mean	**0.018**
StDev	**0.011**

4.4.2 DOM qualitative analysis

Dissolved organic matter (DOM) was characterised using ultra-violet and fluorescence spectroscopy. These methods require smaller sample volume, a factor that is of paramount importance for wetland pore water samples. They also present many other advantages, including being less labour-intensive and time-consuming, and being relatively inexpensive to run per sample. Thus, they have been characterised as valuable techniques in DOM characterisation. Both techniques provide insights into DOM composition, based on the property of different DOM molecules to absorb and reflect light at different wavelengths (McKnight et al., 2003, Vasilas et al., 2013).

Samples were stored at 4^{o}C and analysed within ten days (Peacock et al., 2015). Samples were allowed to reach room temperature prior to analysis. Fluorescence was monitored at Millersford samples from January to December 2012, as the fluorescence spectrophotometer equipment available before January was faulty and returned to the manufacturer. Fluorescence was monitor at Ebbesbourne from November 2012 until March 2013, because of unavailability of spectrophotometer equipment during the remaining study period.

4.4.2.1 UV absorbance

The UV absorption spectra were obtained using a Varian Cary 300 Bio UV-VIS spectrophotometer equipped with a 1cm path-length cuvette over the 200-800nm wavelength range at 1nm intervals. Ultrapure water was used before each set of measurements so that the instrument was set up and corrected for scattering and baseline fluctuations. The average absorbance between 700 and 800nm was subtracted from each spectrum. This was done as coloured DOM absorbance above 700nm is assumed to be zero. Hence this method, introduced by Green and Blough (1994), corrects for offsets that arise due to instrument baseline shift, temperature, scattering, and refractive effects.

The spectral slopes were calculating by fitting log transformed absorption coefficient data (a) to a linear regression. Helms et al. (2008) have showed that this method produces results that differ by less than 1% from the ones produced using nonlinear regression of a spectra. Absorption coefficients (m^{-1}) were calculated from the following equation:

a=2.303A/l, where A is absorbance and l is the path length (m).

The cuvettes were rinsed with ultrapure water in between samples and after every run. To make sure that the cuvettes remained clean of any organic material and microbes, they were stored in a dark container filled with 10%HCl solution at 4^{o}C.

4.4.2.2 Excitation-Emission Matrix Fluorescence

Fluorescence is a type of luminescence i.e. emission of UV or visible light by a substance not due to thermal radiation. Light emission is the result of electronically excited states, when an electron is excited to a higher energy level and returns to the ground state, as illustrated in. First molecules increase their energy by absorbing radiation by photon absorption, in a process that happens instantaneously. Through absorption electrons reach a vibrationaly and electronically excited state (second electronic state) from their original electronic state (ground electronic state). The molecule will then return from the excited state to the ground state, losing energy. The transition between the states occurs via thermal deactivation through collision of the excited molecules with solvent molecules. This thermal deactivation is so slow for some molecules that light is emitted, depending on their molecular structure. Typical fluorophores or fluorescent substances are aromatic molecules (Aiken, 2014, Lakowicz, 2006).

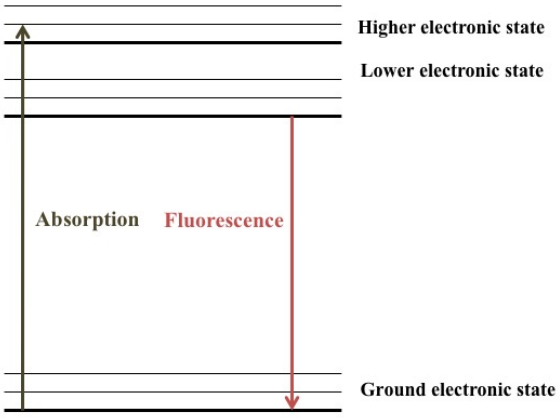


Figure 4.6 Simplified Jablonski diagram of molecule-radiation interaction.

DOM fluorescence is performed by light irradiation of samples in a fluorescence spectrophotometer for a range of excitation wavelengths. The instrument is recording the corresponding emitter wavelengths and light intensity and an excitation-emission matrix (EEM) is produced. To extrapolate EEM datasets, Parallel factor analysis (PARAFAC) is a way of deconvoluting spectra in underlying individual fluorescent phenomena (Bro, 1999). In our study, drEEM toolbox for MATLAB was used (Murphy et al., 2013). Using the same toolbox inner filter and blank correction, Raman normalisation were performed. All the steps are described in the MATLAB code given in appendix A.

EEMs were produced using a Varian Eclipse Fluorescence spectrophotometer (Agilent Technologies). The fluorescence intensity was measured at excitation wavelengths ranging from 240 to 400 nm at 10-nm increments and at emission wavelengths ranging from 300 and 600 nm at 2-nm increments. The instrument was set at a scan speed of 600 nm/min and a response time of 0.1 s.

4.5 Statistical analysis

All statistical analysis including correlation coefficients, testing of significant differences and graphs were done using MATLAB software. Differences between groups were checked using ANOVA, after testing ANOVA's assumptions. Homogeneity of variance was checked using the Bartlett's test, whereas normality was checked using the Shapiro–Wilk test. When assumptions were not met, log transformation of the data was followed. Failing the assumptions after log transformations a non-parametric Kruskal–Wallis ANOVA test was performed. In the case of multiple (more than 2) groups

comparison, a Tukey's multi-comparison test followed the ANOVA tests to reveal significant pairwise differences. An example is given in appendix C.

Chapter 5- DOM and inorganic nutrients in two wetlands with contrasting characteristics: The effect of topography, seasonality, sub catchment land use and wetland type.

5.1 Overview

DOM plays a very important role in aquatic ecosystems. Riparian wetlands commonly accumulate high quantities of DOM. One of the aims of the current study, as discussed in Chapter 1, is to study the spatial distribution of DOM in two wetlands with contrasting characteristics (described in Chapter 3). This chapter attempts a quantitative analysis of three DOM components, DOC, DON and SUP and links the results with different parameters. The effect riparian topography on the spatial distribution is investigated. The vertical distribution of DOM is also reported. Contrasts in wetland type and land use of the catchments are used to explain differences in DOM and inorganic species of carbon, nitrogen and phosphorus levels. Finally, the effect of seasonality is also investigated.

Key findings include:

- Riparian topography, specifically hillslope, affects DOM transport and thus accumulation in areas with lower hydraulic conductivity.
- Sampling strategy in wetlands needs to incorporate adequate representation of the local variation of DOM levels as proved by the strong spatial variability of DOM levels.
- DOM showed seasonal variation, with lowest concentrations reported in winter. The results can be partly explained by correlation with temperature.
- Differences between the two types of wetlands was reflected in different levels of DOC, with levels at Millersford being generally higher.
- Differences in land use of the study catchments affected the nutrient levels of the two wetlands, with levels at Ebbesbourne being higher.

5.2 DOM spatial distribution and vertical profiles

5.2.1 DOC

Mean DOC values in Millersford at 40 cm and 60 cm depth are summarised in Table 5.1. DOC levels at 40 cm depth showed high variability across the Millersford wetland, as shown in Figure 5.1 . Mean DOC concentrations ranged from 4.8 ± 2.8 mg/L at B4 to 35.1 ± 4.3 mg/L at C3. The vast majority of the mean concentrations were found to be significantly different. ANOVA tests were performed for every unique pair created from the 10 samplers. Only 9 out of the 45 pairwise comparisons performed showed no evidence of significant statistical difference. B4 was the sampling point where the minimum concentration was measured throughout the study and the mean concentration was significantly different to any other sampling point except C4.

All Millersford results for 60cm are presented in Figure 5.5 . Similar to the 40cm depth values, the lowest concentration was measured in B4 (4±2.5 mg/L) and the highest in B2 (40±4 mg/L). The concentrations at 60cm were found to be significantly different in the 5 sampling points except between B4 and C4. The concentrations in both depths showed a gradient with the minimum concentrations up the hillslope. Hence spatial distribution in relation to the hillslope elevation is studied in 5.2.1.1.

Mean DOC values in Ebbesbourne at 20 cm, 40 cm and 60 cm depth are summarised in Table 5.5. DOC concentrations at 40 cm in the Ebbesbourne wetland are presented in Figure 5.2. The variability was not as high as in the Millersford wetland. No significant difference was found between the sampling points within each zone. The mean concentrations ranged from 4.9±1.7 mg/L in zone 3 to 16.1±5.2 mg/L in zone 1. The mean differences amongst the zones were found to be significant, showing a gradient with the highest values closer to the river. Ebbesbourne results from different depths are presented in Figure 5.6. No significant difference was found within each zone. DOC concentrations in zone 1 were significantly higher than all other zones, with differences between zone 2 and 3 not found significant. Ebbesbourne values were much lower and could only be compared with the values of the two samplers at the edge of the Millersford wetland with the highest elevation (B4 and C4).

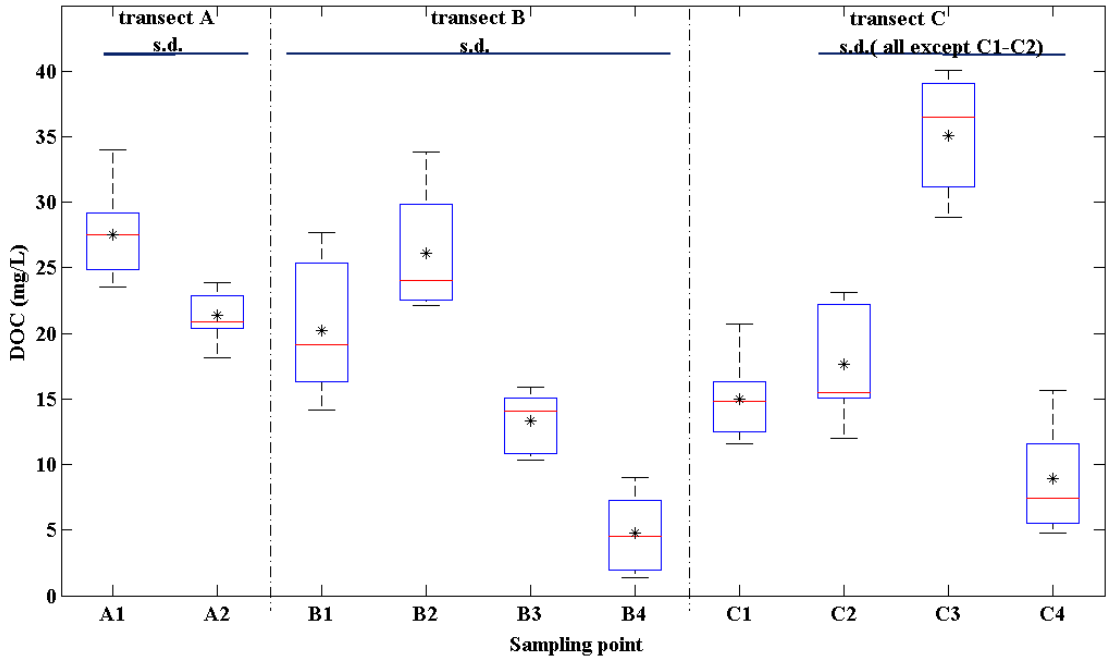


Figure 5.1 Boxplot of Millersford DOC (mg/L) concentrations at 40cm depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the red line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with an asterisk. Significant differences are indicated with s.d.

The differences in DOC levels in the two wetlands reflect wetland type differences. Temperature and water saturation conditions in wetlands impede plant material decay resulting in high levels of carbon. This phenomenon is not evident to the same extent at all wetland types. It is more pronounced in peat forming systems like Millersford (Clymo, 1984, Dise, 2009). Ebbesbourne wetland was studied after high flows of the adjacent stream had caused the wetland to flood. This flooding and the high permeability of the soils described in Chapter 3, can result in the flushing of DOC to the adjacent stream (Mladenov et al., 2005, Worrall and Burt, 2008).

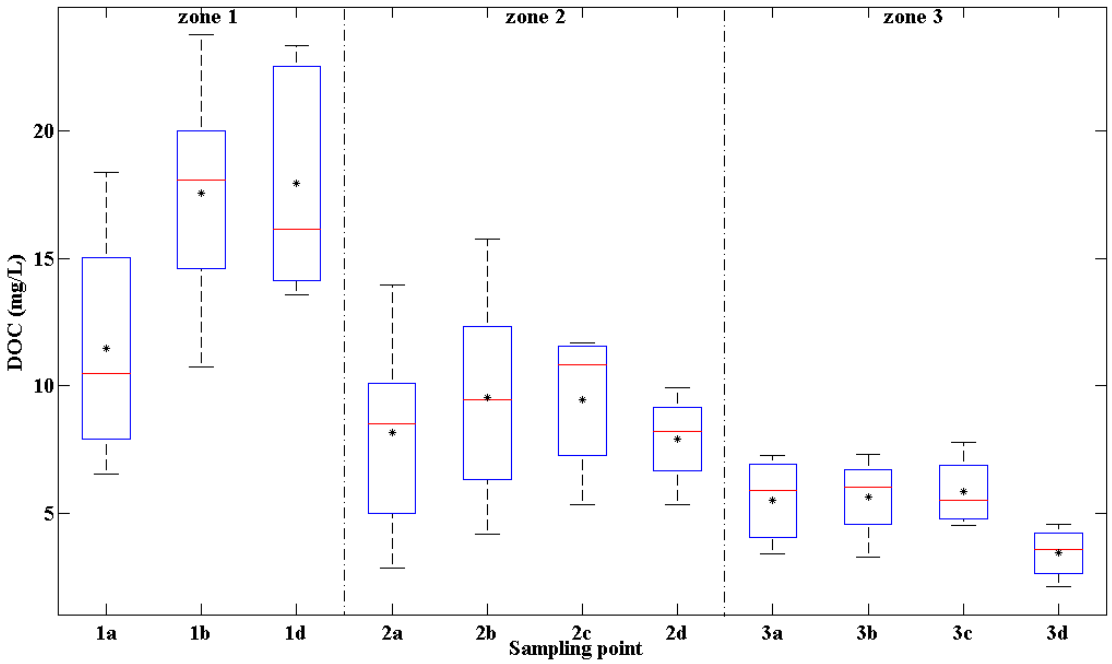


Figure 5.2 Boxplot of Ebbesbourne DOC (mg/L) concentrations at 40cm depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the red line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with an asterisk. Significant differences are indicated with s.d.

The DOC concentrations measured in Ebbesbourne fall in the range reported by Mann and Wetzel (1995) at the 30cm depth of a riverine wetland (6.3 -21.5 mg/L). The Millersford range is similar to those reported by Clark et al. (2008) for peat soils at 20 to 50cm depth (15.1 to 31.3 mg/L) and the values reported by Fellman et al. (2008) for bog and fen soil solution samples from piezometers at 25cm depth. Many papers report wide ranges of DOC concentrations but comparison is difficult as average values across all depths sampled are given; also, the sampling grid includes only a few sampling points and the wetland types are not identical (for example, Dalva and Moore (1991), D'Amore et al. (2010), Kane et al. (2014), Moore (2003), Ulanowski and Branfireun (2013).

The variability of the results at the Millersford site confirm the complexity of the biogeochemical processes involved in DOC transformations and transfers within a wetland. This variability underpins the need to incorporate spatial variability in wetland sampling strategies, as under-sampling could lead to inaccurate conclusions. This study of two wetlands with contrasting characteristics showed different levels of DOC and spatial variability. This difference illustrates the need to treat any extrapolation from studied to non-studied wetlands with extreme caution.

Table 5.1 Millersford wetland soil porewater DOC concentrations (mg/L) ± standard deviations. Range of values is shown in brackets.

40cm										60cm				
A1	**A2**	**B1**	**B2**	**B3**	**B4**	**C1**	**C2**	**C3**	**C4**	**B2**	**B3**	**B4**	**C2**	**C4**
27.5±2.9 (23.5-34)	21.4±1.9 (18.2-23.9)	20.2±5.1 (14.2-27.7)	26.1±4.5 (22.2-33.8)	13.4±2.2 (10.4-15.9)	4.8±2.8 (1.4-9)	15±2.8 (11.6-20.8)	17.6±4.2 (12-23.1)	35.1±4.3 (28.8-40.1)	8.9±4 (4.8-15.7)	40±4.1 (31-45.5)	16.7±3.5 (12.5-24)	4±2.5 (1.6-8.3)	21.5±3.8 (16.8-28.1)	5.9±1.8 (4.4-8.7)

Table 5.2 Millersford wetland soil porewater DON concentrations (mg/L) ± standard deviations. Range of values is shown in brackets.

40cm										60cm				
A1	**A2**	**B1**	**B2**	**B3**	**B4**	**C1**	**C2**	**C3**	**C4**	**B2**	**B3**	**B4**	**C2**	**C4**
1.5±0.9 (0.4-3.1)	1.6±0.9 (0.3-2.6)	1.3±0.9 (0.1-2.6)	1.2±0.6 (0.5-2.3)	0.9±0.5 (0.2-1.6)	0.6±0.5 (0.1-1.5)	0.6±0.3 (0.1-1)	1.1±0.6 (0.4-2.3)	1.7±0.8 (0.5-2.9)	0.6±0.5 (0-1.53)	1.7±0.6 (0.8-2.7)	1.2±0.6 (0.5-2)	0.7±0.6 (0.1-2)	1.1±0.4 (0.5-1.9)	0.8±0.7 (0-1.9)

Table 5.3 Millersford wetland soil porewater SUP concentrations (mg/L) ± standard deviations. Range of values is shown in brackets.

40cm										60cm				
A1	**A2**	**B1**	**B2**	**B3**	**B4**	**C1**	**C2**	**C3**	**C4**	**B2**	**B3**	**B4**	**C2**	**C4**
0.07±0.04 (0-0.16)	0.06±0.04 (0-0.1)	0.07±0.04 (0-0.13)	0.05±0.03 (0-0.09)	0.06±0.04 (0-0.11)	0.07±0.02 (0.05-0.11)	0.05±0.03 (0-0.11)	0.06±0.03 (0.01-0.11)	0.06±0.03 (0.01-0.09)	0.06±0.03 (0.01-0.11)	0.07±0.03 (0.01-0.12)	0.07±0.04 (0.01-0.12)	0.07±0.03 (0.02-0.13)	0.05±0.04 (0.01-0.13)	0.06±0.03 (0.01-0.09)

Table 5.4 Millersford wetland soil porewater DIC concentrations (mg/L) ± standard deviations. Range of values is shown in brackets.

40cm										60cm				
A1	**A2**	**B1**	**B2**	**B3**	**B4**	**C1**	**C2**	**C3**	**C4**	**B2**	**B3**	**B4**	**C2**	**C4**
6.1±4.6 (0.7-15.5)	7.5±3.2 (3-11.9)	6.3±3.9 (1.8-14.3)	3.4±3.7 (0.2-9.8)	4±1.7 (2.3-7.6)	4.3±3 (0.7-10.2)	3.3±1.6 (1-6.3)	5.2±3.7 (0.5-12.6)	3.5±2 (0.6-6.7)	3.5±2.1 (0.4-6.1)	6±4 (0.2-13.2)	3.6±1.7 (0.7-6.1)	2.8±2.3 (0.2-6.9)	5.5±2.7 (2-9.7)	4.9±3.3 (0.2-9.1)

Table 5.5 Ebbesbourne wetland soil porewater DOC concentrations (mg/L) ± standard deviations. Range of values is shown in brackets.

20cm			40cm			60cm		
zone 1	zone 2	zone 3	zone 1	zone 2	zone 3	zone 1	zone 2	zone 3
13.6±5 (2.7-13.6)	12.4±5.5 (5.5-23.4)	8.9±3.9 (2.1-16.3)	16.1±5.2 (6.5-23.8)	8.8±3.3 (2.9-15.8)	4.9±1.7 (2.1-7.8)	18.1±4 (11.8-25.9)	5.9±1.4 (3.4-8.8)	9.4±3 (5.9-15.6)

Table 5.6 Ebbesbourne wetland soil porewater DON concentrations (mg/L) ± standard deviations. Range of values is shown in brackets.

20cm	40cm	60cm
2.4±0.6 (1.3-3.6)	2.1±0.5 (1±3.2)	1.9±0.4 (1.1-1.9)

Table 5.7 Ebbesbourne wetland soil porewater TDN and its fractions mean concentrations (mg/L).

	20cm	40cm	60cm
DON	2.43	2.11	1.93
Nitrates	0.37	0.66	0.46
Ammonium	0.08	0.03	0.03
TDN	2.89	2.80	2.42

Table 5.8 Ebbesbourne wetland soil porewater SRP concentrations (mg/L) ± standard deviations. Range of values is shown in brackets.

Zone1			Zone2			Zone3		
20cm	40cm	60cm	20cm	40cm	60cm	20cm	40cm	60cm
0.07±0.05 (0.02-0.07)	0.23±0.21 (0.02-0.7)	0.12±0.05 (0.02-0.18)	0.03±0.02 (0.01-0.06)	0.014±0.01 (0-0.03)	0.02±0.002 (0-0.07)	0.05±0.04 (0-0.13)	0.02±0.02 (0-0.06)	0.02±0.01 (0-0.05)

5.2.1.1 *Hillslope elevation effects on DOC spatial distribution*

In order to explain the spatial variability of DOC levels within the Millersford wetland, DOC correlation with elevation was also investigated. The correlation plots of DOC and DON at 40 and 60cm depth are shown in Figure 5.3 and Figure 5.4. DOC concentrations at both depths showed strong negative correlation with elevation, as indicated by correlation coefficient (R) values of -0.7 for 40cm and -0.9 for 60cm ($p<0.05$). Similarly, R values for DON were 0.6 for 40cm and 60cm ($p<0.05$). It should be noted that if data from C3 were to be excluded the correlation coefficient would drastically increase to 0.9 for DOC and 0.8 for DON at 40cm depth. Further investigation is needed to understand the hydrological pathways that influence DOM level at that point of the wetland. However, the exception of C3 confirms that other factors and not only elevation affect DOM variability.

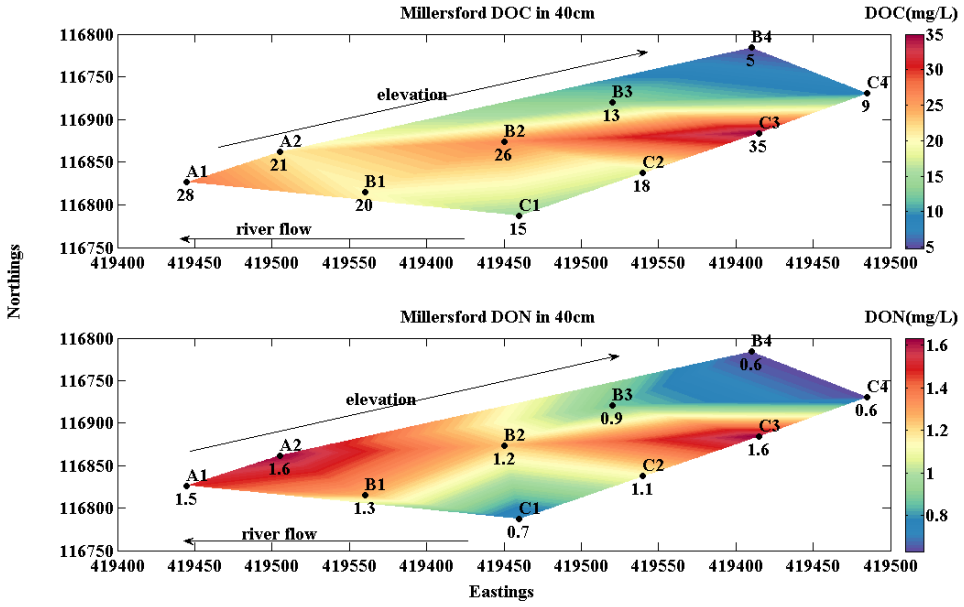


Figure 5.3 Contour plot of DOC (top) and DON (bottom) concentrations at 40cm depth (both in mg/L) along the Millersford wetland.

Hillslope position affects carbon levels in soils. Elevation affects drainage and redox status. Other workers have shown increases in soil carbon moving downslope, showing a strong correlation with distance down the hillslope profile, slope and elevation (Hancock et al., 2010). In contrast, Boothroyd et al. (2015) measured DOC in soil pore water samples collected from 1m deep dipwells across two peatland hillslopes. They reported that hillslope position significantly affected DOC concentration, with lower DOC levels at bottom-slope positions and higher at steeper mid-slopes compared to up-

slope positions. They attributed DOC behaviour to flushing out of the system from bottom slope and increased DOC production and extended residence times at the mid-slope, leading to build-up of humic-rich DOC compounds. However, in their study only the bottom slope part of the wetland was water saturated, resulting in higher DOC production in the aerobic zones of the other parts of the wetlands.

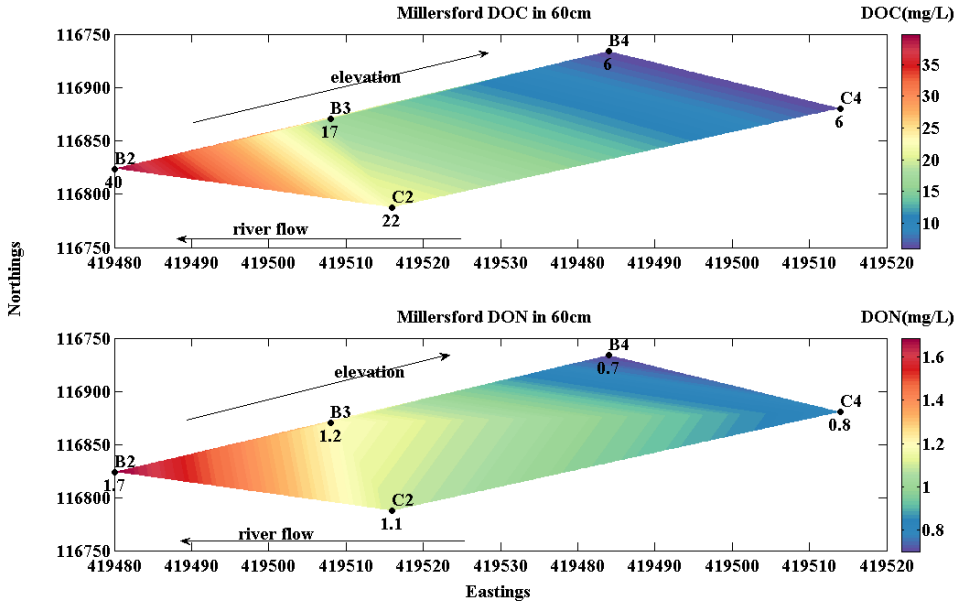


Figure 5.4 Contour plot of DOC (top) and DON (bottom) concentrations at 60cm depth (both in mg/L) along the Millersford wetland.

These results indicate DOM accumulation, as soil pore water flows are created because of hydraulic pressure in a direction from the more elevated parts of the hillslope towards the flatter regions; in the Millersford case, the riverbank (e.g. sampling point A1). Indeed, transport of DOM affects its accumulation, as the DOM produced or introduced in the more elevated areas is flushed because of increased ground water and soil pore water flows (Schiff et al., 1998). This is also confirmed by studies on the residence times hillslope soil water, showing that soil water aged in a downslope direction (Uchida et al., 2006).

5.2.1.2 DOC vertical profiles

DOC levels at 60cm were compared with concentrations at 40cm. In B4, C2 and C3 the concentrations did not vary significantly between the depths. However, in B2 and B3 DOC concentration increased significantly at 60cm.

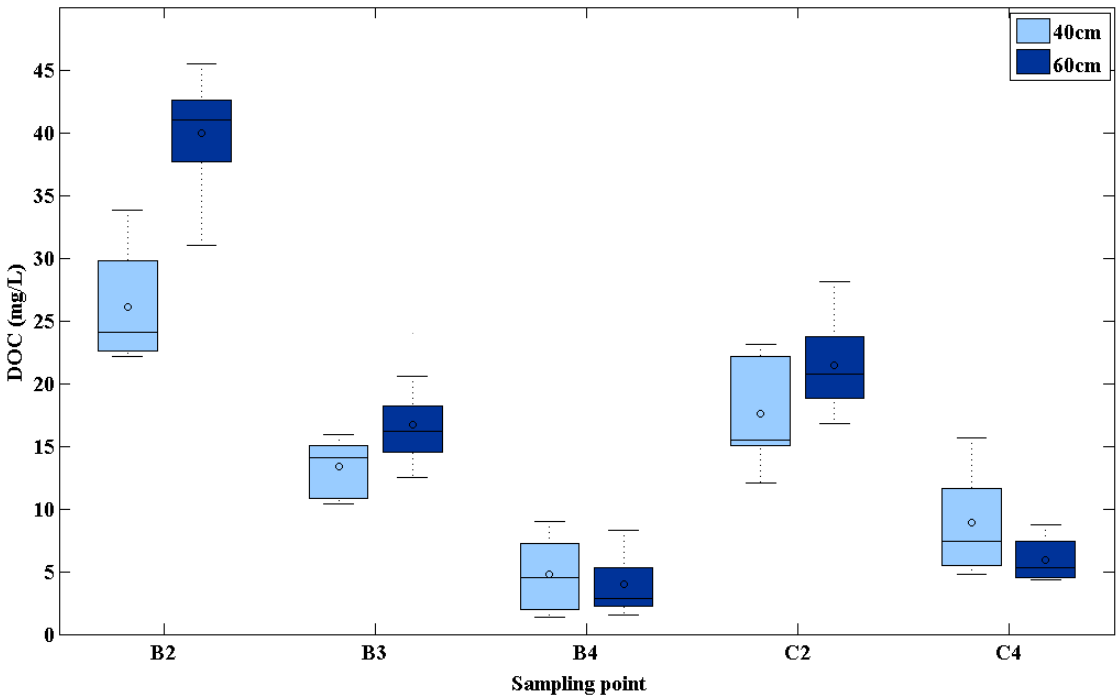


Figure 5.5 Boxplot of Millersford DOC concentration (mg/L) at 40cm (light blue) and 60cm (dark blue) depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the central horizontal line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with a circle.

Differences in each zone between the different depths sampled at Ebbesbourne were also investigated (Figure 5.6). No evidence of significant difference was found for zone 1. In zone 2 the 60cm mean was significantly lower than 20cm and 40cm. In zone 3, the 40cm mean was significantly lower than 20cm and 60cm means. High permeability of the soil in Ebbesbourne (Chapter 3) could possibly prevent the formation of stratigraphy in DOC levels. Further research is needed to investigate possible trends.

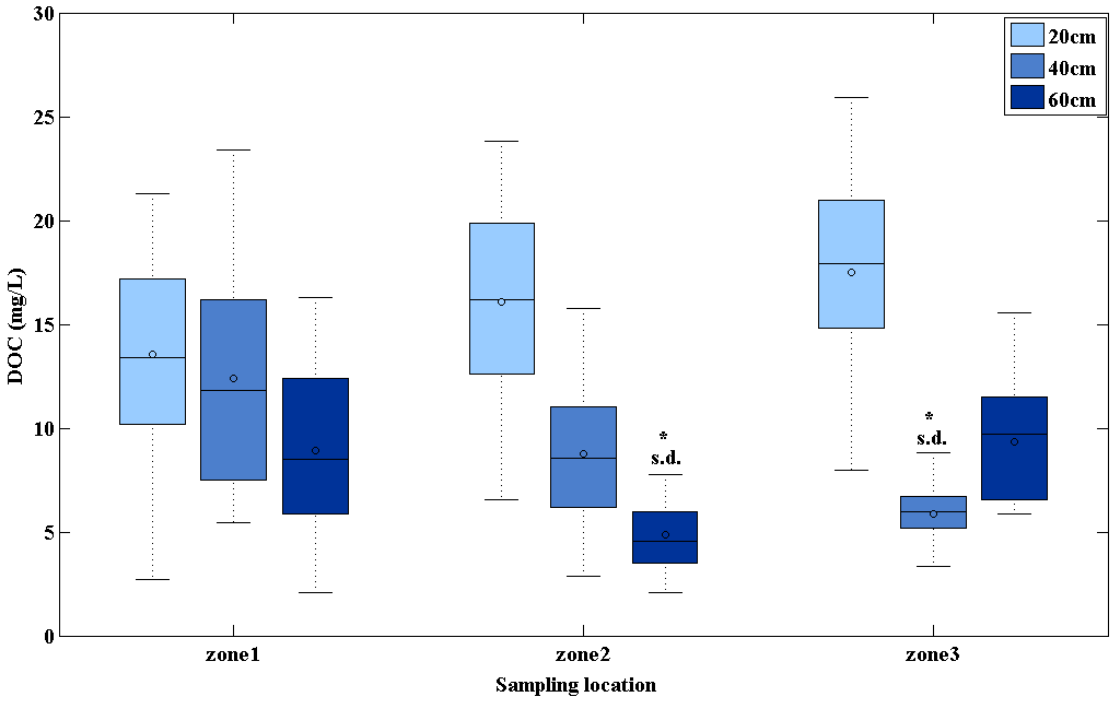


Figure 5.6 Boxplot of Ebbesbourne DOC concentration (mg/L) at 20cm (light blue), 40cm (darker blue) and 60cm (darkest blue) depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the central horizontal line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with a circle. Significant differences are indicated with s.d.

The vertical DOC profile at Millersford is in agreement with the literature. Schiff et al. (1998) explained that although DOC production in wetlands is higher closer to the surface, the maximum concentration occurs in the depth of highest accumulation. It is in the same depth as the low hydraulic conductivity layers. Mann and Wetzel (1995) reported DOC values in samples collected using porous ceramic cups at 0, 20, 30, 60, 90 and 130cm depths. Their study was inconclusive regarding DOC behaviour with depth. In parts of the riverine wetland pond, DOC increased significantly with depth. In other parts, maximum values were observed in 60cm, 20cm and 90cm (hill slope). Similarly, Orem et al. (1997) reported a variety of DOC profiles with depth in Florida Everglades. Some of the sites showed multiple DOC maximum values. More often the DOC concentration increased with depth reaching a maximum value and then decreased constantly. Xi et al. (2007) studied DOC in water samples extracted from soils in a ring-shaped wetland in China. In all plant communities of the wetland, DOC decreased initially with depth, reaching a minimum at 40-60cm depth and increased again at 60-80cm.

This study agrees with those workers who believe that further research is needed to study DOC profile with depth below the 30cm surface soil layer; e.g. Olson and Al-Kaisi (2015); Hiederer (2009). The differences in the vertical DOC profile among the different sampling points indicate potential subsurface preferential flow patterns and possible effects of microtopography. Average values across different sampling depths in wetlands, that are reported widely in the literature, could hide important information on DOC vertical distribution.

5.2.2 DON

Mean DON values in Millersford at 40cm and 60cm depth are summarised in Table 5.2. DON 40cm concentrations varied from 0.63±0.29 in B4 to 1.65±0.84 mg/L in C3, as shown in Figure 5.7 . The differences between DON levels in each of the three transects were not found to be significant except for two sampling points along the third transect (C3 and C4).

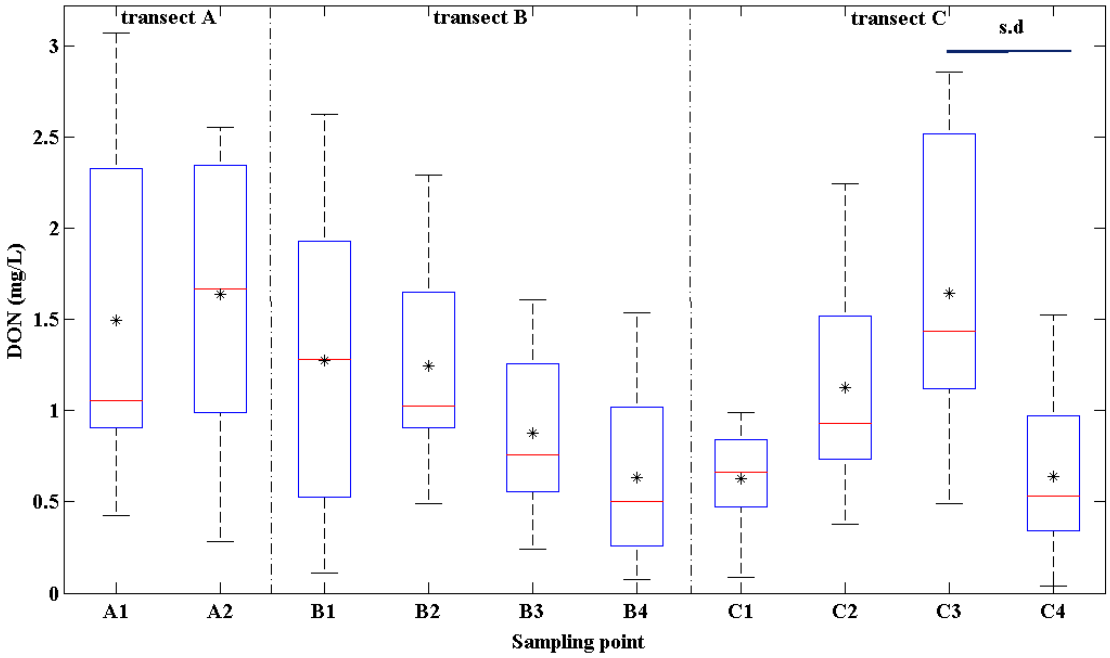


Figure 5.7 Boxplot of Millersford DON (mg/L) concentrations at 40cm depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the red line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with an asterisk. Significant differences are indicated with s.d.

All Millersford results for 60cm are presented in Figure 5.8 . The lowest mean concentration (0.72 mg/L) was measured at B4, whereas the highest (1.66 mg/L) at B2.

Pairwise comparisons showed significant differences among means that did not however reveal spatial trends. DON concentrations in 60cm were compared with the ones measured in 40cm depth. There was no evidence of change in DON levels with depth.

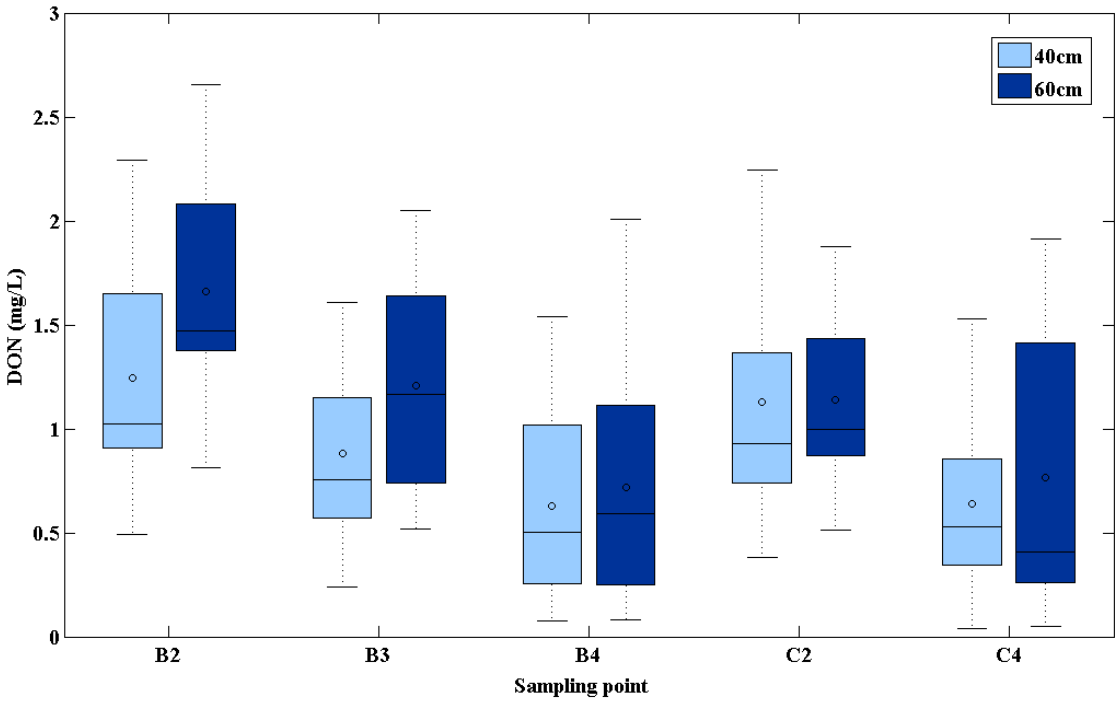


Figure 5.8 Boxplot of Millersford DON concentration (mg/L) at 40cm (light blue) and 60cm (dark blue) depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the central horizontal line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with a circle.

Mean DON values in Ebbesbourne at 20cm, 40cm and 60cm depth are summarised in Table 5.6. All Ebbesbourne results for 40cm are presented in Figure 5.9 . No significant difference was observed among the DON levels in the different sampling points of each zone. No evidence of significant different was found among the zones either.

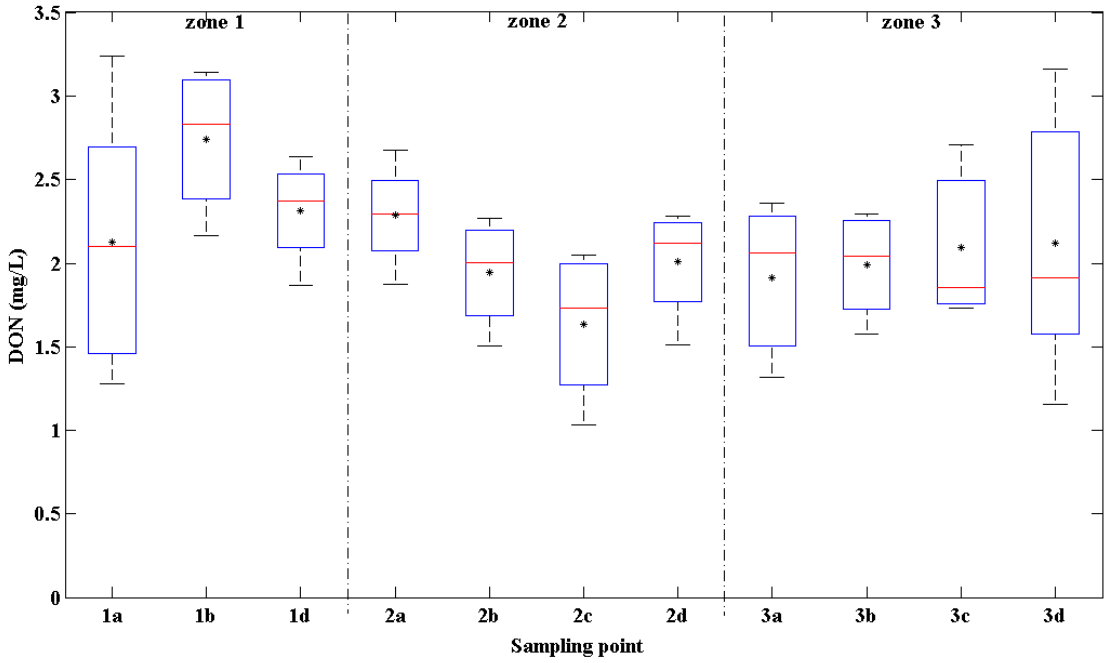


Figure 5.9 Boxplot of Ebbesbourne DON (mg/L) concentrations at 40cm depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the red line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with an asterisk.

Ebbesbourne results for all depths are presented in Figure 5.10. The results are summarised per depth as no significant difference was observed among the different sampling points. Concentrations ranged from 1.93±0.37 mg/L in 60cm to 2.43±0.62 mg/L in 20cm. DON levels in 20cm were significantly higher than the ones in 40cm and in 60cm. No significant difference was found between 40 and 60cm.

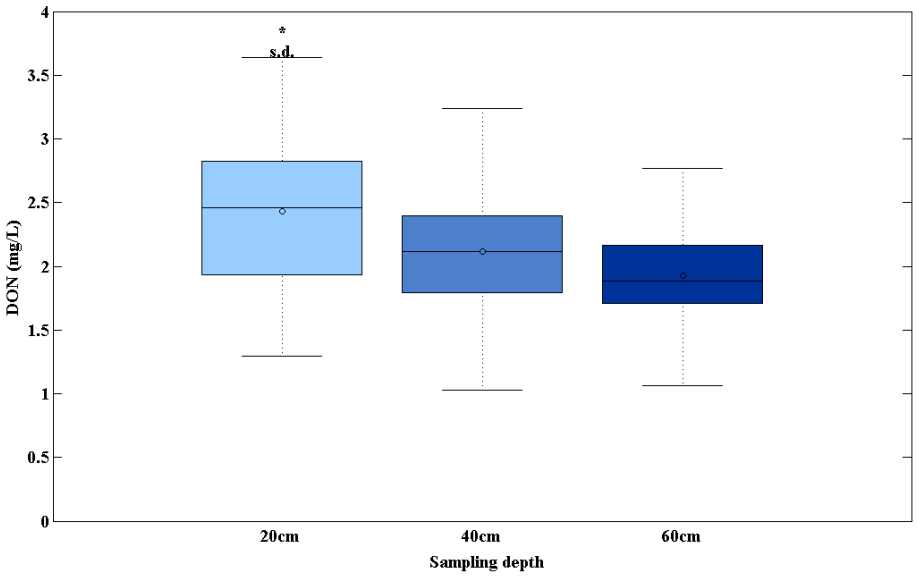


Figure 5.10 Boxplot of Ebbesbourne DON concentration (mg/L) at 20cm (light blue), 40cm (darker blue) and 60cm (darkest blue) depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the central horizontal line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with a circle. Significant differences are indicated with s.d.

The aforementioned difficulty of comparing DOC values with published ones (methods used) was experienced also for DON. In addition, DON is considered to be an understudied parameter, especially in agricultural soils (van Kessel et al., 2009). The DON values measured at Millersford are higher than those reported for soil pore waters of a peat blanket, the latter ranging from 0.515±0.008 in 10cm to 0.379±0.011 mg/L in 50cm (Adamson et al., 1998). The values reported here are similar to ones estimated by D'Amore et al. (2010) for wetland soils (up to 20cm depth) that ranged from 0.03 to 2.4 mg/L. Spatial distribution at Millersford did not reveal patterns and was not correlated to hillslope position. This could be explained by the role of nitrogen as a limiting nutrient. The elevated concentrations at Ebbesbourne could be linked to the human-impacted nature of the catchment and the presence of septic tanks.

5.2.3 SUP

Mean SUP values In Millersford at 40cm and 60cm depth are summarised in Table 5.3. SUP values at 40cm depth are presented in Figure 5.11. The mean concentration of SUP ranged from 0.052±0.033 mg/L in B2 to 0.073±0.019 mg/L in B4. The differences between the concentrations in the different samplers were tested statistically and no evidence of significant difference was found.

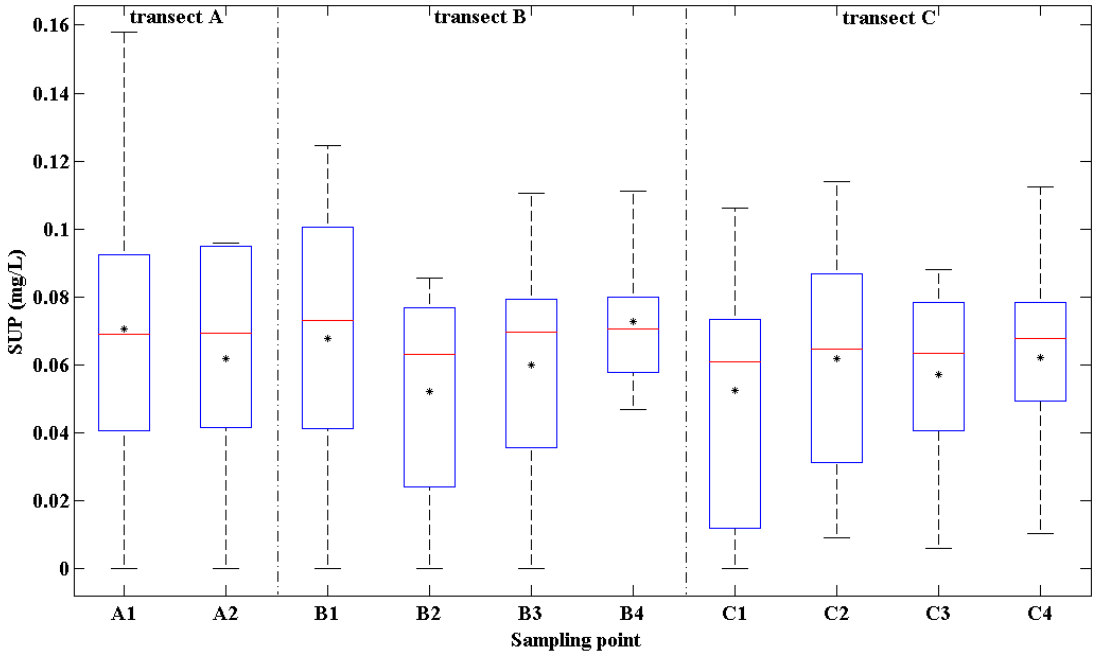


Figure 5.11 Boxplot of Millersford SUP (mg/L) concentrations at 40cm depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the red line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with an asterisk.

The vertical distribution of SUP was also investigated. All results in 60cm are presented in Figure 5.12. The mean concentration of SUP in 60cm depth ranged from 0.051±0.04 to 0.071±0.033 mg/L. No evidence was found that the mean concentrations in the 5 different samplers varied significantly. The concentrations were compared to the ones measured in 40cm and there was no evidence that the concentration of SUP varied with depth.

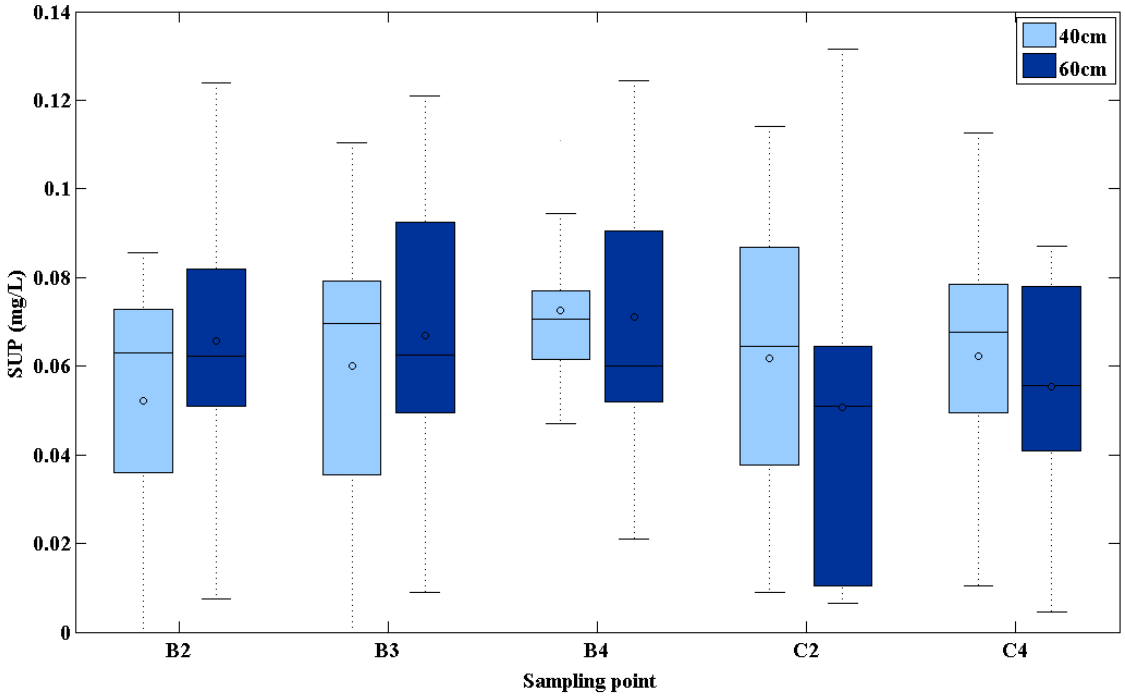


Figure 5.12 Boxplot of Millersford SUP concentration (mg/L) at 40cm (light blue) and 60cm (dark blue) depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the central horizontal line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with a circle.

All Ebbesbourne SUP results for 40cm sampling depth are presented in Figure 5.13. SUP mean concentrations ranged from 0.07±0.03 to 0.08±0.02 mg/L. No evidence of significant difference was found amongst the means of all samplers in 40cm. Ebbesbourne SUP concentration in the three depths studied is shown in Figure 5.14. The concentrations did not vary with sampling location within the same depth, or with depth. Mean SUP concentration was 0.08±0.02 mg/L in all depths studied.

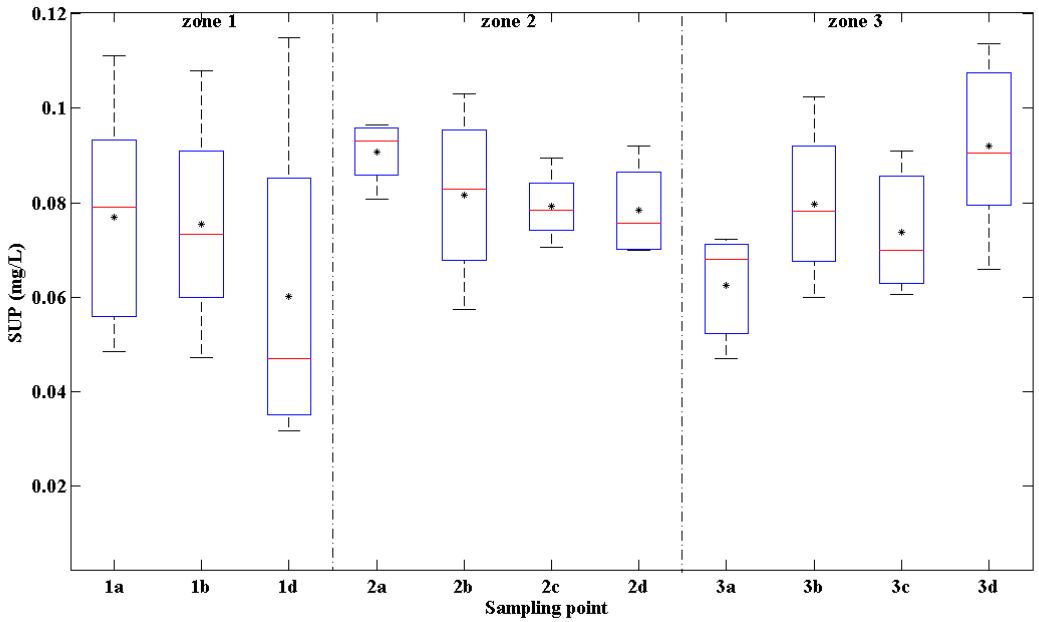


Figure 5.13 Boxplot of Ebbesbourne SUP (mg/L) concentrations at 40cm depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the red line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with an asterisk.

Phosphorus is the primary limiting factor in wetlands. The values reported agree with earlier studies. Orem et al. (1997) measured unreactive phosphate in squeezed core pore water samples that were then filtered. They reported that in freshwater marshes the concentrations were generally less than 60 µg/L. Maximum concentrations were observed in the upper 20cm and then gradually decreased. However, many sites had different profiles with maximum values at different depths, and multiple depths with higher values. According to Orem et al. (1997), this was attributed to "local anomalies in substrate characteristics, microbial community structure in the sediments, or the dissolved reactive phosphate concentration of groundwater infiltrating the sediments".

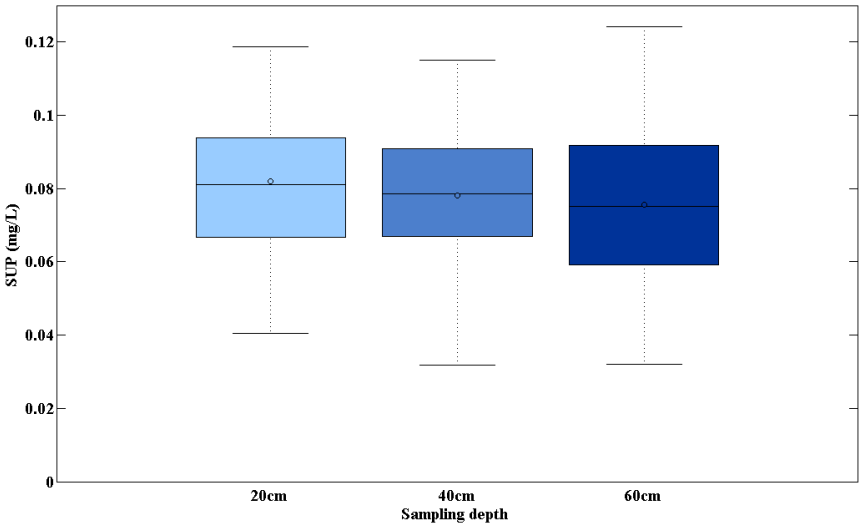


Figure 5.14 Boxplot of Ebbesbourne SUP concentration (mg/L) at 20cm (light blue), 40cm (darker blue) and 60cm (darkest blue) depth. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the central horizontal line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with a circle.

5.3 DOM temporal variability

Seasonal patterns of DOM were evaluated. The results are not biased by the spatial variability or vertical distribution of DOM, as the samplers were equally represented for each season. The results summarise data from all sampling points and depths per wetlands and are shown in Figure 5.15.

DOC in Millersford samples dropped from 22 mg/L in summer to 17 mg/l in winter. None of the differences, amongst the seasons, were found to be significant. In Ebbesbourne, DOC levels decreased from 15 mg/L in summer to 8 mg/L in winter. Mean DOC concentration was found to be significantly lower in winter. Both wetlands had DOC minimum concentrations in winter and maximum in summer, with Ebbesbourne data showing stronger seasonal variability.

Millersford DON levels dropped from 1.5 mg/l in summer to 0.6 mg/L in winter. All differences were found to be significant, indicating clear seasonal dynamics. Maximum Ebbesbourne DON concentrations were measured in summer (3.1 mg/L) and minimum in winter (2 mg/L). Pairwise comparisons revealed significant statistical difference only between summer and winter.

SUP concentrations in Millersford showed a different pattern. Similar to DOC and DON, minimum concentrations were observed in winter (0.05 mg/L), but maximum concentrations were measured in autumn (0.08 mg/L). There was no significant difference between summer and autumn or winter and spring means. The rest of pairwise comparisons showed significant statistical differences. Ebbesbourne SUP levels dropped from 0.36 mg/L in summer to 0.08 mg/L in winter. Similar to Millersford, statistical analysis revealed significant differences except between summer and autumn and between winter and spring means.

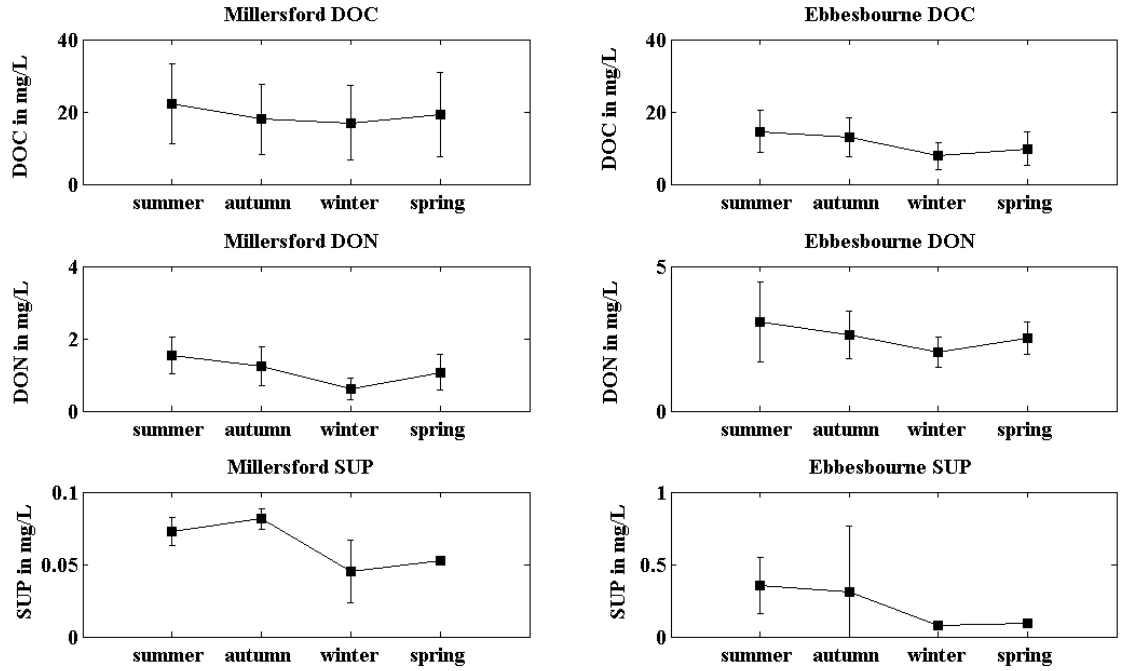


Figure 5.15 Line graph of mean DOC, DON and SUP (mg/L) concentrations for each season in boxes with standard deviation error bars.

Seasonal dynamics in both wetlands agree with the literature. Kalbitz et al. (2000) reviewed numerous field studies and laboratory experiments that showed seasonal variability in DOM concentrations in soil solution. Those studies reported higher concentrations of DOC in summer than in winter. The main driver identified was temperature. Studies on the temporal dynamics of DOM in wetlands confirm the same pattern. D'Amore et al. (2010) reported peak summer DOC concentrations in soil porewaters of a forested wetland and a sloping bog. They concluded that water table and soil temperature were significant factors associated with DOC levels. Dalva and Moore (1991) reported on maximum DOC concentrations in swamp soil during summer resulting from thermal and hydrological regimes. Mann and Wetzel (1995) measured

higher DOC during summer in the surface water of a riverine wetland and suggested that this is due to macrophyte and periphyton primary production as well as microbial activity converting particulate organic matter to DOC. Bonnett et al. (2006) showed that the key factor for the observed DOC seasonality in a valley-bottom riparian peatland was temperature. According to the authors the correlation between temperature and DOC might be because of plant rhizodeposition and microbial activity.

The association of temporal dynamics with temperature, reported in the studies mentioned above, was confirmed. Correlation of DOM with temperature was investigated for both wetlands. In Millersford only DOC showed a good correlation (R^2 0.91, $p<0.1$). In Ebbesbourne both DON (R^2 0.97, $p<0.1$) and DOC (R^2 0.9, $p<0.1$) exhibited strong correlations with temperature.

Although during the period of study both wetlands were water saturated, as proven by dipwell observations, Ebbesbourne sampling followed a particularly dry year. Rewetting of the wetland had possibly contributed to stronger seasonal variation of DOC. In contrast, Millersford wetland was studied during a particularly wet summer that could be related to the resulted moderate seasonal variation. Kalbitz et al. (2000) also reviewed the "rewetting effect" after dry periods. Both field and laboratory studies concluded increased concentration of DOC after dry periods that resulted from anaerobic conditions. Large rainfall events contribute to flushing out stored or absorbed DOM. Tipping et al. (1999) showed that the combination of warming and drying has a more pronounced effect on DOC production than warming alone. However, in comparison to DOC, fewer reports deal with DON and SUP dynamics. DON studies show similar patterns to DOC (Chow et al., 2013, Kalbitz et al., 2000).

5.4 Dissolved carbon, nitrogen and phosphorus speciation

The method of analysis provided data also on inorganic nutrients. Those data were used as added-value to the study, in order to evaluate the effect of external sources e.g. land use on the nutrient levels of the wetlands.

5.4.1 Dissolved carbon

Dissolved carbon speciation data are available only for Millersford because of the methods used to measure DOC (see Chapter 4). Mean DIC values in Millersford at 40cm

and 60cm depth are summarised in Table 5.4. DIC concentrations at 40cm depth are presented in Figure 5.16 . DIC mean concentrations ranged from 3.33 in C1 to 7.47 mg/L in A2. There was no evidence that the concentrations were significantly different. Similarly, the concentrations of DIC in 60cm depth were not found to differ significantly. The range of mean DIC levels was 2.81 mg/L in B4 to 5.98 mg/L in B2. There is no evidence that the concentrations in 40cm are statistically significant different to the ones measured in 60cm in any of the samplers.

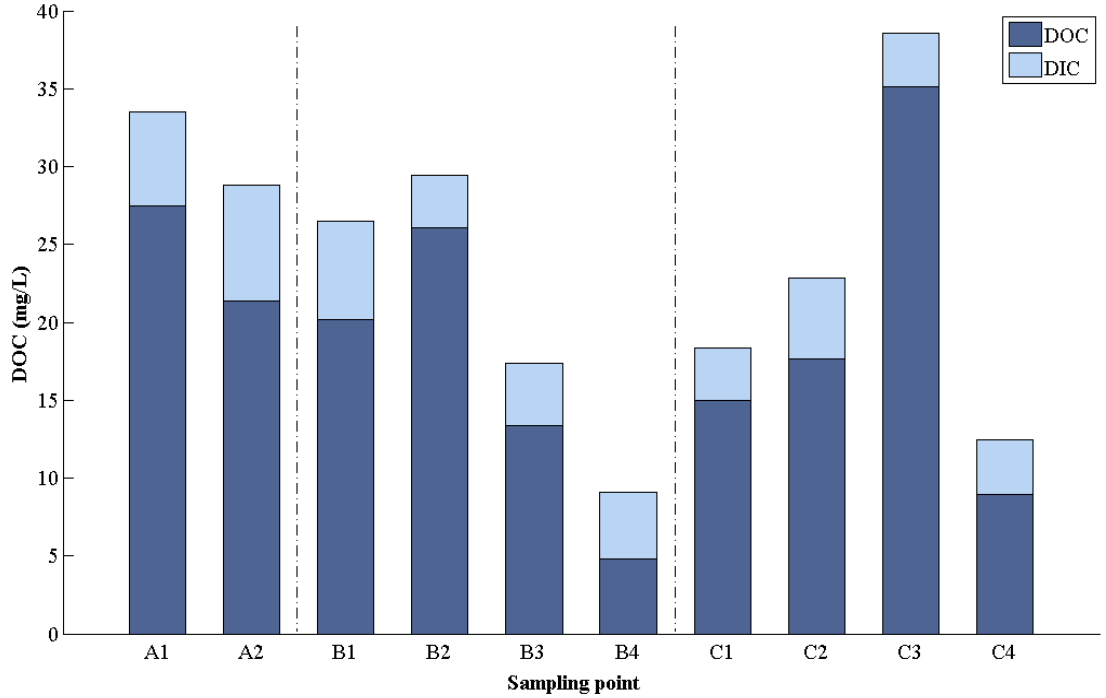


Figure 5.16 Bar graph of Millersford mean TDC concentration (mg/L) at 40cm depth. DOC is shown in dark blue and DIC in light blue.

Dissolved organic carbon was the larger fraction in all 40cm samplers, as illustrated in Figure 5.16 . DOC contributed 54 to 91% of total dissolved carbon. The organic fraction of total dissolved carbon (TDC) did not differ significantly with sampling location, except B4 that was significantly lower than a few but not all sampling points. The DOC fraction of TDC at 60cm was between 0.63 in B4 and 0.88 in B2 (see Figure 5.17), with B2 significantly higher than B4 and C4. The mean fractions in those samplers where both depths were included were tested statistically to investigate whether the fraction of TDC changed with depth. There was no evidence that the fractions of DOC in the two depths measured differ from each other.

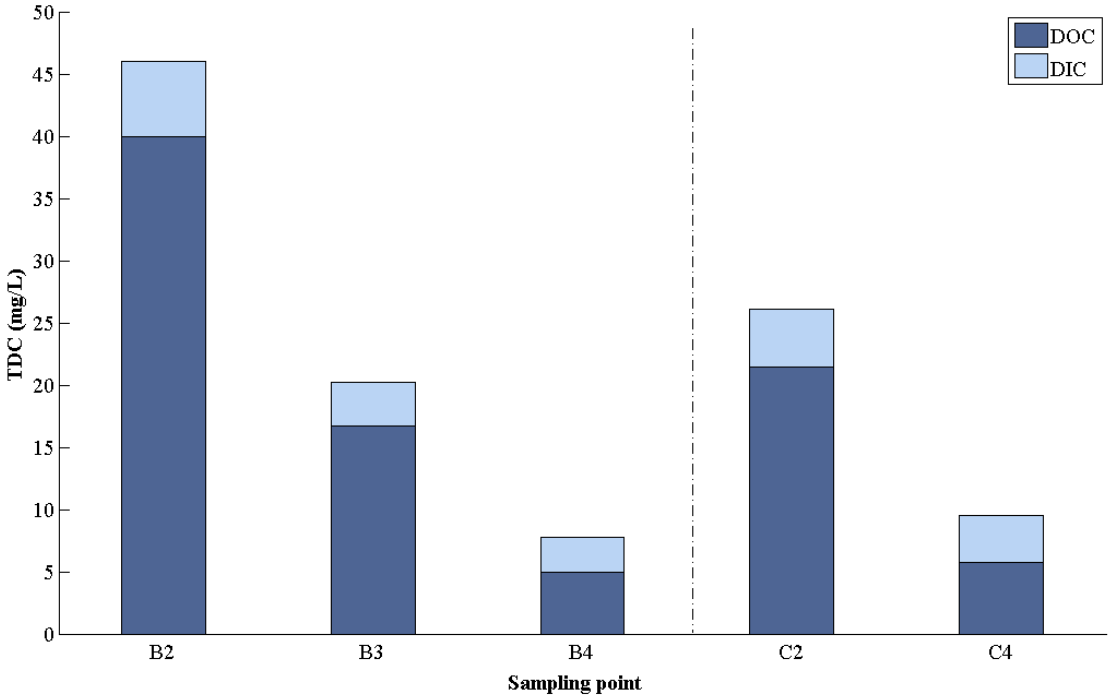


Figure 5.17 Bar graph of Millersford mean TDC concentration (mg/L) at 60cm depth. DOC is shown in dark blue and DIC in light blue.

DIC studies in wetlands are generally not reported and inorganic carbon estimates in soil are limited. It is now accepted that this area needs further research in order to gain better understanding of global carbon cycling; e.g. Wang et al. (2013), Mi et al. (2008), Rawlins et al. (2011). In the upper 1m of soil globally, inorganic carbon represents one third of soil carbon (Batjes, 1996). Mi et al. (2008) studied soil inorganic carbon in different climatic regions of China. They found that SIC (DIC) levels follow the descending order: desert, grassland, cropland, marsh, shrubland, meadow, forest, with SIC in marsh following mixed patterns with depth. Bradley et al. (2007) reported much higher DIC concentrations, ranging from 61.9±18.9 in 0-45cm to 63.1±12.8 mg/L in 50-100cm. They too noted that trends in DIC have received little attention. The levels of DIC reported here are between the values reported by Webster and McLaughlin (2010) for poor fen (3.2 mg/L) and intermediate fen (7.7 mg/L). The authors reported these average values from water samples collected using piezometers at 25, 50, and 100cm depths and found that DIC concentrations increased with depth.

DIC concentration depends on underlying geology groundwater sources and organic matter decomposition. However, the results did not lead to identification of areas of groundwater recharge or of higher organic matter decomposition rates.

5.4.2 Dissolved nitrogen

In Millersford DON comprised the largest fraction of total dissolved nitrogen in all samplers. Nitrate levels were mostly below the detection limit (76% of the data). Ammonium concentrations were below the detection limit with the exception of A1, A2 and B1 samples that had mean concentrations of 1.08, 1.07 and 0.79 respectively.

Ebbesbourne dissolved nitrogen fractions (Table 5.7) in all depths were statistically tested for significant differences. None was detected for all fractions or TDN in all three depths. Therefore, the data are summarised for each depth in Figure 5.18 . The vast majority of Ebbesbourne samples had ammonium concentrations below detection limit (93%). Nitrate in 40cm mean was significantly higher than 20cm.Total dissolved nitrogen mean in 60cm was significantly lower to both 20cm and 40cm means. DON represented between 77% and 85% of TDN in the three depths studied.

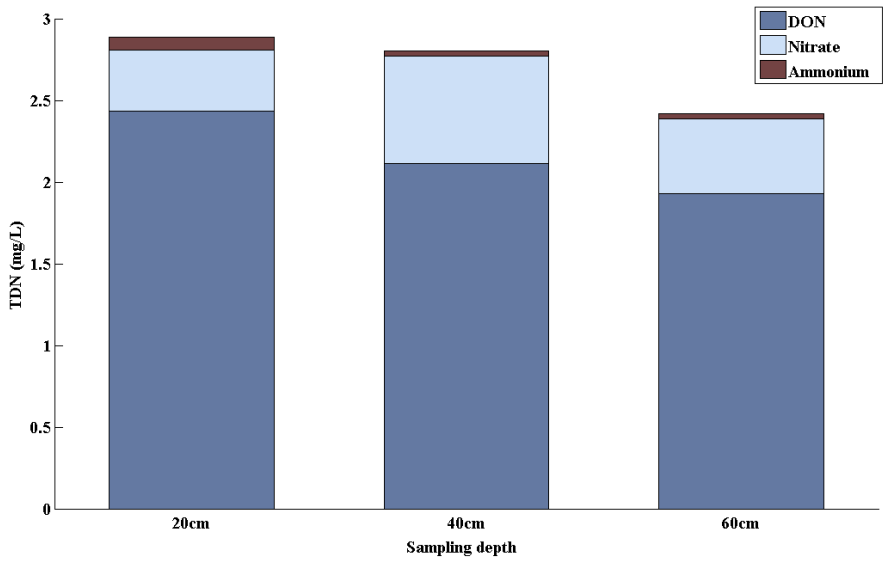


Figure 5.18 Bar graph of Ebbesbourne mean TDN concentration (mg/L) at 20cm, 40cm and 60cm depth. DON is shown in dark blue, nitrates in light blue and ammonium in brown.

In this study, DON was the main TDN component. This was expected because organic forms of nitrogen in wetlands soils represent the largest pool of nitrogen (Adamson et al., 1998, Maltby and Barker, 2009). D'Amore et al. (2010) reported that in all water samples collected from a forested wetland and a bog (using zero tension lysimeters), DON constituted more than 85% of TDN at all depths sampled (10 and 20cm). Christou et al. (2005) studied DON on soil solutions from seven contrasting agricultural land use types. DON levels followed the sequence

citrus>vegetable>forest=arable>grassland=wetland>heathland and represented on average 57±8% of TDN. The highest percentage was estimated for wetlands, at 94±1%. The authors noted that DON is less sensitive to land use compared to DIN.

Nitrate in Ebbesbourne were higher in all depths, signifying the use of inorganic fertilisers and the pottencial presence of septic tanks in the area. Ammonium in A1, A2 and B1 at Millersford can be related to the presence of grazing cattle. Adamson et al. (1998) measured nitrogen in soil pore waters using suction samplers at 10cm and 50 cm depth. They reported NO_3^- values for both depths that were not 'appreciable' (0.003±0.001 mg/L in 10cm and 0.001±0 mg/L in 50cm). In the 10cm samplers NH_4 values were low (0.019±0.004) and much greater in 50cm (0.411±0.015). Prior and Johnes (2002) measured nitrogen in soil pore waters in 20,40 and 60cm depth. They found that maximum concentration for all N species occurs in 60cm. Stanley and Ward (1997) measured inorganic nitrogen in wetland soil pore water. Concentration of NH_4-N ranged from less than 2 to 3177μg/L; NO_3-N ranged from less than 2 to 16μg/L. Depth profiles were not the same across the wetland. At most sites in the Everglades, Florida, ammonium levels in pore waters were between 100 and 400μg/L, and vertical profiles were different for each site (Orem et al., 1997).

5.4.3 Dissolved Phosphorus

In Millersford, SUP was the main fraction of TDP, with SRP values being below detection limit. Ebbesbourne TDP speciation in all depths is shown in Figure 5.19 . SRP means (Table 5.8) in zone 1 were found significantly higher than zone2 and 3. Some variation with depth was also observed but did not reveal a constant pattern across the zones. No significant difference was found in zone 3 whereas 20 and 40cm means differ in zones 1 and 2 but in the reverse order. As no significant difference was found among SUP means in the different sampling points and depths sampled, the differences in proportions of TDP as SUP and TDP fractions mirrored the ones of SRP. The SUP fraction of TDP ranged from 37% to 82%.

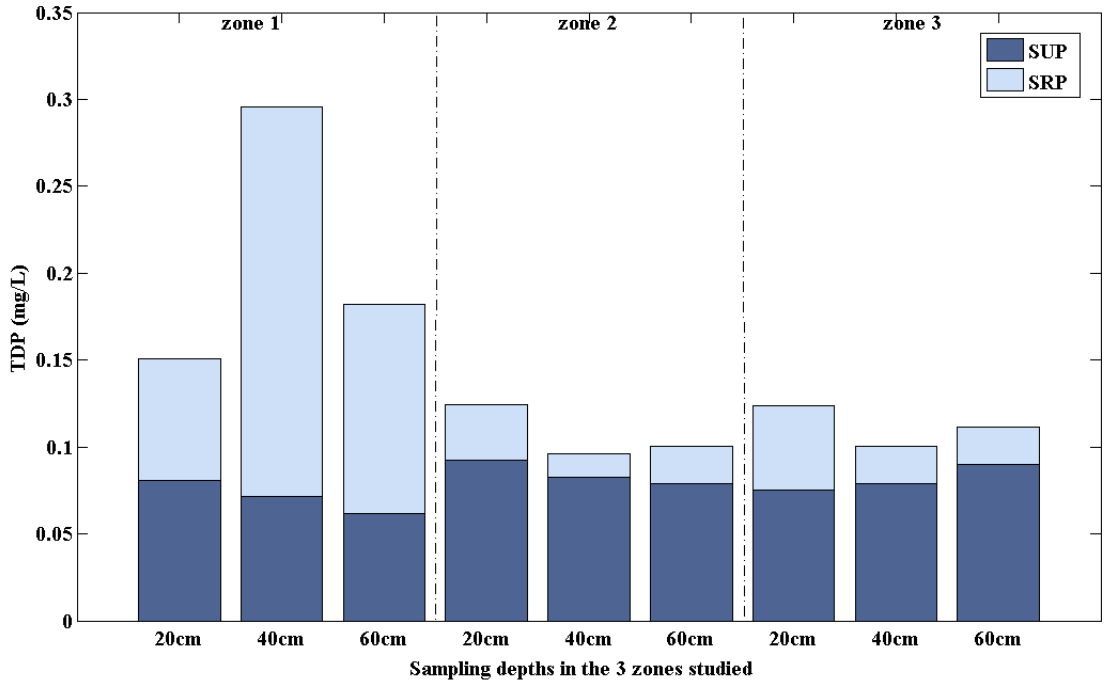


Figure 5.19 Bar graph of Ebbesbourne mean TDP concentration (mg/L) at 20cm, 40cm and 60cm depth. SUP is shown in dark blue and SRP in light blue (both in mg/L).

SUP in Millersford represented the larger part of the TDP pool in all locations and depths, reaching 99%. In Ebbesbourne the percentage was lower, indicating possible influence of the arable nature of the catchment. Phosphorus is a key element that limits ecosystem processes in both freshwater and wetland ecosystems (Daniel et al., 1998, Maltby and Barker, 2009, Rejmankova, 2001, Richardson, 1999, Wetzel, 1983). A large part of phosphorus in wetland soils is in organic form (Davelaar, 1993, Newman and Robinson, 1999). Reddy and DeLaune (2008) reported that organic P accounts for 50-90% total soil P in wetland organic soils, and 10-50% in mineral wetland soil.

5.5 Conclusions

To better understand DOM dynamics within wetlands, spatial and vertical distribution of DOM within two wetlands were studied. The effects of environmental factors, land use and wetland type were examined in correlation with temporal variability, nutrient speciation and comparison of two wetlands with contrasting characteristic.

DOC and DON concentrations in Millersford increased at the bottom of the hillslope and were linearly correlated with elevation, suggesting that hydrology is an important driver controlling DOM dynamics. Spatial distribution of DOC in Millersford indicates DOC accumulation as soil pore water flows are created due to hydraulic pressure with a direction from the more elevated parts of the hillslope towards the flatter regions. At the Millersford site, this direction is towards the riverbank (e.g. sampling point A1). This is in agreement with the research of other workers (Boothroyd et al., 2015, Hancock et al., 2010). Transport of DOC affects its accumulation, as the DOC produced in the more elevated areas is flushed because of increased ground water, soil pore water flows (Schiff et al., 1998). This is confirmed by studies on the residence times hillslope soil water, showing that soil water aged in a downslope direction (Uchida et al., 2006). Hillslope position of wetlands is a factor affecting DOC transfers and transformations that has been overlooked (Boothroyd et al., 2015). Riparian topography has been shown to dictate the transport of DOC in Millersford.

The Ebbesbourne wetland DOC levels showed similar spatial trend with highest concentrations closer to the river. The Ebbesbourne wetland is a more typical example of the mutual influence between the stream, the groundwater flow and the riparian wetland that has been also reported elsewhere (Vidon et al., 2010). Flow paths transporting elements, contaminants and suspended matter in and out of the riparian wetland include groundwater flow, subsurface flow and overbank flow. So, the wetlands' surrounding area, the stream and the riparian zone between the wetland and the stream need to be investigated as a continuum (Fisher et al., 1998, Kasahara and Hill, 2007, Meyer et al., 1998). Riparian zones act as a source of DOM not only for streams but also wetlands (Wetzel, 1992). Thus, spatial distribution of DOC in Ebbesbourne is affected by all the parameters mentioned above plus additional sources as any dead plant material and grass from mowing were more likely disposed in that end of the wetland. The results from both

wetlands underpin the need to incorporate spatial variability in wetland sampling strategies, so that adequate representation of DOM levels is achieved.

Both wetlands showed DOM temporal trends. In both wetlands DOC and DON levels were lower in winter and maximum in summer. Seasonal dynamics were stronger for DOC in Ebbesbourne and DON in Millersford. SUP levels were concentrations were minimum in winter for both wetlands, with not such clear seasonal patterns. One of the main factors reported in the literature to affect seasonal dynamics, temperature, was shown to affect DOM levels, especially DOC. The results are also believed to be affected by the rewetting of the Ebbesbourne wetlands after a particularly dry year and the particularly wet year during the Millersford sampling year.

Riparian wetlands are often hot spots of biogeochemical transformations with high levels of DOM as they are commonly in low land areas with low subsurface flow velocities, low oxygen or anoxic conditions and slow DOM decomposition rates (Vidon et al., 2010). The differences between the two types of wetlands was reflected in different levels of DOC, with levels at Millersford being generally higher. Relatively stagnant conditions of water saturation in wetlands impede plant material decay resulting in high levels of carbon. The importance of these conditions is not the same for all wetland types. It is more pronounced in peat forming systems similar to Millersford. On the other hand, the Ebbesbourne wetland was studied following high flow rates of the adjacent stream. Although this flooded the wetland, the high permeability of the soils described in Chapter 3, may have resulted in the flushing of DOC to the adjacent stream (Mladenov et al., 2005, Worrall and Burt, 2008).

Differences in land use of the study catchments affected the nutrient levels of the two wetlands. The levels of nitrogen and phosphorus, the inorganic fraction in particular, at the Ebbesbourne wetland were higher than the ones at Millersford. This was especially true for inorganic phosphorus (SRP) which was 5 to more than 100 times higher in the Ebbesbourne. These results show that the arable nature of the catchment (Addiscott et al., 1992, DEFRA, 2009) and anthropogenic impacts such as septic tanks have affected the nutrient levels (Withers et al., 2012). This is in contrast with the Millersford wetland where the surroundings are less disturbed.

Vertical distribution of DOC and nutrients was not uniform and showed variability among wetlands and elements. This study agrees with those workers who believe that further research is needed to study DOC profile with depth below the 30cm surface soil layer; e.g. Olson and Al-Kaisi (2015); Hiederer (2009)). The differences in the vertical profile of DOC among the different sampling points indicate potential subsurface preferential flow patterns and possible effects of microtopography. Average values of different sampling depths in wetlands, that are used widely in the literate, could hide important information on DOC vertical distribution.

These findings raise the following questions that are addressed in subsequent chapters:

1 Are the differences in DOM levels reflected in DOM quality? The questions are being addressed in Chapter 6
2 Do riparian wetlands act as sources of DOM to the adjacent streams? (Chapter 8)
3 How is the toxicity of contaminants, affected by DOM exported from riparian wetlands? (Chapter8)

Chapter 6- Qualitative analysis of DOM in soil pore waters of two wetlands with contrasting characteristics

6.1 Overview

Differences in DOM levels at the study wetlands have already been presented in Chapter 5. However, these findings provide little information on the ecologically relevant characteristics of DOM, such as microbial degradability. To assess these characteristics, this Chapter studies the differences in aromaticity, molecular weight and biodegradability of DOM in the study wetlands. Qualitative analysis of DOM from the soil pore waters of the study wetlands is presented, based on spectroscopic techniques and elemental ratios.

Spectroscopic analysis was performed on filtered samples stored in glass bottles in the dark at 4^{o}C, employing a Varian Cary 300 Bio UV-VIS spectrophotometer and Varian Eclipse Fluorescence spectrophotometer (Agilent Technologies) as described in Chapter 4. PARAFAC modelling was done using the drEEM toolbox (Murphy et al., 2013). All significant differences referred to $p<0.05$ level. Ebbesbourne data are presented as means of all zones, as no significant difference with location was observed.

Key findings include:

- Lower DOC:DON ratio values indicate higher biodegradability of DOM at Ebbesbourne. Ratio values at Millersford are closer to the ones reported for pristine wetlands, whereas Ebbesbourne values are closer to those of agricultural systems.
- The spectroscopic indicators confirm higher molecular weight, more aromatic and less biodegradable DOM at Millersford compared to Ebbesbourne. This signifies distinct differences between the two DOM pools, with ecological implications.
- At Millersford, the spectroscopic indicators were correlated with elevation-position on the hillslope, indicating export of higher molecular weight DOM to the adjacent stream.
- Five components were identified by the PARAFAC model, revealing differences in the composition of coloured DOM. Protein-like fluorophores were significantly more abundant in Millersford. The

relative abundance of the components identified confirmed that DOM in Millersford is of higher molecular weight and less biodegradable compared to Ebbesbourne.

- The fluorescence index indicated that Ebbesbourne DOM is of lower aromaticity and more microbially derived compared to Millersford DOM.

6.2 C:N ratio

All results for both wetlands are presented in

Figure 6.1. At Millersford, the DOC:DON ratios did not vary significantly with sampling location at 40cm. The ratio ranged from 14.55 (sampling point C4) to 27.22 (sampling point C3). At 60cm, the mean DOC:DON ratio ranged from 6.97 (B4) to 23.87 (B2). Sampling point B4 was significantly different from B2 and C2, and B2 from C4. The differences between the means at the two different depths sampled were also checked. No evidence was found that the ratio varies with depth.

In the Ebbesbourne wetland, the ratio at 40cm depth ranged from 2.50 (Zone 3) to 6.83 (Zone 1). The differences between the mean ratios of the 3 zones in 40cm were found to be significantly different, revealing a gradient with the highest ratios closer to the river (Zone 1). At 60cm depth, Zone 1 values were significantly higher than those in Zones 2 and 3. No significant difference in values was found between the samplers at 20cm depth, ranging between 3.88 (Zone 3) and 5.30 (Zone 1). At 60cm depth, the ratio ranged from 3.64 (Zone 2) to 7.82 (Zone 1). The vertical behaviour of the ratio was not uniform among the zones. In Zone 1 the mean ratio decreased in the order: 60cm > 40 cm > 20 cm; in Zone 2 there was no significant difference, whilst in Zone 3 values followed the order: 40cm > 20 cm = 60 cm. The lack of uniformity in vertical profiles of the C:N ratio has also been reported by other researchers (Siemens and Kaupenjohann, 2002). The same workers noted that careful interpretation of the absolute values of the ratio is needed because of the expected errors resulting from four determinations.

Differences in the DOC:DON ratios between the two wetlands can be explained by differences in the levels of DOC and DON concentrations in the two wetlands, as described in Chapter 5. DOC concentrations were higher in the Millersford wetland (4.8-35.1 mg/L compared to 4.9-16.1 mg/L at the Ebbesbourne) whereas DON were higher in the Ebbesbourne wetland (2.1 mg/L compared to 0.63 to 1.65 in the Millersford wetland).

As discussed in Chapter 5, wetland type and land use affected the levels of DOC and DON respectively. The DOC:DON ratios can be distinguished according to site as shown in Figure 6.2. Association of the DOC:DON ration with the nature of the wetlands and land use of the catchments are confirmed by the values reported in literature. The ratios reported here, for samples collected at 40cm at the Millersford wetland, fall within the range reported by Wiegner and Seitzinger (2004) for pristine wetlands (22-39, average 32±10). These workers measured the ratio in samples collected from 50cm deep wells. Ebbesbourne ratios fall within the range reported by van Kessel et al. (2009), 3-24, after reviewing studies of agricultural systems.

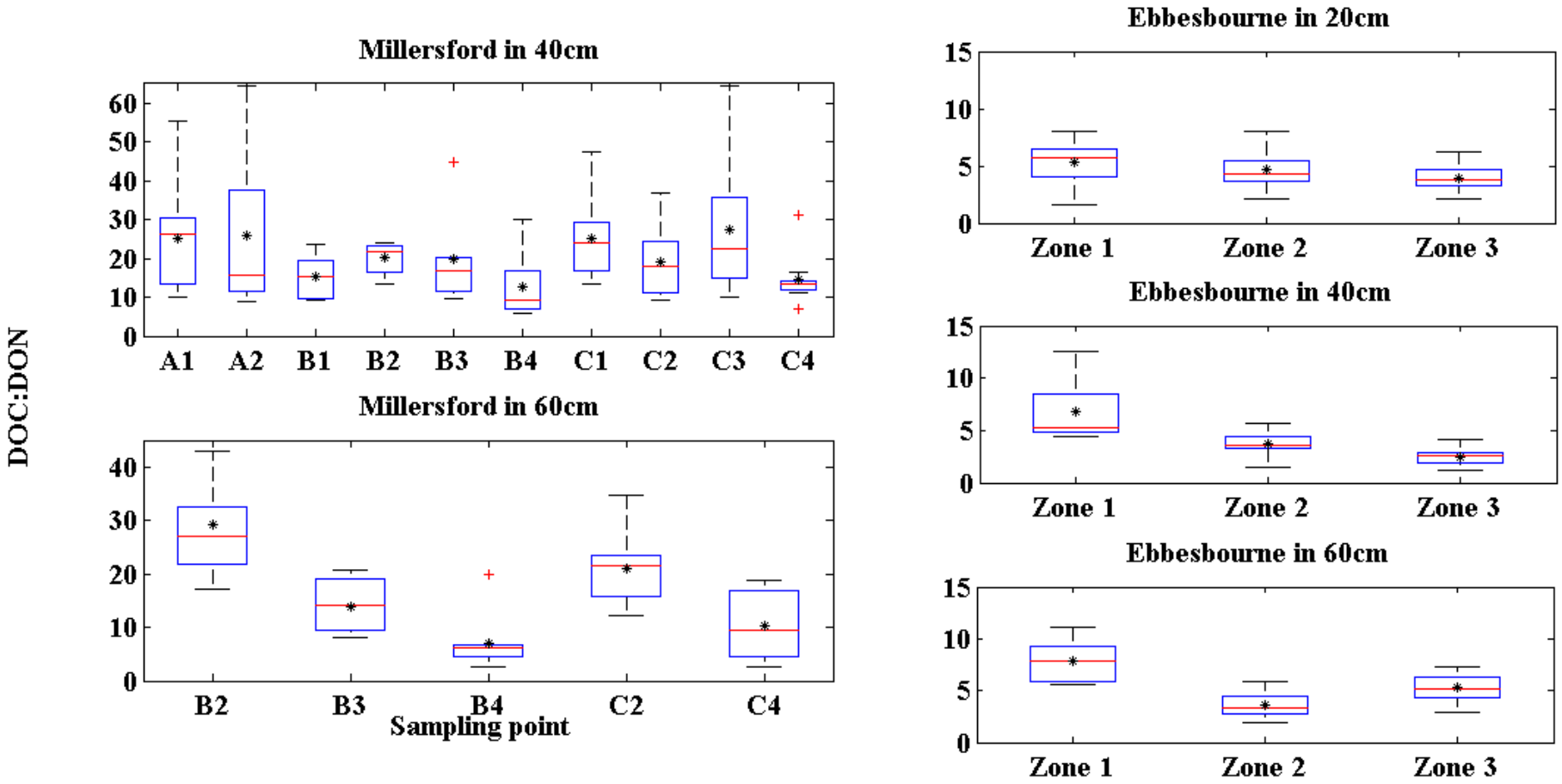


Figure 6.1 Boxplots of DOC:DON ratios in both studied wetlands. Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the red line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with an asterisk. Outliers are plotted individually, using a red cross.

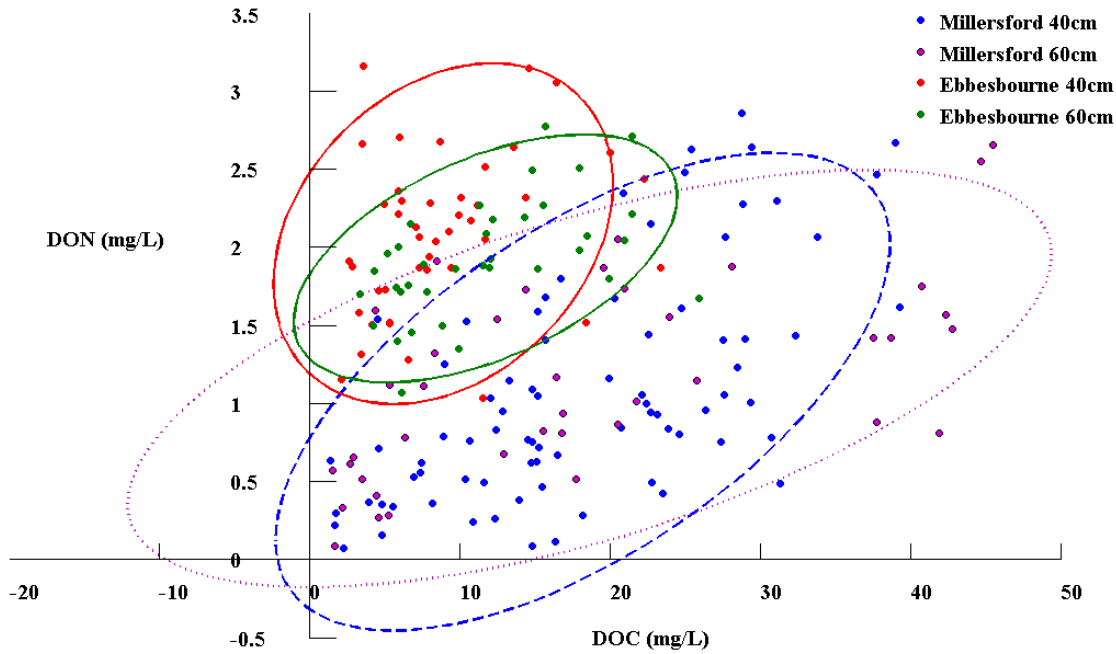


Figure 6.2 Scatter plot of DOC against DON values in both studied wetlands at the 40cm and 60cm sampling depths. Ellipses represent the 90% confidence interval.

The C:N ratio is regarded as a good indicator of DOM biodegradability; specifically, it has been linked to the bioavailability of DOM to microorganisms (Fellman, 2008, Fellman et al., 2008, Hunt et al., 2000, Meyer et al., 1987). The DOC:DON ratio is negatively correlated with DOM biodegradability, indicating that the Ebbesbourne wetland DOM in soil porewaters can be more easily degraded by microorganisms. As discussed in Chapter 2, degradation of DOM can release a significant amount of carbon, nitrogen and phosphorus that is delivered to the adjacent streams and affect their ecosystem. Advances in optical spectroscopy have given rise to a variety of methods to assess DOM structure and bioavailability. A range of these methods are used in the following sections.

6.3 Ultraviolet spectroscopy

UV-Vis spectroscopy has been used for quantifying DOC in water samples, but also for DOM quality estimation. A very wide range of absorption wavelengths has been reported for this purpose. This is due to the presence of a large variety of chromophores in natural organic matter. Absorption coefficients, spectral ratios and specific ultraviolet absorbance (SUVA) have all been used to access DOM composition. An overview of some of the methods reported in the literature is presented in Table 6.1.

Table 6.1 Overview of methods used to assess DOM quality and quantity in aquatic samples.

Method	Indicator
Single wavelength	250 (De Haan and De Boer, 1987), 254 (Edzwald et al., 1985), 260 (Banoub, 1973), 270 (Timperley, 1985), 280-400 (Lawrence, 1980), 300 (McKnight et al., 1997), 330 (Moore, 1987a), 340 (Tipping et al., 2009), 355 (Muller and Tankere-Muller, 2012), 360 (Collier, 1987), 365 (Carpenter and Smith, 1984), 400 (Wallage and Holden, 2010), 410, 436 and 450 (Hongve and Akesson, 1996), 480,465 (Hautala et al., 2000), 562 (Carpenter and Smith, 1984)
Wavelength ratio	250:365 (Peuravuori and Pihlaja, 1997), 250:400 and 270:350 (Peacock et al., 2014), 252:452 (Graham et al., 2012), 254:436 (Selberg et al., 2011), 254:465 (Park et al., 1999), 400:600 (Moore, 1987b), 450:650 (Wilson et al., 2011), 460:660 (Thurman, 1985b), 465:665 (Wallage et al., 2006)
SUVA	254 (Traina et al., 1990, Weishaar et al., 2003), 280 (Duirk and Valentine, 2006), 400 (Worrall et al., 2007)
Slope ratio	275-295 slope:350-400 slope (Helms et al., 2008)

Peacock et al. (2014) evaluated the different methods that can be used as indicators of peatland DOC quantity and quality. The samples included pore water, surface water from ditches and overland-flow, as well as stream water. Methods that have been linked with DOC quality, namely 250nm:365nm (E2:E3), 250nm:400nm, 465nm:665(E4:E6) ratios and $SUVA_{254}$ were investigated. They suggested that the E4:E6 ratio is more useful for seasonal and weather-driven changes in DOC quality. All of the

other three methods can detect DOC quality differences between sites. Based on their findings, all three methods were employed in this study.

6.3.1 Interferences

Inorganic species can interfere with DOM UV absorbance. The American Water Environment Federation identifies iron, nitrate, nitrite and bromide as the UV-absorbing inorganics more likely to interfere with organic compound absorbance at 254nm. They also note that pH values below 4 and above 10 could affect organic matter UV absorbance (Rice et al., 2012). It has been reported that ferric (Fe^{3+}) iron, absorbing light of 200-400nm, affects DOM absorbance of samples, whereas ferrous (Fe^{2+}) has negligible effects. Increased concentrations of ferric iron could result in increased absorbance and SUVA, reduced E2:E3(250:365) and S_R values. $SUVA_{254}$ and spectral slopes (S275−295, S350−400) are more sensitive to interferences compared to E2:E3 and S_R (S275−295: S350−400). $SUVA_{254}$ is less sensitive compared to $SUVA_{280}$ (Doane and Horwath, 2010, Poulin et al., 2014, Weishaar et al., 2003). The magnitude of the effect of interference depends on the concentration of ferric iron. For example, Weishaar et al. (2003) showed that ferrous iron concentrations up to 0.5 mg/L have negligible interference on DOC UV absorbance. Nitrate absorbance has been shown to be additive to DOC absorbance, although it has a lesser effect compared to iron. Weishaar et al. (2003) showed that an increase in DOC absorbance of 0.01 required more than 100 mg/L NO_3^- but only 1 mg/L Fe^{3+}. Significant increases in DOC absorbance can occur in nitrate concentrations higher that 40 mg/L (Weishaar et al., 2003).

Trace element analysis (ICP-OES, see appendix D) revealed that samples from the Millersford wetland have iron concentrations that warrant considerations of potential interference. However, according to the U.S. Environmental Protection Agency (Potter and Wimsatt, 2012), apropos interferences that could occur due to the presence of iron, nitrate, nitrite and bromide: "*The concentration of the interferences and their effect on the UVA cannot be determined as each unique sample matrix may produce a different UVA response for the same concentration of interference or combination of interferences. This method does not treat or remove these interferences. Therefore, suspected or known interferences may affect results and must be flagged in the SUVA result as 'suspected UVA interferences'.*

6.3.2 a250:a365 ratio

Peuravuori and Pihlaja (1997) introduced the use of the 250nm:365nm ratio (E2:E3) for characterising DOM as this ratio correlates strongly with total aromaticity and average molecular weight of humic solutes. Similarly, Uyguner and Bekbolet (2005) asserted that the E2:E3 ratio exhibits molecular size-specific distribution with respect to the source and type of humic acids. The same authors did not recommend the 465nm:665 (E4:E6) ratio for natural organic matter monitoring.

The values of a250:a365 (E2:E3) ratio at 40cm depth at Millersford, presented in Table 6.2, ranged from 3.76 at A1 to 7.62 in C4. Significant differences were observed at sampling point A1 that had a lower E2:E3 ratio compared to the ratios of most sampling points. Also, at sampling point C4 that had significantly higher E2:E3 ratio compared to the ratios of A1, A2, B1 and B3. At 60cm depth, the ratio ranged from 2.43 (C2) to 6.48 (C4), as shown in Table 6.3. The ratio at C4 was found to be significantly lower than the ratios of all other sampling points except B1. At Millersford, E2:E3 ratio did not correlate with sampling depth but was strongly correlated with elevation as shown in Table 6.5.

For all UV parameters Ebbesbourne values did not show significant differences with location and are summarised by depth (Table 6.4). Values ranged from 6.55 at 20cm depth to 7.09 at 60cm depth and were weakly correlated with depth ($r=0.36$, $p=0.006$).

Most values reported for Millersford, were similar to the ones reported in literature. Strack et al.(2015) reported values of 2.96 to 3.79 for restored and 4.03 for natural wetland sampled using dip wells. Peacock et al. (2014) estimated E2:E3 ratio for peatland porewater at 10cm depth to be 3.7±0.14. The values at Ebbesbourne and some of the sampling points at Millersford (e.g. B4 and C4) are higher. These values exceed the ones reported by Ohno et al. (2005) for soils of cropping systems (4.85-5.53). Peuravuori and Pihlaja (1997) showed that the E2:E3 ratio is negatively correlated with aromaticity and molecular weight. The equations that linked them (e.g. aromaticity= 52.509-6.780 E2:E3) are not relevant here as they were produced from humic substances isolated from lakes and rivers. As shown in Figure 6.3, values at Ebbesbourne are higher in both depths studied, indicating that DOM at this site is less aromatic, with lower molecular weight. This conclusion is in agreement with DOC:DON ratio results showing higher biodegradability of DOM at Ebbesbourne.

6.3.3 $SUVA_{254}$

SUVA is estimated by dividing the UV absorbance at a given wavelength by DOC concentration. It is reported in units of litre per milligram Carbon per meter (Potter and Wimsatt, 2012). The reporting of absorbance at 254nm and $SUVA_{254}$ has been linked to UV absorption by organic compounds in aquatic samples (Nollet, 2014, Rice et al., 2012, Vasilas et al., 2013). Indeed it has become a common method for wetland DOM characterisation (Vasilas et al., 2013). Using NMR data, Weishaar et al. (2003) showed that $SUVA_{254}$ is strongly correlated to percent aromaticity.

$SUVA_{254}$ in the Millersford wetland at 40 cm depth ranged from 1.61 (C4) to 6.16 $L*mgC^{-1}*m^{-1}$ (B3), as shown in Table 6.2. The only significant difference detected was between $SUVA_{254}$ values of A1 and B4. Values at 60cm (Table 6.3) ranged from 2.21 (B4) to 7.61 (B2) $L*mgC^{-1}*m^{-1}$. $SUVA_{254}$ values at B2 were significantly higher than B4 and C4. Correlation was found with elevation but not with depth (Table 6.5). Ebbesbourne results are shown in Table 6.4. $SUVA_{254}$ values ranged from 4.19 (20cm) to 2.44 (60cm) and were found to be strongly negatively correlated with sampling depth ($r=-0.62$, $p=0$).

It is generally accepted that $SUVA_{254}$ values in natural waters that are higher than 4 L mg $C^{-1}m^{-1}$ reflect high content of complex heterogeneous macromolecular organic compounds that are rich in aromatics (Edzwald et al., 1985, Nollet, 2014). $SUVA_{254}$ has been used for soil solution samples by Fellman et al. (2008); its levels varied among wetland soil types and mineral soil with highest values reported for forested wetland (4.4) and lowest for fen (3.5). The sequence was fen<bog<upland forest<forested wetland. However, the values are not directly comparable with the ones reported here, as they were measured in 25cm piezometers. Weishaar et al. (2003) linked $SUVA_{254}$ with percent aromaticity ($y=6.52x+3.63$) using NMR spectroscopy. Based on this relationship, percent aromaticity at the Millersford site reaches 44% at 40cm depth and 53% at 60cm. However, these percentages could overestimate aromaticity because of iron interference (section 6.3.1). At Ebbesbourne, percentage aromaticity ranges from 19.5% at 60cm to 31% at 20cm. The results confirm the E2:E3 ratio findings that DOM in Millersford pore water is more aromatic than in Ebbesbourne, as shown in Figure 6.3.

Table 6.2 Millersford wetland soil porewater UV parameters mean values and standard deviation at 40cm depth.

	Sampling point									
	A1	A2	B1	B2	B3	B4	C1	C2	C3	C4
E2:E3	3.76	3.95	4.13	5.36	3.87	5.40	5.00	4.72	5.32	7.62
std(±)	0.12	0.16	0.32	0.41	0.42	0.36	0.77	0.39	0.52	1.25
$SUVA_{254}$	6.02	5.27	4.53	4.70	6.16	2.79	4.27	5.12	5.33	1.61
std(±)	1.64	0.79	1.33	0.73	1.49	1.15	1.24	0.25	0.75	0.53
S275-295 $*10^{-3}$	9.74	9.91	10.90	14.03	10.20	14.01	10.10	11.55	13.46	16.20
std(±)	0.34	0.38	0.20	0.82	1.10	1.66	0.59	0.79	0.92	0.85
S_R	0.55	0.59	0.63	0.71	0.56	0.69	0.55	0.57	0.65	0.63
std(±)	0.02	0.02	0.08	0.04	0.03	0.11	0.10	0.09	0.06	0.05

Table 6.3 Millersford wetland soil porewater UV parameters mean values and standard deviation at 60cm depth.

	Sampling point				
	B2	B3	B4	C2	C4
E2:E3	4.02	4.41	6.38	2.43	6.48
std(±)	0.14	0.85	2.49	0.41	2.06
$SUVA_{254}$	7.61	5.71	2.21	6.60	2.70
std(±)	0.57	2.50	2.74	2.01	0.73
S275-295 $*10^{-3}$	10.44	10.21	13.61	5.64	15.20
std(±)	0.25	0.64	1.92	1.45	2.64
S_R	0.53	0.51	0.72	0.38	0.69
std(±)	0.01	0.06	0.33	0.05	0.17

Table 6.4 Ebbsbourne wetland soil porewater UV parameters mean values and standard deviation at 20cm, 40cm and 60cm depth.

	20cm	40cm	60cm
E2:E3	6.55	7.09	7.48
std(±)	0.46	1.15	1.38
$SUVA_{254}$	4.19	3.34	2.44
std(±)	0.66	0.95	1.15
S275-295 $*10^{-3}$	15.02	15.88	16.17
std(±)	1.06	1.17	1.89
S_R	0.74	0.73	0.73
std(±)	0.06	0.04	0.03

Table 6.5 Millersford correlation coefficients of UV parameters with elevation and depth. The p values are given in parenthesis (3 decimal places are shown).

	Elevation		Depth
	40cm	60cm	
E2:E3	0.64 (0)	0.68 (0)	-0.28 (0.03)
$SUVA_{254}$	-0.51 (0)	-0.74 (0)	0.28 (0.05)
S275-295	0.73 (0)	0.69 (0)	-0.39 (0.002)
S_R	0.31 (0.019)	0.59(0.004)	-0.98 (0)

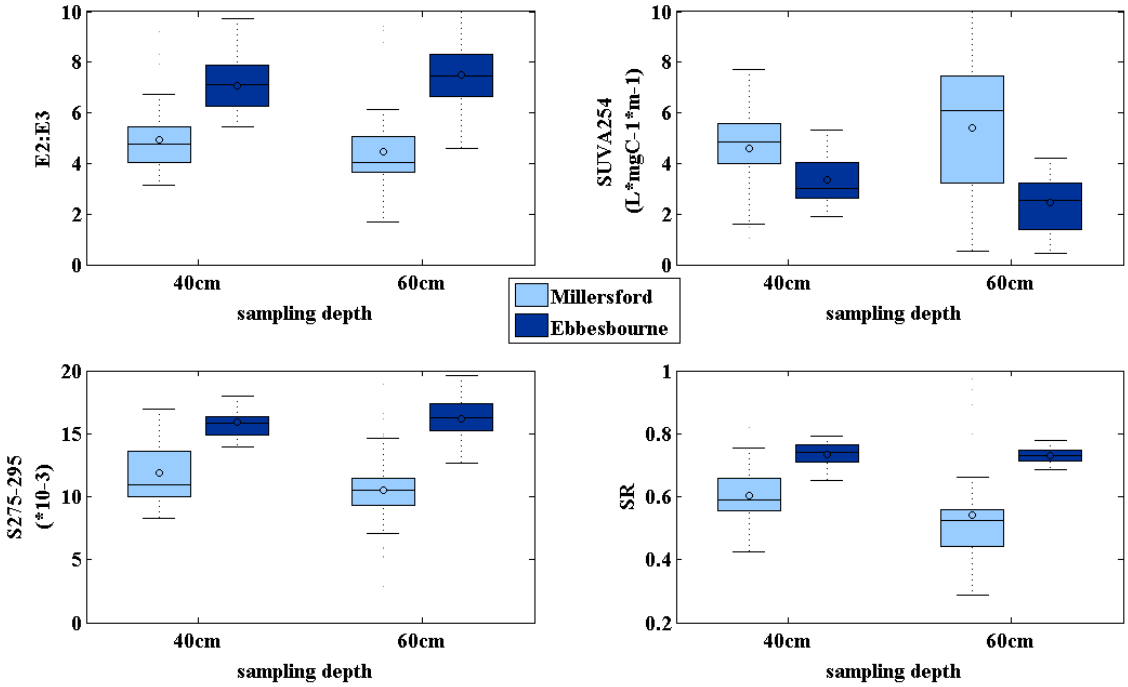


Figure 6.3 Boxplot of E2:E3, $SUVA_{254}$ ($L*mgC^{-1}*m^{-1}$), $S_{275-295}$ ($*10^{-3}$) and SR in Millersford (light blue) and Ebbesbourne (dark blue). Top and bottom edges of each box indicate the 25th percentile and 75th percentile respectively. On each box, the central horizontal line indicates the median. Whiskers extend to the maximum and minimum data points. Mean concentrations are indicated with a circle.

6.3.4 Spectral slopes and spectral slope ratio

Helms et al.(2008) presented a method using two distinct spectral slope regions (275-295nm and 350-400nm) within log-transformed absorption spectra. Using a wide range of aquatic samples, including wetland originated, they showed that the slope of the 275-295nm ($S_{275-295}$) and the ratio of the two slopes (slope 275-295nm: slope 350-400nm = S_R) are linked to DOM molecular weight and photochemically induced shifts in molecular weight. The term S_R differed by a factor of 13 between wetland waters and marine samples. They showed that S_R and $S_{275-295}$ can provide a reliable and accurate proxy of DOM molecular weight.

Mean spectral slope ($S_{275-295}$) values at Millersford, at 40cm depth, are presented in Table 6.2. Slope values ranged from $9.74x10^{-3}$ (A1) to $16.2\ x10^{-3}$ (C4). Pairwise comparisons revealed significant differences between: C4 and A1, A2, B3, C1; C1 and B2, C3, C4; A1 and B2, B4, C3, C4. At 60cm depth (Table 6.3), values ranged from 5.64 (C2) to 15.2 (C4). At 60cm (Table 6.3), spectral slope values ranged from $5.64\ x10^{-3}$ (C2)

to 15.2 x10^{-3} (C4). Values at the C2 sampling point were significantly lower than the ones at B4 and C4. As shown in Table 6.5, correlation with elevation but not with depth was found. Spectral slope ratio (S_R) at Millersford, averaged from 0.55 (A1) to 0.71 (B2) at 40cm depth (Table 6.2), with values at B2 being significantly lower to the ones at A1 and C1. At 60cm (Table 6.4), values ranged from 0.38 (C2) to 0.72 (B4), with values at C2 being significantly lower to the ones at C4. As shown in Table 6.5, S_R was strongly correlated to depth. Correlation with elevation was strong only for the 60cm samples.

In the Ebbesbourne wetland, mean spectral slope values ranged from 15.02 at 20cm to 16.17 at 60cm, with the difference between 20cm and 60cm being significant. Correlation of the slope with depth was weak (r= -0.3464, p= 0.0047). S_R values ranged from 0.73 at 20cm depth to 0.74 at 60cm (Table 6.4).

The values reported here are in agreement with the literature, although reports on pore water samples from wetlands are lacking. The values indicate high molecular weight DOM. Helms et al. (2008) reported S_R values of 0.7 and $S_{275-295}$ 13.1 x10^{-3} for the Great Dismal Swamp, Virginia canal system samples. In agreement with these findings, Yamashita et al. (2010) reported S_R values between 0.83 and 1.07 in surface waters collected from marshes close to the Everglades National Park, Florida, USA. Chen et al. (2013) presented a multi-year DOM characterisation of surface samples within the greater Everglades. In freshwater marshes with peat-based soils, S_R was 0.9 during all seasons. Pennington and Watmough (2015) measured S_R in metal-contaminated peatlands in Canada. They sampled pore water from shallow (0-30cm) wells and reported a mean of 1.07, with a range between 0.67 and 2.04.

Spectral slope ($S_{275-295}$) and spectral slope ratio (S_R) has been shown to inversely relate to DOM molecular weight (Helms et al., 2008). S_R values range from values lower than 1 for higher coloured DOM (DOM that absorbs UV-vis light) representative of terrestrially dominated samples to much greater than 1 with the highest values found in marine samples. The results indicate higher molecular weight DOM content in Millersford compared to Ebbesbourne, but also in 60cm in Millersford compared to 40cm.

6.3.5 Seasonal changes in DOM chemical quality

Seasonal dynamics of the optical properties of DOM were examined and are presented in Figure 6.4. There was little variation among the seasons for the different DOM quality indicators, with no significant differences reported. $SUVA_{254}$ in the Millersford samples ranged from 5 in the summer to 5.3 in the autumn; whereas in Ebbesbourne it ranged from 3.4 in the winter to 3.6 in the summer. E2:E3 values in the Millersford ranged from 4.3 in the autumn to 4.6 in the summer; in the Ebbesbourne, values ranged from 6.7 in the summer to 7 in the autumn. Spectral slope ($S_{275-295}$) ranged in Millersford from 10.7×10^{-3} in winter to 10.9×10^{-3} in the summer; in Ebbesbourne from 15.2×10^{-3} in winter to 15.7×10^{-3} in spring. Spectral slope ratio (S_R) ranged in Millersford from 0.58 in the winter to 0.59 in the summer and, in Ebbesbourne, from 0.73 in the autumn to 0.75 in the summer.

Most studies on seasonal changes in the chemical quality and biodegradability of DOM in soils and in wetlands, refer to colder climates. Hence studies during winter are restricted by ice formation and winter data are lacking. Many of these studies report minimum biodegradability in summer (Marschner and Kalbitz, 2003). These seasonal pattern in wetlands has been attributed to freezing and thawing, a process that is linked with microbial cell lysis and root mortality (Fellman et al., 2009; Wiegner and Seitzinger, 2004). The lack of seasonal pattern in DOM quality in both Millersford and Ebbesbourne is consistent with a study in a fen (Fellman et al., 2008) and a bog (Broder et al., 2017). Embacher et al. (2007) also concluded after analysing water extractable DOM from arable top soils that unlike the strong temporal variability in DOM quantity, DOM quality variation was linked with site and soil and not with seasonality. The results of this study show that seasonality in DOM levels is not necessarily followed by similar patterns in DOM quality.

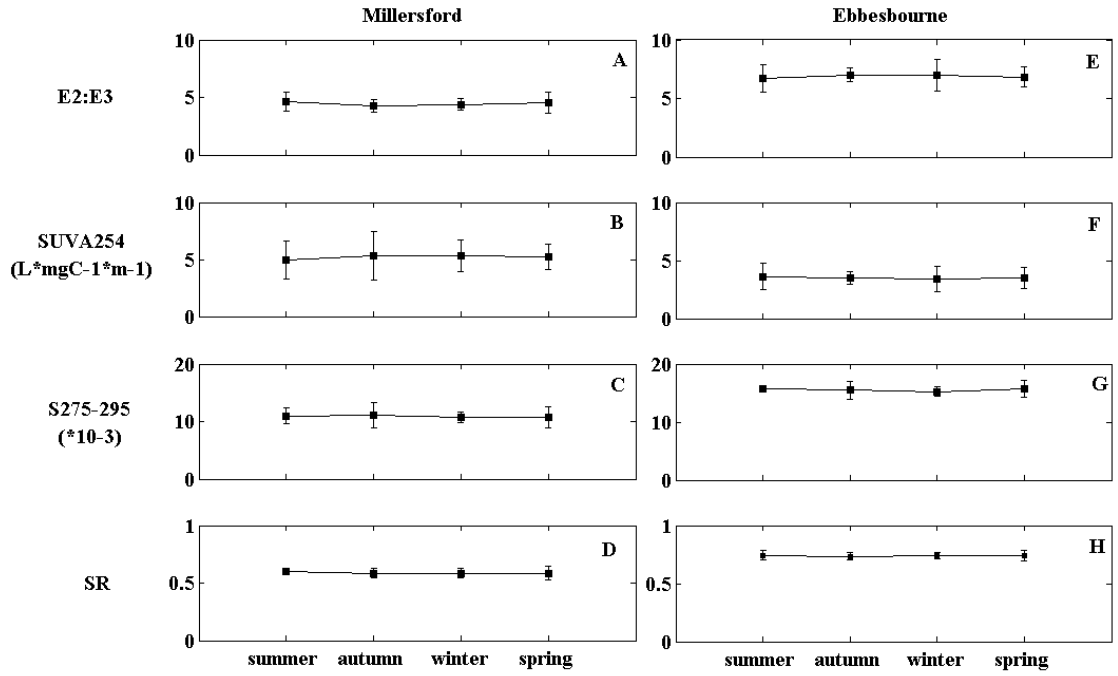


Figure 6.4 Line graph of mean E2:E3, $SUVA_{254}$ ($L*mgC^{-1}*m^{-1}$) , S275-295 ($*10^{-3}$) and SR values for each season in both wetlands, in boxes with standard deviation error bars.

6.4 Fluorescence

EEM data were corrected and PARAFAC analysis was undertaken using drEEM toolbox for MATALAB software (Murphy et al., 2013). Similar to the interferences described in section 6.3.1, iron interferes with fluorescence measurements. Iron ions can cause underestimation of fluorescence due to quenching of the fluorescence signal as a result of DOM-iron complexation (Ohno et al., 2008). Thus, Millersford fluorescence results are likely to be underestimated.

The PARAFAC model identified five components that are described in Table 6.6. All of the components are reported in literature in studies using either the PARAFAC model (Stedmon 2003,2005, Fellman 2008, 2009,2010 Yamashita 2010) or visual analysis of EEMS (Coble 1996,1998,2007, Parlanti 2000). DOM has two major component groups that fluoresce, the humic type and the protein type. The protein type fluorescence is observed at an emission of 300-350nm from an excitation of 220 and 270 (Coble, 1996, Mopper and Schultz, 1993). The humic-type fluorescence is observed at and emission of 430-450 from an excitation of 230-160nm and 320-350nm (Coble, 1996, Mopper and Schultz, 1993). However due to different methods used for peak identification, differences in the position of the excitation and emission maxima occur

amongst different studies (Stedmon et al., 2003). Three of the five components identified in this study are humic like (1,2,3) and two protein like (4,5).

Table 6.6 Characteristics of the five components identified by the PARAFAC model. Secondary maxima are shown in parenthesis.

Component	Excitation max (nm)	Emission max (nm)	Previous studies identifying similar components	Description
1	<240	458	Parlanti et al. (2000), Stedmon et al. (2003), Stedmon and Markager (2005), Coble (2007), Fellman et al. (2008, 2009, 2010), Yamashita et al. (2010)	Humic-like fluorophore
2	<240 (330)	436	Stedmon et al. (2003), Stedmon and Markager (2005), Fellman et al.(2008), Yamashita et al.(2010)	Humic-like fluorophore
3	290	408	Parlanti et al. (2000), Coble (2007), Fellman et al. (2008, 2009)	Humic-like fluorophore
4	270	312	Parlanti et al. (2000), Stedmon and Markager (2005), Coble (2007), Fellman et al. (2008, 2009)	Tyrosine-like fluorophore
5	280	338	Parlanti et al. (2000), Stedmon and Markager (2005), Coble (2007), Fellman et al. (2008, 2009), Yamashita et al. (2010)	Tryptophan-like fluorophore

The dominant component in Millersford was component 1 as shown in Figure 6.5. Component 1 is described as a humic fluorophore group, high molecular weight, aromatic humic that is highest in wetlands and forested environments that corresponds to peak A (Coble, 2007, Fellman et al., 2010, Parlanti et al., 2000, Stedmon and Markager, 2005). Fellman et al. (2008, 2009) found this component to be the dominant one for bog, fen and

forested wetland soil DOM that is negatively correlated with biodegradable DOC. The dominant component in Ebbesbourne was component 3, a component similar to peak M originally thought to correspond to marine humic like fractions of DOM (Coble et al., 1998, Parlanti et al., 2000). Fellman (2008,2009) reported the presence of this component in terrestrial DOM with a relative contribution of 3.5- 5% in wetland samples. Similar components were described as low molecular weight that can be found in wastewater, wetland and agricultural environments (Fellman et al., 2010, Stedmon and Markager, 2005, Stedmon et al., 2003). Component 2 was identified in both wetlands. Similar components have been documented elsewhere (Fellman et al., 2008, Stedmon and Markager, 2005, Stedmon et al., 2003) and are believed to correspond to peak C (Stedmon and Markager, 2005, Stedmon et al., 2003). Stedmon and Markager (2005) described the component as fulvic acid fluorophore group present in all environments.

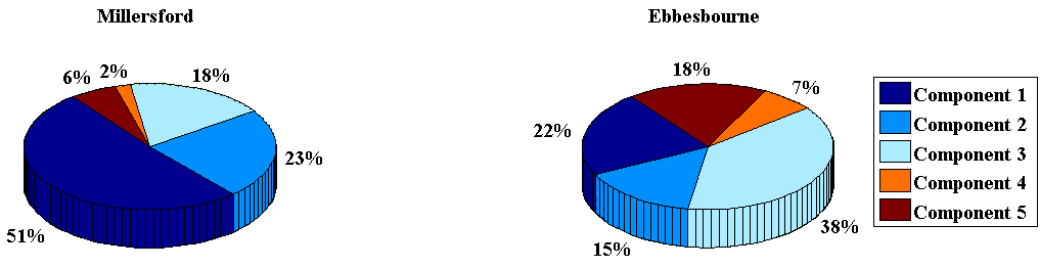


Figure 6.5 Pie chart showing the relative abundance of the five components identified by the PARAFAC model in Millersford (left) and Ebbesbourne (right).

Protein-like fluorescence i.e. the sum of tyrosine and tryptophan-like PARAFAC components is significantly more abundant in Ebbesbourne compared to Millersford (25% and 8% respectively). Protein like fluorescence has been linked with anthropogenic DOM sources like farm wastes, sewage treatment or sewerage (Baker, 2001, Baker, 2002) and correlated with nitrate, phosphate, ammonia, biochemical oxygen demand and dissolved oxygen (Baker and Inverarity, 2004). The abundance of protein like components has been shown to be a strong indicator of biological activity and DOM biodegradability (Balcarczyk et al., 2009, Fellman et al., 2008, Fellman et al., 2009). The composition of humic like fluorophores and the abundance of protein like components in the two wetlands, agree with the rest of the findings of this study describing DOM in Millersford as higher molecular weight, less biodegradable.

One of the most common indices used for qualitative analysis of fluorescence is the fluorescence index (Vasilas et al., 2013). McKnight et al. (2001) defined the fluorescence as the ratio of the emission intensity at a wavelength of 450 nm divided by that at 500 nm, obtained with an excitation of 370 nm. Cory and McKnight (2005) modified the ratio for instrument-corrected spectra, to emission intensity at 470 to that of 520nm at 370nm excitation. Here the latter is used.

In both wetlands, the fluorescence index did not differ significantly with sampling location or depth or season. In Millersford, index values were 1.19±0.4 at 40cm, and 1.13±0.3 at 60cm. In Ebbesbourne the fluorescence index was 2.38±1 at 20cm, 1.56±0.8 at 40cm, and 1.32±0.6 at 60cm. The fluorescence index indicates if DOM is of low aromaticity microbially derived (~1.8 in river waters) or of high aromaticity terrestrially derived (~1.2 in river waters) (McKnight et al., 2001).Wilson and Xenopoulos (2009) studied riverine DOM in 34 watersheds and found that the fluorescence index increased with the proportion of continuous cropland in the watersheds and the autochthonous carbon production. Broder et al. (2017) reported FI values of 1.58 in water samples collected from a German bog during the snow free months. These results indicate that DOM in Millersford Brook is more terrestrially derived, originating from degraded plant and soil organic matter and of higher molecular weight and aromaticity. At Ebbesbourne, microbial sources contribute more to DOM.

6.5 Conclusions

Further to the study of DOM levels in the two wetlands (Chapter 5), the study of DOM quality gave a better insight of the DOM pools under investigation. DOM quality analysis revealed distinct characteristics that differentiate the two wetlands studied. The two DOM pools proved to differ in composition and compound molecular structure. The use of not only one single UV indicator but of a range of relevant ones and the PARAFAC modelling enabled the comparison with various studies that made the interpretation of the results more powerful. The DOM quality variation interpretation can provide a link with site specific characteristics and anthropogenic activity effect. The Millersford wetland can be described as of higher molecular weight, more aromatic, and less biodegradable compared to Ebbesbourne as confirmed by the DOC:DON ratio, UV and fluorescence analysis. Anthropogenic DOM sources such as agriculture and septic tanks are reflected in the DOM characteristics of Ebbesbourne.

Understanding DOM quality variation among wetland soil waters is critical for many biological, chemical and physical processes. Biodegradable fractions of DOM affect aquatic ecosystems function and structure, as microbial metabolism can release significant amounts of carbon nitrogen and phosphorus to aquatic ecosystems (Findlay and Sinsabaugh, 2008, Wetzel, 2001). Hence levels of DOM in the streams adjacent to the wetlands will be studied in Chapter 7 to evaluate the effect of the wetlands on the streams. Aromatic and humic fractions of DOM influence complexation and transport of metals (Aiken et al., 2011). The effects of DOM on the toxicity of metals is studied in Chapter 8.

Chapter 7- Dissolved organic matter and inorganic nutrients in streams: an evaluation of riparian wetlands as mitigation features.

7.1 Overview

Current legislative measures on the ecological and chemical status of rivers and streams are presented. The objectives of the Demonstration Test Catchment (DTC) programme for the study area are also explained. This provides the context for the present study: linking observed data with diffuse agricultural pollution (DAP) mitigation measures. Results from the hydrological monitoring and evaluation of the ecological status of the Ebble river are presented before assessing the role of the wetland in determining the nutrient levels of the Ebble. Throughout the chapter, the results are compared with corresponding data from the Millersford site. Finally, recommendations on DAP mitigation measures are given.

Key findings include:

- Identified factors influencing nutrient levels include hydrology, wetlands, cattle grazing and septic tanks.
- Flow is an important factor and storm events should be incorporated in the monitoring programmes.
- SRP levels in the Ebble are above the *good ecological status* threshold.
- Ebbesbourne wetland acted as source of SRP, DON and DOC.
- Millersford wetland only affected DOC levels in the adjacent stream, acting as a source.
- Recommended mitigation measures are discussed.

7.2 Freshwater ecological status and ecological quality standards: The response of policy makers, researchers and stakeholders.

It is widely accepted that freshwater ecosystems are under multiple pressures. Human impacts on discharge of pollution, water flow regime and morphology of rivers, undermine their biodiversity and ecological functioning. Numerous legislative measures,

being national, European or international, have attempted to protect the water - a "heritage which must be protected, defended and treated as such" (European Union, 2000).

The Water Framework Directive 2000/60/EC (WFD) is widely recognised as the most substantial and ambitious piece of European environmental legislation to date. It integrates several pre-existing European directives, briefly described here. The Drinking Water Directive (98/83/EC) on the quality of water intended for human consumption, targets human health protection. It is based on the World Health Organisation's (WHO) guidelines for drinking water (World Health Organization, 2017). The WHO identified the nitrate pathways to surface water, as follows: agricultural activity, waste- water disposal and oxidation of nitrogenous waste products in human and other animal excreta, including septic tanks. The Drinking Water Directive adopted standards for nitrate (50 mg/l) and nitrite (0.5 mg/l). The Nitrate Directive (91 / 676 / EEC) deals with the protection of waters against pollution caused by nitrates from agricultural sources. Surface freshwater with nitrate concentrations of 50 mg/l or more were characterised as water polluted, or at risk of pollution. The directive promotes the use of good farming practices that lead to prevention of nitrogen losses. WFD is complemented by many other directives. Some examples are the Habitats Directive (92/43/EEC) whose objective is natural habitats and wild fauna and flora preservation; the Marine Strategy Framework Directive (2008/56/EC) on marine environment protection; The Sewage Sludge Directive (86/278/EEC) on the protection of the soil, when sewage sludge is used in agriculture; and The Birds Directive (79/409/EEC) on the conservation of wild birds.

The WFD sets the goal of achieving good chemical- and ecological status for all EU rivers by 2015. EU member states could be granted an extension until the second (2015-2021) and third (2021-2027) management cycles. Good chemical status is met in accordance with the environmental quality standards set by The Mercury Discharges Directive (82/176/EEC), The Cadmium Discharges Directive (83/513/EEC), The Mercury Directive (84/156/EEC), The Hexachlorocyclohexane Discharges Directive (84/491/EEC), The Dangerous Substance Discharges Directive (86/280/EEC) and other relevant legislation. The WFD includes an indicative list of the main pollutants for the estimation and identification of significant point source or diffuse source pollution resulting from urban, industrial, agricultural and other installations and activities. The list

includes organophosphorus compounds, persistent hydrocarbons and persistent and bioaccumulable organic toxic substances, substances which contribute to eutrophication (in particular, nitrates and phosphates).

The general definition of good ecological status given by the WFD is "The values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions". Whereas high ecological status is defined as "There are no, or only very minor, anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water body type from those normally associated with that type under undisturbed conditions. The values of the biological quality elements for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion. These are the type-specific conditions and communities". A more detailed description is provided for biological quality elements - i.e. phytoplankton, macrophytes and phytobenthos, benthic invertebrate fauna, fish fauna; hydromorphological quality elements - i.e. hydrological regime, river continuity, morphological conditions; and physico-chemical elements - i.e. general conditions, specific synthetic pollutants, specific non-synthetic pollutants.

Despite the ambitious goals of the WFD, Grizzetti et al. (2017) estimated that only one third of the EU's territory rivers meet good ecological status standards. After examining a number of indicators of the major pressures acting on European rivers, they found that ecological degradation is better predicted by urbanisation and nutrient pollution (nitrogen and phosphorus concentration). They also showed that wetland restoration measures as in the example of Denmark can lead to achieving WFD goals.

7.2.1 Freshwater ecological status and standards in the UK.

Each individual Member state of the EU takes measures to underpin the implementation of the WFD. In the UK, this is organised by a group of experts, the UK Technical Advisory Group on the WFD (UKTAG). The UKTAG sets the relevant environmental conditions and standards for UK freshwaters to meet good or high ecological status.

With regards to water quality standards for rivers, the physico-chemical conditions and the relevant key parameters prioritised are oxygen (biological oxygen demand, dissolved oxygen), ammonia, acid (pH) and nutrient (phosphorus) (UKTAG, 2008). The prioritisation was based on the sensitivity of biological elements to such condition and key parameters, i.e. macro-invertebrates to oxygen and ammonia, fish to acid and diatoms to phosphorus. The standards set are presented in Table 7.1., SRP standards are not included as the revised threshold are presented below.

Table 7.1 Key Parameter levels to reach good and high ecological status (UKTAG, 2008)

	Ecological status			
Key parameter	**High**	**Good**	**Moderate**	**Poor**
Upland and low alkalinity				
Dissolved oxygen (% saturation)	80	75	64	50
Total ammonia (mg/L)	0.2	0.3	0.75	1.1
Lowland and high alkalinity				
Dissolved oxygen (% saturation)	70	60	54	45
Total ammonia (NH_3 in mg/L)	0.3	0.6	1.1	2.5
All rivers in England				
pH	≥ 6 to ≤ 9		4.7	4.2

Taking into account existing knowledge, the UKTAG confirmed that nutrient levels in UK freshwaters are one of the main reasons of failure to achieve legislative goals and prioritised setting phosphorus standards (UKTAG, 2008, UKTAG, 2012b). In 2013 UKTAG published the updated recommendations on phosphorus standards for rivers Basin Management for the period 2015-2021 (UKTAG, 2013). Table 7.2 shows the revised standards proposed. No standards have been published by UKTAG for nitrogen. The Joint Nature Conservation Committee (2014) suggested that when values of total inorganic nitrogen are around 10 times greater than the soluble reactive phosphorus target, TIN will exert equivalent control to nutrient availability to the phosphorus targets. They proposed that site-specific targets for TIN should be applied where nitrogen-mediated eutrophication occurs and cannot be addressed by applying phosphorus targets alone. Dissolved organic carbon levels are also considered in connection to acidification,

acid neutralising capacity (Joint Nature Conservation Committee, 2014) and metals toxicity, copper and zinc in particular (UKTAG, 2012a).

Table 7.2 Revised standards for phosphorus, representing medians from 456 lowland, high alkalinity sites; 137 lowland, low alkalinity sites; and 97 upland, low alkalinity sites. The upper and lower 5th and 95th percentiles of the standards are shown in parentheses (UKTAG, 2013).

Site Type	Annual mean of reactive phosphorus (mg/l)			
	High	Good	Moderate	Poor
Lowland, low alkalinity	0.019 (0.013-0.0026)	0.040 (0.028-0.052)	0.114 (0.087-0.140)	0.842 (0.752-0.918)
Upland, low alkalinity	0.013 (0.013-0.020)	0.028 (0.028-0.041)	0.087 (0.087-0.117)	0.752 (0.752-0.851)
Lowland, high alkalinity	0.036 (0.027-0.050)	0.069 (0.052-0.091)	0.173 (0.141-0.215)	1.003 (0.921-1.098)
Upland, high alkalinity	0.024 (0.018-0.037)	0.048 (0.028-0.070)	0.132 (0.109-0.177)	0.898 (0.829-1.012)

To achieve good ecological status a catchment-specific approach is required. The Environment Agency has adopted River Basin Management Plans with the goal of coordinating organisations, stakeholders and communities work on the water environment improvement and WFD requirements fulfilment (Environment Agency and DEFRA, 2016). The ecological and chemical 2015 classification for surface waters is presented in Table 7.3. By 2015, 17% of English surface waters were at good or high ecological status or potential (Environment Agency, 2015).

Table 7.3 Ecological and chemical classification for surface waters (rivers, canals, surface water transfers, lakes, coastal and estuarine waters included) (Environment Agency, 2015).

Ecological status or potential					Chemical status	
Bad	Poor	Moderate	Good	High	Fail	Good
136	765	2,966	805	7	137	4,542

7.2.1.1 The Hampshire Avon catchment

The Hampshire Avon failed to achieve Good Ecological Status. Based on the South West river district River Basin Management Plan, out of the 50 water bodies (39 river, canals and surface water transfers and 11 lakes) in the River Avon catchment, only 16 were classified in good ecological status or potential and none in high ecological status. Figure 7.1 below shows the ecological status of the main rivers and streams of the catchment. Only 24% of Hampshire Avon river length achieved good ecological status (DEFRA, 2015).

The ecological status of the Hampshire Avon river has been associated with several factors. Approximately 85% of the catchment has been included in the Nitrate Vulnerable Zones (NVZs), i.e. areas designated as being at risk from agricultural nitrate pollution (81/676/EEC). Agricultural pollution causing increased phosphorus, nitrate and sediment pressures, has contributed to nutrient enrichment (Jarvie et al., 2005), siltation issues (Walling et al., 2008) and the existence of 'chalk stream malaise' i.e. the deterioration of the classic chalk stream habitat (DEFRA, 2003).

With regard to the current study sites, the river Ebble has been classified at good ecological status from 2009 till 2016, apart from the lower part of the Ebble that reaches the Avon river that was in moderate status from 2009 until 2012. The Ebble Trib (the part of the Ebble shown in yellow in Fig. 7.1) has been characterised at moderate ecological status since 2015 and was in good ecological status from 2009 till 2014. The Millersford brook has not been classified. However, all the brooks within the New Forest have been classified at moderate ecological status apart from the Ditchend brook that is in good ecological status in 2016 but also of moderate status from 2009 to 2015.

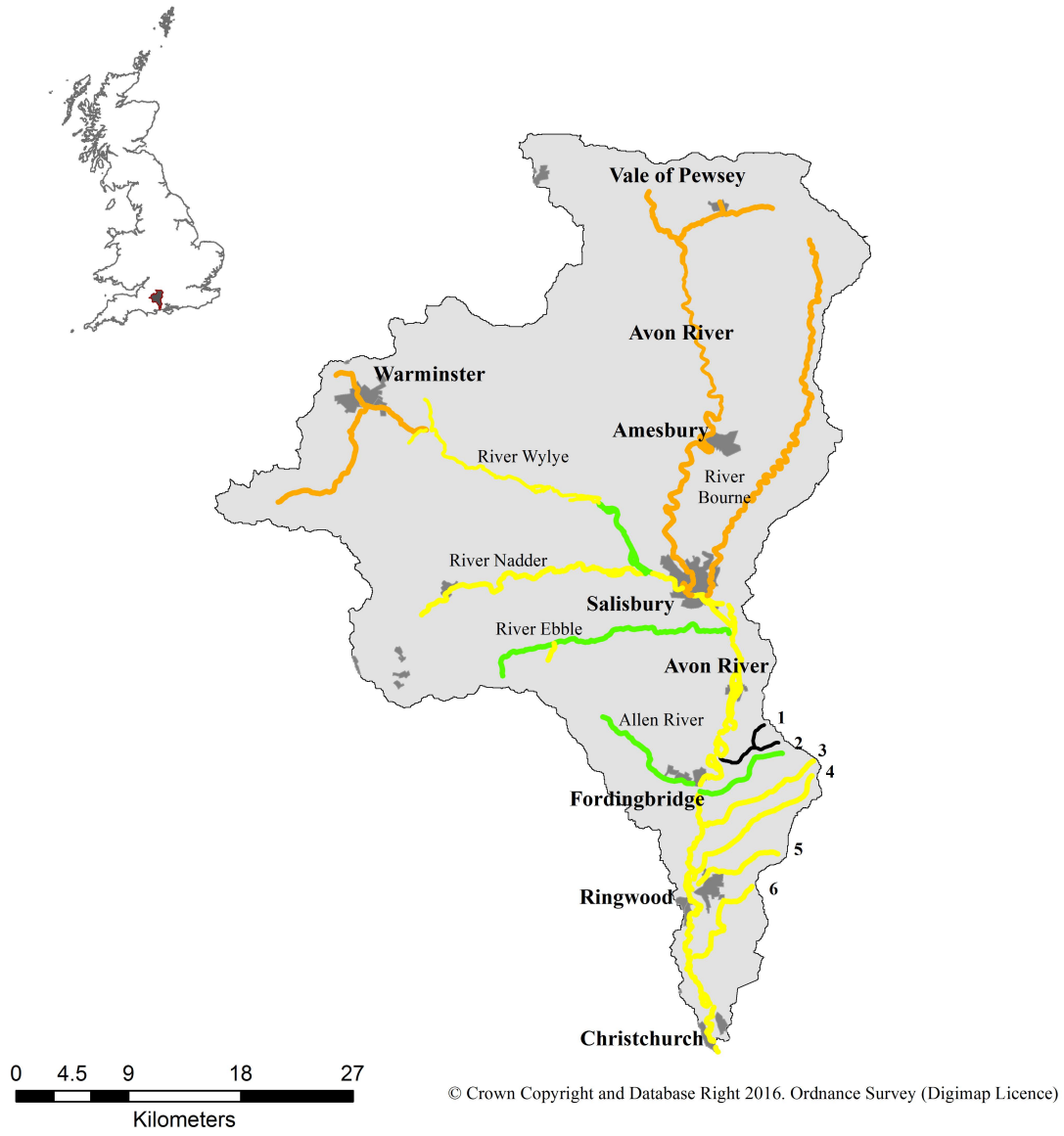


Figure 7.1 Map of the Hampshire Avon catchment, showing the Ecological Status of the river Avon and its tributaries. The numbered streams are 1. Millersford Brook (not classified), 2. Ditchend Brook, 3. Huckles Brook, 4. Dockens Water, 5. Linford Brook, 6. Ripley Brook. The grey areas represent large settlements. Good ecological status is presented in green, moderate in yellow and poor in orange (Environment Agency and DEFRA, 2016).

7.2.2 Demonstration test catchments

The fact that agricultural source pollution is the main reason for 30% of English water bodies failing to comply with the WFD standards led to the development of demonstration test catchments (DTCs) as a research platform on diffuse agricultural water pollution in England. An overview of the DTC programme was produced by

McGonigle et al. (2014) and also by (DEFRA, 2015). Here the key points of the programme are discussed. The research themes of DTCs are presented in Figure 7.2. The DTC project was commissioned by DEFRA in December 2009 and consists of four English river catchments, which represent 80% of UK soil/rainfall combinations and the major farm types across England and Wales. These are the Eden (Cumbria), Wensum (Norfolk), Tamar (Devon/Cornwall) and the Hampshire Avon. It provides research at a range of scales, from farm to catchment that aims to reduce agricultural diffuse pollution and improve ecological status of freshwaters, as well as bringing together researchers, stakeholders and policymakers in the process. In the case of the Hampshire Avon, the study areas involved 98 landowners.

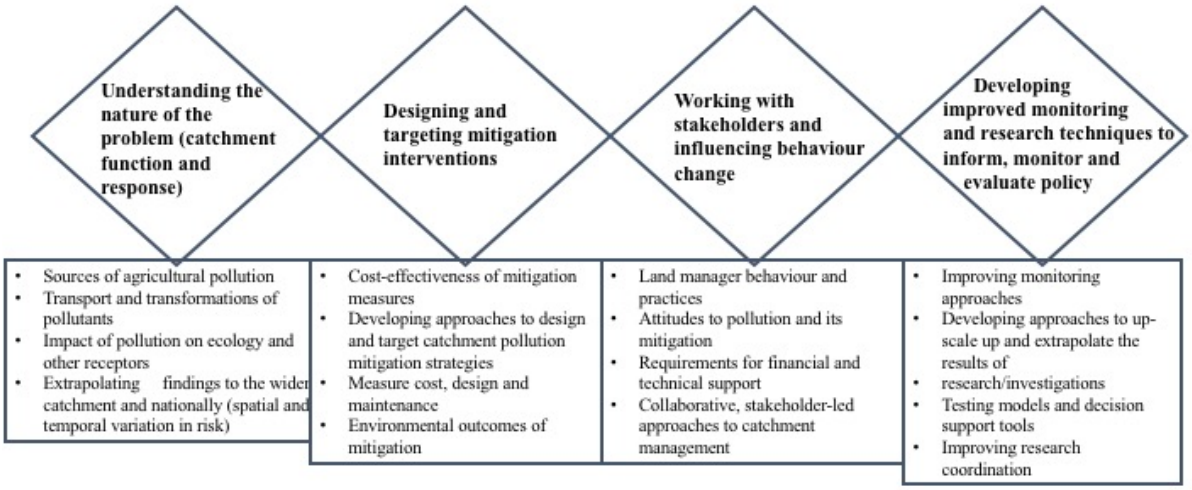


Figure 7.2 Demonstration test catchments main research themes (McGonigle et al., 2014)

In order for the UK water bodies affected by agricultural pollution to reach "good ecological status" progress should be made in reducing the delivery of nitrogen, phosphorus and sediment. The DTC programme identified principal issues for the Hampshire Avon, the pressures prioritised were nitrates and pesticides.

To meet its objectives, the DTC aimed to fill the knowledge gap on how catchments respond to on-farm mitigation measures. Conventional monitoring similar to that undertaken by national agencies could not capture the high episodic nature of agricultural diffuse pollution, the processes involved, nor unveil the effects of on-farm mitigation measures. Thus, test sub-catchments were chosen to be studied that enabled research on a smaller scale (up to 10km). The small streams chosen were equipped for monitoring immediately downstream the manipulated areas. The sampling scheme

followed the "Before and after control impact" (BACI) design (Stewartoaten et al., 1986), where the before impact was assessed using pre-mitigation instream data and the after impact using post-mitigation instream data. The DTC BACI design considered wetlands to be the mitigation area and upstream monitoring of the mitigation area to isolate a control area (DEFRA, 2015).

One of the three sub-catchments studied in the Hampshire Avon was the Ebble. The main concerns were linked with arable and livestock farming and comprised elevated nutrient and sediment inputs. The riparian wetland was studied as a mitigation feature to reduce diffuse pollution. The BACl design used is shown in Figure 7.3.

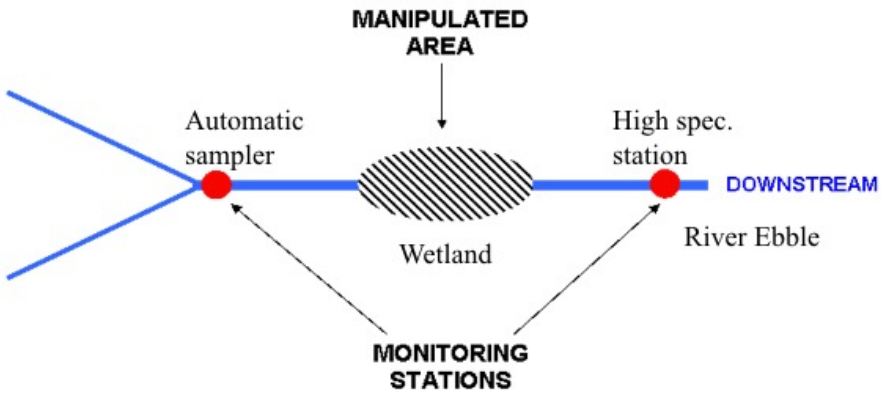


Figure 7.3 BACI design used to assess the impact of the wetland in Ebbesbourne for controlling agricultural diffuse pollution

In the present study, in order to better evaluate the role of riparian wetlands in diffuse pollution reduction, a comparison with the relevant results from Millersford Brook is attempted.

7.3 Hydrological monitoring and ecological status of the Ebble river

The Ebble is an ephemeral stream that was affected by the particularly dry weather conditions at the start of the study. Water started to flow during summer 2012 and was continually flowing from October 2012 till July 2013 when it dried up again. Flow levels resemble the characteristics of a small stream with maximum flow recorded at 0.66 m^3/s and mean flow 0.21 m^3/s (Meybeck et al., 1996).Turbidity values were generally low, close to 1 nephelometric unit (NTU), signifying a clear pristine stream. However, the large peaks that were monitored, contributed to a mean value of 5.7NTU,

with minimum of 0.4 and maximum of 152.6NTU. The large turbidity peaks are linked with high flow events (Figure 7.1). High flow events have the reverse effect on conductivity. This is to be expected as precipitation input dominates during high flow events. Rainwater dilution of dissolved solids and inorganic species results in conductivity decline (Vogt et al., 2010).

The Ebble sub-catchment is categorised as a lowland, high alkalinity site (UKTAG, 2008) and the relevant thresholds are presented in Table 7.1.Dissolved oxygen mean saturation levels were 80±10%. This is within the high ecological status standards set. However, 25% of the measurements were lower than the 70 % threshold, with a minimum of 53%. The Ebble is characterised by high alkalinity (Jarvie et al., 2005) and by alkaline pH with mean value over the study period 7.7±0.2. The 5 and 95 percentiles are 7.5 and 8 respectively, signifying high ecological status.

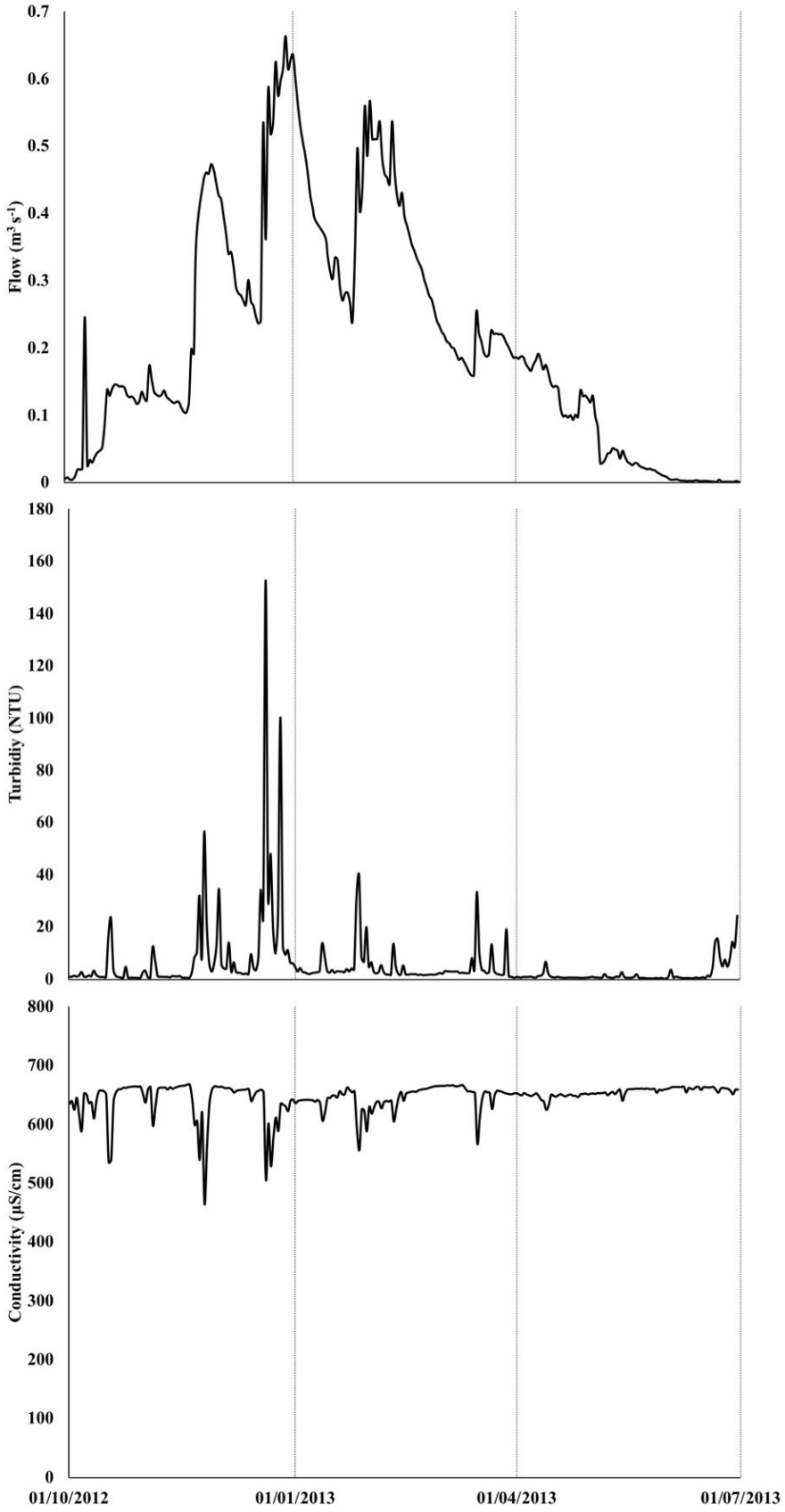

Flow (m3 s-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Turbidity (NTU)
180
160
140
120
100
80
60
40
20
0
Conductivity (µS/cm)
800
700
600
500
400
300
200
100
0
01/10/2012
01/01/2013
01/04/2013
01/07/2013

Figure 7.4 Line graphs of water flow (m^3 s^{-1}), turbidity (NTU) and conductivity (µS/cm) in the Ebble river during the period from 1/10/2012 to 1/7/2013.

Flow peaks seem to have a noticeable effect on the inorganic, particulate and total levels of nitrogen and phosphorus, as illustrated in Figure 7.5. During flow events significant proportions of sediment and pollutants were transferred, resulting in particulate nitrogen and phosphorus, SRP, total nitrogen and total phosphorus peaks. The resemblance of their behaviour indicates similar mobilisation pathways and mechanisms. All maximum concentrations occurred in response to the first storm event following drought conditions, when flushing of the nutrients and pollutants is more pronounced, a phenomenon described as "first flash" effect (Stutter et al., 2008). Nitrate behaved differently with nitrate levels correlating negatively with flow, signifying a dilution effect. Base-flow nitrate concentration was diluted by low-nitrate concentration rainwater. This is typical of agricultural areas where inorganic nitrogen flux is strongly influenced by nitrate leaching to groundwater (Durand et al., 1999, Durand and Torres, 1996, Kemp and Dodds, 2001). The effect of flow on nutrient loss from agricultural soils to surface waters has been reported in the literature. Stutter et al. (2008) showed that peak flows preceded drop of nitrates concentrations and peak dissolved and particulate phosphorus concentrations in agricultural catchments. Heathwaite et al. (2005) identified hydrological flow paths as a key parameter related to the diffuse nutrient loses for agricultural fields. Sharpley et al. (2008) proved that storms play an important role in phosphorus loss from agricultural watersheds. The results of the present study confirm flow to be an important driver of agricultural diffuse pollution flux.

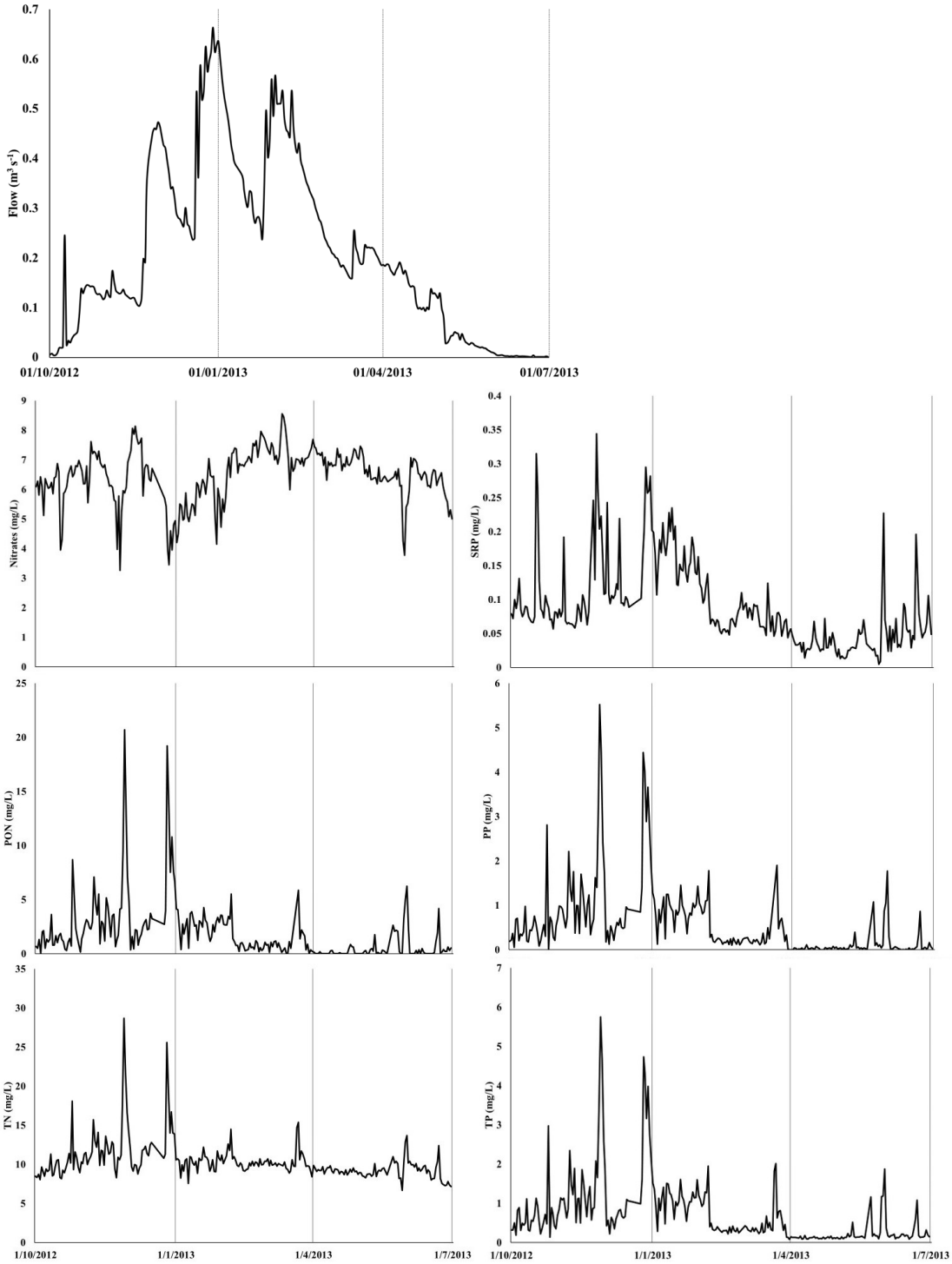


Figure 7.5 Line graphs of the Ebble River water flow (m^3 s^{-1}) and concentrations of nitrates, SRP, PON, PP, TN and TP (all in mg/L) during the period from 1/10/2012 to 1/7/2013.

A ratio widely used to check nutrient limitation, is the Redfield ratio. Redfield (1958) found that marine algal cells average N:P ratio to be 16:1, reflecting the water stoichiometry needed to meet primary production requirements. Therefore, aquatic

environments with a ratio of less than 16 are N limiting and when ratio is higher than 16 primary production is limited by P (Redfield, 1958). It is important to recognise that species-specific N:P ratios span over a wide range of values, meaning that different species can utilise N and P when deviations from the ratio occur. Additionally, N and P are not the only factors that can limit primary production. Nonetheless, the Redfield ratio can be used as a 'rule of thumb' for estimating nutrient limitation in aquatic environments. When taking into account ecological conditions, Klausmeier et al. (2004) showed that the optimal N:P ratio is between 8.2 and 45. The ratio reported here for Ebble river is 67±27. As shown in Figure 7.6, results indicate that phosphorus is the limiting nutrient for primary production. Typically, in UK upland waters P is a limiting nutrient with SRP being minor (Neal et al., 2003, UKTAG, 2012b). Thus, eutrophication prevention needs to target phosphorus sources.

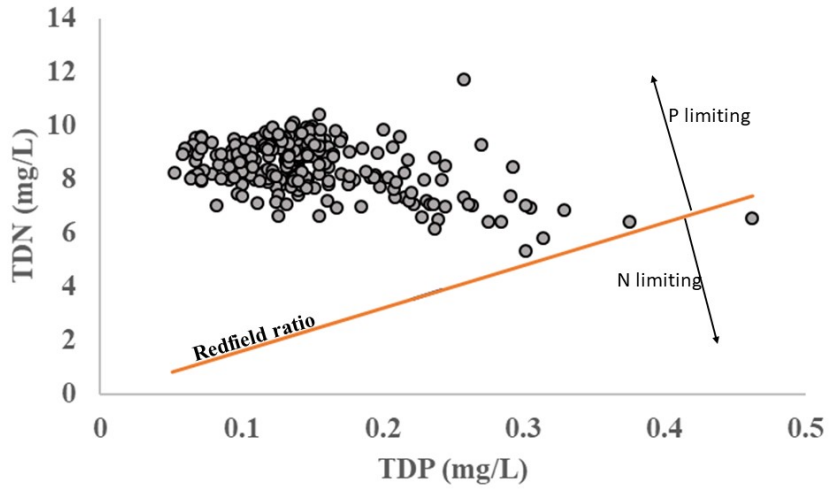


Figure 7.6 Scatterplot of TDN (mg/L) versus TDP (mg/L) in the river Ebble. Red line represents the Redfield ratio 16:1.

Organic levels of nitrogen, phosphorus and carbon show less pronounced peaks with no correlation with flow. Flow does not fully explain the variability observed in Figure 7.5, as proved by the correlation coefficients reported in Appendix E. Hence indicating flow independent sources. Peaks that do not coincide with the flow peaks could be explained by cattle grazing around the wetland. The cattle entered the site over a bridge almost above the downstream sampling location.

Results show storm induced nutrient transfer and verify the environmental effects on water quality. This fact demonstrates the challenges to be addressed by mitigation measures aiming to control nutrient levels in streams exposed to diffuse agricultural

pollution. The significance of such nutrient transfers is higher given the predictions for extreme weather events in the future.

7.4 Nutrient flux upstream and downstream of two riparian wetlands

As already explained, the control of nutrient levels in streams is of paramount importance. In this section, nutrient flux upstream and downstream of the two studied wetlands is investigated. All of the results are presented in Figure 7.7. The scope is to evaluate the role of riparian wetlands on the nutrient levels. Conclusions and recommendations on mitigation measures are presented in 7.5.

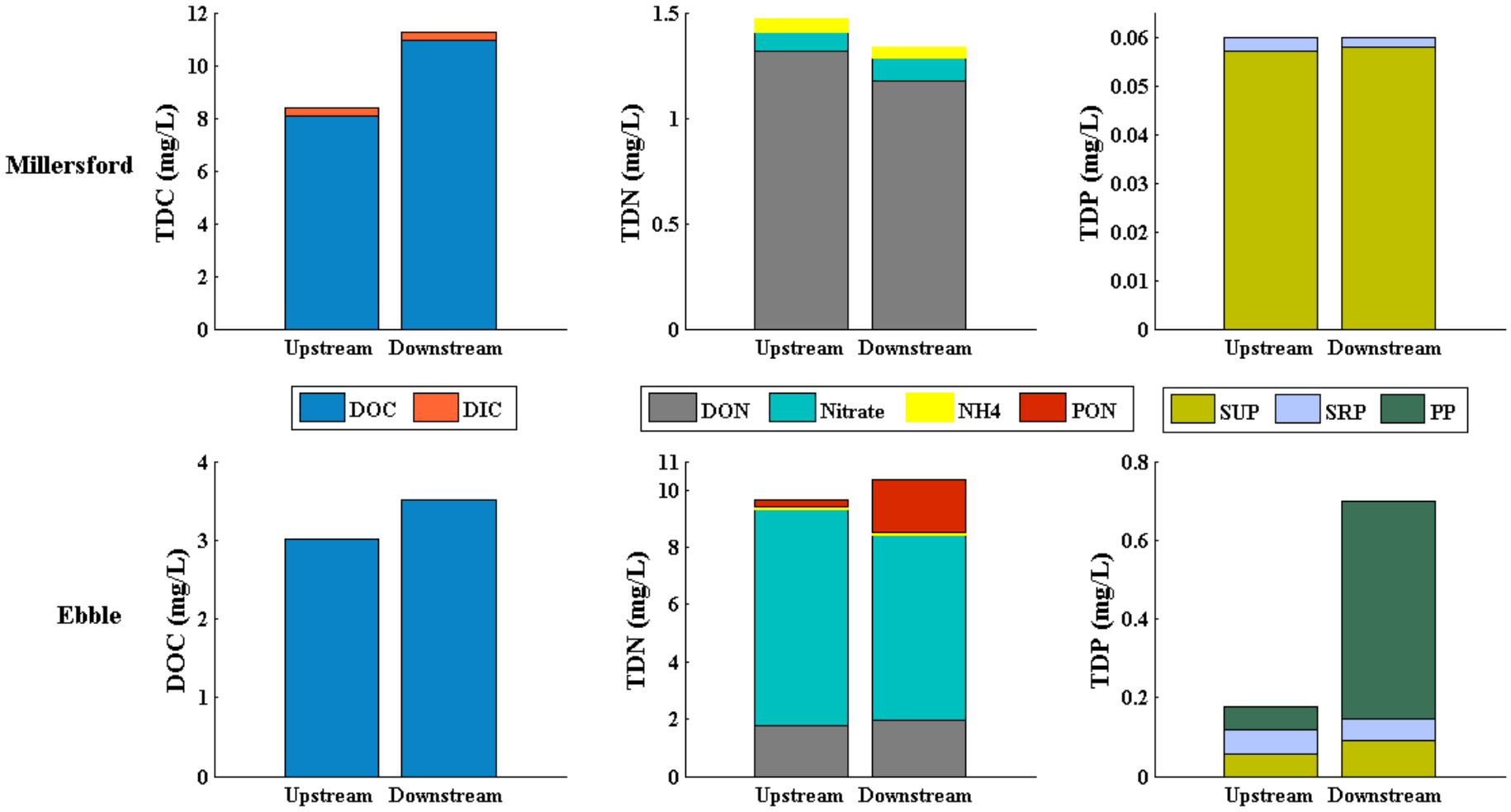


Figure 7.7 Bar charts of mean concentrations of TDC, TDN and TDP and their fractions (mg/L) in river water upstream and downstream of the Millersford Brook and Ebble river wetlands.

7.4.1 Phosphorus

Total P levels in the Ebble increased significantly downstream of the wetland, increasing from 0.2±0.1 upstream to 0.7±0.3 mg/l downstream the wetland (Figure 7.7). This increase is strongly associated with the significant increase of particulate P (10-fold), a form of P that can become bioavailable in aquatic ecosystems over time (Mainstone and Parr, 2002). Phosphorus in the rivers is transformed from particulate to dissolved and vice versa, while even firmly held phosphorus within the particles can slowly diffuse in the river water (Mainstone and Parr, 2002). PP increased from 0.06±0.1 to 0.6±0.5 mg/L, accounting for 34% and 79% of TP upstream and downstream the wetland, respectively (Figure 7.7). However, the effect of the wetland on the P flux is difficult to evaluate. Cattle grazing in the area around the wetland and the access point of the cattle over a bridge situated beneath the sampling point, may have biased the results. Particulate P flux in surface and subsurface flows as a result of grazing has been described in literature (Drewry et al., 2008, Greenwood and McKenzie, 2001, Haan et al., 2006, McConnell et al., 2016). Bilotta et al. (2007) studied the P flux, as well as the rates of erosion, the suspended soils and sorbed contaminants delivery to surface waters in grazed grasslands. They found levels that do not deviate much from arable land, whereby P flux was dominated by suspended and colloidal non-diluted forms. Bilotta et al. (2007) also identified the main processes related to livestock grazing that affect water quality, as follows: 1. Defoliation and vegetation cover reduction 2. Treading, compacting, pugging, poaching of soil and 3. Excretion. McConnell et al. (2016) showed that P losses to surface waters corelate with soil moisture content and herbage cover. The current results indicate that wetlands' ability to act as mitigation feature and control nutrient flux in surface waters can be overshadowed by site management practices.

The dissolved P fractions did not follow the same pattern. Soluble reactive P accounted for 49% and 62% of the dissolved P upstream and downstream the wetland, respectively. There was no evidence that SUP means differed (0.06±0.02 mg/L upstream and downstream). The SRP concentration upstream (0.06±0.03 mg/L) was significantly lower than the mean downstream (0.09±0.05 mg/L). The increase of SRP in the stream indicates possible flushing of wetland porewaters. These findings question the capacity of the Ebble wetland for nutrient retention. The downstream levels of SRP are higher than

the good ecological status threshold (0.069 mg/L) set by UKTAG for lowland high alkalinity streams. Downstream of the wetland, the 5th percentile is 0.024 mg/L; i.e. within the high ecological status standards. However, the 95th percentile is 0.227 mg/L; i.e. considerably higher than the moderate ecological status threshold.

Compared with the Ebble, Millersford brook shows markedly lower concentrations of SRP. The SRP concentration was much lower both at the upstream and downstream sampling sites and fell below the detection limit (0.002±0.3 mg/L). The SUP levels (0.06±0.02 mg/L) at Millersford did not differ with location in respect of the wetland or compared to the Ebble. Moreover, SUP was by far the major fraction of dissolved phosphorus in the Millersford brook. Yates et al. (2014, 2016) agreed with the dominance of SUP in the dissolved phosphorus pool, reporting even higher values for both SUP and SRP further downstream of the wetland.

The marked difference in SRP levels between the Ebble and Millersford Brook (more than 30 times higher in the Ebble) reflects the contrast in the land use of the two catchments and the potential sources of inorganic nutrients. Agricultural diffuse pollution has proven to be a major source of inorganic phosphorus to surface waters (e.g. Carpenter et al., 1998, Withers et al., 2014). It was therefore expected to find increased levels of SRP in the Ebble catchment area compared to the more pristine Millersford Brook. Additionally, anthropogenic impact on inorganic levels in the Ebble includes the presence of septic tanks in streamside properties upstream the study area. There are various sources of P that contribute to raw domestic sewage input in streams and rivers, namely faeces, urine, food waste, mains supply (phosphate addition to reduce lead in drinking water), toothpaste and dishwasher detergent (May et al., 2015). Reports commissioned by Natural England show that septic tanks are a considerable source of phosphorus, mainly SRP, especially during the common occurrences of functionality failure (Dudley and May, 2007, May et al., 2015). Withers et al. (2011) reported soluble P concentrations of 1–14 mg/L in septic tank effluent, with the SRP fraction dominant (70–85% of TDP).

Compared with the Ebble, Millersford Brook shows a much larger fraction of the P in organic form. Whitton and Neal (2011), after studying 18 sites in UK for 1-3 years, found that the SUP/TDP percentage is higher as the analysis moves upstream within the studied sites and as TDP levels decline. Whitton and Neal (2011) proved that SRP should be an indicator for monitoring not only lowland contaminated sites but also upland rivers,

especially those draining peat-rich soils. Their findings are supported by the Millersford results, showing larger fractions of organic phosphates in lower TDP levels and in draining peat systems.

7.4.2 Nitrogen

Concentrations of TN in the Ebble increased from 9.7±0.9 to 10.4±2.4 mg/L between the locations upstream and downstream of the wetland. Similar to PP, PON in the Ebble increased significantly downstream the wetland, from 0.24±0.52 mg/L upstream to 1.83±2.65 mg/L downstream the wetland. The corresponding increase in mean DON concentration increased significantly from 1.78±0.65 mg/L to 1.98±0.58 mg/L (Figure 7.7). The organic forms of nitrogen formed 37% of TN downstream, compared to 21% upstream. The increase in PON can be explained by the presence of cattle. Cattle manure is largely composed of organic nitrogen (DEFRA, 2010, Finch et al., 2014). The effect was enhanced because of the access the cattle had to the stream, as defecation can increase nitrogen levels (Davies-Colley et al., 2004). Total nitrogen comprised mainly by soluble N (97% upstream and 82% downstream) and dominated by nitrates (78% of TN upstream and 62% downstream). Mean nitrate levels decreased significantly from 7.56±0.72 mg/L upstream to 6.46±0.90 mg/L downstream of the wetland, whilst ammonium levels were below the detection limit.

At Millersford, the mean concentrations of DON upstream and downstream of the wetland were 1.32±0.68 mg/L and 1.18±0.70 mg/L, respectively. Nitrate increased from 0.09±0.06 mg/L upstream to 0.11±0.07 mg/L downstream of the wetland. The dissolved nitrogen pool is dominated by the organic fraction in both locations. On average, dissolved organic nitrogen accounts 82% of TDN in Millersford Brook, reaching a maximum of 97%. Ammonium was below the detection limit. Earlier studies (Yates, 2014, Yates et al., 2016) of nitrogen levels in daily samples of Millersford Brook, confirm DON dominates not only in the TDN pool but also in total nitrogen (66%). They reported ranges of 0-1.302 mg/L DON and 0-0.906 mg/L TON for the downstream sampling location, with corresponding mean concentrations of 0.547±0.202 mg/L and 0.132±0.145 mg/L. The levels of both TON and DON increased at sampling points further downstream; indeed, both of these fractions continued as equal contributors to total dissolved nitrogen up to 2km downstream of the wetland (Yates, 2014, Yates et al., 2016)

Nitrogen in rivers and streams is affected by land use, ecosystem type and soil type. However, DON has been shown to be relatively stable and independent of these parameters, whereas inorganic nitrogen is strongly correlated (Arheimer and Liden, 2000, Willett et al., 2004). Willett et al. (2004) related DON behaviour to its source, recalcitrant soil pools that have slow turnover. According to the European Nitrogen Assessment, both DON and nitrates concentrations increase along a gradient from ultra-oligotrophic to hyper-trophic water (Durand et al., 2011). Based on the same report, along the same gradient, the nitrates proportion of total N increases whilst the DON proportion decreases. Hence DON dominance is representative of less enriched, undisturbed catchments, and can account for over 60% of total N. On the contrary, in lowland, intensely farmed agricultural catchments, the proportion of total N as DON is up to 30% (Durand et al., 2011). These findings are consistent with those observed at the Millersford and Ebble sites. Typical DON values range from <0.15 mg/L in low nutrients status upland waters to >3 mg/L in highly enriched waters (Durand et al., 2011, Johnes and Burt, 1991, Kortelainen et al., 2006, Skoulikidis and Amaxidis, 2009, Willett et al., 2004).

The differences between the Millersford and Ebbesbourne catchments in terms of land use are reflected in the dissolved nitrogen levels and composition. The relative dominance of DON in the dissolved N pool at the Millersford site was expected owing to the undisturbed, heather grassland and woodland nature of this New Forest catchment. In contrast, the results presented for the river Ebble are consistent with the arable nature of the catchment where anthropogenic input is higher. PON levels in the river Ebble are associated with the presence of cattle. The findings show that the wetland in Ebbesbourne can buffer TON.

7.4.3 DOC

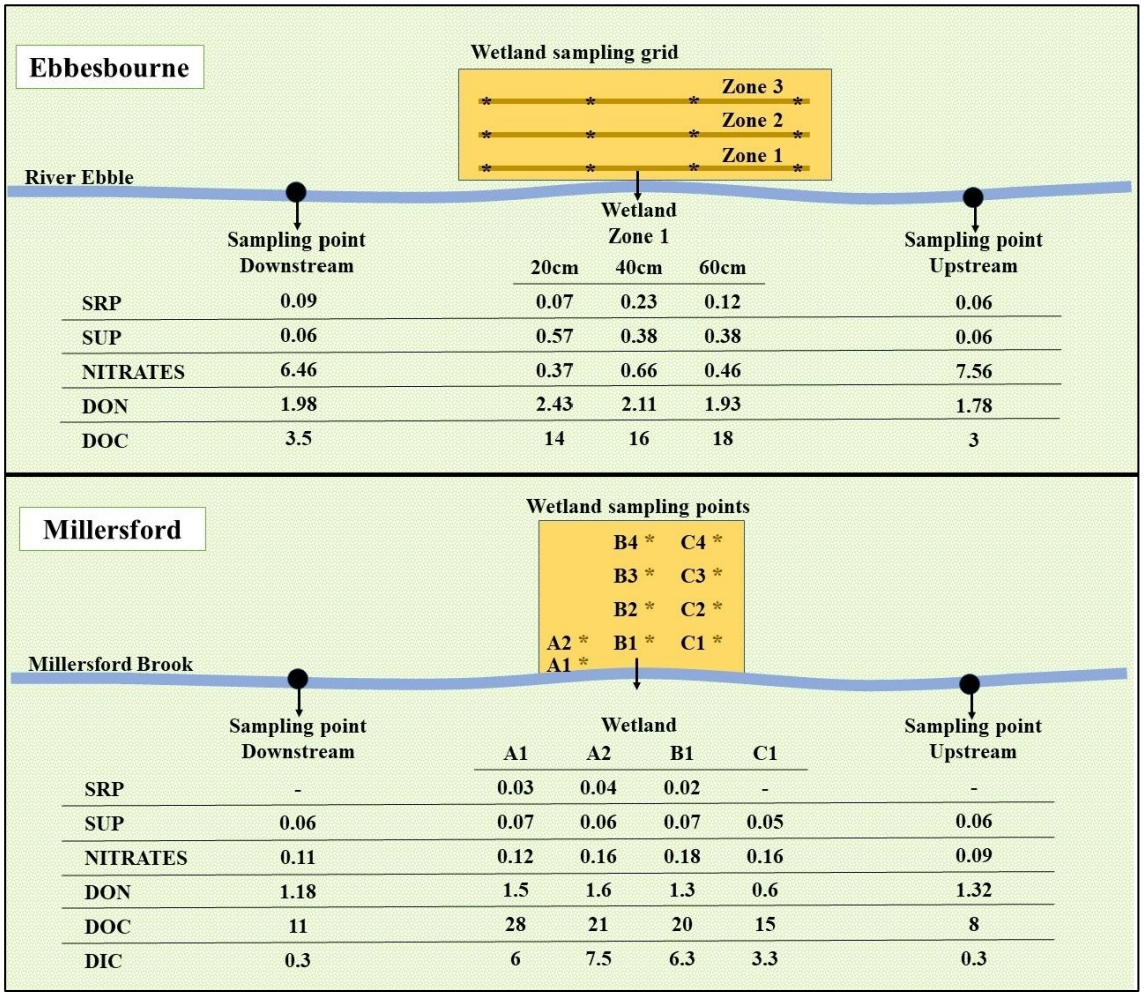
Concentrations of DOC in the Ebble downstream of the wetland (3.5±1.5 mg/L) are significantly higher than those downstream (3±1.4 mg/L) (Fig. 7.7). Dissolved organic carbon represented a minor proportion of the dissolved carbon pool, hence the non-purgeable organic carbon method was used as described in Chapter 4. Because of the method used, no data on DIC in the Ebble River are available. In the Millersford, however, DOC levels were relatively high: 8.08±1.68 mg/L and 10.97±3.86 mg/L upstream and downstream of the wetland, respectively (Fig. 7.7). The organic fraction of dissolved carbon at Millersford was dominant. The inorganic fraction of C accounted for

only 3.9% and 2.8% of the TDC at the upstream and downstream locations, respectively. The above concentration range in Millersford is in agreement with Yates et al. (2014, 2016) who reported a range of 3.88 to 14.5 mg/L for annual results from daily samples. It is noteworthy that the concentrations reported for downstream of the wetland (8.56±2.61 mg/L) declined further downstream, reaching 7.77±2.39 mg/L DOC 3.5km beyond the sampling point (Yates, 2014, Yates et al., 2016).

Thurman (1985a) reported typical values of DOC as follows; pristine streams: 1-3 mg/L, rivers: 2-10 mg/L, cool temperate climate: 2-8 mg/L, warm temperate climate: 3-15 mg/L, rivers draining swamps: and wetlands: 5-60 mg/L. Drever (1997) estimated that fluvial DOC concentration equal or exceeding 10 mg/L corresponds to distinctly coloured river waters. Fluvial DOC concentration is mainly controlled by climate (precipitation) and landform (wetland presence) (Mulholland, 2008). The findings of this study confirm that organic-rich soils, such as the ones in the Millersford wetland, result in higher DOC concentrations in the adjacent Millersford Brook. The Ebbesbourne wetland appears to have a less pronounced effect on DOC concentrations in the Ebble river. The effect of the wetlands on the adjacent river DOM is discussed in 7.5.

7.5 The effect of riparian wetlands on adjacent stream Nitrogen, Phosphorus and Carbon

The data for stream and wetland (as discussed in Chapter 5) nutrient levels are summarised in Figure 7.8. The evaluation of riparian wetlands as mitigation measures is challenging as the “noise” from other sources must be eliminated.



	Sampling point Downstream	Wetland Zone 1 20cm	40cm	60cm	Sampling point Upstream
SRP	0.09	0.07	0.23	0.12	0.06
SUP	0.06	0.57	0.38	0.38	0.06
NITRATES	6.46	0.37	0.66	0.46	7.56
DON	1.98	2.43	2.11	1.93	1.78
DOC	3.5	14	16	18	3

	Sampling point Downstream	Wetland A1	A2	B1	C1	Sampling point Upstream
SRP	-	0.03	0.04	0.02	-	-
SUP	0.06	0.07	0.06	0.07	0.05	0.06
NITRATES	0.11	0.12	0.16	0.18	0.16	0.09
DON	1.18	1.5	1.6	1.3	0.6	1.32
DOC	11	28	21	20	15	8
DIC	0.3	6	7.5	6.3	3.3	0.3

Figure 7.8 Summary of the Ebbesbourne and Millersford mean nutrient concentrations (mg/L) in wetlands and at the river sampling points upstream and downstream of the wetlands.

Wetlands are known to act as sources, sinks or transformers of nutrients and other chemicals. Their role depends on wetland type, hydrology and nutrient loading (e.g.Mitch and Gosselink, 2000, Reddy et al., 1999). Some of these variables explain the contrasting behaviour of the two wetlands here. For example, in Millersford, inorganic forms were low and with little evidence that their concentrations changed throughout the wetland. As a result, inorganic nutrient (SRP, DIC and nitrates) levels in the Millersford Brook remained unaffected by the presence of the wetland. Low inorganic nutrient concentrations in Millersford are attributed to the absence of anthropogenic sources of pollution in the Millersford sub-catchment. On the contrary, there is spatial variation across the Ebble wetland pore waters with regard to concentrations of SRP; e.g. zone 1 (sampling points closer to the river bank) SRP concentrations were significantly higher in all depths ranging in all depths. It is speculated that the elevated SRP concentrations

downstream of the Ebble wetland (0.09 mg/L), compared with 0.06 mg/L upstream, is attributed to the wetland acting as a SRP source. Studies have shown that riparian wetland sediment can export SRP to porewaters and surface waters. Nichols (1983) and Richardson (1999) concluded that wetlands are not a limitless sink of SRP and that wetland soils release SRP to the water. Surridge et al. (2007) showed that riparian wetland sediments release SRP after flooding, due to anaerobic conditions releasing P through the reductive dissolution of Fe bound phosphates. This mechanism largely controls the P buffering ability of wetlands. Fisher and Acreman (2004) reviewed data from 57 wetlands and found that riparian wetlands are more likely to increase soluble P loading. In the same study, these researchers showed that the majority (more than 70%) of riparian wetlands exhibited retention of nitrate. The Ebbesbourne wetland appeared to remove 15% of nitrate, decreasing the concentration from 7.56 mg/L upstream to 6.46 mg/L downstream (Fig. 7.8). Denitrification is the main mechanism of nitrogen retention, followed by nitrogen sedimentation and uptake by aquatic plants (Saunders and Kalff, 2001). Since denitrification is an anaerobic process, the wetland waterlogged conditions enhance nitrate reduction. Thus, riparian wetlands can play a vital role in stream water quality through nitrate retention (e.g.Gilliam, 1994, Johnston, 1991).

Nutrients in both wetlands were mainly in organic form. Results from Chapter 5 showed that the Millersford wetland accumulates DOM in the lower part of the hillslope. Minor part of it is DON, whereas SUP is very limited mostly below detection limit. DOC increases in the adjacent Millersford brook by 38%, suggesting DOC export from the wetland. The Ebbesbourne wetland contributed to a 17% increase of DOC in the Ebble river. In the Millersford Brook DON did not increase significantly, whereas in the Ebble river the concentration increased by 11%. SUP in the adjacent streams was not affected by the presence of the two wetlands. As discussed, the levels of organic nutrients in Ebbesbourne was possibly affected by cattle grazing near the sampling point.

The elevated downstream DOC concentrations at Millersford could be due to the organic-rich soil that prevails in the near surface flow paths. The dominance of such flow paths is characteristic for streams draining wetlands (Mulholland, 2008). The influence of the Millersford wetland on the adjacent stream is also supported by Baker et al. (2008) who showed that DIC in rivers is typically greater than DOC, except for sites with peat-rich headwaters. Moreover, they illustrated that the fluvial dissolved carbon pool consists

largely of DOC where soil water dominates (Millersford) and DIC where groundwater dominates (Ebbesbourne). Based on these observations, it is reasonable to assume that the Millersford wetland has a significant effect on the fluvial dissolved carbon pool.

As described in Chapter 6, soil porewater DOM in Millersford was less bioavailable to microorganisms, with it being of higher molecular weight and aromaticity. The results from Millersford Brook show changes in the DOM bioavailability downstream of the wetland. The DOC:DON ratio increased more than 50% (from 6.1 to 9.3), indicating less bioavailable DOM. The same ratio was not affected in Ebbesbourne (1.8). These findings suggest that compared to Ebbesbourne, Millersford wetland had a more pronounced effect not only on DOM levels but also on the quality of DOM in the adjacent Millersford Brook.

7.6 Conclusions. Mitigation recommendations.

Identification of nutrient sources and pathways to different catchments is essential for planning mitigation measures. The results from the two studied catchments can lead us to valuable recommendations. Identified factors influencing nutrient levels in the studied streams include hydrology, wetlands, cattle grazing and septic tanks.

Storm events influenced nutrient levels, whereby peak flows coincided with turbidity, SRP, particulate and total nutrient peaks. This confirms the highly episodic nature of diffuse pollution attributed to surface sources. This was further confirmed by the observation of the "first flash effect" after a dry period when nutrient enrichment was at a maximum. Nitrates showed dilution effect of high flow events and displayed the importance of groundwater nitrate levels.

In conclusion, flow is an important driving factor in the studied sub-catchments and storm events should be incorporated in monitoring programs. Mitigation measures in the Ebble need to control soluble P sources that are mobilised through flow. Additional measures should be taken to control phosphorus enrichment originating from septic tanks. P mitigation measures should also be dictated by the high proportion of soluble P upstream of the wetland (66%) and the SRP levels that are higher than the good ecological status threshold. As results confirmed P to be the limiting nutrient, such mitigation measures would prevent the risk of eutrophication. Mitigation measures should impede leaching of nitrate to groundwater aquifer by targeting source mobilisation of nitrate.

Additionally, mitigation measures need to target particulates as well. Particulate P and N peaks corresponding to high flow events, showed overland transport. Furthermore, the severe increase of particulate nutrients downstream of the wetlands points to the effect of cattle grazing and cattle access to the stream. Hence, mitigation measures should target the reduction of manure deposits to the stream. It is also very important to note that in order to successfully implement mitigation measures, the study area should be managed accordingly. In Ebbesbourne, the decision of the landowner to allow cattle grazing diminished the effect of the mitigation feature on water quality. This issue highlights the need to engage with the stakeholders, so that the benefits of the mitigation measures are fully supported.

Wetlands were evaluated as mitigation features. The current study focused on diffuse agricultural pollution and, based on the above conclusions, SRP and nitrates reduction need to be targeted by mitigation measures. The Ebbesbourne wetland acted as a source of SRP, showing that not all wetlands act as buffers. The wetland showed an ability to transform nitrates thus reducing nitrate levels in the adjacent stream. Although the nitrate results are promising, there is not enough evidence to recommend the Ebbesbourne wetland as mitigation feature. Both wetlands acted as sources of DOC. As shown in Chapter 6, DOC exported from the two wetlands is not only quantitively but also qualitatively different. As discussed in 2.2.2 DOC plays a distinctive important role in aquatic ecosystems. The ecological effect of DOC export from the two wetlands is examined in the next chapter.

The results indicate that in order to reach the anticipated ecological status in rivers and streams, multiple water quality stressors need to be taken into account. The variety of challenges in the effort to improve water quality was confirmed. Even at the small scale of sub-catchments, isolation of a single or few parameters to draw conclusions on water quality status and mitigation measures can be misleading.

Chapter 8- The effect of wetland exported DOM on tungsten toxicity

8.1 Overview

Wetlands are known to export DOM in adjacent streams and rivers. The effect of the studied wetlands on DOM levels of Millersford Brook and the Ebble river was evaluated in Chapter 7. Chapter 8 attempts to evaluate the effect of DOM of the two streams on the toxicity of Tungsten. The choice of Tungsten is relevant because of the following:

- concerns raised about tungsten toxicity over the last 2 decades, disputing the perception of an inert, non-toxic metal
- increased concentrations of Tungsten in the vicinity of military grounds. This has been confirmed in the UK. Millersford is located approximately 20 miles away from the Salisbury military base.
- lack of reports on DOM effect on tungsten toxicity

Key findings include:

- Dissolved organic carbon exported from the Millersford wetland, in combination with an increase in pH and water hardness, decreased Tungsten toxicity.
- Toxic effects were observed in concentration that have been reported in surface water elsewhere.

8.2 Tungsten

Tungsten properties, applications occurrence and toxicity are reviewed in the following sections.

8.2.1 Physical and chemical properties of Tungsten

Tungsten was named by the Swedish chemist and mineralogist Baron Axel Fredrik Cronstedt (1722 – 1765) when in 1757 he discovered the mineral Scheelite, a calcium tungstate mineral. Its name English translation is "heavy stone" and was given because of the high density of the mineral. Pure metal tungsten was isolated by the Spanish brothers, chemists and mineralogists, Juan Jose de Elhuyar (1754-1796) and

Fausto de Elhuyar (1755-1833) in 1783. They named it Wolfram, a name used for tungsten minerals since the Middle Ages in the tine mines of Saxony-Bohemian Erzgebirge in Germany. Wolfram means wolf's foam and describes the presence of tungsten minerals in the tin ore that resulted in reduced tin yield during smelting and foam forming on the tin melt. The element is named tungsten in English and is represented by the letter W from the name Wolfram.

Tungsten (W) is a metallic transition element of group VI, period 6 of the periodic table. Among the properties of the transition elements are their hardness with high melting and boiling points indicating strong bonding, high density metals, heat and electricity conductivity (Mido and Satake, 2010). It also belongs to refractory metals, a group that possess the highest melting temperature and lower vapour pressure of all metals (Davis, 1997). Tungsten has an atomic number 74 and atomic weight of 183.84.

The element tungsten forms compounds having a wide range of oxidation states (from -2 to +6) and coordination numbers (maximum 9) (Lassner and Schubert, 1999). A wide range of soluble complexes can be formed with aqua-, oxo-, halide-, organo- and mixed ligands (Lassner and Schubert, 1999). The higher oxidation states (+5, +6) result in the most stable compounds. Tungsten has a very low resistance to oxidation (Lassner and Schubert, 1999). In nature, it is found in the form of tungstates (Deltombe et al., 1974).

According to Lassner and Schubert (1999), in aqueous solutions tungstate ions only occur monomerically in alkaline or neutral solutions. At pH<6.2, condensed, complexed isopolytungstate ions tend to be formed. Polymerisation is a common ability for Groups 6&7 of the Periodic table. The formation of polytungaste is described with the following equation and the coefficients and respective tungsten speciation is shown in Table 8.1(Kim et al., 1968, Lassner and Schubert, 1999).

$$A\ [H_3O]^{+} + 12\ [WO_4]^{-2} \rightarrow B\ [W_xO_yH_z]^{n} + C\ H_2O$$

Table 8.1 Tungsten speciation and corresponding coefficients

Coefficients			Tungsten speciation
A	B	C	
4	1	6	$[W_{12}O_{46}]^{20-}$
8	1	12	$[W_3O_{11}]^{4-}$
	4	4	$[H_4W_3O_{13}]^{4-}$
14	1	16	$[H_{10}W_{12}O_{46}]^{10-}$
	2	20	$[HW_6O_{21}]^{5-}$
16	1	24	$[W_{12}O_{40}]^{8-}$
18	1	26	$[H_2W_{12}O_{40}]^{6-}$
	2	24	$[H_3W_6O_{21}]^{3-}$
24	12	24	$[WO_3 \bullet 2H_2O]$

Tungsten has unique physical and mechanical properties. It has the highest melting point of all elements except carbon (3410^{o}C), the lowest thermal expansion and vapour pressure (4,27Pa) of all metals, one of the highest densities (19.1g/cm^3) of all metals, equal to that of gold, high thermal and electrical conductivity (Koutsospyros et al., 2006, Lassner and Schubert, 1999).

8.2.2 Tungsten applications

Tungsten properties make it a metal of high strategic importance (Smith, 1994), suitable for a very wide range of applications. The major uses of tungsten are categorised based on the contributory tungsten properties and are described in (Graedel et al., 2015). High hardness and high compressive strength of tungsten cemented carbides make them suitable for metal cutting and forming tools, and mining and construction equipment. That is the main tungsten application. Mills products such as light filaments, electrodes, and welding applications relate to tungsten high melting point. Tungsten resistance to mechanical and thermal shock is relevant to specialty steels application such as tool steels and dies. Superalloys application such as turbine engine components are feasible because of tungsten's corrosion resistance and high temperature strength. Low vapour pressure and high density of tungsten applies in other uses, for example pigments and counterweights.

Following concerns over environmental impacts of lead shotshell ammunition and lead fishing weights, tungsten was introduced as a non-toxic alternative especially for bird hunting (Scheuhammer and Norris, 1995, Scheuhammer and Norris, 1996). In

the mid-1990s the US Army replaced the lead core with tungsten alloys in military bullets (Petkewich, 2009). Tungsten and tungsten alloys have since then been used in large quantities for the manufacture of ammunition systems (especially anti-armour munitions) used by the British army as well (Doust et al., 2007).

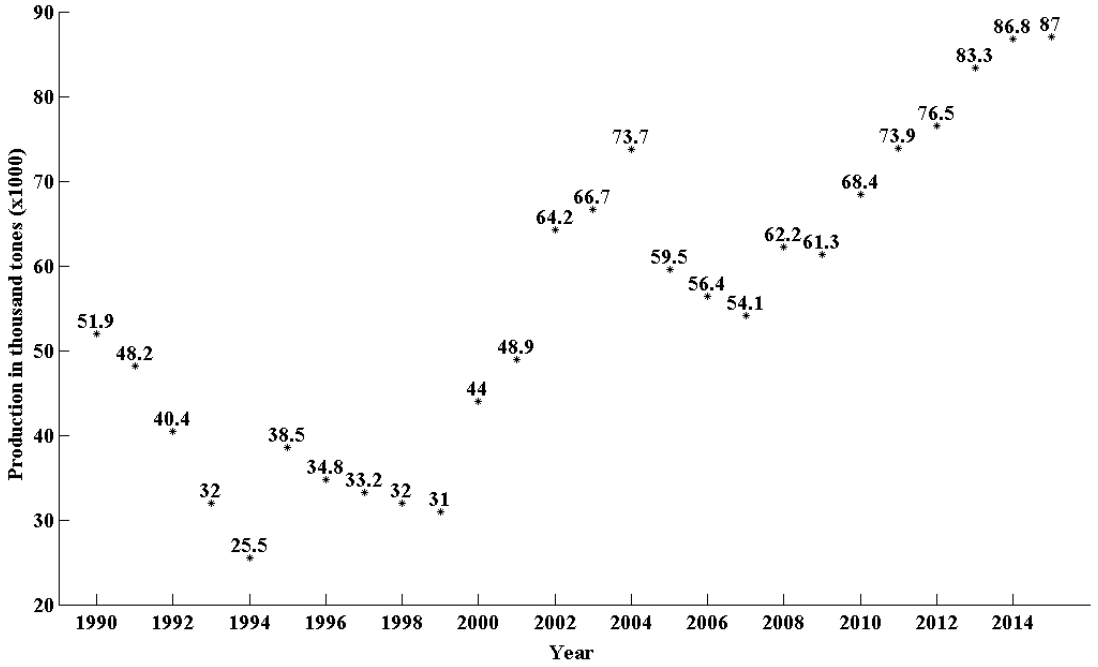


Figure 8.1 Tungsten world annual production (data collected from (Amey, 1994, Amey, 1995, Shedd, 1999, Shedd, 2004, Shedd, 2009, Shedd, 2014, USGC, 2016).

Hence the consumption of Tungsten was further increased in many countries. Figure 8.1 shows the increase in annual world production that reached 87 thousand tonnes in 2015. These data predict that Tungsten levels will increase in the environment.

8.2.3 Tungsten levels in environmental systems

Tungsten, being the least volatile of all metals, has low concentrations in the atmosphere. Concentrations reported range from sub-nanogram in the Arctic and South Polar atmosphere (Maenhaut et al., 1979, Sheridan and Zoller, 1989) to 6ng/m^3 in urban areas of Indiana (Dams et al., 1972) and 23ng/m^3 in copper smelting plants of Arizona (Small et al., 1981). Sahle et al. (1996) reported much higher levels of 8 mg/m^3 in the air of hard metal production plants using tungsten in Sweden.

The earth's crust consists of tungsten by 0.00013%, corresponding to background levels of 1.3 mg/kg (Koutsospyros et al., 2006, Smith, 1994). Senesi et al. (1988) reported on the usual ranges of tungsten in soils. They described the levels of tungsten as very low

particularly in surface soils (0.68-2.7 mg/kg dry mass) and in soils (0.5-83 mg/kg dry mass). However, by examining the levels of tungsten in commercial inorganic fertilisers they concluded that application of the studied fertilisers could significantly affect the status of tungsten in soils. A report by the US Air force research laboratory (Ramos et al., 1998) estimated the levels of tungsten in firing range soils after tungsten-tantalum penetrator munitions usage to be 5500 mg/kg. Pyatt and Pyatt (2004) reported on tungsten values of 78.4 mg/kg is soils and 59.3 in top soil close to a wolframite mine that has not been operating in the recent years.

Typical concentration of tungsten in the ocean is 0.2 ng/kg (Koutsospyros et al., 2006, Kunzendorf and Glasby, 1992) but much higher concentrations have been reported for Northern Atlantic (100 ng/L) and Pacific (8 ng/L) oceans (Merian and Clarkson, 1991). Kishida et al. (2004) estimates levels of tungsten in hydrothermal vent fluids in the deep oceans and found that they were 4 times higher compared to the ambient level in seawater. Johannesson et al. (2000) showed that tungsten values in multiple points of three rivers systems ranged from 0.15 to 189.7 μg/L. Elevated values of 224.5 μg/L were reported in samples from hot springs close to areas with scheelite and wolframite occurrences (Hall et al., 1988).

Tungsten is not part of routine testing analysis as there is no regulation for tungsten. Therefore, reports are scarce. Though it is evident that anthropogenic tungsten releases can significantly affect tungsten levels in the environment. An indication of the effects of military use of Tungsten are the findings of Clausen and Korte (2009). They studied military training grounds where lead was replaced with tungsten and found soil surface concentrations of up to 2080 mg/kg. They also described migration to groundwater (30 m in approximately 5 years) and suggested that migration in areas with high precipitation is expectable. Well samples from 30 m below the ground surface showed tungsten levels of up to 0.56 mg/L. Tungsten concentrations in samples collected from Lulworth, within the Armour Centre of Bovington, Dorset, have been reported in a study published by the Ministry of Defence (Doust et al., 2007). The background concentration in surface soils were less than 0.1 mg/kg but tungsten levels ranged between 1.2 mg/kg and 112 mg/kg in a radius of 10 m around the target. Water sample concentrations ranged from <0.1 μg/L at some distance from the target to 38.25 μg/L near

the target. The results indicated enhanced tungsten concentration by a magnitude between 1 and 3 orders in surface soils close to the target and dissolution in surface water.

The concentrations of Tungsten in the Topsoil of the catchment under study are currently low as illustrated in Figure 8.2.

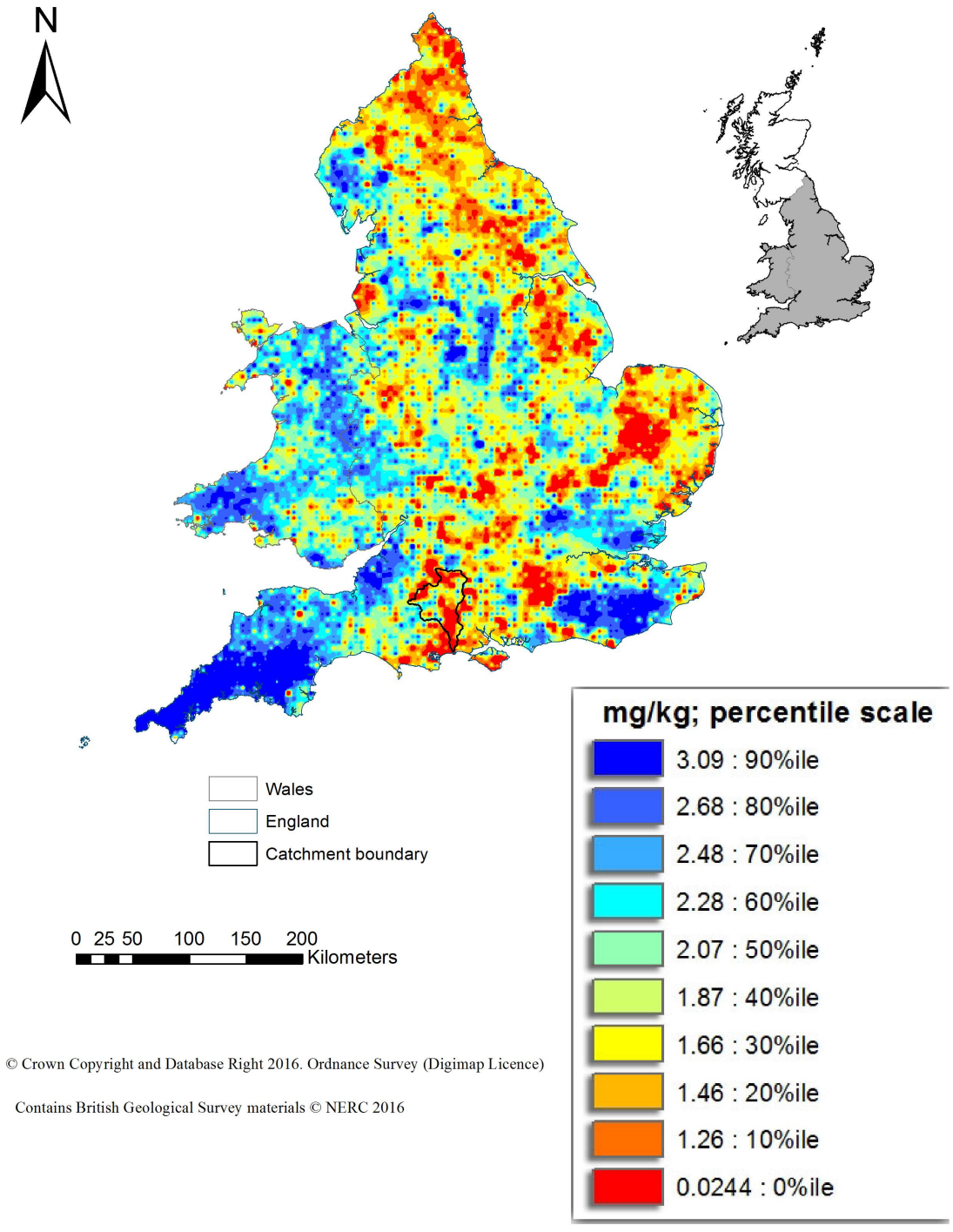


Figure 8.2 Map of tungsten concentration (mg/kg) in the Topsoil of England and Wales.

8.2.4 Tungsten toxicity

Tungsten is one of the least regulated metals as it was considered inert and non-toxic until recently. However, during the past two decades increasing evidence of the adverse effects on animals and humans has arisen.

Miller et al. (2001) showed that heavy metal tungsten alloys used mainly in military applications cause human cell transformation to the neoplastic phenotype, indicating risk of cancer induction. Miller et al. (2004) later studied the mechanism that heavy metal tungsten alloys may be involved in toxicity and tumorigenicity. In a study conducted by Kalinich et al. (2005) rats that were implanted with pellets of weapons-grade tungsten alloy, to imitate shrapnel wounds, developed tumours that in some cases were extremely aggressive. The authors concluded that "These results point out the need for further studies investigating the health effects of tungsten and tungsten-based alloys". A number of studied were undertaken after "A unique cluster of childhood leukemia" that occurred around the city of Fallon in Churchill County, Nevada, from 1999 to 2001 (Steinmaus et al., 2004). Tungsten was found to be unusually elevated in blood, urine, and cheek cell samples of children diagnosed with leukaemia (Rubin et al., 2007). Investigations on the leukaemia cluster link with tungsten exposure were not conclusive (Rubin et al., 2007, Steinberg et al., 2007). However, Sheppard et al. (2007) confirmed that the time of the onset of excessive childhood leukaemia corresponded to the time of tungsten and cobalt rise. The findings of Machado et al. (2010, 2011) supported severe, short-term, human toxicity potential for inhaled ballistic aerosol created by kinetic energy penetrator rods of tungsten heavy alloys. Sodium tungstate was linked with increased apoptosis in human peripheral blood lymphocytes, alteration of cell cycle progression and reduction of cytokine production (Osterburg et al., 2010). Guilbert et al. (2011) illustrated DNA damage following tungsten exposure. Laulicht et al. (2015) reported on the carcinogenic potential of tungsten, by carcinogenic related endpoints induction. Following an accidental exposure of breast cancer patients to tungsten, Bolt et al. (2015) using an animal model, accused tungsten of enhancing metastasis.

Although tungsten was traditionally considered as a non-toxic, environmentally inert metal by the regulators in EU and US, that was not the case in the former USSR where intense research studies started in 1950. As a result, tungsten was classified as a highly dangerous chemical compound in water reservoirs and the maximum allowable

concentration was set at 0.0008 mg W/L for aquatic systems used for fishing and 0.05 mg W/L for drinking water reservoirs. These standards are followed to date (Strigul et al., 2009b).

Eco-toxicological studies are very limited. Toxicity has been studied in laboratory animals. The oral LD_{50} in rats was 1928.4 mg/kg and in mice 1904.1 mg/kg , whereas intravenous LD_{50} was 61 mg/g in rats and 107.1 mg/kg in mice (Fernandez-Alvarez et al., 2000). Strigul et al. (2005) studied the effects of tungsten on environmental systems. They showed that tungsten powder in soils resulted in soil microbial community changes and fungal biomass increase, red worms and plants death. Tungsten ions in soils were taken up by plants and worms, indicating that tungsten compounds could be introduced to the food chain. The LD50 values after 48hours *Daphnia* immobilisation tests were 0.106 gW/L for sodium metatungstate and 0.344 gW/L for sodium tungstate, illustrating greater toxicity of sodium metatungstate to *Daphnia (Strigul et al., 2009a)*. Sodium metatungstate at concentrations higher than 0.05 gW/L inhibited algae (*Selenastrum capricornutum*) growth by 75%, and was more than 25 times more toxic than sodium tungstate (Strigul et al., 2009a). Mortality and LD_{50} values were reported for red worms exposed to sodium metatungstate, whereas no mortality was observed in sodium tungstate worm toxicity tests (Strigul et al., 2009a). The same authors attempted to assess the effect of tungsten to aquatic ecosystems using aquarium with fish and aquatic plant species. The only effect reported was fish mortality. Khangarot and Ray (1989) estimated the EC_{50} value for W^{6+} to be 89.39 mg/L for *Daphnia*. Sodium metatungstate was classified as moderately toxic to fish, unlike sodium tungstate that exhibits low toxicity (Strigul et al., 2010). The LD_{50} estimated after exposure of *Poecilia reticulate* to sodium metatungstate was 0.13 gW/L after 14 days and 0.85 gW/L after 1 day. LD_{50} were much higher for sodium tungstate. Clements et al. (2012) reported sodium tungstate EC 50 values of 95.5 mg W/L after *Daphnia* acute toxicity test and 25.9 mg W/L no observable effect concentration (NOEC) after 21 days toxicity test. Zebrafish acute testing resulted in LC50 of 106 mg W/L, while green algae ErC50 was 31 mg W/L. Kennedy et al. (2012) studied the toxicity of sodium tungstate and an aged tungsten powder-spiked soil containing monomeric and polymeric tungstates to the cabbage *Brassica oleracae* and snail *Otala lacteal*. The results suggested bioaccumulation of tungsten in the two trophic levels. Bioaccumulation is reported elsewhere in reports. For example, Kerley et al. (1996) estimated a concentration factor as the ratio of tungsten concentration in the edible plant

part to that in soil, of 0.3. Plant species have been reported to excessively bioaccumulate tungsten with possible negative effects on herbivorous species (Pyatt and Pyatt, 2004). Tungsten expected bioaccumulation factor in fish (edible parts) is 30 for freshwater and 12000 for marine (Karlsson et al., 2002). It is evident that poly-tungstates are significantly more toxic compared to mono-tungstate (Strigul et al., 2009a, Strigul et al., 2010).

8.3 Materials and methods

Actual toxicity to organisms is not measured directly by instruments, but by living organisms themselves. The dose-response concept is fundamental in toxicology. The response of living organisms and the dose received are measured in order to assess toxicity of substances and determine the predicted zero- effect concentration. This is an essential step towards evaluating safety levels, and is integrated within the ecological risk assessment. Acute toxicity tests are widely used in ecotoxicology to measure toxicity in the short-term; e.g., within 45h in the case of invertebrates. The results are usually expressed as LC50, the lethal concentration to 50% of the test population during the test.

8.3.1 Test species

Because of their sensitivity to a wide range of chemicals, water fleas have been widely used over the years as test organisms for aquatic toxicity screening (Tatarazako and Oda, 2007). *Daphnia magna* is widely used because of its short life cycle, small size, mode of reproduction and relatively easy and inexpensive way of being manipulated. It is a recommended test species by the Organisation for Economic Cooperation and Development (OECD, 1998, OECD, 2004) and has been characterised as the most important test-species in freshwater toxicology (Persoone and Janssen, 2009).

Figure 8.3 *Daphnia* image by Przemyslaw Gaj @2010-2016 Art-de-Viant.

Daphnia is a valuable organism for ecosystem studies as invertebrates make up more than 95% of the animal species and their role is crucial for ecosystem structure and function (Verslycke et al., 2007); it is a model representative of zooplankton and it provides a link between primary production (phytoplankton grazers) and secondary production (major part of fish and invertebrates diet). Essentially, it is a critical species in the aquatic food web (Dodson and Hanazato, 1995), and can cause ecosystem level responses (Flaherty and Dodson 2005). *Daphnia magna* Straus is a freshwater crustacean, shown in Figure 8.3. It is a small (<3mm) filter feeding water flea that can be found in most permanent water bodies (LeBlanc, 2007).

The reproduction cycle of *Daphnia* is illustrated in Figure 8.4. *Daphnia* reproduces asexually. The parthenogenetic mode of reproduction enables the provision of identical test organisms, a factor that is crucial for toxicity testing. At 20ºC, *Daphnia* deposits the first eggs in the brood chamber within 5 to 10 days. The eggs are hatched and the embryos are released after 3 days. *Daphnia magna* can produce more than 100 eggs, with mature females producing eggs every 3 to 4 days (Ebert, 2005). *Daphnia* can switch to sexual reproduction and produce males under environmental stress (Hebert 1978; Lynch and Gabriel 1983). Females can also produce dormant eggs (ephippia) that require fertilisation by males (Ebert, 2005).

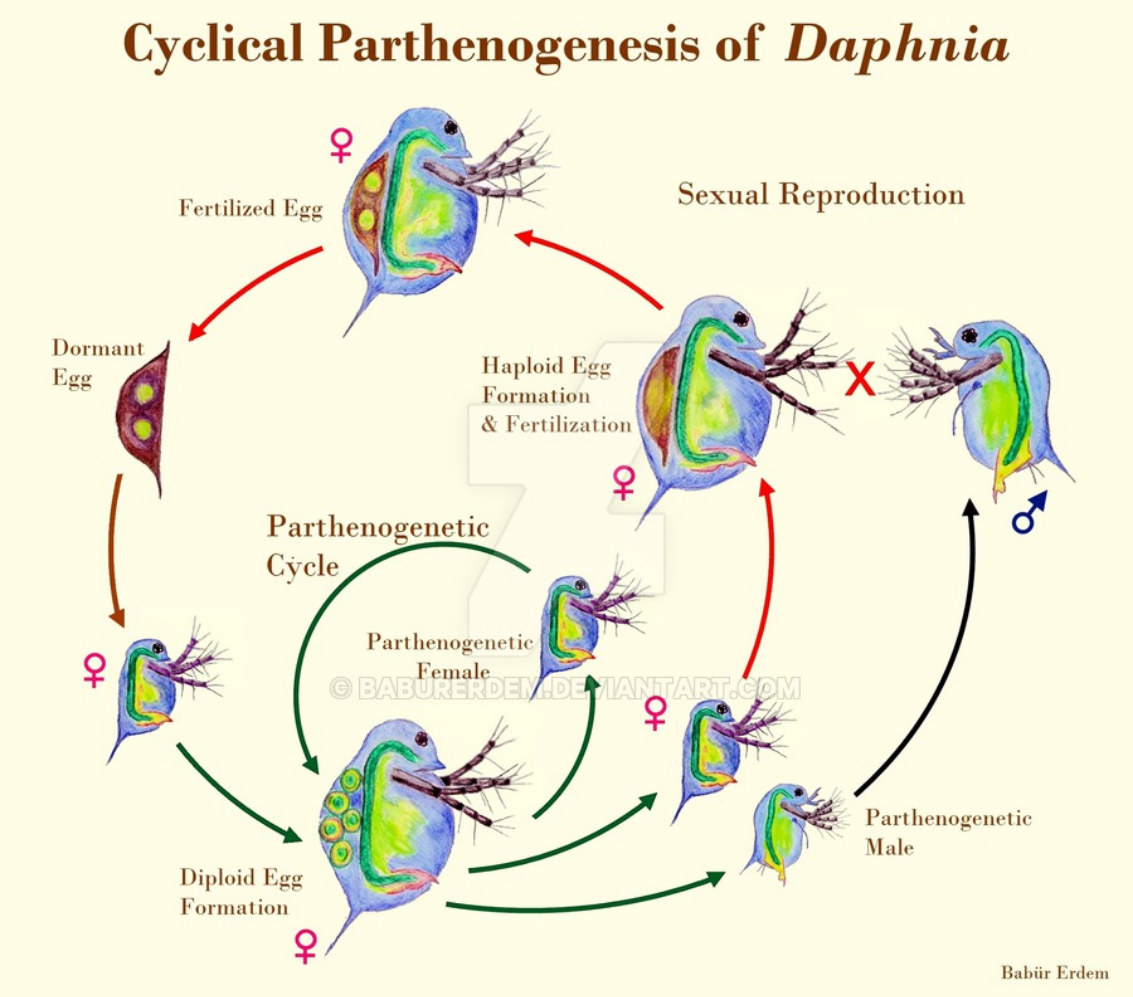


Figure 8.4 Reproduction cycle of *Daphnia* illustrated by Babür Erdem @baburerdem.

8.3.2 Culturing conditions

For the current research, *Daphnia magna* cultures were supplied by the Water Research Centre (WRC), Medmenham, UK, and originated from the National Institute for Applied Chemical Research (IRCHA), France. The cultures have been maintained at the University of Reading since March 1999, under the supervision of Dr A. Callaghan (School of Biological Sciences). The initial neonates originated from a single female. The cultures were maintained and toxicity tests performed following the OECD guidelines (OECD, 1998, OECD, 2004).

The cultures were maintained in 2L plastic beakers containing 1.2L ISO water (Figure 8.5). Each culture started with 15 young daphnids per beaker, aged under 24hours, and which were 3rd to 5th generation of the parental culture. These generations were chosen to ensure that healthy organisms with well-known life history were chosen. New cultures were set-up every 2-3 weeks. The vessels were covered with Perspex disk

to minimise evaporation and to reduce contamination. *Daphnia* were cultured in a 16:8 hr light: dark photoperiod and a temperature of 20 ± 1ºC.

Figure 8.5 Photograph of *Daphnia* cultures.

The temperature was checked and recorded regularly, using 5 temperature probes placed in water-filled beakers that were located in different areas of the lab. Detailed observation records of the cultures were also kept daily; these included diet, media change, diseased and dead individuals, and signs of distress. Diseased or dead individuals were removed daily. In order to ensure that the adults receive an even and adequate amount of food, and to avoid crowding, juveniles were removed daily before feeding.

8.3.2.1Media

ISO water was prepared according to the OECD guidelines (OECD, 1998, OECD, 2004). The substances listed in Table 8.2 were diluted in ultrapure water so that the DOM content was kept to a minimum. The solution was used for a maximum of one month. The media had a pH range of 7.5 to 8.2, conductivity between 360 to 480 µS/cm and water hardness between 130 to 160 mg/L.

Table 8.2 Substances used for media preparation.

Substance	Concentration
Calcium chloride $CaCl_2,2H_2O$	11.76g/L
Magnesium sulphate	4.93g/L
Sodium bicarbonate $NaHCO_3$	2.59g/L
Potassium chloride KCl	0.23g/L
Sodium selenite Na_2SeO_3	40µg/ml

Media was renewed weekly and supplemented with an organic seaweed extract, Marinure (Glenside Organics Ltd Throsk, UK) in concentrations of 0.2 ml/L (Baird et al., 1989). The media used for the exposure experiments received no marinure.

8.3.2.2 Daphnia nutrition

Chlorella vulgaris was cultured in Bolds Basal Medium (BBM), according to OECD guidelines (OECD, 1998, OECD, 2004). The culture was kept in 5L fermenter (see Figure 8.6), constantly aerated (filtered through 0.2μm), so that gas exchange was facilitated and algal cells were kept in suspension. The fermenter was placed under photosynthetic light. In order to avoid contamination, aseptic techniques were employed and the fermenter and tubing autoclaved.

Figure 8.6 Photo of the fermenter used.

After three weeks, the culture was centrifuged and part of it was stored on agar slopes as a reserve and to inoculate future fermenters. The culture was centrifuged and re-suspended in demineralised water. The concentration was checked using a spectrophotometer and the cultures were then stored in -80^{o}C. A small amount was kept at 4^{o}C for the daily feed. *Daphnia* were fed daily with 1 mg of carbon for the first 6 days, 1.5 mg until the 8th day and 2 mg thereafter, with suspensions of Chlorella *vulgaris*.

Daily feed was supplemented with 0.05 mg of Alison's dried baker's yeast (Westmill Foods Ltd, Maidenhead, UK).

8.3.3 Acute toxicity tests

Exposure experiments were performed using the OECD guidelines for testing chemicals (OECD, 1998, OECD, 2004). Third brood juveniles aged less than 24 hours, originating from a healthy stock, were exposed to a range of sodium polytungstate concentrations ($3Na_2WO_4.9WO_3$, Sigma Aldrich) for 48 hours. Range finding tests were conducted in order to determine the range of tungsten concentrations to use in the toxicity tests. For this purpose, five neonates were exposed to a wide range of concentrations. The concentrations chosen were in a geometric series, with a separation factor smaller or equal to 2.2. At least five different concentrations were tested per experiment, aiming to reach a maximum concentration that resulted in 100% immobilisation and a minimum that causes 0%. Immobilisation is defined as those animals unable to swim within 15 seconds, even if they are able to move their antennae after gentle agitation of the vessel. For each concentration, 5 groups of 5 daphnids (25 animals in total) were exposed. The daphnids were transferred to 50-ml glass vials containing 40ml of test solution and covered with a Perspex disk to minimise evaporation and reduce contamination. An example is shown in Figure 8.7. Another set of 25 daphnids was transferred to control vials containing media without tungsten to check the validity of the test (<10% mortality).

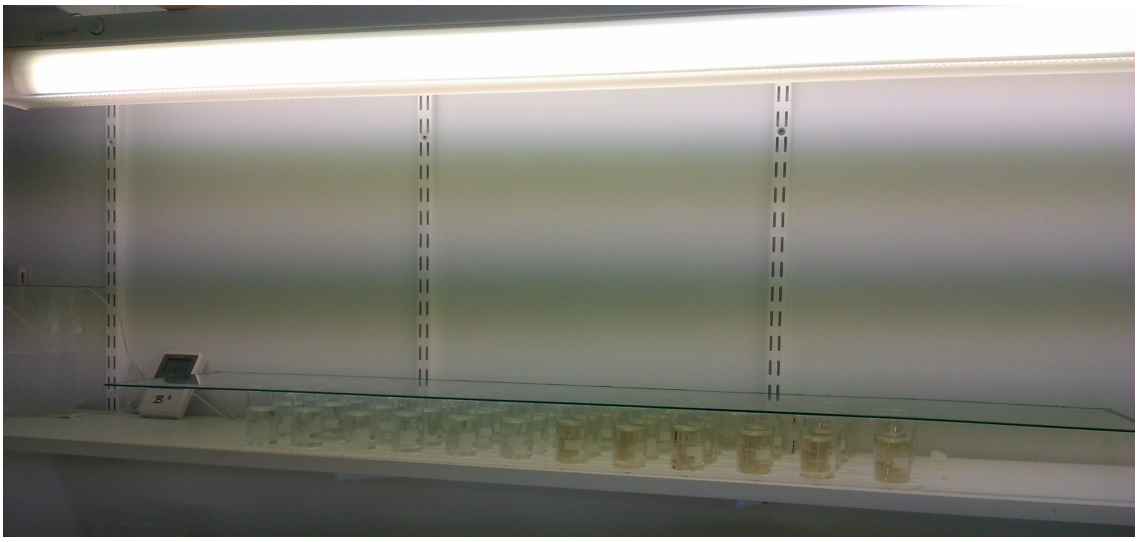


Figure 8.7 Photograph of laboratory set-up during one of the tungsten exposure experiments.

Immobilisation records were kept at 24 and 48 hours. Conductivity (conductivity meter HI3292, Hanna) and hardness (colourimetrically, using benchtop sceptrophotometer DR2800, Hach Lange) were measured in all media. Dissolved oxygen (oxygen meter HI9142, Hanna) and pH were recorded at the start and end of every

experiment in the control and highest concentration vials. All tests were completed with oxygen levels higher than 3 mg/L (validity criterion) and pH did not defer by more than 1.5 units from the initial value.

8.3.4 Estimation of LC50 value

Exposure concentrations were log-transformed. The percentage of *Daphnia* mortality at each exposure concentration was converted to probits using Table 8.3 (Finney, 2009). The percentage mortality rates of 0% and 100% (not in controls) were corrected and then transformed to probits. Zero mortality was corrected to 100(0.25/n) and 100% to 100(n-025/n), where n is the number of *Daphnia* exposed to the particular concentration (Ghosh, 1984).

Table 8.3 Transformation of percentages to probits.

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

The probit values were plotted against log-transformed concentrations and the 50% mortality (LC50) was identified as the exposure concentration corresponding to the probit value 5. The standard error(SE) of LC50 was calculated as SE= (LogLC84-LogLC16)/ √(2n), where LC84 and LC16 are the lethal concentrations of 84% and 16% mortality, respectively (Miller and Tainter, 1944).

8.3.5 Test substance

Sodium polytungstate powder was obtained from Sigma Aldrich, with declared WO_3 content ≥85% and density 3.1 g/cm^3 at 20 °C. Analysis (ICP) of substance samples (see appendix B) revealed WO_3 content of 89.9% and W content of 71.26%.

8.4 Results

Most of the toxicological studies on the effect of DOM have used commercial humic acids. However it has been proven that commercial humic acids differ significantly to natural ones and their usage is further limited as their source, method of isolation, or other pre-treatment is unknown (Malcolm and Maccarthy, 1986). Thus, it is advisable not to use them to replicate humic substances in natural waters.

The exposure experiments in this study were performed using natural water samples from the Millersford Brook (NF) and the Ebble River, filtered through 0.45μm cellulose filters. The same sampling locations as the ones used for Chapter 7 river samples downstream the wetlands points were used. Acclimation of *Daphnia* in natural waters preceded the tests, to make sure that any observed effects are due to exposure to Tungsten and not related to stress. The acclimation duration was at least one generation, i.e. about 3 weeks. The water chemistry of the natural waters differs as presented in Table 8.4.

In order to identify which of the parameters affected toxicity, exposure experiments were performed with Millersford Brook water adjusted for pH (NaOH) and for pH and hardness ($CaCl_2$) to match ISO media (referred to as NFpH and NFhard respectively). This was not possible for the Ebble river water samples as dilution of the samples would eliminate the effects of DOC that is the focus of this study.

Table 8.4 Water chemistry of the media used

	pH	hardness (mg/L $CaCO_3$)	DOC mg/L
ISO	7	164	0.36
Ebble	7.54	342	3.41
NF	3.58	20.7	9.16
NFpH	7	20.7	9.16
NFhard	7	164	9.16

All dose-response graphs are presented in Figure 8.8. Toxicity of sodium polytungstate increased in the order of NFhard<NFpH<NF<ISO<Ebbs. The LC50 and 95% confidence levels in mg/L were for Ebbs 22.76 and 16.91-28.59, ISO 78.83 and 59.95-97.65, NF 107.98 and 82.17-133.79, NF pH 273.84 and 213.17-334.51, NF hard 472.52 and 396.06-548.06.

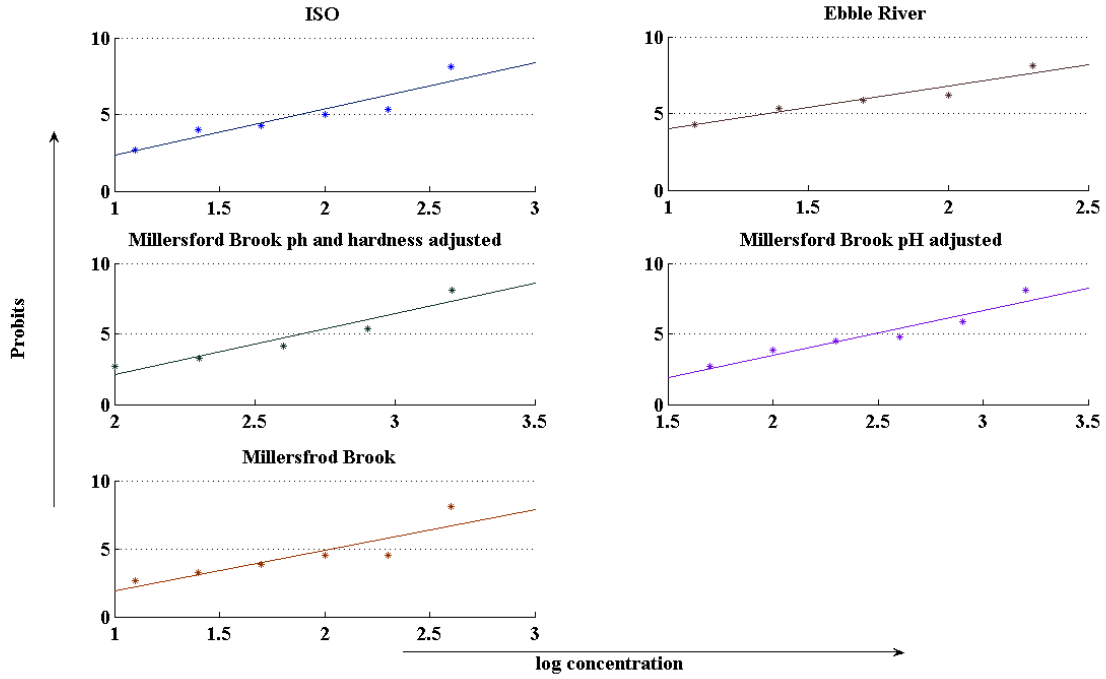


Figure 8.8 Scatterplots of log transformed exposure concentrations against probits for all the media used.

To the best of our knowledge, the only LC50 value published in the literature after exposure of *Daphnia* to poly-tungstate is the one by Strigul et al. (2009a). They reported a LC50 value of 106 mg/L and confidence levels of 78 to 144 mg/L. However, they used a commercially available kit (Daphtockit bioassay, MicroBioTests Inc.) and *Daphnia* eggs that implement the methodology could differ slightly from those used here. It should be noted that in their study, as well as the current, LC50 is higher because of the purity of the powder not being 100%. These LC50 values are environmentally relevant, as levels of 64-135 mg/L W was reported for tap water in Fallon, Nevada (Koutsospyros et al., 2006).

The reported LC50 values of mono-tungstates are by Khangarot and Ray (1989) 89.39 mg/L, by Strigul et al. (2009a) 344 mg/L, and by Clements et al. (2012) >95.5 mg/L. Hence our estimated LC50 value for ISO media, supports previous findings that poly-tungstates are more toxic than mono-tugstates.

The LC50 values were compared, as shown in Figure 8.9. The LC50 value increased almost 6 times with increased DOC in NFhard compare to ISO media. The results indicate that DOC can play a protective role in Tungsten toxicity. This is in agreement with the DOC effect on metal toxicity described in Chapter 1. Increased pH

and hardness also decreased toxicity, based on the comparison of NF with NFpH and NFpH with NFhard respectively. Further research is needed to explain increased toxicity of Tungsten in Ebble river water. This could be linked to increased hardness, above the recommended for *Daphnia* range (OECD, 2004). Although control media showed 0% mortality and *Daphnia* were acclimatised, combined effects could have created lethal effects.

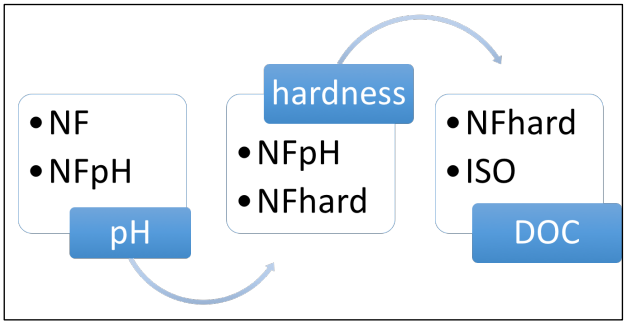


Figure 8.9 Comparison of LC50s and the responsible parameter indicated.

The effects of pH, hardness on metal toxicity have been widely studied in the literature (for example Sprague (1985), Yim et al.(2006), De Schamphelaere and Janssen (2004), Park *et al.* (2009)). Not all metals have been shown to be affected by those two parameters. Strigul (2010) described how pH affects Tungsten speciation in water, showing that in alkaline conditions, and possibly in pH7-10, tungsten is present only as monomeric tungsten oxyanion in the absence of other ions or complexation agents. In contrast, polyoxotungstates can form naturally in acidic conditions. Consequently, toxicity of Tungsten is expected to decrease in alkaline compared to acidic conditions. Metal toxicity is generally higher in soft waters compared to hard, as Ca^{+2} and Mg^{+2} ions compete with metals for aquatic organisms' uptake. No evidence was found in the literature of a hardness effect on tungsten toxicity.

The effect of an organic-rich wetland, such as Millersford, on the transport and toxicity of tungsten can be significant. Transport of tungsten from soils to surface waters has been studied in a limited number of studies. Soil water partition coefficient (K_d) is calculated as the ratio of the concentration of tungsten in the soil divided by the concentration of tungsten in soil water. Due to the lack of available data, Kerley et al. (1996) estimated K_d from soil to plant concentration factors. They concluded that tungsten was 15 times more mobile than uranium in terms of its release from from soil to

water. Clausen et al. (2010) reported a wider range of K_d values that were dependent on the length of contact time. They showed that metallic tungsten and tungsten oxides are rapidly dissolved, and that tungstate and polytungstate species movement to the vadose zone, aquifer and the surface waters depends on precipitation intensity. Solubility and mobility of tungsten in soils was also confirmed by Dermatas et al (2004). Clausen et al. (2011) reported that polytungstates are soluble in soils and water and particularly mobile resulting in shallow groundwater and surface water contamination.

Studies on the mobility and solubility of tungsten raise an issue on the environmental impact of tungsten exposure as concentrations of tungsten in the environment are increasing, especially close to mines and military grounds and tungsten is no longer considered as non-toxic. An organic rich wetland such as the one in Millersford can influence the environmental impact of tungsten exposure in surface waters in two ways. Directly affecting the transport and indirectly by exporting DOM that influence toxicity. Mobility of tungsten is significantly reduced in organic soils, suggesting complexes of tungsten with humic substances (Dermatas et al., 2004). Petruzzelli and Pedron (2017) showed that organic matter is the most important soil parameter to reduce tungstate mobility. Sorption of polytungstate to organic rich soils restrains migration (Clausen et al., 2011). These findings might suggest that organic-rich soils, such as Millersford wetland, can act as sinks for tungsten.

Furthermore, the DOM exported from the Millersford wetland, as shown by the acute toxicity tests presented in this study, can play a protective role in tungsten toxicity. This is an important finding because of the evidence on the toxicity of tungsten as described in 8.2.4. Transition metals have been shown to catalyse oxidative deterioration of biological macromolecules - leading to oxidative tissue damage (Stohs and Bagchi, 1995). Tungsten molecular mechanisms of toxicity include reactive oxygen species generation, increased oxidative stress, direct DNA damage, epigenome modulation, B lymphocyte differentiation alteration, T cell mediated immunity decrease (Bolt and Mann, 2016).

8.5 Conclusions

Organic rich wetlands, such as Millersford, are capable of acting as metal sinks, thereby reducing metal transport to adjacent streams. Furthermore, by acting as DOM sources to the adjacent streams, those wetlands can play a protective role in metal toxicity.

Indeed, DOM exported from Millersford resulted in decreased tungsten toxicity, suggesting possible humic-tungsten complexation.

The results presented in this chapter provide much needed information on tungsten toxicity. Studies on tungsten toxicity are essential, since exposure levels are rising, especially close to mines and military grounds; moreover, the production and uses of tungsten are fast expanding. This is true not only in the US but also in the UK. The current work has shown that DOM exported from the Millersford wetland, as well as increases inf pH and water hardness, decreased Tungsten toxicity. The LC50 value reported is environmentally relevant and challenges the previous "non-toxic" perception of tungsten.

Chapter 9- Conclusions

This thesis reports on field and laboratory studies that were aimed at understanding the DOM concentration dynamics and chemical composition in riparian wetlands and investigate implications for water quality in adjacent water course.

In Chapter 5, DOC concentrations were estimated and found to vary significantly within the two study wetlands (mean concentration range at 40cm depth was 5-35 mg/L in Millersford and 5-16 mg/L in Ebbesbourne). Unravelling this spatial variability of DOC involved investigation of the correlation of DOC with location in relation to the river bank. Both wetlands showed accumulation of DOC closer to the riverbank. The drivers of this accumulation are likely to be different. Land elevation in Millersford explained some of DOC and DON variability. However, not all variation could be explained, even after studying the soil profiles using GPR. The vertical profile of DOC in Millersford confirmed lack of uniform behaviour across the wetland. DOC spatial and vertical distribution findings indicate complex subsurface preferential flow patterns and effects of microtopography. It is difficult to pinpoint the main driver behind the spatial dynamics of DOC in the almost flat wetland in Ebbesbourne. Sources and transport of DOC in Ebbesbourne include flooding of the entire wetland, groundwater flow plus the riparian zone between the wetland and the stream where mowing products were disposed. Spatial variability even at the scale of 5.5m (distance between sampling zones in Ebbesbourne) has implications for the sampling strategy of wetlands.

DOM was found to display seasonal trends in both wetlands. DOC, DON and SUP concentrations were minimum in winter. Maximum concentrations were measured in summer, except SUP in Millersford that was maximum in Autumn. Temperature was identified as a driver of the differences in DOM levels in different seasons. The seasonal trends were more pronounced in Ebbesbourne compared to Millersford. For example, in Ebbesbourne DOC dropped from 15 mg/L in summer to 8 mg/L in winter. This finding could be linked to differences in the periods of monitoring of the two sites. Indeed, the period of monitoring in Millersford was characterised by a particular wet summer and a relatively dry winter that resulted in relatively small seasonal differences. Ebbesbourne monitoring started after that dry winter, when DOM concentrations were possibly higher because of the rewetting of the wetland. These findings may be important in addressing

the existing gap of knowledge regarding the DON and SUP temporal dynamics. Additionally, these findings indicate possible changes in the temporal behaviour of DOM as a result of climate change.

Studying two wetlands with contrasting characteristics was helpful in understanding the controls of wetland type and land use on DOM and inorganic nutrients. DOC concentrations in the points of maximum accumulation was double in Millersford compared to Ebbesbourne, although both wetlands had regions where the concentrations were similar. Wetland type was suggested as the main cause, with peat forming systems like Millersford generally having higher DOC levels. Land use of the two sub-catchments studied was reflected on the levels of inorganic nutrients in the wetlands. Indeed, concentrations of nitrates and SRP were much lower in Millersford, in levels below the detection limit. Anthropogenic impacts such as agriculture and septic tanks have resulted in higher concentrations of nitrogen and phosphorus in Ebbesbourne.

The effect of the land use was studied in further detail in Chapter 6. Fluorescence was employed to identify sources of DOM. Anthropogenic sources contributed to DOM significantly more in Ebbesbourne compared to Millersford, as indicated by the protein-like fluorescence. Fluorescence findings also indicated more biodegradable, less aromatic, more microbially derived DOM in Ebbesbourne. Supplementary information on the quality of the DOM pools were obtained using UV indicators and the DOC:DON ratio. The use of a range of UV indicators enabled comparison with multiple studies. The results showed that DOM in Millersford is less bioavailable to microorganisms, more aromatic, of higher molecular weight, containing complex organic compounds. Based on the findings of Chapter 6, the two wetlands have distinctive DOM characteristics that are associated with the ecological role of DOM in aquatic environments.

This study demonstrates that fluorescence can be used as a rapid and sensible tool to distinguish DOM origins in wetlands. Also, UV indicators provide valuable information on DOM quality, and proved to be more sensitive compared with FI to variations within the Millersford wetland, showing correlation with elevation. Therefore, UV studies can complement fluorescence. Unlike DOM concentrations, DOM quality was not linked with seasonality but only with the two wetlands.

Chapter 7 evaluated and compared the two wetlands and their adjacent streams with respect to DOM and inorganic nutrient dynamics. Concentrations of the parameters monitored in the rivers showed differences related to land use irrespective of the presence of the wetlands. Both wetlands demonstrated a capacity to act as DOC sources, as DOC increased from 8 to 11 mg/L in Millersford brook and from 3 to 3.5 mg/L in the Ebble river downstream the wetlands. Based on the findings of Chapter 6, DOC export in Ebbesbourne is composed of bioavailable compounds that could affect the ecology of the Ebble river. Other sources, such as livestock grazing around the wetland, could have very likely influenced the DOM levels in the Ebble, as well as the particulate P and N levels. SRP and nitrates were affected downstream the Ebble but not the Millersford wetland. As phosphorus was proved to be a limiting factor in the Ebble, the increase of SRP above the good ecological status threshold downstream of the wetland (0.09 mg/L) is an alarming finding. This finding questions the ability of the Ebbesbourne wetland to reduce or buffer phosphorus reaching the Ebble river. It is suggested that mitigation measures in the Ebble catchment should target phosphorus reduction to prevent eutrophication. Nitrate decreases in the presence of the Ebble wetland suggests a possible beneficial effect of the wetland. Loss of SRP and particulate P and N to the Ebble river was found to be driven by flow. Flow also affected nitrates by creating a dilution effect. Hydrological flow paths are therefore suggested as being key to diffuse inorganic nutrient loss in the Ebble river.

In Chapter 8, the effect of DOC on the toxicity of tungsten was investigated. Tungsten is one of the least regulated metals that has a wide range of applications leading to increased consumption. Evidence has grown over the last two decades on the toxicity and bioaccumulation of tungsten. The toxicity tests performed in this study show that DOC can reduce tungsten toxicity (LC50 473 mg/L compared to 79 mg/L in the control). This finding indicates that organic rich wetlands, such as the one in Millersford, can play a protective role in tungsten toxicity. It is evident that DOC and elevated pH and hardness reduce tungsten toxicity.

The results of this study show DOM varies, in time and space, quantity and character, within and among different wetlands. The study of the indicative dynamics between wetlands and surface waters is challenging. Findings of this study showed that wetlands should not be considered as passe-partout for mitigation measures targeting

diffuse pollution. Extrapolation from studied to non-studied wetlands should be treated with extreme caution.

9.1 Further research

Limitations of this study form the basis for future research. A means of improving our understanding of DOM spatial variability would be to study the subsurface water flowpaths in Millersford in more detail. This would enable us to learn more about the biogeochemical cycles, the DOM production hot spots of the wetland, and the processes and pathways by which DOM it is transferred. GPR was a useful tool in identifying the impermeable clay layer but more data on the stratigraphy would give a fuller picture on how this is linked with the biogeochemical processes. Future studies should incorporate soil profile data in interpreting DOM levels in wetlands.

While the usual approach is to sample the top soil to 30 cm depth, the necessity for more studies on deeper samples exists. Although this study presented data from 40 and 60cm depths, the vertical profile of DOM would be more complete with data on 20 and 80 cm sampling depths in Millersford. Future studies could overcome the practical difficulties faced here and identify the depth of maximum DOM accumulation.

The scale of resolution needs to be improved, both spatially and temporally. It is important to study the effect of the wetlands at the catchment level. This would involve more sampling points upstream and further downstream of the wetland, as well as soil samples outside the wetland. As wetlands interact with the terrestrial and aquatic bodies surrounding them, the study of wetlands cannot be performed in isolation. This is especially the case for the wetlands studied here. Critically, the study period was characterised by unusually dry weather that caused the Ebbesbourne wetland to dry up. Essentially, having data for a longer period of time would lead to more concrete assumptions.

Evaluating wetlands as mitigation features for targeting the reduction or buffering of inorganic nutrients in surface waters is complex. Future research should collect more preliminary data before choosing a key site. The choice of Ebbesbourne as the study site for the DTC programme, where all the monitoring equipment was installed, proved to be a significant obstacle for the current research. Not only did the site remain dry for part of this study, but changes in the land management introduced new influences on the

parameters studied. Hence, stronger engagement with the landowners and historical data can and should assist in site selection.

Lastly, future ecotoxicology studies should explore the toxicity of metal mixtures, rather than metals is isolation; this would reflect more closely the environment in which aquatic organisms are exposed. The current research also highlights the need to study further the solubility and mobility of tungsten, as well as other contaminants, in wetland soils, with a specific focus on the influence of natural DOM on ecological toxicity.

Appendix A MATLAB code for PARAFAC analysis

```
%read raw eems
[X,Emmat,Exmat,filelist_eem,outdata]=readineems(2,'csv','A3..AR153',[0
1],1,2);
Ex=Exmat(1,:);
Em=Emmat(:,1);

%read blanks
[X_b,Emmat_b,Exmat_b,filelist_b,outdata_b]=readineems(2,'csv','A3..AR1
53',[0 1],1,2);
Ex_b=Exmat_b(1,:);
Em_b=Emmat_b(:,1);

%import Raman files
[S_R,W_R,wave_R,filelist_R]=readinscans('R350','csv','A3..B53',1,2);

%read absorbance scans
[S_abs,W_abs,wave_abs,filelist_abs]=readinscans('Abs','csv','A1..B601'
,0,2);

%import correction files
Excor=csvread('excor.csv');
Emcor=csvread('emcor.csv');
save corr

%create samplelog from fileleists of eems and blanks
% import samplelog
SampleLog=readlogfile('samplelog.csv',[0 1 1 1 1 1 0]);

%align
sample=alignds(SampleLog,{'EEMfile',filelist_eem},{'index'});
dilfac=alignds(SampleLog,{'EEMfile',filelist_eem},{'dilutionfactor'});
[Sabs,newabslist]=
alignds(SampleLog,{'EEMfile',filelist_eem},{'Absfile',filelist_abs,S_a
bs});
Sr=
alignds(SampleLog,{'EEMfile',filelist_eem},{'Ramanfile',filelist_R,S_R
});
B=
alignds(SampleLog,{'EEMfile',filelist_eem},{'Blankfile',filelist_b,X_b
});
A=[wave_abs;Sabs];
W=[Emb';squeeze(B(:,:,Exb==350))];

%correction. Inner filter and blank substraction
[XcRU Arp IFCmat BcRU XcQS
QS_RU]=fdomcorrect(X,Ex,Em,Emcor,Excor,W,RamOpt,A,B,[],[],[]);

%corect for diluted samples
XcRU_df=undilute(XcRU,dilfac);

%Fluorescence index calculation
FI=XcRU_df(:,86,14)./XcRU_df(:,111,14);

%assemble relevant variables
mydata=assembledataset(XcRU_df,Ex,Em,'RU','Index',sample,filelist_eem)
;
```

```
%preproccessing
%remove noisy parts
SubData=subdataset(mydata,[],mydata.Em>600,mydata.Ex<250);
%remove scatter regions
Xs=smootheem(SubData,[18 15],[15 15],[17 18],[19 18],3);
%normalise dataset
Xpre=normeem(Xs)
%outlier test
Test1p=outliertest(Xpre,[1,1],4:7,'nonnegativity',[],'at once');
loadingsandleverages(Test1p,5)
loadingsandleverages(Test1p,6)
loadingsandleverages(Test1p,7)
Test2=outliertest(Xpre,[2,2],5:7,'nonnegativity',[],'at once');
%to decide number of components
specsse(Test2,2:7)
%model
[LSmodel7,convg7,DSit7]=randinitanal(Xpre,7,10,'nonnegativity');
%visualise
fingerprint(LSmodel7,7)
```

Appendix B Sodium polytungstate powder analysis

Weight(mg) dillutedx2	W concentration measured in solution (mg/L)		W concentration in solid	
		re-run	mg/kg	%
9.7	38.58	38.01	783670	78.37
9.7	38.86	36.37	749913	74.99
7.5	26.19	26.41	704295	70.43
7.5	26.50	26.89	717107	71.71
5.1	18.47	17.83	699238	69.92
5.1	19.05	19.01	745303	74.53
7.3	24.14	24.14	661414	66.14
7.3	24.65	24.16	662015	66.20
6.5	27.61	26.97	829798	82.98
6.5	27.95	26.82	825262	82.53
9.1	32.52	31.15	684689	68.47
9.1	32.66	31.13	684070	68.41
8.3	30.17	28.40	684287	68.43
8.3	29.48	28.24	680489	68.05
7.8	24.94	24.86	637461	63.75
7.8	25.55	25.46	652764	65.28

Mean %W:71.26

Mean %WO_3:89.86

Appendix C Example of statistical analysis

DOC in 40cm at Millersford

ANOVA test results

Source	SS	df	MS	F	Prob>F
Groups	7540.98	9	837.887	64.83	5.26641e-35
Error	1137.34	88	12.924		
Total	8678.33	97			

Homogeneity of variance Barlett's test results

Group	Count	Mean	Std Dev
1	12	27.4917	2.91752
2	7	21.3571	1.92154
3	10	20.209	5.09848
4	11	15.0136	2.74504
5	8	26.105	4.51381
6	10	17.63	4.14795
7	9	13.3567	2.1701
8	9	35.0911	4.28383
9	12	4.8055	2.8028
10	10	8.9367	3.99841
Pooled	98	18.5188	3.59504
Bartlett's statistic	13.7003		
Degrees of freedom	9		
p-value	0.1334		

Normality Shapiro-Wilk test results

Sampler	H	pValue	W
A1	0	0.249	0.921
A2	0	0.795	0.957
B1	0	0.210	0.898
B2	0	0.080	0.843
B3	0	0.095	0.860
B4	0	0.185	0.905
C1	0	0.515	0.940
C2	0	0.065	0.854
C3	0	0.209	0.892
C4	0	0.142	0.883

Tukey's multi-comparison test results

group compared		upper limit	upper limit
A1	A2	0.584	11.685
A1	B1	2.286	12.280
A1	B2	-3.940	6.714
A1	B3	8.989	19.281
A1	B4	17.922	27.451
A1	C1	7.606	17.350
A1	C2	4.865	14.859
A1	C3	-12.746	-2.453
A1	C4	13.558	23.552
A2	B1	-4.603	6.899
A2	B2	-10.788	1.292
A2	B3	2.119	13.882
A2	B4	11.001	22.102
A2	C1	0.701	11.986
A2	C2	-2.024	9.478
A2	C3	-19.615	-7.853
A2	C4	6.669	18.172
B1	B2	-11.432	-0.360
B1	B3	1.490	12.215
B1	B4	10.406	20.401
B1	C1	0.096	10.295
B1	C2	-2.640	7.798
B1	C3	-20.244	-9.520
B1	C4	6.053	16.492
B2	B3	7.077	18.419
B2	B4	15.973	26.626
B2	C1	5.669	16.514
B2	C2	2.939	14.011
B2	C3	-14.657	-3.315
B2	C4	11.632	22.704
B3	B4	3.405	13.697
B3	C1	-6.902	3.589
B3	C2	-9.636	1.089
B3	C3	-27.236	-16.233
B3	C4	-0.942	9.782
B4	C1	-15.080	-5.337
B4	C2	-17.822	-7.827
B4	C3	-35.432	-25.139
B4	C4	-9.128	0.866
C1	C2	-7.716	2.483
C1	C3	-25.323	-14.832
C1	C4	0.978	11.176
C2	C3	-22.823	-12.099
C2	C4	3.474	13.913
C3	C4	20.792	31.517

Appendix D Metal analysis

Results from 2 ICP-MS runs. Units ug/L.

1st run	Al 396.153	Ba 455.403	Ca 317 radial	Cd 228.802	Co 230.786	Cr 205.560	Cu 324.752	Fe 259 radial
Ebble	4.02	12.01	98525.41	0.02	0.04	-0.04	-0.29	1.40
Millersford	183.30	6.37	1139.21	0.05	0.57	0.21	25.33	343.60
Standard deviation	0.33	0.01	1.35	0.02	0.01	0.01	0.12	0.17
Detection lmit	0.99	0.04	4.04	0.06	0.03	0.04	0.36	0.51
Limit of quantification	3.30	0.12	13.47	0.20	0.11	0.15	1.21	1.70
2nd run	Al 396.153	Ba 455.403	Ca 317 radial	Cd 228.802	Co 230.786	Cr 205.560	Cu 324.752	Fe 259 radial
A1 40cm	363.53	14.65	1399.19	0.17	2.43	2.36	0.45	7328.22
A2 40cm	208.40	20.55	1143.97	0.10	1.83	0.70	1.94	4313.64
B1 40cm	167.15	24.45	1553.74	0.08	2.36	0.90	1.30	3884.16
C1 40cm	124.46	27.66	1714.55	0.06	1.97	0.89	0.07	3213.53
B2 60cm	497.82	10.53	3405.76	0.03	2.25	5.93	2.55	23772.99
C2 40cm	386.64	18.78	802.45	0.03	2.41	2.36	0.11	8106.29
B3 40cm	206.05	15.75	1915.66	0.03	1.17	4.57	0.17	8027.22
B3 60cm	159.89	13.49	2038.97	0.05	1.76	2.13	4.22	10119.53
B4 60cm	36.50	17.79	955.77	0.06	1.00	1.31	0.79	864.32
Standard deviation	0.03	0.01	0.44	0.03	0.01	0.01	0.02	0.05
Detection limit	0.08	0.04	1.32	0.10	0.03	0.04	0.06	0.15
Limit of quantification	0.28	0.14	4.39	0.34	0.11	0.15	0.19	0.49

1st run	K 766 radial	Li 670 radial	Mg 279 radial	Mn 257.610	Na 589 radial	Ni 231.604	Pb 220.353	Sr 407 radial	Zn 206 radial
Ebbs	2842.20	3.21	1738.25	1.30	25489.44	0.48	-0.55	301.21	1.85
NF	3488.04	0.53	520.42	20.26	25713.37	0.60	0.75	6.65	85.70
Standard deviation	3.92	0.07	0.06	0.01	331.27	0.05	0.07	0.01	0.23
Detection limit	11.76	0.22	0.17	0.04	993.80	0.14	0.20	0.04	0.69
Limit of quantification	39.20	0.73	0.56	0.14	3312.66	0.47	0.67	0.12	2.28
2nd run	K 766 radial	Li 670 radial	Mg 279 radial	Mn 257.610	Na 589 radial	Ni 231.604	Pb 220.353	Sr 407 radial	Zn 206 radial
A1 40cm	702.48	0.80	3335.39	17.85	15930.84	2.42	1.27	16.55	6.05
A2 40cm	674.60	0.59	2391.41	7.52	13684.47	1.34	1.57	13.85	10.88
B1 40cm	607.88	0.78	2763.79	12.78	15625.22	1.45	1.18	18.26	2.98
C1 40cm	655.94	1.25	2911.96	14.28	13617.82	1.28	0.95	21.23	1.24
B2 60cm	412.37	0.06	3464.09	60.91	13330.31	5.40	1.90	23.12	2.22
C2 40cm	296.70	0.41	2235.94	8.34	14579.05	5.72	0.98	10.91	15.31
B3 40cm	287.31	0.09	2716.91	8.96	13349.70	2.18	1.41	18.51	2.18
B3 60cm	141.77	0.15	2108.93	10.27	13120.07	3.21	8.18	15.84	24.89
B4 60cm	58.15	1.08	2232.08	7.98	11312.22	1.21	0.53	13.02	6.83
Standard deviation	2.08	0.03	0.07	0.02	3.16	0.02	0.12	0.01	0.29
Detection limit	6.25	0.08	0.20	0.05	9.47	0.05	0.37	0.04	0.87
Limit of quantification	20.82	0.27	0.67	0.15	31.57	0.18	1.23	0.15	2.91

Appendix E Correlation matrix of correlation coefficients between flow and fractions of N and P.

	flow	TON	NH4	DON	TDN	PON	TN	SRP	SUP	TDP	PP	TP
TON	-0.512*											
NH4	-0.044ns	0.004 ns										
DON	0.050 ns	-0.403*	0.172**									
TDN	-0.505*	0.777*	0.224*	0.254*								
PON	0.425*	-0.497*	-0.085 ns	0.038 ns	-0.502*							
TN	0.295*	-0.276*	-0.014 ns	0.139 ns	-0.198*	0.947*						
SRP	0.605*	-0.743*	0.168**	0.120 ns	-0.677*	0.549*	0.370*					
SUP	-0.317*	0.105 ns	-0.053 ns	0.272*	0.282*	-0.205*	-0.125 ns	-0.362*				
TDP	0.519*	-0.754*	0.157 ns	0.241*	-0.610*	0.505*	0.347*	0.924*	0.023 ns			
PP	0.449*	-0.542*	-0.088 ns	0.065 ns	-0.532*	0.979*	0.913*	0.603*	-0.230*	0.552*		
TP	0.467*	-0.572*	-0.074 ns	0.079 ns	-0.552*	0.975*	0.901*	0.642*	-0.218*	0.599*	0.998*	
NPOC	0.372*	-0.524*	0.035 ns	0.244*	-0.381*	0.278*	0.173**	0.439*	-0.201*	0.388*	0.323*	0.336*

**correlation is significant at the 0.01 level; *correlation is significant at the 0.05 level; ns correlation is not significant

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