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Riparian areas

Delineation and contribution to ecosystem service provision

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´Riparian areas: Delineation and contribution to ecosystem service provision

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Thesis Summary

Riparian areas, the transitional areas between terrestrial and aquatic ecosystems, are considered cornerstones within the landscape due to their potential contribution to ecosystem service provision. However, they have been subject to a multitude of disturbances, mainly as a consequence of anthropogenic activities such as land use changes, pollution or intense grazing. Restoring and enhancing the wellbeing of these systems have become a priority for progressing towards a sustainable use of our resources and maximizing the delivery of ecosystem services. The overall thesis aims are to critically evaluate different methodologies to delineate riparian areas, especially considering their subsequent use for management purposes and ascertain their relative contribution to the provision of ecosystem services at the local and landscape scale. First, I started by critically evaluating the relative accuracy of different riparian delineation approaches and the impact of data quality and data types on predictions of riparian typologies. I concluded that different delineation methods greatly influenced the prediction of riparian typologies and the potential ecosystem service provided. Therefore, aspects such as economic viability of the buffer or their inclusion within priority habitats should be considered. In subsequent studies, I explored the contribution of riparian areas to deliver a broad range of ecological processes related to water quality enhancement and how riparian vegetation across different habitat types contributed to the provision of shade. My findings revealed that habitat type was the main driver explaining riparian soil physicochemical variability and that riparian function can be largely predicted from neighbouring land use/soil type. Additionally, I identified through a GIS approach that watercourse shading was maximal in afforested areas. Subsequent studies focused on specific regulating services (e.g. C sequestration and N cycling) in riparian areas in semi-natural ecosystems at a finer scale. Firstly, we critically evaluated the influence of five factors (i.e. nutrient stoichiometry, substrate quality and quantity, variations in microbial community due to proximity to the river and soil depth) on C mineralization rates. Differences in the immediate and long-term response of C mineralization suggested different microbial C use efficiency strategies through the soil profile. However, the influence of riparian area vs. non-riparian was minimal. The next experiment focused on denitrification in riparian wetlands with the aim to elucidate the effect of environmental factors, vegetation and microbial communities and N cycling gene abundance, regulating denitrification activity. This identified major changes in soil physicochemical properties, microbial community abundance and structure across the riparian transect, most likely influenced by the hydrology of the site. Additionally, I identified areas close to the river as a potential source of N_2O emission whereas distal areas could become a sink. The last chapter aimed to assess the legal framework affecting riparian areas and identify knowledge gaps in current research. Results showed that the legislation concerning riparian areas was highly fragmented and often contained untargeted measures. In contrast, research tended to focus on specific ecosystem functions (e.g. N removal) in agricultural systems. Our study illustrated that past and current research lacks a multi-ecosystem service based approach that legislative policies promote. This mismatch is due to the complexity of undertaking holistic research and the lack of resources and economic support. This research provides a more detailed understanding of riparian ecosystem functioning. The thesis provides essential information that allows location of where, when and how we might expect the provision of pivotal ecosystem services in riparian areas, especially if the ultimate management goal is their protection, reinstatement of their pristine state or enhancing their resilience in a continuously changing climate.

Thesis Contents

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Abbreviations

°C - Centigrade(s)

µL - Microlitre

AIT - Acetylene inhibition technique

Al - Aluminium

AMN - Anaerobically mineralizable nitrogen

***amo*A -** Ammonia monooxygenase gene

AMOC - Anaerobically mineralizable organic carbon (AMOC).

ANOVA - Analysis of variance

AOA - Ammonia oxidising archaea

AOB - Ammonia oxidising bacteria

ARIES - Artificial Intelligence for Ecosystem Services

ATP - Adenosine triphosphate

BSP - Basic payment scheme

C - Carbon

C_2H_2 - Acetylene

CH_4 - Methane

cm - Centimetre (s)

Cmin - Carbon mineralization

CO_2 - Carbon dioxide

d - Day(s)

DEFRA - Department of Environment Food and Rural Affairs

DEM - Digital elevation model

DIC - Dissolve inorganic carbon

DNA - Deoxyribonucleic acid

DOC - Dissolved organic carbon

DON - Dissolve organic nitrogen

DTM - Digital terrain model

DW - Dry weight

DWAF - Department of Water Affairs and Forestry

EC - Electrical conductivity

EU - European Union

FAO - Food and Agriculture Organization

Fe - Iron

g - Gravitational acceleration

GAEC - Good Agricultural and Environmental Conditions

GC - Gas chromatograph

GHG - Greenhouse gas

GIS - Geographic infomation system

h - Hour(s)

h^{-1} - Per hour

H_2O - Water

ha - Hectare(s)

InVEST - Integrated Valuation of Ecosystem Services

IPCC - Intergovernmental Panel on Climate Change

K - Potassium

K_2SO_4 - Potassium sulfate

kBq - Kilobecquerel

KCl - Potassium chloride

Kg - Kilogram(s)

KNO_3 - Potassium nitrate

K_{ow} - Partition coefficient

L - Litre

LCM - Land cover map

m - Metre(s)

MC - Moisture content

MEA - Millenium Ecosystem Assessment

mg - Milligram(s)

min - Minute(s)

ml - Millilitre

mM -Millimolar

Mn - Manganese

MW - Molecular weight

N_2 - Nitrogen molecule

N_2O - Nitrous oxide

Na - Sodium

NaOH -Sodium hydroxide

NH_4^+ - Ammonium

NIF - Nitrogen fixation

NIR - Nitrite reductase

NO - Nitric oxide

NO_2^- - Nitrite

NO_2 - Nitrogen dioxide

NO_3^- - Nitrate

NOR - Nitric oxide reductase

NOS - Nitrous oxide reductase

NVZ -Nitrate Vulnerable Zones

O_2 - Oxygen

ORP - Oxidation reduction potential

P - Phosphorus

PCA -Principal component analysis

PLFA - Phospholipid fatty acid

PO_4^{3-} - Phosphate

qPCR - Quantitative polymerase chain reaction

SEM - Standard error of the mean

SEPA - Scottish Environment Protection Agency

SMR - Statutory Management Requirements

SOM - Soil organic matter

TC -Total carbon

TDN - Total dissolved nitrogen

TN - Total nitrogen

TOC - Total organic carbon

TXRF - Total reflection x-ray

UK - United Kingdom

USEPA - U.S. Environmental Protection Agency

w/v - Weight to volume

WFD - Water Framework Directive

WFPS - Water-filled pore space

WRB - World Reference Base

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Chapter 1

Introduction

Riparian areas: Challenges and need for research

1.1 Riparian areas: Challenges and need for research

Despite the small area they occupy, riparian zones, the interface between aquatic and terrestrial ecosystems, have long been recognized for their value in supporting and regulating key environmental processes such as nutrient cycling and retention, flood control and stream shading (Naiman et al., 2005; Zaimes et al., 2007; McVittie et al., 2015). In this sense, maximizing their functioning is of importance to mitigate the environmental impacts generated as a consequence of agricultural intensification and industrial pollution, land use changes and modification of watercourses, and rapid population growth (UK NEA, 2011; Darch et al., 2015; Broetto et al., 2017). However, the lack of a universal definition of 'riparian' and strict physical boundaries (i.e. distance away from the watercourse), their inherent complexity and heterogeneity at the landscape scale and their sensitivity to disturbances, have made their regulation, management, preservation and delineation challenging (Naiman and Decamps, 1997; Ilhardt et al., 2000; Naiman et al., 2010).

The identification of relatively homogeneous spatial patterns within riparian areas is an essential task to develop robust classification and delineation systems. Historically, there have been a variety of methods to delineate riparian areas. These range from simplistic models in which a fixed width buffer is implemented (Hawes and Smith, 2005; Stoffyn-Egli and Duinker, 2013), to more complex holistic approaches where inherent riparian characteristics such as vegetation, hydrology or soil type are integrated (McVittie et al., 2015; Alaibakhsh et al., 2017; Zhang et al., 2017). However, different delineation systems tend to over-emphasize specific riparian characteristics, depending on the research discipline (e.g. hydrologists tend to favour hydrogeomorphic delineation models), and the ecosystem services they are trying to preserve (e.g. narrow fixed width buffers for sediment trapping). An evaluation of the different riparian delineation approaches is therefore necessary to successfully provide the basis for protecting, preserving and improving the environmental condition of riparian areas (Holmes and Goebel, 2011; Klemas, 2014; Belletti et al., 2017; Tompalski et al., 2017).

Despite their natural heterogeneity, riparian areas typically possess similar ecological functions such as water quality enhancement, storing and recycling of organic matter and nutrients, or climate regulation by provision of shade (Jorgensen et al., 2000; Clerici et al., 2011; Aguiar et al., 2015). Because of their key role in ecosystem functioning, the riparian zone has become an important component of the growing study of ecosystem services (MEA, 2005; Naiman et al., 2010; Pert et al., 2010). Moreover, due to their strategic position between terrestrial land and aquatic ecosystems, there is a general assumption about their ability to deliver riparian ecosystem services (Naiman et al., 2010; Zaharescu et al., 2017), however, empirical evidence has revealed contradictory results. For example, Osborne and Kovacic (1993) and Stutter et al. (2009) stated that vegetative buffer strips could lead to the increased release of nutrients such as phosphorus (P) to waters. This contrasts with the widely held belief that riparian areas help prevent nutrients flowing into freshwaters. Martin et al. (1999) reported no clear spatial patterns in denitrification rates within riparian areas, and Sullivan et al. (2007) indicated that the size of the vegetated buffer was irrelevant in the efficiency of bacterial removal. Further, Blackwell et al. (1999) and references therein, explained that riparian effectiveness in improving water quality might be compromised by the size, location, hydrology, vegetation, soil type and the nature of threat. Therefore, alternative locations for riparian buffers should be considered. This is also endorsed by studies like Burt et al. (1999) which relate riparian effectiveness in nitrate removal to the hydrology of the site, or Polyakov et al. (2005) who stated that riparian buffers often fail to accomplish their protective functions as a consequence of the low adaptability of their designs to local conditions. Their results stress the importance of furthering our understanding in the underlying mechanistic and biological processes that compromise the capacity of riparian areas to deliver ecosystem services.

Policy-makers need a robust evidence base to produce specific measures that assist in protecting and capturing the ecosystem service delivery that riparian zones might offer. However, there is a general perception that policies usually lack sound foundation of research and that a closer link is needed between scientists, policy-makers and how they deliver the information in practice (Sutherland et al., 2004, 2006; House of Lords, 2012). Therefore, identifying the gaps and conflicting areas between science and policy has become an important task in facilitating the academic understanding of ecosystem functioning and the decision-making authorities.

Uncertainties and knowledge gaps presented above have motivated the need for further research undertaken in this thesis. Using a combination of experimental work, GIS-based analysis and legislation and research review, we will aim to elucidate the incertitude around riparian areas to guarantee the best level of protection and functionality.

1.2 Thesis aims and objectives

This section details the aims and objectives of this thesis, followed by a brief description of the relevant chapters and experimental work referring to each objective. This thesis is divided into eight chapters as a series of four experimental papers and one review paper. A list of the experimental chapter titles is presented in section 1.3. Individual hypotheses and objectives are described in the each of the prepared manuscripts.

1.2.1 Thesis aims

This PhD thesis broadly focuses on riparian areas dynamics across contrasting habitat types in an ecosystem services context, with special consideration given to their delineation, provision of regulating services such as water quality enhancement or carbon storage and regulation being used to achieve policy objectives.

1.2.2 Objective 1

Evaluate the implications of different approaches of varying complexity to delineate riparian areas and the impacts of data quality and type on the prediction of riparian typologies and ecosystem service delivery.

Numerous approaches of varying complexity to delineate riparian areas have been undertaken and their repercussion for riparian management could be essential for riparian management and protection (Abood and Maclean, 2011; Stoffyn-Egli and Duinker, 2013). In Chapter 3, we critically assessed to what extent fixed-width riparian buffers provide a different outcome than functionally-targeted variable-width riparian buffers and their protection and management inferences. The quality of nationally-available digital information is critically evaluated, and implications are considered for the prediction of land cover distribution within the riparian area and future management activities to target riparian ecosystem services. The fixed-width riparian buffer produced in Chapter 3 was used as the buffer width reference in Chapter 4 to test and ground-truth the delivery of riparian ecosystem services.

1.2.3 Objective 2

Determine how soil properties and microbial community across different habitat types are affected by distance from the river and soil depth.

The spatial variability of soil physicochemical properties within the riparian zone was investigated at a broad scale across contrasting habitat types in Chapter 4 and ecosystem service-land cover targeted across a riparian transect in Chapters 5 and 6. Additionally, the impact of soil depth on soil physicochemical properties and ecosystem processing is also explored in Chapters 4 and 5.

1.2.4 Objective 3

Quantify the contribution of riparian areas to the provision of ecosystem services and determining their controlling factors.

In Chapter 4, we assessed the contribution of riparian areas to the delivery of a wide range of ecological processes related to the ecosystem service of water quality enhancement. Additionally, we evaluated how riparian vegetation across different habitat types contributed to the provision of shade.

In Chapter 5, the influence of riparian areas in carbon (C) dynamics was assessed. In particular, we examined the effects of nutrient stoichiometry, substrate quality and quantity and possible variations in microbial diversity and structure due to the proximity of the river and soil depth on C mineralization.

In Chapter 6, we investigated in more detail the role of riparian areas in denitrification as a permanent pathway of nitrogen (N) removal. Specifically, we explored environmental factors, vegetation communities and N cycling gene abundance controlling denitrification activity in riparian areas and how they contribute to explaining the spatial and temporal variability of N cycling in semi-natural ecosystems.

1.2.5 Objective 4

Investigate the legal framework relative to riparian areas and identifying knowledge gaps in current research.

In Chapter 7, we aimed to highlight the riparian regulatory framework within the UK and link it with trends of current research. The aim was to offer new insights into understanding the failure to stop riparian degradation and providing new goal-based strategies to maximize their function.

1.3 Experimental chapter information

The experimental chapters of the current thesis have been prepared in the style of journal article manuscripts. The title page of each experimental chapter includes details of the authors, author contributions to the manuscript and the current progress of each manuscript (e.g. published / accepted / submitted / not yet submitted). The thesis consists of four experimental chapters and one review chapter, located in Chapters 3-7 of the current document. For continuity and clarity, the experimental chapters will be referred to as they appear in this thesis. The titles of the experimental chapters are as follows:

Chapter 3: Delineating and mapping riparian areas for ecosystem service assessment

Chapter 4: Quantifying the contribution of riparian soils to the provision of ecosystem services

Chapter 5: Stoichiometric constraints on microbial community behaviour with soil depth along a riparian hillslope

Chapter 6: Spatial zoning of microbial function and plant-soil nitrogen dynamics across a riparian area

Chapter 7: Riparian research and legislation are they working towards the same common goals? A UK case study

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Chapter 2

Literature Review

2.1 Riparian definition and typology

Riparian zones were first scientifically defined in the early 1970s (Zaimes et al., 2007). Since then, they have been receiving increasing attention as we understand more about the wide range of key ecological processes to which they contribute (Verry et al., 2004). Riparian definitions have largely developed to address specific environmental problems and changing pressures (industrial, agriculture, etc). Definitions have ranged from over-simplified descriptions such as 'river bank' based on its Latin origins, through to more technical and detailed descriptions depending on the perspective of the author and user (e.g. hydrology, biodiversity, ecology, etc.) (Fischer et al., 2001). The use of discipline-specific terminology for riparian areas, the lack of strict physical boundaries (i.e. distance away from the watercourse), and their natural heterogeneity at the landscape scale have led to the development of a confusing and disparate mix of definitions (Naiman and Decamps, 1997; Ilhardt et al., 2000). For the purpose of this review, we will consider riparian zones as "the transitional areas occurring along land and freshwater ecosystems, characterized by distinctive soil, hydrology and biotic conditions strongly influenced by the stream water" (Naiman et al., 2005).

It is worth noting that the terms riparian area and buffer strip are often used interchangeably in the literature even though buffer strips are often just management tools frequently implemented in agricultural managed systems (Stutter et al., 2012) and whose concept do not reflect the complexity of riparian areas. Together with buffer strips, the term 'wetland' has also been a source of confusion as both areas perform important ecological and hydrological functions and share many similar characteristics (DWAF, 2005; USEPA, 2005). Wetlands can be defined as the ecosystem that arises when inundation by water produces hydromorphic soils dominated by anaerobic processes and forces the biota, particularly rooted plants, to exhibit adaptations to tolerate flooding (Keddy, 2010). Common examples of wetlands are swamp, fen or bog. In addition, riparian areas are usually highly connected zones shaped by high-energy disturbance regimes, whereas wetlands are relatively lightly connected and less physically dynamic (Naiman et al., 2010). Despite this, there is no shortage of authors who continue to mix the two (Hanson et al., 1994; Lowrance et al., 1995; Matheson et al., 2002). Nevertheless, in this study riparian zones are considered independent zones from wetlands.

Understanding and identifying the inherent characteristics of riparian areas and their spatial variability are essential tasks in order to ensure their correct management, preservation and delineation (Tompalski et al., 2017). Unfortunately, as far as riparian zones are concerned there is no universally accepted riparian classification system due to their inherent complexity and heterogeneity (Naiman et al., 2010). This is a topic which will be directly addressed within this thesis.

Factors such as habitat, vegetation, geomorphology and hydrology associated with riparian areas usually provide an operationally-defined way of stratifying these ecosystems into recognizable and repeatable units in order to develop a classification system (Kovalchik and Clausnitzer, 2004). In general, riparian typology is based largely on two broad disciplines: 1) fluvial geomorphic-based approach that classifies the structure and dynamics of river corridors (Gregory, 2012), and 2) biological-based approach that identifies and classifies plant communities on a physical pattern associated with river corridors (Naiman et al., 2010). Both of these approaches, however, should not be treated separately as it is usually the interaction of both that determines riparian evolution and spatial variability (Hupp and Osterkamp, 1996; Quinn et al., 2001; Murphy, 2010).

Although riparian classification is challenging and tends to have developed in narrow, context-specific ways (i.e. based on local conditions), several classification schemes have been attempted. For example, Johnson et al. (1984) proposed three different types of riparian areas (hydroriparian, mesoriparian and xeroriparian) according to the duration of the presence of water, soil moisture conditions and type of vegetation present (obligate, facultative or non-riparian) in the area. Delong and Brusven (1991) provided an alternative to the system developed by Platts et al. (1987) in which a really intense field survey is required (e.g. plant diversity, floodplain geomorphology, shading). This classification system identified seven different categories such as riparian vegetation type, bank slope and usage classified as discrete categorical units. Most recent classification systems, such as the one proposed by Quinn et al. (2001), try to better link riparian geomorphological forms with their potential functions and human uses.

2.2 Riparian delineation

Riparian classification and delineation procedures are usually coupled and complement each other. They both assist in identifying spatial relatively homogeneous patterns that offer potential support for policy makers, land managers and ecosystem functioning.

Numerous approaches have been developed in order to delineate riparian areas. Commonly, riparian delineation is advocated as environmental management tools based on simplistic models in which a fixed width buffer is implemented (Hawes and Smith, 2005; Stoffyn-Egli and Duinker, 2013). Depending on the extension of this buffer a smaller or larger number of ecosystem functions will be assured and protected (Jontos, 2004). The choice of a particular buffer width is often directed to maximize its effectiveness towards certain ecological functions such as sediment filtering, nutrient retention or shading (Wenger, 1999; Fischer and Fischenich, 2000; Hickey and Doran et al., 2004). However, the most recent delineation approaches tend to disregard fixed width buffers arguing that they lack consistency and mechanistic process level understanding (Aunan et al., 2005; Abood and

Maclean, 2011; Abood et al., 2012). A vast number of models are currently available in order to delineate riparian areas using Geographic Information Systems (GIS) and other kind of remote sensing data or satellite imagery that allow to incorporate inherent riparian characteristics (Alaibakhsh et al., 2016; Zhang et al., 2017). The different delineation systems try to preference some riparian characteristics depending on the research discipline. Some disciplines such as hydromorphology, vegetation or shading are the most frequently used (Holmes and Goebel, 2011; Klemas, 2014; Belletti et al., 2017; Tompalski et al., 2017).

2.3 Riparian ecosystem service provision

The term ecosystem service was first used in the late 1960s (MEA, 2003) and refers to natural goods or services (benefits) obtained from the ecosystem and utilized by humans, such as clean air, water, food and materials. They contribute to social and cultural well-being and can have high economic value (Fisher et al., 2009; Georgiou and Turner, 2012). This concept, which was popularized by the Millennium Ecosystem Assessment (MEA), has become an important model for linking the functioning of ecosystems to human welfare (Fisher et al., 2009). It aims to encompass the tangible (goods) and the intangible (services) benefits humans obtain from ecosystems (MEA, 2003). The understanding of this link will be a key factor in managing and maintaining riparian areas. The 2005 Millennium Ecosystem Assessment (MEA, 2005) identifies four broad categories of ecosystem services which in turn, provide us with a classification framework for the different benefits riparian areas produce: 1) supporting services (those that are necessary for the production of all other ecosystem services such as nutrient recycling or soil formation); 2) provisioning services (those that cover the material or provisioning from the ecosystems like water, food or minerals); 3) regulating services (the way ecosystems regulate other environmental media processes, such as carbon sequestration or climate regulation); 4) cultural services (those related to the cultural or spiritual needs of people such as aesthetic or recreational services).Although, riparian areas can differ considerably in their structure and functions from site to site, these zones are widely recognized for their contribution to the provision of ecosystem services (Clerici et al., 2011, 2014; McVittie et al., 2015). Some of the more recognizable functions and values of riparian areas identified here are within the MEA ecosystem service classification, however, it should be taken into consideration that some overlaps between categories could exist due to the high interrelations among the different functions riparian areas provide.

2.3.1 Supporting services

Biodiversity

Riparian areas are ecotones and as such, they are considered areas of particular high biodiversity (Naiman et al., 1993; Verry et al., 2004). Sabo et al. (2005) found a 50% increase in regional species richness in riparian habitats across the globe. They provide a natural corridor in a fragmented landscape where a great number of species can find refuge and food sources (Gregory et al., 1991). Thus, the provision of shade that limits water temperature fluctuations, primary food base energy (plant materials), and the dense root system of aquatic plants creates a whole set of different scenarios from the adjacent land that enhances biodiversity (Gregory et al., 1991; Goforth et al., 2002; Naiman et al., 2010). The role of riparian areas in maintaining invertebrates, birds, reptiles, amphibian and mammal communities has been well documented in the literature (Naiman et al., 1993; Nilsson and Svedmark, 2002; Sabo et al., 2005). Some authors like Bell et al. (1999) identified the high affinity of spiders for riparian areas or Cole et al. (2015) described higher populations of insect pollinators within the riparian areas provide some examples of biodiversity in this zone.

Organic matter storage

Riparian soils have been documented to be able to store a substantial amount of organic matter (OM) which, apart from being the storehouse for energy and nutrients used by plants and soil organisms, increases water retention and greatly affects the chemical and physical properties of the soil (McCarty, 2002; Lewis et al., 2003; Norton et al., 2011). Soil organic matter accumulation in riparian areas is often facilitated by the high water table which represses OM decomposition. In addition, due to their strategic position between terrestrial and aquatic systems, they are able to intercept organic litter and sediments rich in nutrients and organic matter coming from both upslope areas and adjacent waterbodies (Naiman et al., 2010).

2.3.2 Provisioning services

Habitat and food supply

Riparian areas constitute the refuge and food source for many terrestrial and aquatic species (Pusey and Arthington, 2003). For aquatic species (e.g. fishes), vegetation, roots and woody debris are particularly important in providing good water quality, food, and necessary habitats for all stages of their life cycles (Gregory and Ashkenas, 1990). Leaves and other plant materials play an important role in storing/recycling organic material in rivers, which in turn feeds invertebrates and therefore all the species which are dependent on them (Baxter et al., 2005).

2.3.3 Regulating services

Riparian areas as sediment and nutrient filters

Sediments can have a pronounced effect on water quality by increasing turbidity within the stream/river (Grabowski and Gurnell, 2016). Excessive sedimentation can reduce the amount of sunlight entering the water increasing the survivability of bacteria and potential pathogens (Droppo et al., 2009). As well as soil sedimentation, other substances such as pesticides, metals, sewage and animal wastes can also be transferred to streams and water bodies causing water quality problems and seriously damage fish and wildlife resources (Holden et al., 2017). Riparian vegetation has been shown to act as buffers and filters of suspended sediments, thus improving the water quality by reducing the amount of sediment eroding from the river bank (Sovell et al., 2000; Aguiar et al., 2015). This can act to dissipate the water's energy (by increasing surface roughness and therefore reducing runoff velocity) and enhancing infiltration in the soil due to vegetation roots (reducing runoff volume). As a result, this causes suspended sediments to settle out or be transformed instead of being transported further downstream. However, it is noteworthy that their effectiveness will probably be dependent upon many factors such as incoming flow rates, nature of the pollutant, sediment particle size, topography, vegetation cover and type (Vigiak et al., 2007). In turn, riparian vegetation along with the soil and microbes, are also able to intercept and store, by similar mechanisms, nutrients which despite being essential elements to the normal development of ecosystems, can turn into a pollution source too (e.g. nitrogen or phosphorus from agricultural fertilizers) (Lowrance and Altier, 1997). Once nutrients enter a riparian area, they are exposed to mechanisms (like denitrification) that may use or change them controlling their cycling within the system. This is particularly important in areas dominated by agriculture which is a major source of contamination (e.g. release of significant amounts of nitrogen, phosphorus and organic matter to aquatic ecosystems) (Malanson, 1993;

European Environment Agency, 2005). This can lead to an excessive growth of algae and other aquatic plants in response to high levels of nutrients enrichment (eutrophication) (USEPA, 1995) and may induce a loss of biodiversity and water quality among other problems (Carpenter et al., 1998).

Moderating stream temperature

One of the most obvious effects of riparian vegetation is the regulation of water temperature by the provision of shade (Davies-Colley and Rutherford, 2005). In some cases, riparian shading has been found to be more effective than reducing nutrient pollution in order to halt river phytoplankton growth (Hutchins et al., 2010). Vegetation strips can reduce inputs of solar radiation and thereby minimize temperature fluctuations of the stream water (Osborne and Kovacic, 1993). This fact is important as temperature can influence metabolism, growth, solubility of gases as well as many other biophysical processes and interactions (e.g. at warmer temperatures, salmonids become more susceptible to disease) (Gregory and Ashkenas, 1990; Broadmeadow et al., 2011). Bonnett et al. (2013) also found a positive exponential relationship between temperature and denitrification rates and net N_2O production in riparian areas. Nevertheless, the intrinsic characteristics of riparian vegetation such as, density and height will determine the effectiveness of this function.

Controlling flooding and recharge aquifers

Well-developed riparian vegetation might be able to attenuate the negative impacts of flooding by slowing down surface runoff during heavy rain events (Noordwijk et al., 2017). These areas also provide a natural basin where floodwater may spread out horizontally reducing water's velocity (Bukaveckas, 2007). They also have the potential to reduce the water's erosive potential and increase the time available for water to infiltrate into the soil and be stored for subsequent use by both plants and humans (Gregory et al., 1991). The infiltration of water, in turn, promotes the percolation of pollutants into the soil profile removing or degrading them by a variety of physical, chemical, and biological processes (Wilson et al., 2015).

Bank stabilisation

Vegetation roots can help bind river banks, reducing the potential for erosion to occur and therefore protecting adjacent lands. During flooding periods, or by the action of the rain, stream banks tend to erode which is a major source of sediments and nutrients (McCloskey, 2010). Without the vegetation, which increases soil cohesion and resistance, river banks are much weaker and prone to be eroded excessively by the river or rain (SEPA, 2009).

2.3.4 Cultural services

Recreation and aesthetics

Riparian zones can offer a wide range of aesthetics and cultural values associated with different features such as physiognomy, relief or vegetation formations (Gregory and Ashkenas, 1990). Recreational activities such as fishing, hiking or canoeing have been undertaken in riparian settings for millenia and provide a possible source of income for landowners and communities (Hein et al., 2006).

2.3.4 Possible adverse effects

Despite the many benefits that riparian areas can contribute, they can also have a number of negative impacts on the surrounding environment that should also be taken into consideration (Forestry Commission, 2004). These include:

- Some riparian plant species, particularly conifers, can provide too much shade reducing excessive water temperature gains, therefore causing a direct impact on reducing growth of fish and aquatic fauna in general.

- The input of too much woody debris into water bodies can restrict fish movement and cause blockage of the stream with implications for flooding.
- Some riparian species such as willow and poplar have a high demand for water which can compromise water supplies during dry summer periods and also reduce stream water levels.
- Some recreational activities in riparian areas can enhance the spread of invasive species (e.g. Himalayan balsam and Japanese knotweed) (Tanner et al., 2013).
- Riparian areas can harbour disease-causing organisms (e.g. liver fluke; Chai et al., 2013).
- Riparian areas can attract livestock to shelter near watercourses increasing their risk of contamination by faecal organisms and increasing sedimentation (Kauffman and Krueger, 1984; Line, 2003).

As highlighted in this section, riparian areas provide a wealth of ecosystem services. Management and/or restoration strategies are therefore required to ensure the continued delivery of these many ecological and social functions. The challenge is that these also need to be both economically feasible and socially acceptable if they are to be adopted and implemented at the landscape scale (Osborne and Kovacico, 1993). This is especially the case since it may involve changes in age-old practices, involve the coordinated action of landowners, and induce social conflict (e.g. between anglers and canoers, or between anglers and those associated with reintroducing higher trophic levels such as otters/beavers) (Maseyk et al., 2017).

2.4 Riparian soil biogeochemistry

Within a catchment context, riparian zones are known to undertake unique ecological functions relating to biogeochemical cycling and biodiversity (Decamps et al., 2004). Soils in these zones display distinct properties which differentiate them from those in adjacent areas. These properties typically include high water contents and organic matter, fine textured, low oxygen status, low redox potential and a bacterial dominated microbial community (Mayer et al., 2007; Zaimes et al., 2007; Naiman et al., 2010; Graf-Rosenfellner, 2016). The permanent or fluctuating anaerobic conditions among other features, frequently cause the fundamental biogeochemical cycles to be vastly different in comparison to surrounding upslope areas (Green and Kauffman, 1989).

The concept of 'biogeochemical cycling' can be defined as the basic elements that occur in living organisms moving through the environment in a series of naturally occurring physical, chemical, geological and biological processes. In particular, it is the study of chemical cycles, such as carbon, nitrogen and phosphorus (all of them of significance in riparian areas) which are either driven by or have an impact on biological activity. Many of these processes are highly influenced by vegetation and microbial populations in riparian areas (Tabacchi et al., 1998; Lewis et al., 2003).

Riparian ecosystems can performe a myriad of biogeochemical processes that impact on the structure and the composition of the streamside biota as well as aquatic systems (Green and Kauffman, 1989; McClain et al., 2003). The combination of microbial activity together with the slow diffusion of oxygen in waterlogged riparian soils produces anoxic conditions and a reduction in oxidation/reduction (redox potential). Redox potential is a measure of the intensity of the anaerobic conditions and provides an indication about which chemical transformations are occurring as well as the biogeochemical pathway functioning in riparian areas (Lewis et al., 2003).

As far as geology is concerned, upland and riparian soils can be derived from a wide range of parent materials, display distinguishing characteristics that allow their identification and delimitation. Riparian soils for their part, are largely influenced by water (Mikkelsen and Vesho, 2000), and usually altered by the reception of sediments and other materials from the catchment that may be deposited in stratified bands with contrasting textures. Consequently, the parent material associated with riparian soils tends to be more heterogeneous in mineral character than their adjacent areas (Mikkelsen and Vesho, 2000). This heterogeneity in soil conditions represents a major factor regulating plant productivity and diversity (Naiman et al., 2005). In addition, riparian soils also contain organic matter and are home to an active biotic community that underpins the long-term vitality of riparian zones. The soil biota largely influences soil properties such as hydrology, aeration and gaseous composition (all of them crucial for primary production and decomposition of organic matter) (Glenn, 2003). Although for the purpose of this review, we will refer mainly to microbial assemblages rather than soil fauna, it is noteworthy that burrowing animals, earthworms, nematodes amongst others, play a key role in riparian soils (Miller et al., 2014). They maximize the function of riparian soils by providing conduits for oxygen and water movement in addition to helping form aggregates and adding nutrients to the soil (Lewis et al., 2003).

Several characteristics of riparian areas make them important sites for subsurface transformations of nutrients and other chemicals (Hill, 1996; Glenn, 2003). With respect to nutrient cycling and transformations, the cycling of carbon, nitrogen and phosphorus are of particular interest due to their abundance and their key role within living organisms. Further, elements that are highly responsive to redox (e.g. S, Mn, Fe) are also important (Chi et al., 2016). However, it should be noted that the biogeochemical features of any riparian zone are critically dependent on their position within the broader landscape.

2.4.1 Carbon cycle

Riparian areas are believed to have a high potential for carbon (C) sequestration (Robert, 2002). The first step in managing C storage is to understand where it occurs and the processes that enhance and maintain it. Soil is the largest reservoir of C of the terrestrial C cycle (FAO, 2004) and contains about three times more C than is present in the overlying vegetation and double the C content of the atmosphere (Batjes and Sombroek, 1997). Soil organic matter (SOM) includes plant and animal residues, substances synthesized through microbial chemical reactions and the microbial biomass (Bot and Benites, 2005; Hernandez-Soriano et al., 2013). However, not all SOM will perform the same functions since its functionality will be largely influenced by its chemical form and residence time (retention in soil before returning to the atmosphere or exported as CO_2 or as dissolved organic C (DOC) in drainage water). For instance, the residence time for C stored in litterfall is considerably shorter in comparison to humified organic matter which is closely linked to the mineral fraction forming aggregates (Lewis et al., 2003).

Fluxes between soil organic C (SOC) and the atmosphere are large but may be positive in the form of sequestration (CO_2 taken up and transformed by plants during photosynthesis to be mainly stored in plant tissue) or negative as CO_2 and CH_4 emissions (respiration). The release of CO_2 through soil respiration comes from two different pathways: (1) respiration from plant roots, and (2) respiration from heterotrophic microorganisms (Robert, 2002). The production rate of CO_2 is an important parameter since it gives us an indication of total below-ground biological activity, the decomposition rate of SOM and therefore the rate of C loss from the soil (Robert, 2002).

This microbial community is especially unique in riparian areas due to their natural adaptation to fluctuating aerobic/anaerobic conditions (Lewis et al., 2003). Many of these microorganisms are heterotrophic, obtaining energy from organic matter to support growth, metabolism and reproduction (Vepraskas et al., 2000). In that respect, many studies have highlighted the great diversity of microbes in riparian soils by using sole C source utilization methods (Clinton et al., 2002). Both bacteria and fungi are important in decomposition and nutrient cycling processes in riparian soils (Lewis et al., 2003). Bacteria are numerically the most abundant decomposers (per gram of soil) whilst fungi are more abundant in terms of biomass (Paul and Clark, 1996). In anaerobic conditions, bacterial processes prevail over fungi or plants, being the ones mainly responsible for breaking down and simplifying complex organic molecules (Paul and Ladd, 1981). However, it should be taken into account that vegetation may affect soil respiration by influencing soil microclimate and structure, the quantity and quality of detritus supplied to the soil and the overall rate of root respiration (Raich and Tufekciogul, 2000). Via their aerenchyma, plant roots can also facilitate the rapid transfer of greenhouse gases such as CH_4 and N_2O from soil to the atmosphere (Machacova et al., 2013). Other factors such as temperature, soil moisture and physical and chemical properties of the soil will also influence soil respiration (Martinez et al., 2001). The type of decomposition (aerobic or anaerobic) is mainly determined by the balance of organic matter load and the demand of oxygen (Csaba, 2011). Thus, if the oxygen transfer rate is enough to satisfy oxygen demand, aerobic decomposition will prevail. Otherwise, anaerobic conditions will result being, as a general rule, a slower process that results in the accumulation of organic matter within the detritus layer (Csaba, 2011). Figure 2.1 shows the main C transformation in riparian areas.

In adjacent upslope soils, organic matter decomposition is mostly an aerobic process where oxygen is the terminal electron acceptor, converting organic C to CO_2 as a final product. When decomposition occurs via an anaerobic pathway, fermentation can occur and other electron acceptors such as oxidized iron (Fe^{3+}), manganese (Mn^{4+}), nitrate (NO_3^-) sulphate (SO_4^{2-}) or CO_2 itself (Vepraskas et al., 2000) are used by bacteria. These anaerobic reactions yield very small amounts of energy in comparison with aerobic decomposition (Lewis et al., 2003). During fermentation, complex organic molecules are broken down into simple compounds (formate, acetate or ethanol) which can be used by plants. Numerous fermentation compounds can be produced including simple alcohols, organic acids and CO_2 that serve as substrates for facultative and obligate anaerobic bacteria, such as denitrifiers, Fe- Mn- or S-reducers and methanogens (Vepraskas et al., 2000). These compounds are unique in that they possess a distinct energy potential, which is a function of how easily they can be reduced. Each process of reduction or oxidation occurs at a particular redox potential (Lewis et al., 2003). Methanogenesis which occurs relatively frequently in riparian areas, refers to a very specific type of anaerobic microbial respiration and results in the production of CH_4 by CO_2 reduction (Kemnitz et al., 2004). Methanogens (organisms in charge of this process) do not use oxygen as the terminal electron acceptor and they are often responsible for the final step in the decomposition of organic matter under highly anaerobic conditions. Without methanogenesis, a large amount of C (in the form of fermentation products) would accumulate in anaerobic environments (Deppenmeier, 2002). In addition, riparian soils may also be a consumer of CH_4 depending on the redox status of the soil and the relative abundance of methanotrophic bacteria (Bodelier et al., 2012, 2013). Of significant interest is the role of riparian areas in controlling the flux of dissolved organic C (DOC) to watercourses and whether they are partly responsible for many of the long-term changes in riverine DOC concentrations (Camino-Serrano et al., 2016). It is clear that riparian areas can operate as both a source and sink of DOC depending upon a wide range of conditions including soil and vegetation type, local hydrology, time of year etc. (Wang et al., 2015; King et al., 2016; Ledesma et al., 2016).

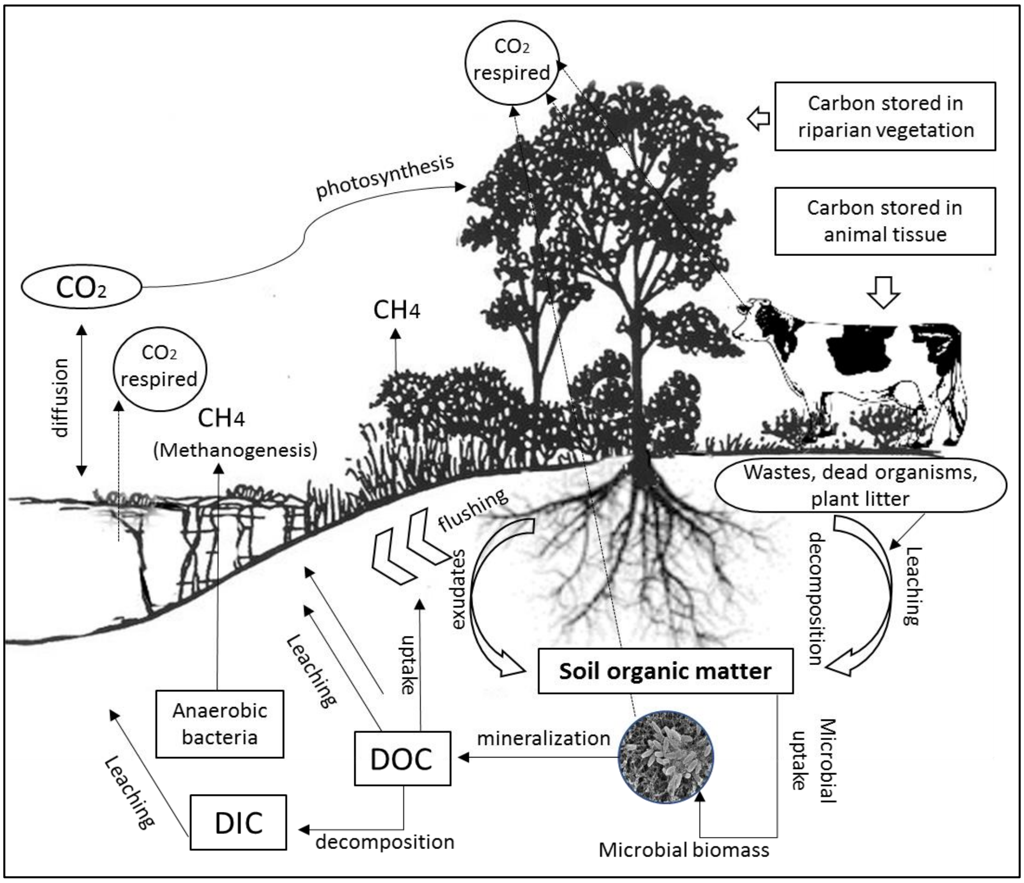

Figure 2.1 Schematic drawing showing the most important transformations of C in riparian areas. Dissolved organic carbon (DOC), Dissolved inorganic carbon (DIC).

2.4.2 Nitrogen and phosphorus cycle

Riparian zones frequently provide a sink for N and P and are typified by high rates of P and N cycling (Green and Kauffman, 1989; Lyons et al., 1998; Hefting et al., 2004). Nitrogen is a vital component of all amino acids and proteins and plays a crucial role in many cellular functions such as plant photosynthesis. On the other hand, P is important in molecules such as nucleic acids or adenosine triphosphate (ATP) (Glenn, 2003). In spite of this, however, researchers point out that the overuse of fertilizers and livestock manures on adjacent land has often caused P and N saturation of riparian soils leading to P and N losses, inducing eutrophication (Randall and Donnison, 2015).

Nitrogen can be found in a wide range of chemical forms within riparian soils and its transformation implies a variety of complex interrelated processes which are mainly controlled by microbial activity and the redox status of the soil (Gambrell and Patrick, 1978; Gresswell et al. 1989). These transformations are shown in the generalized diagram of the N cycle for oxidized and reduced zones in Figure 2.2. The more abundant forms of N within riparian areas are: (1) Organic N (N in plants, microbes and sediments), (2) Soluble N available in water and sediments (mainly NO_3^-, NH_4^+ and dissolved organic N; DON), and (3) N_2 and N_2O gases (Vepraskas et al., 2000; Clilverd et al., 2008; Flint and McDowell, 2015).

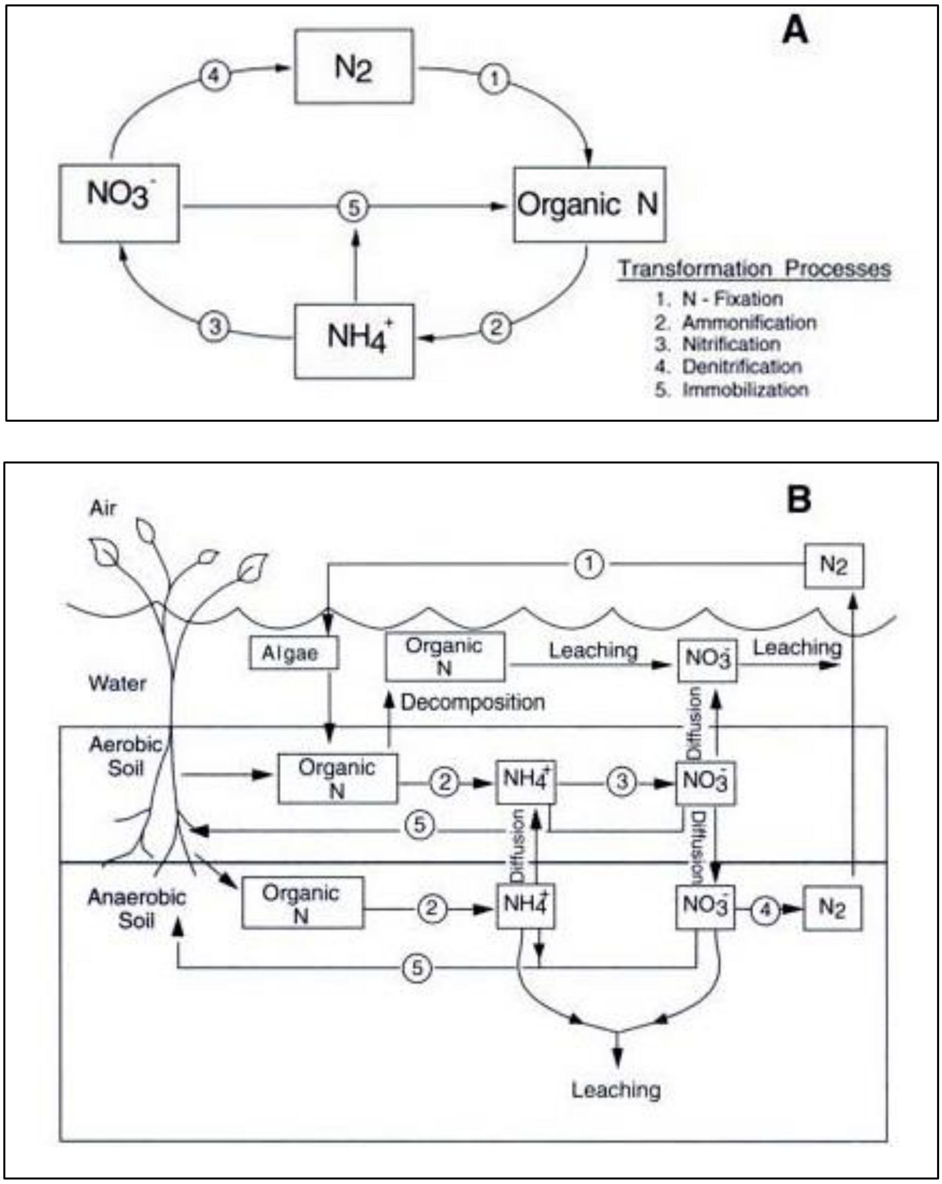

Figure 2.2 Schematic of the N cycle in riparian soils. Source: Vepraskas et al. (2000).

In riparian areas, a major input of N comes from atmospheric deposition (NO_3^- and NH_4^+), N_2 fixation and groundwater inputs of DON and NO_3^- mainly originating from agriculture (Carpenter et al., 1998; Vepraskas et al., 2000). Atmospheric N_2 is converted by diazotrophs (bacteria and archaea) to NH_3 and NH_4^+ (Glenn, 2003) and usually incorporated to organic tissue (Vepraskas et al., 2000). Some common bacteria in riparian areas such as *Nostoc* and *Clostridium* conduct this process anaerobically (Lewis et al., 2003). Another form of N transformation in riparian soils occurs through ammonification or mineralization. Organic N is converted to NH_4^+ when organic matter is oxidized and the N content of the substrate is greater than the N requirement of the decomposer community (Vepraskas et al., 2000). This process can happen both aerobically and anaerobically with the aerobic pathway being much faster, resulting in faster losses of N in part because nitrification can occur (Patrick, 1982). Nitrification is the aerobic production of NO_3^- from NH_4^+ by specific bacterial species such as *Nitrosomonas*, *Nitrosococcus* and *Nitrobacter* (Vepraskas et al., 2000). N is transported mainly in a dissolved form (DON, NO_3^-) through both surface and subsurface routes and its flux through riparian ecosystems is chiefly controlled by vegetative uptake and microbial immobilization and denitrification (Haycock et al., 1993). Denitrification is the biological conversion of NO_3^- to N_2, NO and N_2O using organic C from root exudates and vegetative litter as a source of energy (Decamps et al., 2004) and driven by denitrifying bacteria such as the genera *Pseudomonas*, *Bacillus* and *Escherichia* (Fenchel et al., 2012). Denitrification is believed to be highly effective in removing NO_3^- from subsurface flow when conditions are favourable (rich organic matter content and anaerobic conditions) (Glenn, 2003). This process can occur by two pathways (Madigan et al., 1997):

1. Reduction of NO_3^- under anoxic conditions resulting in the liberation of N_2 from the water column. In order for this to occur, there must be insufficient molecular or dissolved oxygen present so that the bacteria use the NO_3^- rather than the oxygen.

2. Reduction of NO_3^- under aerobic conditions results in the assimilative pathway or accumulation of N into the biomass.

In riparian zones the first pathway operates most frequently, however, denitrification rates can vary with soil organic matter, available P, temperature, and water- filled pore space (Decamps et al., 2004).

Riparian vegetation indirectly influences N cycling through transpiration and other effects on water flow. However, the most direct effect is through the uptake or excretion of N containing solutes by roots as well as symbiotic associations with bacteria or fungi that drive important N processes (Glenn, 2003). Plant uptake can also lead to either short- or long-term N storage, depending on whether it is stored in woody biomass or lost as leaves and twigs that ultimately return back to the soil surface (Glenn, 2003). Despite the doubtless influences that riparian vegetation has on the N cycle, no shortage of studies support the hypothesis that the primary mechanism for N removal in riparian areas is denitrification (Klapproth and Johnson, 2009).

The P soil cycle, for its part, is also remarkable in riparian areas as it is often a limiting nutrient in these systems (Chapin et al., 2004) and, as mentioned before, riparian zones can represent important removal mechanism for elevated nonpoint sources of P levels in many urbanized and agricultural landscapes (Vepraskas et al., 2000). Nevertheless, they are generally less effective in removing P than in the mitigation of sediment or N flow to freshwaters (Parsons and Gilliam, 1994).

Phosphorus can be found in three major forms in riparian areas (Vepraskas et al., 2000). They are: organic P, fixed mineral P and available orthophosphate (ortho-P). Orthophosphate exists mainly in an anionic form as H_3PO_4, $H_2PO_4^-$ and HPO_4^{2-} depending on the pH (the pH is rarely high enough to allow PO_4^{3-} to be present). Fixed mineral P consists of ortho-P attached to an oxide or hydroxide containing Al or Fe^{3+} (e.g. goethite, ferrihydrite, gibbsite) or cations such as Ca^{2+} or Mg^{2+} to form relatively insoluble substances at certain pHs (e.g. apatite). When this occurs, the P is considered fixed or tied up distinguishing it from NO_3^- which even though it also has a negative charge does not form insoluble metal complexes. Thus, in the presence of other elemental redox forms such as ferric iron, bioavailable phosphate can be immobilized by precipitation (Bailey, 2006). Otherwise, it may be released as bioavailable phosphate when, for example, ferric iron is reduced to ferrous iron by anaerobic microbial respiration (Mitsch and Gosselink, 2000) turning riparian areas from a potential sink for P to a possible source (Stutter et al., 2009). Other removal mechanisms include adsorption onto clay or organic particles and incorporation into living biomass via direct uptake, primarily by microbes and to a lesser extent by plants (Bailey, 2006).

Organic forms of P can be found in humus and other organic material (microbial and plant residue). It is predominantly transported in association with fine sediment (Decamps et al., 2004) by overland flow but can also be stored in plant tissue or in soil solution. In addition, dissolved organic P may also be present in high concentrations in some riparian areas, although its functional significance remains poorly understood (Wang et al., 2014). These P transformations differ significantly from the N cycle due to the absence of a gaseous phase that helps P removal (Vepraskas et al., 2000). Phosphorus in organic material is released by a mineralization process involving soil organisms and phosphatase enzymes (Wang et al., 2014; Geng et al., 2017). This process is highly important due to the fact that plants are not able to readily absorb organic forms of P (Lyons et al., 1998) requiring the action of microbial breakdown prior to assimilation. After microbial mineralization, the organic P applied to soils is subject to the same fates as inorganic P (Figure 2.3).

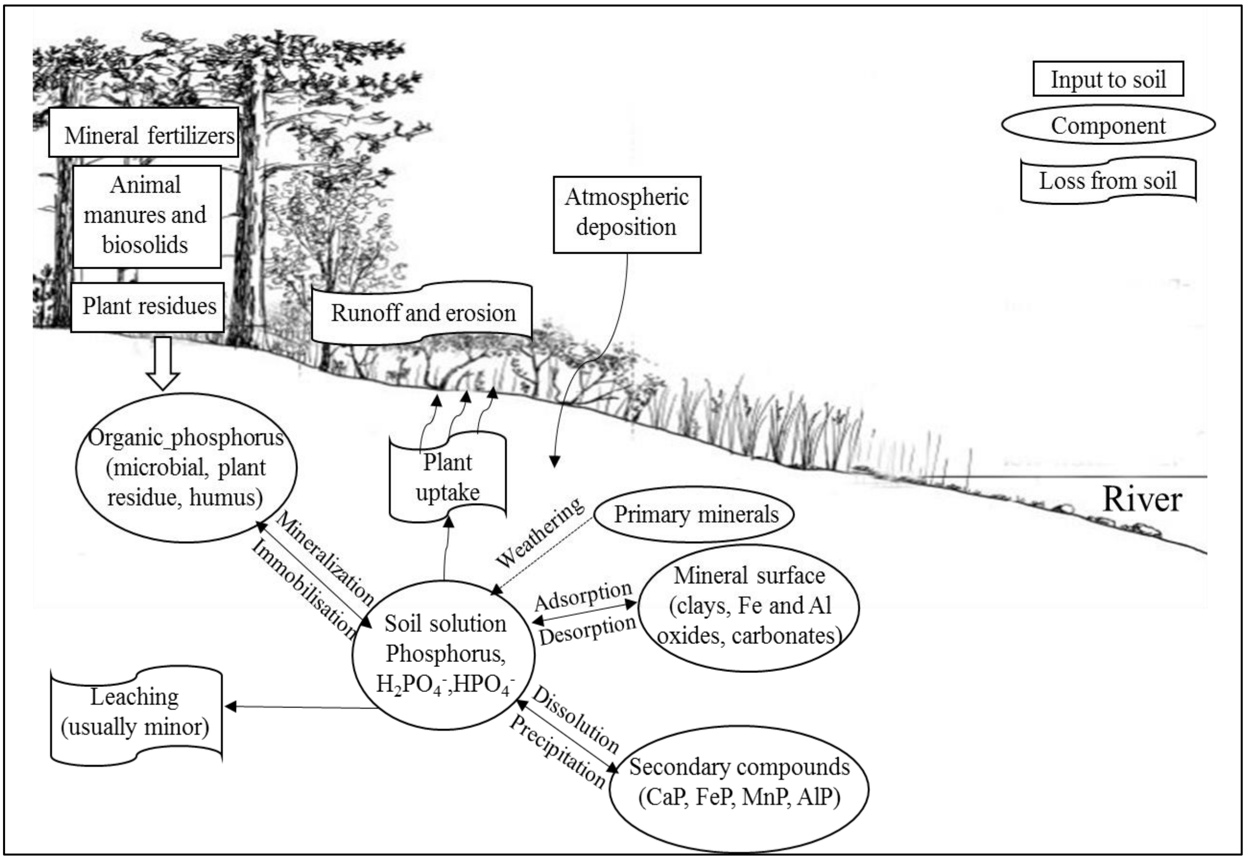

Figure 2.3 Schematic representation of the phosphorus cycle in riparian areas.

Reduction of iron is particularly important in lower energy riverine systems where oxygen is a limiting factor, due to it being the first readily observable stage of reduction in riparian soils (Lewis et al., 2003). Once the microbial community has exhausted the available reserves of oxygen, NO_3^- and Mn^{4+} in soil, Fe^{3+} reduction takes place if there is still an organic mineralizable source available (Figure 2.4). Although Fe^{3+} is an abundant element in many ecosystems, its low solubility in aerobic conditions usually makes it limited (Planquette et al., 2007; Sorichetti et al., 2014). However, in anaerobic conditions, Fe^{3+} is frequently used as an electron acceptor (turning to the reduced form Fe^{2+}) constituting a major pathway for organic matter decomposition in anaerobic sediments (Kostka et al., 2002; Neubauer and Emerson, 2002). In turn, transformation from oxidized to reduced Fe forms causes changes in its solubility in water. Hence, in a reduced state, iron becomes highly water-soluble and therefore becomes subject to translocation within the soil profile. This can lead to areas of high concentration (risk of toxicity) as well as areas of exhaustion (Lewis et al., 2003). In addition to this, it has also been documented that Fe plays an important role in P (influencing its adsorption and precipitation) and N cycling (blocking organic N mineralization at NH_4^+ step) (Clément et al. 2005; Kang et al., 2009) and therefore it should be taken into consideration within riparian zones.

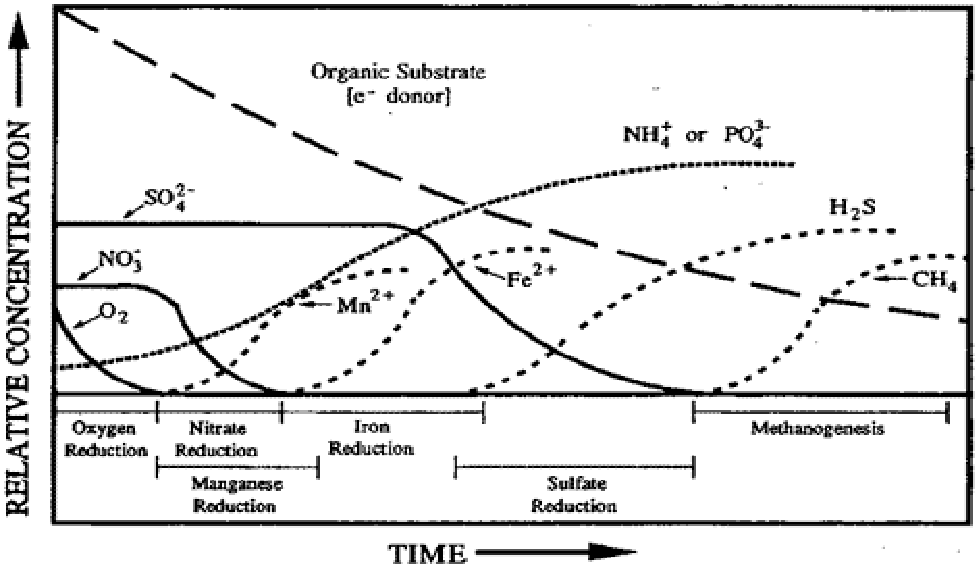

Figure 2.4 Schematic representation of the redox series of organic compounds and other electron donors within riparian areas. The time axis is synonymous with the onset of waterlogging. Source: Lorah et al. (2012).

Given the critical role biogeochemical cycles play in riparian ecosystems, it should be taken into consideration that despite the fact that all of the biogeochemical processes and soil physical and chemical characteristics have been broken down and explained separately, they are intimately related and often occur simultaneously (Jordan et al., 1998; Mikkelsen and Vesho, 2000). In order to ensure these ecosystems are functioning in a dynamic equilibrium, management strategies will need to be designed accordingly.

2.5 Major threats to riparian areas

Throughout history, riparian areas have been subject to a multitude of disturbances, mainly produced by anthrophogenic activities (Seavy et al., 2009; Poff et al., 2011). For example, river channelisation and dredging was of the main causes of rivers and their associated floodplain alteration in the mid-20th Century in the UK (Brookes et al., 1983). Further in England and Wales, it is estimated that 28% of lowland rivers are present in a semi-natural condition (Raven et al., 1998). Figure 2.5 shows the most common activities that have caused major alterations to riparian areas over the last century. Apart from the direct alterations of rivers, riparian areas have also been heavily affected by agricultural practices which are a major source of water pollution and nutrient enrichment, and land use changes in order to increase productivity (European Environment Agency, 2005). On the other hand, livestock intensification has also been acknowledged as a principal cause of sedimentation in rivers (Grabowski and Gurnell, 2016), which is especially important considering that sediments are now classified as a diffuse pollutant under the Water Framework Directive (WFD) (WFD, 2000). In addition, riparian areas are particularly vulnerable to invasion by exotic plant and animal species arising from human activities (e.g. deliberate introduction or spread of ornamental plants such as Himalayan Balsam and the escape of farmed animals such as mink; Bonesi and Macdonald 2004; Roy et al., 2015). These factors potenially make riparian ecosystems less resilient and more prone to further degradation (Dudgeon et al., 2006). Non-native species introduction can dramatically change native ecosystems and affect the water regimes (Tickner et al., 2001; Hejda et al., 2009). The crayfish plague in Europe or Japanese Knotweed are two remarkable examples of this (Gerber et al., 2008; Vanderklein et al., 2014; Faller et al., 2016).

Identifiying the causal factors and impacts of physical alteration affecting riparian areas is crucial in order to develop the most effective restoration strategy (Addy et al., 2016).

For example, Line (2003) identified a decrease up to 65% in bacterial numbers in the stream after limiting livestock access to river. The restoration of the river Eddleston (tributary of the River Tweed in the Scottish Borders) also constitutes a good example of how modifications and habitat degradation can be reversed. Throughout an exhaustive monitoring of species communities, ecological responses to the restoration actions, the commitment of landowners in collaboration with public bodies, the water body status under the WFD classification system improved from 'bad' status to 'moderate' which represents a great achievement for the area (Addy et al., 2016).

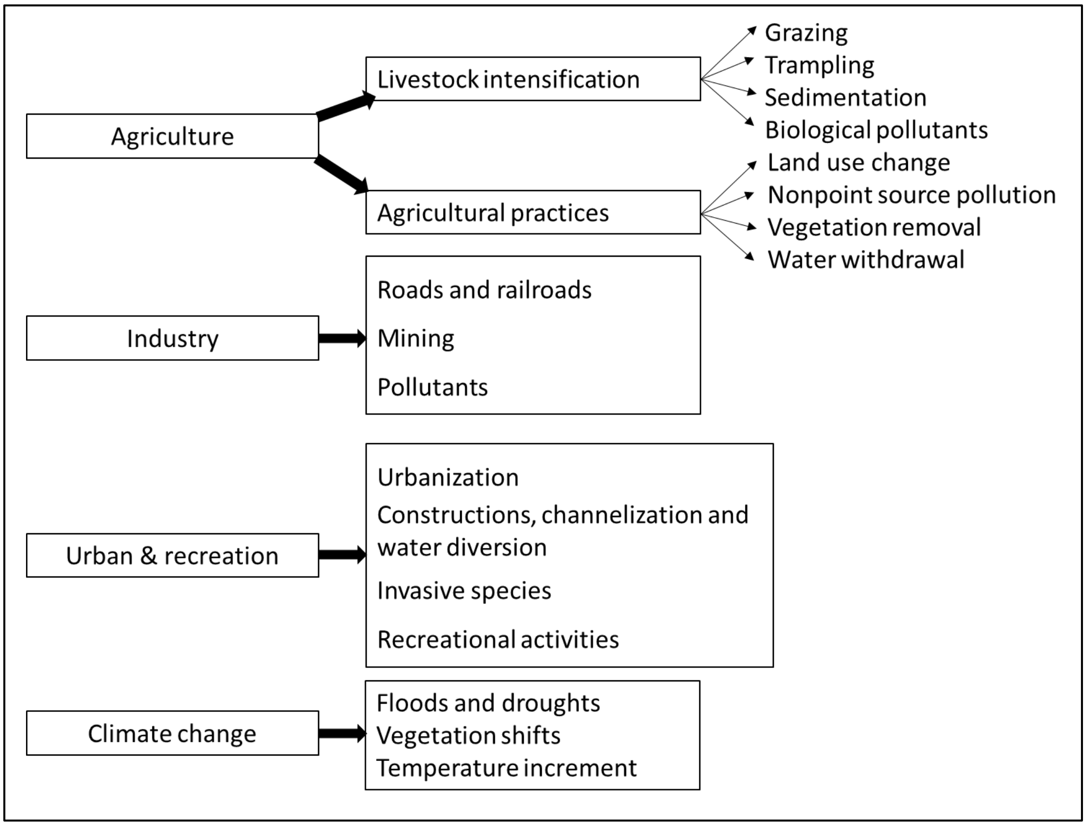

Figure 2.5 Summary of the major threats that can cause alterations in riparian areas.

2.5 Conclusions

In summary, this literature review highlights the importance of riparian areas in delivering a wide range of ecosystem services. It also highlights where significant knowledge gaps exist including: (1) classification and delineating riparian systems, (2) quantitative understanding of the factors controlling the potential role and effectiveness of riparian areas in alleviating agricultural emissions, controlling non-point source pollution or increasing nutrient cycling rates, (3) understanding their spatial complexity and heterogeneity at the landscape scale, (4) designing socially-acceptable and economically viable large-scale restoration schemes for degraded riparian ecosystems.

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Chapter 3

Delineating and mapping riparian areas for ecosystem service assessment

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L.L.S and D.L.J designed and conceived the experiment. L.L.S conducted the analysis of the data and prepared the manuscript. S.A.A developed the functional riparian model used in this study. All authors discussed results and contributed to preparation of the manuscript.

Abstract

Riparian buffers, the interface between terrestrial and freshwater ecosystems, have the potential to protect water bodies from land-based pollution, and also for enhancing the delivery of a range of ecosystem services. The UK currently has no defined optimal width or maximum extent of riparian buffers for specific ecosystem services. Here, we present the first study which attempts to 1) compare and critique different riparian buffer delineation methods, 2) investigate how ecological processes e.g. pollutant removal, nutrient cycling and water temperature regulation are affected spatially by proximity to the river and also within a riparian buffer zone. Our results have led to the development of new concepts for riparian delineation based on ecosystem service-specific scenarios. Results from our study suggest that choice of delineation method will influence not only the total area of potential riparian buffers, but also the proportion of land cover types included, which in turn will determine their main ecosystem provision. Thus, for some ecological processes (e.g. pollutant removal), a fixed–distance approach will preserve and protect its ecosystem function whereas for processes such as denitrification, a variable width buffer will reflect better riparian spatial variability maximizing its ecological value. In summary, riparian delineation within UK habitats should be specific to the particular ecosystem service(s) of interest (e.g. uptake of nutrients, shading, etc.) and the effectiveness of the buffer should be ground-truthed to ensure the greatest level of protection.

Key Words: ecosystem services, freshwater corridors, GIS, land use mapping, riparian zone modelling, riverbanks, wetlands

3.1 Introduction

Riparian areas are defined as the interface between land and freshwater ecosystems and are characterized by distinctive soil, hydrology and biotic conditions (Naiman et al., 2005). Riparian areas have been widely recognised for decades as having great potential to accomplish specific ecological functions such as alleviating agricultural runoff, promoting nutrient cycling and retention, flooding control or stream shading (Malanson, 1993; Wenger, 1999; Zaimes et al., 2007; Vigiak et al., 2016). However, due to the lack of a universal definition of 'riparian' and development of holistic classification systems (Verry et al., 2004; Naiman et al., 2010), their spatial complexity within the landscape as transitional zones and their sensitivity to disturbance have made their integration for management and delineation challenging.

Despite their importance, there is little guidance on how to reliably integrate the main riparian features such as vegetation or floodplain extension when delineating their boundaries (Salo et al., 2016). Delineating riparian areas may assist in improving our understanding of how these areas might benefit ecosystem service provision by: 1) identifying patterns in land use and their importance in the landscape, 2) characterising soil types and habitat distributions within the riparian areas, 3) reducing the anthropogenic pressures to which they are subject, 4) preserving their intrinsic value, and 5) establishing a common framework for their classification. Numerous approaches to delineate riparian areas have been undertaken ranging from simplistic models in which a fixed width buffer is implemented (Hawes and Smith, 2005; Stoffyn-Egli and Duinker, 2013), to more complex holistic approaches where the most relevant riparian characteristics such as soil properties, associated floodplain extent, vegetation type or hydrologic parameters are integrated into delineation models of varying complexity. These are subsequently used to generate a variable width riparian buffer (Lyons et al., 1998; Baker et al., 2006; Abood and Maclean, 2011; Momm and Bingner, 2014;

Belletti et al., 2017). However, recent approaches are more inclined to disregard fixed width buffers as they can be grossly inaccurate due to the poor and inconsistent relationship between riparian width and its ecological functionality (Aunan et al., 2005; Abood and Maclean, 2011; Abood et al., 2012). Furthermore, the use of geographic information systems (GIS) for conducting riparian estimations and the recent availability of high resolution data and imagery have resulted in the variable width buffer gaining more popularity over the past ten years (Xiang, 1993; Goetz et al., 2003). This allows the integration of a large amount of variables to characterise the potential riparian area. Hence, different GIS-based methods are already available which attempt to integrate multiple physical riparian attributes such as land cover (Baker et al., 2006), soil characteristics (Palik et al., 2004) and flood height (Mason, 2007) for riparian delineation. Approaches including biological attributes (e.g. amphibian habitat or vegetation type) have also been applied (Perkins and Hunter, 2006; Mac Nally et al., 2008). It is worth noting that the number of variables incorporated into the riparian area modelling process greatly affect its data-intensiveness and computational complexity by increasing data pre- and post-processing and increasing the number of interactions into the model. Thus, the delineation process should only incorporate spatial data at appropriate resolutions which allows capture of riparian versatility while maintaining the effectiveness and efficiency of the modelling process.

Ultimately, the spatial delineation of riparian areas remains critically dependent upon the ecosystem service being studied. For example, this could involve mapping of services directly adjacent to the river (e.g. shading, habitat), while other services may extend for considerable distances away from the watercourse (e.g. nutrient attenuation, flood risk management). Legal or policy adoption of a specific riparian buffer methodology could therefore potentially lead to the inclusion or exclusion of a particular area as being “riparian”. This could in turn determine the implementation and success of future management activities designed to optimise riparian functioning or in the assessment of riparian performance. Fundamental to this, will be to understand the relationship between land cover strongly influenced by physical attributes such as soil type or hydrology, and ecosystem service provision, as studies have indicated a link between land cover and its capacity to provide specific ecosystem services (Burkhard et al., 2009; Sheldon et al., 2012; Clerici et al., 2014).

The aim of this study was to critically evaluate the relative accuracy of different riparian delineation approaches and explore the impact of data quality and data types on predictions of riparian typologies. Specifically, our objectives are; 1) to evaluate to what extent fixed-width riparian buffers provide a different outcome than functionally-targeted variable-width riparian buffers, and 2) to determine how the quality of nationally-available digital information influences the prediction of functional variable-width riparian buffers?.

3.2 Materials and Methods

3.2.1 Study area

The study was conducted in the Conwy catchment, North Wales, UK (3°50’W, 53°00’N; Figure 3.1). The catchment comprises a total land area of 580 km^2 and its main river (River Conwy) runs for 43 km from its southern source to its subsequent estuarine discharge point into the Irish Sea (Emmett et al., 2016). The river rises in the Snowdonia National Park and the upper reaches of the river cross a wide range of habitats including upland bog, improved and unimproved grazed grasslands and coniferous and deciduous woodlands. Within this catchment, five sub-catchments were selected representing the dominant land-use types and riparian typologies in the catchment. A detailed description of the catchment is provided in Emmett et al. (2016). Main features of the sub-catchments are provided in Table 3.1 and in the Supplementary Information (Figures S1-S5).

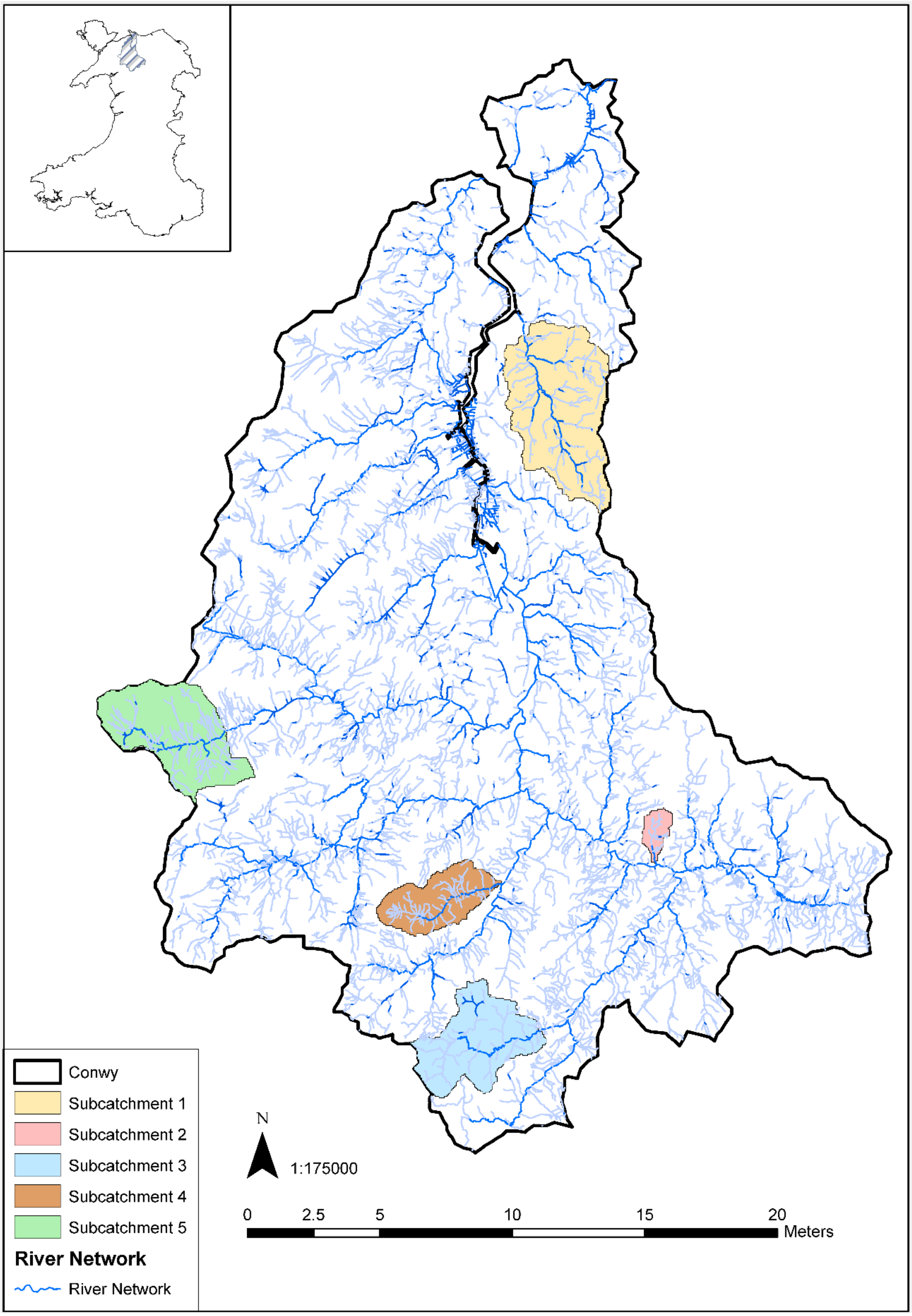

Figure 3.1 Representation of the Conwy catchment and the five sub-catchments used in this study. Inset shows the location of the main catchment within Wales.

Table 3.1 Main features of the sub-catchments selected in this study. More information is provided in the Online Supplementary Information.

	Sub-catchment 1	Sub-catchment 2	Sub-catchment 3	Sub-catchment 4	Sub-catchment 5
Area (km^2)	20.6	1.46	12.0	7.45	14.8
Stream network length (km)	60.0	6.05	34.5	32.1	60.8
Main channel length (km)	9.90	2.29	8.17	5.58	5.86
Average slope (%)	25.8	14.2	10.7	35.2	29.7
Dominant land use	Intensive livestock grazing	Intensive livestock grazing	Light livestock grazing	Light grazing and forestry	Light grazing
Dominant habitat type	Improved grassland	Improved grassland	Blanket bog	Coniferous woodland	Acid grassland

3.2.2 Riparian delineation methodology

All riparian modelling and data manipulation were undertaken using ArcGIS Desktop 10.2 (ESRI Inc., Redlands, CA). A schematic representation of the three different methodological approaches undertaken in this study can be seen in Figure 3.2.

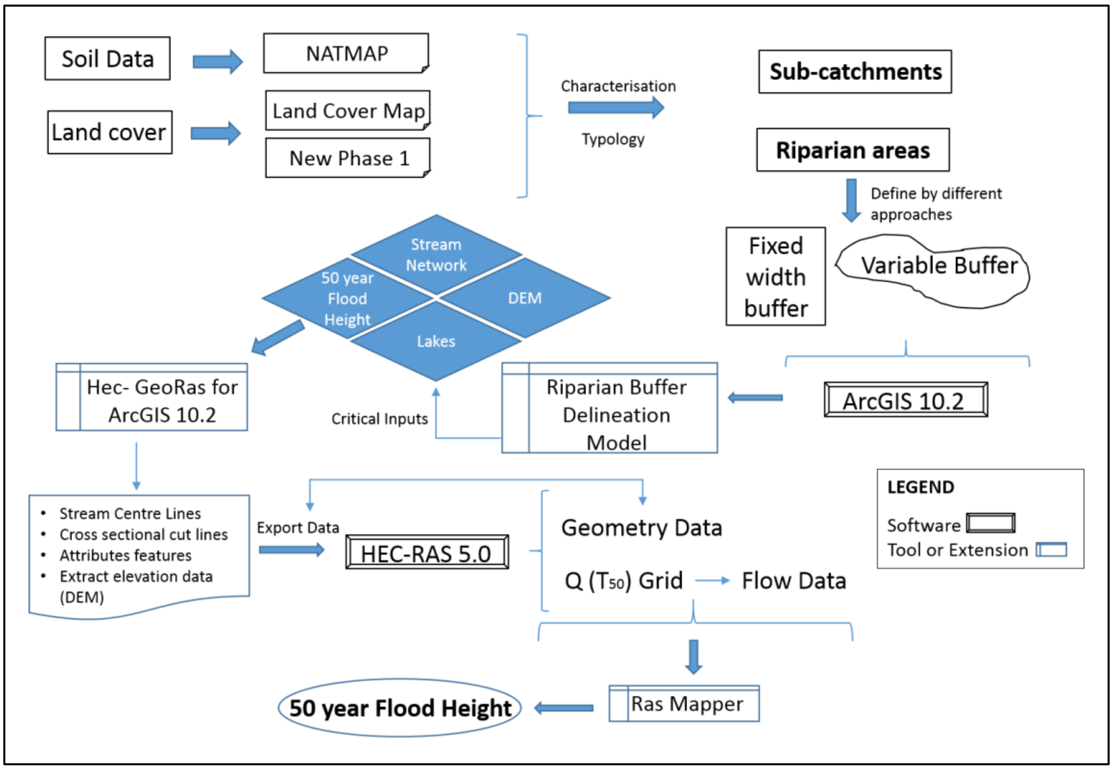

Figure 3.2 Flowchart describing the methodology used to delineate riparian areas within this study.

The different riparian delineation approaches were evaluated as follows:

Method 1. Fixed-width riparian buffer approach: Two buffer strips contiguous to the watercourse, 10 m and 50 m width respectively, were defined to assess the influence of proximal and distal riparian buffer delineation. There is no consensus on the most appropriate fixed buffer width for riparian area delineation (Wenger, 1999), however, as a broad recommendation, studies have indicated that efficient buffer widths should range between 3 m to >100 m depending on what resource they are trying to preserve (Hawes and Smith, 2005). For this study we chose a distance of 10 m following the absolute minimum buffer width suggested by Wenger (1999), and 50 m based on the recommendation of Peterjohn and Correll (1984) for agricultural catchments.

Method 2. Variable-width riparian buffer approach: Variable-width riparian buffer strips were spatially quantified using a modified version of Riparian Buffer Delineation Model v2.3 (Abood et al., 2012; *https://www.riparian.solutions/*) to work with the data available for this study. The model was implemented as an ArcGIS toolbox connected to ArcMap. The model generates riparian ecotone boundaries based on four critical inputs: stream and lake locations, digital elevation model (DEM) and the 50-year flood height. The specific sources and data inputs are listed in Table 3.2. The locations of streams and lakes are critical inputs into the model as they represent the drainage network associated with the riparian areas. In addition, the DEM provides the height information of the floodplain. Alongside the river network and DEM, the model also establishes the 50-year flood height as a required input on the assumption that this parameter represents the optimal hydrologic descriptor of a riparian area throughout the watercourse based on the research of Ilhardt et al. (2000). The 50-year recurrence interval was also indicated as the most likely elevation to intersect the first terrace or other upward sloping surface and in most cases, present the same microclimate and geomorphology as the stream channel

Table 3.2 Data inputs and sources used in the characterisation of the sub-catchments and delineation of the riparian areas.

Dataset	Scale or resolution	Data type	Source	Description
Digital Soil Data	1:250,000 1:63,000	Shapefile	National Soil Resources Institute (NSRI) LandIS soil classification http://www.landis.org.uk/index.cfm	Digital Soilscape based on the National Map Soil; 1:63,000 soil maps only available for sub-catchment 1.
Land Cover Map 2007 (LCM2007)	25 m	Raster	Centre for Ecology & Hydrology (LCM2007) http://www.ceh.ac.uk/services/land-cover-map-2007.html	LCM2007 includes 23 categories derived from satellite images and digital cartography.
New Phase 1 Land Cover	1:25,000	Shapefile	Natural Resources Wales (Lucas et al., 2011)	Updated Phase 1 Survey comprising 105 specific habitat types grouped into 10 broad habitat types.
Network-wide FEH flood peak estimates (Q (T) grids)	50 m	Raster	Centre for Ecology & Hydrology http://www.ceh.ac.uk/services/peak-river-flows-qt-grids (Robson and Reed, 1999; Morris, 2003)	Flood peak river flows estimated for different return periods at 50 m intervals along the UK river network. The flood peak estimates have been produced using a fully automated version of the Flood Estimation Handbook statistical procedures.
Detailed River Network (DRN)		Shapefile	UK Environment Agency (2008)	DRN derived from Ordnance Survey Mastermap features.
Inland lakes	1:10,000	Shapefile	Ordnance Survey (OS) Master Map https://www.ordnancesurvey.co.uk/business-and-government/products/mastermap-products.html	Lakes and open water bodies extracted from OS Master Map.
Catchment and sub-catchments		Shapefile	Centre for Ecology & Hydrology, D. Cooper	Catchment and sub-catchment boundaries.
Flood Zone 3	1:10,000	Shapefile	UK Environment Agency (2004) http://www.environment-agency.gov.uk/homeandleisure/37837.aspx	Shapefile with the Environment Agency best-estimate of the areas of land with a 1% or greater chance of flooding each year from rivers.
Annual rainfall (SAAR 61-90), mm	5 km	Raster	Natural Environment Research Council (NERC, 2012)	Annual rainfall 5 km x 5 km gridded datasets covering the UK based on Met Office Standard Average Annual Rainfall 1961-1990.
Digital Elevation Model (DEM)	2 m	Raster	Centre for Environmental Data Archival (Landmap Earth Observation collection); http://www.ceda.ac.uk/	DEM photogrammetrically derived from aerial photography by GetMapping and acquired by the Landmap project.
Digital Elevation Model	5, 10, 30 and 50 m	Raster	UK Environment Agency	Lidar composite DEM

Previous studies have addressed this task by performing regression equations between periodic measurements of flow rate, velocity and channel width obtained from river gauging stations (Mason, 2007; Abood et al., 2012). In this study, due to the lack of river gauge data for all sub-catchments, an alternative approach was used. Briefly, river hydraulic modelling was performed using HEC-GeoRAS (US-ACE, 2005) with a high resolution DEM to obtain required cross-sectional data and then the HEC-RAS (US-ACE, 2014) software used to generate surface water elevation (Figure 3.2). The model utilized several input parameters that influence flow behaviour: Manning's values (data based on the recommended design values of the Manning Roughness coefficients of McCuen (1998)) and boundary conditions (the channel bed slope of the first two cross-sections at the upstream boundary and the last two cross-sections at the downstream boundary as a starting value for a mixed flow regime). Once the river cross-sections were defined, the Network-wide Flood Estimation Handbook (Q(T) grid flood estimates; Robson and Reed, 1999) was used to derive the 50-year flood discharge (flow data in the HEC-RAS) (Table 3.1) for the major rivers in each sub-catchment.

As an estimate of flood extent, the Flood Zone 3 map for a 100-year event provided by the UK Environment Agency was used to compare the resultant floodplain area in each sub-catchment. Results from the HEC-RAS simulations, which include the locations of the cross-sectional cut lines together with water surface profile data, were processed in the HEC-RAS Mapper utility where the profile data is outputted as water surface elevations (depth grid). A detailed description of the process can be found in Ackerman (2011). Flood height results for the main rivers in all sub-catchments ranged between 1.4 and 2.2. However, in order to implement the same flood height for all study sites and to facilitate model development, a single average flood height of 1.6 m was used for all sub-catchments.

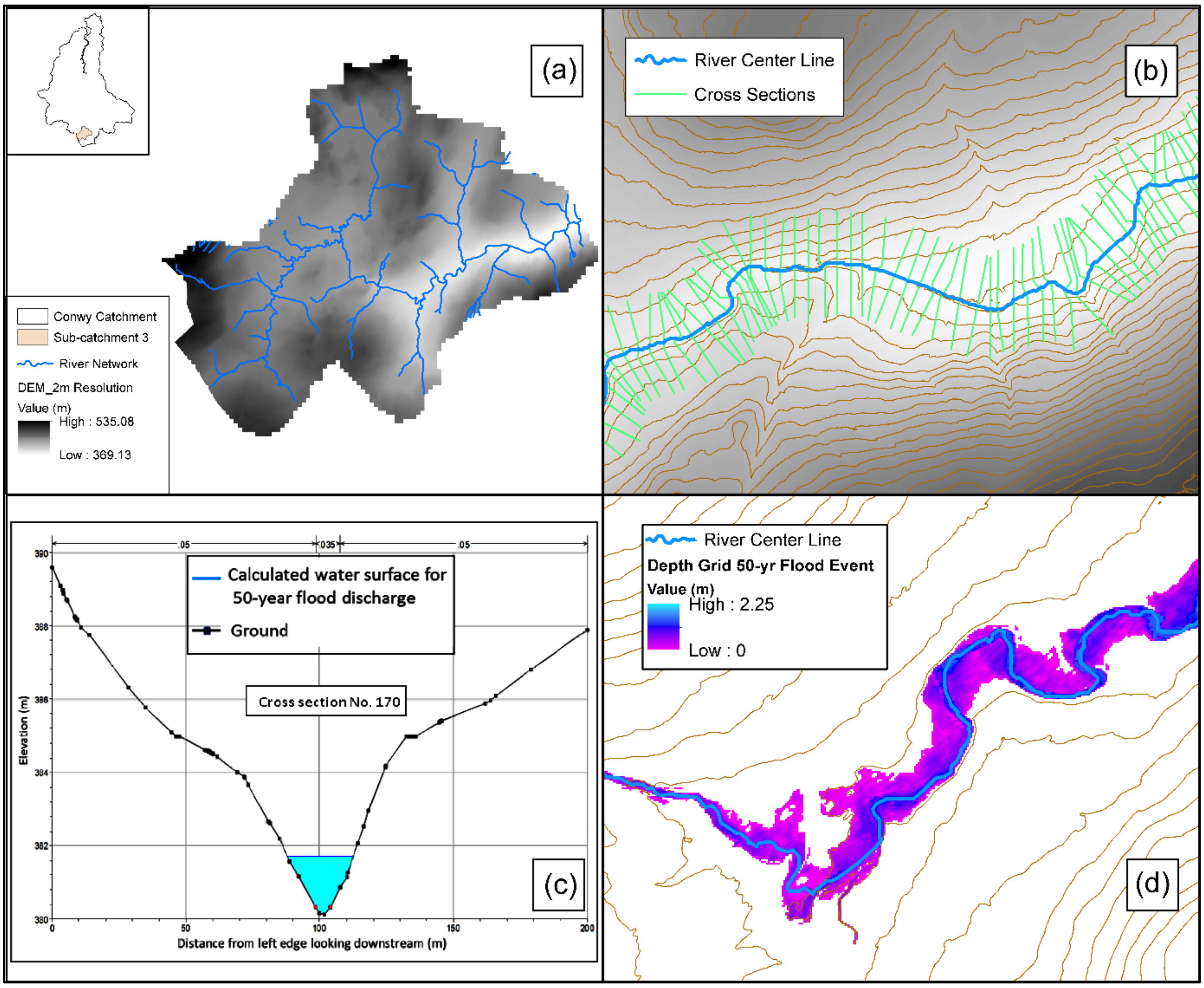

Figure 3.3 Illustration of the river network over the digital elevation model (a) and cross sections along the river centre lines (b) at the same location. (c) An example of a HEC-RAS cross section, looking downstream, and (d) the RAS Mapper depth grid for the 50-year floodplain.

Once all the inputs were introduced into the model, sample points along streams and transects around those sample points were built. For the study area, a maximum transect length of 250 m was imposed to improve the processing efficiency and to account for the spatial variation in height within our study (Abood et al., 2012). The model detected the change in elevation between the sample and the transect points and determined if the point should be included inside the riparian buffer. A detailed description of model performance can be found in Abood et al. (2012). As the DEM is one of the crucial model inputs, we also tested the influence of different DEM spatial resolutions on model output (2, 5, 10, 30 and 50 m). As optional data we include wetlands (according to New Phase 1 classification (Lucas et al., 2011) and soil data from the National Soil Map of England and Wales (National Soil Resources Institute, Cranfield, UK; NATMAP; http://www.landis.org.uk/data/natmap.cfm).

Method 3. Fixed-width legislative riparian buffer approach: One fixed-width buffer of 2 m was defined along minor rivers and the same distance was manually digitalized along the main rivers. As the buffer automation was created from the centre line of the river, manual digitalization was necessary in order to prevent the buffer from ending in the middle of major rivers considering the small size of the buffer. The digitization was accomplished using orthophotos and satellite imagery. The distance was chosen following the main requirements found in national and European-level policies in which a minimal buffer of 2 m is established for riparian areas (i.e. SMR 1; GAEC 1, 2016). This is also in agreement with common riparian fencing practices in the catchment, most of which are undertaken under the auspices of Welsh Government agri-environment schemes (e.g. Tir Gofal, Glastir).

3.2.3 Datasets

The datasets used in the study are presented in Table 3.1. Where possible, the best nationally available datasets were used. For lakes and open water bodies (>2 ha in area), a 30.5 m fixed buffer was used according to Ilhardt et al. (2000). Typically, these riparian areas only constituted <1% of the total riparian area within each sub-catchment. Lastly, the riparian buffers in each of the sub-catchments were overlaid onto soil type and two independent land cover datasets (LCM2007 and New Phase 1; Table 3.1). This was used to evaluate and characterize the percentage of land use and soil type within the riparian areas delineated using each of the three methods. For ease of comparison, different habitat types were aggregated into common land cover categories. These included: (1) broadleaved woodland, (2) coniferous woodland, (3) arable and horticulture, (4) improved grassland, (5) semi-natural grassland, (6) mountain, heath and bog, (7) freshwater, and (8) other, including built-up areas and gardens. A summary of how they were grouped is presented in the On-line supplementary information (Table S1).

3.3 Results

3.3.1 Estimate of riparian area using different delineation methodologies

The different approaches used to delineate stream riparian boundaries differed substantially in terms of their ability to predict the spatial distribution of riparian areas (Figure 3.4) and the total land area they covered in the sub-catchment (Figure 3.5). Of all the study areas, sub-catchment 1 showed the largest differences in terms of the total riparian area delineated by the different methods. For example, the fixed buffer approach (50 m) mapped the largest land area, encompassing 5.5 km^2 (26.6% of the total area), while the variable buffer approach only predicted a total area of 4.1 km^2 (19.7%). In contrast, the fixed (10 m) and the legal (2 m) approaches gave much lower estimates of 1.2 km^2 (5.6%) and 0.26 km^2 (1.2%), respectively. In the case of sub-catchment 2, no major difference was apparent between the fixed buffer (50 m) method (0.50 km^2, 34.3% of the area) and the variable buffer approach (0.52 km^2, 35.8%). Within the same sub-catchment, the legal based approach produced a very small riparian area, probably as it consisted predominantly of minor rivers. Similar to sub-catchment 2, the riparian predictions for the fixed buffer (50 m) method (3.0 km^2, 25.0%) and variable buffer (3.4 km^2, 28.1%) were close for sub-catchment 3. Sub-catchments 4 and 5 were intermediate, giving a discrepancy between the fixed buffer (50 m) and variable buffer of 0.99 km^2 and 0.27 km^2 respectively.

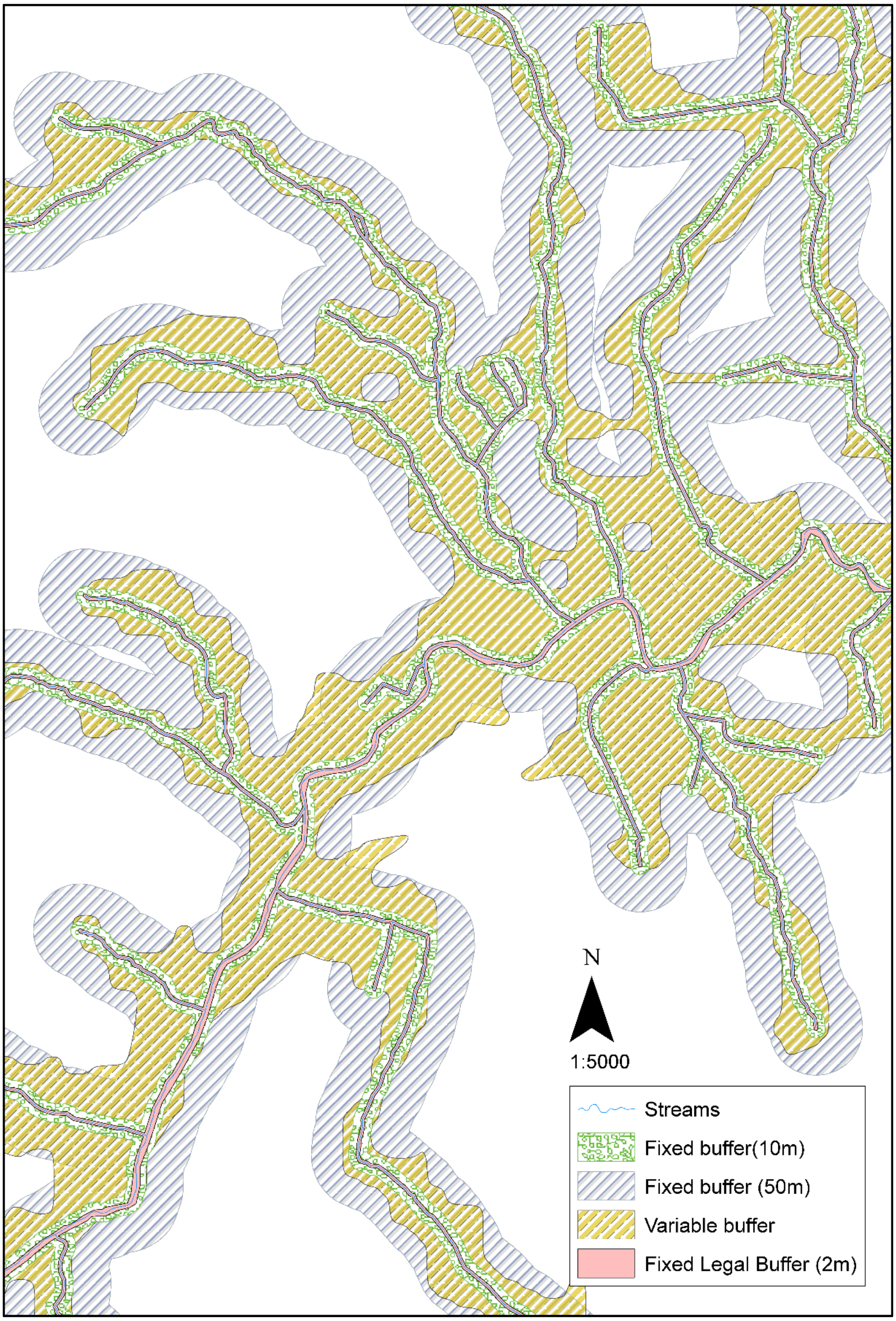

Figure 3.4 GIS comparison of all the different approaches for delineating riparian buffers within sub-catchment 5.

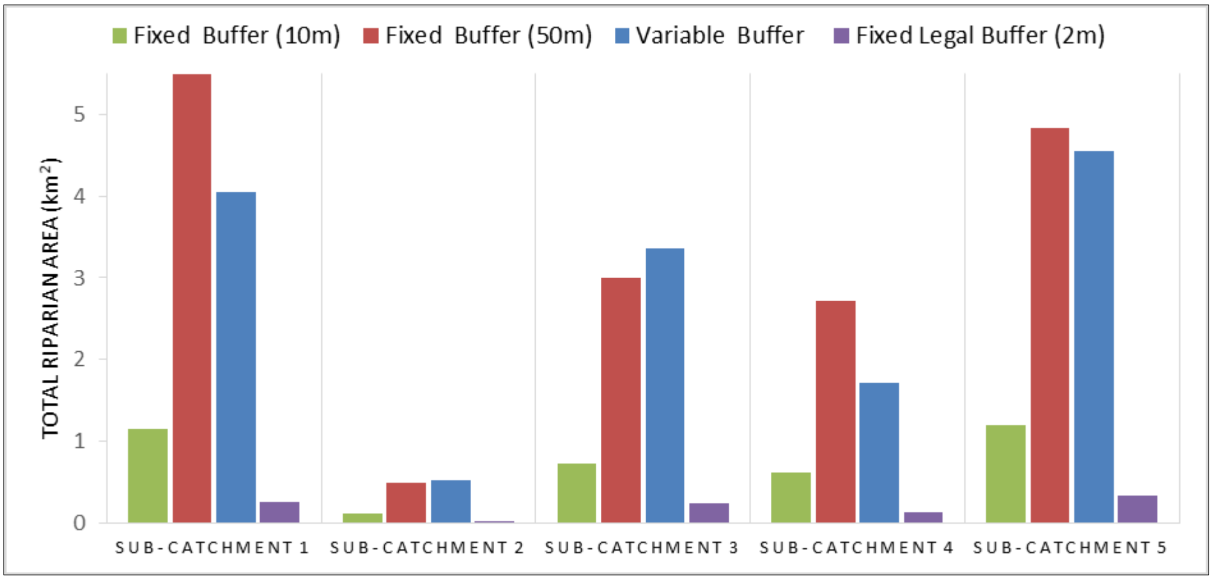

Figure 3.5 Comparison of the four different GIS-based methods on the total amount of riparian area delineated within each of the five sub-catchments within the Conwy catchment.

3.3.2 Agreement between the areas delineated with the fixed and variable width buffer approach

Due to the similarity of the results, in terms of total area delineated, shown by the fixed (50 m) and variable width buffer approaches, we compared whether they actually mapped the same areas. This was achieved by analysing the spatial agreement of pixels identified by both methods. The fixed width buffer (50 m) displayed clear differences when compared with variable width buffer predictions with nearly 30% of the digital pixels in spatial disagreement for sub-catchment 1, 21% for sub-catchment 2, 24% for sub-catchment 3, 27% for sub-catchment 4 and 17% for sub-catchment 5 (Figure 3.4).

3.3.3 Effect of digital elevation model (DEM) resolution on variable width riparian area predictions

Resolution of the DEM (i.e. sources and creation method of the DEM) was tested as it indicates the level of elevation details that are captured within the floodplain topography. A comparison of the impact of DEM resolution (2, 5, 10, 30 or 50 m) on the spatial mapping/distribution of riparian zones is shown in Figure 3.6, while its effect on the total riparian area delineated is shown in Figure 3.7.

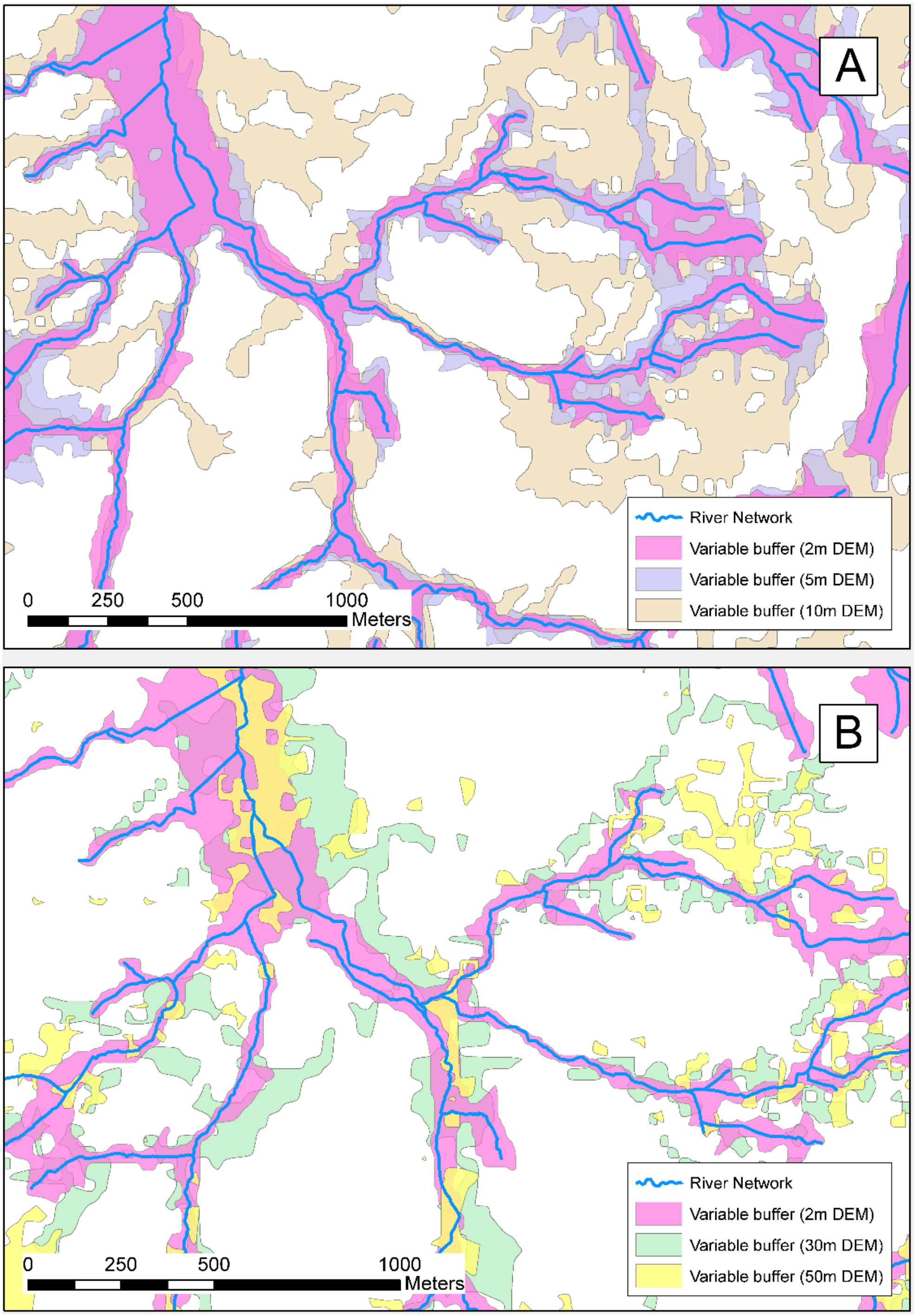

Figure 3.6 Example area comparing the riparian variable width model result using 2 m resolution DEM with 5 and 10 m resolution DEM results (Panel A) and 30 and 50 m resolution DEM results (Panel B) in sub-catchment 1.

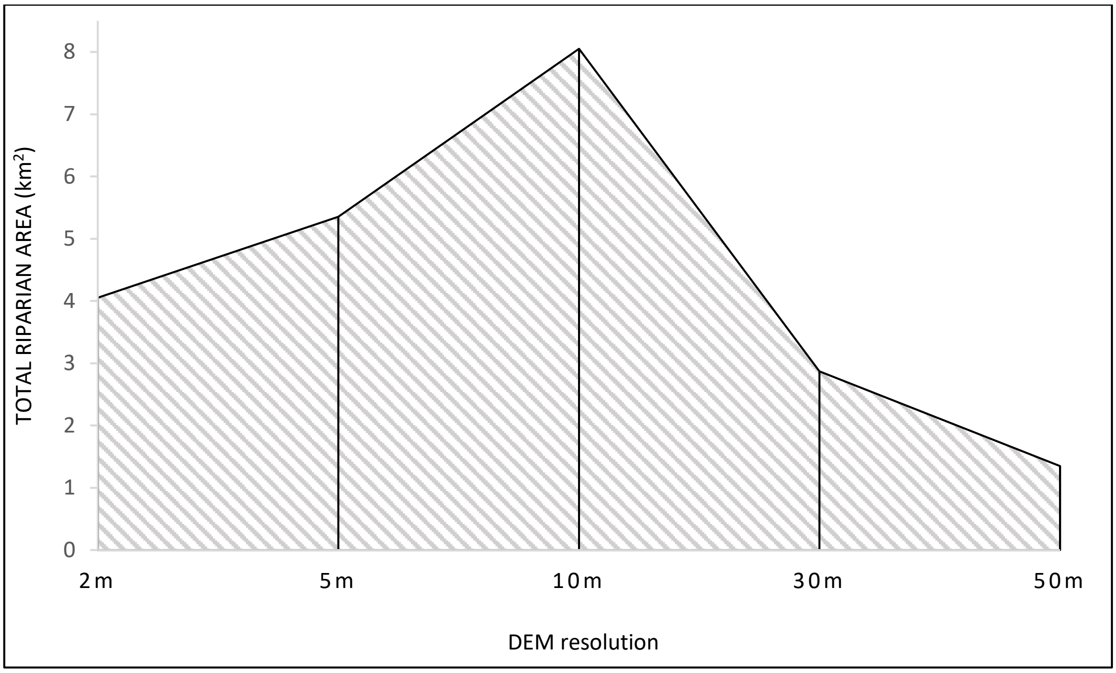

Figure 3.7 Comparison of the total amount of riparian area delineated when running the model with DEM resolutions ranging from 2 m to 50 m for sub-catchment 1.

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The results showed that the variable riparian buffer model calculated from the 2 m DEM produced a range of significantly smaller riparian areas than those calculated with the 5 and 10 m DEMs (Figure 3.6a). The spatial pixel disagreement between the variable width buffer from the 2 m resolution DEM versus the variable width buffer from 5 and 10 m resolution DEM was also noticeable with 24% and 45% disagreement, respectively. In contrast, comparison of the variable width buffer from a 2 m resolution DEM versus the results obtained from 30 and 50 m resolution DEMs showed a decreasing trend in terms of total surface area (Figure 3.6b, Figure 3.7). Both the 30 and 50 m model outputs displayed discontinuous and dispersed riparian area boundaries. The spatial pixel disagreement between riparian area from 2 m resolution and the two coarser DEMs resulted in 67% of disagreement for the 30 m resolution DEM and 74% for the 50 m resolution DEM. The changes observed in riparian surface area according to the different DEM spatial resolutions in sub-catchment 1 are shown in Figure 3.7. The results obtained using the 10 m DEM produced the greatest surface area with an area of 8.05 km^2. A similar trend was found for the other sub-catchments (data not presented).

3.3.4 Effect of delineation method on riparian land cover predictions

Differences in delineation methodology might not only influence the total riparian area, but also the prediction of soil distribution and the proportion of land cover types included within them. We overlaid the different riparian boundaries obtained with the different delineation methodologies onto the most detailed national soil map and the two most widely used national land cover maps (LCM2007 and New Phase 1). It should be noted that the comparison of soil distribution was only undertaken for sub-catchment 1, as it was the only area mapped at sufficient accuracy (1:63,000).

Overall, the Denbigh and Sannan soil series comprised the greatest land area regardless of the delineation approach (Figure 3.8). A description of the different soil series and their equivalent in the FAO World Reference Base (WRB) is shown in Table S2. In general, the total amount of each soil series predicted within the riparian zone was relatively similar for all four delineation methods. Only the variable width buffer showed a >5% discrepancy in the main soil categories compared to the rest of the methodological approaches.

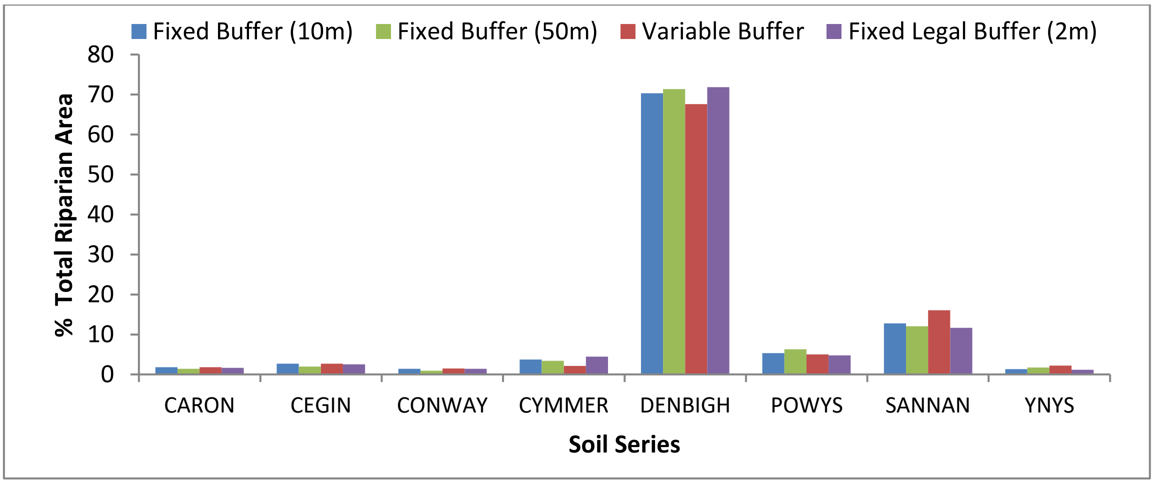

Figure 3.8 Distribution of different soil types (series) estimated by four different riparian delineation methods for sub-catchment 1. A description of the different soil series and their equivalent in the FAO World Reference Base (WRB) is shown in Table S2.

Land cover datasets (LCM2007 and New Phase 1) were intersected with all riparian delineations separately and are presented in Figures 3.9-3.13. It should be noted that some of the least abundant categories (those comprising <1% of the total riparian area) are not presented. In general, both land use datasets gave good agreement with 'improved grassland' and 'mountain, heath and bog' being the dominant habitats within the riparian buffer zones. However, strong contradictions in terms of habitat classification are noticeable in some sub-catchments (e.g. sub-catchment 2 and 3). For instance, while 'improved grassland' and 'mountain, heath and bog' were the dominant habitat types according to the New Phase 1 classification, 'semi-natural grassland' comprised the most abundant habitat type for the LCM2007 classification in sub-catchment 2 (Figure 3.10). It is worth noting that some of the habitat types present in some of the sub-catchments (e.g. sub-catchment 3 and 4) according to the New Phase 1 map are missing for the LCM2007 results (Figure 3.11 and 3.12). Our results suggest that the New Phase 1 land cover map tended to provide the information at a finer resolution than the LCM2007 as it identified a higher number of habitat types within riparian zones with the different modelling approaches (e.g. fixed or variable width buffer).

Sub-catchments 1 and 2 displayed the strongest discrepancy in terms of the proportion of different riparian habitat types identified using the different methodologies with the New Phase 1 habitat map. For example, in sub-catchment 1, 'broadleaved woodland' only compromised 26% of the total variable width buffer area while it accounted for 51% when using the legal approach. Similarly, in the same sub-catchment, 'improved grassland' represented approximately 56% of the total variable buffer approach in contrast with only 18% obtained with the legal buffer approach. In addition, sub-catchment 2 showed the percentage of 'improved grassland' was over 50% for the total variable width buffer, while for the legal buffer this decreased to 35% of the total riparian area. In contrast, sub-catchment 3 gave a similar distribution for the riparian plant communities for both methods of classification. Both datasets indicated that 'mountain, heath and bog' and 'semi-natural grassland' were the dominant land cover classes. However, the LCM2007 dataset estimated that 'mountain, heath and bog' constituted 90% of the total riparian area, whereas the New Phase 1 dataset predicted a coverage range of only 65-72% for the same habitat category. For 'semi-natural grassland' in sub-catchment 3, the LCM2007 predicted that it only covered 5% of the total riparian area compared with 13-20% for the New Phase 1 map. Sub-catchment 4 showed a similar distribution of habitat types across both land cover datasets and all buffer delineations. However, 'freshwater' and 'broadleaved woodland' exhibited the greatest discrepancies in percentage riparian area cover when selecting more restrictive buffer strips (e.g. fixed width 10 m buffer and legal fixed buffer). It is also worth noting that the New Phase dataset included 'freshwater' and 'other' in its habitat categories while these are not present in LCM2007. Sub-catchment 5 displayed a discrepancy between both land cover datasets of 5-10% between the main habitat types.

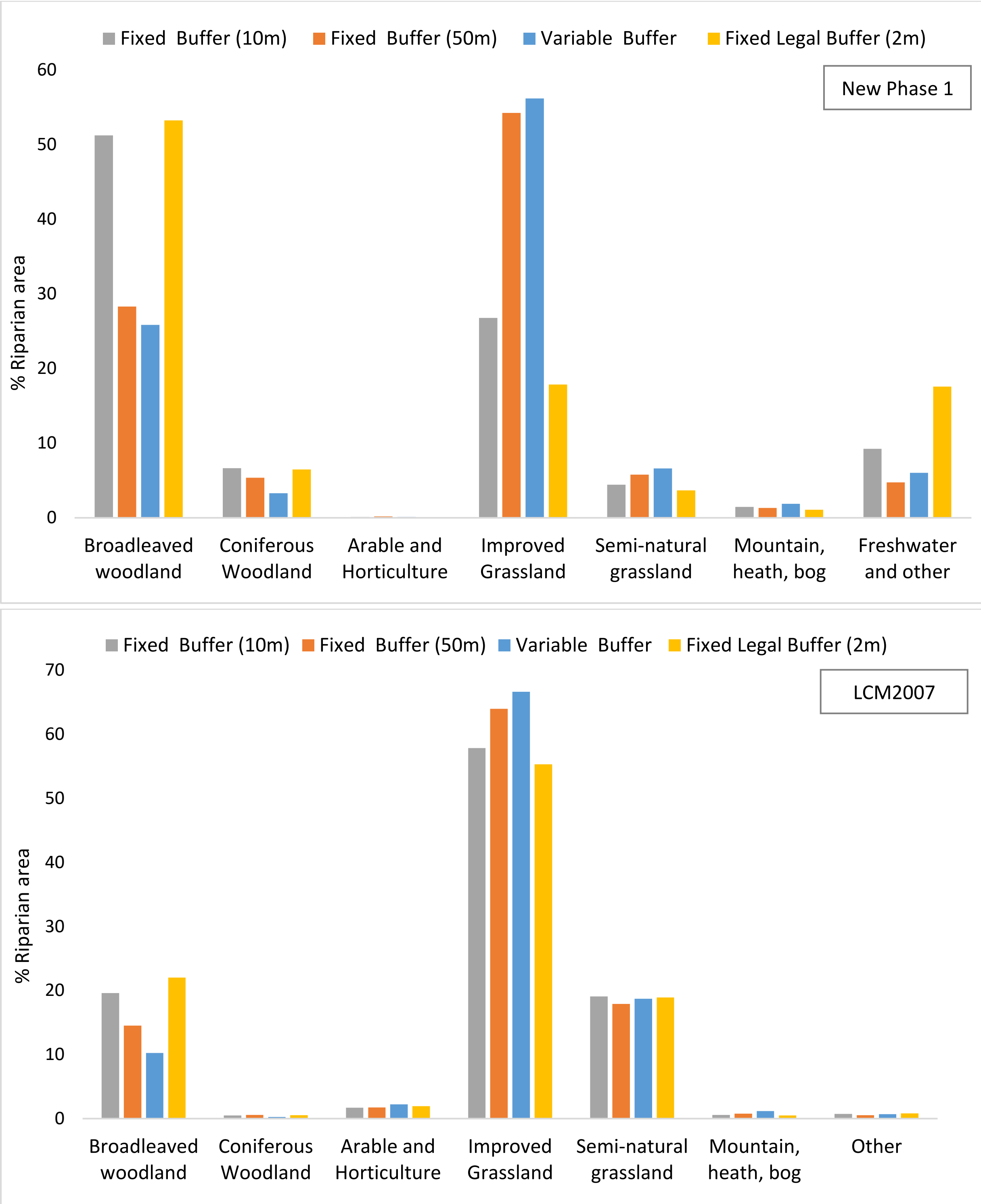

Figure 3.9 Comparison of the area of riparian habitat types determined using either New Phase 1 (Panel A) or LCM2007 (Panel B) national vegetation mapping datasets using four different riparian delineation methods for sub-catchment 1.

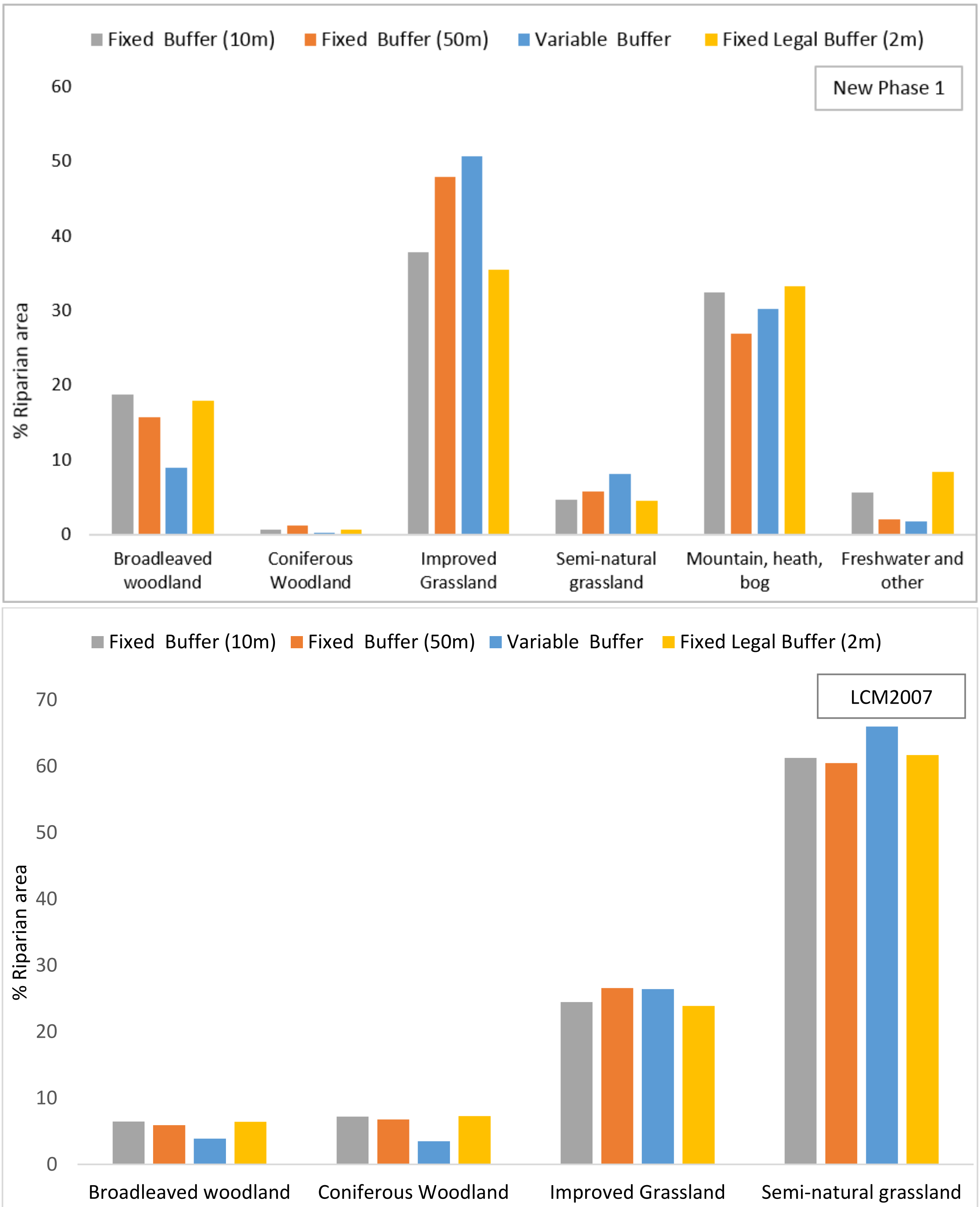

Figure 3.10 Comparison of the area of riparian habitat types determined using either New Phase 1 (Panel A) or LCM2007 (Panel B) national vegetation mapping datasets using four different riparian delineation methods for sub-catchment 2.

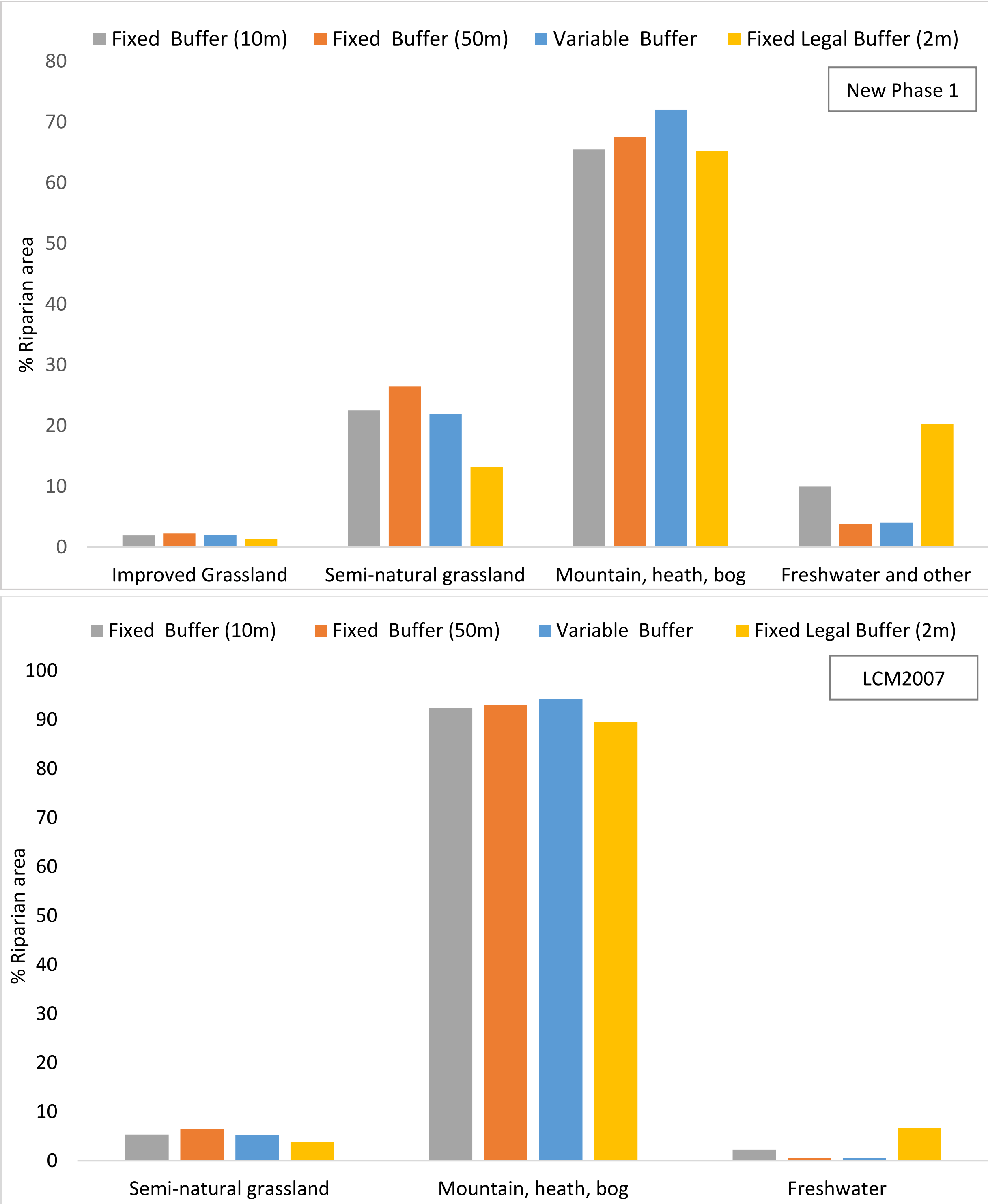

Figure 3.11 Comparison of the area of riparian habitat types determined using either New Phase 1 (Panel A) or LCM2007 (Panel B) national vegetation mapping datasets using four different riparian delineation methods for sub-catchment 3.

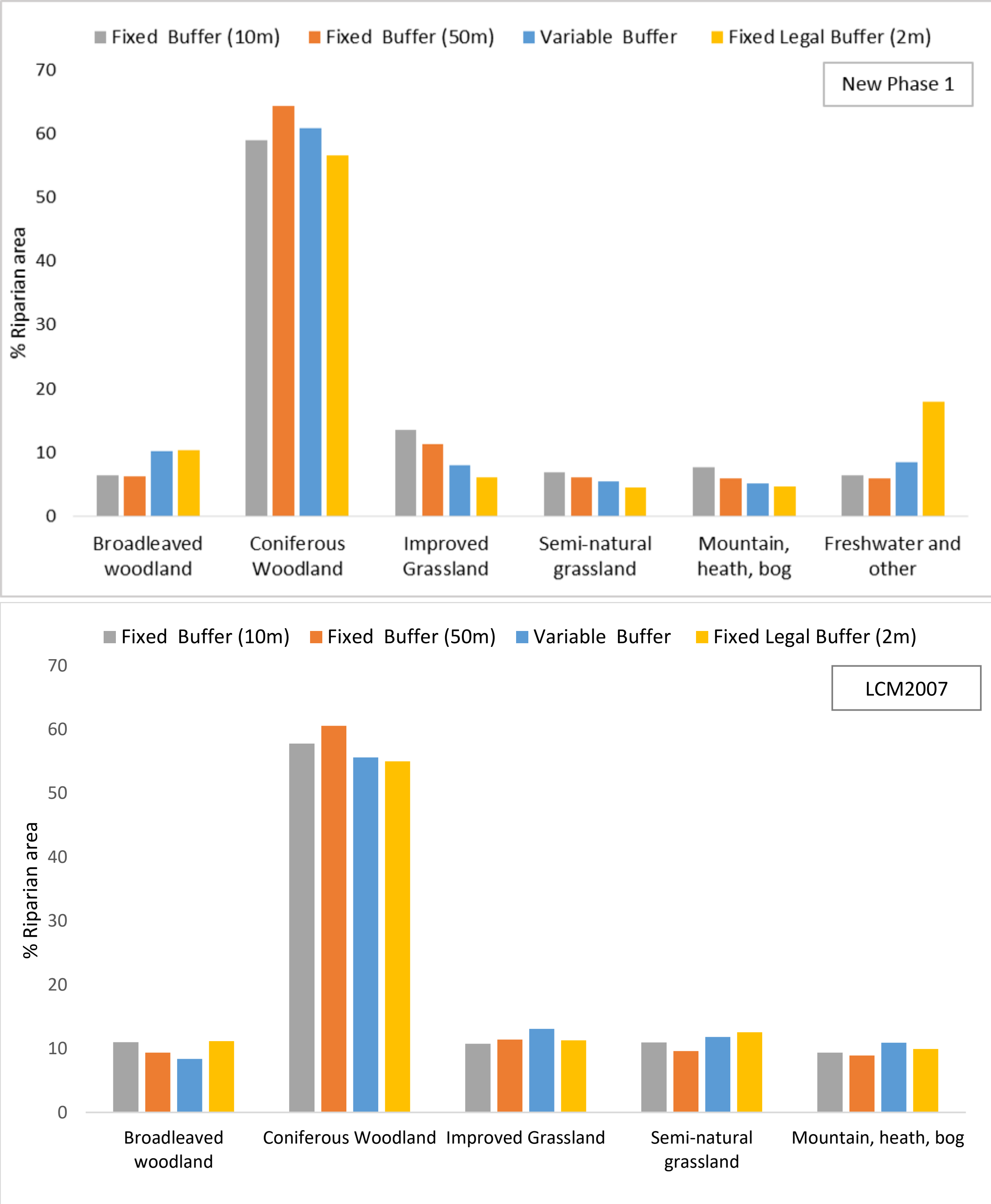

Figure 3.12 Comparison of the area of riparian habitat types determined using either New Phase 1 (Panel A) or LCM2007 (Panel B) national vegetation mapping datasets using four different riparian delineation methods for sub-catchment 4.

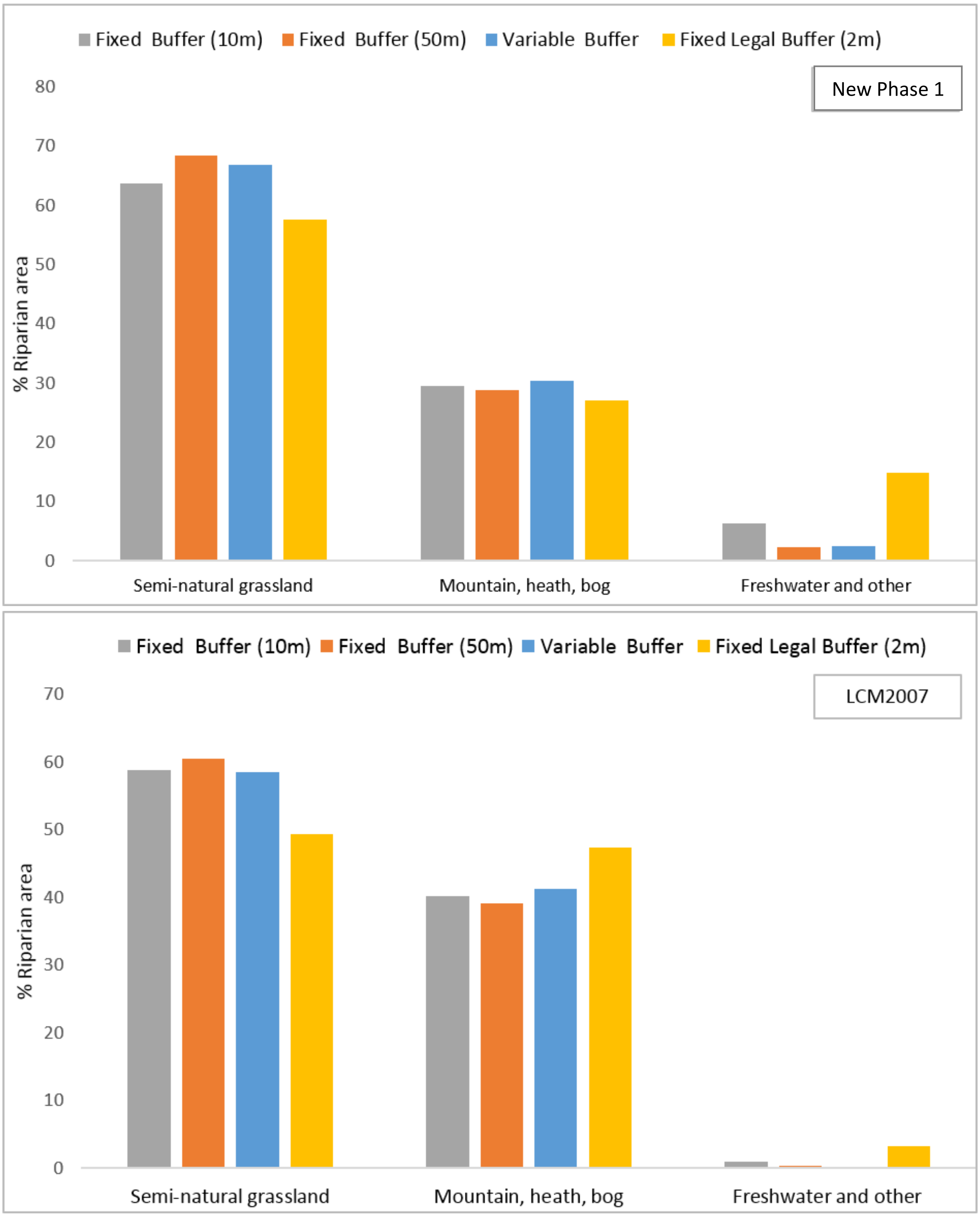

Figure 3.13 Comparison of the area of riparian habitat types determined using either New Phase 1 (Panel A) or LCM2007 (Panel B) national vegetation mapping datasets using four different riparian delineation methods for sub-catchment 5.

3.4 Discussion

3.4.1 Critical evaluation of the differing riparian delineation approaches

Previous studies have attempted to determine the most efficient way to identify riparian areas and the multiple ecosystem services they provide (Hawes and Smith, 2005; Holmes and Goebel, 2011; Fernández et al., 2012). In this work, we show that different delineation approaches greatly influence the total predicted riparian area within a sub-catchment, their spatial land patterning and the subsequent distribution of habitats present within these areas. In reality; however, riparian boundaries are rarely discrete, and no single approach can be expected to adequately capture all the features of riparian areas, particularly as our mechanistic and quantitative understanding of some riparian functions is still lacking (e.g. hyporheic filtering of nutrients, groundwater flow and recharge rate, riparian biodiversity; Hanula et al., 2016; Hathaway et al., 2016; Doble and Crosbie, 2017; Swanson et al., 2017). Further, riparian zones are typically both spatially heterogeneous (vertically and horizontally) and temporally dynamic with strong interactions between the aquatic and terrestrial component (Broder et al., 2017). This frequently results in diffuse and continuously changing riparian limits (Lindenmayer and Hobbs, 2008), in contrast to our riparian boundaries which are both static in time and spatially discrete. Moving forward, it would be useful to agree on a universal definition for riparian areas and the identification for reference values for riparian functions, similar to those which exist for agriculture (Gregory et al., 1991; Fischer et al., 2001; Hawes and Smith, 2005; Naiman et al., 2010; Xiang et al., 2016). Until this is established, and as evidenced here, estimating the spatial extent of riparian areas will be subject to considerable uncertainty and user bias. Establishing a common riparian framework is not impossible. McVittie et al. (2015) proposed a model applied to riparian areas that integrated physical attributes (land cover, soil type, rainfall), terrestrial and aquatic process (e.g. erosion, river flow) and management intervention using Bayesian Belief

Networks (BBN). Thus, the parameters introduced will ultimately aim to outline the fundamental ecological processes that deliver ecosystem services within riparian areas.

In achieving an effective riparian delineation, some theoretical and practical limitations in favour of, or against the fixed-width versus variable-width option were considered. The fixed-width riparian approach has been suggested by some authors to be inadequate for delineating riparian areas as it fails to take into account crucial factors such as geomorphology or stream order (Skally and Sagor, 2001; Holmes and Goebel, 2011). Consequently, some land areas might be incorrectly included or excluded in the buffer delineation. Additionally, this approach does not reflect the magnitude of the river and its associated floodplain (i.e. major and minor rivers). In this sense, some studies such as Peterson et al. (2011) have shown how stream order could be relatively easily incorporated into riparian models by using the strength of a decay function to weight the important of vegetation from close to the stream to further away. However, the results from this study arguably showed a close similarity in terms of surface area and patterns of land cover distribution between the fixed 50 m width approach and the variable-width riparian buffer, even though the latter was constructed more robustly by including digital elevation data, soil and hydrologic descriptors of riparian areas (Abood et al., 2012). Moreover, the digital spatial comparison of the above-mentioned buffers revealed a spatial agreement of ca. 70-83% between the two methods. Whether this percentage is acceptable or sufficient depends on the goals of the study undertaken in terms of ecosystem service provision and the potential value that a particular riparian area can achieve. For instance, this percentage disagreement could be pivotal for those areas designated as being at risk from agricultural pollution (i.e. Nitrate Vulnerable Zones, NVZ) which might require a higher level of protection and precision in their delineation. Moreover, from a management perspective, riparian areas often constitute zones excluded from productivity which greatly affect stakeholders (e.g. farmers) considering the profound impact on the costs associated with the buffer width chosen (Ahnström et al., 2009; Roberts et al., 2009). Additionally, it is worth noting that some riparian areas responsible for important ecosystem services within agricultural catchments such as nutrient cycling or water regulation, might require a more thorough assessment than those with recreational and aesthetic values as the main ecosystem service outcome.

Few riparian delineation studies have highlighted drawbacks associated with the variable-width buffer approach. These may include, however, the heavy dependency of these methodologies on accurate and precise digital information (e.g. DEM, soil data), the need for up-to-date datasets and some technical expertise to reality check the predictions (Phillips et al., 2000; Aunan et al., 2005). In our study, the determination of the 50-yr flood height as a crucial parameter for the model led to additional time-consuming tasks due to the lack of available hydrological data (e.g. flow rate, velocity or channel width) for our sub-catchments. As we were unable to get this hydrological parameter from existing methodologies (Mason, 2007; Abood et al., 2012), manual tracing of the cross-sections along the main rivers and a computation of the 50-yr flood discharge to generate the water surface elevation was required. This additional, component greatly increased the time required to successfully define the riparian boundary by comparison with the fixed-width approach. However, as better digital data (e.g. high-resolution soils and land cover datasets or real-time water quality and flow data) become available, variable-width approaches will become much more efficient and precise than the fixed-width approach.

3.4.2 Influence of DEM on model outcome

The clear need for using a precise digital elevation dataset in the variable-width model was demonstrated here. Abood et al. (2012) observed an increase in the riparian land included in the delineation process when using a coarser spatial resolution of the DEM. A similar finding was also reported by Papaioannou et al. (2016) when flood risk mapping. The difficulty arises in detecting incremental changes in elevation, especially in steep areas where the elevation usually changes abruptly. Our study also supports these conclusions for the 5 and 10 m spatial resolution DEMs. However, in our case, the results from the 30 and 50 m spatial resolution DEMs encompassed between 2 and 5 times smaller total riparian surface (km^2) respectively than obtained at a 2 m spatial resolution. Analysis of the 2 m resolution DEM compared to the 30 m resolution DEM revealed a discordance in elevation of up to 290 m in some cases. As a result, the stream network obtained from much higher resolution data failed to match the coarser resolution DEM. Consequently the 50 year flood height estimation was probably underestimated, directly impacting upon the final riparian delineation. In addition, the maximum transect length of 250 m was clearly insufficient for such a coarse resolution. The same was also true for the 50 m resolution DEM.

3.4.3 Limitations of riparian soil mapping

The National Soil Map at 1:250,000 scale was the only available dataset with full coverage in our study area (SSEW, 1983). During characterisation of the sub-catchments and on assessment of model performance, it became clear that its resolution was inadequate for small-scale applications, such as riparian delineation. The best-available soil maps for the UK are at 1:63,000 scale, however, these only have limited coverage and may still contain significant errors, particularly for soil types of limited spatial extent, as exemplified by riparian soils (Mayr et al., 2008). Of these national 1:63,000 maps, most were completed over 50 years ago and have never been updated. Over time, it can be expected that some soil features may also have changed due to changes in policy and land management regime (e.g. afforestation, fencing, drainage, riverbank stabilization). Further, climate change may also have altered their properties (e.g. changes in soil C content or hydrological regime; Keay et al., 2014). The impact of these factors on riparian soil classification remains unknown, but it adds extra uncertainty to the model outputs. Based on the cost of undertaking ground-based soil surveys, however, it is unlikely that the poor availability of soil data will improve in the near future. The recent availability of high-spatial-resolution satellite and high-spectral-resolution aircraft imagery has significantly improved the capacity for mapping riparian buffers, wetlands, and other ecosystems and potentially the soils contained within them (Makkeasorn et al., 2009; Forzieri et al., 2010). However, satellite sensors still do not have the combined spatial and spectral resolution to reliably identify buffer vegetation types and conditions, let alone soils (Klemas, 2014).

3.4.4 Riparian habitat mapping

Comparison of the two national land cover datasets raised some interesting issues. Firstly, we noted that regardless of riparian delineation method, both datasets produced noticeable differences in the coverage of different habitat types within riparian areas. For instance, there is evidence that in the sub-catchment 2, the criteria used for the classification of the habitat type is different for both datasets (e.g. Mountain, heath and bog versus Semi-natural grassland). This variability is most likely due to the much finer scale resolution of the Phase 1 map in which habitat surveying is both ground- and digital-based (nominal resolution 5 m), compared to LCM2007 that is based largely on remote sensing and digital processing. This fact reveals that comparison of outputs from models run using different underpinning datasets may be problematic and could have severe implications. It should also be noted that small areas of vegetation (<0.01 ha) will also be missed by most land cover maps. In this sense, ecosystem services may be incorrectly assigned due to strong correlation between land cover type and ecosystem service provision (Burkhard et al., 2009; Peterson et al., 2011; Maes et al., 2011). For example, Sgouridis and Ullah (2014) established a link between land cover and land use management with denitrification potential. The importance of accurate habitat identification is also endorsed by studies like Tscharntke et al. (2005) which showed that local habitats might be essential to improve the delivery of ecosystem services, enhancing local diversity and providing a natural corridor of special importance in simple landscapes dominated by arable fields. On the other hand, Fisher et al. (2009) stressed that ecosystem services were not homogeneous across landscapes. Therefore, if riparian models rely on accurate datasets, able to capture the landscape heterogenity, we could better predict the way that services can be managed, protected and monitored across spatial and temporal scales. From this point of view, De Groot et al. (2010) also added that furthering our understanding of the threats and underlying mechanisms at the landscape scale will help better target our resources where the enhancement of the service is needed most.

Differences in the precision and accuracy of digital data could lead to a misinterpretation of the relative position and structure of a particular habitat within riparian zones. This may be particularly problematic for very narrow riparian areas whose habitat type will not be captured (Scholefield et al., 2016). Previous studies have reported that minimal changes in land use might affect ecosystem service provision (Bennett et al., 2009; Raudsepp-Hearne et al., 2010). Brenner et al. (2010) identified that small boundary habitat adjustment could heavily influence the estimation of ecosystem services. Therefore, the over- or under-estimation of the habitats included within riparian areas might influence the ecological and economic value and could lead to an improper use as well as its need for protection.

It is also worth mentioning that although it is important to include riparian physical features into models (i.e. 50-year flood height optimal hydrologic descriptor of a riparian ecotone) that help us to predict their location, a thorough assessment of the resource to be addressed and the particular ecosystem provision being targeted should also be incorporated. The majority of the models follow the trend described in Verry et al. (2004) where it is suggested that the functional riparian delineation (named here as the variable-width approach) is a probabilistic approach based on a most likely predicted extent of riparian areas which are connected with physical patterns (e.g. stream valley geomorphology to predict flood-prone areas). However, apart from physical patterns, we strongly believe that there is a need to link riparian buffers with the ecosystem services they provide and ensure that the width selected is adequate to undertake the function. Results from different studies support this statement. For example, Peterjohn and Correll (1984) established that sediment removal rates by riparian buffers in agricultural catchments only increased by 4% despite more than doubling the buffer width. This suggests that approaches such as a fixed-width buffer (10 m) or the legal approach (2 m), might be sufficient to accomplish certain ecological functions. On the contrary, other studies have showed that a 10% increase in phosphorus removal could be accomplished by extending the buffer width by a factor of 2.5 (Wenger, 1999). Therefore, the implementation of a more restrictive buffer might not preserve the habitat requirements. Consequently, using functional models which detect physical attributes in riparian areas in addition to the incorporation of the spatial supply of ecosystem services, that is its functionality, would greatly strengthen not only riparian delineation but also its understanding.

3.5 Conclusions

The results of this study revealed substantial differences in terms of spatial distribution, total riparian area delineated and land cover patterns depending on the delineation method employed and the spatial data available. Although simple, the single-width buffer approach lacked both consistency and any underpinning scientific rationale for mapping and classifying riparian areas. We conclude that this approach is likely to lead to gross inaccuracies and therefore should not generally be used. The exception to this is where the buffer strip is made sufficiently wide to allow capture of some site-specific ecosystem services, at which point it could prove valuable for assessment and planning purposes without requiring much investment in money or time. In contrast, the variable-width buffer approach, despite being robust enough to recognise the multiple interactions that take place within riparian areas, relies heavily on accurate and up-to-date digital datasets and is more difficult to implement. Nevertheless, the possibility of incorporating a specific dataset into the model to predict riparian zones allows the opportunity to tailor a riparian area for every catchment according to its specific characteristics. The selection of a particular method to delineate riparian areas and the accuracy of the underpinning datasets heavily influences the predicted land cover distribution within the riparian area. This will in turn determine future management activities to target riparian ecosystem services. Our results have led to the development of new concepts for riparian delineation based on ecosystem service-specific scenarios. Outcomes from our study suggest that riparian delineation within UK habitats should be specific to the particular ecosystem service(s) of interest (e.g. uptake of nutrients, shading, etc.).

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Chapter 4

Quantifying the contribution of riparian soils to the provision of ecosystem services

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L.L.S and D.L.J and P.A.W designed and conceived the experiment. L.L.S conducted the experimental work, analysed the data and prepared the manuscript. All authors discussed results and contributed to preparation of the manuscript.

Abstract

Riparian areas, the interface between land and freshwater ecosystems, are considered to play a pivotal role in the supply of regulating, provisioning, cultural and supporting services. Most previous studies, however, have tended to focus on intensive agricultural systems and only on a single ecosystem function. Here, we present the first study which attempts to assess a wide range of ecological processes involved in the provision of the ecosystem service of water quality regulation across a diverse range of riparian typologies. Specifically, we focus on 1) evaluating the spatial variation in riparian soils properties with respect to distance from the river and soil depth in contrasting habitat types; 2) gaining further insights into the underlying mechanisms of pollutant removal (e.g. pesticide sorption/degradation, denitrification, etc) by riparian soils; and 3) quantify and evaluate how riparian vegetation across different habitat types contribute to the provision of watercourse shading. All the habitats were present within a single large catchment and included: (i) improved grassland, (ii) unimproved (semi-natural) grassland, (iii) broadleaf woodland, (iv) coniferous woodland, and (iv) mountain, heath and bog. Taking all the data together, the riparian soils could be statistically separated by habitat type, providing evidence that they deliver ecosystem services to differing extents. Overall, however, our findings seem to contradict the general assumption that soils in riparian areas are different from neighbouring (non-riparian) areas and that they possess extra functionality in terms of ecosystem service provision. Watercourse shading was highly habitat specific and was maximal in forests (ca. 52% shade cover) in comparison to the other habitat types (7-17%). Our data suggest that the functioning of riparian areas in less intensive agricultural areas, such as those studied here, may be broadly predicted from the surrounding land use, however, further research is required to critically test this across a wider range of ecosystems.

Key Words: *E. coli* O157; freshwater corridors; land use; riverbanks, nutrient removal; wetlands.

4.1 Introduction

Ecosystem service-based approaches have been increasingly used to reduce pressure on natural resources and implement better land-management practices with respect to the environment (Van Looy et al., 2017). Riparian areas, the interface between land and freshwater ecosystems, are considered to play a pivotal role in the supply of regulating, provisioning, cultural and supporting services (Jones et al., 2010; Clerici et al., 2011; Aguiar et al., 2015). However, despite the fact that the number of studies referring to ecosystem services has increased by 38% in Europe over the last 20 years (Adhikari and Hartemink, 2016), riparian zones have received less attention than other land use types from an ecosystem services perspective. The few publications which have integrated an ecosystem service approach to the assessment of riparian areas have tended to address this from a modelling perspective (Clerici et al., 2014; Tomscha et al., 2017; Sharps et al., 2017). McVittie et al. (2015) proposed a model which aims to outline the fundamental ecological processes that deliver ecosystem services within riparian areas. Models provide a powerful and cost-effective tool to assess and map ecosystem services at the landscape scale; however, they do not always provide a mechanistic process-level understanding. It is therefore important that models are supported and developed with robust underpinning data to correctly identify and describe the main factors affecting ecosystem services delivery within complex landscapes (i.e. those which may contain a diverse array of different riparian typologies). Little is known, however, about how inherent riparian properties and ecosystem functioning vary across different habitats within a catchment area (Burkhard et al., 2009). This uncertainty is largely due to the majority of riparian studies being focused on single sites, typically intensive agricultural systems (e.g. arable and grasslands) as these represent a major source of pollution (e.g. from fertilizers, livestock and pesticides) and because riparian zones associated with agriculture present pollution mitigation potential (Pierson et al., 2001; Rasmussen et al., 2011; Broetto et al., 2017). However, these studies tend to overlook the fact that riparian areas are inter-related systems and therefore changes (both natural and anthropogenic) occurring in headwater riparian zones across different habitat types could also affect riparian processes occurring downstream (Harper and Everard, 1998; Charron et al., 2008).

Among the many ecosystem services attributed to riparian areas, their role in water quality enhancement has grown in recognition over the years. Water quality has become a universal problem (Stephenson and Pollard, 2008) and is nowadays considered a priority objective for EU environmental sustainability (EEA, 2012). Increased loss of phosphorus (P) and nitrate (NO_3^-) from agricultural fertilizers has led to extensive eutrophication of surface and groundwaters (EEA, 2005), and contamination by pesticides and biological contaminants (e.g. bacteria) are regularly reported (Klapproth and Johnson., 2009; Troiano et al., 2001). Riparian areas are frequently proposed as a management strategy to reduce freshwater nutrient pollution (e.g. Coyne et al., 1995; O'Donnell and Jones, 2006; Stutter et al., 2009; Aguiar et al., 2015; Sgouridis and Ullah, 2015) and could also reduce the cost of drinking water purification (Klapproth and Johnson., 2009; Meador aand Goldstein, 2003; Chase et al., 2016). This pollution mitigation potential is often attributed to specific characteristics within riparian soils (Mikkelsen and Vesho, 2000; Naiman et al., 2010). Table 4.1 summarizes the link between riparian soil properties and the provision of ecosystem services found in the literature. A better understanding of the causal factors for ecosystem services delivery will provide an improved knowledge base on which to make land management decisions and protection policies.

Table 4.1 Summary of riparian soil characteristics and their associated provision of ecosystem services.

Ecosystem services	Causal factor	Resulting soil characteristics
Supporting services Soil formation Nutrient cycling **Regulating services** Water purification by reducing non-point source pollutants Flood and erosion regulation by slowing and spreading flood water	• Periodic sediment deposition together with flushes of organic litter during floods events • Large variation of soil chemical composition mainly due to filtration and nutrient removal from terrestrial upland and aquatic ecosystems	Heterogeneity (Mikkelsen and Vesho, 2000)
Supporting services Biodiversity **Regulating services** Carbon sequestration **Provisioning services** Shading by vegetation	• High vegetation density and diversity associated with higher moisture and organic matter content which leads to more microbial activity • Provide (roots, fallen logs) refuge for aquatic and terrestrial fauna	Biological diversity (Naiman et al., 2010)
Supporting services Soil formation **Regulating services** Carbon sequestration	• New material (organic matter fluxes and sediments) being deposited by flood events and water fluctuation • Regular inundation of soils by river water preventing horizon formation	Undeveloped soils (Zaimes et al., 2007)
Regulating services Water storage	• Their proximity with the river enhances water storage and infiltration	High moisture content (Lewis et al., 2003)
Regulating services Fast engineering resilience[1]	• Anthropogenic activities such as farming, water abstraction, livestock and deforestation • Frequent environmental disturbances such as floods or droughts	Disturbance driven (Klemas, 2014)

[1] Speed with which a system returns to equilibrium after a disturbance (Holling, 1996).

Many regulating services are highly affected by environmental conditions. For example, temperature is known to directly and indirectly affect biological activity through its impact on gaseous concentrations in soil (e.g. CO_2/O_2) and in the water column (Beschta, 1997; Verberk et al., 2016). It also plays an important role in determining the rate of key ecosystem processes such as denitrification (Bonnett et al., 2013). Riparian buffers have increasingly been used as a eutrophication mitigation tool by temperature regulation through provision of shade (Nisbet and Broadmeadow, 2004; Burrell et al., 2014; Johnson and Wilby, 2015). Ghermandi et al. (2009) suggested that shading could viably be used as a management option to improve water quality conditions in small and moderately-sized watercourses. However, finding a cost-effective way to target vulnerable areas is challenging and has been poorly explored to date.

The main focus of this study is to assess the link between riparian areas and the regulating service of water purification through a wide range of ecological processes. In particular, we aim to: 1) evaluate the spatial variation in riparian soils properties (e.g. general nutrient status, soil acidity and conductivity, and microbial community size) with respect to distance from the river and soil depth in contrasting habitat types; 2) gain further insights into the underlying mechanisms of pollutant removal (e.g. pesticide sorption/degradation, denitrification, etc) by riparian soils; and 3) quantify and evaluate how riparian vegetation across different habitat types contribute to the provision of shade. This could help identify areas especially vulnerable to excessive solar radiation and offer a cost-effective way to improve ecosystem service provision (Ghermandi et al., 2009; De Groot et al., 2012). We hypothesized that riparian areas would support a greater delivery of ecosystem services in comparison to the upslope area, but that the balance of these services would be land use specific within a catchment area.

4.2 Materials and methods

4.2.1 Site description

The Conwy catchment was chosen as a demonstration test site for this study due to its extensive use in previous ecosystem service monitoring studies (Emmett et al., 2016). It is located in North Wales, UK (3°50’W, 53°00’N) and comprises a total area of 580 km^2 (Figure. 4.1). The elevation ranges from sea level to 1060 m, with rainfall ranging between 500 to 3500 mm y^{-1} and the catchment has a mean annual temperature of 10 °C. Together, the topography, parent material and climate have given rise to a wide range of soil types within the catchment of which the dominant ones include Eutric Cambisols, Endoskelectic Umbrisols, Albic Podzols and Sapric Histosols (WRB, 2014). It is predominantly a rural catchment, with livestock farming (sheep and cattle) being the main land-uses. The two main habitat types are improved (predominantly limed and fertilised) and unimproved grassland in the lower altitudes to the east and mountain (exposed rock), heathland and bog in the western part of the catchment. Extensive areas of coniferous (plantation) forestry and semi-natural deciduous woodland can also be found in the upper reaches of the catchment.

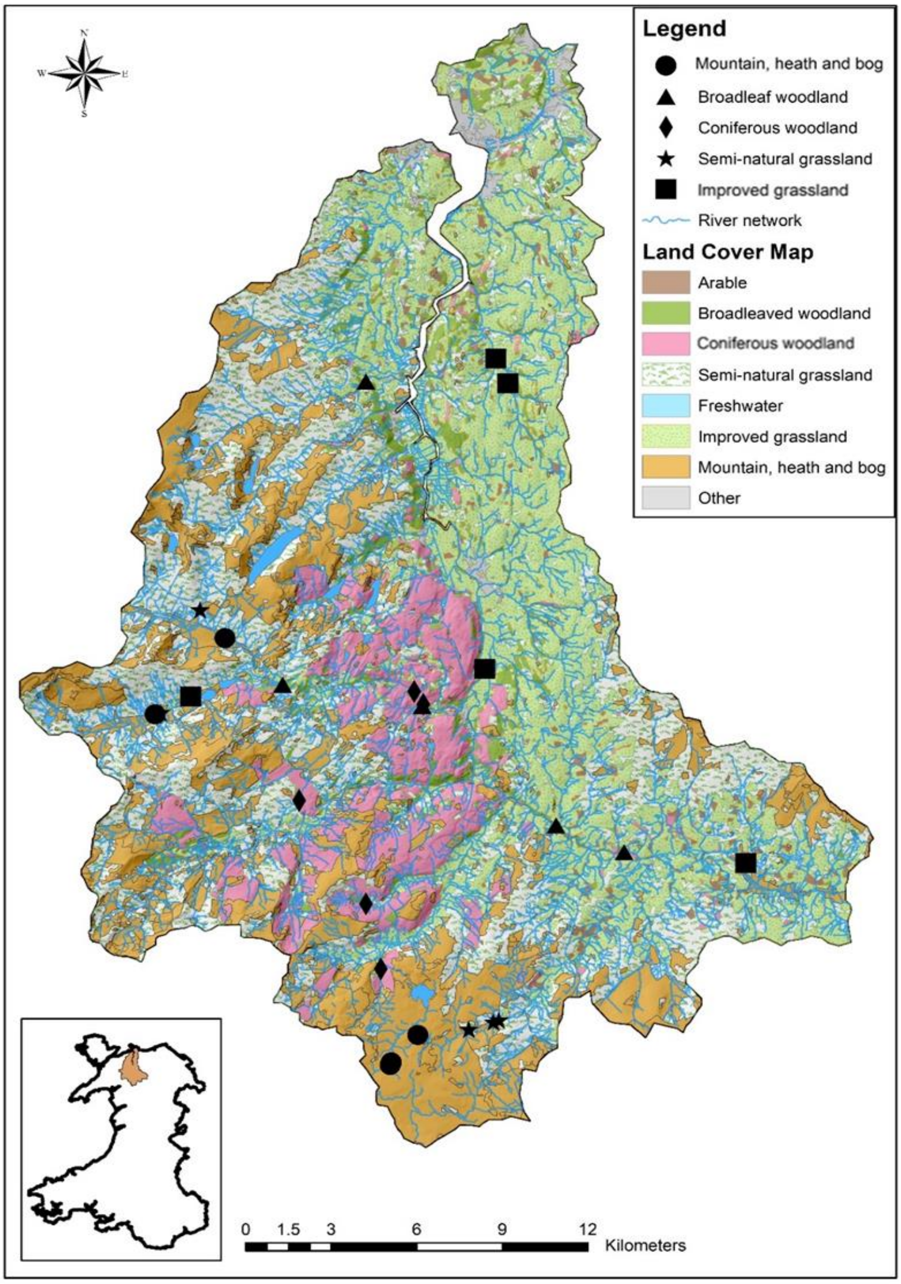

Figure 4.1 The Conwy catchment, North Wales, UK showing location of sample points, land cover classes (Lucas et al., 2011) and river network. Sample sites were distributed within the five dominant habitat types in the Conwy catchment (mountain, heath and bog, broadleaf and coniferous woodlands, semi-natural grassland and improved grassland) and each symbol represents a pair of sample points, one at 2 m and another at 50 m distance from the river system (*n* = 10).

4.2.2 Field sampling

Five dominant habitat types (MHB = mountain, heath and bog; BW = broadleaf woodland; CW = coniferous woodland; SNG = semi-natural grassland; IG = improved grassland) were selected for soil sampling throughout the catchment. Habitat classification was derived from the new Phase 1 National Vegetation Survey (Lucas et al., 2011) and subsequently grouped, for simplicity, into the same broad habitat classes (see Appendix 1 for details of groupings) defined in the UK's Land Cover Map 2007 (Morton et al., 2014).

Independent riparian sampling areas (*n* = 5) were selected from each of the 5 dominant habitat types. At all sites, soil was collected at 2 m distance from a river and 50 m from a river, which is regarded as the maximum extent of the riparian buffer zone and which contained a different vegetation from that close to the river (de Sosa, 2017). The sampling was designed to enable a direct comparison of how soil properties are influenced by proximity to the river.

Intact soil cores (5 cm diameter, 30 cm long) were collected using a split tube sampler (Eijklekamp Soil and Water, Giesbeek, The Netherlands) and separated into top- and sub-soil fractions (0-15 cm and 15-30 cm depths respectively), stored in gas-permeable plastic bags and transported to the laboratory for immediate analysis. These depths reflect the main rooting zones in the soil profile (Glanville et al., unpublished data). In addition, the depths were chosen to be consistent with those used in the national surveys for assessing changes in soil ecosystem service delivery and which are used to directly inform land use policy at the national-level (Countryside Survey, Glastir Monitoring and Evaluation Programme; Emmett et al., 2010, 2016; Norton et al., 2012).

4.2.3 Soil characterisation

Soil samples were sieved (< 2 mm) to remove stones and any visible plant material and to ensure sample homogeneity (Jones and Willett, 2006). Samples were then stored at 4 °C prior to laboratory analysis. Soil water content was determined gravimetrically (24 h, 105 °C) and soil organic matter (SOM) content was determined by loss-on-ignition (LOI) (450 °C, 16 h). Soil pH and electrical conductivity (EC) were measured using standard electrodes in a 1:2.5 (w/v) soil-to-deionised water mixture. Total available ammonium (NH_4-N) and nitrate (NO_3-N) were determined with 0.5 M K_2SO_4 extracts (Jones and Willett, 2006) with colorimetric analysis following the salicylate-based procedure of Mulvaney (1996) and the VCl_3 method of Miranda et al. (2001), respectively. Available P was quantified with 0.5 M acetic acid extracts (1:5 w/v) following the ascorbic acid-molybdate blue method of Murphy and Riley (1962) and total C (TC) and N (TN) were determined with a TruSpec® elemental analyser (Leco Corp., St Joseph, MI). Dissolved organic C (DOC) and total dissolved N (TDN) were quantified in 1:5 (w/v) soil-to-0.5 M K_2SO_4 extracts using a Multi N/C 2100 TOC analyzer (AnalytikJena, Jena, Germany) (Jones and Willett, 2006). Microbial biomass C and N was assayed by chloroform fumigation-extraction after a 72 h incubation using conversion factors of $k_{ec} = 0.45$ and $k_{en} = 0.54$ (Vance et al., 1987).

4.2.4 Process-level studies to measure ecosystem services

A series of process-level studies were conducted to investigate how soils across different habitats contribute to the regulation of important ecosystem services involved in pollutant attenuation. In addition, we aimed to assess how habitat influences the provision of shade and the impacts on temperature regulation. For all experiments, field-moist soil (n = 5) was used to best represent field conditions.

4.2.4.1 Phosphorus sorption to soil

P adsorption isotherms were determined to estimate the soil's capacity for removing dissolved P from solution, and hence assess the potential for soils to reduce the amount of P entering freshwaters. Sorption of P was determined following an adapted method of Nair et al. (1984). In brief, 2.5 g of field-moist soil was shaken in 0.01 M $CaCl_2$ (1:5 w/v soil-to-extractant ratio) containing known concentrations of P (0, 0.3, 1, 5, 10, 20 mg P l^{-1} as KH_2PO_4) spiked with ^{33}P (PerkinElmer Inc., Waltham, MA) (0.2 kBq ml^{-1}). These concentrations were selected due to their likelihood of being encountered in the catchment (DeLuca et al., 2015). Samples were shaken (2 h, 150 rev min^{-1}, 25 °C) on an orbital shaker. This time was chosen to assess intermediate equilibrium conditions (Santos et al., 2011). After 2 h, 1.5 ml of supernatant was removed, centrifuged (10,000 g, 5 min), and subsequently, 1 ml of supernatant was mixed with 4 ml of Optiphase HiSafe 3 liquid scintillation fluid (PerkinElmer Inc.). The amount of ^{33}P activity remaining in solution measured using a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK) and the total amount of P adsorbed was determined as the difference between the initial ^{33}P activity added and the final amount of ^{33}P remaining in solution. Any P not recovered in the solution was assumed to be sorbed onto the soil's solid phase.

Sorption isotherms were examined according to the linearized form of the Langmuir equation to estimate the P adsorption maxima and the P sorption binding energy for P (Reddy and Kadlec, 1999; Mehdi et al., 2007):

$$C/S = (1 / k \times S_{max}) + (C/S_{max}) \qquad \text{(Eqn. 1)}$$

where S is the amount of P adsorbed (mg P adsorbed kg^{-1}), C is the equilibrium solution concentration after 2 h (mg P l^{-1}), S_{max} is the P adsorption maximum (mg kg^{-1}), and k is a constant related to the bonding energy (l mg^{-1} P).

4.2.4.2 Bacterial pathogen survival

Soils from different habitat types were inoculated with human-pathogenic *Escherichia coli* O157:H7 to investigate pathogen persistence in soils with respect to proximity to waterbodies. Faecal samples, collected from a commercial beef farm in North Wales in January 2016, were inoculated with *E. coli* O157:H7 to reproduce the natural vector by which the pathogen is introduced into the environment (Jones, 1999; Williams et al., 2008). Samples were transported to the laboratory and stored at 4.0 ± 0.1 °C prior to use. Both faecal and soil samples were previously screened for the background *E. coli* O157:H7 cells using an enrichment technique (Avery et al., 2008) and absence of *E. coli* O157:H7 was confirmed by latex agglutination (Oxoid DR620; Oxoid Ltd., Basingstoke, UK). Prior to the start of the experiment a basic characterization of the faecal samples was undertaken, and moisture content, organic matter, EC, pH, NO_3-N, NH_4-N and P determined as previously described. The bacterial inoculum was prepared from a fresh overnight culture (LB broth; 18 h, 37 °C, 150 rev min^{-1} on an orbital shaker) of two environmental isolates of *E. coli* O157:H7 (strains #2920 and #3704) (Campbell et al., 2001; Ritchie et al., 2003). A 40 ml aliquot of the *E. coli* O157:H7 was added to 360 g of cow faecal samples and thoroughly mixed to deliver a final concentration of approximately 10^8 cfu g^{-1} faeces (to reproduce the highest natural concentration encountered; Besser and Richards, 2001; Fukushima and Seki, 2004). In brief, 5 g of faeces spiked with *E. coli* O157:H7 was added to 5 g of soil in a sterile 50 ml polypropylene tube and incubated at 10 °C (mean annual temperature for the catchment) for 1, 3, 7 and 14 d. After each incubation time, samples were placed on an orbital shaker (150 rev min^{-1}, 15 min, 37 °C) with 20 ml of sterile quarter-strength Ringers solution (Oxoid Ltd.), followed by 4 × 3 s bursts on a vortex mixer. Serial dilutions were plated in duplicate onto Sorbitol MacConkey agar (SMAC) (Oxoid Ltd.), then incubated (37 °C, 20 h) and colonies enumerated. Presumptive *E. coli* O157:H7 colonies were confirmed via latex agglutination as described previously.

4.2.4.3 Pesticide sorption and degradation in soil

The s-triazine herbicide, simazine ($C_7H_{12}ClN_5$; Water solubility, 5 mg l^{-1}; K_{ow}, 2.2; pKa, 1.6), was selected to investigate the fate of a common pesticide when applied to soils influenced by different environmental factors.

Simazine sorption followed the procedure of Jones et al. (2011). Briefly, 5 ml of ^{14}C-labelled simazine (final concentration 0.5 mg l^{-1}; 0.02 kBq ml^{-1}) was added to 2.5 g of soil contained in 20 ml polypropylene vials. The samples were then shaken (15 min, 200 rev min^{-1}) to reflect instantaneous equilibrium conditions (Kookana et al., 1993). The extracts were then centrifuged (10,000 *g*, 5 min) and the supernatant mixed with Scintisafe 3® scintillation cocktail (Fisher Scientific, Leicestershire, UK). The ^{14}C activity remaining in solution was then determined as described before. The simazine partition coefficient, K_d, was determined as follows:

$$K_d = C_{ads}/ C_{sol} \qquad \text{(Eqn. 2)}$$

where C_{ads} is the amount of simazine sorbed (mg kg^{-1}) and C_{sol} is the equilibrium solution concentration (mg l^{-1}).

To determine how soil influences pesticide degradation, 5 g of soil was placed in individual 50 ml polypropylene tubes and ^{14}C-labelled simazine was added to the soil at a rate of 0.05 mg l^{-1} (0.25 µM; 0.2 kBq ml^{-1}). A 1 ml NaOH trap (1 M) was then placed into the tube to capture any $^{14}CO_2$ evolved. The tubes were hermetically sealed and placed at room temperature (25 °C). The first NaOH traps were replaced after 24 h and then every 5 d for 30 d. On removal, NaOH traps were immediately mixed with Optiphase HiSafe 3 scintillation fluid (PerkinElmer Inc.) and the amount of $^{14}CO_2$ captured was determined using a Wallac 1404 liquid scintillation counter. Total simazine degradation was calculated as the cumulative percentage of ^{14}C labelled CO_2 evolved at the end of the incubation period.

4.2.4.4 Nitrate loss from soil

Loss of nitrate via denitrification represents a major N loss pathway (Sgouridis and Ullah, 2015). Denitrification capacity was estimated using the acetylene inhibition technique (AIT) as described in Abalos and Sanz-Cobena (2013). Although the application of this technique presents limitations (i.e. poor diffusion of C_2H_2 into the soil and inhibition of NO_3^- production via nitrification), it has been widely used to give a qualitative estimate of denitrification activity (Groffman and Altabet, 2006; Estavillo et al., 2002; Tellez-Rio and García-Marco, 2015).

In brief, 20 g of field-moist soil was placed in 150 ml gas-tight polypropylene containers. Subsequently, KNO_3 (8 ml, 42.9 mM) was added to the soil to remove NO_3^- limitation, the containers sealed and placed under vacuum and filled with O_2-free N_2 gas to induce anaerobic conditions. Ten percent of the container headspace was then replaced with acetylene to block the conversion of N_2O to N_2 gas. The containers were put on a reciprocating shaker at 25 °C. After 0, 8 and 24 h, gas samples (10 ml) were removed with a syringe and stored in pre-evacuated 20 ml glass vials, refilled with O_2-free N_2 gas. Nitrous oxide was analysed by gas chromatography (GC) using a Clarus 500 GC equipped with a headspace autoanalyzer Turbomatrix (HS-40) (PerkinElmer Inc.). Emission rates and cumulative fluxes were determined as described by MacKenzie (1998) and Menéndez et al. (2006), respectively.

4.2.4.5 Water temperature regulation and riparian shading provision

A GIS-based methodology was used to determine the extent to which vegetation contributes to water channel shading in the different habitats. Based on the UK Environment Agency 'Keeping River Cool' programme (Lenane, 2012), a LiDAR dataset (2 m resolution Natural Resources Wales composite dataset) (Table 4.2) was used to provide a riparian shade map to quantify how different habitat types and their associated riparian zones contribute to shade provision. Using the ArcGIS Solar Radiation tool, we calculated the difference in average incoming solar radiation during the summer months (1st May to 30th Sept.) between two different elevation datasets to produce a measure of relative shade for the catchment. A Digital Terrain Model (DTM) provided the 'bare earth elevation' whereas a Digital Surface Model (DSM) provided the earth´s surface data including all objects on it. Differences in incoming solar radiation between these datasets indicate the likely amount of shade created by vegetation. Although the relative shade was calculated for the whole catchment, only the parts which overlap with rivers were considered. The Zonal Statistics function (Arc GIS) was used to attach the difference in solar radiation from the DTM and DSM to the water body features (clipped using a 25 × 25 m grid in order to make small but similar sized units to attach results) extracted from the OS Open Rivers dataset (Ordnance Survey, Southampton, UK). The resultant shapefile was exported to Excel where shading differences were ranked (1-20, with 1 being the least shaded and 20 the most shaded). The term "relative shading" was used to refer to those areas that appear to have more or less than others due to the effect of the vegetation. Finally, those areas which scored >10 on the ranking scale (higher provision of shade) were then analysed to assess the influence of the habitat type on shade provision. A 2 m margin was applied to each river, to ensure accurate intersection with the adjacent Phase 1 habitat classification (Lucas et al., 2011) to estimate the percentage occurrence of each habitat in relation to provision of shade.

Table 4.2 Data inputs and sources for the computational GIS tool.

Dataset	Scale	Data Type	IPR holder	Description
Digital Terrain Model	2 m	Raster	Natural Resources Wales	This dataset is derived from a combination of all data that is at 2 m resolution or better which has been merged and re-sampled to give the best possible coverage. Available at: https://data.gov.uk/dataset/lidar-terrainand-surfaces-models-wales
Digital Surface Model	2 m	Raster	Natural Resources Wales	This dataset is derived from a combination of all data that is at 2 m resolution or better which has been merged and re-sampled to give the best possible coverage. Available at: https://data.gov.uk/dataset/lidar-terrainand-surfaces-models-wales
OS Open Rivers	1:25,000	Shapefile	Edina Digimap	Water bodies polygons within the catchment.

4.2.6 Statistical analysis

For physicochemical soil properties, principal component analysis (PCA) was used to explore the spatial relationships of selected soil properties for the different habitat types. A two-way ANOVA was used to evaluate the interactions between physicochemical properties with distance from river and soil depth within each habitat type. For each ecosystem process, an independent t-test was performed to assess the influence of proximity to the river in terms of ecosystem service provision. Pearson correlations were used to explore the relationships between physicochemical properties and the results from the processing studies. All data were analysed for normality and homogeneity of variance with Shapiro Wilk's tests and Levene's statistics, respectively. Transformations to accomplish normality were done when necessary. For all statistical tests, $P < 0.05$ was selected as the significance cut-off value. Statistical analyses were performed with SPSS version 22 for Windows (IBM Corp., Armonk, NY).

4.3 Results

4.3.1 Soil properties

Principal Component Analysis (PCA) of the soil physicochemical variables of all samples across the five dominant habitat types (see Methods for acronyms) (n = 100, irrespective of distance or depth) identified two principal components (PC) which, together, explain 66% of the total variance within the dataset (Figure 4.2). Soil pH, available P, total C, total N, DOC and TDN correlated significantly (P < 0.001) with the positive axis of PC1, whilst microbial-N correlated significantly (P < 0.001) with the positive axis of PC2. Soil moisture, organic matter, available NH_4-N and microbial-C correlated significantly (P water table fluctuation < 0.01) with both PC1 and PC2.

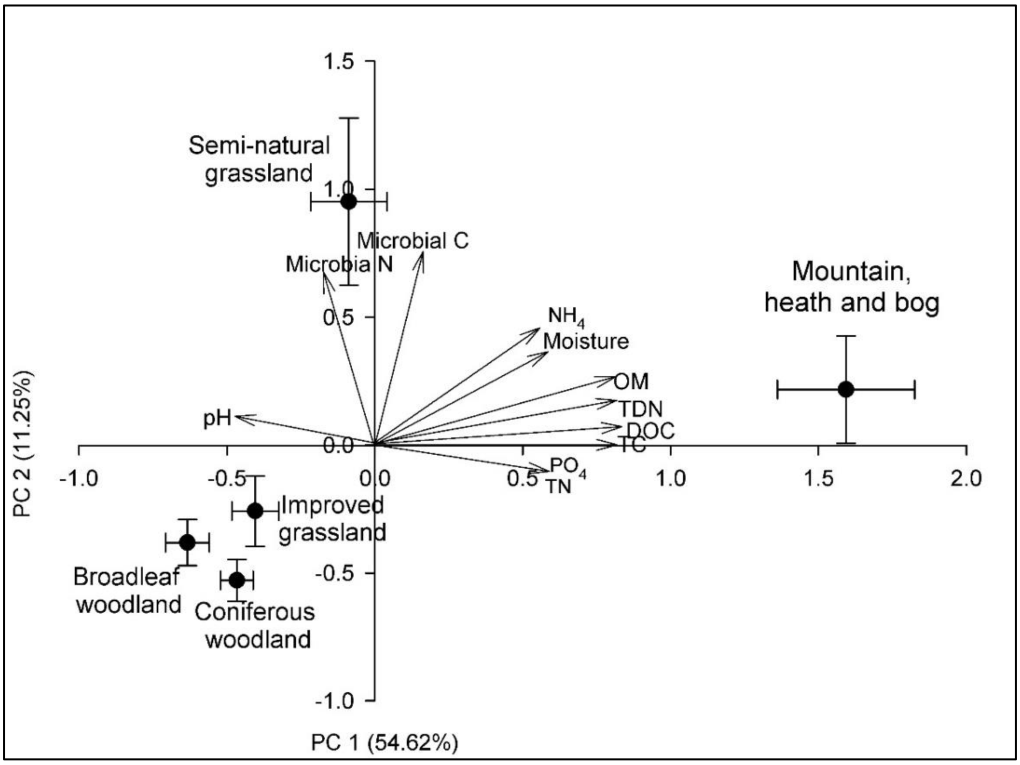

Figure 4.2 Correlation bi-plot from the principal component analysis (PCA) on soil physicochemical variables according to their dominant habitat type and irrespective of distance and depth (n = 100). Correlation of soil properties with the main axes are given by arrows and habitat types by cluster centroids (average score on each horizontal principal component (PC1) and vertical principal component (PC2) with standards errors). Organic matter (OM). Total carbon (TC). Total nitrogen (TN). Dissolved organic carbon (DOC). Total dissolved nitrogen (TDN).

Results of the PCA showed that habitat type (represented by cluster centroids, average score on each PC1 and PC2 with standard errors) was an important predictor of soil physicochemical variables. In terms of soil properties, broadleaved and coniferous woodlands (BW and CW) and improved grassland (IG) were closely associated to each other in the Conwy catchment, although IG displayed overall higher total C and N content (Table 4.3). At the other end of the spectrum (positive axis of PC1), the mountain, heath and bog habitat (MHB) was driven by moisture content (2.5 times more compared to woodlands and IG and 1.5 times greater than semi-natural grassland; SNG) and total C (ranging between 3.5 times greater than IG and 9.5 for BW) (Table 4.3). The SNG habitat resembled MHB in the sense that it had a greater moisture content, total C and N compared to woodlands and IG habitats. However, they were more influenced by microbial biomass showing larger variability in their vertical component. The sites IG, SNG and BW were characterized by more alkaline pH values (ca. 5.2), whilst MHB and CW displayed a more acidic pH (ca. 4.5) (Table 4.3).

Table 4.3 Main soil physicochemical characteristics for the five different habitat types. Sampling depth and distance from the river were amalgamated together as there were no significant differences from the result of a factorial analysis with habitat, depth and distance as the main factors (see Tables S1-S5). Data are mean values (n = 10) ± standard error of the mean (SEM).

	Mountain, heath and bog	**Broadleaf woodland**	**Coniferous woodland**	**Semi-natural grassland**	**Improved grassland**
pH	4.5 ± 0.1	5.2 ± 0.1	4.6 ± 0.1	5.1 ± 0.1	5.3 ± 0.1
EC (µS cm^{-1})	32.5 ± 3.3	31.8 ± 2.9	35.7 ± 3.6	33.3 ± 3.0	93.1 ± 20.5
Bulk density (g cm^{-3})	0.08 ± 0.01	0.74 ± 0.06	0.43 ± 0.1	0.23 ± 0.06	0.66 ± 0.07
Moisture content (%)	86.6 ± 0.6	32.2 ± 1.5	31.9 ± 3.0	64.1 ± 5.0	35.5 ± 2.7
Organic matter (%)	82.4 ± 2.6	10.6 ± 0.8	14.6 ± 2.2	35.3 ± 5.7	11.4 ± 1.4
NH_4^+-N (mg kg^{-1} soil)	18.0 ± 0.76	4.77 ± 0.39	5.06 ± 0.38	12.48 ± 2.21	4.47 ± 0.75
NO_3^--N (mg kg^{-1} soil)	50.3 ± 8.32	3.07 ± 0.47	5.31 ± 0.76	10.6 ± 1.42	12.7 ± 3.14
P available (mg kg^{-1} soil)	4.92 ± 1.28	0.31 ± 0.07	0.32 ± 0.06	0.78 ± 0.14	1.27 ± 0.31
Total C (g kg^{-1} soil)	522 ± 27	54 ± 5	73 ± 12	121 ± 24	149 ± 31

	Mountain, heath and bog	Broadleaf woodland	Coniferous woodland	Semi-natural grassland	Improved grassland
Total N (g kg^{-1} soil)	20.5 ± 1.11	3.45 ± 0.26	4.01 ± 0.55	6.86 ± 1.00	9.10 ± 1.58
Dissolved organic C (g kg^{-1} soil)	1.01 ± 0.11	0.19 ± 0.02	0.27 ± 0.02	0.39 ± 0.05	0.17 ± 0.01
Total dissolved N (g kg^{-1} soil)	0.15 ± 0.02	0.03 ± 0.003	0.03 ± 0.002	0.06 ± 0.01	0.05 ± 0.01
Microbial biomass C (g kg^{-1} soil)	2.31 ± 0.44	0.93 ± 0.07	1.31 ± 0.19	3.58 ± 1.03	1.63 ± 0.22
Microbial biomass N (g kg^{-1} soil)	0.34 ± 0.07	0.23 ± 0.03	0.16 ± 0.02	0.47 ± 0.09	0.29 ± 0.04

As the objective of this work was to assess the influence of the river and soil depth in terms of ecosystem service provision and not to compare different habitats, from this point onwards we will focus on the influence of these factors within each habitat type.

The influence of soil depth and distance from river on physicochemical properties within each habitat type is summarised in Tables S1-S5. Overall, soil depth showed no significant effect on any of the soil physicochemical properties across habitat types, with some exceptions. Microbial biomass-C was three times greater in the topsoil than subsoil in MHB ($P < 0.01$) while microbial biomass-N differed approximately two-fold in the topsoil compared to the subsoil in CW and SNG ($P < 0.05$). Total C showed a 72% change from top- to sub-soil in IG ($P < 0.001$).

Available P was three times greater close to the river than 50 m away ($P < 0.01$) in MHB but it was in the topsoil where the most noticeable difference was seen. The BW habitat displayed the greatest difference when comparing physicochemical properties with respect to distance. The BW habitat displayed 1.5 times greater EC away from the river, whereas total N decreased by 1.5 times with distance from the river. Inorganic N (NH_4-N and NO_3-N) showed a statistically significant increase (27% ($P = 0.042$) and 64% ($P = 0.004$) respectively) away from the river whereas microbial biomass-N was 1.7 times less close to the river.

The pH within the CW habitat showed a significant variation ($P = 0.002$) with a 10% increase close to the river, whereas DOC was 1.5 times greater away from the river. Distance had no effect in physicochemical properties in SNG and IG habitats with the exception of microbial biomass-C in SNG which was 6-times greater close to the river, although the standard error was quite high. Total N within the IG habitat showed an increase of 62% close to the river ($P < 0.05$).

As depth was shown to have very little effect on soil physicochemical properties, this factor was removed from the subsequent assessment of ecosystem services delivery.

4.3.2 Ecosystem service provisioning

4.3.2.1 Phosphorus sorption to soil

P sorption across all habitat types was generally well described by the Langmuir model ($r^2 = 0.92 \pm 0.01$). P sorption maxima, S_{max}, ranged on average from 85 to 382 mg P kg^{-1} across the five habitat types, showing the lowest sorption capacity with BW and the highest in MHB. Results showed that MHB had consistently higher values of maximum P sorption than the other habitats. Nonetheless, the binding parameter, *k*, that reflects the strength of P sorption, was found to be highly variable and reduced for MHB whilst the rest of the habitat types displayed a similar trend (Table 4.4).

Table 4.4 Maximum adsorption values (S_{max}), binding energy constant (k) and correlation coefficients (R^2) as estimated by Langmuir isotherm with respect to distance from the river.

	Langmuir model				
	Maximum P sorption S_{max} (mg kg^{-1})		Binding strength k (l kg^{-1})		R^2
	Close to river	Far from river	Close to river	Far from river	
Mountain, heath and bog (MHB)	379 ± 74	385 ± 137	3.6 ± 2.5	7.3 ± 5.1	0.90 ± 0.03
Broadleaf woodland (BW)	88 ± 10	82 ± 7	42.2 ± 8.0	28.7 ± 9.6	0.87 ± 0.04
Coniferous woodland (CW)	81 ± 6	114 ± 15	31.6 ± 5.3	25.3 ± 5.1	0.91 ± 0.04
Semi-natural grassland (SNG)	246 ± 62	172 ± 55	22.8 ± 8.1	23.7 ± 6.8	0.95 ± 0.04
Improved grassland (IG)	148 ± 68	86 ± 9	14.6 ± 5.1	19.9 ± 3.2	0.97 ± 0.01

Although river proximity did not have a significant effect on S_{max} (P > 0.05), SNG and IG showed a tendency of greater P sorption closer to the river (Table 4.4). Significant positive correlations (P < 0.001) were observed between S_{max} and moisture content, organic matter, available forms of N and P, C content and microbial biomass. In contrast, S_{max} correlated negatively with bulk density (P < 0.001). The most striking relationship was between S_{max} and DOC and TDN, suggesting that organic matter might play a key role in P sorption capacity.

4.3.2.2 Human bacterial pathogen survival in soil

Overall numbers of *E. coli* O157:H7 declined significantly (P < 0.001) between the first and the second harvest dates across all habitat types. After 24 h post-inoculation, a decrease of ca. 20% of pathogen numbers were observed at all sites. Numbers then remained relatively stable in the soil for all habitat types with the exception of SNG in which the final percentage (49 ± 2%) differed significantly from the rest of the habitat types. The final percentage decrease across the other sites was ~ 70%, suggesting different controlling factors within SNG sites. In terms of distance from river, there was no significant effect (P > 0.05) on persistence of *E. coli* O157:H7 colony counts and therefore, both values (close and far) were amalgamated (Figure 4. 3).

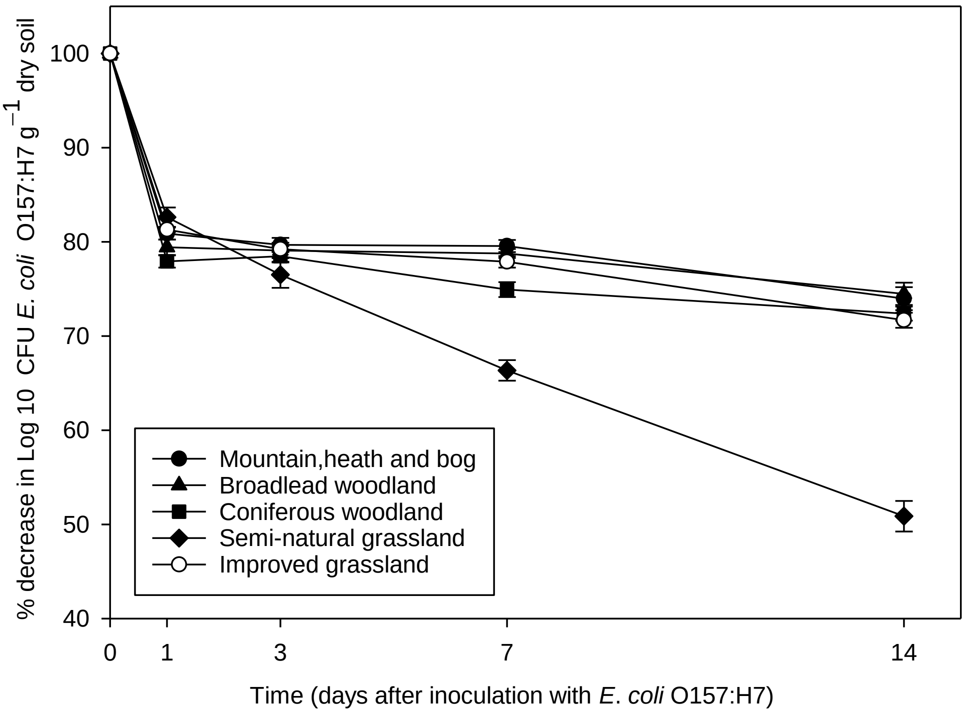

Figure 4.3 Survival of *Escherichia coli* O157:H7 following the application of pathogen-contaminated cattle slurry to the soil from different habitat types amalgamating distance from the river. Data points represent mean values (n = 10) ± standard error of the mean (SEM).

4.3.2.2 Pesticide sorption to soil

Average K_d values, irrespective of distance to river, ranged from 11 to 484 l kg^{-1} across all habitat types. The pesticide sorption capacity in MHB soils was 45 and 23 times greater than in the woodland (BW and CW, respectively) soils and between 6 and 30 times greater than SNG and IG sites (Figure 4.4). Woodland (BW, CW) and IG habitats showed similar K_d values (11 ± 2, 21 ± 3 and 16 ± 6 kg^{-1}, respectively) and the average K_d value for SNG was 79 ± 28 kg^{-1} which is midway between the MHB and woodland habitats. K_d values displayed fairly similar trends (P > 0.05) when comparing results from close and far away from the river (Figure 4. 4). Organic matter and moisture content correlated significantly (P < 0.001) with K_d which might explain the higher sorption capacities within MHB and SNG habitat types.

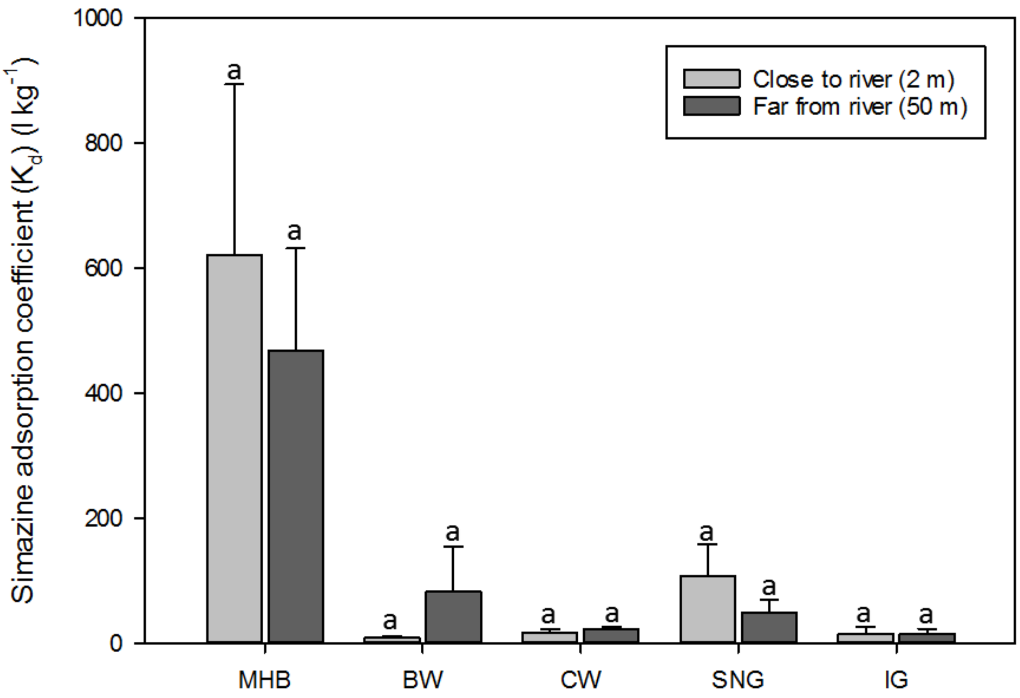

Figure 4.4 Simazine adsorption coefficient (K_d) across habitat types (MHB: mountain, heath and bog; BW: broadleaf woodland; CW: coniferous woodland; SNG: semi-natural grassland; IG: improved grassland) with respect to distance from the river. Same lower-case letters indicate no significant difference ($P > 0.05$) between distance from the river and simazine adsorption coefficient according to independent *t*-test within each habitat type. Bars represent mean values ($n = 5$) ± standard error of the mean (SEM).

4.3.2.4 Pesticide degradation in soil

After 30 d of incubation, the total percentage of simazine degradation ranged from 2.7 to 8.8% of the total ^{14}C-simazine activity added across habitat types irrespective of distance from the river. The amount of simazine mineralized was noticeably less in the MHB sites compared with the rest of the habitats. Across all habitats and distances, the rate of simazine mineralization was maximal in the first week of incubation and then progressively decreased over the 30 d incubation period. No significant differences were noted for MHB and IG with respect to distance from the river. In contrast, significant differences with distance from the river were observed in the two woodland habitats (Figure 4.5; $P = 0.041$ for BW and $P =$

0.035 for CW). However, while the final percentage of simazine mineralized tended to be higher close to the river in CW, the opposite trend was seen for BW. Across habitat types, the most striking relationships between simazine degradation and soil physicochemical properties were a positive correlation with pH (P < 0.01) and negative correlation with DOC (P < 0.001). Simazine degradation also correlated negatively with N inorganic forms (NH_4-N, P = 0.002, NO_3-N, P = 0.003) and available P (P = 0.008).

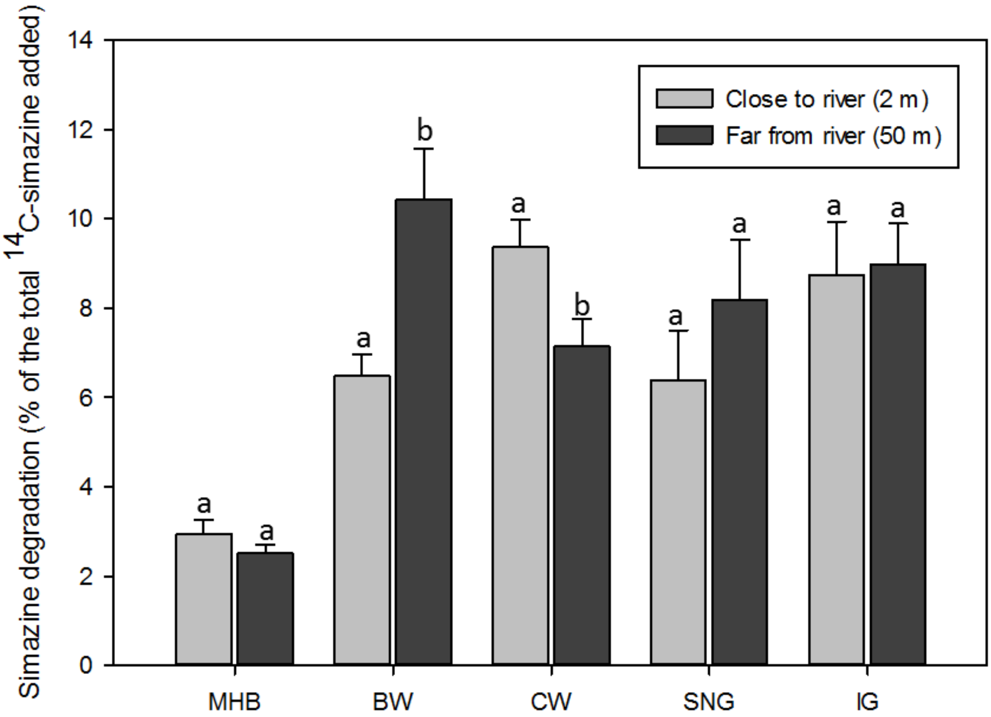

Figure 4.5 Simazine degradation across habitat types (MHB: mountain, heath and bog; BW: broadleaf woodland; CW: coniferous woodland; SNG: semi-natural grassland; IG: improved grassland) with respect to distance from the river. Values are expressed as the cumulative percentage of the total ^{14}C-simazine added. Same lower-case letters indicate no significant difference (P > 0.05) between distance from the river and simazine degradation according to independent t-test within each habitat type. Bars represent mean values (n = 5) ± standard error of the mean (SEM).

4.3.2.5 Denitrification potential in soil

Denitrification potential (DP) ranged between 0.25 and 1.94 mg N_2O-N m^{-2} d^{-1} across habitat types based on a 24 h incubation. Overall, IG showed the highest DP, being 3 and 7.5 times higher than the MHB and the woodlands, respectively.

The influence of river proximity revealed no significant differences in N_2O emissions ($P > 0.05$). Very different emission patterns were observed within each habitat, as indicated by the large error bars in Figure 4.6, reflecting the spatial complexity and the presence of denitrification hot spots across all habitat types. When hot spot values were removed from the analysis, N_2O emissions were the same irrespective of proximity to the river for MHB, BW and CW habitat types. Although not significant, emissions rates tended to be higher further away from the river for SNG and CW whereas the opposite trend was found for MHB and BW.

Overall, significant positive correlations ($P < 0.05$) were found between N_2O emissions ($n = 50$) and bulk density and pH. Higher denitrification rates were found between pH 5 and 6 and bulk densities of 0.6 and 0.8 g cm^{-3}.

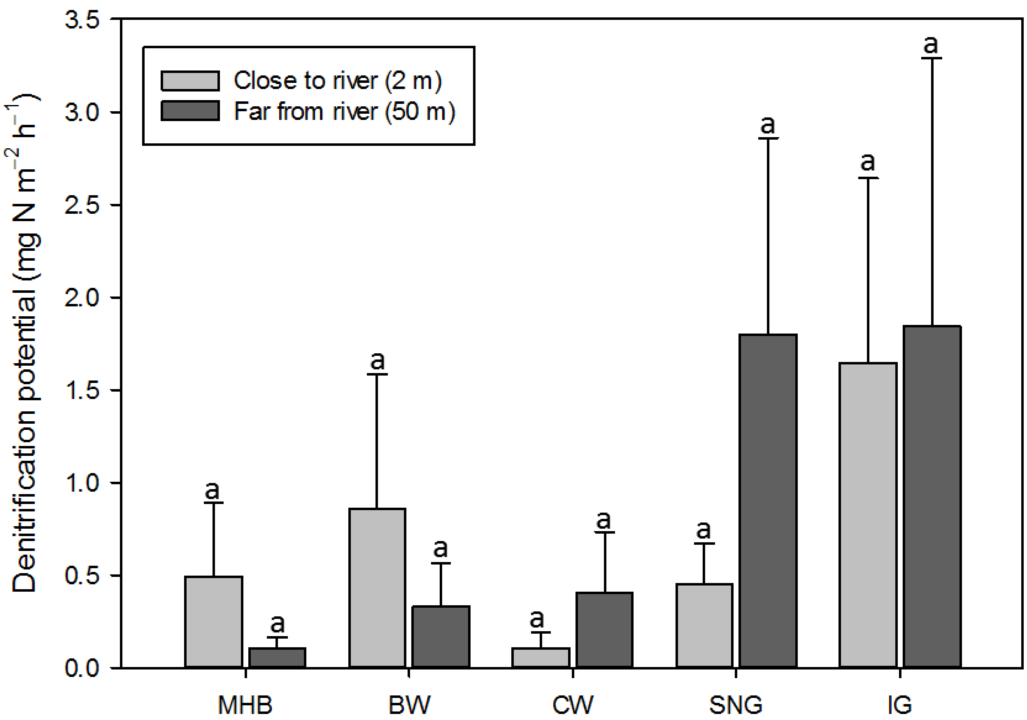

Figure 4.6 Rate of potential denitrification after 24 h across dominant habitat types (MHB: mountain, heath and bog; BW: broadleaf woodland; CW: coniferous woodland; SNG: semi-natural grassland; IG: improved grassland) with respect to distance from the river. Same lower case letters indicate no significant differences ($P > 0.05$) respective distance from the river according to the independent *t*-test. Bars represent mean values ($n = 5$) ± standard error of the mean (SEM).

4.3.2.6 Provision of riparian shade

When evaluated across the whole catchment, the presence of woodland (CW and BW) shaded 52.4% of the water channel. In contrast, in the MHB habitat the vegetation only provided 7.6% shade cover. In the IG and SNG habitats the vegetation provided 17.4% and 12.9% shading respectively, however, this was partially due to the presence of isolated hedges, trees and shrubs which were present within these habitats (Figure 4.7).

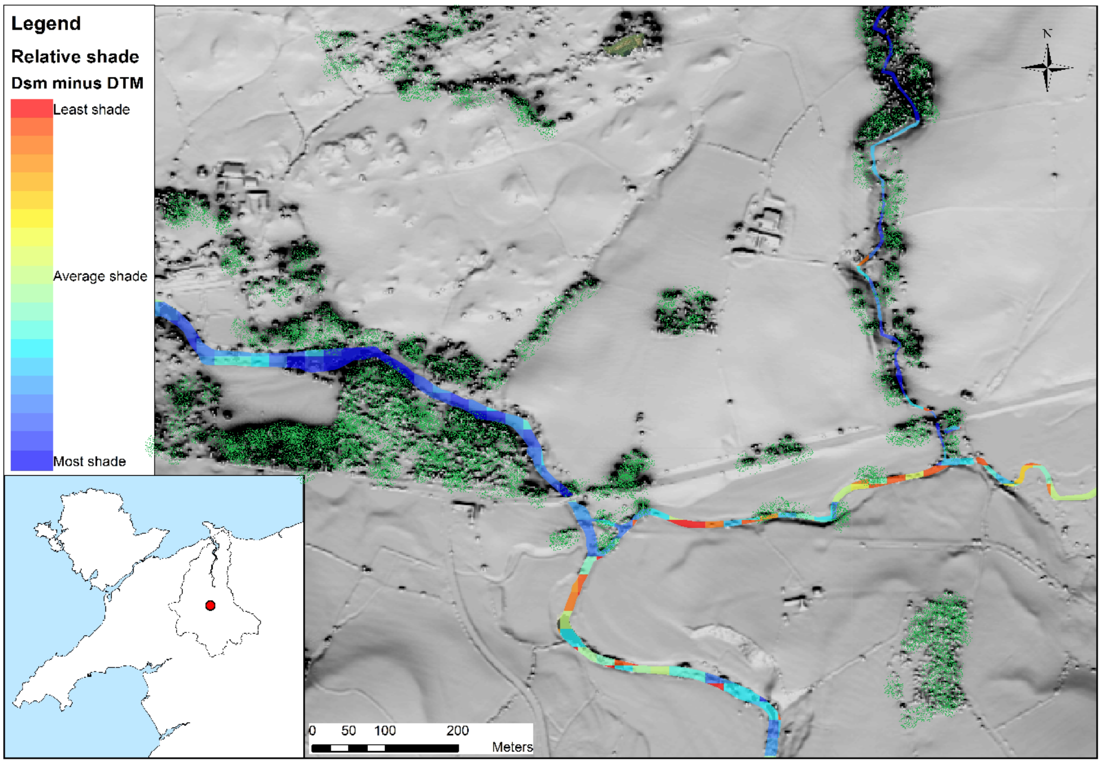

Figure 4.7 An example image showing the areas with the least (red) and greatest (blue) amount of shade from solar radiation, generated using a Digital Elevation Model (DEM) to represent the bare surface without objects (i.e. vegetation and other objects) and the Digital Surface Model representing the earth's surface including vegetation and other objects. Areas with dense vegetation are coloured in green.

4.4 Discussion

4.4.1 General approach

Our study investigated the spatial diversity of riparian soils and the delivery of ecological processes that regulate the ecosystem service related to improving water quality. Soil physicochemical properties were compared between samples taken close to (2 m) and distant (50 m) from the river to further our understanding of how riparian specific soil characteristics vary across different habitat types. Additionally, we explored different mechanisms of pollutant removal (e.g. sorption, degradation and denitrification) and shading involved in water quality enhancement with respect to riparian areas. We acknowledge that significant gradients may exist across riparian areas, however, our sampling approach was designed to simply compare soils in and out of the riparian zone. This approach reflects existing broad-scale soil surveys which are used to measure and predict ecosystem service delivery at the national scale (Emmett et al., 2010, 2016; Norton et al., 2012; Jones et al., 2014).

4.4.2 Riparian soil physicochemical properties

Many studies have linked the provision of riparian ecosystem services to their unique intrinsic characteristics (Vought et al., 1994; Natta and Sinsin, 2002; Groffman and Crawford, 2003). Riparian soils may have higher organic C contents (Figueiredo et al., 2016; Graf-Rosenfellner, 2016), greater amounts of nutrients and fine-grained sediments (Lee et al., 2000; Mayer et al., 2007), increased moisture contents (Lewis et al., 2003; Zaimes et al., 2007) and microbial biomass (Naiman et al., 2010) than adjacent non-riparian areas. Contrary to expectations, our findings contradict the frequently held assumption of riparian area 'uniqueness'. We observed little or no effect of the proximity to the river on the soil physicochemical properties measured, despite major differences in vegetation community composition and exposure to different hydrological regime. General soil physicochemical properties across habitat types followed the same trends as previous studies undertaken in the catchment (Ullah and Faulkner, 2006; Sgouridis and Ullah, 2014, 2015) and the inherent habitat characteristics proved to be the main drivers explaining soil physicochemical variability in riparian areas. In support of our findings, Richardson et al. (2005) also noticed little difference in soil properties between riparian and upslope areas along small streams in temperate forested areas of the Pacific Northwest. In addition, riparian studies have commonly focussed on agriculturally-managed grasslands and more specifically on riparian buffer strips as management tools (Pierson et al., 2001; Hefting and Bobbink, 2003; Hickey and Doran, 2004), even though this habitat type has shown less value in terms of ecosystem service provision (Maes et al., 2011, 2012). Stutter et al. (2012) and Smith et al. (2012) found significant differences when comparing soil physicochemical properties of riparian buffers versus adjacent fields. However, the comparison was undertaken between areas which possessed vastly different management regimes and in which the vegetation cover changed dramatically. Similarly, Burger et al. (2010) also showed differences in soil properties between agriculturally impacted riparian areas and ones conserved in pristine natural conditions. Most of the habitats assessed in our study have little or no management intervention so natural or semi-natural habitat conditions remained consistent across the upslope and riparian area. This was true even for the areas subject to agricultural practices (improved and to a lesser extent semi-natural grassland), although it should be stated that these agricultural areas generally have good soil quality (unlike those under arable cropping; Emmett et al., 2016). It is possibly for this reason that we did not identify any significant change in soil physicochemical properties as reported by others. Further studies are therefore needed to take into account management intensity and to include seasonal patterns as they may also represent an important component in riparian dynamics (Dhondt et al., 2002; Greet et al., 2011).

4.4.3 Ecosystem service provision

In comparison to the surrounding region, riparian areas are usually considered to have extra functionality in terms of ecosystem service provision through enhanced flood control, water purification or biodiversity (Salo and Theobald, 2016; Sutfin et al., 2016; Xiang et al., 2016) . However, in our study there was no evidence that fundamental differences exist between riparian zones and the adjacent land. This is supported by the clear segregation of results according to habitat types and not by riparian areas (Figure 4.8).

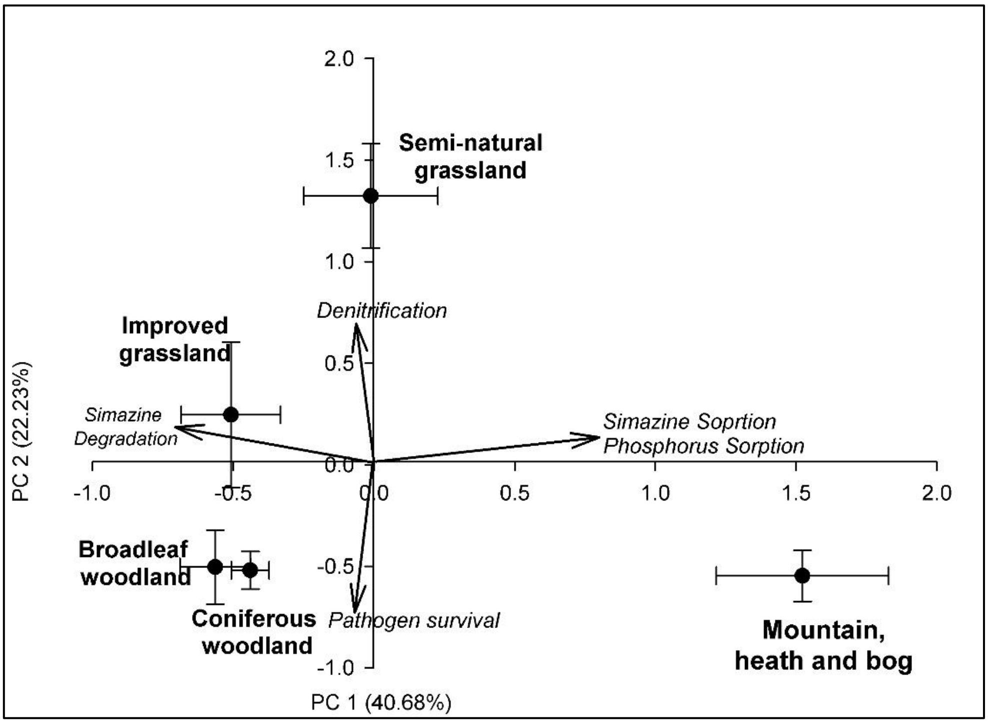

Figure 4.8 Correlation bi-plot from the principal component analysis (PCA) on ecosystem services evaluated in this study irrespective of the distance from the river. Correlation of ecosystem services with the main axes are given by arrows and habitat types by cluster centroids (average score on each horizontal principal component (PC1) and vertical principal component (PC2) with standards errors, *n* = 10).

Main habitat characteristics and not distance from the river was the driving factor in all cases. In this respect, Table 4.5 summarizes the soil habitat physicochemical properties which are most likely to be driving the ecosystem service delivery in this study. Together with that, we also include other factors that, despite not being measured, should be considered in future riparian studies to predict the spatial and temporal variation in ecosystem service delivery. These processes could be responsible for creating 'hot spots and moments' within riparian zones (McClain et al., 2003; Vidon et al., 2010). For example, erosion is more prevalent in riparian areas due to the exposure to a more dynamic water regime (McCloskey, 2010). This can cause a large release of N, P and C into the water column producing similar loads to those induced by fertilizer application (Quinton et al., 2010). Likewise, water table fluctuations that modify oxygen levels and nutrient availability, and the presence of macrophytes are also good examples that could potentially alter ecosystem service delivery dynamics in riparian areas (Naiman and Decamps, 1997; Hill, 2000; Lewis et al., 2003; Ng and Chan, 2017).

Table 4.5 Controlling factors affecting the performance of the ecosystem services selected in this study accompanied by unmeasured factors that most likely influence the behaviour of riparian areas in accomplishing ecosystem functioning.

Ecosystem service	Habitat physicochemical property found	Process likely to occur in riparian areas affecting the delivery of the ecosystem services
Phosphorus and simazine sorption	Organic matter Moisture content Bulk density Available forms of N and P Microbial biomass[1] C content	Erosion processes Rapid uptake by macrophytes Fluxes of organic matter from upland and streams creating 'hot moments' Changes in moisture content and pH controlling pollutant solubility
Simazine degradation	Microbial competition and specialisation pH Total carbon	Changes in pH and redox potential which control pesticide hydrolysis and bioavailability
Denitrification activity	High spatial variation Bulk density pH	Carbon and nitrogen sources provided by the stream Oscillation of anoxic and oxic conditions due to hydrographic regime
Pathogen survival	-	More exposure to animal waste events due to livestock attraction to watercourses
Shade provision	Habitat type canopy	Land change use

[1]Controlling factor only identified for P adsorption

4.4.3.1 Pollutant removal via sorption

Values of S_{max} (P sorption) and K_d (simazine sorption) resulted in good agreement with other values found in the literature across habitat types (Dunne et al., 2005; Flores et al., 2009). Analysis suggested that simazine and P sorption was driven by high organic matter content as has been highlighted in previous studies (Li et al., 2003; Hogan et al., 2004; Kang et al., 2009; Alister and Kogan, 2010). Particularly for P sorption, some authors attribute this affinity of P for organic matter to the co-occurrence of Al and Fe oxides, which can sorb high amounts of P (Pant et al., 2001; Kang et al., 2009). We had expected that the riparian areas would be wetter, have a lower redox status and would contain a lesser amount of oxidised forms of Fe and thus a lower P retention capacity, however, this was not apparent in our soils. Barrow (2017) illustrated different pathways for P sorption according to soil pH but due to the relatively small shifts in pH relative to the distance to the river, no such effect was found in this study.

Comparing the results obtained in this study is challenging as most studies within riparian areas try to identify the most cost-effective buffer width depending on the pollutant load in agricultural systems or constructed wetlands. This is motivated by the fact that land managers do not want to sacrifice more productive land than they have to (Wenger, 1999; Shearer and Xiang, 2007). Consequently, the centre of attention has been on comparing inputs versus outputs of pollutants in runoff through vegetative buffer strips (Schultz et al., 2000; Maillard and Imfeld, 2014). Results found in the literature about the long-term effectiveness of riparian buffers in trapping pollutants are contradictory as riparian areas can vary from being sources to sinks depending mostly on physicochemical soil properties and hydrology (Hickey and Doran, 2004; Fisher and Acreman, 2004; Stutter et al., 2009; Maillard and Imfeld, 2014). Some studies (e.g. Miller et al., 2016) reported different P retention capacities with distance from the river. However, it was only true for samples included inside a concentrated flow path that was visually identified prior to sampling. In contrast, samples outside this concentrated flow path did not reveal any differences in P retention across the transect.

The similar pollutant sorption capacities relative to distance from the river found in this study, combined with fact that simazine and P retention by soil can only occur when they are in direct contact with the adsorbent suggest that the soil potential data alone is not very useful in predicting the pollutant retention capacity (Reddy and Kadlec, 1999). Thus, the study of transport pathways, potential sources of pollutant loads, ease of degradation, desorption potential from the soil, shifts in temperature that controls simazine solubility or pH that controls P precipitation may contribute more efficiently to understanding riparian pollutant attenuation.

4.4.3.2 Pollutant removal through degradation

Degradation, together with sorption, is one of the main processes determining the fate of pollutants within the environment (Gunasekara et al., 2007; Maillard and Imfeld, 2014). In our study, we investigated the degradation of a pesticide and loss of the biological contaminant, *E. coli* O157, which are of concern in terms of their impact on human health (Holden et al., 2017). Sorption and transport of pollutants, and the extension of buffer strips on agricultural and wetland systems has often been the focus of attention (Vellidis et al., 2002; Hickey and Doran, 2004; Rasmussen et al., 2011), but processes influencing pollutant degradation in riparian areas are much less well understood (Vidon et al., 2010). Microbial activity has long been identified as a critical factor determining the fate of pesticides in the environment (Kaufman and Kearney, 1976; Anderson, 1984), and it is suggested that microbial populations within riparian areas are able to degrade pesticides due to their continuous exposure to such chemicals through runoff from agricultural lands (Vidon et al., 2010). Overall, simazine degradation in this study showed a similar percentage decrease (of the total of ^{14}C-simazine added) to other studies (Laabs et al., 2002; Gunasekara et al., 2007;

Jones et al., 2011). Laabs et al. (2002) and Cox et al. (2001) found a negative correlation between simazine degradation rates and organic matter content due to the residue binding to organic matter reducing herbicide movement in the soil. This fact could explain the minimal amount of simazine degraded in MHB sites in this study. Previous studies have demonstrated enhanced pesticide degradation within riparian areas (Mudd et al., 1995; Staddon et al., 2001). However, the riparian buffer strips in these previous studies differed considerably from the adjacent habitat (i.e. bare or highly modified fields versus vegetated buffer strips). In our study, only the woodlands showed a different pattern in terms of pesticide degradation when comparing sites close and distal to the river. However, we hypothesized that the negative correlation between simazine degradation and N and P inorganic forms content could explain this spatial variability as the use of pesticides as a source of energy in areas with low nutrient status has been identified (Błaszak et al., 2011). In addition, it has been shown that some organisms (e.g. *Pseudomonas*) are able to mineralise simazine more rapidly (Regitano et al, 2006; Błaszak et al., 2011) and therefore a more diverse microbial population associated with a higher above-ground plant diversity could be involved in different ecosystems. Our results may therefore reflect the spatial heterogeneity of microbial populations within these habitat types rather than a specialization of microbial population in riparian areas. This fact is endorsed by studies like Widenfalk et al. (2008) where an effect on microbial composition due to pesticide exposure could not be identified. Our results reveal that there is a need for linking functional soil biota groups with the maintenance of ecosystem services to better explain the inherent spatial heterogeneity (Brussaard, 1997; Graham et al., 2016).

Along with pesticides, biological contaminants, in particular faecal coliform bacteria (FCB), have become an important source of water contamination from human and animal wastes applied to land (Bai et al., 2016). Although the use of riparian buffer strips for reducing FCB transport into streams has been explored (Coyne et al., 1995; Parkyn et al., 2003; Sullivan et al., 2007), bacterial survival and behaviour in terrestrial systems has received less attention than in water ecosystems (Jones, 1999). Our results corroborate previous studies that show *E. coli* O157 can survive for long periods (more than 120 d) in a diverse range of soils and under a wide range of environmental conditions (Bogosian et al., 1996; Kauppi and Tatini, 1998; Jones, 1999). Some studies have suggested that moisture status and organic matter are the principal factors controlling *E. coli* survival (Jamieson et al., 2002). However, the lack of correlation between soil properties and pathogen survival in this study suggest that other factors, such as predation or the presence of elements highlighted in other studies (Al, Zn; Avery et al., 2008), might better explain the lower survival rate found in semi-natural grassland sites.

4.4.3.3 Pollutant removal through denitrification

Denitrification, as a mechanism for permanent removal of NO_3^- from ecosystems, has important implications for both water quality and greenhouse gas emissions (Groffman et al., 2009). It has been extensively studied in riparian areas due to the frequency of locally anoxic conditions and labile organic C which trigger denitrification (Bettez and Groffman, 2012). In our study, rates of N_2O emissions across habitat types followed similar trends to those described in Sgouridis and Ullah (2014). However, we could not find any clear evidence that leads us to identify more efficient patterns of NO_3^- removal by denitrification with proximity to the river. We also observed a high degree of spatial variability in denitrification with some extremely high rates as has been observed in other studies and described as ‘hot spots or moments’ controlled by oxygen, NO_3^- and C availability (Parkin, 1987; McClain et al., 2003; Groffman et al., 2009; Vidon et al., 2010). Previous riparian studies have also reported no clear spatial patterns in denitrification rates (Martin et al., 1999). In our study, it was clear that the addition of NO_3^- was not sufficient to trigger large amounts of N_2O, indicating that factors other than NO_3^- limitation were playing a key role. Sgouridis and Ullah (2015) describe significant relationships between denitrification rates and pH and bulk density, and the same pattern was found in our study. However, those factors do not explain the high variability encountered within habitat types, and it was not possible to demonstrate significantly increased N_2O production rates within riparian areas as demonstrated in previous studies (Hanson et al., 1994; Groffman et al., 2000; Groffman and Crawford, 2003). Further research is therefore required to better understand why denitrification is so spatially variable and the spatial/temporal existence of 'hot spots or moments'.

4.4.3.4 Riparian shading

Riparian shading is gaining increased recognition for its potential to alleviate water pollution (Ghermandi et al., 2009; Warren et al., 2016). For example, Hutchins et al. (2000) found that the reduction of nutrient pollution was less effective at suppressing phytoplankton growth than establishing riparian shading. Bowes et al. (2012) also noticed a potential reduction of 50% of periphyton accrual rate through shading in the River Thames.

The shade mapping approach presented here provides an easy tool to identify watercourse exposure to solar radiation. As described in Lenane (2012), the maps generated using this approach, offer the guidance necessary to help with riparian management plans and decision-making strategies. Identifying whether riparian vegetation is providing effective shade is fundamental for environmental protection. Furthermore, the size of this area required to provide shade has economic implications as it takes the land out of production (Sahu, 2010). The shade evaluation undertaken in this study differs from others in which field monitoring are required (Boothroyd et al., 2004; Halliday et al., 2016) and consequently it avoids excessive costs associated with field measurement campaigns. However, it does not predict water quality changes as proposed by Ghermandi et al. (2009) which combines available flow measurements with biochemical and shade models.

As expected, in our study the effects of shading were more significant in woodlands than in any other habitat type. Woodland riparian zones are likely to offer the greatest influence on water temperature within a catchment. Any assessment, however, should also consider excessive shading, mostly caused by abandoned woodlands (Suzuki, 2013) which can be detrimental to aquatic ecosystems by excessively reducing water temperature. This can have a direct impact on aquatic fauna and result in a loss of shade-intolerant plants (Forestry Commission, 2004; Hédl et al., 2010). Shading may also reduce the UV radiation-induced photooxidation of many pesticides within the water column.

4.5 Conclusions

Recommendations and guidance about riparian zone management are frequently undertaken without an accurate evaluation of their status and the ecosystem services that they actually provide. Consequently, many previous environmental protection measures involving riparian management remain too general and untargeted and may offer little environmental benefit. Through a series of laboratory experiments and GIS-based mapping, this study has shown that across a diverse range of habitats, riparian soils diverge from their capacity to deliver the specific ecosystem service of water purification. However, contrary to expectation, riparian soils did not differ greatly in their ability to provide this service in comparison to neighbouring upslope (non-riparian) soils. We ascribe this to our habitats being in a close to natural or semi-natural state rather than the more frequently studied riparian areas in degraded agricultural systems. Further work should focus on validating our findings using an even greater range of ecosystem services (e.g. inclusion of CH_4/CO_2 emissions, metal attenuation, biodiversity), using in situ measurements, encompassing inter-annual variation and over a wider range of ecosystem types.

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Chapter 5

Stoichiometric constraints on microbial community behaviour with soil depth along a riparian hillslope

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H.C.G and M.R.M and D.L.J designed and conceived the experiment; H.C.G and M.R.M and L.L.S conducted experimental work and L.L.S analysed results and prepared the manuscript. All authors discussed results and contributed to preparation of the manuscript.

Abstract

Soil organic matter (SOM) content is a key indicator of riparian soil functioning and the provision of ecosystem services such as; water retention for flood alleviation, pollutant attenuation and carbon (C) sequestration for climate change mitigation. In these soils, the microbial community and availability of nutrients are hypothesized to be key factors regulating SOM turnover rates. In addition, C stabilisation in soil is expected to vary both vertically down the soil profile and laterally across the riparian zone. However, our understanding of the underlying mechanisms controlling SOM decomposition remains poor due to the broad range of physicochemical and biological processes operating simultaneously and the environmental disruptions caused by anthropogenic activities. In this study, we critically evaluated the influence of five factors on C mineralization (C_{min}) rates: (i) substrate quality, (ii) substrate quantity, (iii) nutrient (C, N and P) stoichiometry, (iv) variations in microbial community structure due to the proximity to the river (2 to 75 m), (v) variations in microbial diversity and structure as a function of soil depth (0 – 3 m). Rates of C turnover and C use efficiency (CUE) were evaluated using high and low molecular weight ^{14}C-labelled dissolved organic (DOC) while microbial communities were assessed by phospholipid fatty acid (PLFA) profiling. Overall, soil depth proved to be a major factor influencing microbial biomass, community structure and rates of C_{min}. Differences in the immediate and long-term response of C_{min} suggested different microbial C use efficiency strategies through the soil profile. Nutrient addition (inorganic N and/or P) had little or no effect on C_{min} suggesting that the microbial community growth and activity was always C limited. Similarly, proximity to the watercourse also had relatively little effect on C_{min}. Further work is required to estimate the role of seasonal water table fluctuations on soil C turnover and its link to differences in microbial community composition.

Key Words: humic substances, nitrogen, phosphorus, nutrient cycling, subsoil.

5.1 Introduction

Agricultural grasslands represent one of the biggest managed stores of carbon (C) in the terrestrial biosphere (Jones and Donnelly, 2004). Further, it is widely accepted that soil organic C (SOC) underpins a range of regulating, provisioning, cultural and supporting ecosystem services in these habitats (Adhikari and Hartemink, 2016). It is therefore vital that we preserve SOC levels in grassland landscapes to ensure continual delivery of these services. To achieve this, however, necessitates a good understanding of the factors that regulate C turnover and identification of management practices that promote greater SOC retention.

While below-ground respiration represents a good general indicator of SOC turnover, it provides little indication of whether the C is of plant or microbial origin and from where in the soil profile the CO_2 originates (Robert, 2002; Van Hees et al., 2005; Rui et al., 2016). Recent research, however, suggests that C dynamics differ through the soil profile and, albeit controversial, the processes regulating C storage in topsoils and subsoils may be different (Salome et al., 2010; Sanaullah et al., 2011; Jones et al., 2018). Some authors have suggested that different microbial patterns at depth are due to a decrease in substrate quality (more recalcitrant and less biodegradable) and are thus only able to support small microbial populations (Rovira and Vallejo, 2002; Salomé et al., 2010). Other authors support the idea that subsoil microbial communities are more C efficient due to a permanent limitation of available substrate (Fierer et al., 2003; Blagodatskaya et al., 2007). Studies comparing C responses within the soil profile, however, have often found contradictory results. For example, C addition has been shown to induce both positive and negative priming of native SOC (Kuzyakov, 2002; Zhang et al., 2015; Wordell-Dietrich et al., 2016). This highlights our lack of knowledge about how, and to what extent, differences in microbial community structure and substrate quality influence C and nutrient turnover within the soil profile.

The availability of inorganic nutrients (e.g. N, P, S) in the soil has also been shown to be a key factor regulating rates of SOC turnover (Creamer et al., 2016). In this context, fertiliser addition to grasslands can be expected to significantly alter the ratio of C to other essential nutrients (nutrient stoichiometry). If the stoichiometry (e.g. C:N:P ratio) approaches the optimal ratio required for microbial cells, and there are no other limiting factors (e.g. pH, water, oxygen availability), then microbial growth will occur leading to C storage (Cleveland and Liptzin, 2007; Fierer et al., 2003, Sinsabaugh et al., 2013). As the stoichiometry of microbial groups in soils is different (e.g. fungi versus bacteria), this implies that the microbial response to fertiliser may differ both horizontally and vertically (topsoil vs. subsoil) in response to local shifts in microbial community composition. This is supported by microbial diversity, as well as biomass, being an important predictor of SOC turnover (Graham et al., 2016). In this respect, the transition area between aquatic and terrestrial ecosystems (e.g. riparian areas) are thought to play a key role in SOC decomposition due to a potentially greater microbial diversity which has evolved in response to high-frequency disturbance regimes such as, fluctuation of aerobic/anaerobic conditions (Gregory et al., 1991; Clinton et al., 2002; Lewis et al., 2003). Additionally, flood pulses spreading out across the riparian zone have been shown to be the precursor for intermittent cycles of OM accumulation or abrupt removal (Acuna et al., 2004; Naiman et al., 2010).

Within the context of a grassland riparian transect, the main objectives of our study were: (1) to test how nutrient supply (C, N and P) and their stoichiometry affects rates of C_{min} down the soil profile; (2) to explore the influence of stoichiometry on the turnover of both labile and more recalcitrant C; (3) to assess the influence of microbial diversity on rates of C_{min}; and (4) to assess the influence of proximity to the river on C turnover rates. We hypothesized that nutrient limitation would be a greater constraint to C turnover in subsoils relative to topsoils and that this would be most apparent for labile forms of C which should drive faster microbial growth. We also hypothesised that C turnover would be greatest closest to the river due to it being a zone of higher nutrient enrichment.

5.2 Materials and methods

5.2.1 Study site

The area of study is located within the Conwy Catchment, North Wales (UK) (53°12'5.33"N 3°46'54.66"W) (Figure 5.1). A detailed description of the catchment can be found in Emmett et al. (2016), Sharps et al. (2017) and de Sosa et al. (2017). The experimental site comprised a 3 ha typical improved grassland hillslope (mean slope of 20%) used for intensive livestock (sheep and cattle) production. The soil is free draining and classified as a Eutric Endoleptic Cambisol (WRB, 2007) and the dominant vegetation consists of *Lolium perenne* L. and *Trifolium repens.* The mean annual rainfall is 1230 mm (based on 30-year average 1961–1990 data from the UK Met Office) and the mean annual temperature (at 30 cm depth) is 8°C (based on 30-year average 1981–2010 data from the UK Met Office).

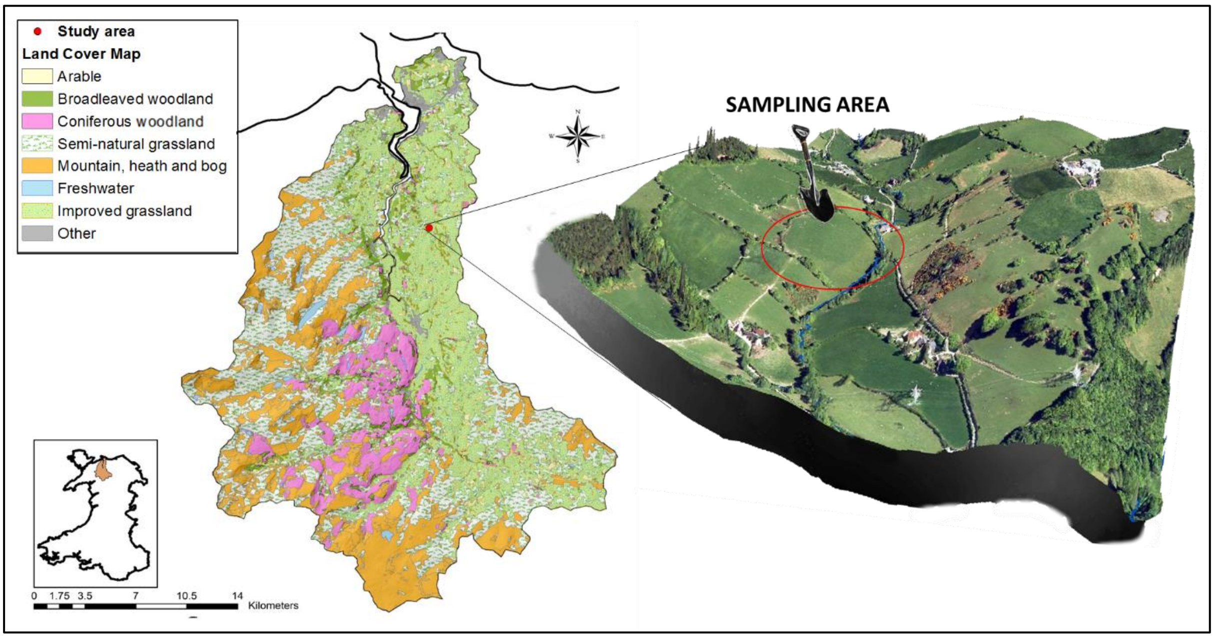

Figure 5.1 The Conwy catchment, North Wales, UK showing location of sampling area and land cover classes based on Phase 1 classification.

5.2.2 Soil core sampling

Three 75 m long transects, 20 m apart, were identified across the hillslope, running perpendicular to the river (Figure 5.2). Along each transect, intact soil cores were extracted at 2, 12 and 75 m (from this point onwards in the manuscript, these are referred to as row 1, 2 and 3 respectively) using a Cobra percussion hammer corer (Van Walt Ltd, Haslemere, Surrey, UK) in May 2016. The total length of extractable core was determined according to the maximum depth of the soil profile (presence of bedrock) or until an impermeable (e.g. clay layer) boundary as determined by a geophysical survey (data not presented) was reached (row 1 = 1 m core length, row 2 = 2 m core length and row 3 = 3 m core length; n = 18 × 1 m core lengths). Intact soil cores were extracted in 1 m lengths (4 cm diameter; total cores n = 18) and wrapped in thin-walled polyethylene (PE) sleeves to maintain core integrity and immediately transferred to the laboratory and stored at 4°C prior to analysis (Jones and Willett, 2006).

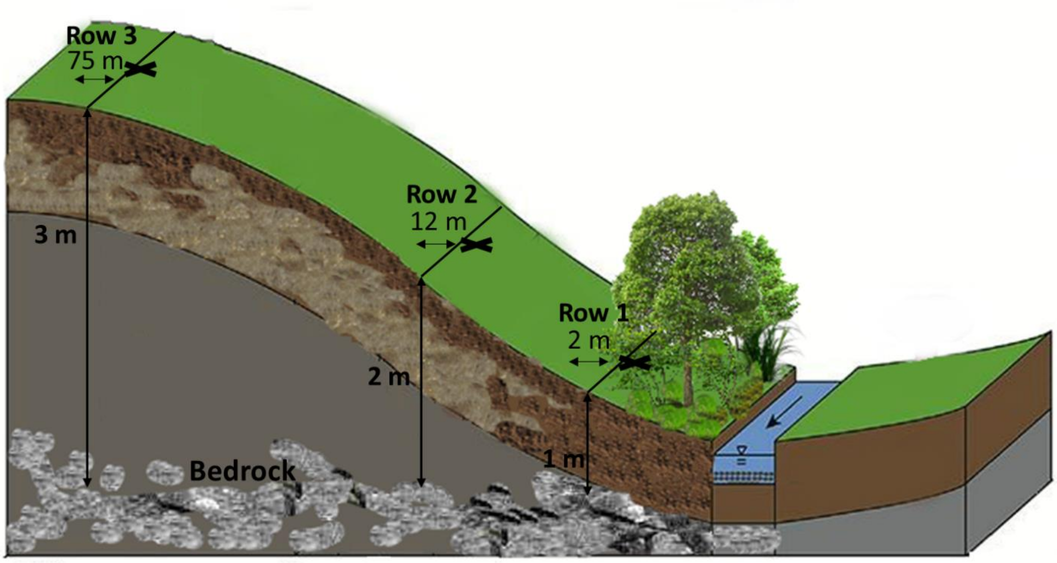

Figure 5.2 Location of sample points across the riparian hillslope. Horizontal arrows indicate distance from the river (these are referred in the manuscript as row 1, 2 and 3). Vertical arrows indicate the total length of extractable core, determined according to the maximum depth of the soil profile (presence of bedrock) or until an impermeable (e.g. clay layer) boundary as determined by a geophysical survey was reached.

5.2.3 General soil characterisation

Soil cores were divided into depth intervals of 0-15, 15-30, 50-100, 100-150, 150-200 and 250-300 cm (from this point onwards in the manuscript, these are grouped and referred to as topsoil (0-30 cm), midsoil (50-100 cm), and deep soil (100-300 cm), respectively), and passed through a 5 mm sieve in order to remove stones and any plant material and to ensure sample homogeneity. This mesh size was chosen as it minimizes changes to the activity of the soil microbial community (Jones and Willet, 2006). Soil water content was determined gravimetrically (24 h, 105 °C) and soil organic matter content (OM) was determined by loss-on-ignition (LOI) (16 h, 450 °C). Soil pH and electrical conductivity (EC) were measured using standard electrodes in a 1:2.5 (*w*/*v*) soil-to-deionised water mixture. Total available ammonium (NH_4-N) and nitrate (NO_3-N) in soil were determined with 0.5 M K_2SO_4 extracts (1:5 *w*/*v*) via the colorimetric procedure of Mulvaney (1996) and the vanadate method of Miranda et al. (2001), respectively. Available phosphate (P) was quantified with 0.5 M acetic acid extracts (1:5 *w*/*v*; Fisher et al., 1998) following the ascorbic acid-molybdate blue method of Murphy and Riley (1962) and total C (TC) and N (TN) were determined with a TruSpec® elemental analyser (Leco Corp., St Joseph, MI). Dissolved organic C (DOC) and total dissolved N (TDN) were quantified in 1:5 (*w*/*v*) soil-to-0.5 M K_2SO_4 extracts using a Multi N/C 2100 TOC analyzer (AnalytikJena, Jena, Germany). Microbial biomass C and N were assayed by chloroform-fumigation (Vance et al., 1987) after 72 h incubation (k_{ec}=0.45 and k_{en} =0.54). Samples were analysed for phospholipid fatty acid (PLFA) analysis according to the 96-well format, high throughput method of Buyer and Sasser (2012) (Microbial ID Inc., Newark, DE). Phosphorus (P) sorption was determined following an adapted method of Nair et al. (1984). In brief, 1.0 g field moist soil was shaken in 0.01 M $CaCl_2$ (1:25 *w*/*v* soil-to-extractant ratio) containing known concentrations of P (0, 0.5, 1, 5, 10, 50 mg P l^{-1} as Na_2HPO_4) spiked with ^{33}P (0.06 kBq ml^{-1}; PerkinElmer Inc., Waltham, MA) to determine the amount of P adsorbed onto the soil phase. These concentrations were selected due to their likelihood of being encountered in natural systems. Samples were shaken for 2 h (150 rev min^{-1}, 25°C) on an orbital shaker. This time was chosen in order to assess intermediate equilibrium conditions (Santos et al., 2011). After 2 h, 1.5 ml of supernatant was removed and centrifuged (10,000 *g*, 5 min). Subsequently, 1 ml of supernatant was mixed with 4 ml of Optiphase HiSafe 3 liquid scintillation fluid (PerkinElmer Inc.) and the amount of ^{33}P activity remaining in solution measured using a Wallac 1404 liquid scintillation counter (Wallac EG & G, Milton Keynes, UK). The total amount of P adsorbed was determined by the difference between the initial ^{33}P activity added and the final amount of ^{33}P remaining in solution. Any P not recovered in the solution was assumed to be sorbed onto the soil solid phase. To estimate the soil absorption maxima of P, sorption isotherms were examined according to the linearized form of the Langmuir equation (Reddy and Kadlec, 1999; Mehdi et al., 2007).

5.2.4 Preparation of nutrient solutions

To investigate how nutrient stoichiometry affected C mineralization (C_{min}) rates, soil samples collected from the hillslope were incubated with N, P and N+P together, in combination with three different C amendments, namely:

(1) High (hotspot) dose of labile DOC

(2) Low (natural abundance) dose of labile DOC

(3) High MW recalcitrant DOC (HMW DOC)

We tested four different nutrient additions for each C amendment

(1) C only addition (C)

(2) C and N addition (CN)

(3) C, N and P addition (CNP) and

(4) C and P addition (CP)

C, N and P treatments were added in mass ratios of C:N = 9 (N in the form of NH_4NO_3) and C:P = 85 (P in the form of Na_2HPO_4) to represent the average stoichiometric ratios of the soil microbial biomass in grassland systems (Cleveland and Liptzin, 2007).

The different C amendments were chosen to simulate distinct soil C conditions within the soil. For the low (natural abundance) C experiment, a total of 6 µM C (specific C addition of 0.72 ng C g^{-1} dry soil) was added to simulate the background C concentrations found under natural conditions (Boddy et al., 2007). For the hotspot C experiment, 300 mM C (specific C addition of 36 µg C g^{-1} dry soil) was chosen to represent soil C released during root cell lysis and would likely stimulate microbial growth (Jones and Darrah, 1994; Tabuchi et al., 2004). Glucose was selected as our C source for the low and high (hotspot) conditions as it represents a common root exudate dominating the low molecular weight (MW) DOC pool and is known to be important in soil C cycling (van Hees et al., 2005). It is also capable of being assimilated by almost all soil microorganisms. For the long-term (recalcitrant) C experiment, 47.4 mM of high MW (>1 kDa) recalcitrant DOC (specific addition of 18.2 µg C g^{-1} dry soil) was selected to represent the compounds remaining once the labile fractions have been utilised by microbial populations (Gillis and Price, 2016). The recalcitrant DOC was obtained following the incubation and subsequent decomposition of ^{14}C-labelled *Calluna vulgaris* (L.) Hull. shoots in a Sapric Histosol for 2 years and recovery of the soil solution (Jones et al., 2015).

5.2.5 Preparation of isotopically labelled solutions

Nutrient solutions, as described above, were spiked with uniformly ^{14}C-labelled D-glucose (PerkinElmer, UK) for the low (native) and high (hotspot) C conditions experiments only. For both C treatments, the specific activity added was 0.2 kBq ml^{-1}. The concentration of ^{14}C added (< 10 nM) did not significantly alter the C concentration of the unlabelled (^{12}C) nutrient solutions. For the recalcitrant DOC, nutrient solutions were spiked with ^{14}C-labelled DOC (specific activity 0.07 kBq ml^{-1}). To ensure the plant-derived DOC solution was only composed of high MW material, the solution was purified using a Amicon 8050 stirred cell equipped with a 1 kDa ultrafiltration membrane (Millipore UK Ltd., Hertfordshire, UK).

5.2.6 Carbon mineralization

To measure the rate of ^{14}C-substrate mineralization, 5 g soil (dry weight equivalent to account for soil water content variability down the soil profile) was placed into sterile 50 ml polypropylene tubes. To determine the rate of $^{14}CO_2$ evolution, 50 µl of ^{14}C-glucose labelled nutrient solution for the low (native) and high (hotspot) C treatments, and 160 µl of the recalcitrant ^{14}C-DOC labelled nutrient solution (higher volume used to account for the lower specific activity of this solution) was added to the soil surface. Immediately after nutrient addition, a 5 cm^3 polypropylene vial containing NaOH (1 ml, 1 M) was added into the tubes to capture $^{14}CO_2$ evolved. The tubes were hermetically sealed and incubated at 10 °C to represent the mean annual temperature of the catchment. The NaOH traps were changed after 0.5, 1, 2, 4, 6, 24, 48, 72, 96, 120, 144, 168, 192, 336 h and then weekly up to 6 weeks after initial ^{14}C-labelling for both glucose-C additions. For the recalcitrant DOC experiment, traps were changed at 1, 6, 24, 48, 72, 168, 336, 504, 672, 840, 1176, 1512, 1680 h due to the slower mineralization rates. On removal, the NaOH traps were mixed with Optiphase HiSafe 3® liquid scintillation fluid (PerkinElmer Inc.) and the amount of $^{14}CO_2$ captured determined using a Wallac 1404 liquid scintillation counter (Wallac EG & G).

5.2.7 Data and statistical analysis

To assess if C dynamics were regulated by different microbial strategies through the soil profile and distance from the river, initial (immediate) C_{min} rates and cumulative C mineralized at the end of the incubation period were calculated for all treatments and C amendments. The specific initial C_{min} rate was calculated for a 6 h incubation period or when the linear phase was achieved for the experiments involving the low and high doses of ^{14}C-

glucose (low MW DOC) and for 72 h for the high MW recalcitrant DOC. An r^2 value of >0.90 was deemed acceptable for assessing linearity rates. Due to large differences in microbial biomass down the soil profile (assessed by total PLFA), C_{min} rates results were normalized according to biomass size. Both the normalized, and the actual respiration rates per soil unit are reported. For normalization of the data, PLFA biomass was chosen over microbial biomass determined by $CHCl_3$ fumigation-extraction due to stronger correlations (r^2 > 0.92). Further, this was considered to be more representative of the viable microbial community present in the soil (Moore-Kucera and Dick, 2008).

Cumulative C mineralized was calculated as the C cumulative percentage at the end of the incubation period respective to the amount of C added at the beginning of the experiment.

Statistical analysis was performed with SPSS version 22 for Windows (IBM Corp., 2013, Armonk, NY) and R (R Core Team, 2012). All data were analysed for normality and homogeneity of variance with Shapiro Wilk's tests and Levene's statistics, respectively. Transformations to accomplish normality were done when necessary. For all statistical tests, $P < 0.05$ was selected as the significance cut-off value. Separate analysis of variance (one-way ANOVA) tests were performed to explore differences in soil physicochemical properties with respect to: (1) distance from the river, followed by Tukey's post-hoc test, and (2) depth, followed by Games-Howell post-hoc test. A principal component analysis (PCA) was used to explore the spatial (depth and distance) relationships of soil physico-chemical properties. Effects of depth, distance from the river, and treatment on mineralization were tested using a mixed-effects model with depth, row and treatment as fixed effects and transects as random effects. Interactions between variables were included for each model when a significant improvement of the model ($P < 0.05$) was observed. A significant improvement in the model was tested by performing an analysis of variance (ANOVA) of the full model both with, and without, inclusion of the effect being tested. Both F and P-value are reported to assess variability between groups. Differences amongst soil depth, nutrient treatment, and distance from the river were tested with Tukey post-hoc tests. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. To assess if different soil properties might be useful predictors of soil C_{min}, a step-wise multiple regression was conducted analysing relationships between C_{min} rates, final percentage respired and specific soil properties.

5.3 Results

5.3.1 Soil physicochemical properties

Principal Component Analysis (PCA) of all soil physicochemical variables across the hillslope (n = 42, irrespective of distance or depth) identified two principal components (PC) which together, explain 64.2% of the total variance within the dataset (Figure 5.3). Organic matter (OM) content, available $NH4^{+}$-N, P maximum adsorption (Pmax) and C:N ratio correlated significantly ($P < 0.05$) with the positive axis of PC1, whilst available PO_4-P, N adsorption (Nads), pH and EC correlated significantly ($P < 0.05$) with both PC1 and PC2. The spatial segregation of samples within the PCA revealed the strong effect of depth on physicochemical properties irrespective to distance from the river.

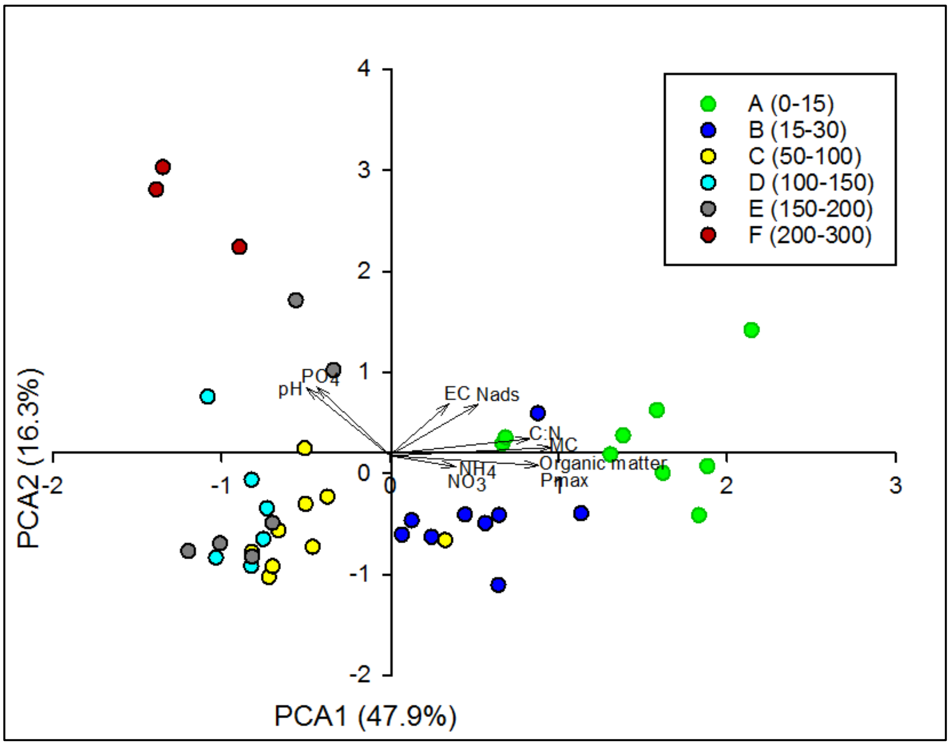

Figure 5.3 Correlation bi-plot from the principal component analysis (PCA) on soil physicochemical variables across the hillslope (*n* =9 for A, B, C; *n* = 6 for D, E; *n* = 3 for F). Correlation of soil properties with the main axes are given by arrows and sample points by colour dots. Nitrogen adsorption (Nads). Phosphorus maxima adsorption (Pmax). Ratio carbon (C)/ nitrogen (N) (C:N). Moisture content (MC). Electrical conductivity (EC).

However, some physicochemical properties differed ($P < 0.05$) according to proximity to the watercourse, but only within certain sampling depths (Table S1). The topsoil (0-30 cm) for row 3 showed an increase of almost a third in OM content compared to row 1. Similarly, DOC was 2 times greater in row 3 in comparison with row 1 for topsoil. The midsoil depth (50-100 cm), again for row 3, showed higher, more alkaline, pH vales compared to rows 1 and 2. Inorganic $NH4^+$-N tended to increase by almost 4 times with distance from the river for the topsoil whereas from the midsoil down 4 fold higher inorganic $NH4^+$-N content was found in areas closest to the river (Table S1). P adsorption maxima increased on average by 29% and 37% from row 1 to row 2 and 3 respectively for the top layer whereas it was 25% and 34% greater from row 1 to row 2 and 3 at 15-30 cm sampling depth (Table S2).

With respect to depth, a decrease of most of the physicochemical properties was identified, except for pH and available P (Table S1). Amongst all physicochemical properties, soil water, OM, DOC, TDN and microbial C displayed the greatest differences from topsoil to deep soil for all rows.

5.3.2 High dose of labile DOC

5.3.2.1 Cumulative C mineralized

On average, the total percentage of C mineralized was 40.7% ± 0.9 irrespective of row, depth or treatment. Cumulative final percentage of C mineralized was higher overall in deeper layers than topsoil (Table 5.1) but was not affected by treatments ($P > 0.05$). After 42 days of incubation, the total amount of C mineralized increased by 36.8% and 26.8% from the top layer to the midsoil and deep soil (250-300 cm) respectively, and irrespective of treatment and row (Table 5.1). Soil influenced by distance from the river also responded differently to the addition of treatments but only row 3 was significantly different from the other two ($P > 0.001$). Overall, row 3 mineralized lower percentages of C for all treatments and depths (Table 5.1). Particularly at sampling depth 50-100 cm, the percentage of C mineralized was on average 35% higher in row 1 than row 4. This effect was especially noticeable for the C only treatment (Table 5.1) which could be due to the inherent nutrient variability within rows (Table S1).

Table 5.1 Total $^{14}CO_2$ production following the addition of a high dose of labile ^{14}C-DOC to soil either in the presence or absence of inorganic nutrients (N and/or P) as a function of soil depth and distance from the river. Soils were incubated with the ^{14}C-labelled substrate for 42 d. The ANOVA results (F and *P*-value) are shown for a mixed effects model with depth, distance from the river and treatment as fixed effects and transect as a random effect. Interactions were only included when a significant improvement ($P > 0.05$, bold) of the model fit was observed. Value are means ± standard errors ($n = 3$). ND equates to no data due to hitting bedrock (Figure 5.2).

Total $^{14}CO_2$ (mg C kg^{-1} DW soil)	**Distance from the river**	**Soil depth**					
		0-15 cm	**15-30 cm**	**50-100 cm**	**100-150 cm**	**150-200 cm**	**250-300 cm**
DOC only	2 m	12.8 ± 1.4	14.7 ± 0.1	21.4 ± 0.5	ND	ND	ND
	12 m	11.9 ± 0.4	13.7 ± 0.1	16.7 ± 2.5	17.9 ± 1.2	16.7 ± 2.4	ND
	75 m	11.0 ± 0.2	13.0 ± 0.2	8.76 ± 4.6	14.3 ± 2.2	10.1 ± 3.3	15.6 ± 0.7
DOC + N	2 m	11.8 ± 1.2	13.3 ± 0.5	19.5 ± 1.2	ND	ND	ND
	12 m	12.3 ± 1.2	13.6 ± 0.6	16.8 ± 2.1	17.1 ± 0.9	17.9 ± 1.9	ND
	75 m	11.2 ± 0.2	15.5 ± 2.6	16.2 ± 2.3	18.7 ± 0.7	14.8 ± 3.7	14.3 ± 4.2
DOC + N + P	2 m	14.2 ± 1.8	13.8 ± 0.6	20.4 ± 1.6	ND	ND	ND
	12 m	10.5 ± 0.5	15.2 ± 1.6	18.1 ± 1.1	17.7 ± 0.5	17.9 ± 2.2	ND
	75 m	10.3 ± 0.1	11.9 ± 0.1	16.4 ± 1.3	16.7 ± 1.0	14.7 ± 3.5	18.5 ± 0.1
DOC + P	2 m	11.2 ± 1.5	14.1 ± 0.5	20.5 ± 1.3	ND	ND	ND
	12 m	10.8 ± 0.2	16.3 ± 1.4	16.8 ± 1.5	18.7 ± 1.1	15.6 ± 2.2	ND
	75 m	10.7 ± 0.2	11.2 ± 1.0	11.2 ± 3.9	11.5 ± 1.7	9.9 ± 3.3	14.8 ± 1.5

ANOVA results	**Soil depth**		**Distance from the river**		**Nutrient treatment**		**Soil depth * Nutrient treatment**		**Distance * Nutrient treatment**	
	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value
	21.33	**<0.001**	21.52	**<0.001**	2.17	0.09	-	-	2.98	**0.008**

5.3.2.2 Initial C mineralization rates

Soil depth was the main factor controlling C_{min} rates regardless of treatments (Table 5.2). Overall, C_{min} rates significantly decreased from the soil ($P < 0.001$) down to 100 cm whereas no significant effects ($P > 0.05$) were identified below that depth (Figure 5.4). Regardless of treatment or row, the amount of C evolved decreased by 62%, 88% and 92% from the top layer to 30-50, 50-100 and 100-300 cm sampling depths respectively (Figure 5.4). A lag phase of about 4 days corresponding to microbial growth was displayed in some sampling depths below 50 cm after the addition of C and/or nutrients whereas no such effect was observed above 50 cm (Figure S1). The effect of distance from the river also affected

C_{min} rates but only row 2 and 3 were significantly different from each other ($P < 0.001$). The addition of N or P separately, and combined had little or no effect on C_{min} rates irrespective to the distance from the river and depth ($P > 0.05$). The multiple regression analysis identified PLFA soil biomass, OM and MC as the best soil physicochemical predictors for C_{min} rates. Significant positive correlations were found between C_{min} rates and the aforementioned physicochemical properties ($r^2 > 0.69 \pm 0.01$ for MC, $r^2 > 0.83 \pm 0.01$ for OM and $r^2 > 0.85 \pm 0.01$ for PLFA biomass, $P < 0.001$ in all cases).

Table 5.2 Results of ANOVA (F and *P*-value) for the mixed effects model with soil depth, distance from the river and nutrient treatment as fixed effects, transect as a random effect and initial C mineralization rate as the independent variable. Interactions were only included when a significant improvement ($P > 0.05$, bold) of the model fit was observed. High and low doses of labile dissolved organic carbon (DOC) refer to the amounts of low MW C added to the soil in the experiment (see section 5.2.4). ND equates to no data due to hitting bedrock (Figure 5.2).

ANOVA results	**Soil depth**		**Distance from the river**		**Nutrient treatment**		**Depth * nutrient treatment**		**Row * Nutrient treatment**	
	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value
High dose of low MW labile DOC	395	**<0.001**	8.92	**<0.001**	2.39	0.07	-	-	-	-
Low dose of low MW labile DOC	178	**<0.001**	21.0	**<0.001**	2.82	**0.04**	1.87	**0.03**	-	-
High MW recalcitrant DOC	57.3	**<0.001**	10.5	**<0.001**	3.69	**0.01**	11.0	**<0.001**	4.73	**<0.001**

Due to large differences in total microbial biomass estimated by the PLFA data through the soil profile, results were normalized by the PLFA soil biomass data in order to identify different trends (µg CO_2 µmol^{-1} PLFA h^{-1}). Neither treatment nor row had a significant effect on C_{min} rates, leaving the factor of depth as the main significant driver ($P < 0.001$) affecting C depletion (Figure S2). No interactions between the fixed effects were found.

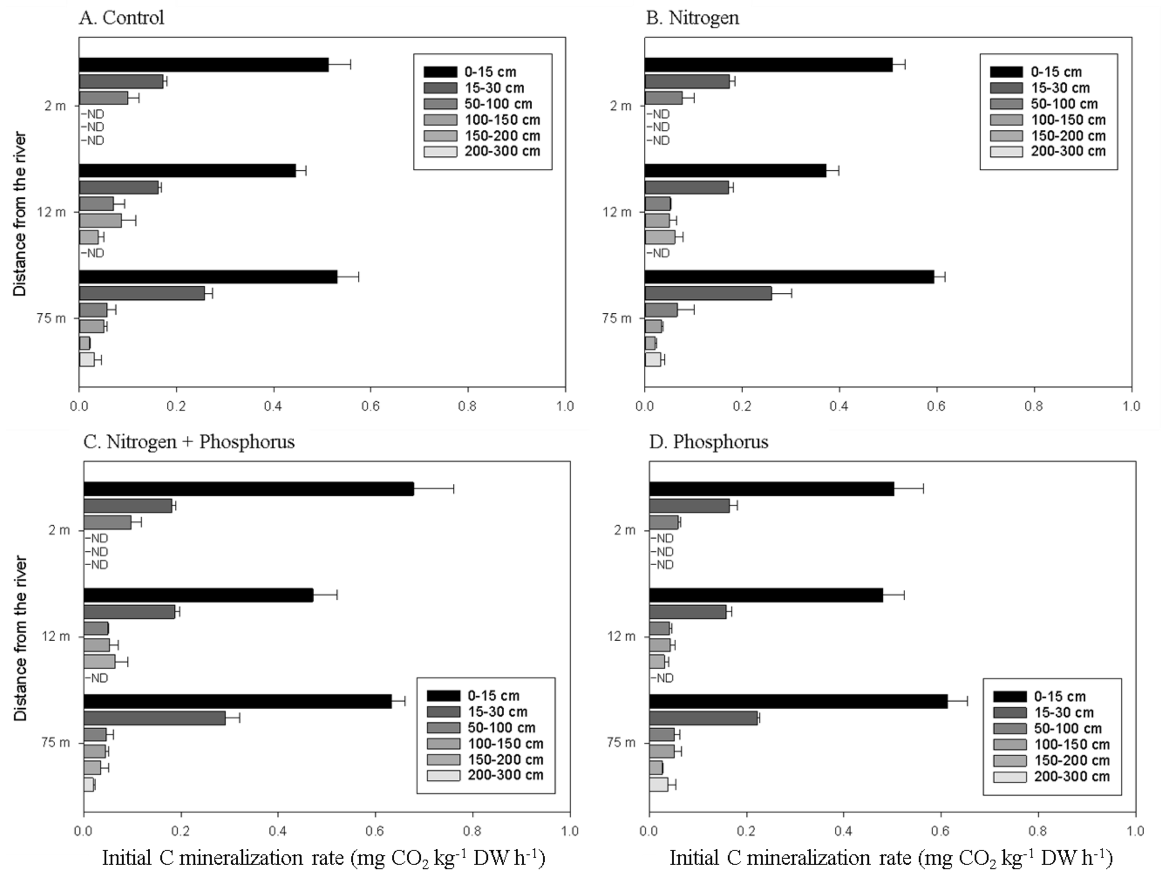

Figure 5.4 Initial C mineralization rates measured during the initial linear phase (between 0-6 h) after the addition of a high dose of labile DOC either alone or in combination with N, P or N+P. Values are presented for three different distances from the river (2, 12 and 75 m) and for 6 different soil depths. Soil depths were grouped into topsoil (0-30 cm), midsoil (50-100 cm) and deep soil (100-300) in the manuscript for the description of the factors assessed. Bars represent mean values (n = 3) ± standard errors. ND equates to no data due to hitting bedrock (Figure 5.2).

5.3.3 Low dose of labile DOC

5.3.3.1 Cumulative C mineralized

After 42 days of incubation, a 30.4% ± 0.5 of C was mineralized regardless of row, depth and treatment (Table 5.3). The total C mineralized generally increased with depth for the N and P treatments (P < 0.001) whereas the control (C only addition) showed a decrease of 18% from topsoil (0-15 cm) to the deepest layer in row 3 (Table 5.3). The overall effect of treatment was exacerbated with depth (P < 0.001). From the top layer to the deepest layer, total C mineralized increased by 30%, 25% and 24% for N, NP and P treatments, respectively. However, although NP and P-only treatment were different from the control (P < 0.001) they did not differ from each other.

Table 5.3 Total $^{14}CO_2$ production following the addition of a low dose of labile ^{14}C-DOC to soil either in the presence or absence of inorganic nutrients (N and/or P) as a function of soil depth and distance from the river. Soils were incubated with the ^{14}C-labelled substrate for 42 d. The ANOVA results (F and P-value) are shown for a mixed effects model with depth, distance from the river and treatment as fixed effects and transect as a random effect. Interactions were only included when a significant improvement (P > 0.05, bold) of the model fit was observed. Values are means ± standard errors (n = 3). ND equates to no data due to hitting bedrock (Figure 5.2).

Total $^{14}CO_2$ (µg C kg^{-1} DW soil)	**Distance from the river**	**Soil depth**					
		0-15 cm	**15-30 cm**	**50-100 cm**	**100-150 cm**	**150-200 cm**	**250-300 cm**
DOC only	2 m	0.19 ± 0.00	0.18 ± 0.01	0.22 ± 0.02	ND	ND	ND
	12 m	0.19 ± 0.02	0.19 ± 0.00	0.21 ± 0.02	0.17 ± 0.03	0.20 ± 0.02	ND
	75 m	0.17 ± 0.03	0.19 ± 0.01	0.21 ± 0.01	0.20 ± 0.00	0.18 ± 0.04	0.14 ± 0.04
DOC + N	2 m	0.23 ± 0.01	0.25 ± 0.04	0.27 ± 0.01	ND	ND	ND
	12 m	0.21 ± 0.00	0.21 ± 0.02	0.22 ± 0.02	0.25 ± 0.01	0.30 ± 0.01	ND
	75 m	0.21 ± 0.00	0.21 ± 0.00	0.24 ± 0.02	0.25 ± 0.01	0.25 ± 0.03	0.31 ± 0.01
DOC + N + P	2 m	0.22 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	ND	ND	ND
	12 m	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.27 ± 0.01	ND
	75 m	0.20 ± 0.00	0.20 ± 0.02	0.18 ± 0.03	0.23 ± 0.01	0.25 ± 0.01	0.28 ± 0.02
DOC + P	2 m	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	ND	ND	ND
	12 m	0.24 ± 0.03	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.27 ± 0.01	ND
	75 m	0.18 ± 0.01	0.23 ± 0.05	0.20 ± 0.01	0.23 ± 0.02	0.27 ± 0.02	0.27 ± 0.00

ANOVA results	**Soil depth**		**Distance from the river**		**Nutrient treatment**		**Soil depth * Nutrient treatment**		**Distance * Nutrient treatment**	
	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
	12.08	**<0.001**	2.66	0.07	35.66	**<0.001**	3.95	**<0.001**	-	-

5.3.3.2 Initial C mineralization rates

Values of C_{min} were strongly influenced by depth (P < 0.001, Table 5.2) ranging from 0.07 ± 0.004 in the top layer to 0.01 ± 0.001 µg C-CO_2 kg^{-1} h^{-1} for the deepest sampling depth, irrespective of treatment and row (Figure 5.5). Significant differences were only identified within depth intervals between 0-100 cm, between 100-300 cm, no differences were apparent. Treatment also showed an effect on Cmin rates although this effect was influenced by depth as the interaction (P = 0.04). Across the full range of sampling depths, Cmin was 3 times greater in the top layer than the midsoil for the control and 5, 4 and 2 times greater for the rest of the treatments, CN, CNP, CP respectively. Carbon mineralization rates in the deep soil were almost 8 times lower than the top layer for the control and N addition treatments but only 5 times lower for the treatment with P alone. Distance from the river also influenced Cmin rates but only the row closest to the river was different compared to the other two rows (P < 0.001). In particular, the midsoil showed on average, and irrespective to the treatment, 50% faster Cmin rates compared with the other two rows (Figure 5.5). Rates of Cmin were strongly correlated with moisture content MC (r^2 = 0.74 ± 0.02), OM (r^2 = 0.68 ± 0.05) and PLFA biomass (r^2 = 0.59 ± 0.06) (P < 0.001 in all cases) irrespective of the treatment.

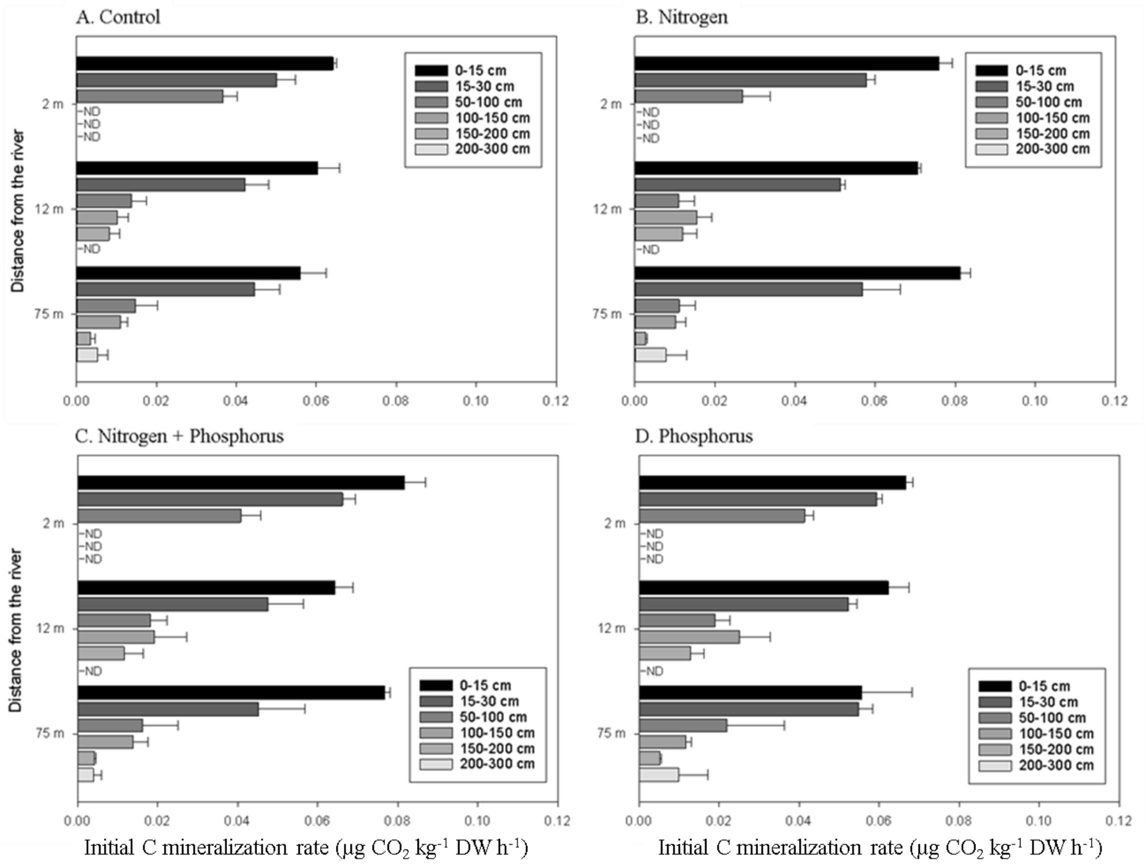

Figure 5.5 Initial C mineralization rates measured during the initial linear phase (between 0-6 h) after the application of a low concentration of labile DOC either alone or in combination with N, P or N+P. Values are presented for three different distances from the river (2, 12 and 75 m) and for 6 different soil depths. Soil depths were grouped into topsoil (0-30 cm), midsoil (50-100 cm) and deep soil (100-300) in the manuscript for the description of the factors assessed. Bars represent mean values (n = 3) ± standard errors. ND equates to no data due to hitting bedrock (Figure 5.2).

As with the high DOC addition, treatment showed no effect on C_{min} rates after adding a low dose of DOC when data was normalized with the PLFA biomass ($P > 0.05$). However, the effect of row and depth still had an overall significant effect on C_{min} rates ($P < 0.001$) (Figure S4).

5.3.4 High MW recalcitrant DOC

5.3.4.1 Cumulative C mineralized

Overall, the total amount of C mineralized was 11.7% ± 0.6 regardless of row, depth and treatment after 70 days of incubation (Table 5.4). In general, total C_{min} decreased with depth up to 100 cm below which, total C remained relatively consistent regardless of treatment or row. Distance from the river and treatment did not show an effect on the total percentage mineralized overall (Table 5.4). However, a significant effect of treatment with respect to depth was identified ($P < 0.001$). The addition of P only decreased C_{min} 5-fold in row 1 and by 2-fold in row 2 in the top layer in comparison with the rest of the treatments (Table 5.4).

Table 5.4 Total $^{14}CO_2$ production following the addition of recalcitrant high MW ^{14}C-DOC to soil either in the presence or absence of inorganic nutrients (N and/or P) as a function of soil depth and distance from the river. Soils were incubated with the ^{14}C-labelled substrate for 70 d. The ANOVA results (F and P-value) are shown for a mixed effects model with depth, distance from the river and treatment as fixed effects and transect as a random effect. Interactions were only included when a significant improvement ($P > 0.05$, bold) of the model fit was observed. Values are means ± standard errors ($n = 3$). ND equates to no data due to hitting bedrock (Figure 5.2).

Total $^{14}CO_2$ (mg C kg^{-1} DW soil)	**Distance from the river**	**Soil depth**					
		0-15 cm	**15-30 cm**	**50- 100 cm**	**100-150 cm**	**150-200 cm**	**250-300 cm**
DOC only	2 m	4.75 ± 0.27	3.42 ± 0.08	1.78 ± 0.47	ND	ND	ND
	12 m	4.69 ± 0.90	3.06 ± 0.10	1.29 ± 0.36	1.11 ± 0.27	0.85 ± 0.12	ND
	75 m	5.06 ± 0.93	3.90 ± 0.54	0.86 ± 0.19	0.86 ± 0.33	0.71 ± 0.07	0.71 ± 0.04
DOC + N	2 m	4.29 ± 0.25	3.36 ± 0.06	1.47 ± 0.23	ND	ND	ND
	12 m	3.15 ± 0.37	2.95 ± 0.04	1.82 ± 0.41	0.99 ± 0.09	0.87 ± 0.08	ND
	75 m	4.27 ± 0.32	3.55 ± 0.12	0.88 ± 0.20	0.98 ± 0.09	0.69 ± 0.07	0.62 ± 0.04
DOC + N + P	2 m	4.03 ± 0.14	3.02 ± 0.16	1.61 ± 0.35	ND	ND	ND
	12 m	3.00 ± 0.22	2.64 ± 0.10	1.42 ± 0.10	1.05 ± 0.19	0.83 ± 0.09	ND
	75 m	3.77 ± 0.27	3.07 ± 0.24	0.70 ± 0.20	0.76 ± 0.22	0.66 ± 0.01	0.63 ± 0.04
DOC + P	2 m	0.83 ± 0.19	4.14 ± 0.78	3.20 ± 0.55	ND	ND	ND
	12 m	1.68 ± 0.60	3.00 ± 0.17	4.02 ± 0.21	0.91 ± 0.07	0.79 ± 0.06	ND
	75 m	2.76 ± 0.75	3.81 ± 0.42	2.28 ± 1.26	0.72 ± 0.06	0.66 ± 0.07	0.62 ± 0.04

ANOVA results	**Soil depth**		**Distance from the river**		**Nutrient treatment**		**Soil depth * Nutrient treatment**		**Distance * Nutrient treatment**	
	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value
	98.91	**<0.001**	0.97	0.38	1.81	0.14	11.16	**<0.001**	-	-

5.3.4.2 Initial C mineralization rates

The recalcitrant C was mineralized at a maximum rate of 0.28% h^{-1}, decomposing C at an 85% and 97% slower rate than the high and low labile C addition respectively after 72 hours across all depth, treatments and rows (Table 5.2, Figure 5.6). Topsoil displayed, on average, 6.5 times greater C_{min} rates than deeper layers (>50 cm) for the control, N and NP treatments irrespective of row. However, the P only treatment resulted in a decrease of 30% in C_{min} rates from topsoil to deeper layers, although this effect was especially remarkable in row 1 and 2 (Figure 5.6). Regarding the treatment effect in the topsoil, the addition of P alone or in combination with N caused a decrease in C_{min} rates of 93% and 33% compared to the control and N alone treatments respectively. Distance from the river also caused different responses in C_{min} rates ($P < 0.001$) but this effect was mainly produced within the top layer and in response to the addition of P which appeared to have a repressive effect on C_{min}. As for the previous C amendments, MC, OM and PLFA biomass ($r^2 < 0.65 \pm 0.02$, $r^2 < 0.76 \pm 0.01$, $r^2 < 0.68 \pm 0.03$ respectively, P<0.001 in all cases) explained a large part of Cmin variability for all treatments except for the P only addition which only correlated with available P ($r^2 = 0.34$, $P < 0.05$). Values of Cmin rates normalized by the PLFA biomass showed no effect with row and a strong effect with depth (Figure S5; $P < 0.001$). Additionally, treatment also influenced C depletion but only the treatment with P only resulted different from the other three.

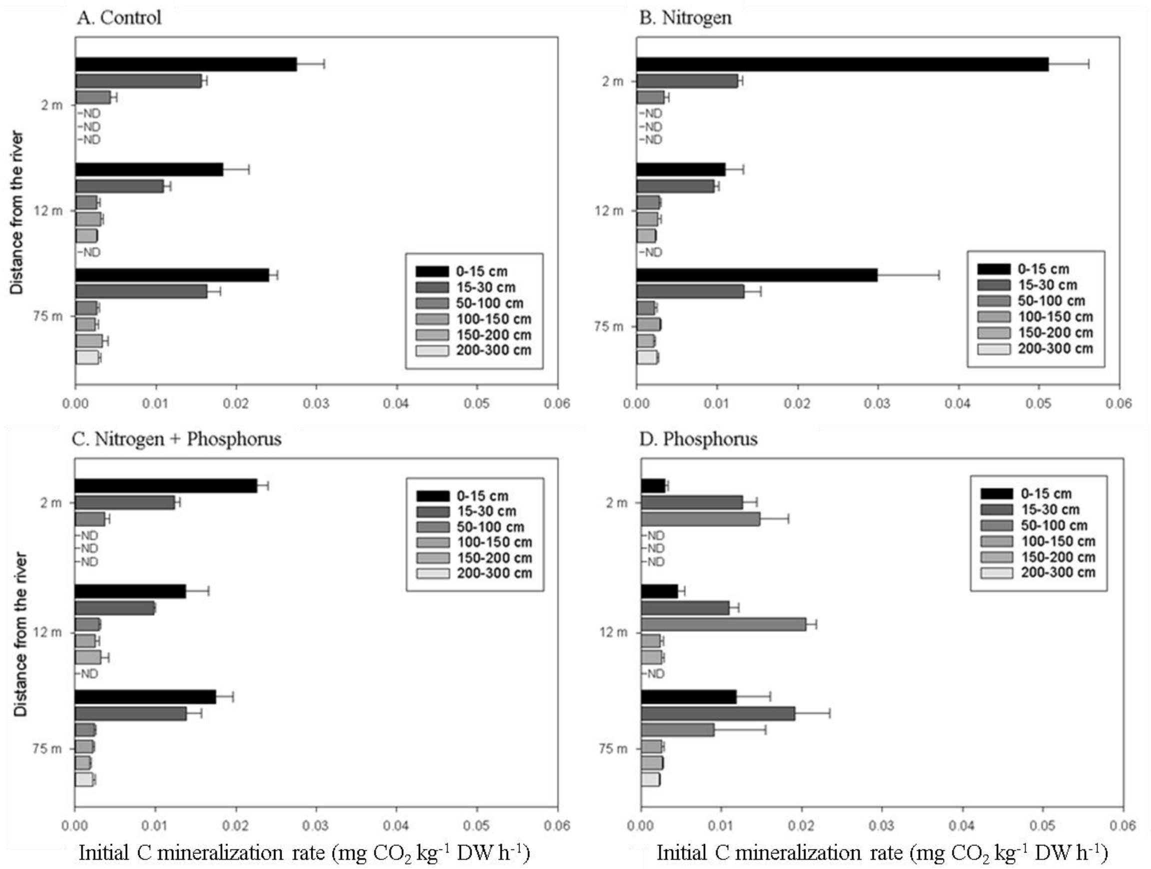

Figure 5.6 Initial C mineralization rates measured during the initial linear phase (between 0-48 h) after the application of a high dose of recalcitrant (high MW) DOC either alone or in combination with N, P or N+P. Values are presented for three different distances from the river (2, 12 and 75 m) and for 6 different soil depths. Soil depths were grouped into topsoil (0-30 cm), midsoil (50-100 cm) and deep soil (100-300) in the manuscript for the description of the factors assessed. Bars represent mean values (n = 3) ± standard errors. ND equates to no data due to hitting bedrock (Figure 5.2).

5.4 Discussion

Inherent differences in soil microbial community structure and physicochemical properties driven by soil depth and distance from the river were tested to investigate their influence in C dynamics and nutrient availability. Here, we explored whether microbial communities from different soil depths and distance from the river displayed the same underlying mechanisms and strategies during organic matter decomposition by analysing initial (immediate) and cumulative mineralization responses.

5.4.1 Effect of soil depth and substrate quantity on C mineralization

Depth showed to have the most striking effect on C mineralization irrespective of the amount, or type, of C added or the incubation time. The fact that microbial communities are regulated by different controlling factors and nutrient limitations at depth has been endorsed before by the few studies that explored C dynamics at depth (Fierer et al., 2003; Tian et al., 2017). Salome et al. (2010) identified greater spatial heterogeneity in soil physicochemical properties at depth and Manzoni et al. (2012) indicated soil moisture as an important constraint in C turnover. Rey et al., 2005 also indicated soil moisture as a crucial parameter in C_{min} rates and van Hees et al. (2005) and references therein reported similar decomposition percentages. Our results endorsed these findings during the total length of the experiment. Both high and low C additions showed faster decomposition rates in the topsoil compared to the subsoil during the first hours of the experiment which is in good agreement with studies such as Rey et al. (2007) or Sanaullah et al. (2010). Some argue that this effect could be due to a more active microbial community in response to regular C (rhizodeposition) and nutrient inputs (N_2 fixation and fertilizers) in grassland systems (Fontaine et al., 2003; Treseder, 2008). However, it is worth noting that although topsoil in our study was initially more responsive to the labile source of C, the size of the microbial population, which was highly correlated to C_{min} rates, was on average 87-fold greater in the top layer compared to the deepest layers (Table S1). Therefore, when C_{min} rates are expressed on a per unit PLFA basis (Figure S2-S4) a much faster use of C was seen at depth irrespective the source of C. Fierer et al. (2003) described the opposite pattern in respiration rates when results were normalized by the water potential and temperature respective to soil depth. Zhang et al. (2016) found the same negative correlation between PLFA biomass and moisture content as this study and also described a shift in basal respiration rates when microbial biomass was taken into account. Surprisingly, not many studies take into account the size of the microbial population when assessing C mineralization rates, however, that may be a reflection that most studies have only focussed on the topsoil horizons (Rey et al., 2005; Boddy et al., 2007; Creamer et al., 2016).

Interestingly, even though topsoil had a faster immediate response to the labile C addition, we observed higher cumulative percentages of C mineralized in deeper layers than topsoil at the end of the experiment. There are some studies that have reported similar results. For example, Sanaullah et al. (2010) found higher decomposition rates in the topsoil compared to deeper layers for over 6 months while the amount of C remaining after 3 years was similar in both horizons. Work presented by Salome et al. (2010) described an increase of 75% in mineralization rates in the soil subsurface with respect to the surface soil after disrupting the soil structure. Some studies have remarked upon the fact that different microbial strategies, r- and K-strategist, with different microbial growth and metabolism efficiencies may be involved in the mineralization process down the soil profile (Six et al., 2006; Fierer et al., 2007). In this sense, we did observe different patterns of microbial growth characterized by an initial lag phase before initiating decomposition (Sanaullah et al., 2011, Blagodatskaya et al., 2014). The addition of the high dose of C started to induce microbial growth in the midsoil and subsoil which suggests a more efficient population at depth (Figure S2). We hypothesized that in the topsoil, as a result of a more abundant and competitive microbial population (presumably r-strategists), it exhausted the available substrate quicker and then became dormant or unable to use SOM or became limited by some other factor (Fointaine et al., 2003). In the subsoil, there is potentially a greater abundance of K-strategists which are better adapted to surviving under limiting conditions and are more able to metabolise complex compounds, therefore remaining active in the latter stages of OM decomposition when the composition of OM is dominated by polymerised- instead of energy-rich compounds (Pianka, 1970; Fontaine et al. 2003).

Our results contradict studies by Heitkötter et al. (2017), in which a higher cumulative C mineralized in the form of organic acids was reported for the topsoil. However, from this study and others found in the literature there is evidence that supports the hypothesis that microbial communities have different substrate preferences and nutrient limitations which may control the intensity of degradation rates (Fountaine et al. 2007; Chen et al., 2012).

Contrastingly, the addition of a recalcitrant C source caused a noticeable decrease in C_{min} at depth. Kemmitt et al. (2008) indicated that SOM mineralization rates at depth were independent of microbial biomass size and that the abiological processes such as C association with mineral surfaces or, spatial isolation within soil aggregates (Jastrow et al., 2007) that made SOM unavailable for degradation were the limiting factors. In our case, the addition of a recalcitrant source of C, low in readily available energy, enhanced C storage rather than mineralization at depth. This fact was confirmed by the presence of a Fe-rich clay layer identified at depth (Table S3) which combined with the SOM source to restrict microbial access (Allison, 1973; Oades, 1988; Bergaya and Lagaly, 2006).

5.4.2 *Effect of nutrient addition on C mineralization*

Currently, there is a wide disparity on the effects of nutrient addition on C_{min}. While some studies report a priming effect of SOM decomposition after the addition of nutrients, others found negative or no effect (Liljeroth et al., 1994; Conde et al., 2005; van Hees et al.,2005; Janssens et al., 2010). In our study, the addition of nutrients (N and P) had no immediate or long-term effect when high amounts of C were supplied (Table 5.2, Figure 5.4). Conde et al. (2005) observed a priming effect of the SOM after the addition of nutrients. This lack of an overall effect suggests that microbial access to organic C is the most important factor determining uptake (both in the topsoil and subsoil) especially when C is released during root cell lysis or exudation. However, under low inputs of labile C (background content), greater CO_2 fluxes (both initial and total) were observed after N and NP addition, particularly for the top and midsoil, indicating greater nutrient limitation than in deeper layers (Figure 5.5). On the contrary, our data suggests that soils at depth must be C limited and therefore N and P amendments did not show any effect on C turnovers. This fact could reflect a more active and abundant microbial community whose maintenance requirements are higher due to their adaptation to a permanent supply of available substrate and therefore more C is used for respiration (root exudation and fertilizers) (Fontaine et al., 2003; van Bodegom, 2007; Treseder, 2008; Paterson et al., 2009).

Regarding the HMW compounds, the addition of nutrients had minimal immediate effect and little or no effect in the long run suggesting that regardless of the nutrient addition the nature of molecule is not preferential for microbial anabolic and catabolic use.

Interestingly, the addition of P only together with the HMW form of C had a suppressive effect on C mineralization rates in the top soil (0-15 cm) being especially remarkable in rows 1 and 2 (Figure 5.6). Bauhus et al., (1994) found a similar response on C depletion after the addition of P and Amador and Jones (1995) reported either a lack of effect or a depression on C mineralization rates. Although this effect has been rarely explored, it has been attributed to long-term P exposures and consequently high P concentrations in the soil (Keller et al., 2006). The high P availability and the reduction of P adsorption capacities found in this study in areas close to the watercourse (Table S1-S2) seemed to be in good agreement with this theory, although further work should be conducted to gain further insight on this inhibition.

5.4.3 Effect of substrate quality on C mineralization

The quality of the C source (meaning susceptibility to microbial enzyme degradation) has also been identified as a main driver controlling mineralization rates (Voroney et al., 1989; Chen et al., 2014; Rui et al, 2016). The use of complex and low-quality substrates requires high activation energies (extracellular enzymes) (Bosatta and Ågren, 1999) and because of this, a very low percentage of the HMW C added was used for microbial respiration (Figure 5.6). Mechanistically, this suggests that although decomposers are able to break down the recalcitrant SOC, the energy gained is lower than the energy needed to catabolise such substrate and therefore long-term storage is preferred (Fontaine et al., 2007).

5.4.4 Effect of distance from the river on C mineralization

Areas adjacent to watercourses are assumed to play a key role in C dynamics mainly due to the influence of hydrologic regimes and riparian vegetation that: 1) controls import/export OM fluxes between the watercourse and the floodplain 2) creates fluctuations of anaerobic/aerobic conditions regulating C source/sink balance 3) and encourages more diverse microbial communities (Gurtz et al., 1988; Lewis et al., 2003;Camino-Serrano et al., 2016; King et al., 2016). However, as far as soil physicochemical properties are concerned, our results stand in disagreement with the general assumption of greater potential C storage within the riparian zone. Stutter et al. (2012) indicated a greater OM content in areas close to the river whereas we found less. Nevertheless, these areas corresponded to unmanaged vegetated buffer strips, mostly fenced and subject to agricultural use. In our case, the first sampling distance from the edge of the river (2 m) fell outside of this very narrow vegetated buffer strip, preventing us therefore from seeing if any difference existed. Giese et al. (2000) could not establish a relationship between percent carbon in the soil and distance from the main channel across the riparian transect.

However, we did identify some interesting patterns that suggest different microbial responses with respect to distance from the river. It should be noted that although the statistical analysis showed a significant effect with respect to row (i.e. distance from the river) across the full range of C and nutrients amendment, it cannot be assumed that this effect reflects the influence of the riparian zone. Thus, the addition of a high dose of labile C exhibited differences in C_{min} rates respective of distance from the river, however, the differences were between the areas more distal to the river (row 2 and 3) suggesting that this fact is more related to the inherent physicochemical variability among rows rather the influence of the riparian zone. Similarly, for the HMW treatment, an effect of distance from the river was also displayed. However, this effect was more related to the suppressive effect of P on C_{min} as explained in section 4.1 than the influence of the riparian zone.

We consistently detected faster C turnover at soil depth of 50-100 cm after the addition of low labile C (treatments showed little or no effect). Previous work by Wilson et al (2011) illustrated the importance of flooding for C dynamics and microbial community structure. Our results suggest that this soil layer which was highly connected to fluctuating hydrology and nutrients, may have developed a more diverse microbial population that literature often generally attributes to riparian zones (Naiman and Decamps, 1997). However, contrasting these results is still challenging because research in riparian areas usually targets processes rather than microbial communities of interest (Gutknecht et al., 2006) and relegates C_{min} to a complement in N cycle (Seitzinger, 1994; Chen et al., 2012).

5.5 Conclusions

Global warming and the increases in CO_2 emissions from land use change and fossil fuel burning could considerably influence SOM residency time (i.e. increase root exudation and microbial activity). Results from our study revealed higher decomposition potential in the subsoil after labile substrate addition, even though the topsoil exhibited faster immediate decomposition rates which might indicate different microbial strategies through the soil profile. Nutrient addition had little or no effect on C_{min} suggesting that overall soil was C limited. Therefore, fast cycling of SOM is likely to be expected in the subsoil if any change in the land use or agricultural management increase the input of labile C along the soil profile. Under a recalcitrant source of C, different mechanisms were activated in the topsoil and subsoil. Whereas a slow-cycling C decomposition prevailed in the topsoil, microbial immobilization dominated in the subsoil which supports previous studies showing that microbial substrates preferences and nutrient limitation control the intensity of rates of degradation. In our study, the effect of the proximity to the river was overall tenuous for all the experiment, mainly focusing on the soil depth directly in contact with river dynamics. While this study provided underpinning information about C dynamics through the soil profile for managerial and modelling future work, further work is required to reliably estimate soil C fluxes in the field to correctly evaluate seasonal patterns and link it with microbial functional groups.

5.6 Acknowledgements

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Chapter 6

Spatial zoning of microbial function and plant-soil nitrogen dynamics across a riparian area

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L.L.S and D.L.J designed and conceived the experiment; L.L.S conducted the practical work, analysed the data and prepared the manuscript.B.A conducted the gene abundance analysis. All authors discussed results and contributed to preparation of the manuscript.

Abstract

Anthropogenic activities have significantly altered global biogeochemical nitrogen (N) cycling leading to major environmental problems such as freshwater eutrophication, biodiversity loss and enhanced greenhouse gas emissions. The soils in the riparian interface between terrestrial and aquatic ecosystems may prevent excess N from entering freshwaters (e.g. via plant uptake, microbial transformations and denitrification). Although these processes are well documented in intensively managed agroecosystems, our understanding of riparian N removal in semi-natural systems remains poor. Our aim was to assess the spatial zoning of soil microbial communities (PLFA), N cycling gene abundance (archaeal and bacterial *amoA*, *nifH*, *nirK*, *nirS*, *nosZ*), N processing rates and plant N uptake across an extensively sheep grazed riparian area. As expected, soil properties differed greatly across the riparian transect, with significant decreases in organic matter, NH_4^+, carbon (C) and N content closer to the river (< 10 m). In addition, different microbial community structures were found along the transect. The abundance of N fixation (*nifH*) increased with distance from the river (> 10 m), while ammonia oxidising archaea (AOA) increased in abundance towards the river. N_2O emissions rates were shown to be limited by C and to a lesser extent by N with greater emissions close to the river. Plant uptake of urea-derived ^{15}N was high (ca. 55-70% of that added to the soil) but 30-65% of the N was potentially lost by denitrification or leaching. It also suggests that the spatial patterning of plant and microbial N removal processes are different across the riparian zone. Our study provides novel insights into the underlying mechanisms controlling the spatial variability of N cycling in semi-natural riparian ecosystems.

Key Words: buffer strip, ecosystem services, DON, nitrification, heathland, wetlands

6.1 Introduction

The overuse of nitrogen (N) fertilizers, alongside land use change, has caused the N saturation of many terrestrial ecosystems worldwide (Gruber and Galloway, 2008). Further, the resultant N loss from these agroecosystems is contributing to many major environmental problems such as marine and freshwater eutrophication, loss of biodiversity, climate change and ecosystem acidification (Canfield et al., 2010; Erisman et al., 2013). Strategies are therefore needed to better retain, or sustainably remove, excess N from land under agricultural production. One potential mechanism is the active management of riparian areas at field margins to intercept and mitigate excess N from migrating towards freshwaters. Within these areas, a range of interrelated biotic and abiotic processes may be involved in N attenuation including, nitrification, denitrification, mineralization, plant and microbial uptake, mass flow/diffusion and sorption-desorption (Matheson et al., 2002; Vyzamal, 2007). The importance of each process, however, is expected to vary greatly between ecosystems and also from the landscape down to the micrometre scale within the plant-microbial-soil system (Burt et al., 1999; Sanchez-Pérez et al., 2003).

Denitrification has been shown to be of particular importance for riparian wetland biogeochemistry because of the predominance of anoxic conditions, high concentrations of dissolved organic carbon (DOC) and the high rates of N fixation (Groffman and Hanson, 1997). It also represents the ultimate removal mechanism for reactive N (e.g. NO_3^-, NO_2^-, N_2O) from terrestrial and aquatic ecosystems (Seitzinger et al., 2006; Jacinthe and Vidon, 2017). In some cases, however, complete denitrification to N_2 may not occur due to a lack of N_2O reductase in the microbial community or if certain environmental conditions remain sub-optimal (e.g. soil moisture, O_2 content), leading to the potential release of environmentally damaging N_2O (Butterbach-Bahl et al., 2013). Additionally, denitrification is strongly coupled, both spatially and temporally, with other environmental processes such as N fixation, nitrification and anaerobic ammonium oxidation (anammox) (Vyzamal, 2007; Groffman et al., 2009).

To optimise N removal by riparian areas and to implement active management, requires a good understanding of the key factors which regulate N cycling across these zones. Fundamental to this, is understanding the spatial abundance and behaviour of the underlying microbial communities which control how and when the different N transformations occur (Herbert, 1999; Chon et al., 2011). In this respect, few studies have tried to combine the analysis of key N cycling genes (abundance and transcription) and quantification of N_2O:N_2 production to gain a better insight into the spatio-temporal factors regulating N_2O fluxes (Bakken et al., 2012; Di et al., 2014). However, contradictory studies showing a clear relationship between gene copy number and N_2O emission rates or a total lack of it, are commonly presented, highlighting the need for further research in this area (Avrahami and Bohannan, 2009). Additionally, research in wetland biogeochemistry has frequently focused on single-ecosystem processes (i.e. denitrification) rather than providing a more holistic view of microbial community functioning (Gutknech et al., 2006).

Alongside the microbial community, wetland vegetation also plays a major role in regulating N losses via denitrification (Schnabel et al., 1996; Veraart et al., 2011). For example, it has been shown that plants can alter the size and composition of the soil microbial community, stimulate microbial activity via C rhizodeposition, and change soil oxidation status (Nijburg and Laanbroek, 1997; Tabuchi et al., 2004; Groffman et al., 2009). In addition, wetland plants employ numerous physiological adaptations to overcome anoxia in waterlogged soils including: shallow rooting, dumping of respiratory by-products into the rhizosphere (e.g. lactic acid) and the formation of aerenchyma (Wheeler, 1999). In light of this, the choice of plant species is likely to be very important for improved riparian management and freshwater protection.

While much work has been undertaken on N removal in riparian areas adjacent to intensive cropping systems, comparatively little work has been undertaken in extensively grazed livestock systems (Wells et al., 2016). In these systems, urine hotspots represent the major input of reactive N and are expected to greatly modify soil microbial communities involved in N cycling (Di et al., 2010). In this context, the main objectives of the present study were: (1) to gain further insight into the environmental factors controlling riparian soil N cycling and how they contribute to explaining the spatial and temporal variability of N cycling in semi-natural ecosystems; (2) to estimate the role of different vegetation communities in N uptake across the riparian zone; and (3) to link N cycling gene abundance to N removal processes.

6.2 Materials and methods

6.2.1 Study site

The experimental site is located in the upper, southern area of the Conwy catchment, North Wales, UK (52° 59' 8.90"N, 3° 49' 15.99"W; Figure 6.1; Figures S1 and S2). The study area is classified as blanket bog according to the New Phase 1 habitat survey (Lucas et al., 2011) and is considered a Special Area of Conservation (SAC) under the EC Habitats Directive (94/93/EEC). The climate of the upper reaches of the Conwy catchment is characterized by relatively high rainfall and cool temperatures (mean annual rainfall of 2180 mm, and mean annual soil temperature at 30 cm depth is 8 °C; based on 30-year average 1981-2010 data from the UK Met Office). The area is subject to sheep (*Ovis aries* L.) grazing at a low stocking density (0.1 ewe ha^{-1}). A detailed description of the Conwy catchment and land use can be found in Emmett et al. (2016) and Sharps et al. (2017).

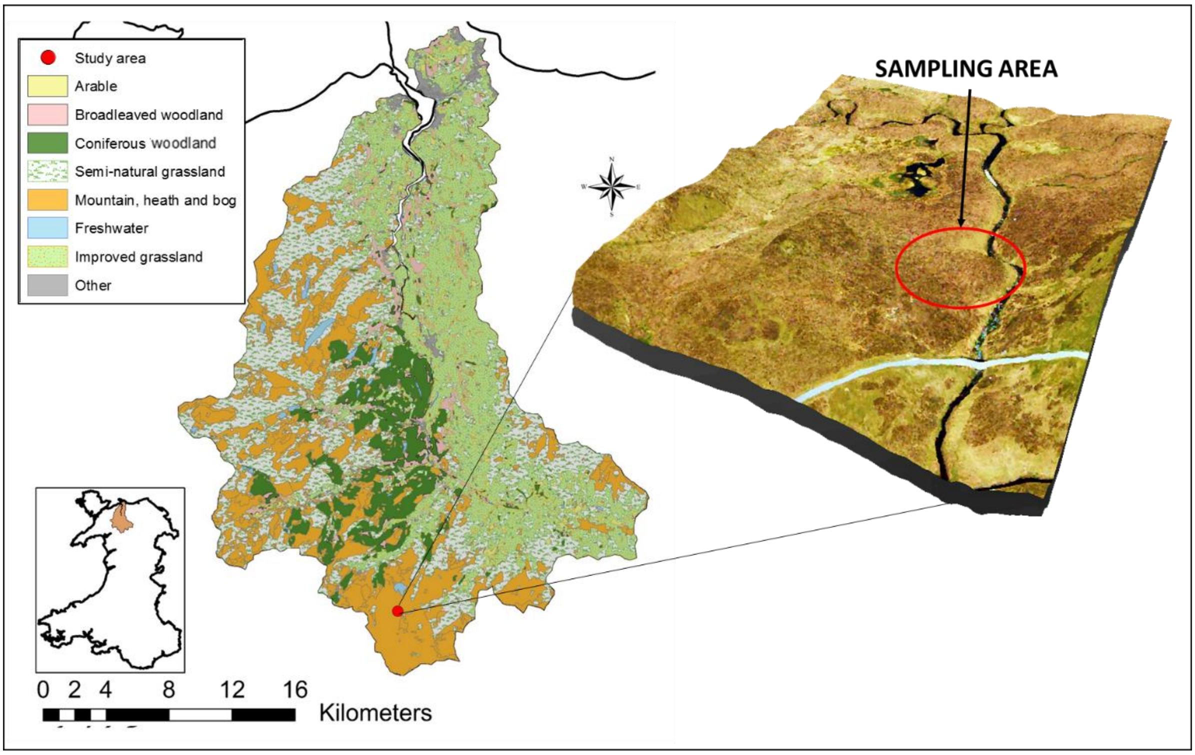

Figure 6.1 The Conwy catchment, North Wales, UK showing the location of the riparian sampling area and the major land cover classes.

6.2.2 Sampling strategy

Four 25 m long transects, 5-10 m apart, and perpendicular to a headwater stream of the Conwy River, were delineated for sampling during the month of October 2016 (Figure 6.2). The maximum length of the transects was decided according to the extent of the riparian zone as defined by the variable buffer delineation method (de Sosa et al., 2017). Intact soil cores (5 cm diameter, 0-15 cm depth) were collected at three different zones (from this point onwards in the manuscript, these are referred to as zones 1, 2 and 3), selected according to their dominant vegetation cover (Figure 6.2). Zone 1 is dominated by thick tufts of soft rush (*Juncus effusus* L.) and is located < 5 m to the river. Zone 2 corresponds to the transitional area between the grasses and the heathland (5-10 m) and zone 3 (> 10 m) represents the area dominated by typical peat-forming heathland species such as bog-mosses (*Sphagnum* spp.), *Calluna vulgaris* (L.) Hull, *Erica tetralix* L. and *Scirpus cespitosus* L. (Figures S1-S2). Along each transect, two sample points were located within zone 1 (2 and 5 m from the edge of the river), one sample point was located within zone 2 (5-10 m), and two sampling points were located in zone 3 (i.e. 15 and 25 m; Figure 6.2).

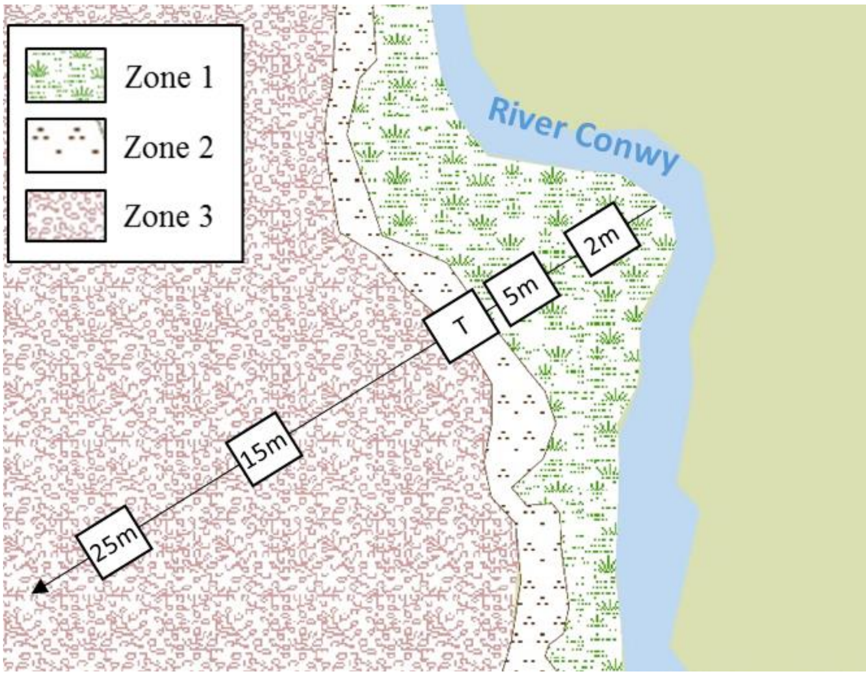

Figure 6.2. Location of sample points across the riparian area. Different colours indicate changes in vegetation. Zone 1 represents the area dominated by *Juncus effusus*, zone 2 corresponds to the transitional area between the grasses and the heath, and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species.

Intact soil cores were taken with a Russian auger (5 cm diameter, 15 cm in length; Eijkelkamp Soil & Water, Giesbeek, The Netherlands) to conduct the main denitrification experiment. Additional intact soil cores were taken for analysis of soil physicochemical properties prior to conducting the laboratory study and a further 20 cores for bulk density determination. All soil samples were stored at 4 °C prior to analysis except for subsamples (~25 g) which were used for Phospholipid Fatty Acid analysis (PLFA) and DNA extractions. These samples were stored immediately at -80 °C.

6.2.3 General soil characterization

Soil samples were passed through a 2 mm sieve to remove any plant material and to ensure sample homogeneity. They were held at field moisture for all subsequent analyses to represent field conditions. Soil water content was determined gravimetrically (24 h, 105 °C) and soil organic matter content was determined by loss-on-ignition (LOI) (450 °C, 16 h). Soil pH and electrical conductivity (EC) were measured using standard electrodes in a 1:2.5 (*w*/*v*) soil-to-deionised water mixture. Total available ammonium (NH_4-N) and nitrate (NO_3-N) in soil were determined within 0.5 M K_2SO_4 extracts (1:5 *w*/*v*) via the colorimetric salicylate procedure of Mulvaney (1996) and the vanadate method of Miranda et al. (2001), respectively. Available phosphate (P) was quantified with 0.5 M acetic acid extracts (1:5 *w*/*v*) following the ascorbic acid-molybdate blue method of Murphy and Riley (1962) and total C (TC) and N (TN) were determined with a TruSpec® elemental analyser (Leco Corp., St Joseph, MI). Dissolved organic C (DOC) and total dissolved N (TDN) were quantified in 1:5 (*w*/*v*) soil-to-0.5 M K_2SO_4 extracts (Jones and Willett, 2006) using a Multi N/C 2100 TOC analyzer (AnalytikJena, Jena, Germany). Total soil porosity was determined using the equation of 1-(bulk density/particle density for organic soils) and percent water-filled pore space (WFPS) was obtained from the relationship between the volumetric water content and total soil porosity. Anaerobic mineralizable N (AMN) was determined by the anaerobic incubation of soil samples for 14 days at 25-30 °C in the dark, followed by extraction with 1 M KCl and measurement of NH_4-N produced as described above (Bundy and Meisinger, 1994). Anaerobically mineralizable organic C (AMOC) was calculated as described in Ullah and Faulkner (2006). Briefly, moist soil samples were placed in gas-tight containers and NO_3^- was added to remove any soil limitation. Containers were purged with N_2 gas to induce anoxic conditions and stored in the dark at room temperature (25 ºC). The headspace of the containers was sampled after 1, 24, 48 and 72 h of incubation and analysed for CO_2

concentration on a Clarus 500 gas chromatograph with a TurboMatrix headspace autoanalyzer (Perkin-Elmer Inc., Waltham, CT).

6.2.4 Phospholipid fatty acid analysis

Microbial community structure was measured by phospholipid fatty acid (PLFA) analysis following the method of Buyer and Sasser (2012). Briefly, samples (2 g) were freeze-dried and Bligh-Dyer extractant (4.0 ml) containing an internal standard added. Tubes were sonicated in an ultrasonic bath for 10 min at room temperature before rotating end-over-end for 2 h. After centrifuging (10 min) the liquid phase was transferred to clean 13 mm × 100 mm screw-cap test tubes and 1.0 ml each of chloroform and water added. The upper phase was removed by aspiration and discarded while the lower phase, containing the extracted lipids, was evaporated at 30 °C. Lipid classes were separated by solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well (Phenomenex, Torrance, CA). Phospholipids were eluted with 0.5 ml of 5:5:1 methanol:chloroform:H_2O (Findlay, 2004) into glass vials, the solution evaporated (70 °C, 30 min). Transesterification reagent (0.2 ml) was added to each vial, sealed and incubated (37 °C, 15 min). Acetic acid (0.075 M) and chloroform (0.4 ml each) were then added. The chloroform was evaporated just to dryness and the samples dissolved in hexane. The samples were analysed with a 6890 gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with autosampler, split-splitless inlet, and flame ionization detector. Fatty acid methyl esters were separated on an Agilent Ultra 2 column, 25 m long × 0.2 mm internal diameter × 0.33 µm film thickness. Standard nomenclature was followed for fatty acids (Frostegård et al., 1993). A detailed description of PLFA markers and taxonomic microbial groups is provided in Table S1.

6.2.5 Denitrification and potential N_2O emissions

Denitrification rates were measured using the acetylene (C_2H_2) block method based on the intact core technique developed by Tiedje et al. (1989). Although this technique presents limitations such as the poor diffusion of C_2H_2 into the soil, it has been found to produce similar results to experiments using ^{15}N tracers (Aulakh et al., 1991).

In brief, intact soil cores (approximately 37 ± 1.5 g dry weight soil) were placed in PVC tubes (10 × 15 cm) to maintain soil structure. These tubes were then placed in gas-tight containers (1.4 dm^3 volume; Lock & Lock Ltd., Seoul, Republic of South Korea).

To measure denitrification, 20 ml of 4 different C and N amendments were applied to individual soil cores (n = 20 per amendment):

1) Control (distilled water addition only)

2) Glucose-C addition (glucose solution containing 4 g C l^{-1}; 55 mM glucose)

3) Urea-N addition (artificial sheep urine containing 2 g N l^{-1}; Selbie et al., 2015)

4) Urea-N + glucose-C addition (artificial urine plus glucose solution containing 2 g N l^{-1} and 4 g C l^{-1} respectively).

Urea was selected as it represents one of the main N inputs to upland grazed ecosystems. The N concentration was chosen according to the concentration range in urine under a light grazing regime (Selbie et al., 2015). The ratio of C-to-N was chosen based on experimental values presented in Her and Huang (1995). Glucose was chosen as it represents a labile C substrate that can be utilized by almost all soil microorganisms (Gunina and Kuzyakov, 2015). The concentration of added C also reflects a typical sugar concentration that would occur in soil upon root cell lysis (Jones and Darrah, 1996).

All cores were directly injected with 5 ml of C_2H_2 into the middle of the soil volume. The cores were then placed into gas-tight containers and 10% of the headspace replaced with C_2H_2 to block the conversion of N_2O to N_2 gas. The control cores were only amended with 20 ml of distilled water without C_2H_2 addition. The containers were stored at 10 ºC in the dark to prevent C_2H_2 breakdown. Headspace gas was sampled at 0, 2, 6 and 24 h and stored in pre-evacuated 20 ml glass vials before being analysed for N_2O concentration on a Clarus 500 gas chromatograph with a TurboMatrix headspace autoanalyzer (Perkin-Elmer Inc., Waltham, CT). Prior to gas sampling, the headspace was homogenised by gently mixing with a syringe. At the end of the experiment, each individual core was weighed and N_2O fluxes corrected accordingly. The rate of N_2O production was calculated in µg N-N_2O g^{-1} dw h^{-1}. Cumulative N_2O emissions were calculated by integration using the trapezoidal rule.

6.2.6 Nitrogen uptake by vegetation

The role of vegetation in N uptake was measured in the field using ^{15}N-labelled urine. Two independent sets of plots (50 × 50 cm^2) were randomly selected within each replicate vegetation zone, one set received no N additions (herein referred to as the control set) while the second received ^{15}N-labelled artificial urine.

Prior to addition of the ^{15}N-labelled treatment, turfs (20 × 20 cm^2) and associated soil (0-15 cm depth) were taken from the centre of each of the control plots to obtain ^{15}N natural abundances for each plant and soil component. After harvest, the samples were transferred to the laboratory and separated into soil, roots, shoots and mosses for ^{15}N determination. Subsequently, in each ^{15}N-labelling plot, 250 ml of artificial urine labelled with ^{15}N urea (15 atom %) at a rate of 2 g N l^{-1} was applied (equivalent to 20 kg N ha^{-1}). Ten pulses of ^{15}N-labelled urine (each pulse was 25 ml in volume) were injected with a syringe (0.84 mm bore × 5 cm long) into the soil underneath the plants (0-15 cm depth) in the centre of the plot. The volume and concentration of N added followed that of a typical sheep urine event (Marsden et al., 2016). Immediately after the final ^{15}N pulse addition, the area was protected with individual wire mesh cages to prevent livestock trampling and grazing. One week after ^{15}N addition, a 20 × 20 cm^2 turf and associated soil (0-15 cm depth) was harvested from the middle of each plot, transferred to the laboratory and separated into soil and plant components as described above for ^{15}N determination.

Soil for ^{15}N analysis was passed through a 2-mm sieve and subsamples (ca. 40 g) were oven-dried (48 h, 80 °C) before being weighed and ground for ^{15}N analysis. Plant shoot and root material followed the same drying procedure after being washed with distilled water to remove any exogenous isotope label. The same procedures were followed for the control samples one week before to avoid any cross-contamination with the ^{15}N-urea labelled samples. All fractions were analysed separately for ð^{15}N at the UC Davis Stable Isotope Facility (UC Davis, Davis, CA). Values of ^{15}N are presented directly as the atom% of ^{15}N in the sample. The ^{15}N atom% excess was calculated as the ^{15}N atom% difference between enriched samples and values of background natural abundances (control). Recovery of tracer ^{15}N (%) was calculated by multiplying the N content in the pool by it mass per square metre and ^{15}N atom% excess divided by total added ^{15}N per square metre (Xu et al., 2011).

6.2.7 DNA extraction and quantitative PCR

A subsample of soil (ca. 25 g) was taken from each of the cores used for physicochemical analysis and stored at -80 °C prior to DNA extraction. The DNA was extracted from three 250 mg subsamples using an UltraClean® Microbial DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA) following the manufacturer's instructions. Triplicate DNA extractions for each soil sample were pooled together to give a total volume of 150 µl. Extractions of DNA were concentrated to give a final volume of 50 µl using a Savant SVC100H SpeedVac Concentrator (ThermoFisher Scientific Inc., Waltham, MA). Extracted DNA was visualized by 0.9% agarose gel electrophoresis and nucleic acid staining with SafeView® (NBS Biologicals, Huntingdon, UK). The concentrations of DNA where checked using Quant-iT™ dsDNA Assay Kit (ThermoFisher). Samples were then stored at -80 °C prior to further analysis.

Microbial N cycling gene abundance was investigated by quantitative-PCR (qPCR) targeting specific genes or genetic regions. Bacterial and archaeal communities were targeted via the 16S rRNA genes, while the fungal community abundance by the ITS region. The different communities involved in N-cycling were investigated: N fixation (*nifH* gene); nitrification by targeting the ammonia oxidising bacteria (AOB) and archaea (AOA) (*amoA* gene), and denitrifiers via the nitrite reductase (*nirK* and *nirS* genes) and the nitrous oxide reductase (*nosZ* genes clade I and II) (Table S2).

Quantitative-PCR amplifications were performed in 10 µl volumes containing 5 µl of QuantiFast (Qiagen, Manchester, UK), 2.8 µl of nuclease-free water (Severn Biotech, Kidderminster, UK), 0.1 µl of each primer (1 µM) and 2 µl of template DNA at 5 ng µl^{-1}, using a CFX384 Touch® Real-Time PCR Detection System (Bio-Rad, Hemel Hempstead, UK). The standards for each molecular target were obtained using a 10-fold serial dilution of PCR products amplified from an environmental reference DNA (also used as positive control) and purified by gel extraction using the Wizard® SV Gel and PCR Clean Up System (Promega, Southampton, UK) following the manufacturer's instruction and quantified by fluorometer Qubit® 2.0 dsDNA BR Assay Kit (Thermo Fisher Scientific). Standard curve template DNA and the negative/positive controls were amplified in triplicate. Amplification conditions for all qPCR assays consisted in 2 steps: first denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C for 10 s and 60 °C for 30 s that included annealing, elongation and reading. Each amplification was followed by melting curve (increase in temperature from 60 °C to 95 °C, with a reading every 0.5 °C) to assess the specificity of each assay. The efficiency of the qPCR varied between 81.5% and 94.5%, and r^2 between 0.996 and 0.999. The melting curves showed specificity for all the genes, except as expected for the fungal ITS, that showed the amplification of products of different lengths, due to the variability in length of the ITS region between different fungal taxa (Manter and Vivanco, 2007).

6.2.8 Statistical analysis

Statistical analysis was performed with SPSS v22 for Windows (IBM Corp., Armonk, NY). All data were analysed for normality and homogeneity of variance with Shapiro Wilk's tests and Levene's statistics, respectively. Transformations ($\log_{10}$ or square root) to accomplish normality and homogeneity of variance were done when necessary. For all statistical tests, $P < 0.05$ was selected as the significance cut-off value. Analysis of variance (one-way ANOVA) was performed to explore the difference of soil physicochemical properties, gene copy numbers, PLFA ratios of microbial groups respective to distance from the river followed by Tukey's post-hoc test to assess differences across the riparian transect. Principal component analysis (PCA) was used to explore the spatial relationships of PLFA microbial groups (%) relative to distance from the river. Cumulative N_2O emissions after treatment application across the riparian transect were compared by Welch's test followed by Games-Howell post-hoc test, due to the data not conforming to homogeneity of variance even after data transformation. In contrast, a one-way ANOVA followed by Tukey's post-hoc test was performed to assess differences in cumulative N_2O emissions between treatments for each sampling distance from the river (i.e. 2, 5, 10, 15 and 25 m). Two separate analyses were conducted to explore differences in ^{15}N recovery. A one-way ANOVA and Tukey's post-hoc test was performed to explore differences in the percentage allocation of ^{15}N to the different fractions (e.g. shoots, roots, moss and soil) across the different riparian zones. A second one was used to assess how ^{15}N recovery differed within each specific fraction across the three zones.

Spearman rank correlation coefficients (ρ) were used to evaluate the relationship between soil physicochemical properties and cumulative N_2O emissions, gene copy number, or PLFA biomarkers ratio whereas linear regressions (r^2) were used between soil physicochemical properties and PLFA biomarker ratios.

6.3 Results

6.3.1 General soil characterization

Significant differences in all soil properties, except for NO_3^- and total dissolved N concentration and anaerobic mineralizable N (AMN), were found across the riparian transect relative to distance from the main river channel (Table 6.1). Zone 2 showed an increase in pH values by 0.66-0.85 unit in comparison with zone 1 and zone 3. Likewise, EC was approximately 2-fold greater in zone 1 and 3 relative to zone 2. In addition, soil organic matter (SOM) tended to increase with distance from the river being 60% higher at the distal points (15 and 25 m) compared with those closer to the river. The high SOM levels associated with soils furthest away from the river contributed to lower bulk densities, higher soil porosities and increased soil water content. Available NH_4^+ concentrations were 3.6 greater in soil from zone 3 in comparison with zone 1 and 1.8 times greater than the soil in zone 2, while NO_3^- did not show any significant differences. Similarly, available P was 10-times greater in zone 3 relative to zones 1 and 2. Total C, total N and the C-to-N ratio were greater in zone 3 relative to zones 1 and 2 and a similar trend was also observed for DOC.

Anaerobic incubation of soils across the transect showed that the amount of AMOC in zone 3 was significantly greater than in zone 1 and 2 (~ 3 and 1.5 times, respectively) (Table 6.1). In contrast, AMN showed little trend across the transect.

Table 6.1. Soil physicochemical properties across the riparian transect. Different zones indicate changes in vegetation community with zone 1 being closest to the river. Values represent means ± SEM (n = 4). Same lower-case letters indicate no significant differences (P > 0.05) with regard to distance from river according to One-way ANOVA and Tukey or Games-Howell post-hoc test. Results are expressed on a soil dry weight basis.

Soil property	Zone 1		Zone 2	Zone 3	
	2 m	5 m	10 m	15 m	25 m
pH	4.18 ± 0.08^{a}	4.24 ± 0.05^{a}	4.90 ± 0.09^{b}	4.12 ± 0.02^{a}	4.05 ± 0.01^{a}
EC (μS cm^{-1})	23.4 ± 3.2^{a}	21.1 ± 2.0^{a}	11.6 ± 1.0^{b}	23.3 ± 2.4^{a}	26.3 ± 1.8^{a}
Bulk density (g cm^{-3})	0.31 ± 0.019^{a}	0.20 ± 0.026^{a}	0.09 ± 0.005^{b}	0.09 ± 0.004^{b}	0.09 ± 0.008^{b}
Total porosity (cm^3 cm^{-3})	0.78 ± 1.33^{a}	0.86 ± 1.88^{ab}	0.94 ± 0.36^{b}	0.94 ± 0.32^{b}	0.93 ± 0.54^{b}
Soil gravimetric water content (g kg^{-1} soil)	659 ± 28^{a}	720 ± 5^{a}	793 ± 34^{a}	892 ± 2^{b}	899 ± 0.6^{b}
Organic matter (g kg^{-1} soil)	364 ± 20^{a}	470 ± 12^{b}	542 ± 87^{ab}	953 ± 5^{c}	965 ± 4^{c}
NH_4^+-N (mg kg^{-1} soil)	5.06 ± 0.95^{a}	4.75 ± 0.70^{a}	9.50 ± 1.56^{ab}	18.5 ± 1.94^{b}	16.7 ± 3.43^{b}
NO_3-N (mg kg^{-1} soil)	9.38 ± 0.92^{a}	12.6 ± 2.40^{a}	8.12 ± 3.09^{a}	10.5 ± 1.95^{a}	8.00 ± 0.91^{a}
Available P (mg kg^{-1} soil)	5.82 ± 3.60^{a}	3.10 ± 1.11^{a}	5.99 ± 3.68^{a}	56.0 ± 10.1^{b}	50.5 ± 13.7^{b}
Total C (g kg^{-1} soil)	215 ± 9^{a}	281 ± 8^{b}	330 ± 57^{abc}	576 ± 4^{c}	588 ± 17^{c}
Total N (g kg^{-1} soil)	8.58 ± 0.61^{a}	12.0 ± 0.57^{b}	15.5 ± 2.58^{abc}	17.1 ± 0.11^{c}	15.7 ± 0.38^{c}
C-to-N ratio	25.3 ± 0.77^{a}	23.5 ± 0.92^{ab}	21.3 ± 0.53^{b}	33.8 ± 0.32^{c}	37.0 ± 1.96^{c}
Dissolved organic C (g kg^{-1} soil)	0.24 ± 0.02^{a}	0.36 ± 0.02^{bc}	0.38 ± 0.07^{ab}	1.31 ± 0.18^{cd}	1.09 ± 0.14^{cd}
Total dissolved N (g kg^{-1} soil)	0.04 ± 0.005^{a}	0.05 ± 0.005^{a}	0.06 ± 0.009^{a}	0.44 ± 0.29^{a}	0.11 ± 0.025^{a}
Microbial biomass PLFA (mmol kg^{-1} soil)	1.12 ± 0.21^{a}	2.02 ± 0.27^{a}	3.83 ± 1.25^{ab}	7.58 ± 0.54^{b}	7.29 ± 1.70^{b}
AMOC (mg C-CO_2 kg^{-1} soil h^{-1})	0.23 ± 0.04^{a}	0.41 ± 0.06^{ab}	0.61 ± 0.16^{ab}	0.92 ± 0.14^{b}	0.98 ± 0.20^{b}
AMN (mg kg^{-1} soil)	69.0 ± 12.2^{a}	116 ± 13.7^{a}	104 ± 15.2^{a}	96.0 ± 8.27^{a}	97.8 ± 30.0^{a}

Electrical conductivity (EC). Phospholipid Fatty Acid Analysis (PLFA). Anaerobically mineralization organic carbon (AMOC). Anaerobically mineralization nitrogen (AMN).

6.3.2 Microbial community structure and abundance

Microbial biomass determined from total PLFA content showed a general decline across the riparian transect towards the river channel. Principal Component Analysis (PCA) of PLFA microbial groups (% abundance) across the transect explained 72.6% of the total variance within the dataset on the first two principal components (PC) (Figure 6.3). The spatial segregation of cluster centroids within the PCA indicates that in zone 1 the most influential components were anaerobes and putative arbuscular mycorrhizal fungi (AM fungi). In contrast, Gram (+) and Gram (-) bacteria were the dominant groups in zone 2 and 3, respectively. Zone 2 showed the greatest microbial variability.

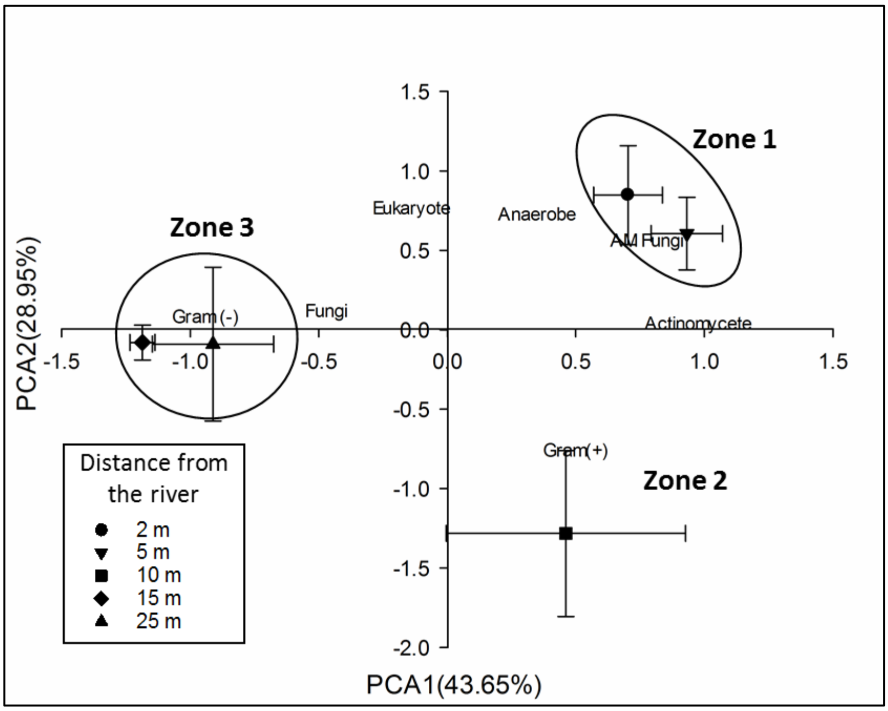

Figure 6.3 Correlation bi-plot from the principal component analysis (PCA) on PLFA microbial groups (%) with respect to distance from the river (*n* = 4). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (2 and 5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m), and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (15 and 25 m). Correlation of PLFA microbial groups with the main axes are given by their specific names and distance from the river by cluster centroids (average score on each horizontal principal component (PC1) and vertical principal component (PC2) with standards errors). Circles represents sample points within the same zone.

The fungi/bacteria ratio decreased by 2 to 2.5 times from zone 1 to zone 2 and 3 (Table S3, P = 0.008). The ratio of Gram (+)/Gram (-) was over 2 times greater in zone 2 than zone 3 but it did not differ from zone 1 (P = 0.001). On average, 16w/17cyclo and 18w/19cyclo ratio (indicative of an actively growing community under low stress conditions) was 2.5-fold greater in zone 3 than zone 1 and 2 (Table S3, P < 0.0001). There were highly positive relationships between fungi/bacteria ratio with bulk density and negatively with total porosity and soil water content (r^2 = 0.60, P < 0.001 for bulk density and total porosity, r^2= 0.45, P = 0.001, soil water) whereas Gram (+)/Gram (-) ratio was negatively correlated to NH_4-N, soil water content, SOM, available-P and DOC content (r^2 > 0.68 for available P and DOC, r^2 > 0.53 the rest, P < 0.001 in all cases). In contrast, a positive correlation was found between 16w/17cyclo and 18w/19cyclo ratios and DOC, soil water, C-to-N ratio and SOM content (r^2 > 0.61, for DOC, r^2 > 0.71, for soil water and C-to-N ratio, r^2 > 0.82, for SOM, P < 0.001 in all cases).

The archaeal 16S rRNA gene abundance tended to increase with distance from the river but the results were not significantly different (P > 0.05) (Figure 6.4). In contrast, the fungal ITS region abundance showed the opposite trend but was also not significant. The bacterial 16S rRNA gene abundance displayed on average 2 times greater bacterial copies in zone 2 than the distal area but it was not significant (Figure 6.4).

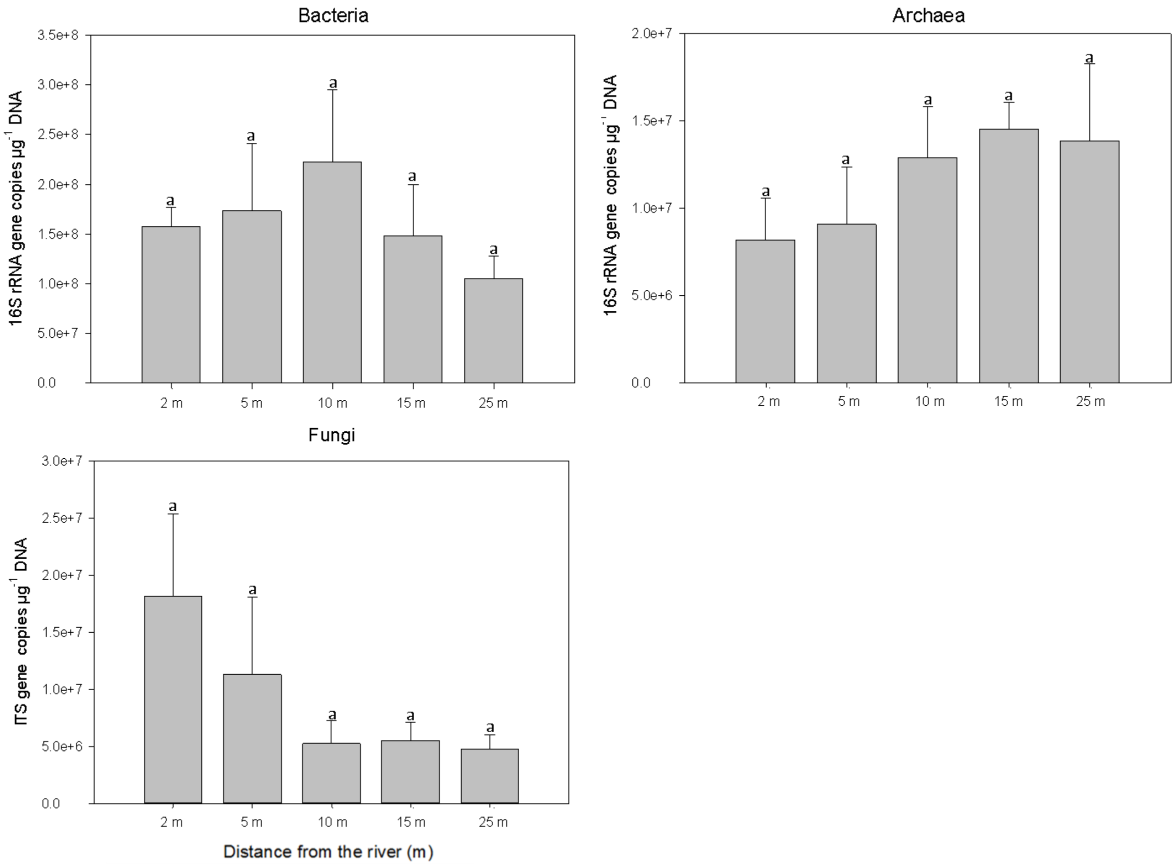

Figure 6.4. Total bacterial, archaeal and fungal gene copy numbers relative to distance from the river. Same lower case letters indicate no significant differences (P > 0.05) with respect to

distance from the river according to one-way ANOVA and the Tukey post-hoc test. Bars represent mean values (n = 4 for 10, 15 and 25 m, n = 3 for 2 m and n = 2 for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 6.2.

Significant positive correlations were found between bacterial *16SrRNA* and pH and EC (ρ = 0.48, -0.45, respectively) whereas archaeal *16SrRNA* correlated negatively with soil bulk density and positively with total porosity (ρ = -0.57, 0.56, respectively; Table 6.2).

Table 6.2. Spearman's rank correlation coefficients and *P*-values between soil physicochemical properties and abundance of functional genes (gene copies μg^{-1} DNA). Significant correlations are shown in bold.

Functional genes	Bacterial *16SrRNA*	Archaeal *16SrRNA*	Fungal *ITS*	*nifH*	Bacterial *amoA*	Archaeal *amoA*	*nirK*	*nirS*	*nosZ*
pH	**0.478**	0.131	0.071	-0.055	0.066	**0.614**	0.275	**0.515**	**0.495**
p-value	**0.033**	0.583	0.788	0.833	0.801	**0.009**	0.286	**0.034**	**0.043**
EC	**-0.450**	-0.128	-0.018	-0.151	-0.170	-0.471	**-0.522**	**-0.522**	**-0.627**
p-value	**0.047**	0.590	0.944	0.563	0.513	0.057	**0.031**	**0.031**	**0.007**
Bulk density	-0.040	**-0.568**	0.440	**-0.699**	0.419	**0.723**	-0.450	0.368	0.184
p-value	0.867	**0.009**	0.077	**0.002**	0.094	**0.001**	0.070	0.146	0.480
Total porosity	0.043	**0.562**	**-0.427**	**0.704**	-0.414	**-0.726**	0.454	-0.365	-0.168
p-value	0.856	**0.010**	**0.087**	**0.002**	0.098	**0.001**	0.067	0.150	0.519
Soil water content	-0.057	0.378	-0.249	**0.592**	-0.215	**-0.907**	0.215	-0.407	-0.316
p-value	0.810	0.101	0.335	**0.012**	0.408	**0.000**	0.408	0.105	0.216
Organic matter	-0.171	0.338	-0.218	**0.597**	-0.244	**-0.907**	0.261	-0.421	-0.360
p-value	0.471	0.144	0.400	**0.011**	0.345	**0.000**	0.311	0.093	0.155
NH_4^+-N	0.135	0.427	-0.108	**0.582**	-0.195	**-0.669**	0.297	-0.387	-0.333
p-value	0.571	0.060	0.680	**0.014**	0.453	**0.003**	0.247	0.125	0.191
NO_3-N	-0.236	-0.237	0.400	-0.173	-0.147	0.071	-0.387	-0.240	-0.184
p-value	0.317	0.314	0.112	0.507	0.573	0.786	0.124	0.352	0.480
Available P	0.103	**0.485**	-0.081	**0.457**	0.129	**-0.618**	0.116	-0.166	-0.218
p-value	0.665	**0.030**	0.757	**0.065**	0.622	**0.008**	0.656	0.525	0.400
Total C	-0.161	0.407	-0.294	**0.577**	-0.258	**-0.869**	0.253	-0.412	-0.440
p-value	0.497	0.075	0.252	**0.015**	0.318	**0.000**	0.328	0.100	0.077
Total N	-0.023	0.358	-0.007	**0.795**	-0.300	**-0.632**	**0.490**	-0.317	-0.105
p-value	0.925	0.121	0.978	**0.000**	0.241	**0.006**	**0.046**	0.216	0.687
Dissolved organic C	-0.029	0.343	-0.106	**0.580**	-0.201	**-0.674**	0.200	-0.361	-0.439
p-value	0.902	0.139	0.687	**0.015**	0.439	**0.003**	0.442	0.155	0.078

Functional genes	Bacterial *16SrRNA*	Archaeal *16SrRNA*	Fungal *ITS*	*nifH*	Bacterial *amoA*	Archaeal *amoA*	*nirK*	*nirS*	*nosZ*
Total dissolved N	-0.062	0.236	0.058	**0.544**	-0.217	**-0.610**	0.201	-0.374	-0.341
p-value	0.796	0.317	0.826	**0.024**	0.403	**0.009**	0.439	0.139	0.181
Microbial biomass PLFA	-0.026	0.276	-0.044	**0.639**	-0.229	**-0.806**	0.256	-0.373	-0.203
p-value	0.912	0.238	0.866	**0.006**	0.376	**0.000**	0.321	0.140	0.434
AMOC	-0.229	-0.263	0.314	0.256	-0.294	-0.181	-0.009	-0.276	0.108
p-value	0.331	0.263	0.220	0.321	0.252	0.486	0.974	0.283	0.680
AMN	-0.033	0.057	0.171	**0.611**	-0.181	**-0.544**	0.316	-0.222	-0.049
p-value	0.890	0.810	0.513	**0.009**	0.486	**0.024**	0.216	0.392	0.852

Electrical conductivity (EC). Phospholipid Fatty Acid Analysis (PLFA). Anaerobically mineralizable organic carbon (AMOC).

6.3.3 ^{15}N uptake by the vegetation

No significant differences were found between the recovery of ^{15}N in the different plant and soil fractions across the riparian transect (zone 1, 2 and 3; P > 0.05). Similar percentages of total ^{15}N recovery of added ^{15}N were obtained for plants and soil in zones 2 and 3 (71.9 % and 79.3%, respectively), whereas only 56.8% was recovered in the plants and soil within zone 1 although it was not significant (Figure 6.5). Generally, there were very few differences between the amounts of ^{15}N recovered in the different plant-soil fractions within each zone. Only in zone 2, were four times more ^{15}N was recovered in the shoots compared to the soil (P = 0.012; Figure 6.5).

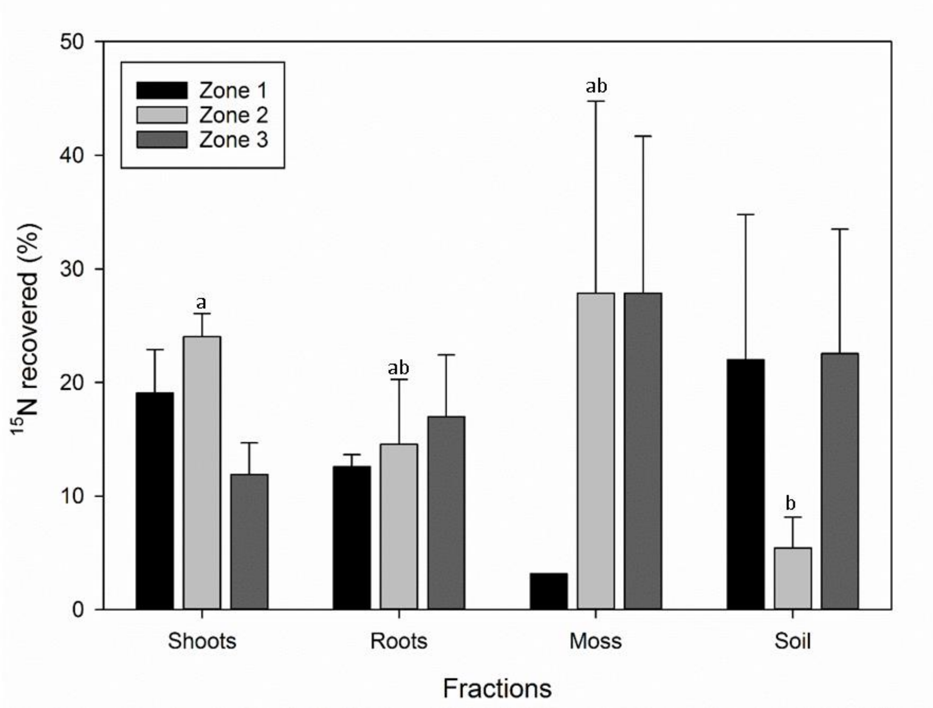

Figure 6.5 Recovery of ^{15}N (% of total applied) from within the different fractions (shoots, roots, mosses and soil) represented by bars (*n* = 3 except moss in zone 1 where *n* = 1). Zone 1 represents the area dominated by *Juncus effusus* and is closest to the river (5 m), zone 2 corresponds to the transitional area between the grasses and the heath (10 m) and zone 3 represents the heathland with *Calluna vulgaris* and *Sphagnum* mosses as the dominant species and the farthest points from the river (25 m). Same lower case letters or the lack of it indicate no significant differences ($P > 0.05$) with respect to the different fractions within each zone according to one-way ANOVA and the Tukey post-hoc test.

6.3.4 Potential denitrification and N_2O emissions

In response to the addition of labile C and/or N to the soil, greater cumulative N_2O emissions were only observed within zone 1, showing little or no effect in zones 2 and 3 (Figure 6.6). In zone 1, the addition of labile C-only increased N_2O emissions by a factor of 1000, from 0.004 ± 0.001 to 4.07 ± 0.14 mg N kg^{-1} h^{-1} relative to the control in the area closest to the river (e.g. 2 m, $P < 0.001$). Similarly, the addition of C and N together also increased N_2O emissions relative to the control (0.004 ± 0.001 to 2.95 ± 0.14 mg N kg^{-1} h^{-1}) at 2 m from the river. After the addition of labile C alone or in combination with N, emissions of N_2O were 78 and 45 times higher, respectively than the control at 5 m from the river (Figure 6.6). Although urea-N addition also increased N_2O emissions in zone 1 (0.24 ± 0.06 mg N kg^{-1} h^{-1} at 2 m, and 0.61 ± 0.36 mg N kg^{-1} h^{-1} at 5 m), fluxes were not significantly different from the control ($P > 0.05$).

N_2O emissions across the riparian transect significantly differed for all treatments with respect to the distance from the river ($P < 0.001$, treatments with C addition alone or in combination with N addition; $P < 0.05$, urea-N only addition). Basal emissions of N_2O from the control cores did not show significant differences with distance from the river ($P > 0.05$). Carbon-only addition greatly stimulated emissions of N_2O with distance from river, with the area closest to the river (2 m) emitting on average 80 times more N_2O than the distal point of the transect (25 m). The addition of C together with N increased N_2O emissions at 2 m from the river by 60, 90 and 101% in comparison to the amount emitted at 5 m and zone 2 and 3, respectively (Figure 6.6).

Significant positive correlations were found between N_2O emissions and bulk density, whereas soil water content, total N, total porosity and AMOC correlated significantly but negatively with N_2O production for all treatments except the control (Table 6.3).

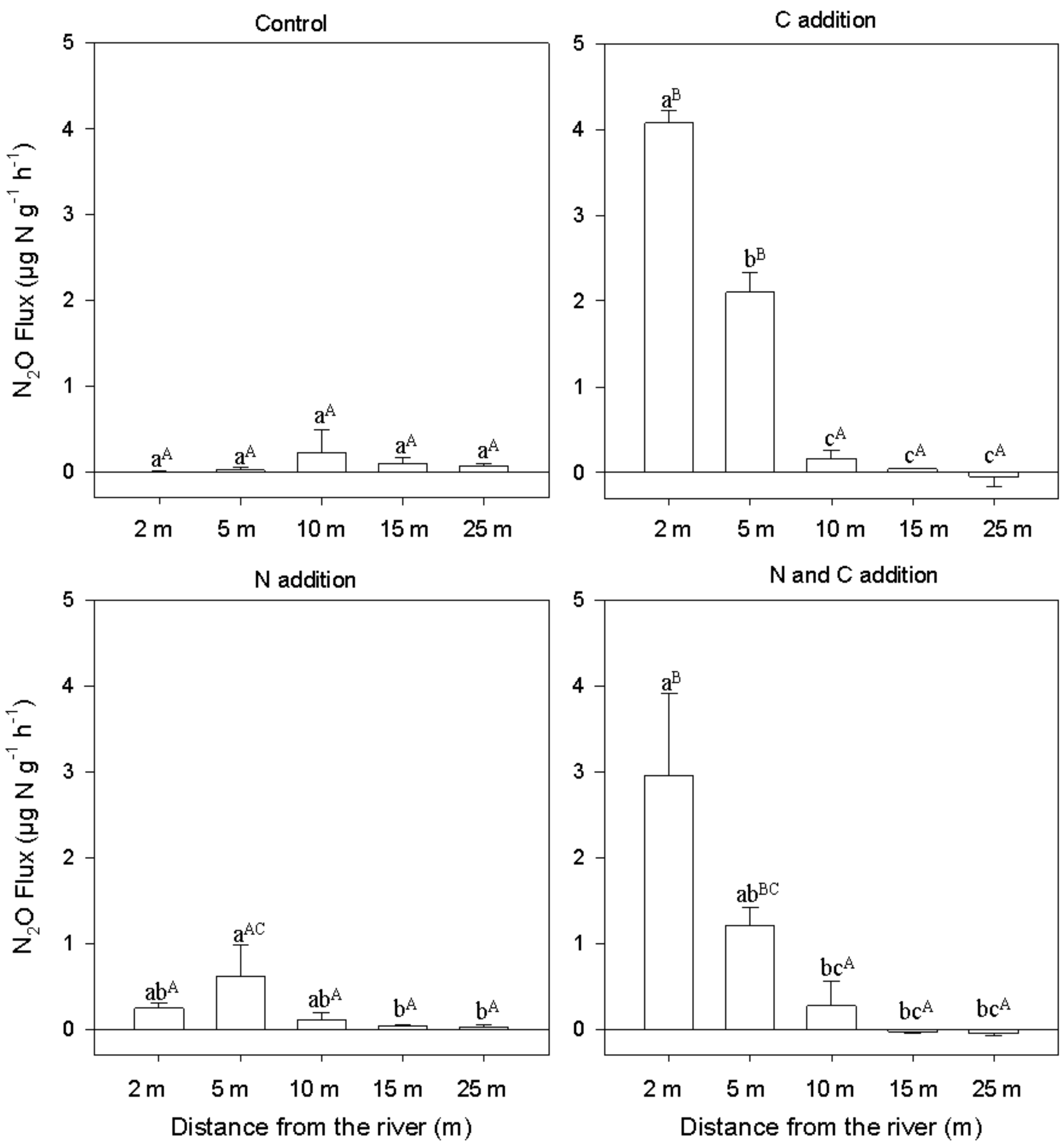

Figure 6.6 Cumulative N_2O emissions via denitrification in unamended soil (control) or after the application of labile C (glucose) and N (urea) either alone or in combination. Same lower case letters indicate no significant differences (P > 0.05) with respect to distance from the river according to Welch's test and the Games-Howell post-hoc test. Same capital letters indicate no significant differences (P > 0.05) between treatments for each distance from the river according to one-way ANOVA and Tukey post-hoc test. Bars represent mean values (n = 4) ± SEM.

Table 6.3 Spearman's rank correlation coefficients and *P*-values between soil physicochemical properties and N_2O emission (mg N kg^{-1} h^{-1}) in unamended soil (control) or after the addition of labile C and N. Anaerobically mineralizable organic carbon (AMOC).

Soil property	**N_2O emissions (Control)**	**N_2O emissions (C addition)**	**N_2O emissions (N addition)**	**N_2O emissions (C and N addition)**
Water content	0.24	-0.80	-0.71	-0.81
p-value	0.316	<0.001	<0.001	<0.001
Bulk density	-0.33	0.73	0.70	0.79
p-value	0.152	<0.001	0.001	<0.001
Total nitrogen	0.19	-0.89	-0.65	-0.74
p-value	0.431	<0.001	0.002	<0.001
Total porosity	0.33	-0.74	-0.69	-0.80
p-value	0.152	<0.001	0.001	<0.001
AMOC	0.31	-0.86	-0.70	-0.82
p-value	0.179	<0.001	0.001	<0.001

6.3.5 N cycling gene abundance

Ammonia oxidizing bacteria (AOB) and archaea (AOA) showed different abundance patterns with respect to distance from the river (Figure 6.7). While the proximity of the river had no effect on the bacterial *amoA* gene numbers, archaeal *amoA* gene copy number significantly decreased ($P = 0.001$) on average by up to 84% from zone 1 closest to the river to zone 2 and by 98% with respect to zone 3. The archaeal-to-bacterial *amoA* gene ratios were approximately 5 and 46-fold greater in zone 1 relative to zone 2 and 3 respectively (Figure S3). In contrast, the *nifH* gene abundance significantly increased ($P = 0.001$) from close to the river to the distal point by 67-82%, whereas a difference with respect to zone 2 was only found for 2 m. Zone 1, specifically the closest point to the river, displayed the lowest value for *nirS* gene abundance which represents 3.5 lower values than zone 2 ($P = 0.038$) (Figure 6.7). In contrast, *nirK* and *nosZ* gene copy numbers did not change significantly across the transect ($P > 0.05$). The clade II of the *nosZ* gene could not be amplified despite the positive control being amplified (data not shown)

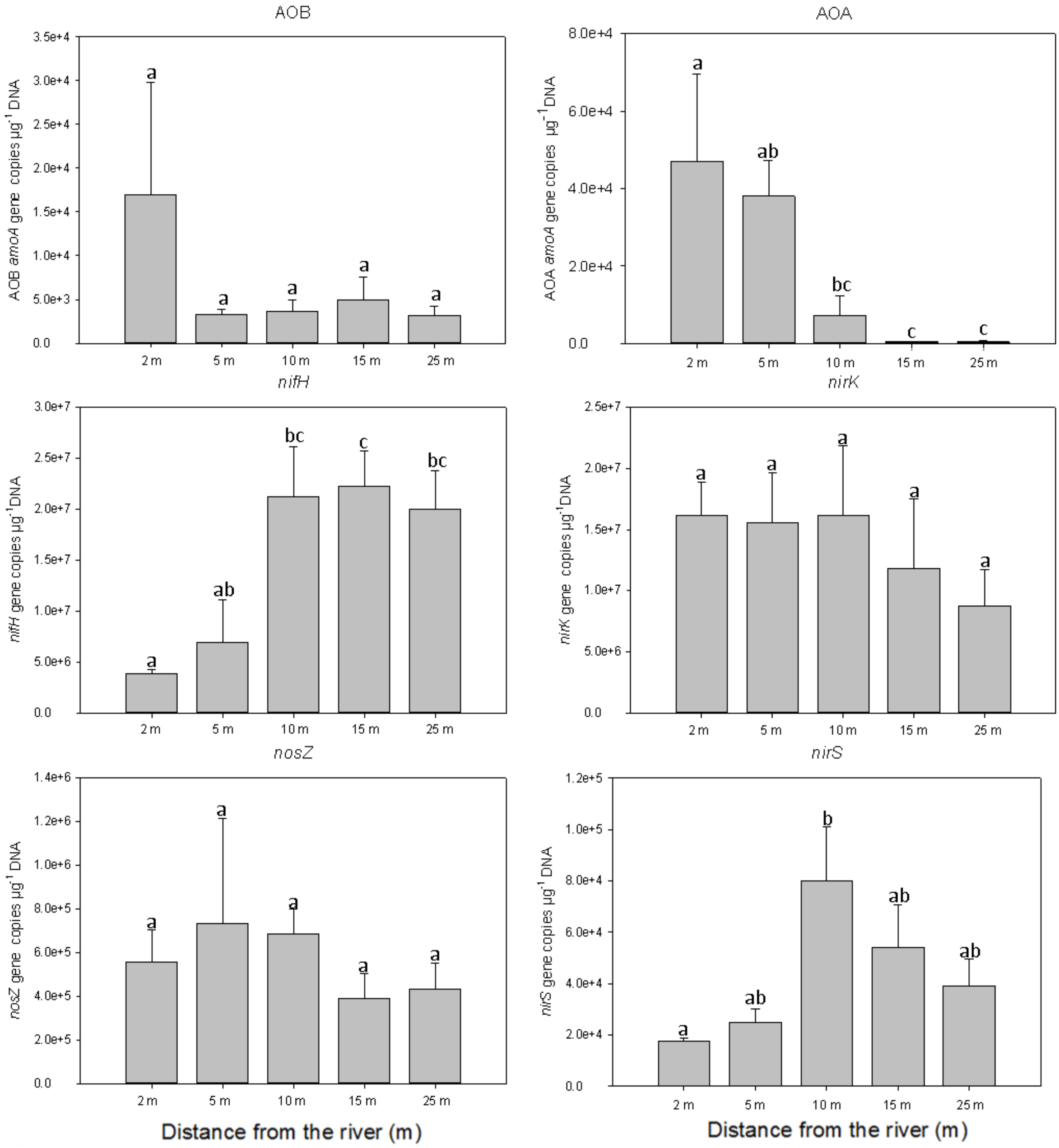

Figure 6.7. Bacterial *amoA* (AOB), archaeal *amoA* (AOA), *nifH*, *nirS*, *nosK*, *nosZ* gene copy numbers relative to distance from the river. Same lower case letters indicate no significant differences ($P > 0.05$) relative to distance from the river according to one-way ANOVA and the Tukey post-hoc test. Bars represent mean values ($n = 4$ for 10, 15 and 25 m, $n = 3$ for 2 m and $n = 2$ for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 6.2.

Abundance of *nirS* and *nosZ* genes correlated positively with pH (ρ ~0.5) but negatively with EC (ρ = -0.52, -0.63, respectively) (Table 6.2). A negative correlation was found between *nifH* and soil bulk density while archaeal *amoA* was correlated positively with bulk density (Table 6.2). Significant positive correlations were found between *nifH* and soil water content, AMOC, total porosity, NH_4^+ content and microbial PLFA whereas archaeal *amoA* abundance correlated negatively to the same soil properties (Table 6.2). The bacterial *amoA* and *nirS* genes did not show any significant correlations.

A positive strong correlation was found between copies of bacterial *16SrRNA*, bacterial *amoA* and *nirK* ($\rho > 0.73$, $P < 0.001$ in all cases) whereas *nifH* showed a highly positive correlation with *nirK* ($\rho > 0.52$, $P = 0.001$).

6.4 Discussion

6.4.1 Soil biology and biogeochemistry across the riparian zone

The riparian zone showed distinct spatial patterns in soil properties, despite the relatively short length of the transect. Results from this study clearly showed that vegetation, influenced in turn by the prevailing hydrodynamic conditions, had a striking effect on most of the soil's physicochemical properties. This finding is supported by a range of studies which have established that mean high water level together with the frequency of water fluctuation is a critical factor controlling species diversity and abundance close to watercourses (Wierda et al., 1997; Lou et al., 2016). In our study, there were lower amounts of soil organic matter and nutrients (N and P) in soils close to the river in comparison to those further away. These can be ascribed to differences in erosion-depositional processes occurring along the transect. Alongside differences in water table depth, this has led to the formation of two very distinct vegetation communities: one that contains species that can tolerate extreme waterlogging and anoxia (via aerenchyma formation and organic acid excretion) and high levels of exogenous Fe^{2+} and Mn^{2+} (e.g. *Juncus effusus*; Visser et al., 2006; Blossfeld et al., 2011), and another that relies on obligate aerobic symbionts, which lacks aerenchyma and can only tolerate mild hypoxia (e.g. *Calluna* heathland; Gerdol et al., 2004; Rydin and Jeglum, 2013). These differences in plants are likely to be a key driver in shaping rhizosphere microbial communities and the dominant N cycling pathways.

The microbial community structure was different in the three riparian zones due to the distinct soil physicochemical properties, and plant cover that are highly dependent on local hydrological regime (Gutknecht et al., 2006; Balasooriya et al., 2007). For example, the fungal-to-bacterial ratio was very low indicating a clear dominance of bacteria community over fungi. Nevertheless, the higher ratios in areas close to the river suggests a zonation pattern in fungal communities across the transect, probably linked to plant type and poor nutrient conditions (Bohrer et al., 2004; Six et al., 2006). The Gram (+)/Gram (-) ratio decreased in zone 3 ($\geq$ 15 m) in relation to the increase in SOM, total C and N content. Gram (-) bacteria are thought to be copiotrophic organisms with a high growth rate, using labile substrate such as in zone 3, while Gram (+) bacteria are thought to be oligotrophic organisms that are better decomposers of less labile soil organic matter but have a lower growth rate (Fierer et al., 2007). Furthermore, the greater relative abundance of cyclopropanes close to the river (64% more than distal areas), which indicates the growth rate in the bacterial community and has been linked to changes in nutrient availability, infers that the most rapid growth or turnover rates will occur in distal areas of the river as a result of higher nutrient availability and lower stress conditions (i.e. water fluctuation) (Ponder and Tadros, 2002; Bossio et al., 2006).

6.4.2 N cycling across the riparian transect

The balance between the different steps of the N cycle varied along the riparian transect, while the plant and soil retention potential was constant, showing the varying potential of riparian wetland for N attenuation. The amount of N added did not exceed N plant demand, however, the total higher plant recovery of ^{15}N (ca. 30-40%) indicated a relatively high rate of removal. A similar amount of N was retained in the moss layer or soil (either in solution, sorbed to the solid phase, or immobilized in the microbial biomass) indicating that approximately 30-65% was lost by denitrification (as NO, N_2O or N_2), mass water flow, or translocated by roots out of the ^{15}N addition area. Our results are consistent with short-term ^{15}N recovery by vegetation in other non-riparian studies (e.g. grasslands; Nordbakken et al., 2003; Wilkinson et al., 2015). However, the high variability in ^{15}N recovery between replicates, most likely due to inherent heterogeneity in riparian areas, made it difficult to identify any consistent spatial patterns in N uptake across the riparian transect (Williams et al., 2015). Additionally, only short-term fate of urea-N was studied and differences in mass flow under the vegetation (and therefore ^{15}N residence time) was not accounted for (Weaver et al., 2001).

The genes abundance of the different steps of the N cycle showed niche differentiation along the riparian transect. The *nifH* gene spatial distribution showed a strong link to areas with lower soil water content, bulk density and higher porosity and NH_4^+ concentrations, indicating the potential role of N fixation in zones 2 and 3 to accumulate NH_4^+ in soil. This is consistent with these plant communities (e.g. *Calluna-Eriophorum* and *Sphagnum* species) being severely N limited (Leppanen et al., 2015). In contrast, AOA abundance followed the opposite trend than *nifH* gene, with the same factors explaining their distribution. Thus, we conclude that nitrogen fixation and nitrification are not coupled in the riparian wetland. This also implies that the archaea are the main microorganisms involved in nitrification over bacteria (Caffrey et al., 2007; Erguder et al., 2009). Thus, despite AOA and AOB delivering the same function, the two communities live in distinct niches with different drivers. The low abundance of AOB is likely due to the low soil pH (4.05 – 4.90), that favour AOA (Leininger et al., 2006), while the drop in AOA abundance in the distal zone could be related to the higher concentration of NH_4^+ (Verhamme et al., 2011) or the change in soil water content.

Thus, the variation in ammonia oxidisers along the riparian transect will directly affect the rate of denitrification. The constant NO_3^- concentration along the transect, indicate that denitrification is occurring close to the river, which was confirmed by the potential denitrification rates, highly stimulated by C addition (glucose), and to a lesser extent by N (urea) in this area. It is well established that denitrification rates are usually enhanced by anoxic conditions, high NO_3^- availability and labile organic C (Weier et al., 1993). This is supported by the oligotrophic nature of the habitat, the high C-to-N ratio of the soil, and the recalcitrant nature of the plant litter produced by the vegetation (Witt and Setala, 2010). Although the *Calluna* heath soil possessed high levels of DOC, this has previously been shown to be largely resistant to microbial attack due to its high aromatic content (Stutter et al., 2012). Interestingly, N_2O production was stimulated greatly in the *Juncus effusus* zone when labile C was added, however, there was not a cumulative effect after the addition of C and N together. The low concentrations of NO_3^- in this zone also suggests that any NO_3^- produced could be lost to the river or is absorbed by plants. Overall, nitrification appears to be the rate limiting step in N cycling within the riparian wetland studied here.

With respect to the functional genes of the denitrifier community, none of the genes studied showed high abundance close to the river. Only the *nirS* gene displayed a higher abundance within zone 2, related to the increase in soil pH by less than a pH unit, highlighting the sensitivity of *nirS* gene abundance to pH (Liu et al., 2010). However, the relatively higher abundance of *nirS* was not translated into higher N_2O, although it should be noted that *nirS* and *nirK* code for nitrite reductase. The fungi, could also play a role in the denitrification as they possess *nirK* and *nirS* genes, which were not captured by the primers used. Some studies have indicated that N_2O emissions from fungal communities can be significant as they lack the *nosZ* gene to reduce N_2O to N_2; their contribution in riparian areas remains uncertain and further work is needed to explore their role further (Ma et al., 2008; Seo and DeLaune, 2010).

It is difficult to conclude on the potential N_2O emissions because the acetylene assay used in the study block the reduction of N_2O into N_2. The higher N_2O emissions close to the river after C and N addition could then be reduced. However, the constant *nosZ* clade I gene abundance and the absence of *nosZ* clade II gene along the transect, might indicate that N_2O is more likely to be emitted from the area close to the river, while the distal zone might be a sink for N_2O. Therefore, from a management perspective, restricted access to grazing and OM amendments which are commonly used for wetland restoration to accelerate soil development and regulate soil moisture fluctuation, would be recommended to avoid future potential greenhouse gas emissions in wetlands under grazing regimes (Bruland et al., 2009).

6.5 Conclusions

In terms of preventing freshwater pollution, riparian areas represent one of the most valuable management tools for preventing excess nutrient loss from land to water. Most studies to date, however, have focused on N and P cycling and transformations in riparian soils adjacent to arable and intensively managed grasslands. Given the heterogeneous nature of land use in many catchments, and the trend towards modelling ecosystem services at the catchment scale, we need to gain a better understanding of riparian N transformations across a variety of habitats and under different land use intensities. Our study in an extensively managed agricultural system clearly showed that changes in environmental factors such as breaks in vegetation or soil water saturation provide strong indicators of the relative importance of different biotic and abiotic processes involved in N cycling. However, our results also revealed hidden gradients in microbial community structure and N cycling gene abundance across the riparian strip. This reflects differences in key soil properties (e.g. organic matter content, redox) and also possibly the source of nutrients flowing through the soil (i.e. in hyporheic water flow versus lateral flow from upslope areas) and N_2O fluxes. This type of spatial information can be used for more accurate mapping of ecosystem services at the catchment scale and the design of better livestock management systems (e.g. prevention of grazing in riparian areas to avoid N_2O emissions). While we have provided novel insights into the dominant pathways for N removal in riparian zones, further work is required to investigate if seasonal patterns exist and how closely gene abundance is related to gene expression.

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Chapter 7

Riparian research and legislation, are they working towards the same common goals? A UK case study

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Abstract

The value of riparian areas has long been recognised due to their contribution in supporting wildlife diversity and their capacity to deliver a wide range of ecosystem services. Their multiple uses (e.g. flood prevention, biodiversity, pollutant attenuation) combined with an inconsistent use of terminology (e.g. river bank, floodplain, wetland, buffer strip), however, has led to the development of fragmented policies associated with riparian areas. This review brings together current EU and UK legislation alongside research publications focused on riparian areas. We critically evaluate the current legislative framework relating to riparian areas and identify key scientific knowledge gaps which need to be addressed to support future decision-making. Our findings revealed several major problems associated with riparian policy and management, including: (i) the fragmented nature of legislation concerning riparian areas; (ii) the presence of redundant policy instruments, (iii) a lack of practical objectives, (iv) contradictory measures, and (v) unachievable targets. Further, our results suggest that most research is focused on agricultural systems and single ecosystem attributes or functions, rather than supporting an ecosystem-service approach that is widely aspired to in policy statements. We recommend that future research could better support riparian protection policies by focusing less on what the different ecosystems 'are', and more on what they can 'offer' by way of multiple benefits.

Key words: Ecosystem services; freshwater protection; riparian management; buffer strip, multiple benefits, river restoration

7.1 Introduction

The value of riparian areas has long been recognised due to their abundant vegetation, ability to support wildlife diversity and capacity to provide a range of ecosystem services (Hawes and Smith, 2005; Clerici et al., 2011; Aguiar et al., 2015). The riparian zone was first described a century ago (Clements, 1905) and its definition has been continually evolving as our understanding of different ecological and hydrological processes has improved (Verry et al., 2004). Historically, they have been the subject of numerous legal conflicts over water rights, partly because there has been no consensus about their delineation and the challenges faced by different owners and water users (Fischer et al., 2001).

There have been many attempts to improve the way that riparian zones are managed and regulated to provide multiple simultaneous benefits (e.g. biodiversity, flood control, cultural services). Furthermore, the growing demand for water, the decline in water quality due to agricultural intensification and industrial pollution, the increasing abstraction for domestic and industrial use and the modification of watercourses over the last 200 years (UK NEA, 2011; Broetto et al., 2017), have made protection of riparian zones increasingly important.

National and regional UK regulations established that riparian landowners (i.e. any landowner whose property is adjoined, above or with a watercourse running through it; NRW, 2017) are ultimately responsible for preserving and managing the riparian zone in collaboration with local organisations. However, inconsistent use of terminology and fragmented policies around riparian areas make it difficult to identify which specific management applications are effective under different scenarios, particularly regarding prevention of land degradation.

Efforts to engage and collaborate with key stakeholders, especially farmers, have been encouraged through European Union (EU) legislation and national initiatives to ensure farming strategies contribute to the sustainable management of riparian areas. It has been found that clear and targeted support is required to assist farmers to develop a focus on conservation and broader sustainability alongside agricultural production (Kaine et al., 2017). This requires policy-makers to appreciate the tight financial situation that farmers usually operate within and make up for the fact that riparian areas provide services that are not directly traded in markets (Orr and Colby, 2004). Key to the success of agri-environment schemes is to have farmer input into their design. Ahnström et al. (2009) highlighted that the lack of integration of “farmers’ perceptions and knowledge of nature” in the design of agri-environment schemes was a major problem that needs addressing.

Another major issue is the lack of dialogue between scientists and policy-makers which has resulted in the popular perception that policies lack an evidence base, with both parties often in disagreement with each other (Sutherland et al., 2004, 2006). Therefore, identifying knowledge gaps between scientists and policy-makers and understanding the way information is exchanged has become an essential task in the design of effective legislation.

The impending departure of the UK from the EU, through which much of the legislation and initiatives protecting our environment have derived, highlights the need for careful consideration of alternatives and the development of strong new policies that set a clear direction. Recently, the EU has set an ambitious target of which the UK is a signatory country, to halt biodiversity decline and to ensure well-functioning of ecosystems to provide essential services to people by 2020 (Maes et al., 2016). Although a considerable effort has been made in recent decades to stop further ecosystem decline in the UK (e.g. increase of 12.9 million ha of protected areas from 2012 to 2017; Defra and JNCC, 2017), recent reports do not suggest a positive picture of the current state of biodiversity. For example, the recent publication of the ‘Biodiversity Intactness Index’, which is an indicator of how intact a country’s biodiversity is, places the UK in the 29th lowest position out of 218 countries assessed (Scholes and Biggs, 2005; Hayhow et al., 2016). Regarding riparian areas, one of the most diverse and valuable ecosystems in terms of services to people, there is evidence that suggests that disturbance factors such as anthropogenic activities (e.g. land use changes, pollution), changes in hydrological regimes or invasion of non-native species, have heavily degraded and made them less resilient and more prone to further degradation (González del Tánago and García de Jalón, 2006; Dudgeon et al., 2006; Sinnadurai et al., 2016). Therefore, scientific research could greatly assist in identifiying driving factors of riparian degradation and guiding new policy instruments to develop the most effective restoration strategies (Maltby et al., 2013).

This paper brings together legislation and associated regulations and guidance relative to riparian areas from the EU and the UK with the aim to determine how current conservation efforts can be improved and to guide the development of new strategies. Additionally, we conduct a comprehensive analysis of scientific publications focused on riparian areas within the UK, in order to identify scientific gaps that will likely need to be addressed to support future decision-making.

7.2 Materials and Methods

7.2.1 Literature review of legislation

Sources from the EU and the UK were used to evaluate the most recent legislation either directly or indirectly related to riparian areas. We acknowledge that there is a vast body of legislation applicable to riparian areas which may not be presented in this study, however, our aim was to present a general legislative framework highlighting the most important actions. Four areas of particular legislative importance were identified: i) biodiversity, as riparian areas are considered one of the most diverse and priority habitat types as expressed in national biodiversity strategies (Clerici et al., 2011; Forestry Comission, 2017); ii) nutrients and water quality as riparian zones can help control non-point pollutant sources in freshwaters (Jontos, 2004; Aguiar et al., 2015); iii) water dynamics and modelling due to riparian areas potentially modifying natural flow regimes, thus altering biotic communities, river systems and their associated floodplain (McKay and King, 2006); and iv) future outlook, current status and impacts (e.g. influence of climate change on riparian dynamics) (Seavy et al., 2009). We also considered riparian guidance and best management practices as they usually refer to certain binding actions required by public organisations to qualify for Common Agricultural Policy (CAP) payments.

7.2.2 Literature review of scientific research

Three major scientific search engines (i.e. Web of Science, Science Direct and Jstor) were used to locate scientific publications with 'riparian' or 'buffer strip' and 'UK' as keywords. The search was refined according to each engine's advanced search options (Table S1). Firstly, we classified publications according to their country of origin to identify any trends in the geographical focus of riparian studies. A paper was included in the category 'UK' if it addressed different regions of UK or covered broad topics such as reviews or habitat surveys. Additionally, publications were divided with respect to the dominant land cover on which the research was based. The UK NEA Broad Habitat categories (UK NEA, 2011) were used as a classification framework for the different land cover types described in each publication. A detailed description of the broad habitat types considered here is provided in Table S2. Two additional categories ('Contrasting land cover' and 'General') were added to encompass studies conducted across multiple habitat types and studies that by the nature of the research could not be included within any specific habitat category (e.g. general reviews, models, studies on specific species).

Secondly, the publications were grouped into four thematic categories according to their subject matter (paralleling those used for the legislative review). In addition, subcategories were added to these to provide a further level of detail (Table 7.1). It should be noted that some publications covered more than one category.

Table 7.1. Main categories and subcategories used to itemize the publications relating to riparian areas within the UK.

Category	Subcategory
1. Biodiversity	1.1. Ecology 1.2. Vegetation
2. Nutrients and water quality	2.1. Riparian buffer strips 2.2. Nonpoint diffuse (NPD) pollution 2.3. Denitrification 2.4. Shading
3. Water dynamics and modelling	3.1. Modelling of riparian interactions with abiotic parameters (i.e. geology, climate, hydrology, vegetation). 3.2. Hydrological dynamics and interactions with groundwater
4. Future outlook and impacts	4.1. Land use change and restoration 4.2. Climate change 4.3. River and habitat survey

7.3 Results and discussion

7.3.1 Legislative review

Riparian regulation covered a broad range of disciplines as it is influenced by both terrestrial and aquatic regulations. At a European scale, the legal framework concerning riparian areas is built via a number of mechanisms such as strategies, directives and regulations (Table 7.2, see also supplementary information for key legislative concepts). However, although these pieces of legislation normally establish the goals that all EU countries must achieve, they do not usually include mandatory and standardised measures, leaving the way goals are incorporated into national legislation up to each Member State. For example, Regulation (EU) No 1307/2013 stipulates the creation of buffer strips along watercourses but leaves the decision of the buffer width to the discretion of each Member State. Another similar example is the specific requirement for buffer strips according to the Nitrates Directive (91/676/EEC) if the land is included inside National Vulnerable Zones

(NVZs) defined by Member States. Further, the introduction of the EU Water Framework Directive (WFD) greatly encouraged the study of riparian areas as they were identified as key elements involved in the determination of good ecological status of water bodies. Thus, a broad range of methods to evaluate riparian conditions and their main physical features came into being (González del Tánago and García de Jalón, 2006). However, the most recent legislation relating to environmental issues, seems to be switching the emphasis towards a more functional side of ecosystems requiring an assessment and mapping of physical attributes but relating them with the multiple services they provide and their interactions with adjacent ecosystems. Hence, it is now possible to create conceptual models which allow ecosystem services to be linked to human wellbeing (Maes et al., 2016). However, it is worth noting that while the regulatory system encourages the uptake of a multidisciplinary ecosystem services-based approach, the legislative information is supplied by fragmented policies spread across over different issues and sectors (e.g. biodiversity, flooding, Table 7.2)

Table 7.2. Compilation of legislation affecting riparian areas both directly and indirectly at a European, national (UK) and regional (England, Scotland, Wales, Northern Ireland) scale.

Legislation name	Scope of application	Year	Objective	Type	Action applied by
1. Biodiversity					
Council Directive 92/43/EEC	Europe	1992	• Protecting natural habitat both terrestrial and aquatic. • Designation of Special Areas of Conservation (SAC) of sites selected (Annex I habitat) (Annex II species). • Creation of Natura 2000 as a network of special areas of conservation.	Directive	Member States
EU Biodiversity Strategy to 2020	Europe	2015	• Target 1. Reinforce the implementation of Natural 2000. • Target 2. Maintenance of ecosystem services. Map and evaluate the status of ecosystems along with their economic value. • Cross-compliance, which includes Statutory Management Requirements and Good Agricultural and Environmental Condition.	Strategy	Member States
Environment (Wales) Act	Regional (Wales)	2016	• Duty on conserve biodiversity and enhancing the resilience of ecosystems and the benefits they provide. • UK Biodiversity Action Plan (UK BAP) which entails the creation of a list of priority habitats. • Greenhouse emissions (CO_2, N_2O) at least 80% lower than the baseline year (1990).	Act	Natural Resources Wales Local and regional authorities
The Natural Environment and Rural Communities (NERC) Act	Regional (England)	2006	• General duty on all public bodies office-holders to conserve biodiversity which includes restoring or enhancing a population or habitat. • UK Biodiversity Action Plan (UK BAP) which entails the creation of a list of priority habitats. • Providing codes of practice to offer recommendations, advice and information on how to stop the damage caused by non-native animals and plants.	Act	Environment Agency Local and regional authorities
Nature Conservation Act 2004	Regional (Scotland)	2004	• General duty on all public bodies to conserve biodiversity which includes restoring or enhancing a population or habitat. • UK Biodiversity Action Plan (UK BAP) which entails the creation of a list of priority habitats. • Duty to give notification of sites of special interest.	Act	Scottish Environment Protection Agency Local and regional authorities
Wildlife and Natural Environment Act 2011	Regional (Northern Ireland)	2011	• General duty on all public bodies to conserve biodiversity which includes restoring or enhancing a population or habitat. • UK Biodiversity Action Plan (UK BAP) which entails the creation of a list of priority habitats. • Power of wildlife inspector to examine specimens and take samples if there is evidence of a relevant offence against biodiversity.	Act	Northern Ireland environment agency Local and regional authorities

Legislation name	**Scope of application**	**Year**	**Objective**	**Type**	**Action applied by**
2. Nutrients and water quality					
Nitrates Directive (91/676/EEC)	Europe	1991	• Halting water pollution, specifically nitrates, through the use of good farming practices that can be either voluntary or compulsory in NVZs. • Designate Nitrate Vulnerable Zones" (NVZs). • National monitoring and reporting.	Directive	Member States
Directive 2000/60/EC (Water Framework Directive (WFD))	Europe	2000	• Assessing river and riverine habitats ecological conditions. • Establishing river basin management plan (RBMP) tool to guaranteeing that the highest ecological and chemical status possible is achieved. • Monitoring programs to check the river status.	Directive	Member States
Regulation (EU) No 1307/2013	Europe	2013	• Common rules for direct support schemes for farmers under the Common Agricultural Policy (CAP) (Title III). • Management of landscape features (riparian woody vegetation). • Buffer strips along the watercourse (Annex IX) but without define a width.	Regulation	Member States
Water Abstraction and Impoundment (Licensing)	Regional (Northern Ireland)	2006	• The abstraction of less than 10 m^3 of water in any one day.	Regulation	Northern Ireland Environment Agency Landowner
The Water Environment (Controlled Activities)	Regional (Scotland)	2011	• General Binding Rule 2. Limitation of river water abstraction of less than 10 m^3 of water in any one day. • General Binding Rule 19. Prevention of significant erosion or poaching of land within 5 m of any surface water or wetland. • General Binding Rule 20. It stablishes a buffer strip at least 2 m wide to be left between surface waters and wetlands and cultivated land.	Regulation	Scottish Environment Protection Agency Landowner
The Environmental Permitting	Regional (England and Wales)	2016	• The erection of fencing is not located on the bed or banks from the river. • The repair and protection of main river banks using natural materials if the length of the bank is not more than 10 m and other circumstances expose in article 13.2. • Construction of bankside wildlife refuge structures.	Regulation	Natural Resources Wales Environment Agency Landowner

Legislation name	Scope of application	Year	Objective	Type	Action applied by
2. Nutrients and water quality					
Basic Payment Scheme (BPS)	Regional (general)	2016/ 2017	• Statutory Management Requirements (SMR) 1. Nitrate Vulnerable Zones (NVZs). • Good Agricultural and Environmental Condition (GAEC) 1. Water-Establishment of buffer strips (minimum of 2 m). • GAEC 5. Soil and carbon stock. Monitoring excessive bank erosion alongside watercourses where livestock have access.	Scheme	Natural Resources Wales Environment Agency Scottish Environment Protection Agency Northern Ireland environment agency Landowner
Other schemes Glastir	Regional (Wales)	2016	• Commitment to cross-compliance (Basic Payment Scheme). • Commitment to the Whole Farm Code (WFC). • Paid management options: buffer to control erosion and rough grass buffer zone.	Agri-environment scheme	Natural Resources Wales Landowner
3. Water dynamics and management					
Directive 2007/60/EC	Europe	2007	• Identifying the river basins and associated coastal areas at risk of flooding. • Elaborating flood risk maps and establish flood risk management plans focused on prevention, protection and preparedness. • Monitoring programs to check river status.	Directive	Member States
Land Drainage Act	National (UK)	1991	• Regulating land drainage and water abstraction. • Creation of Internal Drainage Boards (IDB) to maintain water levels and secure the provision of water. • Securing flood protection.	Act	Natural Resources Wales Environment Agency Scottish Environment Protection Agency Northern Ireland environment agency
The Water Environment (Floods Directive) Regulations	Regional (Northern Ireland)	2009	• Development of flood risk map of protected areas which potentially could be affected if any flood scenario. • Identifying the flood extent and flood conveyance routes and areas which have the potential to retain flood water such as natural flood plains. • Assessing natural features (for example flood plains, wetlands or woodlands) which can assist in the retention of water.	Regulation	Northern Ireland environment agency
Flood Risk Management Act 2009	Regional (Scotland)	2009	• Creation of flood risk assessment, maps and plans at a proper scale specifying land and water management actions. • Considering measures to manage flood water by altering (including enhancing) or restoring natural features and characteristics. • Local flood risk management plan to supplement the relevant flood risk management plan	Act	Scottish Water Local authorities

Legislation name	Scope of application	Year	Objective	Type	Action applied by
3. Water dynamics and management					
Flood and Water Management Act 2010	Regional (England and Wales)	2010	• Creation of a strategy for flood and coastal erosion risk management in England and Wales. • Enhancing the constitution of local flood authorities. • Assessing flood risk from surface runoff, groundwater and ordinary watercourses.	Act	Natural Resources Wales Environment Agency Local authorities
River Basin Plan Management (specific for each River Basin District (RBD))	Local (RBD, general)	2015/ 2016	• Monitoring rivers water ecological status. • Manage ecosystem services at the most appropriate scale. • Commitment of engaging and promoting collaboration with stakeholders, including local authorities, communities, developers and industry.	Strategic documents	Natural Resources Wales Environment Agency Scottish Environment Protection Agency Northern Ireland environment agency RBD
4. Future outlook and impacts					
Paris agreement on climate change	Global	2016	• Limit the amount of greenhouse gases emitted by human activity to the same levels that trees, soil and oceans can absorb naturally. • Keeping average warming below 2°C. • Establishing a global goal of "enhancing adaptive capacity, strengthening resilience and reducing vulnerability to climate change".	Treaty	Parties to the Convention
Climate change Act	National (UK)	2008	• Reducing emissions from the devolved administrations (Scotland, Wales and Northern Ireland) by at least 80% of 1990 levels by 2050. • Legally-binding 'carbon budgets' set by the UK Government.	Act	Regional governments
Wales passed the Environment (Wales) Act	Local (Wales)	2016	• Sustainable management of natural resources (e.g. air, water, soil, geological and physiographical features and processes). • Enhancing a biodiverse natural environment with healthy functioning ecosystems. • Assessing and reporting diversity between and within ecosystems as well as their conditions and connections.	Act	Welsh Ministers Natural Resources Wales Local authorities
The Climate Change Act	Regional (Scotland)	2000	• Commitment of a 56% of reduction of greenhouse emissions by 2020. • Creation of programmes for adaptation to climate change giving clear objectives to enhance resilience of the system. • Duty to produce a land use strategy where sustainable objectives are indicated.	Act	Scottish Environment Protection Agency

Together with EU legislation, UK legislation (primary and secondary legislation or subordinate legislation), as well as common law, also support riparian regulatory processes. In the case of environmental issues, this is largely the responsibility for devolved administrations within different parts of the UK. Therefore, each nation is responsible for setting their own policies and providing incentives as well as designating public bodies (e.g. The Environment Agency in England or NRW in Wales) to ensure the delivery of measures agreed by each Government for the protection and enhancement of the environment. Although legislation related to riparian areas follows a common framework between the different parts of the UK, there are clear regional differences in policy (House of Lords, 2017). For example, Wales has set its own targets with respect to climate change mitigation, while Scotland explicitly specified binding rules within it's Water Environment Regulation to limit specific activities from taking place within riparian areas.

Based on the legislative information gathered, riparian legislation within the UK seems to be more incentivised (through the use of different agri-environment schemes and good management practices) rather than by enforcement. The Basic Payment Scheme (BPS) or specific documents provided by each nation (e.g. 'A guide to your rights and responsibilities of riverside ownership in Wales'; NRW, 2017) provide specific binding actions (cross-compliance measures) that the landowner is required to follow in order to benefit from direct payment schemes.

Most of the EU and UK-based policies reviewed here address the protection of riparian areas in two ways: i) limiting activities that can be undertaken within the riparian buffer zone, e.g. limiting fertilizer application (2 m from the edge of the river) (Nitrates Directive 91/676/EEC) or limiting water abstraction from rivers and lakes to <20 m^3 day^{-1} (Land Drainage Act, 1994), or ii) monitoring, mapping and evaluating the ecological and chemical status of riparian zones and adjacent ecosystems. Examples of initiatives that include monitoring programs are the WFD (2000), Nitrates Directive (1991), EU Biodiversity Strategy

(2020) and River Basin Plan Management (RBPM). They seek to ensure the sustainable management through effective monitoring and reviewing actions implemented by the Member States to achieve the wider objectives of other EU Directives. In recent years, 70% of the measures adopted to address the environmental pressures of agriculture involved the establishment of riparian buffer strips funded via agri-environmental payment schemes (Dworak et al., 2009). For example, the European Council Regulation No 1698/2005 stipulates that 'support shall be granted annually and per hectare to farmers in order to compensate for costs incurred and income foregone resulting from disadvantages in the areas concerned related to the implementation of Directives 79/409/EEC, 92/43/EEC and 2000/60/EC'. Hence, at a national scale, this translates for example into a compensation of £301 to £400 (per hectare per year) if a 4 m to 6 m buffer strip on the edge of cultivated land is established in England (Natural England, 2015) or the entitlement to the BPS of a variable income with the commitment to a 2 to 10 m buffer strip and Good Agricultural and Environmental Condition (GAEC) and Statutory Management Requirements (SMR) (BSP, 2017). However, it is worth noting that to be able to claim for these payments at least 5 ha of eligible land is required.

An important point presented within the River Basin Management Plans (RBMPs), and commonly stressed within legislation affecting riparian areas, is the commitment and the importance of engaging and promoting collaboration with stakeholders, including local authorities, communities, developers and industry. The importance of stakeholder collaboration is crucial, as for example in Wales, only 7% of the land is owned or managed by the competent authority itself (NRW, 2015). Current riparian management policies strongly promote landowner collaboration and participation, often via the different payment schemes (e.g. BPS, Glastir), which are subject to compulsory cross-compliance measures to promote sustainable farming techniques. However, studies such as Ahnström et al. (2009) or Ingram (2008) report contradictory responses from land managers. While they claim to be technically well informed and willing to embrace good ecological practices (e.g. application of manures outside the riparian zone or the establishment of a riparian buffer), evidence shows there is a need for clearly articulated information to better communicate costs and benefits of the measures applied and how they will be recompensed for services provided (Holden et al., 2017). In this respect, the report by DEFRA (2004) on catchment-sensitive farming also indicated that when landowners were provided with the right and precise information (often face-to-face) their actions were much more effective, costs were reduced and as a result they become less dependent on subsidies.

There is no shortage of reports (EA, 2004; UK NEA, 2011; EU Technical Report No 9/2015, EU Biodiversity, 2020) that warn about the decline of ecosystem service provision associated with riparian areas (e.g. river water quality, biodiversity). Some argue this may be due to the lack of linkage between the many different elements that feed into policy (ecology, geomorphology, soil science, hydrology and fisheries science, etc.) (Kohm and Franklin, 1997; Hickey and Doran, 2004). Most of the recent EU and UK legislation acknowledges this and attempts to halt or reverse this loss of ecosystem service provision. The EU Biodiversity Strategy 2020 and the Environment Wales Act (2016) are two recent European and regional examples of this, respectively. However, policy-makers, researchers and scientists need to work together to better understand the effectiveness and potential impact of decisions (Holden et al., 2017).

7.3.2 Research review

The search yielded a total of 820 publications addressing the topic of riparian areas from 1997 to 2017 in the UK. The scientific publications were scrutinised and 161 articles of pertinent material with respect to 'riparian studies in the UK' were selected. We acknowledge that we may have missed some publications focused on riparian areas due to the multiple terms used to refer them (i.e. floodplain, buffer strip, riverine systems). Despite this, we feel that our broad cross-section was sufficient to identify general trends.

7.3.2.1. Riparian studies by geographical scope within the UK and land cover focus

The largest number of papers on riparian areas within the UK were associated with England (59.6%), followed by articles considering the whole of the UK (20.5%) while Scotland and Wales contributed significantly fewer papers (ca. 10% each) (Figure 7.1). No studies were found from Northern Ireland with the search criteria used in this review. Research based on Scotland tended to focus equally on the habitat types ‘Enclosed Farmland’ and ‘Mountains, Moorland and Heaths’ even though the latter covers 44% of its land area. In contrast, Wales focused primarily on ‘Woodlands’ which only accounts for ca. 15% of its territory (UK NEA, 2011). Riparian research from England was concentrated on ‘Enclosed Farmland’ reflecting its important contribution within the landscape (55.3% of its total land; UK NEA, 2011).

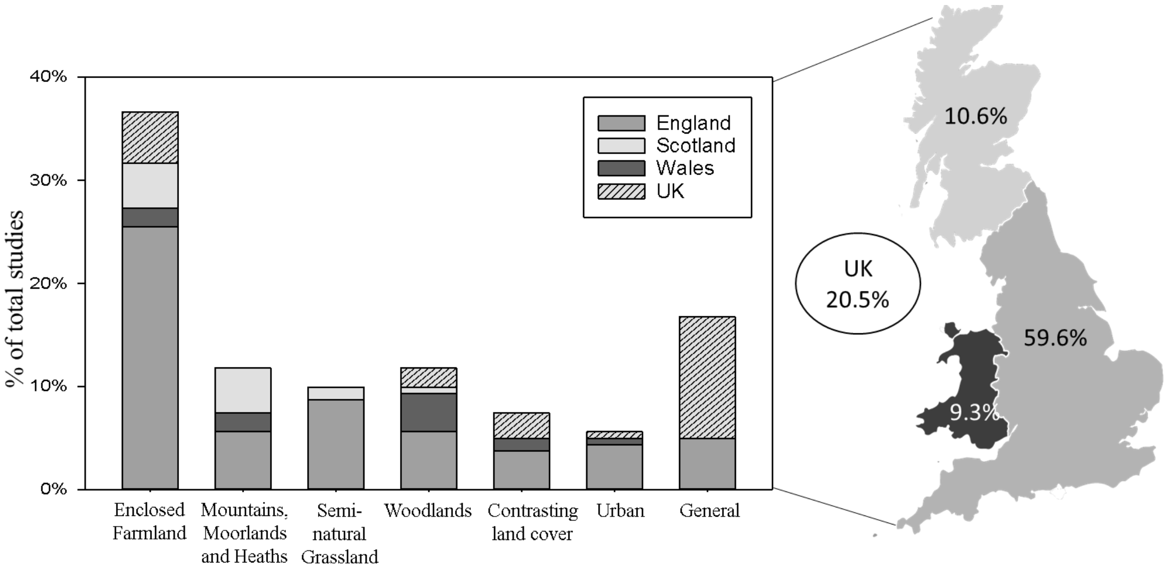

Figure 7.1. Percentage of total number of studies on riparian areas by country (right) and land cover target (left) according to the UK NEA Broad Habitat categories (based on papers published from 1997 to 2017). Different bar colours represent the individual contribution of each country to that specific category. Two additional categories named ‘Contrasting land cover’ and ‘General’ were added to encompass studies conducted across different habitat types (minimum two habitat types) and studies that by the nature of the research could not be included within any specific habitat category (e.g. general reviews, models, studies on specific

species), respectively. Studies developed across different regions of UK or focus on topics such as reviews or habitat surveys were categorized within the 'UK' category.

With respect to land cover, apart from papers based on Wales, most of the riparian publications focused their research on enclosed farmland (i.e. mostly arable and improved grassland). The rest of the habitat types contributed about 10% of the total number of papers except for 'Contrasting land cover' and 'Urban' categories whose percentage of contribution were slightly lower (7.5-5.6% respectively). Overall, the percentage contribution of each habitat type to riparian research seemed to reflect two things: firstly, the relative importance of each UK NEA Broad Habitat within the UK, and secondly, that agriculture and farming have been recognised as the major source of freshwater ecosystem decline within the UK and other developed countries (UK NEA, 2011; McGonigle et al., 2012). Thus, it is not surprising that 'Enclosed Farmland' which accounts for 55%, 19% and 41% of England, Scotland and Wales respectively was the primary focus of riparian research across the UK. However, although it is important to work on strategies that help us to mitigate the negative effects of agriculture, we cannot overlook the pivotal role in provisioning services that minority habitats (such as wetlands or semi-natural grasslands) accomplish, despite the relatively small surface area they cover. Evidence to support this also comes from studies such as De Groot et al. (2012) where it was estimated that globally, inland wetlands possess a value of \$25,682 ha^{-1} y^{-1}, 9 times greater than the estimate for grasslands based on the ecosystem services market price. Morris and Camino (2011) also provided an estimated value of £467 ha^{-1} y^{-1} for inland wetlands due to their contribution to water quality improvement. In addition, Tscharntke et al. (2005) also highlighted that local habitats different from grassland ecosystems might be essential to improve the delivery of ecosystem services, enhancing local diversity and providing a natural corridor of special importance in simple landscapes dominated by arable fields. Hence the importance of their study.

7.3.2.2. Riparian studies by subject matter

Based on subject matter, the studies were categorized according to four broad themes and several subcategories (Table 7.1). The largest number of publications were associated with 'Nutrients and water quality' (33%), followed by 'Biodiversity' (29%). The categories 'Water dynamics and modelling' and 'Future outlook, current ecological status and impacts' contributed similar amounts (ca. 19%) of the total articles published (Figure 7.2).

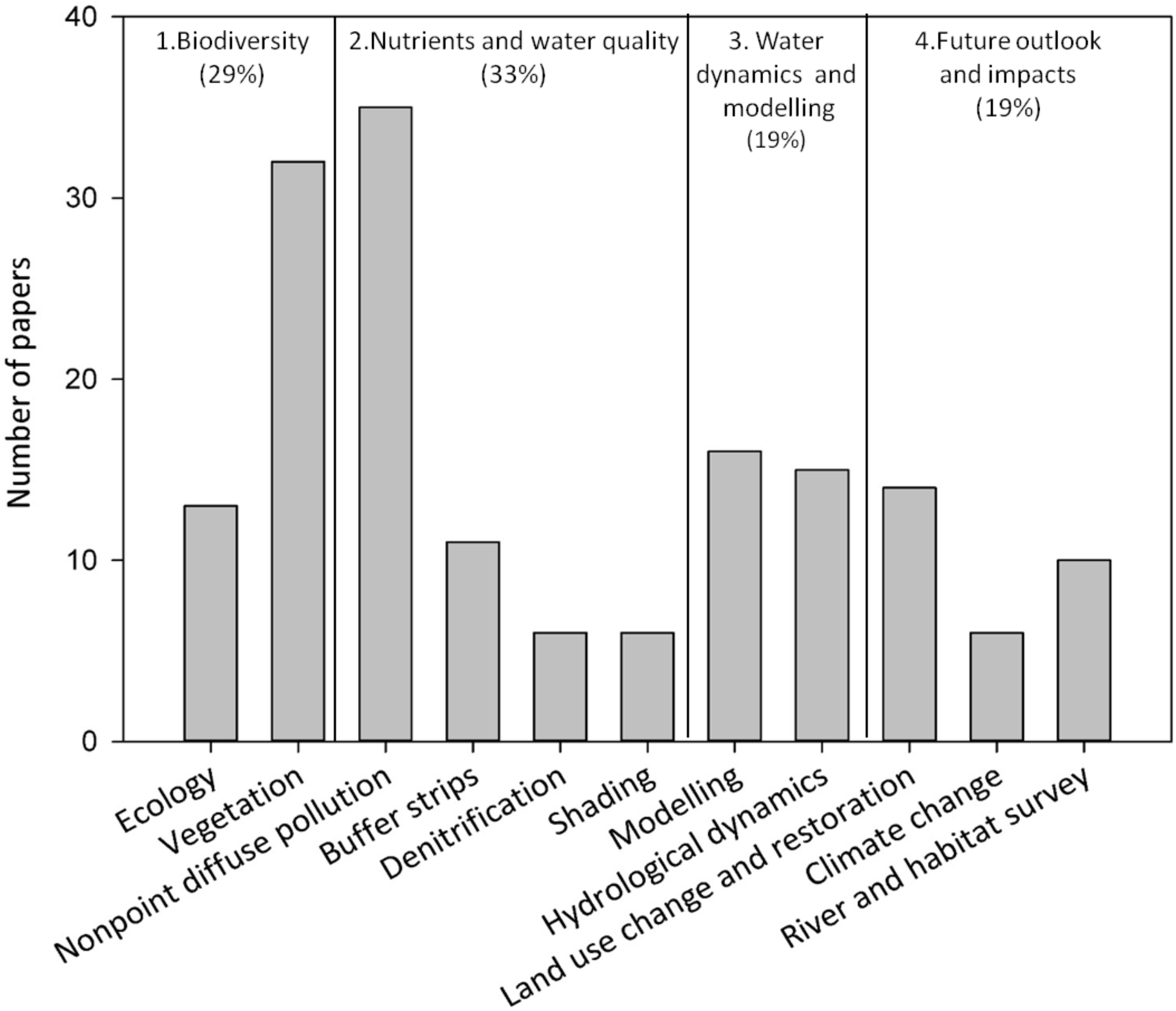

Figure 7.2 Number of papers related to riparian areas in the UK over the period of 1997-2017. Graph based on 161 individual papers. Subcategories grouped according to the subject matter as explained in section 7.2.2

I. "Biodiversity" publications

The study of biodiversity accounted for 29% of the total number of papers on riparian areas (Table S3). The largest number of papers (21%) within this category focused on riparian vegetation (Figure 7.2). It is worth noting that a large number of these studies were focused on the impacts of the spread of non-native species on other communities (e.g. invertebrates (Tanner et al., 2013), native flora (Bradford et al., 2007; Truscott et al., 2008; Tanner and Gange, 2013)) or ecosystem functioning (Hulme and Bremner, 2006; Hladyz et al., 2011). The propagation and distribution of non-native species is also a recurring theme within this subcategory (Wadsworth et al., 2000; Tickner et al., 2001; Maskell et al., 2006; Walker et al., 2009). Manchester and Bullock (2000) detailed the principal non-native species introduced in the UK and their possible impact on UK native biota. However, they also revealed that although they are major plant invaders along streams and rivers, the supportive evidence about their effects on aquatic habitats and species is often contradictory and scarce (Stockan and Fielding, 2013). Additionally, there was no shortage of studies focused on vegetation propagules, distribution and diversity, ecological successions and hydrogeomorphological dynamics (Moggridge and Gurnell, 2010; Cockel and Gurnell, 2012; Gurnell and Grabowski, 2016). Historically, riparian research has largely focused on vegetation because it is relatively easy to assess, exerts a strong influence on the soil microbial community and even influences the nearby air around it (Verry et al., 2004; Lymperopoulou, et al., 2016). However, evidence suggests that other factors such as land use history or management practices have a stronger effect in driving microbial diversity and abundance in the soil and that these factors are not being as extensively studied (Millard and Singh, 2010; Jangid et al., 2011; García-Orenes et al., 2013).

In contrast, ecological papers examining relationships between biota and the environment only represented 8% of the total publications (Figure 7.2). Research within this subject matter addressed changes to the distribution and conservation of populations of invertebrates, small mammals or birds (Sadler et al., 2004; Moro and Gadal, 2008; Sinnadurai et al., 2016). However, most of the studies are focused on particular species or agricultural systems, with little perspective of the ecosystem as a whole.

II. “Nutrients and water quality” publications

Of all papers published between 1997 and 2017, about 33% related to nutrients and water quality (Table S4). Within this body of work, the largest number of publications (20%) explored non-point source (NPS) pollution and its effect on water quality within riparian zones (Nisbet, 2001; Jarvie et al., 2008; Hutchins et al., 2010; Wilkinson et al., 2014); particularly, phosphorus and sediments (Steiger et al., 2001; Roberts et al., 2013; Osei et al., 2015; McCall et al., 2017; Vinten et al., 2017). This focus of attention responds principally to the need to meet environmental standards imposed by the WFD that requires good ecological and chemical status and drinking water standards without increasing the costs of treatment that have to be paid by consumers (Kay et al., 2009). Pretty et al. (2000) estimated that the annual costs of removing contaminants such as pesticides, nitrates, phosphorus (and sediment), and organic carbon losses in water for drinking in the UK to be £120 M, £16 M, £55 M and £106 M, respectively on average for 1996. In this regard, agriculture (diffuse pollution) has been highlighted for special attention because of the pressure it exerts on UK freshwaters, particularly in rivers England and Wales (Defra, 2004; European Commission, 2012). Maltby et al. (2013) estimated an increase of 40% of cultivable area in England between 1940 and 1980, whilst 88% of the land area of Wales was utilised as agricultural land in 2015 (Armstrong, 2016). In view of this pressure, agricultural stewardship schemes (e.g. Glastir, BPS), may offer an effective way to halt riparian degradation. However, although there must be a common framework for protecting riparian areas (e.g. no cropping within riparian area), there is a need to identify context-specific solutions rather than expecting a one-size buffer fits-all solution (i.e. setting a fixed riparian buffer width of 2 m from the watercourse) (Kay et al., 2009). For example, Bergfur et al. (2012) found that the replacement of a septic tank was just as effective as implementing a riparian buffer to stop N and other nutrients entering into watercourses in a monitored catchment.

Together with phosphorus and sediments, nitrogen (N) also represents a major contributor to global environmental problems such as freshwater eutrophication and greenhouse gas emissions (Canfield et al., 2010; Erisman, 2013). Because of this, and due to the fact that denitrification represents a permanent removal of NO_3^-, 3% of the publications focused on this topic. Specifically, they tended to assess the role of hydrology on denitrification as well as other environmental issues (Hefting et al., 2004; Machefert and Dise, 2004; Sgouridis and Ullah, 2015). However, despite the major contribution of denitrification to greenhouse emissions and the UK commitment to reduce emission by at least 80% by 2050 (from the baseline year 1990) (e.g. Climate change Act, 2008), the numerous technical challenges and the cost of accurately measuring it in the field have probably reduced the volume of research in the UK.

The impact of cattle on water quality is also a recurring theme within this subcategory (Bond et al., 2012; Terry et al., 2014). Livestock management is considered a keystone for achieving the required 'good ecological status' required by the WFD since the effects of mismanagement on riparian areas are becoming increasingly apparent (e.g. erosion and destabilization of rivers banks) (Belsky et al., 1999; Bond et al., 2012; Terry et al., 2014). The importance of restricting livestock access to watercourses is especially relevant in the UK context, considering that agriculture is heavily focused on grazing livestock (Armstrong, 2016). However, although livestock restrictions to watercourse constitute a strong advisable measure against water pollution, there is no enforcement in this respect in the UK to date.

The implementation of riparian buffer strips is a well-established tool to protect surface and ground water quality from anthropogenic activities (Blackwell et al., 1999; Kaila et al., 2012; Stutter et al., 2012). Research has tended to determine the effectiveness of the buffer for removal of nutrients. However, it was only covered by 6% of the total studies concerning riparian areas in the UK. It could be argued that the lack of research on this topic is due to the fact that this management tool was advocated in the UK just two decades ago (Muscutt et al., 1993) whereas in some parts of North America its use goes back to the 1950s (Richardson et al., 2012). Although it was not one of the most recurrent topics for riparian research within the UK, there is an extensive body of literature (mainly from the Unite States) focused on riparian buffer strips. In this sense, it is interesting to note that most of these studies and the ones gathered here, focused on evaluating variable widths for riparian buffers to maximize benefits. However, using variable buffer widths would require a regulatory system that is flexible and site-specific based, instead of implementing a uniform buffer width at landscape scale as is currently being done. Some studies have shown that applying a mandatory buffer at the landscape scale is an ineffective policy to target nutrient removal (Kronvang et al., 2011). Rather they recommended that buffer strips (in this case 10 m-wide) should be targeted to critical areas where they would have been much more cost-effective.

An additional effect of a well-structured vegetative buffer strip is the provision of shade. The role of riparian areas in providing shade is being increasingly explored because of its potential to alleviate water pollution (Warren et al., 2016). Recently, some studies have shown that riparian shading could become a valuable tool to mitigate river nutrient enrichment, being in some cases, even more effective than reducing nutrient loads in reducing eutrophication risk (Hutchins et al., 2010, 2012). Shade helps reduces incoming solar radiation thereby preventing excess warming and exposure to sunlight which reduces the opportunity for excessive in-stream plant growth. This suggests that riparian shading could offer a cost-effective alternative to reduce the estimated damage costs of freshwater eutrophication which for England and Wales is expected to cost between £75.0−114.3 million yr^{-1} (Pretty et al., 2003). However, this topic only accounted for 4% of the total publications, with some highlighting it as an area that needs further research (Orr et al., 2015). In that respect, guidelines, as shown in Table 2, are a common approach to raising awareness of the importance of riparian shade. However, it isn't always the case that altering conditions to support riparian vegetation will entail beneficial environment consequences (e.g. channel widening, excessive shade, limit the growth of macrophytes) (Collier et al., 1995; Parkyn et al., 2005). Consequently, riparian owners and managers should carefully assess the impacts of restoration measures before undertaking action.

III. *Water dynamics and modelling*

Water dynamics and modelling accounted for 19% of the total publications (Table S5). Modelling and hydrology within riparian areas produced similar number of papers (10%). These studies tended to explore hydrological interactions within riparian areas in order to predict further sources of variation (Soulsby and Tetzlaff, 2008; Del Tánago et al., 2016; House et al., 2016b). Previous studies have emphasised that understanding the underlying processes between riparian areas and hydrology could provide essential information due to the intertwined relationship with biogeochemical cycles, vegetation type and flood processes (Décamps, 1995; Bendix and Hupp, 2000; Grabowski and Gurnell, 2016). Notably, the potential of riparian areas to reduce and mitigate flood events has been extensively documented (Anderson et al., 2006; Johnson et al., 2008). This has particular relevance for England and Wales, where the expected average cost of flood damage is of the order of £1.2 billion per year (Ramsbottom et al., 2012). However, only one study focused on riparian areas and flood management from a modelling perspective (McLean, 2013).

Table 7.3. Chronological compilation of riparian guidelines at the national (UK) scale.

GUIDELINES					
Name	**Agency**	**Year**	**Objective**	**Type**	**Action applied by**
Engineering in the Water Environment Good Practice Guide: Riparian Vegetation Management	Scottish Environment Protection Agency	2009	• Manage riparian vegetation across contrasting habitat types • Creation of buffer strips with recommended widths. • Management of non-native plant species	Technical guidance	Landowner Competent authority
Planting trees to protect water. The role of trees and woods on farms in managing water quality and quantity	Woodland Trust	2012	• Raise awareness of main water quality problems related to agricultural practices: causes-cost effect. • General recommendations for water quality improvement as (i.e. margin of 10 m from any water body to establish cattle feeders). • Emphasizing the role of riparian trees and recommendations for species choice.	Research report and guidance	Landowner
New Guidance on Aquatic and Riparian Plant Management – Controls for Vegetation in Watercourses	Environment Agency, DEFRA[1], CEH[2] Private parties	2014	• Developing good practice guidance on the management of aquatic plants and vegetation both in and alongside watercourses. • Providing field guide in order to identify non-native species. • Providing a decision-making tool applying site-specific knowledge.	Technical guidance	Natural Resources Wales Internal Drainage Boards Lead Local Flood Authorities/local authorities Canal & River Trust
Keeping Rivers Cool	Woodland Trust	2016	• Creating riparian shade for climate change adaptation. • Providing shade maps for most of England and part of Wales in order to identify where planting and fencing will be more beneficial. • Assisting in the species selection and plantation structure.	Guidance	Landowner Public authorities

[1] Department for Environment, Food & Rural Affairs
[2] Centre Ecology and Hydrology

GUIDELINES					
Name	**Agency**	**Year**	**Objective**	**Type**	**Action applied by**
River Restoration and Biodiversity	IUCN[3] NCUK[4]	2016	• Raising awareness about why rivers and their associated floodplain are important for UK biodiversity. • Identifying causes by which they have been altered. • Recommendations and practice guidance for river restoration.	Report	Researchers and policy-makers
The UK Forestry Standard	Forestry Commission	2017	• Recommendation of a mix of shaded and lightly shaded habitat within the riparian zone to enhance biodiversity. • Control the spread of invasive and non-native species. • Provide and maintain defined buffer areas along watercourses and water bodies.	UK Forestry Standard Guidelines	Forest and woodland managers (Natural Resources Wales is the organisation in charge of public forests in Wales)
A guide to your rights and responsibilities of riverside ownership in Wales[5]	Natural Resources Wales	2017	• Explanation of rights and responsibilities of riparian landowners. • Flood risk management assessment. • Maintaining the bed and banks of the watercourse and the vegetation growing on the banks.	Guidance	Landowner

[3] International Union for the Conservation of Nature

[4] National Committee UK

[5] The same type of guidance is provided by the Environment Agency for England

Predictive models, particularly related to the delivery of ecosystem services, are increasingly informing European and national legislation (Maltby et al., 2013; Adhikari and Hartemink, 2016). Nonetheless, only one study was found that explored riparian areas from this perspective (McVittie et al., 2015). Results from that study showed how models could be used efficiently to integrate physical attributes (land cover, soil type, rainfall), terrestrial and aquatic process (e.g. erosion, river flow) and management intervention using Bayesian Belief Networks (BBN). Thus, the parameters introduced will ultimately aim to outline the fundamental ecological processes that deliver ecosystem services within riparian areas. This kind of riparian model could inform more integrated policies.

With respect to hydrology, research has tended to focus on the interactions between stream and groundwater or the relationship between the hyporheic zone and biogeochemical processes (Lapworth et al., 2009; Allen et al., 2010; Canfield et al., 2013). Although many report how management of buffer strips can assist in reducing nutrient loads entering streams, some (e.g. Hill 1996; Vidon and Hill, 2004) argue that we first need to understand riparian hydrology to better predict the fate of contaminants in riparian zones.

IV. Future outlook, current ecological status and impacts

Riparian areas are sensitive ecosystems as they are coupled tightly with hydrological regimes, connected to longitudinal and lateral fluxes of energy and nutrients that in turn are under strong climatic influence and frequently distubed by anthropogenic activities (Wipfli, 2005). Twenty percent of the publications found focused on the future outlook, current ecological status and impacts of riparian zones (Table S6) with land use change and restoration contributing the largest number of papers, representing 10% of the total. Studies within this category explored the effect of restoration and land use change on invertebrates (Harrison et al., 2004; Petersen et al., 2004), vegetation and floodplain dynamics (Clarke and Wharton, 2000; Clilverd et al., 2016), amongst others. There is evidence throughout history that riparian areas have been heavily affected by land use changes in order to increase agricultural productivity (Seavy et al., 2009; Poff et al., 2011). Flood incidents can increase where intense use reduces the time available for water to infiltrate and therefore, the frequency and magnitude of flood peak flows increase (Nagasaka and Nakamura, 1999). That may be the reason why, researchers within this category usually approach the restoration of riparian areas as a way to return the natural defences for flood protection. Studies such as Stromberg et al. (2007) have also stressed the importance of flood restoration for native riparian vegetation and their consequences for sediment transport. Others highlight the importance of riverine ecosystem restoration including riparian zones for improvements in physico-chemical and biological status (Addy et al., 2016).

Alongside riparian restoration, there is growing evidence that managed adaptation could reduce the impacts of climate change on ecosystems (Thomas et al., 2016). In this respect, climate change was the focus of 4% of the papers which mostly dealt with the role of riparian trees in water cooling and eutrophication (House et al., 2016a; Halliday et al., 2016). There is evidence that further increases in global temperature cannot now be prevented (IPCC, 2014). Therefore, strategies such as the EU Biodiverstiy Strategy 2020 aim to increase resilience of key resources and provide legal protection to minimise the impacts of, and adapt ecosystems to, climate change. However, by definition, riparian zones are transition areas between land and freshwater ecosystems and are therefore affected by both aquatic-terrestrial remedial and mitigation measures. It is therefore difficult to identify which specific actions are directed specifically towards riparian areas.

River and habitat surveys accounted for 6% of the total publications. Studies tended to use the standard riverine hydromorphology survey in the UK (River Habitat Survey; RHS) in order to characterise stream reaches by recording physical characteristics and thus evaluate their conservation status (Davenport et al., 2004; Erba et al., 2006; Vaughan et al., 2010). This category aims to meet the EU desire to assess an ecosystem’s ecological status. Despite this, Maltby et al. (2014) stated that approaches taken to date in mapping and assessing different freshwater ecosystems as ‘priority habitats’ do not necessarily reflect their actual or potential contribution to ecosystem services, thereby impeding the legislative work to protect them.

7.3.2.3. Riparian future research

There are limited examples of studies which have attempted to account for the multiple functions that interact (often in a complex way) within riparian areas. The analysis of riparian studies suggests that research is largely focused on single features (e.g. specific riparian species) or functions of riparian areas. Specifically, a lot of effort has been made on the study of riparian vegetation and nutrient dynamics. Although there is no doubt that studies focused on single species or nutrients offer underpinning information to help us to understand how the ecosystem as a whole works, there is a need to guide future research and managerial activities towards a more multidisciplinary integrated approach. In this way, the whole range of ecosystem services could be maximised, and we could reduce or avoid less desirable outcomes. For example, the restriction of livestock to the watercourse is being increasingly recommended to halt P and sediments loads into the river. However, seasonal grazing is beneficial to maintain a good level of biodiversity within riparian areas so both functions should be considered. In turn, this much more realistic view of the ecosystem which considers that the different environmental processes do not occur in isolation, could offer a better understanding of management actions required to ensure the continuation of multiple benefits (Figure 7.3). We present some key questions that should be considered when assessing riparian areas either for restoration purposes, management or research that can increase the range of services provided by riparian areas.

7.5 Conclusions

Improving and enhancing the communication between scientists and policy-makers is essential to help form policies that are based on robust scientific evidence. Results from this study revealed that legislation concerning riparian areas appears fragmented, contains redundant policy instruments and in places lacks practical objectives or contains contradictory measures or unachievable targets.

On the other hand, most recent EU and UK legislation calls for integration and a more ecosystem service based approach to riparian management to maximise, value and preserve not only the physical ecosystem attributes and individual services but also the set of services that could be provided. Our study indicates riparian research tends to focus on single ecosystem processes (i.e. N cycle, riparian species) or attributes (e.g. specific species or nutrients). More integrated research could help support better policy making in this area by developing a better holistic understanding of riparian functioning and that helps us value less what ecosystems are and more what they can offer.

7.6 Acknowledgements

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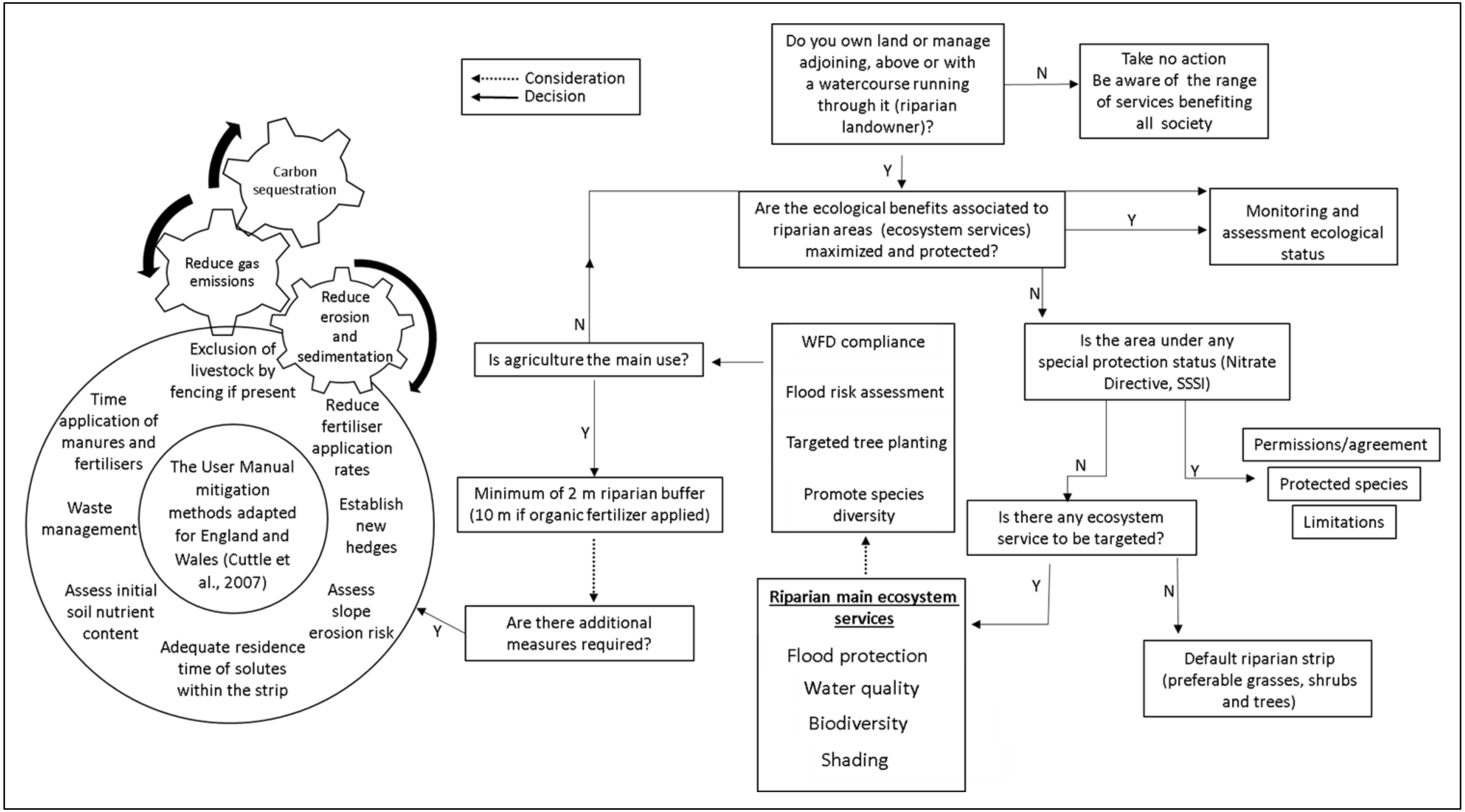

Figure 7.3 Flow chart assessment and prescription procedures that promote ecosystem conservation and services within riparian areas. The flow chart provides key questions and prioritization measures with the aim to guide riparian users and owners throughout the process of riparian assessment.

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Chapter 8

Discussion and recommendations for future research

8.1 Introduction

This section reviews the experimental work presented in Chapters 3-7, summarising the main findings and discussing them in relation to the common theme within this thesis. A detailed discussion of the results from the individual experiments are provided in each experimental chapter. An overview of the strengths of the approach used in this thesis, alongside the possible caveats are also presented. Finally, an assessment of the challenges and uncertainties identified in this thesis that need to be addressed by future research is also provided.

8.2 Synthesis of findings

The overarching aim of this thesis was to address two fundamental issues relative to riparian areas; (i) how can we effectively delineate them, and how does this impact on their subsequent use for management purposes, and (ii) ascertain their relative contribution to the provision of ecosystem services at the local and landscape scale.

Evidence from Chapter 3 showed that the different delineation methods greatly influenced the total area of potential riparian buffers. Therefore, the selection of the most appropriate delineation method should consider: 1) the economic viability of the buffer; 2) the need to strengthen the set of ecosystem services within priority areas (e.g. areas close to a drinking water sources), and 3) the inclusion of the area within a critical status of potential future risk (e.g. from erosion or pollution). Moreover, we identified that the different methods and datasets used to delineate riparian areas could have significant repercussions on the prediction of their potential functionality as riparian ecosystem functioning is closely linked to adjacent land cover and position within the landscape (Burkhard et al., 2009; De Groot et al., 2010; Maes et al., 2012). However, as the performance of the riparian zone also depends on local environmental conditions, such as hydrology or type of vegetation, we concluded that the effectiveness of the buffer should be ground-truthed to ensure the greatest level of protection.

In Chapter 4, we explored these findings further, investigating the extension of influence of the river on soil physicochemical properties, microbial community and the provision of ecosystem services related to water quality (e.g. attenuation of microbial pathogens, pesticides, phosphorus and nitrate) across contrasting habitat types. In parallel, we also quantified and evaluated how riparian vegetation across different habitat types contributes to the provision of shade as recent research suggest that riparian shading could be one of the most effective tools to halt river eutrophication (Hutchins et al., 2010). To do so, the fixed-width riparian buffer from Chapter 3 provided the extension of the buffer at which soil samples were collected (e.g. 2 and 50 m away from the river). Subsequently, a wide range of process-level studies were undertaken to estimate ecosystem service provision. Overall, our findings suggested that the functioning of riparian areas in less intensive agricultural areas may be broadly predicted from the surrounding land use as the habitat type was the main driver explaining riparian soil physicochemical variability and ecosystem service provision. Further, we also identified that watercourse shading was highly habitat-specific and was maximal in forests (ca. 52% shade cover) in comparison to the other habitat types (7-17%). These results therefore validated assumptions about the link between land cover type and ecosystem service provision made in Chapter 3.

Recent research has stressed the importance of small-scale heterogeneity in driving riparian ecosystem functioning (Kuglerová et al., 2014; Kuzyakov and Blagodatskaya, 2015). Thus, there is evidence that specific system characteristics can shift the magnitude and the importance of riparian beneficial services (Groffman et al., 2009; Vidon et al., 2010). Consequently, we investigated the provision of ecosystem services by riparian areas at a finer scale in Chapters 5 and 6.

Chapter 5 explored the role of riparian areas in carbon (C) storage and turnover alongside the influence of other factors such as soil depth, substrate quality and quantity, and nutrient stoichiometry. We observed that the influence of riparian area and nutrient addition on

C turnover was generally minimal. However, the soil microbial community was strongly influenced by soil depth and this had the most striking effect on C turnover rate. Comparison of the initial rate of C mineralization and the total amount of C mineralized suggested different microbial strategies in the topsoil and the deep soil. In addition, evidence also suggested different pathways of C transformations through the soil profile (e.g. respiration/immobilization) when a more recalcitrant source of C was added. This implies the presence of different zones of microbial functioning within the soil profile and that more research is clearly needed in this area (Graham et al., 2016).

Chapter 6 explored the environmental factors controlling riparian soil denitrification activity and how they contribute to explaining the spatial and temporal variability of nitrogen (N) cycling in semi-natural ecosystems. We also assessed the role of different vegetation communities in N uptake and the link of N cycling gene abundance to N removal processes. Once again, we found that major environmental changes such as breaks in vegetation or soil water saturation could be used as broad predictors of the different biotic and abiotic processes involved in N cycling. However, as we anticipated, our results also revealed hidden gradients in microbial community structure and N cycling gene abundance across the riparian transect. These results led us to conclude that there is an urgent need to adopt a gene-to-ecosystem approach to better understand riparian function. Further work is also needed to determine how gene abundance relates to actual process rates and whether they can be used as reliable proxies for function (Morales et al., 2010; Maltby et al., 2014).

Chapter 7 integrated findings from prior studies in riparian research, undertaken across the UK with the aim to detect knowledge gaps and conflicting areas between scientists and policy-makers. We also assessed how the information is subsequently employed by individuals implementing management activities. Our study revealed that legislation and guidelines concerning riparian areas were fragmented and contained a range of redundant policy instruments. Further, the legislation is widely spread over a wide range of policy areas making it difficult to manage riparian areas in a coordinated way. Conversely, we also found that while policies were moving towards adoption of holistic approaches that recognise the multi-ecosystem services that nature provide, research was still largely focused on single ecosystem processes or features (e.g. N cycling, riparian species).

In combination, these results demonstrated the importance of the scale and resolution in the assessment of riparian areas. Priority habitats, such as riparian zones, that are crucial for maintaining key ecosystem functions need to be carefully identified and managed at local-to-regional scales (Chan et al., 2006; Egoh et al., 2007). Catchment land cover may play a pivotal role in broadly predicting the provision of ecosystem services accomplished by riparian areas, but its influence appears relatively less important for individual ecosystem functions, for which microbial functional groups might become overriding (Wissmar and Beschta, 1998; Graham et al., 2016). Figure 8.1 summarises this idea, showing from a broad scale (meteorology and climate) to micro-scale (genes) the contribution of different environmental factors in shaping and defining riparian area typologies. Thus, the interaction of climate and meteorology has created a variety of landscapes drained by river networks and their associated riparian zone. The relative position of riparian areas within the landscape will broadly determine their potential contribution to the provision of ecosystem services, creating functional gradients depending on their position within the catchment. In turn, the specific interactions between the plant and soil microbial community and associated animals will determine the main nutrient flow pathways and their residence time in the environment. However, these interactions will ultimately be determined at a deep molecular genetic level that will be responsible for the delivery of a specific ecosystem services.

Effective management, restoration and protection of riparian areas requires an in-depth understanding of the biophysical environmental components and ecosystem services and functioning, all of it integrated in an economic and social context. However, over the years, a vast body of information has been produced by the scientific community and maybe it is time to start putting the disparate pieces of the complex puzzle together.

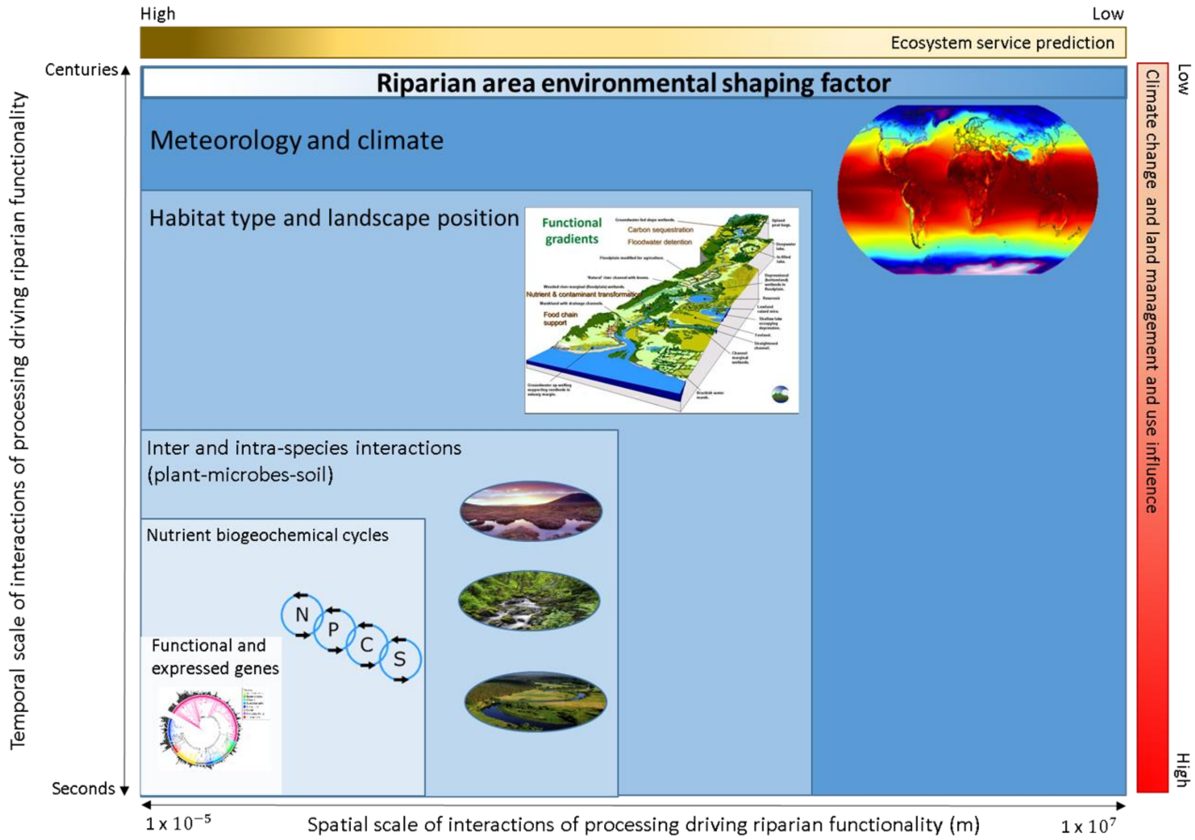

Figure 8.1 Riparian shaping factors influencing riparian typologies across a range of temporal and spatial scales. The size and colour of the boxes indicate the level of influence on defining riparian typologies. Red vertical bar shows the level of impact of climate change and land use management on shaping factors. The horizontal bar indicates the probability of ecosystem prediction of the different shaping factors.

8.3 Strengths of the approach used and potential caveats

The experimental chapters contained in this thesis involved soil sampling across contrasting habitat types. Sampling strategies were designed to capture landscape spatial heterogeneity as well as determining possible variations in soil physicochemical properties and ecosystem service provision within the riparian zone. This was facilitated by the mosaic of habitat types being well distributed and clearly differentiated across the target catchment used here. Further, the streams associated with the riparian areas were generally similar in size allowing direct comparison of habitats. As demonstrated in Chapter 7, most previous studies have tended to ignore the functionality of riparian areas outside the limits of agricultural practices. The approach undertaken in this thesis, however, provided a good range of different scenarios at a catchment scale which could offer underpinning information to inform catchment-level management practices. However, in some cases, the large spatial variability as a result of the relative large scale of the catchment (560 km^2), hampered the recognition of clear patterns within riparian areas for some specific ecosystem services. For example, in Chapter 4, very different emission patterns were observed within each habitat type for 'denitrification potential'. Denitrification is widely recognised to be an extremely challenging process to measure and model, mainly due to the difficulty in measuring the dominant end product (N_2) against the high atmospheric background of this gas, its spatially and temporally variability and its sensitivity to soil structural disturbance (Groffman et al., 2006). Moreover, riparian areas are considered even more challenging in terms of denitrification assessment due to oscillation of conditions that may or may not favour denitrification (e.g. water table level, hyporheic flow, C inputs). Therefore, we concluded that new experimental designs, applied at a smaller scale, are needed to account for these large variations (McClain et al., 2003; Groffman, et al., 2009). The findings from Chapter 6 were more robust and gave clear denitrification patters across a riparian transect. However, we recommend that in future, these measurements should be performed *in situ* over a full annual cycle. If the flux of N into the riparian area can be determined, then it may be possible to calculate accurate greenhouse gas emission factors for this ecosystem type (i.e. the proportion of N entering that is subsequently transformed to N_2O).

A similar caveat was found in the assessment of N uptake by vegetation (Chapter 6). While the approach used was potentially more representative of natural environmental conditions, the inherent complexity of the ecosystem (variable mass flows, heterogeneous distribution of the vegetation and soil particles) caused high variability in ^{15}N recovery between replicates. Therefore, it was difficult to identify any consistent spatial patterns in N uptake across the riparian transect. This represents a major drawback of field studies involving undisturbed soil, however, there are few alternatives available apart from increasing the number of replicates (Barraclough and Puri, 1995; Well et al., 2003). In addition, we only focused on one form of N, however, the behaviour of other N forms was not considered (e.g. dissolved organic N in the form of amino acids or peptides, NH_4^+ and NO_3^-). Similarly, it would have been good to have carried out the potential of plants to acquire N at different times of the year.

Overall, the detailed nature of the experiments carried out in this project did not allow for a seasonal comparison of our results. Therefore, it will be advisable for future studies to look at the temporal dynamics of the processes studied here as this is expected to have a large impact on the seasonal performance of riparian areas.

8.4 Future outlook

Studies presented in this thesis have provided pivotal information about riparian dynamics in an ecosystem service context. However, several research gaps have also been identified during the work. It is clear from the results presented here that a much deeper analysis of many areas covered in this project are required. Some of these are detailed below:

1. Major hurdles such as the lack of agreement on a riparian universal definition or strict physical boundaries (i.e. distance away from the watercourse) needed to be overcome at the beginning of this project. In order to address this, we firstly assessed the implications of using a particular delineation method for riparian areas. However, there are important limitations about how to reliably integrate physical riparian attributes and more importantly, how to relate this to ecosystem functioning. Further work is required to develop models which are able to link ecosystem structure with functionality in both space and time. In addition, it

would be useful to incorporate an economic (i.e. buffer cost incurred) and social element to assess the resultant boundary feasibility in reality.

2. We identified that some riparian functions could be largely predicted from neighbouring land use/cover. However, this statement needs to be tested across a wider range of ecosystems and seasons to fully validate this assertion, particularly in light of the gradients observed in Chapter 6. In this sense, as shown in Chapter 7, the vast majority of riparian studies are focused on intensive agricultural systems, therefore the inclusion of more habitat types in their assessment could provide a more robust framework to guarantee better riparian management and protection.

3. Another important point that should be addressed with future work is the design of more *in-situ* based experiments which can provide a better understanding of key variables that might drive riparian functionality. For example, similar pollutant sorption capacities (e.g. simazine and P) relative to distance from the river found in Chapter 4, did not provide fundamental information about the performance of riparian pollutant attenuation. Therefore, although field-based studies are more difficult to control, increase the effects of confounding variables and are less accessible and reproducible, they could offer greater insights into regulating services such as nutrient and contaminant transport pathways or sources of pollutant loads. In addition, it is likely that different habitats are better or worse at attenuating different inorganic pollutant types (e.g. heavy metals vs NO_3^-) and organic pollutants (e.g. pesticides vs hormones). Greater focus on a wider range of pollutants is therefore required.

4. My studies on the influence of microbial communities in ecosystem service provision also highlighted the need for further research. In the work presented here, environmental variables were often strong predictors of the processes measured. However, some processes such us bacterial pathogen survival or C immobilization in the deep soil, remain unexplained by the environmental variables measured here. In this sense, better establishing the link between the abundance of different biota groups their functional genes (especially transcription

levels) with ecosystem functioning could greatly assist in understanding the complex nature of riparian dynamics and strengthen the prediction of ecosystem service provision (Graham et al., 2016). Once such remaining uncertainties associated with functional genes and ecosystem functioning have been resolved, further studies should address the factors which trigger gene expression in riparian areas and its potential link to rhizosphere processes and seasonality.

5. The assessment of denitrification raised some interesting issues. Firstly, I observed low or negative rates of N_2O production in the distal areas far from the river suggesting a potential role for riparian wetlands as N sinks. Further studies could validate these findings as well as determine the gross rates of N_2O consumption both vertically and horizontally across riparian zones. When done, these findings could have a great applicability in riparian management as potential greenhouse gas N_2O sinks. Secondly, the impossibility of quantifying the contribution of the different processes (e.g. denitrification/nitrification) to the N_2O production, calls for studies in which the importance of these different environmental pathways can be determined. In this sense, the use of multiple isotopic tracers could offer a promising tool to gain a more detailed mechanistic understanding of the processes involved and their relative importance. However, its cost is still prohibitive for large scale experiments.

6. The suppressing effect of P addition in combination with a high MW recalcitrant DOC source on C turnover, also identified a new area that needs further investigation. Future research should test this phenomenon in a wider range of soil types to ascertain under what specific circumstances this effect might occur.

7. Finally, one of the specific objectives of Chapter 5 was to gain further insight into soil organic matter (SOM) decomposition after the addition of different doses of labile C and nutrients. First-order reaction kinetics have frequently been used within multi-component models to describe SOM turnover (Glanville et al., 2016). However, the high dose of labile C caused very different patterns of microbial growth through the soil profile (e.g. sigmoidal, mono- and bi-phasic exponential decay) preventing the use of a single modelling approach

with common theoretical parameters (i.e. half-life of the substrate). This prevented a direct comparison of the C turnover rates for the different soil depths. Previous studies have tried to overcome this issue by using mathematical models that incorporate a first order exponential plus a logistic model (Gillis and Price, 2011; Creamer et al., 2016). However, these models had a poor fit in our case due to the large variation of shapes describing mineralisation and therefore they could not be applied. Whilst some recent work has illustrated the possible application of gamma distributions to achieve a more accurate and flexible description of growth rates (Akkermans et al., 2017), further work is required to demonstrate its applicability with soil systems and to provide a biological explanation for the different parameters within the model.

8.5 General conclusions and management implications

In this thesis, I addressed fundamental questions related to riparian areas such as their delineation, contribution to ecosystem services provision and regulation that could have key implications for conservation and management purposes. Our data suggests that the influence of riparian areas may be reflected in a small spatial scale. Further, when ecosystems were present in relatively unaltered states (semi-natural), their potential function may be broadly predicted from the surrounding land use. However, in more intensive agricultural areas, the data gathered highlighted that there is an urgent need to adopt an ecosystem-based approach to help understand the link between multi-ecosystem services operating as a whole and how best to manage their interactions. This thesis provided essential information that shows where, when and how we might expect the provision of pivotal ecosystem services in riparian areas to take place. As the ultimate management goal is the protection of freshwater habitats, this thesis will aid in the reinstatement of their pristine state or enhancing their resilience in a continuously changing climate.

8.7 References

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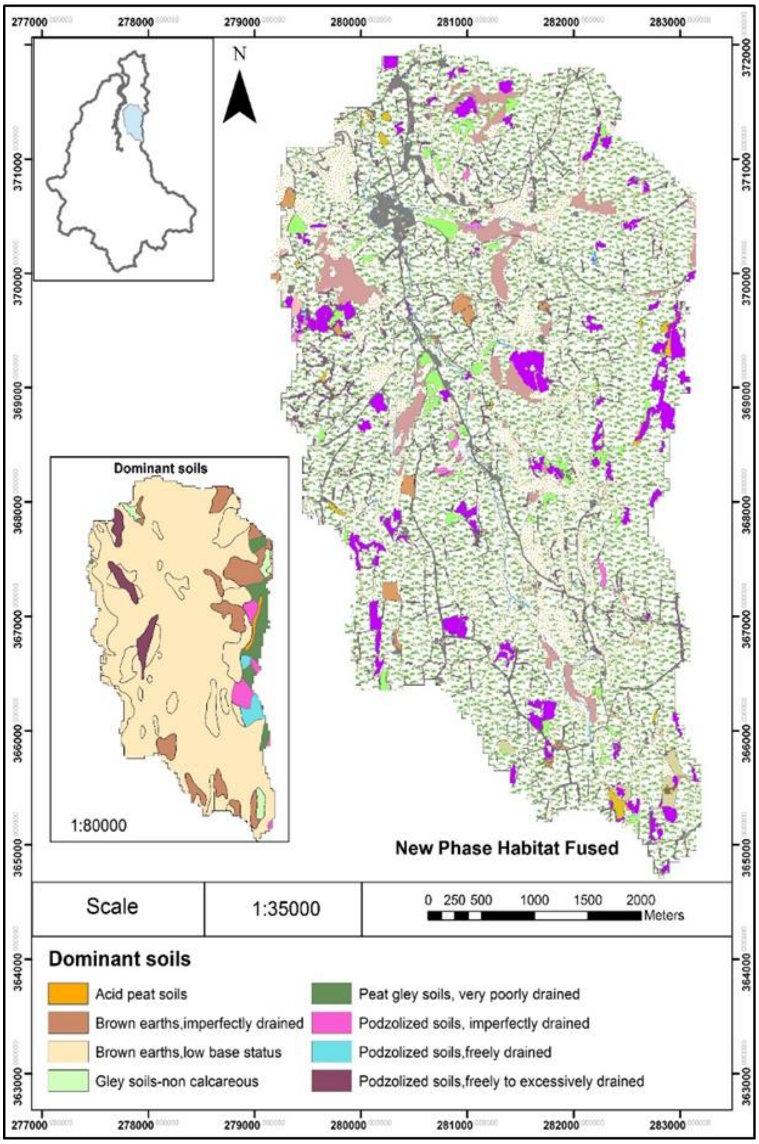
Appendix 1

Supplementary material for Chapter 3

Delineating and mapping riparian areas for ecosystem service assessment

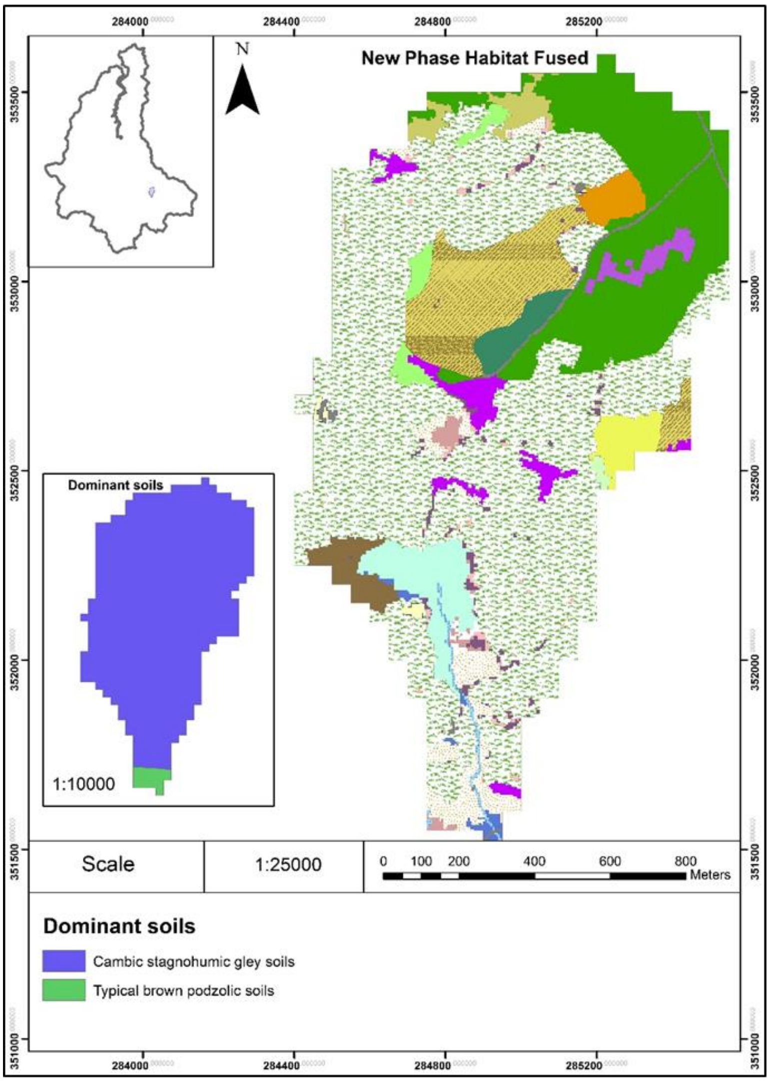
Laura L. de sosa, Helen C. Glanville, Miles R. Marshall, Sinan A. Abood, A. Prysor Williams, Davey L. Jones

1.1 | Detailed description of the sub-catchments used in the study



Coordinates	3°46′ W, 53°12′ N
Area (km2)	20.60
% of the whole catchment	3.55
Perimeter (km)	26.7
Stream network length (km)	60
Main channel length (km)	9.90
Elevation (m)	**Max**: 388.42 **Min:** 33.09
Average Slope (%)	25.83
Rainfall (mm)	850-1400
Dominant geology	**Geologic period:** silurian **Bedrock geology:** nantglyn flags formation (NGF), mudstone and siltstone **Superficial deposits:** till devensin, diamicton
Dominant soil	Brown earths, low base status
Dominant land use	Intensive agriculture
Dominant habitat type	Improved grassland

Figure S1. Detailed description of the main characteristics of sub-catchment 1.



Coordinates	3°43' W, 53°30' N
Area (km²)	1.46
% of the whole catchment	0.25
Perimeter (km)	6.7
Stream network length (km)	6.05
Main channel length (km)	2.29
Elevation (m)	**Max:** 321.47 **Min:** 193.64
Average Slope (%)	14.23
Rainfall (mm)	1200-1600
Dominant geology	**Geologic period**: silurian **Bedrock geology:** bron y graig formation (BGG), sandstone and siltstone **Superficial deposits**: peat, till devensian, diamicton
Dominant soil	Cambric stagnohumic gley soils
Dominant land use	Cattle farm
Dominant habitat type	Improved grassland

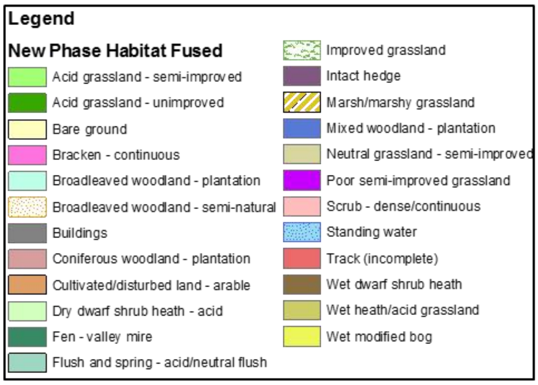
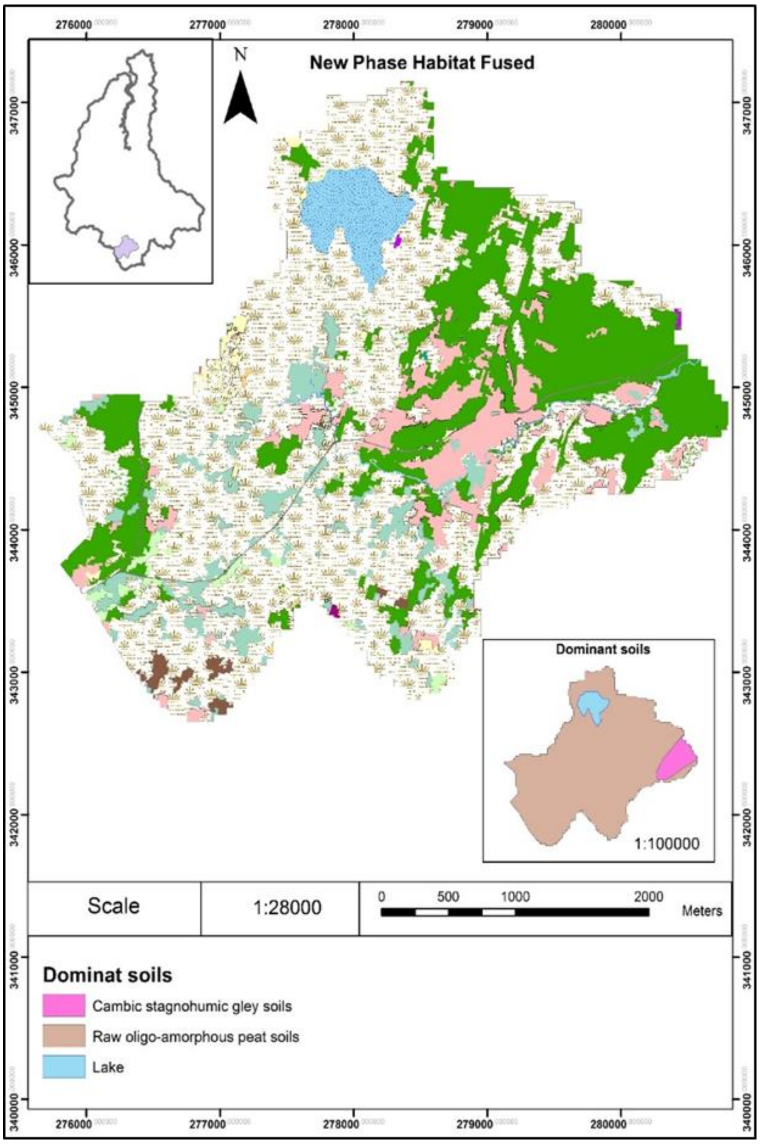


Figure S2. Detailed description of the main characteristics of sub-catchment 2.



Coordinates	3°49′ W, 52°59′ N
Area (km²)	11.98
% of the whole catchment	2.06
Perimeter (km)	22.5
Stream network length (km)	34.50
Main channel length (km)	8.17
Elevation (m)	**Max:** 535.08 **Min:** 369.13
Average Slope (%)	10.68
Rainfall (mm)	2100-2500
Dominant geology	**Geologic period:** ordovician **Bedrock geology:** nant ffrancon subgroup (NFR), siltstone **Superficial deposits:** peat
Dominant soil	Raw oligo-amorphous peat soils
Dominant land use	Some grazing
Dominant habitat type	Blanket bog

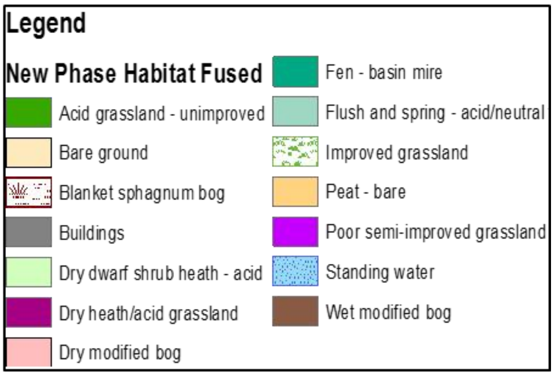
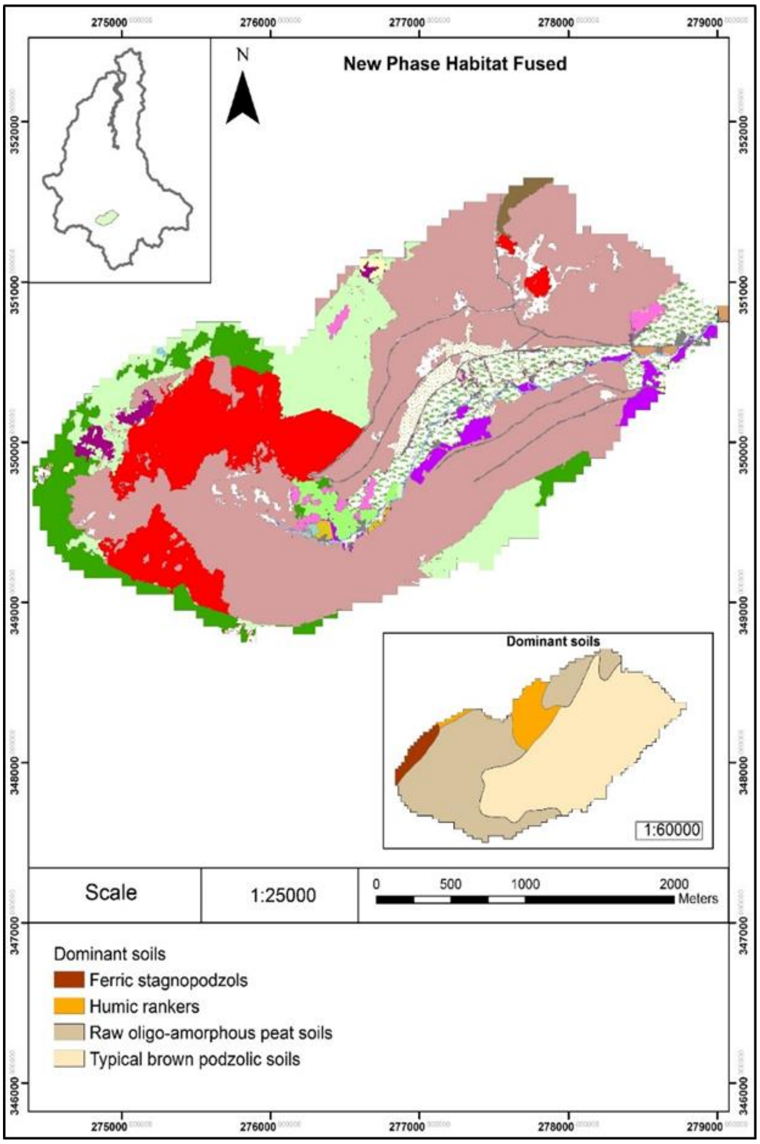
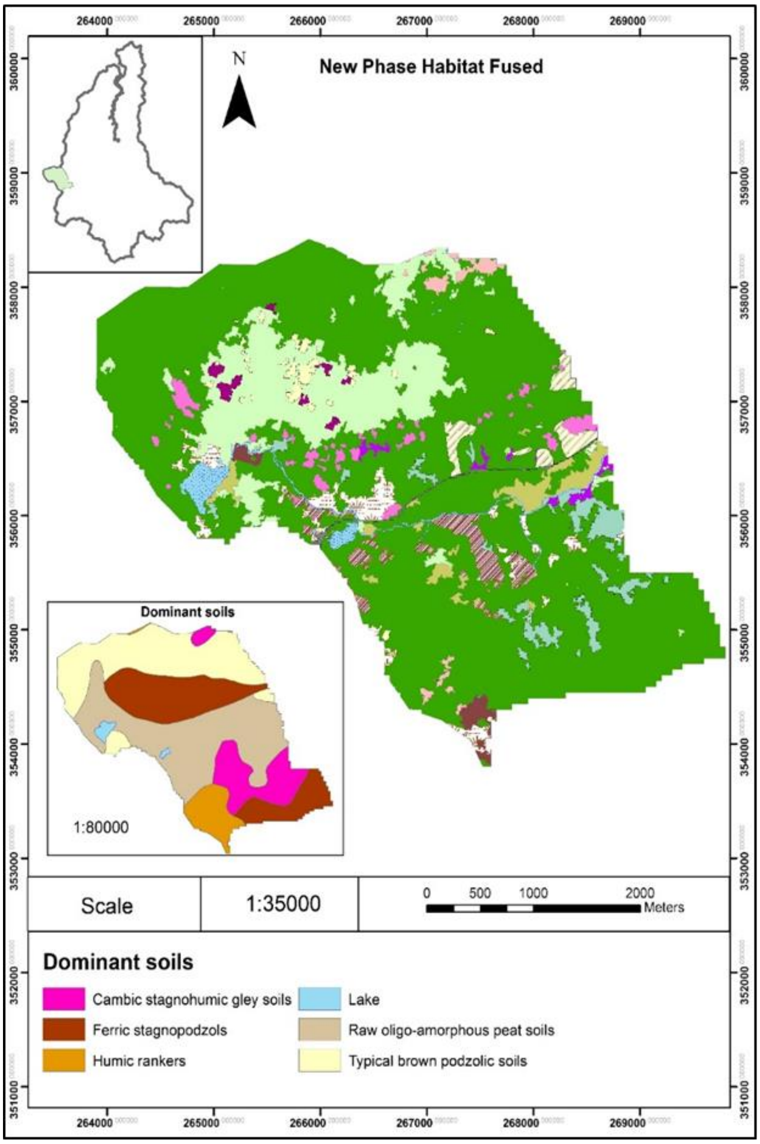


Figure S3. Detailed description of the main characteristics of sub-catchment 3.



Coordinates	3°50′ W, 53°20′ N
Area (km^2)	7.45
% of the whole catchment	1.28
Perimeter (km)	16
Stream network length (km)	32.08
Main channel length (km)	5.58
Elevation (m)	**Max:** 598.34 **Min:** 144.43
Average Slope (%)	35.17
Rainfall (mm)	1900-3000
Dominant geology	**Geologic period:** ordovician **Bedrock geology:** nant ffrancon subgroup (NFR), siltstone **Superficial deposits:** head, clay, silt, sand and gravel
Dominant soil	Tipical brown podzolic soils
Dominant land use	Some grazing and forestry
Dominant habitat type	Coniferous woodland

Figure S4. Detailed description of the main characteristics of sub-catchment 4.



Coordinates	3°59′ W, 53°50′ N
Area (km^2)	14.76
% of the whole catchment	2.55
Perimeter (km)	22.6
Stream network length (km)	60.81
Main channel length (km)	5.86
Elevation (m)	**Max:** 998.56 **Mix:** 197.16
Average Slope (%)	29.66
Rainfall (mm)	3000-3500
Dominant geology	**Geologic period:** ordovician **Bedrock geology:** cwm eigiau formation (CEI), mudstone and siltstone **Superficial deposits:** head, clay, silt, sand and gravel
Dominant soil	Raw oligo-amorphous peat soils
Dominant land use	Some sheep grazing
Dominant habitat type	Acid grassland

Figure S5. Detailed description of the main characteristics of sub-catchment 5.

1.2 | Habitat types grouping categories

Table S1. Summary of aggregated habitat categories.

New Phase habitat code	New Phase habitat description	Land cover categories
A1.1.1	Broadleaved woodland - semi-natural	Broadleaf woodland
A1.1.2	Broadleaved woodland - plantation	Broadleaf woodland
A1.2.2	Coniferous woodland - plantation	Coniferous woodland
A1.3.1	Mixed woodland - semi-natural	Broadleaf woodland
A1.3.2	Mixed woodland - plantation	Broadleaf woodland
A2.1	Scrub - dense/continuous	Broadleaf woodland
A4	Recently felled woodland	Broadleaf woodland
A4.1	Broadleaved woodland - recently felled	Broadleaf woodland
A4.2	Coniferous woodland - recently felled	Coniferous woodland
B1.1	Acid grassland - unimproved	Semi-natural grassland
B1.2	Acid grassland - semi-improved	Semi-natural grassland
B2.2	Neutral grassland - semi-improved	Semi-natural grassland
B3.1	Calcareous grassland - unimproved	Semi-natural grassland
B3.2	Calcareous grassland - semi-improved	Semi-natural grassland
B4	Improved grassland	Improved grassland
B5	Marsh/marshy grassland	Mountain, heath and bog
B5.1	Marshy grassland Juncus dominated	Mountain, heath and bog
B5.2	Marshy grassland Molinia dominated	Mountain, heath and bog
B6	Poor semi-improved grassland	Semi-natural grassland
C1.1	Bracken - continuous	Mountain, heath and bog
C3.1	Other tall herb and fern - ruderal	Mountain, heath and bog
C3.2	Other tall herb and fern - non ruderal	Mountain, heath and bog
D1.1	Dry dwarf shrub heath - acid	Mountain, heath and bog
D2	Wet dwarf shrub heath	Mountain, heath and bog
D5	Dry heath/acid grassland	Mountain, heath and bog
D6	Wet heath/acid grassland	Mountain, heath and bog
E1.6.1	Blanket sphagnum bog	Mountain, heath and bog
E1.7	Wet modified bog	Mountain, heath and bog
E1.8	Dry modified bog	Mountain, heath and bog
E2.1	Flush and spring - acid/neutral flush	Mountain, heath and bog
E2.2	Flush and spring - basic flush	Mountain, heath and bog

New Phase habitat code	New Phase habitat description	Land cover categories
E3	Fen	Mountain, heath and bog
E3.1	Fen - valley mire	Mountain, heath and bog
E3.1.1	Modified valley mire	Mountain, heath and bog
E3.2	Fen - basin mire	Mountain, heath and bog
E4	Peat - bare	Mountain, heath and bog
F1	Swamp	Mountain, heath and bog
F2.2	Marginal and inundation - inundation vegetation	Mountain, heath and bog
G1	Standing water	Freshwater
I1.1.1	Inland cliff - acid/neutral	Other
I1.2.1	Scree - acid/neutral	Other
I1.4	Other rock exposure	Other
I1.4.1	Other exposure - acid/neutral	Other
I2.1	Quarry	Other
I2.2	Spoil	Other
J1.1	Cultivated/disturbed land - arable	Arable
J1.2	Cultivated/disturbed land - amenity grassland	Other
J1.5	Gardens	Other
J2.1	Intact hedge	Broadleaf woodland
J3.4	Caravan site	Other
J3.6	Buildings	Other
J3.7	Track (incomplete)	Other

1.3 | Description of soil series

Table S2. Description of soil series and their equivalent in the FAO World Reference Base 2006.

Series	Major soil group	Subgroup	Parent material	Drainage class	WRB 2006[1]
Caron	Organic soils	Acid hill peat soils	Peat	Very poorly drained	Ombric Sapric Histosols
Cegin	Gley soils	Non-calcareous	Drift (shale)	Poorly drained	Dystric Stagnosols
Conway	Gley soils	Non-calcareous	Alluvium(shale)	Poorly drained	Fluvic Eutric Gleysols
Cymmer	Podzolized soils	–	Colluvium of shale	Freely to excessively drained	Podzol
Denbigh	Brown earths	Low base status	Drift (shale)	Freely drained	Eutric Endoleptic Cambisols
Powys	Brown earths	Low base status	Shales	Excessively drained	Eutric Endoleptic Cambisols
Sannan	Brown earths	With gleying	Drift (shale)	Imperfectly drained	Eutric Endostagnic Cambisols
Ynys	Gley soils	Peaty gley	Drift (shale)	Very poorly drained	Umbric Stagnosols

[1]IUSS Working Group WRB (2006) World Reference Base for Soil Resources. World Soil Resources Report No 103. FAO Rome.

Appendix 2

Supplementary material for Chapter 4

Quantifying the contribution of riparian areas to the provision of ecosystem services

Laura L. de sosa, Helen C. Glanville, Miles R. Marshall, A. Prysor Williams, Davey L. Jones

Table S1. Soil physicochemical properties in mountain, heath and bog (MHB) land use type with respect to the distance from the river and soil depth in the Conwy Catchment. Data are mean ± SEM (n = 5). Significant differences are shown according to two-way ANOVA (One-way ANOVA for bulk density) with distance and depth as main factors. No interactions between depth and distance were found in the analysis. No significant differences were found by the interaction of distance with depth.

	Riparian distance				*P*-values	
	Close to river (2 m)		Far from river (50 m)			
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	Distance	Depth
pH	4.85 ± 0.40	4.92 ± 0.40	4.34 ± 0.20	4.46 ± 0.20	ns	ns
EC (μS cm^{-1})	33.2 ± 6.3	26.8 ± 5.4	37.1 ± 4.4	24.0 ± 5.0	ns	ns
Bulk density (g cm^{-3})	0.07 ± 0.01		0.09 ± 0.02		ns	ns
Moisture content (%)	87.7 ± 0.8	87.4 ± 0.5	87.4 ± 1.7	84.2 ± 1.1	ns	ns
Organic matter (%)	78.7 ± 6.8	86.1 ± 5.6	86.3 ± 3.5	78.6 ± 5.9	ns	ns
NH_4^+-N (mg kg^{-1} soil)	19.8 ± 1.3	18.4 ± 1.2	20.7 ± 4.0	18.1 ± 1.2	ns	ns
NO_3^--N (mg kg^{-1} soil)	51.5 ± 18.7	50.5 ± 19.3	56.8 ± 15.1	42.5 ± 12.1	ns	ns
Available P (mg kg^{-1} soil)	10.8 ± 4.04	3.11 ± 1.49	3.42 ± 0.53	2.29 ± 0.72	0.002	ns
Total C (g kg^{-1} soil)	453 ± 102	456 ± 147	545 ± 30	524 ± 40	ns	ns
Total N (g kg^{-1} soil)	17.8 ± 3.1	21.6 ± 1.8	13.8 ± 4.5	21.1 ± 2.2	ns	ns
Dissolved organic C (g kg^{-1} soil)	0.95 ± 0.30	1.00 ± 0.30	1.07 ± 0.20	1.01 ± 0.20	ns	ns
Total dissolved N (g kg^{-1} soil)	0.14 ± 0.03	0.16 ± 0.04	0.17 ± 0.04	0.14 ± 0.01	ns	ns
Microbial biomass C (g kg^{-1} soil)	3.20 ± 0.89	1.04 ± 0.41	3.81 ± 1.07	1.20 ± 0.19	ns	0.005
Microbial biomass N (g kg^{-1} soil)	0.26 ± 0.11	0.28 ± 0.11	0.43 ± 0.24	0.38 ± 0.08	ns	ns

EC, electrical conductivity.

Table S2. Soil physicochemical properties in broadleaf woodland (BW) land use type with respect to the distance from the river and depth in the Conwy Catchment. Data are mean ± SEM (n = 5). Significant differences are shown according to two-way ANOVA (One-way ANOVA for bulk density) with distance and depth as main factors. No interactions between depth and distance were found in the analysis. No significant differences were found by the interaction of distance with depth.

	Riparian distance				*P*-values	
	Close to river (2 m)		Far from river (50 m)			
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	Distance	Depth
pH	5.14 ± 0.30	5.18 ± 0.20	5.07 ± 0.30	5.24 ± 0.30	ns	ns
EC (μS cm^{-1})	26.6 ± 5.0	25.2 ± 4.2	42.9 ± 6.2	31.5 ± 5.4	0.047	ns
Bulk density (g cm^{-3})	0.74 ± 0.11		0.73 ± 0.06		ns	ns
Moisture content (%)	30.0 ± 3.0	27.2 ± 5.0	41.0 ± 7.8	34.3 ± 2.8	ns	ns
Organic matter (%)	14.3 ± 4.8	8.4 ± 1.9	24.8 ± 12.5	10.1 ± 0.7	ns	ns
NH_4^+-N (mg kg^{-1} soil)	3.75 ± 0.8	4.25 ± 0.7	6.37 ± 0.5	4.70 ± 0.8	0.042	ns
NO_3^--N (mg kg^{-1} soil)	1.99 ± 0.6	1.77 ± 1.1	7.01 ± 1.6	3.49 ± 1.0	0.004	ns
P available (mg kg^{-1} soil)	0.31 ± 0.11	0.41 ± 0.20	0.57 ± 0.28	0.19 ± 0.12	ns	ns
Total C (g kg^{-1} soil)	57 ± 13	44 ± 10	76 ± 8	42 ± 6	ns	ns
Total N (g kg^{-1} soil)	3.38 ± 0.60	4.47 ± 0.30	2.72 ± 0.40	3.21 ± 0.20	0.016	ns
Dissolved organic C (g kg^{-1} soil)	0.19 ± 0.05	0.19 ± 0.05	0.26 ± 0.06	0.14 ± 0.02	ns	ns
Total dissolved N (g kg^{-1} soil)	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.005	0.02 ± 0.002	ns	ns
Microbial biomass C (g kg^{-1} soil)	0.26 ± 0.11	0.28 ± 0.11	0.43 ± 0.24	0.38 ± 0.08	ns	ns
Microbial biomass N (g kg^{-1} soil)	0.16 ± 0.03	0.18 ± 0.02	0.26 ± 0.03	0.32 ± 0.11	0.024	ns

EC, electrical conductivity.

Table S3. Soil physicochemical properties in coniferous woodland (CW) land use type with respect to the distance from the river and depth in the Conwy Catchment. Data are mean ± SEM (*n* = 5). Significant differences are shown according to two way ANOVA (One way ANOVA for bulk density) with distance and depth as main factors. No interactions between depth and distance were found in the analysis. No significant differences were found by the interaction of distance with depth.

	Riparian distance				***P*-values**	
	Close to river (2 m)		**Far from river (50 m)**			
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**	**Distance**	**Depth**
pH	4.75 ± 0.20	4.95 ± 0.10	4.23 ± 0.10	4.52 ± 0.10	0.002	ns
EC (μS cm^{-1})	28.9 ± 4.8	27.0 ± 3.2	43.6 ± 7.5	45.0 ± 10.1	ns	ns
Bulk density (g cm^{-3})	0.45 ± 0.15		0.41 ± 0.16		ns	ns
Moisture content (%)	36.4 ± 9.9	36.2 ± 10.7	39.3 ± 5.8	32.9 ± 7.5	ns	ns
Organic matter (%)	13.5 ± 5.8	12.9 ± 6.6	18.9 ± 3.4	13.3 ± 1.6	ns	ns
NH_4^+-N (mg kg^{-1} soil)	5.62 ± 0.90	4.79 ± 0.60	5.08 ± 0.90	4.75 ± 0.80	ns	ns
NO_3^--N (mg kg^{-1} soil)	4.95 ± 1.2	4.11 ± 1.4	7.54 ± 2.2	4.63 ± 5.9	ns	ns
Available P (mg kg^{-1} soil)	0.27 ± 0.08	0.34 ± 0.20	0.40 ± 0.08	0.28 ± 0.03	ns	ns
Total C (g kg^{-1} soil)	71 ± 33	56 ± 36	109 ± 13	58 ± 11	ns	ns
Total N (g kg^{-1} soil)	4.21 ± 1.40	5.38 ± 0.50	3.32 ± 1.60	3.11 ± 0.40	ns	ns
Dissolved organic C (g kg^{-1} soil)	0.22 ± 0.04	0.22 ± 0.04	0.32 ± 0.03	0.33 ± 0.03	0.011	ns
Total dissolved N (g kg^{-1} soil)	0.03 ± 0.004	0.03 ± 0.005	0.04 ± 0.004	0.04 ± 0.004	ns	ns
Microbial biomass C (g kg^{-1} soil)	1.09 ± 0.38	0.85 ± 0.41	2.15 ± 0.23	1.15 ± 0.28	ns	ns
Microbial biomass N (g kg^{-1} soil)	0.20 ± 0.05	0.10 ± 0.02	0.22 ± 0.03	0.13 ± 0.04	ns	0.019

EC, electrical conductivity.

Table S4. Soil physicochemical properties in semi-natural grassland (SNG) land use type with respect to the distance from the river and depth in the Conwy Catchment. Data are mean ± SEM (n = 5). Significant differences are shown according to two-way ANOVA (One way ANOVA for bulk density) with distance and depth as main factors. No interactions between depth and distance were found in the analysis. No significant differences were found by the interaction of distance with depth.

	Riparian distance				***P*-values**	
	Close to river (2 m)		**Far from river (50 m)**			
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**	**Distance**	**Depth**
pH	4.95 ± 0.20	5.07 ± 0.10	5.25 ± 0.40	5.27 ± 0.20	ns	ns
EC (μS cm^{-1})	35.1 ± 5.3	26.9 ± 4.6	44.4 ± 8.4	28.1 ± 4.6	ns	ns
Bulk density (g cm^{-3})	0.16 ± 0.05		0.31 ± 0.12		ns	ns
Moisture content (%)	73.0 ± 7.6	68.9 ± 10.1	62.7 ± 9.3	51.7 ± 13.0	ns	ns
Organic matter (%)	41.4 ± 11.6	39.9 ± 12.2	33.9 ± 11.4	25.9 ± 13.2	ns	ns
NH_4^+-N (mg kg^{-1} soil)	15.5 ± 4.9	14.1 ± 4.4	12.9 ± 5.9	7.40 ± 2.3	ns	ns
NO_3^--N (mg kg^{-1} soil)	14.6 ± 5.6	14.7 ± 4.2	13.7 ± 5.1	9.10 ± 1.9	ns	ns
Available P (mg kg^{-1} soil)	1.06 ± 0.36	0.64 ± 0.25	0.63 ± 0.21	0.57 ± 0.24	ns	ns
Total C (g kg^{-1} soil)	74 ± 35	218 ± 67	101 ± 25	83.3 ± 20	ns	ns
Total N (g kg^{-1} soil)	5.47 ± 1.9	7.46 ± 1.5	11.03 ± 3.7	12.28 ± 4.0	ns	ns
Dissolved organic C (g kg^{-1} soil)	0.40 ± 0.10	0.41 ± 0.14	0.42 ± 0.10	0.35 ± 0.1	ns	ns
Total dissolved N (g kg^{-1} soil)	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.008	ns	ns
Microbial biomass C (g kg^{-1} soil)	6.84 ± 2.40	5.50 ± 2.68	1.05 ± 0.38	0.94 ± 0.30	0.050	ns
Microbial biomass N (g kg^{-1} soil)	0.90 ± 0.23	0.29 ± 0.08	0.43 ± 0.11	0.27 ± 0.10	ns	0.014

EC, electrical conductivity.

Table S5. Soil physicochemical properties in improved grassland (IG) land use type with respect to the distance from the river and depth in the Conwy Catchment. Data are mean ± SEM (*n* = 5). Significant differences are shown according to two way ANOVA (One way ANOVA for bulk density) with distance and depth as main factors. No significant differences were found by the interaction of distance with depth.

	Riparian distance				***P*-values**	
	Close to river (2 m)		**Far from river (50 m)**			
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**	**Distance**	**Depth**
pH	5.19 ± 0.30	5.28 ± 0.30	5.39 ± 0.10	5.43 ± 0.20	ns	ns
EC (μS cm^{-1})	104 ± 37	34 ± 7	131 ± 55	101 ± 47	ns	ns
Bulk density (g cm^{-3})	0.60 ± 0.11		0.71 ± 0.10		ns	ns
Moisture content (%)	39.0 ± 6.9	35.4 ± 8.9	44.0 ± 5.3	30.6 ± 2.8	ns	ns
Organic matter (%)	13.3 ± 3.7	12.6 ± 6.3	20.0 ± 4.4	10.0 ± 2.0	ns	ns
NH_4^+-N (mg kg^{-1} soil)	5.18 ± 1.7	3.42 ± 1.1	5.87 ± 2.1	3.39 ± 1.1	ns	ns
NO_3^--N (mg kg^{-1} soil)	9.78 ± 3.4	6.96 ± 1.8	22.7 ± 9.1	21.4 ± 12.1	ns	ns
Available P (mg kg^{-1} soil)	2.08 ± 1.06	1.05 ± 0.55	1.84 ± 0.75	0.93 ± 0.48	ns	ns
Total C (g kg^{-1} soil)	270 ± 65	87 ± 59	223 ± 65	56 ± 8	ns	0.001
Total N (g kg^{-1} soil)	14.8 ± 3.4	14.2 ± 3.3	3.31 ± 0.5	6.10 ± 1.9	0.017	ns
Dissolved organic C (g kg^{-1} soil)	0.17 ± 0.02	0.18 ± 0.05	0.20 ± 0.02	0.15 ± 0.02	ns	ns
Total dissolved N (g kg^{-1} soil)	0.04 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.02	ns	ns
Microbial biomass C (g kg^{-1} soil)	1.90 ± 0.55	1.54 ± 0.77	2.49 ± 0.31	1.19 ± 0.20	ns	ns
Microbial biomass N (g kg^{-1} soil)	0.18 ± 0.04	0.30 ± 0.10	0.38 ± 0.06	0.31 ± 0.11	ns	ns

EC, electrical conductivity.

Aerial photographs sample points

1. Aerial photograph of sample point nº 1 within broadleaf woodland habitat type.

2. Aerial photograph of sample point nº 2 within broadleaf woodland habitat type.

3. Aerial photograph of sample point nº 3 within broadleaf woodland habitat type.

4. Aerial photograph of sample point nº 4 within broadleaf woodland habitat type.

5. Aerial photograph of sample point nº 5 within broadleaf woodland habitat type.

1. Aerial photograph of sample point nº 1 within coniferous woodland habitat type.

2. Aerial photograph of sample point nº 2 within coniferous woodland habitat type.

3. Aerial photograph of sample point nº 3 within coniferous woodland habitat type.

4. Aerial photograph of sample point nº 4 within coniferous woodland habitat type.

5. Aerial photograph of sample point nº 5 within coniferous woodland habitat type.

1. Aerial photograph of sample point nº 1 within improved grassland habitat type.

2. Aerial photograph of sample point nº 2 within improved grassland habitat type.

3. Aerial photograph of sample point nº 3 within improved grassland habitat type.

4. Aerial photograph of sample point nº 4 within improved grassland habitat type.

5. Aerial photograph of sample point nº 5 within improved grassland habitat type.

1. Aerial photograph of sample point nº 1 within mountain, heath and bog habitat type.

2. Aerial photograph of sample point nº 2 within mountain, heath and bog habitat type.

3. Aerial photograph of sample point nº 3 within mountain, heath and bog habitat type.

4. Aerial photograph of sample point nº 4 within mountain, heath and bog habitat type.

5. Aerial photograph of sample point nº 5 within mountain, heath and bog habitat type.

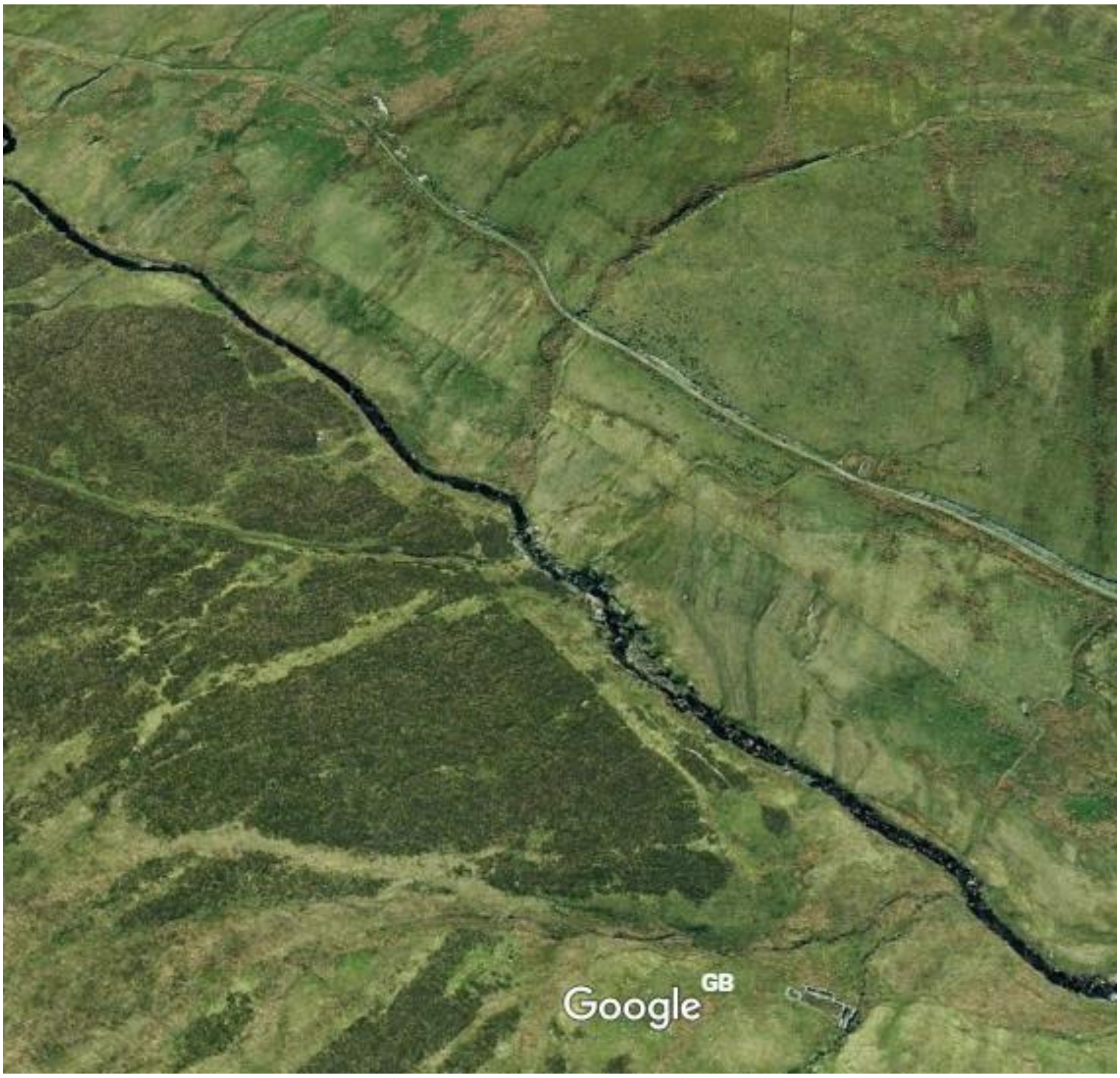
1. Aerial photograph of sample point nº 1 within semi-natural grassland habitat type.

2. Aerial photograph of sample point nº 2 within semi-natural grassland habitat type.

3. Aerial photograph of sample point nº 3 within semi-natural grassland habitat type.

4. Aerial photograph of sample point nº 4 within semi-natural grassland habitat type.

5. Aerial photograph of sample point nº 5 within semi-natural grassland habitat type.



Appendix 3

Supplementary material for Chapter 5

Stoichiometric constraints on microbial community behaviour with soil depth along a riparian hillslope

Laura L. de Sosa, Helen C. Glanville, Miles R. Marshall, Andrea Schnepf, David M. Cooper, A. Prysor Williams[a], Davey L. Jones

Table S1. Soil physicochemical properties according to soil depth and distance from the river (row 1, 2 m; row 2, 12 m; row 3, 75 m). Different upper-case letters indicate significant differences (P < 0.05) according to One-way ANOVA with depth as the main factor followed by a Games-Howel post-hoc test. Different lower-case letters indicate significant differences (P < 0.05) with respect to distance from the river according to One-way ANOVA followed by a Tukey post-hoc test. Value are means ± standard errors (n = 3). All the PLFA biomass values below a soil depth of 100 cm were combined due to the low abundance of organisms present. Only PLFA soil biomass up to 100 cm was included in the statistical analysis. Missing values indicate no samples due to hitting bedrock.

Soil property	**Distance from the river**	**Soil depth**					
		0-15 cm	**15-30 cm**	**50-100 cm**	**100-150 cm**	**150-200 cm**	**250-300 cm**
pH	2 m	5.58 ± 0.18	5.87 ± 0.19	6.16 ± 0.09 ab			
	12 m	5.34 ± 0.20^{A}	5.35 ± 0.23^{A}	6.03 ± 0.05^{AB} b	6.48 ± 0.10^{AB}	7.03 ± 0.28^{B}	
	75 m	5.52 ± 0.04^{A}	5.73 ± 0.13^{AB}	6.57 ± 0.14^{B} a	6.36 ± 0.29^{AB}	6.28 ± 0.34^{AB}	6.74 ± 0.32^{AB}
EC (μS cm^{-1})	2 m	63.0 ± 13.1	25.2 ± 5.4	34.1 ± 14.5			
	12 m	33.6 ± 8.5	58.5 ± 41.9	18.6 ± 1.5	34.5 ± 8.4	47.7 ± 13.4	
	75 m	77.3 ± 44.3	28.0 ± 8.4	14.5 ± 1.12	18.7 ± 1.4	20.2 ± 3.9	22.1 ± 1.9
Soil water (g kg^{-1} soil)	2 m	296 ± 17^{A}	240 ± 10^{AB}	179 ± 19^{B}			
	12 m	333 ± 9^{A}	257 ± 8^{B}	216 ± 4^{ABC}	133 ± 2^{C}	133 ± 29^{BC}	
	75 m	304 ± 9^{C}	236 ± 9^{A}	136 ± 16^{AB}	125 ± 11^{B}	110 ± 2^{B}	107 ± 4^{B}
Organic matter (g kg^{-1} soil)	2 m	62.2 ± 6.3^{A} a	3.71 ± 3.1^{A} a	16.5 ± 3.4^{B}			
	12 m	76.7 ± 3.6^{C} ab	5.11 ± 1.5^{A} ab	25.4 ± 6.8^{AB}	1.11 ± 1.3^{B}	11.2 ± 2.4^{B}	
	75 m	89.4 ± 3.5^{A} b	5.61 ± 5.7^{AB} b	18.8 ± 1.5^{B}	1.43 ± 0.6^{B}	12.8 ± 1.0^{B}	13.9 ± 0.7^{B}
Ammonium (NH_4^+-N) (mg kg^{-1} DW soil)	2 m	1.71 ± 0.12 a	1.13 ± 0.23 a	0.95 ± 0.33 a			
	12 m	3.45 ± 0.69^{A} b	3.62 ± 1.33^{AB} b	0.85 ± 0.09^{AB} a	0.98 ± 0.21^{AB} a	0.77 ± 0.06^{B}	
	75 m	7.39 ± 1.53^{AB} c	4.02 ± 0.46^{A} b	0.23 ± 0.07^{B} b	0.17 ± 0.08^{B} b	0.24 ± 0.13^{B}	0.32 ± 0.07^{B}
Nitrate (NO_3-N) (mg kg^{-1} DW soil)	2 m	4.08 ± 2.40	2.90 ± 1.55	2.61 ± 1.38			
	12 m	4.33 ± 2.65	3.87 ± 3.74	0.52 ± 0.38	2.81 ± 1.51	0.45 ± 0.12	
	75 m	1.91 ± 1.04	3.57 ± 3.02	0.90 ± 0.42	0.16 ± 0.08	1.74 ± 0.93	0.40 ± 0.22
P available (PO_4-P) (mg kg^{-1} DW soil)	2 m	22.5 ± 1.93^{A} a	2.73 ± 0.91^{B}	16.4 ± 6.14^{AB} a			
	12 m	5.51 ± 1.74 b	1.02 ± 0.17	1.11 ± 0.31 b	57.1 ± 32.5	41.4 ± 17.8	
	75 m	3.08 ± 0.29 b	1.10 ± 0.31	6.61 ± 1.93 a	8.22 ± 2.15	18.9 ± 5.06	80.7 ± 21.3
C:N ratio	2 m	8.39 ± 3.34	4.46 ± 0.23 a	2.58 ± 0.59			
	12 m	11.1 ± 1.67	6.38 ± 1.06 b	1.71 ± 0.34	3.08 ± 1.21	4.02 ± 0.77 a	
	75 m	9.68 ± 1.58^{AB}	6.44 ± 0.29^{A} b	1.39 ± 0.26^{B}	1.09 ± 0.17^{B}	1.01 ± 0.18^{B} b	0.94 ± 0.04^{B}
Dissolved organic C (mg kg^{-1} DW soil)	2 m	111 ± 18.5^{A} a	73.9 ± 7.68^{A} a	20.3 ± 8.09^{B}			
	12 m	186 ± 3.11^{A} ab	110 ± 5.50^{B} b	43.4 ± 24.4^{ABC}	14.7 ± 9.36^{C}	3.56 ± 1.34^{C} a	
	75 m	238 ± 23.8^{A} b	148 ± 20.2^{AB} b	38.3 ± 14.2^{B}	24.4 ± 6.08^{B}	11.9 ± 2.13^{B} b	5.30 ± 0.63^{B}
Total dissolved N (mg kg^{-1} DW soil)	2 m	30.7 ± 4.28^{A}	17.4 ± 2.04^{A}	4.71 ± 1.74^{B}			
	12 m	48.5 ± 8.08^{AB}	21.7 ± 2.31^{A}	7.15 ± 4.35^{AB}	3.76 ± 2.46^{B}	2.69 ± 1.03^{B}	
	75 m	46.2 ± 2.01^{A}	25.0 ± 3.25^{B}	6.26 ± 0.67^{B}	5.32 ± 0.92^{B}	8.34 ± 4.94^{B}	2.77 ± 0.47^{B}
Microbial biomass C (mg kg^{-1} DW soil)	2 m	853 ± 258^{A}	264 ± 44^{B}	102 ± 18^{B} a			
	12 m	739 ± 67^{C}	217 ± 31^{AB}	92.2 ± 3.4^{A} a	59.1 ± 4.3^{B}	52.1 ± 9.5^{AB}	
	75 m	670 ± 23^{A}	238 ± 47^{B}	36.9 ± 9.8^{B} b	37.4 ± 2.2^{B}	35.8 ± 12.5^{B}	31.3 ± 2.9^{B}
PLFA biomass (μmol kg^{-1} soil)	2 m	210 ± 11^{A} a	52.1 ± 7.2^{B}	9.57 ± 1.53^{C}			
	12 m	269 ± 29^{A} ab	113 ± 61^{AB}	5.15 ± 1.68^{B}		3.05 ± 1.29	
	75 m	322 ± 8^{A} b	113 ± 7^{B}	4.53 ± 2.07^{C}			

Table S2. Maximum sorption (S_{max}) and binding energy constant (k) describing the binding of inorganic P to the soil with respect to distance from the river and soil depth. S_{max} and k were estimated using the Langmuir equation fitted to experimental data ($r^2 > 0.9$, $p < 0.001$ for all cases). Different lower-case letters indicate significant differences ($P < 0.05$) with distance from the river according to One-way ANOVA followed by a Tukey post-hoc test. Values are means ± standard errors ($n = 3$). Missing values indicate no samples due to hitting bedrock.

	Distance from the river	**Soil depth**					
		0-15 cm	**15-30 cm**	**50-100 cm**	**100-150 cm**	**150-200 cm**	**250-300 cm**
Maximum P sorption S_{max} (mg kg^{-1})	2 m	730 ± 73^{a}	646 ± 47^{a}	356 ± 55			
	12 m	1037 ± 37^{b}	859 ± 25^{b}	500 ± 306	303 ± 99	268 ± 39	
	75 m	1157 ± 46^{b}	976 ± 67^{b}	582 ± 65	462 ± 41	403 ± 26	327 ± 25
Binding strength k (l kg^{-1})	2 m	0.72 ± 0.06^{a}	0.92 ± 0.14^{a}	0.55 ± 0.05			
	12 m	1.49 ± 0.16^{b}	2.17 ± 0.75^{a}	2.16 ± 1.19	1.54 ± 0.90	0.80 ± 0.05	
	75 m	2.02 ± 0.13^{b}	2.40 ± 0.18^{b}	1.76 ± 0.43	1.90 ± 0.96	1.29 ± 0.58	0.74 ± 0.1

Table S3. Total iron concentration as a function of soil depth. Iron was measured by total reflection X-ray fluorescence (TXRF) analysis. Values represent means ± standard errors (for each sampling depth with the range 0-100 cm, *n* = 9; 100-200, *n* = 6; 250-300 cm, *n* = 3).

Soil depth (cm)	**Fe (g kg^{-1} soil)**
0-15	19.3 ± 0.84
15-30	23.3 ± 1.41
50-100	26.2 ± 2.79
100-150	27.9 ± 2.08
150-200	29.4 ± 0.98
250-300	55.0 ± 5.85

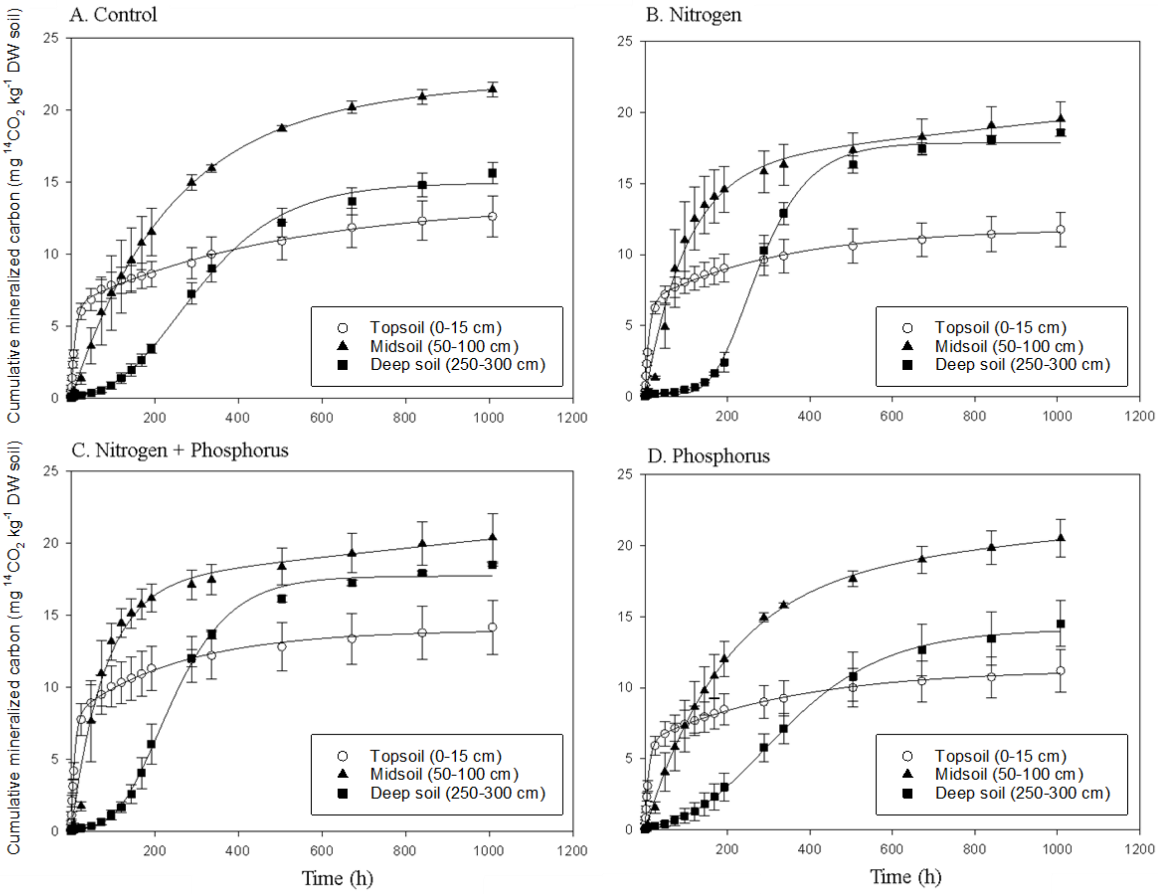


Figure S1. Example of different microbial growth patterns as evidenced from the cumulative mineralization of substrate-C after the addition of a high dose of labile DOC either alone or in combination with N, P or N+P during a 42 d incubation at three different soil depths. The curves are only presented for row 1 (2 m from the river) in the riparian transect. Bars represent mean values (n = 3) ± standard errors.

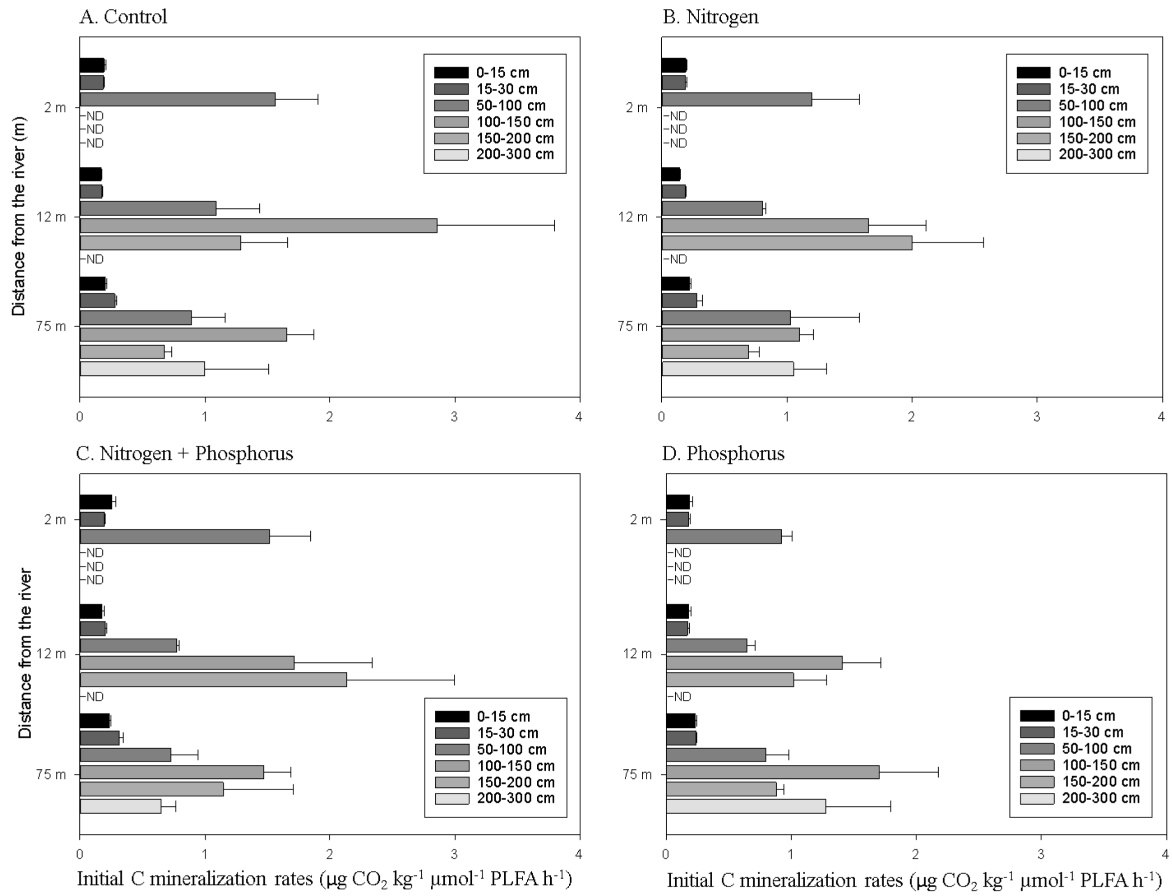


Figure S2. Initial C mineralization rates measured during the initial linear phase (between 0-6 h) after the addition of a high dose of labile DOC either alone or in combination with N, P or N+P. Values are presented for three different distances from the river (2, 12 and 75 m) and for 6 different soil depths. Bars represent mean values (n = 3) ± standard errors. ND equates to no data due to hitting bedrock (Figure 5.2).

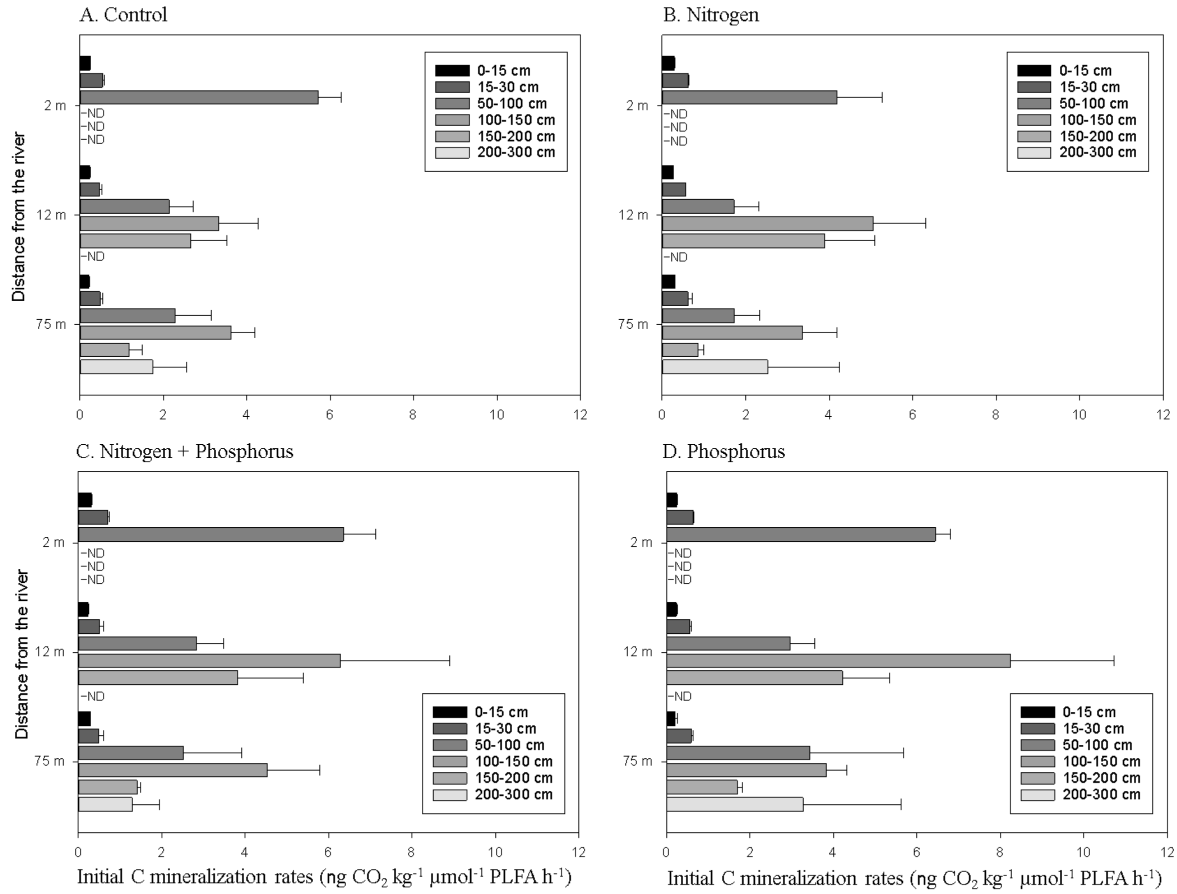


Figure S3. Initial C mineralization rates measured during the initial linear phase (between 0-6 h) after the application of a low concentration of labile DOC either alone or in combination with N, P or N+P. Values are presented for three different distances from the river (2, 12 and 75 m) and for 6 different soil depths. Bars represent mean values (n = 3) ± standard errors. ND equates to no data due to hitting bedrock (Figure 5.2).

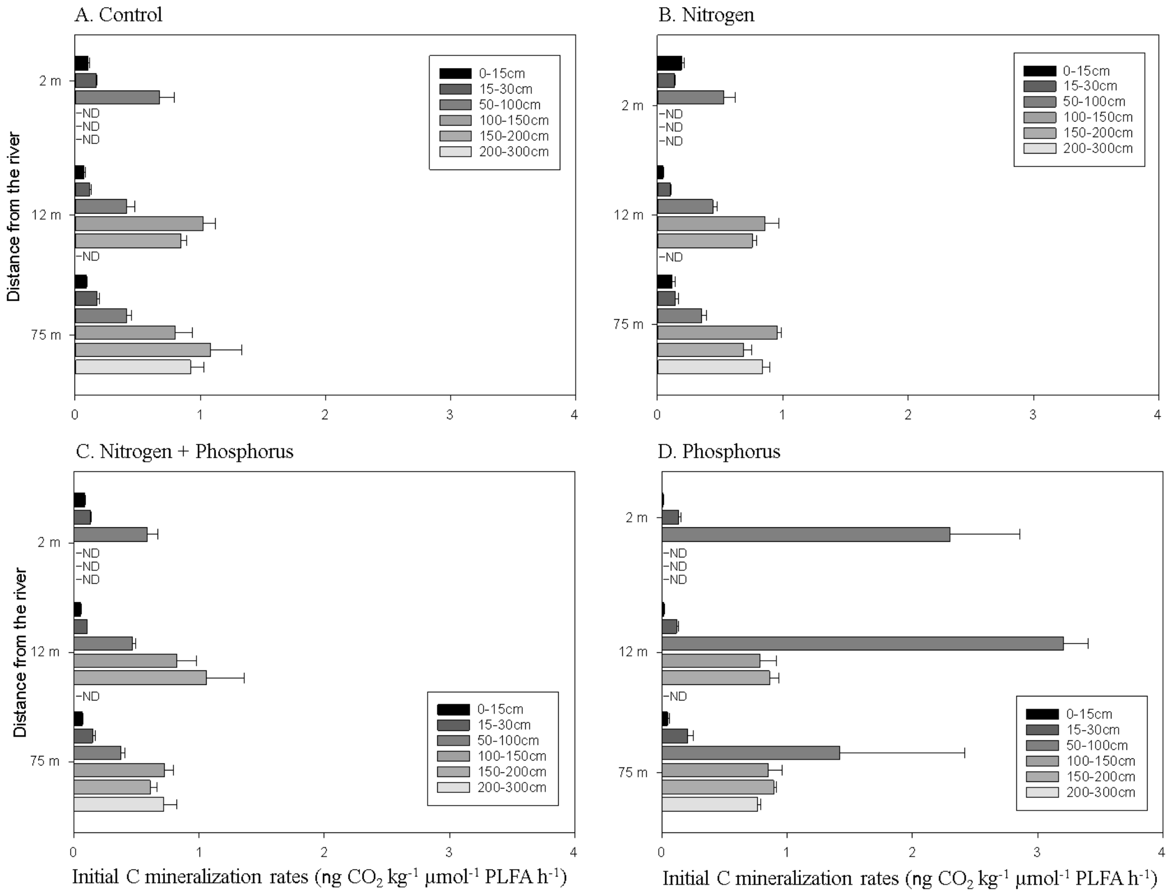


Figure S4. Initial C mineralization rates measured during the initial linear phase (between 0-48 h) after the application of a high dose of recalcitrant (high MW) DOC either alone or in combination with N, P or N+P. Values are presented for three different distances from the river (2, 12 and 75 m) and for 6 different soil depths. Bars represent mean values (n = 3) ± standard errors. ND equates to no data due to hitting bedrock (Figure 5.2).

Appendix 4

Supplementary material for Chapter 6

Spatial zoning of microbial function and plant-soil nitrogen dynamics across a riparian area

Laura L. de Sosa, Helen C. Glanville, Miles R. Marshall, A. Prysor Williams, Maïder Abadie, Ian M. Clark, Aimeric Blaud, Davey L. Jones

Supplementary on-line information

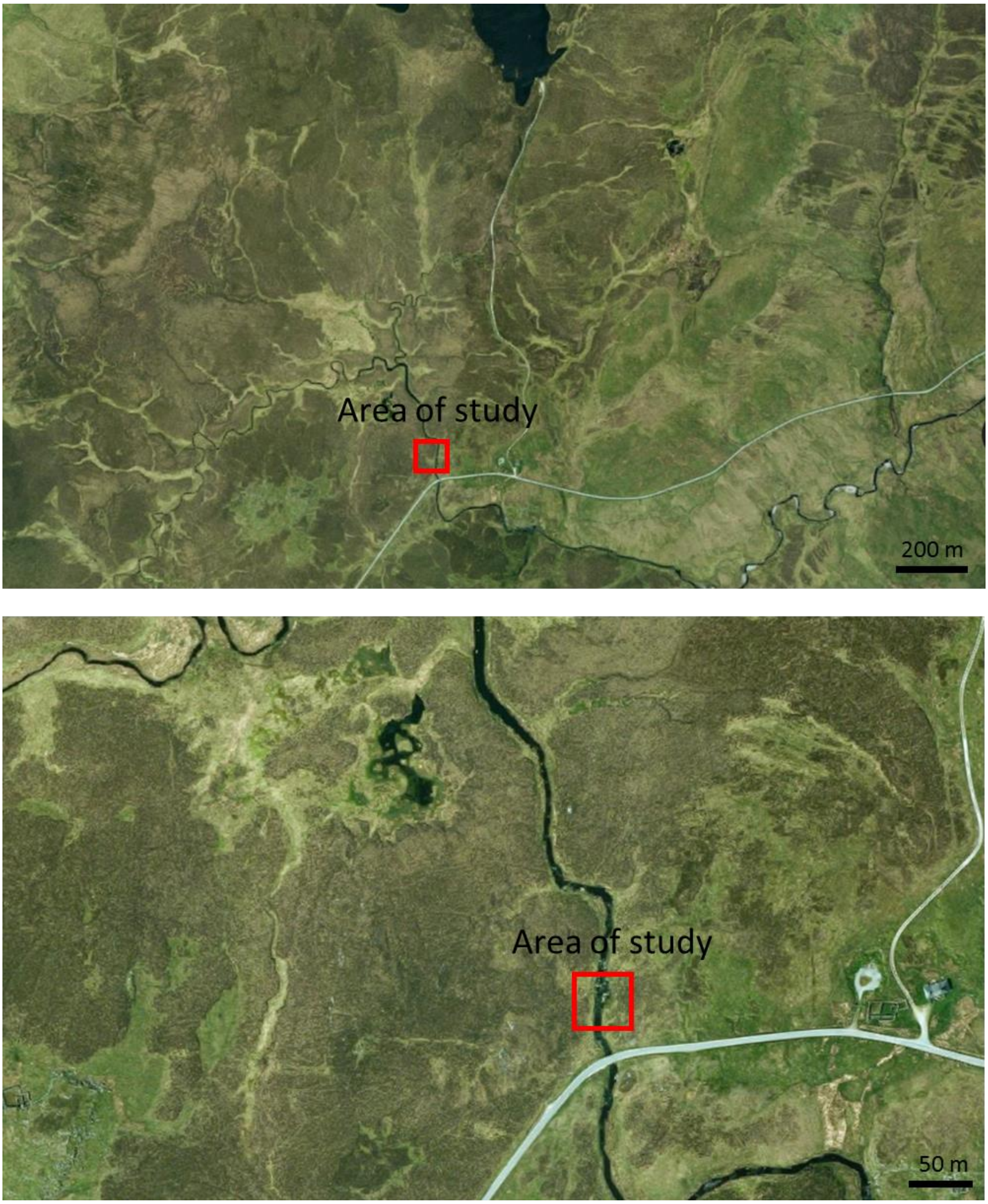


Figure S1. Aerial photography of the area of study.

Figure S2. Detailed photographs of vegetation in the area of study.

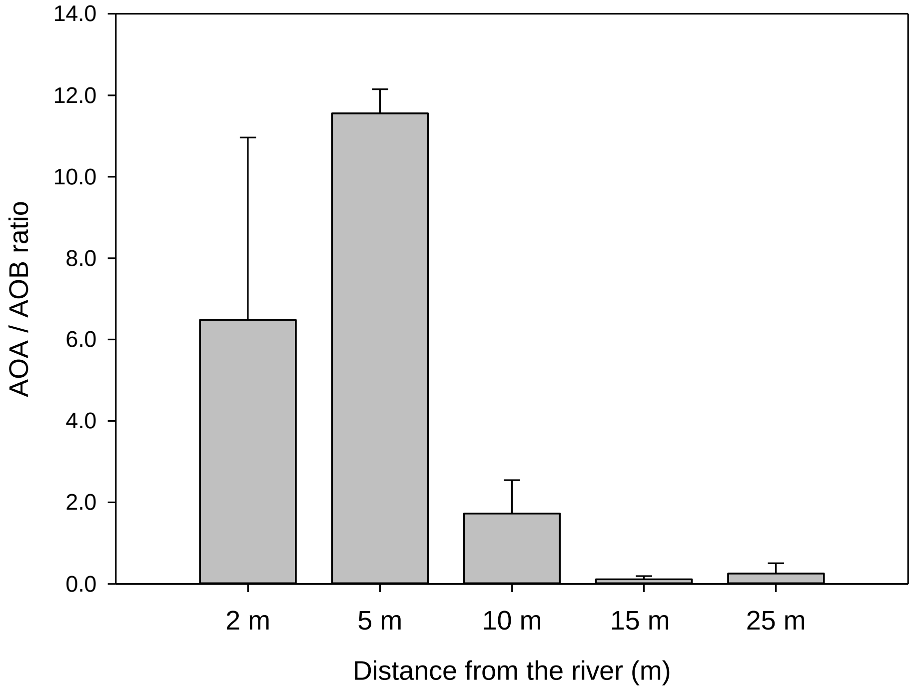


Figure S3. Ratios of AOA to AOB *amoA* copy numbers relative to distance from the river. Bars represent mean values (n = 4 for 2, 10, 15 and 25 m and n = 2 for 5 m) ± SEM. Distance from river corresponds to a change in the vegetation as shown in Figure 3.

Table S1. PLFA biomarkers used for taxonomic microbial groups

Microbial group category	PLFA specific fatty acids			
AM Fungi	16:1 w5c			
Saprophytic Fungi	18:2 w6c			
Gram Negative	10:0 2OH	14:0 2OH	18:1 w6c	21:1 w8c
	10:0 3OH	16:1 w9c	18:0 cyclo w6c	21:1 w6c
	12:1 w8c	16:1 w7c	18:1 w3c	21:1 w5c
	12:1 w5c	16:1 w6c	19:1 w9c	21:1 w4c
	13:1 w5c	16:1 w4c	19:1 w8c	21:1 w3c
	13:1 w4c	16:1 w3c	18:1 w5c	22:1 w9c
	13:1 w3c	17:1 w9c	19:1 w6c	22:1 w8c
	12:0 2OH	17:1 w8c	19:0 cyclo w9c	22:1 w6c
	14:1 w9c	17:1 w7c	19:0 cyclo w7c	22:1 w5c
	14:1 w8c	17:1 w6c	9:1 w17c	22:1 w3c
	14:1 w7c	17:1 w5c	20:1 w9c	22:0 cyclo w6c
	14:1 w5c	17:1 w4c	20:1 w8c	24:1 w9c
	15:1 w9c	17:1 w3c	20:1 w6c	24:1 w7c
	15:1 w8c	16:0 2OH	19:0 cyclo w6c	11:0 iso 3OH
	15:1 w7c	17:0 cyclo w7c	20:1 w4c	14:0 iso 3OH
	15:1 w6c	18:1 w8c	20:0 cyclo w6c	
	15:1 w5c	18:1 w7c	21:1 w9c	
Methanotroph	16:1 w8c			
Eukaryote	15:4 w3c	19:3 w3c	22:5 w6c	23:3 w3c
	15:3 w3c	20:4 w6c	22:6 w3c	23:1 w5c
	16:4 w3c	20:5 w3c	22:4 w6c	23:1 w4c
	16:3 w6c	20:3 w6c	22:5 w3c	24:4 w6c
	18:3 w6c	20:2 w6c	22:2 w6c	24:3 w6c
	19:4 w6c	21:3 w6c	23:4 w6c	24:3 w3c
	19:3 w6c	21:3 w3c	23:3 w6c	24:1 w3c
Gram Positive	11:0 iso	14:0 iso	16:0 iso	17:1 anteiso w7c
	11:0 anteiso	14:0 anteiso	16:0 anteiso	19:0 iso
	12:0 iso	15:1 iso w9c	17:1 iso w9c	19:0 anteiso
	12:0 anteiso	15:1 iso w6c	17:0 iso	20:0 iso
	13:0 iso	15:1 anteiso w9c	17:0 anteiso	22:0 iso
	13:0 anteiso	15:0 iso	18:0 iso	
	14:1 iso w7c	15:0 anteiso	17:1 anteiso w9c	
Anaerobe	12:0 DMA	15:0 DMA	16:1 w5c DMA	18:1 w7c DMA
	13:0 DMA	16:2 DMA	16:0 DMA	18:1 w5c DMA
	14:1 w7c DMA	17:0 DMA	18:2 DMA	18:0 DMA
		16:1 w9c DMA	18:1 w9c DMA	
	14:0 DMA	16:1 w7c DMA		
	15:0 iso DMA			
Actinomycetes	16:0 10-methyl	18:1 w7c 10-methyl	22:0 10- methyl	
	17:1 w7c 10-methyl	18:0 10-methyl	20:0 10- methyl	
	17:0 10-methyl	19:1 w7c 10-methyl		

Table S2. List of the primers used to target each community.

Target gene	Primer	Sequence 5'-3'	References
Bacterial *16SrRNA*	341F	CCT AYG GGR BGC ASC AG	Glarling et al. (2015)
	806R	GGA CTA CNN GGG TAT CTA AT	
Archaeal *16SrRNA*	Parch519F	CAG CMG CCG CGG TAA	Øvreaset al. (1997)
	Arch1060R	GGC CAT GCA CCW CCT CTC	Reysenbach and Pace, (1995)
Fungal *ITS*	ITS1f	TCC GTA GGT GAA CCT GCG G	Gardes and Bruns (1993); Vilgalys and Hester (1990)
	5.8s	CGC TGC GTT CTT CAT CG	
nifH	PolF	TGC GAY CCS AAR GCB GAC TC	Poly et al. (2001)
	PolR	ATS GCC ATC ATY TCR CCG GA	
amoA Bacteria	amoA-1F	GGG GTT TCT ACT GGT GGT	Rotthauwe et al. (1997)
	amoA-2R	CCC CTC KGS AAA GCC TTC TTC	
amoA Archaea	Arch-amoAF	STA ATG GTC TGG CTT AGA CG	Francis et al. (2005)
	Arch-amoAR	GCG GCC ATC CAT CTG TAT GT	
nirK	nirK876F	ATY GGC GGV CAY GGC GA	Henry et al. (2004)
	nirK1040R	GCC TCG ATC AGR TTR TGG TT	
nirS	cd3aF	GTS AAC GTS AAG GAR ACS GG	Throbäck et al. (2004)
	R3cdR	GAS TTC GGR TGS GTC TTG A	
nosZ	nosZ1F	CGC RAC GGC AAS AAG GTS MSS GT	Henry et al. (2006)
	nosZ1R	CAK RTG CAK SGC RTG GCA GAA	
nosZII	nosZ-II-F	CTI GGI CCI YTK CAY AC	Jones et. al (2013)
	nosZ-II-R	GCI GAR CAR AAI TCB GTR C	

Table S3. Phospholipid fatty acid (PLFA) ratios of main microbial groups. Values represent means ± SEM (n = 4). Same lower case letters indicate no significant differences (P > 0.05) with respect to distance from the river according to one-way ANOVA and the Tukey post-hoc test.

PLFA ratio	Zone 1		Zone 2	Zone 3	
	2 m	5 m	10 m	15 m	25 m
Fungi/Bacteria	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.04 ± 0.004^{b}	0.03 ± 0.005^{b}	0.04 ± 0.006^{b}
Predator/Prey	0.03 ± 0.003^{a}	0.03 ± 0.001^{a}	0.02 ± 0.004^{a}	0.03 ± 0.004^{a}	0.03 ± 0.005^{a}
Gram +/Gram -	0.76 ± 0.03^{ab}	0.83 ± 0.04^{b}	0.88 ± 0.10^{b}	0.56 ± 0.01^{c}	0.61 ± 0.03^{ac}
Saturated/Unsaturated	1.01 ± 0.09^{a}	1.05 ± 0.10^{a}	1.38 ± 0.26^{a}	0.74 ± 0.04^{a}	0.88 ± 0.18^{a}
Mono/Poly	13.5 ± 1.18^{a}	14.4 ± 2.68^{a}	15.9 ± 1.76^{a}	17.6 ± 1.50^{a}	16.6 ± 2.44^{a}
16w/16 cyclo	3.31 ± 0.19^{a}	3.89 ± 0.23^{a}	4.11 ± 0.53^{a}	9.65 ± 0.54^{b}	9.20 ± 0.69^{b}
18w/19 cyclo	$0.70 \quad 0.07^{a}$	$0.64 \quad 0.04^{a}$	$0.85 \quad 0.11^{a}$	$1.93 \quad 0.08^{b}$	$2.06 \quad 0.25^{b}$

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Appendix 5

Supplementary material for Chapter 7

Riparian research and legislation, are they working towards the same common goals? A UK case study

Laura L. de Sosa, A. Prysor Williams, Harriet G. Orr, Davey L. Jones

Table S1. Refined search according to engine advanced search options

Search engine	**Refined search**	**Number of papers from the search**
Web of Science	Full text: Riparian, buffer strip, UK Years: 1997-2017	162
Science Direct	All fields: Riparian Abstract, keywords, abstract: UK Years: 1997-2017	230
Jstor	Abstract: Riparian Full text: UK Years: 1997-2017	434

Table S2. Broad Habitat types used in this study for the classification of the land cover in which different publications have based their research in. The UK Biodiversity Action Plan specified the subcategories included within each Broad Habitat.

UK NEA Broad Habitat	UK Biodiversity Action Plan (BAP)[1]	Land use	Reference for description
Enclosed farmland	Arable and Horticultural Improved Grassland	Crops and grazing	Firbank et al. (2011)
Mountains, Moorlands and Heaths	Bracken Dwarf Shrub Heath Bog Fen, Marsh and Swamp Montane; and Inland Rock	Light grazing	Van der Wal et al. (2011)
Semi-natural Grassland	Acid, Neutral and Calcareous Grassland Purple Moor-grass and Rush Pastures	Grazing	Bullock et al. (2011)
Woodland	Coniferous woodland Broadleaved mixed and yew woodland	Forestry and grazing	Quine et al. (2011)
Urban	Built-up areas and gardens	Recreational	Davies et al. (2011)

[1] Jackson, 2000

Table S3. Compilation of studies on riparian areas within 'Biodiversity' category.

Sub-category	Article titles
Ecology	Macroinvertebrate community composition and diversity in ephemeral and perennial ponds on unregulated floodplain meadows in the UK (2017)
	Riparian buffer zones in intensive grassland Agri-systems are not necessarily a refuge for high conservation value species (2015)
	Species turnover and geographic distance in an urban river network (2013)
	Metapopulation dynamics of a burrowing herbivore drive spatio-temporal dynamics of riparian plant communities (2013)
	Novel management to enhance spider biodiversity in existing grass buffer strips (2013)
	Riparian field margins: Their potential to enhance biodiversity in intensively managed grasslands (2012)
	Making agricultural landscapes more sustainable for freshwater biodiversity: A case study from southern England (2011)
	Benefits of habitat restoration to small mammal diversity and abundance in a pastoral agricultural landscape in mid-Wales (2007)
	Livestock trampling reduces the conservation value of beetle communities on high quality exposed riverine sediments (2007)
	Where new farm woodlands support biodiversity action plans: A spatial multi-criteria analysis (2005)
	The hydro-ecological controls and conservation value of beetles on exposed riverine sediments in England and Wales (2004)
	Dispersal of adult aquatic insects in catchments of differing land use (2004)
	Waterways Bird Survey: evaluation of population monitoring and appraisal of future requirements (1997)
Vegetation	The role of riparian vegetation density, channel orientation and water velocity in determining river temperature dynamics (2017)
	Vegetation-hydrogeomorphology interactions in a low-energy, human-impacted river (2016)
	Phenological responses of ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*) to riparian thermal conditions (2016)
	n-Alkane biosynthetic hydrogen isotope fractionation is not constant throughout the growing season in the riparian tree *Salix viminalis* (2015)
	The early impact of large wood introduction on the morphology and sediment characteristics of a lowland river (2015)
	Genetic diversity, population structure and phenotypic variation in European *Salix viminalis* L. (Salicaceae) (2014)
	Instream and riparian implications of weed cutting in a chalk river (2014)
	Impacts of an invasive non-native annual weed, *Impatiens glandulifera*, on above- and below-ground invertebrate communities in the United Kingdom (2013)
	The impact of two non-native plant species on native flora performance: Potential implications for habitat restoration (2013)
	An investigation of the composition of the urban riparian soil propagule bank along the River Brent, Greater London, UK, in comparison with previous propagule bank studies in rural areas (2012)
	Changing river channels: The roles of hydrological processes, plants and pioneer fluvial landforms in humid temperate, mixed load, gravel bed rivers (2012)
	Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs (2011)
	Hydrological controls on the transport and deposition of plant propagules within riparian zones (2010)
	Propagule input, transport and deposition in riparian environments: The importance of connectivity for diversity (2009)
	Population genetics of an invasive riparian species, *Impatiens glandulifera* (2009)
	Plants intertwine fluvial landform dynamics with ecological succession and natural selection: A niche construction perspective for riparian systems (2009)

Sub-category	**Article titles**
Vegetation	Assessing the vulnerability of riparian vegetation to invasion by *Mimulus guttatus*: Relative (2008)
	Consequences of invasion by the alien plant Mimulus guttatus on the species composition and soil properties of riparian plant communities in Scotland (2008)
	Three seedling emergence methods in soil seed bank studies: Implications for interpretation of propagule deposition in riparian zones (2007)
	The river-bed: A dynamic store for plant propagules? (2007)
	Impacts of invasive plant species on riparian plant assemblages: Interactions with elevated atmospheric carbon dioxide and nitrogen deposition (2007)
	The distribution and habitat associations of non-native plant species in urban riparian habitats (2006)
	Assessing the impact of *Impatiens glandulifera* on riparian habitats: Partitioning diversity components following species removal (2006)
	Riparian forestry management and adult stream insects (2004)
	The response of macroinvertebrates to artificially enhanced detritus levels in plantation streams (2004)
	Dynamics and management of plant communities in ditches bordering arable fenland in eastern England (2004)
	Interactive effects of soil moisture, vegetation canopy, plant litter and seed addition on plant diversity in a wetland community (2003)
	Evidence for hydrochory and the deposition of viable seeds within winter flow-deposited sediments: The River Dove, Derbyshire, UK (2003)
	Riparian seed banks along the lower River Dove, UK: Their structure and ecological implications (2002)
	Hydrology as an influence on invasion: Experimental investigations into competition between the alien *Impatiens glandulifera* and the native *Urtica dioica* in the UK (2001)
	Simulating the spread and management of alien riparian weeds: Are they out of control? (2000)
	Effects of late summer cattle grazing on the diversity of riparian pasture vegetation in an upland conifer forest (2000)
	Predicting the spatial distribution of non-indigenous riparian weeds: Issues of spatial scale and extent (2000)
	The Distribution and abundance of riparian trees in English lowland floodplains (1997)

Table S4. Compilation of studies on riparian areas within 'Nutrients and water quality' category.

Sub-category	**Article titles**
Nonpoint diffuse pollution	Impacts of phosphorus concentration and light intensity on river periphyton biomass and community structure (2017)
	Scaling effects of riparian peatlands on stable isotopes in runoff and DOC mobilisation (2017)
	A tool for cost-effectiveness analysis of field scale sediment-bound phosphorus mitigation measures and application to analysis of spatial and temporal targeting in the Lunan Water catchment, Scotland (2017)
	Sensitive areas, vulnerable zones and buffer strips: A critical review of policy in agricultural nitrate control (2017)
	Analysis of fundamental physical factors influencing channel bank erosion: Results for contrasting catchments in England and Wales (2017)
	Diagnosing problems of fine sediment delivery and transfer in a lowland catchment (2016)
	Identifying multiple stressor controls on phytoplankton dynamics in the River Thames (UK) using high-frequency water quality data (2016)
	The role of large wood in retaining fine sediment, organic matter and plant propagules in a small, single-thread forest river (2015)
	Modelling the impacts of agricultural management practices on river water quality in Eastern England (2015)
	Costs and benefits of erosion control measures in the UK (2015)
	The Glasgow consensus on the delineation between pesticide emission inventory and impact assessment for LCA (2015)
	Nested monitoring approaches to delineate groundwater trichloroethene discharge to a UK lowland stream at multiple spatial scales
	Temporal dynamics between cattle in-stream presence and suspended solids in a headwater catchment (2014)
	Enhancing soluble phosphorus removal within buffer strips using industrial by-products (2014)
	Microbial biomass phosphorus contributions to phosphorus solubility in riparian vegetated buffer strip soils (2013)
	A framework for managing runoff and pollution in the rural landscape using a catchment systems engineering approach (2012)
	Gradients in the biophysical structure of urban rivers and their association with river channel engineering (2012)
	Temperature-driven river utilisation and preferential defecation by cattle in an English chalk stream (2012)
	Water quality targets and maintenance of valued landscape character - Experience in the Axe catchment, UK (2012)
	Which offers more scope to suppress river phytoplankton blooms: Reducing nutrient pollution or riparian shading? (2010)
	A preliminary investigation of the efficacy of riparian fencing schemes for reducing contributions from eroding channel banks to the siltation of salmonid spawning gravels across the south west UK (2010)
	Impeded drainage stimulates extracellular phenol oxidase activity in riparian peat cores (2008)
	Stream water chemistry and quality along an upland-lowland rural land-use continuum, south west England (2008)
	Sourcing, transport and control of phosphorus loss in two English headwater catchments (2007)

Sub-category	Article titles
Nonpoint diffuse pollution	Seasonal variations in decomposition processes in a valley-bottom riparian peatland (2006)
	The water quality of the River Dun and the Kennet and Avon Canal (2006)
	The exchange of phosphorus between riparian wetland sediments, pore water and surface water (2005)
	Overland flow transport of pathogens from agricultural land receiving faecal wastes (2003)
	Regulation of surface water quality in a Cretaceous Chalk catchment, UK: An assessment of the relative importance of instream and wetland processes (2002)
	Sediment deposition along the channel margins of a reach of the middle River Severn, UK (2001)
	The role of forest management in controlling diffuse pollution in UK forestry (2001)
	Mitigation options for diffuse phosphorus loss to wáter(1998)
Buffer strips	Riparian buffer strips: Their role in the conservation of insect pollinators in intensive grassland systems (2015)
	Riparian buffer hydrology: Representing catchment-wide implementation and the influence on flood risk (2015)
	Riparian buffer strips as a multifunctional management tool in agricultural landscapes: Introduction (2012)
	Vegetated buffer strips can lead to increased release of phosphorus to waters: A biogeochemical assessment of the mechanisms (2009)
	Evaluation of contrasting buffer features within an agricultural landscape for reducing sediment and sediment-associated phosphorus delivery to surface waters (2007)
	Field-based evaluation tool for riparian buffer zones in agricultural catchments (2003)
	The use of conventionally and alternatively located buffer zones for the removal of nitrate from diffuse agricultural run-off (1999)
	Grassed buffer strips for the control of nitrate leaching to surface waters in headwater catchments (1998)
	Nitrogen and phosphorus in runoff from grassland with buffer strips following application of fertilizers and manures (1998)
Denitrification	Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in a temperate re-connected floodplain (2011)
	Hydrological controls on denitrification in riparian ecosystems (2004)
	Wetland nutrient removal: A review of the evidence (2004)
	Water table elevation controls on soil nitrogen cycling in riparian wetlands along a European climatic gradient (2004)
	Denitrification in riparian buffer zones: The role of floodplain hydrology (1999)
Shading	Riparian shading controls instream spring phytoplankton and benthic algal growth (2016)
	Seeing the landscape for the trees: Metrics to guide riparian shade management in river catchments (2015)
	16S rRNA assessment of the influence of shading on early-successional biofilms in experimental streams (2015)
	What impact might mitigation of diffuse nitrate pollution have on river water quality in a rural catchment? (2012)
	Nutrient and light limitation of periphyton in the River Thames: Implications for catchment management (2012)
	The influence of riparian shade on lowland stream water temperatures in southern England and their viability for brown trout (2010)

Table S5. Compilation of studies on riparian areas within 'Water dynamics and modelling' category.

Sub-category	**Article titles**
Hydrology	Long-term Holocene groundwater fluctuations in a chalk catchment: Evidence from Rock-Eval pyrolysis of riparian peats (2016)
	Derivation of lowland riparian wetland deposit architecture using geophysical image analysis and interface detection (2014)
	Stormflow hydrochemistry of a river draining an abandoned metal mine: The Afon Twymyn, central Wales (2013)
	Sustainable surface water management and green infrastructure in UK urban catchment planning (2013)
	Channel bar dynamics on multi-decadal timescales in an active meandering river (2011)
	Revealing the temporal dynamics of subsurface temperature in a wetland using time-lapse geophysics (2011)
	Interaction between groundwater, the hyporheic zone and a Chalk stream: A case study from the River Lambourn, UK (2010)
	Controls on the spatial and temporal variability of Rn-222 in riparian groundwater in a lowland Chalk catchment (2009)
	Understanding groundwater, surface water, and hyporheic zone biogeochemical processes in a Chalk catchment using fluorescence properties of dissolved and colloidal organic matter (2009)
	Near-stream soil water-groundwater coupling in the headwaters of the Afon Hafren, Wales: Implications for surface water quality (2006)
	Evidence for deep sub-surface flow routing in forested upland Wales: Implications for contaminant transport and stream flow generation (2004)
	Water table fluctuations within the floodplain of the River Severn, England (2002)
	Water table fluctuations in the riparian zone: comparative results from a pan-European experiment (2002)
	A perspective on the abiotic processes sustaining the ecological integrity of running waters (2000)
	Bench development along the regulated, lower River Dee, UK (1999)
	The characteristics of overbank deposits associated with a major flood event in the catchment of the River Ouse, Yorkshire, UK (1998)
Modelling	Incorporating catchment to reach scale processes into hydromorphological assessment in the UK (2016)
	Modelling groundwater/surface water interaction in a managed riparian chalk valley wetland (2016)
	Modelling the impacts of agricultural management practices on river water quality in Eastern England (2016)
	Indicators of river system hydromorphological character and dynamics: Understanding current conditions and guiding sustainable river management (2016)
	Operationalizing an ecosystem services-based approach using Bayesian (2015)
	Discrete wetland groundwater discharges revealed with a three-dimensional temperature model and botanical indicators (Boxford, UK) (2015)
	An improved Cauchy number approach for predicting the drag and reconfiguration of flexible vegetation (2015)
	Spatial variability of suspended sediment yield in a gravel-bed river across four orders of magnitude of catchment area (2015)
	The role of large wood in retaining fine sediment, organic matter and plant propagules in a small, single-thread forest river (2015)
	Natural Flood Management in the UK: Developing a Conceptual Management Tool (2013)

Sub-category	**Article titles**
Modelling	The Growing Hierarchical Self-Organizing Map (GHSOM) for analysing multi-dimensional stream habitat datasets (2009)
	Characterisation of river reaches: The influence of rock type (2008)
	Towards simple approaches for mean residence time estimation in ungauged basins using tracers and soil distributions (2008)
	Initial adjustments within a new river channel: Interactions between fluvial processes, colonizing vegetation, and bank profile development (2006)
	Using factor analysis and end-member mixing techniques to infer sources of runoff generation (2006(
	Riparian zone influence on stream water chemistry at different spatial scales: a GIS-based modelling approach, an example for the Dee, NE Scotland (2001)

Table S6. Compilation of studies on riparian areas within 'Future outlook and impacts' category.

Sub-category	Article titles
Land use and restoration	Coupled hydrological/hydraulic modelling of river restoration impacts and floodplain hydrodynamics (2016)
	Squeezed out: The consequences of riparian zone modification for specialist invertebrates (2016)
	The early impact of large wood introduction on the morphology and sediment characteristics of a lowland river (2015)
	Making sense of landscape change: Long-term perceptions among local residents following river restoration (2014)
	Linking the restoration of rivers and riparian zones/wetlands in Europe: Sharing knowledge through case studies (2013)
	Runoff attenuation features: A sustainable flood mitigation strategy in the Belford catchment, UK (2012)
	Riparian zone creation in established coniferous forests in Irish upland peat catchments: Physical, chemical and biological implications (2011)
	Appraising riparian management effects on benthic macroinvertebrates in the Wye River system (2010)
	Evaluating the effects of riparian restoration on a temperate river-system using standardized habitat survey (2010)
	Management approaches to floodplain restoration and stakeholder engagement in the UK: A survey (2008)
	The effect of instream rehabilitation structures on macroinvertebrates in lowland rivers (2004)
	Dispersal of adult aquatic insects in catchments of differing land use (2004)
	An investigation of marginal habitat and macrophyte community enhancement on the River Torne, UK (2000)
	A catchment-scale approach to the physical restoration of lowland UK rivers (1999)
	The role of habitat enhancement in the return of the European otter (Lutra lutra) to Northumberland (1999)
	Land-use history and sediment flux in a lowland lake catchment: Groby Pool, Leicestershire, UK (1998)
Habitat and river survey	Stories from the English riverbank: How riparian communities interpret, articulate and action water resource sustainability (2016)
	The challenges and implications of linking wetland science to policy in agricultural landscapes - experience from the UK National Ecosystem Assessment (2013)
	Optimizing agri-environment schemes to improve river health and conservation value (2013)
	Habitat indices for rivers: Derivation and applications (2010)
	Preliminary testing of River Habitat Survey features for the aims of the WFD hydro-morphological assessment: An overview from the STAR Project (2006)
	Habitat survey and classification of urban rivers (2004)
	Why should the habitat-level approach underpin holistic river survey and management? (1998)
	Quality assessment using River Habitat Survey data (1998)
	A system for evaluating rivers for conservation (SERCON): Development, structure and function (1997)
Climate change	Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms (2016)
	Beyond cool: Adapting upland streams for climate change using riparian woodlands (2016)

Sub-category	**Article titles**
Climate change	Projecting impacts of climate change on hydrological conditions and biotic responses in a chalk valley riparian wetland (2016)
	Temperature response of denitrification rate and greenhouse gas production in agricultural river marginal wetland soils (2013)
	Nocturnal river water temperatures: Spatial and temporal variations (2013)
	Evidence needed to manage freshwater ecosystems in a changing climate: Turning adaptation principles into practice (2010)
	Microthermal gradients and ecological implications in Dorset rivers (1999)

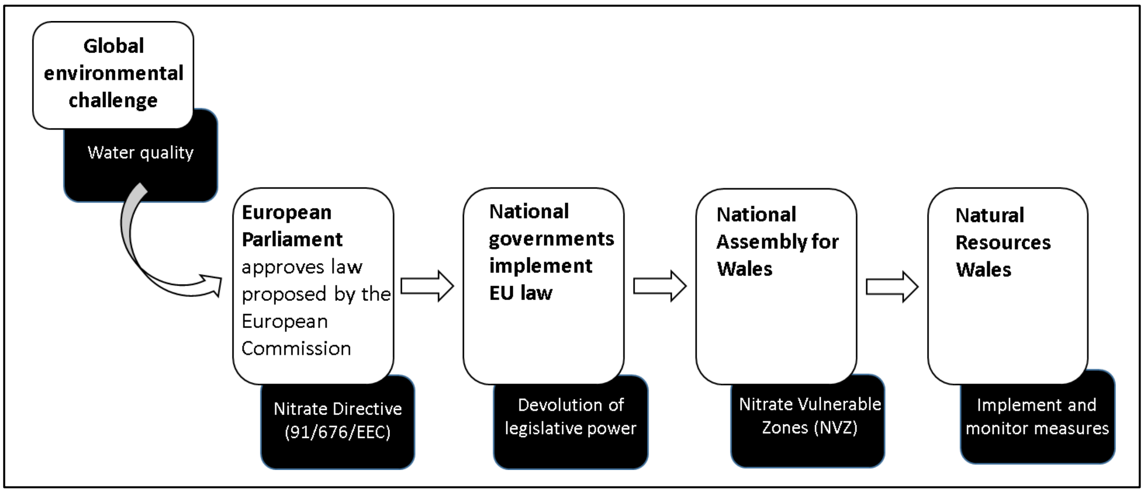

Figure S1. A flow-chart of the legislative procedure in the UK and devolution of legislative power to Wales.

Glossary

Cross-compliance: It is a mechanism that links direct payments to compliance by farmers with basic standards concerning the environment, food safety, animal and plant health and animal welfare, as well as the requirement of maintaining land in good agricultural and environmental condition.

Directive: A "directive" is a legislative act that sets out a goal that all EU countries must achieve. However, it is up to the individual countries to devise their own laws on how to reach these goals.

Regulation: A "regulation" is a binding legislative act. It must be applied in its entirety across the EU.

Act: An Act is a Bill that has been approved by both the House of Commons and the House of Lords and been given Royal Assent by the Monarch.

Primary UK legislation: Acts of parliament or status which have been approved by the House of Commons, the House of Lords (legislative branch of government) and granted by Royal Assent. The principal examples are:

- Acts of the UK Parliament
- Acts of the pre-UK Parliaments
- Acts of the Scottish Parliament
- Measures of the National Assembly for Wales
- Acts of the National Assembly for Wales
- Acts of the Northern Ireland Assembly (and other primary legislation for Northern Ireland)
- Church of England Measures (legislation for the established church in England passed by the General Synod of the Church of England)

Secondary UK legislation (also called "subordinate legislation"): It is delegated legislation made by a person or body under authority contained in primary legislation. The main types of secondary legislation are:

- Statutory Instruments
- Scottish Statutory Instruments
- Welsh Statutory Instruments
- Statutory Rules of Northern Ireland
- Church Instruments
- Bye-laws

Bill: Proposal for a new law, or a proposal to change an existing law that is presented for debate before Parliament.

Common law: Legal system made from the precedent judge-made law in courts.

Treaty: A formal agreement or contract between two or more states, such as an alliance or trade arrangement

Basic payment scheme: Direct subsidy payments to landowners meeting environmental, public, animal and plant health and animal welfare standards together with maintaining land in good agricultural and environmental condition.

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