

Chapter 1: General Introduction

1.1 Introduction

The biogeochemical cycling of key elements within ecosystems is comprised of a complex array of abiotic and biotic processes. The stoichiometry or the mass-balance of elements is a measure of their total mass present within an ecosystem. Ecological stoichiometry theory recognizes that the ratio of ecosystem elements controls the rates of the biotic ecological processes which recycle these elements (Sterner & Elser 2002, Cross et al. 2005). However, the rates at which these ecological processes occur can also be influenced by many other abiotic parameters and processes.

At the basal level of aquatic systems, autotrophic and heterotrophic communities are responsible for some of the main biotic processes that control the cycling of the key ecosystem elements, carbon (C), nitrogen (N) and phosphorus (P) (Sterner & Elser 2002, Cross et al. 2005). Photosynthesis is the dominant process that produces carbon, while autotrophic respiration or the heterotrophic decomposition of organic matter are the main pathways for carbon utilisation in aquatic systems. As both of these processes require N and P, the concentrations at which they are available to consumers will control the rate at which these elements are retained and recycled within the system (Sterner & Elser 2002, Cross et al. 2005).

In lotic systems, the cycling of key elements is also controlled through abiotic ecosystem processes and their interaction with biotic processes (Brookes et al. 2005). The main abiotic factors that can affect the biotic process of autotrophic production in streams are light (Bunn et al. 1999, Bott et al. 2006), substrate (Cardinale et al. 2002, Atkinson et al. 2008), and hydrology (Uehlinger 2000, Acuña et al. 2004). In contrast, rates of heterotrophic decomposition and respiration are influenced by the quantity and chemical composition or quality of organic matter (Aldridge et al. 2009, Hladyz et al. 2009, Gibson & O'Reilly 2012).

The quantity of organic matter present within streams is controlled through the balance of import, retention, breakdown and export processes (Webster et al. 1999, Lepori et al. 2005). Riparian vegetation has been identified as a major source of allochthonous organic matter

contributing leaves, bark and woody fractions that are transported laterally to streams (Webster & Meyer 1997, Reid et al. 2008, França et al. 2009). Stream reaches can also receive allochthonous organic matter from upstream areas via material that is transported longitudinally when surface water is present (Gurtz et al. 1988, Boulton & Lake 1992). Organic matter that enters stream reaches through longitudinal or lateral links will be retained through abiotic processes associated with channel geomorphology and hydrology (Lepori et al. 2005, Quinn et al. 2007). The rate of organic matter breakdown through abiotic (i.e. physical fragmentation) and biotic (i.e. heterotrophic decomposition) processes will also control the quantity of organic matter retained in reaches (Webster et al. 1999). These processes can in turn be related to the quality of the retained organic matter, which is largely determined from its source (Findlay & Sinsabaugh 1999). High quality low-molecular weight organic matter is characterised as having low lignin:cellulose (LCI) ratios and is generally from autochthonous sources, while low quality high-molecular weight organic matter has high lignin:cellulose ratios and originates from allochthonous sources (Melillo et al. 1992, Gessner & Chauvet 1994, Royer & Minshall 2001). The quantity and quality of organic matter can play an important role in providing habitat and energy for invertebrates, fish and heterotrophic communities (Erhman & Lamberti 1992, Jones 1997), which subsequently affect the retention and cycling of nutrients within reaches (Aldridge et al. 2009, Gibson & O'Reilly 2012).

Channel geomorphology is controlled through a combination of local and catchment factors including riparian vegetation, hydrological regime, climate and geology (Gregory et al. 1991, Stoffel & Wilford 2012). In upland streams, channel geomorphology is generally considered to be complex with steep gradients and distinct riffle, run and pool areas. Channels are usually constrained by bedrock that can be exposed in large areas. In comparison to lowland streams, the channels are narrower and are not connected to wide floodplains, so the sources of organic matter to channels are predominantly from adjacent and surrounding vegetation (Gregory et al. 1991, Campbell et al. 1992). Upland streams are also characterised by having lower discharges and higher surface water velocities in comparison to lowland streams (Beschta & Platts 1986). In some catchments, the hydrological regime in upland streams is temporally variable ranging from floods to cease-to-flow events depending on the timing and intensity of rainfall (Boulton

& Lake 1992, Acuña et al. 2004). The high-surface water velocities are important for the import, retention, and export of nutrients and organic matter within reaches during periods of connectivity (Boulton & Lake 1992, Harms & Grimm 2010, Stewart & Ryder 2012 - see Appendix 1). During cease-to-flow events, the complex geomorphology of reaches can cause surface water to fragment into disconnected pools, with stream energetics in pools relying on retained organic material (Ylla et al. 2010).

Knowledge of the abiotic and biotic processes that control the biogeochemical cycling of key elements is important for understanding the functioning of stream ecosystems. These abiotic and biotic processes are strongly linked to organic matter dynamics (Cross et al. 2005), which can interact with channel geomorphology and hydrological parameters (Tzoraki et al. 2007). Therefore, exploring the interaction among organic matter and nutrient dynamics, geomorphology and hydrology will offer further understanding of the drivers of stream function in intermittent upland streams.

1.2 Anthropogenic effects to upland streams

Within agricultural landscapes, land-use management practices have led to the widespread removal of riparian vegetation along upland streams (Robertson et al. 1999, Allan 2004). In combination with other anthropogenic influences including the application of fertiliser, the removal of large woody debris from channels, planting of exotic species of vegetation, and the alteration to flow regimes, these impacts have cumulatively led to major impacts on biogeochemical processes within these systems (Sabater et al. 2000, von Schiller et al. 2007, Hadwen et al. 2010). Over the last twenty years, there has been an improved effort to restore biogeochemical processes and/or stream functions through a range of biophysical restoration techniques (Lake et al. 2007, Bernhardt & Palmer 2011). These management interventions commonly focus on the replanting of streambank and riparian vegetation at reach-scales, as this is the spatial scale at which effective on-ground restoration activities can be implemented (Mika et al. 2010, Bernhardt & Palmer 2011). However, there has been little research into whether these efforts to restore the structural complexity of riparian habitats have been

successful in restoring biogeochemical processes in adjacent streams (Mika et al. 2010, Bernhardt & Palmer 2011).

1.3 Riparian vegetation

Riparian zones represent the interface between terrestrial and aquatic ecosystems. Spatially, the riparian zone encompasses the area that runs laterally from the main stream channel to the wetted area defined during flood events, and horizontally from the soil subsurface to the height of the streamside vegetation (Gregory et al. 1991). The vegetation present in the riparian zone can be compositionally and structurally diverse ranging from herbs, to shrubs and larger trees. The abundance and composition of riparian vegetation is governed by both spatial and temporal processes including; the local climate, soil and topographic conditions, and the recovery from disturbances such as floods, fire, storms/wind, and plant diseases (Gregory et al. 1991, Stoffel & Wilford 2012).

Riparian vegetation plays several important functions in stream biogeochemical processes. In particular, vegetation is an important source of allochthonous organic matter and nutrients to aquatic ecosystems (Fisher & Likens 1973, Cummins 1974). Coarse, fine and dissolved fractions of organic matter are an essential food resource and habitat for aquatic heterotrophic and autotrophic communities, and for higher trophic levels including invertebrates and fish (Fisher & Likens 1973, Cummins 1974, Erhman & Lamberti 1992). The rate of organic matter processing and utilisation by aquatic biota can facilitate the longitudinal cycling of nutrients and the stoichiometric balance of nutrients within reaches (Newbold et al. 1981, Cross et al. 2005). In particular, aquatic heterotrophic communities including bacteria and fungi actively take up nutrients (predominantly N and P) from the water column in order to decompose organic matter and acquire C for energy and growth (Bärlocher et al. 2011, Bärlocher et al. 2012 - see Appendix 1). Organic matter that has a high C:N:P (also known as LCI – lignin:cellulose index) will lead to an increase in uptake of N and P from the water column by heterotrophs (Aldridge et al. 2009, Gibson & O'Reilly 2012). The C:nutrient stoichiometry (C:N:P) of organic matter is dependent on its source and state of decomposition, therefore organic matter from different species of riparian vegetation will have varying C:nutrient stoichiometry (Esslemont et al. 2007).

In Australia, deciduous exotic species of riparian vegetation such as *Salix babylonica* have replaced evergreen native species in many riparian zones that were previously cleared (Schulze & Walker 1997, Miller & Boulton 2005). The leaves of these exotic species are of a high-quality with low C:nutrient and lignin:cellulose ratios compared to locally-endemic native riparian species such as *Casuarina cunninghamiana* and *Eucalyptus spp.*, which are considered low-quality with high C:nutrient and lignin:cellulose ratios (Esslemont et al. 2007). As heterotrophic communities can decompose high-quality organic matter at a faster rate and only require low quantities of N and P for this process, the rate at which nutrients are removed from the water column is reduced and their spiraling lengths become longer (Gibson & O'Reilly 2012). This leads to longitudinal nutrient spiraling processes that are referred to as 'leaky' (Newbold et al. 1981), due to poor retention of allochthonous organic matter within stream reaches. The stream length at which nutrients are taken-up and retained within streams is a measure of how retentive the system is and will be reflected in the mass-balance of elements. Generally, shorter spiraling lengths and greater retention ability are associated with streams that have complex abiotic and biotic processes (Brookes et al. 2005).

Trees that grow in the riparian zone can also provide shade over the stream channel (Gregory et al. 1991). Subsequently, the removal of these trees can lead to increased light or photosynthetic active radiation (PAR) reaching the stream channel (Bunn et al. 1999, Fellows et al. 2006a). As autotrophs are reliant on PAR for photosynthetic processes, the increase in PAR penetrating the water column can enhance autochthonous photosynthetic production and therefore, the quantity of high-quality dissolved organic matter (DOM) (McTammany et al. 2007, Julian et al. 2008). However, increases in PAR can also lower the quality of dissolved organic matter through photochemically transforming autochthonous produced DOC to recalcitrant products (Wetzel et al. 1995, Tranvik & Bertilsson 2001). The changes to the quantity of high-quality organic matter available to heterotrophic communities may lead to reduced rates of respiration and uptake of nutrients from the water column (Aldridge et al. 2009, Gibson & O'Reilly 2012), essentially increasing nutrient spiralling lengths in streams. However, as photosynthetic processes by autotrophs also require nutrients, the effects of increased photosynthesis on C:nutrient stoichiometry (also referred to as C:nutrient ratios) may

counteract the reduced rates of respiration (Bernhardt et al. 2003). Similarly, the increased biomass and production of submerged and emergent macrophytes is also a consequence of increased PAR reaching stream channels (Bunn et al. 1998, Julian et al. 2010), while the combination of increased PAR (Rier & Stevenson 2002) and the presence of stable substrates such as bedrock and logs can promote the metabolic activity of biofilms (Ryder et al. 2004, Atkinson et al. 2008). The increase in rates of macrophyte production and biofilm productivity and respiration may also lead to increased uptake and retention of nutrients (Ryder et al. 2006, Julian et al. 2010). In addition, macrophytes (Horvath 2004, Quinn et al. 2007) and biofilms (Fischer 2003) have been shown to physically entrain particulate and dissolved organic matter, respectively, which may lead to increased heterotrophic respiration within reaches.

Streams that are considered geomorphically complex will have spatially heterogeneous features such as pools, riffles, exposed bedrock, logs and flood debris accumulation points (also known as log jams) within channels (Brookes et al. 2005). Channel morphology and geomorphic features can influence the longitudinal connectivity of water in streams as well as the lateral and vertical connections with riparian and hyporheic zones, respectively (Gregory et al. 1991, Boulton 2007). These geomorphic features can be considered as interception pathways for the retention of organic matter and uptake of nutrients (Brookes et al. 2005). Features that protrude through the water column such as logs and flood debris accumulation points are pivotal in trapping and retaining particulate allochthonous organic matter (Moutka & Laasonen 2002, Quinn et al. 2007). Stream channels that are geomorphically complex can therefore enhance the number of interception pathways within reaches, which may increase organic matter and nutrient retention (Brookes et al. 2005).

At the whole-reach scale, the enhanced retention of allochthonous organic matter can lead to increased surface water C:nutrient ratios. Heterotrophic decomposition of coarse and fine fractions of organic matter produces DOM that is released into the water column (Gessner et al. 1999, Webster et al. 1999). Other abiotic processes such as leaching and physical fragmentation can also facilitate the release of DOM to surface water (Webster et al. 1999). Therefore, reaches that have enhanced geomorphic complexity and/or increased macrophyte

and biofilm biomass will be able to retain larger masses of organic matter (Brookes et al. 2005, James & Henderson 2005), which may increase C:nutrient ratios at whole-reach scales.

The removal of riparian vegetation has been shown to reduce reach geomorphic complexity and led to a decrease in the retention of organic matter and therefore the quantity within reaches of upland streams. For example, reaches that lack riparian vegetation may have fewer logs and other large woody debris accumulations (Quinn et al. 1997, Lester & Boulton 2008) that are pivotal in retaining organic matter (Brookshire & Dwire 2003, Quinn et al. 2007). The removal of riparian vegetation has also shown to destabilise and erode banks that can smother protruding instream features reducing their ability to retain organic matter (Pusey & Arthington 2003, Allan 2004). Subsequently, alteration to these physical conditions may reduce organic matter loads being retained within reaches (Brookshire & Dwire 2003, James & Henderson 2005). A reduction in organic matter within stream reaches may decrease rates of heterotrophic respiration and the uptake and retention of available nutrients (Tank et al. 1998, Aldridge et al. 2009), and potentially decrease C:nutrient ratios at the whole-reach scale.

1.4 Hydrology

The hydrological regime of aquatic systems is defined by the frequency and duration of the presence of water and the pattern of its connectivity (Gordon et al. 2004). Although geography and climate obviously play a large role in determining the hydrological regime (Gordon et al. 2004), channel geomorphology can also have significant influence over the defining hydrological parameters. For example, channels that are greater in width will have a larger wetted area and lower surface water velocities (James & Henderson 2005, Julian et al. 2010).

Channel geomorphology can interact with changes in hydrology to create new in-stream habitats that can alter biogeochemical pathways and rates of processes. As discharge increases, the wetted area can extend to previously dry channel areas and riparian zones. This inundation can initiate or increase rates of biogeochemical processes such as microbial denitrification (Claret & Boulton 2003) and organic matter decomposition (Boulton 1991, Langhans & Tockner 2006). The spatial and temporal mosaic of these processes is referred to 'hot spots and hot

moments' (McClain et al. 2003), and may lead to a localised increase in nutrient uptake to support these processes (Bernal et al. 2005, von Schiller et al. 2011a).

The depth of surface water height will also change with discharge. Depending on their morphology and placement within the stream channel, geomorphic features can vary in how far they protrude into the water column (Frazier et al. 2011 – see Appendix 1). The change in water height infers that the importance of particular geomorphic features in the retention of organic matter will be altered. As increases in discharge can import large quantities of organic matter to reaches (Gurtz et al. 1988, Boulton & Lake 1992, Mollá et al. 2006), the interaction of hydrology with changes in retention pathways will determine the reach-scale retention or export of organic matter (Quinn et al. 2007). An increase in organic matter mass retained can lead to an increase in the C:nutrient ratio and may also enhance rates of heterotrophic respiration (Aldridge et al. 2009). However, reaches that lack riparian vegetation may not have the geomorphic complexity to increase the quantity of organic matter retained and therefore there may be no change in C:nutrient stoichiometry or heterotrophic respiration.

Upland streams that periodically cease to flow have three distinct hydrological phases: contraction of surface water, fragmentation of surface water into pools, and periods of surface water connectivity (Larned et al. 2010). The predictability of these phases is used to characterise these systems and includes: ephemeral, episodic, intermittent and seasonal rivers and streams (Larned et al. 2010). Flow variability is thought to be one of the most important drivers of ecological processes in temporary streams. Similar conceptual models of temporary-river ecology theory relating to biogeochemical processes have been proposed by Stanley et al. (1997), Dent & Grimm (1999), and Larned et al. (2010). These models suggest that different biogeochemical processes will be present and/or occur at different rates during the three hydrological phases of temporary rivers.

During phases of contraction and fragmentation when reaches become isolated, ecological or biogeochemical processes will become more heterogeneous between isolated reaches and pools, and the influence of local environmental parameters at reach scales including, riparian vegetation and geomorphology, will be more dominant (Stanley et al. 1997, Dent & Grimm

1999, Larned et al. 2010). In some intermittent streams during periods of surface water fragmentation, there are reduced quantities of high-quality organic matter, which requires heterotrophic communities to switch to low-quality sources of organic matter (Ylla et al. 2010). This suggests that if there are differences in the quantity and quality of organic matter retained within surface-water disconnected reaches, then there will be concomitant variation in the C:nutrient ratios, and rates of production and respiration between reaches.

Once surface water is connected, local environmental parameters will be of less importance and reach-scale organic matter dynamics become more homogeneous. During these periods of connectivity, the surrounding catchment may have more influence over reach biogeochemical processes (Stanley et al. 1997, Dent & Grimm 1999, Larned et al. 2010). Precipitation events and increases in wetted area can lead to larger quantities of allochthonous organic matter being transported laterally to streams (Gurtz et al. 1988, Raymond & Saiers 2010), as well as particulate nutrients that have been desorbed from soils moving vertically (Drewry et al. 2006, Blanco et al. 2010). Increases in discharge can also have a disturbance effect on ecosystem processes. Stream biota such as biofilms and macrophytes can be removed through scouring or smothered by sediment as discharge increases, both of which may reduce rates of reach-scale ecosystem metabolism (Biggs et al. 1999, Uehlinger 2000, Acuña et al. 2011). Retained benthic organic matter may also be exported from reaches at higher discharges, leading to a reduction in reach-scale heterotrophic decomposition (Acuña et al. 2007). This suggests that the biogeochemical processes will also become more homogeneous between reaches and that the C:nutrient ratios between reaches could become more similar (Stanley et al. 1997, Dent & Grimm 1999, Larned et al. 2010).

Using the theory of temporary-river ecology as a conceptual basis, it is predicted that reach-scale organic matter dynamics and other biogeochemical processes will be dominated by the effects of riparian vegetation during periods of low and no-flow when reaches are disconnected, resulting in increased heterogeneity between reaches. In contrast, during periods of connectivity, there may be less effect of riparian vegetation and therefore less variation in rates of biogeochemical processes among vegetated and non-vegetated reaches (Stanley et al.

1997, Larned et al. 2010). However, there have been a limited number of studies that have tested these predictions on reach-scale biogeochemical processes, and fewer that link changes to stream function as a response to reach-scale riparian restoration.

1.5 Stream restoration

Over recent years there has been considerable effort to address the detrimental impacts of anthropogenic impacts on streams and rivers. Most stream restoration projects are now focused on changing the physical environment and/or hydrological regime to restore biochemical processes (Lake et al. 2007). This stream restoration practice has been referred to as the 'Field of Dreams' hypothesis (*sensu* Palmer et al. 1997) where reinstating physical habitats or hydrologic regime provides the basis for the return of higher level ecosystem functions. In unregulated upland streams, riparian revegetation techniques are commonly used to facilitate restoration as the hydrological regime cannot be controlled. Riparian revegetation is commonly implemented at the reach-scale in stream restoration projects with the aim to provide physical benefits of bank stability and habitat creation, as well as facilitating the return of in-stream processes such as increased nutrient and organic matter retention. The majority of these projects use techniques such as grass buffer strips that effectively filter nutrients and sediment from water transported laterally to the stream channels (Osborne & Kovacic 1993, Newbold et al. 2010, Roberts et al. 2012), often ignoring the role of longitudinal transport and retention processes.

Riparian revegetation has also been applied to streams with the aim to enhance channel heterogeneity and subsequent increase in nutrient retention and restoration of other biogeochemical processes (Bernhardt & Palmer 2011). Few studies have investigated whether this method can influence the biogeochemical processes in water that moves longitudinally through the reaches. However, two recently published studies reporting on the outcomes of restoration through reach-scale (<200 m) channel reconfiguration in urban streams have explored the longitudinal uptake and retention of N at reach (Sudduth et al. 2011) and catchment scales (Filoso & Palmer 2011).

Monitoring the success of restoration projects requires sufficient data appropriate to the goals of the project. Previously, there have been issues in the appropriateness of measuring structural ecosystem parameters for monitoring projects that aim to restore ecosystem processes or functions (Ryder & Miller 2005). It has been suggested that the presence of structural parameters such as nutrient concentrations, may not be an accurate indicator of whether there is variation in the processes associated with these parameters (i.e. biotic retention) and whether the fundamental processes supporting stream foodwebs are sustainable (Ryder & Miller 2005). Some of the structural parameters that are related to the aims of riparian revegetation are increased number of geomorphic features and increased retention and accumulation of organic matter. Although these structural parameters are linked to biogeochemical processes (i.e. functional indicators), measuring the quantity of organic matter cannot provide insight into the whether there are differences in heterotrophic respiration. Similarly, revegetation techniques aim to enhance C:nutrient ratios through the increases in heterotrophic respiration, however, simply measuring organic matter biomass cannot determine whether the increases result from biogeochemical processes such as primary production or abiotic absorption. This highlights the need for further research into biogeochemical processes to provide insight into the success of restoration.

Enhanced nutrient and organic matter retention is a biogeochemical process that many restoration projects have as stated goals (Bernhardt & Palmer 2011). A method for quantifying the retention of nutrients and organic matter is to use a mass-balance approach. This approach estimates the total mass or load of organic matter or nutrients being imported, retained and exported through the reach, as well as identifying key biogeochemical pathways for retained organic matter and nutrients (von Schiller et al. 2011b). As geomorphic complexity is a structural feature of upland streams associated with nutrient and organic matter retention (Roberts et al. 2007, Watson & Barmuta 2010), quantitative data on the physical processes of retention are also required to explore the relationship between these parameters.

Despite the large number of stream restoration projects that employ reach-scale riparian revegetation techniques and the expense associated with these efforts, there have been

limited reports of success (Williams et al. 2011). In addition, much attention has been drawn to the lack of scientific input into the design process and the limited amount of monitoring and assessment post-restoration (Bernhardt et al. 2005, Mika et al. 2010, Williams et al. 2011). As the key to understanding the restoration of biogeochemical processes is linked to hydrology, alteration of physical habitats, and organic matter and nutrient dynamics, an interdisciplinary approach is required to monitoring success and guide future projects (Thoms & Parsons 2002, Palmer et al. 2005, Elosegi et al. 2010). Quantitative data that describes the relationship between geomorphology, hydrology and ecological processes is essential to develop meaningful conceptual and empirical models of nutrient cycling within stream reaches.

1.6 Thesis aims and structure

Riparian vegetation can play a pivotal role in the cycling of nutrients through changes to the quantity and quality of organic matter, which can also interact with channel geomorphology and hydrology. The goal of this thesis is to examine in-stream biogeochemical processes in intermittent upland streams in agricultural landscapes of the upper Gwydir River catchment, northern Murray Darling Basin Australia. The Gwydir River catchment area is dominated by agriculture, mainly sheep and cattle grazing, which has led to the removal of much of the native riparian vegetation species along many of the main-stem and tributaries of the Gwydir River. The main-stem of the Gwydir River and its tributaries are characterised as having an intermittent hydrological regime and short (<200 m) patches of riparian vegetation. Structural parameters such as channel geomorphology play a pivotal role in the cycling of organic matter and nutrients in streams, however, differences in these parameters may not provide insight into in-stream ecosystem functions or biogeochemical processes such as ecosystem metabolism. Additionally, there have been few studies that have explored the role of riparian vegetation on biogeochemical processes in intermittent streams, where discharge varying from floods to cease-to-flow events over relatively short time-frames can drive biogeochemical pathways and rates of processes. Quantifying rates of biogeochemical processes is essential to identifying the long-term success of reach-scale restoration that aims to reinstate ecosystem function. This thesis aims to explore the interactions among riparian vegetation, channel

geomorphology and hydrology, and their influence on dominant in-stream biogeochemical processes that regulate nutrient cycling and ecosystem metabolism.

The logical flow of this thesis, beginning with a description of the physical features of study reaches, through to nutrient and organic matter retention, and their influence on rates of ecosystem metabolism, is described below:

Chapter 2: Using high-resolution digital elevation models (DEMs), the objective of this chapter is to map channel geomorphic features and complexity within vegetated and non-vegetated reaches of upland streams in the study region. I aim to show whether there are differences in geomorphology and hydrology between vegetated and non-vegetated reaches which will provide initial insight for predictions of differences in organic matter mass-balance and retention. I also aim to use channel parameter data from the DEMs to estimate discharge that will then be used to transform nutrient and organic matter point concentration data into reach-scale loads. This transformation is essential to estimate the mass-balance and retention of nutrients and organic matter.

Chapter 3: The objective of this chapter is to investigate reach-scale nutrient dynamics between vegetated and non-vegetated reaches during different hydrological conditions. Specifically, I aim to determine whether there are significant differences in nutrient stoichiometry and retention between vegetated and non-vegetated reaches. These data will provide the basis for predictions on differences in organic matter and metabolic processes between these treatment reaches.

Chapter 4: In this chapter, I aim to investigate reach-scale organic matter dynamics between vegetated and non-vegetated reaches during different hydrological conditions. I aim to determine whether there are differences in the total mass and composition of benthic organic matter present between vegetated and non-vegetated reaches, and relate these data to reach nutrient stoichiometry. I will also explore differences in the mechanisms of retention and relate this to the quantity of organic matter retained within reaches.

Chapter 5: This chapter will report on the use of extracellular enzyme analysis techniques to explore the dominant sources of dissolved organic carbon within vegetated and non-vegetated reaches during different hydrological conditions. These data will determine whether heterotrophic communities are dependent on allochthonous or autochthonous sources of carbon, and if this varies between vegetated and non-vegetated reaches. The results of this chapter will also provide insight into the role of nutrient limitation on dominant metabolic processes within reaches.

Chapter 6: The objective of this chapter is to investigate reach-scale ecosystem metabolic processes in vegetated and non-vegetated reaches during different hydrological conditions. I aim to explore the relationships between nutrients, quantity and quality of organic matter, and habitat with reach-scale whole-system rates of productivity and respiration.

Chapter 7: This chapter will present a synthesis that discusses the specific influence of riparian vegetation, geomorphology, and hydrology on the reach-scale biogeochemical processes investigated in Chapters 3, 4, 5 and 6. I will also discuss the advantages and limitations of methods and techniques used in this study and suggest implications for future restoration efforts in upland intermittent streams.

Chapter 2: Hydro-geomorphic features of vegetated and non-vegetated reaches.

2.1 Introduction

Fluvial geomorphology defines the morphological traits of stream and river channels, and forms the physical template for ecological processes. The morphology of channels can be highly variable and is influenced locally by a combination of hydrological regime, sediment regime and riparian vegetation (Gregory et al. 1991, Stoffel & Wilford 2012). Catchment influences such as climate and geology can interact with these local factors to determine channel morphology and complexity (Gregory et al. 1991, Stoffel & Wilford 2012).

The geomorphic template is a logical starting point for river restoration (Ward et al. 2001, Brierley et al. 2008) because the availability of physical habitat can govern key ecological processes such as organic matter and nutrient cycling, and resources such as ecological habitat and food for aquatic biota (Harper and Everard 1998, Poole et al. 2006, Elsoegi et al. 2010). This approach necessitates understanding a stream's physical character and behavior in its specific landscape setting (Wiens 2002), and requires an understanding of interactions among stream ecology, hydrological regime (Poff et al. 1997) and geomorphic processes (Vannote et al. 1980).

The removal of riparian vegetation has been shown to reduce channel geomorphic complexity through reducing the amount of woody debris and logs that fall into the stream channel (Allan 2004, Lester & Boulton 2008). The stream channel can also become more incised from increases in sediment and streambank erosion (Hendry et al. 2003). Channel incision can reduce geomorphic complexity through smothering protruding features and pools in sediment, and eroding riffles, consequently decreasing habitat availability for biota (Brooks et al. 2004, Shields et al. 2004). Grazing by cattle and sheep in agricultural streams can also decrease bank stability promoting erosion and sedimentation in channels, as well as altering bank morphology (Myers & Swanson 1991). These alterations to channel geomorphology and complexity can have significant impacts on in-stream biogeochemical processes (Sovell et al. 2000, Lunt et al. 2007).

Digital elevation models (DEMs) have previously been used within environmental studies to link land-use effects to catchment health, and have the potential to accurately delineate land-use zones and catchments (Sponseller & Benfield 2001, Maloney et al. 2005, Lefebvre et al. 2007). For studies at catchment and sub-catchment scales, remote sensing techniques such as aerial photography and LiDAR, and high-resolution satellite imagery application such as, QuickBird and IKONOS (Marcus & Fonstad 2007), are able to provide enough topographic detail to develop the DEMs. At sub-metre scales, these mapping techniques are unable to provide data to develop high-resolution DEMs of in-stream channel morphology (Marcus & Fonstad 2007). Several factors including shading from riparian vegetation, turbidity in deep waters and pixel size have shown to decrease the accuracy of mapping in several habitat and channel morphology studies (Marcus et al. 2003, Leckie et al. 2005, Frazier et al. 2011). LiDAR has been successfully used to map fluvial geomorphic units in river valleys with 80% accuracy (Jones et al. 2007), however turbidity and shading from vegetation still limit this method (Marcus & Fonstad 2008). Remote sensing techniques including aerial photography cannot map channel morphology or features below the water surface (Marcus & Fonstad 2008). Traditional surveying techniques collecting point-coordinates has the ability to create accurate high-resolution DEMs of in-stream habitats. High-resolution (sub-metre) DEMs of stream channel and habitats developed through topographic surveys holds the most potential to link biogeochemical processes to physical habitats within reaches.

At the reach scale, the biogeochemical cycling and stoichiometry of C, N and P are closely linked to the number of abiotic and biotic interception pathways within reaches (Brookes et al. 2005). An increase in geomorphic complexity and protruding features within reaches can enhance organic matter retention (Brookshire & Dwire 2003, Quinn et al. 2007), which has been shown to alter the mass-balance and retention of in-stream nutrients (Aldridge et al. 2009). To accurately determine the mass-balance and retention of C, N and P, point concentration data must be transformed to loads. Loads are a measure of the total mass present within the reaches and calculations require an estimate of discharge for the particular sampling period (von Schiller et al. 2011b). Estimates of discharge require the dimensions of channel cross-sectional area, which may be derived from high-resolution DEMs. Therefore, data derived from

the high-resolution DEMs and habitat maps can be used to develop hypotheses about effects of riparian vegetation on biogeochemical processes and their interaction with hydrology at reach and sub-reach scales.

Aims

The main objectives of this chapter are to:

- 1) Using traditional surveying techniques, I aim to develop high-resolution DEMs for each study reach that can effectively identify and display sub-reach scale in-stream features and channel morphology. I predicted that there would be more channel geomorphic complexity in vegetated reaches compared to non-vegetated reaches due to a larger number of in-stream features.
- 2) I also aim to quantify the rates of discharge and other hydrological parameters at each study reach during each sampling period.
- 3) The final aim is to compare and discuss differences between the hydro-geomorphic parameters from vegetated and non-vegetated reaches.

2.2 Methods

Study area

The study took place in the upper Gwydir River Catchment, located in the north-eastern corner of the Murray-Darling Basin. The catchment drains an area of around 26,600 km², flowing west from the Great Dividing Range to meet the Barwon River. The major water storage is Copeton Dam (capacity 1,364 GL), which is located about 90 km from the headwaters of the Gwydir River. Five pairs of study reaches (i.e. ten reaches in total) were selected upstream of Copeton Dam and the township of Bundarra, four pairs were located on tributaries of the Gwydir River and a pair on an equivalent upland section of the Gwydir River main-stem (Figure 2.1). The geology is complex and dominated by variable contributions of acid and basic igneous rocks and marine sediments (Banens 1987). All study reaches were located upstream of Copeton Dam and were not regulated, thus discharge could greatly vary.

Typically, the study area receives the majority of its rainfall between October and February, the austral spring and summer period. The average annual rainfall recorded at the Bundarra Post Office (<http://www.bom.gov.au/climate/data/weather-data.shtml>) was approximately 776 mm from 1970 to 2010. However, the annual rainfall within the area was only 581 and 532 mm during 2008 and 2009 respectively. During 2010, when the main sampling for this study took place, the annual rainfall increased to 927 mm.

The annual discharge estimated at the Gwydir River gauge downstream of Bundarra (Gauge #418014) was approximately 120,777,83 m³ during 2009. The maximum daily discharge during 2009 was 3,658,987 m³ and 39% of flows were under 1000 m³ day⁻¹. During 2010, when the study took place, annual discharge was approximately 534,746,193 m³ with a maximum daily discharge of 29,948,090 m³ and 75% of flows under 1000 m³ day⁻¹. After almost 11 months of mean monthly discharge below 1,500,000 m³, monthly discharge increased to 20,000,000 m³ during January 2010 (Figure 2.3). Significant rainfall events during June 2010 (Figure 2.2) increased discharge during July through to December 2010 (Figure 2.3).

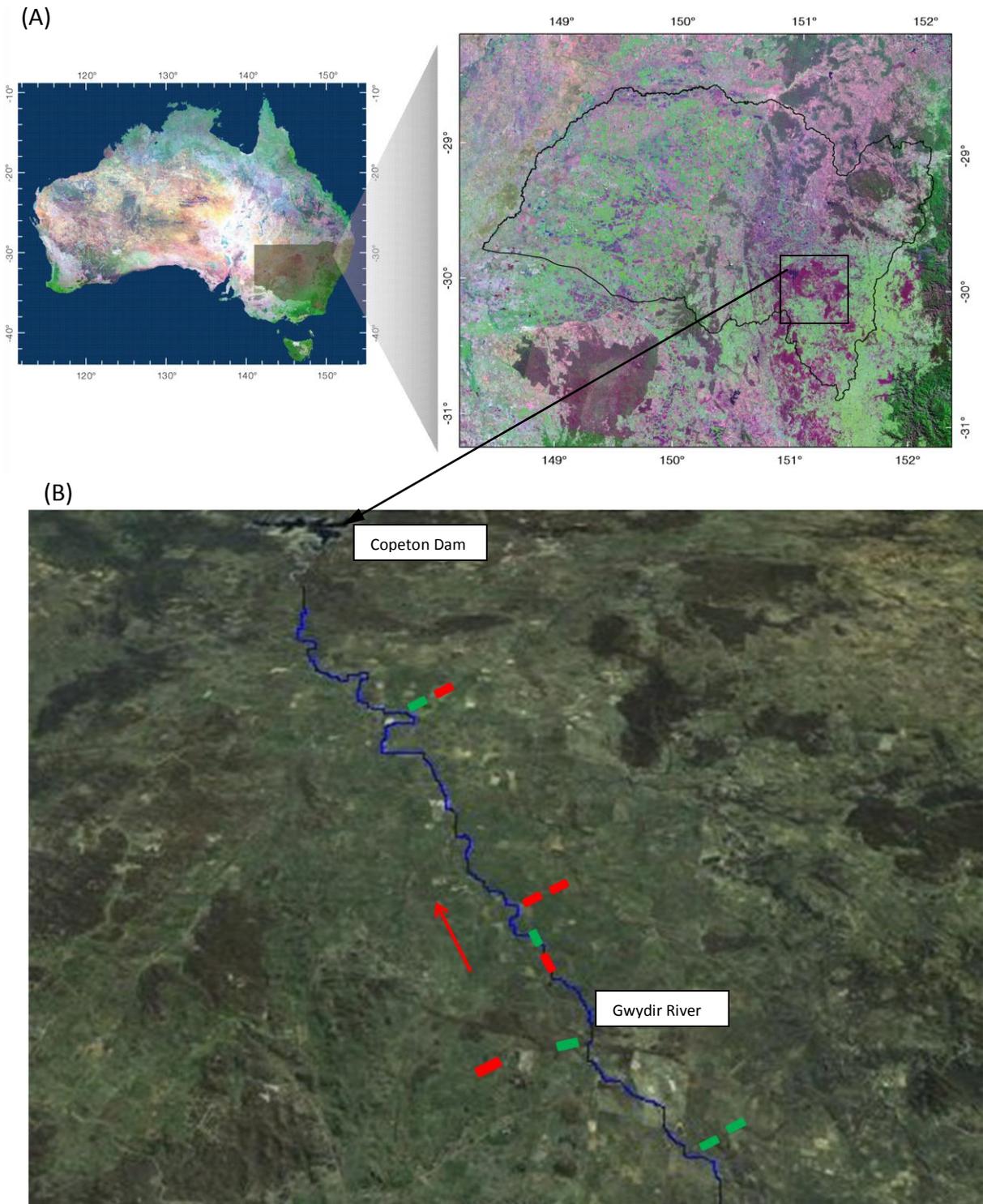


Figure 2.1: (A) The Gwydir River Catchment and field location area and (B) distribution of vegetated (green) and non-vegetated (red) reaches within the field area. The red arrow indicates the direction of flow of the Gwydir River. The catchment map was obtained from the NSW Office of Water and the field area map was obtained from Google Earth.

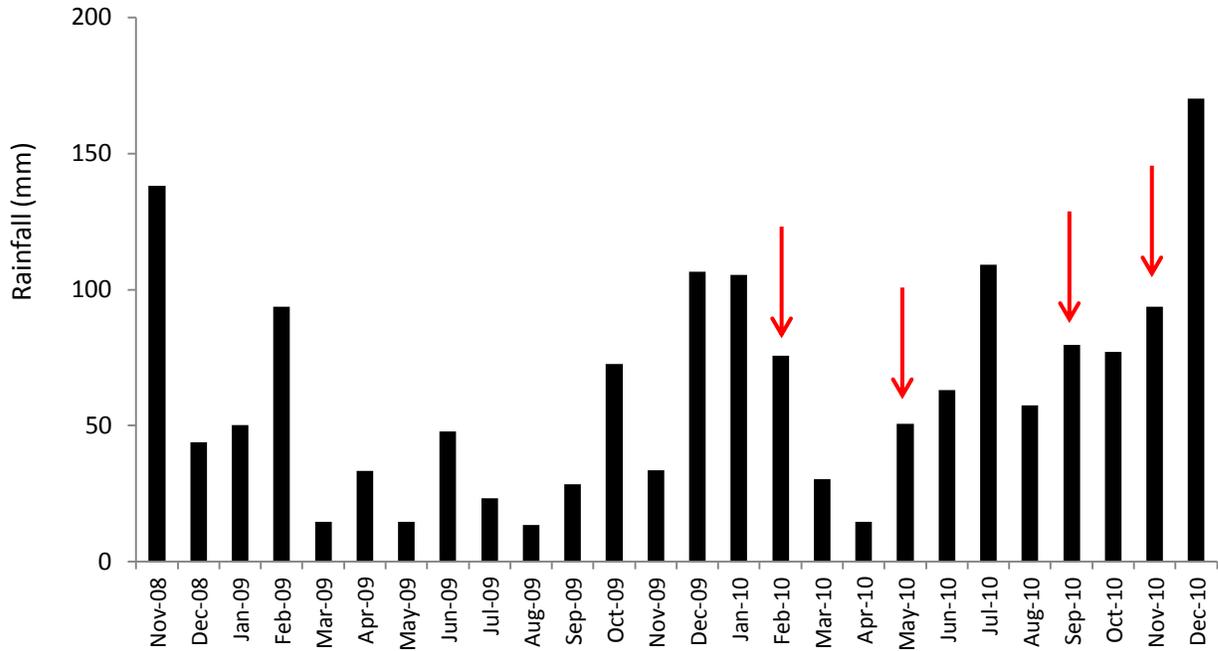


Figure 2.2: Mean monthly rainfall (mm) recorded at the Bundarra Post Office, from November 2008 to December 2010. Red arrows indicate the sampling periods.

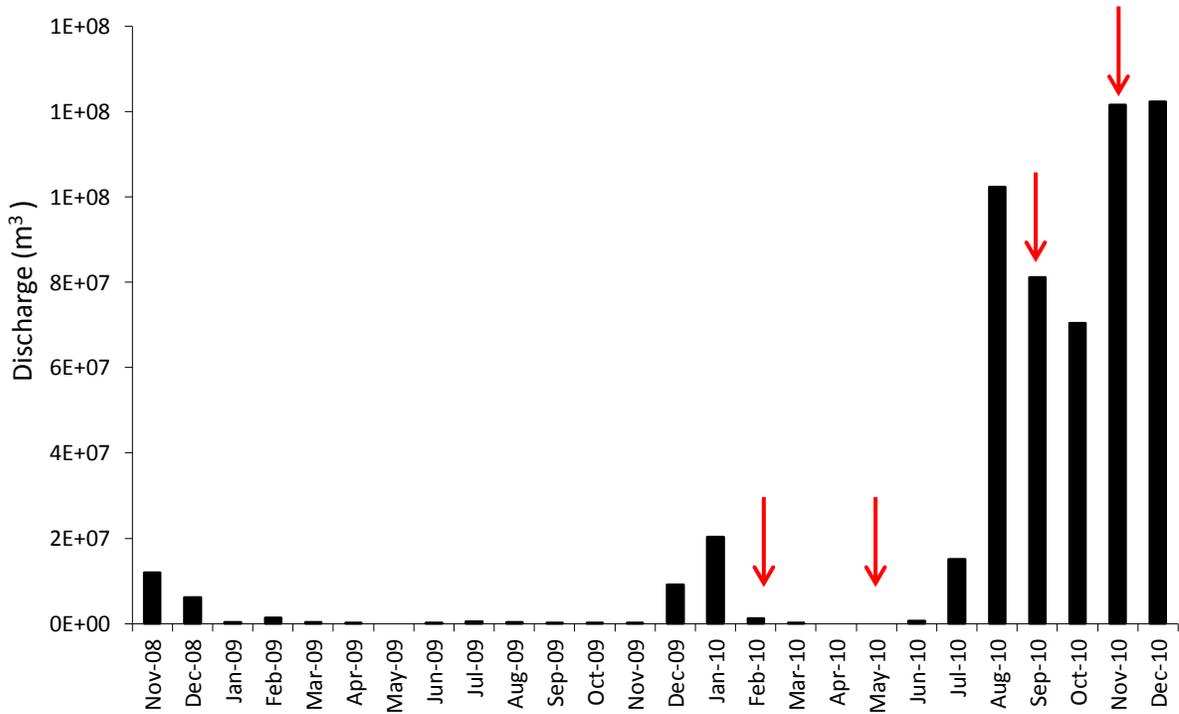


Figure 2.3: Mean monthly discharge (m³) of the Gwydir River at Bundarra, from November 2008 to January 2011. Red arrows indicate the sampling periods.

Study design

The study design followed a matched-pairs approach. Three pairs of reaches were located on Moredun Creek, Roumalla Creek and the Gwydir River, and considered treatment pairs. Each pair consisted of an upstream non-vegetated reach and a downstream vegetated reach. The remaining two pairs located on Booralong and Laura Creek were considered control pairs, which consisted of a pair of vegetated and non-vegetated reaches, respectively (Figure 2.1). Riparian vegetation along the Gwydir River and its tributaries is dominated by *Casuarina cunninghamiana* Miq. and *Eucalyptus spp.*. A minimum of a single line of *C. cunninghamiana* trees were present on either side of the main channel in vegetated reaches, while trees were absent or very sparse within the riparian zone of non-vegetated reaches.

There were four main sampling periods for this study, which were conducted at different discharges during 2010 (Figure 2.1). The four sampling periods each had different combinations of discharge and temperature and included: February 2010 (low flow, high temperature), May 2010 (no flow, moderate temperature), September 2010 (high flow, low temperature), and November 2010 (moderate to high flow, moderate temperature).

Digital Elevation Models and Habitat Mapping

The study reaches of approximately 100 m in length were mapped using a total station (TopCon GPT 7500, 3" precision) during March 2009. The total station was moved at least twice within each reach during survey to reduce errors from increased range of error and line of site obstruction. The coordinates of the new position of the total station within the reach were recorded by the total station before relocating.

At each reach, the embankments and side channels were mapped according to the changes in topography. The profiles of the wetted channels were recorded at transects perpendicular to the channel at 1 – 2 m intervals. The location of geomorphic features and channel complexities including exposed bedrock, logs, flood debris accumulation points, macrophyte beds, pools and riffles were also mapped. Logs were defined as woody debris that had a diameter larger than 20 cm and at least 100 cm in length.

To develop the DEMs, the coordinates were imported into ArcMap (version 9.3, ESRI Inc.) and interpolated using the ArcMap Spatial Analyst Topo to Raster tool. This interpolation method uses an iterative finite difference interpolation technique, based on the ANUDEM program (Hutchinson, 1989). The coordinates of the geomorphic features and channel complexities were transformed into polygons to show the location of these features within the reach, and the water channel perimeter coordinates were transformed into lines. To derive the channel cross-sectional area (m^2), the cross-section coordinates were plotted against the recorded water height to produce a polygon shape. Cross-sectional area was then calculated using the vertices of the polygon. Other common channel parameters such as channel width and slopes were derived using the elevation and distance changes between topographic coordinates.

Hydrological parameters

During each sampling period, the average water height and flow velocity (Marsh McBirney Flowmate, model 2000) across a defined downstream transect was recorded at approximately 1 m intervals to calculate discharge in $m^3 s^{-1}$. Discharge was calculated using the equation $Q = vA$, where v is equal to velocity ($m s^{-1}$) and A is equal to cross-sectional area (m^2) (Gordon et al. 2004).

Flow duration curves (flow percentiles) were calculated for each reach using discharge data collected during 1984 when there was a functioning gauge measuring stage height on each of the five study streams. This was thought to be a suitable analogue for the study period as 1984 had a similar pattern of mean monthly rainfall and there is no evidence for substantial change in riparian vegetation between 1984 and the present study period.

The surface areas (m^2) and volumes (m^3) of each reach inundated under different stages of discharge were estimated using the 3D Analyst, Surface Volume tool in ArcMap (Version 9.3, ESRI Inc.). The water height at a known transect was used to estimate a new elevation (z) point and then the Analyst tool estimated the surface area and volume below this point.

2.3 Results

Moredun Creek

The Moredun Creek subcatchment drains an area of approximately 683 km². The reaches are approximately 2.6 km apart and both are located within a cattle grazing property (Table 2.1). There were 10 logs present at the non-vegetated reach (Table 2.1, Figure 2.4) while only two were present within the vegetated reach (Table 2.1, Figure 2.5). Macrophyte beds were only present within the non-vegetated reach and flood debris accumulation points were only present within the vegetated reach. A gully entered the stream channel approximately 10 meters downstream of the permanent upstream transect at the vegetated reach (Figure 2.5). Discharge from this gully was only evident during episodic rainfall events.

Discharge ranged from 0 to 5.73 m³ s⁻¹ at both reaches, which corresponded to a flow range of 100 to 1.5 percentiles (Tables 2.2 and 2.3). During September and November, the high-flow periods, average surface water velocity was higher at the vegetated reach (0.65 and 0.50 m s⁻¹) compared to the non-vegetated reach (0.39 and 0.27 m s⁻¹). There was no surface water present at the vegetated reach during May, the no-flow sampling period. With the exception of the May sampling period, the vegetated reach (2420 to 3801 m²) had a consistently higher total wetted area compared to the non-vegetated reach (1788 to 2287 m²). This also resulted in the vegetated reach having higher surface area:volume ratios (Tables 2.2 and 2.3).

Table 2.1: Details of location, substrate type, channel slope and number of features present at the Moredun Creek reaches.

	Upstream non-vegetated	Downstream vegetated
Coordinates	-30.14592, 151.11863	-30.15001, 151.09177
Elevation (m)	656	646
Length (m)	116	119
Substrate	Cobble, some sand	Cobble, some sand
Channel slope (m)	0.003	0.001
<u>Number of features</u>		
Logs	10	2
Bedrock	3	3
Flood debris	0	3
Macrophyte beds	2	0
Total	15	8



Plate 1: Photograph of Moredun non-vegetated reach looking downstream.

Table 2.2: Hydro-geomorphic features of Moredun non-vegetated reach during the four different sampling periods.

Sampling time	February Low-flow	May No-flow	September High-flow	November High-flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.06	0	5.61	3.33
No. features inundated	11	11	11	11
Flow percentile	63	100	1.7	2.8
Average velocity (m s^{-1})	0.01	0	0.39	0.27
Channel volume (m^3)	758	228	1685	1464
Wetted area (m^2)	1788	1286	2287	2159
Surface area:volume	2.36	5.63	1.36	1.47

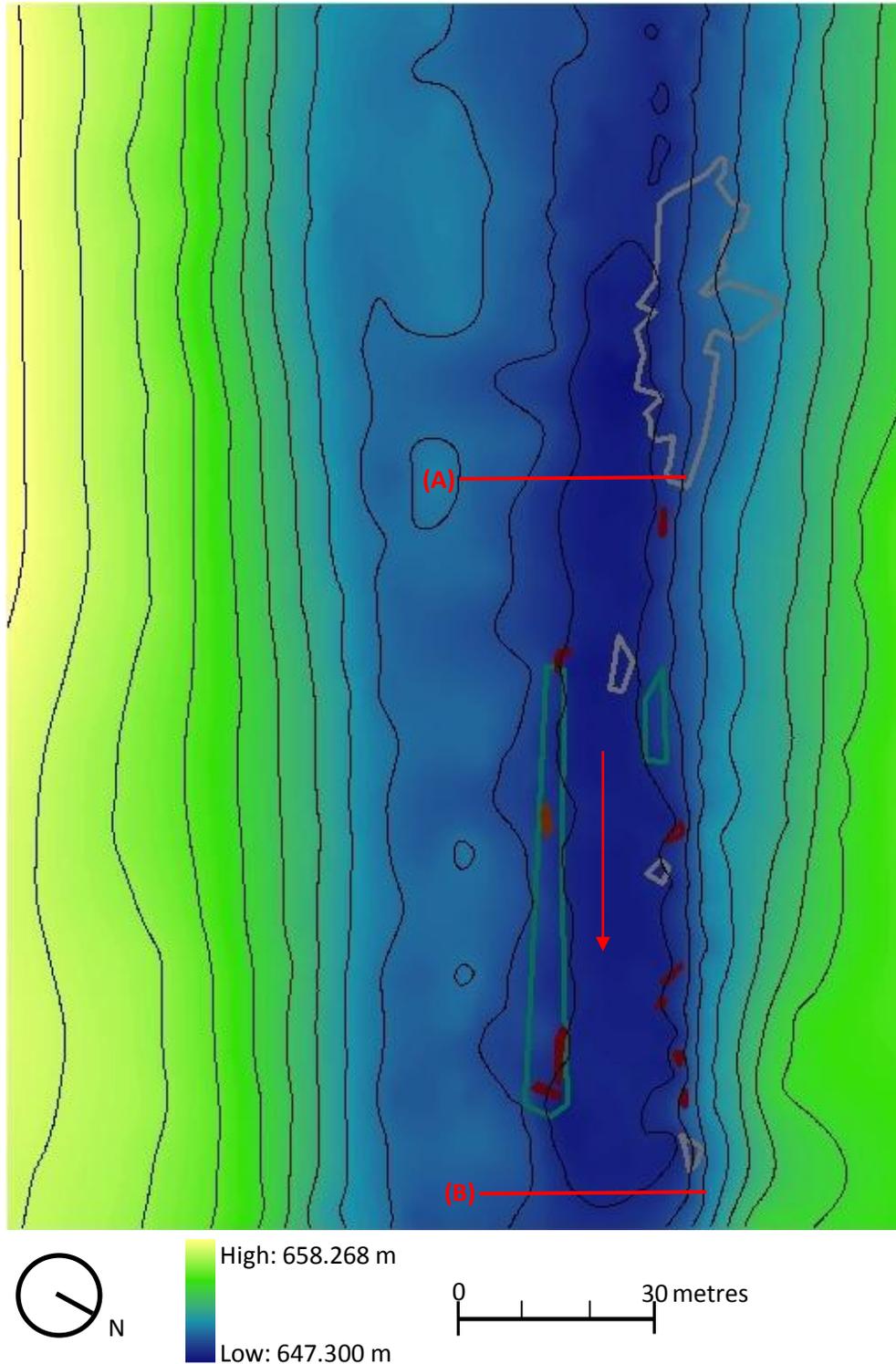


Figure 2.4: DEM of Moredun non-vegetated reach, riparian zone and channel features including bedrock (grey), logs (brown) and macrophyte beds (green). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.



Plate 2.2: Photograph of Moredun vegetated reach looking downstream.

Table 2.3: Hydro-geomorphic features Moredun vegetated reach during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.02	0	5.73	3.60
No. features inundated	5	0	5	5
Flow percentile	83	0	1.5	2.5
Average velocity (m s^{-1})	0.01	0	0.65	0.50
Channel volume (m^3)	538	0	1963	1596
Wetted area (m^2)	2420	0	3801	3545
Surface area:volume	4.5	0	1.94	2.22

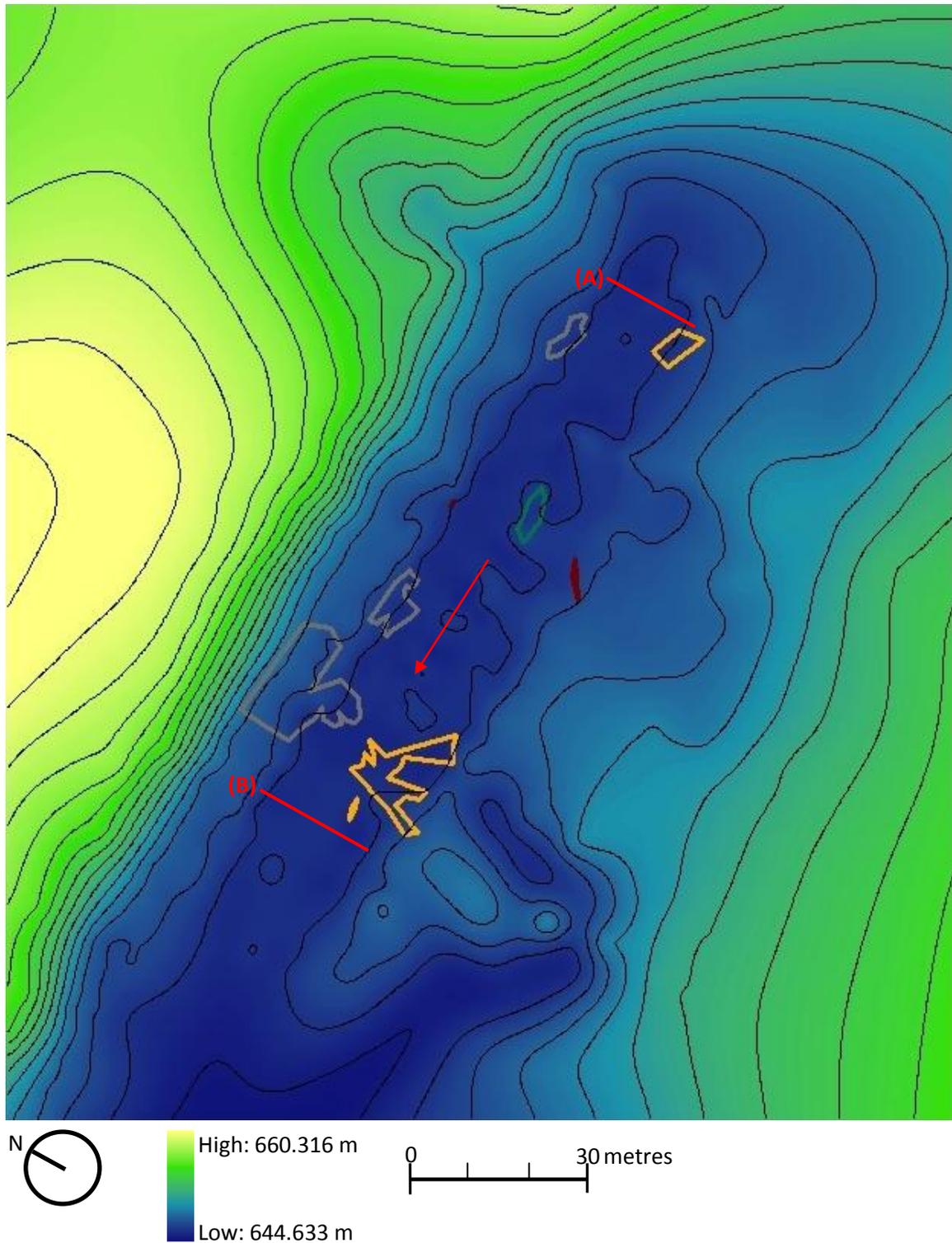


Figure 2.5: DEM of Moredun vegetated reach, riparian zone and channel features including bedrock (grey), logs (brown), flood debris accumulation points (orange) and macrophyte bed (green). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.

Gwydir River

The Gwydir River catchment drains an area of approximately 26,600 km². The reaches were approximately 2.2 km apart and both located within same cattle grazing property. The channel bed substrates at both reaches was primarily sand (Table 2.4). There were eight logs present at the non-vegetated reach (Table 2.4, Figure 2.6) while only five were present within the vegetated reach (Table 2.4, Figure 2.7). Macrophyte beds were only present within the non-vegetated reach and flood debris accumulation points were only present within the vegetated reach.

The rates of discharge sampled ranged from 0 to 5.73 m³ s⁻¹, which corresponded to flow range of 100 to 7.1 percentile (Tables 2.5 and 2.6). The average surface water velocities were higher within the vegetated reach (0.7 and 0.6 m s⁻¹) compared to the non-vegetated reach (0.5 and 0.46 m s⁻¹) during the high-flow sampling periods. The total reach volume and wetted area at the non-vegetated ranging between 1343 to 3236 m³ and 870 to 4387 m², respectively. At the vegetated reach, total reach volume and wetted area ranged between 24.9 to 407 m³ and 171 to 1323 m², respectively. This led to lower surface area:volume ratios within the non-vegetated reach compared to the vegetated reach (Tables 2.5 and 2.6).

Table 2.4: Details of location, substrate type, channel slope and number of features present at the Gwydir River reaches.

	Upstream non-vegetated	Downstream vegetated
Coordinates	-30.28122, 151.13689	-30.25617, 151.12563
Elevation (m)	665	663
Length (m)	104	100
Substrate	Sand	Sand, some cobble
Channel slope (m)	0.003	0.003
<u>Number of Features</u>		
Logs	8	5
Bedrock	6	3
Flood debris	0	3
Macrophyte beds	2	0
Total	18	11



Plate 2.3: Photograph of Gwydir non-vegetated reach during May 2010, the low-flow sampling period, looking downstream.

Table 2.5: Hydro-geomorphic features of Gwydir non- vegetated reach during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.40	0	5.25	5.53
No. features inundated	9	9	10	10
Flow percentile	54	100	7.4	7.3
Average velocity (m s^{-1})	0.12	0	0.5	0.46
Channel volume (m^3)	1343	1365	2852	3236
Wetted area (m^2)	2977	870	4092	4387
Surface area:volume	2.22	1.21	1.43	1.36

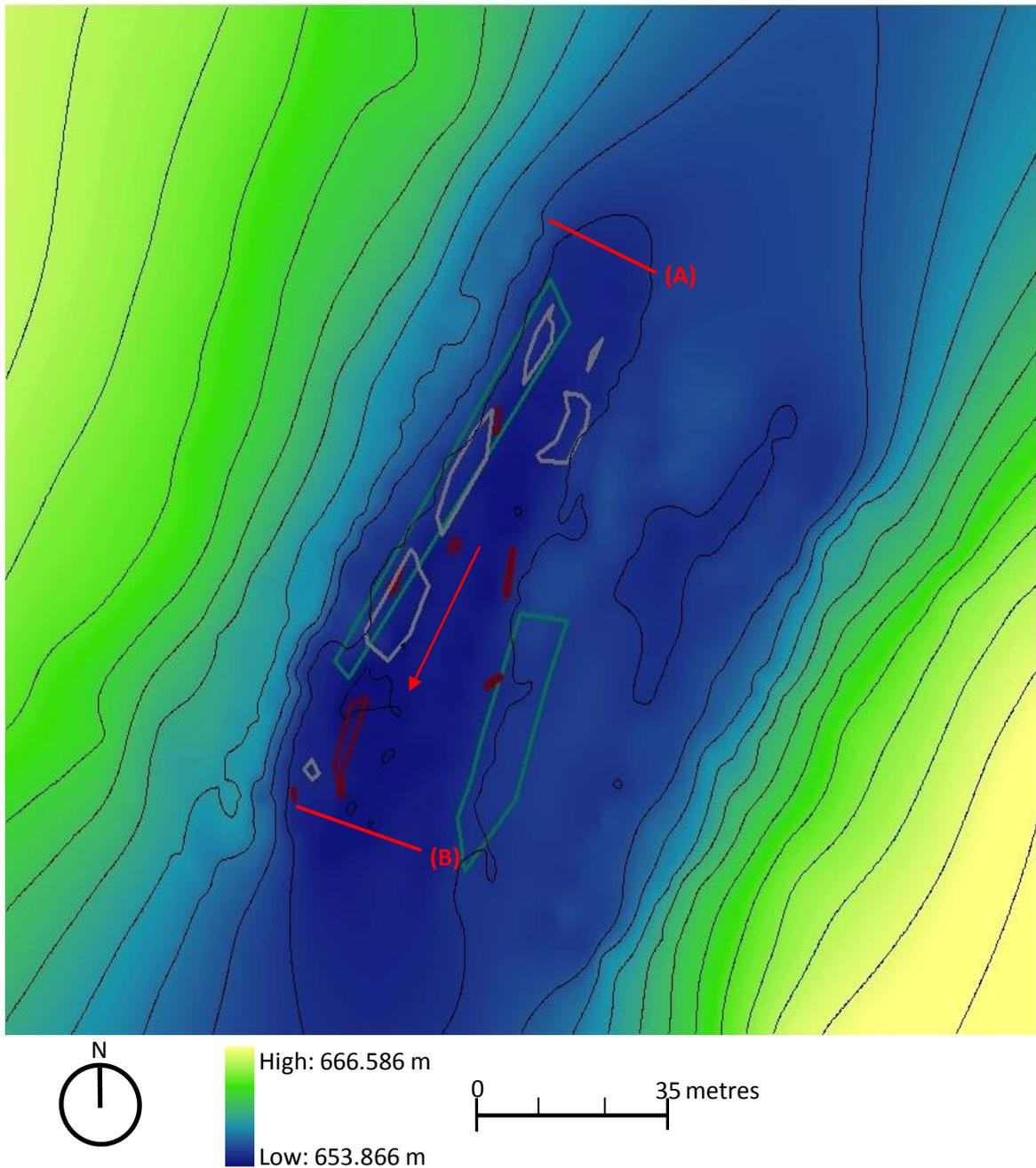


Figure 2.6: DEM of Gwydir non-vegetated reach, riparian zone and channel features including bedrock (grey), logs (brown) and macrophyte bed (green). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.



Plate 2.4: Photograph of Gwydir vegetated reach, looking downstream.

Table 2.6: Hydro-geomorphic features of Gwydir vegetated reach during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.36	0	5.73	5.49
No. features inundated	8	8	8	8
Flow percentile	56	100	7.1	7.4
Average velocity (m s^{-1})	0.1	0	0.7	0.6
Channel volume (m^3)	161.5	24.9	407	407
Wetted area (m^2)	772	171	1323	1323
Surface area:volume	4.78	6.87	3.25	3.25

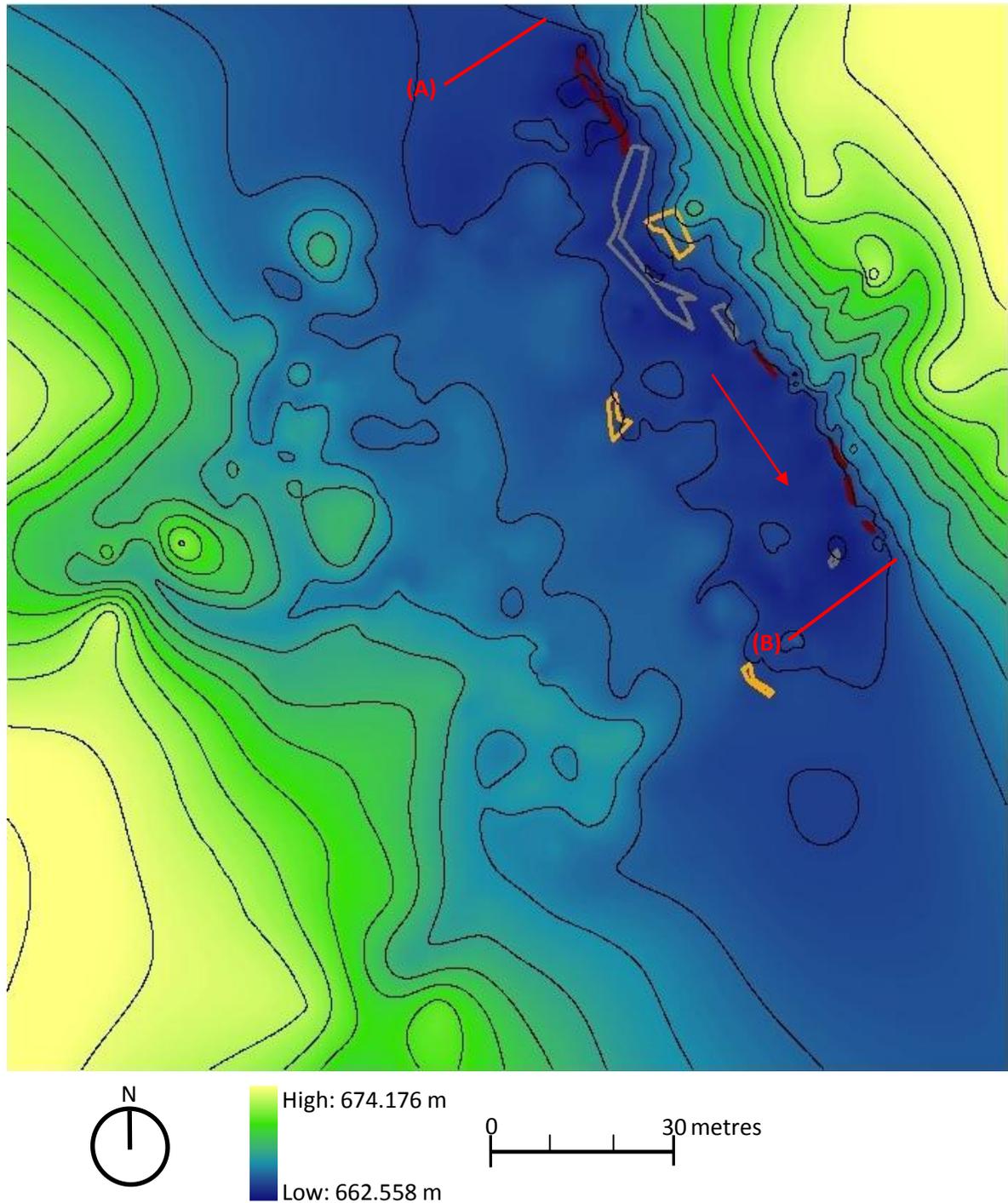


Figure 2.7: DEM of Gwydir vegetated reach, riparian zone and channel features including bedrock (grey), logs (brown) and flood debris accumulation points (orange). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.

Roumalla Creek

The Roumalla Creek catchment drains an area of approximately 560 km². The reaches are approximately 5 km apart. The non-vegetated reach is located within a sheep and cattle grazing property while the vegetated reach is located within a travelling stock route, so there is less predictable grazing by cattle at this reach. The channel bed substrate at the non-vegetated reach was sand, while a mixture of sand and cobbles were found within the vegetated reach (Table 2.7). There were single gullies present at both the vegetated and non-vegetated reaches. There were nine logs present at the vegetated reach (Table 2.7, Figure 2.9) and only two present within the non-vegetated reach (Table 2.7, Figure 2.8). Macrophyte beds were only present within the non-vegetated reach and flood debris accumulation points were only present within the vegetated reach.

Rates of discharge at the reaches ranged from 0 to 1.77 m³ s⁻¹ across the four sampling periods. These rates corresponded to a flow range of 100 to 2.2 percentile (Tables 2.8 and 2.9). During the high-flow sampling periods, average surface water velocity was higher at the vegetated reach (0.25 and 0.11 m s⁻¹) compared to the non-vegetated reach (0.06 and 0.025 m s⁻¹). Total reach volumes and wetted areas at the non-vegetated reach ranged between 250 to 3119 m³ and 870 to 3686 m², respectively (Table 2.8). At the vegetated reach, the total reach volumes and wetted areas ranged between 162 to 2655 m³ and 385 to 2846 m², respectively (Table 2.9). With the exception of the May sampling period, the surface area:volume ratios were lower within the non-vegetated reach compared to the vegetated reach (Tables 2.8 and 2.9).

Table 2.7: Details of location, substrate type, channel slope and number of features present at the Roumalla Creek reaches.

	Upstream non-vegetated	Downstream vegetated
Coordinates	-30.4532, 151.16789	-30.42764, 151.19586
Elevation (m)	674	646
Length (m)	101	115
Substrate	Sand	Cobble, some sand
Channel slope (m)	0.002	0.005
<u>Number of Features</u>		
Logs	2	9
Bedrock	2	3
Flood debris	0	5
Macrophyte beds	3	0
Total	7	18



Plate 2.5: Photograph of Roumalla non-vegetated reach, looking downstream.

Table 2.8: Hydro-geomorphic features of Roumalla non-vegetated reach during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.10	0	1.77	0.55
No. features inundated	4	3	4	4
Flow percentile	45	100	2.2	8.5
Average velocity (m s^{-1})	0.01	0	0.06	0.025
Channel volume (m^3)	1003	250	3119	2413
Wetted area (m^2)	2225	870	3686	3315
Surface area:volume	2.22	3.48	1.18	1.37

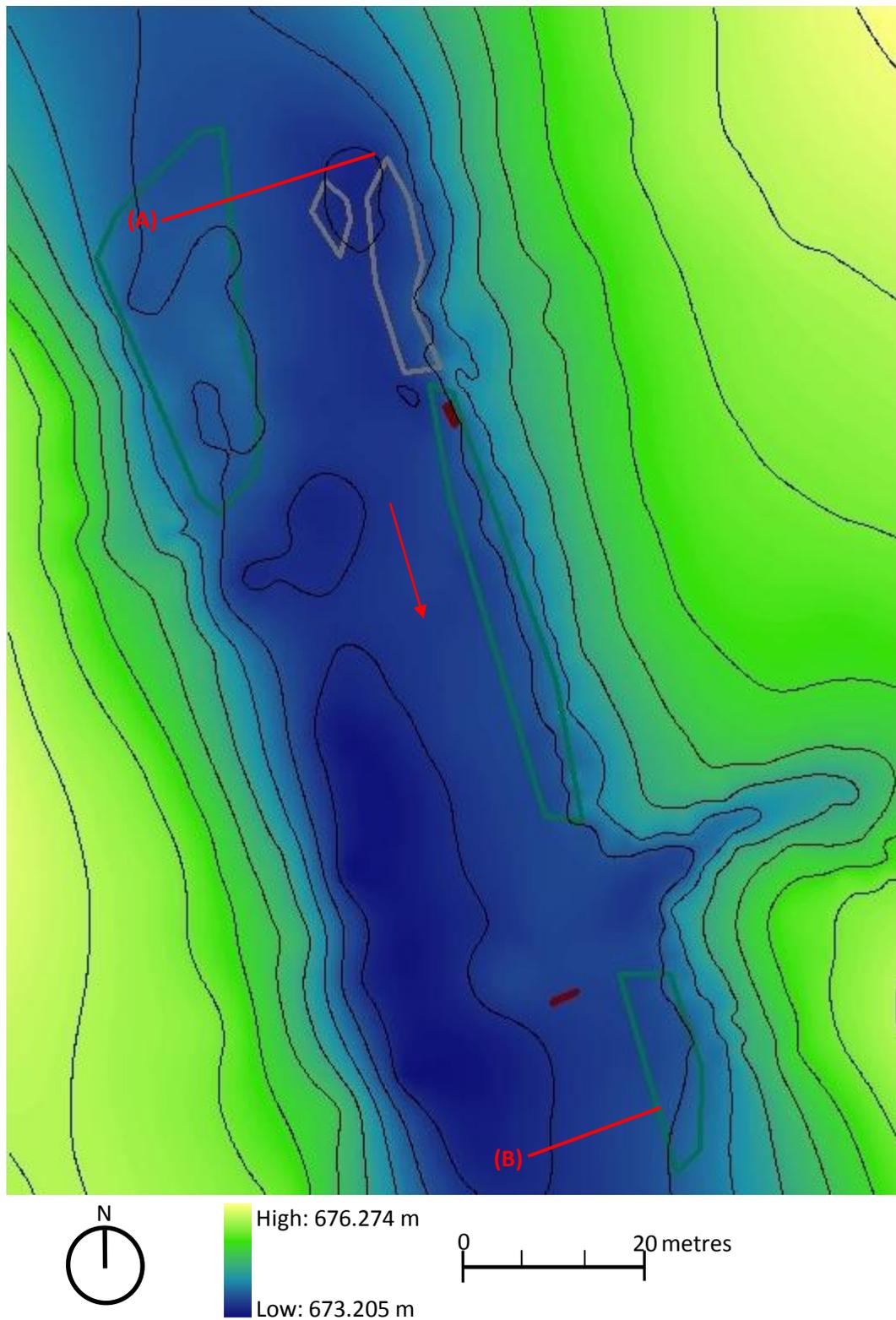


Figure 2.8: DEM of Roumalla non-vegetated reach, riparian zone and channel features including bedrock (grey), logs (brown) and macrophyte beds (green). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.



Plate 2.6: Photograph of Roumalla vegetated reach, looking downstream.

Table 2.9: Hydro-geomorphic features of Roumalla vegetated reach during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.04	0	1.76	0.51
No. features inundated	10	6	10	10
Flow percentile	70	100	2.4	9
Average velocity (m s^{-1})	0.01	0	0.25	0.11
Channel volume (m^3)	162	250	2655	1995
Wetted area (m^2)	2248	385	2846	2427
Surface area:volume	1.29	4.95	1.07	1.22

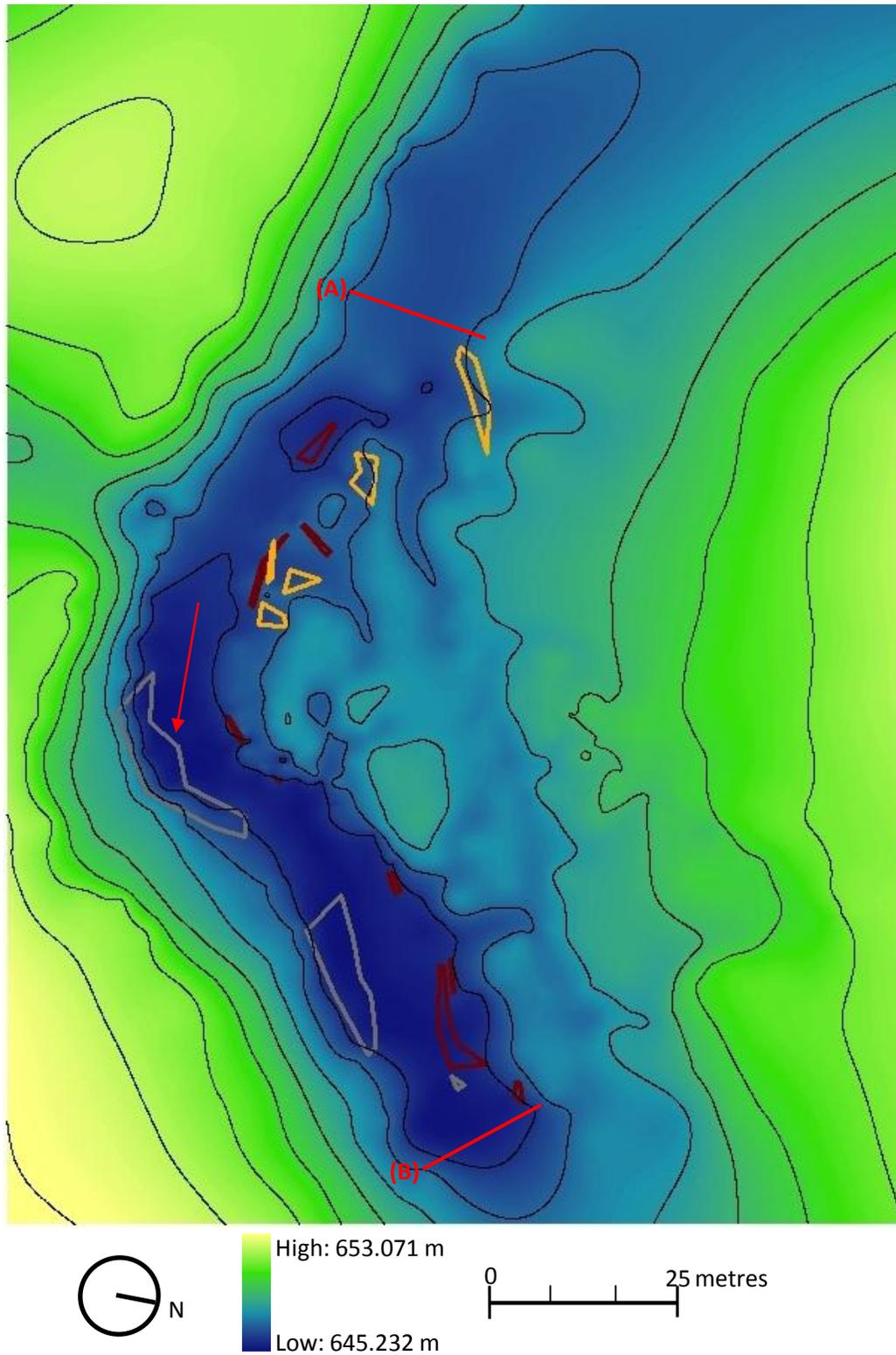


Figure 2.9: DEM of Roumalla vegetated reach, riparian zone and channel features including bedrock (grey), logs (brown) and flood debris accumulation points (orange). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.

Booralong Creek

The vegetated control stream, Booralong Creek, drains a catchment area of approximately 302 km². The paired reaches are approximately 1.5 km apart with the upstream reach located within a sheep grazing property and the downstream reach is located within a travelling stock route. The channel bed substrates are primarily cobble interspersed with boulders at both reaches and some sand was present at the downstream reach, Booralong Bridge (Table 2.10). There were five logs present at the downstream reach (Table 2.10, Figure 2.11) and only a single large tree or log was present at the upstream reach (Table 2.10, Figure 2.10). Flood debris accumulation points were present within both reaches.

The rates of discharge at reaches ranged between 0 and 1.13 m³ s⁻¹, which corresponded to flow range between 100 and 4.5 percentile (Tables 2.11 and 2.12). Average surface velocities during the high-flow periods were higher at the downstream reach, Booralong Bridge (0.15 and 0.06 m s⁻¹), compared to the upstream reach, Booralong CS (0.10 and 0.03 m s⁻¹). During May, the no-flow period, there was no surface water present at the upstream reach, Booralong CS. Total reach volumes and wetted areas at the upstream reach, Booralong CS, ranged between 0 to 1087 m³ and 0 to 2144 m², respectively (Table 2.11). At the downstream reach, Booralong Bridge, the total reach volumes and wetted areas ranged between 84 to 1338 m³ and 307 to 2028 m², respectively (Table 2.12). With the exception of the May sampling period, the surface area:volume ratios were higher at the upstream reach, Booralong CS, compared to the downstream reach, Booralong Bridge (Tables 2.11 and 2.12).

Table 2.10: Details of location, substrate type, channel slope and number of features present at the Booralong Creek reaches.

	Upstream vegetated	Downstream vegetated
Coordinates	-30.47862, 151.43805	-30.48366, 151.42651
Elevation (m)	791	745
Length (m)	100	95
Substrate	Cobble, boulder	Cobble, boulder, sand
Channel slope (m)	0.003	0.001
<u>Number of Features</u>		
Logs	1	5
Bedrock	1	14
Flood debris	2	1
Macrophyte beds	0	0
Total	4	20



Plate 2.7: Photograph of vegetated upstream control reach, Booralong CS, looking downstream.

Table 2.11: Hydro-geomorphic features of vegetated upstream control reach, Booralong CS, during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.05	0	0.93	0.21
No. features inundated	4	0	4	4
Flow percentile	48	100	5.8	17
Average velocity (m s^{-1})	0.01	0	0.1	0.03
Channel volume (m^3)	445	0	1087	785
Wetted area (m^2)	1493	0	2144	1896
Surface area:volume	3.36	0	1.97	2.42

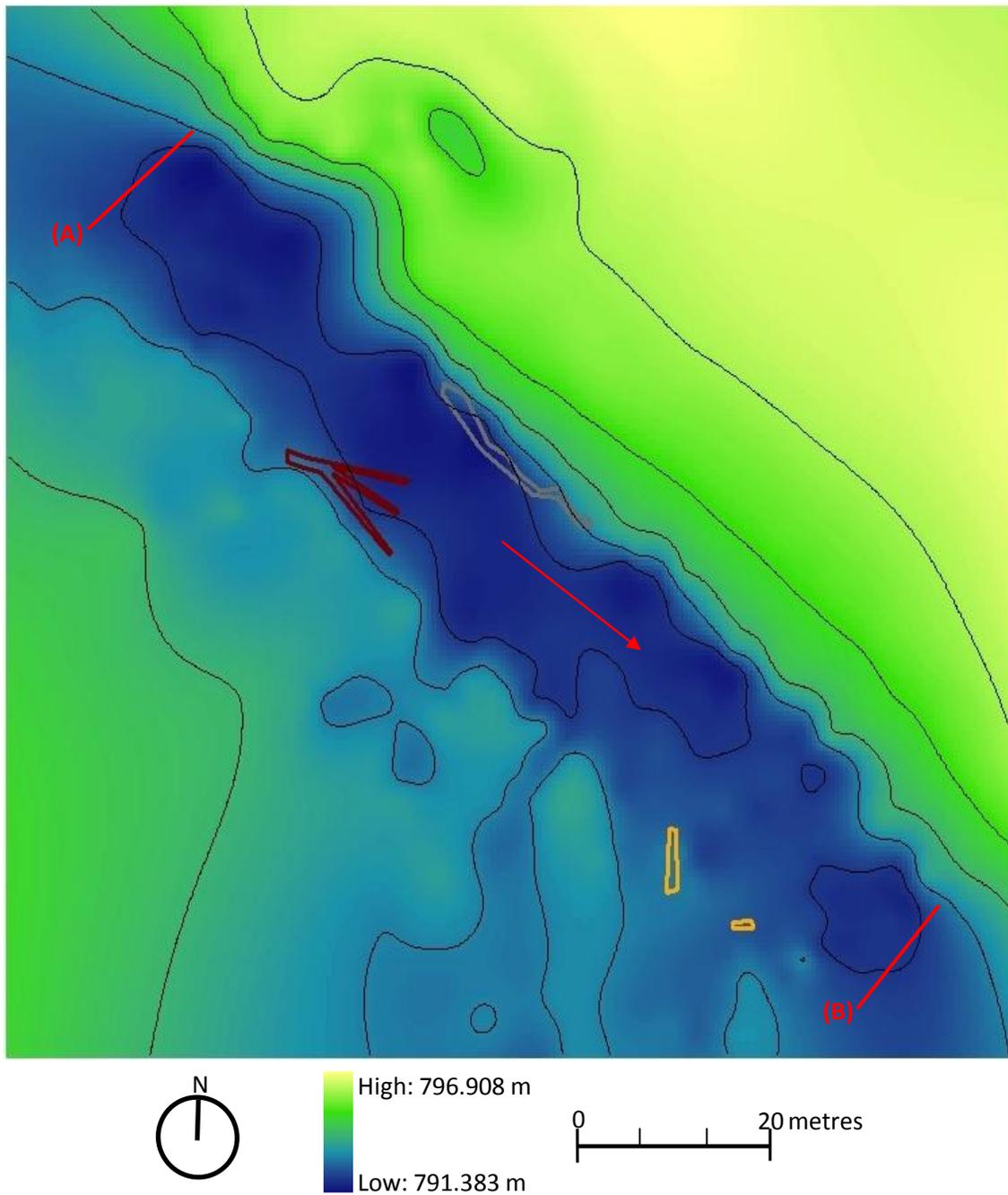


Figure 2.10: DEM of the upstream vegetated control, Booralong CS, reach, riparian zone and channel features including bedrock (grey), logs (brown) and flood debris accumulation points (orange). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.



Plate 2.8: Photograph of vegetated downstream control reach, Booralong Bridge, looking downstream.

Table 2.12: Hydro-geomorphic features of vegetated downstream control reach, Booralong Bridge, during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.04	0	1.13	0.30
No. features inundated	18	4	18	18
Flow percentile	54	100	4.5	14
Average velocity (m s^{-1})	0.01	0	0.15	0.06
Channel volume (m^3)	717	84	1338	869
Wetted area (m^2)	1643	307	2028	1744
Surface area:volume	2.29	3.65	1.52	2.01

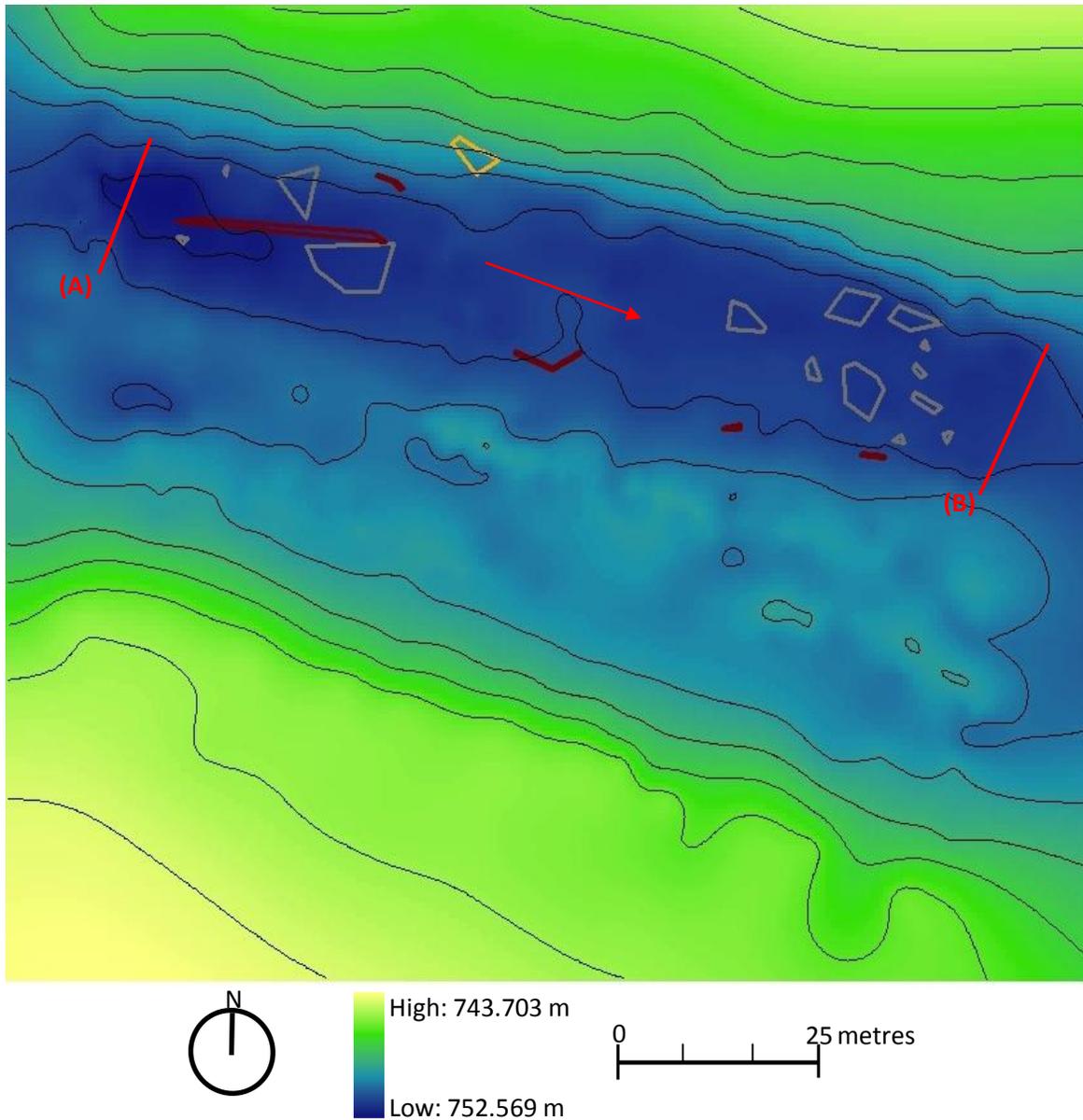


Figure 2.11: DEM of the downstream vegetated control, Booralong Bridge, reach, riparian zone and channel features including bedrock (grey), logs (brown) and flood debris accumulation point (orange). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.

Laura Creek

The Laura Creek catchment drains an area of approximately 384 km². The reaches are approximately 3 km apart and both are located within a sheep grazing property. The channel bed substrate at both reaches was primarily sand and fine sediment with some small areas of cobbles present (Table 2.13). There were seven logs present at the upstream reach, Laura HD (Table 2.13, Figure 2.12), and ten present within the downstream reach, Laura Bridge (Table 2.13, Figure 2.13). Macrophyte beds were only present at the downstream reach, Laura Bridge. Flood debris accumulation points were not present within either of the reaches.

The rates of discharge ranged between 0 and 2.28 m⁻³ s⁻¹, which corresponded to flow values between 100 to 11.8 percentile (Tables 2.14 and 2.15). During the flow periods, average surface water velocities were higher at the downstream reach, Laura Bridge, ranging between 0.08 and 0.26 m s⁻¹ (Table 2.15), compared to the upstream reach, Laura HD, which ranged between 0.02 and 0.18 m s⁻¹ (Table 2.14). The channel volumes at the upstream reach were consistently higher (1118 to 1922 m³) than the downstream reach (728 to 1963 m³). This led to the downstream reach, Laura Bridge having larger surface area:volume ratios than the upstream reach, Laura HD (Tables 2.14 and 2.15).

Table 2.13: Details of location, substrate type, channel slope and number of features present at the Laura Creek reaches.

	Upstream non-vegetated	Downstream non-vegetated
Coordinates	-30.23427, 151.15263	-30.23443, 151.13251
Elevation (m)	661	659
Length (m)	125	125
Substrate	Fine sediment, cobble	Sand, fine sediment, cobble
Channel slope (m)	0.003	0.002
<u>Number of Features</u>		
Logs	7	10
Bedrock	1	7
Flood debris	0	0
Macrophyte beds	0	1
Total	8	18



Plate 2.9: Photograph of non-vegetated upstream control reach, Laura HD, looking downstream.

Table 2.14: Hydro-geomorphic features of non-vegetated upstream control reach, Laura HD, during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.17	0	2.28	1.27
No. features inundated	7	4	7	7
Flow percentile	25.6	100	11.8	16.8
Average velocity (m s^{-1})	0.02	0	0.18	0.13
Channel volume (m^3)	1922	1118	2626	2078
Wetted area (m^2)	2563	2172	2858	2630
Surface area:volume	1.33	1.94	1.09	1.27

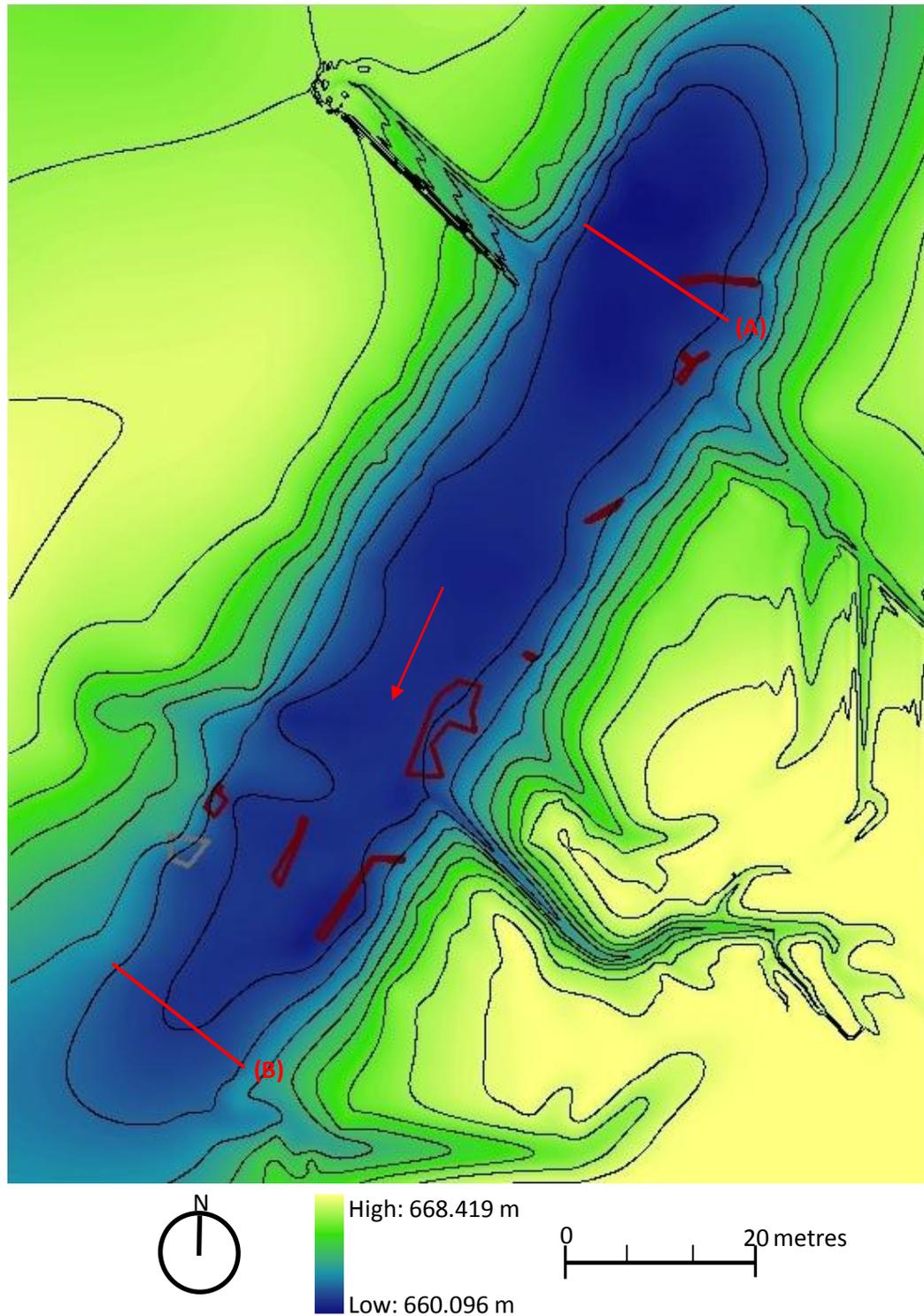


Figure 2.12: DEM of the upstream non-vegetated control, Laura HD, reach, riparian zone and channel features including bedrock (grey) and logs (brown). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.



Plate 2.10: Photograph of non-vegetated downstream control reach, Laura Bridge, looking downstream.

Table 2.15: Hydro-geomorphic features of non-vegetated downstream control reach, Laura Bridge, during the four different sampling periods.

Sampling time	February low flow	May no flow	September high flow	November high flow
Discharge ($\text{m}^3 \text{s}^{-1}$)	0.19	0	2.16	1.16
No. features inundated	10	10	12	12
Flow percentile	33	100	14.5	21.3
Average velocity (m s^{-1})	0.08	0	0.26	0.17
Channel volume (m^3)	1251	728	1963	1596
Wetted area (m^2)	2590	2148	3773	3575
Surface area:volume	2.07	2.95	1.22	1.31

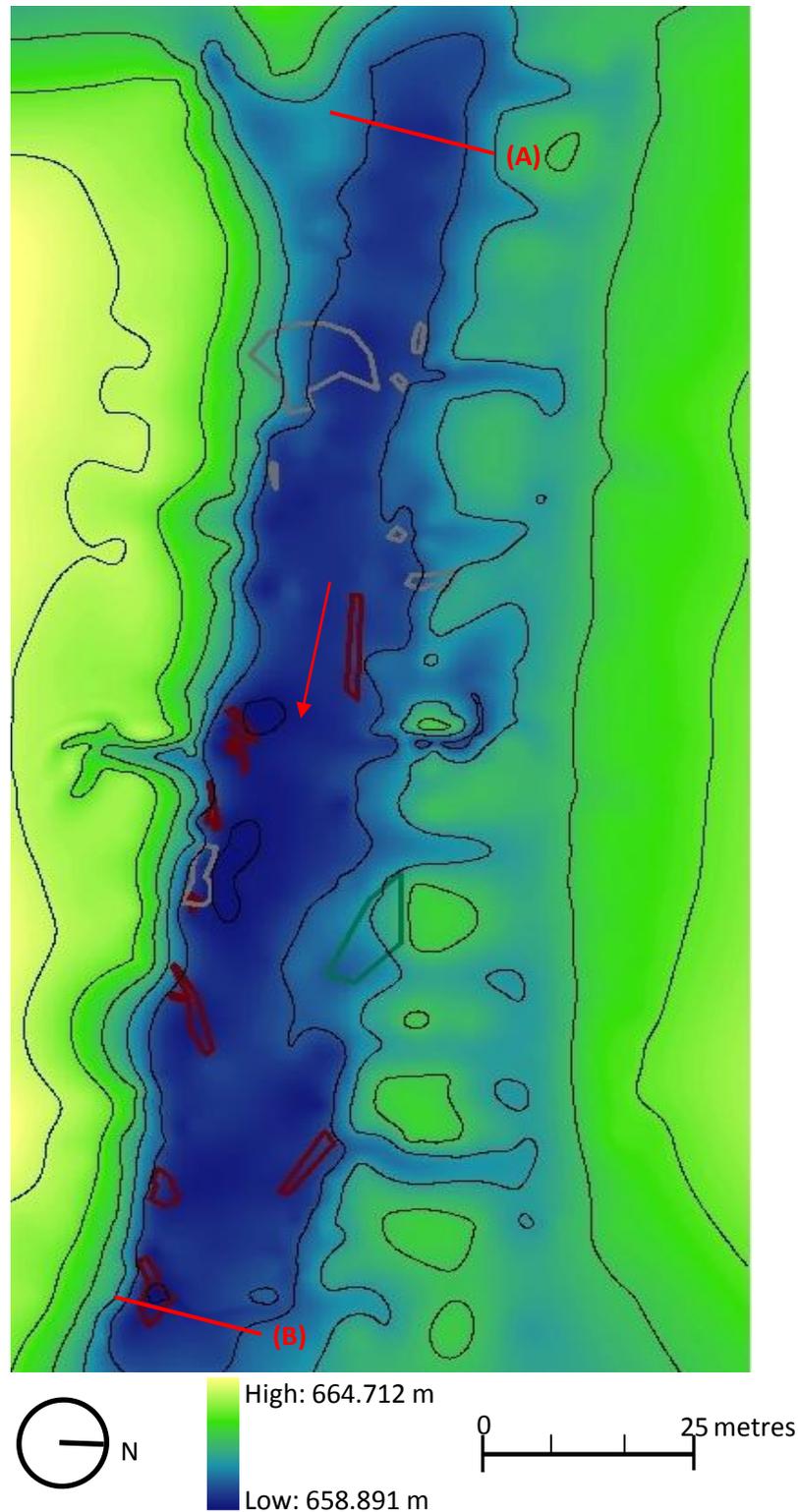


Figure 2.13: DEM of the downstream non-vegetated control, Laura Bridge, reach, riparian zone and channel features including bedrock (grey), logs (brown) and macrophyte bed (green). Contours are set to 1 m interval. Red lines represents the import/upstream (A) and export/downstream (B) transects. Direction of flow is indicated by the red arrow.

2.4 Discussion

Using ground-based surveying equipment allowed for the development of sub-metre scale high-resolution DEMs that could show the morphology of channels in areas that were inundated. These data allowed for discharge, channel volume and wetted area to be calculated at each reach during the four different sampling periods. Spatially explicit maps were also developed using ground-based coordinates and together with the DEMs, the different geomorphic features and complexity of vegetated and non-vegetated reaches were indicated. The ground-based surveying method was able to remove potential interference from aspect, vegetation and turbidity inherent in DEMs created using remotely sensed data. Satellite imagery and LiDAR are compromised in these environments because these methods of data capture rely on interpretation of pixels and the reflection of light from objects respectively (Marcus & Fonstad 2008).

Reach morphology and features

Flood debris accumulation points were only present within the vegetated reaches. Flood debris accumulation points are formed when logs become wedged in between standing trees and/or through interaction with the river and riparian zone morphology (Pettit et al. 2005, Moulin et al. 2011). These features have been shown to increase soil nutrient concentrations and also trap particles and larger woody debris, such as logs (Pettit & Naiman 2005, Wohl & Jaeger 2009). A larger number of trees were present within the vegetated reaches compared to non-vegetated reaches and it appeared that the flood debris accumulation points were formed when logs became wedged in standing trees (*pers. obs.*). This highlights that even a single line of riparian trees can be important in contributing channel geomorphic heterogeneity at reach scales.

Despite flood debris accumulation points only being present within vegetated reaches, this did not always lead to a larger number of single logs present at these reaches compared to non-vegetated reaches. Other studies have shown that the number of logs present within reaches can increase as channel slope increases and channel width decreases (Bilby & Ward 1989, Beechie et al. 2000, Wohl & Jaeger 2009), however, the non-vegetated reaches in the present

study had larger wetted areas and lower channel slope than their paired vegetated reaches. Alternatively, in-stream wood recruitment and decay rates have also been shown to influence the number of logs within reaches (Warren et al. 2007). For example, a study comparing organic matter retention between logged and forested upland streams in Tasmania found that there was an increase in wood present within streams because of the excess debris associated with logging (Watson & Barmuta 2010). Flood events in combination with bank erosion can also destabilise trees within the riparian zone causing them to fall into the channel (Lundström et al. 2008). Long-term grazing by sheep and cattle has shown to result in large amounts of erosion from riparian zones (Myers & Swanson 1991, Prosser et al. 2001), which suggests that the large number of logs within the non-vegetated reaches may have been due to increased erosion at these reaches causing streambank vegetation to fall into the channels. However, as there is no long-term history available on the differences in grazing between the vegetated and non-vegetated reaches it is difficult to validate this suggestion. Despite this lack of history, the larger number of logs present within the non-vegetated reaches will not be sustainable in the long-term as the absence of trees in the riparian zones means these reaches cannot continually supply logs to the main channel.

There were several reaches where only one or two logs were present, despite two of these reaches being vegetated. The active removal of wood from streams (desnagging) was widespread in Australia up until the mid-1990's (Erskine & Webb 2003). Desnagging practices were employed by landholders to increase maximum flow velocity to decrease the frequency of flooding and therefore erosion (Erskine & Webb 2003). It is likely that the small number of logs present in some reaches used in this study, particularly within the vegetated reaches, have been due to desnagging practices by local landholders. The presence of logs in streams has been shown to be important for many biogeochemical processes such as physical entrainment of organic matter and sediment (Erhman & Lamberti 1992, Quinn et al. 2007, Flores et al. 2011), providing substrate for biofilms (Tank & Winterbourn 1995, Ryder et al. 2006) and increasing the hyporheic exchange of water and nutrients (Boulton 2007, Hester & Doyle 2008), which can affect in-stream nutrient stoichiometry. This suggests that the different number of

logs present between reaches could lead to differences in the rates of important biogeochemical processes at the reach scale.

In many of the non-vegetated reaches, there were macrophyte beds of *Eleocharis spp.* lining the main reach channel. In contrast, the macrophytes present within the vegetated reaches were single culms of *Carex spp.* and *Juncus spp.* scattered within the riparian zone. Biomass of emergent macrophyte species such as *Eleocharis spp.* have been shown to increase with decreasing riparian vegetation due to increased PAR within reaches (Bunn et al. 1998). Increases in wetted area and low surface water velocities have also shown to positively influence macrophyte biomass (Carr et al. 1997). Macrophytes have been shown to facilitate sediment deposition, which can increase the abiotic retention of nutrients, particularly P (see Reddy et al. 1999). The fronds and stems of macrophytes have also shown to be important in trapping organic matter (Horvath 2004, Quinn et al. 2007), which may facilitate the biotic uptake of nutrients by heterotrophs. In addition, macrophytes as primary producers can also provide substrate for epiphytic algae, which can further contribute to rates of primary production within reaches (Viches & Giorgi 2010). Therefore, it is likely that a combined effect of increased PAR and low surface water velocity within the non-vegetated reaches has led to the augmentation of macrophyte beds, and this could have implications for the retention of nutrients and rates of whole-system metabolism.

Hydrological parameters

Typically, the non-vegetated reaches had a larger wetted area and volumes of surface water present during each of the four sampling periods. These differences meant that some of the vegetated reaches had higher surface area:volume ratios compared to non-vegetated reaches. Larger wetted areas and high surface area:volume ratios are typical of non-vegetated reaches, where the combination of erosion and vegetation removal has led to increased channel widths (Triska et al. 1984, Hendry et al. 2003). When habitats and soil surfaces become inundated, different ecosystem processes such as microbial denitrification (Clément et al. 2003) can be activated, therefore differences in wetted area between the vegetated and non-vegetated

reaches may suggest there will be an increase in the rates of these processes evident at the whole-reach scale.

Lower surface area:volume ratios and increased roughness from macrophytes can also lead to lower surface water velocities (Bunn et al. 1998, Champion & Tanner 2000), which was evident in the non-vegetated reaches, particularly during the high-flow periods. These conditions can lead to higher surface water temperatures within reaches, which may affect ecological processes that are influenced by temperature such as rates of ecosystem productivity and respiration (Mulholland et al. 2001, Demars et al. 2011). Alternatively, higher surface water velocities can deliver larger concentrations of nutrients to reaches which can also increase ecological processes such as biofilm metabolism (Ryder 2004).

Interaction of hydrology with channel features and morphology

The DEMs were also able to be used to show how the size of the wetted area expanded during the high-flow periods and how the number and type of features inundated changed with discharge. For example, the flood debris accumulation points and macrophyte beds were placed in the outer areas of the main reach channels so an increase in wetted area during the high-flow events meant that these areas would become inundated. As these features can play important roles in the retention of organic matter and the uptake of nutrients (Clarke 2002, Pettit & Naiman 2005), the increase in inundation could lead to larger quantities of organic matter and nutrients retained within the reach. In addition, increased water depths could mean that other geomorphic features such as logs within the main channel could become less important for retention. These potential differences in retention processes highlight the importance of understanding the interaction of geomorphic features with hydrology.

Control streams

There were several differences in the number of geomorphic features and hydrological characteristics present between upstream and downstream reaches within each of the vegetated and non-vegetated control streams. There was a larger number of logs and areas of exposed bedrock and lower surface area:volume ratios at the downstream vegetated control

reach, Booralong Bridge, compared to the upstream vegetated control reach, Booralong CS. Within the non-vegetated control stream, the downstream reach, Laura Bridge, also had more logs present compared to the upstream reach, Laura HD; however, the downstream reach had larger surface area:volume ratios compared to the upstream reach. This indicates that there is natural variability in geomorphic features and hydrological characteristics between reaches on the same stream regardless of the presence of riparian vegetation and may suggest that there also will be natural variability in the rates of some biogeochemical processes such as organic matter retention arising between reaches on the same stream.

Methodology considerations

Collecting topographic points through on-ground surveying techniques is obviously labour-intensive and could potentially be seen as economically inhibiting for many projects. In this study using traditional surveying techniques, it only took 1 day to collect approximately 1300 coordinates, enough to produce a high-resolution DEM for a single reach. A similar surveying study, mapping the topography of a braided gravel-bed river, was able to record 2000+ coordinates in one day (Brasington et al. 2000). Satellite imagery and LiDAR can produce datasets of larger spatial areas more quickly, but to create maps of in-stream channel morphology, geomorphic features and habitats, on-ground surveying is the only technique that can achieve this resolution and allow for the accurate interpretation of areas that are inundated (Frazier et al. 2011 – see Appendix 1).

Reach-scale riparian revegetation is employed by many restoration projects with the aim to restore biogeochemical processes such as organic matter and nutrient retention, through enhancing reach geomorphic complexity (Bernhardt et al. 2005). Different in-stream features and habitats can play specific roles in the cycling of nutrients and organic matter, therefore identifying various features and habitats can create an understanding of which processes will be present at vegetated and non-vegetated reaches. High-resolution DEMs and spatially explicit habitat maps have the ability to identify these features, the complexity of channels and their interaction with the hydrological regime. This mapping is essential to understanding

whether revegetation has facilitated the restoration of biogeochemical processes and in-stream functioning as opposed to the vegetation just acting as a structure in riparian zones.

2.5 Summary

The topographic points collected using on-ground surveying techniques in this study were used to develop high-resolution DEMs and spatially-explicit habitat maps. The high-resolution data allowed for discharge to be estimated at each reach during different sampling periods, facilitating the calculation of reach-scale mass balances. The DEMs also allowed for total volume, wetted area and surface area:volume ratios to be estimated during the different discharges. Overall, the vegetated reaches did not always have a larger number of logs or total number of features present compared to non-vegetated reaches. However, flood debris accumulation points were only found at vegetated reaches, while the non-vegetated reaches had a larger number of macrophyte beds present presumably due to increased light to the streambed. Surface water velocities and surface area:volume ratios were generally higher in vegetated reaches compared to non-vegetated reaches. Geomorphology and hydrology can interact with different ecological or biogeochemical processes such as abiotic and biotic retention of organic matter and nutrients, and ecosystem metabolism. Therefore, the differences in hydro-geomorphic parameters between vegetated and non-vegetated reaches could also lead to differences in ecological processes; each of which will be explored in Chapters 3, 4, 5 and 6.

Chapter 3: Nutrient dynamics within vegetated and non-vegetated reaches.

3.1 Introduction

Surface water nutrients are an essential resource for stream biota to facilitate growth and maintenance. The presence, form and concentration of nutrients and trace elements within streams reflect the catchment geology, soil processes and upstream land use. Within agricultural landscapes, land-use practices such as the widespread removal of vegetation and application of fertilizer has decreased important soil biogeochemical processes and subsequently led to increased nutrient and sediment loads to streams (Harris 2001, Prosser et al. 2001). At the reach-scale, the adjacent riparian zone can also largely influence nutrient dynamics by filtering nutrients from laterally transported water, which then undergo different biochemical transformations (Gregory et al. 1991, Newham et al. 2011). The removal of riparian vegetation has led to a decline in the occurrence and rates of these processes, which has not only contributed to increased in-stream nutrient concentrations, but also resulted in alteration and deterioration of in-stream habitats and channel morphology (Pusey & Arthington 2003, Allan 2004). The combination of these issues can change in-stream ecological processes and the longitudinal ecological functioning of the whole stream (Sabater et al. 2000, von Schiller et al. 2007, Hadwen et al. 2010). Stream restoration projects that aim to reduce in-stream nutrient concentrations commonly use riparian revegetation in the form of vegetative buffer strips. Consequently, monitoring the effectiveness of revegetation and restoration has focused on the quantity of nutrients retained within the riparian zone (see Hoffmann et al. 2009b); rather than the effects on nutrients transported longitudinally in surface water, and potentially exported to non-restored downstream reaches. Therefore, this chapter will investigate the effects of riparian vegetation on surface water nutrient dynamics within intermittent upland streams in an agricultural landscape.

Nutrient concentrations and stoichiometry

Ecological stoichiometry theory recognizes that the ratio of ecosystem elements controls the rates of ecological processes which recycle the elements (Sterner & Elser 2002, Cross et al. 2005). Variation in surface water nutrient concentrations can alter stream nutrient

stoichiometry and related ecological processes. In particular, deviations in the key ecosystem elements carbon (C), nitrogen (N) and phosphorus (P) from the Redfield ratio of 100:16:1 have found to increase or decrease autotrophic growth within oceanic and freshwater systems (Redfield 1958, Elser et al. 2007). Similarly, microbial and fungal decomposition of organic matter is influenced by the nutrient stoichiometry of the organic matter (Hladyz et al. 2009), and the ratio of available nutrients within the system (Sridhar & Bärlocher 2000, Gulis & Suberkropp 2003, Menéndez et al. 2011).

Deviations in nutrient stoichiometry from the Redfield ratio to determine trophic status and autotrophic nutrient limitation have been suggested by a number of studies (Dodds & Welch 2000, Dodds 2003, von Schiller et al. 2008a, Johnson et al. 2009). However, a wide range of other environmental variables such as hydrology (Biggs 1995, Uehlinger 2006) and benthic substrate type (Cardinale et al. 2002, Hoellein et al. 2009), can also indirectly affect ecosystem processes including rates of production and respiration. Consequently, there is some debate as to whether indicators such as nutrient availability can predict autotrophic production in some ecosystems (Biggs 1995, Francoeur et al. 1999, Biggs 2000). The removal of riparian vegetation in agricultural streams can cause variation in many other environmental variables such as increasing light availability and surface water temperature, which can promote autotrophic production (Mosisch et al. 2001, Johnson et al. 2009). It is therefore necessary to establish the interaction between the influence of riparian vegetation and nutrient stoichiometry on ecosystem processes such as autotrophic production.

Bioassays are commonly performed to support the predictions of nutrient limitation within freshwater systems. The majority of assays use inorganic N and P, and their combination, as indicators of the source of nutrient limitation (see Mulholland & Webster 2010). In freshwater systems, only a single study has published the results of bioassays that included trace elements (Chessman et al. 1992), while organic C has been included in several bioassays specifically relating to autotrophic not heterotrophic limitation (Fairchild & Everett 1988, Munn et al. 1989). Dissolved organic carbon (DOC) can be acquired by autotrophs through photoassimilation (Neilson & Lewin 1974) or through algal heterotrophism (Abeliovich &

Weisman 1978, Ellis & Stanford 1982) to be used in addition to their own C-containing metabolic products for growth (Neilson & Lewin 1974, Mayo & Noike 1994). Some autotrophs can become organic-C limited when photosynthesis is limited and results in low stores of C-containing metabolic products (Fairchild & Everett 1988, Barnese & Schelske 1994). Shading by riparian vegetation can obviously limit algal photosynthesis, but alternatively, its' removal can produce extreme high light conditions causing photoinhibition (Üveges & Padisák 2011) and/or damage to their metabolic products (Köhler et al. 2002). In order to develop a better understanding of the effects of riparian vegetation on the sources of autotrophic nutrient limitation, a wider range of nutrient combinations need to be investigated.

Nutrient retention and export

The mass-balance or stoichiometry of C, N, and P in an ecosystem is an estimate of the total mass present. The difference between the total imported and exported masses or loads, determines whether a system is acting as a source or sink of nutrients (von Schiller et al. 2011b). An ecosystem's nutrient stoichiometry or mass-balance is controlled by the biotic and abiotic pathways of nutrient uptake within streams, and forms the important process of longitudinal nutrient spiraling within streams (Newbold et al. 1981). Ecosystems that are acting as nutrient sinks are likely to have a number of abiotic and biotic interception pathways to retain nutrients and organic matter (Brookes et al. 2005). The rate of biotic processes such as autotrophic production and heterotrophic decomposition of organic matter can control the quantity and velocity of in-stream nutrient uptake (Hall & Tank 2003, Bernot et al. 2006, Gibson & O'Reilly 2012), and form the biotic pathways of nutrient retention. In-stream habitat heterogeneity and channel complexity has shown to increase the frequency of abiotic interception pathways for organic matter and nutrients (Wallace et al. 1995, Moutka & Laasonen 2002, Gooseff et al. 2007).

Biotic and abiotic pathways of nutrient uptake have the potential to interact (Brookes et al. 2005) demonstrating that ecological processes are tightly linked to the physical template. For example, protruding channel features such as exposed bedrock can influence the quantity of organic matter retained within a reach (Lepori et al. 2005), which affects the rate of

heterotrophic decomposition and therefore the uptake of surface water dissolved nutrients (Aldridge et al. 2009). Additionally, different channel features and habitats can be responsible for different ecological processes. Macrophyte beds can accumulate large amounts of fine particles that increases nutrient retention and acts as a nutrient sink (Schulz et al. 2003, Kleeberg et al. 2010), while also acting as a source of organic matter and nutrients during decomposition and annual senescence (Agrandi 1991). Flood debris accumulation points at the stream-riparian interface can trap particles and increase local soil nutrients (Pettit & Naiman 2005). The removal of riparian vegetation can alter and decrease in-stream habitat heterogeneity and channel complexity, potentially reducing the number of interception pathways (Brookes et al. 2005).

The link between ecological processes and the physical template within streams is largely affected by the hydrological regime. Changes in the frequency, duration and magnitude of discharge can alter the nutrient and organic matter loads and sources within the system (Drewry et al. 2006, Acuña et al. 2007, Blanco et al. 2010, Raymond & Sayers 2010), which can affect the rate of in-stream ecological processes and the number of interception pathways (McClain et al. 2003, Quinn et al. 2007, Vidon et al. 2010). Temporary-river ecology suggests that an increase in discharge will connect in-stream habitats and homogenize ecological processes between reaches on the same stream (Larned et al. 2010). During periods of low-flow and no-flow when there is contraction and fragmentation of surface water, habitats between reaches become more heterogeneous and there is the potential for different ecological processes to be present in different reaches, for example, higher rates of respiration in reaches with large amounts of organic matter. When surface water expands and flows providing longitudinal connectivity and habitat re-wetting, different biotic and abiotic pathways such as longitudinal nutrient spiralling processes can be initiated (Stanley et al. 1997, Dent & Grimm 1999, Larned et al. 2010). This suggests that the contrast in ecological processes which affect nutrient retention between vegetated and non-vegetated reaches will be most evident during low and no-flow periods.

Aims

The objective of this chapter is to investigate the stoichiometry, mass-balance and retention of nutrients within vegetated and non-vegetated reaches, during different hydrological conditions.

- 1) Through water column nutrient surveys I aim to investigate whether there are differences in the concentration and stoichiometry of C, N and P between vegetated and non-vegetated reaches. I predict that there would be higher C:N and C:P ratios within vegetated reaches compared to non-vegetated reaches, and that this difference will be more pronounced during low-flow periods.
- 2) Past research has shown that deviations from the Redfield nutrient ratios can indicate autotrophic nutrient limitation (of production). To test the application of this theory in upland agricultural streams, I will use nutrient enrichment experiments (bioassays) to determine if the stoichiometric ratios were indicative of autotrophic limitation. I also aim to determine if there is related reach-scale retention of the most limiting nutrient to autotrophic production.
- 3) The final objective is to determine if the presence of riparian vegetation can alter the potential for a reach to act as a nutrient source or sink within agricultural streams. I compare the retention or export of C, N, and P at vegetated and non-vegetated reaches across three different discharges, by quantifying the nutrient mass-balances (imported and exported loads) at each reach. I hypothesise that there will be less nutrient retention associated with high discharge, but that high discharge events could change the magnitude of the transported load.

3.2 Methods

Experimental design

To determine the effect of riparian vegetation, hydrology and their interaction on nutrient stoichiometry and retention, samples were collected from ten different stream reaches, at four different discharges. It is important to note that the four different sampling times also had four different combinations of discharge and temperature and included: February 2010 (low flow, high temperature), May 2010 (no flow, moderate temperature), September 2010 (high flow, low temperature), and November 2010 (high flow, moderate temperature). The spatial design of the study followed a matched-pairs approach that included a pair of reaches from five different streams - an approach that allowed control of specific sub-catchment differences. Three streams, Moredun Creek, Gwydir River and Roumalla Creek were considered treatment streams, and consisted of an upstream non-vegetated reach and a downstream vegetated reach. The non-vegetated reach was located upstream to ensure that sampling remained spatially independent of any effects arising from the treatment (the vegetated reach) during periods of hydrologic connectivity. To discriminate between the main treatment effect of vegetation, and the natural longitudinal change in nutrient dynamics inherent in flowing systems, two control streams were also selected. These streams were Laura Creek and Booralong Creek, and consisted of a pair of non-vegetated and vegetated reaches respectively. The descriptions of each reach are outlined in Chapter 2.

Nutrient stoichiometry and retention surveys

Water column samples were collected in triplicate from fixed upstream and downstream locations within each 100 m reach. Samples were collected for analysis of total nitrogen (TN), total phosphorus (TP), dissolved inorganic nitrogen (NO_x), soluble reactive phosphorus (SRP), dissolved organic carbon (DOC), and total suspended solids (TSS). The NO_x and SRP samples were filtered in the field through glass fibre filters (0.7 µm pore size Whatman GF/F). Additionally, aliquots of filtered water were refiltered through membrane filters (0.45 µm pore size, Millipore) in the field for DOC analyses. All samples were kept cool and dark until return to the laboratory. On each sampling occasion, pH, temperature, conductivity (mS cm⁻¹), and

dissolved oxygen (mg L^{-1}) were measured in the field using a WP-81 meter (TPS, Brisbane, Australia) and an Orion Star Plus meter (USA). The temperature, TSS, pH, conductivity and dissolved oxygen (DO) results are listed in Appendix 2. Flow velocity was also measured during periods of flow using a flowmeter (Marsh McBirney Flowmate, model 2000), following the methods outlined in Chapter 2 (page 22).

The NO_x and SRP concentrations were determined spectrophotometrically, using standard laboratory methods (NO_x – Woods et al. 1967; SRP – Murphy & Riley 1962). The SRP samples were refiltered before analysis to ensure that there would be no interference from particulates formed from freezing. The TP and TN samples were digested with persulfate (Hosomi & Sudo 1986), and the concentrations analysed using a Skalar autosampler attached to a Shimadzu UV-110 spectrophotometer. The concentration of DOC was determined using a Sievers InnovOx Laboratory TOC Analyser. All nutrient analyses took place within laboratories at the University of New England.

The TSS water samples were processed within 24 hours of collection. Samples were processed by filtering a known volume of water through an ashed (3 hours at 540°C), pre-weighed glass fibre filter ($0.7 \mu\text{m}$ pore size Whatman GF/F), which was then stored in separate foil envelopes at -4°C , until further analysis. Each filter was dried for 24 hours at 70°C and weighed to four decimal points, to determine TSS concentration (mg L^{-1}).

Nutrient enrichment experiment

To determine if autotrophic production within each treatment was limited by C, N, and/or P, nutrient enrichment experiments were conducted at each site, during each sampling period. Algal biomass net accumulation (measured as chlorophyll *a*) were measured in response to different nutrient treatments, diffusing from amended agar in small plastic pots (80 mL volume, 4.2 cm diameter).

Agar pot preparation

The different nutrient enrichment treatments were prepared by amending 1% agar solution with different combinations of salts (Table 3.1). The agar and agar plus nutrient solutions were heated to boiling point using a microwave (NEC 800W N728E) and poured into the 80 mL plastic containers. I ensured that the level of the set agar was above the lip of the pot, so there would be maximum diffusion from the agar upon deployment. Each pot was covered with a damp glass fibre filter (0.7 µm pore size Whatman GF/F), and a 5 sq. cm piece of material (650 mm pore-size, red waterproof polyester, Broadway Textiles, Sydney), and was secured using the rims cut out from each container lid. All pots were stored at 4°C between 24 and 38 hours before deployment at the sites.

Table 3.1: List of the eight different nutrient treatments (C = carbon, N = nitrogen, P = phosphorus) and the combination of salts used to amend the 1% agar. Note that no nutrients were added to the procedural control.

Treatment	0.5 M C ₆ H ₁₂ O ₆	0.5 M NaNO ₃	0.5 M KH ₂ PO ₄ ⁻	1 % Agar
C	X			X
N		X		X
P			X	X
C + N	X	X		X
C + P	X		X	X
C + N + P	X	X	X	X
N + P		X	X	X
Control				X

Deployment and collection of agar pots

A total of 24 agar pots (8 x treatments, 3 x replicates) were deployed at each site for 21 days during February 2010, May 2010, September 2010 and November 2010. At each site the pots were randomly arranged into six lengths of rigid, plastic mesh (5 mm mesh size). Each length of mesh had four pre-cut holes, and the pots were secured into the mesh using rubber bands. The mesh and pots were placed in a straight line across a randomly selected section of each

channel, perpendicular to the direction of flow. Four tent pegs were used to secure the mesh and pots to the stream bed.

Water column samples were collected from each site at each deployment and collection time. The concentration of TN, TP, NO_x, SRP, DOC, chlorophyll *a*, and TSS were determined using the methods outlined above. Measurements of pH, temperature, conductivity (mS cm⁻¹), and dissolved oxygen (mg L⁻¹) were also made at these times, using a WP-81 meter (TPS, Brisbane, Australia) and an Orion Star Plus meter (USA).

During the May 2010 deployment, both the Booralong CS and Moredun Vegetated reaches were completely dry, however, the pots were still deployed in the dry streambed. Due to the unexpected force of the high discharges during the September 2010 deployment, replicates from specific nutrient treatments at five of the ten reaches were lost, and all of the agar pots deployed at the Gwydir vegetated reach were lost during September and November 2010 periods. A high rainfall event occurred during the collection of the November 2010 experiment, which meant that three reaches (Gwydir non-vegetated, Gwydir vegetated, and Roumalla non-vegetated) could not be retrieved until 25 days after deployment.

The mesh and pots were removed from the stream bed, with care not to disturb any biofilm growing on the polyester material. While still in the field, the material was lifted from the pots and cut into 4 cm diameter disks, which were then cut in half and placed into labelled tubes. All samples were kept dark and cool while being transported back to the laboratory. Samples were frozen until analysis.

As a measure of net algal accumulation, chlorophyll *a* was extracted from each agar pot sample (one half of the material disks) using 90% acetone and the concentration determined spectrophotmetrically, following the method of Parsons et al. (1984).

*Statistical analyses**Nutrient concentrations*

To identify significant differences in the concentration of specific nutrients between vegetated and non-vegetated reaches and across time, the water column concentration of each component were analysed using a mixed-model ANOVA. The model consisted of three factors: i) Treatment – vegetated and non-vegetated ($a=2$; fixed), ii) Stream – Moredun Creek, Gwydir River, Roumalla Creek, Booralong Creek and Laura Creek ($b=5$; random), iii) Time – four sampling occasions ($c=4$; random).

Triplicate water column samples were collected from each reach at each time. All analyses used nutrient concentration values from downstream sampling points of each reach ($n=3$). During periods of no-flow, the samples were taken from the largest pool.

The term ‘treatment’ was classed as a fixed factor because the vegetated reaches at the three treatment streams had to be located downstream. Secondly, the vegetated reaches had to specifically have *Casuarina cunninghamiana* Miq. trees lining the stream channel edge.

The mixed-model ANOVA included the term ‘stream’ to discriminate between sub-catchment effects that may compromise any treatment effects, for example, high nutrient loadings during a sub-catchment specific high rainfall event. The term ‘stream’ was classed as a random factor because the streams were chosen as a representative of upland agricultural streams present within the Gwydir River catchment.

The term ‘time’ was classed as a random factor and was chosen to represent any combination of temperature and stream discharge. It was never intended to sample at specific times or flow events and as such, the sampling times occurred randomly with an aim to represent only a range of conditions.

To test whether any significant differences in nutrient concentration could occur from natural longitudinal changes, data for each nutrient component collected from the two control streams was first analysed using three separate one-way ANOVAs with ‘location’ as the effect. The term

'location' consisted of two levels – upstream and downstream ($a=2$) and was a fixed factor. Two of the ANOVA analyses were for each of the two control streams, where concentrations from the upstream reaches were compared to the downstream reaches, and data were pooled across all times within each reach. A third one-way ANOVA test of location was also performed, where the data from both control streams were combined, to provide further evidence and information of location effects (tables of formulae are listed in Appendix 3, tables of results are listed in Appendix 4). A significant difference in location meant that any differences arising in the main test of the treatment (vegetation) may be compromised by location, and would consequently have to be interpreted with caution.

The analyses of the main test and location test were conducted using the PERMANOVA program (PERMANOVA+ for Primer, ePrimer, Plymouth, UK), which performs non-parametric permutational tests on univariate data. A distance or dissimilarity matrix of the data is first constructed using a selected measure (i.e. Euclidean distance for environmental data), and a pseudo- F test statistic is calculated from sums of squares partitioned from the matrix. The distribution of the pseudo- F statistic is then achieved through permutations of the matrices. It is similar to the original F statistic determined in traditional ANOVA where, as the pseudo- F statistic gets larger, the probability that the null hypothesis is true decreases. Each PERMANOVA test was set at 999 permutations, which is recommended to interpret the data at a significance level of 0.05 (Anderson et al. 2008). During several of the one-way tests of location the number of unique permutations was considerably less, but the validity of each test was considered individually as opposed to ignoring each test with low permutations. Euclidean distance was used to construct the matrices as it is generally accepted that this measure is suitable for physico-environmental variables (Anderson et al. 2008). Although testing univariate data using pseudo- F statistics developed from Euclidean distance matrices in PERMANOVA will yield the same F -statistic generated in traditional ANOVA tests (Legendre & Anderson 1999), the PERMANOVA test was selected because it could easily handle unbalanced designs (missing observations), which occurred when sampling could not occur at dry reaches during May (and also because of high flows removing nutrient enrichment experiments during September and November, see Chapter 2). While PERMANOVA tests can handle non-normal datasets, the test

is sensitive to differences in dispersions (i.e. heteroskedasticity) and outliers. The nutrient concentration data were transformed to meet these assumptions using the following transformations: TN – $\log_{(x)}$; TP – $1/x$; NO_x – Box-Cox; SRP – $1/x$. A test within the PERMANOVA program that is specifically designed to test for differences in dispersions, PERMDISP, was used to test whether differences in dispersions could be contributing to the differences detected by the PERMANOVA test. Only significant differences in dispersions are reported.

The statistical power of the main test was increased through replication at the treatment level, whereby the data from both the control and treatment streams were combined so that the analyses included five vegetated and five non-vegetated reaches. The inclusion of the data from the two control streams meant that the statistical design became unbalanced (an unequal number of replicate samples within each factor level) and as such, the degrees of freedom were altered in the tests of interactions between the main factors. The consequences of the unbalanced design mean that the multipliers on individual components of variation in the expected mean squares are not necessarily whole numbers and secondly, the main effects of factors and the interaction terms are no longer independent of each other (i.e. there will be some overlap among terms regarding the individual portions of variation they explain, Anderson et al. 2008). The PERMANOVA program can handle unbalanced designs of this nature, however, it means that the formulae originally developed from first principles for a balanced statistical design (Table 3.2, Underwood 1997) will be incorrect. Instead, the correct multipliers for the expected mean squares will change with the sample size (Anderson et al. 2008) and therefore, the tables of formulae for each different variable analysed in this study are listed in Appendix 5.

Nutrient stoichiometry

Nutrient molar ratios were calculated in order to determine how differences in nutrient concentrations affected water column nutrient stoichiometry. Two sets of C:N:P ratios were calculated; one using the dissolved nutrient fractions (NO_x and SRP), and the other using the total (particulate) nutrient fractions (TN and TP). In both cases, DOC was used as the carbon component. To determine whether there was a significant difference in molar ratios between

vegetated and non-vegetated reaches, data were analysed through multidimensional scaling (MDS) plots and a three-factor statistical model using PERMANOVA. The three-factor model was described in the above section but briefly, consisted of: i) Treatment – vegetated and non-vegetated ($a=2$; fixed), ii) Stream ($b=5$; random) and iii) Time – four sampling occasions ($c=4$; random). The three ratio components (molar concentrations of N, P and C) were treated as three separate variables. The ratios were calculated using an average of the triplicate nutrient concentrations, hence $n=1$ for each reach at each sampling time. Correct formulae for the unbalanced design are listed in Appendix 5.

To test for significant differences in nutrient stoichiometry that may occur from natural longitudinal change, data were combined from two the control streams and analysed using the one-way ANOVA model described above. Data from a single control stream could not be used because there were not enough unique permutations (tables of formulae are listed in Appendix 3, tables of results are listed in Appendix 4). Each nutrient variable was also transformed following the principles and transformations described above.

Nutrient enrichment experiment

The nutrient enrichment experiment was conducted to test whether nutrient stoichiometric ratios were indicative of autotrophic nutrient limitation. I also sought to determine whether autotrophic production was limited by the same nutrients or a combination of nutrients within vegetated and non-vegetated reaches. Autotrophic nutrient limitation was determined by assuming that the highest concentration of chlorophyll *a* had accumulated on the most limiting nutrient or combination. The chlorophyll *a* data were analysed using MDS plots and the three-factor mixed-model ANOVA. Prior to the main test, data from the control streams were tested for the effects of location using the one-way ANOVA model described above, which allowed for any significant differences due to natural longitudinal change to be elucidated. Each replicate pot was considered a single sample, hence $n=3$ per site. The analyses were conducted with the multivariate statistical program, PERMANOVA, and the correct formulae for the unbalanced design are listed in Appendix 5. The data matrix included the chlorophyll *a* concentrations (mg g^{-1}) from the eight treatments (C, N, P, CNP, NP, CN, CP, A - control), which were considered

different variables and Euclidean distance was used as the resemblance measure. The chlorophyll *a* data were transformed (\log_{x+1}) prior to analysis to reduce the effects of heterogeneous dispersions, which can increase type 1 error rates and is an assumption of the PERMANOVA test. PERMDISP was used to test for differences in dispersions.

Nutrient retention and export

To determine the effects of vegetation and hydrology on the retention or export of nutrients (i.e. whether the reach acts as a source or sink of nutrients), water column samples were collected from fixed upstream and downstream locations at each reach. For each sampling occasion the average of triplicate nutrient concentrations were transformed into daily loads by multiplying the mean concentration with total daily discharge ($\text{m}^3 \text{day}^{-1}$). Daily discharge was calculated for each sampling occasion following the methods outlined in Chapter 2. Retention or export was then determined by subtracting the exported (downstream) load from the imported (upstream) load (Von Schiller et al. 2011b). A resulting negative value meant that the reach was retaining nutrients (acting as a sink), while a positive value indicated the reach was exporting nutrients (acting as a source).

The effects of vegetation and time on the daily retained/exported nutrient loads were analysed using the three-factor ANOVA described in the above section. Each nutrient variable was tested individually and also as a multivariate dataset within the PERMANOVA program. All nutrient loads were transformed (TP, NO_x, SRP, DOC – square-root with the sign reinstated; TN – cube-root with the sign reinstated) and normalised before analysis. Euclidean distance was used as the resemblance measure and PERMDISP was used to check for differences in dispersions.

To test for significant differences in nutrient stoichiometry that may occur from natural longitudinal change, data were combined from the two control streams were analysed using the one-way ANOVA model described above. Data from a single control stream could not be used because there were not enough unique permutations (tables of formulae are listed in Appendix 3, tables of results are listed in Appendix 4).

I also wanted to explore whether different hydro-geomorphic variables were correlated with nutrient retention at vegetated compared to non-vegetated reaches. Correlation analyses using the BEST program within Primer (ePrimer, Plymouth, UK) were performed on each nutrient individually and also as a multivariate dataset. Data collected during May were not included in the PERMANOVA data because loads were zero due to no flow, therefore I did not want to compromise the role of other hydrological and physical features during discharge periods. The hydro-geomorphic variables (outlined in Chapter 2) used in the analyses included: combined number of logs and sections of bedrock, flow percentile values (%), $\log_{(x+1)}$ transformed), velocity (m s^{-1} , $\log_{(x+0.01)}$ transformed), surface area:volume ratio, and wetted area (m^2).

All statistical analyses throughout the thesis were interpreted as significant if the resultant p -value was less than 0.05.

Table 3.2: Table of formulae for a balanced statistical design. Treatment is a fixed factor and has two levels ($a=2$), stream is a random factor and has five levels ($b=5$), and time is a random factor with four levels ($c=4$). Tables of correct formulae for the unbalanced design are listed in Appendix 5 and will be referred to in the results section.

Source of variation	Multipliers				Degrees of freedom	Expected mean square	Variance component
	i	j	k	r			
1 Treatment = Tr	0	b	c	n	a-1	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2 + bn\sigma_{\text{TrTi}}^2 + cn\sigma_{\text{TrSt}}^2 + bc n\sigma_{\text{Tr}}^2$	$(MS_{\text{Tr}} - MS_e)/bcn$
2 Stream = St	a	1	c	n	b-1	$\sigma_e^2 + a n\sigma_{\text{StTi}}^2 + a c n\sigma_{\text{St}}^2$	$(MS_{\text{St}} - MS_e)/acn$
3 Time = Ti	a	b	1	n	c-1	$\sigma_e^2 + a n\sigma_{\text{StTi}}^2 + a b n\sigma_{\text{Ti}}^2$	$(MS_{\text{Ti}} - MS_e)/abn$
4 Tr x St	0	1	c	n	$(a-1)(b-1)$	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2 + c n\sigma_{\text{TrSt}}^2$	$(MS_{\text{TrSt}} - MS_e)/cn$
5 Tr x Ti	0	b	1	n	$(a-1)(c-1)$	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2 + b n\sigma_{\text{TrTi}}^2$	$(MS_{\text{TrTi}} - MS_e)/bn$
6 St x Ti	a	1	1	n	$(b-1)(c-1)$	$\sigma_e^2 + a n\sigma_{\text{StTi}}^2$	$(MS_{\text{StTi}} - MS_e)/an$
7 Tr x St x Ti	0	1	1	n	$(a-1)(b-1)(c-1)$	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2$	$(MS_{\text{TrStTi}} - MS_e)/n$
8 Residual = e	1	1	1	1	$abc(n-1)$	σ_e^2	MS_e

3.3 Results

Nutrient concentrations

Dissolved inorganic nitrogen

There was no significant effect of riparian vegetation on NO_x concentrations (Table 3.3). The three PERMANOVA tests for the effect of location in the control streams were also not significant when samples were pooled across time (Appendix 4, Table A4.1). On most sampling occasions, NO_x concentrations were below 0.04 mg L⁻¹ across all reaches (Figure 3.1). There was large variability in NO_x concentration between streams and across the four sampling periods, which was confirmed by the significant interaction between streams (St) and time (Ti) ($p = 0.001$), and the significant difference in NO_x concentration between streams ($p = 0.02$, Table 3.3). During May, a period of no-flow, only three of the eight study reaches (that could be sampled) had detectable concentrations of NO_x. In contrast, the highest NO_x concentration of 0.16 mg L⁻¹ was recorded at Gwydir Vegetated also during May. All reaches recorded detectable concentrations of NO_x during September, a high-flow period, with the exception of the Booralong Creek reaches. There was also a large amount of variation within triplicate samples as indicated by the large standard error bars (Figure 3.1).

Table 3.3: Results of PERMANOVA main test for significant differences in NO_x concentration (mg L⁻¹) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	8.474e ⁶	8.474e ⁶	1.026	0.444
St	4	1.134e ⁹	2.834e ⁸	4.521	0.020
Ti	3	3.599e ⁸	1.120e ⁸	1.743	0.214
TrxSt**	2	4.356e ⁷	2.178e ⁷	0.674	0.529
TrxTi	3	5.184e ⁷	1.728e ⁷	0.535	0.676
StxTi	12	7.775e ⁸	6.479e ⁷	4.680	0.001
TrxStxTi**	5	1.616e ⁸	3.231e ⁷	2.334	0.054
Res	83	1.149e ⁹	1.384e ⁷		
Total	113	3.673e ⁹			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.1 for correct formulae.

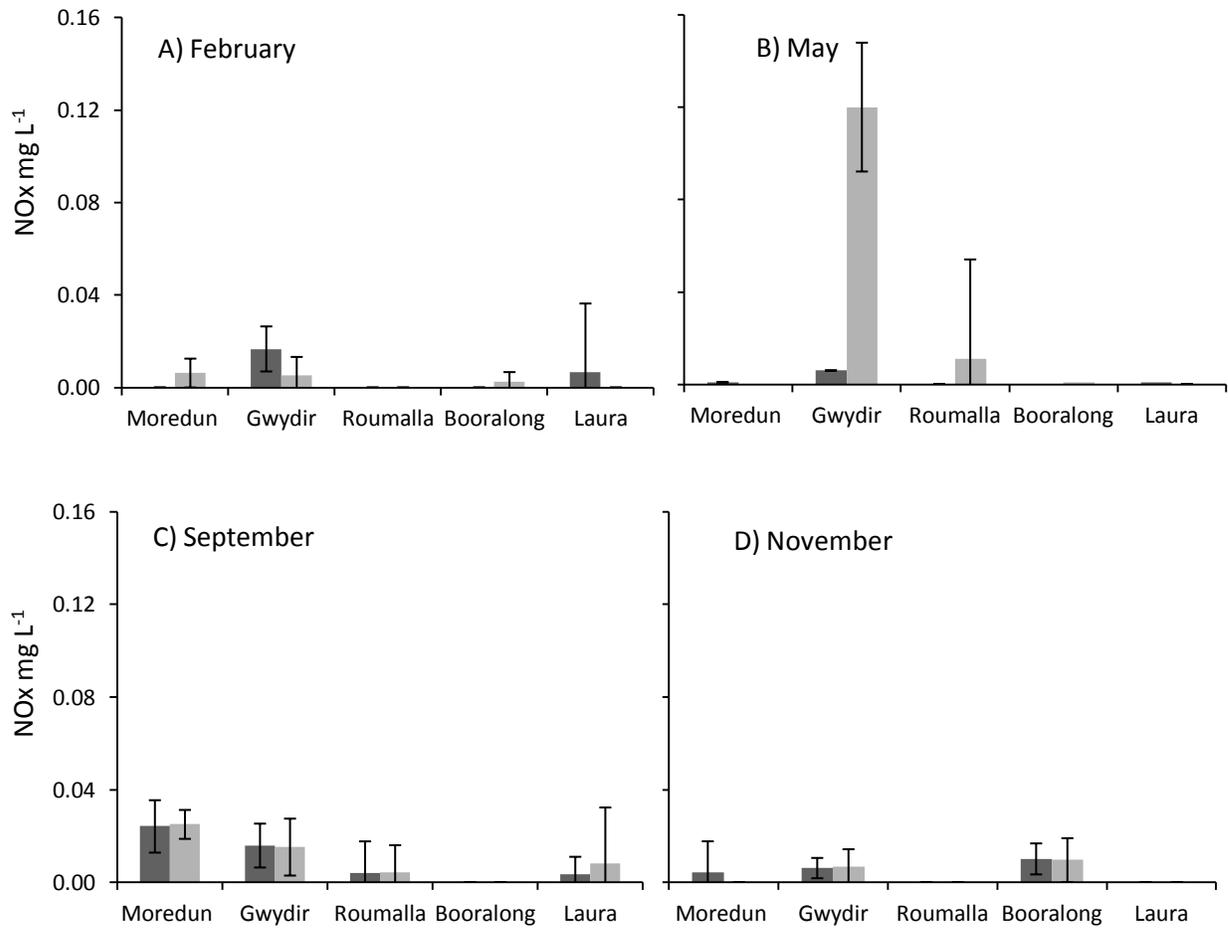


Figure 3.1: Mean surface water NOx concentration (mg L⁻¹) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are standard error of the sample mean (n=3). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Soluble reactive phosphorus

There was no significant effect of riparian vegetation on SRP concentration. The three PERMANOVA tests for the effect of location in the control streams were also not significant when samples were pooled across time (Appendix 4, Table A4.2). The variation in SRP concentration between streams and sampling times was found to be statistically significant ($p = 0.001$, Table 3.4). There was also a significant effect of hydrology (time) on SRP concentration ($p = 0.003$, Table 3.4). The highest mean concentrations of SRP were recorded at all downstream reaches during February (ranging between $0.12 - 0.28 \text{ mg L}^{-1}$), the low-flow period, with the largest within stream differences occurring at Roumalla Creek and Booralong Creek (Figure 3.2). Throughout all other sampling periods, mean SRP concentration was below 0.1 mg L^{-1} , with the exception of Booralong Bridge during May.

Table 3.4: Results of PERMANOVA main test for significant differences in SRP concentration (mg L^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	516.050	516.050	0.993	0.465
St	4	10630.000	2657.400	1.238	0.373
Ti	3	$1.009e^5$	33626.000	14.069	0.003
TrxSt**	2	403.010	201.510	2.258	0.206
TrxTi	3	1266.100	422.030	4.729	0.078
StxTi	12	26789.000	2232.400	14.583	0.001
TrxStxTi**	5	446.180	89.237	0.583	0.700
Res	83	12706.000	153.080		
Total	113	$1.635e^5$			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.1 for correct formulae.

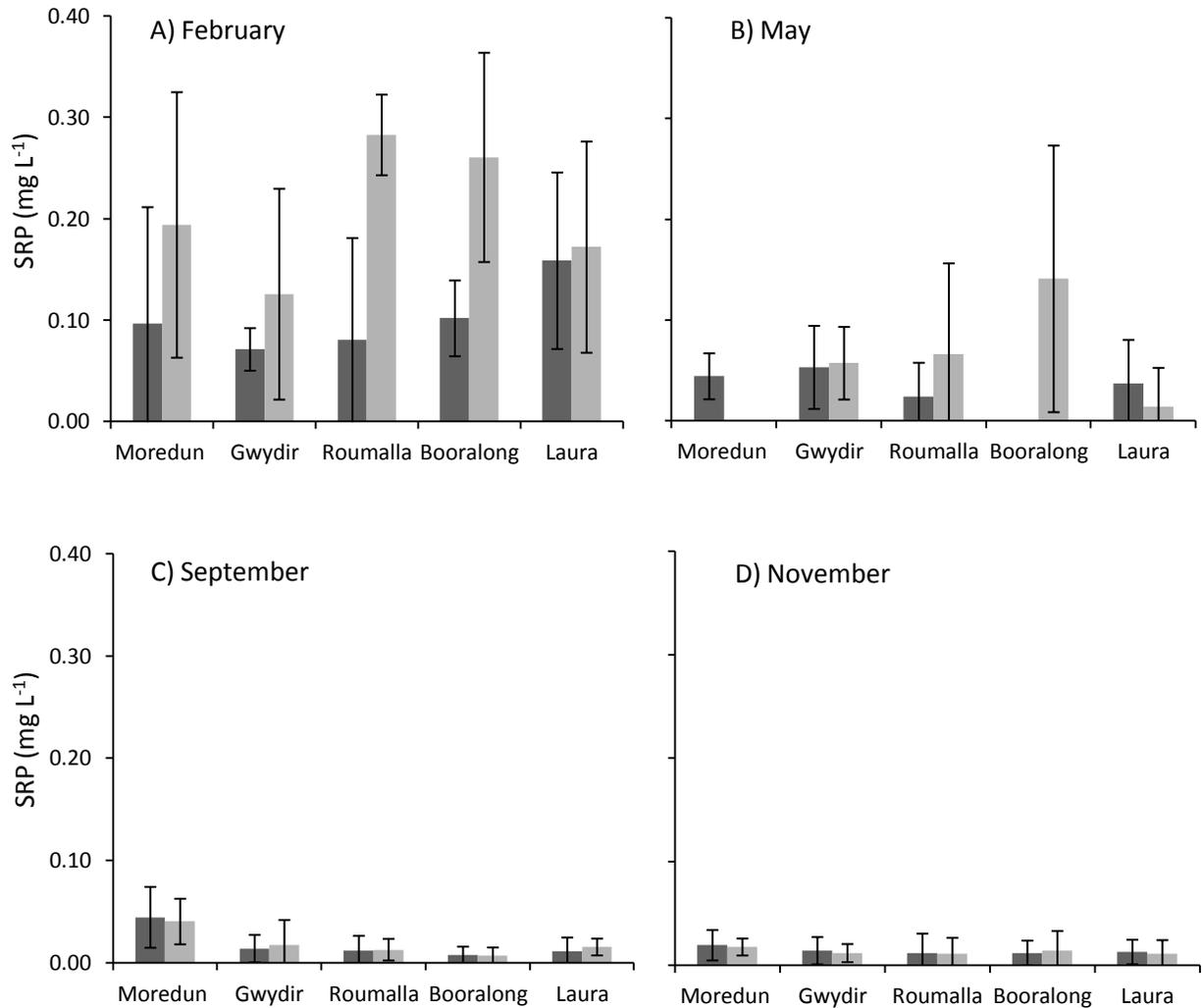


Figure 3.2: Mean surface water SRP concentration (mg L⁻¹) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are standard error of the sample mean (n=3). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Total nitrogen

There was no significant effect of vegetation on TN concentration, however, there was a significant effect of hydrology (time) ($p = 0.001$, Table 3.5). There was also a significant interaction between treatment (Tr), stream (St) and time (Ti) ($p = 0.001$, Table 3.5). The tests of location found that there were no significant differences between reaches on the non-vegetated control stream, Laura Creek; however, there were significant differences between reaches within the vegetated control stream, Booralong Creek ($p = 0.024$). When the two control streams were combined the location test was not significant (Appendix 4, Table A4.3). TN concentrations were highest during the May, the no-flow period, ranging between 1.66 to 3.12 mg L⁻¹ (Figure 3.3). During September and November, the high-flow periods, mean TN concentrations decreased to 0.58 to 0.94 mg L⁻¹ (Figure 3.3). The largest difference in mean TN concentration between the vegetated and non-vegetated reaches occurred during February, the low-flow sampling period, with minimal variation between paired reaches during the high-flow periods (Figure 3.3).

Table 3.5: Results of PERMANOVA main test for significant differences in TN concentration (mg L⁻¹) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	3.127e ⁻²	3.127e ⁻²	1.169	0.428
St	4	0.234	5.837e ⁻²	1.251	0.327
Ti	3	3.444	1.148	22.232	0.001
TrxSt**	2	3.553e ⁻²	1.777e ⁻²	0.265	0.791
TrxTi	3	0.198	6.582e ⁻²	0.982	0.456
StxTi	12	0.579	4.824e ⁻²	4.824	0.001
TrxStxTi**	5	0.335	6.700e ⁻²	6.699	0.001
Res	83	0.830	1.000e ⁻²		
Total	113	6.447			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.1 for correct formulae.

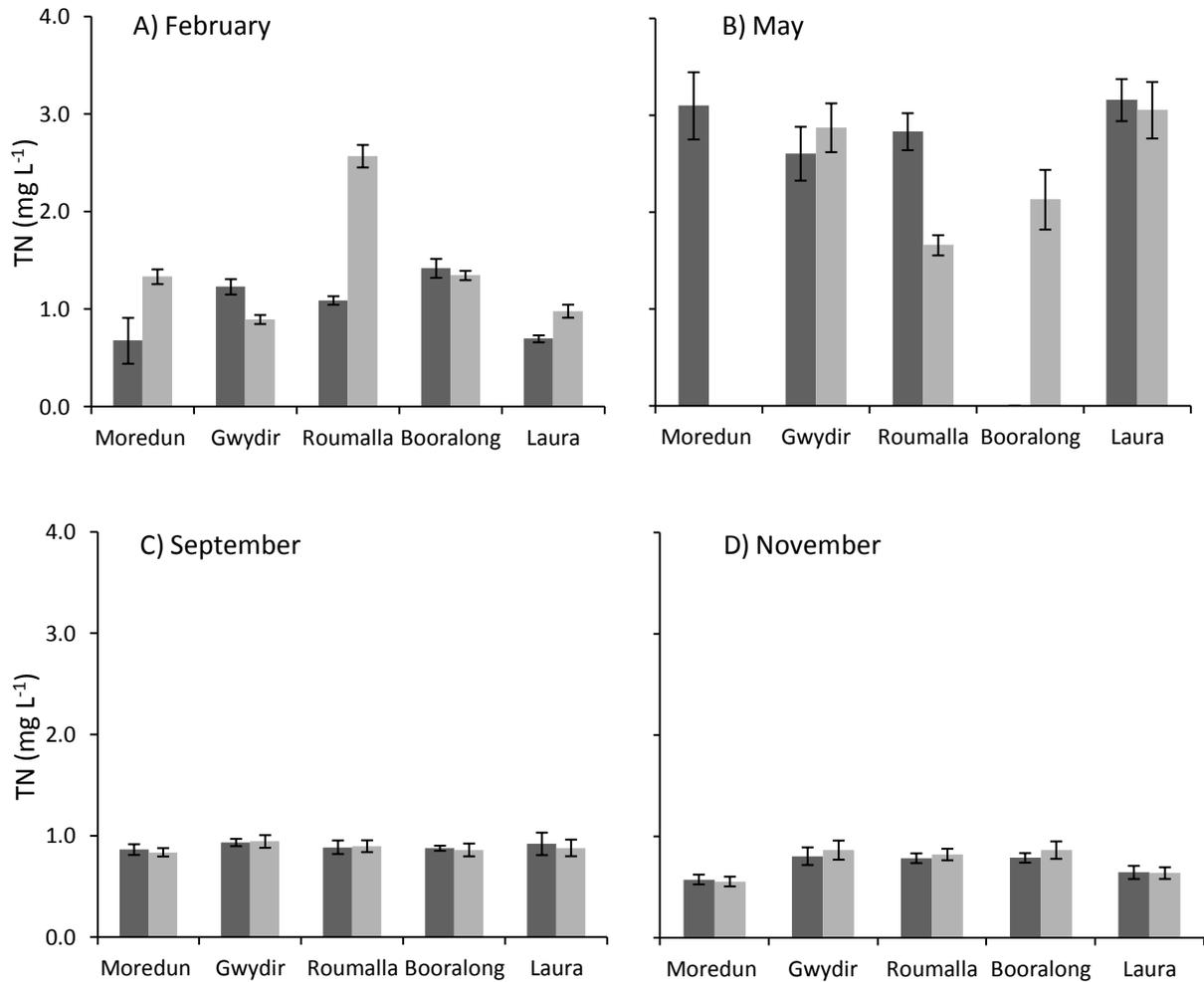


Figure 3.3: Mean surface water TN concentration (mg L⁻¹) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are standard error of the sample mean (n=3). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Total phosphorus

There was no significant effect of riparian vegetation on mean TP concentration. There was a significant effect of hydrology ($p = 0.004$) on TP concentration and there was a significant interaction between streams and hydrology ($p = 0.001$, Table 3.6). The tests for differences in TP concentration between locations on control streams were all non-significant (Appendix 4, Table A4.4). Mean TP concentrations were highest during February and May, the low and no-flow periods, and ranged between 0.08 to 0.20 mg L⁻¹ (Figure 3.4). During the high-flow periods, the mean TP concentration decreased across all reaches and ranged between 0.04 to 0.11 mg L⁻¹ (Figure 3.4). In the three treatment streams, mean TP concentration was higher at all the downstream vegetated reaches during February and conversely, the mean concentration was lower at downstream reaches in both control streams (Figure 3.4). There was little variation in mean TP concentration between reaches on the same stream during September and November, the high-flow periods.

Table 3.6: Results of PERMANOVA main test for significant differences in TP concentration (mg L⁻¹) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	7.178	7.178	0.842	0.538
St	4	134.510	33.627	0.605	0.642
Ti	3	2589.200	863.060	13.949	0.004
TrxSt**	2	3.479	1.739	0.623	0.585
TrxTi	3	30.940	10.313	3.694	0.087
StxTi	12	693.470	57.789	15.444	0.001
TrxStxTi**	5	13.959	2.792	0.746	0.562
Res	83	310.570	3.742		
Total	113	4050.300			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.1 for correct formulae.

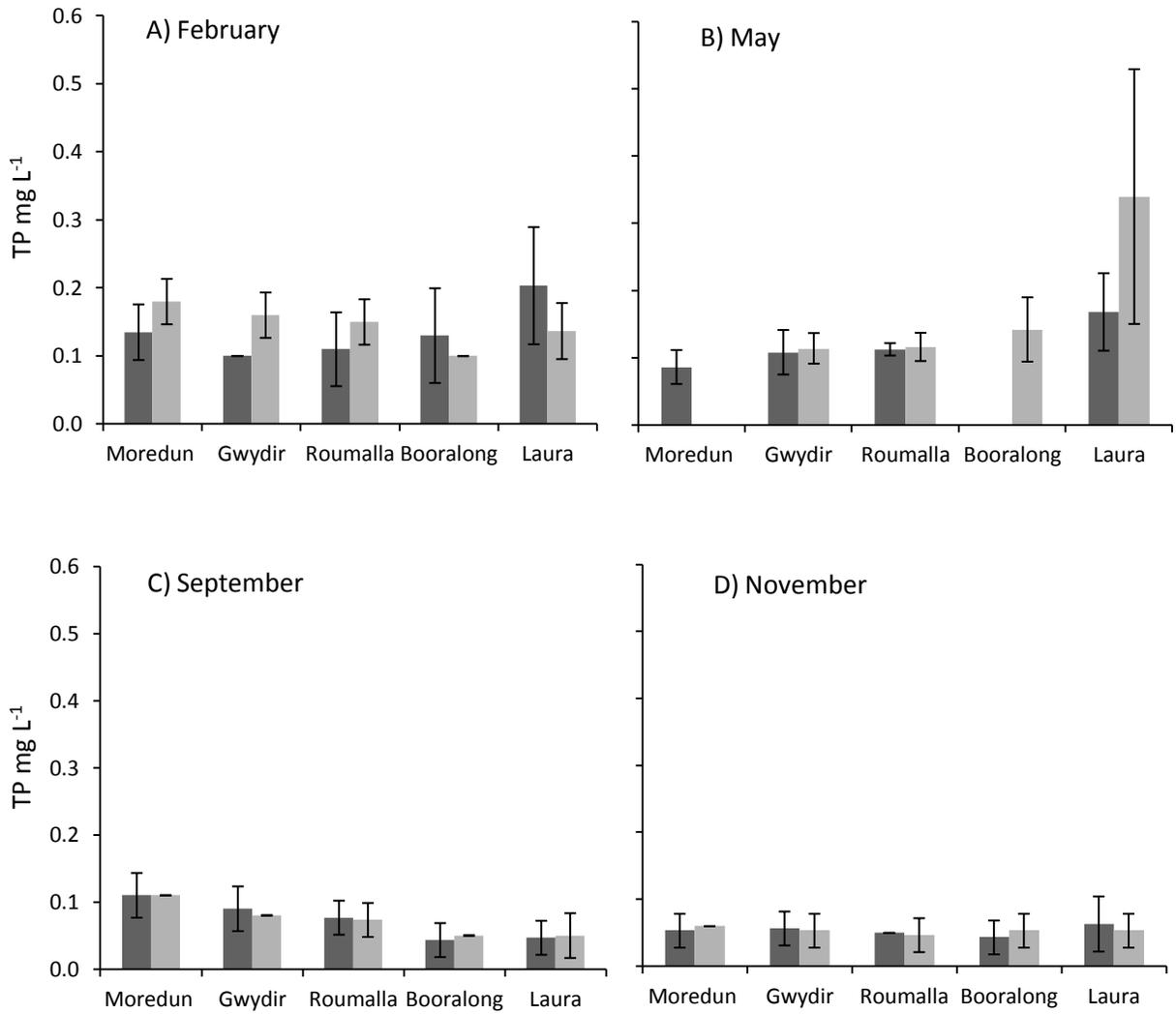


Figure 3.4: Mean surface water TP concentration (mg L⁻¹) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are standard error of the sample mean (n=3). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Dissolved organic carbon

There was no significant effect of riparian vegetation on DOC concentration. There were significant interactions between treatment and stream ($p = 0.006$), treatment and hydrology ($p = 0.001$), and stream and hydrology ($p = 0.001$, Table 3.7). The difference in DOC concentration between reaches on the non-vegetated control stream, Laura Creek, was significant ($p = 0.002$), however, there was no significant difference between reaches on the vegetated control stream, Booralong Creek, or when data from the two control reaches was combined (Appendix 4, Table A4.5). The highest mean DOC concentrations were detected during May, the no-flow period, and ranged between 8.2 to 28.8 mg L⁻¹ (Figure 3.5). The concentration of DOC also decreased during September and November, the high-flow periods, to similar concentrations during the February, the low-flow period (Figure 3.5). The mean DOC concentration across all reaches during the low and high-flow periods, ranged between 12.0 to 17.4 mg L⁻¹. There was minimal variation in mean DOC concentration between reaches on the same stream across all time periods, with the largest within stream difference recorded at Laura Creek during May, the no-flow period (Figure 3.5).

Table 3.7: Results of PERMANOVA main test for significant differences in DOC concentration (mg L⁻¹) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	7.654	7.654	2.077	0.228
St	4	424.380	106.090	2.079	0.142
Ti	3	489.820	163.270	2.871	0.073
TrxSt**	2	0.979	0.489	15.607	0.006
TrxTi	3	10.016	3.339	106.470	0.001
StxTi	12	637.750	53.146	24.089	0.001
TrxStxTi**	5	0.157	3.136e ⁻²	1.421e ⁻²	1.000
Res	83	183.120	2.206		
Total	113	1790.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.1 for correct formulae.

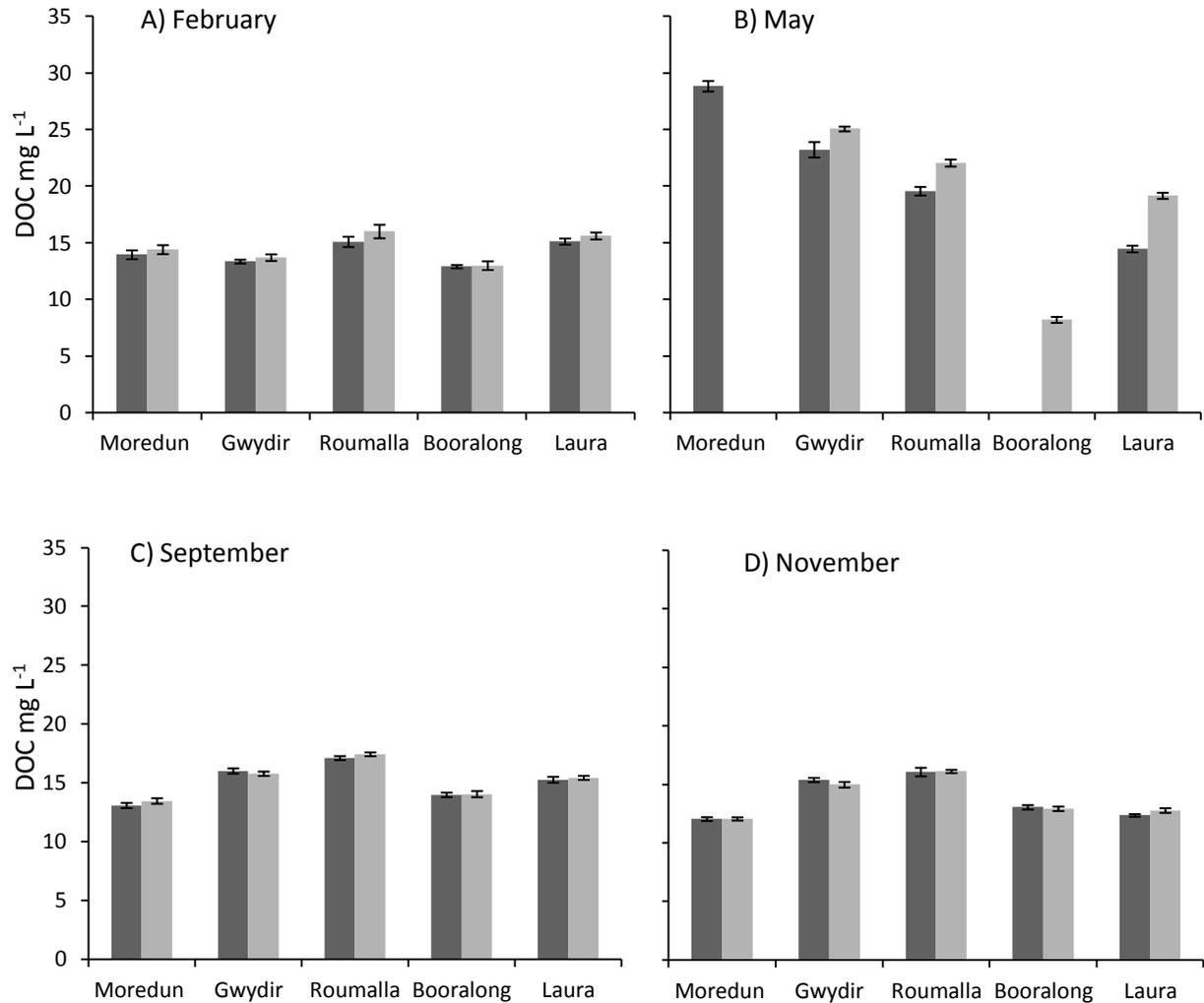


Figure 3.5: Mean surface water DOC concentration (mg L⁻¹) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are the standard error of the sample mean (n=3). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

*Nutrient stoichiometry**Dissolved nutrient fractions*

Within the water column dissolved nutrient fraction, N was the more limiting nutrient compared to P during all four sampling times in both the vegetated and non-vegetated reaches. The nearest N:P ratio to the Redfield N:P ratio of 16:1, was 5:1, which occurred during May at the Roumalla vegetated reach (Table 3.8). All other N:P ratios were between 0 and 3, indicating that N was limited relative to P. The water column C:P ratio at the Booralong Bridge reach was 128:1 and 150:1 during February and May respectively, which was the nearest value to the Redfield ratio of 100:1 (Table 3.8). During May, when there was no flow, all C:P ratios increased to above 1021:1 which was due to an increase in DOC concentration (Figure 3.5) and a concurrent decrease in SRP concentration (Figure 3.2). During September, a high-flow period and after one month of consistent surface water flow, the C:P ratios showed some variability between all reaches. The ratios ranged from 754:1 at the Moredun non-vegetated reach to 5309:1 at the downstream vegetated control reach, Booralong Bridge (Table 3.8). The results of the November sampling, which was another high-flow period and after three months of consistent surface water discharge, showed that the variation in C:P ratios between all ten reaches had decreased. The C:P ratios during November ranged from 1762:1 at the Moredun vegetated reach to 3731:1 at the Roumalla vegetated reach (Table 3.8). The increase in C:P ratios during September and November were driven by decreases in surface water SRP concentration (Figure 3.2) and not due to increases in DOC concentration (Figure 3.5). Overall, the C:P ratios were generally lower within the vegetated reaches compared to the non-vegetated reaches during February, when high SRP concentrations were present (Figure 3.2). Also during February, the C:N ratios were much higher at three vegetated reaches compared to the non-vegetated reaches.

Table 3.8: Molar C:N:P ratios of dissolved nutrient fractions. N and P values were calculated from mean NO_x and SRP concentrations respectively. C values were calculated from mean DOC concentrations. No C:N:P ratio could be calculated for Moredun vegetated and Booralong CS reaches during May as there was no surface water present.

Site	C:N:P ratios			
	February	May	September	November
Moredun non-vegetated	374 : 0 : 1	1692 : 0 : 1	754 : 1 : 1	3237 : 1 : 2
Gwydir non-vegetated	935 : 1 : 2	4501 : 1 : 4	2960 : 3 : 1	2773 : 1 : 1
Roumalla non-vegetated	486 : 0 : 1	2118 : 0 : 1	4909 : 1 : 1	3525 : 0 : 1
Laura Homestead	2680 : 1 : 11	1021 : 0 : 1	5080 : 1 : 1	2512 : 0 : 1
Laura Bridge	235 : 0 : 1	1353 : 0 : 1	2566 : 1 : 1	3042 : 0 : 1
Moredun vegetated	2668 : 1 : 14		854 : 1 : 1	1762 : 0 : 1
Gwydir vegetated	3086 : 1 : 11	1135 : 5 : 1	2344 : 2 : 1	3372 : 1 : 1
Roumalla vegetated	146 : 0 : 1	2326 : 1 : 3	4679 : 1 : 1	3731 : 0 : 1
Booralong CS	305 : 0 : 1		4650 : 0 : 1	2861 : 2 : 1
Booralong Bridge	6014 : 1 : 47	13680 : 1 : 91	5309 : 0 : 1	2393 : 2 : 1

The results of the PERMANOVA analyses showed that there was no significant difference in the dissolved nutrient molar ratios between the vegetated and non-vegetated reaches (Table 3.9), however, there were significant differences between streams ($p = 0.021$, Table 3.9). The PERMANOVA analyses also showed that there was a significant effect of time (hydrology) on C:N:P ratios ($p = 0.003$, Table 3.9) and a significant interaction between streams and time ($p = 0.001$, Table 3.9). PERMDISP tests showed that there were significant differences in dispersions between sampling times ($p = 0.002$) which is also evident from the MDS (Figure 3.6). The MDS shows that during February and November sampling periods, there is little variation in the C:N:P ratios between the ten study reaches. During May and September, there was large variation in C:N:P ratios between the study reaches (Figure 3.6). Together with the results of the PERMANOVA test, this suggests that dissolved C:N:P ratios are sensitive to no-flow conditions and also to the initial stages of surface water re-connectivity or initial high discharge events, and the nutrient ratios will be more variable during these periods.

Table 3.9: Results of PERMANOVA test for significant differences in dissolved molar C:N:P ratios between treatment (Tr), streams (St), and time (Ti). Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	0.285	0.285	1.069	0.431
St	4	27.389	6.847	2.557	0.021
Ti	3	40.051	13.350	4.487	0.003
TrxSt**	2	0.462	0.231	0.849	0.501
TrxTi	3	0.874	0.291	1.072	0.447
StxTi	12	33.381	2.782	10.726	0.001
TrxStxTi**	5	1.359	0.272	1.048	0.448
Res	7	1.816	0.259		
Total	37	111.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.2 for correct formulae.

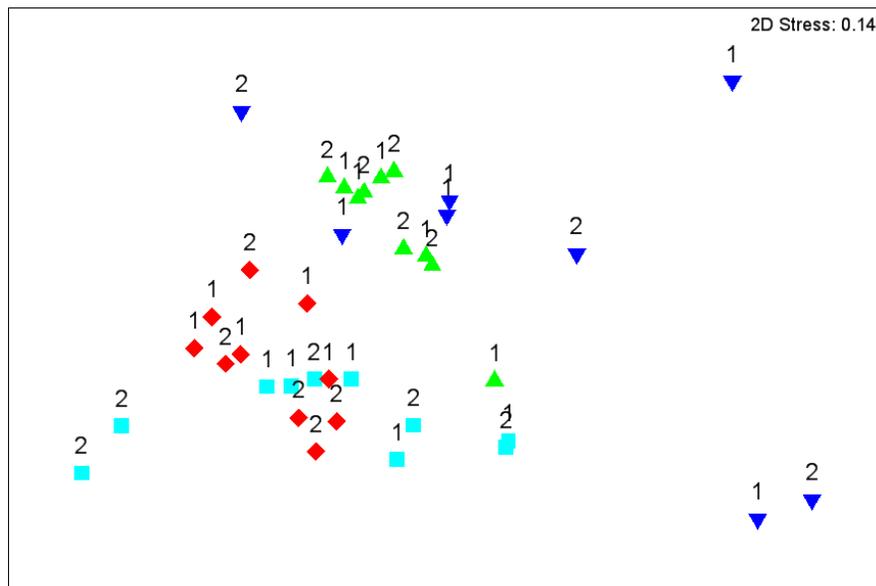


Figure 3.6: MDS of molar C:N:P ratio calculated from the dissolved nutrient fraction. Samples were from all non-vegetated (labeled 1) and vegetated (2) reaches, collected during all four sampling periods (February – green triangles, May – blue inverted triangles, September – light blue squares, November – red diamonds).

Particulate nutrient fractions

In contrast to the dissolved nutrient fraction, P was the more limiting nutrient within the particulate nutrient fraction when compared to Redfield ratios, at both the vegetated and non-vegetated reaches. The highest N:P ratio of 81:1 occurred during February in both the Gwydir vegetated and Gwydir non-vegetated reaches (Table 3.10). During the high-flow periods, September and November, the N:P ratios decreased and ranged between 17:1 and 44:1 in September, and between 23:1 and 40:1 in November (Table 3.10). These N:P ratios are the closest to the N:P Redfield ratios of 16:1. The C:N ratios were much lower and closer to the C:N Redfield ratio of 6.6:1 during the low and no-flow sampling periods, February and May. The ratios ranged from 7:1 at the Roumalla vegetated reach to 23:1 at the Moredun non-vegetated reach during February, and from 4:1 at the Booralong Bridge (downstream vegetated control) reach to 16:1 at the Roumalla vegetated reach during May (Table 3.10). During September and November the C:N ratios increased and ranged between 17:1 and 25:1 (Table 3.10) and was driven by a decrease in TN concentration (Figure 3.3). Compared to the C:P Redfield ratio of 100:1, the C:P ratios within the particulate nutrient fractions were high during all sampling periods. The C:P ratios were lowest during the low and no-flow periods, February and May. The C:P ratios increased during the high-flow sampling periods, September and November (Table 3.10) and ranged from 315:1 to 892:1 during September and November. The C:P ratio increase could be attributed to a decrease in TP concentration (Figure 3.4).

Table 3.10: Molar C:N:P ratios of particulate nutrient fractions. N and P values were calculated from mean TN and TP concentrations respectively. C values were calculated from mean DOC concentrations. No C:N:P ratio could be calculated for Moredun vegetated and Booralong CS reaches during May as there was no surface water present.

Site	C:N:P ratios			
	February	May	September	November
Moredun non-vegetated	824 : 36 : 1	865 : 80 : 1	305 : 17 : 1	583 : 24 : 1
Gwydir non-vegetated	711 : 81 : 1	555 : 53 : 1	459 : 23 : 1	700 : 31 : 1
Roumalla non-vegetated	355 : 22 : 1	450 : 56 : 1	390 : 17 : 1	831 : 35 : 1
Laura Homestead	191 : 15 : 1	223 : 42 : 1	841 : 44 : 1	504 : 23 : 1
Laura Bridge	295 : 22 : 1	145 : 20 : 1	797 : 39 : 1	318 : 27 : 1
Moredun vegetated	918 : 56 : 1		316 : 17 : 1	517 : 20 : 1
Gwydir vegetated	711 : 81 : 1	568 : 56 : 1	508 : 26 : 1	725 : 36 : 1
Roumalla vegetated	276 : 38 : 1	491 : 32 : 1	612 : 27 : 1	889 : 39 : 1
Booralong CS	317 : 39 : 1		830 : 45 : 1	776 : 40 : 1
Booralong Bridge	406 : 45 : 1	150 : 33 : 1	236 : 38 : 1	626 : 36 : 1

There were no significant differences in dissolved and particulate C:N:P ratios between locations on the control streams (Appendix 4, Table A4.6). There was no difference in particulate C:P and C:N ratios between vegetated and non-vegetated reaches during any of the sampling periods. This was supported by the non-significant effect of the main treatment ($p = 0.605$, Table 3.11), however, time (hydrology) was found to have a significant effect ($p = 0.001$, Table 3.11). There was also a significant interaction between streams and times ($p = 0.001$, Table 3.11). The results of the PERMDISP test show that there were significant differences in dispersions between sampling times ($p = 0.018$). The MDS shows that there is large variation in C:N:P ratios between all reaches during February and May. This variation then decreases during September and even more during November (Figure 3.7). This suggests that the particulate C:N:P ratios are affected by hydrology and that the variation in ratios between reaches and streams will decrease after longer periods of surface water connectivity.

Table 3.11: Results of PERMANOVA test for significant differences in particulate molar C:N:P ratios between treatment (Tr), streams (St), and time (Ti). Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	0.241	0.240	0.831	0.605
St	4	12.533	3.133	1.142	0.38
Ti	3	49.229	16.410	5.375	0.001
TrxSt**	2	7.993e ⁻²	3.996e ⁻²	0.185	0.952
TrxTi	3	1.543	0.515	2.375	0.106
StxTi	12	34.240	2.853	12.577	0.001
TrxStxTi**	5	1.083	0.217	0.955	0.531
Res	7	1.588	0.227		
Total	37	111.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.2 for correct formulae.

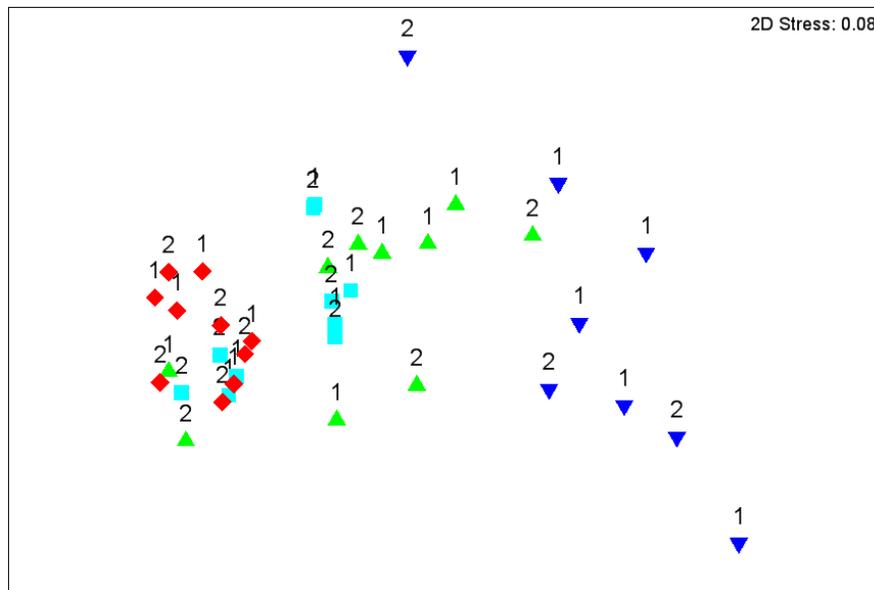


Figure 3.7: MDS of molar C:N:P ratio calculated from the particulate nutrient fraction. Samples were from all non-vegetated (labeled 1) and vegetated (2) reaches, collected during all four sampling periods (February – green triangles, May – blue inverted triangles, September – light blue squares, November – red diamonds).

Nutrient enrichment experiment

Across all deployment times, N and nutrient treatments that included N generally accumulated the highest mean chlorophyll *a* mass within both the vegetated and non-vegetated reaches. This indicates that autotrophic growth at all reaches was limited by N during all deployment times, regardless of temperature or hydrological conditions. The highest chlorophyll *a* mass occurred during February, when there were high temperatures and low-flow conditions, at both vegetated and non-vegetated reaches. The largest mass present during the February deployment period was 14 g m^{-2} at the upstream non-vegetated control reach, Laura Homestead (Figure 3.8). During February within the vegetated reaches, chlorophyll *a* mass was clearly highest on two nutrient treatments, N and NP. In contrast, chlorophyll *a* mass was highest on three nutrient treatments (N, NP and CNP) at the non-vegetated reaches (Figure 3.8). This trend is further evident in the MDS that shows there was larger variation in dominant limiting nutrient combination within the non-vegetated reaches, compared to the vegetated reaches (Figure 3.10). During May, the highest chlorophyll *a* mass occurred on the CNP nutrient treatment pots at Roumalla vegetated and Gwydir non-vegetated reaches (Figure 3.8). The lowest chlorophyll *a* masses occurred during September and November, the high-flow periods. Due to the very low chlorophyll *a* growth during September, it was difficult to identify the limiting nutrient treatment at the majority of reaches. The NP treatment accumulated the most chlorophyll *a* at both of the Moredun Creek reaches during September (Figure 3.9). The N and NP treatments accumulated the most chlorophyll *a* during November at both the vegetated and non-vegetated reaches (Figure 3.9). There was larger mean chlorophyll *a* mass on the C and nutrient treatment that included C at the non-vegetated reaches compared to the vegetated reaches during the February deployment (Figure 3.8). This difference was not observed during May (Figure 3.8). During September and November, mean chlorophyll *a* growth was higher on CNP treatments at non-vegetated reaches compared to vegetated reaches (Figure 3.9).

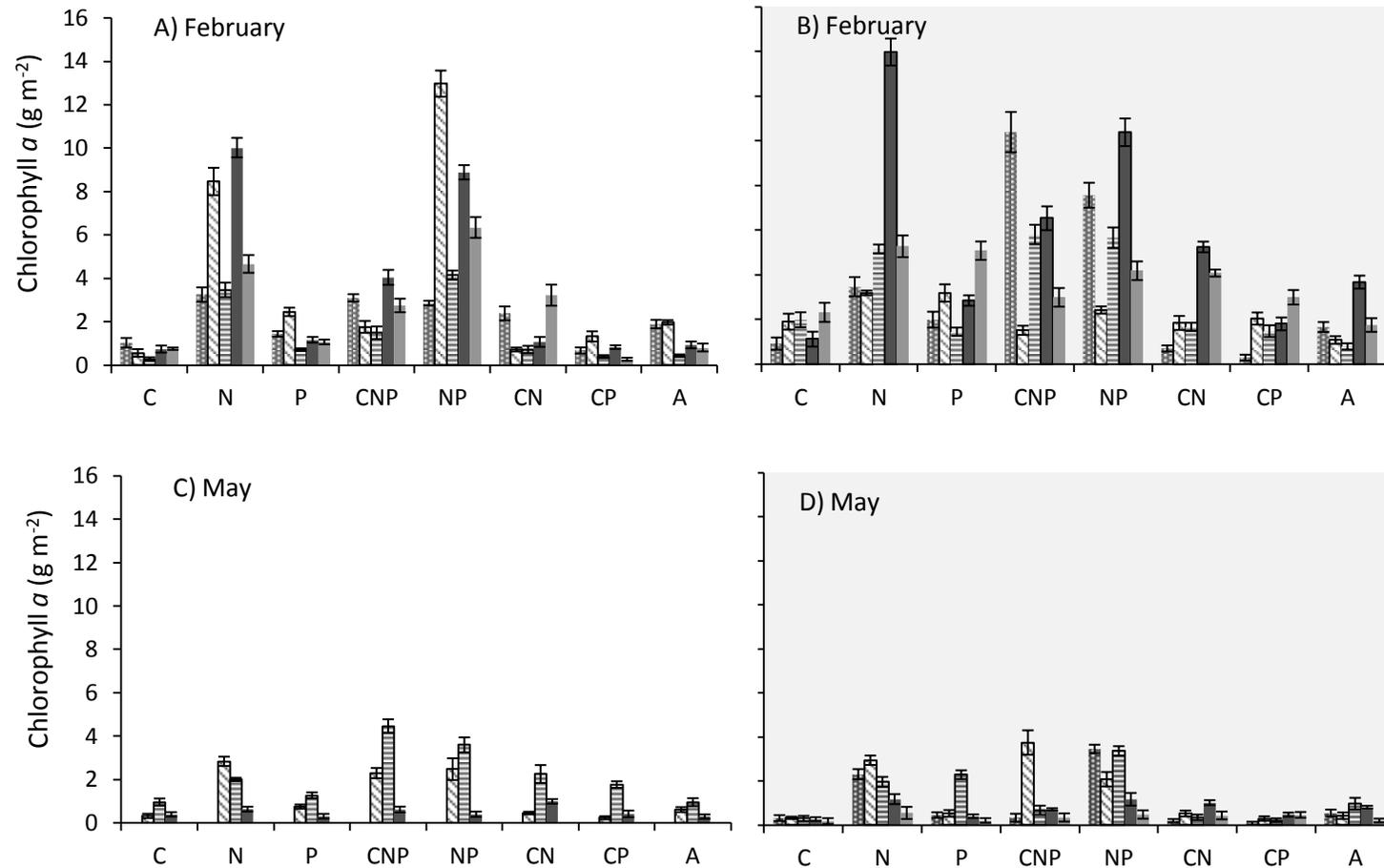


Figure 3.8: Mean chlorophyll *a* mass (g m^{-2}) recovered from each of the eight nutrient treated agar pots at the vegetated (white background, A and C) and non-vegetated (grey background, B and D) reaches, during the February and May deployments. Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura HD, and the light grey bars represent the downstream reaches, Booralong Br and Laura Br, on each of the control streams. The standard error bars represent the standard error of the sample mean ($n=3$).

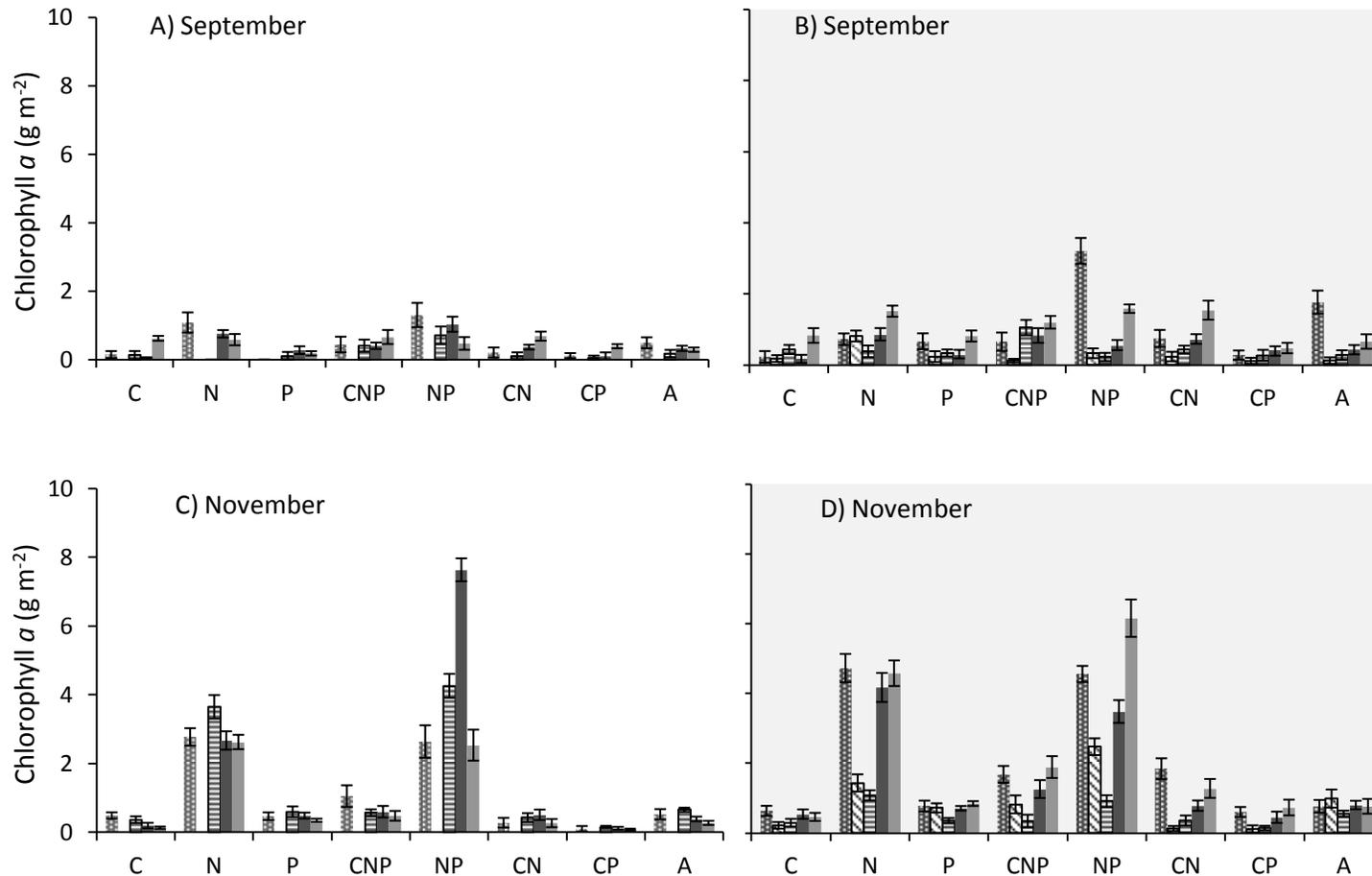


Figure 3.9: Mean chlorophyll *a* mass (g m⁻²) recovered from each of the eight nutrient treated agar pots at the vegetated (white background, A and C) and non-vegetated (grey background, B and D) reaches, during the September and November deployments. Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura HD, and the light grey bars represent the downstream reaches, Booralong Br and Laura Br, on each of the control streams. The standard error bars represent the standard error of the sample mean (n=3). Note the axis values are different to Figure 3.8.

There was no significant effect of location on the source of nutrient limitation (Appendix 4, Table A4.7). The main PERMANOVA test of differences in nutrient limitation, measured as chlorophyll *a* concentration, show there was a significant effect of time (hydrology) ($p = 0.002$, Table 3.12). There also a significant three-way interaction between treatment, stream and time ($p = 0.0012$ Table 3.12), and a two-way interaction between stream and time ($p = 0.001$, Table 3.12). PERMDISP analyses showed that there were significant differences in dispersions between deployment periods ($p = 0.001$).

Table 3.12: Results of PERMANOVA test for significant differences in chlorophyll *a* mass (g m^{-2}) using all eight nutrient enrichment treatments ($n=3$ for each reach at each deployment time). Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	7.409	7.409	0.762	0.619
St	4	47.607	11.902	1.120	0.379
Ti	3	376.340	125.450	10.808	0.002
TrxSt**	2	25.755	12.877	1.049	0.460
TrxTi	3	38.948	12.983	1.058	0.476
StxTi	12	140.400	11.700	5.619	0.001
TrxStxTi**	3	36.817	12.272	5.894	0.001
Res	79	164.500	2.082		
Total	107				

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.3 for correct formulae.

The MDS shows that the significant PERMDISP result is driven by the large variation between the sources of nutrient limitation during the February deployment (Figure 3.10). However, the distance between all deployment times as shown by the MDS does suggest that there were significant differences in the dominant source of nutrient limitation between deployment times (Figure 3.10), and supports the significant effect of time (hydrology) as determined by the PERMANOVA test (Table 3.12). Despite this significant effect, it is still evident from the mean chlorophyll *a* concentration (Figures 3.8 and 3.9) that the autotrophic growth at all reaches was limited by N during all deployment times, regardless of temperature or hydrological conditions.

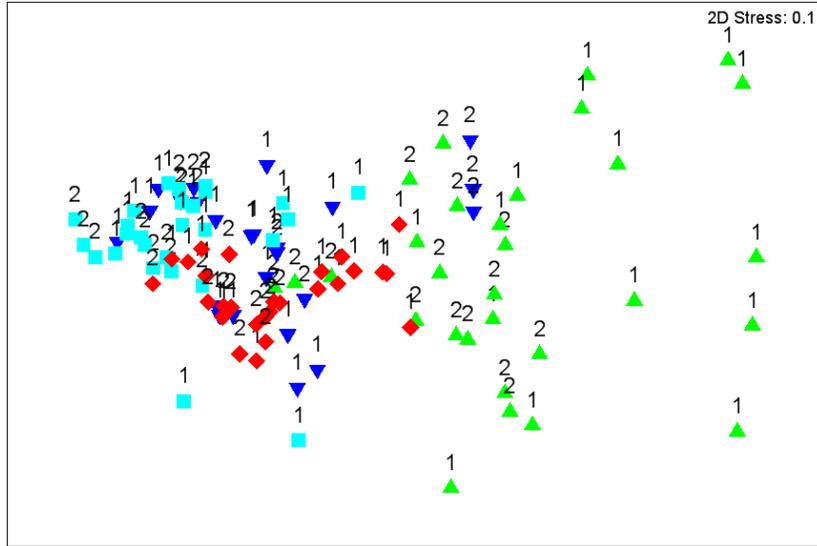


Figure3.10: MDS of chlorophyll *a* mass (g m^{-2}) using all eight nutrient enrichment treatments. Samples were from all non-vegetated (labeled 1) and vegetated (labeled 2) reaches during the four deployment times (February – green triangles, May – blue inverted triangles, September – light blue squares, November – red diamonds). Individual points represent a single sample ($n=3$ for each reach at each deployment time).

*Nutrient retention and export**Dissolved inorganic nitrogen*

Across all sampling times, NO_x was retained at the vegetated reaches, with the exception of the Gwydir vegetated reach during November. It was difficult to identify any trend in the mass-balance and retention of NO_x between vegetated and non-vegetated reaches, and across sampling times because concentrations were consistently below the detection limit at many reaches (Table 3.13, Figure 3.1). The magnitude of the imported and exported NO_x loads were similar between reaches on the same stream. The mass-balance estimates showed that the imported and exported loads of NO_x did increase during the high-flow periods (Table 3.13) The largest loads occurred during September, the initial high-flow period, with the highest imported load at the Moredun vegetated reach (13,508 g day⁻¹).

Table 3.13: The imported and exported loads of NO_x (g day⁻¹) at each reach during the February, September and November sampling periods. Values in parentheses are the standard error of the sample mean (n=3).

	February		September		November	
	Import	Export	Import	Export	Import	Export
Moredun NV	11 (0.4)	0	12,404 (3.1)	11,726 (7.9)	2,765 (14.5)	1,258 (7.19)
Moredun V	15 (0.5)	13 (0.3)	13,508 (10.5)	12,403 (4.39)	986 (9.84)	0
Gwydir NV	531 (1.8)	515 (1.7)	7,410 (10.9)	7,889 (6.7)	2,987 (6.7)	2,939 (3.0)
Gwydir V	155 (1.6)	161 (1.4)	8,879 (11.7)	7,559 (8.7)	3,050 (4.3)	3,255 (5.2)
Roumalla NV	0	0	638 (3.7)	617 (5.4)	41 (2.0)	0
Roumalla V	7 (0.8)	0	1,040 (11.1)	670 (4.6)	92 (3.5)	0
Laura HD	20 (0.6)	94 (3.6)	899 (3.0)	696 (3.4)	0	0
Laura Br	0	0	1,249 (4.2)	1,534 (10.4)	551 (10.3)	0
Booralong CS	19 (0)	0	0	0	201 (0.7)	187 (0.9)
Booralong Br	52 (0.6)	9 (0.3)	0	0	448 (5.6)	250 (1.5)

At the non-vegetated reaches, NO_x was retained during all sampling times, except at the Laura Homestead reach during February and both the Gwydir non-vegetated and Laura Bridge reaches during September (Figure 3.11). There was no significant difference in NO_x retention/export between vegetated and non-vegetated reaches, or between streams and times (Table 3.14). It was evident that the magnitude of NO_x retention or export did change in some reaches during September and November, the high flow periods, compared to February, the low-flow period. For example, the Gwydir and Moredun vegetated reaches retained 1105 and 1320 g day⁻¹ during September compared to 5 and 2 g day⁻¹ during February. The Moredun vegetated reach also retained 759 g day⁻¹ during November. Within the Moredun non-vegetated reach, NO_x retention increased to 678 and 1507 g day⁻¹ during September and November, compared to 11 g day⁻¹ during February (Figure 3.11).

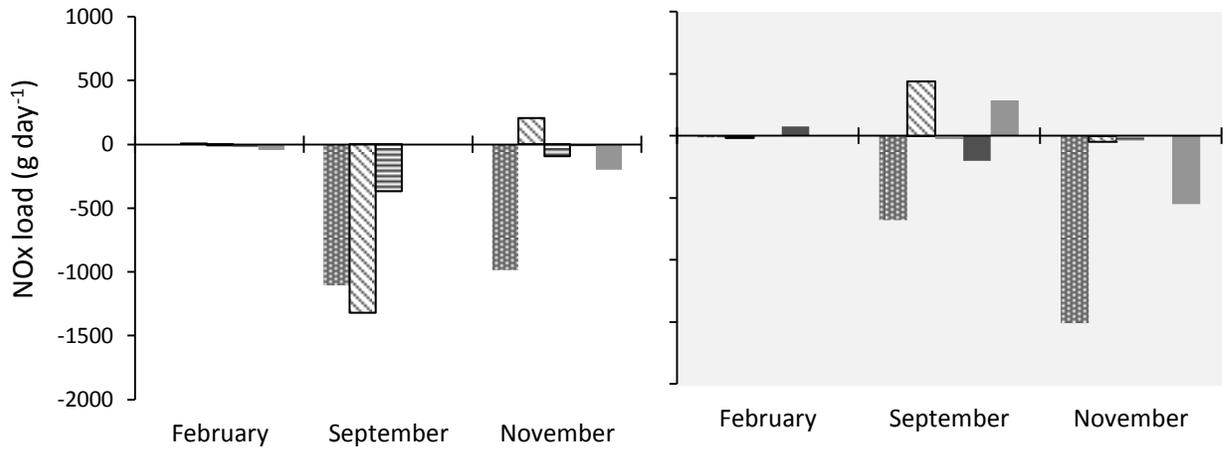
Table 3.14: Results of PERMANOVA main test for significant differences in NO_x retention/export (g day⁻¹) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	127.070	127.070	0.589	0.681
St	4	1746.200	436.550	2.205	0.153
Ti	2	650.480	325.240	1.550	0.273
TrxSt**	2	87.922	43.961	0.216	0.807
TrxTi	2	1033.800	516.890	2.544	0.175
StxTi	8	1584.100	198.010	1.395	0.322
TrxStxTi**	4	812.890	203.220	1.431	0.324
Res	6	852.010	142.000		
Total	29	6519.400			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.4 for correct formulae.

The effect of location could only be tested using the nutrient retention values from both control streams (Laura Creek and Booralong Creek), because tests of location on each individual stream had only 5 degrees of freedom, leading to only 10 unique permutations. There was no significant effect of location on the retention of any particular nutrient (Appendix 4, Table A4.8).

(A)



(B)

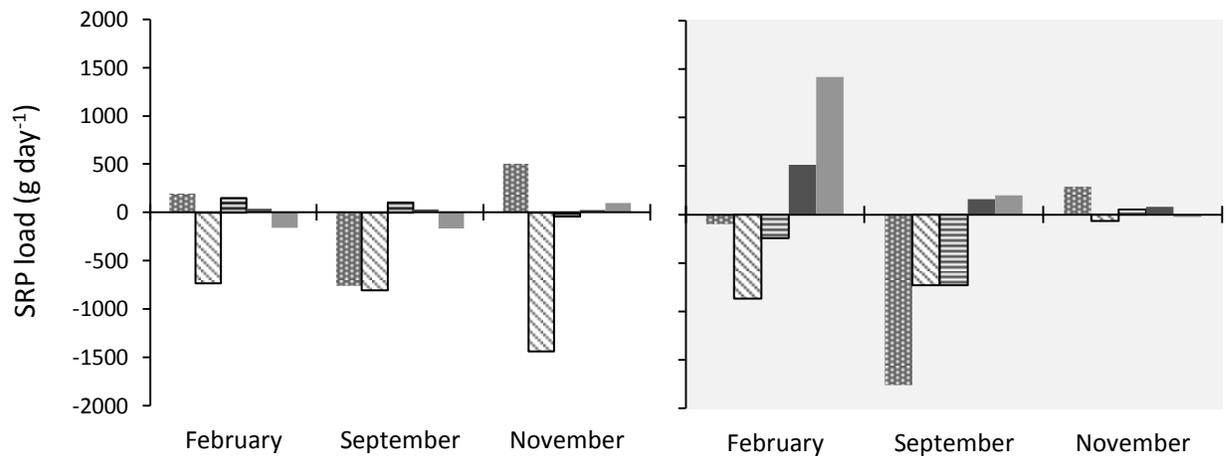


Figure 3.11: Total loads (g day⁻¹) of NOx (A) and SRP (B) retained (negative values) or exported (positive values) by each reach (vegetated – white background, non-vegetated – grey background) during February, September and November sampling times. Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura HD, while the light grey bars represent the downstream reaches, Booralong Br and Laura Br, on each of the control streams.

Soluble reactive phosphorus

The magnitude of the imported and exported SRP loads were generally similar between reaches on the same stream, with the exception of the Gwydir River and Booralong Creek reaches during February, the low flow period (Table 3.15). During February, the SRP load imported to the Gwydir vegetated reach ($4,607 \text{ g day}^{-1}$) was almost twice the load imported to the Gwydir non-vegetated reach ($2,964 \text{ g day}^{-1}$). The SRP load imported to the downstream vegetated control reach, Booralong Bridge ($1,086 \text{ g day}^{-1}$), was nearly three times that imported to the upstream vegetated control reach, Booralong CS (396 g day^{-1}). The imported and exported SRP loads increased during September, the first high-flow period. During November the second high-flow period, the SRP load estimates returned to less than the February low-flow estimates in the Roumalla Creek and Booralong Creek reaches. There were large differences within treatments and between streams during the high-flow periods, with $23,387 \text{ g day}^{-1}$ and $2,566 \text{ g day}^{-1}$ of SRP imported to the Moredun and Roumalla non-vegetated reaches during September.

Table 3.15: The imported and exported loads of SRP (g day^{-1}) at each reach during the February, September and November sampling periods. Values in parentheses are the standard error of the sample mean ($n=3$).

	February		September		November	
	Import	Export	Import	Export	Import	Export
Moredun NV	568 (4.5)	467 (8.0)	23,387 (26.2)	21,626 (20.7)	5,242 (3.6)	5,530 (7.9)
Moredun V	203 (2.0)	400 (6.0)	20,814 (13.7)	20,055 (15.7)	4,949 (8.8)	5,457 (4.6)
Gwydir NV	2,964 (8.4)	2,194 (3.69)	7,740 (5.04)	6,948 (9.43)	6,653 (11.6)	6,590 (9.1)
Gwydir V	4,607 (17.4)	3,875 (18.3)	9,358 (15.6)	8,549 (17.4)	6,938 (8.2)	5,500 (6.0)
Roumalla NV	951 (9.6)	709 (9.5)	2,566 (8.8)	1,836 (5.7)	507 (2.7)	559 (4.1)
Roumalla V	771 (1.5)	920 (2.3)	1,867 (7.4)	1,974 (4.1)	535 (3.4)	492 (3.2)
Laura HD	1,748 (5.4)	2,262 (10.4)	2,074 (2.3)	2,232 (6.0)	1,309 (4.0)	1,385 (3.9)
Laura Br	1,458 (4.7)	2,877 (13.5)	2,715 (3.6)	2,913 (3.6)	1,106 (4.7)	1,079 (4.2)
Booralong CS	396 (4.0)	437 (2.5)	587 (2.0)	619 (2.3)	189 (1.3)	216 (1.6)
Booralong Br	1,086 (4.0)	929 (6.2)	849 (4.1)	679 (2.6)	267 (0.8)	362 (3.1)

There was no significant effect of vegetation or hydrology on SRP retention (Table 3.16), however, there were patterns in SRP retention within reaches on the same stream. For example, both of the Gwydir River reaches were the only reaches to retain SRP during all sampling times. The retention of SRP at the Gwydir non-vegetated reach ranged between 64 g day⁻¹ during November and 863 g day⁻¹ during February. At the Gwydir vegetated reach, SRP retention ranged between 732 g day⁻¹ during February and 1438 g day⁻¹ during November (Figure 3.11). Both of the Moredun Creek study reaches also retained SRP during September and then both exported SRP during November (Figure 3.11). The magnitude of SRP retention and export did not change with increases in discharge. The significant interaction between streams and time shows there was variation in SRP retention among streams ($p = 0.034$, Table 3.16). This interaction may have been driven by a significant difference in dispersions between streams ($p = 0.017$), detected in the PERMDISP test, and further suggests that the differences in retention is due to catchment specific parameters between streams rather than an effect of hydrology (time).

Table 3.16: Results of PERMANOVA main test for significant differences in SRP retention/export (g day⁻¹) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	244.480	244.480	0.384	0.819
St	4	5505.800	1376.400	2.831	0.107
Ti	2	1099.800	549.900	1.037	0.405
TrxSt**	2	660.190	330.090	5.852	0.071
TrxTi	2	905.980	452.990	8.031	0.044
StxTi	8	3890.200	486.280	5.082	0.034
TrxStxTi**	4	225.630	56.408	0.590	0.671
Res	6	574.100	95.684		
Total	29	12069.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.4 for correct formulae.

Total nitrogen

The magnitude of the imported and exported TN loads was similar between reaches on the same stream, with the exception of the Roumalla Creek reaches during February (Table 3.17). In this case, the TN load imported to the Roumalla non-vegetated reach ($9,268 \text{ g day}^{-1}$) was almost three times the load imported to the Roumalla vegetated reach ($3,527 \text{ g day}^{-1}$). The mass-balance estimates showed that the imported and exported loads of TN did increase during the high-flow periods (Table 3.17), despite there being a decrease in TN point concentrations (Figure 3.12). The largest loads occurred during September, the initial high-flow period, with the highest imported load at the Gwydir non-vegetated reach ($478,617 \text{ g day}^{-1}$). There were large differences within treatments and between streams during all sampling periods. For example, the imported TN load within vegetated reaches was $183,645 \text{ g day}^{-1}$ at the Moredun but only $14,510 \text{ g day}^{-1}$ was imported at the upstream vegetated Booralong CS reach during November (Table 3.17).

Table 3.17: The imported and exported loads of TN (g day^{-1}) at each reach during the February, September and November sampling periods. Values in parentheses are the standard error of the sample mean ($n=3$).

	February		September		November	
	Import	Export	Import	Export	Import	Export
Moredun NV	3,991 (2.9)	3,280 (16.4)	455,462 (82.0)	418,314 (36.8)	177,613 (40.5)	166,092 (25.8)
Moredun V	3,144 (8.2)	2,748 (3.4)	415,619 (53.9)	413,970 (29.0)	183,645 (42.8)	173,269 (26.8)
Gwydir NV	36,996 (5.85)	37,921 (13.8)	472,015 (44.0)	462,113 (25.2)	197,540 (41.2)	382,437 (59.9)
Gwydir V	21,992 (32.9)	27,593 (8.1)	478,617 (58.0)	467,064 (44.0)	365,053 (45.9)	410,882 (65.0)
Roumalla NV	9,268 (6.3)	9,621 (4.1)	132,613 (26.2)	135,673 (26.2)	38,643 (14.6)	37,376 (26.8)
Roumalla V	3,527 (4.0)	8,355 (6.6)	132,444 (29.9)	136,504 (22.7)	37,223 (14.0)	36,197 (11.9)
Laura HD	20,910 (7.1)	20,245 (11.6)	174,612 (22.5)	181,177 (49.4)	98,070 (19.3)	70,727 (21.5)
Laura Br	20,559 (13.5)	22,510 (6.2)	173,935 (28.9)	163,996 (35.5)	61,816 (18.9)	64,155 (18.3)
Booralong CS	3,116 (5.2)	2,987 (2.4)	73,139 (22.8)	70,203 (7.2)	15,306 (6.5)	14,510 (6.4)
Booralong Br	3,420 (4.0)	3,492 (4.0)	84,852 (18.7)	84,199 (19.8)	21,817 (11.5)	22,510 (13.9)

The within stream patterns of nutrient retention and export was also evident during September, where paired reaches on each stream were either exporting or retaining TN (Figure 3.12). Another example of this trend was shown during November, where the Gwydir River reaches both exported TN, while both of the Moredun Creek reaches retained TN (Figure 3.12). However, there were no significant effects or interaction between main effects on the retention of TN (Table 3.18). There appeared to be a change in the magnitude of TN retention or export with discharge within the non-vegetated reaches. For example, the retention of TN increased to 37148 and 11521 g day⁻¹ during September and November, compared to 711 g day⁻¹ during February. Total TN export increased to 186291 and 45829 g day⁻¹ at the Gwydir non-vegetated and vegetated reaches respectively, during November, which was when the highest discharge occurred (Figure 3.12).

Table 3.18: Results of PERMANOVA main test for significant differences in TN retention/export (g day⁻¹) between treatment (Tr), streams (Ri), time (Ti), and their significant interactions. All ten reaches were used in the analysis.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	19.219	19.219	0.507	0.725
Ri	4	3008.100	752.030	1.216	0.339
Ti	2	756.610	378.300	0.606	0.596
TrxRi**	2	116.840	58.419	1.159	0.391
TrxTi	2	157.990	78.995	1.5672	0.317
RixTi	8	4949.500	618.680	1.790	0.262
TrxRixTi**	4	201.630	50.406	0.146	0.952
Res	6	2073.700	345.610		
Total	29	11123.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.4 for correct formulae.

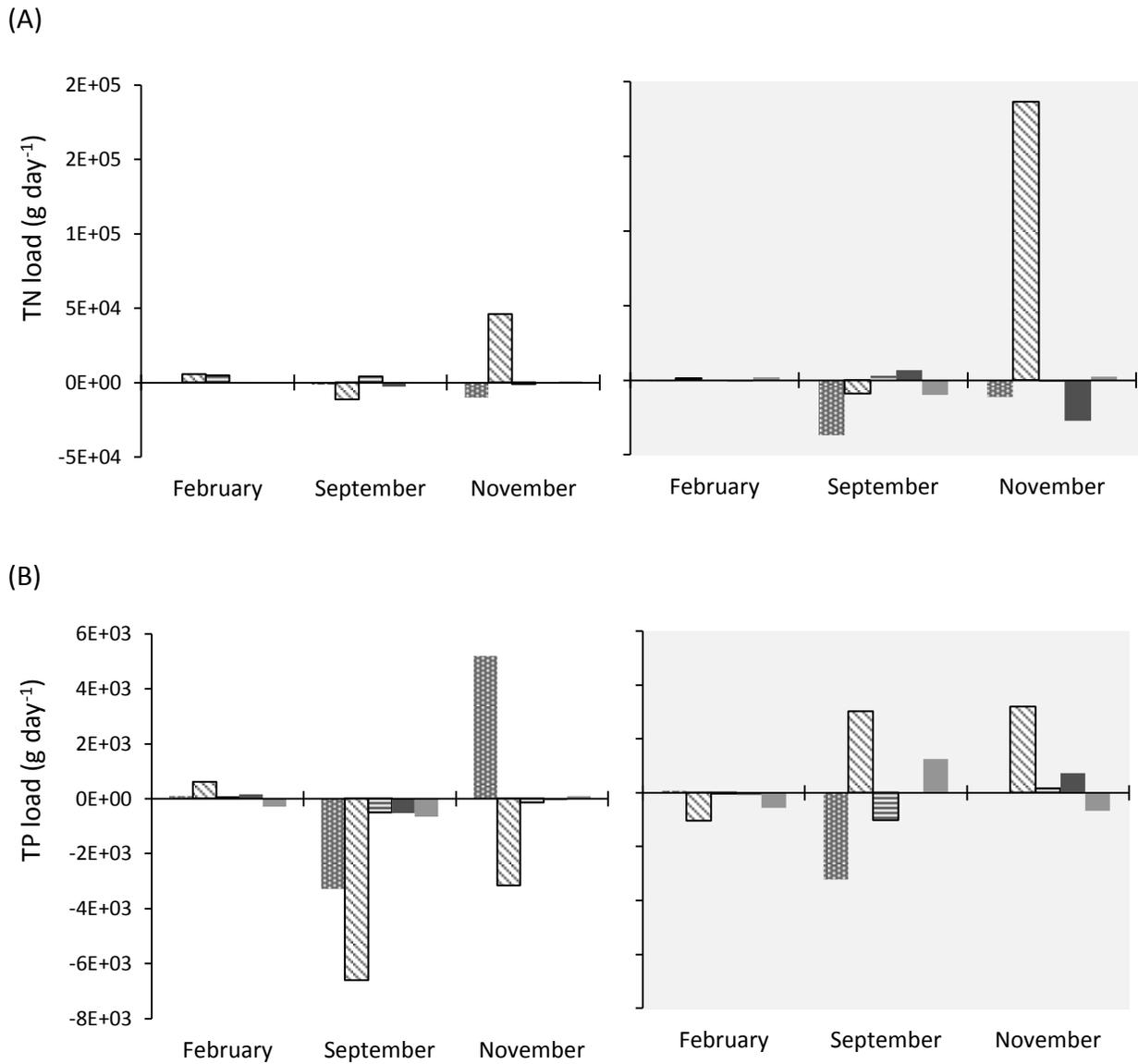


Figure 3.12: Total loads (g day^{-1}) of TN (A) and TP (B) retained (negative values) or exported (positive values) by each reach (vegetated – white background, non-vegetated – grey background) during February, September and November sampling times. Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura HD, while the light grey bars represent the downstream reaches, Booralong Br and Laura Br, on each of the control streams.

Total phosphorus

The magnitude of the imported and exported TP loads were also similar between reaches on the same stream, although during February there was more than twice the mass of TP being imported to the Roumalla non-vegetated (1,015 g day⁻¹) reach compared to the Roumalla vegetated reach (433 g day⁻¹, Table 3.19). The pattern of mass-balance with discharge was similar to the TN loads, with the largest loads being transported through reaches during September, the initial high-flow period. The largest TP loads transported across all streams occurred within the Moredun non-vegetated and vegetated reaches during September (56,529 and 57,725 g day⁻¹, respectively). There were also large differences within treatments and between streams during all sampling periods. For example, the imported TP load was 28,446 g day⁻¹ at the Gwydir vegetated reach but only 2,198 g day⁻¹ was imported at the Roumalla vegetated reach during November.

Table 3.19: The imported and exported loads of TP (g day⁻¹) at each reach during the February, September and November sampling periods. Values in parentheses are the standard error of the sample mean (n=3).

	February		September		November	
	Import	Export	Import	Export	Import	Export
Moredun NV	582 (0)	654 (2.8)	56,529 (25.0)	53,299 (23.2)	15,361 (13.6)	15,361 (13.6)
Moredun V	282 (1.2)	371 (1.5)	57,725 (17.8)	54,426 (0)	13,488 (14.1)	18,676 (0)
Gwydir NV	4,008 (5.9)	3,455 (0)	41,260 (17.8)	44,561 (23.5)	23,705 (0)	26,865 (17.4)
Gwydir V	4,316 (0)	4,933 (5.9)	46,211 (17.8)	39,610 (0)	28,446 (0)	25,285 (17.4)
Roumalla NV	1,015 (2.2)	971 (5.1)	12,751 (9.9)	11,731 (9.9)	2,217 (5.5)	2,376 (0)
Roumalla V	433 (4.3)	488 (1.9)	11,671 (9.9)	11,164 (9.9)	2,198 (0)	2,052 (5.3)
Laura HD	2,970 (5.1)	2,899 (10.3)	9,190 (11.2)	9,190 (11.2)	6,198 (8.4)	6,927 (13.6)
Laura Br	2,842 (4.3)	2,284 (5.3)	8,076 (10.9)	9,318 (14.4)	6,015 (13.9)	5,346 (8.1)
Booralong CS	400 (1.7)	557 (4.6)	4,004 (0)	3,470 (7.2)	857 (3.4)	796 (3.4)
Booralong Br	641 (0)	356 (0)	5,548 (7.9)	4,895 (0)	1,299 (0)	1,385 (4.1)

A difference in nutrient retention between vegetated and non-vegetated reaches occurred during September, where all vegetated reaches retained TP, in contrast to TP being retained at only two non-vegetated reaches (Figure 3.12). The Gwydir River reaches did not follow the same trend across all three sampling periods. This result was further supported by the significant interaction between treatment, stream and time ($p = 0.024$, Table 3.20). The differences in dispersions between streams ($p = 0.004$) was found to be significant, and may have contributed to the three-way interaction. The magnitude of TP retention and export increased with discharge within some of the vegetated reaches. A large change in TP retention occurred within the Gwydir vegetated reach, where TP export was 617 g day^{-1} during February, while TP retention was 6602 and 3161 g day^{-1} during September and November. The Moredun vegetated reach also increased TP export from 89 g day^{-1} during February to 5188 g day^{-1} during November (Figure 3.12).

Table 3.20: Results of PERMANOVA main test for significant differences in TP retention/export (g day^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	811.340	811.340	0.676	0.609
St	4	139.970	34.993	$4.145e^{-2}$	0.994
Ti	2	4140.000	2070.000	2.260	0.153
TrxSt**	2	6196.100	3098.100	1.262	0.383
TrxTi	2	3468.700	1734.300	0.706	0.573
StxTi	8	6754.500	844.310	1.866	0.239
TrxStxTi**	4	9821.000	2455.20	5.426	0.024
Res	6	2714.800	452.470		
Total	29	35647.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.4 for correct formulae.

Dissolved organic carbon

The magnitudes of the imported and exported DOC loads were similar between reaches on the same stream, although again there were differences in the imported loads during February, between reaches on Moredun Creek and Roumalla Creek (Table 3.21). The DOC loads increased more than 100-fold at some reaches during September and November, the high-flow periods, compared to February, the low-flow period. For example, the imported load at the Moredun non-vegetated reach was 62,890 g day⁻¹ during February and increased to 6,488,718 g day⁻¹ during September (Table 3.21). The largest DOC loads transported across all streams occurred within the Gwydir non-vegetated and vegetated reaches during September (7,297,439 and 7,967,322 g day⁻¹, respectively). There were differences within treatments and between streams during all sampling periods, for instance, the imported DOC load was 3,384,247 g day⁻¹ at the Moredun non-vegetated reach but only 801,765 g day⁻¹ was imported at the Roumalla non-vegetated reach during November.

Table 3.21: The imported and exported loads of DOC (g day⁻¹) at each reach during the February, September and November sampling periods. Values in parentheses are the standard error of the sample mean (n=3).

	February		September		November	
	Import	Export	Import	Export	Import	Export
Moredun NV	62,890 (33.3)	67,616 (27.4)	6,488,718 (201.2)	6,323,169 (153.2)	3,384,247 (224.1)	3,468,253 (87.7)
Moredun V	27,703 (25.9)	29,696 (17.9)	6,683,721 (218.7)	6,646,612 (167.1)	3,831,116 (99.2)	3,753,301 (75.9)
Gwydir NV	417,233 (28.5)	411,581 (29.5)	7,959,070 (128.5)	7,913,684 (155.0)	6,929,689 (125.8)	7,293,161 (125.8)
Gwydir V	420,316 (66.7)	422,371 (50.7)	7,967,322 (193.8)	7,798,156 (131.3)	6,870,427 (109.3)	7,099,573 (164.0)
Roumalla NV	182,425 (61.9)	133,214 (42.6)	2,693,064 (96.9)	2,614,006 (66.8)	801,765 (53.0)	763,359 (80.2)
Roumalla V	52,802 (11.6)	52,043 (34.1)	2,665,381 (68.3)	2,650,158 (64.1)	724,673 (57.9)	708,553 (30.4)
Laura HD	199,836 (56.2)	215,518 (32.4)	2,904,732 (79.4)	3,001,557 (112.0)	1,377,176 (75.1)	1,351,656 (37.0)
Laura Br	259,083 (56.2)	261,033 (39.8)	2,854,399 (131.2)	2,869,929 (76.4)	1,288,945 (69.3)	1,280,592 (31.9)
Booralong CS	55,244 (27.5)	55,351 (9.5)	1,092,411 (36.1)	1,117,102 (53.3)	241,128 (30.3)	239,689 (26.2)
Booralong Br	44,864 (17.5)	46,289 (22.8)	1,349,471 (107.6)	1,372,316 (81.0)	323,580 (15.2)	335,701 (30.4)

Both the vegetated and non-vegetated reaches in the treatment streams retained DOC during September sampling. Conversely, all of the reaches on the two control streams exported DOC during this time. Only a small amount of DOC was retained at the vegetated reaches during the February sampling period (Figure 3.13). The temporal variation among streams was supported by the significant interaction between stream and time ($p = 0.001$, Table 3.22), and also between treatment, stream and time ($p = 0.45$, Table 3.22). The results of the PERMDISP tests showed that there were significant differences in dispersions between streams ($p = 0.011$) and times ($p = 0.003$). These results suggest that DOC retention is affected by hydrological regimes specific to each catchment and also suggests that variation in other catchment parameters such as the total quantity of vegetation within the catchment may be affecting DOC mass-balance. Additionally, the magnitude of DOC export and retention changed within almost all reaches with discharge. For example, DOC export within the Moredun non-vegetated reach was 4726 g day^{-1} during February and increased to 84006 g day^{-1} during November (Figure 3.13). The Roumalla non-vegetated reach was the exception to changes in magnitude with discharge, with 49211 g day^{-1} being retained during February, which was the low-flow period compared to 38406 g day^{-1} during November, the high-flow period (Figure 3.13).

Table 3.22: Results of PERMANOVA main test for significant differences in DOC retention/export (g day^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	Df	SS	MS	Pseudo-F	P(perm)
Tr	1	1538.900	1538.900	0.417	0.778
St	4	2.744e^5	68599.000	0.545	0.731
Ti	2	1.063e^5	53169.000	0.386	0.706
TrxSt**	2	59181.000	29590.000	1.053	0.431
TrxTi	2	82860.000	41430.000	1.474	0.349
StxTi	8	1.006e^6	1.258e^5	19.334	0.001
TrxStxTi**	4	1.124e^5	28103.000	4.320	0.045
Res	6	39031.000	6505.100		
Total	29	1.612e^6			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.3 for correct formulae.

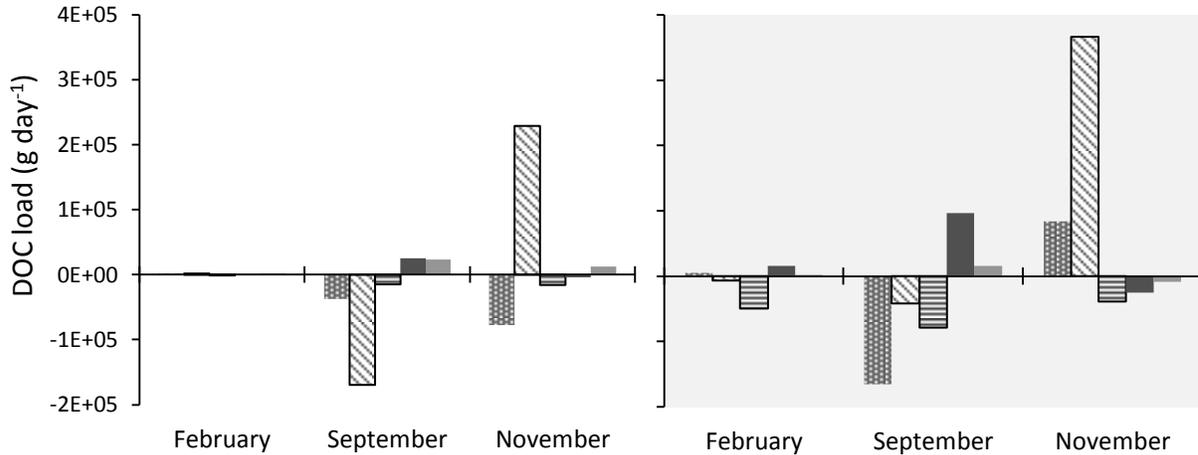


Figure 3.13: Total loads (g day^{-1}) of DOC retained (negative values) or exported (positive values) by each reach (vegetated – white background, non-vegetated – grey background) during February, September and November sampling times. Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura HD, while the light grey bars represent the downstream reaches, Booralong Br and Laura Br, on each of the control streams.

Correlation analyses

The BEST analyses showed that there was a significant positive correlation between nutrient retention (including the results from all five nutrients) and hydro-geomorphic variables in the non-vegetated reaches, but not within the vegetated reaches (Tables 3.23 and 3.24). Tests for correlation between individual nutrients and hydro-geomorphic variables were conducted for both vegetated and non-vegetated reaches. Within the vegetated reaches, the BEST analyses showed that there were no significant correlations between the hydro-geomorphic variables and nutrient retention (Table 3.23). Wetted area and flow percentile (frequency of discharge) were correlated with retention of all five nutrients in the non-vegetated reaches, but individually, nutrient retention was correlated with different combinations of wetted area and other hydro-geomorphic variables (Table 3.24). For example, TN retention was correlated with velocity and wetted area, while NO_x was correlated with discharge, surface area:volume ratio, and wetted area. The individual correlation tests for DOC, TP and NO_x retention with hydro-geomorphic variables at the non-vegetated reaches were not significant.

Table 3.23: Results of the BEST analyses between nutrient retention data from the vegetated reaches and their corresponding hydro-geomorphic variables.

Nutrient	Correlation level	Correlation variables	Significance level
All	0.130	Velocity, wetted area	0.71
TN	0.062	No. of features, velocity, wetted area	0.77
TP	0.188	Velocity, wetted area	0.39
NOx	0.105	No. of features, velocity, wetted area	0.69
SRP	0.123	Velocity, wetted area	0.39
DOC	0.011	Wetted area	0.98

Table 3.24: Results of the BEST analyses between nutrient retention data from the non- vegetated reaches and their corresponding hydro-geomorphic variables. Significant results are in bold type.

Nutrient	Correlation level	Correlation variables	Significance level
All	0.369	Flow percentile, wetted area	0.10
TN	0.380	Velocity, wetted area	0.09
TP	0.232	Flow percentile, velocity, surface area:volume, wetted area	0.25
NOx	0.210	Flow percentile, surface area:volume, wetted area	0.29
SRP	0.351	Surface area:volume, wetted area	0.05
DOC	0.169	Flow percentile, wetted area	0.35

3.4 Discussion

Nutrient concentrations

Nutrient concentrations in agricultural streams have been generally recorded at concentrations higher than those reported in less impacted and forested streams (Harris 2001, Rabalais et al. 2002, Frankforter et al. 2009, Hadwen et al. 2010). This has largely been attributed to an increase in nutrient and sediment loads being transported to these streams from the surrounding landscape (Harris 2001, Rabalias 2002). In this study, the range of TN and TP concentrations were up to 18 times larger than the trigger values estimated for relatively pristine upland streams in south-eastern Australia, while the concentrations of NO_x and SRP fell just above the trigger values (ANZECC 2000). The range of NO_x, TN and TP concentrations were comparable to those reported for Gwydir River reaches downstream of the current study and other agricultural rivers (Logan and Ovens Rivers) in Queensland and Victorian catchments (Hadwen et al. 2010) and also to agricultural streams in west, central and eastern USA (Duff et al. 2008, Frankforter et al. 2009). In contrast, the SRP concentrations during the low and no-flow period were at least an order of magnitude higher than most other studies conducted on agricultural streams in central USA (O'Brien et al. 2007, Duff et al. 2008), although they were similar to those reported for midwestern USA agricultural streams (Figueroa-Nieves et al. 2006). Similarly, the SRP concentrations were an order of magnitude higher than those reported for Gwydir River reaches downstream of the current study and from the Ovens River, however, the concentrations were similar to those reported for reaches in the Logan River (Hadwen et al. 2010). The authors of the study suggested that the elevated SRP concentrations were from enriched effluent derived from the agricultural farms (predominantly chicken and dairy) within the surrounding catchment (Hadwen et al. 2010). The comparable range of nutrient concentrations found in this study and the elevated SRP concentrations suggests that streams in upper Gwydir River catchment have been enriched by agricultural practices.

Table 3.25: Range of nutrient concentrations in this study and the suggested ANZECC trigger value nutrient concentrations.

Nutrient	Concentrations in this study (mg L⁻¹)	ANZECC trigger values for upland streams (mg L⁻¹)
NOx	0.000 – 0.025	0.015
SRP	0.015 – 0.300	0.015
TN	0.750 – 1.800	0.250
TP	0.500 – 0.200	0.020
DOC	13.05 – 28.83	N/A

According to temporary-river ecological theory, processes within reaches on the same stream are expected to increase in heterogeneity during periods of surface water contraction and fragmentation, while during periods of expansion the processes between reaches will become more homogeneous (Stanley et al. 1997, Dent & Grimm 1999, Larned et al. 2010). In this study, the nutrient concentrations show that SRP, TP, TN and DOC concentrations have high variation among reaches during low and no-flow conditions in comparison to high-flow periods. These patterns of nutrient concentrations are also similar to those in other temporary streams (Dent & Grimm 1999, Gómez et al. 2009, von Schiller et al. 2011a). This suggests that during surface water contraction and fragmentation there are different ecological processes occurring at different rates between reaches, each contributing to the variation in nutrient concentrations, thus supporting the temporary-river ecology hypotheses (Stanley et al. 1997, Dent & Grimm 1999, Larned et al. 2010). In addition, as reaches become fragmented the physical habitats are also expected to become more heterogeneous between reaches and therefore the ecological processes that are related to these physical habitats will also be more heterogeneous during these periods and contribute to the variation in nutrient concentrations (Dent & Grimm 1999, Larned et al. 2010). The decrease in variation of nutrient concentrations during the high-flow periods suggests that these rates and processes have become more similar as habitats within reaches become more homogeneous. However, during these periods, it is also likely that these processes have been masked by new or spatially-larger processes that have been initiated through the presence and connectivity of surface water (Dent & Grimm 1999, Larned et al. 2010). The retention and/or export of nutrients within reaches would also be expected to change with variation in ecological processes and the increase in magnitude of DOC and NOx

retention during the high-flow periods compared to the low-flow period in this study may support this suggestion.

The largest difference in SRP and TP concentrations between vegetated and non-vegetated reaches within the treatment streams occurred during the low-flow period, with higher SRP concentrations detected at vegetated reaches. The cycling of P in aquatic ecosystems is closely related to uptake by autotrophs and heterotrophs for growth (Sterner & Elser 2002). Low surface water velocities can promote the growth of many autotrophs including phytoplankton, filamentous and epiphytic algae, and macrophytes (Dewson et al. 2007). However, riparian vegetation has been shown to reduce autotrophic growth during high temperature and low-flow periods by decreasing in-stream light availability (Mosisch et al. 2001, Figueroa-Nieves et al. 2006, Julian et al. 2010, Üveges & Padisák 2011). The lower concentrations of SRP within the non-vegetated reaches compared to the vegetated reaches may be due to an increase in autotrophic growth, which would lead to SRP being 'locked-up' in plant biomass.

Alternatively, the difference in SRP concentration during the low-flow period may also be due to higher microbial demand within vegetated reaches. Microbes release extracellular enzymes to hydrolyse inorganic P to SRP (Olander & Vitousek 2000). The presence of riparian vegetation can increase allochthonous organic matter inputs (Reid et al. 2008), which has been linked to increased heterotrophic activity and therefore the production of SRP (Brookes et al. 2005). This suggests that the higher SRP concentrations within the vegetated reaches in this study may be due to an increase in heterotrophic extracellular enzyme activity. To determine why the difference in SRP concentration is more pronounced during low-flow periods, it is essential to further explore ecological processes such as metabolic rates and extracellular enzyme activity within reaches.

During low and no-flow conditions, there was higher variation in the TN concentration between all reaches compared to high-flow periods. The concentrations of TN were also highest during the low and no-flow periods and may be explained through the increased input of allochthonous organic matter. The peak fall of dried litter within south-eastern Australia is usually during the Austral summer, December to February (Robertson et al. 1999, Reid et al.

2008), which was during and just prior to the low and no-flow sampling periods in this study. *Casuarina cunninghamiana* trees, the dominant riparian vegetation along these streams, contain high concentrations of N within both fresh and dried needles (Esslemont et al. 2007). However, it is unknown how the age or quality of *C. cunninghamiana* needles affects the concentration of N leached from needles upon immersion. The variation in TN concentrations suggests that there may be differences in quantity or age of needles present within the benthic organic matter pool between reaches. Further insight into the quantity and type of benthic organic matter present within vegetated and non-vegetated reaches would be necessary to explore the potential effect of *C. cunninghamiana* needles on water column TN concentrations.

The increased SRP, TN, TP and DOC concentrations during the low and no-flow periods may have occurred through water evaporation, while the increase in surface water volume during the high-flow periods would dilute these concentrations (Gasith & Resh 1999). In contrast, the Gwydir vegetated reach was the only reach that had increased concentrations of NO_x during the no-flow period, while most other reaches had concentrations below the detection limits. The process of N regeneration through excretion by aquatic biota (including primary producers) and mineralisation of organic matter and other organic compounds has been shown to be an important part of N cycling in streams, leading to increased concentrations of N in some agricultural streams (Dodds et al. 2004, von Schiller et al. 2009). During periods of surface water fragmentation, groundwater can also be a source of N to isolated pools, particularly in soft-bottomed reaches with porous bed substrates where the rate of exchange between surface and sub-surface habitats can be enhanced (Boulton et al. 1998). Given that the remnant pools at the Gwydir vegetated reach during the no-flow period had sandy channel bed substrates, the increased NO_x concentrations during this period may be attributed to increased sub-surface input and a concomitant increase in nutrient regeneration processes. Although the majority of nutrient increases during the low and no-flow periods may be due to water evaporation, the NO_x results highlight the importance of exploring the potential sources of nutrients and ecosystem processes controlling their concentrations. Additionally, the changes in concentration will most likely affect other ecosystem processes that are sensitive to nutrient

fluctuations and should be explored to assess the effects of intermittent hydrology and the interaction with riparian vegetation.

Nutrient stoichiometry

The low NO_x concentrations detected during most sampling times resulted in higher C:N and lower N:P stoichiometric ratios within the dissolved nutrient fraction, relative to the Redfield ratios of 6.6:1 and 16:1, respectively. If the implications of the Redfield ratio and ecological stoichiometry theory apply to these freshwater agricultural streams, then the deviations indicate that autotrophic growth is N limited. In this study, detectable concentrations of NO_x were not present at the majority of reaches until the first high-flow event. This pattern is similar to other studies researching the effects of storm-flow or increases in discharge within perennial and intermittent streams (Bernal et al. 2005, von Schiller et al. 2008, Harms & Grimm 2010, von Schiller et al. 2011a). Some studies attribute the increase in NO_x to a decrease in biological demand by autotrophs and plant communities (Bernal et al. 2005, von Schiller et al. 2008) and seems a likely explanation for the results of this study, as the increase in discharge can also decrease algal biomass within agricultural streams (Biggs 1995). However, in addition to biological uptake for growth, the concentration of NO_x is controlled by a number of ecological processes, including nitrification, denitrification and N-fixation (Burgin & Hamilton 2007). The increases in NO_x concentration in several intermittent Mediterranean streams was thought to be partly due to an increase in microbial nitrification of TN leached from accumulated organic matter (von Schiller et al. 2011a). During all sampling periods there were still high concentrations of TN present (compared to ANZECC guidelines) and the particulate C:N ratios were higher than Redfield ratios. The low detection of NO_x despite the high TN concentrations suggests that the nitrification and denitrification processes may not occur at the same rates between sampling periods, and suggests that there should be an increase in N-related extracellular enzyme activity and microbial respiration during the first high-flow period in these study reaches.

Autotrophic nutrient limitation

The dominant source of nutrient limitation for autotrophic growth at all reaches generally included N or combinations of N (predominantly NP), regardless of temperature or hydrological conditions. Comparison of the bioassay results to Redfield proportions shows that the N-limitation to autotrophic growth agrees with the dissolved nutrient stoichiometry, but not the particulate nutrient stoichiometry results.

Particulate nutrient ratios have been suggested to be a better indicator of autotrophic nutrient limitation as it measures the total nutrient content of phytoplankton, microbial seston and nekton, while the dissolved nutrient fraction only represents the rates of nutrient cycling within the system (Dodds 2003). A number of other studies also using bioassays have had limited success in using dissolved nutrient stoichiometry to predict nutrient limitation (Francoeur et al. 1999, Wold & Hershey 1999, Tank & Dodds 2003, von Schiller et al. 2007). There also remains some skepticism as to whether SRP assays are a reliable indicator of available P to autotrophs (Dodd 2003). The results of this study do not agree with these findings. As previously mentioned, the high TN concentrations found in this study might be attributed to leaching from the N-rich *C. cunninghamiana*, however, a significant portion of this TN may be able to be reduced to NO_x and therefore unavailable to the biotic community (Weigner et al. 2006, Petrone et al. 2009). The results of this study highlight the importance of using both dissolved and particulate nutrient concentrations, and stoichiometric ratios to assess autotrophic nutrient limitation. In addition, exploring the uptake and remineralisation processes that influence nutrient concentrations will provide further insight to biotic nutrient dynamics within these reaches.

During high temperature and low-flow conditions there was a large amount of variation in the dominant source(s) of nutrient limitation between vegetated and non-vegetated reaches, and within non-vegetated reaches, compared to the no-flow and high-flow periods. This suggests that autotrophic growth in these agricultural streams is consistently N-limited, but the limiting nutrients will be specific to each reach during high-temperature and low-flow conditions, and the specificity will be more pronounced within non-vegetated reaches. The high-temperature and low-flow conditions are optimal for algal productivity (Dewson et al. 2007), and the results

of this study support the suggestion that controlling light and water temperatures through riparian vegetation may be an effective method of controlling benthic autotrophic production, rather than controlling for a specific suite of nutrients (Mosisch et al. 2001, von Schiller et al. 2007).

During the high-temperature no-flow period, the non-vegetated reaches had higher mean chlorophyll *a* mass on the C and C-combination pots, compared to vegetated reaches. There was also minimal variation in DOC concentration between vegetated and non-vegetated reaches during February. Algae have been shown to become photoinhibited under extremely high-light conditions (Üveges & Padisák 2011), which initiates algal uptake of C (Bouarab et al. 2004). It is likely that the higher chlorophyll *a* mass on C-combination pots deployed at the non-vegetated during February was due to a larger amount of light reaching the streambed compared to the vegetated reaches, causing the autotrophic community to be photoinhibited.

Initially, the higher chlorophyll *a* mass on C-combination amendments at vegetated reaches during May was thought to be due to the DOC pool containing a large amount of non-labile high molecular weight fractions, possibly leached from the *C. cunninghamiana* needles. However, algal growth has been shown to be inhibited by low molecular weight DOC, rather than high molecular weight DOC (Tulonen et al. 1992). During no-flow conditions, surface water light penetration can be reduced through an increase in fine sediment deposition and turbidity from resuspended fine sediments (Wood & Petts 1999, Lake 2003). Large amounts of humic DOC leaching from accumulated allochthonous organic matter can also lower light penetration (Phlips et al. 2000, Rier & Stevenson 2002, Steinberg et al. 2006). Under low-light conditions, algae have been shown to increase their uptake of organic C (Zotina et al. 2003, Tuchman et al. 2006). A larger amount of allochthonous organic matter that is contributing coloured humic DOC to the water column may be present within vegetated reaches compared to non-vegetated reaches, which would decrease the amount of light reaching the streambed in these reaches. This may explain the preference for C-containing pots within the vegetated reaches.

Nutrient retention

The results of this study showed no significant difference in nutrient retention between vegetated and non-vegetated reaches. Therefore, there was no clear effect of riparian vegetation as to whether reaches acted as a nutrient source or sink. According to the nutrient spiraling theory, the biotic community within reaches should retain limiting nutrients and lead to shorter spiraling lengths (Newbold et al. 1981, Fisher et al. 1998). As N was the most limiting nutrient for autotrophic growth during all sampling periods, it would be predicted that NO_x would always be retained within all reaches and the reaches would subsequently act as N sinks. Due to the persistent low concentrations of NO_x it was difficult to identify changes in NO_x mass-balance and to compare across reaches. When detectable concentrations of NO_x were present, which was predominantly during the high-flow periods, it was retained at the majority of reaches, supporting the predictions of the nutrient spirally concept (Newbold et al. 1981). This provides some evidence of both vegetated and non-vegetated reaches acting as nutrient sinks, but no clear mechanism for whether retention is through similar biotic and/or abiotic pathways between the reaches. The uptake of NO_x at reach scales has been related to gross primary productivity (GPP) within open-canopied headwater streams (Fellows et al. 2006b, Valett et al. 2008). In forested streams, NO_x uptake has been related to both ecosystem respiration (ER) (Valett et al. 2008) and GPP (Fellows et al. 2006b). The process of denitrification within streambed sediments has also been identified as an important mechanism for NO_x removal in intermittent streams (Tzoraki et al. 2007) and also in headwater streams across varying land uses (Peterson et al. 2001, Inwood et al. 2005), with some of the highest rates being detected in agricultural streams (Inwood et al. 2005). As these processes can also be affected by temperature (Simon et al. 2005) and discharge (Peterson et al. 2001, Gómez et al. 2009), it is also difficult to assume that the same mechanisms will be controlling NO_x retention between sampling periods in this study and further investigation is needed to identify the primary mechanisms.

The results of NO_x retention (and other nutrients) highlight one of the main limitations of assessing nutrient retention through load calculations. An alternative method of estimating

nutrient retention is through volatile tracer and nutrient addition techniques (Mulholland et al. 2002, Dodds et al. 2004, Fellows et al. 2006b, Claessens et al. 2010). A limitation of both the tracer/nutrient addition techniques and the load calculations is that the concurrent uptake and release of nutrients cannot be determined, or be attributed to biotic or abiotic transformations (von Schiller et al. 2011b). This issue is partly related to scale, as estimates of nutrient mass-balance at the whole ecosystem scale cannot give insight into the processes of retention that occur at smaller spatial scales. However, it can allow for comparisons of nutrient mass-balance across reaches and streams. The tracer and nutrient addition methods can allow for nutrient uptake rates, velocity of uptake and total amount of nutrient(s) retained within a system (Stream Solute Workshop 1990, von Schiller et al. 2011b), and may provide more insight into the mechanisms of retention. However, this alternative technique could not be used in this study as it is also expensive and difficult to perform in large streams and rivers (Stream Solute Workshop 1990). In many nutrient retention studies using volatile tracer and nutrient addition techniques, other variables such as the stoichiometry and metabolism of major functional groups have been measured during experiments (Hall & Tank 2003, Fellows et al. 2006b, Hill et al. 2010a). A similar approach would be needed in this study to elucidate the processes regulating retention.

Using load calculations to estimate nutrient retention and export does not allow for replication at the reach level and as such, it is hard to estimate the variance of the load calculations (Meyer & Tate 1983). Studies that use load estimates can calculate variance as the sum of all potential sources of error (Coser 1989, McKee et al. 2000). This usually includes a combination of error in discharge estimates, which may be up to 15% (Coser 1989), and error in laboratory determination of nutrient concentrations, which can be up to 5% (McKee et al. 2000). For the discharge calculations in this study, error would range between $300 \text{ m}^3 \text{ day}^{-1}$ during the low-flow period to $7126 \text{ m}^3 \text{ day}^{-1}$, during the high-flow period. Volatile tracer and nutrient addition techniques can allow for estimates of error if more than one release is made during each experiment (Stream Solute Workshop 1990). A comparison of these techniques with the load estimate method showed that retention rates of N determined from the load estimates gave

accurate results, but were sometimes higher than those calculated from tracer methods under high ambient N concentrations (von Schiller et al. 2011b).

Variation in mass-balance

The SRP, TN, TP and DOC concentrations in this study decreased with increased discharge, so the difference in magnitude was due to an increase in volume rather than concentration. The magnitude of TP loads increased with discharge across most reaches. During high-intensity rainfall periods, TP can be desorbed from the soil surface and transported as solutes in aqueous run-off or incorporated with eroded soil that is transported to the stream (Blanco et al. 2010, Rodríguez-Blanco et al. 2010). This pattern is dominant in many studies on agricultural streams because of the large application of P-based fertilizer (Blanco et al. 2010, Rodríguez-Blanco et al. 2010). Channel bed sediments can contain large amounts of P, which can also become resuspended during increases in channel discharge (Hoffman et al. 2009a). Although both of these mechanisms were likely to contribute to the increase in TP loads across the study reaches, the increase in total suspended solids load with discharge (Stewart & Ryder 2012 – see Appendix 1) suggests that the majority of load increase was due to increased soil surface run-off.

The magnitude of DOC loads also increased with discharge across most reaches. Dried organic matter can accumulate and remain in the riparian zone or channel for months until high-intensity rainfall occurs (Kaplan et al. 1980, Butturini & Sabater 2000). In this study, there was low rainfall within the study area for several months after the summer peak litterfall period (February to June 2010, see Chapter 2). Surface water contracted during this period at all study reaches, which suggests that the increase in DOC loads could be attributed to leaching from accumulated terrestrial organic matter as the wetted area expands.

Variation in retention

The variation in nutrient retention patterns within vegetated and non-vegetated reaches is surprisingly consistent with many other nutrient retention experiments. Many of these studies suggest that the large variation in other environmental parameters such as nutrient

concentrations and hydrology should indicate that there will also be large variability in reach-scale nutrient retention (Peterson et al. 2001, Simon et al. 2005, O'Brien et al. 2007, von Schiller et al. 2008).

The main source of disturbance in temporary rivers and streams such as those in this study is the hydrologic regime and clearing of riparian vegetation (Lake 2000, Larned et al. 2010, Stanley et al. 2010a). Therefore, it would be intuitive to assume that the variation in nutrient retention and export across reaches is driven by direct and indirect effects of hydrological parameters. The BEST analyses showed that SRP and TN retention were significantly correlated with hydro-geomorphic variables, which does suggest that these nutrient processes are partly driven through the interaction of hydrology and the physical template.

There were no significant correlations between the hydro-geomorphic variables and nutrient retention within the vegetated reaches. This suggests that nutrient retention within the vegetated reaches maybe controlled by processes that were not measured in this part of the study, or is not related to physical template as hypothesised. Additionally, the load data used in this study are essentially only a snapshot of processes that could be occurring on much larger temporal scales. When habitats are re-wetted or inundated, their associated ecological processes are usually activated within minutes to days, which suggests that it is hard to detect the response in the nutrient load data at reaches when the data only provides a snapshot of the processes (see Larned et al. 2010). The high-rainfall events can also cause erratic fluctuations in stream discharge leading to the regular expansion and contraction of surface water, and suggests that the activation and deactivation of some ecological processes will change with these fluctuations (Larned et al. 2010). Finally, the impacts of prior floods on processes that are related to nutrient retention such as organic matter decomposition by heterotrophs or autotrophic growth can also create variation in antecedent conditions among reaches, which may also result in differences in their response to inundation at reach scales (Dent & Grimm 1999).

Although there was no significant difference in the retention of nutrients between vegetated and non-vegetated reaches, the mass-balance results showed that were patterns in retention

between reaches on the same stream, as well as significant differences between streams. For example, DOC was retained within all reaches on the treatment streams while DOC was exported from reaches on the control streams during the high-flow periods. In addition, the quantities of nutrients imported to reaches on the same stream were also similar during the high-flow periods. This suggests that the nutrient mass-balance and retention patterns in these intermittent streams are related to catchment specific factors such as hydrology that override the potential effects of the main treatment of reach-scale vegetation (Dent & Grimm 1999, Bernhardt & Palmer 2011). These results provide further support for the suggestion that hydrology plays a dominant role in nutrient mass-balance and retention in temporary rivers and streams (Dent & Grimm 1999, Larned et al. 2010).

3.5 Summary

Riparian vegetation was found to have no effect on the concentrations of NO_x, SRP, TN, TP or DOC. Increased discharge had a significant negative effect on the concentrations of SRP, TN and TP. Mean SRP and TP concentrations were higher within the vegetated reaches compared to the non-vegetated reaches during the low and no-flow sampling periods.

Riparian vegetation was also found to have no effect on the dissolved and particulate C:N:P stoichiometric ratios. Both dissolved and particulate C:N:P ratios were significantly affected by hydrology, with particulate C:N:P ratios becoming more heterogeneous between reaches during the low and no-flow periods compared to the high-flow periods. The nutrient enrichment experiment showed that all reaches were N limited, regardless of vegetation presence or sampling period. There was large variation in the dominant source of nutrient limitation within and between vegetated and non-vegetated reaches, particularly during the low-flow deployment period.

There was no effect of riparian vegetation or hydrology on in-stream nutrient retention and export. However, there were interactions between streams and hydrology on SRP and DOC retention, and an interaction between treatment, streams and hydrology on TP and DOC retention. This indicates that nutrient retention between streams can be significantly different and the pattern of retention may be related to catchment-specific features. The BEST analyses

showed that 38% of the variation in SRP retention within the non-vegetated reaches was significantly related to variation in stream wetted area and surface area:volume ratio, suggesting that there are interactions between hydrology and the physical environment on SRP retention.

Nutrient concentrations and stoichiometry were more heterogeneous during the low and no-flow periods and more homogeneous during the high-flow periods, which support temporary-river ecology theory. This suggests that a complex array of ecological processes associated with changes in physical habitats will be initiated during channel re-wetting and connectivity, and that some of these processes could be reactivated at different spatial and temporal scales. The rates of some ecological processes that have been associated with nutrient retention will be explored in Chapter 4 (organic matter breakdown), Chapter 5 (extracellular enzyme activity) and Chapter 6 (ecosystem metabolism).

Chapter 4: Organic matter dynamics within vegetated and non-vegetated reaches.

4.1 Introduction

Organic matter is an important basal resource within aquatic foodwebs. The growth and functioning of autotrophic and heterotrophic communities depends on organic matter as a source of carbon and nutrients (Fisher & Likens 1973, Cummins 1974). Benthic organic matter provides habitat and food for invertebrates and fish (Erhman & Lamberti 1992, Jones 1997), and can contribute to in-stream channel complexity through increasing the deposition of sediment and other organic material (Erhman & Lamberti 1992, Flores et al. 2011). The processing and utilization of organic matter can facilitate nutrient cycling within streams (see Chapter 3, Aldridge et al. 2009), and can be influenced by the quantity and quality of available organic matter (Sinsabaugh 1997, Gibson & O'Reilly 2012).

Organic matter retention and storage

The quantity of organic matter present within streams is dependent on import, retention, breakdown and export processes (Webster et al. 1999, Lepori et al. 2005). Riparian vegetation has been identified as the main source of organic matter imported to forested streams worldwide including Australia (Campbell et al. 1992), USA (Webster & Meyer 1997), Spain (Sabater et al. 2001), and Brazil (França et al. 2009). Within southeastern Australia, leaf litter inputs from riparian vegetation have been estimated to be 600-700 g DW m⁻² year⁻¹ within upland streams (Campbell et al. 1992), 550-617 g DW m⁻² year⁻¹ within lowland agricultural streams (Reid et al. 2008), 117-460 g DW m⁻² year⁻¹ across urban streams (Imberger et al. 2011), and between 29-74 g C m⁻² year⁻¹ along the large lowland Murray River (Gawne et al. 2007).

Retention of organic matter is dependent on the interaction between in-stream hydrology and the physical template, which facilitates organic matter transport and interception (Brookshire & Dwire 2003, Quinn et al. 2007, Watson & Barmuta 2010). The quantity of organic matter retained within reaches can depend on the dimensions and frequency of these abiotic interception pathways (Brookes et al. 2005), which can also change with the pattern of inundation and fluctuations in surface water height (James & Henderson 2005, Quinn et al. 2007). Experimental organic matter releases using natural and artificial material have indicated

the pivotal role of channel geomorphic features such as, exposed bedrock, logs, flood debris points, and macrophyte beds intercepting and retaining organic matter (Brookshire & Dwire 2003, Quinn et al. 2007, Cordova et al. 2008). Additionally, an increase in the roughness (i.e. size, shape, porosity, texture) of the channel bed substrate and lower surface area:volume ratios have also been shown to increase the retention of submerged organic matter (Brookshire & Dwire 2003, Cordova et al. 2008).

During periods of surface water expansion in temporary streams, the increase in discharge or storm events can increase the standing stocks of benthic organic matter through larger amounts of organic matter being imported to the stream (Gurtz et al. 1988, Boulton & Lake 1992, Mollá et al. 2006). These events can be especially significant in temporary streams as connectivity of surface water between reaches following a period of fragmentation can initiate and/or alter the rate of ecological processes associated with organic matter, such as denitrification, heterotrophic decomposition and nutrient retention (Acuña et al. 2004, Larned et al. 2007, McIntyre et al. 2009, Newham et al. 2011). Standing stocks of benthic organic matter can also be reduced with increases in discharge and velocity through fragmenting and transporting retained organic matter to riparian zones, floodplains or downstream reaches (Jones & Smock 1991, Wallace et al. 1995, Treadwell et al. 1997). This export of organic matter to downstream reaches is an important longitudinal process providing connectivity of energy sources within streams (Vannote et al. 1980). Although many studies have investigated the total organic matter loads exported from streams and catchments, few have looked at the mass-balance and retention of organic matter loads at the reach scale (but see Gurtz et al. 1988 and Imberger et al. 2011).

The removal of riparian vegetation has contributed to substantially reduced allochthonous organic matter inputs (Campbell et al. 1992, Sabater et al. 2000, Reid et al. 2008), and simplified in-stream geomorphic and channel complexity that form interception pathways important for organic matter retention (Pusey & Arthington 2003, Allan 2004). These effects have been demonstrated to decrease benthic organic matter standing stocks and/or alter the sources of organic matter available to in-stream consumers (Reid et al. 2008, Watson &

Barmuta 2010). However, few studies have investigated the interaction of riparian vegetation and hydrology on organic matter retention and benthic standing stocks in intermittent streams (but see Imberger et al. 2011). In the intermittent agricultural streams used in the present study, the concentration of nutrients has shown to be more variable between vegetated and non-vegetated reaches during periods of low and no-flow (see Chapter 3), potentially reflecting the importance of localized organic matter retention. As organic matter is an important source of carbon and nutrients to the system, it is important to identify the dynamics between organic matter retention and storage with hydrology in vegetated and non-vegetated reaches in these streams.

Organic matter breakdown

The rate of organic matter breakdown affects the length of time organic matter is retained within the reach, and the quantity and quality of organic matter exported downstream (Webster et al. 1999). The breakdown process is a combination of abiotic and biotic mechanisms and is essential for providing higher trophic levels with palatable fractions of organic matter for energy and growth (Peterson & Cummins 1974, Cummins et al. 1989). The abiotic mechanisms of organic matter breakdown include a combination of physical fragmentation and leaching, while the biotic mechanisms include the decomposition by heterotrophic and invertebrate organisms (Gessner et al. 1999). The rate of breakdown is measured as the mass-loss over a defined period of time and reported as a coefficient (e.g. Young et al. 2008). These breakdown rates or coefficients can be influenced by various biophysical parameters (Bunn 1988, Hoover et al. 2006, Ferreira & Chauvet 2011) and the susceptibility (i.e. quality) of organic matter to biotic decomposition (Melillo et al. 1982, Enriquez et al. 1993, Hladyz et al. 2009).

Biotic decomposition of organic matter facilitates in-stream nutrient retention; the longitudinal process of nutrient spiraling (Newbold et al. 1981). Heterotrophic decomposition is known as 'microbial conditioning'; a process where aquatic fungi and bacteria produce a suite of enzymes that digest organic matter to smaller particles (Gessner et al. 1999). Organic matter that has a high lignin:cellulose and carbon:nutrient ratios is known as refractive (versus labile) and will be

decomposed at a slower rate by heterotrophs (Melillo et al. 1982, Gessner & Chauvet 1994). High carbon:nutrient ratios will also increase the demand for heterotrophic uptake of nutrients from overlying surface water during decomposition leading to decreased spiraling lengths (Aldridge et al. 2009). Widespread native riparian vegetation of Australia such as *Eucalyptus* spp. and *Casuarina cunninghamiana* have high lignin:cellulose and carbon:nutrient ratios (Pidgeon & Cairns 1981, Esslemont et al. 2007), and are therefore likely to facilitate local nutrient retention when retained and processed within reaches.

Litterbag experiments have been used to determine rates of organic matter breakdown and the effects of different biophysical variables on breakdown rates. The rate of organic matter mass-loss from the litterbags over time has been used as an indicator of the integrity of stream ecosystem function and resilience to altered biophysical variables (see Gessner & Chauvet 2002, Young et al. 2008, Tank et al. 2010). Removal of riparian vegetation can alter many biophysical variables that affect organic matter breakdown. For example, an increase in temperature can increase the rate of heterotrophic decomposition (Webster & Benfield 1986, Ferreira & Chauvet 2011), while an increase in direct sunlight (UV-B) can increase breakdown through photodegradation of dissolved organic matter (Wiegner & Seitzinger 2001, Howitt et al. 2008). Large amounts of erosion have also been associated with riparian vegetation removal, a process that can cause in-stream habitats to deteriorate and reduce heterogeneity of in-stream features and channel morphology (Wood & Armitage 1997, Pusey & Arthington 2003). The resultant impacts of reduced channel heterogeneity are unstable bed substrates and increased surface water turbidity that can smother organic matter and produce anoxic or reducing habitats that are unsuitable for biota (Bunn 1988, Benfield et al. 2001, Zweig & Rabeni 2001).

Rates of organic matter breakdown can be significantly affected by hydrology. Increases in current velocity have led to higher rates of organic matter breakdown in both perennial (Hoover et al. 2006) and temporary streams (Carvalho & Uieda 2009). It is also evident that hydrology can alter other biophysical parameters that may indirectly control organic matter breakdown, for example, surface water nutrients and temperature (Larned et al. 2010). Several

studies have also shown that the rates of organic matter breakdown will be higher during inundation compared to dry periods in temporary streams and rivers (Boulton 1991, Langhans & Tockner 2006) and wetlands (Baldwin 1999, Watkins et al. 2010), and this has thought to be due to increased leaching from organic matter and higher heterotrophic activity during inundation. A study on the effects of organic matter pre-conditioning within dry stream beds on breakdown rates once flow had recommenced found that there was lower mass-loss from *Populus tremmer* leaves that were pre-conditioned in anoxic pools or placed under high UV-light, which was attributed to lower leaf chemical quality and fungal biomass (Dieter et al. 2011). However, there appears to be little research into the variation in rates of breakdown during the transition from inundated to dry periods or at different magnitudes of flow within the inundated periods.

Using the conceptual basis of temporary-river ecology, the connectivity of habitats through surface water will result in conditions becoming more homogeneous, and therefore the rates of ecological processes such as organic matter breakdown would be also expected to become more similar. Alternatively, when surface water contracts and reaches are fragmented the habitats and conditions will be more heterogeneous (see evidence from nutrients, Chapter 3), and as such the rates of organic matter breakdown will also be more variable between disconnected reaches (Stanley et al. 1997, Dent & Grimm 1999, Larned et al. 2010). Therefore, given that energy (resources) within fragmented pools will be driven by retained organic matter and the adjacent riparian vegetation can affect many biophysical parameters that control the rate of organic matter breakdown, the influence of riparian vegetation on the breakdown process would be expected to be greatest during these low and no-flow periods.

Aims

The objective of this chapter is to investigate the mass-balance, retention and fate of organic matter within vegetated and non-vegetated reaches, during different hydrological conditions.

- 1) Through benthic organic matter surveys, I aim to test whether there is a difference in fine benthic organic matter (FBOM) and coarse benthic organic matter (CBOM) mass, and in the composition of in situ CBOM between vegetated and non-vegetated reaches. I predict that there will be a higher mass of FBOM and CBOM present within vegetated reaches compared to non-vegetated reaches, but that high discharge events could reduce this difference.
- 2) I also aim to measure reach-scale mass-balances of FPOM (fine particulate organic matter) and CPOM (coarse particulate organic matter) across all study reaches to determine if there are differences in retention between vegetated and non-vegetated reaches. I predict that larger quantities of FPOM and CPOM would be retained within vegetated reaches.
- 3) I aim to explore the ability for vegetated and non-vegetated reaches to retain coarse organic matter through experimental CPOM releases, using *C. cunninghamiana* needles and twig analogues. Specifically, I aim to explore the method of retention within vegetated and non-vegetated reaches and predict that there would be less retention of CPOM within non-vegetated reaches.
- 4) A further objective for this chapter is to determine whether there are differences in the breakdown rate of *C. cunninghamiana* between vegetated and non-vegetated reaches, using an experimental litterbag technique. I hypothesise that breakdown rates will be faster in non-vegetated reaches, owing to increased water temperatures. I also aim to explore the main biophysical effects on litterbag breakdown within vegetated and non-vegetated reaches.

4.2 Methods

Organic matter standing stocks

To determine the effects of vegetation, hydrology and their interaction on standing stocks of organic matter in reaches, CBOM (>1000 μm) and FBOM (250 μm – 1000 μm) surveys were conducted at each reach (Chapter 2), at four magnitudes of discharge. On each sampling occasion, a total of thirteen benthic organic matter samples were collected randomly within the main reach channel and were always one metre in from the surface water perimeter. The area immediately in front (upstream) of large geomorphic features such as logs and flood debris accumulation points, was not sampled. The samples were collected on each sampling occasion using the kick-net method, where a benthic area of 20 x 30 cm was agitated and organic matter collected using a 40 x 25 cm net (250 μm mesh size). Samples were placed in sealed plastic bags and kept cool at all times before processing.

Particulate organic matter mass-balance and retention

To determine the effects of vegetation, hydrology and their interaction on reach-scale organic matter mass-balance and retention, triplicate coarse and fine particulate organic matter (FPOM and CPOM) samples were taken at fixed upstream and downstream locations within each 100 m reach, at three magnitudes of discharge. A single net (250 μm mesh size, 52 cm diameter) was deployed three times at each end of the reach for a period of 5-10 minutes, depending on the velocity of the surface water. To ensure true replicates were collected, the net was always deployed at fixed points at permanent upstream and downstream transects, which were perpendicular to the bank. When sampling, the top of the net was 10 cm above the water surface. Samples were taken downstream first, to avoid the issue of overestimating the organic matter collected by stirring up the existing organic matter. The velocity of the surface water at the middle of the net (approximately 20 cm below the water surface) was taken at each fixed point to accurately determine the volume of water passing through the net to estimate transported loads.

Processing of organic matter fractions

Within 24 hours of collection, each of the benthic and transported organic matter samples were sieved into 3 fractions (250 – 500 μm , 500 – 1000 μm , >1000 μm) and oven-dried at 70°C for 48 hours. Samples were not elutriated because of the risk of losing very small organic matter fractions (<250 μm), although all large inorganic material was rinsed and removed by hand. Once dried, the >1000 μm fractions were sorted by hand into leaf material (native and exotic trees), *C. cunninghamiana* needles, bark, wood, *C. cunninghamiana* reproductive material, other reproductive material, macrophytes (including terrestrial grasses), droppings, and unidentifiable fractions. Each separated coarse fraction and the finer organic matter fractions were weighed to four decimal points, ashed (540°C for 3 hours) and then reweighed to estimate the ash-free dry mass (AFDM). For the final analyses, the 500 – 1000 μm fraction was combined with the unidentified fraction from the >1000 μm samples, and considered the unidentifiable fractions in the final analyses. The 250 – 500 μm samples were considered as the FBOM and FPOM fractions.

Experimental organic matter releases

To determine if all reaches and in-stream biophysical habitats had the potential to trap organic matter, CPOM releases were conducted at each of the ten study reaches. To replicate the main source of non-woody CPOM at the treed reaches, strips of water-proof paper (3 mm width, 210 mm length) were used as analogues of dry and fresh *Casuarina cunninghamiana* needles (Plate 4.1). Wooden craft sticks (10 mm width, 100 mm length) were used as analogues for small twigs from *C. cunninghamiana*. It is acknowledged that the simple shape of the craft sticks when compared to natural wood may underestimate the retention of small twigs within the system (Imberger et al. 2011). A small area of each CPOM analogue was spray-painted a different colour depending on their particular release (e.g. first paper release – yellow, second paper release – pink), to enhance their visibility and to ensure that any missed CPOM types from the first releases were not included in the replicate release.

Two temporally replicate releases were conducted at each reach at a single level of discharge (Table 4.1). During each experiment, 500 needle analogues were released over a 10 minute

period (approximately 72 needles per minute) across the wetted channel, immediately upstream of the study reach (corresponding to point A in the DEMs in Chapter 2). After a further 15 minutes, 150 twig analogues were released in a similar process, over a 5 minute period (approximately 30 twigs per minute). A minimum period of 30 minutes was observed to allow the released CPOM to settle before collecting and counting the retained CPOM. To collect the retained CPOM, one person walked upstream from the downstream nets (corresponding to point B in the DEMs in Chapter 2), and recorded the location and the quantity of CPOM retained, in addition to what the CPOM was retained by (pool, riffle, macrophytes, log etc.). All analogues were cleared before the replicate release to ensure the space available for retention was not compromised.



Plate 4.1: Photograph of *C. cunninghamiana* needles attached to branch. Source: http://en.wikipedia.org/wiki/File:Casuarina_cunninghamiana_fruit_and_leaves01.jpg.

Table 4.1: Discharges ($\text{m}^3 \text{s}^{-1}$) for each of the ten study reaches during the experimental CPOM releases.

Upstream reaches	Discharge ($\text{m}^3 \text{s}^{-1}$)	Downstream reaches	Discharge ($\text{m}^3 \text{s}^{-1}$)
Moredun Non-vegetated	0.28	Moredun Vegetated	0.33
Gwydir Non-vegetated	0.60	Gwydir Vegetated	0.64
Roumalla Non-vegetated	0.12	Roumalla Vegetated	0.22
Laura HD	0.20	Laura Bridge	0.09
Booralong CS	0.12	Booralong Bridge	0.09

The downstream nets used to capture the non-retained CPOM were made using 8 metre lengths of rigid plastic mesh (5 cm mesh size), held together with bamboo poles and polyethylene cable ties. In the streams, the nets were deployed across the stream and stabilised with steel posts (Plate 4.2). To ensure that all non-retained CPOM analogues were collected, the nets were stretched across the whole stream channel. A mesh size of 5 cm meant the nets would not collapse from being clogged with debris. The replicate release took place after the retained CPOM from the first release had been collected. The numbers of needle and twig analogues retained by different habitat types at each reach were calculated as a proportion of the total number of analogues retained.



Plate 4.2: Photograph of the downstream nets used to collect the CPOM analogues. Nets were stretched across the whole stream channel and held in place with steel posts.

Organic matter breakdown experiment

Organic matter breakdown was explored using the litterbag technique (Boulton & Boon 1991). *Casuarina cunninghamiana* needles were collected from a population growing along the Gwydir River at Yarrowyck in the New England Tablelands (30° 27' 17" S, 151° 22' 36" E). Branches of

fresh needles were cut from *C. cunninghamiana* trees and air-dried for approximately four weeks prior to each of the four litterbag deployments. The litterbags were constructed using 30 cm lengths of 5 mm diamond mesh, which was doubled over to minimise the effects of physical abrasion and fragmentation by large invertebrates. The resulting mesh size was approximately 1-5 mm. Approximately 7.2 g (\pm 0.05 g) of *C. cunninghamiana* were placed into each litterbag, which was then closed with staples. Each litterbag was then placed in a separate, sealed plastic container for transport to the field. To estimate handling loss, any needles retained in the plastic containers were weighed and used to correct the initial mass of each sample.

At each site, 12 litterbags (3 collection times x 4 replicates) were tethered to the stream bed using polyethylene ties and tent pegs. Rocks or another peg were used to help secure the loose end of the litterbag. Litterbags were randomly placed in a 2 m x 2 m area of each channel, avoiding any areas that altered the flow velocity (i.e. large, protruding boulders and logs). There was a minimum of 20 cm distance between each litterbag to maximise spatial independence.

Sample collection and processing

Initially, I endeavored to collect litterbags at the same three intervals (e.g. on days 7, 21, and 55) across each of the four different deployment periods. However, the high discharges experienced during the last 6 months of the study period restricted access to the study reaches and resulted in amended collection times (Table 4.2). At each collection time, four litterbags were randomly selected and each carefully placed into separate zip-lock bags, with effort to minimise disturbance to any inorganic matter that had settled on or within the bag. Samples were kept cool on return to the laboratory and then frozen until processing. On several occasions, only one or two litterbags could be retrieved because very high discharges had removed both the litterbag and the tent pegs. During the May 2010 litterbag deployment, the upstream vegetated control, Booralong CS, and Moredun vegetated reaches were completely dry. Litterbags were still deployed at these reaches, but their results were not included in the overall statistical analyses.

Table 4.2: The collection schedule for each of the four deployment periods.

Experiment number	Initial deployment date	Days after deployment		
		1 st collection	2 nd collection	3 rd collection
1	2-2-2010	7	21	54
2	30-4-2010	7	21	43
3	1-9-2010	7	21	43
4	22-10-2010	7	21	34

Throughout each deployment, changes in water temperature at each site were measured using StowAway TidbiT (onsetcomp.com) set at hourly logging intervals. At each collection time pH, temperature, conductivity (mS cm^{-1}), were measured using a WP-81 meter (TPS, Brisbane, Australia) and dissolved oxygen (mg L^{-1}) was measured using an Orion Star Plus meter (USA). The concentration of water column TN, TP, NO_x, SRP and DOC were determined from samples collected as part of other experiments (see Chapter 3). If there was more than 7 days between the previously collected samples and the litterbag collection, separate water column samples were collected and analysed using methods outlined in Chapter 3.

Once the samples were defrosted, each litterbag was cut open above a plastic tray, and the remaining needles transferred to a foil container. Any inorganic matter (e.g. sand) that had settled on or between the layers of the litterbag was collected in the plastic tray and added to the needles in the foil container. The needles and inorganic matter in each foil container were dried for 72 hours at 60°C and weighed to two decimal points. To estimate AFDM and mass of inorganic material associated with the litterbag, each total sample was ashed (540°C for 3 hours) and then reweighed. The difference between the dry and ashed weights was the remaining needle mass (g), and the ashed weight was the total mass of inorganic material (g).

Analysis of litterbag decomposition

The respective handling losses were subtracted from each initial litterbag mass. The breakdown coefficient, k , for each litterbag was calculated by regressing the corrected % mass loss against time (days) following (Boulton & Boon 1991), and determined through both exponential and linear curve-fitting (Prism 5.0, www.graphpad.com). To see if there was a detectable effect of

temperature on breakdown rates, the breakdown coefficient, k° , was also calculated using degree days (Hanson et al., 1984), and determined through exponential curve-fitting. Breakdown coefficients determined from poorly fitted breakdown curves cannot provide an unbiased statistical test of the effects of different treatments (Boulton & Boon 1991), so an alternative estimate of mass loss was employed. Using a computer graphing program (SigmaPlot 10.0), the area underneath a line connecting the remaining mass data points was calculated and subtracted from the area established from a horizontal line through the initial 100% (after Bärlocher et al. 2011, Bärlocher et al. 2012).

Statistical analyses

Organic matter standing stocks

A mixed-model ANOVA was used to identify significant differences in FBOM and CBOM mass between vegetated and non-vegetated reaches, and across time. The model consisted of three factors: i) Treatment – vegetated and non-vegetated ($a=2$; fixed), ii) Stream – Moredun Creek, Gwydir River, Roumalla Creek, Booralong Creek and Laura Creek ($b=5$; random), iii) Time – four sampling occasions ($c=4$; random). A total of thirteen organic matter samples were collected from each reach at each time ($n=13$). The statistical power of the main test was increased through replication at the treatment level, whereby the data from both the control and treatment streams were combined so that the analyses included five vegetated and five non-vegetated reaches. This meant that the statistical design became unbalanced (an unequal number of replicate samples within each factor level) and as such, the formulae used for a balanced design (Table 4.3) were invalid. The correct formulae used for the unbalanced design as determined by the PERMANOVA program are listed in Appendix 5 and will be referred to throughout the results section.

To test whether any significant differences in FBOM and CBOM mass could occur from natural longitudinal changes, the data collected from the two control streams was first analysed using three separate one-way ANOVAs with 'location' as the effect. The term 'location' consisted of two levels – upstream and downstream ($a=2$) and was a fixed factor. Two of the ANOVA analyses were for each of the two control streams, where masses from the upstream reaches

were compared to the downstream reaches, and data were pooled across all times within each reach. A third one-way ANOVA test of location was also performed, where the data from both control streams was combined, to provide further evidence and information of location effects (tables of formulae are listed in Appendix 3, tables of results are listed in Appendix 4). A significant difference in location meant that any differences arising in the main test of the treatment (vegetation) may be compromised by location, and would consequently have to be interpreted with caution.

To test for differences in CBOM composition, the different fractions were treated and analysed as a multivariate dataset also using the three-factor model described above. Some fractions were combined so that the final dataset of six different fractions included: *C. cunninghamiana* needles, wood, *C. cunninghamiana* reproductive structures, exotic and native leaves/bark/other reproductive structures, macrophytes, and an unidentifiable fraction that included animal fecal material. Whole-reach estimates of FBOM mass, CBOM mass and CBOM composition were calculated by multiplying the aerial unit average (g m^{-2}) by the corresponding total wetted area (Chapter 2).

All analyses were performed using PERMANOVA. To reduce the effect of outliers and differences in dispersions the FBOM, CBOM and CBOM composition data were $\log_{(x+1)}$ transformed prior to analysis. To test for equal variances across factors, PERMDISP was also used to check for differences in dispersions.

To explore whether different hydro-geomorphic variables were correlated with benthic organic matter mass and composition at vegetated compared to non-vegetated reaches, correlation analyses using the BEST program within Primer were performed on each organic matter fraction individually and also as a multivariate dataset. Data collected during May were not included in the PERMANOVA data because loads were zero due to no flow, therefore I did not want to compromise the role of other hydrological and physical features during discharge periods. The hydro-geomorphic variables used in the analyses included: combined number of logs and sections of bedrock, flow percentile values (%), $\log_{(x+1)}$ transformed), velocity (m s^{-1} , $\log_{(x+0.01)}$ transformed), surface area:volume ratio, and wetted area (m^2).

Organic matter mass-balance and retention

The effects of riparian vegetation and discharge on organic matter retention and export were determined by sampling organic matter at upstream and downstream transects at each reach using seine nets. For each sampling occasion the average mass of triplicate FPOM and CPOM (different fractions) samples were transformed into daily loads by multiplying the concentration with daily discharge. Daily discharge was calculated for each sampling occasion following the methods outlined in Chapter 2. Retention or export was then determined by subtracting the exported (downstream) load from the imported (upstream) load (Von Schiller et al. 2011b).

The effects of vegetation and time on the daily retained/exported FPOM and CPOM loads were analysed using MDS plots and a three-factor ANOVA in PERMANOVA. The model consisted of: i) Treatment – vegetated and non-vegetated ($a=2$; fixed), ii) Stream – Moredun Creek, Gwydir River, Roumalla Creek, Booralong Creek and Laura Creek ($b=5$, random), and iii) Time – four sampling occasions ($c=4$; random). The composition of CPOM retained or exported were also analysed using this model and the six different fractions were treated as a multivariate dataset.

To test for significant differences in organic matter retention that may occur from natural longitudinal change, data were combined from two the control streams were analysed using the one-way ANOVA model described above (tables of formulae are listed in Appendix 3, tables of results are listed in Appendix 4). Data from a single control stream could not be used because there were not enough unique permutations.

The data were checked for differences in dispersions using PERMDISP and significant results were reported. All data were transformed (square-root with the sign reinstated) and normalised before analysis, and I used Euclidean distance as the resemblance measure.

I also wanted to explore whether different hydro-geomorphic variables were correlated with organic matter retention at vegetated compared to non-vegetated reaches. Correlation analyses using the BEST program were performed on each organic matter fraction individually and also as a multivariate dataset. Data collected during May were not included in the PERMANOVA data because loads were obviously zero due to no flow, therefore I did not want

to compromise the role of other hydrological and physical features during discharge periods. The hydro-geomorphic variables used in the analyses included: combined number of logs and sections of bedrock, flow percentile values (%), $\log_{(x+1)}$ transformed), velocity (m s^{-1} , $\log_{(x+0.01)}$ transformed), surface area:volume ratio, and wetted area (m^2).

Experimental organic matter releases

The total proportion of needle and stick analogues retained were tested for the effect of ‘treatment’ using simple one-way ANOVAs in PERMANOVA. To test if there was a difference in the method of retention (type of habitat responsible for retention) of the analogues between vegetated and non-vegetated reaches, a multivariate dataset was created with six different habitats including: exposed bedrock, logs, flood debris accumulation points, macrophytes, pool and bank edges. These were consistent with the main habitat types at the reaches identified in Chapter 2. MDS plots in PERMANOVA were also used to explore the main methods of retention. The proportion (%) of the total number of analogues retained by the habitat type, during each release, was entered into the dataset.

Organic matter breakdown

To test for differences in organic matter breakdown between vegetated and non-vegetated reaches the total litterbag mass losses (%) were analysed using the three-factor ANOVA. Mean daily surface water temperature (measured as the mean daily temperature on the date of sample collection) and total inorganic matter accumulation (measured as the mean mass of inorganic mass attached to the samples collected at time three) were also analysed using the three-factor ANOVA. All analyses were conducted using PERMANOVA.

One-way ANOVAs were used to test for significant differences that may occur from natural longitudinal change. In some cases, Monte Carlo p -values had to be used as there were not enough unique permutations (tables of formulae are listed in Appendix 3, tables of results are listed in Appendix 4). The PERMDISP routine was used to check for significant differences in dispersions. Litterbag mass loss and inorganic matter accumulation data were $\log_{(x+1)}$ transformed prior to analysis.

I used the DISTLM routine within PERMANOVA to explore the effects of several hydro-geomorphic variables, mean daily water temperature and inorganic matter accumulation on litterbag mass loss. Similar to the BEST analysis, the DISTLM routine can identify the relationship between response variable dataset and one or more predictor variables, however, DISTLM can provide a quantitative measure of the variation explained by each predictor variable. Marginal tests explain the relationship of the response variable to a single predictor variable, and conditional tests describe the relationship after sequentially fitting more than one variable (Anderson et al. 2008). I used a step-wise selection procedure to sequentially fit all predictor variables with litterbag mass loss. The selection or stopping criteria used for the model was R^2 . Analyses were conducted separately for data from vegetated and non-vegetated reaches and data collected during May was not included in the datasets. The hydro-geomorphic variables used in the analyses included: combined number of logs and sections of bedrock, flow percentile values (%), $\log_{(x+1)}$ transformed), velocity (m s^{-1} , $\log_{(x+0.01)}$ transformed), surface area:volume ratio, and wetted area (m^2). The inorganic matter data were also $\log_{(x+0.01)}$ transformed prior to the analyses.

Table 4.3: Table of formulae for a balanced statistical design. Treatment is a fixed factor and has two levels ($a=2$), stream is a random factor and has five levels ($b=5$), and time is a random factor with four levels ($c=4$). Tables of correct formulae for the unbalanced design are listed in Appendix 5 and will be referred to in the results section.

Source of variation	Multipliers				Degrees of freedom	Expected mean square	Variance component
	i	j	k	r			
1 Treatment = Tr	0	b	c	N	a-1	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2 + bn\sigma_{\text{TrTi}}^2 + cn\sigma_{\text{TrSt}}^2 + bc n\sigma_{\text{Tr}}^2$	$(MS_{\text{Tr}} - MS_e)/bcn$
2 Stream = St	a	1	c	N	b-1	$\sigma_e^2 + an\sigma_{\text{StTi}}^2 + acn\sigma_{\text{St}}^2$	$(MS_{\text{St}} - MS_e)/acn$
3 Time = Ti	a	b	1	N	c-1	$\sigma_e^2 + an\sigma_{\text{StTi}}^2 + abn\sigma_{\text{Ti}}^2$	$(MS_{\text{Ti}} - MS_e)/abn$
4 Tr x St	0	1	c	N	$(a-1)(b-1)$	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2 + cn\sigma_{\text{TrSt}}^2$	$(MS_{\text{TrSt}} - MS_e)/cn$
5 Tr x Ti	0	b	1	N	$(a-1)(c-1)$	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2 + bn\sigma_{\text{TrTi}}^2$	$(MS_{\text{TrTi}} - MS_e)/bn$
6 St x Ti	a	1	1	N	$(b-1)(c-1)$	$\sigma_e^2 + an\sigma_{\text{StTi}}^2$	$(MS_{\text{StTi}} - MS_e)/an$
7 Tr x St x Ti	0	1	1	N	$(a-1)(b-1)(c-1)$	$\sigma_e^2 + n\sigma_{\text{TrStTi}}^2$	$(MS_{\text{TrStTi}} - MS_e)/n$
8 Residual = e	1	1	1	1	$abc(n-1)$	σ_e^2	MS_e

4.3 Results

Organic matter standing stocks

Fine benthic organic matter

There was no significant difference in FBOM mass between vegetated and non-vegetated reaches (Table 4.4). The results of the PERMANOVA test for the effects of location showed that there was a significant difference between the upstream and downstream reaches on the vegetated control ($p = 0.009$) and non-vegetated control stream ($p = 0.004$; Appendix 4, Table A4.9). However, the streams differed in the location of the largest mass of FBOM, with the upstream Booralong reach (Booralong CS) having the highest FBOM mass during three sampling times, and the largest mass alternating in location within Laura Creek (Figure 4.1). The downstream vegetated reaches in the Gwydir River and Roumalla Creek treatment streams had smaller FBOM mass than the upstream non-vegetated reaches during the high-flow periods, September and November. Conversely, the Moredun Creek treatment stream had a larger FBOM mass at the downstream vegetated reach during these times (Figure 4.1). The significant interaction between treatment, stream and time from the PERMANOVA test further supports the variation in the effect of the treatment in different streams across times (Table 4.4). The results of the PERMDISP analyses showed that there were significant differences in dispersions for all three main factors (treatment $p = 0.012$, stream $p = 0.001$, and time $p = 0.001$) suggesting that there is large natural variation in FBOM mass between reaches and sampling times, regardless of the presence of vegetation.

Across all time periods, the three treatment streams generally had lower FBOM mass than both the vegetated and non-vegetated control streams. The PERMANOVA test found that this difference between streams was significant (Table 4.4). The mean FBOM masses were below 5 g m^{-2} within both vegetated and non-vegetated reaches on treatment streams during all sampling periods. During February and May, the low and no-flow periods, the downstream non-vegetated control reach, Laura Bridge, FBOM mass of 21 and 28 g m^{-2} , respectively, which was five to seven times higher than the nine other reaches. This mass decreased to less than 5 g m^{-2} in September and November when there were high flows. The upstream Booralong Creek

reach (vegetated control stream, Booralong CS) had the largest FBOM mass of 5 g m^{-2} during the high flow periods (Figure 4.1).

Table 4.4: Results of PERMANOVA main test for significant differences in total FBOM mass (g m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	5.282e^{-2}	5.282e^{-2}	0.901	0.547
St	4	62.785	15.696	6.552	0.010
Ti	3	21.406	7.135	2.684	0.104
TrxSt**	2	2.928	1.464	1.071	0.383
TrxTi	3	0.227	7.562e^{-2}	5.244e^{-2}	0.980
StxTi	12	31.540	2.628	11.123	0.001
TrxStxTi**	6	9.317	1.553	6.572	0.001
Res	454	107.280	0.236		
Total	485	263.590			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.5 for correct formulae.

The whole-reach FBOM estimates showed a similar pattern to the per unit area (g m^{-2}) results, with the highest FBOM masses occurring at the downstream non-vegetated control reach, Laura Bridge, during February (38,565 g) and May (53,953 g), and at the upstream vegetated control reach, Booralong CS during September (7,526 g) and November (7757 g, Figure 4.2). Scaling up FBOM mass to the whole-reach scale did not alter the location that had the higher mass within each stream pair, however, it did increase the magnitude of the difference in mass between the paired reaches in some cases. For example, during November, the Gwydir non-vegetated reach had a larger FBOM mass compared to the Gwydir vegetated reach. At the per unit area scale, the vegetated reach had a 20% higher mass (Figure 4.2), while at the reach scale the difference in mass between locations was over 400% (Figure 4.2).

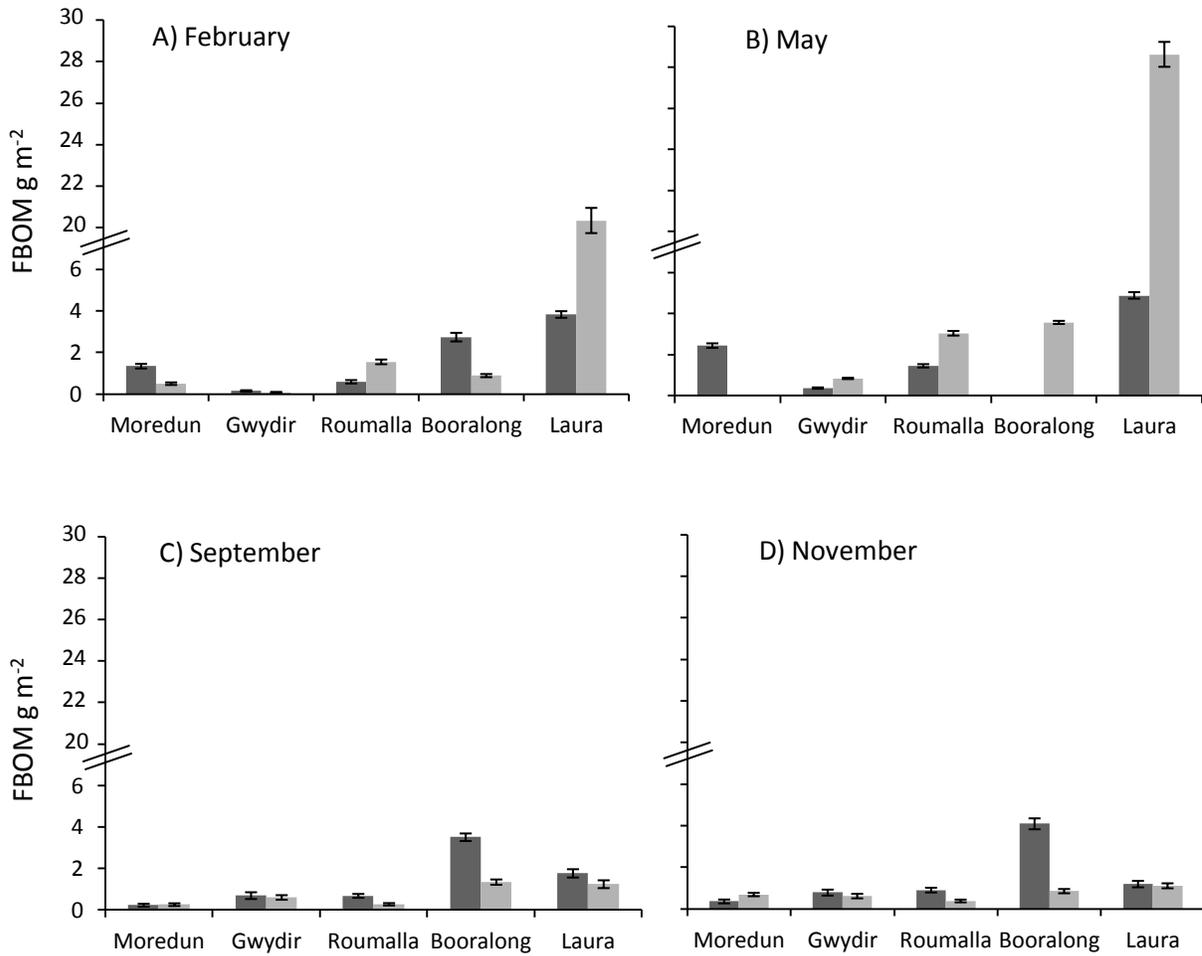


Figure 4.1: Mean FBOM mass (g m^{-2}) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are standard error of the sample mean ($n=13$). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

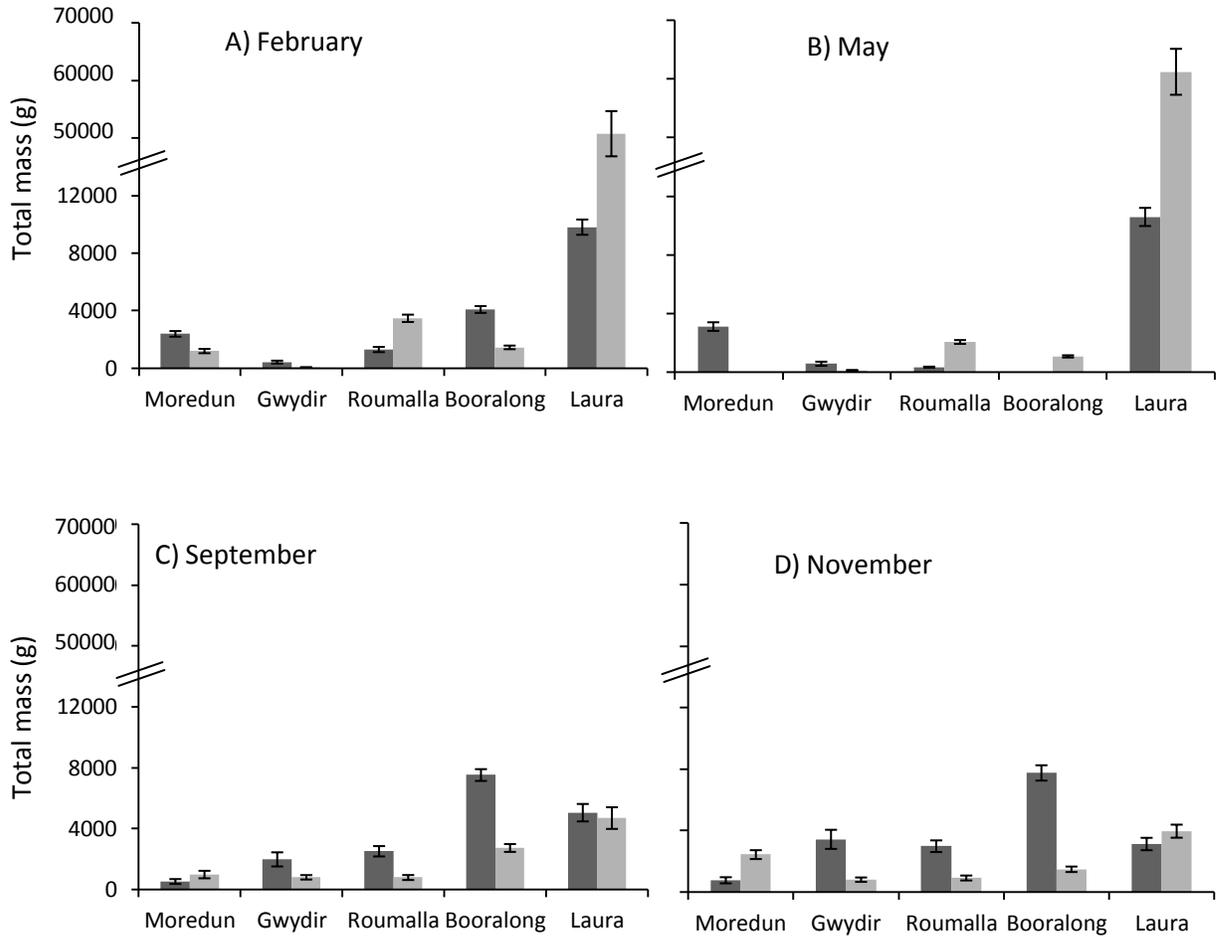


Figure 4.2: Total FBOM mass (g) for each reach during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are standard error of the sample mean ($n=13$). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Coarse benthic organic matter

There was no significant difference in total CBOM mass or CBOM composition between vegetated and non-vegetated reaches (Tables 4.5 and 4.6). The effect of location on CBOM mass was significant in the vegetated control stream, Booralong Creek ($p = 0.045$; Appendix 4, Table A4.10), and the effect of location on the composition of CBOM was significant for both the vegetated control, Booralong Creek ($p = 0.009$) and the non-vegetated control stream, Laura Creek ($p = 0.004$; Appendix 4, Table A4.11). The mass of CBOM was higher at the upstream Booralong CS reach compared to the downstream Booralong Bridge reach during the

February, September and November sampling periods. Similar to the FBOM mass, the largest CBOM mass alternated between the upstream and downstream reaches on the non-vegetated control stream, Laura Creek.

Throughout all sampling periods, the Roumalla vegetated reach had a higher CBOM mass compared to the Roumalla non-vegetated reach. Within the two other treatment streams, Moredun Creek and Gwydir River, the non-vegetated reaches had an overall larger CBOM mass than the vegetated reaches during February, the low-flow period (Figure 4.3). In this case, the unidentifiable fraction contributed to the larger mass. Within these two treatment streams during November, the second high-flow sampling period, the vegetated reaches had the larger CBOM mass compared to the non-vegetated reaches, which was driven by an increase in the woody fraction (Figure 4.4). The variation in total CBOM mass within vegetated and non-vegetated reaches was supported by the significant interaction between treatment, stream and time in the main PERMANOVA test (Table 4.5). The PERMDISP test showed that there were significant differences in dispersions between streams ($p = 0.001$) and sampling times ($p = 0.001$). This indicates that discharge is an important driver of the variation in CBOM mass, and that CBOM mass will vary between streams, regardless of the presence of riparian vegetation.

Table 4.5: Results of PERMANOVA main test for significant differences in average total CBOM mass (g m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	7.347	7.347	1.522	0.336
St	4	75.134	18.784	7.616	0.004
Ti	3	14.302	4.767	1.757	0.232
TrxSt**	2	10.448	5.224	4.616	0.071
TrxTi	3	2.836	0.945	0.799	0.560
StxTi	12	32.198	2.683	6.604	0.001
TrxStxTi**	6	7.519	1.253	3.084	0.005
Res	461	187.320	0.406		
Total	492	349.720			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.6 for correct formulae.

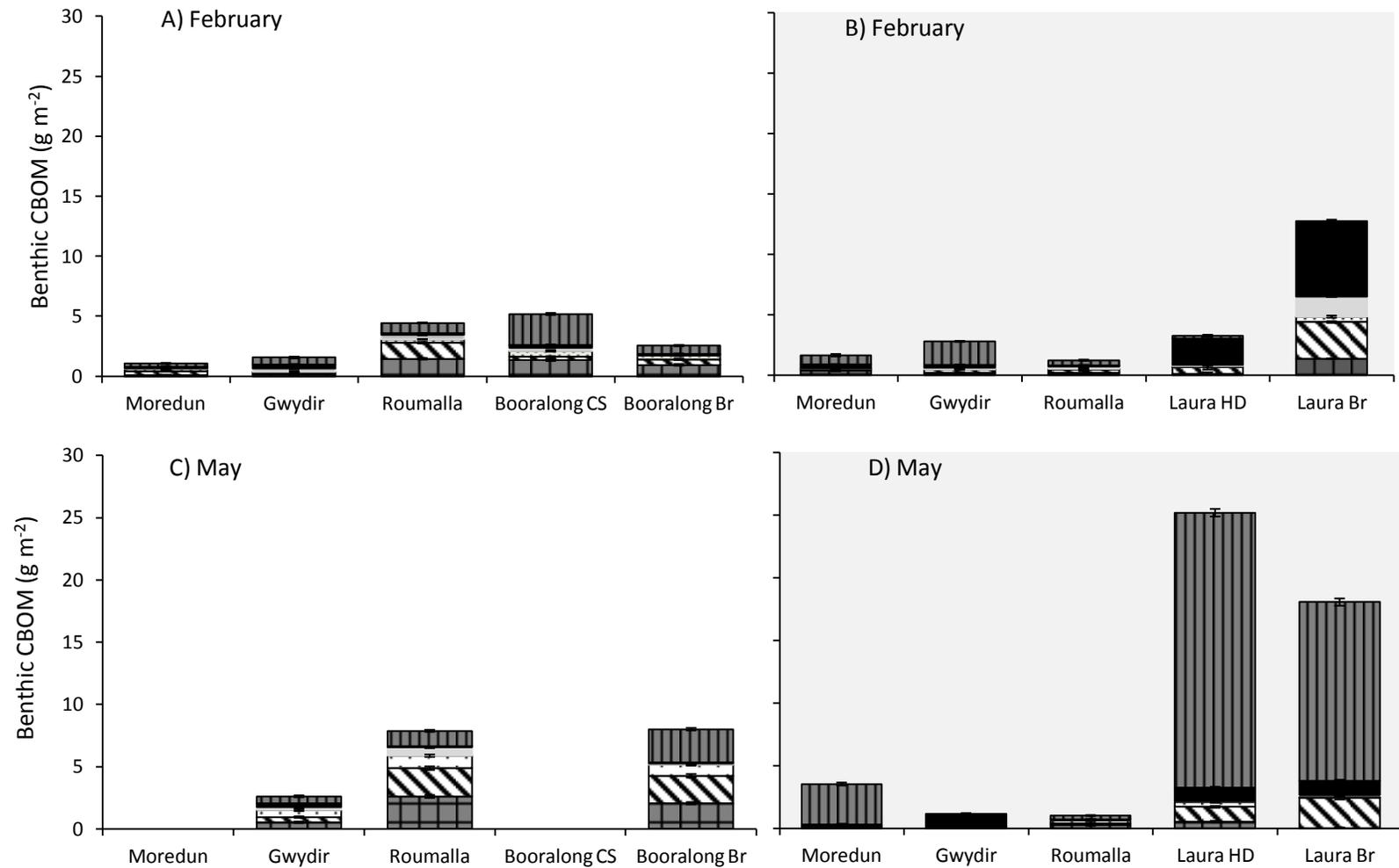


Figure 4.3: Mean CBOM mass (g m⁻²) at the vegetated (white background, A and C) and non-vegetated (grey background, B and D) reaches during February (A-B) and May (C-D). Error bars are standard error of the sample mean (n=13). Patterning in bars represent the six different CBOM fractions – needles (hatching), wood (diagonal stripes), *Casuarina* reproductive structures (dots), leaf (grey), macrophyte (black), and unidentified (horizontal stripes). The unidentifiable fraction (51 g m⁻²) from Laura Br (February) is not presented in this figure.

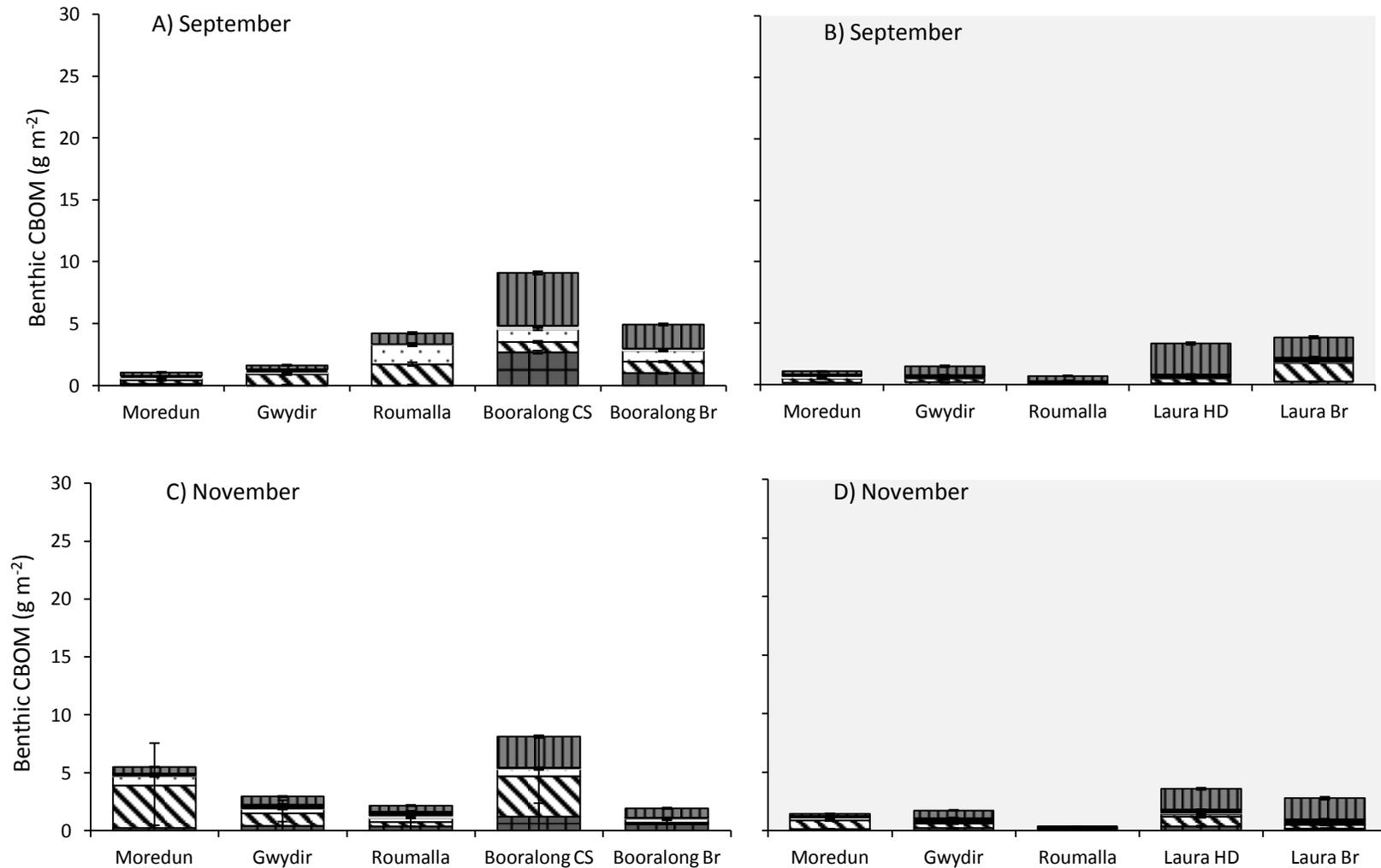


Figure 4.4: Mean CBOM mass (g m⁻²) at the vegetated (white background, A and C) and non-vegetated (grey background, B and D) reaches during September (A-B) and November (C-D). Error bars are standard error of the sample mean (n=13). Patterning in bars represent the six different CBOM fractions – needles (hatching), wood (diagonal stripes), *Casuarina* reproductive structures (dots), leaf (grey), macrophyte (black), and unidentified (horizontal stripes).

With the exception of the Moredun vegetated and Gwydir vegetated reaches during February (the low-flow period) there was generally a higher mass of needles present in vegetated reaches (Figure 4.3). *C. cunninghamiana* needles were not present within CBOM samples from the non-vegetated reaches during May, when there was no surface water flow. During September, the first high-flow sampling period, there were no *C. cunninghamiana* needles present within the CBOM samples from all reaches, with the exception of Booralong Creek. The mass of needles increased at the vegetated reaches during November (Figure 4.4).

There was also a large increase in the wood and *Casuarina* reproductive structure fractions at eight reaches in September and November (Figure 4.4). Within the non-vegetated control reaches, the unidentifiable fraction was very large at both Laura Homestead and Laura Bridge during the low-flow period, February. During May, only the upstream reach, Laura Homestead, had a large unidentifiable fraction.

There was a significant interaction between treatment, streams and times on the composition of CBOM (Table 4.6). The PERMDISP analyses showed that there were also significant differences in dispersions between streams ($p = 0.001$) and sampling times ($p = 0.001$). This indicates that discharge and time are important drivers of the variation in CBOM composition, and that CBOM composition will vary between the study streams, regardless of the presence of riparian vegetation.

Table 4.6: Results of PERMANOVA main test for significant differences in CBOM composition (g m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. The multivariate dataset included masses of: needles, wood, *Casuarina* reproductive structures, leaf/bark, macrophytes, and unidentified fractions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	8.996	8.996	1.804	0.192
St	4	108.310	27.077	4.586	0.003
Ti	3	36.836	12.279	1.885	0.108
TrxSt**	2	1.908	0.954	0.504	0.785
TrxTi	3	15.717	5.239	2.763	0.077
StxTi	12	73.418	6.118	7.527	0.001
TrxStxTi**	5	9.469	1.894	2.330	0.010
Res	457	371.460	0.813		
Total	487	662.240			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.7 for correct formulae.

Whole-reach CBOM estimates

When CBOM mass was estimated at the whole-reach scale, the downstream non-vegetated control reach, Laura Bridge, still had the largest CBOM mass present across all study reaches during February (55,300 g) and May (61,471 g). The upstream vegetated control reach, Booralong CS, also still had the largest CBOM mass present across all study reaches during September (19,518 g, Figure 4.5). During November, when CBOM mass was estimated at the unit area scale, Booralong CS was identified as having the largest CBOM mass (8.1 g m^{-2}) and Moredun vegetated reach had the second largest CBOM mass (5.3 g m^{-2}). However, when CBOM mass was estimated at the whole-reach scale, the Moredun vegetated reach was found to have a higher CBOM mass (18,174 g) than the Booralong CS reach (15,374 g, Figure 4.5).

The CBOM estimates at the unit area scale showed a general decrease with increases in discharge. For example, the CBOM mass at the Moredun non-vegetated reach was 3.9 g m^{-2} during May, the no-flow period, compared to 0.9 g m^{-2} during September, the first high-flow period (Figure 4.6). In some cases, this pattern was still evident when CBOM mass was estimated at whole-reach scales, for example, CBOM mass at the Moredun non-vegetated reach decreased from 5046 g during May to 2139 g during September (Figure 4.6). However, this did not occur in all reaches. At the Roumalla vegetated reach, despite a decrease in CBOM mass from 8.0 g m^{-2} during May to 4.7 g m^{-2} during September, there was an actual increase in the CBOM estimates at the whole-reach scale from 5,488 g in May to 13,478 g during September (Figure 4.6).

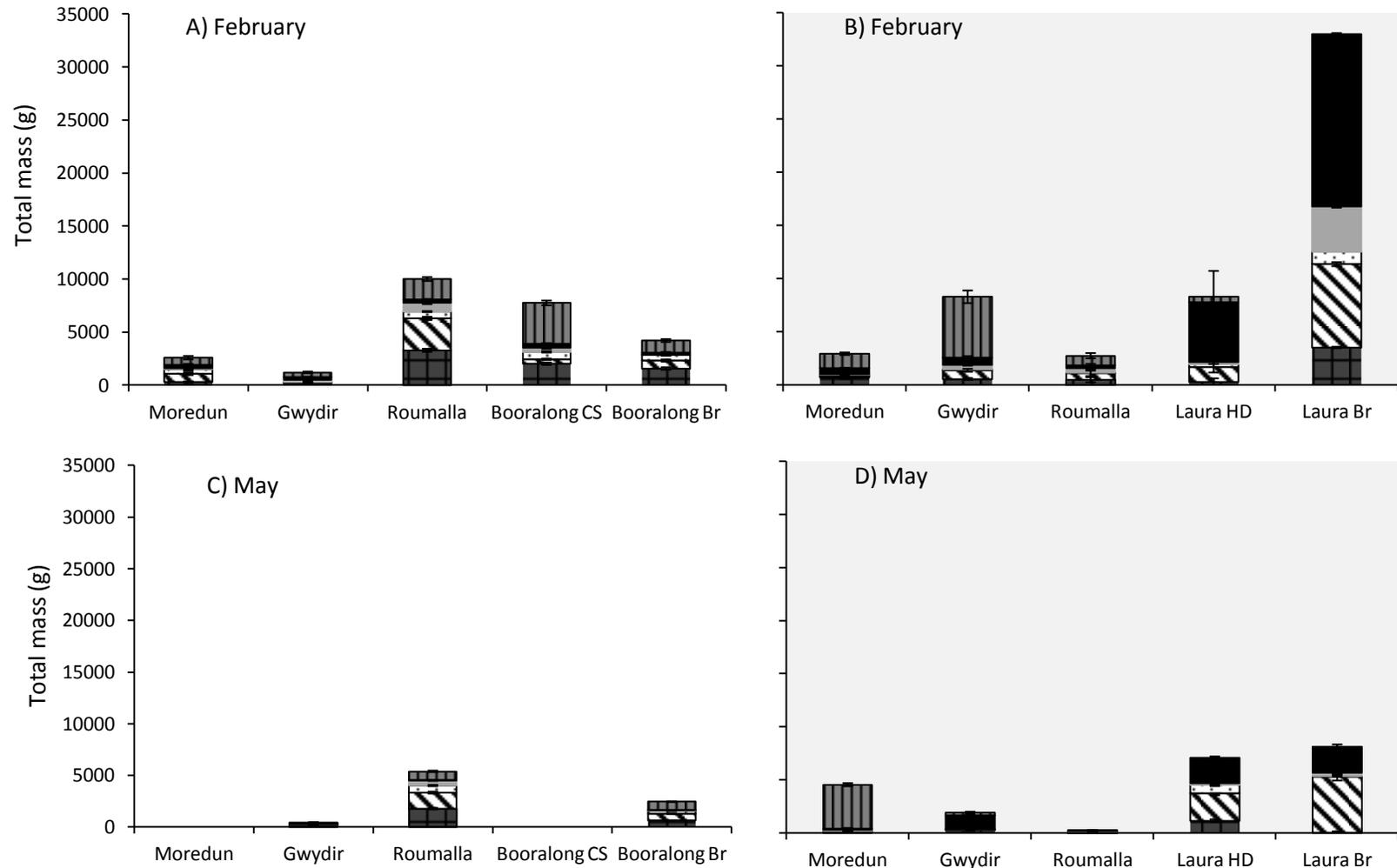


Figure 4.5: Total CBOM mass (g) present at the vegetated (white background, A and C) and non-vegetated (grey background, B and D) reaches during February (A-B) and May (C-D). Error bars are standard error of the sample mean (n=13). Patterning in bars represent the six different CBOM fractions – needles (hatching), wood (diagonal stripes), *Casuarina* reproductive structures (dots), leaf (grey), macrophyte (black), and unidentified (horizontal stripes). The unidentifiable fractions from Laura Br during February (133,107 g) and May (30,604 g), and Laura HD during May (47,584) are not presented in this figure.

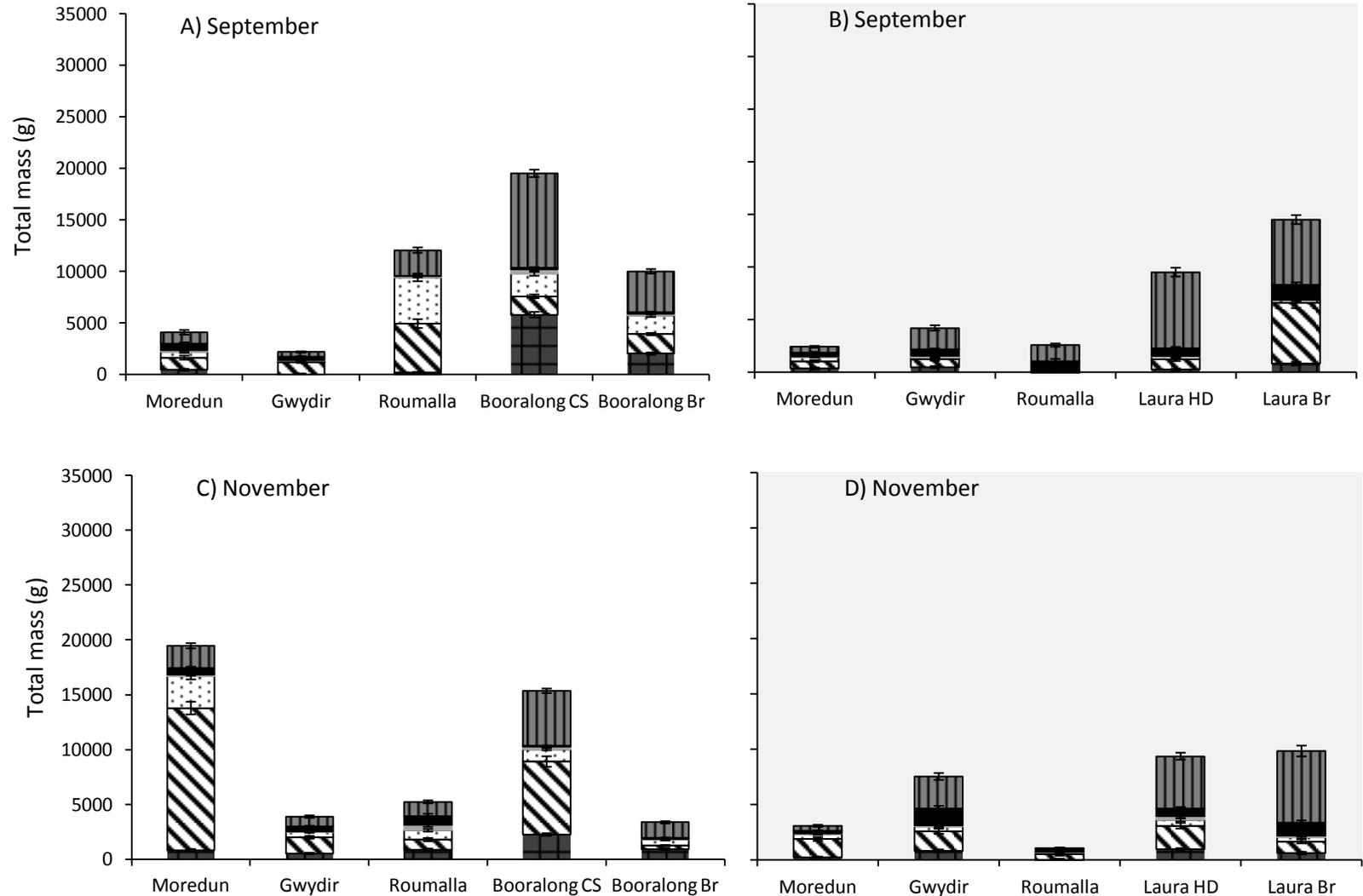


Figure 4.6: Total CBOM mass (g) present at the vegetated (white background, A and C) and non-vegetated (grey background, B and D) reaches during September (A-B) and November (C-D). Error bars are standard error of the sample mean (n=13). Patterning in bars represent the six different CBOM fractions – needles (hatching), wood (diagonal stripes), *Casuarina* reproductive structures (dots), leaf (grey), macrophyte (black), and unidentified (horizontal stripes).

The results of the BEST analyses showed that the whole-reach CBOM mass within the vegetated reaches was correlated with surface area:volume ratio, explaining 35.9% of the variation ($p = 0.06$, Table 4.7). This correlation was driven by the unidentified and *Casuarina* reproductive fractions which were significantly correlated with surface area:volume, explaining 37.4% ($p = 0.03$) and 31.2% ($p = 0.02$), respectively. The *Casuarina* reproductive fraction was also correlated with wetted area (Table 4.7).

Table 4.7: Results of the BEST analyses between total (whole-reach, g) organic matter data from the vegetated reaches and their corresponding hydro-geomorphic variables. Significant results are in bold type. C-repro = *Casuarina* reproductive fraction, UI = unidentifiable fraction.

Nutrient	Correlation level	Correlation variables	Significance level
FBOM	0.225	Surface area:volume	0.27
CBOM	0.359	Surface area:volume	0.06
Needle	0.171	Velocity, surface area:volume	0.25
Wood	0.254	Surface area:volume, wetted area	0.13
C-repro	0.312	Surface area:volume, wetted area	0.02
Leaf	0.168	No. features, surface area:volume, wetted area	0.37
Grass	0.301	Wetted area	0.07
UI	0.374	Surface area:volume	0.03

The BEST analyses for the variation in whole-reach CBOM within the non-vegetated reaches was also correlated with the surface area:volume ratio, however this was slightly less significant than the correlation observed within the vegetated reaches ($p = 0.11$), with only 26.7% of the variation explained by this factor (Table 4.8). The needle fraction was significantly correlated with the number of features and surface area:volume ratio ($p = 0.03$), at a level of 30%. A combination of the number of features, flow exceedence, surface area:volume ratio and wetted area were significantly correlated with the wood fraction ($p = 0.05$, Table 4.8).

Table 4.8: Results of the BEST analyses between total (whole-reach, g) organic matter data from the non-vegetated reaches and their corresponding hydro-geomorphic variables. Significant results are in bold type. C-repro = *Casuarina* reproductive fraction, UI = unidentifiable fraction.

Nutrient	Correlation level	Correlation variables	Significance level
FBOM	0.136	Surface area:volume	0.51
CBOM	0.267	Surface area:volume	0.11
Needle	0.300	No. features, surface area:volume	0.03
Wood	0.294	No. features, flow exceedence, surface area:volume, wetted area	0.05
C-repro	0.352	Flow exceedence, velocity	0.06
Leaf	0.060	No. features	0.72
Grass	0.328	Surface area:volume	0.06
UI	0.068	No. features, flow exceedence	0.67

*Particulate organic matter mass-balance and retention**Fine particulate organic matter*

The smallest loads of FPOM imported to the study reaches occurred during February, the low-flow period and then increased during the high-flow periods (Table 4.9). The range of loads varied from 0.8 g day⁻¹ being exported from the Roumalla non-vegetated reach, to 98 g day⁻¹ being imported to the upstream non-vegetated control reach, Laura Homestead, during February. The largest imported load was recorded at the Moredun non-vegetated reach (345 g day⁻¹) during November, the second consecutive high-flow period. The largest imported load within the vegetated reaches was 190 g day⁻¹ at the Moredun vegetated reach during September (Table 4.9). These changes in imported loads led to an increase in the magnitude of FOM retention and/or export during the high-flow periods.

Table 4.9: The mean imported and exported loads of FPOM (g day⁻¹) during the low-flow (February) and high-flow (September and November) periods. Standard error of the mean are in parentheses (n=3). NV = non-vegetated and V = vegetated.

	February		September		November	
	Import	Export	Import	Export	Import	Export
Moredun NV	1 (0.1)	1 (0.0)	346 (5.3)	176 (3.1)	157 (2.3)	209 (2.3)
Moredun V	4 (1.0)	3 (0.6)	191 (2.4)	156 (1.9)	182 (1.8)	136 (2.8)
Gwydir NV	8 (0.5)	8 (0.6)	314 (2.8)	916 (6.8)	262 (2.4)	811 (3.5)
Gwydir V	8 (0.6)	5 (0.6)	167 (2.2)	231 (2.8)	190 (2.0)	186 (2.2)
Roumalla NV	1 (0.2)	1 (0.2)	78 (1.8)	153 (5.3)	19 (1.2)	84 (2.9)
Roumalla V	7 (0.5)	3 (0.6)	46 (1.1)	55 (1.5)	28 (1.1)	17 (1.1)
Laura HD	98 (3.0)	28 (1.6)	190 (3.6)	106 (2.7)	323 (4.3)	198 (3.8)
Laura Br	15 (1.1)	40 (1.7)	71 (2.2)	85 (3.0)	198 (2.8)	215 (3.3)
Booralong CS	4 (0.5)	8 (0.4)	112 (3.5)	111 (3.1)	68 (5.4)	235 (2.7)
Booralong Br	1 (0.3)	4 (0.4)	105 (1.8)	108 (1.7)	78 (2.0)	45 (1.2)

There was no significant difference in FPOM retention or export between vegetated and non-vegetated reaches, streams, or across time (Table 4.10). The test for differences between locations in FPOM retention on the control streams were non-significant (Appendix 4, Table A4.12). There was also no clear pattern in FPOM retention and export between vegetated and non-vegetated reaches. However, the non-vegetated reaches were consistent in whether they retained or exported FPOM during the three sampling periods. For example, the non-vegetated Gwydir and Roumalla reaches, and the downstream non-vegetated control reach, Laura Bridge, all exported FPOM during the three sampling periods. The upstream non-vegetated control reach, Laura Homestead, consistently retained FPOM (Figure 4.7). Within the vegetated reaches, only the Moredun vegetated reach consistently retained FPOM during the three sampling periods. During the second high-flow sampling period, November, all vegetated reaches except Booralong Bridge, retained FPOM. Additionally, all non-vegetated reaches except Laura Homestead, exported FPOM during November (Figure 4.7).

Table 4.10: Results of PERMANOVA main test for significant differences in total FPOM retention (g day^{-1}) between treatment (Tr), streams (St) and time (Ti), and their interaction. All ten reaches were used in the analysis.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	281.570	281.570	1.977	0.249
St	4	739.910	184.980	2.830	0.104
Ti	2	74.438	37.219	0.643	0.552
TrxSt**	2	113.600	56.798	2.770	0.170
TrxTi	2	191.990	95.996	4.682	0.088
StxTi	8	522.850	65.357	0.7660	0.655
TrxStxTi**	4	82.012	20.503	0.240	0.917
Res	6	511.970	85.328		
Total	29	2202.300			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.8 for correct formulae.

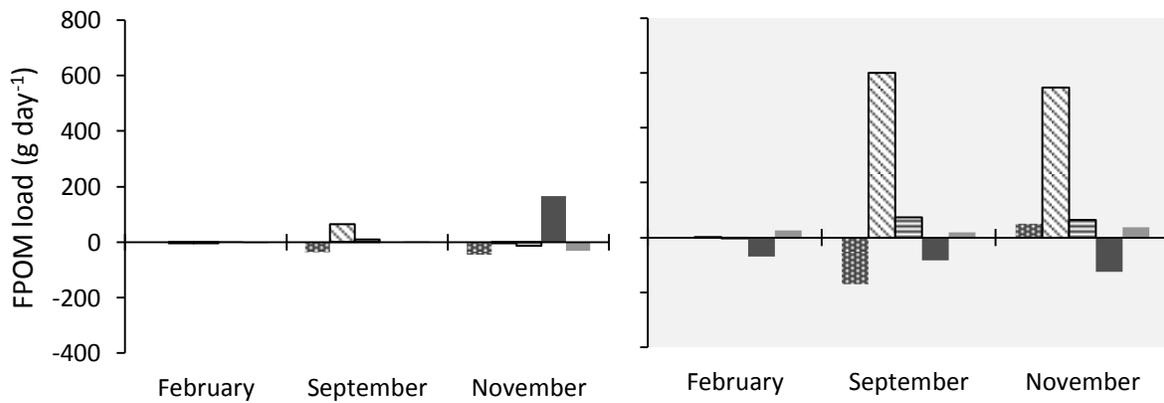


Figure 4.7: Total loads (g day^{-1}) of FPOM retained (negative values) or exported (positive values) by each reach (Vegetated – white background, Non-vegetated – grey background) during February, September and November. Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura Homestead, while the light grey bars represent the downstream reaches, Booralong Bridge and Laura Bridge, on each of the control streams.

Coarse particulate organic matter

The smallest imported CPOM loads occurred during February, the low-flow period and ranged between 3 g day⁻¹ at the Roumalla non-vegetated reach to 239 g day⁻¹ at the upstream vegetated control reach, Booralong CS (Table 4.11). Imported CPOM loads increased across all reaches during September and November, the high-flow periods. The largest imported CPOM load of 11,551 g day⁻¹ occurred at the Moredun non-vegetated reach during September, the first high-flow period, and then decreased to 2,286 g day⁻¹ during November, the second high-flow period. This large decrease in imported CPOM loads was also seen at both the Roumalla vegetated and non-vegetated reaches (Table 4.11).

Table 4.11: The mean imported and exported loads of CPOM (g day⁻¹) during the low-flow (February) and high-flow (September and November) periods. Standard error of the mean are in parentheses (n=3). NV = non-vegetated and V = vegetated.

	February		September		November	
	Import	Export	Import	Export	Import	Export
Moredun NV	21 (1.5)	2 (0.5)	11,551 (31.9)	2,917 (14.1)	2,286 (7.9)	3,128 (9.7)
Moredun V	4 (0.6)	10 (1.3)	5,073 (16.4)	4,713 (12.7)	3,157 (11.0)	2,532 (10.8)
Gwydir NV	439 (4.7)	180 (3.3)	3,051 (8.2)	18,210 (31.5)	2,554 (6.8)	12,225 (23.3)
Gwydir V	533 (3.7)	156 (3.7)	857 (4.4)	4,015 (7.4)	882 (8.4)	1,919 (11.4)
Roumalla NV	3 (0.7)	11 (1.0)	854 (5.3)	196 (2.6)	171 (4.6)	342 (5.1)
Roumalla V	70 (1.6)	36 (2.3)	341 (2.6)	665 (8.3)	99 (3.0)	186 (4.1)
Laura HD	136 (3.4)	12 (1.4)	1,923 (9.8)	759 (3.8)	1,412 (8.8)	1,171 (9.3)
Laura Br	8 (0.7)	22 (0.7)	642 (4.8)	1,552 (10.3)	1,843 (6.0)	1,489 (6.8)
Booralong CS	239 (1.7)	35 (2.0)	628 (5.7)	1,154 (6.1)	995 (10.2)	2,569 (7.0)
Booralong Br	7 (1.0)	4 (0.2)	329 (5.0)	733 (2.6)	917 (7.1)	428 (6.5)

The composition of CPOM imported to each reach was not significantly different between vegetated and non-vegetated reaches, but there were significant differences between sampling times (Table 4.12). A significant interaction existed between streams and times (Table 4.12), and the PERMDISP test showed that there was a significant difference in dispersions between streams ($p = 0.004$). The MDS indicates that differences in imported droppings, bark and macrophyte fractions contributed to some of the variation between streams and reaches, but the difference in imported wood, needle and unidentifiable fractions drove the variation between sampling times (Figure 4.8).

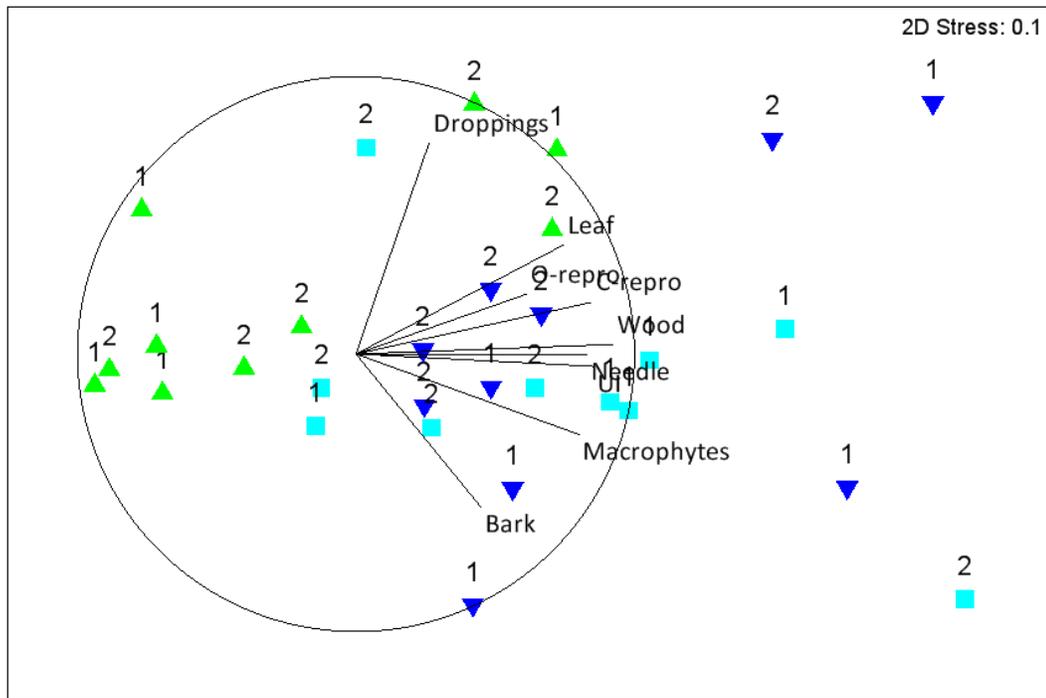


Figure 4.8: MDS of imported CPOM composition including: needles, wood, *Casuarina* reproductive structures, leaf/bark, macrophytes, and unidentified fractions (g day^{-1}). Samples were from all non-vegetated (labeled 1) and vegetated (labeled 2) reaches during the three sampling times (February – green triangles, September – blue inverted triangles, November – light blue squares). Individual points represent the mean of three samples ($n=3$ for each reach at each sampling time).

Table 4.12: Results of PERMANOVA main test for significant differences in the composition of CPOM imported (g day^{-1}) between treatment (Tr), streams (St) and time (Ti), and their interactions. The multivariate dataset included loads of: needles, wood, *Casuarina* reproductive structures, leaf/bark, macrophytes, and unidentified fractions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	Df	SS	MS	Pseudo-F	P(perm)
Tr	1	1.237	1.237	0.322	0.977
St	4	47.343	11.836	1.216	0.337
Ti	2	72.997	36.499	3.417	0.013
TrxSt**	2	8.514	4.257	1.690	0.212
TrxTi	2	14.785	7.393	2.934	0.108
StxTi	8	77.846	9.731	2.454	0.011
TrxStxTi**	4	10.079	2.520	0.635	0.827
Res	6	23.791	3.965		
Total	29	261.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.8 for correct formulae.

The test for significant differences in the import of needles, wood and *Casuarina* reproductive structures showed that there was no difference between vegetated and non-vegetated reaches but there were significant interactions between treatment, streams and time (Table 4.13).

There was also a significant interaction between streams and times (Table 4.13) on the import of needles, wood and *Casuarina* reproductive structures. The results of the PERMDISP test showed that there were also significant differences in dispersions between sampling times ($p = 0.003$).

Table 4.13: Results of PERMANOVA main test for significant differences in *Casuarina* CPOM imported (g day^{-1}) between treatment (Tr), streams (St) and time (Ti), and their interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	Df	SS	MS	Pseudo-F	P(perm)
Tr	1	2.444	2.444	1.103	0.469
St	4	24.078	6.020	1.403	0.278
Ti	2	12.608	6.304	1.326	0.358
TrxSt**	2	2.372	1.186	0.702	0.563
TrxTi	2	5.125	2.563	1.517	0.308
StxTi	8	34.317	4.290	30.973	0.001
TrxStxTi**	4	6.758	1.690	12.199	0.001
Res	6	0.831	0.139		
Total	29	87.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.8 for correct formulae.

Although there was no clear pattern of retention within or between vegetated and non-vegetated reaches, all reaches recorded retention of one fraction at least once (Figure 4.9). It was necessary to separate and calculate the retention or export of each individual fraction of CPOM to determine whether any retention was occurring, as it would have been masked by the change in the total amount of transported CPOM. The results of the PERMANOVA tests showed that there was no significant difference in the total CPOM or composition of CPOM retention between vegetated and non-vegetated reaches or between times (Tables 4.14 and 4.15). However, there was a significant interaction between streams and sampling times on the composition of CPOM retention and the PERMDISP test found that there were significant differences in dispersions between streams ($p = 0.019$) and times ($p = 0.034$).

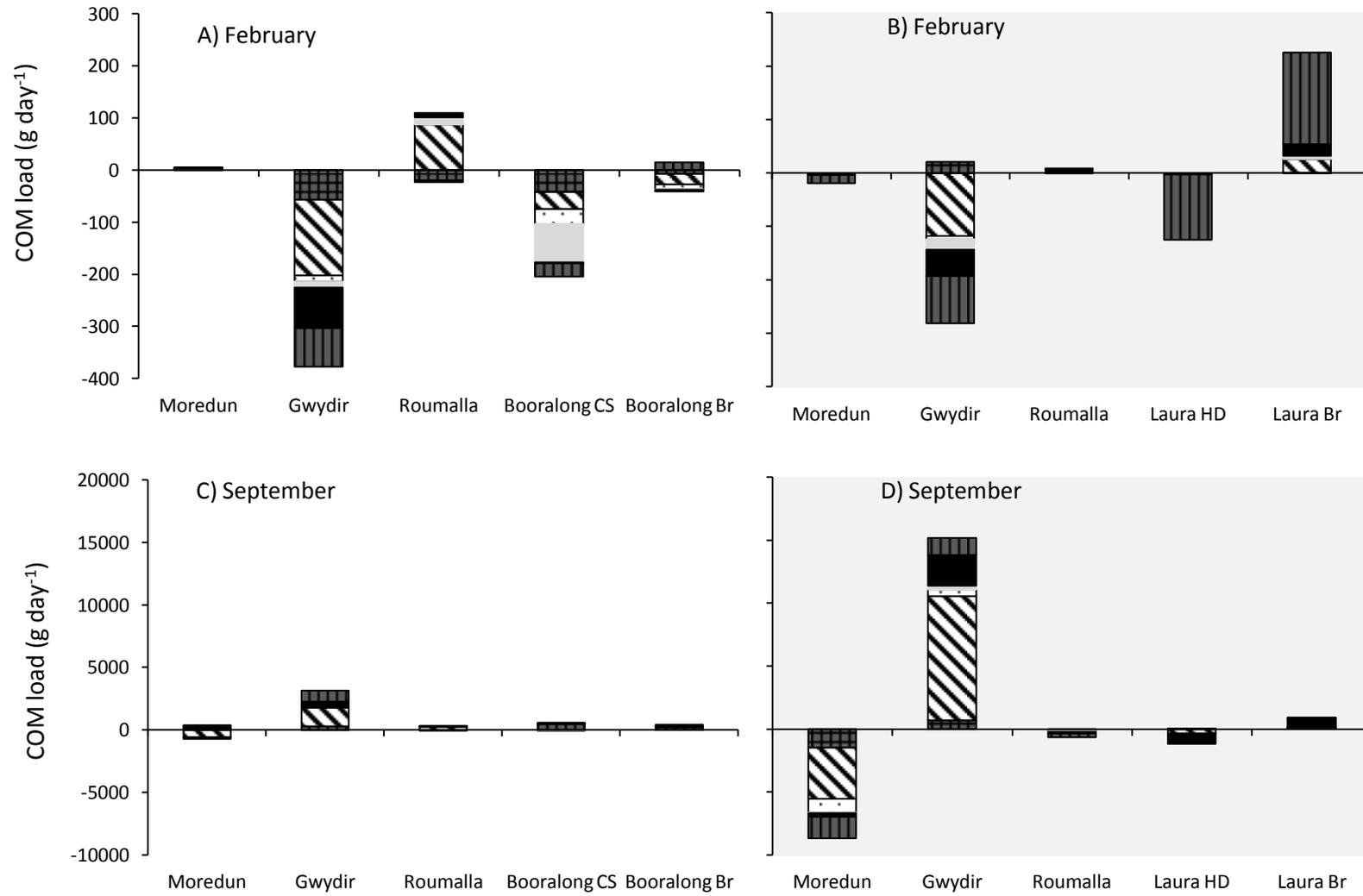
There appeared to be no patterns in which fractions of CPOM were retained or exported across all reaches. There was a similar pattern of retention and export across time within the Gwydir River reaches, where the majority of CPOM was retained during February, the low-flow period, and exported CPOM during September and November, in both the vegetated and non-vegetated reaches. Wood (144 g day^{-1}), macrophyte material (78 g day^{-1}), needles (56 g day^{-1}) and the unidentified fraction (73 g day^{-1}) were the main CPOM fractions retained at the Gwydir vegetated reach during February. These were also main fractions which contributed to the exported loads in September, ranging between 294 g day^{-1} for needles and $1,505 \text{ g day}^{-1}$ for wood, and November, ranging between 129 g day^{-1} for macrophyte material and 361 g day^{-1} for wood (Figure 4.9).

Within the Gwydir non-vegetated reach, wood (147 g day^{-1}), macrophyte material (50 g day^{-1}) and the unidentifiable fraction (89 g day^{-1}) were the main CPOM fractions retained during February. Similar to the vegetated reach, they were also the main contributors to the exported load during September (wood $9,844 \text{ g day}^{-1}$, macrophyte material $2,462 \text{ g day}^{-1}$, unidentified material $1,317 \text{ g day}^{-1}$). During November, wood ($4,282 \text{ g day}^{-1}$), unidentified material ($3,652 \text{ g day}^{-1}$) and needles (684 g day^{-1}) were the main fraction exported from the Gwydir non-vegetated reach (Figure 4.9). During September, wood was retained at both the Moredun

vegetated (568 g day^{-1}) and non-vegetated reaches ($4,070 \text{ g day}^{-1}$), but only the vegetated reach retained wood (560 g day^{-1}) during November.

The largest exported CPOM load was from the Gwydir non-vegetated reach during September ($15,160 \text{ g day}^{-1}$). The largest retained load was at the Moredun non-vegetated reach also during September ($-8,634 \text{ g day}^{-1}$), the first high-flow sampling period (Figure 4.9). During February, the low-flow period, larger masses of CPOM were retained in comparison to those exported across the reaches. For example, the largest CPOM masses retained included 376 g day^{-1} and 259 g day^{-1} at the Gwydir vegetated and non-vegetated reaches, respectively, while the largest exported CPOM load was 87 g day^{-1} from the Roumalla vegetated reach (Figure 4.9).

Changes to the CPOM loads at the vegetated reaches during September and November, the high-flow periods, were driven by the retention or export of the needle fraction. The largest amount of needles exported was from the Booralong CS ($1,505 \text{ g day}^{-1}$), while the largest amount of needles retained was within the Booralong Bridge reach (513 g day^{-1}) during November. The Moredun vegetated reach also retained needles (229 g day^{-1}) during November (Figure 4.9).



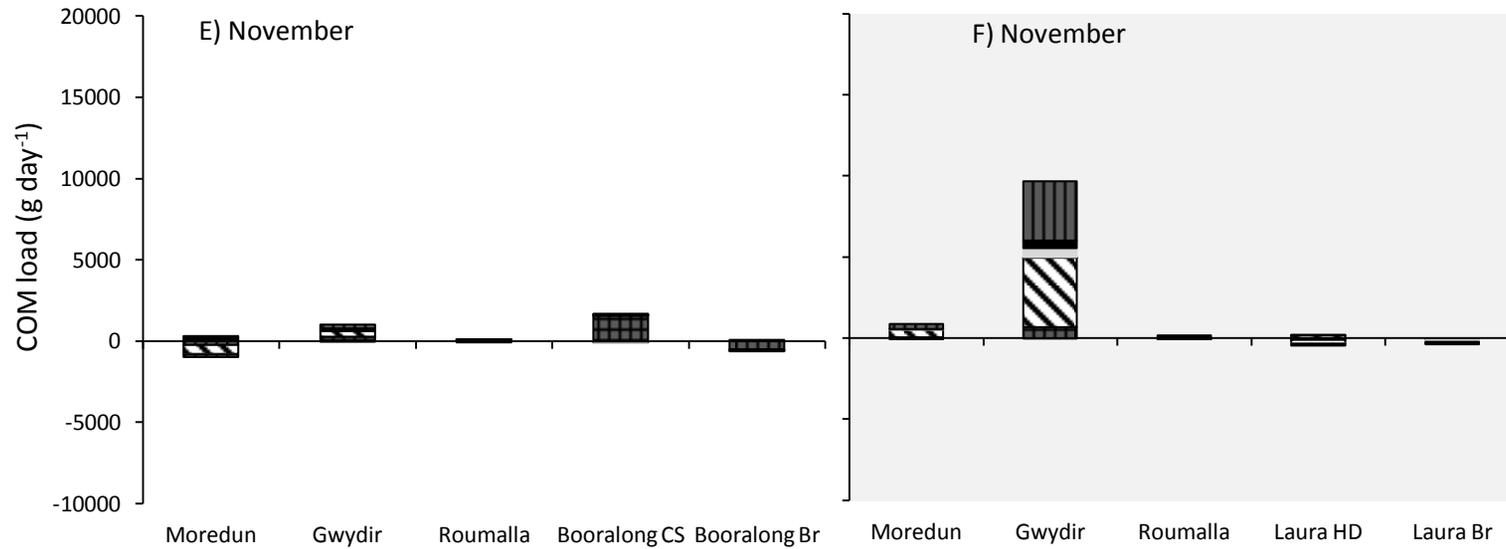


Figure 4.9: Total COM loads (g day⁻¹) retained or exported at the vegetated (white background, A, C, and E) and non-vegetated (grey background, B, D, and F) reaches during February (A-B), September (C-D) and November (E-F). Patterning in bars represent the six different CBOM fractions – needles (hatching), wood (diagonal stripes), *Casuarina* reproductive structures (dots), leaf (grey), macrophyte (bark), and unidentified (horizontal stripes). Note that the axis for February (A-B) figures is different from the September and November axes (C-F).

Table 4.14: Results of PERMANOVA main test for significant differences in total CPOM retention (g day^{-1}) between treatment (Tr), and time (Ti), and their interaction. All ten reaches were used in the analysis.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	222.72	222.72	0.33949	0.824
Ri	2	1966.1	983.05	0.43134	0.673
Ti	4	14342	3585.5	1.6206	0.25
TrxRi	2	2779.4	1389.7	1.6984	0.274
TrxTi**	2	3353.1	1676.6	2.049	0.228
RixTi	8	17700	2212.5	3.179	0.09
TrxRixTi**	4	3273	818.25	1.1757	0.397
Res	6	4175.8	695.96		
Total	29	46282			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.8 for correct formulae.

Table 4.15: Results of PERMANOVA main test for significant differences in the composition of CPOM retained (g day^{-1}) between treatment (Tr), streams (St) and time (Ti), and their interactions. The multivariate dataset included loads of: needles, wood, *Casuarina* reproductive structures, leaf/bark, macrophytes, and unidentified fractions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	3.120	3.112	0.496	0.864
St	4	47.768	11.942	1.123	0.428
Ti	2	8.528	4.264	0.406	0.861
TrxSt**	2	20.895	10.447	1.695	0.243
TrxTi	2	16.519	8.259	1.340	0.313
StxTi	8	85.102	10.638	2.211	0.035
TrxStxTi**	4	24.659	6.165	1.281	0.282
Res	6	28.873	4.812		
Total	29	232.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.8 for correct formulae.

The results of the BEST analyses showed that there was a significant correlation between FPOM retention with the size of wetted area within the non-vegetated reaches ($p = 0.01$, Table 4.16), but not the vegetated reaches. The total CPOM retained in the non-vegetated reach was significantly correlated with wetted area and velocity ($p = 0.03$, Table 4.16). The results for the vegetated reaches showed that velocity and wetted area were significantly correlated with the composition of CPOM retained ($p = 0.01$, Table 4.17).

Table 4.16: Results of the BEST analyses between organic matter retention data (g day^{-1}) from the non-vegetated reaches and their corresponding hydro-geomorphic variables. Significant results are in bold type.

Nutrient	Correlation level	Correlation variables	Significance level
All FOM retention	0.351	Wetted area	0.03
All COM retention	0.401	Velocity, wetted area	0.03
Composition COM retention	0.303	Velocity, wetted area	0.18

Table 4.17: Results of the BEST analyses between organic matter retention data (g day^{-1}) from the vegetated reaches and their corresponding hydro-geomorphic variables. Significant results are in bold type.

Nutrient	Correlation level	Correlation variables	Significance level
All FOM retention	0.094	No. of features, velocity, wetted area	0.85
All COM retention	0.219	Velocity, wetted area	0.23
Composition COM retention	0.572	Velocity, wetted area	0.01

Experimental organic matter releases

The PERMANOVA test identified the difference in the proportion of twig analogues retained between vegetated and non-vegetated reaches as being statistically significant (Table 4.18). However, this difference was most likely driven by a significant difference in dispersion between vegetated and non-vegetated reaches, as determined by the PERMDISP test ($p = 0.002$). There was large variation between the results for the replicate twig releases within all non-vegetated reaches compared to the twig releases at the vegetated reaches. At least one of the twig releases in each of the non-vegetated reaches retained a larger amount of twig analogues compared to the twig releases at the vegetated reaches (Figure 4.10).

There was no clear difference in needle retention between vegetated and non-vegetated reaches (Figure 4.10, Table 4.18). Three reaches showed large variation between duplicate needle releases within the non-vegetated reaches, while only one reach showed large variation in releases within the vegetated reaches (Figure 4.10). The Roumalla vegetated and non-vegetated reaches both retained the largest proportion of needles released in their respective treatments. The Gwydir vegetated reach consistently retained the lowest proportion of both needles and twigs.

Table 4.18: Results of PERMANOVA tests for significant differences in analogue retention (% retained) between treatments (Tr), and releases (Rel). All ten reaches were used in the analysis.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
Twig retention	Tr	1	1332.200	1332.200	4.207	0.041
	Res	18	5700.000	316.670		
	Total	19	7032.200			
Needle retention	Tr	1	260.140	260.140	0.408	0.529
	Res	18	11492.000	638.450		
	Total	19	11752.000			

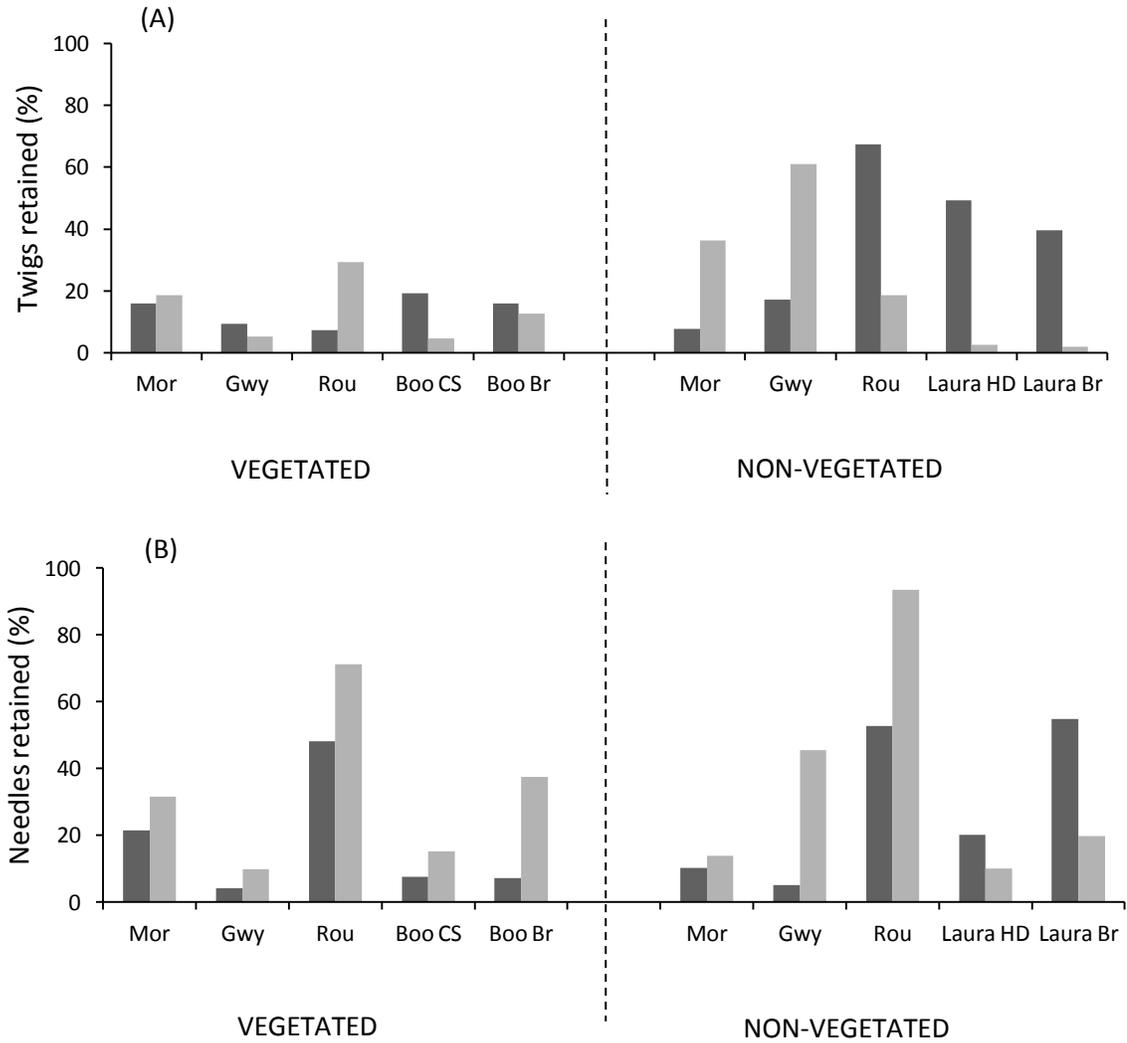


Figure 4.10: The total proportion (%) of twigs (A) and needles (B) retained by each reach for the first (dark grey) and second (light grey) releases. Stream names are abbreviated as: Moredun – Mor, Gwydir – Gwy, Roumalla – Rou, Booralong – Boo.

The MDS graphs show that the difference in methods of retention between vegetated and non-vegetated reaches was very similar for twig and needle releases (Figure 4.11). Macrophytes were an important method of retention for twigs and needles in both vegetated and non-vegetated reaches. The separation in the method of retention between the two reach types was more distinct for the twig releases compared to the needle releases, and was driven by flood debris accumulation points and pools, respectively (Figure 4.11).

Table 4.19: Results of PERMANOVA tests for significant differences in method of retention (% retained by each habitat) between treatments (Tr). All ten reaches were used in the analysis.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
Twig retention	Tr	1	6593.900	6593.900	2.229	0.095
	Res	18	53244.000	2958.000		
	Total	19	59838.000			
Needle retention	Tr	1	3722.900	3722.900	0.965	0.425
	Res	18	69476.000	3859.800		
	Total	19	73199.000			

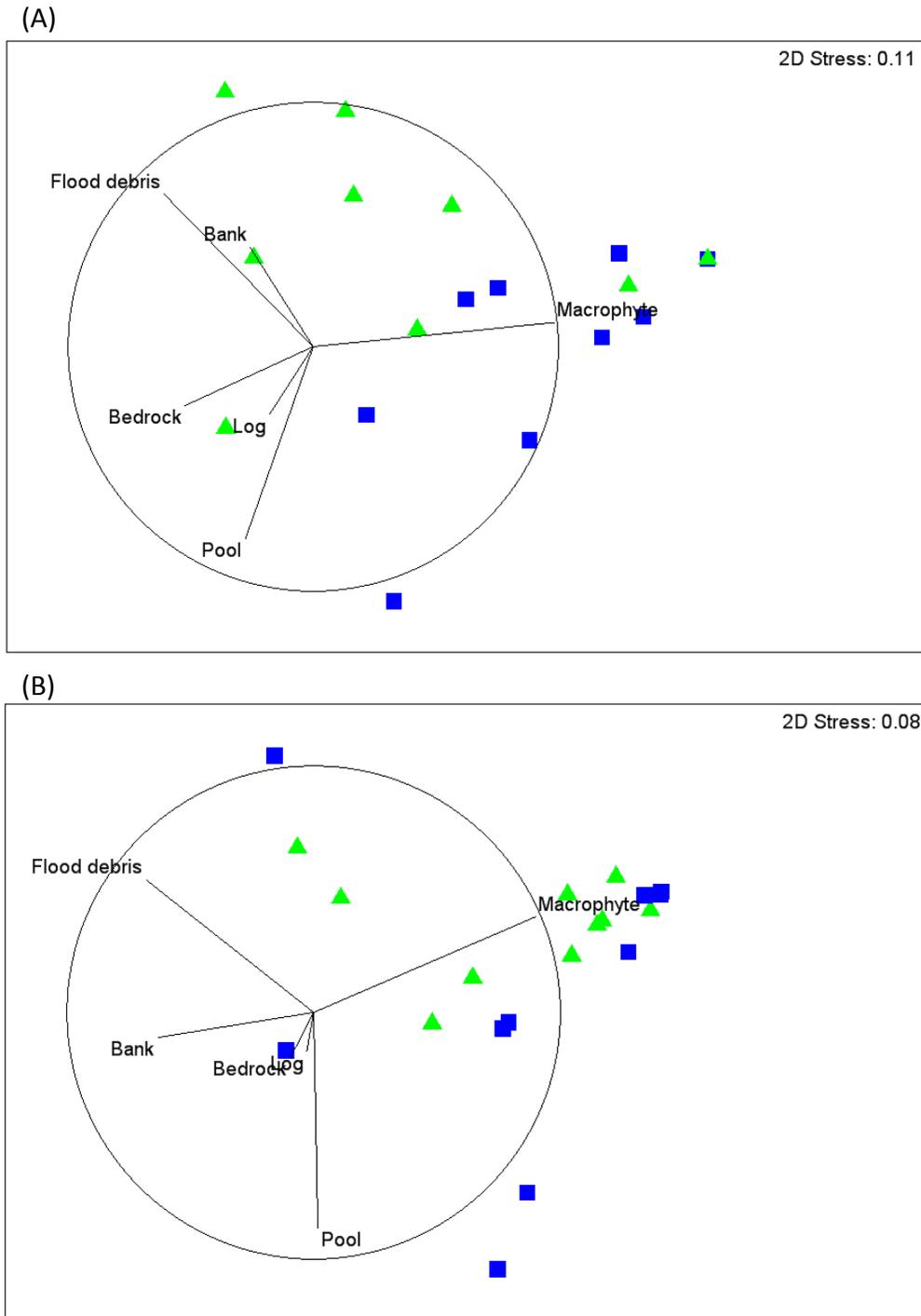


Figure 4.11: The MDS of proportions of twigs (A) and needles (B) retained by different habitats within each reach. Individual points represent a single release at vegetated (triangles) and non-vegetated (squares) reaches.

Organic matter breakdown experiment

The results of the first PERMANOVA test using the mass loss (%) of each litterbag sample showed that there was a large amount of variation among the residuals; however, time was identified as a significant factor (Table 4.20). A subsequent test using the mean mass loss per retrieval time (%; n=3) reduced the residual variation and confirmed that time (hydrology) was the only significant factor or interaction (Table 4.21). There was no effect of location on mass loss (Appendix 4, Table A4.13).

The largest mean mass losses occurred within the vegetated reaches on the treatment streams during September, the first high-flow deployment period. These mass losses ranged from 35 – 60%. During all other deployment periods, the mean mass loss was generally below 30% across all reaches. There was no clear pattern of mean mass loss between vegetated and non-vegetated reaches during February and May (Figure 4.12). The mean mass loss from the litterbags was higher at the vegetated reaches within the three treatment streams (although litterbags were lost from Moredun Creek in November) during the September and November deployment periods.

Table 4.20: Results of PERMANOVA main test for significant differences in litterbag mass loss (% mass lost, determined from area under the curve) between treatment (Tr), streams (St), and time (Ti), and their interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	5.926e ⁻²	5.926e ⁻²	0.393	0.743
St	4	0.255	6.383e ⁻²	0.608	0.680
Ti	3	3.398	1.133	9.299	0.007
TrxSt**	2	0.101	5.059e ⁻²	0.861	0.514
TrxTi	3	0.776	0.259	4.339	0.124
StxTi**	11	1.209	0.110	1.710	0.087
TrxStxTi**	3	0.176	5.878e ⁻²	0.915	0.444
Res	71	4.564	6.428e ⁻²		
Total	98	10.825			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.9 for correct formulae.

Table 4.21: Results of PERMANOVA main test for significant differences in mean litterbag mass loss (% mass lost, determined from area under the curve, n=3) between treatment (Tr), streams (St), and time (Ti), and their interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	64.807	64.807	0.786	0.513
St	4	136.000	34.001	0.712	0.718
Ti	3	852.410	284.140	5.560	0.019
TrxSt**	2	52.094	26.047	0.786	0.548
TrxTi	3	339.440	113.150	3.414	0.163
StxTi**	11	509.010	46.273	0.766	0.684
TrxStxTi**	3	99.418	33.139	0.548	0.669
Res	7	423.050	60.436		
Total	34	2697.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.10 for correct formulae.

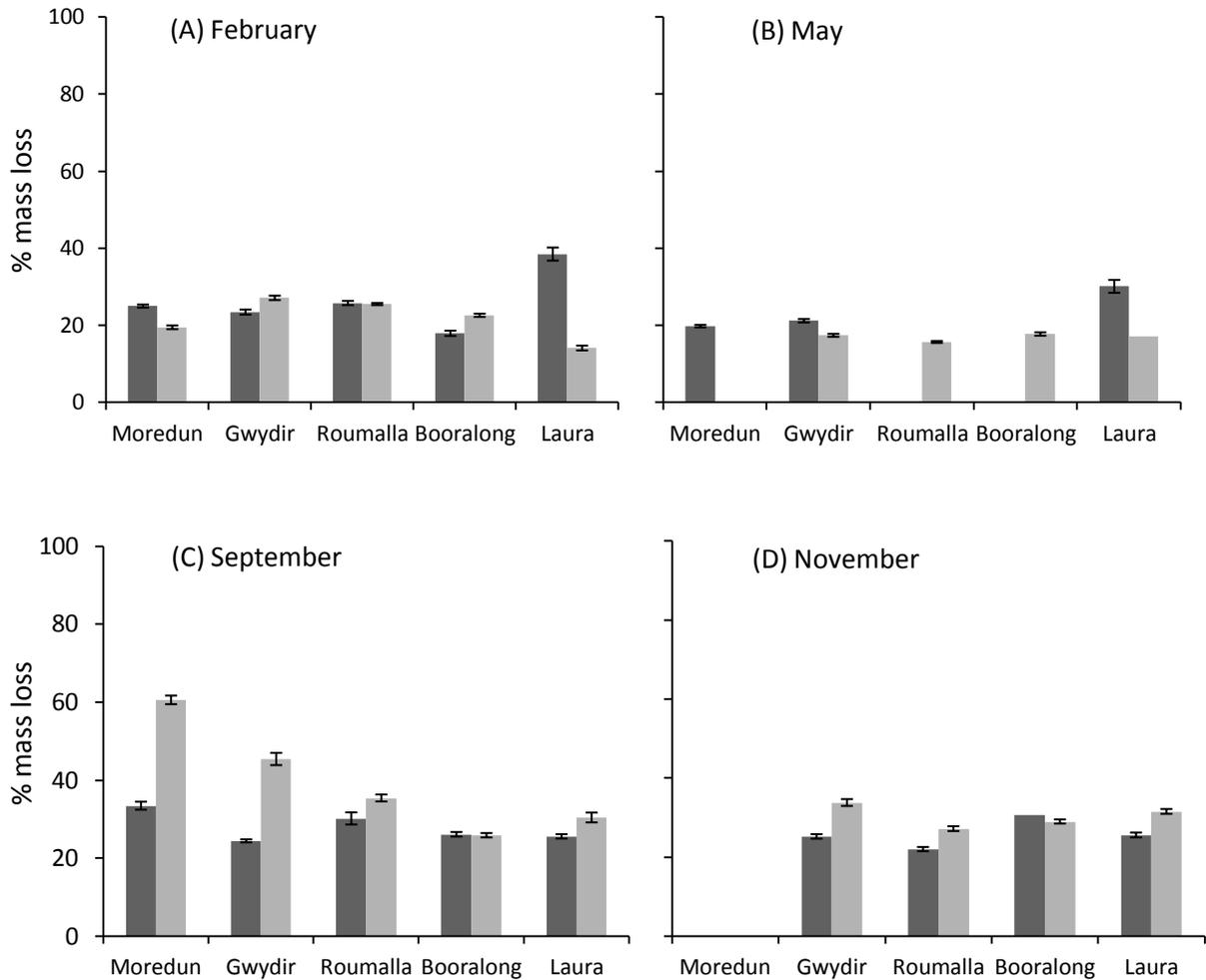


Figure 4.12: Mean proportion mass loss (%) from the *C. cunninghamiana* litterbags during (A) February, (B) May, (C) September and (D) November. Error bars show the standard error of the sample mean (n=3). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Several issues were encountered while attempting to determine breakdown rates. It was evident visually that the pattern of breakdown (exponential vs. linear curve) varied between deployment times. For example, the litterbags deployed at the vegetated reaches showed a linear pattern of breakdown during November (and several reaches during September also), while an exponential breakdown pattern was evident during February and May deployment periods (Figure 4.13). The coefficients of determination (R^2) supported this observation (Table 4.22). Overall, using days as the predictor variable, exponential curve-fitting did provide a good fit on most occasions ($R^2 > 0.75$), but the resulting breakdown rates were usually an order of magnitude higher compared to those reported in the literature. Linear curve-fitting was found to fit poorly in most cases ($R^2 < 0.70$), except during September and November. Using degree-days as the predictor variable, exponential curve-fitting also provided a good fit, but did not reduce any variation in breakdown rates between or within vegetated and non-vegetated samples (Figure 4.13). Therefore, because of the ambiguity of the breakdown rate data, the total mass loss estimates were used in the PERMANOVA analyses to test for differences between vegetated and non-vegetated reaches.

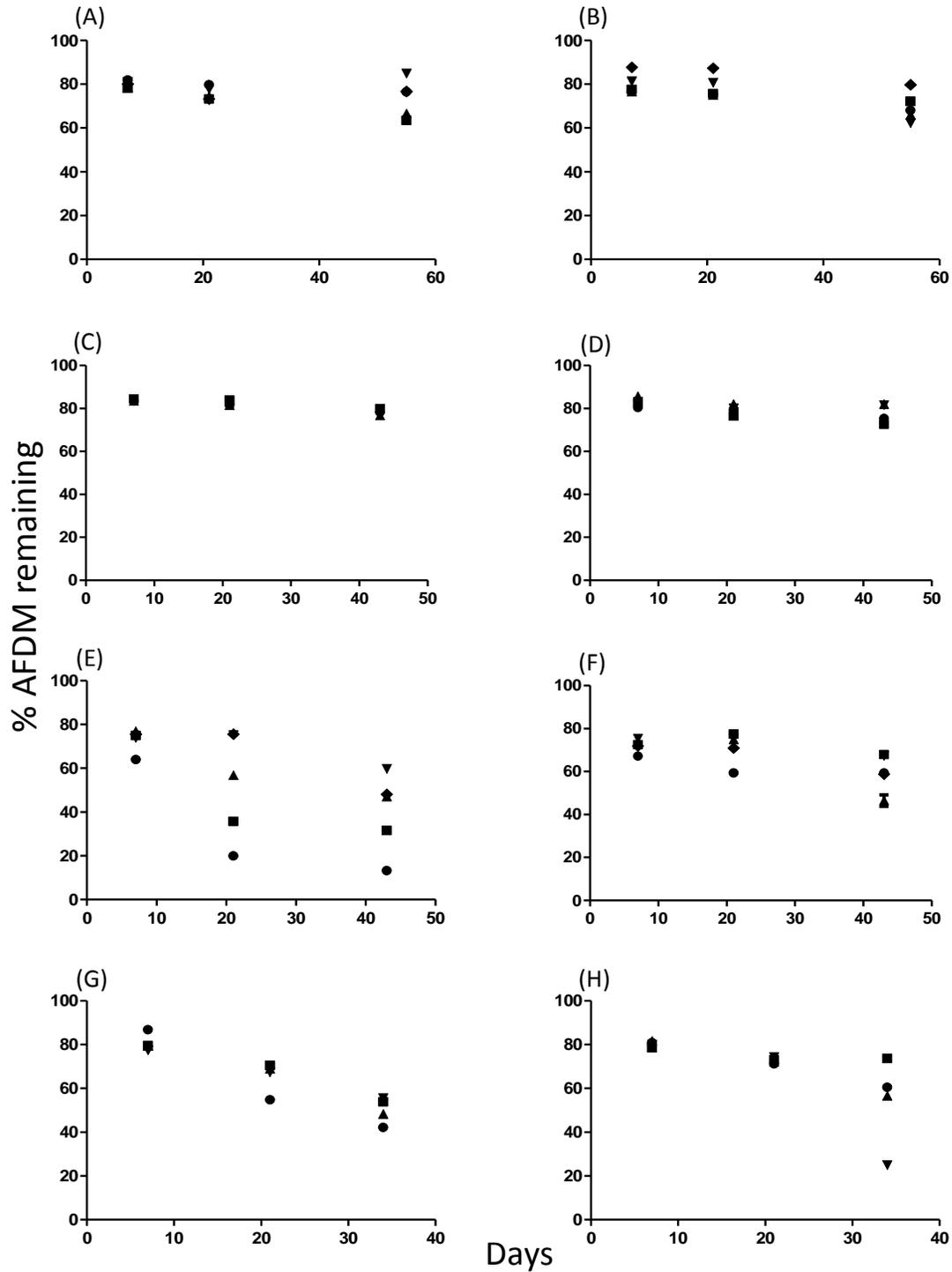


Figure 4.13: Mean and standard error (n=3) of remaining litterbag AFDM versus days for vegetated (A, C, E, G) and non-vegetated (B, D, F, H) reaches for each deployment period: February (A-B), May (C-D), September (E-F) and November (G-H). Symbols represent Moredun (circle), Gwydir (square), Roumalla (triangle), Booralong CS and Laura Homestead (inverted triangle), Booralong Bridge and Laura Bridge (diamond).

Table 4.22: Summary of breakdown rates and coefficient of determination for each reach during each deployment time.

Reach	Days		Days		Degree days	
	Exponential	R ²	Linear	R ²	Exponential	R ²
February						
Moredun vegetated	0.237 ± 0.059	0.95	0.004 ± 0.001	0.53	0.008 ± 0.002	0.95
Gwydir vegetated	0.115 ± 0.028	0.93	0.008 ± 0.002	0.73	0.004 ± 0.001	0.94
Roumalla vegetated	0.146 ± 0.027	0.96	0.007 ± 0.001	0.70	0.005 ± 0.001	0.96
Booralong CS	**		0.002 ± 0.001	0.11	0.012 ± 0.003	0.97
Booralong Bridge	0.234 ± 0.045	0.97	0.004 ± 0.001	0.38	0.010 ± 0.002	0.97
Moredun non-vegetated	0.224 ± 0.062	0.93	0.006 ± 0.001	0.61	0.007 ± 0.002	0.93
Gwydir non-vegetated	0.271 ± 0.100	0.91	0.005 ± 0.002	0.48	0.009 ± 0.003	0.91
Roumalla non-vegetated	0.190 ± 0.061	0.90	0.007 ± 0.002	0.64	0.006 ± 0.002	0.90
Laura Homestead	0.040 ± 0.027	0.78	0.008 ± 0.002	0.75	0.001 ± 0.0003	0.76
Laura Bridge	0.094 ± 0.045	0.76	0.004 ± 0.001	0.65	0.001 ± 0.0002	0.77
May						
Gwydir vegetated	0.233 ± 0.054	0.95	0.005 ± 0.001	0.61	0.014 ± 0.003	0.95
Roumalla vegetated	0.269 ± 0.081	0.94	0.004 ± 0.001	0.59	0.016 ± 0.005	0.95
Booralong Bridge	0.199 ± 0.038	0.96	0.005 ± 0.001	0.68	0.014 ± 0.003	0.96
Moredun non-vegetated	0.273 ± 0.068	0.96	0.006 ± 0.001	0.58	0.015 ± 0.003	0.96
Gwydir non-vegetated	0.139 ± 0.026	0.96	0.007 ± 0.001	0.71	0.008 ± 0.002	0.96
Laura Homestead	0.224 ± 0.097	0.87	0.004 ± 0.001	0.68	0.014 ± 0.006	0.87
Laura Bridge	0.305 ± 0.166	0.86	0.004 ± 0.001	0.38	0.019 ± 0.010	0.86
September						
Moredun vegetated	0.083 ± 0.014	0.97	0.066 ± 0.007	0.96	0.005 ± 0.0009	0.98
Gwydir vegetated	0.073 ± 0.016	0.97	0.033 ± 0.005	0.94	0.004 ± 0.0007	0.99
Roumalla vegetated	0.077 ± 0.023	0.93	0.019 ± 0.003	0.85	0.005 ± 0.001	0.93
Booralong CS	**		0.016 ± 0.001	0.95	0.007 ± 0.003	0.85
Booralong Bridge	0.082 ± 0.036	0.86	0.011 ± 0.002	0.80	0.001 ± 0.001	0.92
Moredun non-vegetated	0.235 ± 0.122	0.85	0.011 ± 0.004	0.52	0.017 ± 0.009	0.85
Gwydir non-vegetated	**		0.007 ± 0.003	0.50	0.008 ± 0.001	0.95
Roumalla non-vegetated	0.038 ± 0.046	0.69	0.016 ± 0.005	0.68	0.003 ± 0.003	0.69
Laura Homestead	0.242 ± 0.069	0.94	0.008 ± 0.002	0.64	0.015 ± 0.004	0.94
Laura Bridge	0.224 ± 0.092	0.88	0.012 ± 0.003	0.67	0.013 ± 0.005	0.88
November						
Gwydir vegetated	0.033 ± 0.029	0.87	0.027 ± 0.004	0.87	0.0005 ± 0.001	0.87
Roumalla vegetated	0.042 ± 0.025	0.90	0.017 ± 0.002	0.89	0.002 ± 0.001	0.94
Booralong CS	0.102 ± 0.024	0.97	0.018 ± 0.002	0.90	0.004 ± 0.001	0.97
Booralong Bridge	0.024 ± 0.022	0.92	0.020 ± 0.002	0.92	0.0015 ± 0.001	0.93
Gwydir non-vegetated	0.071 ± 0.026	0.92	0.015 ± 0.002	0.88	0.003 ± 0.0013	0.87
Roumalla non-vegetated	0.232 ± 0.064	0.94	0.008 ± 0.002	0.60	0.009 ± 0.0026	0.94
Laura Homestead	0.042 ± 0.03	0.88	0.016 ± 0.002	0.87	0.002 ± 0.001	0.89
Laura Bridge	**		0.029 ± 0.005	0.82	**	

**no curve could be fitted for these points.

There was no significant difference in average daily surface water temperatures between vegetated and non-vegetated reaches (Table 4.23). However, during the low and no-flow periods, the average daily temperatures were higher within the non-vegetated reaches (ranging between 14.58 to 27.10°C, Table 4.24) compared to the vegetated reaches (ranging between 13.03 to 23.59°C, Table 4.24), with the exception of the Gwydir vegetated reach during February, the low-flow period (27.49°C, Table 4.24). The range of average daily temperatures during September and November, the high-flow periods, were similar between vegetated (ranging between 13.44 to 21.16°C, Table 4.24) and non-vegetated reaches (ranging between 13.10 and 21.04°C, Table 4.24). There was a significant difference in average daily temperatures between deployment periods (Table 4.23). The mean daily water temperature was also significantly different between rivers (Table 4.23). There were no significant differences in mean daily water temperatures between locations on the control streams (Appendix 4, Table A4.14).

Table 4.23: Results of PERMANOVA main test for significant differences in average daily water temperature (°C, n=24) between treatment (Tr), streams (St), time (Ti), and their significant interactions. The multivariate dataset included mean daily temperature for the three collection times. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	6.802	6.802	1.099	0.441
St	4	91.848	22.962	4.084	0.021
Ti	3	2133.600	711.210	103.350	0.001
TrxSt**	2	7.843	3.922	1.420	0.329
TrxTi	3	17.727	5.909	2.139	0.178
StxTi**	11	66.642	6.058	3.112	0.033
TrxStxTi**	3	8.286	2.762	1.419	0.343
Res	7	13.626	1.947		
Total	34	2485.500			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.10 for correct formulae.

Table 4.24: Average daily water temperatures and average daily minimum and maximum temperatures (°C) for each vegetated and non-vegetated reaches during each deployment time (February n = 53 , May n = 41, September n = 41, November n = 31).

Reach	Average Daily	Average Minimum	Average Maximum
February			
Moredun vegetated	24.60	18.01	27.62
Gwydir vegetated	27.49	20.41	28.15
Roumalla vegetated	23.59	19.68	24.17
Booralong CS	22.97	16.98	27.89
Booralong Bridge	23.62	17.62	23.78
Moredun non-vegetated	25.43	20.19	28.06
Gwydir non-vegetated	27.10	21.26	28.49
Roumalla non-vegetated	25.55	22.31	27.20
Laura Homestead	26.80	22.33	27.68
Laura Bridge	25.04	21.91	25.28
May			
Gwydir vegetated	15.05	11.62	15.65
Roumalla vegetated	13.03	9.54	13.12
Booralong Bridge	13.16	8.54	13.65
Moredun non-vegetated	16.89	10.24	17.65
Gwydir non-vegetated	16.08	12.41	16.29
Laura Homestead	14.88	11.65	15.18
Laura Bridge	14.58	11.54	15.12
September			
Moredun vegetated	13.76	13.45	16.25
Gwydir vegetated	13.44	13.33	17.66
Roumalla vegetated	16.70	13.56	17.09
Booralong CS	14.24	12.53	15.48
Booralong Bridge	14.23	12.64	15.56
Moredun non-vegetated	14.44	14.24	16.86
Gwydir non-vegetated	13.10	14.76	17.32
Roumalla non-vegetated	16.43	14.82	16.64
Laura Homestead	13.43	13.65	16.56
Laura Bridge	13.12	13.63	16.25
November			
Gwydir vegetated	21.16	19.83	22.84
Roumalla vegetated	20.46	18.80	21.91
Booralong CS	16.58	16.63	19.73
Booralong Bridge	17.07	16.19	19.61
Gwydir non-vegetated	21.04	19.30	22.95
Roumalla non-vegetated	20.74	18.81	22.01
Laura Homestead	17.49	18.39	22.29
Laura Bridge	17.39	18.18	22.38

There was no significant difference in mean inorganic mass accumulation on litterbags between vegetated and non-vegetated reaches across the deployment periods (Table 4.25). The largest mass of inorganic mass to accumulate over the full deployment period occurred at the Roumalla vegetated reach during the September (43.6 g, Figure 4.14) and November (28.4 g, Figure 4.14) deployments, which were the high-flow periods. Although it appeared that more inorganic mass accumulated during September and November at several reaches, time was not found to be a significant factor (Table 4.25). The difference in inorganic mass accumulation between rivers was determined to be close to significant (Table 4.25, $\beta=0.05$). There were no significant differences in mean total inorganic mass accumulated on litterbags between locations on the control streams (Appendix 4, Table 4.15).

Table 4.25: Results of PERMANOVA main test for significant differences in mean total inorganic mass accumulated on litterbags (g, n=3) between treatment (Tr), streams (St) and time (Ti), and their interactions. All ten reaches were used in the analysis.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	1.558	1.558	1.822	0.256
St	4	7.904	1.976	2.891	0.090
Ti	3	3.622	1.207	1.535	0.270
TrxSt**	2	2.162	1.081	0.660	0.589
TrxTi	3	1.940	0.647	0.395	0.798
StxTi**	11	7.930	0.721	1.962	0.170
TrxStxTi**	3	4.913	1.638	4.457	0.051
Res	7	2.572	0.367		
Total	34	27.482			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.10 for correct formulae.

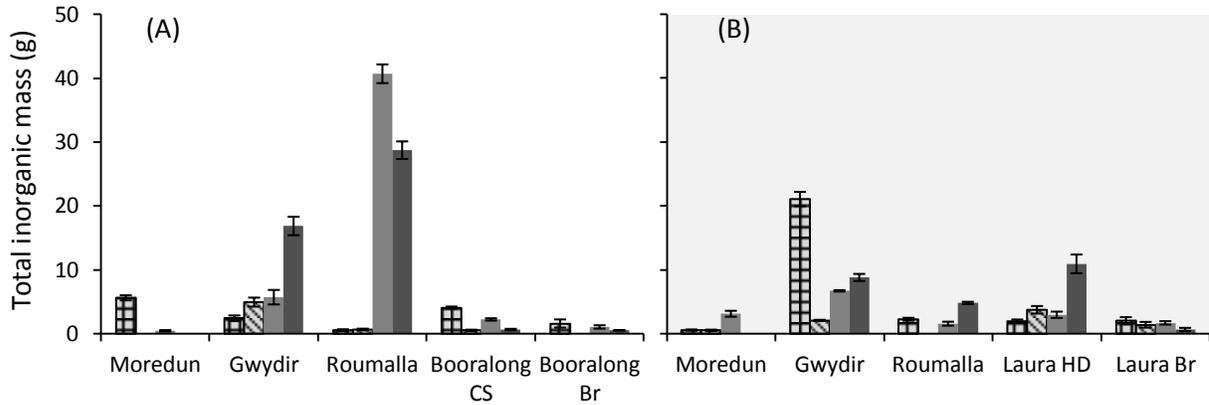


Figure 4.14: The mean total inorganic mass (g) present on litterbags at the final collection times (time 3) at vegetated (A) and non-vegetated reaches (B). Patterning on bars corresponds to the deployment periods: February (hatching), May (diagonal stripes), September (light grey) and November (dark grey). Error bars represent the standard error of the mean ($n=3$).

The results of the marginal test (relationship to a single predictor variable) showed that flow percentile and velocity cumulatively explained 48.9% and 63.9% of the variation in litterbag mass loss at vegetated reaches, respectively (Table 4.26). These individual relationships were significant (flow percentile: $p = 0.004$, velocity: $p = 0.001$, Table 4.26). The conditional test, which sequentially fits the best combination of predictor variables, identified velocity alone as being the best variable model and was highly significant (Table 4.27). The step-wise selection procedure identified velocity, wetted area, temperature and sediment accumulation as being the best combination (89.5%) of four variable model that was still statistically significant in explaining the majority of variation in mass loss between vegetated reaches (Table 4.27).

The variation in litterbag mass loss at the non-vegetated reaches was not significantly correlated with any of the biophysical variables, sediment accumulation or temperature (Table 4.26). The results of the conditional tests showed that fitting each predictor variable does not significantly explain the variation in mass loss between the non-vegetated reaches (Table 4.28).

Table 4.26: Results of marginal tests from DISTLM for total litterbag mass loss (% mass loss, determined from area under the curve, n=3) from vegetated and non-vegetated reaches with their respective biophysical variables. SA:V = surface area:volume ratio. Significant results are in bold type.

Variable	Vegetated				Non-vegetated			
	SS(trace)	Pseudo-F	P	Prop.	SS(trace)	Pseudo-F	P	Prop.
Feature no.	52.595	0.410	0.576	3.304e ⁻²	18.593	0.532	0.467	4.245e ⁻²
Flow percentile	779.180	11.503	0.004	0.489	4.649	0.129	0.711	1.061e ⁻²
Velocity	1017.900	21.277	0.001	0.639	29.432	0.864	0.375	6.719e ⁻²
SA:V	53.863	0.420	0.515	3.383e ⁻²	6.063	0.168	0.672	1.384e ⁻²
Wetted area	366.150	3.584	0.093	0.230	8.976	0.251	0.634	2.049e ⁻²
Inorganic acc.	3.194	2.413e ⁻²	0.870	2.007e ⁻³	37.180	1.113	0.316	8.488e ⁻²
Temperature	23.827	0.18233	0.658	1.497e ⁻²	23.796	0.68935	0.405	5.433e ⁻²

Table 4.27: Results of conditional test from DISTLM for total litterbag mass loss (% mass loss, determined from area under the curve, n=3) from the vegetated reaches with biophysical variables. SA:V = surface area:volume ratio. Significant results are in bold type.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Velocity	1017.900	21.277	0.001	0.639	0.639	12
Wetted area	163.640	4.386	0.045	0.103	0.742	11
Inorganic acc.	135.550	4.931	0.053	8.514e ⁻²	0.827	10
Temperature	108.160	5.838	0.039	6.794e ⁻²	0.895	9
Feature no.	27.238	1.562	0.255	1.711e ⁻²	0.912	8
Flow percentile	2.809	0.144	0.676	1.764e ⁻³	0.914	7
SA:V	10.020	0.475	0.498	6.294e ⁻³	0.920	6

Table 4.28: Results of conditional test from DISTLM for total litterbag mass loss (% mass loss, determined from area under the curve, n=3) from the non-vegetated reaches with biophysical variables. SA:V = surface area:volume ratio.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Inorganic acc.	37.180	1.113	0.299	8.488e ⁻²	8.488e ⁻²	12
Temp at time 3	22.685	0.660	0.474	5.179e ⁻²	0.137	11
Velocity	29.371	0.842	0.406	6.705e ⁻²	0.204	10
SA:V	49.021	1.472	0.269	0.112	0.316	9
Flow percentile	68.597	2.374	0.155	0.157	0.472	8
Feature no	11.304	0.360	0.574	2.581e ⁻²	0.498	7
Wetted area	8.440e ⁻²	2.304e ⁻³	0.963	1.927e ⁻⁴	0.498	6

4.4 Discussion

Organic matter standing stocks

Riparian vegetation

The presence of riparian vegetation did not significantly increase standing stocks of FBOM or CBOM within study reaches. This result is in contrast to previous studies that compared standing stocks of organic matter between vegetated and non-vegetated stream reaches. In a survey of intermittent agricultural streams in southeastern Australia, CBOM standing stocks during base-flow conditions were found to increase in reaches with riparian vegetation cover (Reid et al. 2008). A separate study in Joyce's Creek, another intermittent agricultural stream in southeastern Australia, found that CBOM was significantly higher in reaches with riparian vegetation compared to non-vegetated reaches (Gilling et al. 2009). Surveys of clear-felled and forested upland streams in Tasmania also found that CBOM standing stock was higher within the forested streams (Watson & Barmuta 2010). In several headwater streams in New Zealand (North Island), standing stocks of CBOM were higher within forested reaches compared to those that had been cleared for pasture (Scarsbrook & Halliday 1999). These disparate findings suggest that there may be other important factors influencing standing stocks of organic matter in the present study reaches, rather than only the presence of riparian vegetation.

With the exception of organic matter estimates from Laura Bridge (the downstream non-vegetated control reach), the quantities of both CBOM and FBOM were much lower than those reported for forested biomes (CBOM: 0 – 5000 g m⁻²; FBOM: 0 – 2000 g m⁻²; Jones 1997), but within the range of quantities reported in other semi-arid and arid streams (CBOM: 0 – 500 g m⁻²; FBOM: 0 – 50 g m⁻²; Jones 1997). In addition, the quantities of CBOM standing stocks found in this study were much lower than those recorded in temporary streams and in those studies that compared CBOM between vegetated and non-vegetated reaches (see review in Table 4.29). For example, CBOM mass ranged from 1 – 25 g m⁻² in the present study, while in other intermittent agricultural streams CBOM estimates ranged from 134 – 999 g m⁻² (Gurtz et al. 1988), 634 – 2083 g m⁻² (Reid et al. 2008), and 19 – 279 g m⁻² (Gilling et al. 2009).

Table 4.29: Standing stocks of CBOM (g m^{-2}) reported in other studies. V = vegetated or non-vegetated riparian zone.

Study	Biome/Study area	V	CBOM
Acuña et al. 2004	Mediterranean intermittent	Yes	6.9 - 54
Imberger et al. 2011	Temperate urban intermittent	Yes	400 - 3720
Imberger et al. 2011	Temperate urban intermittent	No	204 - 2240
González & Pozo 1996	Mediterranean intermittent	Yes	21 - 74
González & Pozo 1996	Mediterranean intermittent	No	10 - 48
Boulton & Lake 1992	Temperate intermittent	Yes	10 – 70 (pools) 2.5 – 20 (riffles)
Reid et al. 2008	Temperate agricultural intermittent	Yes	1741 - 2083
Reid et al. 2008	Temperate agricultural intermittent	No	634 - 979
Schade & Fisher 1997	Temperate intermittent	Yes	1 - 7
Gurtz et al. 1988	Temperate intermittent	Yes	134 - 999
Gurtz et al. 1988	Temperate intermittent	No	291 - 341
Gilling et al. 2009	Temperate agricultural intermittent	Yes	208 - 259
Gilling et al. 2009	Temperate agricultural intermittent	No	19 - 279
Scarsbrook & Halliday 1999	Temperate agricultural	Yes	5 - 104
Scarsbrook & Halliday 1999	Temperate agricultural	No	20 - 110
Colón-Gaud et al. 2008	Tropical intermittent	Yes	48 – 113
This study	Temperate agricultural intermittent	Yes	1 - 9
This study	Temperate agricultural intermittent	No	1 - 25

It is important to note that the studies described above were only conducted at a single discharge, usually base-flow conditions (Scarsbrook & Halliday 1999, Reid et al. 2008, Gilling et al. 2009, Watson & Barmuta 2010). In this study, it was predicted that the largest difference in CBOM mass between vegetated and non-vegetated reaches would occur during the low and no-flow periods. During these periods, three of the five non-vegetated reaches had higher total CBOM mass than vegetated reaches, and it was actually during the high flow period sampling periods that total CBOM mass was slightly higher within the vegetated reaches compared to

non-vegetated reaches. A study within an intermittent prairie stream showed a similar inconsistent pattern in CBOM mass between vegetated and non-vegetated reaches (Gurtz et al. 1988). CBOM mass in the downstream vegetated reach was found to be nearly three times higher compared to the upstream non-vegetated reach, however, following the flow period the non-vegetated reach had almost twice the quantity of CBOM than the downstream vegetated reach (Gurtz et al. 1988). There were differences in the type of CBOM present in their study, with grass being the main component within the non-vegetated reach while leaves and wood were the main components within the vegetated reaches (Gurtz et al. 1988). The authors concluded that the non-vegetated reaches were highly retentive despite the lack of riparian vegetation and that the hydrologic regime reduced the predictability of the CBOM mass within both the vegetated and non-vegetated reaches (Gurtz et al. 1988). In combination with the results of this study, which showed that organic matter analogues could be retained within non-vegetated reaches as well as large variation in CBOM mass with hydrology within both vegetated and non-vegetated reaches, this emphasises the importance of conducting benthic surveys across a range of discharges or periods of connectivity.

During the low and no-flow sampling periods, the CBOM and FBOM masses present at the downstream non-vegetated control reach, Laura Bridge, was six to ten times larger compared to the other nine study reaches, including the upstream non-vegetated reach within the same stream. These quantities were comparable to those present in other intermittent and/or forested streams (Jones 1997, Table 4.29). However, approximately 70 and 85% of the CBOM mass present at the Laura Bridge reach was attributed to the unidentifiable fraction during the low and no-flow periods, respectively, which appeared to be mostly highly processed animal droppings. This highlights the importance of looking at different organic matter fractions, as the presence of some fractions may not necessarily have any relationship to or be the direct result of riparian vegetation, and may still influence ecosystem processes.

Although the differences in CBOM composition were not significantly different, the dataset did show some variation in composition. The fractions of wood and *C. cunninghamiana* reproductive structures were larger within the vegetated reaches during both of the high-flow

events. This difference between vegetated and non-vegetated reaches was attributed to an increase in these fractions within the vegetated reaches. Reid et al. (2008) found that there were significantly larger quantities of benthic woody material within vegetated reaches compared to non-vegetated reaches, however, the study found no significant difference in the quantity of woody material vertically and laterally entering the reaches. Similarly, Gurtz et al. (1988) found larger quantities of woody material were also found within vegetated reaches compared to the single non-vegetated reach used in their study. In contrast to the pattern in this study, Gurtz et al. (1988) found the quantity of wood in-stream decreased within the vegetated reaches after the flow period, and that the wood was redistributed throughout the riparian and bank areas. This discrepancy may be due to differences in sampling regime as Gurtz et al. (1988) only conducting sampling once the channel had dried whereas sampling in this study took place while flow events were occurring or while pools were disconnected. In contrast, CBOM was found to increase in some habitats immediately after flow commenced in an intermittent stream in southeastern Australia and was attributed to mobilization from upstream areas and riparian zones (Boulton & Lake 1992). As *C. cunninghamiana* reproductive structures are connected to small branches and stems, an increase in both may suggest that the wood fraction is derived from *C. cunninghamiana* trees within the adjacent riparian zone and is being retained within the vegetated reaches.

Casuarina cunninghamiana needles are known to contain a large amount of nitrogen (Esslemont et al. 2007) and high concentrations of total nitrogen (TN) found during the no-flow period suggest leaching from *C. cunninghamiana* needles as a potential source (Chapter 3). The largest quantity of *C. cunninghamiana* needles were present within the Roumalla vegetated and the downstream vegetated control reach, Booralong Bridge, during the no-flow periods (both approximately 2.5 g m^{-2}). The upstream reach on the vegetated control stream, Booralong CS, also had approximately 3 g m^{-2} during the first high-flow sampling period. However, the concentrations of TN at the Roumalla vegetated and Booralong Bridge reaches during the no-flow period were actually the two lowest recorded from all the reaches. The TN concentration at Booralong CS during the first high-flow period was similar to the other reaches (see Chapter 3). This indicates that the high TN concentrations and the variation between TN

concentrations during the no-flow period cannot be exclusively attributed to the presence of *C. cunninghamiana* needles. During periods of surface water fragmentation, groundwater can be a potential source of N to isolated reaches and pools (Boulton et al. 1998). Alternatively, TN concentrations have been shown to increase in agricultural streams from animal excrement (Vitousek et al. 1997). Considering that the area surrounding the streams in this study has a long history of grazing and the large loads of droppings being imported to the reaches, this is likely to be a potential source of TN in these study streams.

Highly refractive organic material is also thought to have a lower rate of decomposition and therefore reaches may have a higher rate of heterotrophic respiration (Webster & Benfield 1986, Esslemont et al. 2007). In laboratory experiments, *C. cunninghamiana* needles have been found to have lower rates of aerobic mineralization than most emergent macrophytes and aquatic herbs (Esslemont et al. 2007). In addition, in-stream retention and uptake of SRP has found to increase with additions of allochthonous CBOM (Aldridge et al. 2009). This would suggest that there may be an increase in the rate of respiration and nutrient uptake (see Chapter 3) within the vegetated Roumalla and Booralong Creek reaches, which had the largest quantity of needles present at the whole-reach and aerial unit scales, compared to the other study reaches that had more labile CBOM present such as, macrophytes. However, in this study the largest loads of SRP retained were not found within the vegetated Roumalla or Booralong Creek reaches (see Chapter 3). Further exploration is needed to assess the effects of the *C. cunninghamiana* needles on ecosystem functions as alternative and preferential sources of carbon for consumers may still be available within vegetated reaches.

Hydrology

There were no significant effects of discharge on FBOM or CBOM mass however, there were interactions between treatment, streams and hydrology (time). There was a general decrease in FBOM and CBOM mass at some reaches during the high-flow periods, which together with the significant interaction of between treatment, streams and hydrology suggests that the influence of hydrology on organic matter retention will be reach specific. Studies of intermittent streams have shown decreases in organic matter standing stocks with increases in

discharge, as peak litterfall can occur prior to the onset of flow in northern hemisphere streams that have deciduous riparian vegetation (Schade & Fisher 1997, Acuña et al. 2004, Acuña et al. 2007, Colón-Gaud et al. 2008). Boulton and Lake (1992) found that although CBOM standing stocks were lowest during periods when surface water was connected and flowing, they did note that standing stocks were not always depleted and there was less variation in CBOM mass within pool habitats. Similarly, there was no seasonal pattern in CBOM standing stocks within an intermittent upland stream in South Africa (Stewart & Davies 1990). These authors suggested that the increase in wetted area during the winter months when discharge was highest may have meant that material within previously dry areas of the stream bed would have been incorporated in sampling (Stewart & Davies 1990). In this study, the results of the BEST analyses showed that surface area:volume ratio explained 37% of the variation in the unidentified fraction and total CBOM mass (whole-reach scale) within the vegetated reaches. Surface area:volume ratio and the number of features inundated explained approximately 30% of the variation in needle and wood mass within the non-vegetated reaches. This again suggests that there is an interaction between CBOM mass with hydrology and the physical environment.

There are few other studies that have described the pattern of FBOM mass with changes in discharge in intermittent streams, which could be in part due to FBOM not being separated from the total organic matter mass (e.g. Acuña et al. 2007). However, FBOM standing stocks were reported to increase during the dry season in an intermittent upland Panamanian stream (Colón-Gaud et al. 2008). In contrast, Boulton & Lake (1992) found that there was a decrease in FBOM standing stocks in some riffles with increases in discharge, but overall there was no clear pattern in FBOM mass. The transport, retention and resuspension of in-stream FPOM has been shown to be poorly related to hydrological variation alone (Minshall et al. 2000), and it is thought to be controlled through a combination of biotic interactions such as, uptake by biofilms and filter feeders, as well as particle size (Minshall et al. 2000, Monaghan et al. 2001, Thomas et al. 2001). An interbiome study of 31 streams found that there was a significant positive relationship between in-stream wood and FBOM mass (Jones 1997). There was no significant correlation between the hydrogeomorphic variables and whole-reach scale FBOM estimates within vegetated or non-vegetated reaches in this study. This suggests that there are

a range of other variables that were not measured in this study such as, particle size of FBOM or biotic uptake, that are influencing FBOM mass. The lack of predictability between discharge with CBOM and FBOM also suggests the need for other studies to sample during periods or connectivity and fragmentation to accurately quantify organic matter masses within both vegetated and non-vegetated reaches.

Particulate organic matter mass-balance and retention

There was no significant difference in total FPOM or CPOM retention between vegetated and non-vegetated reaches. This is an important finding as it provides a further explanation as to why there were not significantly larger standing stocks of FBOM and CBOM present within vegetated reaches compared to the non-vegetated reaches. Most studies that have investigated the effects of riparian vegetation on stream particulate organic matter mass-balance have been at larger catchment and sub-catchment spatial scales (Meyer & Tate 1983, Webster & Meyer 1997, Acuña et al. 2007), making it difficult to compare the findings of this reach-scale study (Johnson & Covich 1997, Tank et al. 2010). A synthesis of particulate organic matter mass-balance across different biomes did use a mixture catchment and reach-scale studies data, however, the overall mass-balance estimates were calculated as aerial units ($\text{g m}^{-2} \text{y}^{-1}$) as opposed to the volume units used in this study (g day^{-1}) (Webster & Meyer 1997). As riparian revegetation is commonly carried out at the reach scale (Bernhardt et al. 2005), the results from this reach scale study are more relevant for guiding and monitoring restoration projects.

The COM mass-balance results of this study showed that all reaches were capable of retaining at least one COM fraction, and there did not appear to be any difference in which fractions were retained between the vegetated and non-vegetated reaches. This again offers further support as to why there was no significant difference in standing stocks of CBOM mass and composition between vegetated and non-vegetated. It also highlights the importance of using mass-balances to estimate retention rather than only CBOM mass, which has been used by many studies and is reported at the area unit scale (e.g. Reid et al. 2008). For example, the upstream vegetated control reach, Booralong CS, had the largest CBOM mass present during

September for both the aerial unit and whole-reach scale estimates. However, the Moredun non-vegetated reach actually retained the largest CPOM load during this period. This highlights that CBOM surveys alone cannot provide insight into capacity of reaches to retain CPOM. In addition, it is necessary to take into account the longitudinal import of CPOM from upstream (Webster & Meyer 1997), as larger loads retained may be because more CPOM is entering the reaches rather than a difference in retention capacity.

In this study, there was no significant effect of hydrology on the retention of FPOM, but there was an increase in magnitude of FPOM import, retention and export with discharge. As previously mentioned, the transport, retention and re-suspension of FPOM can be controlled by a combination of hydrological and biological processes with the physical properties of FPOM such as particle size (Minshall et al. 2000). During high-flow events, FPOM that has accumulated on the riparian zone topsoil can be eroded as the wetted area expands (Wallace et al. 1995, Bass et al. 2011), and this may be exacerbated at reaches that lack riparian vegetation (Laubel et al. 2003). This may offer some explanation for 35% of the variation in FPOM export being significantly correlated with wetted area in non-vegetated reaches. The lack of any significant correlation between FPOM retention and the hydro-geomorphic variables within the vegetated reaches suggests FPOM is being sourced differently between vegetated and non-vegetated reaches and that the mass-balance of FPOM is under the control of different processes, which were not measured in the present study.

The magnitude of imported CPOM increased during the high-flow periods. In addition, there was larger variation in the composition of CPOM being imported into the reaches during the high-flow periods compared to low-flow periods. It has been widely acknowledged that allochthonous organic matter accumulates on stream beds during dry periods in intermittent streams, which causes a peak in imported CPOM mass at the onset of flow (Boulton & Lake 1992, Schade & Fisher 1997, Acuña et al. 2004, Acuña et al. 2007, Colón-Gaud et al. 2008). In the present study, the main fractions that increased during the high-flow periods were wood, needles and unidentifiable fractions, indicating that the presence of *C. cunninghamiana* trees provide a large amount of allochthonous CPOM transported through these reaches. However, it

is important to note that the first high-flow sampling period occurred approximately one-month after surface water had reconnected so the initial 'flush' of organic matter was not sampled, but would be essential in providing further details of the mass-balance of organic matter within reaches (Webster & Meyer 1997).

Processes important in retaining organic matter

Experimental organic matter releases

The results of the experimental organic matter analogue releases confirmed that both vegetated and non-vegetated reaches have the potential to retain coarse organic matter, and the majority of both the twig and needle analogues were retained by macrophytes within both reach types. Several other studies have shown the importance of macrophytes in retaining organic matter (Koetsier & McArthur 2000, Quinn et al. 2007, Imberger et al. 2011). In this study, there was a difference in the physical form of macrophytes present between the vegetated and non-vegetated reaches, however, this difference was not accounted for in the statistical analyses. Within the non-vegetated reaches, the edges of the main channel were lined with stands of semi-submerged *Eleocharis* spp., and submerged macrophytes grow in the in the wetted channel, which have long fronds touching the water's surface. This is a common characteristic of non-vegetated or 'open' reaches (Julian et al. 2010). Macrophytes within the vegetated reaches usually consisted of a single submerged plant within the channel or trailing vegetation from terrestrial plants overhanging from the adjacent riparian zone (see Chapter 2, Koetsier & McArthur 2000). These results suggest that although the physical form of macrophytes can change between vegetated and non-vegetated reaches, they are both important in retaining organic matter.

Within the vegetated reaches, the flood debris accumulation points and the bank edges were also important in retaining the twig analogues. Flood debris accumulation points are formed when logs become wedged in between standing trees and/or through interaction with the river and riparian zone morphology (Pettit et al. 2005, Moulin et al. 2011) and were only present at the vegetated reaches in this study (see Chapter 2). Bank edges that repeatedly cut in and away from the main channel can create small backwaters and hydraulic heterogeneity, which can

lead to an increase in organic matter retention (James & Henderson 2005). Within the vegetated reaches in this study, the contours of the channel bank most likely evolved through an undercutting erosive interaction between the roots of the *C. cunninghamiana* and the surface water. The bank edges were not as effective at retaining the needle analogues compared to the twig analogues within the vegetated reaches. This may be attributed to the twig analogues being more inflexible than the needles, as the type of habitat for retention has been shown to vary with the flexibility of organic matter (Cordova et al. 2008, Hoover et al. 2010). Differences in discharge will also interact with the flexible characteristics of the organic matter, for example, increases in the force of surface water will physically fragment more flexible organic matter fractions such as needles (Cordova et al. 2008, Hoover et al. 2010).

In contrast to the vegetated reaches, in-stream pools were another important physical feature for retaining twigs and needles within the non-vegetated reaches. Reaches that lack riparian vegetation can widen, which reduces the velocity of surface water (see Chapter 2, Julian et al. 2010, Deegan et al. 2011). The pools formed in the non-vegetated reaches in this study were usually in the centre of the main channel, which were wider and had reduced surface water velocity. There were also macrophytes growing in these sections within four of the non-vegetated reaches (Chapter 6), which are also known to reduce surface water velocity (Gurnell & Midgley 1994, Julian et al. 2010).

There was a significantly larger amount of twigs retained within the non-vegetated reaches compared to the vegetated reaches however, the large variation in retention between the replicate releases means confidence in this result is low and repeated releases would be necessary to confirm this significant result. Only a single release of analogues for each of the two replicate (temporal) experiments could be used in this study due to time and equipment limitations. Many organic matter retention studies use triplicate releases or more to produce a mean retention value (e.g. James & Henderson, Quinn et al. 2007). However, Imberger et al. (2011) also conducted duplicate releases in their study of organic matter retention in urban streams and found that there was only moderate variability between releases with an average coefficient of variation of 30.67%. A further limitation in this study, may be that the needle and

twig analogues had a more simple shape and different buoyancy to natural *C. cunninghamiana* needles and twigs. Although, a study comparing organic matter analogues, similar to those used in this study, to represent white pine needles (tied in bunches of five) found that the retention and travel distances of the analogues was similar to the natural needle bundles (Cordova et al. 2008).

It also important to note that the organic matter releases in this study were conducted during discharges which were similar to those occurring during the low-flow sampling period (Chapter 2), and could mean that these would not be effective methods of retention under larger discharges. During the high-flow periods in this study, the faster surface water velocities removed many of the in-stream macrophytes (Chapter 6) and increased depths would reduce the likelihood of interception with the remaining in-stream macrophytes. The non-vegetated reaches in this study have areas of exposed bedrock and logs in the riparian zones (Chapter 2) and the correlation between CPOM retention and wetted area suggests that these features within the riparian zone will become more important for organic matter retention during high-flow periods. Temporary-river ecology theory would offer support to the suggestion of retention occurring through different pathways during different levels of inundation (Larned et al. 2010). However, the overall results of the experimental organic matter releases indicate that both vegetated and non-vegetated reaches can retain organic matter, but the primary mechanism of retention will vary between vegetated and non-vegetated reaches.

Organic matter breakdown

Riparian vegetation

The litterbag experiment showed that there was no significant effect of riparian vegetation on organic matter breakdown (measured as total mass-losses over the deployment periods) in this study. Studies on the effects of riparian vegetation on total organic matter breakdown and breakdown rates (measured as rates of breakdown over the deployment periods) have found similar results. The results from an experiment using different *Eucalyptus spp.* deployed within vegetated and non-vegetated reaches of a single temperate stream in southeastern Australia found no difference in total breakdown rate, however, there were differences in the process of

breakdown (Danger & Robson 2004). The study showed that organic matter breakdown within non-vegetated reaches was attributed to microbial decomposition and physical fragmentation, as macroinvertebrates were absent from the litterbags (Danger & Robson 2004). A large-scale field experiment within 30 streams in the European Union found no difference in total breakdown of oak and alder leaves between streams with cleared riparian zones and native deciduous woodland (Hladyz et al. 2010). Although, the study did find differences that breakdown was mediated by macroinvertebrates in some woodland streams, while microbial breakdown was higher in the non-vegetated streams (Hladyz et al. 2010). The results of a study of leaf breakdown along an agricultural land-use gradient also showed that breakdown rates were not significantly different between the four land-use categories, which was attributed to the high variability in breakdown rates among streams and the influence of multiple confounding factors (Hagen et al. 2006). In contrast, a number of studies have found differences in organic matter breakdown rates between vegetated and non-vegetated streams or streams across land-use gradients, which have been driven by the effects of increased surface water nutrients (Young et al. 1994, Gulis & Suberkropp 2003, Bärlocher et al. 2010) and temperature (Webster & Benfield 1986, Menéndez et al. 2003, Ferreira & Chauvet 2011) on macroinvertebrate and/or microbial activity.

Although there were no significant differences in surface water nutrient concentrations between vegetated and non-vegetated reaches in this study, there were higher SRP concentrations within the vegetated reaches during the low and no-flow periods (Chapter 3). A study of organic matter breakdown in 10 streams across a nutrient gradient in Portugal found that organic matter breakdown rates were positively correlated with SRP, as well as NO_x (Gulis & Suberkropp 2003). However, the results of the litterbag experiment did not show any consistent difference in breakdown during these periods, which suggests that SRP was not an important driver of organic matter breakdown in this study.

To account for differences in temperature between the reaches, breakdown coefficients were normalised to degree days (Hanson et al. 1984, Boulton & Boon 1991). This also did not minimise any of the variation between the vegetated and non-vegetated breakdown rate

estimates. Although this adds further support as to why no difference in organic matter breakdown was detected between vegetated and non-vegetated reaches, unfortunately no other measurements relating to breakdown were made in this study and therefore it is difficult to elucidate if there were differences in the main biological drivers (microbes and invertebrates) of organic matter breakdown.

Removal of riparian vegetation and other land-use effects can produce large quantities of inorganic matter or sediment (Wood & Armitage 1997, Pusey & Arthington 2003). The accumulation of inorganic matter has been found to have confounding effects on other biophysical variables such as nutrient concentrations and temperatures, that would be expected to alter organic matter breakdown (Hagen et al. 2006). A related study conducted between August and October 2010 within a subset of six reaches used in this study, showed some variation in the rate of breakdown of litterbags containing *Eucalyptus viminalis* leaves between vegetated and non-vegetated reaches (Bärlocher et al. 2012 – see Appendix 1). Litterbags at three of the four vegetated reaches were found to have higher breakdown rates than the two non-vegetated reaches, and mass loss estimates were positively correlated with hyphomycete conidia and it was suggested that sediment accumulated on the leaves had inhibited hyphomycete colonisation (Bärlocher et al. 2012 – see Appendix 1).

There was no significant difference in the total inorganic matter mass accumulated on litterbags between vegetated and non-vegetated reaches, and the largest accumulation was found on the litterbags deployed at the Gwydir and Roumalla vegetated reaches during September, the first high-flow period. In this case, this did not result in a decreased mass-loss, as one of the highest overall mass-losses occurred at the Gwydir vegetated reach during September. It is important to note that in contrast to this study, the *E. viminalis* litterbags were deployed in the water column as opposed to being attached to the benthos (Bärlocher et al. 2012 – see Appendix 1). This means that the inorganic matter accumulated in this study would be from benthic substrate rather than suspended solids in the water column. It is common for unstable streambed substrates such as sand to move during high-flow events (Uehlinger 2000, Atkinson et al. 2008). The channel substrate within the Gwydir and Roumalla vegetated reaches

was a mixture of sand and cobble prior to the high-flow events, however, it was evident that the sand substrate had shifted and there were new layers of sand that had been deposited from upstream during the high-flow events. These results suggest that there is an interaction between hydrology and the stability of the physical environment on the total mass of inorganic matter material covering organic matter within these reaches. Furthermore, this variation in inorganic matter accumulation provides further support for the inconsistent results in organic matter breakdown between vegetated and non-vegetated reaches.

Hydrology

In this study, there was a significant positive effect of hydrology on organic matter breakdown and the largest difference in mass-loss between vegetated and non-vegetated reaches also occurred during the high-flow deployment periods, September and November. The effect of increased surface water velocity on organic matter breakdown has been primarily explored through litterbag deployment between riffle and pool habitats (Heard et al. 1999, Lepori et al. 2005, Hoover et al. 2006). The increase in surface water velocity can increase physical fragmentation directly through shear stress or through the abrasion of sediments as they are transported in-stream (Heard et al. 1999). In some cases, the increase in surface water velocity has been found to be the most important determining factor in variation of organic matter breakdown (Lepori et al. 2005). In contrast, velocity has only been an important determining factor during high-flow events, with more emphasis on other biophysical parameters during baseflow conditions (Hoover et al. 2006). This suggestion needs to be considered when exploring the breakdown rates in this study.

The results of the regression models using multiple variables showed that the combination of surface water velocity, discharge, temperature and sediment accounted for 89.5% of the variation between litterbag mass-loss within vegetated reaches with surface water velocity accounting for 65% of the variation. In the context of temporary-river ecology, this suggests that parameters such as surface water velocity during the expansion phase will be more important in controlling organic matter breakdown within vegetated reaches compared to other biophysical parameters. However, there was lack of significant differences in breakdown

between vegetated and non-vegetated reaches and no significant relationships between organic matter breakdown at non-vegetated reaches with the hydro-geomorphic variables. This adds support to the suggestion from Hoover et al. (2006), that other biophysical parameters may be more important in determining organic matter breakdown within non-vegetated reaches and also during periods of low and no-flow. Further investigation into other biophysical parameters such as, microbial activity is necessary to elucidate the mechanisms of breakdown during these periods.

Unfortunately breakdown coefficients could not be confidently estimated with either linear or non-linear curve fitting for all litterbag deployments. The ambiguity of the coefficients means it is difficult to compare breakdown rates with other studies. However, there are only two reported studies that have used *C. cunninghamiana* needles in organic matter breakdown experiments. The breakdown coefficient for *C. cunninghamiana* in an upland intermittent stream in northern New South Wales, Australia, was estimated in degree days at 0.0009 (Pidgeon & Cairns 1981), and macroinvertebrates and surface water velocity were found to be the main drivers of breakdown. The breakdown of *C. cunninghamiana* needles deployed in pools and riffles was also explored between two second-order rivers in southeastern Australia (Wolfenden 2009). The breakdown coefficients were estimated between 0.0008 – 0.0017 in pools and 0.0024 – 0.0076 in riffles (degree days), and microbial activity (driven through differences in temperature) and surface water velocity were the main drivers of breakdown. Generally, the breakdown coefficients in this study are higher than those found in the other studies. Despite this difference, the findings of these earlier studies add further support that discharge and surface water velocity were the main drivers of *C. cunninghamiana* breakdown and may have compromised the confidence of *C. cunninghamiana* breakdown estimates in this study.

4.5 Summary

This chapter showed that there was no significant difference in FBOM or CBOM mass or CBOM composition between vegetated and non-vegetated reaches. The mean FBOM and CBOM masses at all reaches were generally higher during the low and no-flow periods compared to

the high-flow periods. There were patterns in whether reaches consistently exported or retained FPOM, with four of the five non-vegetated reaches exporting FPOM across the three different sampling periods with surface flow. There was no significant effect of hydrology on FPOM retention, but the magnitude of the FPOM loads increased with discharge within the vegetated and non-vegetated reaches. The magnitude of the CPOM load retained or exported increased at some reaches during the high-flow periods, but there was no significant effect of hydrology.

Both vegetated and non-vegetated reaches were able to retain organic matter and experimental leaf releases showed that non-vegetated reaches primarily retained CPOM through stands of macrophytes and pool habitats, while vegetated reaches retained CPOM through bank edges and flood debris accumulation points. The magnitude and composition of CPOM was largely controlled by the change in discharge and the interaction with the riparian zones within the reaches. Organic matter breakdown was not significantly different between the vegetated and non-vegetated reaches, however, surface water velocity had a significant positive effect on breakdown within vegetated reaches.

The ability of both vegetated and non-vegetated reaches to retain organic matter is evident and, in addition to the variation in organic matter standing stocks and breakdown during the high-flow periods, this is particularly important for the promoting heterogeneity in in-stream food resources and habitats for aquatic organisms. Exploration as to the sources of the finer and dissolved organic matter fractions will provide further insight as to whether these fractions are from allochthonous or autochthonous sources and the further implications that organic matter dynamics may have on the metabolic functioning of intermittent streams.