UTILITY OF STREAM MESOCOSMS FOR CLIMATE CHANGE RESEARCH

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ABSTRAT

The utility of stream mesocosms was examined in a study of replicability of water physicochemstry and benthic macroinvertebrate assemblages in an array of artificial flumes near the River Itchen in southern U.K. High quality groundwater supply and similar exposure to the environment lead water physicochemistry to be highly replicate across all channels. The within- and between-flume replicate design reduced macroinvertebrate assemblages' variability temporally, but the structure of macroinvertebrate assemblages in mesocosms shift seasonally. The highly temporal replicability of mesocosms allowed a long-term (i.e. 1- year) study of drought in these stream mesocosms.

Seven water depth treatments were applied in a series (n=21) of artificial flumes to construct a linear varying drought gradient so that each treatment was replicated three times. The drought experiment lasted a course from August, 2013 to August, 2014. Algal growth and the abundance of three grazer taxa were negatively correlated with both drought intensity and drought duration. Additionally, the drought intensity impact on algal growth shifted with drought duration. Conversely, drought intensity had a fixed negative impact on decomposition process. Shredder community structure was altered by drought impact reducing shredder abundance and shredding efficiency. However, the shredding efficiency in freshwater ecosystem was more related to shredding efficiency of specialist shredder rather than shredder abundance.

The mesocosms could mimic freshwater ecosystem physiochemistry environment and macroinvertebrate assemblage effectively and comprehensively, which provided an access to study the impact of natural disturbance on freshwater ecosystem. This study developed the understanding of the drought effect on the entire freshwater ecosystem.

For Mum

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CHAPTER 1

General Introduction: The Utility of Stream Mesocosms and Drought Impact on Lotic Freshwater Ecosystems

1.1 Utility of mesocosms

A mesocosm (*meso-* or 'medium' and *-cosm* 'world') is a large multispecies system (e.g. artificial stream, pond or soil system > 1L volume) that can be used as a venue for experimentation under semi-controlled / controlled conditions (Lamberti & Steinman, 1993). The experiences from field survey and microcosm research has informed that ecological experiments need to be undertaken in mesocosms: larger and more realistic simulation systems. Researchers can use mesocosms to mimic hydrological disturbance (i.e. drought treatment; Chase, 2007; Ledger et al., 2011; Boersma et al., 2014), temperature regime (Kim et al., 2001; Riemann et al., 2001; Liboriussen et al., 2005), water physical and chemical quality (Havens et al., 1989; Hichey et al., 1999; Caquet et al., 2007) and habitat diversity (Batzer, 1998; Roussel et al., 2008; Korajkic et al., 2013). Moreover, mesocosms have been employed widely to investigate ecological processes and consequences of environmental stress since 1950s (Schindler, 1998), such as the chemical and biological impact on biofilm (Cardinale & Palmer, 2002; Battin et al., 2003), the sedentary filter-feeders impact on freshwater function (Vaughn et al., 2004), and drought impact alters food web size (Brown et al., 2011).

However, the results of mesocosms ecological studies are widely questioned, those result may not be able to be repeated and replicated in natural freshwater ecosystem (Schindler, 1998; Sanderson, 2002). It may be due to the limited unrealistic experimental environment and conditions, such as limited water volume in mesocosms flumes, unnatural lighting (e.g. 12 hr light, 12hr dark) and low trophic level colonization (Prieto et al., 2016). Outdoor semi-controlled mesocosms have been developed in order to mimic the stream environments more realistically. Mesocosm facilities have evolved with changing research priorities over time, through three principal steps. Firstly, during the

1950s to 1960s laboratory streams were designed and tested by scientists such as Odum et al. (1956) and then further developed by Warren et al (1958) at Oregon State University during the 1960s – a decade which saw the establishment of multiple lotic mesocosm facilities internationally. For instance, McIntire et al. (1964) developed a no-exchange closed stream system in 1964. Secondly, in the 1970s, partially closed stream systems were developed, with exchange of water with local water sources. By the late 1970s, in order to solve flow problems specifically, ecologists used flumes instead of tanks (aka artificial streams) to better mimic the hydrology of running water ecosystems, both outdoor and indoor artificial channels then became commonplace, in a wide variety of shapes and sizes. But artificial channel applications were still limited. Between 1980 and 1990, approximately 20% lotic studies were contrasted of total mesocosms in freshwater mimic applications (Lamberi, 1993; Stewart et al., 2013) Due to the large temporal and spatial scale, high trophic level and various habitat simulation, semi-controlled mesocosms have been used as a research tool to study climate change, especially since 1995 (Stewart et al., 2013).

Nevertheless, many ecologists have challenged the utility of mesocosms as venues for experimentation for decades. For instance, Swift et al. (1993) argued that whole ecosystem manipulation is the only way to test hypotheses about natural systems. The rationale underpinning this view was that aquatic ecosystems are highly complex and threatened by multiple global impacts (e.g. climate change, nutrients cycle, biotic interaction, Zimmerman et al., 2008) and these complexities cannot be mimicked effectively at small scales. Over the past 20 years, views have shifted and there is now a growing consensus that mesocosm studies provide a useful bridge between the realism of field surveys and control and replicability of laboratory experiments (Petersen &

Englund, 2005), and, that they provide valuable opportunities to test the effect of stressors (e.g. pesticides) and change (e.g. future climate change scenarios) in ways that would simply not be possible in nature. Mesocosms proponents argue that they capture key aspects of natural systems, whilst providing the control necessary to eliminate covariation that blights field studies, thus establishing ecologically meaningful cause and effect relationships.

Although mesocosms, stream mesocosms in particular, as an experimental tool, have been widely used. The limited replicability (Schindler, 1998; Steele, 2013) and the semi-controlled natural variation suggest that the statistical power of stream mesocosms is low. In addition, outdoor mesocosms have strong spatial and temporal characteristics (e.g. regional seasonal pattern). Hence, to investigate the replicability of key factors, such as water physicochemistry (Caquet et al, 2001) and benthic assemblages (Ledger et al., 2009; 2013) in stream mesocosm is required, in order to improve the reliability of the experiment results.

In the first part of this study, the focus is on the replicability of stream mesocosms, physicochemical and biological replicability separately. Limited studies focus on the replicability of pond mesocosms. Caquet et al., (2001) found that moderate inter-mesocosm variability was found in physical, biological parameters. Kraufvelin (1998) suggested that there was high error in individual and community variable in pond mesocosm ecosystem. Very few researches focus on the replicability of stream mesocosm. Harris et al., (2007) found that both physicochemistry and benthic community were highly replicated in flumes. Wong et al., (2004) investigated the temporal and spatial variability of macroinvertebrate assemblages in an outdoor stream mesocosm. They found that the within and between channels variation of mesocosm community was

controlled by species sensitivity. It suggested that those variation should be considered in the further risk assessment. Here, I assess how water physicochemistry and benthic community replicability varies on spatial and temporal scale.

1.2 Climate change and extreme events

The drivers of climate change - global warming associated with increased greenhouse gas emissions - has been ongoing since last century (NASA, 2018). Climate change acts as compound stressor with potentially profound consequences for the structure and functioning of freshwater ecosystems across the globe. As the climate changes, model predict that the intensity, frequency and duration of extreme water-related events (e.g. floods, droughts) will increase (Beniston et al., 2007). The occurrence of extreme events is also expected to vary markedly across climate regions according to local atmospheric process (Williamson et al., 2009; Fischer & Schär, 2010; Dai, 2012). For instance, multiple climatic models suggest that by 2050, 10-30% of stream runoff will have decreased as a result of climatic variation at mid-latitudes (Milly et al., 2005; IPCC, 2014). At northern high latitudes, however, stream flow may not decrease due to the melting of permafrost (Dai, 2010). Over recent decades, drought events haven't received enough focus as other extremes such as flooding, perhaps because of their slow and unnoticed development (Van Loon, 2015). There is no doubt that extreme events like drought can have significant negative impacts on freshwater ecosystems, but there remains a lack of long-term (more than 12 months) data for catchments, and hence understanding of consequences these extreme disturbance is still limited (Boulton & Lake, 2007; Ledger et al., 2012).

There are three classical types of drought, meteorological drought, agricultural drought and hydrological drought (Wilhite, 2000). Meteorological drought is defined as less precipitation than regional average annual precipitation (Wayne et al., 1965; Keyantash et al., 2002). Soil water deficiency causes plant water stress and reduces biomass and yield called agricultural drought (Boken et al., 2005). Hydrological drought is observed water reduction in stream, lakes, reservoirs, ponds and groundwater (Nalbantis et al., 2009; Van Loon, 2015). Many studies have shown that the impact of meteorological, agricultural and hydrological drought on freshwater resource is significant (IPCC, 2014). Hydrological drought – the focus here - affects freshwater resource directly. Due to the increased water demand, the less surface water and groundwater was observed globally (IPCC, 2014). Beniston et al. (2007) predict increases in drought duration and intensity by end of 21 Century. The general consequences of such stream runoff reduction include increased water temperature, decreased dissolved oxygen concentration and elevated levels of pollutants (Ficke et al., 2007; Whitehead et al., 2009; Woodward et al., 2011; Ledger et al., 2013). Meteorological drought has no direct relationship with hydrological drought, but it does affect groundwater recharge (IPCC, 2014, but see Lake, 2000; 2007; 2011; 2013). Furthermore, increasing water demand, land-use change and agricultural drought interact synergistically to influence groundwater level and storage. Hence, meteorological and agricultural drought reduces streamflow of groundwater-fed rivers (e.g. English chalk streams).

Although Hisdal et al. (2001) found that drought events have not become more severe or frequent in Europe, drought magnitude does vary regionally. In the U.K., climate change has significantly influenced air temperature and precipitation, with air temperature in central England increasing by approximately 1°C since 1970s (Bardossy

& Caspary, 1990). Compared with precipitation records since 1766, U.K. has less precipitation observed in summer (Jenkins et al., 2008). Recently, field surveys, laboratory experiments, data analysis and process-based modelling found that over 50% of chalk stream and 25% of the rivers in England are at high risk of drought (WWF, 2017). Increasing water demand and poor river management are further intensifying drought impacts in U.K.

The WWF (2017) report analysed the record from 1962 to 1990 and found that increasing trend of drought gradient and duration in large part of U.K. (Hisdal et al., 2001). In recently years, according to the historical data from Environment Agency, summer were wetter than normal. For instance, 2007 has been report as the wettest year on the record. However, due to climate change, Environment Agency warned that the water demand in U.K. may exceed water supply around 2045.

According to historical data, April has been the one of the driest month in U.K., suggesting this month may be a likely start point for drought events, which subsequently develop during summer months (WWF, 2017).

1.3 Drought and its impact on freshwater resource

In this study, drought is defined as a hydrological event, specifically a significant low-flow period in a specific location. Typically, the description of drought includes the specific detail of the catchment reach, duration and intensity (Humphries & Baldwin, 2013). Water volume reduction is a relative concept, which depends on comparison of flow variation relative to the long-term flow record of the hydrological regime (McMahon & Finlayson, 2003). Duration is another important element of drought in river systems, drought duration divides drought into two types seasonal/ predictable and supra –

seasonal/ unpredictable (Humphries & Baldwin, 2003). The long-term drought increased recently (e.g. Boulton, 2003; Stubbington et al., 2009). For instance, the supra seasonal drought has been observed in permanent river (IPCC, 2014). Frequency is another a factor to define drought. For example, the frequency of short-term hydrological drought was increased reported in U.K. (Jenkins et al., 2009). Intensity of drought is a term associated with the extent of impact, including the longitudinal connectivity between reaches as well as changes in the vertical connection with hyporheic zone and groundwater as a result of flow reduction (Lake, 2003).

Due to the three factors above, it can be difficult to investigate drought effects in natural waters, since it does not have a precise start point and often ends abruptly with a flood. Drought research has been regarded as largely phenomenological, opportunistic and restricted (Lake, 2000, 2003): impacts on water quality are relatively well understood whereas ecological consequences are less well understood.

Freshwater environments are especially vulnerable to drying climates and drought conditions (Kundzewicz et al., 2009; Woodward et al., 2010), because of they are geographically isolated and fragmented habitats. Physiochemistry (e.g. water quality, Sangiorigo et al., 2007; Dewson et al., 2007) and ecological processes (e.g. river ecosystem function and structure, Lake, 2003;2011; Rolls et al., 2012) and aquatic habitats (e.g. lose longitudinal connectivity, Boulton, 2003; Lake, 2003; Ormerod et al., 2010; Lake, 2011) were altered by extreme drought events (e.g. long-term duration, serve intensity).

Studies on the ecological responses of aquatic biota to drought have gradually increased in recent years (e.g. Boulton &Lake, 2007; Dewson et al., 2007a; Lake, 2008). Stream biota responses to drought vary with the intensity, duration and timing of drought

(Boulton, 2003; Dai, 2013). Where drought is predictable, for instance occurring annually in summer, biota is typically well an adapted (Lake, 2003; Bond et al., 2008), and often both resistant and resilient to drying events (Lake, 2003; Ledger et al., 2013). A number of studies have shown how macroinvertebrates have greatest resistance in intermittent streams and can quickly recover after drought (del Rosario & Resh, 2000; Boulton, 2003; Leigh et al., 2016; Sánchez-Montoya et al., 2018). However, by contrast, less is known about the consequences of drought where they occur unpredictably, most probably because these events are difficult to study, with pre-impact data often lacking.

1.4 Drought impact on English chalk streams

Chalk streams are a type of groundwater-dominated waterbody fed by chalk aquifer, > 70 % of which are found in England, a few in France (WWF, 2017). Aquifers underlying English chalk streams are recharged by rainfall during winter and these then sustain river flow throughout the summer (Sear et al., 1999). The conservation value of chalk streams is high – they are characterised by stable discharge, high physicochemical water quality and contain multiple habitat types that sustain populations of macrophytes, macroinvertebrates, fish and other animals. The research presented in this thesis was undertaken in artificial streams located on the bank of the Candover Brook, an important headwater of a major chalk system, the River Itchen. The River Itchen is designated as a Site of Special Scientific Interest (SSSI) on the basis that it provides high quality habitat for range of protected species, such as southern damselfly, freshwater crayfish and Atlantic salmon (Natural England, 2019). Despite the ecological importance of the SSSI, there has been a few of studies on the impacts of stressors affecting the ecology of river. Water quality decline (Hopwood et al., 2015), instream vegetation reduction (Zhang et

al.,2017), macroinvertebrate community change (Fung et al., 2013) were observed in the River Itchen with drought condition.

Many English chalk streams are particularly sensitive to low rainfall (WWF, 2017) and the expectation is that their rich flora and fauna are less resistant to drought than communities found in intermittent river ecosystems (Langhans et al., 2006; Datry et al., 2011). For perennial rivers, like many chalk streams, unpredictable drought may exceed certain critical thresholds, with profound consequences for the structure and functioning of these systems. Hence, there is an urgent need to improve our understanding of the impact of drought in these perennial waters. Stream biota are likely to exhibit relatively low resistance and a variable resilience to the unpredictable supra-seasonal droughts that occur in the U.K. (Boulton et al., 2003; Lake, 2003). Due to the different region, varied drought intensity may occur under climate change in the future (IPCC, 2004). Major mesocosm studies were constructed one certain drought level (Ledger et al., 2011; 2013), however water physicochemical quality and habitat heterogeneity differs in response to drought intensity (Lake, 2003; Boulton & Lake, 2007). Additionally, as relatively low resistance of permeant river ecosystem, even minor water reduction may alter benthic community and ecosystem function. Hence, this study created a shift of drought intensity (from non-drought to moderate to intense drought) treatment to investigate stepped ecological response associated with the sequence of hydrological alternation (Lake,2000; Boulton & Lake, 2007).

Drought may alter macroinvertebrate communities markedly, with recovery post-drought taking years to reach pre-drought levels (Wright, 1992; Covich et al., 2003). Since 1970s, ecologists have researched summer drought in chalk streams in England and found that long-term drying (e.g. over 6 months) severely influenced the whole

ecosystem, including macroinvertebrate, fish and macrophytes (Wright & Berrie, 1987; Wright, 1992). However, since these drought events are uncommon, these studies remain scarce (Ledger et al., 2013; IPCC, 2014). Most of this past research has focused on effects of low flow on community structure – the presence and abundance of species, especially macroinvertebrates - in running waters, whereas the consequences for ecosystem functioning – the rates of the many ecological processes such as primary production and detrital decomposition – remain rare.

This study focus on ecosystem macroinvertebrate community structure relative to two key freshwater functional processes, biofilm primary production, algal growth in particular, and leaf decomposition. Few studies have explored drought effects on the rate of these processes, but it could be that at certain threshold (e.g. water reduction < 50%), drought may increase water temperature and the concentration of nutrients, which may enhance growth and production of benthic algae (Tayor et al., 2004; Katharina & Fabriclus, 2005; Gruner et al., 2008; Raven, 2017; Bestová et al., 2018). However, more severe droughts that cause major dewatering may strongly reduce algal production. Additionally, drought may affect the abundance and diversity of macroinvertebrate herbivores, which associate with consequences for biofilm production (Rosemond et al., 1993; Wallace & Webster, 1996; Rutherford et al., 2000; Hillebrand, 2009). By comparison, drought impacts on decomposition processes are relatively well known for rivers where droughts are predictable (summer water reduction; Schilief et al., 2009; Datry et al., 2011; Pinna et al., 2016). Here, drying can reduce leaf litter decomposition rates as well as alter the density and richness of macroinvertebrate shredders (Boulton, 1991; Gessner et al., 1999; Garca, 2001). There remains a lack of knowledge regarding drought effects on this ecological process in permanent rivers. Here, I assess how both

the algae-grazer and detritus-detritivore systems respond to droughts of contrasting intensity simulated in a mesocosm experiment.

1.5 Focus of thesis research

This thesis outlines the design and performance (i.e. replicability) of an outdoor stream mesocosm facility in southern England, together with the application of an environmental change treatment (hydrological drought) in a mesocosm experiment conducted at the facility. Mesocosm replicability is assessed in space and time, assessing variation within and among mesocosms, both initially and through time during a 12-month experiment. The drought experiment takes a gradient approach, applying drought of contrasting intensities to determine the freshwater ecosystem functional and structural response to changing hydrology in a warming, drying world. This research provides new insights, into both the replicate behaviour of mesocosm facilities to inform future experimental design, and, the likely response of running water ecosystems to increased drought intensity predicted to occur in the future through climate change.

This thesis comprised four data chapters, the first of which**, Chapter 2,** describes the experimental design and outlines how the water reduction was applied, creating seven treatments including controls treatment in the experimental mesocosm. All treatments and basic water quality information are described in this chapter, providing a background to the following chapters.

Although stream mesocosms have been widely used in ecological studies, there is a question about the balance between mesocosm replicability and realism, especially the large-size model stream systems (Schindler, 1998; Stewart et al., 2003). Large mesocosms have been questioned for their lack of replicability, which can reduce the

power to detect cause-effect relationships in these systems. Replicability of lotic mesocosms is addressed in terms of physicochemistry and biodiversity in **Chapter 3** and **Chapter 4**, respectively.

In this experiment the physicochemical conditions among treatments was used to construct an index of drought intensity to provide an integrated assessment of ecosystem function and macroinvertebrate responses. The impact of the drought treatments is then assessed for a) the structure of herbivore assemblage and function of algal primary production and its herbivory (**Chapter 5)** and b) the structure of the detritivores and their processing of detritus in the benthos (**Chapter 6**).

The following key questions are addressed by the research programme:

Experimental design

(1) Can water physicochemical condition be successfully mimicked and replicated in stream mesocosms during a long-term experiment?
(2) How are macroinvertebrate assemblages in mesocosms replicated in space and time?

Experimental insights

(3) How is the biomass of benthic algae influenced by drought intensification?
(4) How do macroinvertebrate herbivores and grazer respond to drought?
(5) How is the process of leaf litter decomposition altered by drought?

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CHAPTER 2

Outdoor Lotic Mesocosm Design and Set-up

2.1 Study area

This mesocosm was conducted from February 2013 to January 2015 on floodplain of the Candover Brook, a 1.2 Km chalk stream in Hampshire of Unite Kingdom (51.167°N, 1.134°W; Figure 2.1) and it is a tributary of the River Itchen Catchment (350 km^2; Figure 2.2).The hydrological regime of the Candover Brook (Figure 2.3) is a typical groundwater fed river, with some degree of buffering from meteorological extremes. The Candover Brook has distinctive features and biological richness of this area, and the clean water supporting a rich diversity of mammal, bird, fish, invertebrate and plant communities (Willson, 2009). Mean annual discharge is 5.3 m^3s^{-1}, and mean rainfall is 853 mm (Marsh et al., 2008). The mean air temperature is 18°C in summer, and 8°C in winter (Durance & Ormerod, 2009).

2.2 Experimental design

The mesocosm facility conducted of 21 stainless steel channels (dimensions 15 m length × 0.5 m width × 0.5 m height; Figure 2.4) installed on a flat gravel area beside the riverbank. Groundwater was pumped from a borehole and suppled through PVC drainage pipes to feed each channel. Each channel streambed consisted of uniformly alternating sections of riffle and pool habitat (three riffles and four pools). Channels were filled by mixed coarse and fine clean gravels (mainly 10-54 mm width). The depth of riffle (from riffle peak to the steel channel bottom) is 25cm. The depth of pool (from pool bottom to the steel channel bottom) is 15 cm (Figure 2.5). Before water reduction treatment was applied into mesocosms. In each channel, there are same amount of macrophytes (*Ranunculus penicillatus subsp. Pesudoflutians* (Syme) S.D. Webster),

macroinvertebrates, benthic algae and fish (bullhead, *Cottus gobio*) were seeded in each channel. All those biological materials were collected from the Candover Brook. *Ranunculus* were selected into similar condition, each plant has same length of leaves and root. Seven plants were planted in each riffle- pool habitat in each experimental channel. Same amount kick samples of macroinvertebrate (10 × 5 kick samples) was transferred into each mesocosms. Algae was seeded by the biofilm coated cobbles. Cobbles were placed in the channel bed. Seven bullheads (mean body length = 5cm) were seeded into each channel. From Jan.2018 to Jul. 2018, the channels were then left with full flow conditions (water depth = 35cm) for additional natural colonization and for the community to stabilise before the drought experiment was initiated.

2.3 Drought experiment set-up

In August 2013, water flow was manipulated by the mesocosm inlet pipes sluice of each channels. A series gradient of surface water loss was applied to cover the critical threshold of ecosystem function response were adjusted (Kayler et al., 2015). Seven water depth Treatments (water depth = 0, 2, 5, 7, 15, 25, 35cm, measure from bottom of pool to water surface) were randomly assigned to 21 channels to eliminate spatial basis. Each treatment was applied into three channels (Figure 2.6).

A Tinytag temperature logger (Gemini Data Loggers Ltd, Chichester, UK) was placed in the final pool (d) of each channel to collect temperature data at 15 min intervals. An Optical dissolved oxygen sensor (MiniDOT logger, PME Inc., Vista, CA, USA) was placed midway of channels, and the sensor was kept moisture through water column. The dissolved oxygen (DO) was recorded every five minutes over 24-hour period from August

2013 to August 2014 (Harris et al., 2007). Temperature data was used to calculate temperature variability (i.e. the range of temperature annual variation) and oxygen data was used to calculate the man daily minimum DO concentration. Water temperature and DO concentration in extreme condition are more serious to influence water ecology rather than average condition (Thompson et al., 2013; Vázquez et al., 2017).

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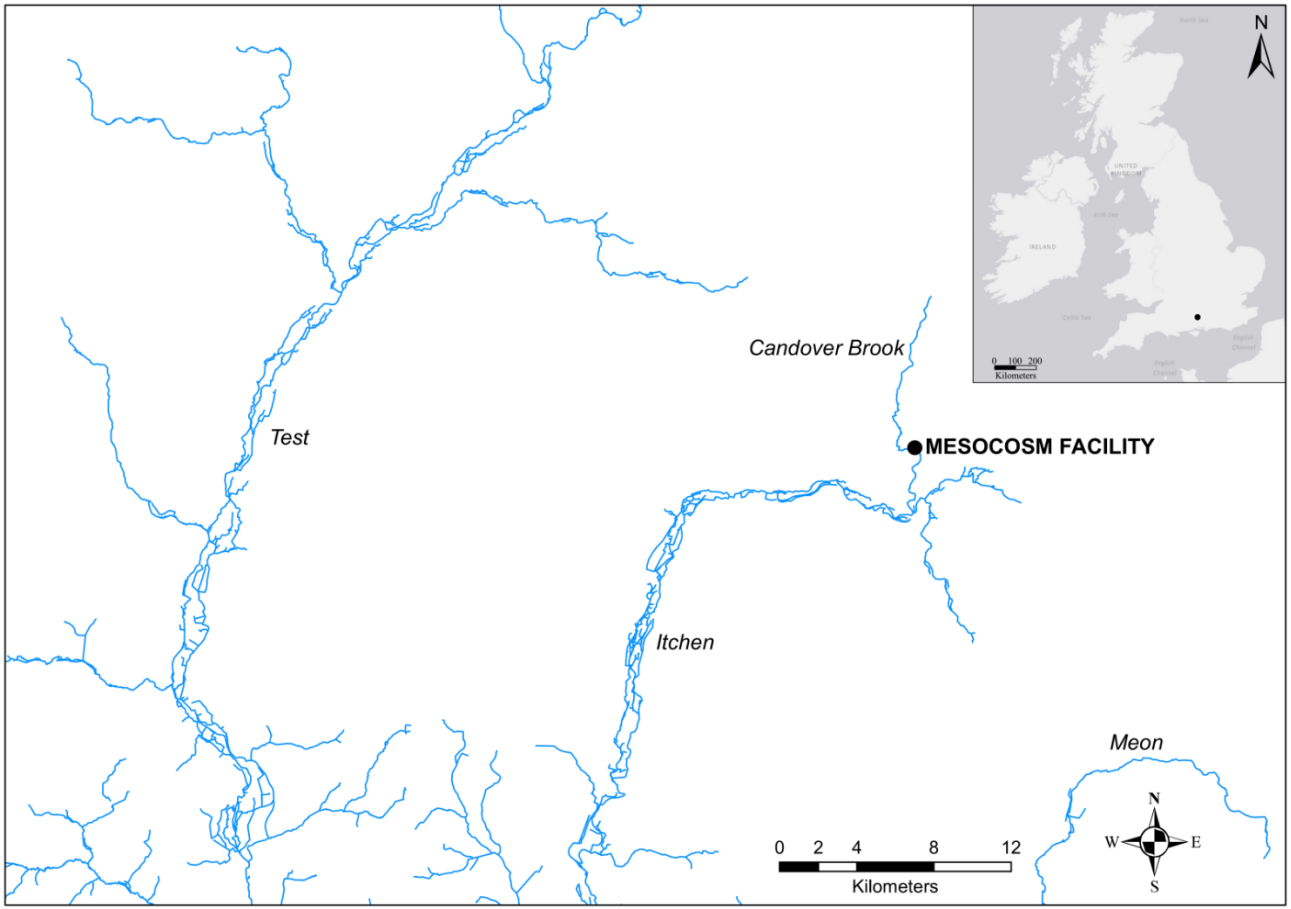

Figure 2.1 Location of the River Itchen in the U.K. and the location of stream facility near the Candover Brook

Figure 2. 6 River Itchen

Figure2. 7 Candover Brook

Figure2. 8 Mesocosms facility

Figure2. 9 Mesocosm flume set-up

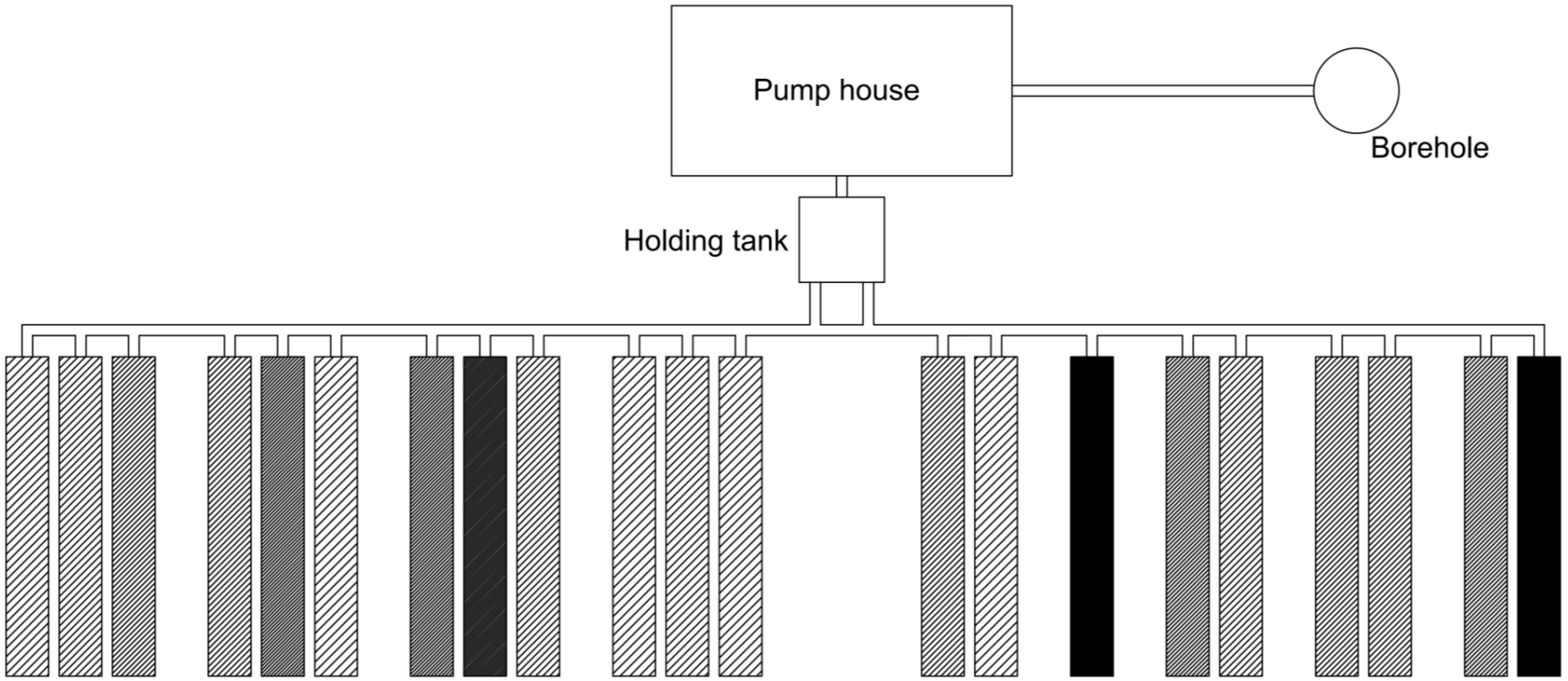

Figure 2. 10 Seven drought treatment were randomly assigned into 21 channels. Feeding water was abstracted from borehole nearby and transferred to holding tank. All of mesocosms was supplied from holding tank uniformly.

CHAPTER 3

Spatial and Temporal Variation of Physicochemistry in Replicated Outdoor Stream Mesocosms

3.1 Summary

1. This study focuses on the utility of stream mesocosms, using statistical methods to assess the extent of the variation among nine physicochemical determinants. The analysis sheds light on the likely statistical power of mesocosm experiments, with high statistical power being the foundation of establishing precise and accurate cause-effect relationships in experiments.
2. The result of the statistical analysis revealed that water physicochemistry in mesocosms was highly replicable in space and time. It is probable that the outdoor venue and consistent groundwater supply common to all flumes underpinned thisy replicability of mesocosms.
3. Temperature, conductivity, pH and dissolved oxygen were less variable than dissolved greenhouse gases (CO_2, N_2O and CH_4) and macronutrients (NO_3^- and PO_4^-). CH_4 and NO_3^- were the least replicable of the variables measured.
4. Mesocosms replicability increased with time, as the flumes settled following initial founder effects.

3.2 Introduction

Semi-controlled outdoor mesocosms (SCOM) combine the benefits of laboratory experiment and field survey and have been widely used in ecological assessment since 1990s (Clements, 1991; Graney, 1993; Belanger, 1997). As an experimental tool, mesocosms should be highly replicate (Wong et al., 2004; Cañedo-Argüelles et al., 2012; Grantham et al., 2012), but several studies reveal that the characteristics of mesocosms vary in space (e.g. study site) and with time (e.g. study period), experimental method or unexpected disturbance. Mesocosm performance can also be affected by design features such as size, design and experimental set-up (Giesy & Allred, 1985; Belanger, 1997; Harris et al., 2007). This study examines the patterns of variability in a large array of mesocosms to assess the extent to which they are able to provide satisfactory venues for experimentation. Increasingly, mesocosm studies are being constructed and used in freshwater ecological studies, but there few studies evaluate the scales of variability in these systems. Here, I specifically examined the spatial replicability in physicochemistry across an array of large semi-controlled mesocosms and establish whether this replicability varies through time.

The term "replicate" is simply defined as a repeating scientific experimental unit used to obtain a consistent result. Replicated experiments allow experimental procedures and findings to be repeatable (same procedure in same location) and/or reproducible (same procedure in different location), enhancing experimental reliability (Caquet et al., 2001; Harris et. al., 2007). Additionally, "replicate" is a term associated with the provision of more than one experimental unit in one treatment (Giesy & Allred, 1985; Pestana et al., 2009). High replication of mesocosm units is often desirable to ensure there is sufficient statistical power to detect treatment effects in these systems. For

instance, Giesy & Allred (1985) suggested that mesocosms should contain 13 replicates minimum to cover 90% variation. Yet, due to funding and logistical constraints, two to five replicates in more typical for each treatment of outdoor mesocosm arrays. For instance, two treatments were applied in four mesocosms tanks (3.2 m diameter × 1.2 m high), each replicated twice (Seguin et al., 2002); Eight mesocosms channels (40 m length × 3.4 m width × 0.5 m depth) applied three treatments, each replicated two times, control channels replicated four times (Van den Brink et al., 1996); Taylor et al. (2018) used 12 artificial flow-through streams (18.3 m length ×0.61m width) applying three treatments, each replicated four times. There is therefore a need to understand the scale of spatial and temporal variation within and between these mesocosm units so that methodologies for mesocosm establishment can be refined to limit sources of statistical error for experimenters (Hurlbert, 1984; Giesy & Allred, 1985; Suter, 1996). Where sources of error are understood, methodological and statistical approaches can be used to manage these. For instance, both Muñoz et al., (2018) and Harris et al. (2007) used block effects to account for sources of variability in mesocosm units that were not of primary interest to the experimenters. Usually, there are two replicate types found in SCOM. For lentic mesocosms, it only requires replicate between ponds (channels). For the lotic system, the ecosystem is also affected by the flow longitude connection (Drago, 2007; Aschonitis et al., 2016), the present SCOM requires both replicates within channels and replicates between channels. In this study, those mesocosm channels constructed four pool-riffle sections (a, b, c, d) along channel length as replicates within channel and three repeating channels under same treatment as replicates between channels.

Historically, mesocosm experiments were undertaken to test the effect of stressors and change on single species populations and/or simple species mixes, for instance, basic

food chains. In laboratory settings, mesocosms were used in ecological studies on algae (e.g. Pickhard et al., 2002, 2005; Song et al., 2018), invertebrates (e.g. Dick et al, 2002; Vaughn et al., 2008; Elbrechtetal et al., 2016; Folegot et al., 2017) and fish (e.g. Garvey et al.,1994; Lefebure et al., 2013; Evans et al., 2016)and are still perhaps most frequently employed in ecotoxicology research (e.g. Mohr et al., 2005; Piggott et al., 2012; Berghahn et al., 2012; Cadmus et al., 2018;). Although laboratory-scale mesocosms are often highly replicated, enabling statistical analysis of formal experiments, they are sometimes criticised as being of low realism, since they simplify environmental conditions, explore effects of single stressors rather than natural cocktails, and limit natural biodiversity (Giesy & Allred, 1985; Carpenter, 1996; Crane, 1997; Petersen & Englund, 2005). More recently, mesocosm research has evolved, using larger systems to increase realism, thereby capturing more complex ecological responses typical of field studies. Within this context it has been possible to use more elegant experimental approaches to test, for instance, for the independent and interactive of multiple stressors that occur in natural systems (Lefebure et al., 2013; Stewart et al., 2013; Beermann et al., 2018).

Outdoor mesocosms, semi-controlled lotic mesocosm in particular, are typically open systems in more environmentally realistic settings than laboratory mesocosms (McIntire, 1993; Belanger, 1997; Stewart et al., 2013). Therefore, researchers were using large size mesocosms to investigate the ecological consequences of climate change in freshwater ecosystem (Stewart et al., 2013; Piggott et al., 2015), which make the results closer to the natural response and make it more credible. However, due to the inverse relationship between replicate and realism, the results are also questionable statistically (Schindler, 1998; Ledger et al., 2009; Stewart et al., 2013).

System replicability can be assessed as the extent of variation of physical, chemical and biological factors among replicate experimental units under the same experimental treatment (Harris et al., 2007). The magnitude of replicability may be influenced by multiple aspects of operation and is likely to be context dependent. For instance, replicability of experimental units may fade away with increasing experimental period if individual units develop contingent communities. Because of the semi-controlled experimental conditions in an outdoor setting, mecososm replicability vary with local seasonal patterns (e.g. temperature, light) (Ledger et al., 2013). Additionally, variability is attributed with measurement error associated with specific operators and equipment. Therefore, to establish a reliable cause-effect relationship in a SCOM, the initiation and performance of experimental units must be evaluated.

From preview studies, mesocosm replicability is always represented as the coefficient of variation (CV) of environmental data among experimental units. Relatively few studies have explored replicate behaviour in stream mesocosms, especially for physicochemistry. From preview studies, Giddings & Eddlemon (1979) defined that the CV of basic water quality parameters (e.g. pH, water temperature) in a high replicability facility is 10-30%, which was found in whole system pond mesocosm. However, the variability of nutrient CV (e.g. N, P) was observed much higher than others chemical variables in mesocosm systems (Caquet et al., 2001; Harris et al., 2007). For example, in Crane's (1985) review the mean CV of PO_4^{3-} and NO_3^- was 62.94% and 72.62%, respectively, which is due to the limited concentration in water (Giddings & Eddlemon, 1979), and unrealistic experimental environment of replicated mesocosms caused (Crane, 1997). Researcher normally defined that CV less than 30% means highly replicability (e.g. Crane, 1997; Caquet et al., 2001), but there are no uniform standards of CV to

estimate replicability. Additionally, the variation of CV depends on variable. Therefore, more advanced indicator is required.

Most pertinent to this chapter is the research by Ledger et al. (2008; 2009; 2012; 2013; 2016) using large mesocosms in lowland chalk streams to test the long-term effects of hydrologic drought. The outdoor mesocosms used in that study were fed by water from groundwater of similar composition to the source of the nearby chalk stream, the River Frome in Dorset, U.K. These mesocosms were artificial streams (each 12m length, 0.33 m width) filled with a gravel substratum to create small patches of habitat similar to gravel mesohabitats in chalk streams. This research served as a foundation for the new mesocosm system established at a watercress farm in Hampshire, U.K. In this chapter, the physiochemical replicability of the Hampshire flumes will be evaluated, with biotic replicability examined in next chapter (see **Chapter 4**). Mesocosm physicochemical replicability was assessed for nine physicochemical variables recorded by researchers across mesocosm units and experimental time, specifically: four water quality parameters measured using in-situ environmental sensors (i.e. temperature (T), conductivity (EC), pH and dissolved oxygen (DO)) and five parameters derived from mesocosm water samples measured in the laboratory, specifically greenhouse gases (i.e. carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O)), and macronutrients (i.e. nitrate (NO_3^-) and phosphate (PO_4^{3-})). Here, high replicability is consistent with limited temporal and spatial variation of each physicochemical parameter.

Temperature (T), conductivity (EC), pH, dissolved oxygen (DO) are four basic indicators of water quality, they have been wildly used in water science studies. The temperature regime is an important parameter in running waters since ecosystem structure and function can be significantly influenced by temperature variation (Lake, 2003;

Sommer et al., 2006). Water temperature affects almost every other water quality parameter, including physical (e.g. water density; Webb et al., 1980), chemical (e.g. dissolved oxygen; Grossman & Ku, 1986), and biochemical parameters (e.g. photosynthesis production; Merz-Preiß & Riding, 1999).

Water conductivity reflects the capacity for an electrical current to flow through water, as determined by the total concentration of available ions in the water (Collier, 1995). Water conductivity is a regular indicator of water quality in aquatic resource. Conductivity reflects the varitivity of discharge and other disturbance may affect biochemical processes (e.g. dissolved oxygen solubility; Kannel et al., 2007) and aquatic organisms (e.g. fish; Squire & Moller, 1982). Additionally, water conductivity is a general indicator of water quality assessment (Kannel et al., 2007).

pH reflects the concentration of free hydrogen and hydroxyl ions water. pH governs the solubility and biological availability of chemical constituents and heavy metals, which influence aquatic assemblages significantly (Ledger & Hildrew, 2001). Most organisms will be killed by too low (acidic) or too high (basic) pH.

Dissolved oxygen is produced by green plants in water and consumed by fish and other aquatic animals, with the balance of production to consumption governing the metabolic balance of the system (Jacobsen, 2008). Unusually low DO levels (e.g. hypoxia) can cause stress and mortality of aquatic organisms (including benthic organisms, fish), and little to no DO (anoxia) can create dead zones in any water body (Davis, 1975).

Mesocosms are increasingly used to explore how global warming could influence the emission of greenhouse gases (GHGs: CO_2, CH_4, N_2O) from freshwater environments

(Stewart et al., 2013). As little data exists on the replicability of GHG emissions in running water resource, in this experiment, we monitored the GHGs from benthic sediment. The main source of CO_2 in freshwater environment is (a) a product of terrestrial organic matter decomposition, (b) exchange of CO_2 between atmospheres and freshwater, and (c) a product of plant respiration (Boutton, 1991). Animal in water depends on oxygen, but aquatic plants depends on CO_2 concentrations in water. Keeping a good balance between DO and CO_2 in water is important to sustain both animals and plants (Nicot, 2008). Methane is a product of detrital decomposition in sediments and a by-product of organism metabolism (e.g. methanogens), which effluxes to the atmosphere from water (roughly 80% transfer rate, Yao et al., 2016). Approximately 34% of CH_4 emissions are from water sources, which is critical to global climate (Bell et al., 2017; Short et al., 2017). Nitrous oxide (N_2O) is a potential greenhouse gas that influences climate (Bastviken et al., 2011). N_2O is product of incomplete denitrification and aquatic ecosystem is a significant source of N_2O (Beaulieu et al., 2015).

Nitrogen and phosphorus are important indicators of water quality and are the main resource of nutrients in freshwater ecosystem (Rabalais, 2002). NO_3^- is one of the main forms of nitrogen in water. pH of water is also affected by nitrogen; large amount of nitrogen causes nitric acid pollution. PO_4^{3-} concentration is extremely important in water. It is an essential plant nutrient, but eutrophication is caused by large amount PO_4^{3-} in water bodies. To monitor NO_3^- and PO_4^{3-} is important to monitor pollution.

In this chapter the research gap identified above is addressed experimentally, by examining the water physicochemitry in a series (n=21) artificial channels. This chapter assesses the extent of this variation within and among an array of stream mesocosms, in order to inform the developing framework for large-scale mesocosm design and

application. To examine the spatial and temporal variation, nine physicochemical parameters were examined in three control channels during the whole experimental period, with the following hypotheses tested:

H1: Physicochemical conditions will be highly replicable (i.e. CV < 30%) between mesocosms;

H2: Physicochemical parameters measured using in-situ environmental sensors (i.e. temperature, conductivity, pH and dissolved oxygen) will be more replicable among experimental units than parameters measured in laboratory settings (i.e. CO_2, CH_4, N_2O, NO_3^-, and PO_4^{3-});

H3: The replicability of the study channels will decline with experiment duration, due to stochastic processes operating at the channel level (e.g. colonisation and species turnover of autotrophs).

3.3 Materials and methods

3.3.1 Sample collection

Physicochemical measurements were taken approximately monthly (Sep. 2013 – Aug. 2014, n = 10 sampling occasions, except May 2014 and June 2014, Table 3.1) in each of three control channels (coded C1, C2 or C3). On each sampling occasion, nine parameters were measured. Four physicochemical water parameters were measured monthly by in-situ environmental sensors. Thus, water temperature (T, Tinytag® data logger model TGP-4017, Gemini Data loggers Ltd., Chichester, U.K.), conductivity (EC, Hanna model HI 8633, Hanna Instruments Ltd, Leighton Buzzard, U.K.) and pH (Hanna

model HI 9024, Hanna Instruments Ltd, Leighton Buzzard, U.K.) were sampled at the outflow pool of each artificial channel. Dissolved oxygen (DO, MiniDOT loggers; PME, CA, USA; ± 0.5 %) was sampled at the mid-point of each channel (7.5 m out of 15 m length) on each sampling occasion.

A 20ml vale of water sample was collect from each pool (a, b, c, d) of control channels (C1, C2, C3), and store in cold and dark and transferred to the Wolfson Aquatic Sciences Laboratory in University of Birmingham to measure five physicochemical parameters. Greenhouse gas concentrations were determined by gas chromatography (Agilent 6890N, Agilent Technologies, Berkshire UK) using a flame ionisation detector (FID). The peak analysis was measured by GC Chemstation (revision A.10.20) software (Agilent Technologies, U.S.A). The 6 ml of groundwater feeding the mesocosms was added to a dry pre-weighed gas tight vials. Vials with sediment were incubated on a reciprocating shaker table at 85 RPM in a 15 °C temperature room. Extra three vials only contained groundwater and another 3 vials only contained gas, were added to analysis. For three gas only vials, 2 vials were using to detect the peak. The last vials contained certified standard mix gas (CO_2/ CH_4/ N_2O, 3699/ 100/ 100 ppm respectively, BOC, special gas mix), was used as the calibration standard. Gas was identified based upon retention time of the standard gas mix. The FID process was repeated three time to ensure gas production had plateaued. Each gas production curve was calculated and corrected for time to determine gas production.

One water sample was collected from each pool (a, b, c, d) for phosphate (PO_4^{2-}) and nitrate (NO_3^-) analysis. A 0.22 μm pore size filter (PES, ANR2522C) was using to filter water sample by 50 ml syringe and subsequently frozen. A segmented flow auto analyser (Skalar, type 5000, Skalar Analytical B.V, The Netherlands) was using to

measure PO_4^{2-} and NO_3^- concentration (Acuña et al., 2005). In this measurement, the limit of detection (LOD) and precision for PO_4^{2-} and NO_3^- was 0.2 µmol L^{-1} ± 1%. The standard was chosen from 0.25 µm, 0.5 µm, 2 µm, 10 µm and 20 µm to produce calibration curve for PO_4^{2-} and NO_3^-. The 2 µm standard solution was used to assess the instrument drift and 5 µm multi standard solution was using to compare against a spiked ground water sample to ensure drift correction during the whole measurement experiment. Double deionised water was used as a blank sample.

In additional, three control channels water temperature and head water temperature were recorded continuously during whole experiment period (Figure 3.1).

3.3.2 Statistical analysis

In order to analyse the replicability of water physicochemistry in space and time, the variation of nine physicochemical parameters was investigated. These nine parameters were assigned into two groups according to sampling methodology (field sensor or laboratory procedure) as we expected contrasting levels of variation among these groups. In order to examine the stability/ persistent of the present SCOM, three control channels instead of 21 artificial channels were used to apply this analysis. For each control channel the mean, maximum and minimum of each physicochemical variable (Temperature, Conductivity, pH, DO, CO_2, CH_4, N_2O, NO_3^-, and PO_4^{2-}) were calculated for each sampling occasion.

The coefficient of variation (CV %) is a statistical index that reflects the level of statistical variability in data. It is the ratio of the standard deviation to the mean (Kraufvelin, 1998; Ippolito et al., 2012). For each of the nine physicochemical variables two coefficient of variation (CV) were calculated to examine the variability a) within four pools within each mesocosm replicate, and b) between three replicate mesocosms (the

unmanipulated controls) on each of six sampling occasions (i.e. month 2, 5, 7, 8, 12, 13). The temporal variation of physicochemistry variables' CV was demonstrated by boxplots. Pearson's product moment correlation was used to analysis temporal variation of nine physicochemical variables (package: ggpubr; Kassambara, 2018).

Temporal and spatial variation in physicochemistry among three control channels was analysed by redundancy analysis – an ordination technique. Redundancy analysis (RDA) is a linear ordination method to analyse multiple response variables that can be explained by a set of explanatory variables (Braak &Smilauer, 2002). Because the gradient lengths on axes of a preliminary detrended correspondence analysis (DCA) were shorter than two standard deviations (axis length = 0.59, Lepš & Šmilauer, 2003), RDA was applied in this study (Ramette, 2007). In the analysis, physicochemical variables were centred and rescaled (0-1), to remove the influence of different units among response variables (Harris et al., 2007, Zuur et al., 2007). Because of missing data, RDA was used to compare physicochemical variation between control channels sampled for six sampling occasions across the experiment (i.e. month 2, 5, 7, 8, 12, 13). Sampling times (6 occasions) were coded as either dummy environmental variables or co-variables. A Monte Carlo permutation test (999 random permutations) was applied to test the statistical significance of each model. High replicability is evidenced as low or non-significant temporal and spatial variation in physicochemstry between three control channels across the experiment period. Spearman's correlation was also used to calculate the association between physicochemistry variable and scores for two ordination axes. The centroid distance was calculated in RDA analyse for each sampling occasion. The value presents the average distance of three sampling sites to the centre of convex hull. Shorter distance means higher spatial replicate between three channels. One-way

ANOVA will be used to check the statistically difference between average centroid distance.

All analyses were applied using the statistical package R (Version 3.5.0, 2018), and 'Vegan' (Oksanen et al., 2017) was used to run ordination analysis. The significance level for all statistical analysis was indicated at the 5% level i.e. a *p-value* <0.05.

3.4 Results

3.4.1 Physicochemical conditions in stream mesocosms

In order to present the variation of each parameters, 95% confidence intervals were constructed to give a certain variation range. According to the Figure 3.2, the variation range of four chemical parameters (T, EC, pH, and DO) in major sampling occasion distributed between (or lower) 95% confidence intervals. There were few outliers of those four chemical parameters, such as temperature variation exceeded 95% confidence intervals only in month 13. On the contrary, the rest five parameters' variation exceeded 95% confidence intervals in most sampling occasions (Figure 3.3). It demonstrated that during the main study period the four chemical parameters measuring by in-situ field sensors (i.e. Temperature, Conductivity, pH and DO) varied less than those determined by laboratory analysis (i.e. CO_2, CH_4, N_2O, NO_3^- and PO_4^{2-}).

According to the sensor in feeder head tank (the detail demonstrated in **Chapter 2**), water temperature variation was lower in the feeder head tank (mean ± SD, 10.32 ± 0.07 °C, range 10.20°C to 10.60°C) than in the three control channels (mean 10.40°C, mean range of three control channels, 9.93 ± 0.11 to 11.47 ± 1.76 °C; Table 3.1; Figure 3.2a). The lowest value was found in winter (month 7, Feb. 2014), 8.42 °C in channel C2.

The highest value was found in summer (month 13, Aug.2014), 14.51°C in channel C3, which is much higher than previous summer (month 2, Sep.2013).

Comparing to the water conductivity in River Itchen (mean = 524 μs/cm; EA, 2018), the water conductivity in control mesocosms was similar during whole study period (mean 531.95 μs/cm, range 391.98 ± 0.99 - 633.08 ± 0.02 μs/cm; Table 3.1; Figure 3.2b). The highest mean conductivity was found at beginning of experiment (month 2, 633.08 μs/cm) then drop sharply to 391.98μs/cm in month 5. Back to 591.08 μs/cm in month 6, the minor decrease trend of mean conductivity was found following months, conductivity decreased to 523.88 μs/cm (month 13).

Mean pH was 7.21 (range 6.88 ± 0.81 to 7.42 ± 0.01, Table 3.1; Figure 3.1c), which was less alkaline than in the River Itchen (EA,2018; mean = 8.2). The lowest mean value was 6.88 in month 2 increased to 7.40 in month 5, decreased to the lowest value, 6.95, in month 12. During 13 months of experiment, pH increased, the water in control channels from weak acid to weak alkalinity, which is more similarity to natural chalk stream (Collingridge, 2002).

Dissolved oxygen concentration (mean 300.60 micromolar, range 275.14 ± 16.13 – 406.50 ± 18.00; Table 3.1; Figure 3.2d) were in typical range of DO in English chalk stream (EA,2018), reflecting substantial in-channel primary production of macrophytes and benthic algae were functioning well to support the mesocosms ecosystem. The highest DO concentration was 406.50 micromolar in month 3, drop to 275.14 micromolar in month 7. There was a minor fluctuation between month 8 and month 13. Generally, DO in control channels decreased for 13 months experimental duration.

According to variation of EC, pH and DO, it found that there has more fluctuation in the early experimental period (before month 7). The variability of water temperature is limited relatively.

Dissolved carbon dioxide was the most abundant of dissolved gases in mesocosms. The mean CO_2 concentration in three control channels was 424.3 micromolar (range 340.42 ± 26.11 - 486.69 ± 19.30; Table 3.1; Figure 3.3a). It was much lower than the CO_2 / HCO_3^- found in River Itchen (EA, 2018). The CO_2 concentration among three control channels varied across drought duration.

Dissolved methane concentration was low but highly fluctuated across experiment period (mean 13.68 micromolar; Table 3.1; Figure 3.3b). The extremely high CH_4 was found in month 4 (33.71 ± 24.77), the lowest concentration was found in month 13 (0.00 ± 0.00).

Dissolved nitrous oxide concentration was the second largest amount gas in water (mean 142.86 micromolar, range 116.76 ± 7.56 – 214.29 ± 22.97; Table 3.1; Figure 3.3c). N_2O decreased a little during main study period.

Nitrogen dioxide concentration was low in water (mean 0.09 micromolar, range 0.00 ± 0.00 – 0.16 ± 0.14; Table 3.1; Figure 3.3d). The extremely high concentration was found in month 2 and decreased sharply in month 3. The total N concentration in mescoscom was roughly 4 mg/L, which was little lower than in natural chalk stream (Collingridge, 2002).

Phosphate concentration was low in mesocosm (mean 0.65 micromolar, range 0.17 – 2.04; Table 3.1; Figure 3.3e), which was approximately one fifth lower than mean concentration in typical chalk stream (mean 0.06 mg/L in study mesocosm, 0.37 mg/L in River Itchen; EA,2018). The PO_4^{2-} - P concentration varied during 13 months.

In summary, according to the data from Environmental Agency measurement, the temperature, conductivity, pH and DO in mesocosm was similar to water quality of River Itchen (EA,2018). Compare to water quality indicators, the dissolved GHG and nutrient concentrations in the mesocosm were relatively lower than those physicochemical parameters found in natural chalk stream. Dissolved gases and nutrient valuables were more various than four basic water quality indicators (EA,2018).

3.4.2 Physicochemical variation among replicate control channels

In this section, two CVs were calculated. Within-channel CV (WCV) demonstrated the parameter variability across pools (a, b, c and d) in each channel, which demonstrated the variation along channel gradient. Between-channel CV (BCV) demonstrated the replicability of each parameter among three control channels. Lower CV means higher replicability and lower variability.

Within-channel CV (WCV)

There was no WCV data of DO, because of only one measurement was taken in each channel in each sampling occasion. The major WCV of T, EC and pH (>90%) was in 95% confidential intervals.

The WCV of temperature within channels was extremely low (range 0.35 to 3.37%.; Table 3.2; Figure 3.4a) except month 13 (10.29% in channel C3). There was an extreme difference from C1 and C2 in month 13. The extreme outlier might be cause by unforeseen disruption (e.g. pump shut down stops water supply), which should be ignored.

The variability of water conductivity and pH in mesocosms were even lower than temperature, mean WCV was 0.94% and 0.54%, separately (conductivity range: 0-4.36%; Table 3.2; Figure 3.4b; pH range: 0-1.19%, Figure 3.4c). Temperature, conductivity and pH were limited varied (i.e. WCV<5%) along artificial channel gradient during whole experiment time.

During the main study period, dissolved gases and nutrient concentration in water varied stronger than the three parameters measured by in-situ environmental sensors. For CO_2 concentration variability was limited (mean = 9.8%), over 60% experiment period, the variability of CO_2 was lower than 10% (Table 3.2; Figure 3.5a). The CH_4 concentration varied dramatically along channel length gradient (mean WCV: 75.66%, range 0 – 173.21%; Table 3.2; Figure 3.5b). The CH_4 had the highest WCV value, which means it was the least replicate variable in mesocosm. Comparing with other gas dissolved in water, N_2O was the most replicate parameter in control channels. The mean WCV of N_2O was 7.28% (range: 0.72 – 31.32%; Table 3.2; Figure 3.5c), which was 5.82 times higher than WCV of temperature. NO_3^- (mean: 62.63%, range 0 – 173.21%; Figure 3.5d) was the second less replicate parameter, the mean WCV was only lower than the WCV of CH_4. PO_4^{2-} concentration varied little during study period (mean: 28.40%), except in month 5, the WCV of PO_4^{2-} among pools was extremely high (Table3.2; Figure 3.5e).

In summary, T, EC, pH, DO, CO_2, N_2O and PO_4^{2-} were highly replicated within channel (WCV < 20%) during main study period. However, CH_4 and NO_3^- displayed low replicability within artificial channels.

Between-channel CV (BCV)

The mean BCV was lower the mean WCV for each physicochemical parameter, which suggests the replicability between channels were higher than within mesocosm replicability. The BCV of temperature, conductivity and pH were extreme low, all of them were lower than 10%, except BCV of temperature in month 13 (15.32%; Table 3.3; Figure 3.6a). The mean BCV of DO was 2.65% (range: 0.44 -6.61%, Table 3.3; Figure 3.6d), and it had the highest variability of four environmental sensors' parameters.

The BCV of dissolved gases demonstrated that dissolved gases varied more than previous four variables. Between three control channels, the spatial variability of CO_2 and N_2O were limited (BCV<20%). As similar situations found in WCV, the BCV of CH_4 was extremely high, 64.5%. PO_4^{2-} was less replicability among channels than CO_2 and N_2O but was higher than NO_3^- replicability (71.4%) (Table 3.3; Figure 3.7).

Overall, physicochemical parameters of control channels had high spatial replicability in both within-channels and between- channels (CV<30%). Additionally, the within- channel and between-channel replicate set-up maintained high spatial replicability of water physicochemical condition during 13-month study period.

3.4.3 Physicochemistry comparisons

In order to examine the temporal variation of multiple physicochemical parameters, an RDA analysis was regressing nine physicochemistry parameters collected from three control channels over the 13 months study on 6 sampling occasions (month 2,5,7,8,12,13). In the ordination, 83.9% variance of water physicochemstry was explained ($p<0.001$, Table 3.4). For each axis, 63.71% and 32.80% were explained by axis 1 and 2 separately. Three control channels in each sampling occasions, the central point of each sampling occasion and physicochemical variable vectors were plotted in Figure 3.8. The

matrix score of each sampling occasion central point and physicochemical variable vector were present in Table 3.5 and Table 3.6.

Due to the missing data, in this study, the spatial variation of water physicochemistry between two/three control channels in each sampling occasion was demonstrated by centroid distance. Centroid distance is the distance between the central point of convex hull and each channel point of each sampling occasion. The coordinate of each central point of convex hull and average centroid distance in each sampling occasion have been showed in Table 3.5. According to the result, the range of mean centroid distance is from 0.0040 (13-Month) to 0.0102 (5-Month) (Table 3.5). The higher spatial variation between channels was found in 5-Month, and the most limited spatial variation between channels in 13-Month. Although, the mean centroid distance of 13-Month is 2.55 times higher than 5-Month, there was no significant statistical difference between average centroid distance in six sampling occasions (*p-value >0.05,* ANOVA).

According to Figure 3.8, six sampling occasion points distribute into three regions. 2-Month point were plotted in negative quadrant (RDA1 < 0). 7-Month, 8-Month, 12-Month and 13-Month points were plotted near origin of axes (RDA1 = 0). 5- Month point was plotted in positive quadrant (RDA > 0). According to axis 1 scores, there was a variation change between 2- Month (-0.63, 0.14) and 5- Month point (0.86, -0.05). Because of axis 1 explained major temporal variation, it suggested that there was limited temporal variation from 7-Month to the end of experiment.

The biggest difference was found between month 2 (RDA1, RDA2 = -0.63, 0.14, Table 3.5; Figure 3.7) and month 5 (0.86, -0.05). The highest water conductivity was found in month 2 (EC=633.08 ±0.66 μs/cm) and the lowest conductivity was found in

month 5 (EC=-0.13 ± 0.05 μs/cm). Compared with physiochemical condition of month 2, extremely high pH was found in month 5 (pH =7.42 ± 0.01).

Physicochemical condition in mesocosms of month 7 (-0.17, -0.22) and month 8 (-0.10, -0.61) were similar. The two sampling occasions increased along axis 1 and decreased along axis 2. The variation of axis 1 was driven by conductivity only. Water conductivity of those two sampling occasions increased back to average level from data in month 5. The variation along axis 2 was driven by CH_4 and PO_4, both of two parameters were found huge increase from month 5 to month 8.

Physicochemistry in month 12 (-0.10, 0.56) and month 13 (-0.02, 0.18) were similar. The two sampling occasions only increased along axis 2. The variation was major driven by CO_2. CO_2 concentration was found the lowest in month 8 and then increased to the highest in month 12 (Figure 3.2a). Although temperature also showed that it was second driver, it was natural seasonal pattern (month 8: March 2013; month 12: July 2014).

Physicochemical variable was present as vector in Figure 3.8, and each variable has been applied statistical check. According to the result, only six of nine P-value is lower than 0.05 (Table 3.6). CH_4, N_2O and NO_2 were non-significant in RDA analysis (P-value >0.05, Table 3.6).

According to vectors' matrix score, CO_2 (0.37,0.95), pH (0.58, -0.36) and Temperature (0.04,0.59) were positive along axis 1. The rest of six variables were negatively along axis 1. Each vectors' Euclidean distances has been calculated (Table 3.6). The result showed that CO_2 (Euclidean distance = 1.02) and Conductivity (Euclidean distance = 0.99) are the main driving force of water physicochemical temporal variation. The variation of axis 1 was negatively driven by water conductivity, the positive driving

force along axis 1 was CO_2. The angle between vectors shows the relationship between variable. The angle between CO_2 and Conductivity is approximate 90^o, which means those two variables uncorrelated each other (Cos $90^o = 0$). Additionally, DO, N_2O, NO_2 and Conductivity were (linear) correlated positively (angle < 90^o).

In conclusion of RDA analyse, it found that the water conductivity, CO_2 were the first main driver to cause temporal variation in control channels, the similar result was found in Harris 'study (2007). Additionally, spatial variation is limited during experimental period.

The temporal variation of each water quality variables was examined by Pearson's product moment correlation. The ρ is Pearson's product moment correlation index that demonstrates similarity of variable between 2 channels. So, three ρ were calculated for each parameter (e.g. C1 and C2, C1 and C3, C2 and C3). Higher ρ value means higher temporal variability of physicochemical variables.

The temporal variation was highly synchronous between control channel for temperature (Pearson's product moment correlation, ρ range: 0.85 – 0.96, $p < 0.001$), conductivity ($\rho = 0.99$, $p < 0.001$), pH (ρ range: 0.79-0.92, $p < 0.05$), DO (ρ range: 0.86-0.98, $p < 0.05$; Table 3.7). Compared with 4 previous parameters, temporal variations of rest 5 parameters were less highly synchronous between control channels. The between-channel correlations of N_2O (ρ range: 0.85-0.93, $p < 0.05$), and PO_4^{2-} (ρ range: 0.78-0.90, $p < 0.05$) were all significant. The significant between-channel correlations of CO_2 were found between C1 and C2, C2 and C3, separately (ρ range: 0.65-0.87, $p < 0.05$), but the correlation between C1 and C3 was not significant ($\rho = 0.39$, $p > 0.05$). The between-channel correlation of NO_2 was only significant between C2 and C3 ($\rho = 0.88$, $p<0.05$).

The temporal variability of CH_4 was non- synchronous between control channels ($p > 0.05$).

Overall, the major water physicochemical variation in control channels were explained as temporal variation. The temporal variation was driven by feeding water physicochemical features (e.g. Conductivity). However, those temporal variability between control channels were highly synchronous during the whole experimental period. Additionally, the spatial variability of control channels was limited.

3.5 Discussion

As an ecological experimental tool, the water phsicochmestry of a SCOM is expected to replicate spatially and temporally (e.g. >1-year experimental period). The aim of the present analysis was to investigate the current research gap of examining the replicability of water physicochemistry among replicated experimental units of a lotic SCOM. The principal objective of the study was to determine if water physicochemical variation of three control channels was consistent. The nine selected water physicochemical parameters were examined individually, the integrated physical and chemical condition were also investigated. Both spatial and temporal replicability of the SCOM were analysed separately. The possible causes for the replicability discovered and discussed below.

3.5.1 The replicability of physicochemical condition among mesocosms

As expected, hypothesis (H1) was supported, water physicochemical condition was highly replicated among mesocosm channels. Seven of nine water physiochemistry parameters (i.e. T, EC, pH, DO, CO_2, N_2O and PO_4^-), were highly replicated (CV < 30%)

in three artificial channels, which suggests that those large-size groundwater-fed lotic mesocosms were highly replicable over the course of the experimental period.

Spatial variability of the entire facility was captured by both the within-channel and the between-channel variation, which were both limited. The high within-channel replicability suggested that there was limited water physicochemical variation along channel length. It may be due to the fact that at 15 m long the artificial channel was sufficiently short to constrain any potential upstream-downstream gradient (Lamberti& Steinman, 1993). Within-channel replicability was lower than the between-channel replicability, suggesting that four replicate riffle-pool sections along each channel length did not markedly alter water quality. Hence, the experimental design of four replicates per channel, three replicates per treatment is sustainable for experimental replicability requirement.

The high among-channel replicability observed suggested that the water physicochemical condition of experimental facility was predominantly governed by the experimental site water supply rather than the spatial distribution of channels within the site itself. The water source is the foundation of the outdoor semi-controlled mesocosm experiments and in this study, borehole water was used instead of surface water to limit unexpected or seasonal variation. Despite an unexpected disturbance (i.e. drastic fluctuation of temperature in month 13), water physicochemical conditions remained stable between the flumes. According to the results, the majority of data variability across the mesocosm array could be explained by temporal variability. Periodic fluctuation of water physiochemistry was found in the flumes, including a seasonal water temperature pattern similar to that found in the natural stream (Candover Brook) running alongside the flumes. Meanwhile, the similar environmental expose decreased spatial variability.

Non-laboratory controlled experimental environment reduced artificial impact (e.g. 12 hr light and 12 hr dark in laboratory), which also improved the replicability of mesocosm units (Harris et al., 2007; Stewart et al., 2013).

The similarity of mesocosm conditions can be attributed to a standardization of protocols for mesocosm set-up and establishment (Giesy & Allred, 1985). For instance, the mesocosm substrate was constructed using uniform washed gravel, such that there was only very limited patchiness among microhabitats beyond the constructed riffle-pool sequence. For instance, the cleared gravel substrate provided a limited amount of organic matter, which reduced the variability of CO_2.

As the mesocosms were fed water from a common borehole on the watercress farm, there was very limited opportunity for physicochemical changes to occur differentially across the mesocoms array (Alexander et al., 2007; Harris et al., 2007). The low surface area-volume ratio (ratio = 2) of the flumes also decreased water surface heat exchange, which reduced water temperature variability (Robert et al., 2012). Similar geomorphic stream bed design (e.g. riffle – pool) and limited hyporheic zone design reduce biochemical process (Harvey et al., 1998).

Giddings & Eddlemon (1979) suggested that mesocosm facility used a period (e.g.7 weeks) to stabilize water physiochemistry. In this study, the six months pre-experimental period were spent to stabilize water physicochemical condition, which reduced the integrated water physicochemistry variability in controls (Jüttner et al., 1995).

3.5.2 The comparison of parameters' replicability

Hypothesis (H2) was supported by the analysis, with the the four basic water parameters measured by in-situ environmental sensors being more replicable than

dissolved gases and nutrient parameters measured by laboratory procedures. Mesocosm variability is likely a result of spatial and temporal heterogeneity. Water temperature, conductivity, pH and DO are primarily controlled by similar outdoor exposure to environmental conditions with their outdoor bankside location maximising realism. The common groundwater source also contributed strongly to the four parameters' replicability (Caquet et al., 2001; Harris et al., 2007).

The replicability of dissolved GHG, CO_2 and N_2O, may be attributable to high replicability of water temperature across the array. Constantly stable water temperature and similar partial pressure limited gases solubility, which reduced dissolved gas variability (Weiss, 1974; Akiya & Savage, 2002; Kritzer et al., 2004). The mesocosm channel design also improved the replicability of dissolved gases. Limited surface area (7.5 m^2) reduced the gas exchange between atmosphere and surface water. The lack of interaction between surface water and ground water reduced CO_2 variation in mesocosm facility (Griffiths et al., 2007). The limited substrate and limited organic matter in substrate reduced incomplete denitrification in mesocosm, which suggested that the high replicability of N_2O was developed by those mesocosm design (Pretty et al., 2006). The high variation of CH_4 was caused by the extreme low concentration inner - mesocosm (Giddings & Eddlemon, 1979). The similar observation of CH_4 was also found in natural chalk stream (Griffiths et al., 2007; Sha et al., 2010; Shelley et al., 2015).

Overall, reduced atmosphere-surface gas exchange, surface-ground water exchange (Griffiths et al., 2007) and standardized stream bed gravels (Shelley et al., 2015) maintained relatively low CO_2 and N_2O variability between the mesocosms.

The variability of nutrient concentration observed in some mesocosm and microcosm experiments can be extremely high (Giddings & Eddlemon, 1979), including

for pond mesocosms (Kraufvelin, 1998; Caquet et al., 2001), laboratory and outdoor stream mesocosms (Giesy & Allred, 1985), which may be attributable to founder effects or concentrations at the limits of detection. A similar observation has been found in some natural rivers, where the concentration of NO_3^- was extremely low (Hill, 1981) and highly variable (Pretty et al., 2006). In this study, the variability of NO_3^- observed was high (up to 115.92%). The nutrient concentrations in water can vary depend on biogeochemical activity, run-off and groundwater legacy. The sediment size, organic matter accumulation (Pretty et al., 2006), vegetation growth (Clarke, 2002) in channels influences biogeochemical activity. In this study, organic matters and vegetation growth varies at each control channel (Figure 1.4). Moreover, the nutrient cycle is also influenced by local seasonality (Bowes et al., 2005). It may explain that NO_3^- was the most variable parameter in this mesocosm.

However, the between-channel variability of PO_4^{2-} was low (except month 5: CV = 48.63%). It may due to the high-quality groundwater supply, there was no P accumulation from water source and upstream (Bowes et al., 2005).

Additionally, as those five parameters were measured in laboratory, analytical error in measurement may contribute to chemical variability (Giesy & Allred, 1985). Differences in detection method do not account for the replicability of parameters. It suggests that in the future SCOM, the first priorities of physicochemical parameter monitor are temperature, conductivity, pH and DO. The real-time monitoring could provide SCOM water condition/ experimental condition monitor timely and effectively.

3.5.3 The comparison of parameters' replicability

In contrast to hypothesis (H3), the RDA result suggested that the replicability of the control channels could be maintained at a high level with a certain experiment duration. Outdoor pond mesocosm studies found that, pond mesocosm is a self-sustainable system for ecological studies (Caquet et al., 1995; Jüttner et al., 1995). However, water characteristics degraded over time in those studies, which was not observed in this lotic system.

In this study, local water resources were used instead of tap water (Caquet et al., 1995)/ surface water (Harris et al, 2006) and the once-through nature of flow in the flumes refreshes water quality continually. According to the result, water conductivity and CO_2 are the main drive of mesocosm physicochemical replicability. Water conductivity is high variable in shallow water depth (i.e.10-20 cm, Pretty et al., 2006). In this study, the water depth is 30 cm in control channel, so conductivity has relative high variable in this mesocosms. The CO_2 concentration was influenced by channel substrate, vegetation and microbe in channels.

This experiment demonstrated that the large lotic mesocosm is a self-sustainable system for two-year period. Although, the larger mesocosm could simulate natural environment more realistic and contain more ecological processes, the simulation cannot instead of real freshwater ecosystem. Therefore, the extremely large lotic mesocosm (e.g. 1000L) may not be required. This mesocosm facility reduced cost and solved the problem of replicates, which proved that a large scale lotic mesocosm considerate both ecosystem realism and ecological study requirements.

In summary, this mesocosm provided a stable and similar water chemistry conditions between control channels, which could guarantee a relatively realistic environment to minimize stochasticity between treatments.

3.6 Reference

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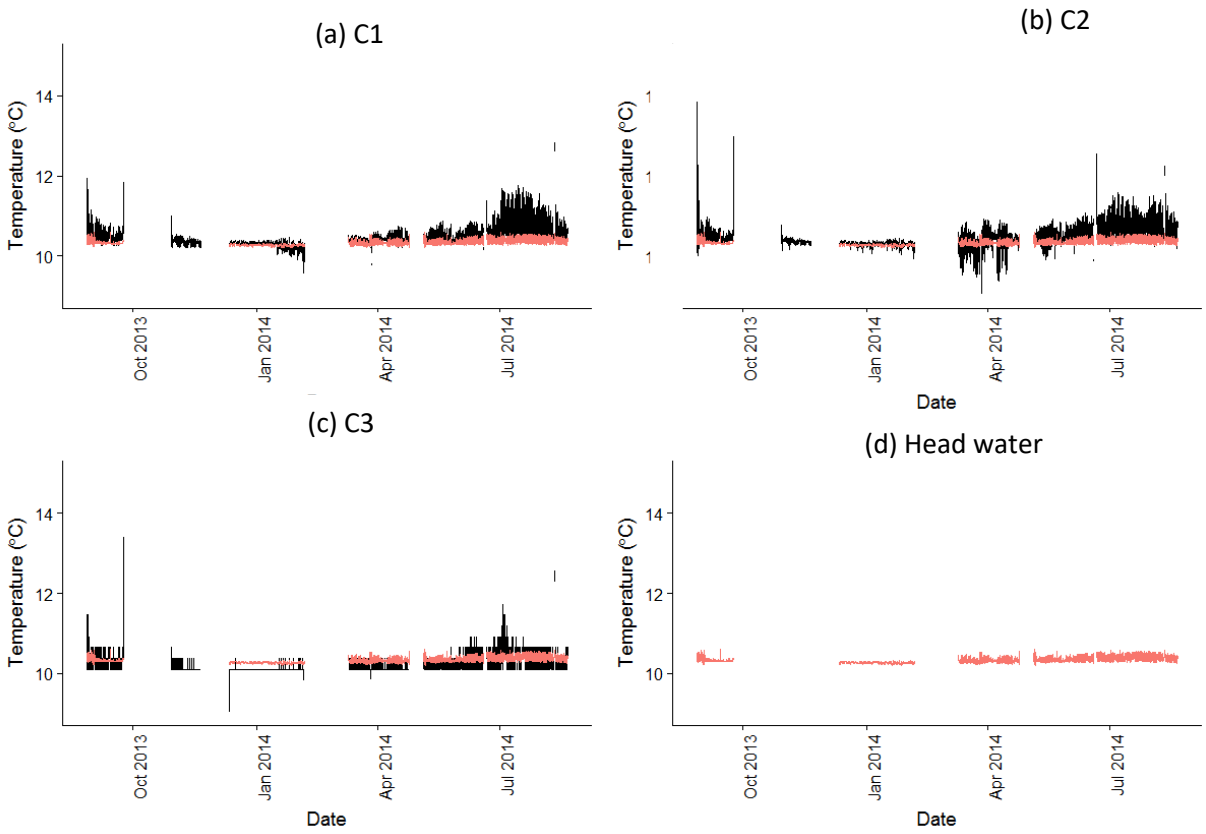

Figure 3.1: Temperature variation in 3 control channels from Month 2 (September 2013) to Month 13 (August 2014).

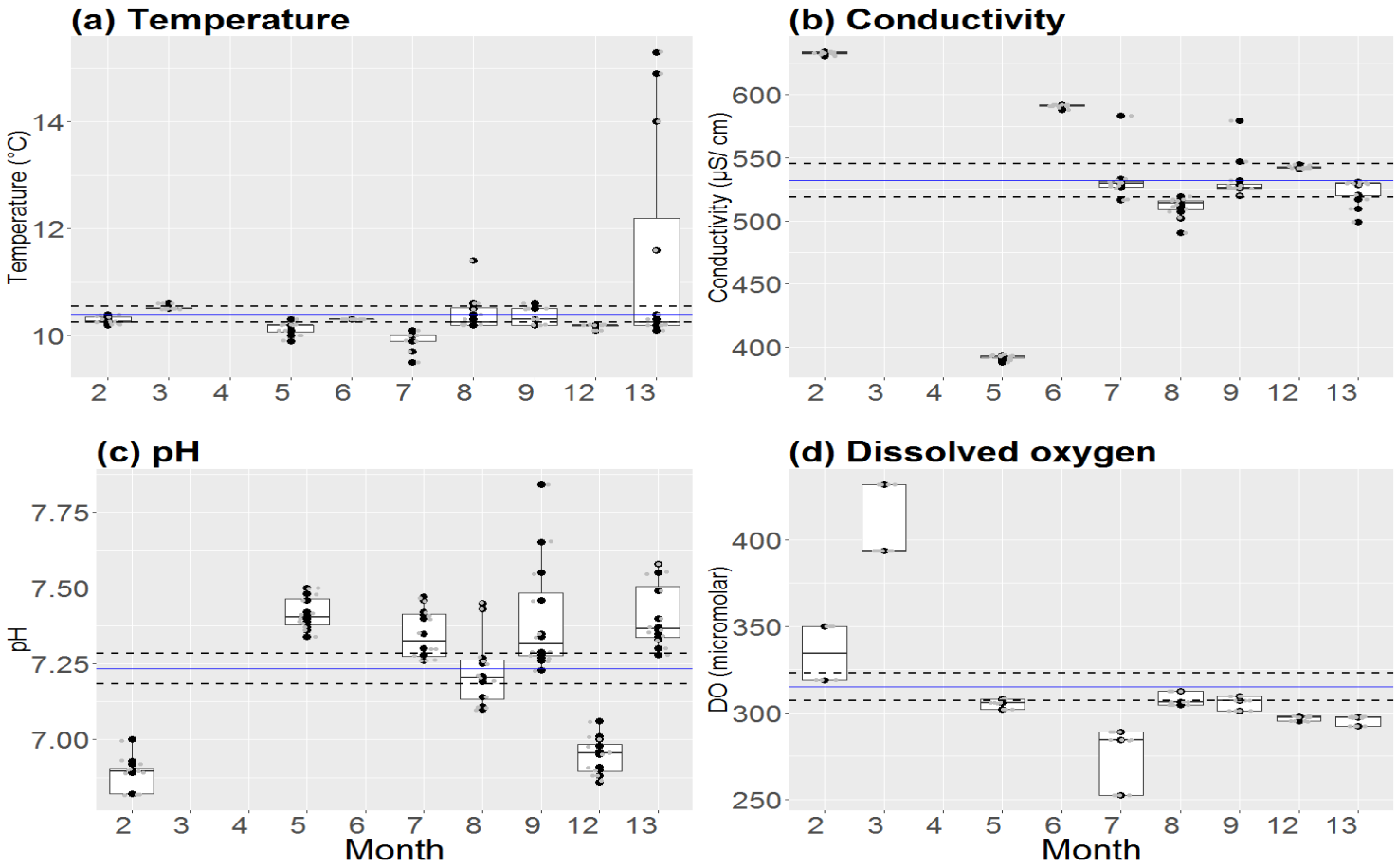

Figure 3.2: Variation of physicochemical parameter (in-situ environmental sensor) among three control channels from Month 2 (September 2013) to Month 13 (August 2014). Mean is presented as blue line. 95% confidence interval is presented as black dash line. Data is presented as black point. Repeat data is presented as grey point.

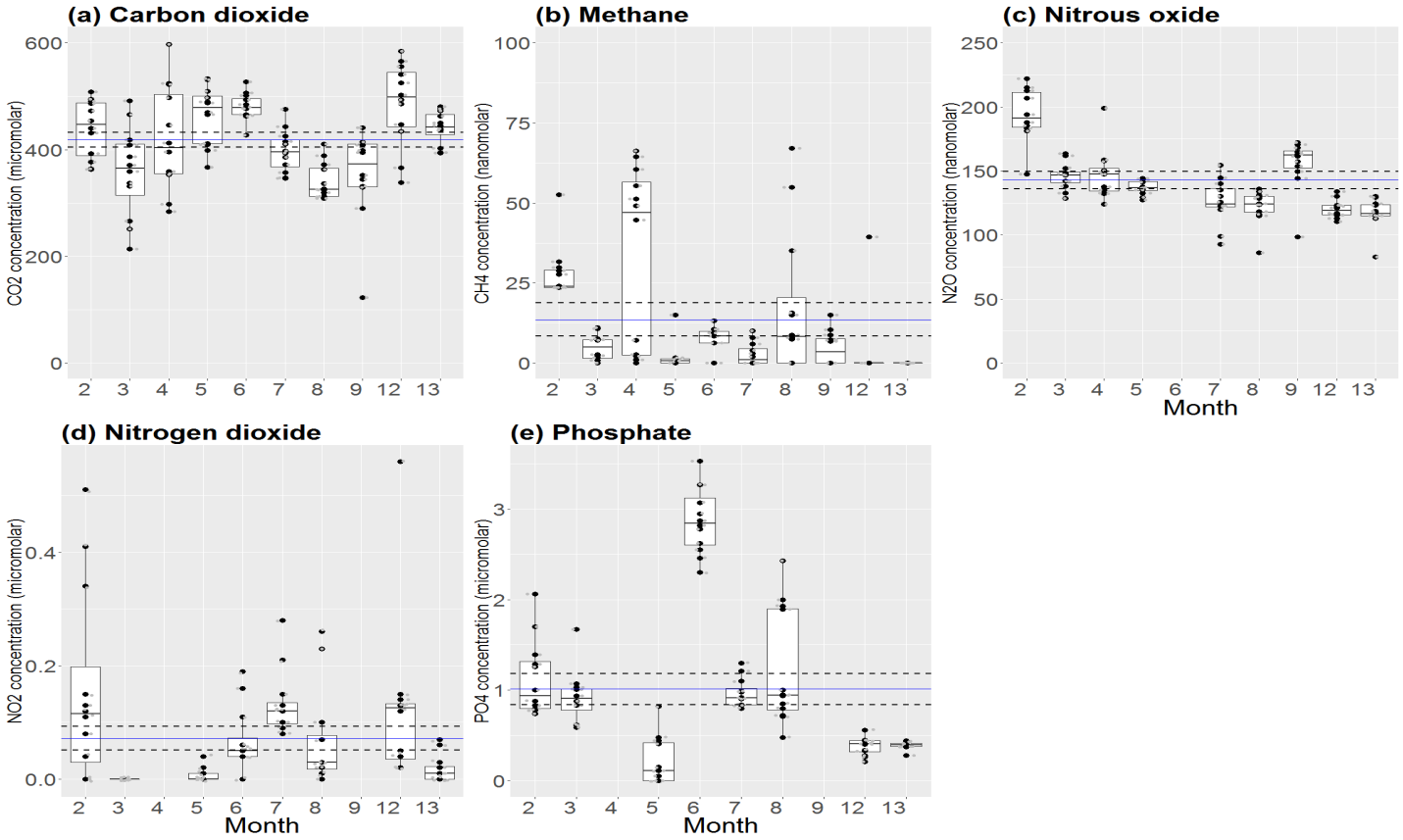

Figure 3.3: Variation in physicochemical parameters (laboratory water sample) between three control channels from Month 2 (September 2013) to Month 13 (August 2014). Mean is presented as blue line. 95% confidence interval is presented as black dash line. Data is presented as black point. Repeat data is presented as grey point.

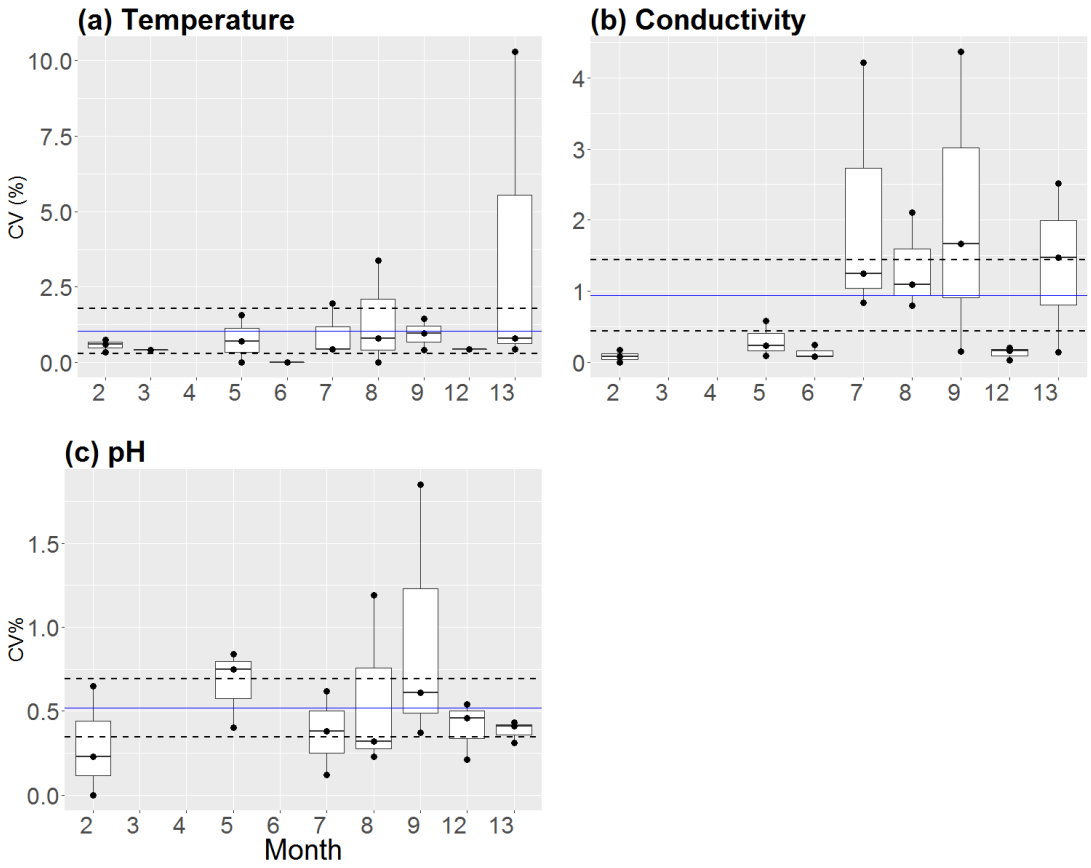

Figure 3.4: Physicochemical parameter (in-situ environmental sensor) CVs variation within pools (a, b, c, d) of each control channels from Month 2 (September 2013) to Month 13 (August 2014). For each channel, 4 nest data were collected along channels. Mean is presented as blue line. 95% confidence interval is presented as black dash line.

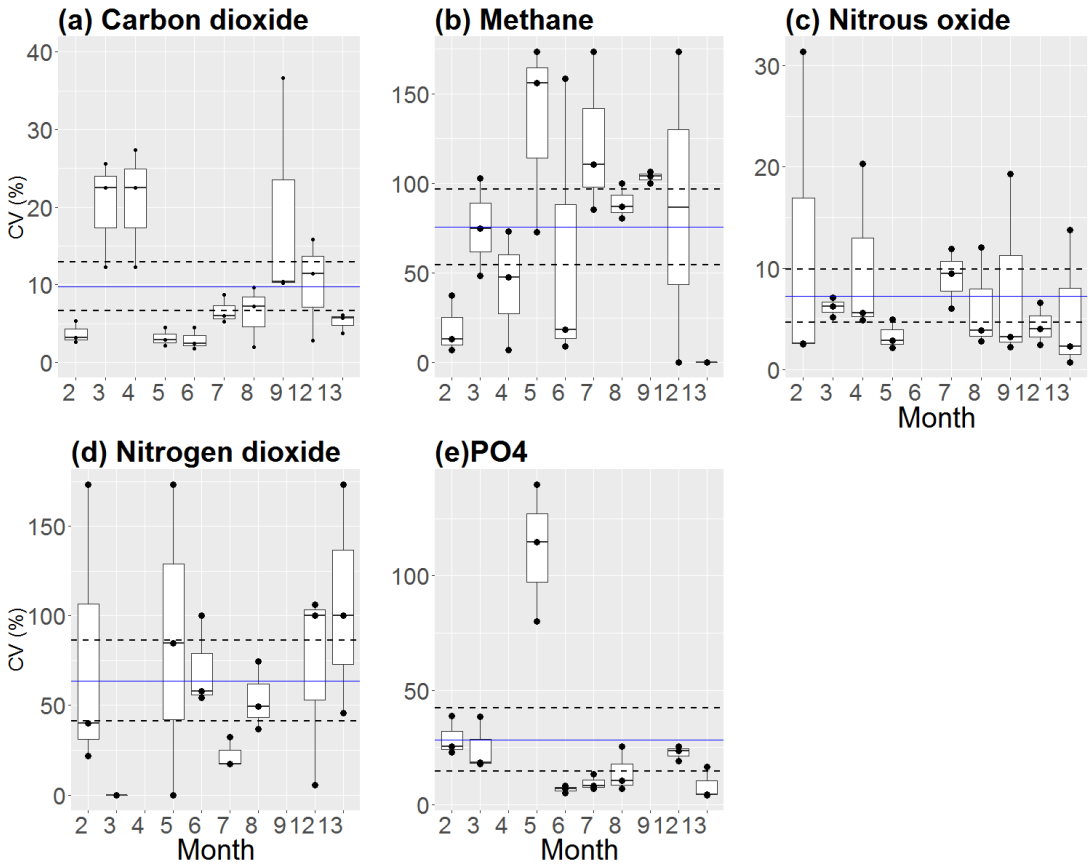

Figure 3.5: Physicochemical parameter (water sample) CVs variation along channel gradient among 3 control channels from Month 2 (September 2013) to Month 13 (August 2014). For each channel, 4 nest data were tested. Mean is presented as blue line. 95% confidence interval is presented as black dash line.

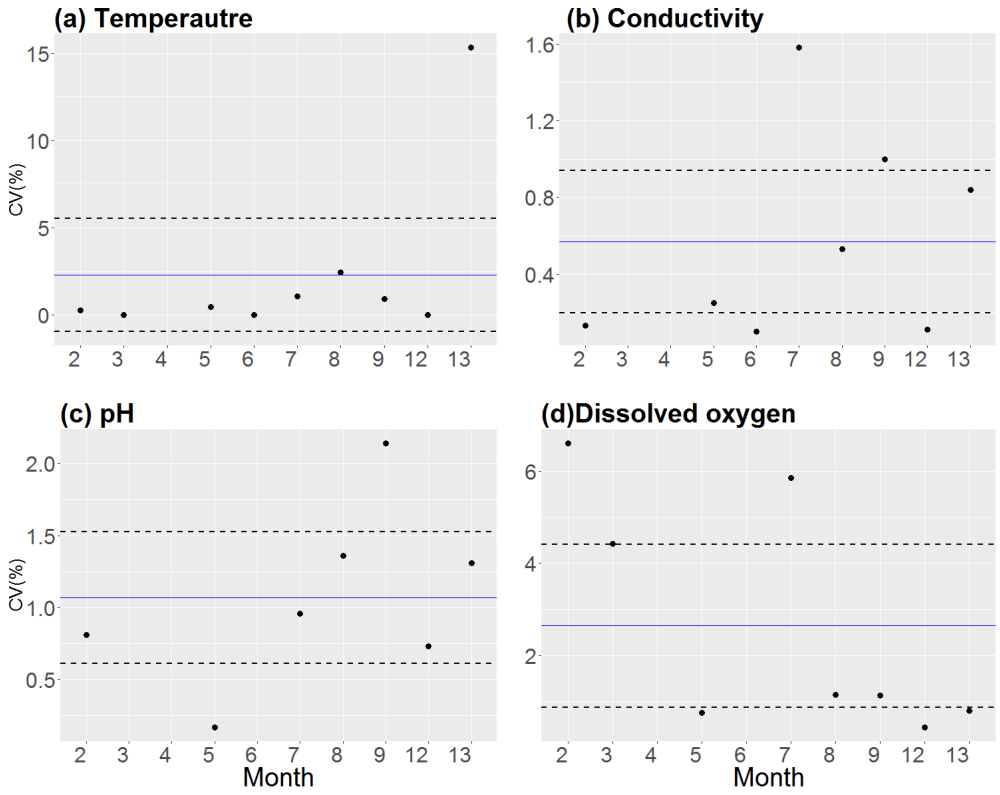

Figure 3.6: Physicochemical parameter (in-situ environmental sensor) CVs variation among 3 control channels from Month 2 (September 2013) to Month 13 (August 2014). For each sampling occasion, the mean value is calculated by each parameter from 3 control channels. Mean value of channel is calculated by 4 samples in each channel. Mean is presented as blue line. 95% confidence interval is presented as black dash line.

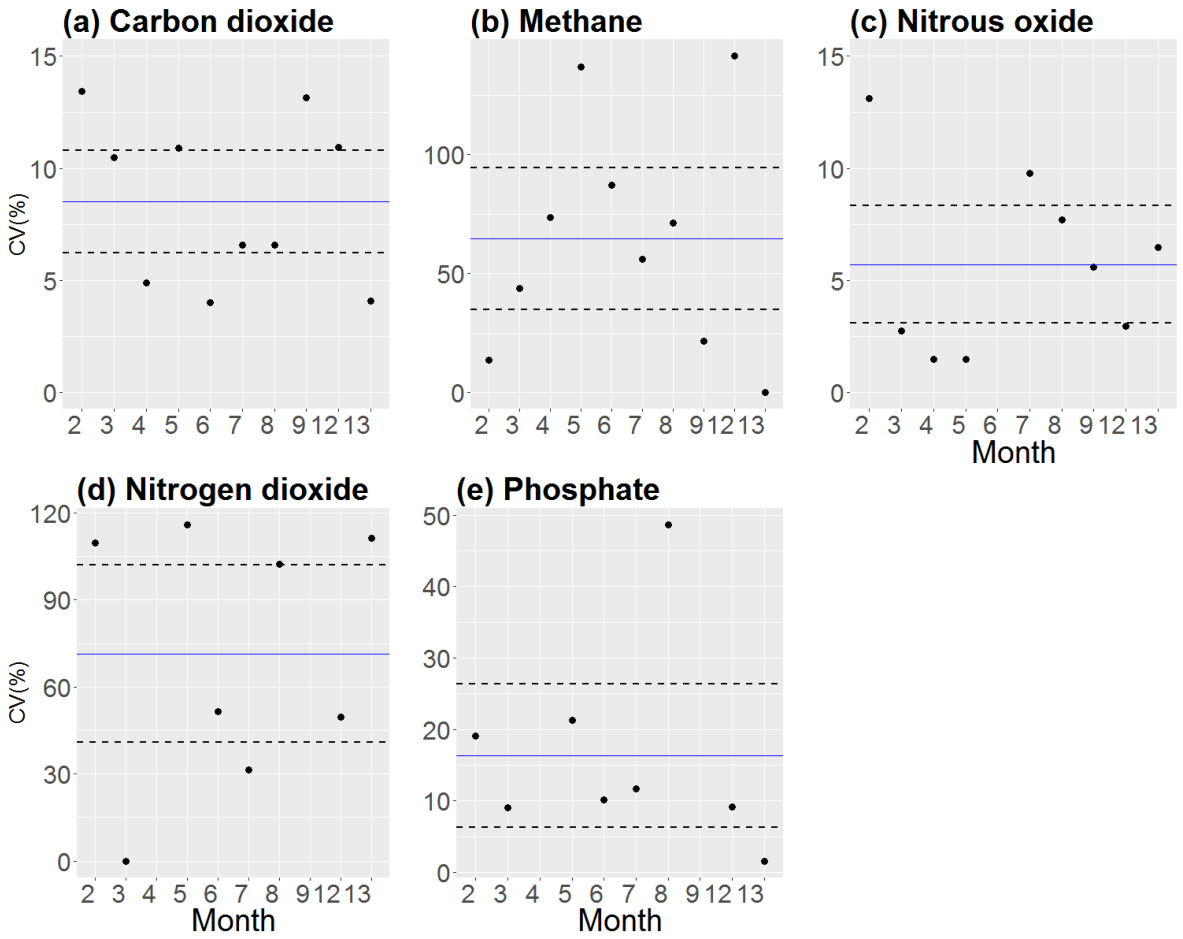

Figure 3.7: Physicochemical parameter (water sample) CVs variation among 3 control channels from Month 2 (September 2013) to Month 13 (August 2014). For each sampling occasion, the mean value is calculated by each parameter from 3 control channels. Mean value of channel is calculated by 4 samples in each channel. Mean is presented as blue line. 95% confidence interval is presented as black dash line.

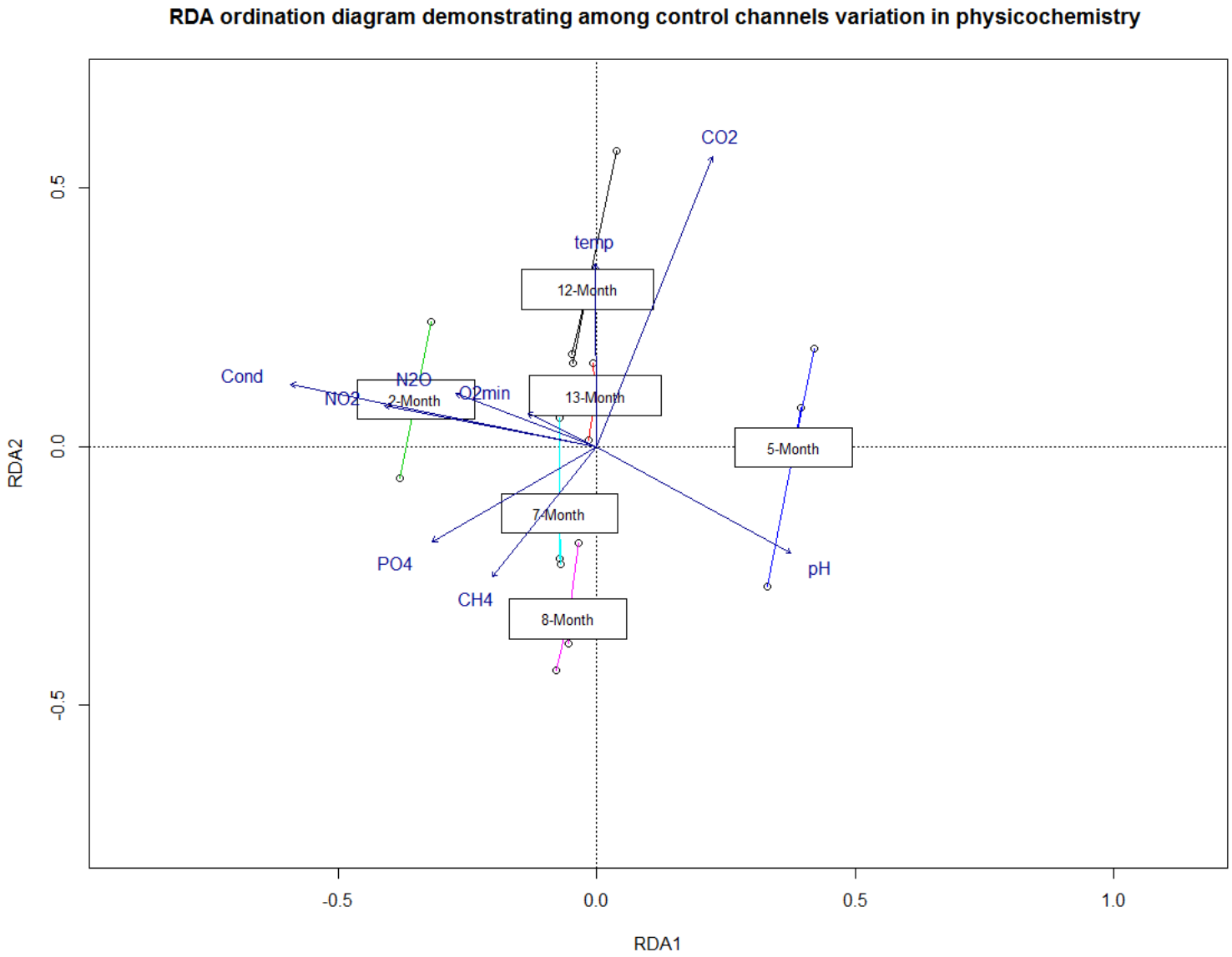

Figure 3.8 RDA ordination diagram demonstrating among control channels variation in physicochemistry during drought experiment. The direction and length of vectors indicates the trend and extent of increase in those variables. Boxes indicate the sampling occasions (2, 5, 7, 8, 12, 13 months) and inter-control channel variation in physicochemistry on each sampling occasions. Open circles are each sample site. Cond is Conductivity; temp is temperature.

Table 3.1 The average value of Physicochemistry factor in three control channels (C1–C3) between Month2 (September 2013) and Month 13 (August 2014). The value is presented as mean ± SD. T is temperature. EC is water conductivity. DO is dissolved oxygen.

Duration	**Date**	**T (°C)**	**EC (**µs/cm)	**pH**	**DO (**micromolar)	**CO_2 (**micromolar)	**CH_4 (**micromolar)	**N_2O (**micromolar)	**NO_3^- (**micromolar)	**PO_4^{2-} (**micromolar)
2-Month	Sep.2013	10.29 ± 0.02	633.08 ± 0.66	6.88 ± 0.81	334.40 ± 15.63	439.73 ± 48.20	28.06 ± 3.12	214.29 ± 22.97	0.16 ± 0.14	1.13 ± 0.18
3-Month	Oct.2013	10.53 ± 0.00	NA	NA	406.50 ± 18.00	358.46 ± 37.61	4.87 ± 2.14	146.02 ± 4.03	0.00 ± 0.00	0.92 ± 0.08
4-Month	Nov.2013	10.41 ± 0.02	NA	NA	NA	420.44 ± 20.55	33.71 ± 24.77	147.71 ± 2.19	NA	NA
5-Month	Dec.2013	10.13 ± 0.05	391.98 ± 0.99	7.42 ±0.01	305.09 ± 2.33	464.53 ± 50.58	18.34 ± 25.11	136.98 ± 2.04	0.01 ± 0.01	0.21 ± 0.05

6-Month	Jan.2014	10.30 ± 0.00	591.08 ± 0.59	NA	NA	480.69 ± 19.30	22.55 ± 19.68	NA	0.07 ± 0.03	2.87 ± 0.29
7-Month	Feb.2014	9.93 ± 0.11	532.22 ± 8.41	7.35 ± 0.07	275.14 ± 16.13	397.47 ± 26.11	2.75 ± 1.54	125.86 ± 12.31	0.13 ± 0.04	0.97 ± 0.11
8-Month	Mar.2014	10.42 ± 0.26	511.28 ± 2.71	7.23 ± 0.10	307.69 ± 3.49	340.42 ± 22.41	17.66 ± 12.58	122.27 ± 9.42	0.07 ± 0.07	1.23 ± 0.60
9-Month	Apr.2014	10.36 ± 0.10	532.68 ± 5.35	7.40 ± 0.16	305.91 ± 3.46	355.78 ± 46.84	4.61 ± 0.99	155.66 ± 8.68	NA	NA
12-Month	Jul.2014	10.18 ± 0.00	542.51 ± 0.58	6.95 ± 0.05	296.82 ± 1.31	486.69 ± 53.25	3.28 ± 4.64	120.19 ± 3.54	0.13 ± 0.06	0.38 ± 0.03
13-Month	Aug.2014	11.47 ± 1.76	523.88 ± 4.39	7.41 ± 0.10	295.73 ± 2.34	441.23 ± 18.05	0.00 ± 0.00	116.76 ± 7.56	0.02 ± 0.02	0.39 ± 0.01

Table 3.2 The Within channel CVs (%) of Physicochemistry factors among 4 pools in three control channels (C1–C3) between Month 2 (September 2013) and Month 13 (August 2014). T is temperature. EC is water conductivity. DO is dissolved oxygen.

Duration	Date	Controls	T	EC	pH	DO	CO_2	CH_4	N_2O	NO_3^-	PO_4^{2-}
		C1	0.60	0.17	0.00	NA	2.64	37.28	2.59	173.21	38.64
2-Month	Sep.2013	C2	0.75	0.08	0.65	NA	5.36	7.03	31.32	21.74	22.95
		C3	0.35	0.00	0.23	NA	3.21	12.88	2.53	40.10	25.38
		C1	0.41	NA	NA	NA	22.48	74.77	7.12	NA	17.86
3-Month	Oct.2013	C2	0.41	NA	NA	NA	12.26	102.74	6.22	0.00	18.47
		C3	0.41	NA	NA	NA	25.63	48.36	5.16	0.00	38.59
		C1	NA	NA	NA	NA	22.48	6.83	4.92	NA	NA
4-Month	Nov.2013	C2	NA	NA	NA	NA	12.26	73.09	5.62	NA	NA
		C3	NA	NA	NA	NA	27.39	47.42	20.32	NA	NA
		C1	0.70	0.24	0.84	NA	2.90	72.63	4.98	84.52	80.15
5-Month	Dec.2013	C2	0.00	0.09	0.40	NA	2.21	173.21	2.13	0.00	114.60
		C3	1.57	0.58	0.75	NA	4.48	155.95	2.90	173.21	139.79

		C1	0.00	0.25	NA	NA	2.46	9.06	NA	54.05	4.97
6-Month	Jan.2014	C2	0.00	0.08	NA	NA	1.81	18.15	NA	57.96	7.17
		C3	0.00	0.08	NA	NA	4.49	158.37	NA	100.00	8.44
		C1	0.43	4.21	0.12	NA	5.99	173.21	6.04	17.42	13.24
7-Month	Feb.2014	C2	1.96	0.84	0.38	NA	8.71	110.55	11.90	32.23	8.38
		C3	0.43	1.25	0.62	NA	5.23	85.35	9.46	17.42	6.88
		C1	0.81	0.80	0.23	NA	7.25	100.07	3.88	74.54	10.46
8-Month	Mar.2014	C2	3.37	2.11	1.19	NA	9.70	87.09	12.07	49.33	7.08
		C3	0.00	1.09	0.32	NA	1.95	80.50	2.78	36.85	25.33
		C1	1.44	4.36	0.61	NA	36.71	104.17	2.22	NA	NA
9-Month	Apr.2014	C2	0.96	1.67	0.37	NA	10.24	106.57	3.25	NA	NA
		C3	0.42	0.15	1.85	NA	10.41	100.02	19.27	NA	NA
		C1	0.43	0.16	0.46	NA	15.88	173.21	4.03	100.28	25.31
12-Month	Jul.2014	C2	0.43	0.03	0.21	NA	11.44	0.00	2.46	5.44	19.15
		C3	0.43	0.21	0.54	NA	2.79	NA	6.59	106.12	23.56

13-Month	Aug.2014	C1	0.43	0.14	0.41	NA	3.76	NA	2.31	173.21	4.49
		C2	0.81	2.52	0.31	NA	5.77	0.00	0.72	45.81	4.29
		C3	10.29	1.47	0.43	NA	6.14	0.00	13.75	100.00	16.52

Table 3.3 The between channel CVs (%) of Physicochemistry factor among three control channels (C1–C3) between Month2 (September 2013) and Month 13 (August 2014). T is temperature. EC is water conductivity. DO is dissolved oxygen.

Duration	Date	T	EC	pH	DO	CO_2	CH_4	N_2O	NO_3^-	PO_4^{2-}
2-Month	Sep.2013	0.27	0.13	0.81	6.61	13.43	13.63	13.13	109.66	19.12
3-Month	Oct.2013	0.00	NA	NA	4.43	10.49	43.92	2.76	0.00	9.02
4-Month	Nov.2013	NA	NA	NA	NA	4.89	73.48	1.49	NA	NA
5-Month	Dec.2013	0.47	0.25	0.17	0.76	10.89	136.94	1.49	115.92	21.23
6-Month	Jan.2014	0.00	0.10	NA	NA	4.02	87.29	NA	51.59	10.12
7-Month	Feb.2014	1.09	1.58	0.96	5.86	6.57	56.04	9.78	31.33	11.70
8-Month	Mar.2014	2.45	0.53	1.36	1.14	6.58	71.23	7.70	102.24	48.63

9-Month	Apr.2014	0.93	1.00	2.14	1.13	13.16	21.52	5.58	NA	NA
12-Month	Jul.2014	0.00	0.11	0.73	0.44	10.94	141.42	2.95	49.53	9.10
13-Month	Aug.2014	15.32	0.84	1.31	0.79	4.09	0.00	6.47	111.27	1.57

Table 3.4 Result of redundancy analysis (RDA) of spatial and temporal variation in the physicochemistry in control channels. The significance of each model was tested using a Monte Carlo permutation test (999 permutations). Physicochemistry data were centred and ranged to (0, 1) to eliminate response variables' dimensionally homogeneous. Analyse 1 is physicochemistry temporal variation of control channels (C1, C2 and C3).

Response Variable	**Explanatory variables**	**F**	**P-value**	**% variation explained**	**Axis 1 explained**	**Axis 2 explained**
1.Physicochemistry	Duration	11.46	<0.001	83.9%	63.71%	32.89%

Table 3.5 Result of matric sores of physicochemical variables of control channels against duration correlates 2 ordination axes and centroid distance.

RDA model	Duration	RDA 1 (Centroid)	RDA 2 (Centroid)	Centroid distance (mean)
.Physicochemistry ~ Duration	2-Month	-0.63	0.14	0.0097
	5-Month	0.86	-0.05	0.0102
	7-Month	-0.17	-0.22	0.0090
	8-Month	-0.10	-0.61	0.0053
	12-Month	-0.10	0.56	0.0067
	13-Month	-0.02	0.18	0.0040

Table 3.6 Result of matric sores of physicochemical variables vector correlates 2 ordination axes, Euclidean distances and P-value for each vector. P<0.05, 0.01**, 0.001***, ns = non-significant (p > 0.05).*

Physicochemical variables	RDA1	RDA2	Euclidean distances	P-value
Temperature	0.04	0.59	0.59	0.001***
Conductivity	-0.97	0.18	0.99	0.001***
pH	0.58	-0.36	0.68	0.001***
DO	-0.21	0.12	0.24	0.001***
CO_2	0.37	0.95	1.02	0.03*
CH_4	-0.31	0.45	0.55	ns
N_2O	-0.44	0.18	0.48	ns
NO_2	-0.67	0.12	0.68	ns
PO_4	-0.50	-0.37	0.62	0.005***

Table 3.7 Result of Pearson's product-moment correlation of physicochemical variables among 3 control channels between Month 2 to Month 13. P<0.05, 0.01**, 0.001***, ns = non-significant (p > 0.05).*

Physicochemical variables	C1-C2	C1-C3	C2-C3
Temperature	0.96***	0.96***	0.85***
Conductivity	0.99***	0.99***	0.99***
pH	0.89**	0.92**	0.79*
DO	0.86*	0.93**	0.98***
CO_2	0.87**	0.39^{ns}	0.65*
CH_4	ns	ns	ns
N_2O	0.93***	0.92**	0.85**
NO_2	0.42^{ns}	0.5^{ns}	0.88**
PO_4	0.90**	0.78*	0.83*

CHAPTER 4

Spatial and Temporal Variation of Biological Parameters in Replicated Outdoor Stream Mesocosms

4.1 Summary

1. The previous chapter has been examined that water physicochemistry highly replicate in flumes. This chapter focus on the mesocosms macroinvertebrate community replicability. As the experimental object, to investigate individual taxa and mesocosms community variability is required before treatment apply. Due to the biological feature of benthos (e.g. life cycle shift), the replicability examination is required to distinguish treatment impact from its own variability (i.e. seasonality). The flumes as an experimental environment and living environment for mesocosm community, both spatial and temporal replicate are both checked.
2. The macroinvertebrate community replicated highly in this large-size lotic mesocosm spatially and temporally. The macroinvertebrate taxa richness shows a distinct up-stream and down-stream distribution, the rare taxa group favour head pool. But the macroinvertebrate community density distribution is controlled by dominate macroinvertebrate taxa groups. Hence, the within- and between-channel replicability are high in this lotic mesocosms.
3. The similar water physicochemistry developed the macroinvertebrate community replicability. Flume design and experiment initial set-up reduced the heterogeneity of each flume at start point. Within-channel and between- channel replicate design guarantee the longitude connectivity of lotic ecosystem and also reduced spatial variability.
4. There are some development of the semi-controlled outdoor mesocosm study should be made in the future, including sampling strategy, artificial channel design and experiment running maintained etc.

4.2 Introduction

Stream mesocosms are valuable tools to investigate environmental stressors threaten macroinvertebrate communities in running waters (Cardinale et al, 2002; Battin et al, 2003; Harris et al., 2007). Many previous mesocosm studies using macroinvertebrates were undertaken at relatively small spatial scales (< 1 m^3, Boyle & Fairchild, 1997; Stewart et al., 2013) and typically for single-species chemical toxicity testing (Cuppen et al., 2000; Liston et al., 2008; Wagenhoff et al., 2012; Cadmus et al., 2018) or ecological investigations of biodiversity-ecosystem function relationships (e.g. shredder breakdown of leaf litter, Teube, 1991; Araujo et al., 2004; MacNeil et al. 2010). However, those mesocosm experiments simplified the interaction among physical, chemical and biological condition of natural freshwater ecosystem which means the response of assemblages does not corresponded with natural ecosystems (Petersen & Englund, 2005). More recently, mesocosms experiments have used more complex assemblages of macroinvertebrates, incorporating multiple taxonomic and functional groups, to test the effect of environmental multiple stressors at the community and ecosystem level (Clements, 2004; Connolly et al., 2004; Ormerod et al., 2010). In order to detect stressor responses, mesocosm units must be highly replicable, with low inter-unit variation. However, the question remains as to whether complex, semi-realistic assemblages can be adequately replicated in outdoor flume facilities, and little is known about the replicate behaviour of individual mesocosm units in space and time (Gisesy & Allred, 1985; Stewart et al., 2013). In this chapter, this gap in knowledge is examined for a flume facility located in southern England on the banks of the River Itchen in Hampshire, with a particular focus on lotic macroinvertebrates characteristic of chalk stream systems.

Although whole ecosystem manipulations would arguably be a better way to investigate consequences of environmental change in fresh waters, low replicability and lack of control can confuse the detection of impact (Schindler, 1998; Petersen & Englund, 2005). Hence, mesocosms have become popular as mimics of natural environments that can be highly replicated, forming a bridge between the realism of real ecosystems and the reproducibility of laboratory experiments (Harris et al., 2007; Stewart et al., 2013). Macroinvertebrate are a useful biological indicator, macroinvertebrate have short life cycle, and responsed to impact immediately, days to months long experiments are enough to investigate the change of biological indicator variation (Muñoz et al., 2018). Additionally, the study of higher trophic level (e.g. macroinvertebrate community) is required recently. Hence, due to the high trophic level of mesocosm macroinvertebrate community, mesocosm studies have been widely used in ecological studies (Shin-ichiro et al., 2009; Woodward et al., 2010; YVON-DUROCHER et al., 2011).

Previous studies have demonstrated that macroinvertebrate community structure in stream mesocosms depends on a host of factors, including artificial channel size and duration of community establishment (Schindler, 1998; Stewart et al., 2013). Developing container / flume size develops mesocosms biological complexity directly. Large size container / channel large (> 1 m^3, Beermann et al., 2017; Folegot et al., 2018) allows introduce more speces of macroinvertebrates to the mesocosms. A review of published mesocosm studies suggests that artificial streams established over long time frames (> 3 months, Van den Brink et al., 1996) contain more species than those that are smaller and younger (Heckmann & Friberg, 2005) and may therefore be the most realistic experimental environments for ecological studies. Additionally, stream mesocosm facilities can be realistic when they use local water sources to supply flow and colonists

to mesocosm units (Ledger et al., 2006; Harris et al., 2007). Large outdoor flume facilities can maximise this realism, yielding physical, chemical and biological characteristics more similar to natural streams and some indoor facilities. In the study of Heckmann & Friberg (2005), mesocosm facility as an in-stream artificial channels colonized majority taxa (89%) of the local river. In these systems, natural colonisation can also occur from the regional species pool, and local weather and climate influence water physiochemistry. Mesohabitats can be created that mimic those of headwater streams and these are critical in shaping mesocosm biodiversity. Thus, outdoor flumes have the potential to be relatively realistic locations to study aquatic ecology (Caquet et al., 2000; Stewart et al., 2013). Because the heterogeneity of each individual mesocosms facility, outdoor mesocosm in particular (i.e. container size, water physiochemistry, regional pattern etc.), the biological complexity of mesocosms vary. Hence, the biological replicability of large – scale and long-term mesocosm study is required.

Replicability is a critical condition to examine the ability of mesocosm arrays to detect treatment effects. Highly replicable mesocosms can be used to control key variables in experimentation (Petersen & Englund, 2005). High replicability can be defined as a limited degree of variation of physicochemistry (had been addressed in **Chapter 3**) and/or biological variables between experimental units (Kraufvelin, 1998; 1999; Caquet et al., 2001). There are two main reasons why it is necessary to investigate replicability of SCOM. On one hand, SCOM can be impacted by local site-specific physical, chemical, weather conditions, such that the replicability of each SCOM varies with microscale conditions. On the other hand, the initial physicochemical and biological condition of outdoor mesocosms should be considered as a factor that may shape system characteristics (Boyle et al., 1997) such that small differences in initial conditions during

mesocosm establishment may magnify over time across the mesocosm array. Although stream mesocosms are used routinely by freshwater ecologists, studies assessing the utility of these systems in terms of their replicate behaviour remain scarce (Harris et al., 2007; Brown et al.,2011).

In this study, the mesocosm facility was developed from the basis of Harris's (2007) study. The set-up of this mesocosm facility contained both within-channel (among riffle and pool patches) and between-channel elements. Many stream mesocosms can potentially have a strong upstream to downstream shift in habitat and water quality, and this may strongly influence macroinvertebrate distribution among patches falling along the length of each mesocosm channel (Boulton, 2003; Lake, 2011). Since each mesocosm sits adjacent to its neighbours in an outdoor setting, factors such as shading and shelter, as well as position within the water distribution network, can all affect conditions of individual mesocosm units. This in turn can create patchiness in macroinvertebrate assemblages across the whole mesocosm array (Boulton, 2003). Here, I will assess both within- and between-mesocosm variation in macroinvertebrate assemblages across the facility based at Fobdown Farm in Hampshire (Aspin et al. 2018; 2019).

The temporal variation of macroinvertebrate community composition and structure under long-term impact is another factor to examine in replicability investigation in SCOM. The high replicability of macroinvertebrate community in mesocosms also means mesocosms as a container is capable to maintain a steady high trophic level of macroinvertebrate (e.g. Community level) for the following experiment. Statistically, to establish a realisable cause-effect relationship, a high temporal replicability is required in mesocosm studies (Giesy & Allred, 1985; Kraufvelin, 1998; 1999).

This chapter assesses the replicability of stream mesocosms located at a watercress farm in Alresford, Hampshire, U.K. the array was used to conduct a drought experiment over 12 months. Before the drought treatment was applied the array was established for a period of six months. Here the replicability of these relatively young macroinvertebrate assemblages (n=19 mesocosm units) is assessed, alongside data collected from a subset of untreated control mesocosms (n=3 units) sampled repeatedly during the drought experiment itself. The following hypotheses were tested:

H1: Given the variability in longitude connection across the channel gradient (i.e. high velocity near the inlet and slower more depositional conditions at the outlet) a distinct upstream - downstream gradient in macroinvertebrate community composition will be apparent;

H2: Macroinvertebrate abundance, diversity and community structure will be highly replicable between channels;

H3: The macroinvertebrate community structure at the experimental start point and endpoint will be similar. The variation of the macroinvertebrate community structure will be explained as temporal biological variation.

4.3 Materials and methods

4.3.1 Sample collection and processing

To compare the benthic macroinvertebrate assemblages across the mesocosm array, replicate channels (n=19 i.e. excepting channels 4, 10 due to sample loss, channel 10 is control channel) were sampled before any drought treatment was applied, in August 2013 (aka month 1). The whole mesocosm array (n=21 channels) was sampled again, after one year of simulated drought (August 2014, month 13), to illustrate the final temporal and

spatial variation of macroinvertebrate community composition. Untreated control channels (n=3) were also sampled repeatedly (4 sampling occasions) between August 2013 - August 2014. At month 1, the treatments haven't been applied in the mesocosm. Hence, at month 1, there were four surber samples taking from each channel (4 × 19 = 76). In next three sampling occasions (month 3, month 6 and month 13), there were four surber samples taking from each of three control channels (4 ×3 ×3 = 36). Thus, during the experiment, 112 surber samples were collected.

On each sampling occasion, benthic macroinvertebrates were collected using a Surber sampler (0.0225 m^2, mesh size 300 μm), with four replicate samples were collected from each channel (one per pool a, b, c, d; see **chapter 2** for overview of the experimental design). All macroinvertebrate samples were immediately preserved in 70% industrial methylated spirit in the field. In the laboratory, macroinvertebrates were sorted, identified to the lowest feasible taxonomic level (species or genus, excepting Oligochaeta) and counted. Richness, abundance and density (individuals per m^2) were average by 4 samples for each artificial channel. The spatial variation of taxa composition along series pool were provided for each mesocosm.

4.3.2 Statistical analysis

To determine the replicability of macroinvertebrate assemblages in model stream is to investigate the spatial and temporal variation. Spatial variation was represented by the within-channel and between-channel variation of macroinvertebrate assemblages in 19 channels in month 1. Within-channel variation is used to check the spatial variation along channel length. Between- channel variation is used to check the spatial replicability of channel distribution. The temporal variation of this mesocosm facility was represented

by the variability of macroinvertebrate assemblage structure in control channels over the course of a year. Both within-channel and between-channel biological indicators are calculated separately. Macroinvertebrate were sign into different taxonomic group, based on their ecological function. Macroinvertebrate distributions were assessed for nine taxonomic groups, specifically Amphipoda & Isopoda, Chironomidae, Oligochaeta, Gastropoda, Tricladida, EPT (Ephemeroptera, Plecoptera and Trichoptera), Hirudinea, Coleoptera, Chironomidae and Other Diptera (excepting Chironomidae).

Spatial replicability analysis

Within–channel replicability is assessed as variation in assemblage structure of each specific pool position (each of pool a, b, c, d) between channels. The richness and density data of each pool position were averaged by 19 channels at start point (month 1). One-way ANOVA was used to test differences of four pool position along channel length to determine whether there was an effect of pool position on assemblage structure. The significance level of one-way ANOVA was set to $p\text{-}value < 0.05$.

Between-channel replicability is addressed as variation in assemblage structure between 19 channels. Macroinvertebrate community composition, taxonomic richness and density in each channel were calculated for four surber samples. The coefficient of variation (CV), is a statistical technique to quantify variability. It is the ratio of the standard deviation to the mean. CVs of macroinvertebrate density in each pool were calculated for each channel in month 1 (Kraufvelin, 1998; Ippolito et al., 2012). Species accumulation curves were also constructed to compare assemblage richness among the 19 channels in month 1. For statistic purpose, the species accumulation curve is used to examine the cumulative effort of macroinvertebrates in each flume.

Temporal replicability analysis

Ordination was used to examine the temporal variation in macroinvertebrate composition during the main study period. The macroinvertebrate samples have been taken in 4 sampling occasions. In month 1 (Aug. 2013), macroinvertebrate sample has been taken for 19 channels before water depth treatments applied (see **Chapter 2**, experimental set-up). The macroinvertebrate abundance data were *log10 (X+1)* transformed to ensure normality. Ordination analysis, specifically non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity (Krraufvelin 1999; Datry et al., 2016), was used to analysis macroinvertebrate community composition and structure along at each sampling occasions (package: vegan 2.4-4, Oksanen, 2017). The Spearman's correlation between each taxa abundance and scores for the first two ordination axes was also calculated. Between-channel replicability at each sampling occasion was also examined by convex hull area variation. Smaller convex hull area means lower macroinvertebrate variability between control channels, which demonstrate limited spatial variability of macroinvertebrate community during long-term experiment period. Due to there was no treatment applying at 19 channels at month 1, all 19 channels can be recognized as control channels. In order to compare the temporal variation convex hull area between three channels, the convex hull between three channels of month 1 was randomly selected (three out of 19) and calculated 20 times. A one-way ANOVA was used to analysis the variation of convex hull area along the study period.

Control channel macroinvertebrate community analysis

The spatial variation is represented as within- channel and between-channel CVs. Both within-channel and between –channel CVs of density in each control channel on each sampling occasion were also calculated separately (Harris et al., 2007, Ledger et al., 2011).

The temporal variability of the macroinvertebrate community composition in control channels was also examined by rank- abundance curve. In addition, temporal variation in taxon richness was examined by CVs during experiment period for each replicate channel. NMDS ordination also examined the temporal variation of macroinvertebrate community in control channels. Because of the one control channel data (channel 10) missing in month 1, centroid distance was calculated in each sampling occasion instead of convex hull. Centroid distance is the mean value of the distances between the present channel points to the central point of line (two channel points) or convex hull (>three channel points). For spatial variability in control channels, the mean centroid distance is used to demonstrate the replicability between control channels, the lower value means higher replicability.

The temporal variation of individual taxa groups in controls was examined by one-way ANOVA, to figure out the significant variation with increasing experimental duration.

All analyses were done in R (Version 3.5.0, R Studio. Inc.), the package 'Vegan' (Oksanen et al., 2017) was used to run the ordination analysis, and 'BiodiversityR' (Kindt, 2018) was used to calculate rank –abundance curve. The significance level for all statistical analysis was checked by *p-value* <0.05. The CVs level was classified as CV < 30% was low.

4.4 Result

In month 1, a total of 25,845 macroinvertebrates were collected from 19 channels. According to the individual taxa abundance value in each pool, 9 taxa groups were classified as core taxa (>100 individuals, or > 30% total abundance) or non-core taxa. The core taxa groups were Amphipoda & Isopoda, Chironomidae and Oligochaeta. The second abundant groups were Gastropoda, Tricladida, EPT (Ephemeroptera, Plecoptera and Trichoptera) and Hirudinea. The least abundant groups were Coleoptera and Other Diptera (i.e. excepting Chironomidae). At the beginning of the experiment, the macroinvertebrate community was dominated by Amphipoda, Isopoda and Chironomidae, which comprised over 90% of total macroinvertebrates abundance.

4.4.1 Benthic assemblage variation between pool sections in individual channel in month 1

The macroinvertebrate community richness and density of each pool varied in month 1 (Figure 4.1). The similar pattern was found in macroinvertebrate assemblage mean richness and density of each pools. The maximum mean richness and density were found in head pool, the minimum of mean richness and density were all found in pool C (Figure 4.1, Table 4.1).

One-way ANOVA demonstrated that the there was no significant difference of mean density between each pool ($p > 0.05$, ANOVA; Table 4.2). By contrast, taxa richness was weak affected by pool position (p = 0.04, ANOVA; Table 4.2), with seven out of nine taxa groups favouring headwater pools, except Gastropoda and Hirudinea. According to those two results, it showed that there was more species in the head pool, however there was not more individual macroinvertebrate.

Pool position had no significant impact on macroinvertebrate assemblages mean density. The mean density of 9 individual taxa group within 4 pools had also been examined by one-way ANOVA. According to the result, the mean density of core groups (Amphipod/Isopoda, Chironomidae and Oligochaeta), Hirudinea and Other Diptera, were not significant different between pool positions ($p > 0.05$, ANOVA; Table 4.2; Figure 4.2). But the rest three taxa groups, Gastropoda, Tricladida and EPT were significantly influenced by pool position ($p < 0.05$, ANOVA; Table 4.2; Figure 4.2). Coleptera was weak influenced by pool postion ($p= 0.02$, ANOVA; Table 4.2; Figure 4.2). Tricladida, EPT and Coleoptera favoured head pools, where their mean density was much higher than the pools downstream (Table 4.1; Figure 4.2). The mean density of Gastropoda varied significantly, which was 7.32 times higher at the end of channel than in the head pool.

The within-channel CV (WCV) was used to analysis macroinvertebrate total density between pool position in individual channels. The mean total density WCV of 19 channels was 34.5 ± 16.7 % (range 5.0 to 76.1%, Table 4.3). The low variability (i.e. WCV < 30%) was found in eight channels, four out of this eight channels' WCV were found less than 20%. The lowest variability was found in channel 5 (CV = 5.0%). The median variability (i.e. 30% < WCV <42.1 %) were found in nine channels. The highest variability was only found in two channels, the WCV was 69.3% in channel 3, 76.1% in channel 9, respectively. Although, there was 42% channels having low WCV, the variability of macroinvertebrate total density within channel should not be ignored.

In summary, there was no significant difference of density between each pool in different channels. Although, richness and some taxa groups' abundance were impacted significantly by pool position and channel distribution. The total abundance and the core taxa groups taxa has no difference between pools. However, based on each individual

channel, the variability of macroinvertebrate community distribution along channel length should not be ignored. It suggested that macroinvertebrate community of 19 channels has limited spatial variability at beginning of experiment.

4.4.2 Benthic assemblage variation between 19 channels in month 1

In month 1, the mean richness and density of each channel were calculated by four surber samples per channel. The mean total density of macroinvertebrates across 19 channels was 15641 individuals per m^2 with a SD 5253 (range: 9445 ± 2308.32 - 35167 ± 9005.62; Table 4.3; Figure 4.3). The extremely high density was found in channel 15, with minimum density in channel 2 was roughly 27% of maximum density. However, the mean density of 58% channels (11 out of 19 channels) fell within the 95% confidence interval. The mean richness between 19 channels was 13 ± 1.65 (range: 11 ± 1.58 -16 ± 1.92, Table 4.3; Figure 4.4).

The channel richness varied significantly between channels. ($p< 0.05$, ANOVA; Table 4.5). However, the channel density and abundance had non-significant variation between channels ($p > 0.05$, ANOVA; Table 4.5). Additionally, the result of one-way ANOVA demonstrated that the channel distribution had no significant impact on the mean density of taxa groups in stream mescososms ($p > 0.05$, ANOVA, Table 4.5).

Overall, the channel distribution has weakly impact on total macroinvertebrate richness. But there was no evidence showing channel distribution has significant impact on macroinvertebrate density and abundance.

4.4.3 Benthic assemblages' composition between 19 channels in month 1

In month 1, there were total 19 macroinvertebrate taxa from 14 taxonomic orders found in 19 mesocosms. The species accumulation/ discovery curve illustrated the accumulation effort with increasing number of flumes. There was a positive relationship between taxa and flume number (slope=1.86; Figure 4.5). More than 95% cumulative number of taxa were recorded in accumulative 14 channels. Hence, 19 mesocosms were qualified to investigate macroinvertebrate community composition.

The composition of Diptera, Amphipoda & Isopoda and Oligochaeta were core taxa (> 30% total abundance or >100 individuals) whereas other taxa groups were rare (<1% total abundance) (Figure 4.6). The most abundant group were Diptera (7 species, mean: 36.3% of total abundance, range: 32.2 – 45.3%). Chironomidae (8 species) accounted for more than 98% of total Diptera. Other Diptera taxa, *Clinocera, Tipula,* and *Palpomyia* were found rare in stream mesocosms. The second abundant groups were Amphipoda (*Gammarus pulex*) and Isopoda (*Asellus aquaticus*) occupied 29.1% (range: 25.4 – 32.0%) of mean total macroinvertebrate density. The third abundant group was Oligochaeta (17.2%). Those 3 taxa groups occupied 82.6% (range: 74.8 – 94.5%) of mean total macroinvertebrate density.

The rest 5 taxa groups occupied 17.4% of mean total macroinvetbrate density. Gastropoda (2 species) occupied 6.7 % macroinvertebrate density. The last abundant group was Ephemeroptera, Plecoptera and Trichoptera (EPT, 4 species) density only accounted for less than 1% (Table 4.5; Figure 4.6). Nine taxa, *Gammarus pulex, Radix balthica, Oligochaeta, Chaetocladius dentiforceps, Macropelopia sp. Micropsectra sp., Synorthocladius semivirens* accounting for over 5% of total density in month 1.

4.4.4 Macroinvertebrate community variation from month 1 to month 13

Non-metric multidimensional scaling (NMDS) was applied to analyse the temporal variation of macroinvertebrate community composition across the 13-month study period. Four sampling occasions of the macroinvertebrate community were analysed: month 1 (August 2013; 2 control channels + 17 pre-treatment channels), month 3 (October 2013; 3 control channels), month 6 (January 2014; 3 controls) and month 13 (August 2014; 3 controls). For each sampling occasion, convex hull was calculated to demonstrate assemblage similarity between three channels, with smaller convex hull area reflecting higher spatial replicability.

The convex hull between 19 channels in month 1 (summer, Aug.2013) was 0.308. Because data of one control channel was missing. Two control channel and one of 17 pre-treatment channels was used to calculate the convex hull in month 1. The mean convex hull between 3 channels in month 1 was 0.020 ± 0.01(mean ± SD, range: 0.011 to 0.043; Table 4.6). The smallest convex hull was found in month 3 (winter, Dec.2013), 0.008. The maximum convex hull area was 0.052 in month 6 (spring, Mar.2014). The final convex hull between three control channels decreased to 0.018 (summer, Aug.2014). There was non-significant variation between four sampling occasions ($p > 0.05$, ANOVA) demonstrating that the spatial replicability between the three control channels was high at each sampling occasions (Table 4.6; Figure 4.7).

The ordination also demonstrated the temporal variability of macroinvertebrate community in stream flumes. The replicability among three control channels was high. However, the macroinvertebrate community structure in flumes varied with each sampling occasion. The temporal variability of macroinvertebrate community was negatively correlated to axis 1 ($\rho = -0.81$, $p < 0.05$, Spearman's correlation test; score = -0.59; Figure 4.6, Non-metric fit $R^2 = 0.97$, Linear fit $R^2 = 0.89$). The macroinvertebrate

community changed with increasing experiment period, the most dissimilarity from month 1 was found in month 6. Then the macroinvertebrate community dissimilarity decreased, the community in month 13 was more similar as initial condition (Figure 4.6). According to the level of Spearman's correlation coefficient, there were 20 macroinvertebrate taxa were high correlated to axis 1 (ρ <-0.4 or >0.4, $p< 0.05$; Table 4.7). Although, the less dissimilarity of macroinvertebrate community was found between month 1 and month 13, the composition of macroinvertebrate changed. During the experimental period, *Gammarus pulex* correlated with axis 1 positively, reflecting decreasing density over time. and this was in contrast to the pattern for *Asellus aquaticus* increased during the 13-month experiment (Table 4.7). The total abundance of Amphipod/Isopoda decreased to month 6 then rebounded (Figure 4.10). However, *Gammarus pulex* was replaced by *Asellus aquaticus,* which explained that the macroinvertebrate community in month 13 (summer, Aug.2014) was different from the macroinvertebrate community in month 1 (summer, Aug.2013).

Diptera (including Chironomidae) correlated positively with axis 1 of the ordination (Table 4.7). Although, other groups (e.g. EPT) were highly correlated with axis 1 positively, those groups were rare in the macroinvertebrate community during whole study period. It suggested that the variation of macroinvertebrate community was driven by core taxa, like Amphipod/Isopoda and Diptera.

The seasonal pattern of macroinvertebrate community was also presented by the convex hull position variation. The positon of convex hull in month 1 (NMDS1=0.3) moved along axis 1 negatively to NMDS1= - 0.8 in month 6 (summer 2013 to winter 2014). Then the community convex hull moved along axis 1 positively, to NMDS1 = -0.4 in month 13, which means the macroinvertebrate community in month 13 (summer

2014) became more similar to the macroinvertebrate community at start point (Figure 4.7). The NMDS ordination result demonstrated that the macroinvertebrate community composition is influenced by experimental duration, which suggested that benthic community biological variability was associated with regional seasonality.

4.4.5 Macroinvertebrate community temporal variation in 3 control channels

Rank abundance of taxa was calculated using mean abundance of four replicate samples in each control channel to compare among between control channels at each sampling time (Figure 4.9). According to the rank abundance curve, approximately six taxa accumulate for over 90 % of macroinvertebrate community abundance in each control channel at each sampling occasion (August 2013, October 2013, January 2014, and August 2014). The maximum overall density was found in C3, 93.0±35.8 (mean ± SD, individuals per m^2) in month 1 (Table 4.8). A trend of decreasing total macroinvertebrate density was observed over the experimental period, with a minimum density was found in C2, 5.2±4.1 (individuals per m^2) in month 6 (Table 4.8), then increased back at end of experiment. In month 1 and month 6, the mean CVs of between-channels (BCV) were 18.8% and 17.6%, respectively, which demonstrated that spatial variation between channels was low (CV<20%). In month 3 and month 13, the BCV were 37.3% and 36.1%, respectively, which means the spatial variation in those two months were low-median (20% < CV< 40%). However, the mean CVs of within-channels (WCV) was higher than the mean CVs of between-channels (BCV) in each sampling occasion, except month 3 (Table 4.8). Macroinvertebrate community in control channels varied within pools during the main study period.

The CV of taxon richness for 19 taxa in control channels has also been calculated at each sampling occasion (Table 4.9). In month 1, the core taxa (Amphipoda, Isopoda, Oligochaeta and Chironomid) variation between channels was low (CV < 20%), whereas rare taxa had high CVs (e.g. CV of Ephemeroptera = 100%; Table 4.9). A similar pattern was found in month 3. In month 6, the taxa richness of Chirononmidae decreased (CV =61.2%) but then increased back in month 13. The rest taxa CVs was relatively high. The highest CVs were found in Coleoptera (rang: 100- 141.4%) and Ephemeroptera (range: 70.7- 141.4%). However, they were rare taxa and low density, which suggested that a small change of taxa richness caused the rise of CVs (Table 4.9). The variation of taxa richness of core taxa group was stable during experiment period, but the rare taxa varied.

In order to examine the temporal variability between three control channels, the NMDS ordination analysis was used and mean centroid distance was also explored. The centroid distance among controls increased from 0.13 in month 1 to 0.29 in month 6 then decreased to 0.20 at the experimental end point (Table 4.10). Additionally, the centroid distance varied little in January over 3 years (mean 0.26 ± 0.04, range 0.21 to 0.29), which demonstrated that the replicability of macroinvertebrate community between 3 control channels was maintained during long-term (e.g. >1-year) experimental duration. The temporal variability of macroinvertebrate community in the same season was limited in 3 control channels.

The taxonomic composition of macroinvertebrate community in controls changed with season (Figure 4.11). In month 1, the abundant taxa groups were Chironomidae (39.8%), Gammurs/Asellus (25.9%) and Oligochaeta (23.3%). The portion of Chironomidae and Oligochaeta decresesed to 4.9% and 9.8% in month 6 (Jan. 2014), respectively. Then two taxa groups increased back to 27.6% (Chironomidae) and 39.5%

(Oligochaeta) in following summer (Aug. 2014). The trend for Garmmurs/ Asellus was different. It increased from 25.9% (month 1) to 48.8% (month 6) then deceased to 17.1% at endpoint. But higher proportions of EPT, other Diptera and other taxa were found at endpoint. Gastropoda disappeared in control channels after 13- month running.

It showed a strong temporal shift of macroinvertebrate composition during 13-month study period. Although, three abundant taxa groups varied with increasing experimental duration, the proportion of three abundant taxa groups, Gammarus/Asellus, Chironomidae and Oligochaeta (total proportion: 84.32%) at start point was as similar as community composition (total proportion: 99.15%) at endpoint.

However, the mean density of two abundant taxa groups, Gammurs/ Asellus and Chironomidae, decreased sharply during experimantal duration. The mean density (individual per m^2) of Gammurs/ Asellus decreased to 34.5% of the mean density in month 1. The similar pattern was found in Chironomidea, which decreased to 36.2% of the mean density in month 1 (Table 4.11). a mean density of Oligochaeta at endpoint was as similar as in month 1 (Table.4.11). According to one-way ANOVA result, there was no significant temporal variation found in each taxa group during main study period ($p>0.05$, ANOVA; Table 4.12).

Overall, in 13-month experiment, the spatial variation of macroinvertebrate assemblages between three control channels was limited at each sampling occasions. However, pool positions have impact on macroinvertebrate richness distribution in individual channels. The temporal variation of macroinvertebrate in control channels was associated with seasonality. Additionally, the rare macroinvertebrate density varied greatly during long-term study.

4.5 Discussion

4.5.1 The replicability in spatial scale

The hypotheses (H1and H2) has been proved. Despite macroinvertebrate taxa pattern was weakly influenced by pools position, macroinvertebrate community present high spatial replicability during the study period.

Although, the water physical and chemical condition is high replicability (see **Chapter 3**), the macroinvertebrate taxa pattern might be affected by experimental channel design, habitat heterogeneity (i.e. sediment accumulation, Wagenhoff et al., 2012), flow alternation (Jones et al., 2015) and water physiochemistry etc.

The channel design could affect macroinvertebrate behaviours. As an experimental tool, macroinvertebrate community is also affected by flumes physical property and flow fluid mechanics. The higher density of macroinvertebrate was found in end pool, which might be influenced by the wall effect of mesocosm channel (Plaut, 2001). Wall effect may provide rich food resource and relative stable environment (e.g. low flow velocity) which attracts macroinvertebrates. Additionally, macroinvertebrates were scoured by the water flow in lotic system, so macroinvertebrate may accumulate in the end of channel. Hence, with increasing the experimental duration, the wall effect should not be ignored in mesocosms ecosystem.

At the beginning of experiment, the experimental set-up eliminated the difference between channels physicochemical (i.e. same source water) and biological feature (i.e. same amount macroinvertebrate colonization, see **Chapter 2**) initially, but the increasing experimental duration might establish individual channel habitat heterogeneity. In this semi-controlled outdoor mesocosm, habitat condition was not controlled completely by researchers except water depth treatment. There was same amount of *Ranunculus* was transferred to each pool of each channel. However, with increasing experimental duration,

the macrophyte was found in the pool b and pool c of channels (e.g. channel 14 and 15). Additionally, due to enter water pressure (pool a) and wall effect (pool d), the *Ranunculus* in pool a was less than in pool d. It suggested that the macrophyte in channel developed habitat condition, such as reduced flow velocity, increased fine sediment deposit and increased canopy, which might cause significant ecological effect, such as relatively high macroinvertebrate density in pool b and pool c (Gregg & Rose, 1984; Wright, 1992; Harrison et al., 2004).

Water physicochemical feature also affect the macroinvertebrate taxa pattern. Although **Chapter 3** has been explained that there was no significant difference of physiochemistry between pools. The water physiochemistry of head pool is better than the other pools, such as the more stable temperature regime and relative high DO concentration, which attracts more macroinvertebrates.

From this study, it found the macroinvertebrate in head pool and end pool is more variability in mesocosms, which suggest that the lotic freshwater mesocosm must set up within – channel replicates. The future sampling strategy in lotic mesocosm should depend on the objective of study. For instance, in the chemical impact study, it suggests that the sampling strategy should avoid collecting macroinvertebrate sample in head and end experimental unit. In macroinvertebrate community composition / structure study, the sampling strategy should collect macroinvertebrate sample in every replicate unit to guarantee that every species is account for. Furthermore, the macroinvertebrate sample should be collected in a similar position in each experimental unit to reduce the habitat heterogeneity impact and promote comparability of macroinvertebrates samples. Actually, this within -channel spatial variation didn't reduce the entire mesocosm system

replicability, it represented the longitudinal nature of lotic ecosystem in artificial channels.

On the other hand. the individual channel variability may increase with increasing experimental duration, which suggests that the between - channel replicates are required in lotic mesocosms to reduce the spatial variability statistically. Furthermore, artificial channel should be maintained regularly. Regular maintain work may reduce the habitat heterogeneity, such as removing extra macrophytes in channels to keep similar sustains condition during study period.

In summary, within -channel and between channel replicates experimental design of this facility maintains the natural feature of lotic ecosystem and shows a high spatial replicability.

4.5.2 The replicability in temporal scale

The hypotheses (H3) was that the macroinvertebrate community in artificial channels was driven by regional seasonality. It suggests that the temporal variation of macroinvertebrate community structure is the factor overriding any stochastic processes operating at the channel level (Townsend, 1986). The macroinvertebrate community in mesocosm is influenced by regional seasonal pattern, which is similar to the macroinvertebrate community living in the stream nearby (Caquet et al., 1996; Harries et al.,2007; Ledger et al., 2009). The temporal dynamic is the important foundation to maintain and develop the similarity between control channels increased during long-term experimental duration. Hence, to monitor the surrounding area physical and chemical condition, such as atmospheris temperature, water physicochemical condition is

necessary in stream mesocosms study, which is using to exclude non-experimental treatment impact and develop precise treatment consequences (Caquet et al., 2000).

Although, the variation of macroinvertebrate community composition obeyed the temporal pattern at family level, the abundant group of Gammurs/Asellus composition changed. Welton (1979) found that the density of *Gammarus pulex* started to reproduce from June and July and may reach the maximum in September in natural chalk stream, Dorset, UK. However, *Gammarus pulex* is sensitive to habitat conditions (e.g. *Ranunculus* or *Callitriche* in habitat; Welton, 1979) and water quality (e.g. water temperature; Welton & Clarke,1980). Furthermore, *Asellus aquaticus* has higher ability to adapt to lower water quality than *Gammarus pulex* (Edwards & Learner, 1960).

Additionally, predation behaviour was another reason that macroinvertebrate community varied. There were 7 bullhead (Cottus gobio) fish transferring to each channel at beginning of experiment (see **Chapter 2).** According to the previous studies, it found bullhead has various macroinvertebrate prey items, such as amphipods, mayfly, stonefly, caddis larvae, and blackfly larvae etc.. (Dahl, 1998; Olson et al., 2003; Meier et al., 2015). For macroinvertebrate core taxa group in this mesocosms, G. *pulex* as one of the important prey items for bullhead, bullhead could reduce *G.pulex* density effectively (Dahl,1998;Macneil et al., 1999; Crisp et al.,2004). For the rare taxa group in this mesocosm, with increasing bullhead prey behaviour might reduce some taxa completely. For instance, Ephemeroptera was totally reduced in two control channels at endpoint. Nevertheless, taxa distribution pattern might be affected by bullhead (Muotka et al., 1999; Fleituch & Amirowicz, 2005; Harrison et al., 2005). Bullhead distribution associated with the high abundance of benthos, which could explain that pool a has high richness, but pool c has high macroinvertebrate density in experimental channels.

The temporal variability of macroinvertebrate community was mainly explained as regional temporal dynamic, which is un-avoided. The other factors (e.g. predation) might also effect macroinvertebrate community structure, but it didn't reduce the mesocosms temporal replicability. The temporal variation of macroinvertebrate community in long-term mesocosm study should be monitored as a foundation to distinguish the treatment impact from natural impact.

4.5.3 Compared to previous studies

Compared to the mesocosm facility in Harris et al., (2007) study (127 taxa from 15 taxonomic orders), this mesocosm contained relatively lower taxa richness macroinvertebrate (maximum richness: 19 taxa from 14 taxonomic orders). In Harris et al., (2007) study, the mesocosm facility used water resource from natural river directly, which supplied macroinvertebrates persistently. The mesocosms in this study introduced the same amount (6 surber samples) of macroinvertebrates to each channel and abstracted groundwater to feed the artificial channels, the macroinvertebrate community in mesocosm could be recognized as offspring of the initial kick-sample from nearby River Itchen. Due to lack of taxa in this mesocosms, the realism is less in this mesocosm. However, this experimental facility is designed to investigate water volume to affect ecosystem function and structure. Sustained macroinvertebrate supply from a river could confuse the consequence caused by drought condition. Hence, the experimental design and set-up strongly relate to experimental aims.

4.5.4 Mesocosm studies in further research

Chapter 3 and **Chapter 4** examined the physical, chemical and biological functions of the mesocosms, which proved that this mesocosms was capable to maintain the relative realism freshwater ecosystem for a long-term (> 1-year) and suitable for the following experiments. However, there were some problems showing in this experimental facility during main study duration. Firstly, the water management should strengthen. For instance, in order to detect the pump stop incident, water velocity should be monitored. Secondly, the channel design could be improved. For example, the water entrance of channel might be improved to reduce the water enter pressure to affect the first riffle-pool habitat features. Thirdly, the plant investigation should process in future mesocosm study which could provide more information to investigate the physicochemical and biological variation in mesocosms. last but not least, the channel maintain work is especial in long-term study, to reduce the interference factor in experiment (e.g. unexpected macrophyte).

Overall, this stream mesocosms provided a high trophic level biology and high replicability experimental environment for drying experiment.

4.6 Reference

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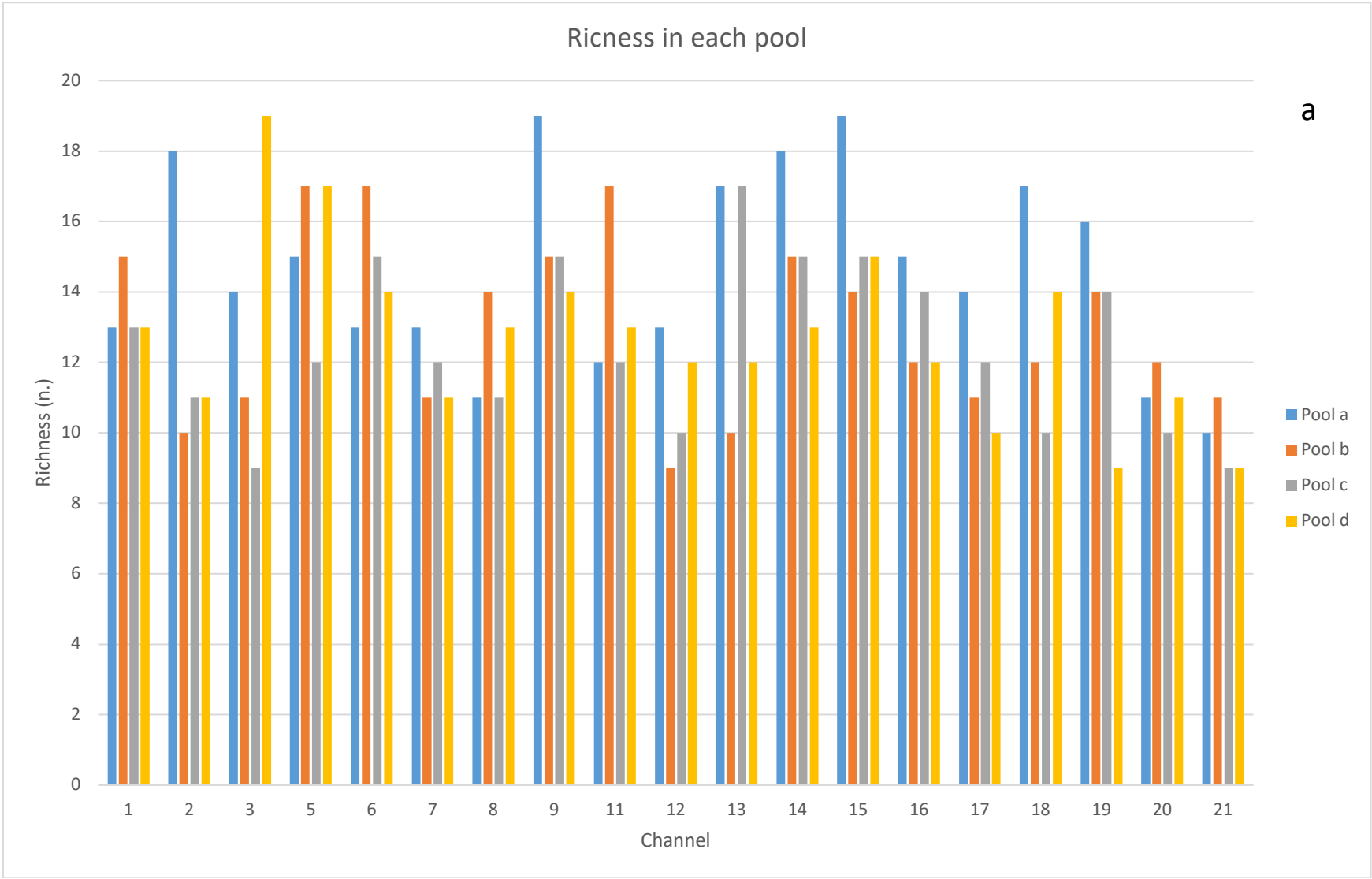
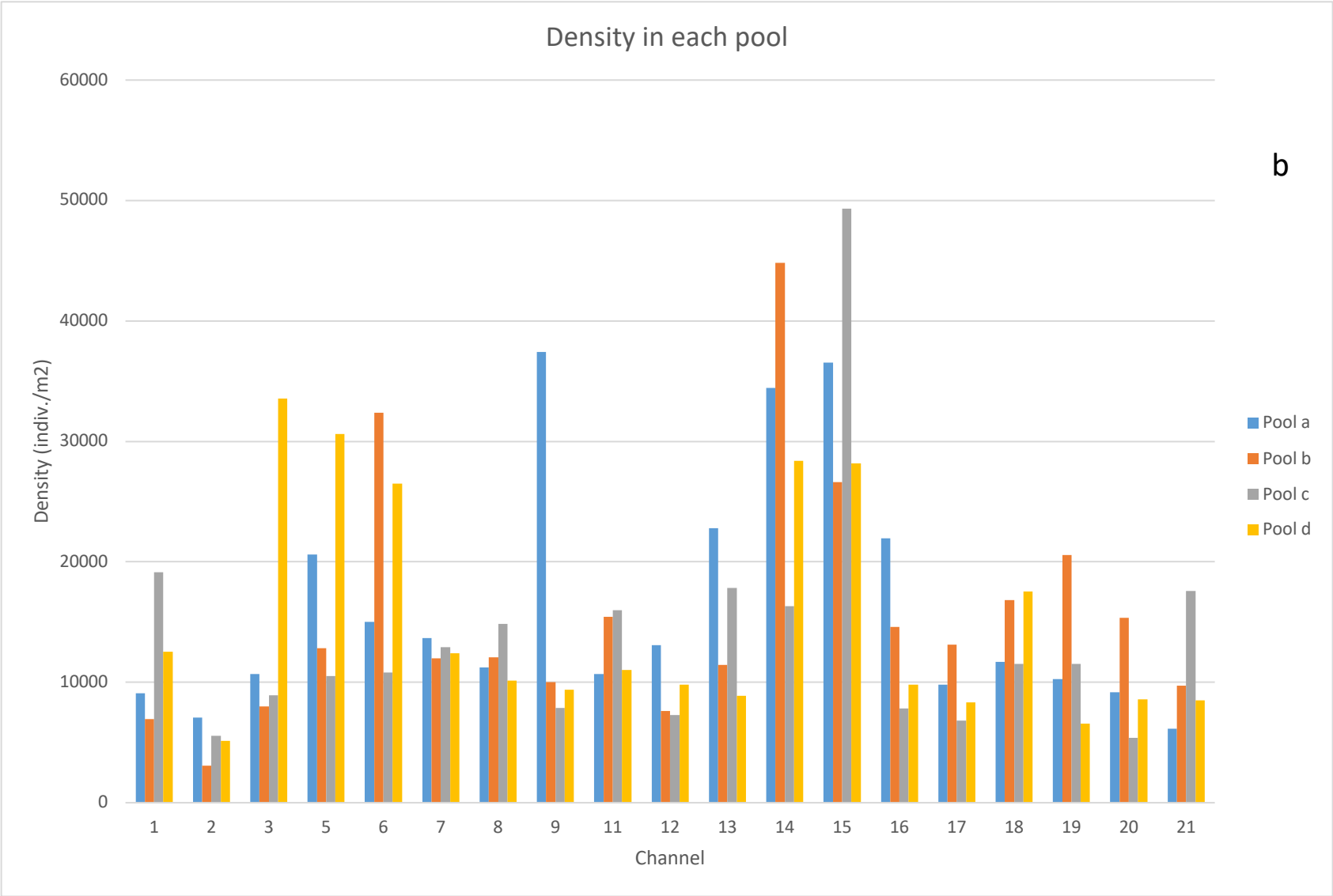

Ricness in each pool
a
Richness (n.)
Channel
0
2
4
6
8
10
12
14
16
18
20
1
2
3
5
6
7
8
9
11
12
13
14
15
16
17
18
19
20
21
Pool a
Pool b
Pool c
Pool d


Density in each pool
60000
50000
40000
30000
20000
10000
0
Density (indiv./m2)
1
2
3
5
6
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8
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15
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17
18
19
20
21
Channel
Pool a
Pool b
Pool c
Pool d

b

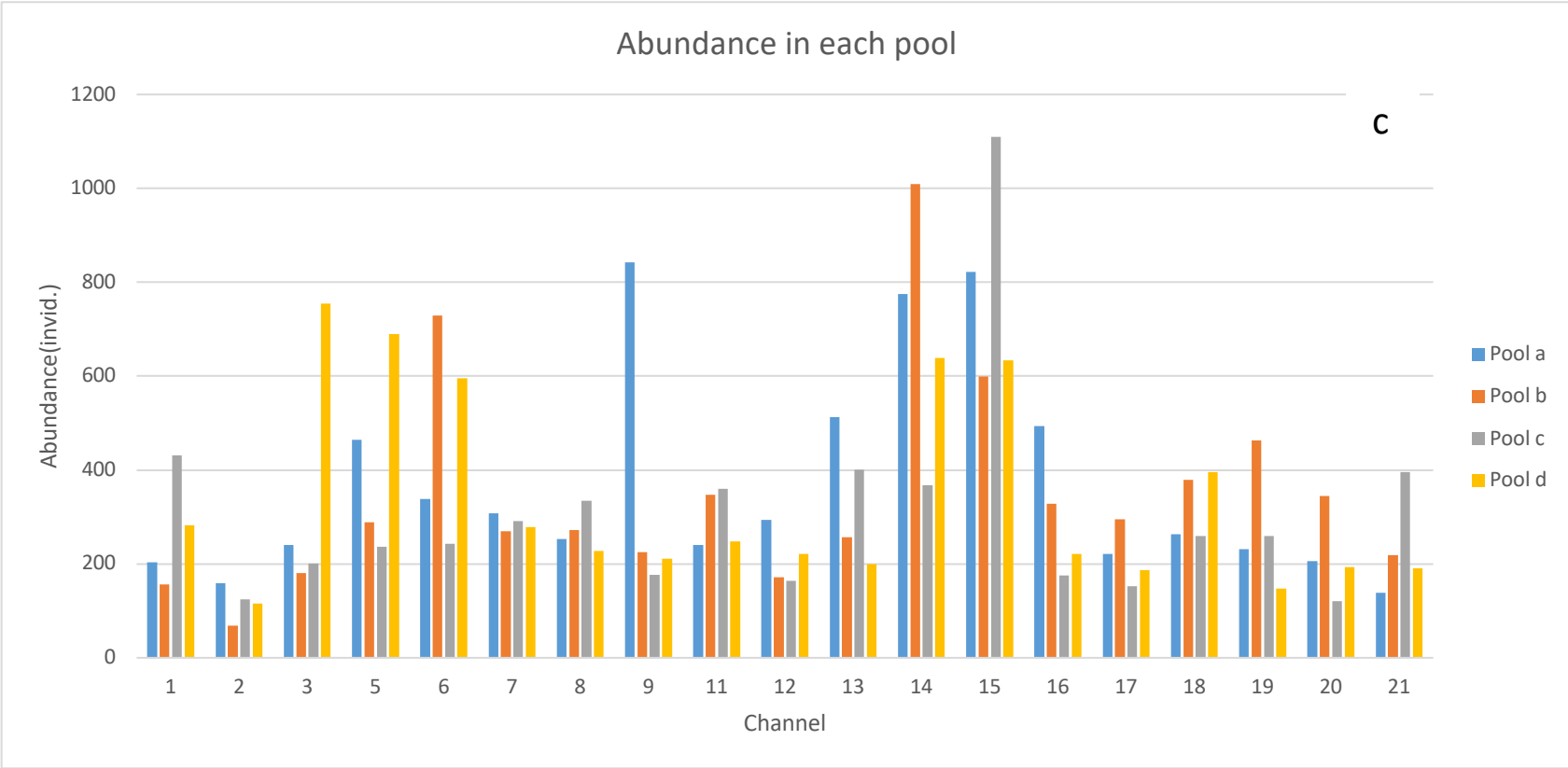

Figure 4.1: Macroinvertebrate community richness(a), density(b) and abundance(c) variation in same pool between channels in Month 1 (August 20 13). For each pool, 19 surber sample were analysed.

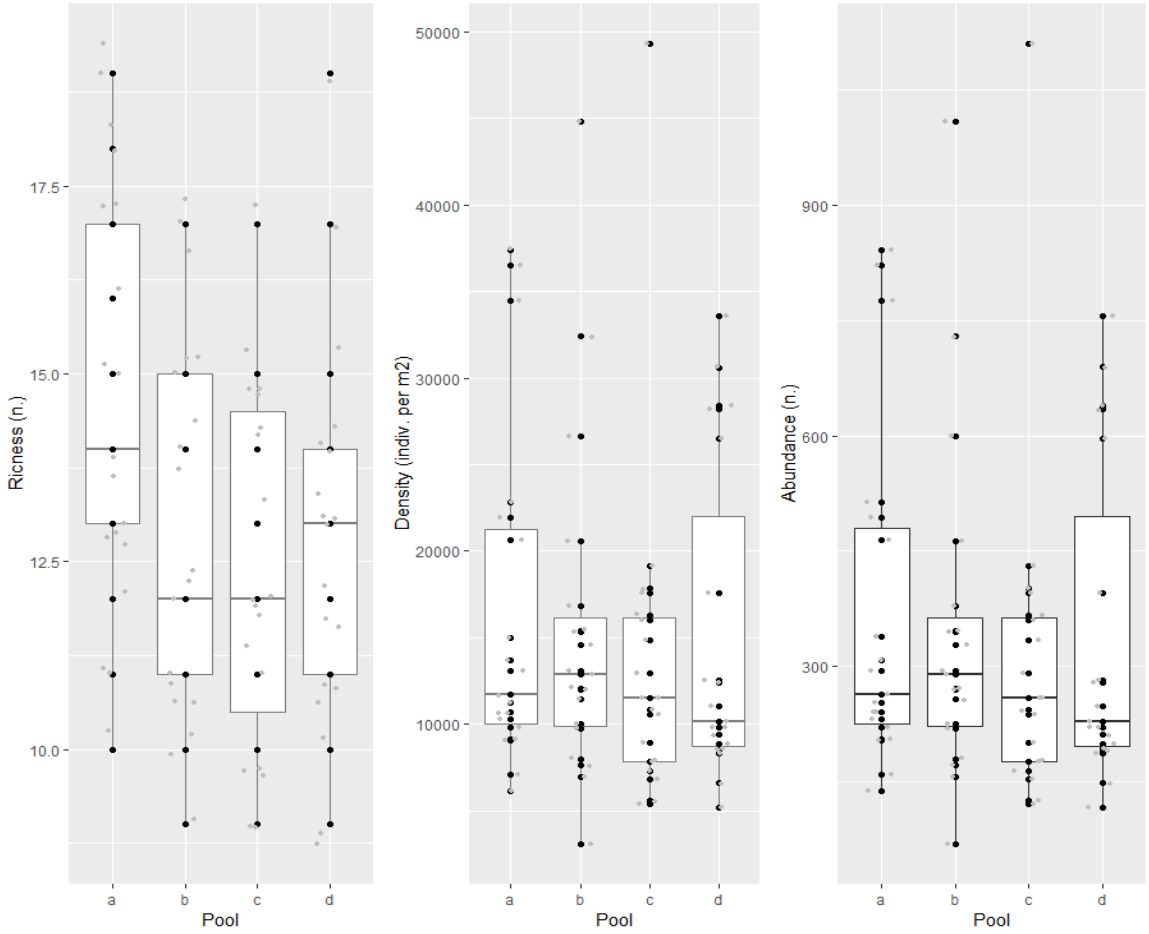

Figure 4.2: Macroinvertebrate community richness, density and abundance variation along channel gradient in Month 1 (August 2013). For each pool, 19 surber samples were analysed.

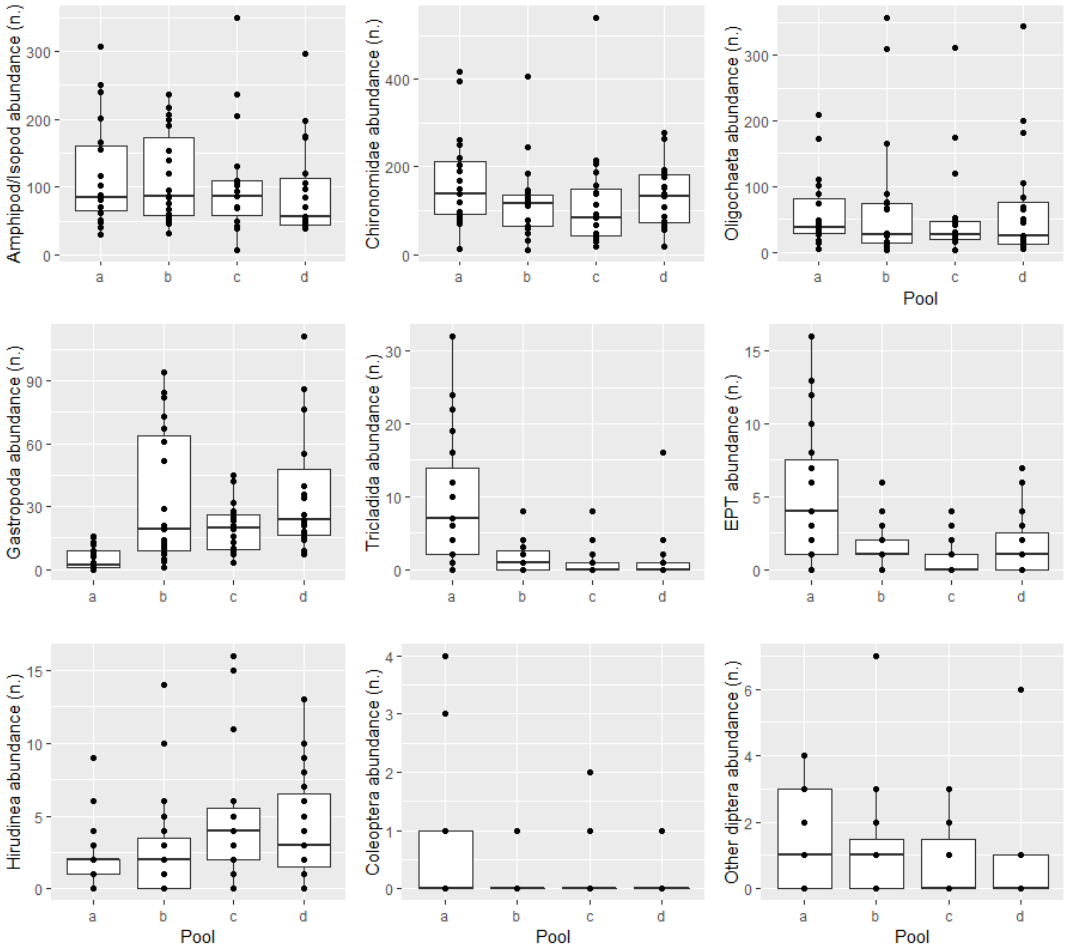

Figure 4.3: Macroinvertebrate composition variation along channel gradient in Month 1 (August 2013). For each pool, 19 surber samples were analysed.

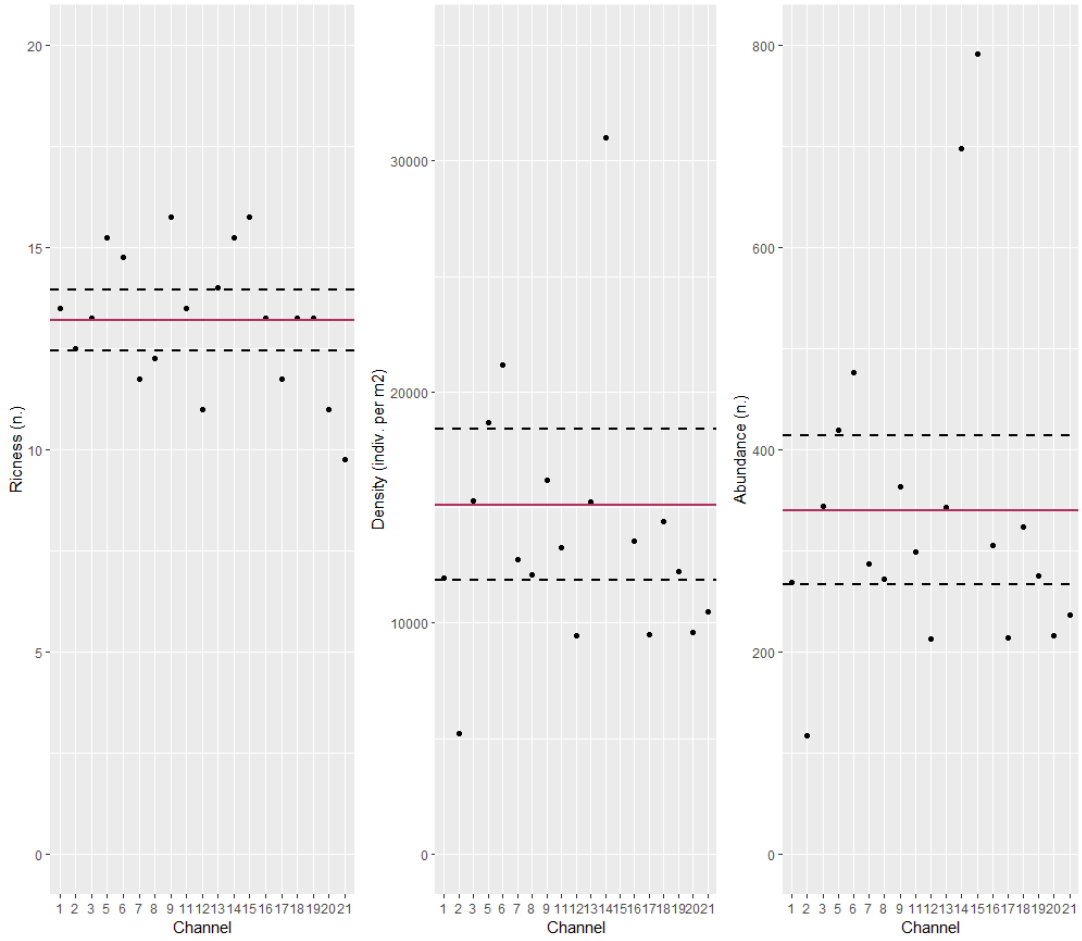

Figure 4.4: Macroinvertebrate community richness, density and abundance variation in each channel in Month 1 (August 2013). For each channel, 4 surber samples were analysed. Mean value was presented as red line. 95% confidence interval was presented as black dash line.

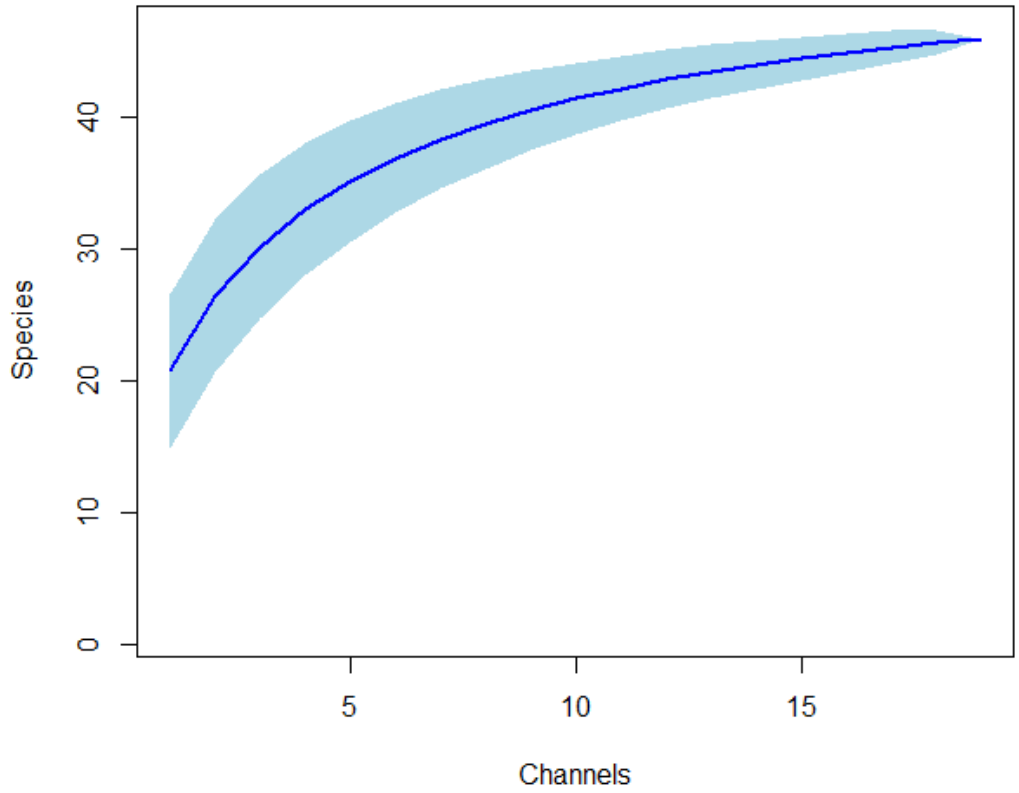

Figure 4.5: Macroinvertebrate richness accumulation curve in Month 1 (August 2013). For each channel, 4 surber samples were analysed. The blue shading shows the range of richness accumulation.

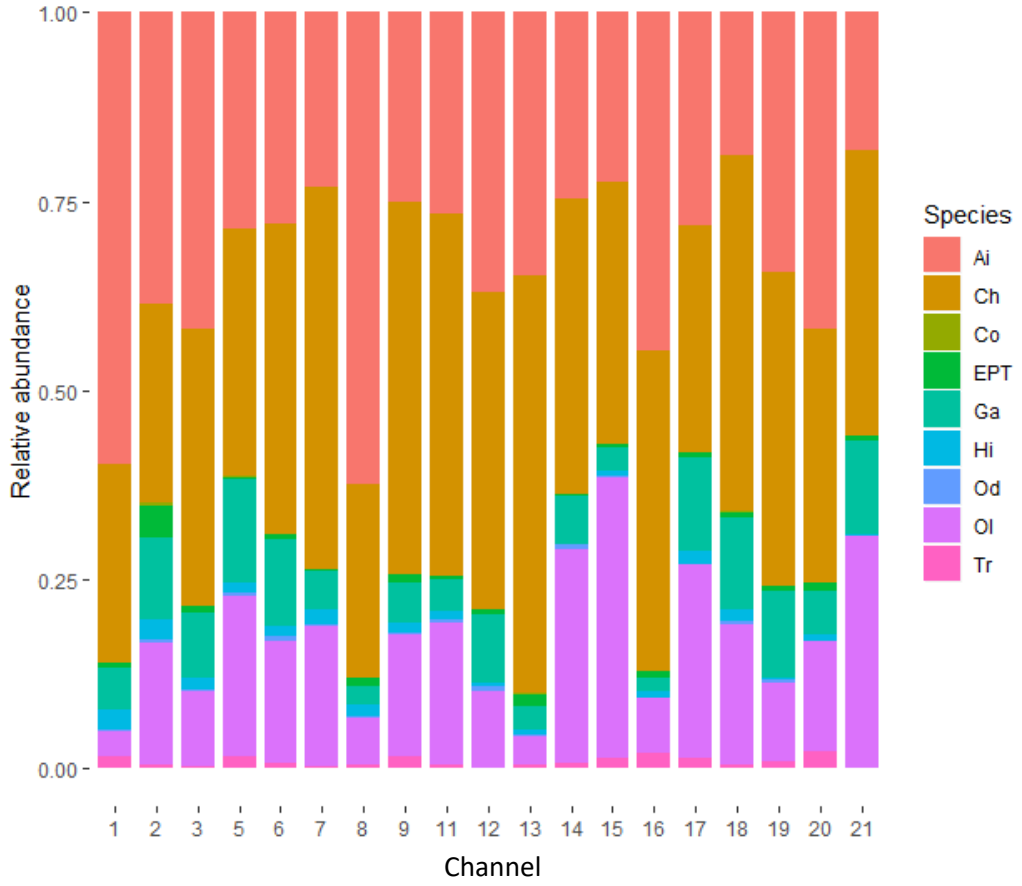

Figure 4.6: Macroinvertebrate composition variation among channels in Month 1 (Aug.2013). For each channel, 4 surber samples were analysed. Ai is Amphipod/Isopoda; Ch is Chironomidae; Co is Coleoptera; Ga is Gastropoda; Hi is Hirudinea; Od is Other Diptera; Ol is Oligochaeta; Tr is Tricladida.

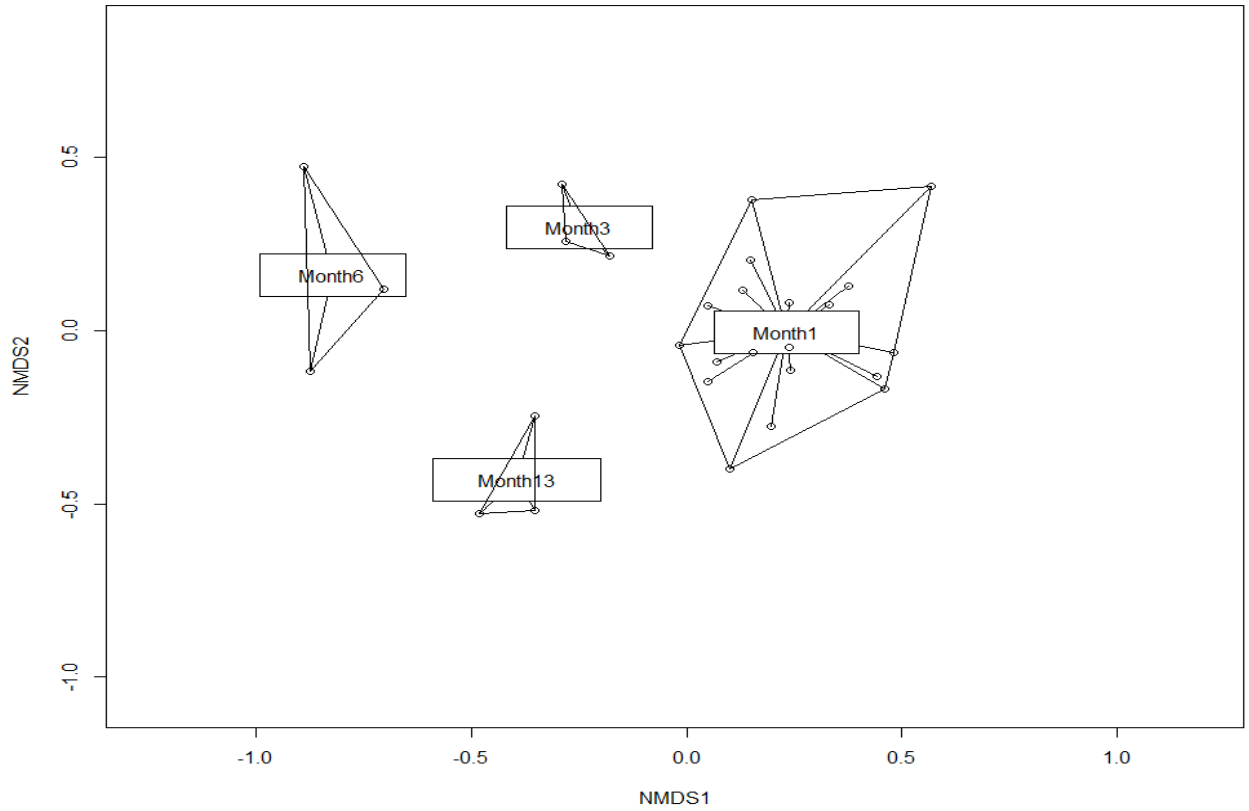

Figure 4.7: Non-metric multidimensional scaling (NMDS) biplots showing 19 mesocosms channels in Month 1(August 2013) and 3 control channels in Month 3(Oct. 2013), Month 6 (Jan. 2014) and Year 1 (Aug.2014). Each biplot presents one channel, sampling occasion was presented in box. In this analysis, mean abundance data were transformed by log10 (x+10) and applying to Bary-Curtis similarity matrices.

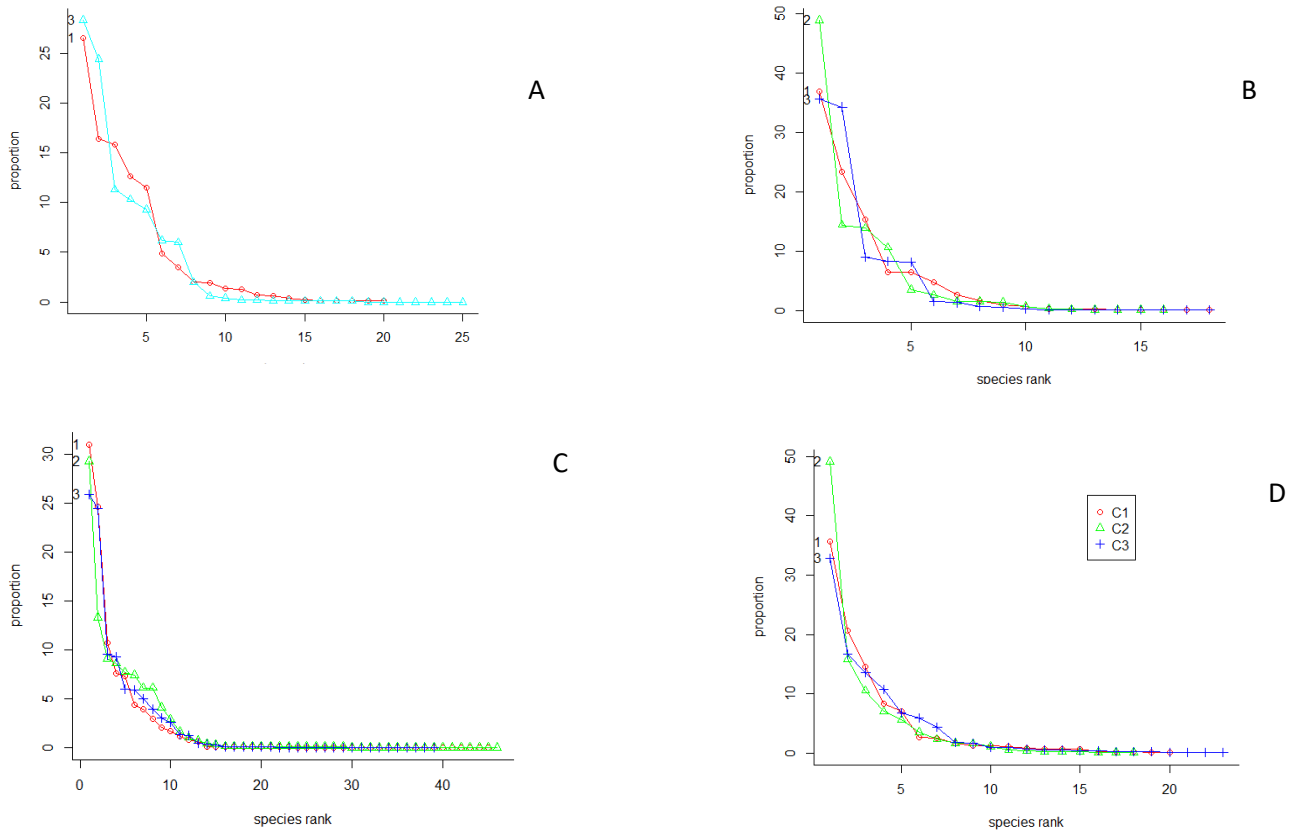

Figure 4.8: Rank-abundance curve on control channel (control channels 6, 10 and 14 are assigned as C1, C2, and C3) between Month 1(Aug.2013) to Year 1(Aug. 2014) (A: August 2013; B: October 2013; C: January 2014; and D: August 2014)

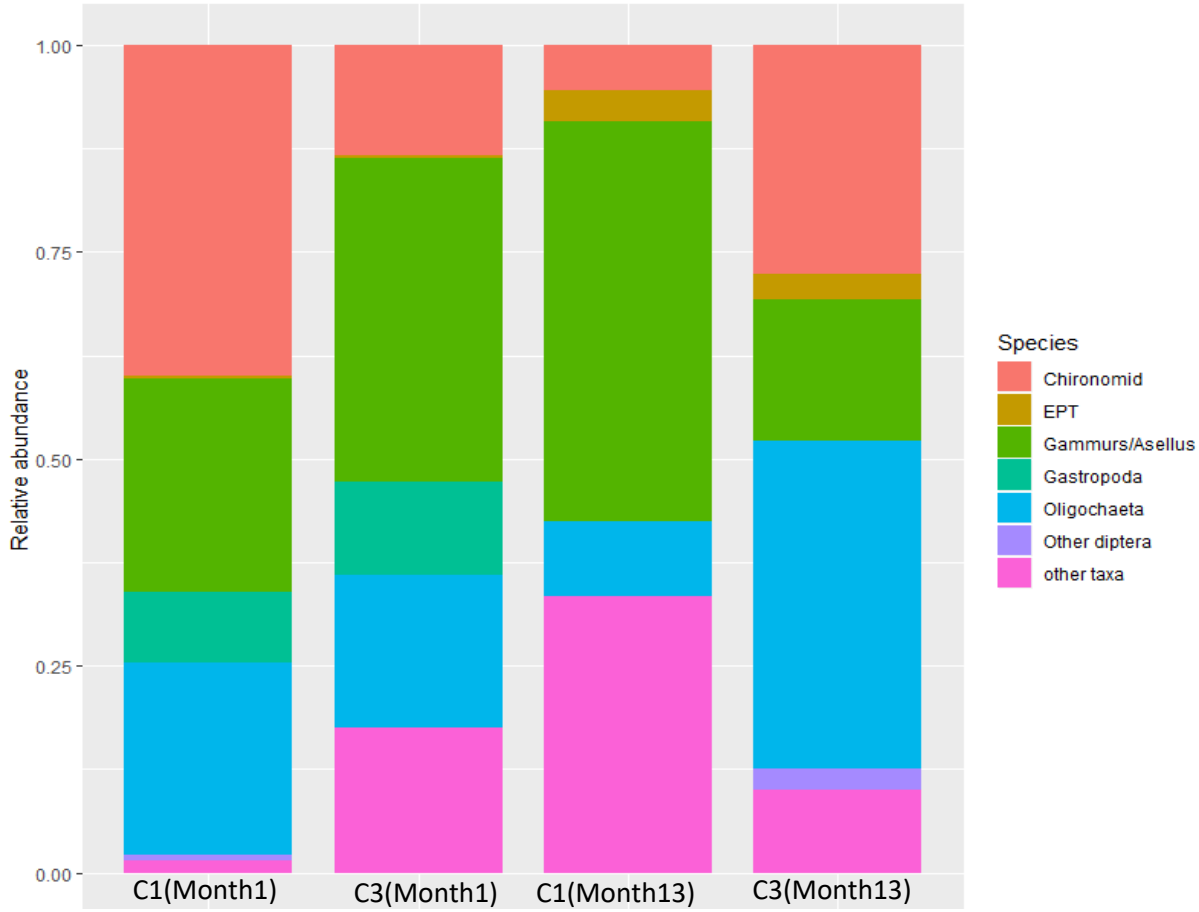

Figure 4.9: Macroinvertebrate community composition variation in control channels (C1, C2) between Month 1(August 2013) and Month 13 (August 2014). Other taxa contain Tricladida, Hirudinea, Hemiptera and Hydrachnidiae.

Table 4.1 Mean biological data for all pool cross 19 channels in month 1. The value is presented as mean ± SD.

Pool	A	B	C	D
Richness (n.)	14.6 ± 2.7	13.0 ± 2.5	12.4 ± 2.3	12.7 ± 2.4
Density (Indiv./m^2)	16386.0±9670.9	15443.2±9630.6	13578.9±9428.5	15048.0±9035.7
Abundance (Indiv.)	368.7 ± 217.6	347.5 ± 216.7	305.5 ± 212.1	338.6 ± 203.3
Amphipod/Isopoda (Indiv.)	118.1 ± 77.5	113.7 ± 65.8	104.3 ± 79.1	93.5 ± 68.6
Chironomidae (Indiv.)	167.0 ± 103.2	119.3 ± 86.4	118.6 ± 116.6	130.7 ± 69.4
Oligochaeta (Indiv.)	59.5 ± 53.8	72.6 ± 98	55.4 ± 72.2	66.8 ± 86.1
Gastropoda (Indiv.)	5.3 ± 5.4	34.5 ± 31.3	19.8 ± 11.7	38.8 ± 32.3

Tricladida (Indiv.)	9.3 ± 9.1	1.5 ± 2.1	1.0 ± 1.9	1.4 ± 3.6
EPT (Indiv.)	4.9 ± 4.7	1.6 ± 1.5	0.7 ± 1.1	1.8 ± 2.0
Hirudinea (Indiv.)	2.1 ± 2.2	2.9 ± 3.6	4.7 ± 4.5	4.4 ± 3.6
Coleoptera (Indiv.)	0.7 ± 1.1	0.2 ± 0.4	0.2 ± 0.5	0.2 ± 0.4
Other Diptera (Indiv.)	1.6 ± 1.5	1.2 ± 1.7	0.7 ± 1.0	0.9 ± 1.8

Table 4.2 One- way ANOVA result of macroinvertebrate community variation along channel gradient in Month 1. Significance value denotation is as follows: ns = non-significant (p > 0.05); p < 0.05; p < 0.01**; p < 0.001***.*

	df	F	p-value
Richness	3	2.8	0.04*
Density	3	0.3	0.84^{ns}
Abundance	3	0.3	0.85^{ns}
Amphipod/Isopoda	3	0.4	0.75^{ns}
Chironomidae	3	1.02	0.40^{ns}
Oligochaeta	3	0.17	0.92^{ns}
Gastropoda	3	7.61	<0.001***
Tricladida	3	11.30	<0.001***
EPT	3	8.00	<0.001***
Hirudinea	3	2.19	0.10^{ns}
Coleoptera	3	3.67	0.02*
Other Diptera	3	1.03	0.38^{ns}

Table 4.3 Macroinvertebrate community biological variation of 19 channels in August 2013. The data is presented as mean ± SD. Mean value and CVs was calculated by 4 surber samples.

Channel	Mean richness (n.)	Mean abundance (Indiv.)	Mean density (individuals per m^2)	CV% (between pool section)
1	14 ± 0.87	268 ± 104.17	11922 ± 4629.94	38.8
2	13 ± 3.20	117 ± 32.14	15212 ± 10589.79	27.4
3	13 ± 3.77	344 ± 88.52	15289 ± 238.27	69.3
5	15 ± 2.05	420 ± 176.74	18655 ± 7855.14	42.1
6	15 ± 1.48	477 ± 70.63	21178 ± 8656.49	40.9
7	12 ± 0.83	287 ± 14.23	12756 ± 632.47	5.0
8	12 ±1.30	272 ±39.18	12078 ±1741.37	14.4
9	16 ±1.92	364 ±276.67	16167 ± 12296.21	76.1
11	14 ± 2.06	299 ± 55.02	13278 ± 2445.04	18.4
12	11 ± 1.58	213 ± 51.94	9445 ± 2308.32	24.4

13	14 ± 3.08	343 ± 122.59	15233 ± 5448.22	35.8
14	15 ± 1.79	698 ± 232.21	31000 ± 10320.42	33.3
15	16 ± 1.92	791 ± 202.63	35167 ± 9005.62	25.6
16	13 ± 1.30	305 ± 122.42	13545 ± 5441.17	40.2
17	12 ± 1.48	214 ± 52.58	9511 ± 2337.00	24.6
18	13 ± 2.59	324 ± 63.27	14400 ± 2812.00	19.5
19	13 ± 2.59	275 ± 115.83	12234 ± 5147.92	42.1
20	11 ± 0.71	216 ± 81.08	9611 ± 3603.25	37.5
21	10 ± 0.83	236 ± 96.85	10489 ± 4304.43	41.0

Table 4.4 Mean density (Indiv./m^2) of each macroinvertebrate taxa group for all 19 channels in Month 1. The value is presented as mean ± SD.

Channel	Amphipod/ Isopoda	Chironomidae	Oligochaeta	Gastropoda	Tricladida	EPT	Hirudinea	Coleoptera	Other Diptera
1	7133.33±5308	3122.22±1299	400±245	655.56±576	200±296	88.89±104	311.11±249	0±0	11.11±19
2	2000±710	1366.67±1196	844.44±283	566.67±384	22.22±39	222.22±208	133.33±100	22.22±22	22.22±22
3	6388.89±3934	5611.11±3934	1522.22±1040	1300±1460	55.56±73	133.33±122	255.56±218	0±0	11.11±18
5	5344.44±1983	6100±2778	3955.56±2540	2577.78±1545	311.11±284	44.44±54	222.22±137	11.11±19	88.89±63
6	5922.22±2151	8711.11±3139	3411.11±2756	2433.33±1678	155.56±132	100±96	300±192	0±0	144.44±145
7	2933.33±587	6466.67±262	2377.78±1153	655.56±228	33.33±19	22.22±39	255.56±278	0±0	11.11±19
8	7533.33±2356	3088.89±1072	755.56±424	300±101	55.56±58	122.22±58	177.78±175	11.11±18	33.33±37
9	4055.56±2840	7944.44±6271	2588.89±2935	877.78±271	266.67±411	177.78±206	200±168	0±0	55.56±48

11	3522.22±1458	6388.89±1514	2488.89±1486	544.44±462	66.67±74	44.44±54	144.44±73	0±0	77.78±32
12	3488.89±640	3977.78±2117	966.67±676	866.67±620	0±0	44.44±44	44.44±32	0±0	55.56±66
13	5300±3684	8400±1458	577.78±169	488.89±452	77.78±135	211.11±290	77.78±85	55.56±58	44.44±44
14	7600±2424	12111.11±5758	8755.56±4412	1944.44±1264	233.33±170	66.67±50	55.56±37	33.33±19	166.67±19
15	7855.56±4064	12188.89±7134	13077.78±2248	1111.11±678	455.56±559	133.33±180	177.78±130	55.56±73	88.89±19
16	6044.44±2216	5744.44±3673	966.50±967	222.22±286	288.89±451	122.22±138	133.33±54	11.11±19	11.11±50
17	2688.89±956	2844.44±778	2433.38±563	1177.78±832	133.33±231	55.56±73	155.56±67	11.11±17	11.11±44
18	2733.33±1029	6766.67±920	2644.44±982	1766.67±1548	88.89±130	88.89±130	222.22±130	11.11±19	66.67±50
19	4200±3658	5077.78±1326	1266.67±485	1388.89±1358	122.22±106	77.78±111	44.44±77	11.11±18	44.44±44
20	4022.22±2687	3222.22±1360	1411.11±957	566.67±246	211.11±366	88.89±63	77.78±58	11.11±19	0±0

21	1922.22±1168	3944.44±3226	3233.33±2621	1311.11±321	0±0	55.56±73	11.11±19	11.11±19	0±0

Table 4.5 One- way ANOVA result of macroinvertebrate community variation among channels in Month 1. Significance value denotation is as follows: ns = non-significant (p > 0.05); p < 0.05; p < 0.01**; p < 0.001***.*

	df	F	p-value
Richness	18	2.07	0.02*
Density	18	4.01	0.08^{ns}
Abundance	18	4.01	0.08 ^{ns}
Amphipod/Isopoda	18	1.63	0.08^{ns}
Chironomidae	18	2.53	0.02^{ns}
Oligochaeta	18	8.84	0.03^{ns}
Gastropoda	18	1.43	0.15^{ns}
Tricladida	18	0.76	0.73^{ns}
EPT	18	0.58	0.90^{ns}
Hirudinea	18	1.24	0.26^{ns}
Coleoptera	18	1.35	0.19^{ns}
Other Diptera	18	2.58	0.07^{ns}

Table 4.6 The convex hull among channels of each sampling occasion in NMDS ordination.

Sampling occasion	Channels	Area
1-Month	19	0.308
1-Month	2 control + 1 pre-treatment (17times)	0.020 ± 0.01(mean ± SD) Range: 0.011-0.043
3-Month	3	0.008
6-Month	3	0.052
13- Month	3	0.018

Table 4.7 NMDS scores between taxa abundance and 2 ordination axes (p<0.01). Only non-rare taxa are displayed. NMDS 1 presents the scores correlated with NMDS model axes 1, and NMDS 2 presents the scores correlated with NMDS model axes 2. According to the level of Spearman's correlation coefficient, in this analysis will choose >0.4 and < -0.4 which mean this taxa has high- moderate positive or negative correlation with sites.

Species	Group	NMDS1	NMDS2
Gammarus pulex	Amphipod/Isopoda	-0.0090	0.1478
Asellus aquaticus	Amphipod/Isopoda	-0.4937	-0.0976
D.lacteum	Tricladida	-0.6794	-0.1807
Planaria (Genus)	Tricladida	-0.8132	0.1943
Polycelis (Genus)	Tricladida	-0.4942	0.1779
Radix peregra	EPT	0.2333	0.1595
Baetidae (Famlily)	EPT	0.9327	0.4502
Ephemera danica	EPT	0.6317	0.0844
Leuctra geniculata	EPT	0.8201	-0.2664
Nemurella picteti	EPT	-0.7391	-0.9975
Drusus annulatus	EPT	-0.3683	-0.0771
Chaetocladius dentiforceps	Chironomidae	0.2985	-0.1394
Cricotopus fuscus	Chironomidae	0.2229	-0.1393
Macropelopia (Genus)	Chironomidae	0.0818	0.0669
Micropsectra (Genus)	Chironomidae	0.1732	0.0202
Synorthocladius semivirens	Chironomidae	0.3859	-0.0024
Clinocera (Genus)	Other Diptera	0.4384	-0.0507
Setacera(Genus)	Other Diptera	0.6532	-0.0561
Tipula (Genus)	Other Diptera	0.5386	-0.2881
Palpomyia (Genus)	Other Diptera	0.4335	-0.0186

Table 4.8 Macroinvertebrate community biological variation across one- year drought duration. Control channels (6, 10, and 14) were assigned as C1, C2, and C3. Mean density was calculated average value of 4 replicate samples in each channel. CVs within pool section were calculated by density of 4 replicate samples in each channel.

Date	Mean density (individuals per m^2)			CVs (between control channel)	CVs (within pool section)			
	C1	C2	C3		C1	C2	C3	Average
Month 1	21178 ± 865.4	NA	31000 ± 10320.4	18.8	40.9	NA	33.3	37.1
Month 3	22323 ± 4350.6	10356 ± 4364.2	22700 ± 5018.6	37.3	19.5	42.1	22.1	27.9
Month 6	3967 ± 1183.3	1722 ± 1170.3	2459 ± 1252.8	17.6	29.8	68.0	51.0	49.6
Month 13	12811 ± 7113.1	19156 ± 12338.6	8989 ± 2233.9	36.1	55.5	64.4	24.9	48.3

Table 4.9 Occurrence of macroinvertebrate taxa in three control channels (C1–C3) between August 2013 and August 2014. Replicability in faunal composition is evidenced by the coefficient of variation (CV%) between the three control channels

Family	Month 1			Month 3				Month 6				Month 13			
	C1	C3	CV%	C1	C2	C3	CV%	C1	C2	C3	CV%	C1	C2	C3	CV%
Amphipoda	1	1	0	1	1	1	0.0	1	1	1	0.0	1	1	1	0.0
Isopoda	1	1	0	1	1	1	0.0	1	1	1	0.0	1	1	1	0.0
Tricladida	2	2	0	3	4	4	12.9	4	4	2	28.3	3	3	3	0.0
Hirudinea	1	1	0	2	2	1	28.3	1	1	0	70.7	1	3	1	56.6
Gastropoda	2	2	0	3	2	2	20.2	0	0	0	0.0	0	0	0	0.0
Oligochaeta	1	1	0	1	1	1	0.0	1	1	1	0.0	1	1	1	0.0
Coleoptera	0	2	100	0	0	1	141.4	0	0	0	0.0	0	0	2	141.4
Ephemeroptera	0	1	100	1	0	0	141.4	1	0	1	70.7	0	0	1	141.4
Megaloptera	0	0	0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0
Plecoptera	0	1	100	0	0	0	0.0	0	0	0	0.0	1	1	1	0.0
Trichoptera	2	2	0	1	1	2	35.4	1	1	2	35.4	3	2	4	27.2
Non chironomid	2	3	20	0	0	0	0.0	0	0	0	0.0	5	4	3	20.4
Chironomid	8	8	0	7	6	7	7.1	4	7	1	61.2	9	9	9	0.0
Hemiptera	0	0	0	0	0	0	0.0	1	0	0	141.4	0	1	1	70.7
Hydrachnidiae	0	1	100	1	0	0	141.4	1	1	1	0.0	0	2	1	81.6

Table 4.10 The centroid distance among 3 control channels of each sampling occasions in NMDS ordination.

Month	Centroid distance
August 2013	0.13
October 2013	0.20
January 2014	0.29
August 2014	0.20
January 2015*	0.28
February 2015*	0.25
January 2016*	0.21

Drought experiment was end at August 2014, but control channels were running until January 2016.

Table 4.11 Mean abundance of macroinvertebrate community in control channels between Month 1(August 2013) and Month 13(August 2014). Other taxa contain Tricladida, Hirudinea, Hemiptera and Hydrachnidiae. The value is presented as mean ± SD.

	Gammurs/Asellus	Chironomid	Oligochaeta	Gastropoda	EPT	Other diptera	other taxa
Month 1	152.13 ± 18.88	234.25 ± 38.25	136.88 ± 60.13	49.25 ± 5.50	1.88 ± 0.38	3.50 ± 0.25	9.13 ± 1.13
Month 3	162.58 ± 34.65	55.17 ± 26.30	76.42 ± 63.00	47.08 ± 20.06	1.17 ± 0.24	0.00 ± 0.00	72.92 ± 44.11
Month 6	29.82 ± 9.04	3.36 ± 1.84	5.64 ± 4.74	0.00 ± 0.00	2.27 ± 0.57	0.00 ± 0.00	20.55 ± 15.69
Month 13	52.17 ± 13.42	84.83 ± 22.89	121.50 ± 60.65	0.00 ± 0.00	9.42 ± 6.10	8.08 ± 5.07	30.58 ± 15.31

Table 4.12 One- way ANOVA result of macroinvertebrate community variation in controls from month 1 to month 13. Significance value denotation is as follows: ns = non-significant (p > 0.05); p < 0.05; p < 0.01**; p < 0.001***.*

Taxa group	df	F	p-value
Gammurs/Asellus	3	0.08	0.80^{ns}
Chironomid	3	4.15	0.18^{ns}
Oligochaeta	3	1.48	0.35^{ns}
Gastropoda	3	8.97	0.74^{ns}
EPT	3	2.94	0.23^{ns}
Other diptera	3	0.49	0.56^{ns}
other taxa	3	0.01	0.94^{ns}

CHAPTER 5

Algal growth responses to drought intensity and drought duration

5.1 Summary

1. Drought intensity was estimated by PCA (Principal Components Analysis), using water velocity, wetted area, wetted volume, max diel temperature and mean diel oxygen concentration from seven water depth treatments mesocosms. Drought intensity scale ranges from 0 (total wet) to 1 (total dry).
2. Algal biomass was strongly reduced by both drought intensity and drought duration. Drought side-effects (e.g. nutrient enrichment) were not observed in the mesocosms contrary to what might be expected in the natural situation.
3. Macroinvertebrate abundance (grazer) was reduced proportional to the drought duration rather than drought intensity.
4. Due to the drought duration reducing grazer abundance gazing activity was found to be significant reduced by drought events.

5.2 Introduction

The impact of drought is defined by drought magnitude and duration of water volume loss (Lake, 2003; 2008; 2011). Meanwhile, a series of physical and chemical consequences are caused by drought disturbance, which influences freshwater ecosystem function and structure. Hence, an integrated drought index to explain both direct impact (i.e. water volume loss) and indirect impact (i.e. habitat loss) needs to be constructed. As such, there has been interest in functional freshwater ecosystem response to integrated drought model (Humphries & Baldwin, 2003; Aldous et al., 2011). Primary production, in particular algal growth serves an important function in freshwater ecosystem and in this study how algal growth will respond to drought impacts with increasing intensity and duration.

Benthic algae play a fundamental and important role in freshwater ecosystems linking the physicochemical environment and the biological community. Benthic algae are fundament to the food web, providing oxygen (Chapman, 2010; Putt et al., 2011; Lowe & LaLiberte, 2017) and organic carbon for consumers (macroinvertebrates and some fish), and also provide habitat for many organisms (O'Brien, 2018). Algae are the dominant food resource for herbivorous macroinvertebrates and fish in chalk streams (Cummins & Michael, 1979; Zah et al., 2001; Dewson et al., 2007; Woodward et al., 2008). Loss of algal habitat typically reduces macroinvertebrate abundance and richness and influences benthic macroinvertebrate community structure (Shamsudin & Sleigh, 1995; Aarnio & Mattila, 2000). In freshwater ecosystem studies, as their short life cycle is sensitive to biochemical disturbance, benthic algal communities are used as a freshwater environmental indicator, for increased nutrient fluxes (Tayor et al., 2004;

Gruner et al., 2008), organic enrichment (Katharina & Fabriclus, 2005; Bestová et al., 2018), and temperature regime (Raven, 2017).

In the freshwater ecosystem, algal growth is governed by physical environmental variables (e.g. flow velocity, Wright et al., 2002; Dewson et al., 2007; Ledger et al., 2007) and biotic variables (e.g. grazing, Lamberti et al., 1983; Dewson et al.,2007; Vincent, 2010). Many investigations about physicochemical and ecological stressors on algal growth have occurred separately but not in combination (Holomuzki et al., 2010). The single physicochemical variable impact on algal growth are widely investigated. For example, primary production increased with higher water temperature and a longer growing season in the USA (Mulholland et al., 1997). Siltation effect may reduce periphytic biomass initially and change the algal community structure during a long-term disturbance in indoor mesocosms (Izagirre et al., 2009). Costa et al. (2014) found that water level reduced by half caused increasing water turbidity, nutrients and conductivity, which increases benthic algae biomass in deep lakes but decreases in shallow lakes. Generally, increased algae growth is associated with warmer water temperature, lower flows and nutrient enrichment with minor water reduction (less < 50%) (Power et al., 2008; Piggott et al., 2012).

Drought intensity, drought duration and frequency are important characteristics to define drought impact (Lake, 2003). The impact of drought frequency on algae is mostly investigated in seasonal/predictable drought intermittent rivers (e.g. Darty et al., 2011). The impact of drought frequency was not investigated in this study; the focus was drought severity and drought duration. Although, drought severity is a key factor to define drought strength, only a few experiments focus on ecological responses to water reduction along a gradient. Many studies' impacted condition was constructed as one level of water

reduction experimental condition. focusing on fish and macroinvertebrates (Ledger et al., 2008; Ledger et al., 2012; Schneider & Petrin, 2017; Fabian et al., 2018). There are some studies focus on algal growth after drought disturbance, such as algal recolonization mechanism (Ledger & Woodward, 2001) and rewetting response (Schere et al., 1984). Dewson et al., (2007) summarized that benthic algal growth increased with water reduction in the freshwater ecosystem. The structure of the algal community is changed by drought, with toxic algae blooms associated with drought impact (Robson & Matthews, 2004; Bond & Lake, 2008).

Besides physicochemical impact, algal biomass is also controlled by macroinvertebrate grazer communities (Rosemond et al., 1993; Wallace & Webster, 1996; Rutherford et al., 2000; Hillebrand, 2009), and herbivorous fish communities (Burkholder et al., 2018). Grazers can reduce periphyton biomass depending on the grazer density, richness, type and grazer efficiency. Algal biomass consumption declines with decreasing grazer abundance and density (Alvarez & Peckarsky, 2005; Liess & Kahlert, 2008). In chalk stream the grazing is influenced by a number of key grazer species (e.g. *Radix balthica;* Ledger et al., 2008).

Drought impacts water physiochemistry in addition to macroinvertebrates (grazer) activity. Hence isolated physicochemical experiments or isolated biotic simulation experiments are not suitable to investigate drought impact on algal growth (Steinman et al., 2017). Drought normally reduces macroinvertebrate abundance (Lake, 2011) by up to 50% in chalk streams (Ledger et al. 2012). Hence, macroinvertebrate and fish top down consumption pressure on algae is typically reduced under drought conditions (Morrongiello et al., 2011). Meanwhile, the macroinvertebrate community is altered with algal habitat fragmentation, which may also reduce algal biomass consumption (Boulton,

2003; Robson & Matthews, 2004; Bonada et al., 2006). However, there is limited knowledge about macroinvertebrate communities' response to the drought of differing severity (e.g. >3 treatments, Boulton, 2003). Drought duration is another important factor, but long-term (e.g. >1-year) investigations of algae and grazer are rare (Schneider & Petrin, 2017).

In this chapter the research gap identified above is addressed experimentally using 21 artificial channels simulating a gradient of drought intensity over a long duration (13 months) and assessing algal growth with relation to macroinvertebrate grazer abundance in these systems. The mesocosms were used to provide a high realism and high degree of control (Ledger & Hildrew, 2001; Stewart et al., 2013) and to investigate the effects of a drought of differing severity and duration on algal biomass.

Five hypotheses were tested in this study:

H1: Algal biomass response will differ according to drought intensity (DI);

H2: Drought duration (DD) will have an interactive impact with DI on algal biomass;

H3: Increasing DI and DD would decrease grazer abundance

H4: DI and DD alter grazer community composition;

H5: Algal growth is dominantly controlled by grazer activity.

5.3 Materials and methods

5.3.1 Algal growth experiment set-up

The detail of design, basic experimental artificial channel set-up and experimental location description have been described in **Chapter 2**.

Terracotta tiles (10cm x 10cm, 100cm^2) were used to simulate epilithic substratum for algae biomass. For the drought experiment, 504 algal tiles (4 per channel per sampling

occasion, a4 × 21 × 6 = 504) were placed. Each artificial channel has 4 pool- riffle sections (a, b, c, d). Two tiles were placed in pool b and two in pool c in each channel on six occasions between August 2013 (month 1) to August 2014 (month 13) (Table 5.1). There were two tiles in each set of tiles, one was suspended (ungrazed tile) and one was on the channel bed (grazed tile). Ungrazed tiles were suspended approximately 3cm above the channel bed in order to minimize any differences in algae growth, without grazer effects. Tiles were placed in channels for one month (30 days) to let algae establish before sample collecting. Algal scrapes were collected every 2-months over the experimental period (Table 5.1). Organic material was collected using a toothbrush to brush the upper surface of each tile, which was washed into a 24ml polypropylene vial. All samples were frozen and stored immediately in the dark (Taylor et al., 2004). Benthic macroinvertebrates were collected by a Surber sampler (0.0225 m^2 mesh size 300 μm) on 4 sampling occasions during the main study period (Table 5.1) from two pools (b and c) in each channel.

5.3.2 Laboratory work

All algal samples were unfrozen and refilled with distilled water to 24ml. Each sample was homogenized and separated into aliquots of 10ml for ash free dry mass (AFDM) estimates and chlorophyll analysis. For each 24ml sample, one 10ml algal sample was oven dried in a crucible and weighted to the nearest 0.001g. Oven dried samples were then placed into a furnace at 450 °C for 2 h and then reweighed to the nearest 0.001g, and the difference assumed to be algal biomass. Another 10ml algae sample was freeze dried for 48h and 10ml of acetone (90%) added to lyse the algal cells and release the chlorophyll. Chlorophyll a (Chl a) b (Chl b) and c (Chl c) were determined

by the absorption of light in a photo spectrometer at 664nm, 647nm and 630nm, respectively. The light absorption of 750nm was also recorded to subtract from each of the chlorophyll readings account for turbidity in the samples.

During the drought experiment period, 168 Surber samples (2 per channel per sampling time, 2 × 21 × 4 = 504) were collected. Macroinvertebrates were sorted and identified by a dissecting microscope and assigned to two functional feeding groups; grazer and non- grazer. Moog (2002) were used for identification. All the macroinvertebrates were identified to the lowest feasible taxonomic level (species or genus, where possible).

5.3.3 Data Analysis

Drought intensity quantification

The DI score was used to present the stepped change of drought impact, which included habitat heterogeneity decline, water volume variation and water quality chemical variation (Boulton, 2003; Lake, 2008). DI provides a more reliable and realistic key variable than individual stress or multiple stressors analysis (e.g. Beermann et al., 2018). Principal Component Analysis (PCA) was used to construct DI score. PCA is a useful statistic method for multiple variables analysis (Anderson & Will, 2003; Zuur et al., 2007). Five variables, flow velocity, wetted area, water volume, max diel temperature and mean diel temperature, are used to construct drought intensity. Those five variables were investigated in seven water treatments (see **Chapter 2**) channels. Those data were applied to PCA, to scale DI score from 0 to 1 explaining drought impact. It integrated drought multiple parameters into one linear explanatory variable.

Algal biomass calculation

Algal biomass was indicated by AFDM (mg/cm^2) and chlorophyll *a* (mg/cm^2). Additionally, an Autotrophic Index (AI) was calculated to indicate biofilm quality by dividing the total AFDM by chlorophyll *a* to reflect the extent to which biofilms consist of heterotrophic organisms (bacteria, fungi, macroinvertebrates) and detritus versus autotrophic pigment-containing algae and cyanobacteria (APHA, 2005).

$$\text{Autotrophic Index} = \frac{\text{AFDM } (mg \cdot cm^{-2} \cdot d^{-1})}{\text{Chlorophyll } a \ (mg \cdot cm^{-2} \cdot d^{-1})} \quad (1)$$

To analyse the grazer effect, grazing was estimated by dividing the difference between ungrazed biomass and grazed biomass by ungrazed biomass. AFDM grazing rate was determined. In the experiment three grazers were used: the gastropod mollusc *Radix* balthica, the mayfly *Serratella ignita* and the cased caddisfly *Agapetus spp.*, all of which are dominant grazers in English chalk stream (Vincent, 2010). Grazing efficiency was calculated by equation (2):

$$Grazing(\%) = \frac{\text{Ungrazed}(mg \cdot cm^{-2} \cdot d^{-1}) - \text{Grazed}(mg \cdot cm^{-2} \cdot d^{-1})}{\text{Ungrazed}(mg \cdot cm^{-2} \cdot d^{-1})} \quad (2)$$

5.3.4 Statistical analysis

Algal biomass indicator analysis

Eight algal biomass indicators were determined, (1-3) AFDM (ungrazed/ grazed/ grazing), (4-6) Chl a (ungrazed/ grazed/ grazing) and (7-8) AI (ungrazed/ grazed). For each indicator, the mean, maximum and minimum were calculated for each sampling occasion. Because of the repeated measurement of each channel over the study period ((Pinheiro & Bates, 2000, Zuur et al., 2009), Generalized Least Squares (GLS) regression models were used to analyse the algal biomass indicators' variation caused by DI and DD. GLS with a compound correlation structure was employed to handle the auto-correlation

of the residuals. First, 4 response variables (i.e. AFDM ungrazed/ grazing; Chl a ungrazed/grazing) fitted with explanatory variables, DI and DD, by GLS model (package *nlmn* 3.1-131, Pinheiro et al., 2017). Second, GLS models were fitted for the response variables, AFDM (grazing) and Chl a (grazing), with biological descriptor, grazer abundance, used as explanatory variables. This analysis was used to study the grazing impact caused the algal biomass variation.

Response variables, AFDM (ungrazed) and AI (ungrazed), were $log_{10}\ (X+0.01)$ transformed to meet normal distribution and grazing efficient (AFDM) was $log_{10}\ (X+0.001)$ transformed. Grazer abundance data was standardized by:

$$x_S = \frac{x - Min(x)}{Max(x) - Min(x)} \quad (3)$$

where x_s is standardized data, x is the raw count data.

A selection of five models (DI + DD, DI * DD, DI, DD and Null model) was tested, and the optimal model structure was chosen on AIC, delta AIC (dAIC) and Pseudo R^2 (Nakagawa et al., 2013). The model of DI+DD and DI*DD were used to study the interaction between DI and DD. DI and DD, individual variable model was used to study the single variable impact. For each model, AIC, dAIC and Pseudo R^2 were calculated (package: *AICcmodavg* 2.1-1; Mazerolle, 2017). According to the delta AIC score, the best model (dAIC =0) or the most effective model (dAIC< 3) was selected and carried forward for all analysis. Further to this, residuals were inspected using a suite of graphical tools to check assumptions (e.g. normality and homogeneity of variance) were not violated (Zuur et al., 2010).

Biological indicator analysis

Three typical grazers (*Radix balthica, Serratella ignita* and *Agapetus spp*) were chosen to estimate grazing impact on algal biomass. The mean, maximum and minimum value of total grazer abundance and individual grazer abundance were calculated for each sampling occasion.

In order to analyse the grazer variation caused by DI and DD, Generalized Estimating Equation (GEE) model was used. Due to the taxa abundance is panel data, GEEs model as an extend Generalized Line Models (GLMs) was chosen to enable model fitting with a range of distributions and correlation structures (Halekoh et al., 2006; Zhang et al., 2012). The optimal model structure was (DI + DD), DI was sign as a main parameter and DD was signed as dumy id. The package *geepack* (1.2-1) (Højsgaard et al., 2016) was used to apply for count responses.

All statistical analyses were performed using R (Version3.5.0, R Core Team). The significance level for all statistical analysis was set at 0.05.

5.4 Results

5.4.1 DI score

The drought intensity was defined as a series score from 0 (totally wet) to 1 (totally drought). There was minimal loss of wetted area and water volume in low drought channels (DI, 0.05 to 0.25). In moderate drought channels (DI, 0.25 to 0.7), habitat fragmented, and isolated pools were established. In high drought channels (DI >0.7), the moisture area was limited. Increasing DI was first characterized by water flow decreasing (from 2.3 L/s to 0.2 L/s), which led to riffles being exposed (Figure 5.1a). Afterwards, wetted area decreased. Two stages were evident, from > 6 m^2 to 3.5 m^2 and from 3.5 m^2 to 0.3 m^2, the riffle habitat was lost, and meanwhile isolated pool habitat was established

(Figure 5.2b). The stepped decrease of water volume was from 1.9 m^3 to 0.4 m^3, which was also associated with habitat fragmentation (Figure 5.1c). Temperature variation and mean daily minimum oxygen concentration increased and decreased linear along DI, respectively (Figure 5.1 d, e).

5.4.2 Algae biomass indicators

AFDM variation

There was general increasing trend of ungrazed AFDM with increasing DD (Table 5.2; Figure 5.2). The initial ungrazed AFDM in month 3 was the lowest (mean ± SD: 0.06± 0.03 mg/cm^2 after 3 months drought), then the final sampling occasion (month 13) mean ungrazed AFDM was 1.77 times higher than the initial value (0.10 ± 0.09). There was higher ungrazed AFDM in lower DI channel in month 13 (Table 5.2). The highest ungrazed AFDM was 0.30 ± 0.28 mg/cm^2 following 11 months of drought. There was no clear trend for AFDM on ungrazed tiles along the drought gradient for the first four sampling occasions (Figure 5.2). There was a negative relationship between DI and ungrazed AFDM, in month 11 and month 13 (Figure 5.2). At the end of the experiment, mean ungrazed AFDM decreased again, which was approximately 34.4% of the mean ungrazed AFDM in month 11 (Table 5.2; Figure 5.2).

A similar pattern was found with AFDM on grazed tiles, increasing with increasing DD (Table 5.2; Figure 5.3). The highest grazed AFDM was 0.21 g ± 0.19 mg/cm^2 (mean ± SD) in month 11, which was 4.6 times higher than the lowest grazed AFDM in month 5 (0.05 ± 0.02 mg/cm^2; Table 5.2). Grazed AFDM was low even at low DI level (e.g. DI < 0.2) in first 4 sampling occasions. After 11 months of drought, AFDM

on grazed tiles were still low at high DI levels (e.g. DI >0.7) but was high at low DI channel.

Generally, grazing activity on AFDM increased with increasing DD (Table 5.2; Figure 5.4). After 11 months of drought impact, grazing rate (0.11 ± 0.13 mg/cm^2) was 5 times higher than after 3-months (0.02 ± 0.02 mg/cm^2). Grazing activity was markedly affected by DI, with low grazing in intense drought channels (DI > 0.7) at every sampling occasion (Figure 5.4).

Chlorophyll a (Chl a) variation

The ungrazed Chl *a* variation could be divided into 3 time periods. For a short period (DD: 3-month and 5 month), the ungrazed Chl *a* decreased sharply from 1.19 ± 1.51 pcm in month 3 to 0.10 ± 0.05 pcm in month 5. Then ungrazed Chl *a* concentration increased with increasing DD until month 11 (1.61 ± 1.72 pcm). At end of the experiment after 13 months, Chl *a* (ungrazed) decreased again to 30 % of mean value in month 11 (Table 5.2; Figure 5.5). Although, there was no clear pattern found in months 5, 6 and 9, ungrazed Chl *a* was low in high DI (>0.7) channels for each sampling occasion (Figure 5.5).

Chl *a* on grazed tiles increased with increasing DD generally (Table 5.2; Figure 5.6). The grazed Chl *a* was relatively low after short drought period (DD< 6 months), the highest was 1.67 ± 0.81 pcm, which is 16.7 times higher than the lowest Chl *a* in month 5 (0.08 ± 0.05 pcm, Table 5.2). The grazed Chl *a* increased in a median drought DI (0.2<DI<0.7) but was near 0 in high DI channels (DI >0.7) (Figure 5.6).

The Chl *a* concentration variation shows that the grazing activity decreased during the 13-month drought experiment (Table 5.2; Figure 5.7) from 1.05± 1.48 pcm in month 3 to 0.04 ± 0.04 pcm in month 5. Following 6 months, grazing activity remained low with

minor fluctuations (i.e. month 5, 6, 7). The highest grazing activity was found in month 11 (1.31 ± 1.63 pcm), then decreased to the end of the experiment (Table 5.2). At each sampling occasion, grazing activity reduced with increasing DI, reaching almost 0 in high DI channel (DI >0.7).

Autotrophic Index (AI)

The AI for ungrazed tiles fluctuated markedly during the 13 months drought period (Table 5.1; Figure 5.8). The highest AI occurred in month 5 (3.10 ± 5.71) and was 7.86 times higher than the initial AI of ungrazed tiles in month 3. Then the ungrazed AI decreased sharply to of 8.06% of the initial value four months later (0.25 ± 0.38 after 9-month drought). At the experiment endpoint, the ungrazed AI increased again (Table 5.2). For each sampling occasion, the ungrazed AI was higher in high DI channel (DI >0.7) and was near 0 in low and median DI channel (e.g. DI =0) (Figure 5.8).

The mean variation of AI on grazed tiles between sampling occasions was dramatical (Table 5.2; Figure 5.9). After 13 months of drought, grazed AI decreased to the lowest value, and was approximately 40% of the initial value (0.76 ± 0.37 after 3-month, 0.31 ± 0.16 after 13-month). The highest value was found in month 5 (2.26 ± 3.82), which was almost 8 times higher than the final grazed AI.

5.4.3 The relationship between primary algal biomass and drought intensity

The ideal model structure for AFDM (ungrazed), based on delta AIC scores (dAIC=0), consisted of an interaction variable of DI and DD (Table 5.3). Significant results were only found after 11- month and 13-month drought impact (p-value <0.05); only 27.41% variation was explained in this model. There was a non-significant relationship between ungrazed AFDM and drought gradient at 3, 5, 6 and 9-month

sampling occasions (Table 5.4), DI had limited impact on ungrazed AFDM in short- term drought duration (e.g. DD < 11 months). After 11 months of drought impact, the mean model slope was -2.70 ± 0.34, which suggests DI affected AFDM on ungrazed tile significantly and negatively (Table 5.4; Figure 5.10). Additionally, the impact of DI was also influenced by DD, the negative strength decreased from -3.23 (month 11) to -2.16 (month 13). After 11 months of drought, the intercept (DI=0) was -0.85 then decreased significantly (intercept = -1.85) and was 2.18 times higher than after 2 months (Table 5.4).

The ideal model structure for Chl a (ungrazed), based on delta AIC scores (dAIC=0), consisted of an interaction variable of DI and DD (Table 5.3). The variation of ungrazed Chl *a* was negatively and significantly related with DI (slope= -1.54 ± 1.22, $p<0.05$) on the first 3 sampling occasions (Table 5.4; Figure 5.11). The GLS model explained 47.52% of the variation, with the slope altered by the interaction with drought duration. DI had significant impact on the first 6 months study period (slope: -3.23 to -0.40). After more than 6 months drought period, DI had no significant effect on Chl *a* level where ungrazed ($p > 0.05$, GLS; Table 5.4). The intercept decreased significantly (DI=0, 0.67 to -2.18, $p<0.05$), which suggests that DD influenced Chl *a* level where ungrazed for less than 6 months drought period.

A GLS model with an interaction of DI and DD was used to analysis ungrazed AI variation. During whole drought period, there was a positive and significant relationship between ungrazed AI and DI (slope: 1.00 ± 1.06), except in month 13 (slope= -0.19). DI had a negative impact on ungrazed AI significant at end of experiment. The ungrazed AI was influenced by interaction of DI and DD. DD altered the intercept increasing with along drought duration length (DI=0, intercept -3.14 to -1.61; Figure 5.12).

5.4.4 Benthic grazer

Bio-descriptor

A total 3450 macroinvertebrate grazers belonging to 3 taxa were identified from the 21 channels (Table 5.5). *Radix balthica* (95.48% of total grazer abundance) was the dominant grazer in the experimental system, as typically found in chalk streams (Ledger et al., 2008). *Serratella ignita* (4.00%) and *Agapetus spp.* (0.52%) were rare in the mesocosms. Mean abundance of *Radix balthica* decreased markedly during the 13 months of drought, with only 0.54 % of the initial mean abundance (38.60 ± 36.60) found at end of experiment (Figure 5.13). *Serratella ignita* also decreased to 5.41 % of initial abundance at endpoint (Figure 5.14). However, *Agapetus spp.* increased to 166.67% of initial abundance after 13 months of drought (0.06 ± 0.13 after 3 months, 0.10 ± 0.37 after 13 months; Figure 5.15). Because *Radix balthica* accounted for more than 95% of total grazer abundance, the variation of total grazer abundance basically reflected the variation of *Radix balthica* (Figure 5.16).

From the result of GEEs model, total grazer abundance and three taxa individual abundance were affected by DD significant, but only grazer abundance was affected by DI and DD significantly (Table 5.6). The slope of GEEs model was -0.0744 indicating grazer abundance was significantly negatively influenced by DI ($p < 0.01$; Table 5.6). DD reduced grazer abundance significantly, as intercepts shifted down with increasing DD (Intercept: 1.24 to 0.88).

5.4.5 The grazing effect

The variability of grazing activity on AFDM was explained by the GLS model with a DI + DD interaction (dAIC = 0, Table 5.3). However, there was no significant

impact of DI on grazing AFDM, except month 11 ($p < 0.01$, Pseudo R^2 =10.23%; Table 5.3). DI influenced the grazing rate negatively and significantly in month 11 (slope = -3.80, $p = 0.01$; Table 5.4; Figure 5.17).

The variation of grazing activity which is demonstrated by Chl *a* concentration, was explained by a GLS model with DI * DD (Table 5.3). The impact of DI on grazing activity was complex (Table 5.4; Figure 5.18). In month 3, grazed Chl *a* was negatively related with DI (slope = -5.84). However, there was positive relationship between grazing Chl *a* and DI (1.06 ± 0.59) following 6 months drought duration. After 9-month drought duration, DI had non-significant impact on grazing activity (Table 5.4). During 9 months of drought, the intercept was altered by DD, a sharply reduction from 0.30 to -4.38 (Table 5.4).

5.4.6 The relationship between algal biomass and bio- descriptor

Chlorophyll *a* (grazing) had a significant positive relationship with grazer abundance (Indiv.) significantly (slope = 0.02; $p < 0.03$; Table 5.7), and there was no interaction with drought duration (Table 5.7; Figure 5.19). There was no significant relationship between the other algal descriptors (e.g. grazing AFDM) and grazer abundance.

Chlorophyll a (grazing) had a significant positive correlation with *Radix balthica* abundance (slope = 0.16, $p<0.01$; Table 5.7) and *Serratella ignita* abundance (slope = 0.13, $p < 0.05$; Table 5.7) abundance significantly. There was no significant relationship between other algal biomass indicator and individual grazer abundance.

The 27.81 % grazing Chl a variation was explained by total abundance of three taxa. For individual taxa, the 31.51% and 27.18 % variation of grazing Chl a was

explained by *Radix balthica* abundance and *Serratella ignita* abundance respectively (Table 5.7).

5.5 Discussion

5.5.1 Drought alters the algal biomass

Hypothesis (H1) was supported as the response of the algal biomass was different according to the drought intensity. Without macroinvertebrate grazing activity, both AFDM (ungrazed) and Chl a (ungrazed) correlated with DI negatively suggesting that water quantity reduction causes a direct impact on algal biomass.

According to the negative correlation between Chl a (ungrazed) and DI in first three sampling occasions, the drought impact reduced algae biomass effectively and directly (Lake, 2003; Dewson et al., 2007). However, there was no expected negative correlation found between AFDM (ungrazed) and DI from 1-month to 9-month. Nutrient enrichment is one of the main consequences of low flow in natural rivers (Flemer & Champ, 2006), and is associated with eutrophication (Smith et al., 1999, 2006). Hence, there was no significant variation of detrital material in the water including living (algae) and non-living organic matter. Additionally, the high AI value (AI >250; Biggs. 1996; EA,2000) shows that the typical nutrient (e.g. N and P) enrichment may occur in high DI channels.

In natural rivers, drought affects water quality including increased water temperature, decreased dissolved oxygen (DO), pH and water conductivity (EC) etc. (Lake, 2003; Dewson et al., 2007; Prathumratana et al., 2008). In natural rivers, algal growth is limited with lower DO, pH and EC (Mosley, 2015). These mesocosms were supplied by water of high quality during entire study period (See **Chapter 3**). Hence, the

high-water quality supply was minor the impact on water quality in the median/high DI channel. It suggests that, water volumes loss is the main reason reduce algal growth in this mesocosms.

The algal growth was also affected by the habitat heterogeneity and water quality variation, which caused by water reduction. In chalk streams, *Ranunculus sp.* is the dominatant macrophyte, typically covering about 75% of the streambed (Wright,1978). Epiphytic algal growth is typically associated with *Ranunculus sp.* growth (Steinman & McIntire, 2011). Based on the experimental set- up, the ungrazed tile was suspended in the middle of channel without touching channel bed, channel wall and any macrophyte inside channels. However, abundant *Ranunculus sp.* and other macrophytes (see **Chapter 4**) in low/median DI channels might cover ungrazed tile surface during incubation period and encourage algal growth. Moreover, in median/ high drought channel, *Ranunculus sp.* was reduced by limited water supply and silt deposition, which might reduce algal growth indirectly.

The two algal biomass indicators response to drought intensity according to the time period. As at early stage of the experiment (DD < 6 months), the negative correlation between chl *a* and drought intensity (Caramujo et al., 2008), and the negative correlation between AFDM and drought intensity at later period (DD: month 11 to month 13) (Mosisch, 2001), suggests that the drought resistance of affected algae varied.

The typical algal community in English chalk stream is constituted by diatom (up to 98%), chlorophytes and cyanophytes (Westlake et al., 1972). The annual peak of chlorophytes (green algae) is in summer, up to 10% of total algal biomass (Shamsudin & Sleigh, 1995). In the early stage of the experiment (Aug.2013), it was assumed that the high percentage of chlorophytes dominated the algal community in the mesocosm

channels before treatments were applied. According to ungrazed algal biomass variation, algae were reduced and replaced by bacteria (Hambrook Berman & Canova, 2007). For the algal community, diatoms replaced the dominated green algae under drought impact (Ledger et al., 2007; 2013) and cyanobacteria (Lake, 2003). Moreover, chlorophytes have lower resistance than diatoms against drought impacts (Barthès et al., 2015). Hence, Chl a (ungrazed) was sensitive to drought over a short period and AFDM response to drought intensity after long-term drought in chalk streams.

Hypothesis (H2) was proven in this study, that drought duration has an impact on algal biomass. The strength of drought impact decreased with experimental period. On the one hand, the decreased trend of drought impact is because the study site seasonality controlled algal biomass. The high value of the ungrazed Chl a indicates that the high green algae grew on Oct.2013(month 3) and Jun. 2014 (month 11) in mesocosms, possibly due to warmer summer weather and strong long-term irradiation. A similar pattern is found in natural chalk streams, where algal biomass reaches its annual peak in summer and second peak in October (Shamsudin & Sleigh, 1995). On the other hand, after long-term drought impact, low resistance algae died out (e.g. chlorophytes), the surviving algae showed a high resistant ability against prolonged drought (e.g. diatom) (Evans, 1958; Barthès et al., 2015). Hence, the drought duration is an important factor in drought studies shaped by drought timing and study site local seasonality.

5.5.2 Drought effects on the macroinvertebrate grazer community

Hypothesis (H3) and (H4), are supported that the total grazer abundance was decreased by drought intensity and drought duration as shown by other studies (Gimm,

1993; Boulton, 2003). However, the DI has no significant impact on three individual grazer taxa. All of three taxa was affect by DD significantly.

In this study, *Radix balthic*a, the dominate grazer, (Ledger et al., 2011) declined markedly from Oct. 2013 to Jan. 2014 and did not increase again. The variation of *Radix balthica* between channel in Oct. 2013 might be caused by diet. In low DI channels (DI<0.2), *Radix balthica* was fed by algae. In high DI channels (DI>0.7), *Radix balthica* may die out with extreme drought condition (e.g. limited wet area; Boulton,2003). There was high *Radix balthica* abundance found in median DI channels (0.2 <DI<0.7; Ledger et al.,2011). Although there was less algae in median channels, warm water temperature might cause *Radix balthica* choose diatom as food instead of their primary food source, periphytic algae (Gordon et al.,2018). Additionally, water minor reduction increases *Radix balthica* refuge use to reduce their vulnerability. Rich refuge area and nutrient enrichment might also increase *Radix balthica* food source in minor water reduction channels (Riseng & Wiley, 2004). *Radix balthica* is very adaptive taxa in variable environment. *Radix balthica* has evolved different adaptive traits against environmental variation (Hedgepeth et al., 2018). However, there was sharp reduction of *Radix balthica* abundance found in Jan. 2014. The long-term hydrological disturbance reduced habitat, water quality and algal biomass in Jan.2014 (Dewson et al., 2007). In month 6, there was 20% of algae in the mesocosms even in the low DI channels. There was an expected *Radix balthica* abundance reduction in this situation, because the *Radix balthica* community might reduce abundance of algae and increase individual body size to against food crisis (Brönmark et al., 2012). Additionally, *Radix balthica* is air-breathing freshwater snail, and evidence exists showing that *Radix balthica* can change their habitat by migrating from open water areas (Hedgepeth et al., 2018). *Radix balthica* also prefers

warmer water temperature. So, food shortage, low water temperature in median DI channel might urge large *Radix balthica* individuals (e.g. >9.00mm) to escape the mesocosms in winter (Jan.2014). No supplement of macroinvertebrates after mesocosms initial set-up, might explain that the dominate grazer *Radix balthica* abundant never increased back in the flowing summer (Aug.2014).

The mayfly species *Serratella ignita*, was found in low densities in the mesocosms during whole study period. In Oct. 2013, Serratella *ignita* was found high abundance in median DI channel. In median drought channel, there was less the pressure of competition from *Radix balthica*. Additionally, nutrient enrichment may provide a plentiful food source, detritus with diatom (López-Rodríguez et al.,2009). Drought might make *Serratella ignita* disappearing in channel. In high DI channel, *Serratella ignita* was killed in the early drought duration. The *Serratella ignita* abundance variation in median DI channel might relate with drought duration. *Serratella ignita* can tolerance regular drought events in temporary river, and there is minor impact on their abundance. However, long-term drought may reduce *Serratella ignita* density (Ledger et al., 2005), individual body size, increase life cycle and increase egg mortality (Everall et al., 2018). Those reason might explain the *Serratella ignita* was not found in mesocosms but in low DI channel.

Agapetus spp. was found in low density in these mesocosms due to lack of algal food resources important this grazer (Alvarez & Pardo,2005). The small caddisfly *Agapetus spp.* showed a limited resistance of drought in aquatic ecosystem (Chester et al., 2015).

Radix balthica is the main grazer taxa in mesocosm channels. *Radix balthica* abundance is more responsive to water reduction than other grazing taxa, but grazer

abundance is not a suitable indicator for drought intensity impact. This finding suggests that the investigations of taxa biological indicators (e.g. body size, life cycle) is required in the further drought density study.

Hypothesis (H5) has not been proved in this study. Drought intensity and drought duration are the main impact on both algae and grazer individual taxa and group. Algae and grazer interacted with each other.

There is no doubt that the grazing activity was mainly driven by grazer abundance (Leham et al.,1985). Because of less grazer especially main grazer taxa in median/high DI channel, there was expected lower grazing activity in drought channel. However, in Jan.2014 and Aug.2014, the grazing was higher in drought channel. The grazed tile was put inside the isolated pool during incubation period. As wet isolated pools was the refuge area for survived macroinvertebrate in drought channel and might be the only food source during drought period. It suggests that the grazing activity is high in refuge area.

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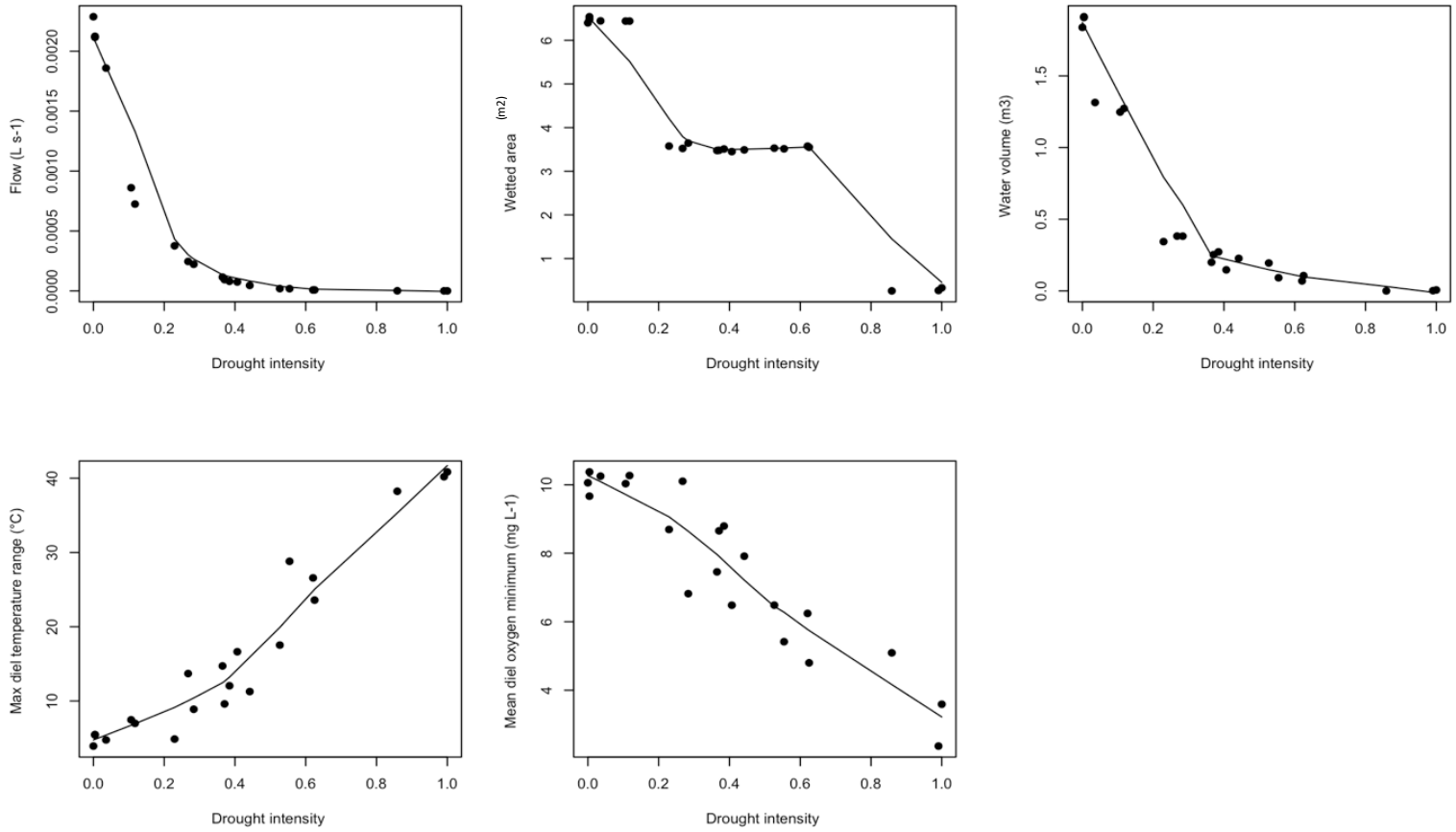

Figure 5.1 Experiment channel physiochemical characteristic against drought intensity. Fitted curves are lines of best fit given by LOESS smoothing (Span=0.5).

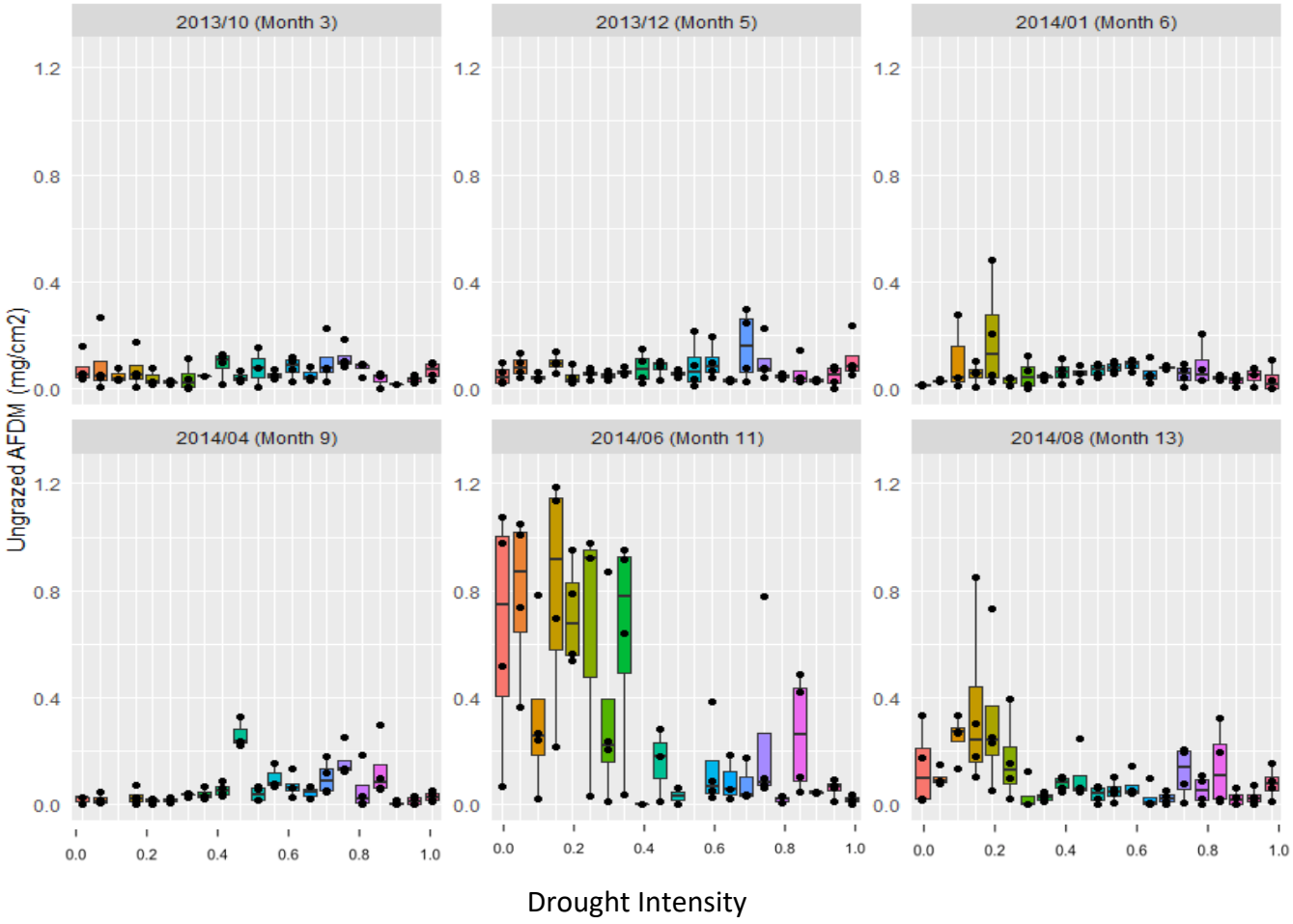

Figure 5.2 Ungrazed AFDM (mg/cm^2) during drought experiment. On each sampling occasion, for each channel 4 ungrazed tiles were analysed.

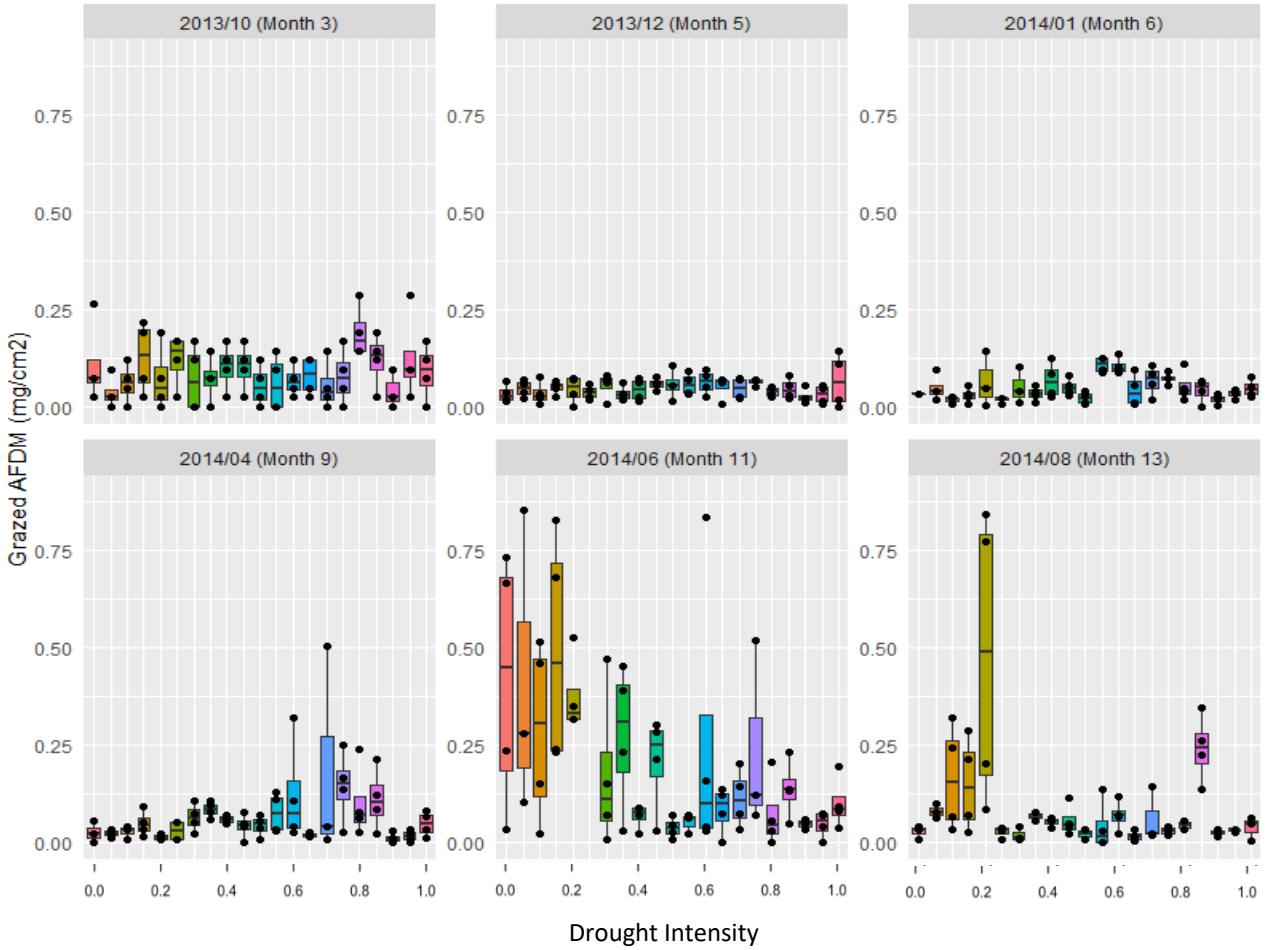

Figure 5.3 Grazed AFDM (mg/cm^2) during drought experiment. On each sampling occasion, for each channel 4 grazed tile were analysed.

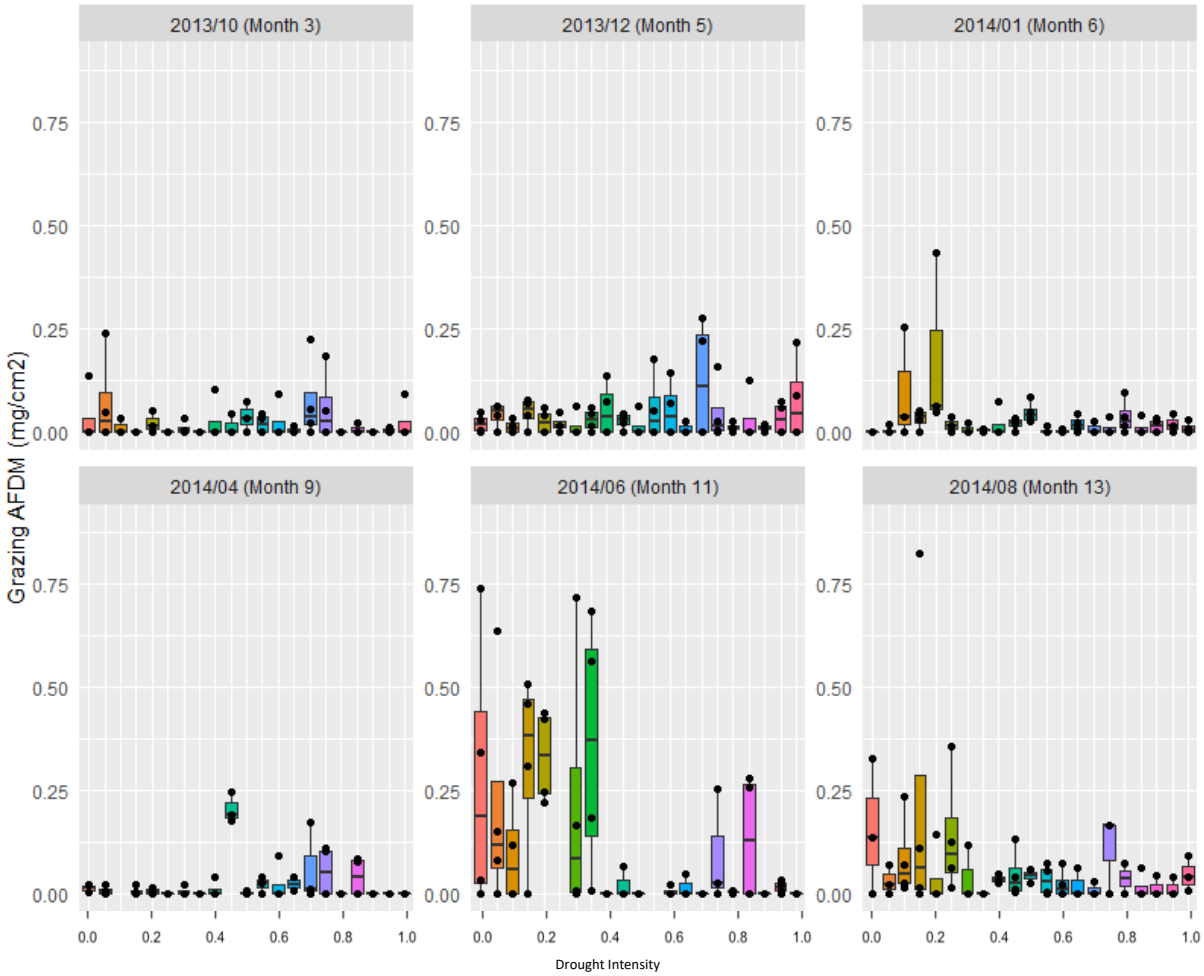

Figure 5.4 Grazing AFDM (mg/cm^2) during drought experiment. On each sampling occasion, for each channel 4 grazing titles were analysed.

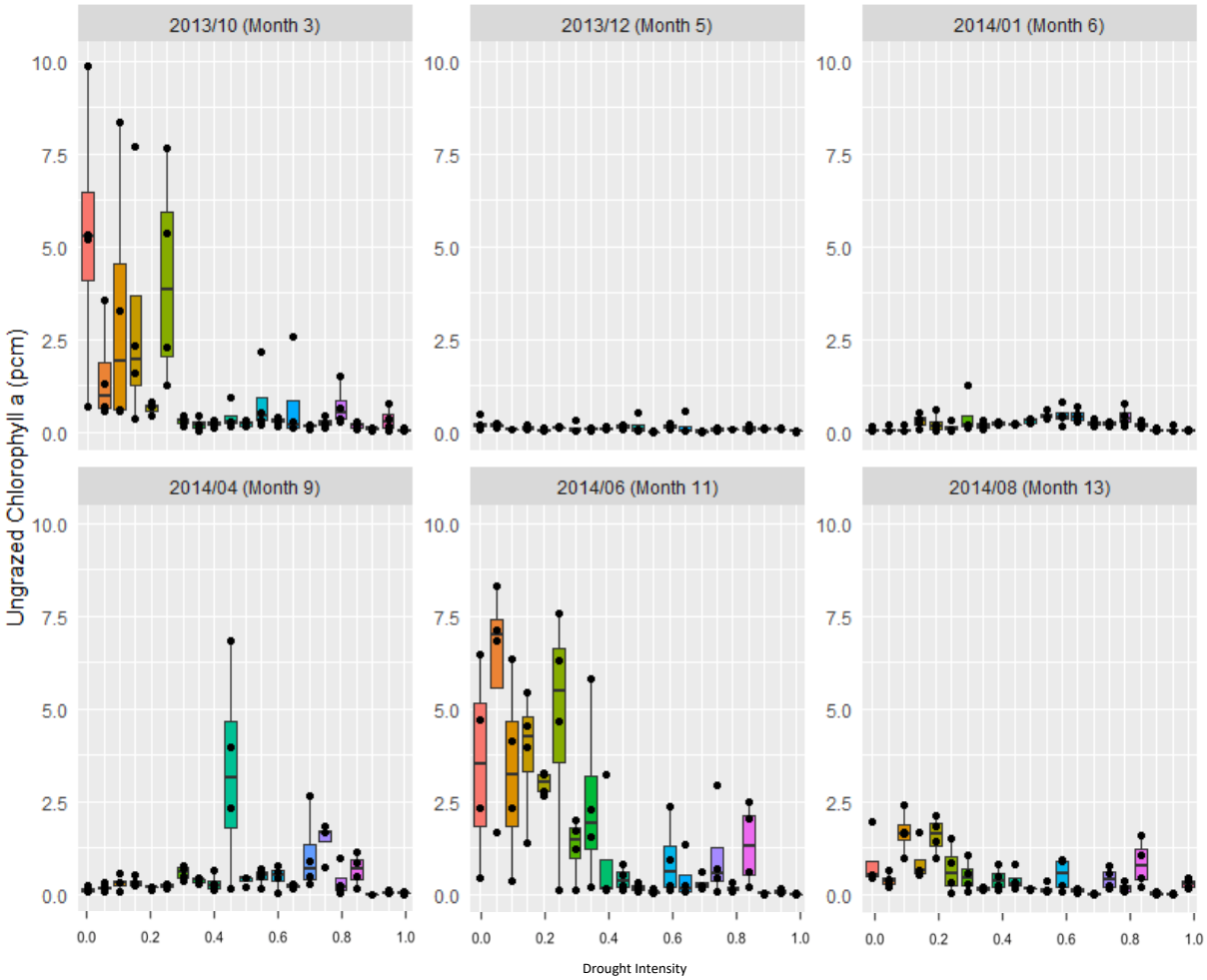

Figure 5.5 Ungrazed Chlorophyll a (pcm) during drought experiment. On each sampling occasion, for each channel 4 ungrazed tile were analysed.

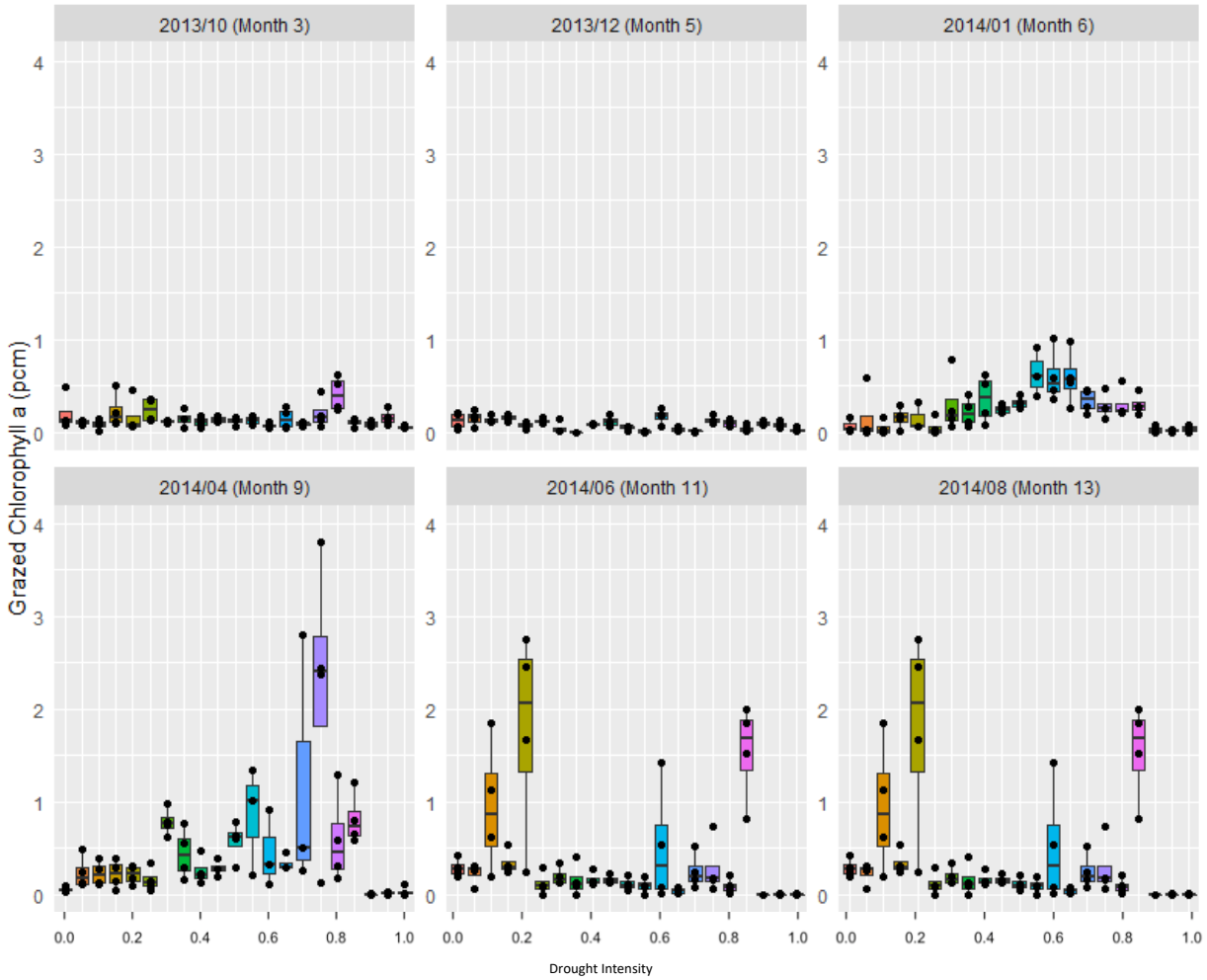

Figure 5.6 Grazed Chlorophyll a (pcm) during drought experiment. On each sampling occasion, for each channel 4 grazed tile were analysed.

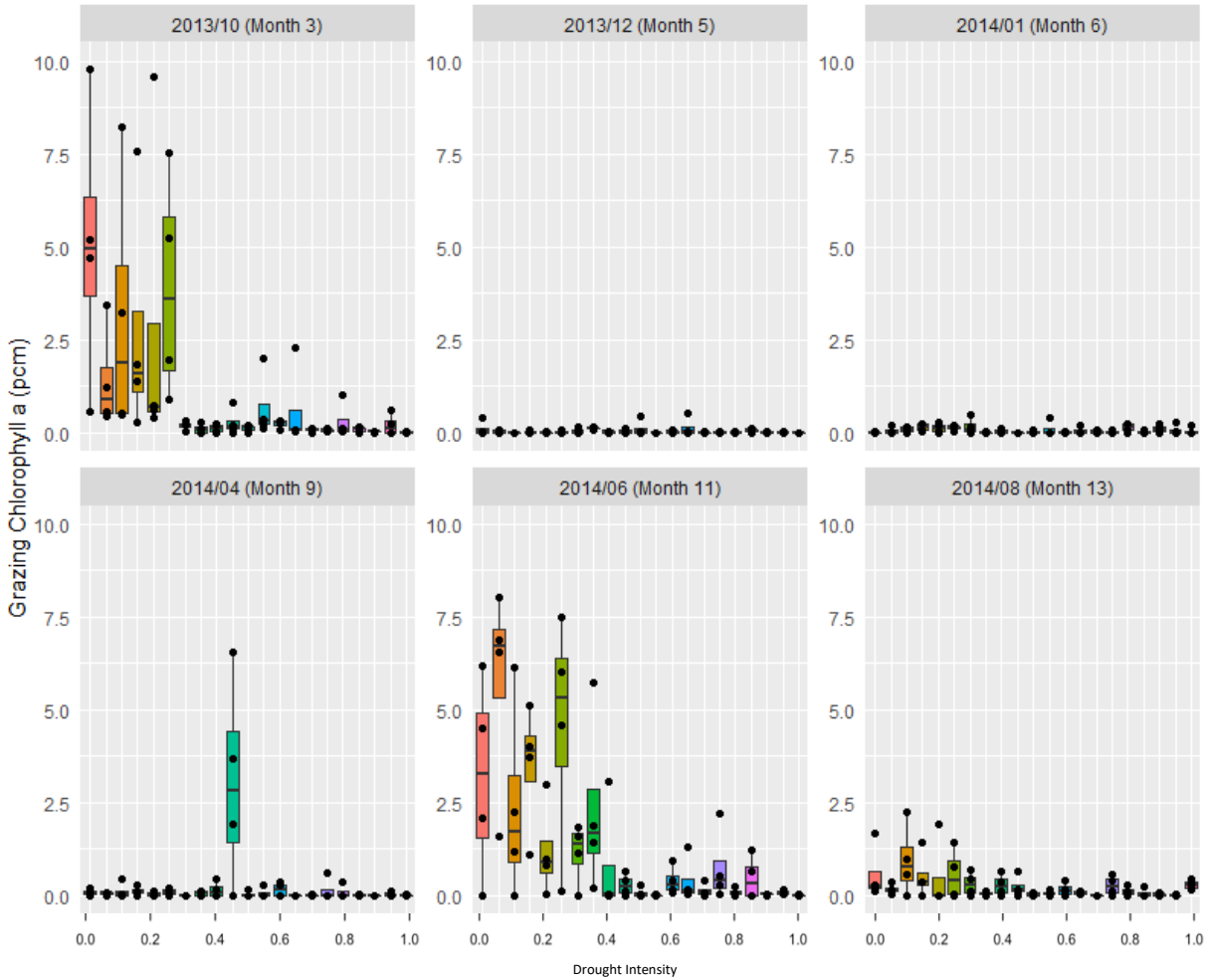

Figure 5.7 Grazing Chlorophyll a (pcm) during drought experiment. On each sampling occasion, for each channel 4 grazing titles were analysed.

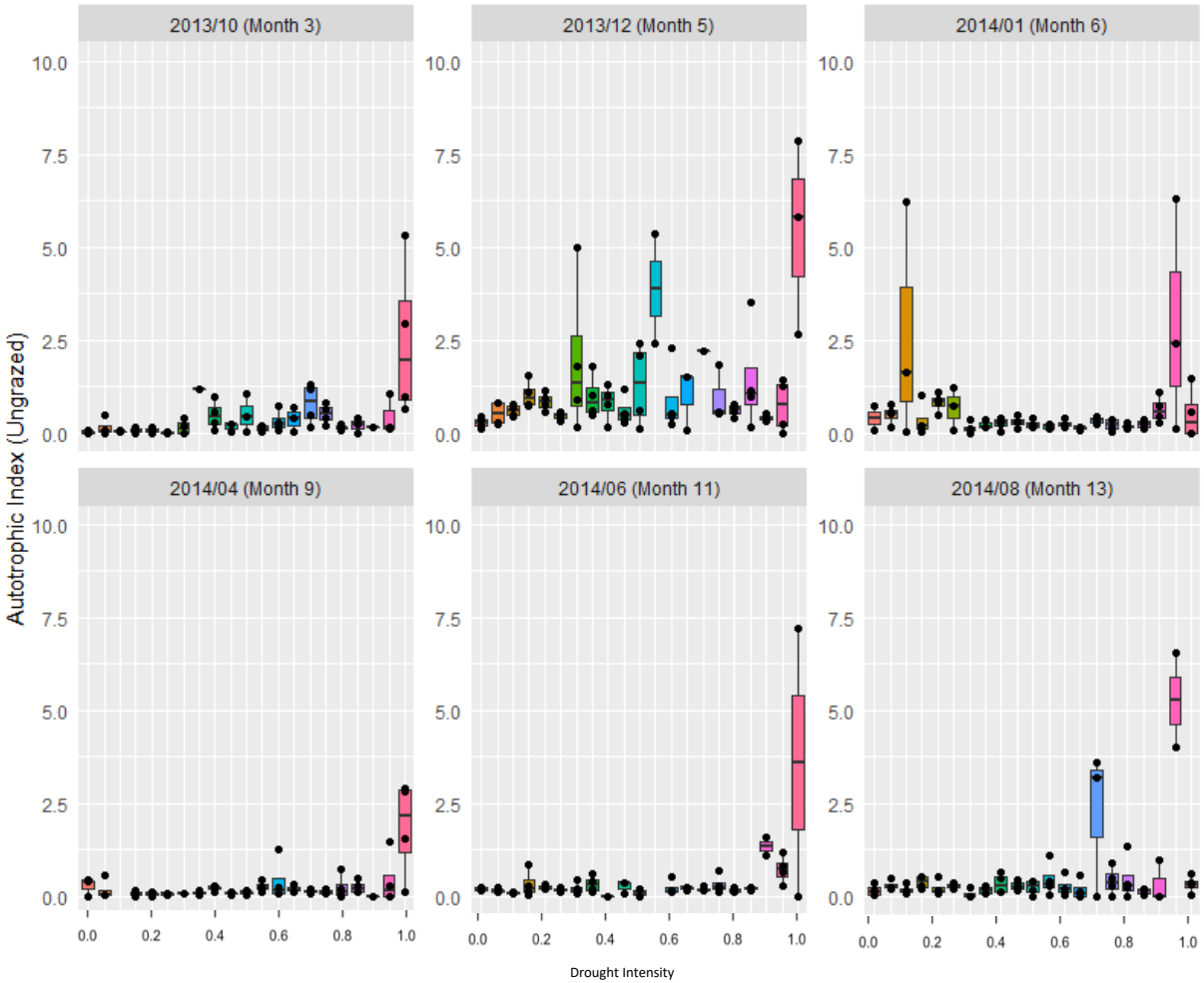

Figure 5.8 The variation of ungrazed Autotrophic Index (AI) during drought experiment. On each sampling occasion, for each channel 4 algal titles were analysed.

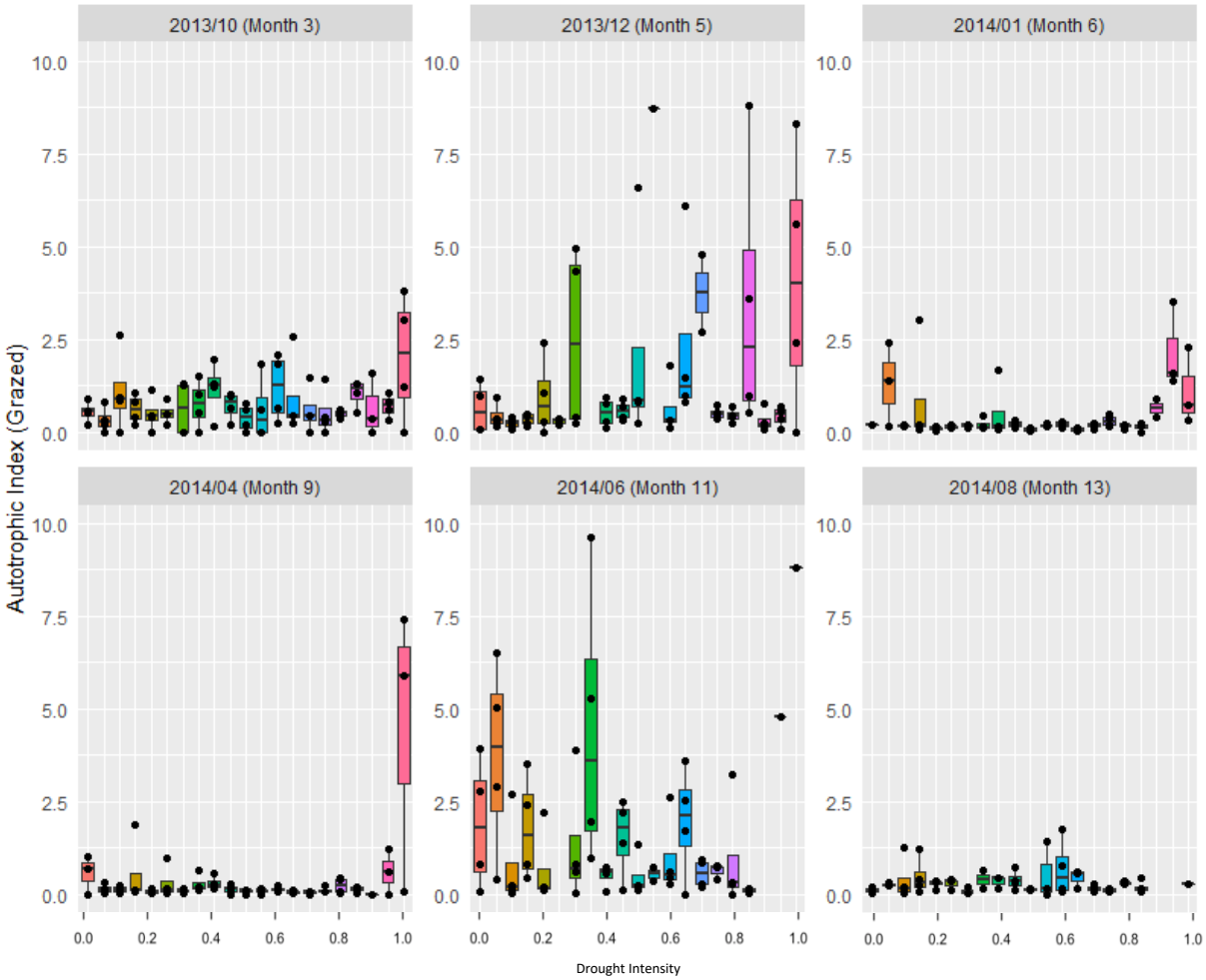

Figure 5.9 The variation of grazed Autotrophic Index (AI) during drought experiment. On each sampling occasion, for each channel 4 algal titles were analysed.

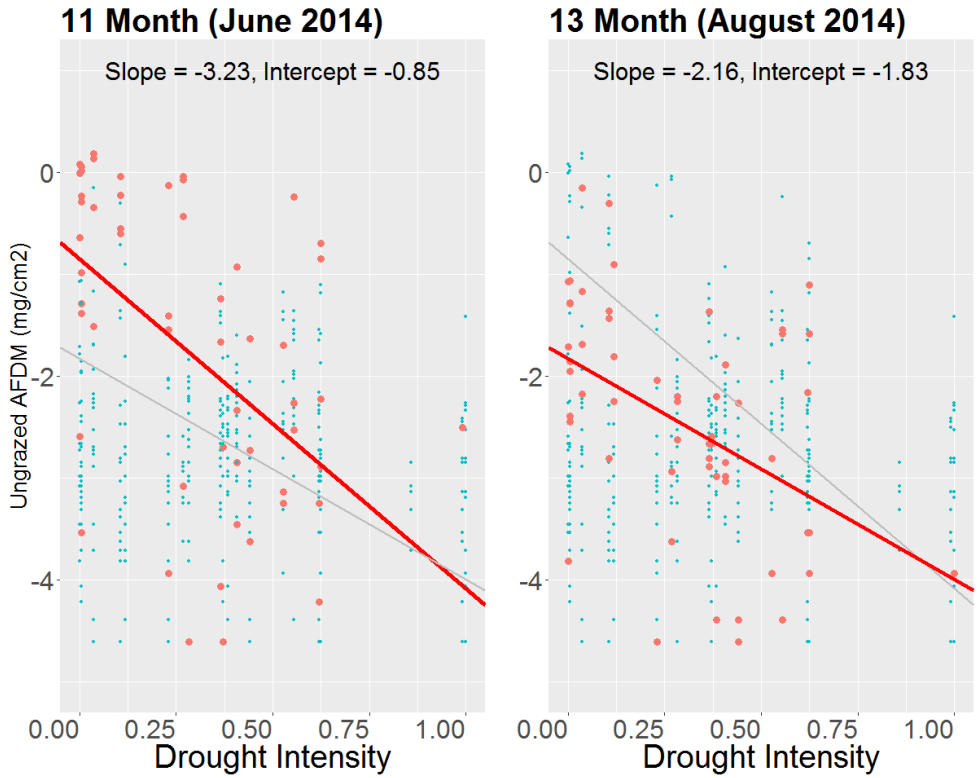

Figure 5.10 GLS model result of AFDM on ungrazed tile (mg/cm^2) across drought intensity during 1 year drought duration. Every figure shows the data from all sampling occasions, the red dots are the data for the current sampling occasion, blue dots are the data from other sampling occasions. The red line is the slope for the specific sampling occasion, and grey lines are the fits for all other months. The coefficient and intercept are present on each figure. Only significant result presented.

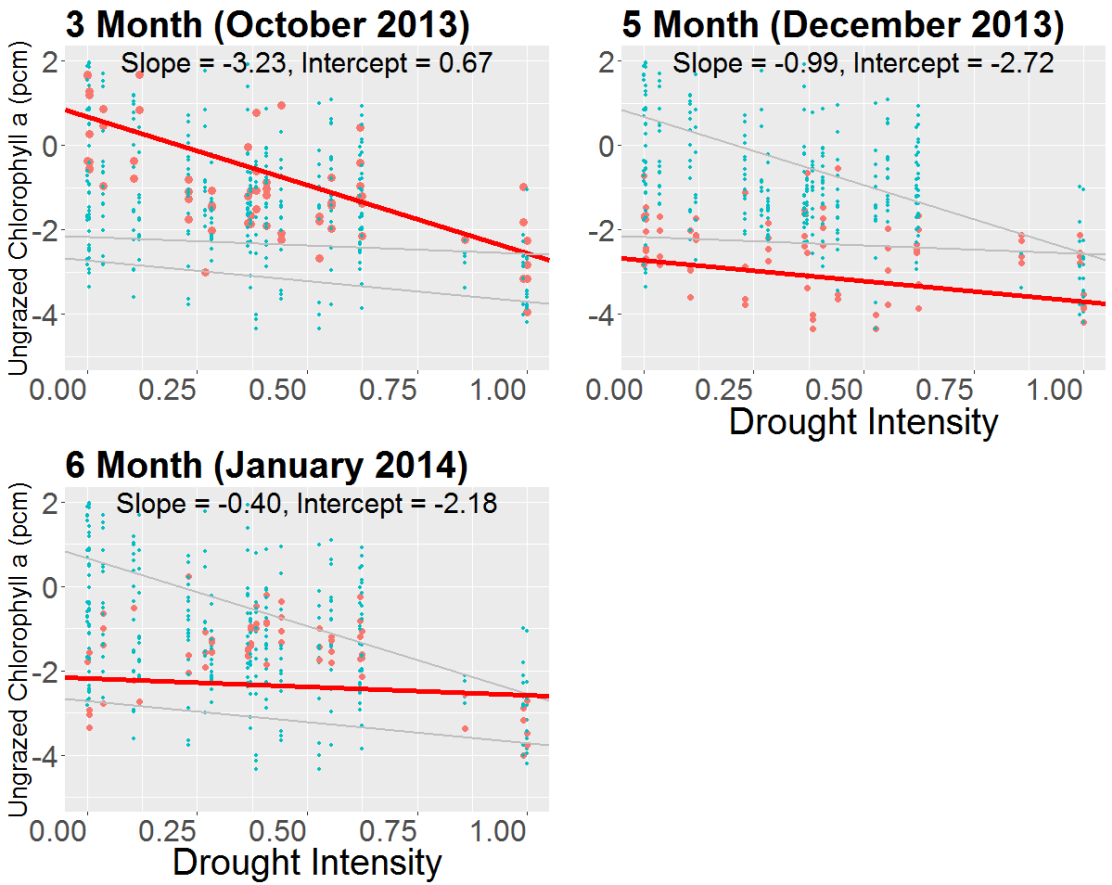

Figure 5.11 GLS model result of ungrazed Chlorophyll a (pcm) across drought intensity during 1 year drought duration. Every figure shows the data from all sampling occasions, the red dots are the data for the current sampling occasion, blue dots are the data from other sampling occasions. The red line is the slope for the specific sampling occasion, and grey lines are the fits for all other months. The coefficient and intercept are present on each figure. Only significant result presented.

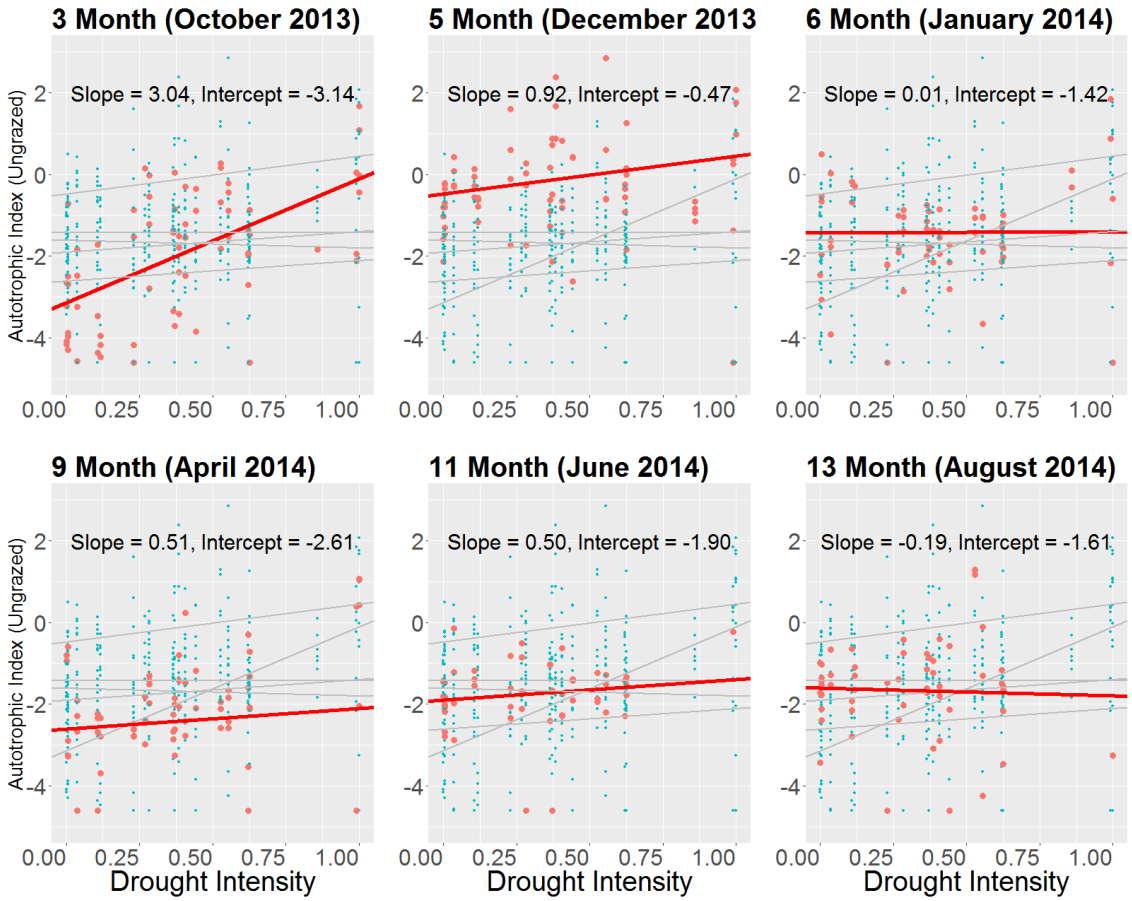

Figure 5.12 GLS model result of ungrazed Autotrophic Index (AI) across drought intensity during 1 year drought duration. Every figure shows the data from all sampling occasions, the red dots are the data for the current sampling occasion, blue dots are the data from other sampling occasions. The red line is the slope for the specific sampling occasion, and grey lines are the fits for all other months. The coefficient and intercept are present on each figure. Only significant result presented.

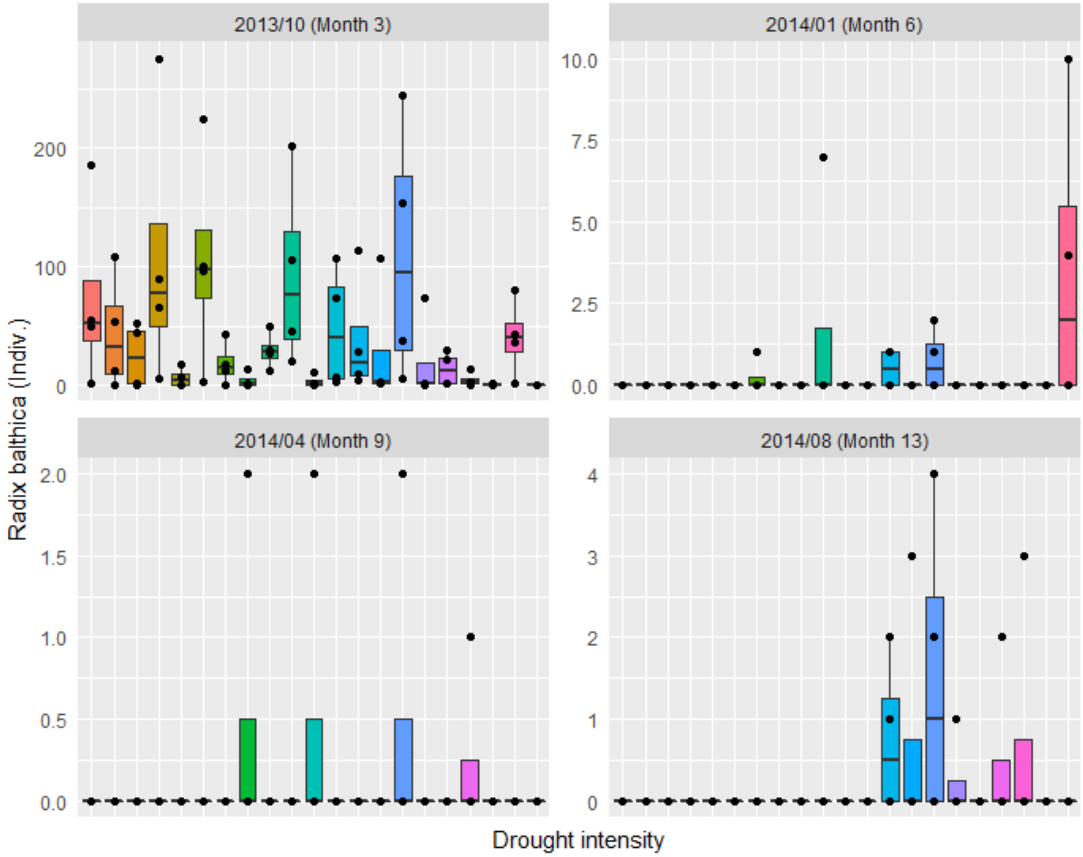

Figure 5.13 The variation of Radix balthica during drought experiment. The variation of Radix balthica was presented along the duration. On each sampling occasion, for each channel 4 samples were analysed.

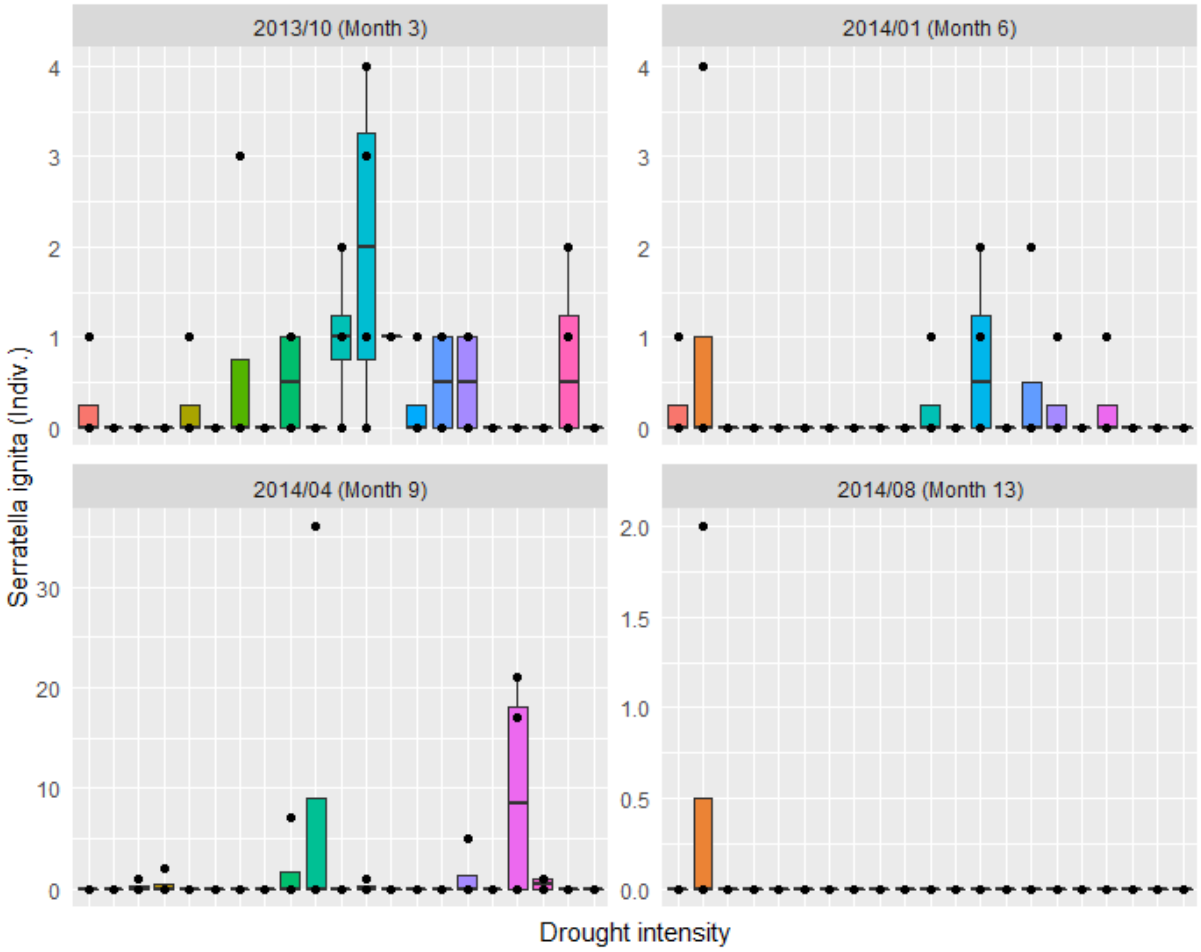

Figure 5.14 The variation of Serratella ignita during drought experiment. The variation of Serratella ignita was presented along the duration. On each sampling occasion, for each channel 4 samples were analysed.

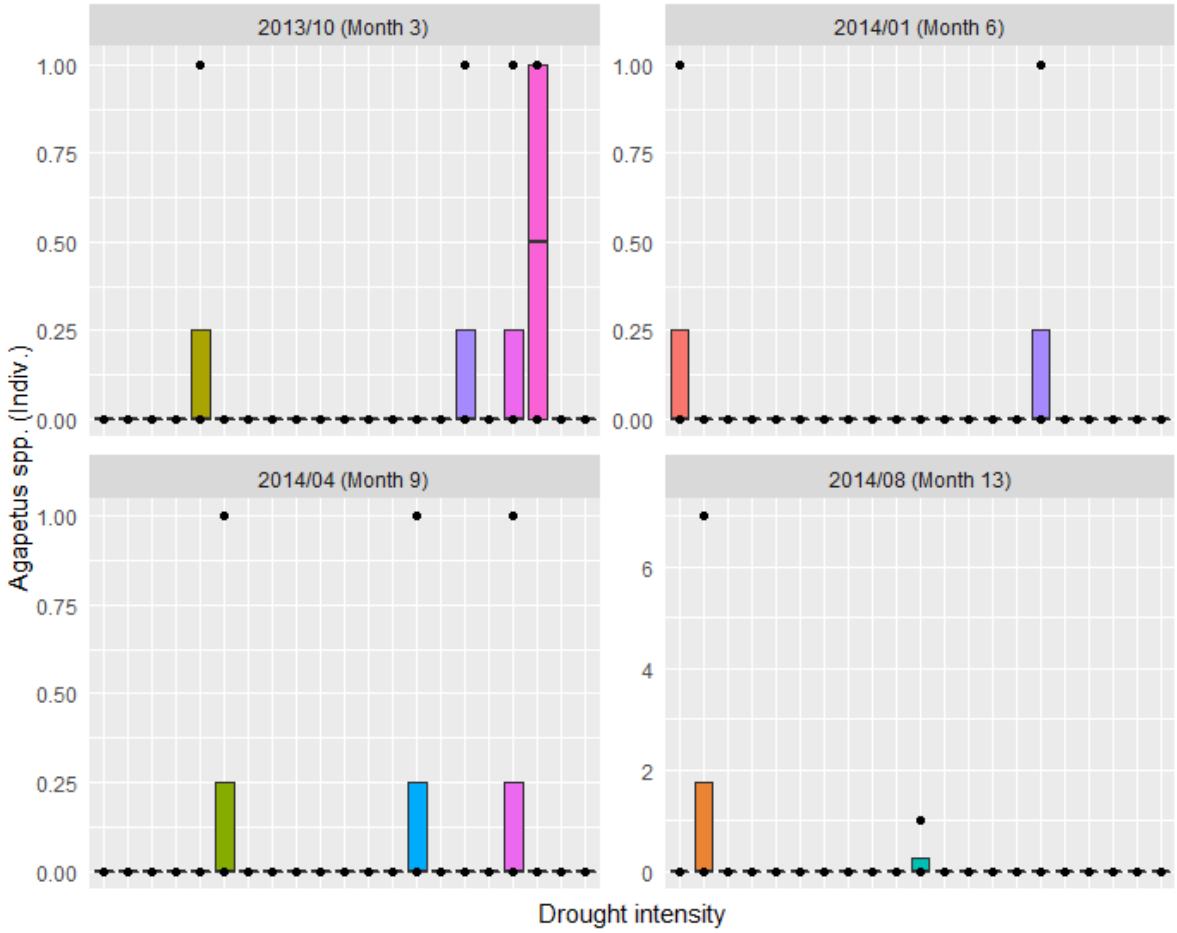

Figure 5.15 The variation of Agapetus spp. during drought experiment. The variation of Agapetus spp. was presented along the duration. On each sampling occasion, for each channel 4 samples were analysed.

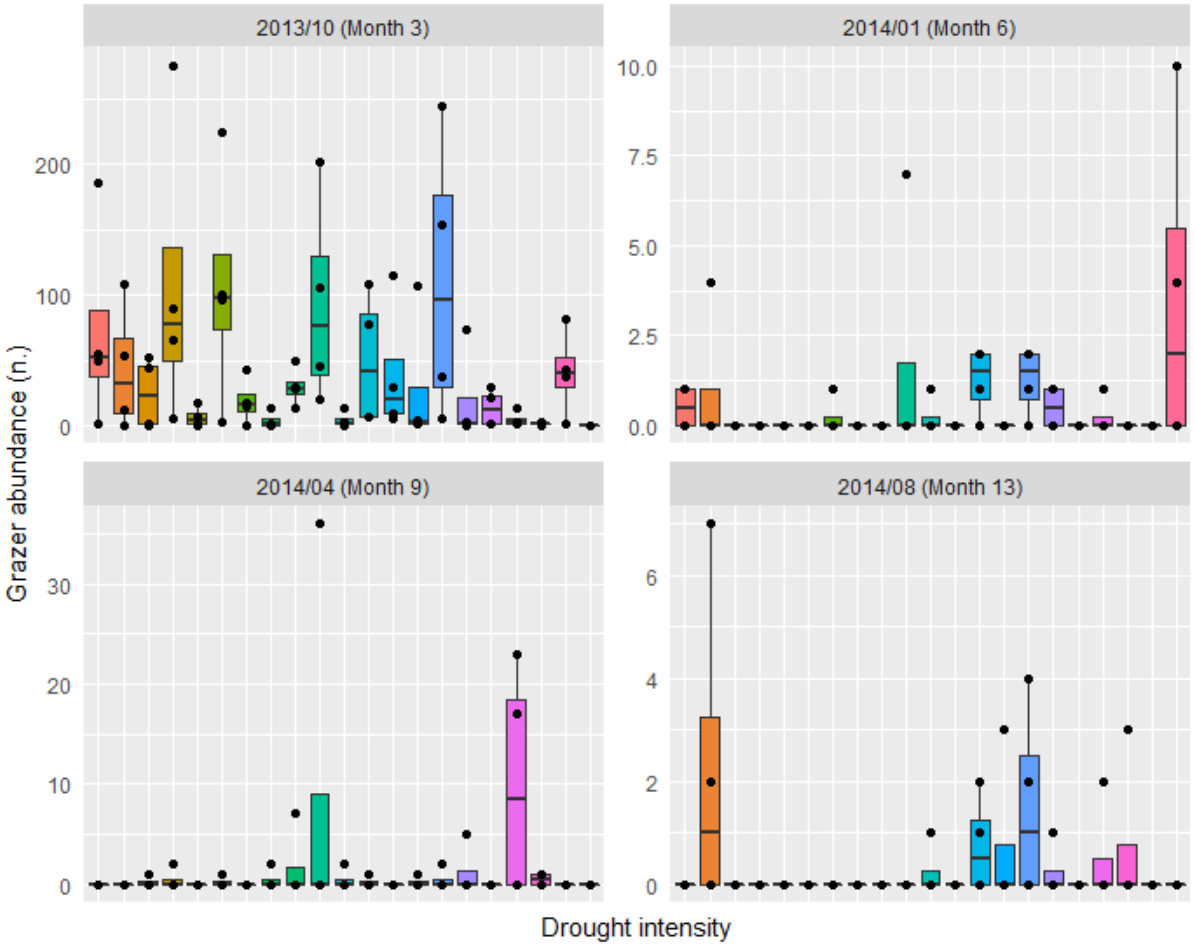

Figure 5.16 The variation of grazer abundance during drought experiment. The variation of grazer abundance was presented along the duration. On each sampling occasion, for each channel 4 samples were analysed.

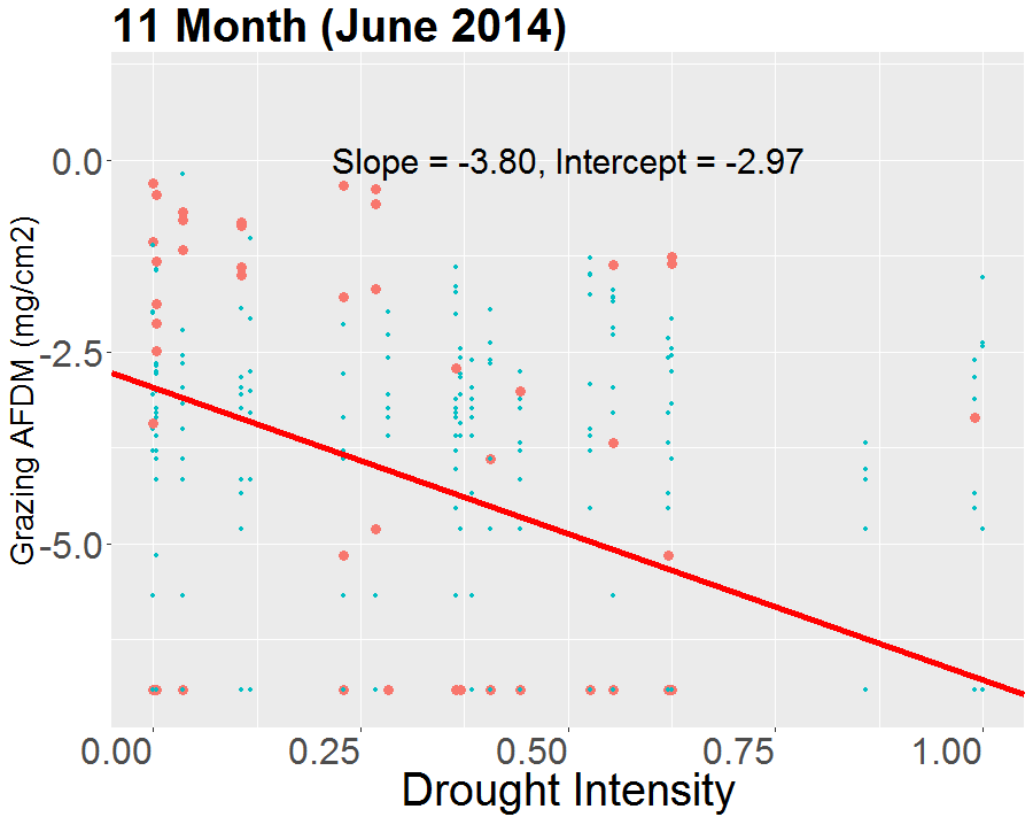

Figure 5.17 GLS model result of grazing activity on AFDM (mg/cm^2) across drought intensity during 1 year drought duration. Every figure shows the data from all sampling occasions, the red dots are the data for the current sampling occasion, blue dots are the data from other sampling occasions. The red line is the slope for the specific sampling occasion, and grey lines are the fits for all other months. The coefficient and intercept are present on each figure. Only significant result presented.

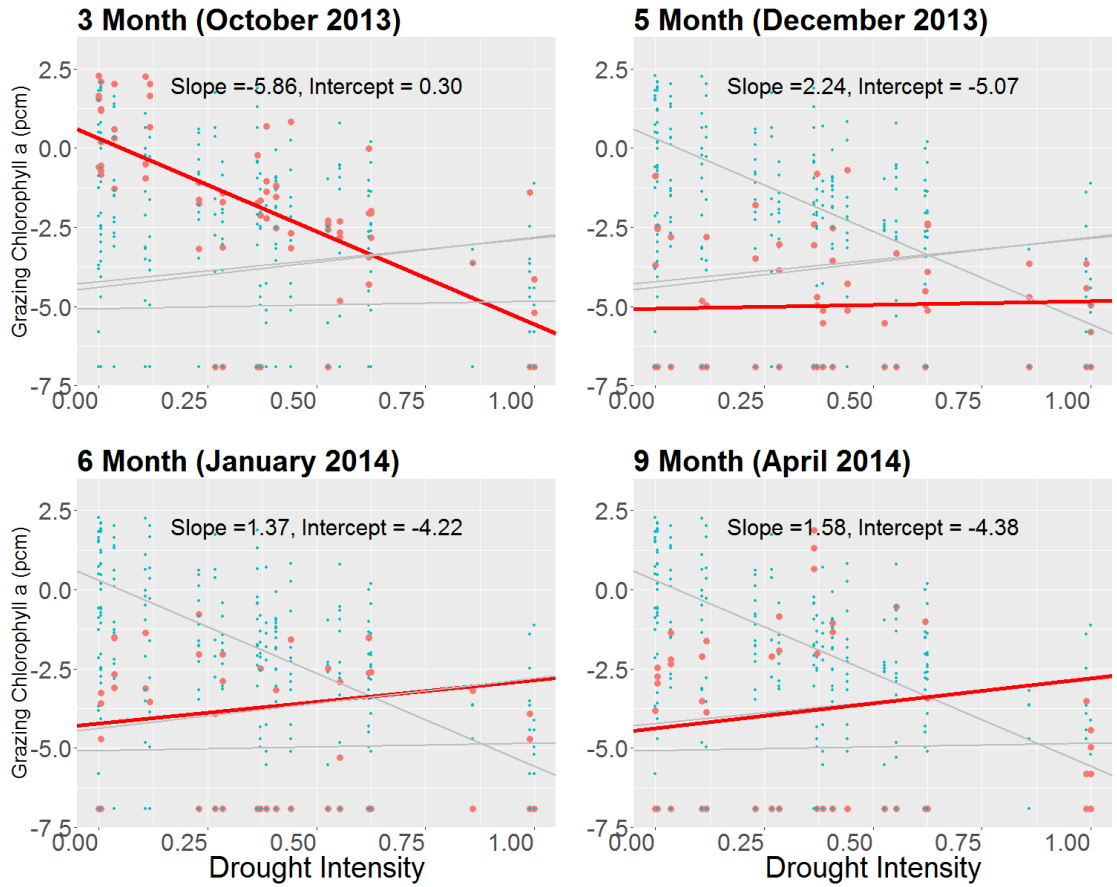

Figure 5.18 GLS model result of grazing Chlorophyll a (pcm) across drought intensity during 1 year drought duration. Every figure shows the data from all sampling occasions, the red dots are the data for the current sampling occasion, blue dots are the data from other sampling occasions. The red line is the slope for the specific sampling occasion, and grey lines are the fits for all other months. The coefficient and intercept are present on each figure. Only significant result presented.

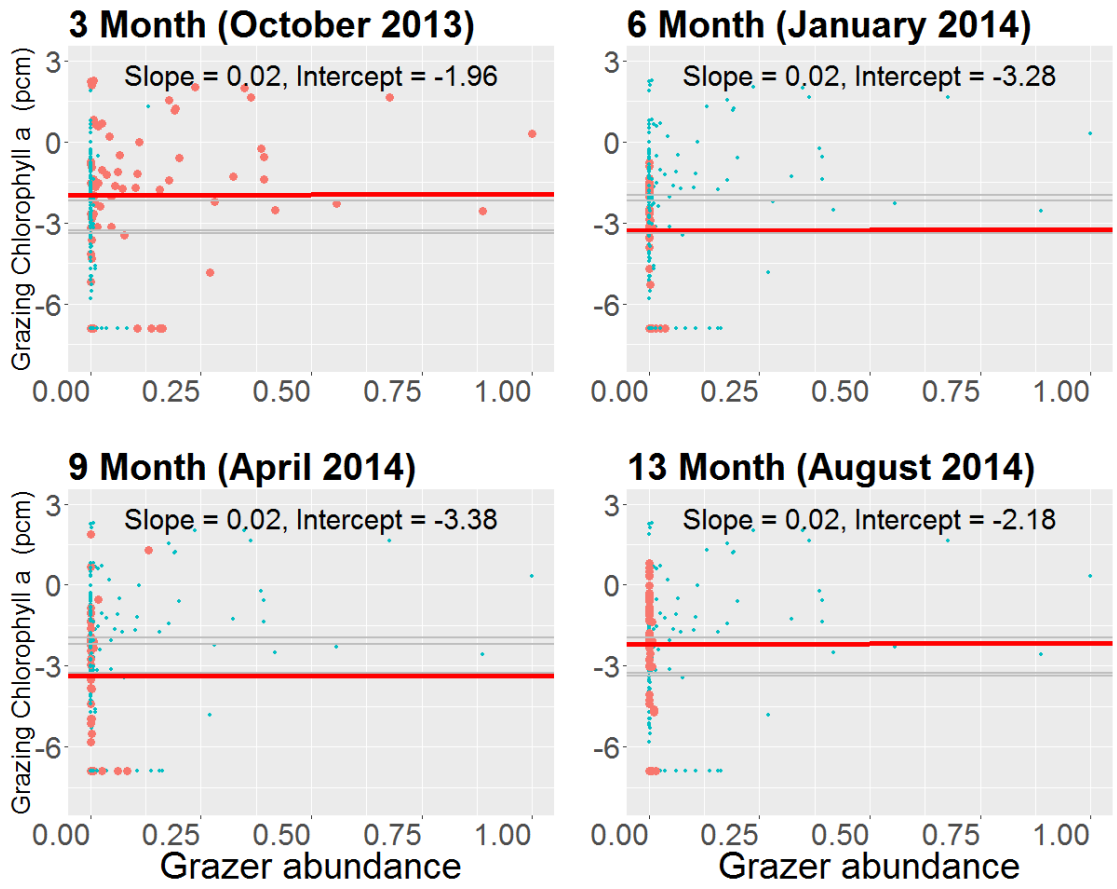

Figure 5.19 GLS model result of grazing Chlorophyll a with grazer abundance during 1 year drought duration. Every figure shows the data from all sampling occasions, the red dots are the data for the current sampling occasion, blue dots are the data from other sampling occasions. The red line is the slope for the specific sampling occasion, and grey lines are the fits for all other months. The coefficient and intercept are present on each figure. Only significant result presented.

Table 5.1 Algae sample and surber sample collection were applied during drought period (August 2013 to August 2014).

Date	Drought duration	Last days	Surber sample
October 2013	3 Months	30	√
December 2013	5 Months	30	
January 2014	6 Months	30	√
April 2014	9 Months	30	√
June 2014	11 Months	30	
August 2014	13 Months	30	√

Table 5.2 Mean value for all algae descriptors in the drought experiment. Each value is presented as mean ± SD.

Duration	AFDM (mg/cm^2)			Chlorophyll a (μg/cm^2)			Autotrophic Index	
	Ungrazed tile	Grazed tile	Grazing	Ungrazed tile	Grazed tile	Grazing	Ungrazed rate	Grazed rate
3 Month	0.06 ± 0.03	0.09 ± 0.04	0.02 ± 0.02	1.19± 1.51	0.15 ± 0.08	1.05 ± 1.48	391 ± 758	760 ± 731
5 Month	0.07 ± 0.03	0.05 ± 0.01	0.04± 0.03	0.10 ± 0.05	0.08± 0.05	0.04 ± 0.04	310 ± 571	226 ± 382
6 Month	0.06 ± 0.04	0.05 ± 0.02	0.03± 0.04	0.23± 0.14	0.25 ± 0.18	0.06 ± 0.05	581± 740	433 ± 521
9 Month	0.06 ± 0.06	0.06 ± 0.05	0.02 ± 0.05	0.52 ± 0.71	0.68 ±0. 81	0.20 ± 0.64	251 ± 387	481 ± 124
11 Month	0.30 ± 0.28	0.21± 0.19	0.11± 0.13	1.61 ± 1.72	0.35 ± 0.47	1.31 ± 1.63	584± 131	192 ± 209
13 Month	0.10 ± 0.09	0.08 ± 0.10	0.06 ± 0.06	0.48 ± 0.46	0.44 ± 0.31	0.24± 0.24	603 ± 113	313± 161

*Table 5.3 GLS model selection results. Chl a is Chlorophyll a, AI is Autotrophic Index. In GLS model, AFDM was transformed by log(x+0.01), Chlorophyll a was transformed by log(x+0.01), and AI was transformed by log(x+0.01). Grazing value was transformed by log(x+0.001). DI is drought intensity (range 0 to 1) and DD is drought duration. (DI + DD) means model was fitted by DI and DD without interaction. (DI * DD) means model was fitted by DI and DD with interaction. Null means model without any explanatory factor. The chosen model was sign by bold. The family was applied for all model is Gaussian.*

Response variable	Model	AIC	Delta-AIC	Pseudo R^2	Model selection
AFDM Ungrazed	DI + DD	1100.10	34.16	19.01%	
	DI * DD	**1065.94**	**0.00**	**27.41%**	√
	DI	1149.67	83.73	3.27%	
	DD	1103.69	37.76	17.47%	
	Null	1159.30	93.36	0%	
AFDM Grazing	DI + DD	1727.80	9.14	8.01%	
	DI * DD	**1718.65**	**0.00**	**10.24%**	√

	DI	1739.75	21.10	2.03%	
	DD	1730.80	12.15	6.78%	
	Null	1745.76	27.11	0%	
	DI + DD	1298.16	21.15	43.75%	
	DI * DD	**1277.01**	**0.00**	**47.52%**	√
Chl *a* Ungrazed	DI	1432.13	155.12	17.47%	
	DD	1377.50	100.49	30.62%	
	Null	1505.79	228.77	0%	
Chl *a* Grazining	DI + DD	1848.58	25.00	33.22%	
	DI * DD	**1823.58**	**0.00**	**37.19%**	√
	DI	1964.21	137.63	8.76%	

	DD	1879.33	55.75	27.44%	
	Null	1996.15	172.57	0%	
	DI + DD	1339.58	18.71	27.72%	
	DI * DD	**1320.87**	**0.00**	**32.05%**	√
AI Ungrazed	DI	1430.89	110.02	4.85%	
	DD	1358.43	37.57	23.55%	
	Null	1447.79	126.93	0%	

Table 5.4 GLS model result for Algae biomass descriptors against DI and DD. Chl a is Chlorophyll a, AI is Autotrophic Index. In GLS model, AFDM was transformed by log(x+0.01), Chlorophyll a was transformed by log(x+0.01), and AI was transformed by log(x+0.01). Grazing value was transformed by log(x+0.001).DI is drought intensity and DD is drought duration. The significant result was sign as bold. nf means non-significant.

Response variable	**Parameter**	**Slope**	**SE**	**Intercept**	**SE**	**t**	**P-value**	**PseudoR²**
ADFM Ungrazed	DI * DD							27.41%
	3 Month	0.18	0.36	-2.93	0.17	0.50	0.61^{nf}	
	5 Month	-0.05	0.48	-2.72	0.23	-0.47	0.63^{nf}	
	6 Month	-0.28	0.50	-2.82	0.25	-0.91	0.36^{nf}	
	9 Month	0.52	0.52	-3.21	0.25	0.46	0.64^{nf}	
	11 Month	**-3.23**	**0.61**	**-0.85**	**0.25**	**-4.97**	**<0.001**	
	13 Month	**-2.16**	**0.62**	**-1.83**	**0.26**	**-3.77**	**<0.01**	
ADFM Grazing	DI * DD							10.23%

	3 Month	-0.13	0.82	-5.55	0.40	-0.16	0.87[nf]
	5 Month	-0.18	1.11	-4.73	0.54	-0.05	0.96[nf]
	6 Month	-0.52	1.16	-4.91	0.57	-0.33	0.73[nf]
	9 Month	-0.52	1.21	-5.41	0.58	-0.32	0.75[nf]
	11 Month	**-3.80**	**1.42**	**-2.97**	**0.59**	**-2.58**	**0.01**
	13 Month	-2.26	1.44	-3.75	0.59	-1.41	0.15[nf]
Chl *a* Ungrazed	DI * DD						47.52%
	3 Month	**-3.23**	**0.47**	**0.67**	**0.23**	**-7.54**	**<0.001**
	5 Month	**-0.99**	**0.63**	**-2.72**	**0.31**	**3.74**	**0.02**
	6 Month	**-0.40**	**0.66**	**-2.18**	**0.33**	**3.84**	**<0.01**
	9 Month	-1.58	0.68	-1.17	0.33	2.94	0.08[nf]
	11 Month	-4.51	0.81	0.77	0.33	-0.29	0.18[nf]
	13 Month	-1.91	0.81	-1.17	0.33	1.26	0.20[nf]

Chl *a* Grazing	DI * DD							37.19%
	3 Month	**-5.86**	**0.94**	**0.30**	**0.45**	**-6.21**	**<0.01**	
	5 Month	**0.24**	**1.27**	**-5.07**	**0.62**	**4.44**	**<0.01**	
	6 Month	**1.37**	**1.33**	**-4.22**	**0.66**	**3.38**	**<0.01**	
	9 Month	**1.58**	**1.38**	**-4.38**	**0.67**	**3.01**	**<0.01**	
	11 Month	-4.56	1.63	0.08	0.67	0.79	0.42^{nf}	
	13 Month	-2.97	1.65	-2.35	0.68	1.75	0.08^{nf}	
AI Ungrazed	DI * DD							32.05%
	3 Month	**3.04**	**0.49**	**-3.14**	**0.24**	**6.15**	**<0.001**	
	5 Month	**0.92**	**0.66**	**-0.47**	**0.32**	**-3.18**	**<0.01**	
	6 Month	**0.01**	**0.69**	**-1.42**	**0.34**	**-4.36**	**<0.01**	
	9 Month	**0.51**	**0.72**	**-2.61**	**0.35**	**-2.43**	**0.02**	

11 Month	**0.50**	**0.85**	**-1.90**	**0.35**	**-2.97**	**<0.01**
13 Month	**-0.19**	**0.86**	**-1.61**	**0.35**	**-3.75**	**<0.01**

Table 5.5: Grazer mean abundance and diversity metrics calculated across all channels in drought experiment. Data was presented by mean ± SD. Total abundance was calculated by the total abundance of three grazers.

Duration	***Radix balthica***	***Serratella ignita***	***Agapetus spp.***	**Total abundance**
3 Month	38.60 ± 36.60	0.37 ± 0.50	0.06 ± 0.13	39.02 ± 36.53
6 Month	0.32 ± 0.82	0.15 ± 0.27	0.02 ± 0.07	0.50 ± 0.84
9 Month	0.08 ± 0.18	1.10 ± 2.68	0.04 ± 0.09	1.21 ± 2.73
13 Month	0.21 ± 0.40	0.02 ± 0.11	0.10 ± 0.37	0.33 ± 0.58

Table 5.6 GEE model result of grazer taxa and abundance variation. Abundance was transformed by log10(x+10) to meet data normal distribution.

Response Variable	Parameter	Slope	SE	Intercept	SE	P-value
Radix balthica	DI					0.3
	Duration					**<0.001**
	3 Month	-0.0768	0.058	1.23	0.57	
	6 Month	-0.0768	0.058	0.87	0.57	
	9 Month	-0.0768	0.058	0.87	0.57	
	13 Month	-0.0768	0.058	0.87	0.57	
Serratella ignita	DI					0.23
	Duration					**<0.001**
	3 Month	0.0054	0.0045	0.85	0.02	

	6 Month	0.0054	0.0045	0.84	0.02	
	9 Month	0.0054	0.0045	0.86	0.02	
	13 Month	0.0054	0.0045	0.84	0.02	
	DI					0.44
	Duration					**<0.001**
	3 Month	0.0018	0.0023	0.84	0.001	
Agapetus spp.	6 Month	0.0018	0.0023	0.84	0.001	
	9 Month	0.0018	0.0023	0.84	0.001	
	13 Month	0.0018	0.0023	0.84	0.001	
	DI					**<0.001**
Abundance	**Duration**					**<0.001**
	3 Month	**-0.0744**	**0.0084**	**1.24**	**0.0033**	

6 Month	**-0.0744**	**0.0084**	**0.89**	**0.0033**
9 Month	**-0.0744**	**0.0084**	**0.90**	**0.0033**
13 Month	**-0.0744**	**0.0084**	**0.88**	**0.0033**

Table 5.7 GLS model between Chlorophyll a grazing value and grazer abundance. Chl a is Chlorophyll a. In GLS model, Chl a was transformed by log(x+0.001) to meet data normal distribution. Other algae grazing descriptors were check by GLS model but non-significant.

Response variable	Parameter	Slope	SE	Intercept	SE	t	P-value	R^2
Grazing Chl *a*	Abundance + DD						**0.03**	**27.81%**
	3 Month	**0.02**	**0.01**	**-1.96**	**0.52**	**2.16**		
	6 Month	**0.02**	**0.01**	**-3.28**	**0.63**	**2.16**		
	9 Month	**0.02**	**0.01**	**-3.38**	**0.63**	**2.16**		
	13 Month	**0.02**	**0.01**	**-2.18**	**0.63**	**2.16**		

Grazing Chl *a*	*Radix balthica* + DD						**<0.01**	**31.51%**
	3 Month	**0.16**	**0.05**	**2.34**	**0.02**	**96.4**		
	6 Month	**0.16**	**0.05**	**2.30**	**0.02**	**96.4**		
	9 Month	**0.16**	**0.05**	**2.27**	**0.02**	**96.4**		
	13 Month	**0.16**	**0.05**	**2.31**	**0.02**	**96.4**		
Grazing Chl *a*	*Serratella ignita* +DD						**0.03**	**27.18%**
	3 Month	**0.13**	**0.02**	**2.39**	**0.02**	**137.5**		
	6 Month	**0.13**	**0.02**	**2.30**	**0.02**	**137.5**		

9 Month	**0.13**	**0.02**	**2.27**	**0.02**	**137.5**
13 Month	**0.13**	**0.02**	**2.32**	**0.02**	**137.5**

CHAPTER 6

The impacts of drought on leaf litter breakdown

6.1 Summary

1. A mesocosm drought experiment was used to analyse the effects of drought duration and intensity on leaf litter decomposition in freshwater ecosystems.
2. The total decomposition rate was negatively affected by drought intensity and drought duration.
3. Shredder community abundance, richness and composition were altered by drought, decreasing with increasing drought intensity and duration.
4. As drought progressed, microbe decomposition exhibited a higher resistance ability, and replaced shredder decomposition as the primary method of decomposition.
5. The decomposition rate mostly depends on abundant specialist taxa rather than on high shredder diversity.

6.2 Introduction

Drought as a hydrological disturbance has been well defined (Lake 2003, 2011). The direct impacts of drought on river ecosystems, such as loss of water volume, loss of aquatic habitat for organisms and loss of stream connectivity can substantially alter freshwater ecosystem structure and function (Lake, 2003; Bond & Lake, 2008). As such, there has been an increased interest in the structural and functional freshwater ecosystem responses to drying; however, little is known about how freshwater ecosystems will respond to the increased drought intensity predicted to occur in the future (Dai, 2013). In the present study, this question is specifically addressed through evaluation of the decomposition process in aquatic ecosystems and assessment of how this process will respond to increasing drought intensity and duration.

Leaf litter breakdown is a fundamental ecological process and decomposition rate (k) is the indicator most often used as a measure of breakdown in the scientific literature (e.g. Darty et al., 2011). In freshwater ecosystems, leaf litter decomposition is the key energy pathway from basal resources upwards and is thus essential to sustain food webs (Schilief et al., 2009; Datry et al., 2011; Pinna et al., 2016). The decomposition process includes three distinct temporal stages (leaching, conditioning, and fragmentation); all of which have been well studied by freshwater ecologists (e.g. Boulton, 1991; Gessner et al., 1999; Garca, 2001). However, all of these stages are potentially sensitive to drying, either directly or indirectly (Cotri et al., 2011), but this has been much less studied.

The decomposition process is often defined as the combined breakdown of leaf litter by microbes and invertebrate shredders (Gessner et al., 1994). In healthy rivers and streams, shredder-mediated leaf breakdown (shredding) is a dominant component of detrital decomposition (e.g. Chauvet et al., 2016). Previous studies have revealed that flow reduction

and stream bed drying decrease the shredder-mediated litter decomposition rate significantly (Monroy et al., 2016; Pesce et al., 2016). For instance, the summer flow reduction in an intermittent river was found to have reduced shredder-mediated decomposition rate by over 50% (Schlief & Mutz, 2009).

Microbial breakdown of leaf litter is also a major component of detrital decomposition in freshwater systems. Microbial communities are generally more tolerant of environmental stressors than higher organisms (Foulquier et al., 2015; Pinna et al., 2016). As such, the impacts of drought on microbial decomposition rates are complicated and uncertain. Some studies have shown that the microbial decomposition process can be reduced by drying (Bruder et al., 2011), possibly because flow reduction can reduce microbial colonization of leaf litter (Gulis & Suberkropp, 2003). Alternatively, the warmer temperatures associated with drying may also enhance the microbial decomposition rate (Boyero et al., 2011).

Previous studies have shown that drought events decrease the rate of leaf litter breakdown (e.g. Monroy et al., 2016), but these studies suffer from a range of limitations. Drought events are typically classified according to drought severity and duration (Lake, 2003, 2011). However, most ecological drought studies only focus on how the impact of a certain aspect of drought (e.g. a certain water volume loss) influences ecosystem processes (e.g. Datry et al., 2011). This is likely due to these studies investigating drought events in natural systems, which is obviously more challenging than in an experimental setting (discussed below). Additionally, the majority of studies are focused on relatively short, isolated seasonal drought events in summer (e.g. Schilef et al., 2009; Pinna et al., 2016), or autumn and winter (e.g. Foulquier et al., 2015; Monroy et al., 2016). Thus, a long-term supra-

seasonal study focused on multiple aspects of drought severity impacts on leaf litter decomposition is needed (Boulton, 2003; Lake, 2003).

Previous studies have demonstrated that the impact of drought on macroinvertebrate community structure varies both spatially and temporally among study sites (Sangiorgio et al., 2005). The linkage between leaf litter decay rates and macroinvertebrate community structure, shredder composition in particular, is uncertain. Some studies have shown that drought alters the decomposition process but that macroinvertebrate assemblage structure remains similar (Sangiorgio et al., 2005). In contrast, a different study found that drought altered macroinvertebrate community composition significantly, but there was limited variation found in leaf litter breakdown rate; this was put down to the resilience of the studied intermittent river ecosystem (Pinna et al., 2016). In another study, Datry et al. (2011) observed a reduced leaf litter breakdown rate and lower shredder abundance following flow reduction.

Most of the aforementioned studies were conducted in intermittent rivers where drought is a periodic hydrological event and thus easier to observe. The impact of drought on freshwater systems is likely to be contingent upon the annual hydrograph and the extent of flow permanence (Dai, 2013). For example, the biota of intermittent rivers characterised by regular flow cession are generally regarded as being particularly resistant to drought events (Leigh et al., 2016). Nevertheless, unpredictable droughts and increasing drought intensity may threaten the ecology of these intermittent waters (Sánchez-Montoya et al., 2018). For perennial rivers, which have been much less studied in the context of drought, unpredictable drought may exceed certain critical thresholds, with profound consequences for the structure and functioning of these systems. For instance, over half of perennial chalk streams in England are affected by drought and are particularly sensitive to low rainfall

(WWF, 2017) as their rich flora and fauna is less resistant to drought than communities found in intermittent river ecosystems (Langhans et al., 2006; Datry et al., 2011). Hence, there is an urgent need to improve our understanding of the impact of drought on freshwater ecosystem structure and function in perennial river ecosystems (e.g. perennial chalk streams).

In this chapter, the research gap identified above is addressed experimentally. A gradient of drought intensity and duration was created in a series (n=21) of artificial channels (aka mesocosms), and the macroinvertebrate shredder assemblages and leaf decomposition rate in these systems was assessed. Mesocosms represent an effective model system that can be controlled and replicated, and which are increasingly being used to study ecological responses to climate change (Stewart et al., 2013). Mesocosm use is underpinned by model-based reasoning to demonstrate the impact of a given phenomenon (i.e. drought) on a quantifiable response variable (i.e. decomposition rate) (Drake & Kramer, 2011). The experimental design used in the current study has been designed to form a gradient of flow reduction so that habitat heterogeneity (Boulton, 2003) and the response of leaf litter breakdown to drought can be determined. The drought process increases habitat heterogeneity, which in turn alters macroinvertebrate (particularly shredder) community structure and composition (Ledger et al., 2012).

Drought impacts can be partitioned into four critical stages based on the extent of habitat loss; these stages are reflected in the mesocosm experiment used in this study. The low intensity channel represents water loss Stage 1: dewatering leads to a decline in lateral connectivity and active surface flow resulting in increased temperature, oxygen decline and riparian habitat loss. Macroinvertebrates that favour fast flow and high oxygen concentration decrease and disappear at this stage. As the water volume falls, longitudinal connectivity

declines and pool habitats begin to form among shrinking, dewatering riffles, which is represented by median drought intensity artificial channels (Stage 2). At this stage, macroinvertebrate communities start to shift from lotic dominated taxa to lentic, and dispersal is constrained. At Stage 3, pool habitats are established completely, and become disconnected from the surrounding wet habitat by dry riffle substratum. Remaining biota are likely to be those adapted to lentic environments. At Stage 4 of drought intensification, surface water is essentially lost, leaving only moist sediment. The last two water reduction stages are represented by the high drought intensity channels in the mesocosm.

Based on the aforementioned points, the study hypotheses are:

H1: Drought duration (DD) and drought intensity (DI) will have additive effects on leaf litter breakdown rate (both shredder-mediated (k_s) and microbial (k_m));

H2: Shredder richness and abundance will display negative linear responses to DI and DD and thus decrease along the drought gradient. However, shredder richness will increase under increasing drought impact;

H3: k_s will be related to the shredding efficiency of the dominant taxa in the community; thus, shredding rate will be driven by the change in community composition rather than a change in shredder abundance.

6.3 Materials and methods

6.3.1 Leaf decomposition experiment set-up

English Oak (*Quercus robur*) leaves were used in the experiment as this is the predominant tree species in the study region. Leaves were collected from Birmingham Botanical Garden in November 2012 just after abscission. All the leaves were air-dried at

room temperature (20°C) and stored dry until needed. Coarse (5mm) and fine (0.1mm) nylon mesh bags were filled with 3.00 g of leaf litter (Petersen & Cummins, 1974). As leaching can influence air-dried leaf litter weight (Gessner et al., 1999), all leaf packs were pre-leached in water (2 hours) before placement in channels.

For the drought experiment, 1176 leaf packs (4 coarse and 4 fine bags per channel per sampling time) were constructed. The initial dry mass of leaves was 3.01 ± 0.009 g (mean ± SD). During the drought period, the leaf packs were placed and secured in each pool (a, b, c, d) in each of the 21 mesocosm channels on seven occasions between August 2013 (Month 1) and August 2014 (Month 13) (Table 6.1). Leaf litter bags were placed in the channels for 30 days and then retrieved. In addition, benthic macroinvertebrates samples were collected using a surber net (0.0225 m^2; mesh size 300 μm) on August and October 2013 and April and August 2014. Surber samples were collected from each pool (a, b, c, and d).

6.3.2 Laboratory work

All the leaf packs were placed in-situ for 30 days. After submersion, leaf packs were collected from the channels and carefully placed in polyethylene bags to avoid loss of leaf material, and then returned to the laboratory in a cool box and stored in a freezer (-18 °C).

In the laboratory, the contents of the litter bags were carefully removed. Whole leaves and identifiable leaf fragments were removed by hand and placed into a container. The remaining material was washed to remove inorganic material, and passed through a 250 μm mesh sieve. Macroinvertebrates retained on the sieve were collected and preserved in 70% industrial methylated spirit. Litter fragments were dried in an oven (70°C, 72 h) and re-

weighted to the nearest 0.001 g. Subsamples of the ground leaf material (250 mg) were ashed at 550 °C (2h) to estimate ash-free dry mass (AFDM).

During the experiment, 336 surber samples (4 surber samples per channel per sampling occasion) were collected. The macroinvertebrates were sorted and identified by a dissecting microscope. Taxa were assigned to coarse functional feeding groups, either shredder or non- shredder. Members of the shredder group were assigned based on various literature sources (e.g. Moog, 2002). All the macroinvertebrates in the shredder group were identified to the lowest feasible taxonomic level (i.e. all Diptera to genus, and 63.2% of all taxa to species) then counted. For each member of the shredder group, affinity to shredding was calculated as a percentage based on affinity scores taken from Moog (2002). This score enabled the shredders to be split into two sub-groups: specialist shredders (affinity > 60%) and facilitation shredders (affinity < 60%).

6.3.3 Data Analysis

Drought intensity quantification

The multivariate index of Drought Intensity outlined in Chapter 5 was used to quantify the intensity across the gradient.

Leaf decomposition calculation

The decomposition (breakdown) rate was modelled as a negative exponential decay function, an approach frequently used in leaf litter breakdown studies (Petersen & Cummins, 1974; Gessner, 1999):

$$M_t = M_0\, e^{-kt} \quad (1)$$

where M_t is the original mass remaining at time (t), M_0 is the initial mass and k is the decomposition rate. The k-value was calculated using equation (1), and 3 breakdown coefficients, total decomposition rate (k_t), shredder-mediated decomposition rate (k_s) and microbial decomposition rate (k_t) were calculated separately to partition microbial and macroinvertebrate leaf litter breakdown (Hieber & Gessner, 2002).

Macroinvertebrate assemblage descriptors

In order to minimize the impact of the artificial channel length gradient, the shredder community composition, and the mean abundance and mean richness of shredders were averaged across 4 surber samples (Graça, 2001). In addition, taxonomic diversity was calculated using the Shannon index (*H'*), Pielou's evenness index (*J'*) and Magelef diversity index (*d*). Each metric was calculated per surber sample (Marini et al., 2013; Pinna et al., 2016).

The Shannon index (*H'*) is a common diversity index in ecological studies (e.g. Ollivier et al., 2018; Wan et al., 2018) and is calculated using the following equation:

$$H' = -\sum_{i=1}^{R} P_i \ln P_i \quad (2)$$

where P_i is the proportion of characters belonging to the *i*th type of letter in the string of interest. In ecology P_i is often the proportion of individuals belonging to the *i*th species in the community of interest. Pielou's evenness index (*J'*) is a ratio of a relatively stable index

to quantify how equal the community of interest is. The index is calculated by the following equation:

$$J' = \frac{H'}{\ln S} \quad (3)$$

where *H'* is the Shannon index, and *S* is the total number of species in the community. *J'* is constrained between 0 and 1. The lower the *J'* value, the less even a community is.

The Margalef diversity index (*d*; Margalef, 1958) is a useful diversity index to examine the ecological status of water bodies (Gamito, 2010), and is calculated using the following equation:

$$d = \frac{S - 1}{\ln N} \quad (4)$$

where *S* is the number of species and *N* is the total number of individuals in the surber sample. *d* is a simple index that is sensitive to changes in both species' evenness and the relative influence of the dominant species (Gamito, 2009).

Statistical Analysis

Decomposition rate analysis

Total decomposition rate (k_t) consists of the shredder-mediated decomposition rate (k_s) and the microbial decomposition rate (k_m). This chapter focuses on both k_s and k_m to determine how they vary according to DI and DD (Figure 6.1, 6.2).

The decomposition rate analysis was divided into two parts. Due to the repeated measurement of each channel over the experiment, a generalized least squares regression

(GLS) model with a compound correlation structure was adopted to handle the auto-correlation of the residuals (Pinheiro & Bates, 2000, Zuur et al., 2009). First, GLS models were fitted (package *nlmn* 3.1-131, Pinheiro et al., 2017) using the response variables k_s and k_m, with two explanatory variables in each case, DI and DD. Second, GLS models were fitted using the response variable k_s, with the biological descriptors, shredder abundance, richness, *H'*, *J'* and *d* used as explanatory variables. As k_m is the microbial decomposition rate, it does not relate to shredding. Hence, the biological descriptors were not used to explain k_m.

Response variable k_s was $log_{10}\ (X+0.001)$ transformed to ensure it was normally distributed, and k_m was $log_{10}\ (X+0.1)$ transformed for the same reason. Abundance and richness were standardized by:

$$x_S = \frac{x - Min(x)}{Max(x) - Min(x)} \quad (4)$$

where x_s is the standardized data, and x is the raw count data.

Then, the best model was chosen from a selection of five models (DI + DD, DI * DD, DI, DD and an intercept-only null model) using AIC. For the analysis including the biological descriptors, the approach was similar (i.e. the five models: Abundance + DD, Abundance * DD, Abundance, DD and Null model). The AIC, delta AIC (dAIC) and Pseudo R^2 (package: *piecewiseSEM* 1.2.1, Lefcheck, 2016) were calculated for each model (Nakagawa et al., 2013). The best model (dAIC =0) was selected and carried forward for further analysis. Model residuals were inspected using a suite of graphical tools to check various assumptions (e.g. normality and homogeneity of variance) were not violated (Zuur et al., 2010).

Macroinvertebrate assemblage analysis

Ordination was used to assess variation in shredder community composition along the drought intensity gradient. Firstly, shredder abundance data were log_{10} *(X+1)* transformed to ensure normality. Secondly, non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity (Furse et al., 1984; Clark & Warwick, 2001; Pinna et al., 2016) was used to compare taxonomic similarity and shredder community composition at the start point (August 2013) and end point (August 2014) of the drought intensity gradient (using package: *vegan* 2.4-4, Oksanen, 2017). The Spearman's correlation between each taxon's abundance and the scores for the first two ordination axes was also calculated.

Generalized Estimating Equation (GEEs) models were used to analyse the biological response variables, shredder abundance and richness, alongside two explanatory variables, DI and DD. GEEs were used as they enable model fitting with a range of distributions and correlation structures (Halekoh et al., 2006; Zhang et al., 2012). A Poisson distribution was used as the shredder abundance and richness data are nonnegative count data. In this model, the main explanatory variable was DI, and DD was assigned as an 'id' as a dummy environmental code (e.g. DD = month 1 was signed as id =1) to distinguish the different duration lengths. The R package *geepack* (1.2-1) (Højsgaard et al., 2016) was used to apply the Poisson family for the response variables.

All statistical analyses were undertaken using R (Version 3.5.0, R Core Team). The significance level for all statistical analyses was set at $p < 0.05$.

6.4 Results

6.4.1 Decomposition rate

Partitioning the total decomposition rate

At the beginning of the experiment, k_s was the major component of k_t, being 5.05 times higher than k_m. In contrast, at the end of the experiment k_t was maintained by k_m, with k_s only representing 26 % of k_m (Table 6.2).

6.4.2 Shredder-mediated decomposition

There was a general trend of decreasing *ks* with increasing DD (Figure 6.2). k_s was highest after a relatively short drought duration (0.0187± 0.0010 after 1-month DD and 0.0136 ± 0.0009 after 3 months DD, mean ± SD). Shredding activity was then severely impaired following 5 months of drought (mean ± SD: 0.0002 ± 0.0002) and stayed low with only minor fluctuations for the remainder of the experiment (i.e. k_s<0.0026).

Shredder activity was severely affected by drought intensity, with a k_s of 0 in the most intense drought channels (DI>0.7) at every sampling period (Table 6.2, Figure 6.1).

The optimum model structure for k_s, based on AIC, consisted of an additive combination of DI and DD (R^2 = 61.8%, Table 6.3). The slope of DI was -0.52 ±0.52 (slope ± SE), highlighting the negative impact of DI on k_s. The best model did not include an interaction between DI and DD, suggesting that the impact of drought intensity on k_s was consistent and did not interact with drought duration (Table 6.4). This suggests that changes at DI = 0 were likely driven by seasonal variability; the decomposition rate (e.g. k_s) in the control non-impact channels (DI=0) can be considered as representing the basic decomposition rate in the mesocosms.

However, as the DD slope shifted with DD, drought duration altered the decomposition rate across the whole study period (Table 6.4). After a short drought duration (1 month to 3 months), there was a slight negative impact on k_s (DD slope = -0.19). After 5 months of drought, the DD impact increased (DD slope = -2.66). After 9 months, the DD slope reduced to -1.13, before increasing again to -2.46 (Table 6.4; Figure 6.3). At the end of the experiment, k_s had again increased, but was still 1.75 times lower than the *ks* value at the beginning of the experiment (Table 6.2; Figure 6.1).

6.4.3 Microbial decomposition

The variation in k_m along the drought gradient was more complex. The lowest k_m was found at the beginning of the experiment (0.0037 ± 0.003, mean ± SD), and the peak k_m was found after 5 months drought impact (0.0101 ± 0.0002). It then dropped to 0.0065 ± 0.0002 after 1 year of drought (Table 6.2, Figure 6.2).

The model selection based on AIC suggested that the variability in k_m was also best explained by an additive combination of DI and DD during the 1-year study period (Table 6.3), and 52.3% of the variance could be explained by this model. Over the drought experiment period, the DI slope was -0.0020 ± 0.0024 (slope ± SE), highlighting that k_m was significantly negatively related to DI (Table 6.4; Figure 6.4; $p < 0.05$, GLS). The variation of the intercept associated with DD highlights the impact of drought duration, and to some extent seasonality, on k_m (DI = 0). The DD slope increased from 0.0229 at the beginning of the experiment to 0.0598 after 5 months, and then remained relatively stable and low until the end (k_m slope > 0.007; Figure 6.4).

6.4.4 Benthic shredder assemblages

Bio- descriptors

A total of 10,353 shredder individuals belonging to 19 taxa were identified from the 21 channels (Table 6.6). Shredder abundance decreased during the 1-year drought event (Table 6.6, Figure 6.5). The maximum shredder abundance was recorded after 2 months (208.44±17.20 individuals) of drought, and the lowest abundance was found after 9-months (25.27±3.80 individuals), which represented 21 % of the abundance at the beginning of the drought experiment. At the beginning of the experiment, specialist shredders were the dominant shredder group (96% of total shredder abundance), but after 1 year of drought this group only constituted 1% of total shredder abundance. No major changes in shredder richness were observed. However, interestingly the peak *H'* and *J*' were found after 1 year of drought, meaning that after a long-term drought disturbance, the shredder assemblage became more even (Table 6.6; Figure 6.6, 7, 8).

According to the GLS model selection using the biological predictors, the best model with k_s as the response variable included an interaction between shredder abundance (indiv.) and drought duration (Table 6.9; Figure 6.9; p-value<0.05, GLS). k_s was negatively and significantly related with *J'*, and the impact of *J'* had an interaction with drought duration (Table 6.9).

In regard to the GEE model analysis, there was no significant relationship between abundance (indiv.) and DI (p >0.05, GEE; Table 6.8). DI had no significant impact on taxa richness or shredder abundance (p > 0.05, GEE; Table 6.8). However, DD significantly decreased shredder abundance and richness (p <0 .001, GEE; Table 6.8).

Shredder community structure

The NMDS ordination method was applied after 1 month and 1 year of the experiment. Compared with the NMDS ordination at the 1 month point (stress=0.11), the shredder community became more dissimilar after 1 year of drought (stress = 0.09).

After 1 month of drought, DI was only weakly correlated with axis 1 of the ordination ($p < 0.05$, Spearman's ρ=0.14), and hence there was no clear relationship between changes in community structure and DI (Figure 6.11(A)). After one year of drought, shredder composition varied considerably across the drought gradient (Figure 6.11(B)). Additionally, DI was significantly negatively correlated with axis 1 ($p < 0.05$, Spearman's ρ =-0.60).

The correlation between taxa abundance and the 2 ordination axes was also calculated (Table 6.10; Figure 6.12). There was an obvious difference between 1 month and 1 year shredder community composition. After 1 year of drought, shredder abundance decreased sharply, but richness increased and shredder assemblages became more even.

DI was not correlated with axis 1 at the 1 month duration point, but low DI sites did contain more specialist taxa (Table 6.10; Figure 6.12(A)). *Tipula* was negatively correlated with axis 1 ($p < 0.05$, ρ =-0.44; Table 6.10). *G. Pulex* and *S. personatum* were positively correlated with axis 1 ($p < 0.05$, $\rho > 0.4$ in both cases; Table 6.10). Obligate taxa were the only shredders recorded in the high DI channels (DI>0.7). For facilitation taxa, *P. antipodarum* was strongly negatively correlated with axis 1 ($p < 0.05$, ρ =-0.84; Table 6.10).

The ordination of the experiment end-point data indicated substantial changes in taxa composition. Facilitation shredder taxa was now the dominant shredder group. The specialist taxa *S. personatum* and *G. Pulex* were highly correlated with axis 1 (ρ >0.6 in both cases; Table 6.10). The facilitation shredder taxa *Pericoma* and *Oxycera* were negatively correlated

with axis 1 ($\rho < -0.4$ in both cases; Table 6.10). *N. picteti* and *D.annulatus* were positively correlated with axis 1 ($\rho > 0.4$ in both cases; Table 6.10). With the exception of *Pericoma* and *Oxycera*, the abundance of all taxa decreased with increasing DI (Figure 6.12 (B); Table 6.10). In contrast with the 1 month period ordination, the facilitation species *P.antipodarum, Oxycera sp., S.palustris* and *Tipula sp.* were still present in the high DI sites.

6.5 Discussion

Climate change induced increases in drought are expected to alter freshwater ecosystems physically (e.g. flow connectivity reduction), chemically (e.g. dissolved oxygen reduction) and biologically (e.g. decreases in macroinvertebrate density). The aim of the present experiment was to address the current lack of investigations examining the effects of drought on leaf litter decomposition in lotic ecosystems. The principal objective of the study was to determine if drought intensity (DI) represents a key control on an important aquatic ecosystem function (decomposition process), and also on community structure (particularly shredder assemblages). DI and drought duration (DD) were examined individually and in combination.

6.5.1 Long term effect of drought on the decomposition rate

As expected, the hypothesis (H1) has been proved. DI decreased both the shredder-mediated (k_s) and microbial (k_m) decomposition rate. Additionally, the leaf litter breakdown rate was negatively related to DD. Overall, these results suggest that DI and DD are both key factors controlling the decomposition process during drought.

Many previous studies have demonstrated that flow reduction slows down the decomposition process, an observation that has been recorded in natural rivers (Datry et al., 2011; Chessman, 2015), lab experiments (Leberfinger et al., 2010), and mesocosm systems (Schief et al., 2009). The study of Hutchens et al. (2002) demonstrated that leaf litter breakdown was primarily driven by flow volume. Moreover, Datry et al., (2011) found that decomposition rate may reduce with decreasing flow permanence, although this relationship may not be linear. In addition, the physicochemical conditions of water impacted decomposition rate in the same study. In this experiment, the DI gradient represents variation in a number of factors related to drought, such as water depth, water physicochemical condition and habitat condition. The results suggest that decomposition rate is sensitive to even minor reductions in water availability, and that the impacts of increased water reduction and water quality deterioration on the decomposition rate are substantial.

Drought duration (DD) is also an important aspect of drought. DD has been examined in preview studies. However, these have been isolated-season studies (e.g. summer), which lacked a long-term view of drought duration impact on decomposition rate. The results of the present study indicate that increasing DD significantly affects the decomposition process. As drought impact is an accumulation process, the impact of drought likely needed 2 or 3 months to exceed the critical resistance of the ecosystem at the beginning of the water reduction period (Lake, 2011); this could explain why the reduction in decomposition was lower in the first two months, and with increasing drought duration the impact of drought became more serious (Bogan et al., 2015). The duration of drought is an important factor that should be evaluated in more detail in future research (Lake, 2011).

It was also observed that there was no statistical interaction between DI and DD (that is, the model without an interaction provided a better fit to the data). This implies that DD has no impact on the DI level.

Although both k_s and k_m were reduced by drought, the pattern of each decomposition rate–drought relationship was different. The decomposition process depends on biotic activity (Pinna et al., 2016), which could explain why at the beginning of experiment, the decomposition function was dominated by shredding activity.

Shredder-mediated decomposition rate, which exhibited a similar pattern to the total decomposition rate, was reduced with increasing drought intensity and duration. In each sampling occasion, the shredder-mediated decomposition rate was negatively correlated with drought intensity. In all drought treatment channels, the shredder-mediated decomposition rate was lower in comparison to that in the control treatment. Thus, the shredding process was very sensitive to water flow variation. A similar result was reported by Northington et al. (2017), who observed that even a minor water reduction alters community- and ecosystem-level shredder density and thus influences decomposition in freshwater ecosystems.

The pattern of microbial decomposition rate differed from the shredder-mediated decomposition rate. The results indicated that the microbial decomposition rate decreased with increasing drought intensity; however, with increasing drought duration, mean microbial decomposition rate increased. Allison et al. (2013) found that drought reduced microbial abundance and changed microbial community structure, which could explain why microbial decomposition process declined in the high drought intensity channels in this study. In addition, Allison et al. (2013) found that drought makes N-addition micro-habitat.

Hence, microbial adaptive mechanisms can make microbes decompose leaf litter more effectively, which could explain why, during the drought period, the mean microbial decomposition rate increased. Another possibility for this observation is that, as shredding declines and special habitat becomes established (e.g. isolated pool; Lake, 2011), an increase in food resources may lead to higher microbial decomposition activity. Thus, after long-term drought, the decomposition process was found to be maintained by microbe activity.

As shredder-mediated decomposition declined and microbial decomposition increased, during the drought period, the total decomposition process was maintained by microbial decomposition towards the end of the experiment (see also Pinna et al., 2016).

6.5.2 Long- term drought impact on macroinvertebrate assemblages

In contrast to hypothesis H2, there was no significant relationship between DI and macroinvertebrate abundance and richness. However, shredder assemblages did show significant responses to DD.

According to Lake (2011), a drought event usually takes 2-3 months to establish. Thus, the two-month duration (month 1 to month 3) of water reduction in this experiment can simply be recognized as a short-term sudden flow reduction period. After two months of drought, the shredder abundance did not decrease as expected. Indeed, it increased, even in the high drought intensity channels. This result may in fact be an artefact of the sudden water reduction increasing macroinvertebrate density in the remaining wet areas, rather than total abundance in the system. The limited substratum design of the mesocosms provided an extreme restricted hypho refuge area for macroinvertebrate, due to the extreme sudden water reduction (Boulton, 2003). The extreme change in habitat availability may have led to the

surviving shredders collecting in the remaining wet areas, such as isolated pools, leading to increased density. Thus, sampling from these remaining wet areas may have resulted in an apparent increase in abundance. This can be thought of as an emergency response mechanism by shredders, resulting in increased abundance in the remaining wet areas (density), but reduced abundance across the system, in high drought intensity channels after short-term drought (Acuña et al., 2005).

Compared with the previous result, after a short-term drought (2 months), the composition of the shredder group was changed significantly and the exact response differed across treatments. In the high drought intensity channels, the shredder community was completely different from that in the low drought intensity channels, where there was no common shredder species present in high intensity channels. This result indicates that the extreme sudden water reduction reduced common shredder species immediately.

Certain shredder taxa (e.g. *Gammarus pulex*) were particularly sensitive to the change in flow conditions and potentially represent useful indicator macroinvertebrate taxa. In the low drought intensity channels, after 2 months of drought, *G. pulex,* a core shredder species in English chalk streams that prefers high flow speed (Wood et al., 1999), was found to decrease in abundance, whilst *A. aquaticus*, a low water velocity species, increased in abundance. As such, *A. aquaticus* replaced *G. pulex* as the most abundant shredder in the flowing channels.

In the intermediate drought channels, after 2 months of drought, channel morphology was changed to a greater degree than in the low drought channels (Dewson et al., 2007; Lake, 2011). In these channels, specialist shredders died out, and taxa with high drought tolerance (e.g. Gastropoda) and facilitation shredders started to replace the lost specialist

shredders (e.g. *G. pulex*). For instance, shallow, well aerated pools were established, which provided a suitable habitat for Gastropoda (e.g. *S. palustris*).

In the high drought intensity channel, after 2 months of drought, the channel dried out and the facilitation shredders had completely replaced the specialist shredders. Only the taxa with the highest tolerance for drought survived, such as Diptera. These results highlight how drought impacts shredder diversity and composition even after only a short time period (Boulton, 2003; Ledger et al., 2012).

As expected, after long-term drought (8 months and 1 year) shredder abundance and richness was significantly reduced; a pattern that has been observed in several previous studies (e.g. Datry et al., 2012). A novel aspect of the present study is that it indicates that both drought intensity and drought duration are important factors driving shredder community structure in freshwater streams (see also Boulton, 2003; Wood et al., 2010). The impact of drought duration on shredder composition was complex. Compared to the short-term drought impact, long-term water loss reduced aquatic habitat area, substantially altered water quality, and reduced the available food resources; together these led to the observed reduction in shredder abundance and richness (Dewson et al., 2007). The regression analysis indicated that DI had no significant impact on shredder abundance and richness. However, a focus on abundance and richness masked changes in community composition (see White et al., 2016). After long-term (8 months and 1 year) drought, specialist shredders were lost due to reduced water volume (high DI channel) and water quality (low DI channel). However, the lost taxa were replaced by facilitation shredders with high drought tolerance (e.g. *Tipula*). This perhaps indicates an ability of the system to respond to drought events, although it should be noted that shredder-mediated decomposition rate declined with DI and DD. It also suggests that integrated biological assessment should be used in ecological

studies of this nature, rather than specific species assessment, which has been widely used in previous river physio-chemistry surveys (e.g. Wei et al., 2009; Ormerod et al., 2010).

6.5.3 Relationships between biotic variables and shredding

As predicted, k_s was strongly related to the shredding efficiency of the dominant taxa. Higher shredder abundance resulted in a greater decomposition rate, but an unexpected finding was that after 2 months of drought disturbance k_s was negatively correlated with abundance. As outlined above, this may be the point after the sensitive specialist shredders have been extirpated under short-term drought disturbance, resulting in specialist shredder abundance reducing dramatically, but before the new shredder community has become established, thus reducing shredding efficiency. Overall, these results show that shredder abundance is an important factor influencing the decomposition process in aquatic ecosystems; however, it is not necessarily always the primary influencing factor.

The positive relationship between *ks* and Pielou's evenness index indicates that higher shredding is found in less even shredder communities. This suggests that the high shredding efficiency of highly abundant specialist species (e.g. *Gammarus pulex*) is the main driver of decomposition in undisturbed streams, which has previously been observed in natural English chalk streams (Wood et al., 2000; Leberfinger et al., 2010).

6.6 Reference

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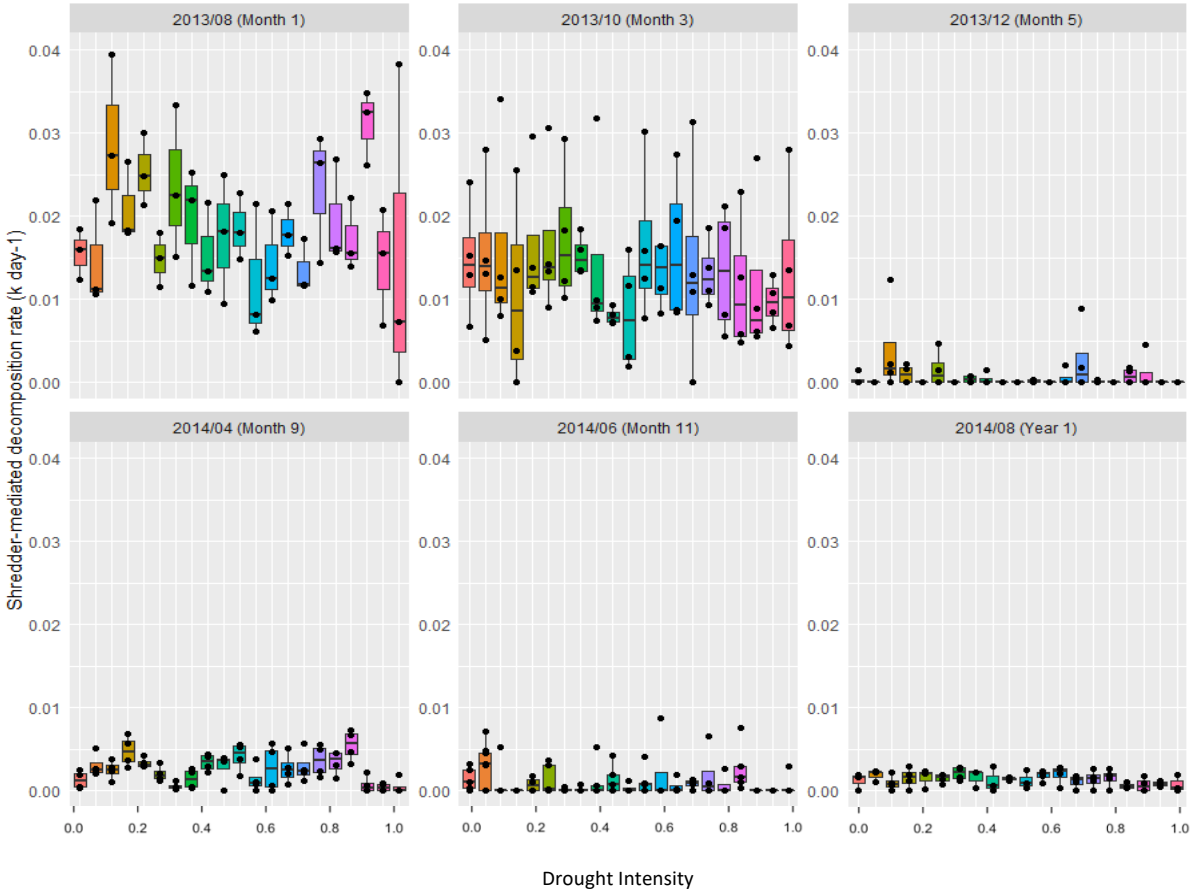

Figure 6.1: Shredder-mediated litter decomposition rate variation (k day^{-1}) during different stages of the drought experiment. On each sampling occasion, and for each channel, 4 leaf-pack samples were analysed.

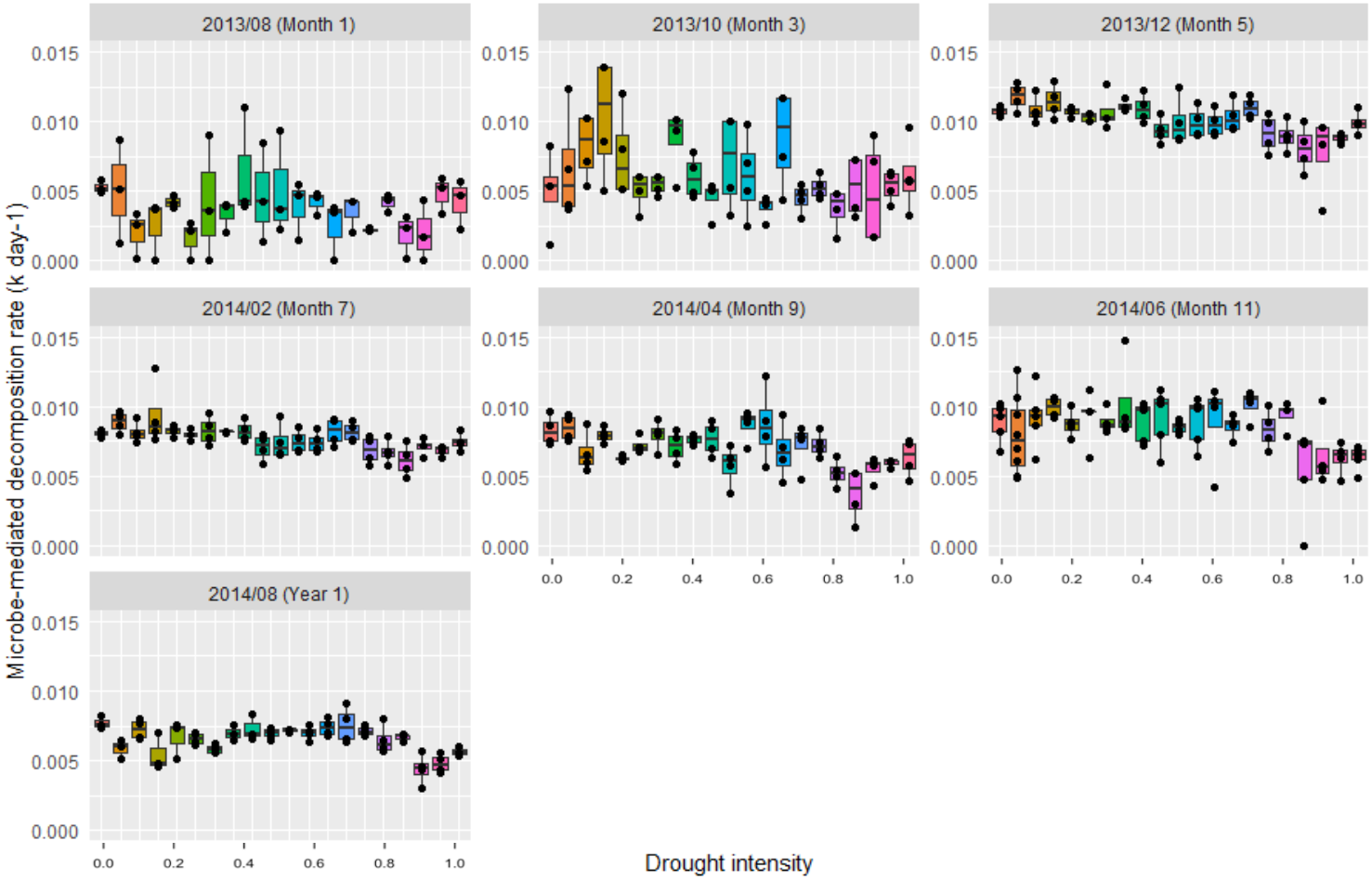

Figure 6.2 Microbe-mediated litter decomposition rate variation (k day^{-1}) during different stages of the drought experiment. At each sampling occasion, and for each DI level, 4 leaf-pack samples were analysed.

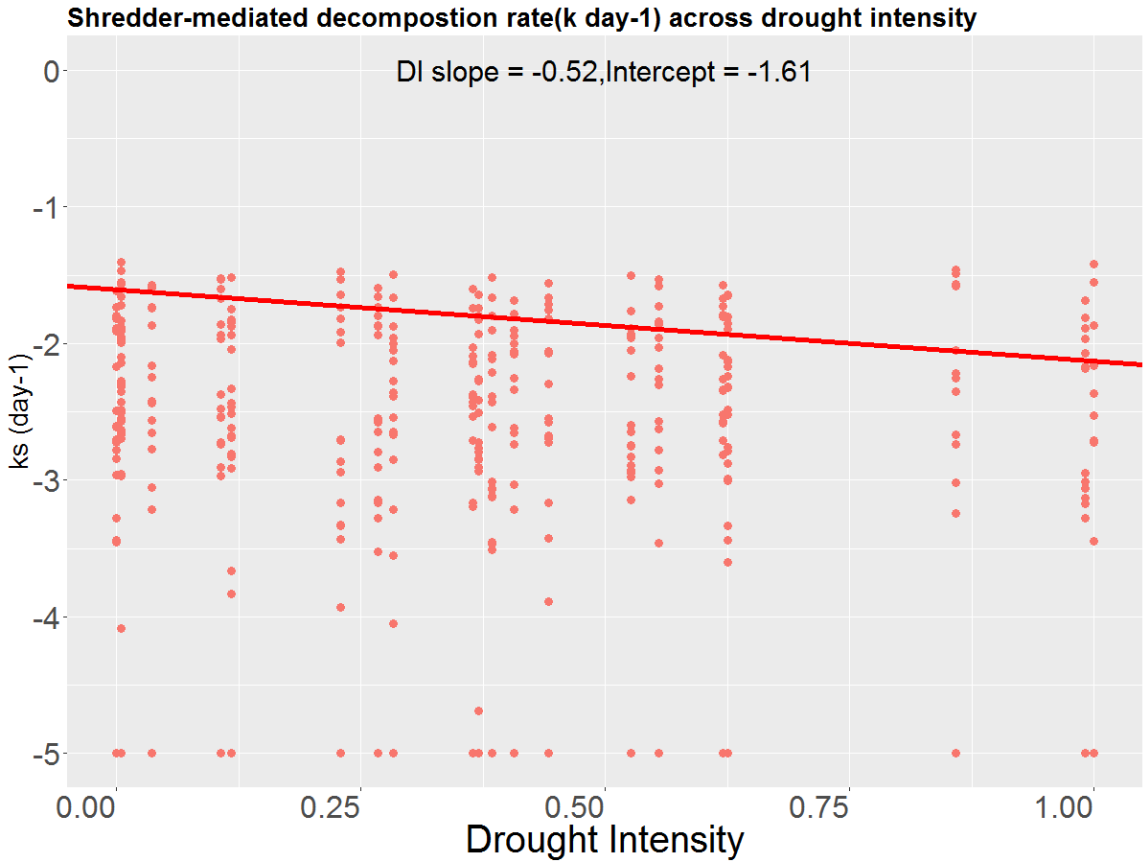

Figure 6.3: GLS model result of the shredder-mediated decomposition rate (k day^{-1}) across the drought intensity gradient, during a 1 year drought duration. Ks is the shredder-mediated decomposition rate. Figure shows the data from all sampling occasions, the red dots are the data for each drought intensity. The red line is the slope for the specific sampling occasion.The coefficient and intercept are present on each figure.

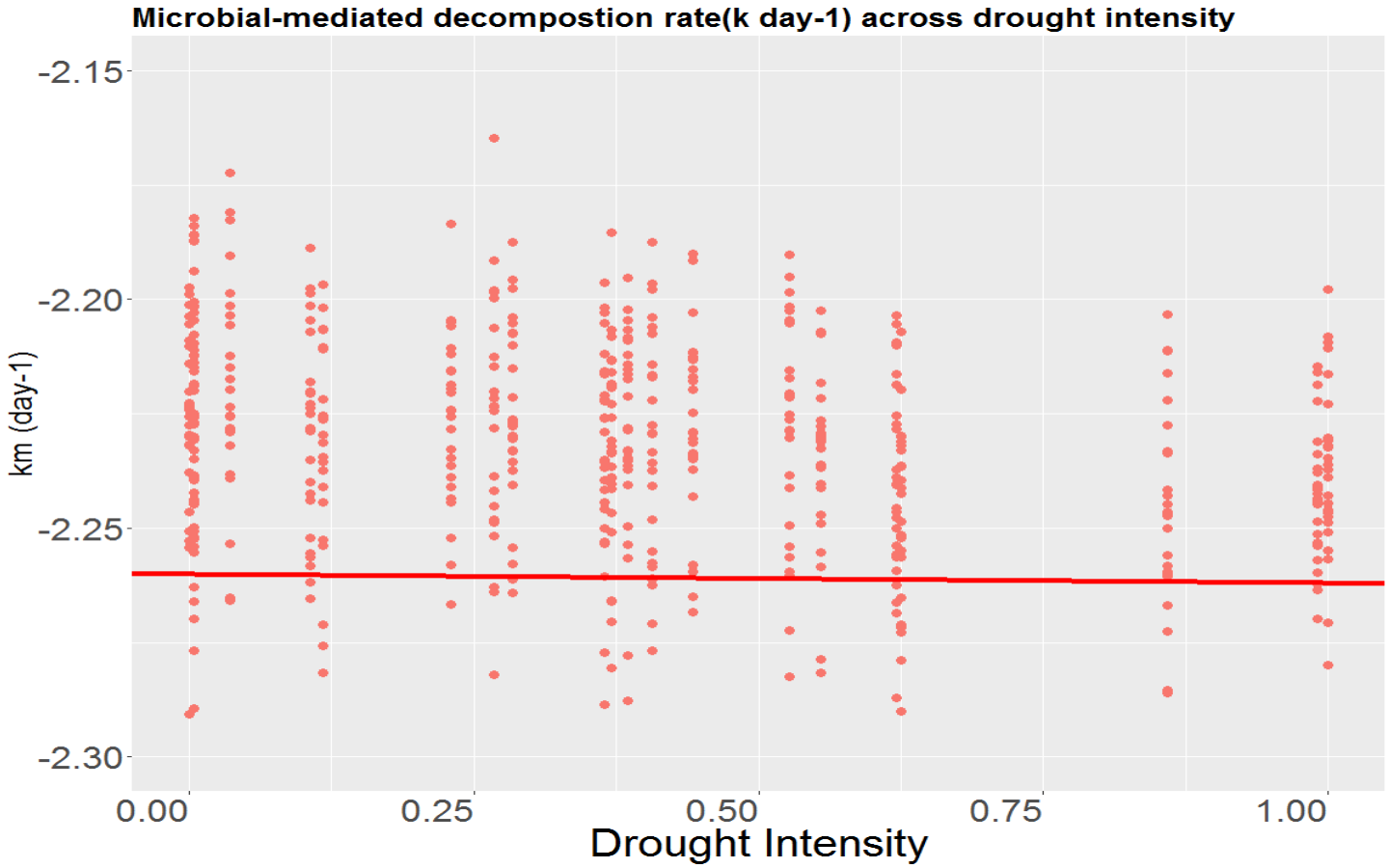

Figure 6.4: GLS model result of microbial decomposition rate (k day^{-1}) across drought intensity. Km is microbial decomposition rate. Every figure shows the data from all sampling with the red. The red line is the slope for the specific sampling occasion.

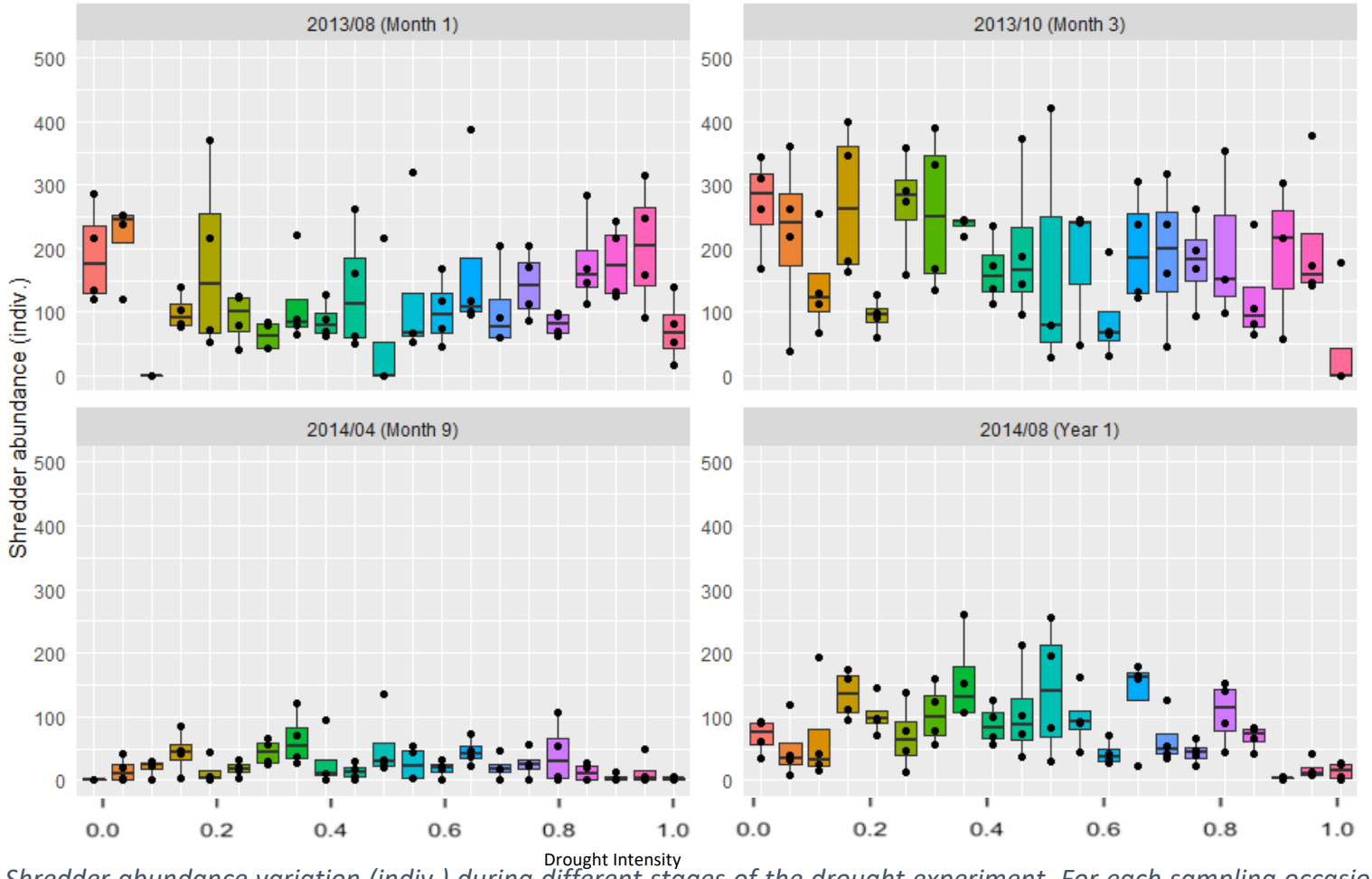

Figure 6.5: Shredder abundance variation (indiv.) during different stages of the drought experiment. For each sampling occasion, the box for each DI level channel (21) was based on the analysis of 4 surber samples (see dots for each individual surber value).

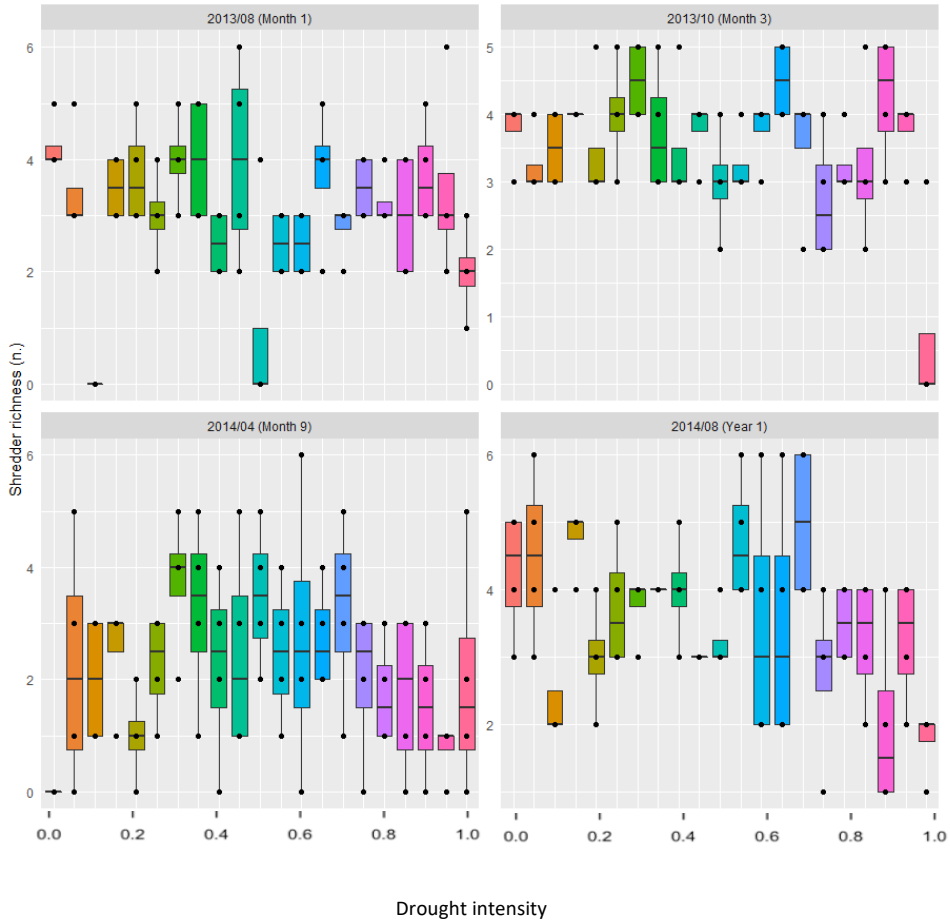

Figure 6.6: Shredder taxa richness (n.) variation during different stages of the drought experiment. For each sampling occasion, the box for each DI level channel (21) was based on 4 surber samples.

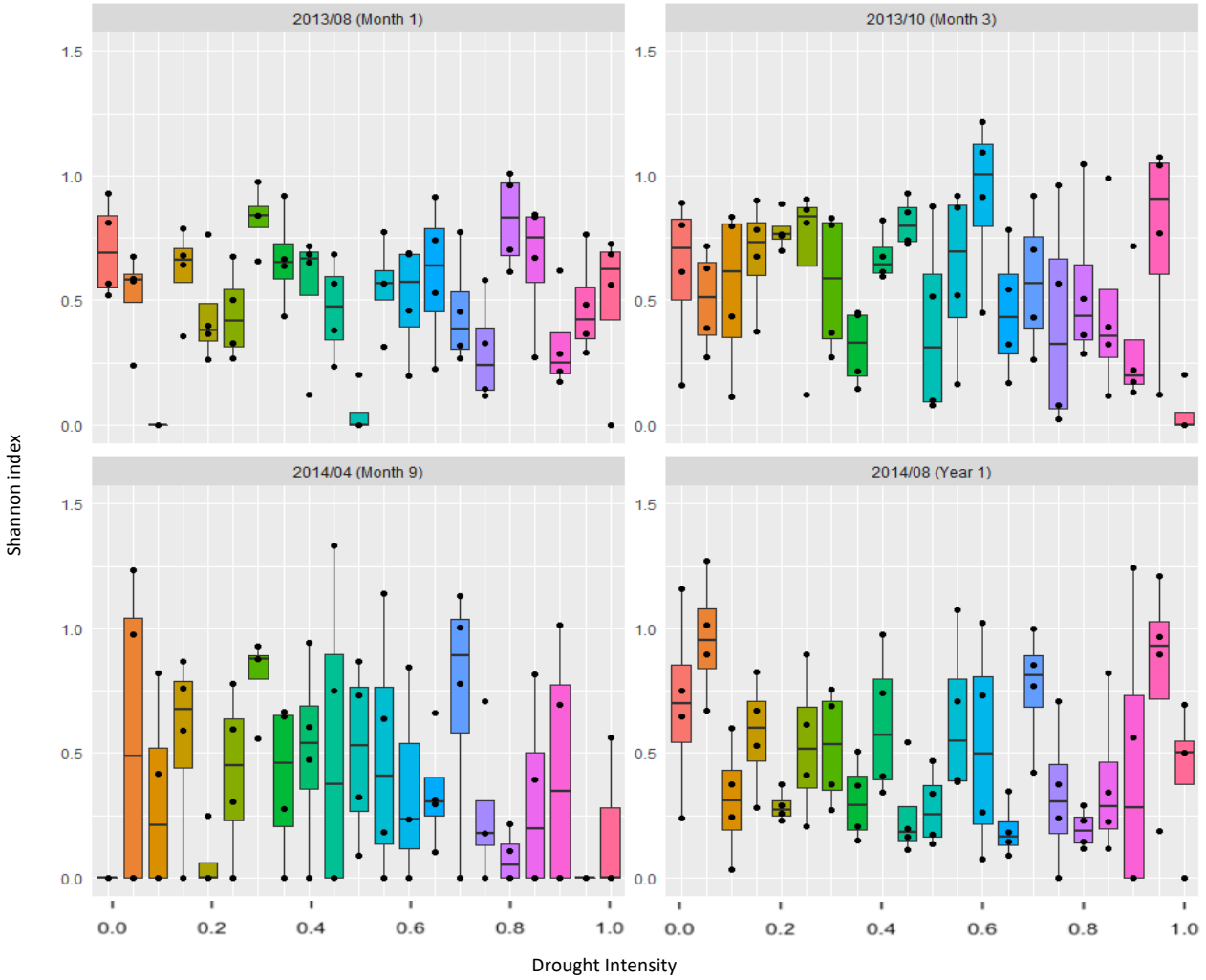

Figure 6.7: Shredder Shannon index variation during different stages of the drought experiment. For each sampling occasion, the box for each DI level channel (21) was based on 4 surber samples.

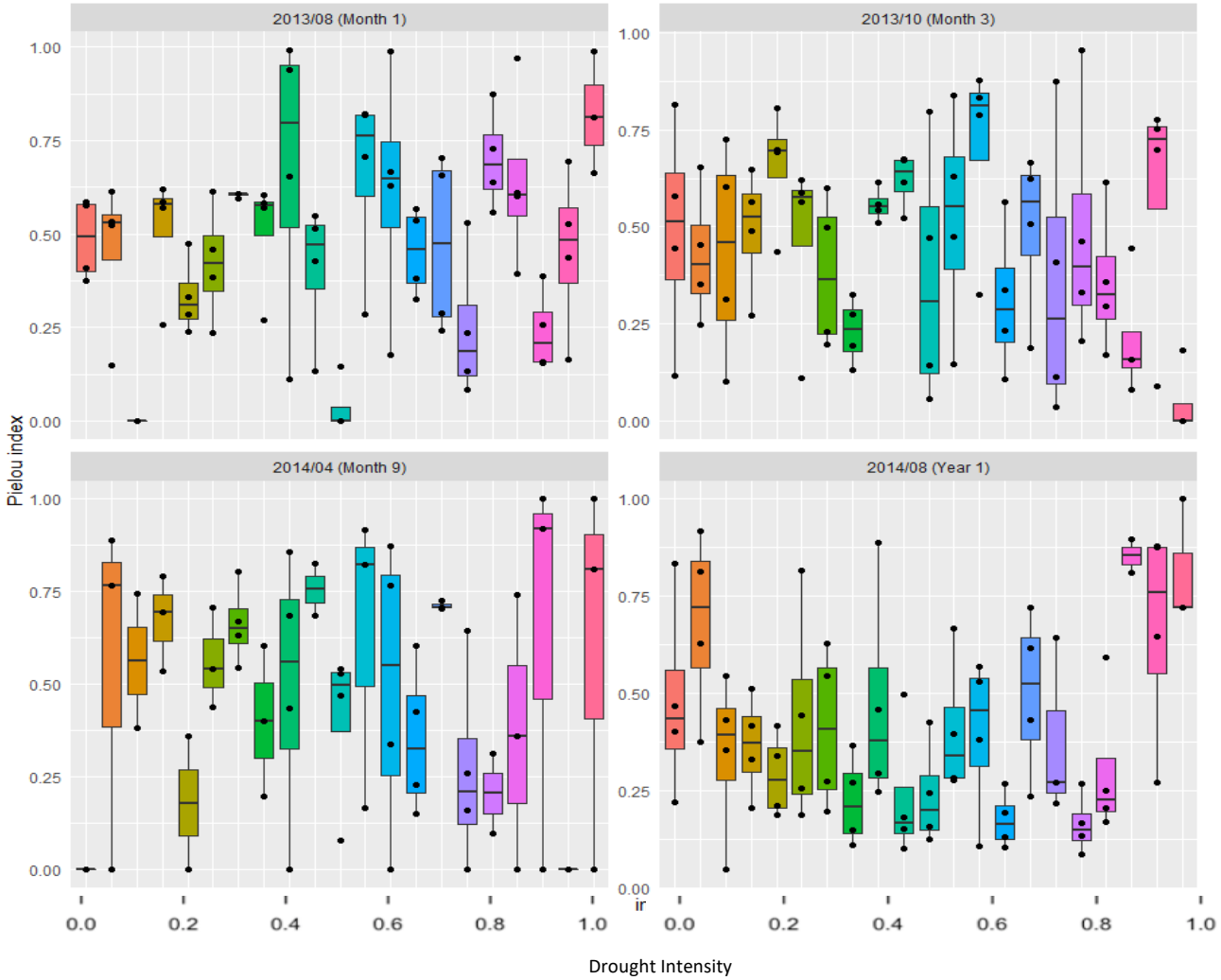

Figure 6.8: Shredder Pielou's evenness index variation during different stages of the drought experiment. The box for each DI level (21) was based on 4 surber samples.

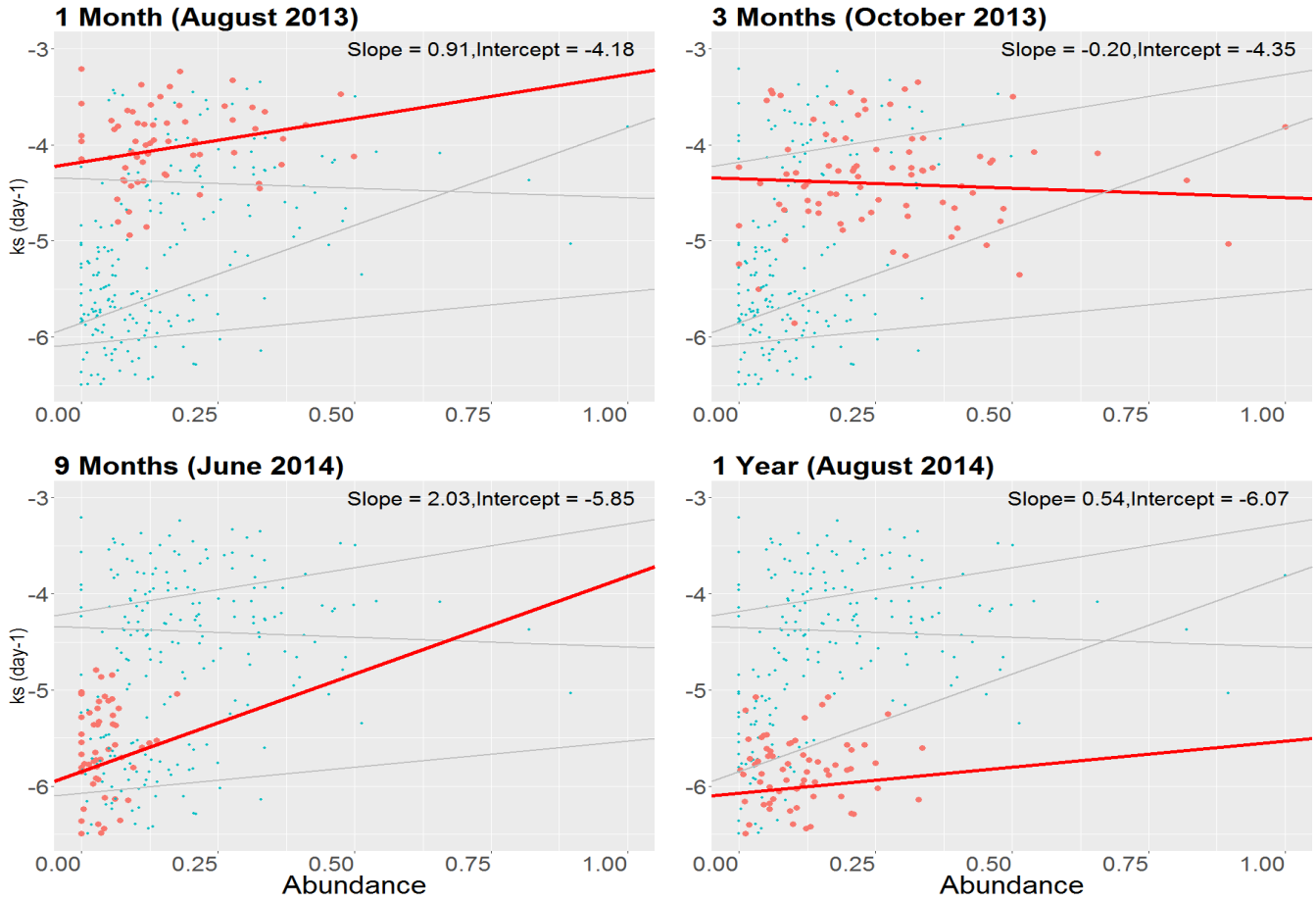

Figure 6.9: GLS model result of shredder-mediated decomposition rate (k day^{-1}) against abundance. Ks is the shredder-mediated decomposition rate. Each figure shows the data from all sampling occasions. Abundance was standardized within the range (0, 1). Red dots are the data at current sampling occasion, blue dots are the data from other sampling occasions. The red line is the GLS model at the current sampling occasion, and the grey lines are the rest GLS model result. The coefficient and intercept are present on each figure.

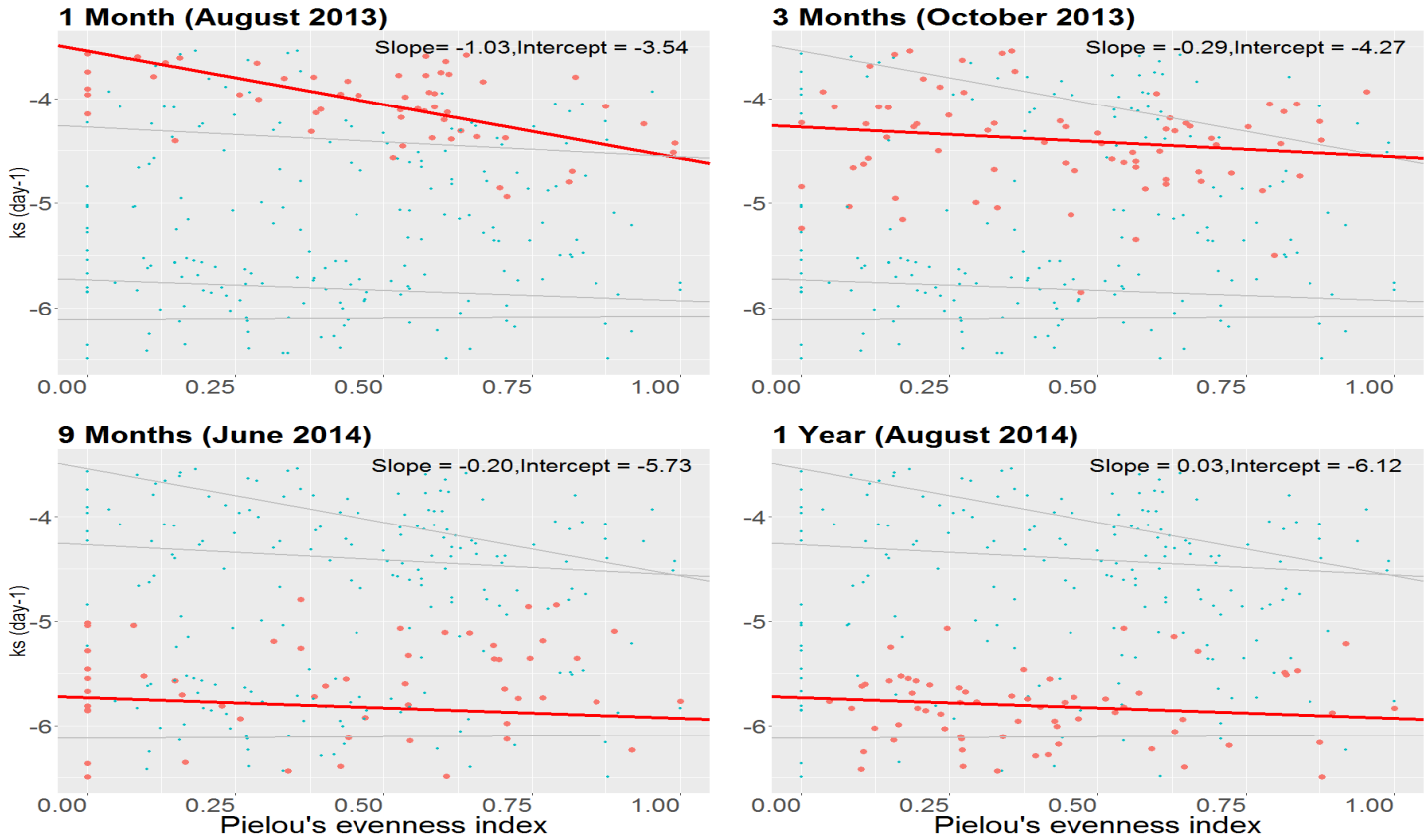

Figure 6.10: GLS model result of shredder-mediated decomposition rate (k day^{-1}) against Pielou's evenness index. Ks is the shredder-mediated decomposition rate. Each figure shows the data from all sampling occasions and all GLS models fit by (Pielou's evenness index + DT). Red dots are the data at current sampling occasion, blue dots are the data from other sampling occasions. Red line is GLS model at current sampling occasion, and grey lines are the rest GLS model result. The coefficient and intercept are present on each figure.

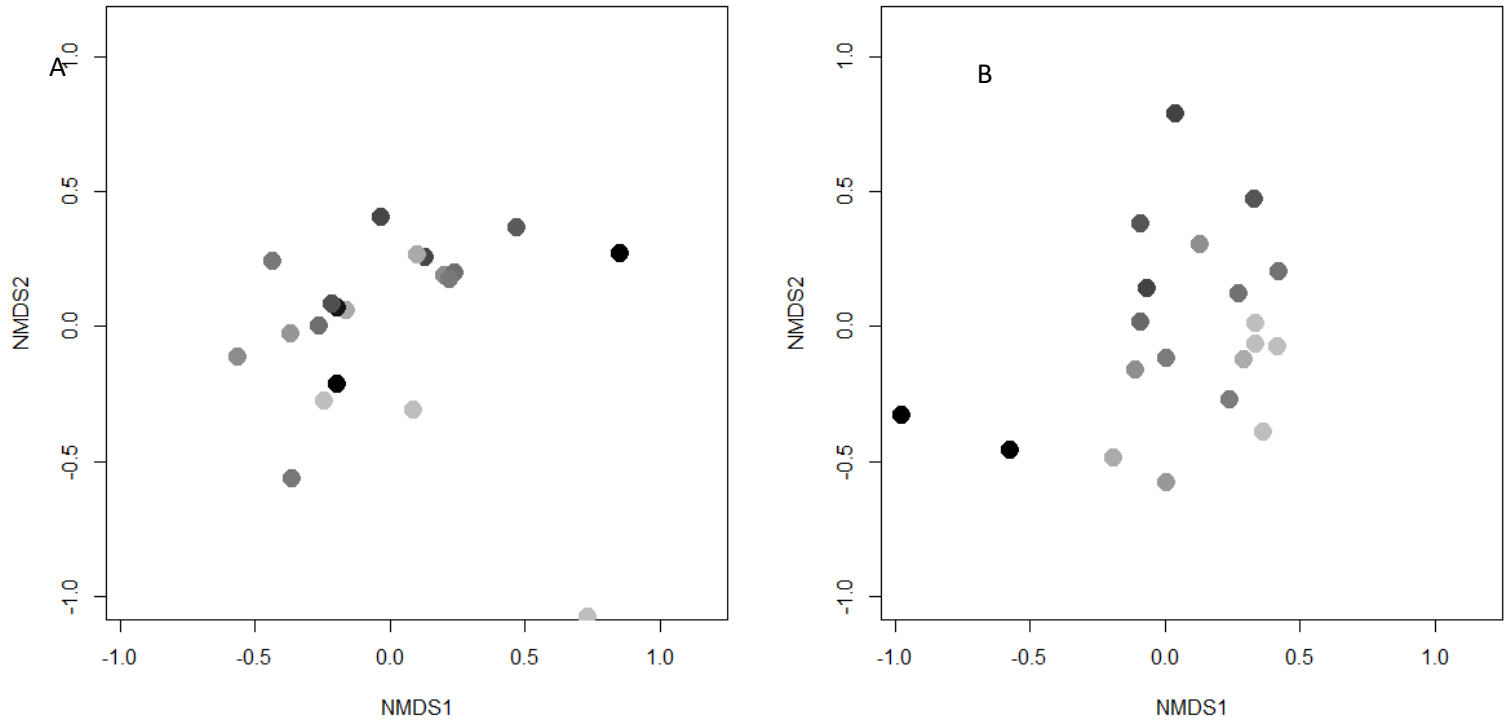

Figure 6.11 Non-metric multidimensional scaling (NMDS) biplots showing variation in the composition of mesocosm channels as a function of DI. Each point is a site, darker colour presents higher DI: (A) 1 Month duration, (B) 1 Year duration. Mean abundance data and Bray-Curtis similarity were used.

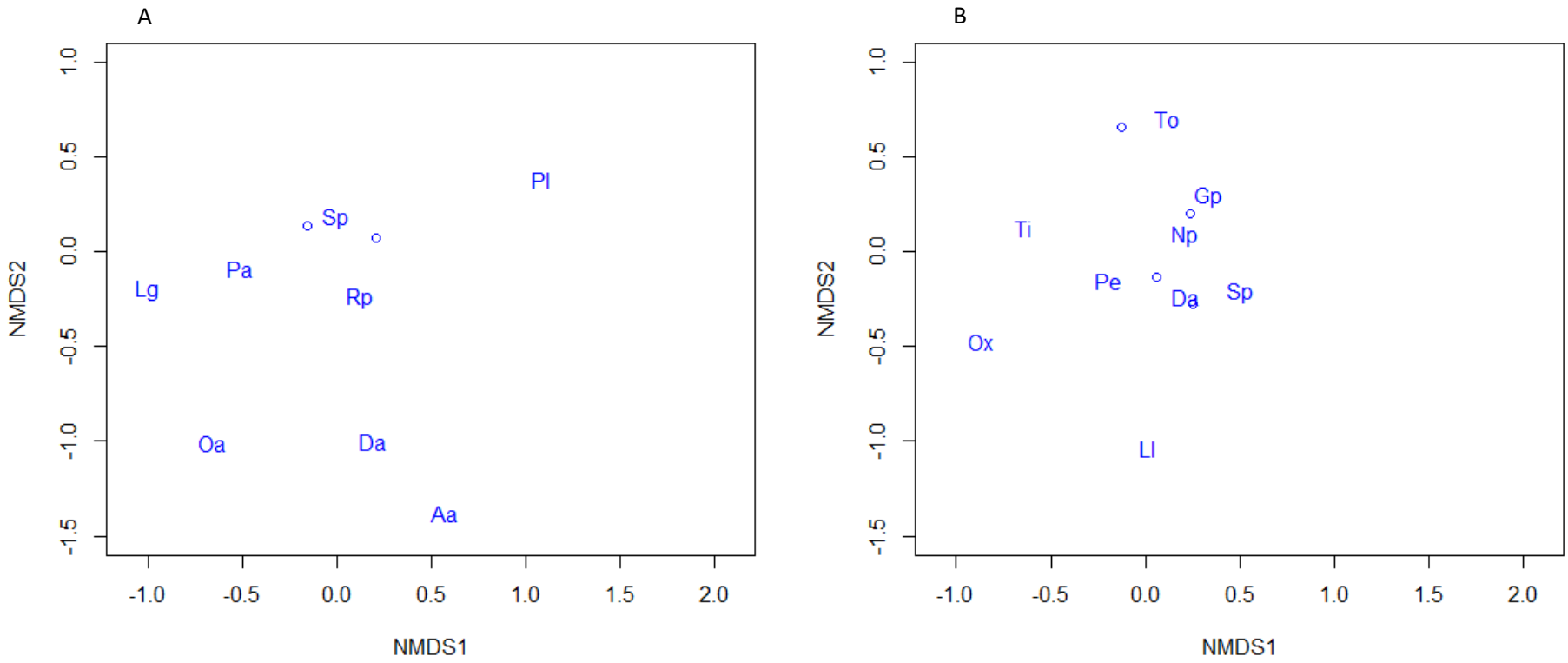

Figure 6.12 Non-metric multidimensional scaling (NMDS) biplots displaying taxa rather than sites: (A) 1 Month duration, (B) 1 Year duration. Mean abundance data and Bray-Curtis similarity were used. Taxa abbreviations are as follows: *Sp= Sericostoma personatum; Ti=Tipula;Gp=Gammarus pulex; Pl = Potamophylax latipennis; Aa= Asellus aquaticus; Rp= Radix peregra; Lg = Leuctra geniculate; Oa = Odontocerum albicorne; Pa= P.antipodarum; Da=Drusus annulatus; Ba= Bagous; Ll=Limnephilus lunatus; Br= Brilla; Pe= Pericoma; To=Tonnoirella; Ps= Psychoda; Ox = Oxycera; Spa= Stagnicola palustris; Np= Nemurella picteti. Only taxa found in at least 3 samples are displayed.*

Table 6.1 The seven drought periods of the leaf litter breakdown experiment (August 2013 to August 2014).

Date	Drought duration
August 2013*	1 Month
October 2013*	3 Months
December 2013	5 Months
February 2014	7 Months
April 2014*	9 Months
June 2014	11 Months
August 2014*	13 Months

*Suber samples were collected at this sampling time.

Table 6.2 Mean decomposition rate for all channels in the drought experiment. Leaf litter decomposition rate is presented as mean ± SD. k_s is the shredder-mediated decomposition rate, k_m is the microbial decomposition rate. NA refers to missing data.

Duration	k_s	k_m
1 Month	0.0187 ± 0.0010	0.0037 ± 0.0003
3 Months	0.0136 ± 0.009	0.0061 ± 0.0003
5 Months	0.0002 ± 0.0002	0.0101 ± 0.0002
7 Months	NA	0.0077 ± 0.0001
9 Months	0.0025 ± 0.0002	0.0070 ± 0.0002
11 Months	0.0007 ± 0.0001	0.0088 ± 0.0002
13 Months	0.0017 ± 0.0001	0.0065 ± 0.0002

*Table 6.3 GLS model selection results. ks is the shredder-mediated decomposition rate, km is the microbial decomposition rate. In the GLS model, ks was transformed using log(x+0.001), and km was transformed using log(x+0.1). DI is drought intensity (range 0 to 1) and DT is drought duration. (DI + DD) means the model was fitted with DI and DD but without an interaction. (DI * DD) means the model was fitted using DI and DD and the interaction between them. Gaussian GLS models were used. The best model and that selected for subsequent analyses is highlighted in bold and in the Model selection column.*

Response variable	**Model**	**AIC**	**Delta-AIC**	**Pseudo R^2**	**Model selection**
k_s	**DI+DD**	**1175.2**	**0**	**61.8%**	√
	DI*DD	1177.5	2.3	62.1%	
	DI	1498.4	294.9	0.65%	
	DD	1208.4	4.9	42.6%	
	Null	1498.9	295.4	0%	
k_m	**DI+DD**	**-5415.6**	**0**	**52.3%**	√
	DI*DD	-5340.8	74.8	52.8%	
	DI	-5125.4	290.2	5.7%	
	DD	-5366.6	49.0	46.5%	
	Null	-5107.3	308.4	0%	

Table 6.4 GLS model results for the analysis of decomposition rate against DI and DD. ks is the shredder-mediated and km the microbial decomposition rate. DI is drought intensity and DD is drought duration.

Response Variable	Parameter	Value	SE	t	P-value	Pseudo R^2
k_s	Intercept	-1.61			**<0.001**	61.8%
	DI	-0.52	0.52	-14.59		
	3 months	-0.19	-0.19	-18.90		
	5 months	-2.66	-2.66	-15.92		
	9 months	-1.13	-1.13	-34.75		
	11 months	-2.46	-2.45	-23.19		
	13 Months	-1.26	-1.26	-24.07		
k_m	Intercept	-2.26			**<0.000**	52.3%
	DI	-0.0020		18.3		
	3 months	0.0229	0.022	26.3		
	5 months	0.0598	0.059	39.3		
	7 months	0.0380	0.038	31.5		
	9 months	0.0308	0.030	29.0		
	11 months	0.0480	0.048	35.1		
	13 Months	0.0271	0.027	27.7		

Table 6.5: The composition of the shredding function group. Shredder species (genus) are ranked by affinity to shredding based on scores from Moog (2002). Those with an affinity weight > 60% are identified as specialist shredders. The other taxa (weight <60%) were identified as facilitation species.

Order/Class	Family	Genus	Species/Type	Weight
Coleoptera	Curculionidae	Bagous		100%
Trichoptera	Sericostomatidae	sericostoma	S.personatum	90%
Diptera	Tipulidae	Tipula		70%
Amphipoda	Gammaridae	Gammarus	G. pulex	60%
Trichoptera	Limnephilidae	Potamophylax	P.latipennis	60%
Trichoptera	Limnephilidae	Limnephilus	L.lunatus	50%
Diptera	Orthocladinae	Brilla		50%
Diptera	Psychodidae	Pericoma		40%
Diptera	Psychodidae	Tonnoiriella		40%
Isopoda	Asellidae	Asellus	A. aquaticus	30%
Gastropoda	Lymnaeidae	Radix	R.peregra	30%
Plecoptera	Leuctridae	Leuctra	L.geniculata	30%
Diptera	Psychodidae	Psychoda		30%
Diptera	Stratiomyiidae	Oxycera		30%
Gastropoda	Lymnaeidae	Stagnicola	S.palustris	20%
Gastropoda	Tateidae	Potamopyrgus	P.antipodarum	20%
Plecoptera	Nemouridae	Nemurella	N. picteti	20%
Trichoptera	Lepidostomatidae	Lepidotoma	L.hirtum	20%
Trichoptera	Limnephilidae	Drusus	D. annulatus	10%

Table 6.6: Shredder mean abundance and diversity metrics calculated across all channels in the drought experiment. Data was presented as the mean ± SD. H' is Shannon-Wiener's index. J' is Pielou's evenness index.

Duration	Total Shredder Abundance	Specialist shredder abundance	Facilitation shredder abundance	Shredder richness	*H'*	*J'*
1 month	123.25±12.29	118.83±11.78	4.41±3.04	3.05±0.23	0.50±0.05	0.46±0.05
2 months	208.44±17.20	156.18±16.46	52.26±8.40	3.50±0.17	0.56±0.04	0.44±0.03
9 months	25.27±3.80	3.49±0.90	21.79±3.70	2.20±0.20	0.40±0.05	0.45±0.05
13 Months	80.17±9.30	1.19±0.35	79.00±9.25	3.53±0.19	0.48±0.05	0.42±0.04

Table 6.7 GLS model selection results for the response variable ks (shredder-mediated decomposition rate). In the GLS models, ks was transformed using log(x+0.001) to normalise the data. Abundance was standardized to the (0, 1) scale. H' is the Shannon-Wiener index. J' is Pielou's evenness index. Gaussian GLS models were used. The models selected for subsequent analyses are highlighted in the Model selection column and in bold.

Response variable	Model	AIC	Delta-AIC	Pseudo R^2	Model selection
k_s	Abundance+DD	531	3.67	68.7%	
	Abundance *DD	**527**	**0**	**68.6%**	√
	abundance	813	285.5	13.1%	
	DD	528	1.2	68.6%	
	Null	852	325.2	0%	
	Richness +DD	530	2.24	68.7%	
	Richness *DD	532	4.23	68.8%	
	Richness	855	326	0%	
	DD	**528**	**0**	**68.6%**	
	Null	852	324	0%	
	H' +DD	536	2.95	68.7%	
	H' *DD	535	6.65	68.9%	
	H'	856	327	0%	
	DD	**528**	**0**	**68.6%**	
	Null	852	324	0%	
	J' +DD	525	1.6	69.3%	
	***J'* *DD**	**523**	**0**	**70.0%**	√
	J'	855	331	0%	

DD	528	4.86	68.6%
Null	852	352	0%
d + DD	-1935	12.6	59.5%
d * DD	-1910	38.3	60.8%
d	-1750	198.1	5.8%
DD	-1947	0	59.3%
Null	-1749	198.3	0%

Table 6.8 GEE model results for the response variables shredder abundance and richness. Abundance was transformed using log10(x+10) to normalise the data. Note that the p-value (>0.05) indicates no significant impact of DI on the responses and DD impact

Response Variable	Parameter	Slope	SE	Intercept	SE	P-value
Abundance	DI					0.30
	Duration					**<0.001**
	1 month	-0.21	0.22	4.89	0.1	
	3 months	-0.21	0.22	5.41	0.2	
	9 months	-0.21	0.22	3.31	0.1	
	12 months	-0.21	0.22	4.46	0.1	
Richness	DI					0.07
	Duration					**<0.001**
	1 month	-0.23	0.10	1.21	0.1	
	3 months	-0.23	0.10	1.30	0.1	
	9 months	-0.23	0.10	0.92	0.1	
	12 months	-0.23	0.10	1.31	0.1	

Table 6.9 GLS model results, with shredder abundance and evenness as predictors. ks is the shredder-mediated decomposition rate and was the response variable. In the GLS models, ks was transformed using log(x+0.001) to normalise the data. Abundance and richness data were standardized to the (0, 1) scale. J' is Pielou's evenness index, which ranges from 0 to 1.

Response Variable	**Parameter**	**Slope**	**SE**	**Intercept**	**SE**	**t**	**P-value**	**Pseudo R^2**
k_s	Abundance*DD						<0.0001	68.6%
	1 month	0.91	0.61	-4.18	0.12	-34.2		
	3 months	-0.20	1.30	-4.35	0.17	-325.8		
	9 months	2.03	2.23	-5.85	0.16	-44.9		
	12 months	0.54	1.60	-6.07	0.17	-45.3		
	*J'*DD						<0.0001	69.2%
	1 month	-1.03	0.13	-3.54	0.14	-24		
	3 months	-0.29	0.23	-4.27	0.27	-27.97		
	9 months	-0.20	0.22	-5.73	0.19	-35.6		
	12 months	-0.03	0.23	-6.12	0.19	-37.2		

Table 6.10 Spearman's correlations between taxa abundance and scores from 2 ordination axes (p=0.02). Only non-rare taxa are displayed. NMDS 1 relates to the coefficient correlated with NMDS model axes 1, and NMDS 2 relates to the coefficient correlated with NMDS model axes 2. The table is separated into two parts: the upper part relates to specialist taxa and the lower part relates to facilitation taxa. Levels of > 0.4 and < -0.4 were taken to represent moderate positive or negative correlations.

	1 Month			**13 Months**	
	NMDS 1	**NMDS 2**		**NMDS 1**	**NMDS2**
DI	0.14	0.53	DI	-0.60	0.37
Sp	-0.20	-0.27	Ba	0.06	-0.17
Ti	-0.44	-0.17	Sp	0.79	-0.10
Gp	0.54	-0.18	Ti	-0.36	0.13
Pl	0.46	0.22	Gp	0.59	0.57
Aa	0.06	-0.68	Ll	-0.04	-0.37
Rp	-0.31	-0.38	Br	0.14	0.37
Lg	-0.38	-0.18	Pe	-0.43	-0.27
Oa	-0.26	-0.34	To	0.06	0.56
Pa	-0.84	-0.62	Aa	0.00	-0.23
Da	-0.00	-0.75	Rp	-0.04	0.69
			Ps	0	0.37
			Ox	-0.43	-0.22
			Spa	-0.37	0.33
			Pa	-0.33	-0.22
			Np	0.51	0.37
			Da	0.46	-0.33

Taxa abbreviations are as follows: Sp= Sericostoma personatum; Ti=Tipula;Gp=Gammarus pulex; Pl = Potamophylax latipennis; Aa= Asellus aquaticus; Rp= Radix peregra; Lg = Leuctra geniculate; Oa = Odontocerum albicorne; Pa= P.antipodarum; Da=Drusus annulatus; Ba= Bagous; Ll=Limnephilus lunatus; Br= Brilla; Pe= Pericoma; To=Tonnoirella; Ps= Psychoda; Ox = Oxycera; Spa= Stagnicola palustris; Np= Nemurella p

CHAPTER 7

General Discussion

7.1 Discussion

The research presented in this thesis a) evaluated the utility of a stream mesocosm facility and b) then assessed how experimental drought applied in these model systems affected benthic algal growth, leaf litter decomposition and the structure of herbivore and detritivore macroinvertebrate assemblages. Stream mesocosms have been used widely to undertake ecological studies, including climate change effects on freshwater ecosystems (Stewart et al., 2013). The replicability of stream mesocosms is an important consideration when designing these studies and here I found that both low variability of the physicochemical and biological conditions in flumes are important for mesocosms application. The low between- channels variability was found in both water physicochemistry and macroinvertebrate assemblages in flumes. Meanwhile, the physicochemical and biological replicability did not fade with experiment duration. Hence, the high replicability of this mesocosms presented in space and time. This result suggests that stream mesocosms may be effective as experimental tools, with high replicability yielding the statistically power necessary to investigate realistic cause-effect relationships.

Climate change-induced droughts are increasing in duration and magnitude (Dai, 2013). In future, many rivers and streams, especially those with normally permanent flow, could experience longer, more prolonged droughts with the potential to alter the structure and functioning of freshwater ecosystems. The direct impact of water reduction is to the hydraulic habitat loss in the channel. In natural river, the hydraulic habitat variation is the most important abiotic factor to control macroinvertebrate community (Wood et al.,2003). The hydraulic habitat loss would lead to dramatic variation in macroinvertebrates abundance, species and community structure. Due to the low drought resistant ability of the macroinvertebrate in permanent river, the drought would destroy the whole assemblages and

might take years to recovery. Compare with the natural river, the hydraulic habitat condition in mesocosms has limited quality, because of hydraulic habitat in mesocosms has less diversity and wet area. Additionally, hydraulic habitat in mesocosms is the petri dish for macroinvertebrates. Hence, hydraulic habitat is the foundation of mesocosm experiment, especially for macroinvertebrate study.

In this thesis some of the effects of drought intensification were explored experimentally at the mesoscale by simulating stream drying of contrasting intensities in stream mesocosms. In this chapter, I will return to the principal research questions asked in **Chapter 1** and discuss the main finding of this research.

7.1.1 Experimental design

Q1: Can current water physiochemistry be successfully replicated in mesocosms during a long-term experiment?

The use of outdoor freshwater mesocosms has increased since the 1990s (Lamberti & Steinman, 1993). The main advantage of freshwater mesocosms is that they can simulate patches of natural environments and/or natural processes such as disturbances at experimentally tractable scales (Harries, 2006; Ledger et al., 2012; 2013). Outdoor mesocosms provides an access to manipulate stream freshwater ecosystem (Schindler, 1998). Hence, it follows that the result of mesocosm studies can reliably be extrapolated back to the scale of whole natural systems (Petersen & Hastings, 2000; Englund & Cooper, 2003).

In the study facility, water physicochemical variability was limited by a once-through water delivery system fed from a borehole (Caquet et al., 1996; 2000), mesocosms location (Harries, 2006; Ledger et al., 2012; 2013) and experimental flume design (Gillespie et al.,

1996; Mohr et al., 2005). It has been shown elsewhere that local water sourcing is the key to sustain mesocosms physicochemical replicability (Harris et al., 2007). A 15m once-through channel reduced physicochemical heterogeneity between patches within individual flumes and continuous flow from the borehole limited the temporal variability of water physicochemistry. Moreover, each mesocosm unit was exposed to common local meteorology, such as solar, precipitation and air temperature. This exposure to natural conditions enhances the realism of mesocosm experiments over those undertaken under unrealistic laboratory conditions (i.e. 12 hr light, 12 hr dark) (Stewart et al., 2013). In addition, aspects of flume design such as limited channel length (15m) reduced upstream-downstream variation (Harries et al., 2007) and clean substrate composition reduced water physicochemical biochemical exchange (Chróst & Rai, 1993; Tanaka et al., 2009) to reduce water physicochemical variability. Meanwhile, three replicates for each treatment, and four replicates for within-channel pool-riffle section design ensure result of this study replicated (Zuur et al., 2009).

Due to the extremely low concentration of greenhouse gas, the dissolved greenhouse gas (i.e. CO_2) were found to have low replicability in stream mesocosms. However, the mesocosms water physiochemical replicability were driven by conductivity in stream mesocosm (Harries et al., 2007). The conductivity, water temperature, pH and dissolved oxygen was determined by the water source. This experimental set-up would expect the four variables to be stable. Hence the low replicability of greenhouse gas could be ignored in this study. Due to the limited spatial and temporal variability, water physicochemical conditions were successfully replicated in stream mesocosms during a long-term experiment.

Q2: How macroinvertebrate assemblages replicate in mesocosms spatially and temporally?

High replicability of physical and chemical experimental conditions is fundamental to establish biological replicability (Connolly et al., 2004; Heckmann & Friberg, 2005; Berghahn et al., 2012; Wagenhoff et al., 2012). In this study, relatively limited variability in water physicochemistry was found in the mesocosms (see **Q1**), and this may in turn explain the similarity of macroinvertebrate assemblages living in each artificial channel. Further, as macroinvertebrate assemblages are affected by stream characteristics such as microhabitat, we ensured that the same amount *Ranunculus* plants were transferred into each mesocosm in an attempt to mimic plant stands in natural chalk streams. *Ranunculus* dominates in many chalk stream reaches and provides food and shelter for many macroinvertebrates (Welton, 1979; Shamsudin & Sleigh, 1995; Flynn et al., 2002). Mesocosms were initially seeded with macroinvertebrates, plants and fish, from other channels within the watercress farm, these were the only sources of biota. As the experiment progressed, mesocosms began to develop more pronounced differences, reflecting unexpected macrophyte colonisation (by seed) found in channels in this study (see also Steele, 2013). This unexpected mesocosm habitat heterogeneity may explain why macroinvertebrate assemblage composition altered with time.

Fish (Cottus gobio) impact is another factor to effect macroinvertebrate assemblage composition. Due to the food limited in the drought condition, fish accelerated the reduction of macroinvertebrate abundance (Meier et al., 2015).

Additionally, initial experimental set-up may help developing biological replicability. For instance, the same amount of macroinvertebrate was introduced into each mesocosm. Groundwater supply replaced river water, preventing unexpected macroinvertebrate drift in

mesocosms. Meanwhile, due to the continuous groundwater supply and local macroinvertebrate assemblage colonization, the mesocosms provided a relatively high degree of realistic simulation of natural environment and benthos in freshwater ecosystem at the beginning of the experiment (Crossland & La Point, 1992; Kraufvelin, 1999). Hence, macroinvertebrate assemblage well replicated in this experiment.

7.1.2 Experimental insight

Q3: How is the biomass of benthic algae influenced by drought intensification?

We applied drought via water depth manipulation and collected drought indicator data, namely flow velocity, wetted area, water volume, water temperature and diel oxygen concentration in each artificial channel. These five data sets were used to calculate a compound drought intensity metric to illustrate conditions in the mesocosms. Thus, drought intensity as an integrated explanatory variable was applied in the experiment. In this study, only water depth treatment was formally controlled, with other responses reflecting consequences of water depth reduction. It is different from other stressor gradient experiments, such as nutrient and sediment gradient constructed by experimental set-up (Wagenhoff et al., 2012; 2013). Hence, the drought intensity is more realistic indicator to explain the drought condition in mesocosms.

Algal growth varied in response to drought intensity and duration. Water loss reduced algal biomass and increaed algal Autotrophic Index. Algal responses can vary markedly among studies, with reports of algal biomass increases, decreases or no detectable response to natural drought (Caramujo et al., 2008). It is possible to get increased, decreased or not effect of drought on algae depending on a range of abiotic (i.e. nutrient enrichment, increased temperature) and biotic factors (i.e. grazing) that are independent of experimental

application, and likewise drought conditions vary from place to place and are not of a standard type (Robson & Matthews, 2004; Rier et al., 2006; Everard, 2010; Mosley, 2015). Hence, isolated water reduction simulation is not sufficient to investigate the drought impact on algal growth. Without indirect effects, algal growth negatively correlated with drought intensification.

The nutrient level, sediment accumulation level is important factor to effect algal growth in drought condition, those indicators should be required in further study.

Q4: How do macroinvertebrate herbivores and grazer respond to drought?

Excepting the total loss of habitat caused by the most intense drought, there was no relationship between grazer abundance and drought intensity (Ledger et al., 2011). Due to the resistance of macroinvertebrates and refuge zones in artificial channel (i.e. isolated pool) (Boulton, 2003; Lake, 2003; Dewson et al., 2007), grazer abundance was mainly decreased by increasing drought duration. Hence, the drought duration is more important to study the drought impact on grazer rather than the certain water volume loss (Power et al., 2008; Ledger et al., 2011).

Because, *Radix balthica* is dominat (occupied over 90% total grazer abundance in this experimental ecosystem) and high efficiency grazer contributes majority grazing in chalk streams (Ledger et al., 2011; 2013). Hence, only three taxa grazers were examined instead of whole grazer functional group in this study. However, there was no significant relationship between *Radix balthica* abundance and grazing. Hence, *Radix balthica* cannot be the only biological variable to study grazing. In this study, only macroinvertebrate abundance and density were used to study the drought impact, which could not satisfy to

explain the trait of grazer in drought condition. The biomass indicators, such as body size should be required in the further study to investigate the variation of grazer community.

In this study, I found that grazing correlated with grazer abundance. Additionally, drought altered grazer taxa abundance, with drought reducing high efficiency grazer taxa; whereas non-specialist grazer maintained grazing activity (Smit & Grant, 2009; Ledger et al., 2011). Hence, the correlation between grazing and drought intensity varied with increasing drought duration.

Q5: How is the process of leaf litter decomposition altered by drought?

In this study, the effects of both drought intensity and duration impact on the decomposition process were evaluated.

The reduced leaf litter breakdown in the drought treatments that was observed indicates that the shredder-mediated litter decomposition process is negatively related to drought intensity and duration (Leberfinger et al., 2010); microbial decomposition is negatively related to drought intensity but positively related to drought duration. Due to release from top-down pressure (Gessner et al., 2010), microbes increased with increasing drought duration, resulting in an increased microbial breakdown rate during long term drought. As such, the results of this experiment indicate that the leaf litter decomposition process under long term drought conditions is mainly driven by microbes.

The response of the benthic shredder community to drought intensity and duration was complex. Due to changes in habitat heterogeneity and physicochemical water conditions, shredder abundance and diversity was reduced following short-term drought (Wood et al., 1999; Lake, 2011). However, after long-term drought, the shredder community became more

even and the majority of specialist shredders were replaced by facilitation shredders (Dewson et al., 2007; Lake, 2011).

7.2 Summary

My research has shown that this stream mesocosm facility was highly replicable for water physicochemistry, but that the replicability of dissolved greenhouse gases (i.e. CO_2) is low. Macroinvertebrate assemblages were highly replicated in mesocosm, but macroinvertebrate richness taxa distributed differently between head and tail of flume. Algal growth was negatively correlated with drought intensity, but grazer abundance was reduced by drought duration. Both drought intensity and drought duration reduced decomposition in this mesocosm.

Due to local water supply, local macroinvertebrate assemblage colonization and local regional weather pattern, the result of stream mesocosm could be applied into local chalk river ecosystem. The stream mesocosm is used to investigate the disturbance impact on specific local chalk stream, due to the study site locates near water source (i.e. river water, groundwater).

Well-designed flume and strict experimental set-up are the key points to success of this study. However, there were some unexpected man-made disturbance happened during experimental period, such as water supply suddenly stopped. Hence, the water supply system should be developed. Moreover, during long-term running, to avoid mesocosms become their own ecosystems rather than replicates, the unexpected flume heterogeneity should be avoided (Steele, 2013). For instance, removing unexpected plants should be done as an environment maintenance inner mesocosms.

Although, the outdoor flumes captured elements of natural variation that enhanced the realism of drought (i.e. macroinvertebrate assemblages), but flumes cannot mimic side-effects of drought disturbance completely, such as nutrient enrichment that is always observed in natural drought. Hence, this stream mesocosm is satisfied to investigate the drought consequences driving by water volume loss rather than the side-effects caused by water volume loss.

Less realism is also found in this mesocosms. As the variation of macroinvertebrate in mesocosms shows that this system is lack of reproduce ability to reproduce the core taxa. Although the function group was not lost, the biocomplexity decreased during experimental period (Stewart et al.,2013). Compared with other mesocosms studies, the researcher used unfiltered river water to feed the artificial channel directly to accept the macroinvertebrate constantly to maintain the realism of mesocosms (Harris et al.,2007; Ledger et al.,2009). Hence, enclosure mesocosms has limited reproduce ability in the long-term duration. This mesocosms system may not be maximize the realism of ecosystem, but it can maintain the relevant ecological information during the long experiment (Landner et al., 1989).

The sampling disturbance is another factor should be considerate. In extremely drought channel, the sampling disturbance might associate with macroinvertebrate density. Due to the limited wet area in drought channels, the hydraulic habitat became the refuge area for macroinvertebrates (Dewson et al.,2007; Lake, 2011). Because the surber sample was collected from the wet area of each pool, the sampling disturbance might destroy the only habitat for biota in mesocosms. The sampling disturbance could explain there was much taxa macroinvertebrate lost in the second summer.

This mesocosm facility had many disadvantages as an experimental tool, but this study has confirmed that this stream mesocsom is useful in climate change research. For the

experimental set-up aspect, it can simulate multiple magnitudes of water depth (i.e. seven water treatment in this study), which allows predict consequences caused by different degree of water reduction. Long-term systems develop the understanding of drought duration impact. Duration as a main impact should not be ignored in the drought study and the long-term study can be operated more in the future. In additional, this stream mesocosms can investigate the disturbance impact on multiple trophic level of freshwater ecosystem, from single taxa to macroinvertebrate community.

As the observations found in this study, drought could alter freshwater ecosystem negatively. The drought condition could affect the macroinvertebrate immediately, even in a minor water reduction (Boulton & Lake, 2008). Macroinvertebrate species loss might cause the partial collapse of food web and trigger the shift in species under drought condition (Ledger et al., 2013; Lu et al.,2016). The shifts in species interaction develops the stability of drought- impact food web. Additionally, drought declines the macroinvertebrate biomass, abundance and alters their relevant key function in freshwater ecosystem (Atkinson et al.,2014). As the abundant core function taxa loss, the facilitation taxa might replace their ecological niche, and maintain the ecological process in freshwater system (Boulton & Lake, 2008). Hence, the complexity macroinvertebrate community is a foundation to develop the resilience to drought.

On the one hand, side-effect of drought, such as warm water temperature, nutrient enrichment, oxygen reduction, also alter freshwater ecosystem. On the other hand, drought accelerates those side-effect (Mosley, 2015). Hence, drought is not an individual impact on freshwater ecosystem, it is a trigger and accelerator of other stressors, such as nutrient pollution. In this mesocosms, the high-quality water supplement could not mimic the post-drought water quality, which should develop in the further researches.

Water reduction and ecological process loss in freshwater has a great impact on human economy, agriculture and society etc. (Banerjee et al., 2013). Meanwhile, increasing human water demand is associated with drought worldwide (Wada et al.,2013). However, there was a limited access to evaluate the impact of drought and predict the exact drought consequence of running water (Bachmair et al., 2016; Crasbay et al., 2017).

In summary, this study approved that mesocosms is a reliable experimental tool and foundation to maintain the suitable experimental environment (Chapter 3) and relatively high tropic macroinvertebrate community (Chapter 4) for the following ecological process studies. Drought intensification integrated multiple variables to provide entire picture to demonstrate drought impact rather than simply multiple (e.g. 2 or 3) variables combination. As the result in Chapter 5, the water reduction shows the negative impact on algal growth and core grazer taxa. Drought also declined the decomposition process in mesocosms and alter the structure of shredder community structure and composition (Chapter 6).

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