Refining biological monitoring of hydromorphological change in river channels using benthic riverfly larvae (Ephemeroptera, Plecoptera and Trichoptera).

Anna Doeser

November 2016

Submitted to Biological and Environmental Sciences
School of Natural Sciences
University of Stirling
Scotland.

For the degree of Doctor of Philosophy

Supervisor:
Prof. Nigel Willby

UNIVERSITY OF
STIRLING

Statement of Originality

I hereby confirm that this PhD thesis is an original piece of work conducted independently by the undersigned and all work contained herein has not been submitted for any other degree.

All research material has been duly acknowledged and cited.

Signature of Candidate:

Anna Doeser

Date

General Abstract

Rivers and their catchments are under mounting pressure from direct channel modification, intensification of land use, and from a legacy of decades of channelisation. Recent legislation, in the form of the EU Water Framework Directive, places a greater emphasis on the management of water bodies as holistic systems, and includes the explicit consideration of hydromorphological quality, which describes the hydrologic and geomorphic elements of river habitats. These are defined specifically as hydrological regime, river continuity and river morphology. This appreciates that sediment and flow regimes, along with the channel structure, provides the 'template' on which stream ecological structure and function is built.

Invertebrate fauna contribute significantly to the biodiversity of rivers, and often form the basis of monitoring river health. However much of the fundamental ecological knowledge base on the response of invertebrates to hydromorphological change needed to make informed decisions and accurate predictions, is either lacking, inadequate or contradictory. This thesis addresses some of the key potential shortcomings in recent bio-assessment that others have alluded to, but which have rarely been explored in the context of direct channel manipulations. By using two case studies of, realignment in a natural upland catchment, and flood protection engineering in an urban stream, this study investigates the sensitivity of hydromorphological impact assessment methods that rely on biodiversity patterns of benthic riverfly (Ephemeroptera, Plecoptera and Trichoptera) larva.

This work employed widely used biomonitoring indices of benthic riverfly larva abundance, species richness, alpha and beta diversity, and community composition, applied over a range of spatial scales, in combination with spatially contemporaneous physical habitat data, to describe and explain community changes in response to disturbance, and patterns of natural variation. The effects of restoration were investigated using a high degree of sample replication within channels and across the wider catchment, as well as contrasting spring and autumn seasons. To assess change in a small urban channel, approaches that explicitly consider spatial elements of community data, using spatial eigenvectors analysis, were applied to spatially detrend community data and directly investigate spatial patterns.

Restoration of the Rottal Burn was found to be successful in restoring habitat diversity and geomorphic processes, and in turn increasing reach scale species richness and beta diversity through the gradual arrival of rare and specialist taxa into novel habitats. Catchment scale replication revealed high variation in diversity indices of modified and undisturbed streams, and

a strong temporal pattern related to antecedent flow conditions. Channels with greater habitat heterogeneity were able to maintain high gamma diversity during times of high flow stress by providing a number of low flow refuges along their length.

The urban Brox Burn had surprisingly high riverfly richness and diversity driven by small scale hydraulic heterogeneity, created by bed roughness resulting in a range of microhabitats. Riverfly community responses to direct channel dredging could not be detected by measurements of average richness and diversity, however distinct changes were seen in gamma diversity, the identity of community members and their arrangement among sample patches. Impacts of sediment pollution release due to engineering were short lived and apparently had little detrimental impact on biodiversity. Strong spatial patterns of community assembly on the stream bed were uncovered, relating to longitudinal, edge and patchy patterns. Significant habitat drivers of community composition were confounded by high amounts of spatial autocorrelation, especially hydraulic variables.

Due to the strongly physical and spatial nature of hydromorphological disturbance, turnover of species between sample locations at a range of scales, and the spatial arrangement of habitats and communities is of more use for detecting these types of subtle changes compared to mean richness or diversity. These findings have implications for the targeting of resources for monitoring of restoration, or engineering disturbances, in order to be sensitive to hydromorphological change. Efforts should target the main area of natural variability within the system, either replicating sampling in time or space to distinguish effects of impact. Spatial patterns, measures of beta diversity and species identity can be better exploited to identify systems with functioning geomorphological processes. Channel typologies proved misleading, and quantification of habitat and selection of control sites using multiple pre-defined criteria should be carried out. Studies of restoration operations and engineering impacts provide considerable opportunities for advancing our knowledge of the mechanisms that drive community response under a range of conditions to improve impact detection.

Acknowledgements

Special thanks to my supervisor Professor Nigel Willby, who has been a constant source of encouragement and fruitful river "nerd-out" sessions over the past four years.

This project would not have been possible without the generous funding from SEPA, and strong initial direction and enthusiastic support from Chris Bromley. Additional funding support through Stirling University Impact Studentship was also gratefully received. Thanks also to Prof. Dave Gilvear for providing a friendly welcome, early support and local allotment (sadly neglected).

Many thanks to the assistance from Marshall Halliday and particularly Steve Hawkins of the Esk Rivers and Fisheries Trust for showing me the lay of the land in Glen Clova. Also the enthusiastic permission of the Rottal Estates landowner Dee Ward.

Thanks to my fellow, past and present colleagues at the Number 1 Freshwater Detective Agency: Alan Law, Colin Bull, Charlie Perfect and Alex Seeney. Thanks to Roni Balfour, Scott Jackson and Helen Ewen for technical and logistical support. Also to Tim Paine for taking the time to get me looping with R, which has saved me many hours or work, and now I cannot stop.

My thanks to those brave people who assisted in the lab and the field over years either for love or money: Lois Campbell, Megan Layton, Mellissa Shaw, David Eastwood, Ross Hepburn, Adam Welsh, Catherine Gibson-Pool, Renee Hermans, Junyao Sun. My special thanks David García Martínez and Clara Gajas Roig for spending a summer wading in the Broxburn, at least I took you to Glen Clova too!

To my treasured Toothless and Abrosius, who have brought me great happiness and much needed exercise in the beautiful scenery of Stirling through stressful times (my bikes..). Finally, to my parents Kathy and Bernie, for always encouraging me to do what makes me happy and never doubt what I could achieve. Also to my brother Dr James Doeser; there may have been a small aspect of sibling rivalry prompting me to pursue this PhD, and my grandma Renee, who supported all my academic endeavours.

Table of Contents

List of Figures

Shannon’s diversity and d) Simpson’s diversity. Asterisks below pairs indicate the result of Mann-Whitney U significance tests (< 0.001 ***, < 0.01 **, < 0.05 *).

Fig 2.9 Correspondence analysis (CA) biplot for whole Rottal Burn community of a) sample locations on axis 1 and 2, with 95% confidence intervals around each sampling occasion x treatment x season combination (n=8), coloured by sampling occasion b) Contribution biplot of riverfly species with a contribution > 1%, location on axis in relation to sample group communities in b), indicates how much a species contributes to the identity of those sites, the size of the symbol is scaled to species abundance. A key to species abbreviations is given in Appendix 2.4

Fig 2.10 Median distance to groups centroid and result of the significance of difference test between each pair, using a) Bray Curtis distance, and b) Chao distance. Asterisks between pairs indicate the result of a permutational MANOVA **<0.01, *<0.05, +<0.1, "ns" not significant

Fig 2.11 Within sampling group mean sample riverfly community dissimilarity (Bray Curtis) and standard error (dot and whisker), below, the partition of the dissimilarity into nestedness (blue) and turnover (red) components per treatment sample period combination. C= Control, R = Restored, S = Spring, A=Autumn.

Fig 2.12 Samples and RHS variables (Table 2.7) plotted in MCA axis 1 and 2. Point and circle shows group centroid (average conditions) and 95% confidence ellipses. The variables are 20 that contribute most to the MCA solution.

Fig 2.13 Samples and measured sediment variables plotted in PCA axis 1 and 2. Large point and circles shows group centroid (average conditions) and 95% confidence ellipses. (see Table 2.1 for description of variables)

Fig 2.14 Biplots of RDA results, species abundance explained by axes over a threshold amount (15% spring, 30% autumn), sample locations, and significant environmental gradients for a) and b) spring, and c) and d) autumn samples. A key to species abbreviations is given in Appendix 2.4

Fig 3.1 Location map of sample rivers in the South Esk Catchment. Straight channel Glenmoye Downstream (GD) and March Burn (MA). Natural channels: Prosen (PR), Moulzie (MO) and Glenmoye Upstream (GU), and restoration and control channel sites from Chapter 2: Rottal Restored (RR) and Rottal Straight (RS).

around each river, inset: Extended CA axis showing locations of MO left of axis and MA below axis b) Contribution biplot of riverfly species with a contribution > 1%. A key to species abbreviations is given in Appendix 2.4.

Fig 3.11. Samples measured sediment variables plotted in PCA axis 1 and 2, for the 7 rivers. Large point and circles shows group centroid and 95% confidence ellipses.

Fig 3.12 Samples and RHS variables plotted in MCA axis 1 and 2. Point and circle shows group centroid and 95% confidence ellipses. The variables shown are the 20 that contribute most to the MCA solution.

Fig 3.13 Results of the CA, showing community composition change over time within each river a) RR, b) RS, c) MA, d) MO ,e) GU, f) GD, g) PR, over the 4 sample periods, Spring 2013, Autumn 2013, Spring 2014 and Autumn 2014. Where significant correlations to environmental variables were found these gradients arrows are plotted, R^2 and the significance of a PCoA model of community composition with year as an explanatory factor are reported above..

Fig 3.14 Median distance to group centroid of community (beta diversity).Result of the significance of river identity (p value), using a) Bray Curtis distance in Spring 2013, b) Spring 2014, c) Autumn 2013, d) Autumn 2014, and e) Chao distance in Spring 2013, f) Spring 2014, g) Autumn 2013, h) Autumn 2014.

Fig 3.15. Daily gauged flow data from Gella Bridge (Fig 2.2a GS) for the duration of the project. May 2013-November 2014. A) October 2012- 2013, b) October 2013 - 2014, and c) October 2014-2015. Sampling windows outlined in black box.

Fig 3.16 Within sampling group mean Bray-Curtis dissimilarity and standard error (dot and whisker), and the partition of the dissimilarity into nestedness and turnover components for the 7 sample rivers for a) Spring 13 - *wet* , b) Spring 14 – *dry*, c) Autumn 13 -*dry*, and d) Autumn 14 *wet.*

Fig 3.17 Habitat heterogeneity (mean distance to group centroid of PCA) plotted against Beta diversity (Median distance to group centroid PCoA using Bray Curtis and Chao dissimilarities). Line is a linear model based on the 7 data points in the sampling period. a) A 2013 Bray, b A 2013 Chao, c) S 2013 Bray, d) S 2013 Chao, e) A 2014 Bray, f) A 2014 Chao, g) S 2014 Bray, h) S 2014 Chao. (S -Spring, A – Autumn).

Fig 3.18 Biplots of RDA results showing species and sample locations, and significant environmental gradients for Spring samples in a) 2013 samples and rivers outlined b) 2014 samples and rivers outlined, and c) 2013 species explained by RDA over 35% and d) 2014 species explained by RDA over 30%. A key to species abbreviations is given in Appendix 2.4.

Fig 3.19 Biplots of RDA results showing species and sample locations, and significant environmental gradients for Autumn samples in a) 2013 samples and rivers outlined b) 2014 samples and rivers outlined, and c) 2013 species explained by RDA over 35% and d) 2014 species explained by RDA over 25%. A key to species abbreviations is given in Appendix 2.4.

Fig 4.1 Effects of fine sediment, deposited and suspended particles, on benthic macroinvertebrates. Reproduced from (Jones et al. 2012)

Fig 4.2 a) Brox Burn geographic location within Scotland b) catchment outline with land use. Urban= grey, arable and horticulture = red, woodland and grassland = green (NRFA 2016), position of study sites (circled).

Fig 4.3 Photo of a) failed sediment stopping net and b) in channel and riparian engineering work upstream of IMP1.

Fig 4.4 Location map of the 6 sampled reaches (purple boxes) and site names (Upstream: UP2, UP1, Impacted: IMP1, IMP2, Downstream: DS1, DS2) on the Brox Burn.

Fig 4.5 Site photos of the 6 study reaches: a) and b) Upstream: UP2, UP1, c) and d) Impacted: IMP1, IMP2, e) and f) Downstream: DS1, DS2.

Fig 4.6 Locating a sediment collection mat on the stream bed.

Fig 4.7. a) diagram outlining reach scale survey design, delineating sample cells, b) sampling features within a cell (2 in each quadrat), red dots indicate location systematic sampling of depth and velocity measurements.

Fig 4.8 Outline of invertebrate sampling scheme, river flows from l-r. Samples taken from cells outlines in brown rectangles, alternate cells for 2 rows with a break of 3 non sampled rows.

Fig 5.8 Biplots of RDA analysis within each site for both 2013 and 2014 illustrating change over time of species composition of samples, and arrows indicate the environmental gradients for a) US2, c) IMP1, d) DS1, and species associations to sites and gradients, for b) US2, d) IMP1 and f) DS2. A key to species abbreviations is given in Appendix 2.4.

Fig 5.9 Plot of model fit - R^2 from RDA of Upstream communities with each environmental predictor (T, using spatially detrended (2013.det, 2014.det) or non detrended data at each focal extent.

Fig 5.10 Plot of R^2 from RDA of Impacted communities with each environmental predictor, using spatially detrended (2013.det, 2014.det) or non detrended data at each focal extent.

Fig 5.11 Plot of R^2 from RDA of Downstream communities with each environmental predictor, using spatially detrended (2013.det, 2014.det) or non detrended data at each focal extent.

Fig 5.12. Selected fractions from variance partitioning. Amount of variance in community assembly explained by unique environmental, unique spatial (MEM, AEM and XY summed) and any shared fraction between environmental and all spatial. * indicates significance of unique fraction of 0.05 or higher.

Fig 5.13. Selected fractions from the variance partitioning. Amount of variance in community assembly explained by unique types of spatial variables: MEMs and AEMs and any shared fraction. * indicates significance of unique fraction of 0.05 or higher.

List of Tables

Chapter 1 - General Introduction

1.1 River systems in modern society

Functioning river systems are valued by society for providing a range of vital services, such as the purification of drinking water, water storage and flood control, sources of energy generation and transportation, aside from their cultural, spiritual, recreational and aesthetic value. From an ecological point of view, rivers integrate many aspects of terrestrial and atmospheric systems, transporting material and energy from the catchment, downstream (Covich et al. 2004). Despite their small spatial footprint, river systems harbour a disproportionately high amount of biodiversity (Strayer and Dudgeon 2010), from the likely underestimated fungi and bacteria to larger fish and mammals.

Invertebrate fauna contribute significantly to biodiversity of streams. They play a central position in the functioning of river ecosystems, regulating primary production, decomposition, and nutrient cycling (Strayer 2006). Due to their ubiquitous presence and range of sensitivities to a number of environmental conditions (acidity, nutrient pollution, thermal regime), they are commonly the foundation of biomonitoring techniques that aim to provide an integrated assessment of overall river condition. Accordingly, there are nearly 100 assessment methods in place across Europe using benthic invertebrates for monitoring surface water condition (Birk et al. 2012). The benthic riverfly larva (Ephemeroptera, Plecoptera and Trichoptera), are commonly used as a measure of stream biological quality, calculating either species richness or abundance, or these as a proportion of full invertebrate community (Townsend et al. 2008, Feld 2013). Aquatic riverfly play a large role in river food webs, feeding on a diverse array of materials, and themselves acting as prey items. Members within the group, occupy a wide diversity of niches, particularly the Trichoptera (Altermatt et al. 2013). Their association with cool, well oxygenated waters, makes them useful indicators of good ecological quality, and as such they are used to detect general degradation, specific changes in flow conditions (Alonso et al. 2013, Silva et al. 2014) or sediment variables (Townsend, Uhlmann & Matthaei 2008). Although using only a subset of the full invertebrate community, riverfly metrics often respond in similar ways to full community metrics (Johnson et al. 2007). Riverfly taxonomy is well documented and keys are widely available, allowing these groups to be consistently and accurately resolved to species, and as such riverfly monitoring contributes to the assessment of broad scale river health (Everard 2008).

Despite improvements in wider river health through remediation of water quality issues (Vaughan and Ormerod 2012), river hydromorphology continues to be impacted directly or indirectly through channelization and other planform modifications, in-channel structures, regulation of flow regime and altered lateral or longitudinal connectivity. The theory that a functioning stream ecosystem is one where biota are sustained by suitable habitats and appropriate physical processes, is well appreciated and understood to a general level, e.g. habitat template theories or fluvial hydrosystems (Townsend and Hildrew 1994, Petts and Amoros 1996). However, an increasing demand is being placed on biomonitoring techniques to be sensitive to hydromorphological impacts and to predict the extent of hydromorphological restoration needed to deliver the sought after ecological benefits.

The process of urban expansion and a historic legacy from industrial use of rivers is a continuing issue for a large number of river systems globally. The altered conditions of hydrology, chemistry and fluvial geomorphology experienced in urban streams has been summarised by Walsh et al. (2005), and termed the Urban Stream Syndrome. Beyond reducing species richness and selecting for tolerant taxa, very little is known about urban stream invertebrate communities and their response to further modification to in-channel habitats. In a rapidly urbanising world in which urban streams are commonly exposed to novel stressors or combinations of stressors this lack of basic knowledge is concerning.

The introduction of statutory requirements through the Water Framework Directive (WFD) (European Commission, 2000) has shifted approaches in water resource appraisal to consider water bodies in a more holistic way. There is now a need to consider not only water quality specifically, but also channel hydraulic conditions, connectivity, integrity of flow regime and fluvial geomorphology, under the general banner of 'hydromorphology'. This is in addition to the biological quality elements comprising phytobenthos and macrophytes, macroinvertebrates, and fish. In 2010 54% of Scotland's streams were classified as being in good or high condition. The Scotland River Basin Management plan sets out the aim for 98% of rivers to be in good or better condition by 2027 (Natural Scotland 2015).

Protecting rivers from further degradation, a specific requirement under the WFD, is enforced by requiring activities that may impact on stream physical structure and biodiversity quality elements to be authorised under the Water Environment (Controlled Activities) (Scotland) Regulations 2011 (“CAR”), and carried out following good practice guidance (SEPA 2009). Projects to restore streams, rivers and their floodplains are proliferating to meet the requirements of the WFD and also to maximise functional benefits such as upstream water storage and downstream flood alleviation. 1079 completed projects were recorded on the UK River Restoration Centre National River Restoration Inventory by Smith, Clifford & Mant (2014), in 2011. By 2016 this number had increased to 2656. A major focus of river restoration involves some form of physical reconfiguring of the channel form (Palmer et al. 2014), to reverse, or mitigate, past or current human activities. However, the degree of success of projects varies considerably, particularly with regard to the aims of increasing abundance and diversity of aquatic macroinvertebrates, or when such metrics form the basis of an ecological evaluation. A wide range of shortcomings in restoration projects have been identified to account for this apparent failure. Of the numerous reviews that have now appeared on the subject of river restoration (e.g. Roni, Hanson & Beechie 2008; Miller, Budy & Schmidt 2010; Matthews et al. 2010; Leps et al. 2016; Muhar et al. 2016; Nilsson et al. 2015) common barriers to success include the remaining presence of physical barriers to invertebrate recolonization, the multiple wider scale pressures that may remain unaddressed by the restoration project and limit species diversity, and the time lag between habitat restoration and ecological response. However, making changes to channel physical morphology remains appealing because results are tangible and visually apparent in the short term, even if not from a biological perspective.

1.2 Knowledge gaps in stream ecology

The rise in popularity of river restoration projects, along with more stringent requirements to achieve good ecological status or protect existing resources, has led to the realisation that much of the fundamental ecological knowledge base on the response of invertebrates to hydromorphological change, needed to make good decisions and predictions is either lacking, inadequate or contradictory (Vinson and Hawkins 1998, Vaughan and Ormerod 2010). This situation has arisen not through a lack of investigation, but more through a neglect of strong inferential tools (Downes 2010). As a result, our ability to identify confidently the causes of biological degradation and to predict the potential effects of proposed modifications is restricted. Furthermore, the developing ideal of restoration goals, as an action that will restore not just natural species and habitat diversity, but also ecological function and fluvial geomorphic processes, including their required disturbance regimes, requires more in depth understanding of how structure indicates, or indeed creates function (Palmer et al. 1997, Strayer 2006, Allan and Castillo 2007). This situation needs urgent remedy if good scientific reasoning is to underpin aquatic resource management and conservation in the presence of multiple and interacting stressors.

The difficulty in quantifying relationships between stream biota and the physical environment stems from a number of inherent properties of rivers that make general rules difficult to define. The importance of the type and arrangement of physical structures that support functioning river ecosystems are complicated by factors of scale dependency, natural variability and dynamics, and the interacting effects of multiple pressures. Existing monitoring approaches can therefore be limited in their ability to capture sensitive elements of the biota.

Rivers and streams are patchy and hierarchical open systems (Frissell et al. 1986, Poole 2002), which are best described by hierarchical patch dynamics concepts. This views ecological systems as nested, discontinuous hierarchies of patch mosaics, with interactions between component patches and their context at broader scales illustrated in Fig 1.1 (Poole 2002).

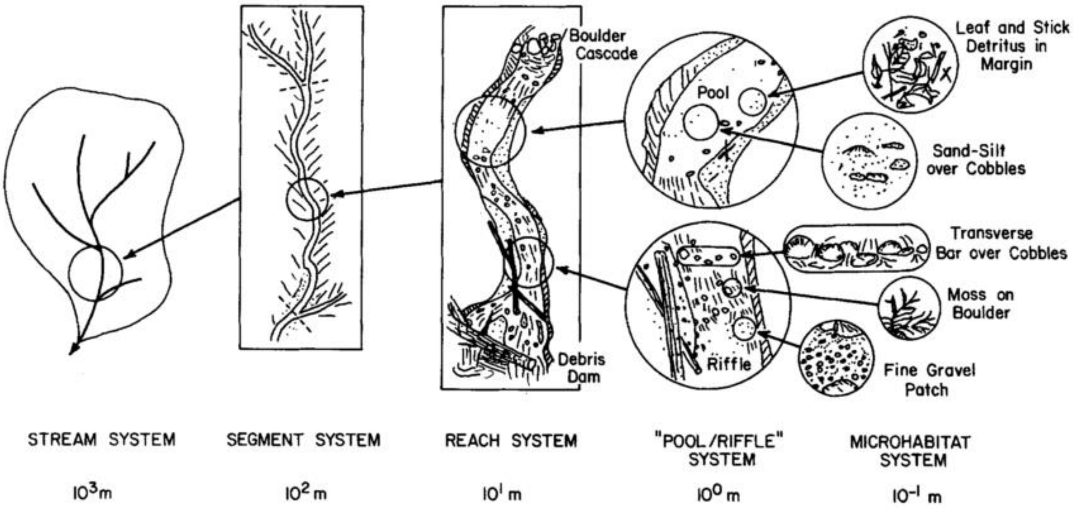


Fig 1.1 The hierarchical arrangement of habitats in a stream system (Reproduced from Frissell et al. (1986))

The detection of natural phenomena such as patterns of species richness or community assembly relies on the scale of measurement that is used, being appropriate to the scale at which the phenomena occurs (Legendre and Legendre 2012). For example, the scale of the UK River Habitat Survey (500m reaches) may be sufficient to observe patterns in riverine birds, but is too coarse to be meaningful for diatoms (Vaughan and Ormerod 2010). The accurate matching of scales between target pattern and process, and that of observation, is essential. Another complication is that many relationships that are observed are scale dependent, meaning that findings from studies at one scale cannot necessarily be applied at coarser or finer scales (Cooper et al. 1998). Therefore, explicit attention must be paid to the scale of relationships when determining the suitability of general ecological rules.

Moss (2000), distinguishes freshwater systems from terrestrial ones as representing a continually changing environment, where a high frequency of disturbance from sources ranging from variable flow to large herbivores, drives turnover in biodiversity. Therefore, heterogeneity of physical features in space and time is often viewed as a good indicator of 'naturalness'. Relationships between biotic and abiotic elements in river systems too, are complex, often having multiple interacting drivers, feedback loops and non-linear relationships mediated by thresholds. This natural dynamism and complexity at a range of spatial and temporal scales presents a challenge when attempting to isolate and apportion the effects of factors such as engineering stresses, or other non-natural disturbances, from natural variation or other unknown or unmeasured factors or sampling error. Space for time substitution survey designs, routine monitoring programmes and current biomonitoring techniques, may not be able to appreciate the full dynamics of natural systems. However, understanding and quantifying local spatial and temporal variability is important for interpreting mechanisms controlling stream ecology and assessing ecological status of impacted sites in comparison to natural ones.

Therefore, we need accurate measurements and descriptions of natural river form and function, including the natural disturbance regime, against which to compare modified states and dynamics. Compared to previous monitoring requirements, where invertebrate communities were used to reveal hidden pressures such as chemical pollution, temperature stress or acidification, physical alterations to channels and floodplains are obvious, but their relevance to ecology and thus to the integrity of river function is subtler and what we now strive to demonstrate clearly. The current debate on how best to assess the physical habitat requirements of aquatic organisms and to use this information to demonstrate stream ecosystem function, requires explicit observation and consideration of the role of environmental factors at a range of scales, and an appreciation of natural river dynamics in both impacted and unimpacted systems.

1.3 Study aims and overview

The work in this thesis aims to show how an approach to survey design, that explicitly appreciates temporal and spatial patterns and scales of measurement, can improve our understanding of the relationship between aquatic riverfly and hydromorphology. By uncovering the biological metrics and sampling approaches that are most sensitive to hydromorphological disturbance, the ultimate goal is to improve predictions of biotic response to disturbance.

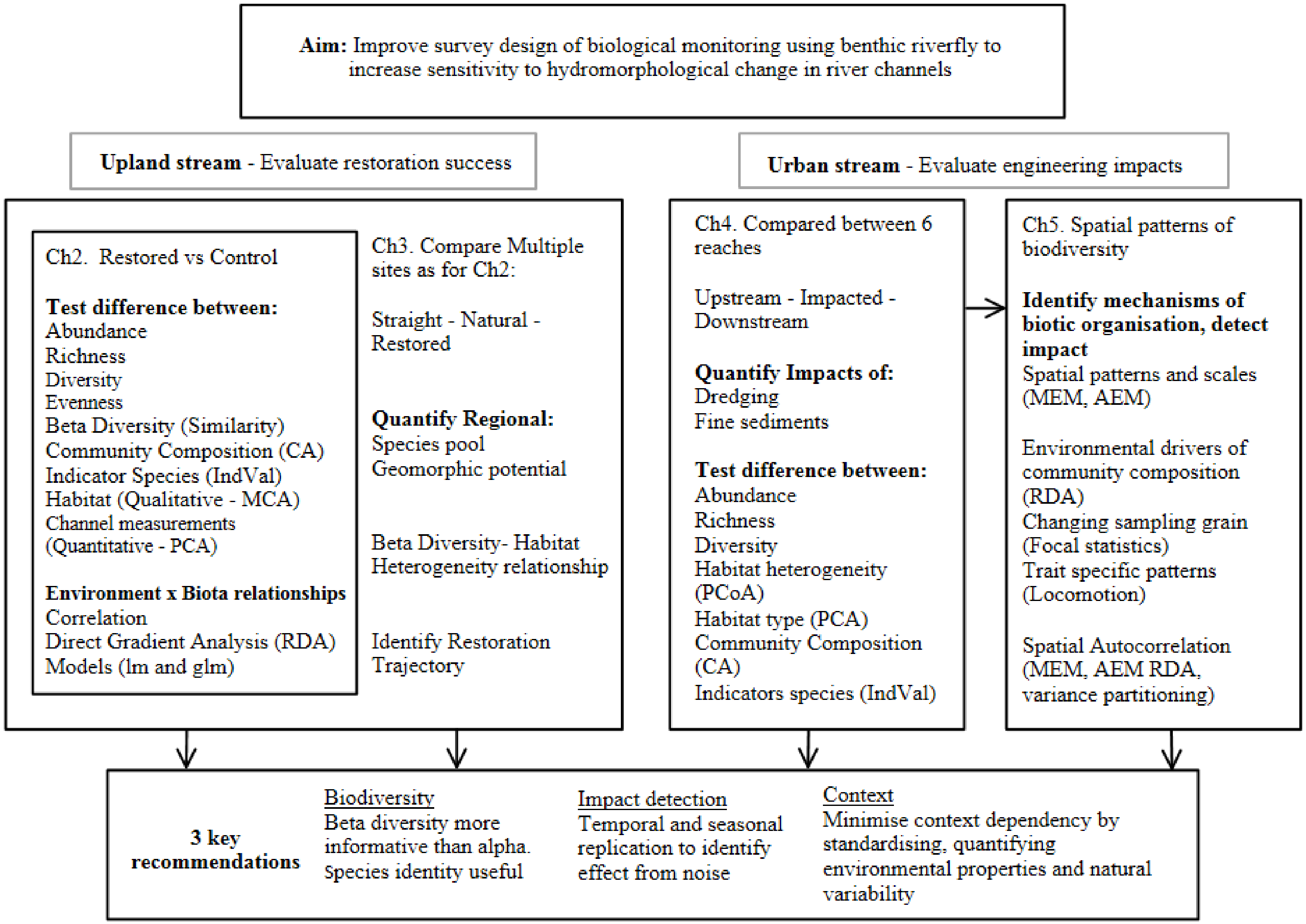


Fig 1.2 Structure and overview of approach in this thesis, outlining chapter contents and relationships between chapters. Abbreviated methods CA – Correspondence Analysis, IndVal – Indicator Value, MCA – Multiple Correspondence Analysis, PCA – Principal Components Analysis, RDA - Redundancy Analysis, PCoA – Principal Co-Ordinates Analysis, MEM – Moran's Eigenvector Maps, AEM – Asymmetrical Eigenvector Maps, lm -linear model, glm – generalised linear model.

The data in this thesis are presented in 4 chapters of primary research, uncovering the spatial patterns and environmental drivers of riverfly biodiversity and community assembly, and how these are altered due to anthropogenic disturbance. The structure of the approach taken is outlined in Fig 1.2.

Chapters 2 and 3 record the physical and biological response of an upland channel realignment project in Glen Clova, a near natural catchment in the eastern Cairngorms, Scotland. Chapter 2 consists of an upstream versus downstream assessment of the realignment project, using a high intensity sampling approach over time, and within study sites, taking spatially contemporaneous physical and biological measurements. A range of biodiversity and community metrics were considered to identify which measure were most sensitive to hydromorphological improvements and their effects on riverflies, and to assess their performance as indicators of restoration success. Chapter 3 considers replicate impacted and references streams in the catchment of the realignment project, to assess the restoration trajectory of the restored channel in relation to regional biological and geomorphic potential.

Chapters 4 and 5 document the physical and ecological impacts of flood defence engineering work in the channel of a small urbanised stream in central Scotland, using a high resolution grid mapping approach to intensively survey a number of short reaches. Chapter 4 describes the habitat availability, riverfly biodiversity and community assembly present in this urban stream and quantifies the physical pressure from engineering and fine sediment release. It then contrasts habitat and biotic change overtime, in impacted and unimpacted reaches, to determine and differentiate between the effects of natural variability and man-made disturbance. Chapter 5 explores the usefulness of taking a spatially explicit approach to sampling and data analysis, to explore the mechanisms that are responsible for biodiversity patterns and biota-environmental relationships. It firstly describes the spatial patterning of invertebrates, including those related to the directional nature of stream flow. This is followed by an assessment of the impact of disturbance on the natural temporal and spatial variability of these patterns. Finally, the role of sampling grain size and taxa locomotion traits on species-environment relationships and how these can be confounded by spatial autocorrelation is assessed. Chapter 6 summarises and discusses a number of significant themes in river research highlighted by both case studies. Suggestions are given on how findings from this thesis can be employed in monitoring designs to improve the mechanistic understanding of how hydromorphology determines properties of riverfly diversity and community composition.

Chapter 2 – Measuring and evaluating success of a restoration project using benthic riverfly larvae, implications for monitoring

2.1 Introduction

2.1.1 History of river modification

Humans have a long history of modifying rivers and the surrounding landscape for their benefit. Now however it is appreciated that this legacy has negative consequences for functioning river ecosystems and as a result people, either directly, through flooding and limits to water resources, or indirectly by the breakdown of wider ecosystem services provided by river processes (Vörösmarty et al. 2010). Modifications to freshwater systems occurred much earlier in the past than can be evident from looking at the landscape today, leading to a distorted appreciation of natural ecosystem function that may underestimate human impacts and make it difficult to set suitable reference conditions. For example historic weir and mill construction affecting migratory fish species (Dudgeon 2010) and the loss of large herbivorous mammals (Moss 2015).

Contemporary threats to freshwater biodiversity remain, with pressure from a growing population resulting in a continued demand for land use change due to urban development, industrial activity, agricultural expansion, water abstraction and damming (Malmqvist and Rundle 2002). These changes and threats should all be seen through the lense of a changing climate, that alters many aspects of the hydrological cycle (Gilvear et al. 2002), that in turn drive spatial and temporal patterns of river habitat and biota. This makes it important to conserve current natural systems and restore degraded or managed rivers to become resilient to future change.

2.1.2 The fluvial landscape

The understanding of river systems requires a holistic perspective considering the disciplines of, hydrology, chemistry, biology and geomorphology. Fluvial systems are complex open systems with a 4 dimensional nature (Ward 1998). The 3 spatial dimensions consist of longitudinal downstream flow, lateral connectivity to the riparian and floodplain area, and the often neglected vertical connection through the bed sediment to the hyporheic zone. The 4th dimension of time, considers the rate of input and movement of water and sediment through the river network.

Elements of the river system are set in a hierarchical framework (Fig 1.1), where physical forms provide structure, and processes act at scales from the catchment down to the mesoscale sediment patch (Poole 2002, Brierley et al. 2010). Within this complex landscape is set the river channel or reach, a focal unit of investigation for stream research. River channel morphology at any location within the catchment is determined by 4 principle conditions, 1) the volume and timing of water from upstream, 2) the volume, timing and calibre of sediment inputs to the channel, 3) the character of the bed and bank material 4) the geological history of the landscape, primarily the topographic gradient (Church 2015).

River channels are naturally dynamic structures at a range of spatial and temporal scales, constantly adjusting to changes in discharge or sediment inputs (Sear et al. 2003). These adjustments through erosion, deposition, channel migration and flooding can lead to conflict with human use of the landscape, resulting in management actions which aim to control natural channel processes and ultimately degrade the river system and stream biodiversity.

2.1.3 Ecological impacts of river modification

The sediment and flow regimes, along with the channel structures provides the 'template' on which stream ecological structure and function is built (Vaughan et al. 2009). Stream communities have over time adapted to these natural structures and dynamic process, meaning that human modifications, either directly to the channel or indirectly through land use changes (Allan 2004) have led to negative impacts on stream biodiversity and ecosystem function. For example, fine sedimentation of the river bed makes substrate conditions unsuitable for some taxa, causing them to evade the stress through drifting down stream (Wood and Armitage 1997). The alteration to flow dynamics through abstractions and flow regulation, for example for energy generation disrupts the complex pattern of wetting and drying that supports specialist taxa (Bradley et al. 2012). Removal of the riparian structures that provide shade and inputs of organic materials, can alter the basal food source from detrital to primary production (Allan 2004).

Simplification or alteration of channel structure through channelisation can remove the spatial and temporal heterogeneity of natural stream channels that provide a range of niches to support diverse communities. Friberg (2009) describes the specific impacts of stream channelization to be the replacement of gently sloping banks with steep ones, creating a uniform cross section and eliminating processes that would create or maintain natural pool and riffle sequences and marginal depositional areas.

In Scotland, the Scottish Environment Protection Agency (SEPA) recognise the severe impact historical engineering can still exert today, not only resulting in reduced habitat quality, but also costly river maintenance and sometimes increased flood risk. They identify 10% of river length morphology to be affected by historical engineering in the Scotland river basin (SEPA 2007). Further to the impacts described above, historic engineering of rivers in Scotland has also resulted in the loss of floodplain wetlands through the construction of embankments, loss of in channel habitat due to increase erosion during floods and also a loss of bank side vegetation.

2.1.4 The need to restore hydromorphology

The introduction of statutory requirements through the Water Framework Directive (WFD) (2000/60/EC) has shifted approaches in water resource monitoring to consider water bodies in a more holistic way. There is now a need to consider not only water quality - which has improved considerably in recent years, but also hydromorphology directly in addition to the biological components of phytobenthos, macroinvertebrates, macrophytes and fish. The WFD introduced the term hydromorphology to describe the hydrologic and geomorphic elements of river habitats, defined specifically by Wolter et al. (2013) as 3 components:

Hydrological regime: the quantity, and dynamics of flow and connectivity to groundwater

River continuity: longitudinal and lateral movement of water, biota and sediment

River morphology: habitat type, structural complexity, substrate, width/depth, bed bank and riparian zone

The capacity of river managers to restore hydromorphology and its function of supporting healthy biota is hindered because the state of knowledge regarding the links between ecology and river hydromorphology is currently poor, consisting of mostly qualitative observations (Vaughan et al 2009). Despite this lack of knowledge a significant proportion of restoration projects state their aims to be to improve biological quality and biodiversity, through physical methods of stabilising channels and improving riparian and in-stream habitat (Palmer et al. 2014). This has the benefit of meeting the hydromorphological WFD requirements directly, however it relies on the common assumption that an increase in habitat heterogeneity, substrate and flow diversity will result in biodiversity gains.

2.1.5 Monitoring and recent outcomes of restoration

Questions have been raised about our level of understanding of biota-environment relationships, (Wolter et al. 2013) since the success of the growing number of in-channel restoration projects has been mixed, with biological recovery not progressing as expected (Feld et al. 2011, Haase et al. 2013). In their review of success of restoring habitat heterogeneity and thus invertebrate richness, Palmer, Menninger & Bernhardt (2010) found that of 78 independent studies, only 2 showed significant increases in biodiversity that could be clearly attributed to increased habitat heterogeneity. Similarly Leps et al. (2016) observed clear improvements in habitat heterogeneity, but benthic communities response was inconsistent.

A major hindrance to the advancement of assessing the success of restoration is a lack of monitoring, or poor quality monitoring of projects, despite the significant amounts of money spent on the improvements themselves (Harris and Heathwaite 2012). For example, Brooks & Lake (2007) found that only 14% of Australian river restoration projects were properly monitored, and Bernhardt et al. (2005) found that only 10% of reviewed studies in the USA had any form of monitoring, pre or post intervention, included in the project design. Where monitoring is in place the results of many studies have been inconclusive due a plethora of reasons (Kail et al. 2015). Suggested reasons include, poor monitoring design (Downes 2010), a mismatch of scale of restoration and scale of degradation, different recovery rates of studies, (Miller, Budy & Schmidt 2010; but see Leps et al. 2016), or a limited pool of species to colonised restored reaches (Ernst et al. 2012). Lepori et al. (2005) found poor success of restoration in Swedish streams with increased habitat heterogeneity and they suggest that biodiversity did not improve because the restoration, while successful in a purely hydromorphic sense, did not target structures relevant to the target taxa. Tullos et al. (2009) meanwhile found restoration sites in North Carolina (USA), to be occupied by species insensitive to disturbance, highlighting that the act of restoration should be seen as a disruption to food and habitat resources.

2.1.6 Areas for improving restoration monitoring.

It is clear then, that our knowledge regarding the physical structure and processes required to support stream biota and their response to change (Roni et al. 2008, Palmer et al. 2010) needs investment. The exact reasons for project failure is an area of ongoing debate where clarity is still needed, especially considering the high financial costs of projects themselves and the current threats to freshwater biodiversity. Reasons suggested for project failures tend to focus on the remaining physical barriers to invertebrate recolonization, or the multiple pressures unaddressed by the restoration project that limit diversity (Friberg 2010, Miller et al. 2010, Haase et al. 2013). Also suggested is the inaccurate targeting of the perceived pressure on the focal taxa (Lepori et al. 2005). Alternatively, biota may respond to changes that were unmeasured and so not quantified, or respond in ways that current methods do not address, or lack sensitivity too. Four approaches are discussed further that have the potential to clarify biotic response to in-channel modification or restoration and improve the use of biomonitoring for impact detection.

Increasing the temporal resolution of monitoring. In the early stage of restoration, recovery can be unstable as this constitutes a short term period of disturbance (Leps et al. 2016). The time lag of the response and establishment of a new dynamic equilibrium can be variable and river specific. Miller et al (2010), found time since restoration to not influence how invertebrate diversity or richness responded to restoration beyond the initial period of disturbance. However time was the most significant driver of restoration outcomes in a review by Kail et al. (2015), with invertebrate metrics showing a non-linear response and worsening over time. Recovery time scale is dependent on a range of factors, initial starting conditions, availability and proximity to invertebrate colonisers (Sundermann et al. 2011), natural dynamics of flow and sediment regimes, that deserve attention in their own right.

Designing a sampling strategy to be sensitive to expected patterns. Many studies lack of before intervention data or a sufficient number of replicates, due to practical limitations. There is heavy reliance on nearby impacted channels as controls through a space for time substitution approach. The use of a more rigorous BACI (Underwood 1991) design is rare, out of 316 studies on the relevant topic of restoring hydromorphology in rivers, only 30 met full BACI requirements (Kail et al. 2015).

Due to the nature of invertebrate lifecycles, patterns in community assembly and richness are strongly seasonal, and comparisons should only be made between equivalent seasons (Reece et al. 2001). As such the choice of sample season for restoration evaluation is likely to affect the values of biodiversity indices, community identities discovered, and their relationships to physical variables. The taxonomic resolution used will also affect the sensitivity of metrics to the changes occurring (Jones 2008). The combination of sampling sufficiency across sites and over time, either years or season, is often a practical choice based on the limited resources available to project monitoring. For example Grönroos et al. (2013) use spring samples, since spring larvae are larger and so can be identified more accurately. Careful consideration must also be given to the spatial and temporal scales, and the degree of concordance between the different elements of sampling. Ensuring relevance of the scale of measurement to the target taxa is essential, as even conditions defined as “local” can be acting at scales much larger than that relevant to invertebrates (Downes et al. 2010), for example variation within a riffle (Pedersen and Friberg 2007).

Measuring and comparing the most sensitive aspect of biotic change. A multi taxa approach is often taken to assess pressures, since different aquatic groups (e.g, diatoms, macroinvertebrates, fish) are sensitive to different pressures over different spatial scales (Friberg 2010). Benthic macroinvertebrates, including the riverfly group, are a popular choice as they integrate many of the habitat conditions and processes that act on and within the reach scale, relevant to the act of restoration of small streams (Friberg et al. 2009).

Retaining information on the taxonomic identification of the biota can yield a greater understanding in addition to using richness and diversity measures (Jones 2008). For example, in German mountain streams, certain orders of taxa were found to be exclusive to either restored multi-channel sites, or unrestored single channel (Jahnig et al. 2009). Knowing which species are present and responsive to environmental gradients allows the identification of indicator species (Podani and Csanyi 2010) or traits that can be used to characterise desirable or undesirable conditions.

Standardising for passive sampling effects, i.e. the relationship between abundance and richness (Gotelli and Colwell 2011), is infrequently encountered in studies of restoration (Louhi et al. 2011), and these limitations of data comparability are rarely considered in restoration impact reviews or meta analyses. Considering the proven reductions to abundance in the early stages of restoration, accounting for these effects on the estimation of species richness could improve sensitivity of evaluation methods.

Moving beyond species richness and abundance, which do not in themselves indicate a more natural biological state (Kail et al. 2015) may prove worthwhile. For example, some studies consider a measure of beta diversity, particularly using sample group dispersion of community data as a measure of beta diversity (Legendre and De Cáceres 2013). Although potentially resource intensive, beta diversity is a measure of the biological heterogeneity between samples, a pattern that could reasonably be expected to follow that of habitat heterogeneity.

Understanding the causes of biotic change requires the measurement of relevant environmental factors. Miller et al. (2010) found that in some cases assessing restoration, changes in habitat heterogeneity were not quantified. The scales of measurement and influence between environmental and biological elements can be mismatched (Downes and Reich 2008). A hierarchical approach is commonly used to investigate this role of scale, for example by considering environmental variables from the smallest microhabitat, up to reach or segment mesoscale, and then catchment macro scales, to test which are most relevant to select for certain species or traits (Lamouroux et al. 2004). Sampling resolution needs to capture the part of the environmental gradient and scale that is of relevance to biota, otherwise results could be unclear or irrelevant.

Geomorphic sampling, when undertaken, is generally limited by being a static snap shot of conditions encountered under low flows. Attributes must be recorded that are relevant to assessing the behavioural regime for that type of river to infer functioning process (Brierley et al. 2010). Hydromorphological restoration is likely to result in the reinstatement of in channel processes of active sediment transport and deposition, which need to be quantified by monitoring of channel attributes, planform, and bed character including grain size and stability.

2.2 Aims

In order to seek new insights into the effects of the restoration on stream biodiversity, this study used a high intensity sampling approach where physical and biological samples were spatially contemporaneous. A wide range of biodiversity measures and habitat features were measured in search of those most relevant.

The aim of this chapter is to quantify how the restored Rottal Burn channel has changed due to the restoration measures, by comparing it to the upstream control. Additionally, to identify which seasons, timescales and biotic indicators were most sensitive to detect these changes in habitat structure? This is achieved by quantifying firstly the effect of restoration on riverfly biodiversity and community composition, and habitat type and diversity. Secondly combining the biological and physical elements to model the environmental drivers of the biotic response. These findings will inform how to refine biomonitoring of restoration projects, to improve certainty of their impacts on their target biotic community, and more generally illustrate how disturbed streams change in the short term. The following research hypotheses are proposed;

Biological

1. Riverfly diversity indices and species assemblages differ between treatment channels. Where the restored channel has higher biodiversity and a unique community.
2. Differences between treatment channels of these measures increase over time.
3. Certain measures and time periods maximally discriminate between treatments and illustrate the effect of restoration.

Environmental

4. Environmental channel properties and habitat diversity differ between treatment channels, restored channels having more natural and diverse habitats.
5. Habitat properties evolve over time in the restored channel, but remain constant in control channels

6. Certain habitat characteristics differ most between treatments, which indicate the restoration of natural channel processes.

Biological – Environment Relationships

7. Riverfly biodiversity indices and community composition are driven by local environmental factors.

8. Local environmental drivers structuring biodiversity indices and community composition, vary between treatments and seasons.

2.3 Methods

2.3.1 Study site: Rottal Burn, Glen Clova, Angus

The Rottal Burn is a minor tributary of the River South Esk and forms part of an upland catchment on the south-east edge of the Cairngorms National Park, 38km north of the city of Dundee (Fig 2.1a and 2.1c). The study site lies at an elevation of 220m and drains a catchment of 17.1km^2. The channel is predominantly cobble/gravel, and surface geology is dominated by alluvium, deposited during historic flooding of the South Esk. Land use in the area is predominantly pastoral farming on the valley floor with rough grazing, plantation forestry and moorland managed for grouse shooting on the valley slopes. The catchment is steep and very responsive to rainfall (Braid 2014). Monitoring after the first year of channel construction indicated a mean annual flow of 0.96m^3/s (EnviroCentre 2014).

The Rottal Burn has been canalised since the 1800s, and is impacted by embankments, dredging, and grazing on the riparian area (Jacobs 2011, Fig 2.1b). Although the South Esk is a designated Special Area of Conservation (SAC) for Atlantic salmon and Freshwater Pearl Mussel and historically, the Rottal Burn was a good site for spawning fish, in recent years, populations of both have declined due to poor habitat availability (Jacobs 2011). The restored channel course is based on historical research, old maps and signs in the landscape of old channels and extended the channel length from 800m to 1.2km. Three energy zones were designed (Fig 2.2a), of decreasing stream power and gradient from the road bridge downstream, and increasing sinuosity in the central zone (Braid 2014).

The new channel was formed in June 2012, with the aim to reverse the effects of straightening and dredging, creating suitable habitat for salmonids. After connection to the upstream channel in August 2012, several spates in the first autumn lead to significant channel change due to active bank erosion and deposition (Esk Rivers and Fisheries Trust 2012). Within a short time active development of channel planform occurred, observed in May 2013, mostly in the upstream section (Day 2013) extending zone 1 length by 50m (EnviroCentre 2014). The approach of the landowners and projects managers is to allow natural channel development unless threatening livestock fences and services on adjacent land. The upstream control section remains straightened, embanked and with a legacy of dredging (Fig 2.2c). Surrounding land use is fenced off grazing, rough grassland and the estate grounds.

2.3.2 Stud*y* design

This study consists of 20 sample locations covering the full extent of both the downstream restored reach, and the immediately upstream control (Fig 2.2a). Samples were distributed within the restored reach in order to provide a high number of replicates, as well as to accommodate the range of habitats present in each of the 3 energy zones. Invertebrate samples and habitat surveys were conducted in each sample location taking in the whole channel width and approximately 5m of river length.

2.3.3 Invertebrate sampling and processing

Invertebrate samples at each site consisted of a 3-minute kick sample, using a standard 30x30cm frame kick net with a 0.5mm mesh size. Each sample covered the full channel width, traversed in "v" shape or "w" depending on the channel width covering all available habitats proportionally. Large boulders were disturbed by hand and any vegetation, mostly mosses was also swept through. Samples were taken in spring (May) and autumn (November), 2013 and 2014, to identify differences in seasonal response as well as evolution of the community over time. This resulted in 80 samples being collected.

In the field samples were bagged and preserved with 100% Industrial Methylated Spirits (IMS), marked with an identifying sample number and date and kept in cold storage until processing. In the lab samples were washed through with water and anything greater than 0.5mm sieve mesh was retained (though smaller invertebrates often remain). All invertebrates were picked from samples and stored with 70%IMS. Only the Riverfly taxa, Ephemeroptera, Plecoptera and Trichoptera (EPT) were identified due to the large number of samples and limited time available. Riverfly were identifies to species where possible and counted using the standard FBA keys. For small individuals that were too immature to resolve to species, genus or family was recorded initially. Once identification was complete all samples were briefly revisited to correct any earlier mistakes in identification, and standardise groupings of non-species data to the lowest resolution possible based on: the presence of larger identified specimens in nearby samples, life history notes pertaining to instar sizes at sample months, as well as range distribution from the NBN gateway online map (National Biodiversity Network 2014).

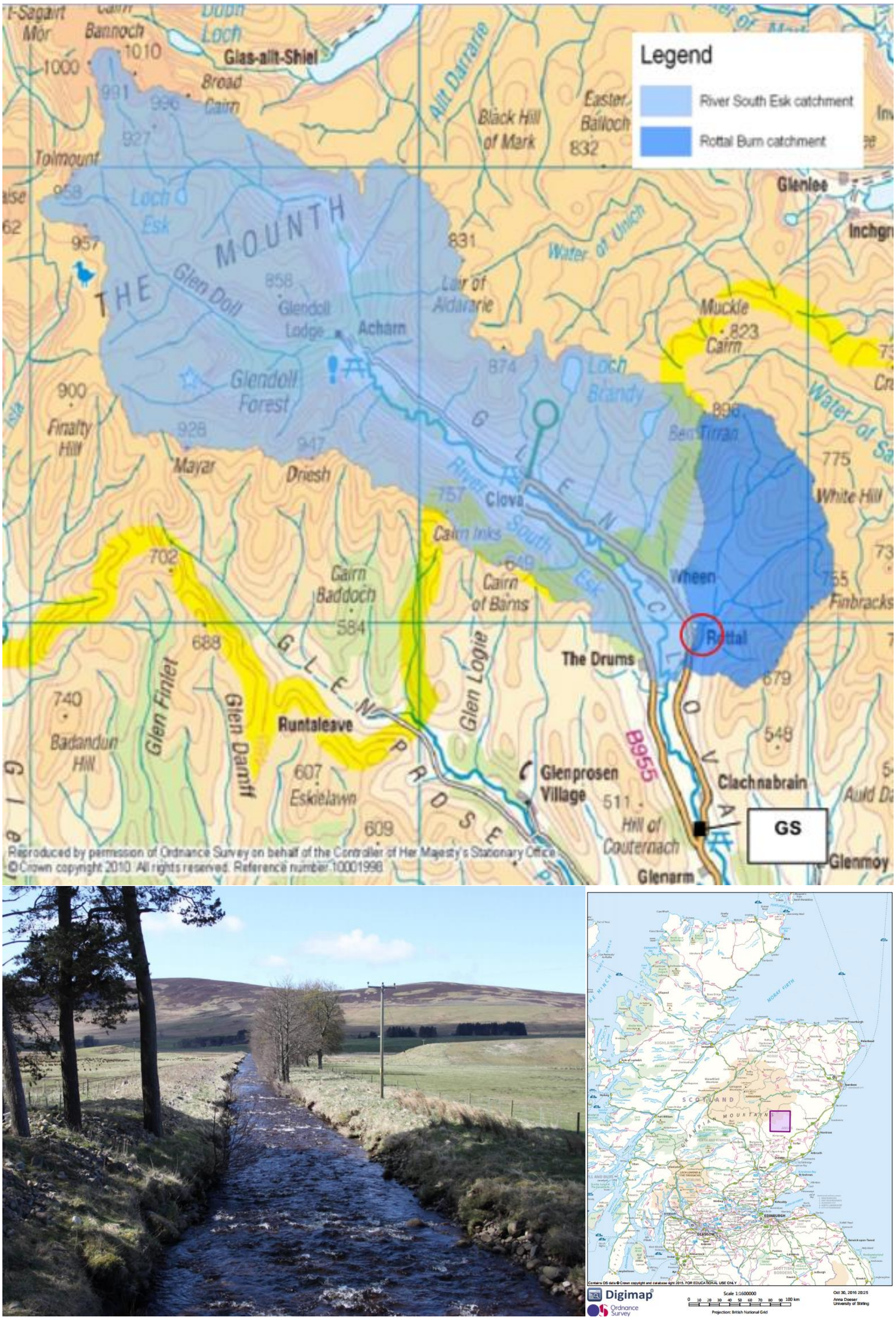


Fig 2.1 a) Location map of the Rottal Burn, showing the extent of the South Esk and Rottal burn catchments (MacDougall 2012). Circle indicates the location of the restoration project, b) photo of the Rottal channel pre restoration (MacDougall 2012), c) Location of the South Esk within Scotland.

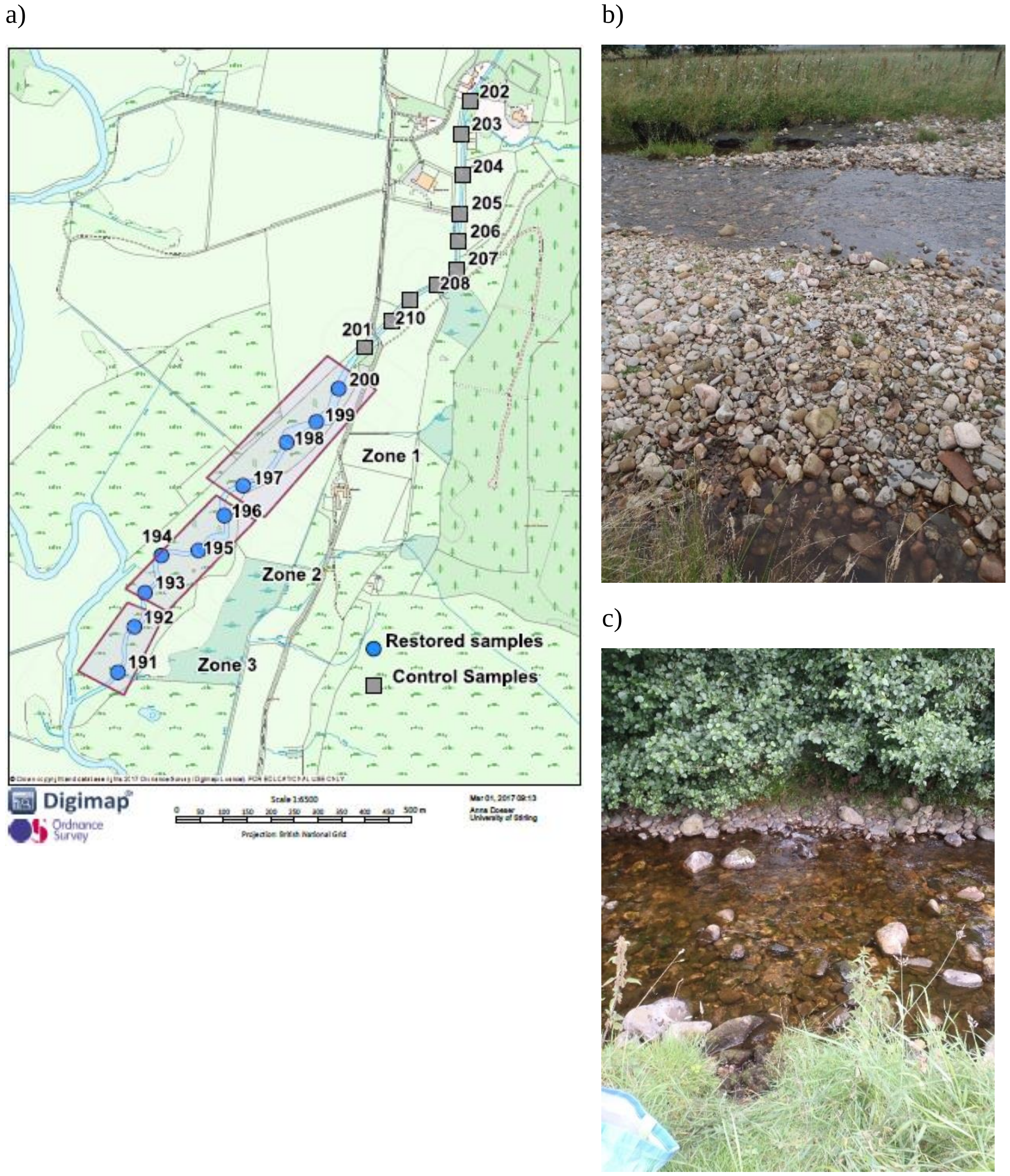


Fig 2.2 a) map of new channel and sample locations, restored (circles) control (square), in the Rottal channel, the 3 energy zones (purple boxes) decreasing in energy from 1-3, b) Photo of example restored channel sample site, and c) Photo of control channel sample site.

2.3.4 Habitat sampling and sediment processing

Physical habitat data were collected during the summer (August and September) of 2013 and repeated in 2014 during low flow periods, when most of the river bed was visible and could be safely accessed. Measurements of fine sediment, particle size estimation, in-channel and riparian habitat and channel dimensions were taken.

Interstitial fine sediment

Resuspension techniques provide a fast and accurate method to quantify surface and subsurface fine sediments that performs equally well over a range of substrates (Duerdoth et al. 2015). Interstitial stored fine sediments were quantified using a modification of the methodology of Lambert & Walling (1988). A flexible plastic bucket with bottom removed was used to enclose 0.1m2 of stream bed (Fig. 2.3), the flexible sides of the bucket enabling it to penetrate between the coarse substrate that is characteristic of streams in this catchment.

Fast flowing sites with cobble-gravel substrate were targeted to avoid naturally depositing areas and to keep habitat consistent between 2 replicates within each site and between sites. Sampling was restricted to water depths of <0.25m to ensure that water loss from the area enclosed was minimal. In placing the bucket some large embedded boulders were removed to reach the sub surface substrate more easily and to secure the equipment in the substrate. The sediment was disturbed by hand and heavier fines could settle by delaying water sampling by 20 seconds. 2 litre water samples were removed, collected in an integrated method by lowering the bottle through the water column. The depth of water was also recorded to standardise for dilution and to calculate the total estimated mass of sediment mobilised per unit area of stream bed.

Fig 2.3 Interstitial fine sediment sampling equipment

The water sample was sieved in the field using a 63 micron sieve. Sediment was retained and subsequently decanted in to a foil tray and dried in the oven at 100 ºC. The final weight of the dried sediment was corrected for the volume of water suspended, to give a standardised value per sample (Appendix 2.1). The mean of the 2 replicates was used in further analysis.

Bed surface sediment estimation

Sediment particle size measurements were taken using a standard Wolman pebble count method. The area sampled was the same 5m river length covered by the invertebrate sample. The surveyor walked heel to toe across the river width channel including areas exposed at the time of sampling such as sand and gravel bars. 100 particles were selected by hand from the river bed at the toe of the surveyor's boot. Measurements of the intermediate axis of each particle were taken to the nearest mm using a metal ruler. During the pebble count, particles were recorded as embedded or not, on the basis of whether they could be picked up freely or force was required to extract them. Pebble count data was converted into percentiles and percentage amount of certain fractions (Table 2.1) for use in the analysis. D50 is the median or most common particle size, used to represent channel sediment size and D85 is an estimation of the size of the large fraction, these large particles are said to be responsible for creating complex hydraulic environments (Brooks et al 2002).

Table 2.1 Sediment variables calculated from the pebble count data.

Name	**Calculation**
Mean pebble size	Sum of sizes/100
D50	50th percentile
D85	85th percentile
% fines	Number of particles < 0.2cm
% Embedded	Number of particles embedded

Habitat survey

At each sample site (n =20) a survey was conducted based on the River Habitat Survey in Britain and Ireland "Spot check" (Environment Agency 2003, Section 2.2). This records a number of habitat features present in the channel, bank and riparian areas. Features recorded for this study are outlined in Table 2.2.

Table 2.2 Description of the habitat variables recorded, based on the River Habitat Survey spot-check.

Zone	Item	Description
Channel	Substrate	Single predominant bed substrate in the wetted channel: *Bedrock, Boulder, Cobble, Gravel, Pebble, Sand, Silt, Clay, Peat, Earth, Artificial*
	Flow type	Predominant flow category based on water surface patterns: *free fall, chute, broken standing waves, unbroken standing waves, chaotic flow, rippled, upwelling, smooth, no perceptible flow, dry*
	Features	Presence of any channel features: *Exposed boulders, vegetated rocks, un-vegetated mid channel bars, vegetated mid channel bars, side bar, none*
	Vegetation	Type and cover (0-absent, 1-present, 2-extensive) of: *Liverworts/mosses/lichens, emergent broad-leaved herbs, emergent reeds, filamentous algae*
Bank - Left and Right	Material	Predominant material making up bank: *Bedrock, Boulder, Cobble, Gravel/Sand, Earth, Peat, Clay*
	Modification	Presence of modified bank structures: *Embankment, Resectioned*
	Features	Presence of any of the following: *Eroding Cliff, Stable Cliff, Side Bar, Vegetated Side Bar, None* (recording if cliff is made of sand or sandy soils)
	Vegetation	Structure of vegetation of both bank face and bank top (within 5m of the bank edge): *Bare, Uniform, Simple, Complex*

Once all habitat data had been recorded, inconsistencies from inexperience during the first survey were corrected for by referring to site photos and photographic guidance (Environment Agency 2003). Ambiguous areas that needed clarification related primarily to location and type of bank vegetation (Fig 2.4).

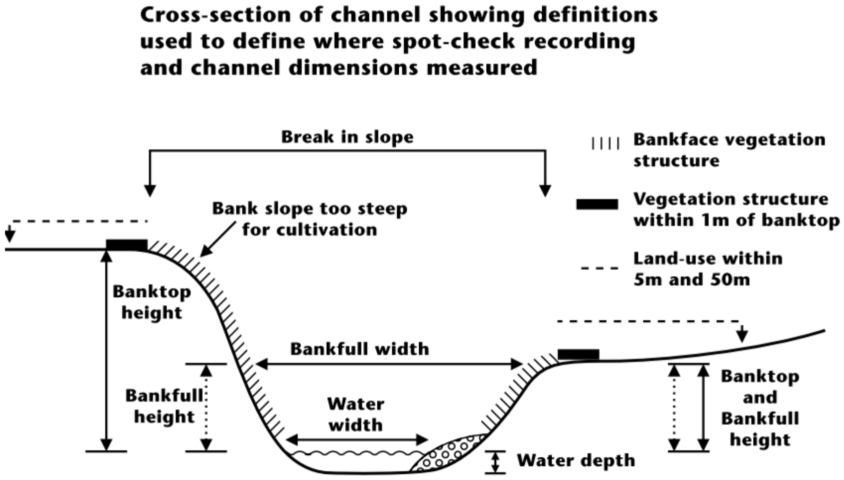


Fig 2.4 Illustration showing definitions of bank face, bank top and channel width used in the habitat survey (reproduced from Environment Agency (2003)).

Channel measurements

Channel bank full width was also measured at each site using a tape measure stretched between bank tops (Fig 2.4). Substrate composition was visually estimated giving the percentage cover of the following sediment size classes that occurred over more than 5% of the channel: Boulder (256-4096mm), Cobble (64-256mm), Pebble (16-64mm), Gravel (2-16mm), Sand (0.25-2mm), Silt and Earth. If potentially influential sediment occurred at less than 5% cover of the bed (mostly the occasional patch of silt, earth or clay due to bank collapse) this was recorded as 1%.

2.3.5 Statistical analysis

2.3.5.1 Biotic indices

Using the invertebrate data, for each sample (n=80) the total EPT abundance and species density (the number of unique species per sample) was calculated. Diversity indices Simpson's and Shannon's were also calculated, including Pielou's evenness as the ratio Shannon diversity/log Species richness. Sample rarefied richness was calculated using the minimum number of individuals found in any one sample (24 individuals in spring 2014 - site 191), to standardise for differences in richness between samples caused by differences in the number of individuals.

To make use of a larger sample size a reach scale rarefied richness was calculated by pooling the 10 samples within each treatment. This uses the minimum abundance of the 10 samples combined (1069 individuals in Autumn Control 2014) to calculate a rarefied species richness estimate for each treatment and sampling occasion. Pairwise comparison tests between treatments within each sampling occasion were made, using rarefaction to the lowest number of individuals within each pair, followed by a randomisation test to assess the significance of the difference in mean rarefied richness. Low sample size may also have limited the accuracy of estimated species diversity for the reach as a whole. Similarly to species richness estimates, the 10 samples in each reach were pooled to produce a reach composite species diversity estimate. Confidence intervals and significant differences between treatments were tested a modified version of a t-test as outlined by Gardener (2014).

Complimentary to rarefaction to standardise sample sizes and to calculate reach scale richness, rarefaction analysis was used to statistically resample the data to produce both individuals and sample based species accumulation curves. Although an equal number of samples were taken for each season and treatment type, this method can be useful to determine if by taking further samples a more accurate estimate of species richness could have been obtained. Estimated species richness was calculated using the bias corrected Chao1 (O'Hara 2005), which can also standardise species richness estimates for unequal sample sizes. This is a nonparametric asymptotic species richness estimator which accounts for unseen species in the sample based on the number of singleton and doubleton species present (Gotelli and Chao 2013).

Each biotic measure was explored for normality and consistent variance between sample groupings (season and restoration treatment). Where these parametric assumptions were violated, non parametric statistical tests were used to compare between sites. Statistical significance of differences between sampling occasions were tested using Mann-Whitney U test (2 groups) and Kruskal -Wallis tests (>2 groups).

2.3.5.2 Community composition and indicator species

The level of uniqueness of riverfly community assembly for each sample period and treatment group was analysed using multivariate ordination techniques. Detrended Correspondence Analysis (DCA), is an indirect gradient analysis technique which can infer an environmental gradient from species composition in a site by species abundance matrix. A long first gradient derived from the technique indicates high turnover of species from one sample to another, and that univariate, not linear, methods are appropriate. In this case DCA indicated a long gradient of species change between samples (approximately 4), suggesting that univariate methods were most appropriate. Therefore the sample by species matrix for the whole Rottal Burn community was analysed using Correspondence Analysis (CA). CA is an unconstrained ordination method summarising each sample as a weighted average of the species present. This is useful as samples can be compared, considering all members of the community simultaneously. In an ordination plot, distances between sample points are their approximate Chi-squared distances between their relative species abundances, indicating community similarity (Greenacre and Primicerio 2013). Species identity is retained in the analyses and the standardised species profiles can be regressed along the ordination axis to determine which species contribute most to sample discrimination. A contribution biplot was used (Greenacre and Primicerio 2013) to illustrate species relationships based on their Chi-squared distance, with species axis scores weighted by their relative abundance in the whole data set. The result is that the species that contribute most to the CA solution, and so community dissimilarity, are shown at the outer edges of the axes.

Invertebrate data was analysed using untransformed count data. A log (x+1) transformation of abundance resulted in equivalent patterns of sample group positions and spread. Since CA is not as sensitive to rare species as some have criticised (ter Braak & Šmilauer 2014; Greenacre & Primerico 2013; Greenacre 2006), and rare species appear important in distinguishing between treatment richness and diversity, raw data was used. Data groupings in CA were identified by the plotting of 95% confidence ellipses, these ellipses indicate, with 5% error rate, the true location of the group mean, equivalent to the average community composition for that group.

Potential indicator species of each channel treatment were determined using their Indicator Values (IndVal). Indicator value analysis determines the specificity and fidelity of each taxa to each a priori defined group (Dufrene and Legendre 1997). Where specificity is defined as the relative abundance of a species in that group over all groups, and fidelity as the proportion of sites within a group that the species is present. IndVal was carried out on the full dataset, using 8 groups defined by the combination of the year x season x treatment. Indicator Values range from 0-1, with the largest values indicating that that species is only found in that group, and occurs in all samples of that group. Taxa that had a score of over 0.25 were deemed good indicators, the significance of this value was tested against a random permutation of the data using 1000 iterations.

2.3.5.3 Beta diversity and partitioning

Beta diversity within each treatment was compared using the Bray-Curtis and Chao dissimilarity coefficients. Bray Curtis distance is a very commonly used method to determine beta diversity, but it has been criticised for being strongly affected by differing sample sizes due to the use of absolute abundances (Jost et al. 2011). The Chao distance, is an abundance based version of the Jaccard index (Chao et al. 2005), a measure of the number of shared species in proportion to the total number of species in 2 sites combined. The Chao distance has a number of properties, not present in Bray Curtis, that make it well suited to the analysis of macroinvertebrate samples. As has been shown species richness was most robustly approximated when corrected for abundance differences between samples, and rarefaction showed that species diversity may be underestimated due to un-sampled and infrequent taxa. The Chao dissimilarity corrects for under sampling, and is invariant to the total abundance of the samples being compared, i.e. density invariant (Jost et al. 2011, Legendre and De Cáceres 2013).

Fluctuation in invertebrate community assembly across space and over time is likely to occur naturally and stochastically. PERMANOVA is a robust technique to analyse and test the significance of differences in multivariate community composition (location) and difference from the average community (dispersion) for pre-determined groups of samples (Anderson and Walsh 2013). The differences in community composition and dispersion were tested, followed by pairwise comparisons between restored and control channel treatments, for each sampling period, using a permutational MANOVA with each dissimilarity matrix (Bray-Curtis and Chao).

Beta diversity can be partitioned into its constitute components, attributable firstly to the change in composition due to the addition and removal of species, and secondly to changes in abundance of common species (Baselga 2010). Within reach heterogeneity, or beta diversity, for each group of samples (8 groups of 10 samples) was analysed individually, determining mean distance to group centre and standard deviation. This measure of beta diversity was further partitioned into; "gradient", the abundance based equivalents of the binary nestedness, and "balance change", the equivalent of turnover (Baselga 2013), hereafter referred to as the more familiar terms of nestedness and turnover. Species turnover refers to the replacement of one species by another from one sample to the next. Nestedness is the loss of species from one sample to another, resulting in the second sample consisting of a subset of the first. The mean dissimilarity and standard error, along with the 2 diversity components were plotted together to make direct comparisons. This method is currently restricted to using the Bray Curtis dissimilarity.

2.3.5.4 Physical environment and habitat

Characterisation and comparison of environmental and habitat characteristics was done using both categorical responses from the River Habitat Survey style data, and continuous data from channel and sediment measurements. The physical differences between the 2 treatments, their change over time, and the gradients driving these differences was analysed using ordination methods Principal Components Analysis (PCA) and its categorical counterpart Multiple Correspondence Analysis (MCA) (Legendre and Legendre 1998). MCA and PCA simultaneously consider multivariate predictors, relating samples to each other based on their environmental profiles, and identify the environmental and habitat characteristics responsible for similarities.

During MCA, the contribution to, and representation of, sites and features on each axis was checked for the influence of dominant sites. Although one site with an unusual profile was observed, its removal from the analysis did not alter the sample groupings and positions. Major environmental gradients in the data were summarised as the PCA and MCA ordination axes, and extracted for later use in constrained ordination and regression models of the community data and biotic indices.

2.3.5.5 Modelling community composition

Since there was an evident change in riverfly community due to season (Fig 2.9), this effect was quantified, then the data divided into Spring and Autumn samples for both years (n=40). For each season, the influence of the environmental variables was tested using Redundancy Analysis (RDA) for their ability to explain variation between community assemblage across samples and ultimately between treatments.

RDA is a constrained ordination method that extracts the variation in one data set that can be explained by another. The procedure combines linear regression and PCA, and is an extension of regression for modelling multivariate response (Borcard et al. 2011). Since RDA has linear assumptions the species abundance data was first Hellinger transformed (Legendre and Gallagher 2001), which is a square root transformation that can normalise Poisson or Poisson like data to meet linear assumptions (Peres-Neto and Legendre 2010). Using 4 environmental variable groups identified initially by PCA (Table 2.9), a model with all group terms was constructed to determine a global model adjusted R squared, this was used as a second stopping criteria (Blanchet et al. 2008a) for variable reduction methods. To remove redundant variables, forward selection methods were applied to the list of variables, aiming to include at least one significant term from each family of variables. The significant group variables were included in a "best model", to which the forward selection process was applied again to lead to a final reduced model.

2.3.5.6 Correlation between biotic indices and environment

Biotic indices calculated and described in section (2.3.5.1) were tested first for correlation with each of the environmental variables using the non-parametric method of Spearman rank using the full dataset. Correlations within each season and river treatment combination were also calculated. The values of significant correlations are displayed.

2.3.5.7 Modelling the variation in species indices using the environmental gradients

The relationship between each environmental variable and biotic measure, was initially explored through a scatter plot. This indicated possible distinct trends that were different between river treatments and between seasons. As a result of this, data for each season was modelled separately, using the environmental variables listed in Table 2.9. The model form was tailored to the properties of the response variable. For instance, species richness and rounded rarefied richness count data, were analysed using a generalised linear model with Poisson distribution. Abundance data indicated over-dispersion, so a negative binomial model was used (Zuur et al. 2009). Shannon's and Simpson's diversity indices were first log10 transformed to approximate a normal distribution, and then analysed using a linear model.

A number of potential model scenarios were possible (Fig 2.5), describing different relationships between the biological response, environmental gradient and effect of restoration treatment. To determine what forms of relationship exist between the environment and riverfly biodiversity each individual biotic metric was modelled using each environmental variable. River treatment (restored, control) was included as an interaction term, appreciating that the environment x biotic response relationship might vary between treatments. Before modelling, all predictor variables were scaled to allow direct comparisons of effect sizes. In total 160 models were generated, each was evaluated for treatment effects, interactions, and treatment specific slopes. Slope estimates and confidence intervals (CI) for treatment specific effects, were extracted from the model covariance matrix. Where the CIs did not extend across 0, this slope was deemed significant, this value also illustrated the direction and magnitude of the relationship. Model R^2, or deviance explained, was recorded, as was the treatment effect: the difference in intercept from the restored to the control (negative indicates restored has higher intercept), and also if the interaction between treatments was significant. PCA analysis and agglomerative hierarchical clustering of model results was used to group the models into types (Fig 2.5). Clustering used the Euclidean distance and "Ward" method. Models were grouped based on the significance and value of: treatment effects, treatment x predictor interaction and treatment slope.

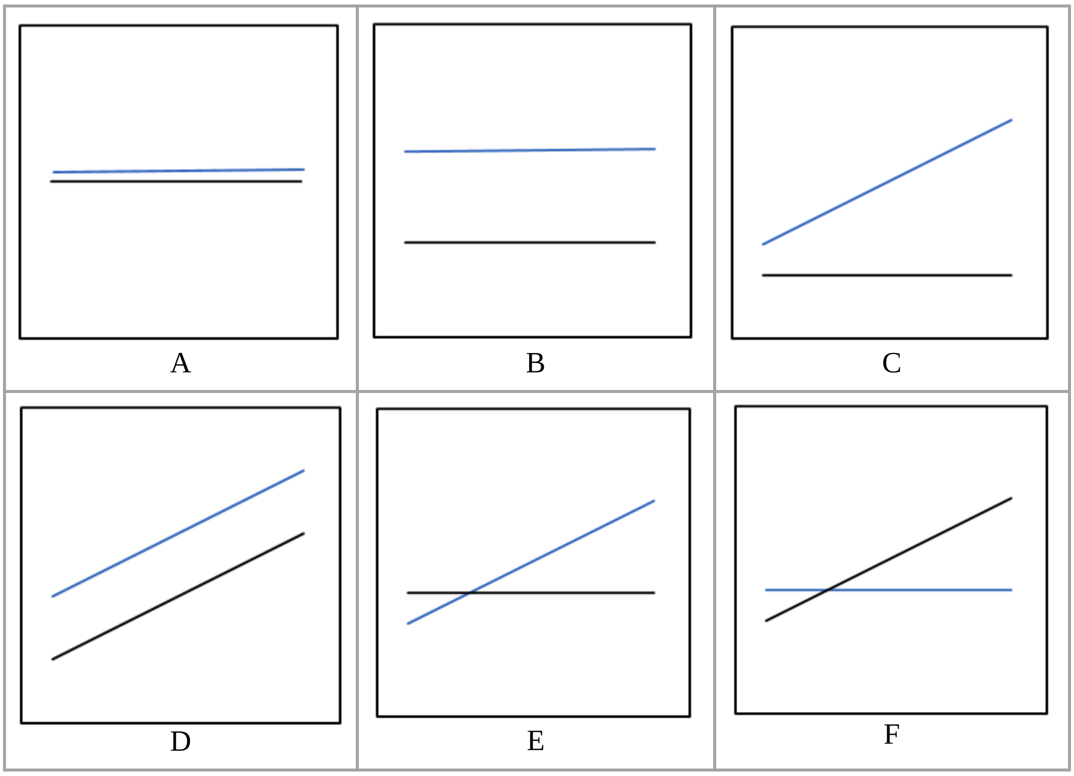


Fig 2.5. Possible model scenarios for observed relationships between the biological metrics and environmental variables and the effect of restoration. Black line = control, Blue line = restored,

A - indices in neither treatment are related to environmental drivers (no slope, no river effect, no interaction), and there is no difference between the treatments

B - indices are not driven by environmental gradient in either treatment, but treatment itself is a strong predictor (no slope, possible interaction, strong river effect)

C- indices in restored channel are linked to environment, control is not, and therefore significant river effect - (restored slope, interaction, river effect)

D- indices in both channels are driven by environmental gradient but one is higher or lower (both slopes, possible interactions, river effect)

E - Restored samples have relationship to the environmental gradient but not control (restored slope, possible interaction, no river effect)

F - Control samples have relationship to the environmental gradient but not restored (control slope, possible interaction, no river effect)

All statistical analyses and graphics were produces using the R environment (R Core Team 2016) in R Studio version 0.99.902 (RStudio Team 2015) with the use of the following packages: rich (Rossi 2011), vegan (Oksanen et al. 2016), Sciplot (Morales and Murdoch 2012), Labdsv (Roberts 2016). betapart

(Baselga et al. 2013) FactoMinerR (Le et al. 2008), ca (Nenadic and Greenacre 2007), Packfor (Dray et al. 2016) , MASS (Venables and Ripley 2002) and cluster (Maechler et al. 2015).

2.4 Results

This first part of this study considers and contrasts the biodiversity, community composition and physical habitat in both restored and control channels over the course of 2 years. The physical and ecological effects of restoration are quantified, identifying when difference are most pronounced, in what season and after how long. A total of 59 riverfly species were found in the Rottal sites over the sampling period, 12 of which accounted for the 5 most abundant species within each treatment reach, season and year sampling occasions (Table 2.3). Identification of the full community to a mixed level: species (Coleoptera), genus and family (Diptera), tribe (Chironomid), family (Mollusca) order (other taxa) was made during the first sampling season, spring 2013. Riverflies made up a large part of this full invertebrate community, 65% and 64% of total species richness and abundance respectively in the restored channel, and 56% and 78% in the control.

Table 2.3 Summary of biological samples, riverfly abundance, richness, diversity, and singly occurring species (mean, maximum and minimum). Mean abundance and standard deviation of 5 most abundant species per season, year and treatment combination.

Year	2013				2014			
Season	Spring		Autumn		Spring		Autumn	
Treatment	Restored	Control	Restored	Control	Restored	Control	Restored	Control
No. Samples	10	10	10	10	10	10	10	10
Abundance	183.8 (45-348)	465.2 (155-793)	470 (170-1131)	225.9 (117-329)	393.1 (24-1058)	423.4 (134-707)	364.8 (145-640)	105.9 (47-351)
Richness	13.1 (5-20)	17 (13-21)	22.8 (18-29)	18.1 (15-22)	18.8 (8-25)	17.4 (12-20)	24.9 (15-31)	14 (11-20)
Shannon	1.67 (1.11-2.16)	1.89 (1.73-2.23)	2.09 (1.79-2.34)	2.10 (1.88-2.42)	2.09 (1.83-2.48)	1.97 (1.65-2.36)	2.19 (1.58-2.59)	1.90 (1.50-2.11)
Simpson	0.72 (0.61-0.84)	0.76 (0.71-0.86)	0.79 (0.70-0.87)	0.80 (0.76-0.87)	0.81 (0.76-0.86)	0.79 (0.72-0.87)	0.80 (0.65-0.89)	0.77 (0.63-0.83)
Singletons	3.7 (1-7)	3.1 (1-6)	5.9 (3-8)	3.2 (1-6)	4.9 (3-7)	3.6 (1-7)	7.8 (1-13)	5.4 (0-11)
Ameletus inopinatus	23.8 (28)	32.9 (19)						
Baetis muticus					17.7 (12)	24.1 (11)		
Baetis rhodani	65.3 (45)	200.2 (129)	41.9 (29)	20.3 (15)	98.1(108)	145.0 (72)	32.0 (30)	32.3 (35)
Brachyptera risi	10.4 (11)							
Chloroperla torrentium					57.3 (39)	34.8 (15)		
Ecclisopteryx guttulata			48.8 (42)				104.4 (94)	
Ecdyonurus venosus				10.9 (5)			22.8 (11)	5.5 (3)
Ecdyonurus venosus				10.9 (5)			22.8 (11)	5.5 (3)
Electrogena lateralis		27.1 (15)						
Electrogena lateralis		27.1 (15)						
Leuctra hipporus/moselyi							18.3 (13)	
Leuctra inermis	44.1 (30)	84.5 (46)	24.1 (21)	17.3(13)	91.7 (94)	106.0 (57)		7.8 (8)
Protonemoura meyeri			36.5 (34)	43.1 (28)				11.0 (12)
Rithrogena semicolorata	15.6 (21)	36.7 (22)	177.3 (124)	78.2 (32)	47.9(54)	36.9(29)	87.8 (70)	28.1(20)

2.4.1 Biotic indices

Abundance and species richness

There were differences in abundance and richness estimates between restored and control channels for many sampling occasions (Fig 2.6 a-e), most often significant between autumn samples. During the first spring season after restoration, and in some autumn samples, riverfly occur at low numbers resulting in unequal sample sizes (2.6a). On the first sampling occasion, abundance, species density, rarefied and estimated species richness was greater in the control channel. However, over time abundance and species richness increased in the restored channel to become greater or equal to that of the control for all subsequent occasions. Whereas species richness remained stable in the unrestored channel. By the last sampling occasion, abundance and richness was significantly greater for all estimation methods in the restored channel.

Samples in autumn consistently showed higher abundance and richness in the restored channel compared to spring (Fig 2.6a and b). Once corrected for differences in abundance and missing species the effect of channel treatment on richness measures was reduced (Fig 2.6c and d), and seasonal differences in the control channel were reduced or removed. However, these patterns remain in the restored channel indicating a true underlying seasonal patterns in species richness. High estimations of the Chao richness (Fig 2.6d) for autumn suggest that these were due to the presence of rare species that occurred as single or double counts.

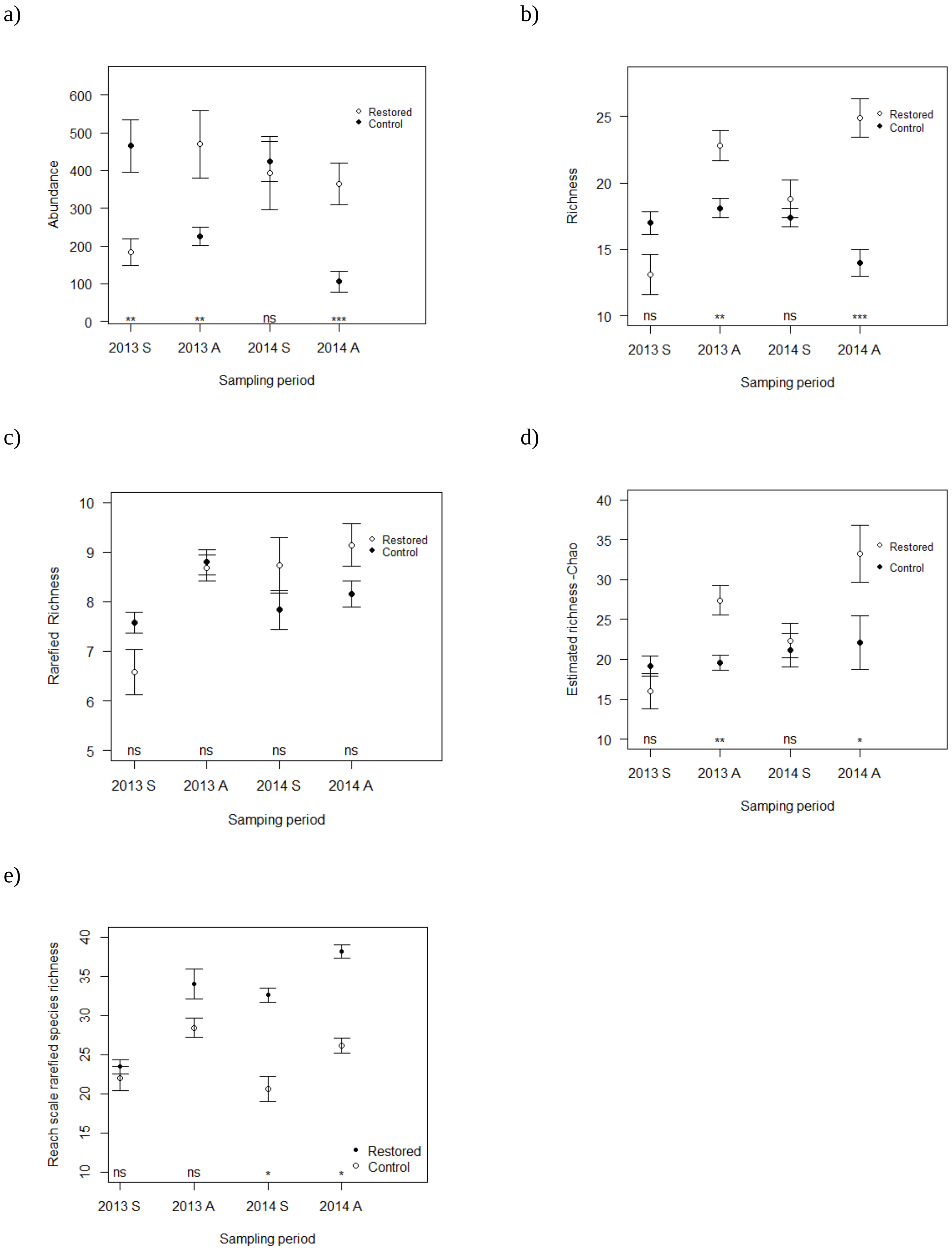


Fig 2.6 Sampling occasion mean value and standard error for a) Riverfly abundance, b) Riverfly richness c) sample rarefied species richness, d) Chao 1 estimated species richness e) reach scale species richness. Asterisks below pairs indicate the result of Mann-Whitney U significance tests (< 0.001 ***, < 0.01 **, < 0.05 *).

The aggregated reach scale measure (Fig 2.6 e) was the only case where restored channel richness was significantly greater than control for the spring 2014 sampling period. This trend was reinforced in the rarefaction curves where the curve for the restored reach was initially steeper than control (Fig 2.7), and consistently showed a greater richness in spring, that increased as more samples or individuals were included. Species richness for spring 2013 restored reach may not have reached its asymptote, as it was limited by the number of individuals captured in each sample. All curves, although levelling off after approximately 1000 individuals do not come to a final asymptote.

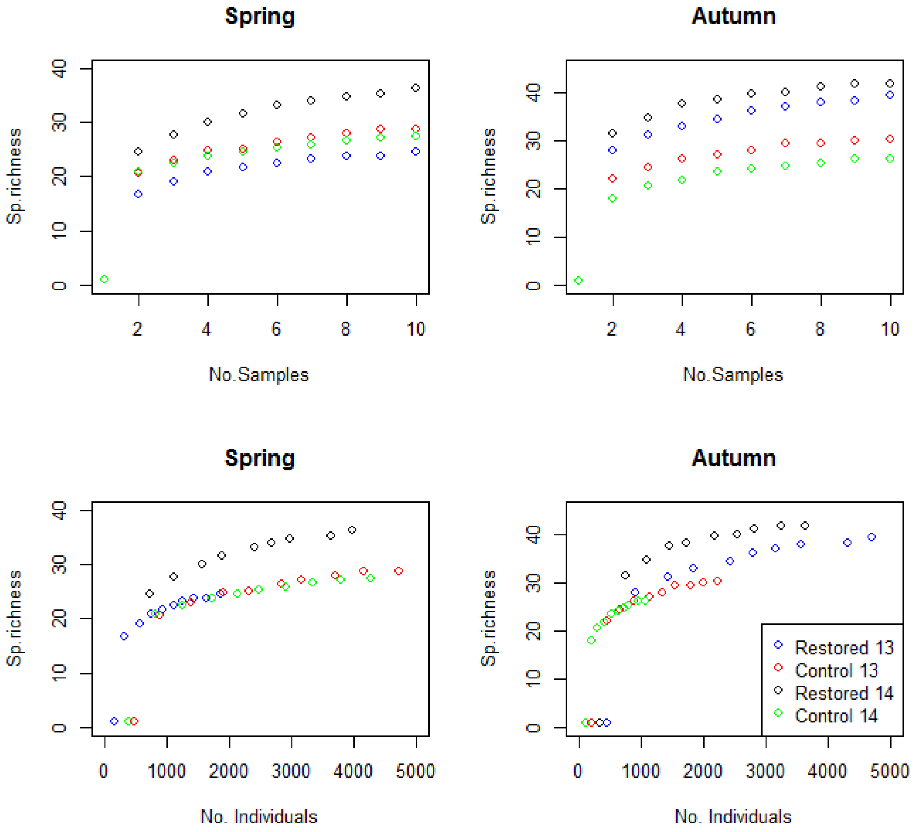


Fig 2.7 Sample based and individual based species richness rarefaction curves for each season, year and treatment reach combination.

Species Diversity

Both Shannon's and Simpsons' diversity indices indicated similar patterns to richness estimates (Fig 2.8). Initially riverfly diversity was lower in the restored sites, gradually increasing over time. However, mean restored sample diversity was only significantly higher for Shannon diversity in the final sampling period (Fig 2.8a). There were no occasions when Simpson's diversity differed between treatments (Fig 2.8 c). Rank evenness between the treatments was variable, controls sites generally exhibited a higher evenness, though this was only significant in Autumn 2013 (Appendix 2.2). Reach scale diversity estimates better differentiated between sites, with the restored reach being more diverse on 3/4 occasions for both Shannon and Simpsons indices (Fig 2.8b and d).

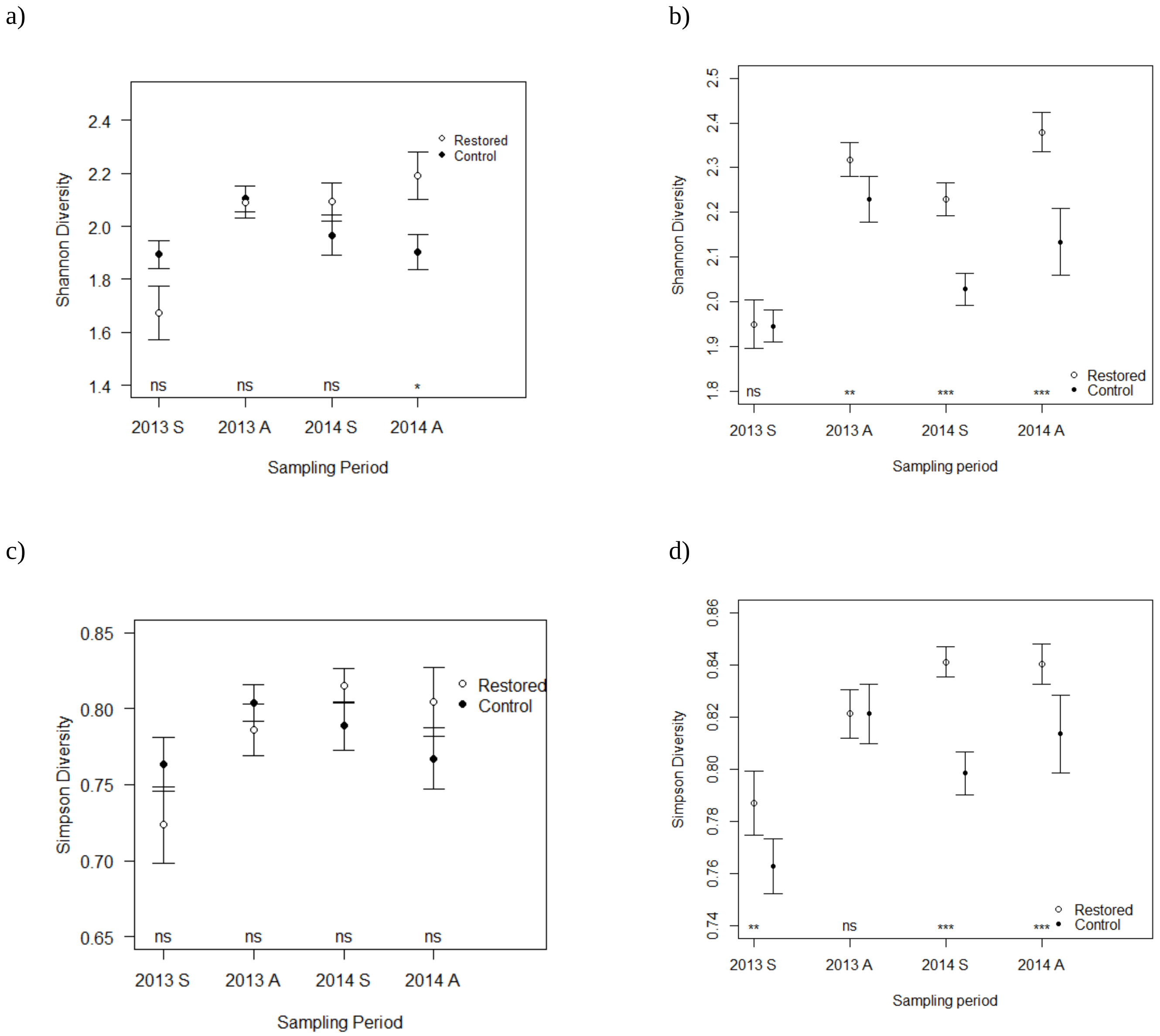


Fig 2.8 Mean sample a) Shannon's diversity, and c) Simpson's diversity with standard error. Reach composite diversity and 95% confidence intervals using pooled reach data for b) Shannon's diversity and d) Simpson's diversity. Asterisks below pairs indicate the result of Mann-Whitney U significance tests (< 0.001 ***, < 0.01 **, < 0.05 *).

2.4.2 Community composition

The ordination analysis of the riverfly community composition identified 9 potentially significant axes, the first 3 accounted for 50% of the variation in the community data (Table 2.4).

Table 2.4. Correspondence Analysis Axis number, inertia and factor interpreted as that axis.

Axis	% Inertia	Gradient
1	25.3	Season
2	13.6	Year and Treatment
3	11.6	Year and Treatment

The strongest gradient in the invertebrate community composition could be attributed to season. Autumn samples being positive on axis 1 and spring, negative (Fig 2.9). The second and third axes divided the sampling years, and in autumn only between the treatments. In spring the restored and unrestored channels overlapped significantly, so could be considered more similar in species composition, however 4 distinct groups could be seen in autumn, and distance between treatments was greater in the second year. There was a larger change in riverfly composition from one year to the next in restored channels.

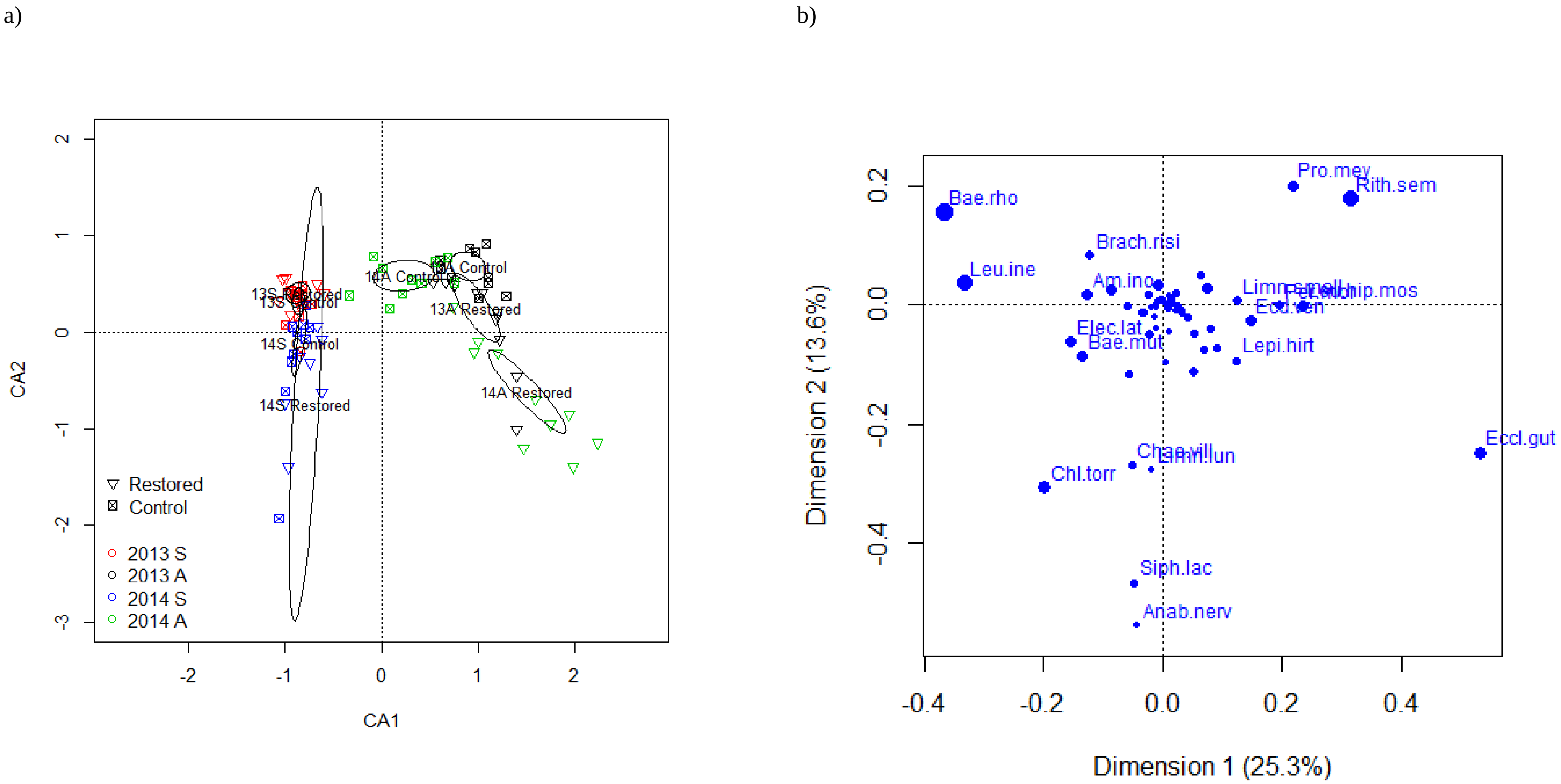


Fig 2.9 Correspondence analysis (CA) biplot for whole Rottal Burn community of a) sample locations on axis 1 and 2, with 95% confidence intervals around each sampling occasion x treatment x season combination (n=8), coloured by sampling occasion b) Contribution biplot of riverfly species with a contribution > 1%, location on axis in relation to sample group communities in b), indicates how much a species contributes to the identity of those sites, the size of the symbol is scaled to species abundance. A key to species abbreviations is given in Appendix 2.4

Difference in species composition between treatments.

The contribution biplot (Fig 2.9a) and IndVal results (Table 2.5) confirm which species were responsible for driving the separation of samples along each ordination axis, or which species were indicative of each year, season and treatment combinations. A species' position on the contribution plot (Fig 2.9b) indicates its association to sample groups and also its co-occurrence in samples with other nearby species. The number of indicator taxa, as identified through the Indicator Value approach, was much higher for the restored reach compared to the control, n = 18 and n= 11 respectively. The number of indicator taxa also increased with each sampling occasion from none in spring 2013, to 8 in autumn 2014 (Table 2.5).

The control channel had very few Trichoptera as indicators (Table 2.5), with the exception of *Hydroptila sp*, which characterised the control samples in spring 2014. In comparison, each sampling season in the restored channel was characterised by at least one Trichoptera species. By the final sampling occasion, the majority of indicators were Trichoptera (6/8), including both cased and uncased. The control channel, however, was characterised mostly by various Plecoptera species. Some species from within the same family indicated different channel treatments, especially for the Ephemeroptera and Plecoptera, for example: *Chloroperla tripuncata* in control and *C.torrentium* in the restored channel, in the Nemouridea family *Amphinemoura sulcicollis* in the restored, and *Protonemoura meyeri* and *P.praecox/montana* in the control channel.

Table 2.5. Significant group Indicators (p> 0.05) and IndVal score of over 0.25, listed in descending order.

Treatment	Spring 13	Autumn 13	Spring 14	Autumn 14
Restored	none	*Leuctra hipporus/moselyi (0.42),* *Amphinemoura sulcicollis,* *Rithrogena semicolorata,* *Limnophilidae small*	*Siphlonurus lacustris (0.67),* *Serratella ignita,* *Agapetus sp,* *Chloroperla torrentium,* *Anabolia nervosa,* *Chaetopteryx villosa*	*Potamophylax latipennis (0.68),* *Ecclisopteryx guttulata,* *Polycentropus flavomaculatus,* *Lepidostoma hirtum,* *Sericostoma personatum,* *Perlodes mortoni,* *Silo pallipes,* *Ecdyonurus venosus*
Control	*Electrogena lateralis (0.46),* *Brachyptera risi,* *Amaletus inopinatus,* *Baetis rhodani,* *Isoperla grammatica*	*Protonemoura praecox montana (0.48),* *Protonermura meyeri*	*Baetis muticus (0.50),* *Chloroperla tripunctata,* *Hydroptila sp,* *Leuctra inermis*	none

2.4.3 Beta diversity

There was a significant difference between group centroids, i.e. community assembly, for a global comparison (all sampling periods) using both the Bray Curtis and Chao distances (R^2 : B-C: 53.1, Chao: 84.5, p = 0.001). Group dispersion was also higher in restored reaches for both distance measures (B-C -p =0.025, Chao p=0.039). Pairwise comparisons within each sampling period illustrated when the differences in reach community were most distinct (Table 2.6). For Bray-Curtis distance all pairwise comparisons except in spring 2014 show that centroid locations of restored and control were different. However, for Chao distance all pairwise comparisons were significant except spring 2013.

Homogeneity of variance between samples for each distance measure and sampling period are shown in Fig 2.10 and the results of significance tests are displayed between each treatment pair. The boxplots show the median distance (Bray-Curtis and Chao) to the centre of the sample group (average community composition, Appendix 2.3). The greater the median distance the more heterogeneous the sample communities within that group. Restored channel samples were consistently more heterogeneous than control, this trend was strongest in spring samples and when compared using the Chao distance.

Table 2.6 Summary of permutation test results of differences in beta diversity between restored and control reaches for each sample period, using Bray-Curtis (B-C), and Chao (C) distance measures.

Sample group	Distance measure	R^2 of grouping factor	Location significance (p)
Spring 2013	B-C	0.28	0.004
Autumn 2013	B-C	0.24	0.002
Spring 2014	B-C	0.11	0.068
Autumn 2014	B-C	0.43	0.001
Spring 2013	C	0.20	0.073
Autumn 2013	C	0.41	0.011
Spring 2014	C	0.31	0.003
Autumn 2014	C	0.86	0.001

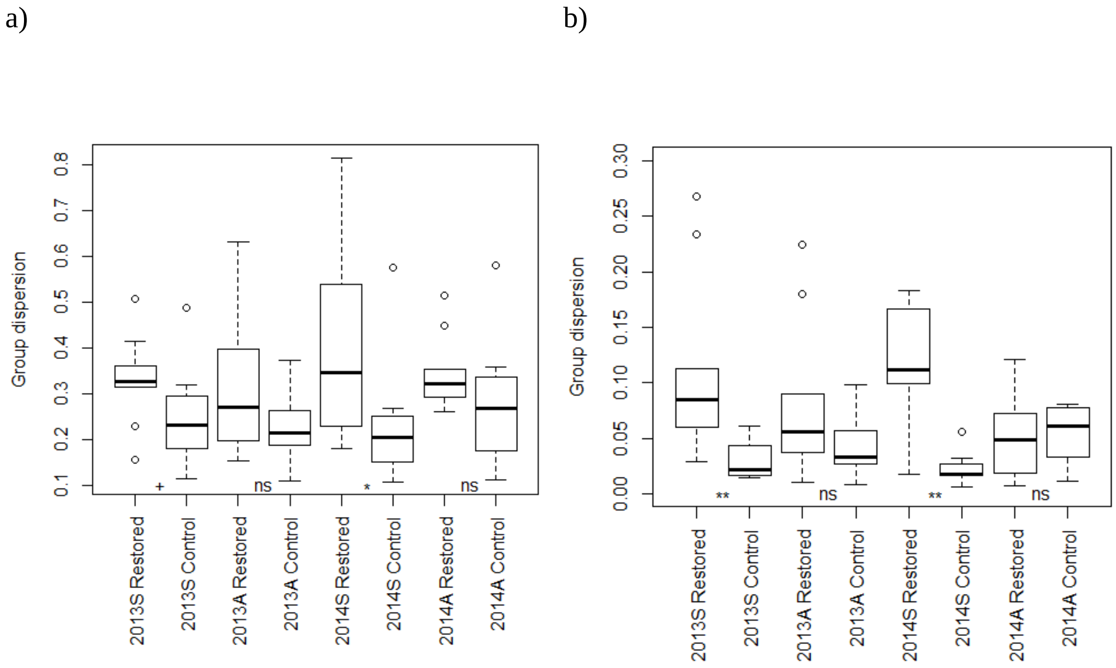


Fig 2.10 Median distance to group centroid and result of the significance of difference test between each pair, using a) Bray Curtis distance, and b) Chao distance. Asterisks between pairs indicate the result of a permutational MANOVA **<0.01, *<0.05, +<0.1, "ns" not significant.

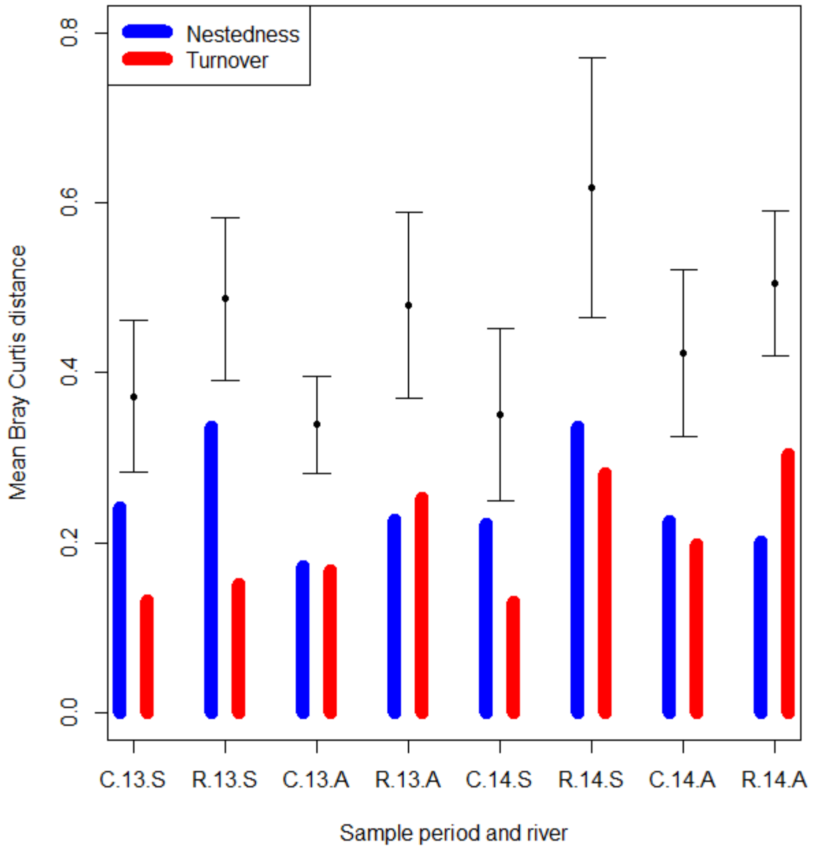


Fig 2.11 Within sampling group mean sample riverfly community dissimilarity (Bray Curtis) and standard error (dot and whisker), below, the partition of the dissimilarity into nestedness (blue) and turnover (red) components per treatment sample period combination. C= Control, R = Restored, S = Spring, A=Autumn.

The pattern for within group community similarity resembled that for group dispersion (Fig 2.11), however now with the relative importation of each beta diversity component also shown as red and blue bars (Fig 2.11). For all sampling periods, restored samples were more heterogeneous compared to controls, this heterogeneity also increased over time. Dissimilarity among control samples fluctuated slightly over time, however overall mean dissimilarity remained constant. Overall nestedness contributed more to the dissimilarity of most sites, compared to turnover. Over time the proportion of turnover increased in restored sites, relative to nestedness, to become the major contributor to beta diversity in autumn 2014. The relative components of beta diversity remained constant in the control reach. The nestedness portion tends to dominate beta diversity in spring, but during autumn, contributions are relatively equal. In the second autumn however there was an increase in contribution from species turnover in the restored reach.

2.4.4 Physical environment and habitat

Strong habitat differences between restored and control channels were revealed by MCA. Axis 1:3 accounted for a total of 26.9% of the variation in the data (10.7%, 8.9% and 8.3% respectively). Each axis accounted for small values, indicating high degree of dimensionality in the habitat conditions. The restored and control channels show different site characteristics primarily based on the gradient described by axis 1 (Fig 2.12). Within reach variability and change over time was captured by axis 2. Axis 3 (not shown) was related to bank erosion present in one of the control samples. Each channel treatment changed by only a small amount over time, and also in similar ways. The categories that describe each axis and characterise the differences between the treatment channels are summarised in Table 2.7 and Fig 2.12.

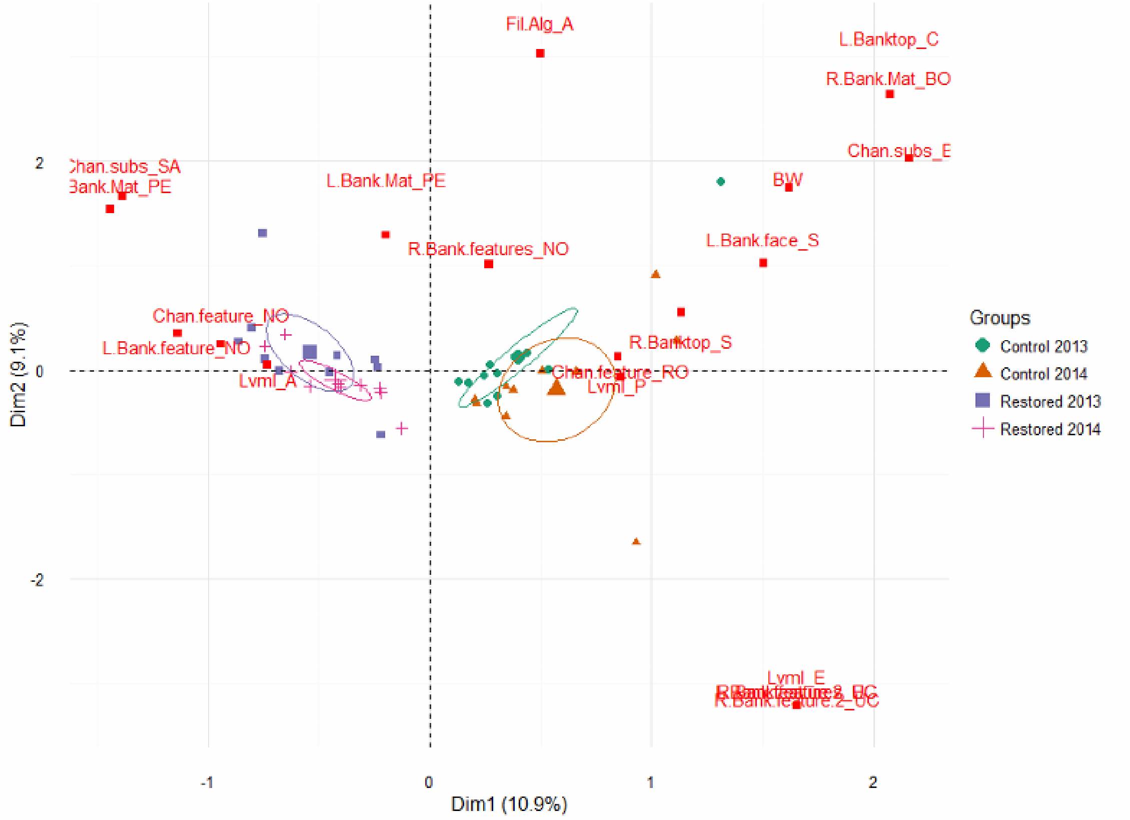


Fig 2.12 Samples and RHS variables (Table 2.7) plotted in MCA axis 1 and 2. Point and circle shows group centroid (average conditions) and 95% confidence ellipses. The variables are 20 that contribute most to the MCA solution.

Restored channels lie on the negative side of axis 1. They were characterised by a lack of distinguishing channel and bank features, small channel sediment consisting of pebbles and sand and an absence of moss cover. Control channel sites lie on the positive side of axis 1, they were characterised by in-channel exposed rock features, with moss cover, simple and complex vegetation on the bank face and bank top, and larger channel substrates including boulders. The most useful descriptors that characterise the differences between the channel treatments were channel sediment size, bank vegetation structure, in channel vegetation and presence of exposed rock features (Table 2.7).

Table 2.7 The habitat features that characterise different areas of the MCA plot and the groups they describe (Corresponding labels from the plot (Fig 2.12) Lvml = Liverworts/Mosses/Lichens).

	Control		Restored
Label	Axis 1 +	Label	Axis 1 -
Chan.feature.RO	Exposed rocks	L/R.Bank.feature_No	No bank features
L/R.Banktop_S/C	Simple and Complex bank top vegetation	Chan.feature_No Lvml_A	No channel features Lvml absent
L/R.Banktop_S	Simple bank face vegetation	Chan.subs_SA	Sand channel substrate
Lvml_P	Lvml present		Pebble bank material
Chan.subs_B	Boulder channel substrate	Bank.Mat_PE	
BW	Broken Water flow type		
	Within Group Variation		
	Axis 2 +		Axis 2 -
Fil.Alg_A	Algae absent	R.Bank.feature_UC	Bank undercut or eroding
L.Bank.Mat_PE	Pebble bank material		
r.Bank.features_NO	No right bank features	Lvml_E	Lvml extensive

Analysis of the quantitative sediment and channel measurements using PCA also revealed strong differences between restored and control channels (Fig 2.13). The first 2 PCA axes accounted for 68% of the variation in site environment (PCA1 = 52.38%, PCA2 = 15.36). Channel treatment groups lie on separate sides of axis 1, and axis 2 partly described within reach variation in the restored channel. There was minor change in the overall reach characteristics over time. The first and strongest gradient combined many of the sediment variables recorded in the pebble count as well as the percentage boulder from the visual assessment. Axis 2 was less strongly correlated to single measured variables, but was most strongly linked to percentage sand from the visual assessment and also to channel width. A third axis (8.3%) was not strongly correlated to any of the measured variables, and is not discussed further. The environmental factors that described each axis and characterised the treatment channels are summarised in Table 2.8.

Table 2.8 The environmental variables that characterise different areas of the PCA plot and the groups they describe. Corresponding labels from the plot (Fig 2.13).

	Control		Restored
Label	Axis 1 +	Label	Axis 1 -
Mean.peb X.Embed X.BO	Large mean pebble size % Embedded particles % Boulders	Fines.stor X.fines X.SA X.GR	More stored fines Fine sediment % Sand % Gravel
	Variation within Restored		
	Axis 2 +		Axis 2 -
X.SA Width.m	% Sand Narrow channel	Width.m	Wide channel

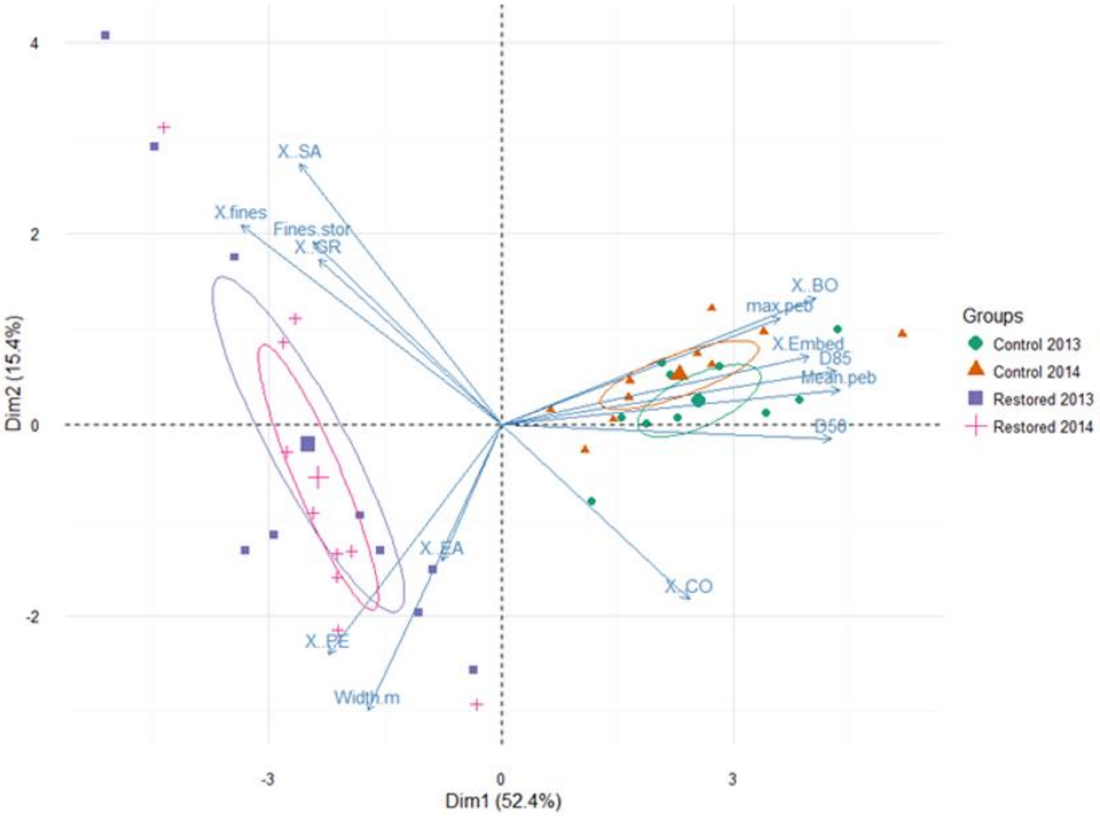


Fig 2.13 Samples and measured sediment variables plotted in PCA axis 1 and 2. Large point and circles shows group centroid (average conditions) and 95% confidence ellipses. (see Table 2.1 for description of variables)

Restored channels were characterised by a range of finer grained sediments and a lower number of embedded particles. There was also greater variation on axis 2 relating to a variety of channel widths and amount of sand deposits among samples. Control channel sites were characterised by a range of larger substrate sizes, more embedded particles and fewer fine sediments, with less heterogeneity between samples. Axis 1 best discriminated between habitats among the 2 treatments, and describes a gradient from large and immobile sediments in the control reach, where finer sediments have been washed out from the control reach, to more active sediment transport processes in the restored reach, with a degree of fine sediment retention. Three groups of correlated environmental variables were identified form the PCA analysis (Table 2.9). These variable groups in addition to the ordination axes scores, were further used to explain and model the physical drivers of riverfly species richness, abundance, diversity and community composition.

Table 2.9 Summary of correlated measured environmental variable groupings, and ordination axes used in further analysis.

Group	Variables
Large Sediment	Max. pebble size, D85, D50, Mean pebble size, % Embedded particles, % Boulder
Fine Sediment	% Sand, Amount Fines Stored, % Gravel, % Fines
Channel Character	% Cobble, % Pebble, % Earth, Width
Ordination	RHS1 and 2, PCA1 and 2

This section reports the results of the use of models the explain the causes of variation in biotic indices and community composition using the measured environmental data. Certain sampling seasons or biodiversity measures may be most sensitive to the response of riverflies to the physical effects of restoration. This was investigated firstly by considering which environmental factors caused the greatest difference in community composition between restored and control sites, and which species were most responsive to these gradients. Secondly, determining which richness or diversity indices were strongly correlated to any environmental variables. Finally, by considering more sophisticated modelling techniques, describe the environment x biological metric relationships, and determine which environmental gradient had the strongest effect.

2.4.5 Drivers of community composition

It was clear that season was a strong driver of community composition (Fig 2.14) accounting for 33.7% of the variation when partitioned from a global Redundancy Analysis (RDA). After accounting for season, 22.2% of the community variation remained, explained solely by the environmental variables indicating that much of the variation attributable to season was different from that explained by the measured variables. There were differences between the models for each season in terms of strength, variables selected, characteristic riverfly species, and the separation of the channel treatment groups. Significant environmental variables identified in the RDA, global and reduced model adjusted R^2 values are shown in Table 2.10. The relationships between the fitted samples, species and significant environmental gradients are shown in Fig 2.14.

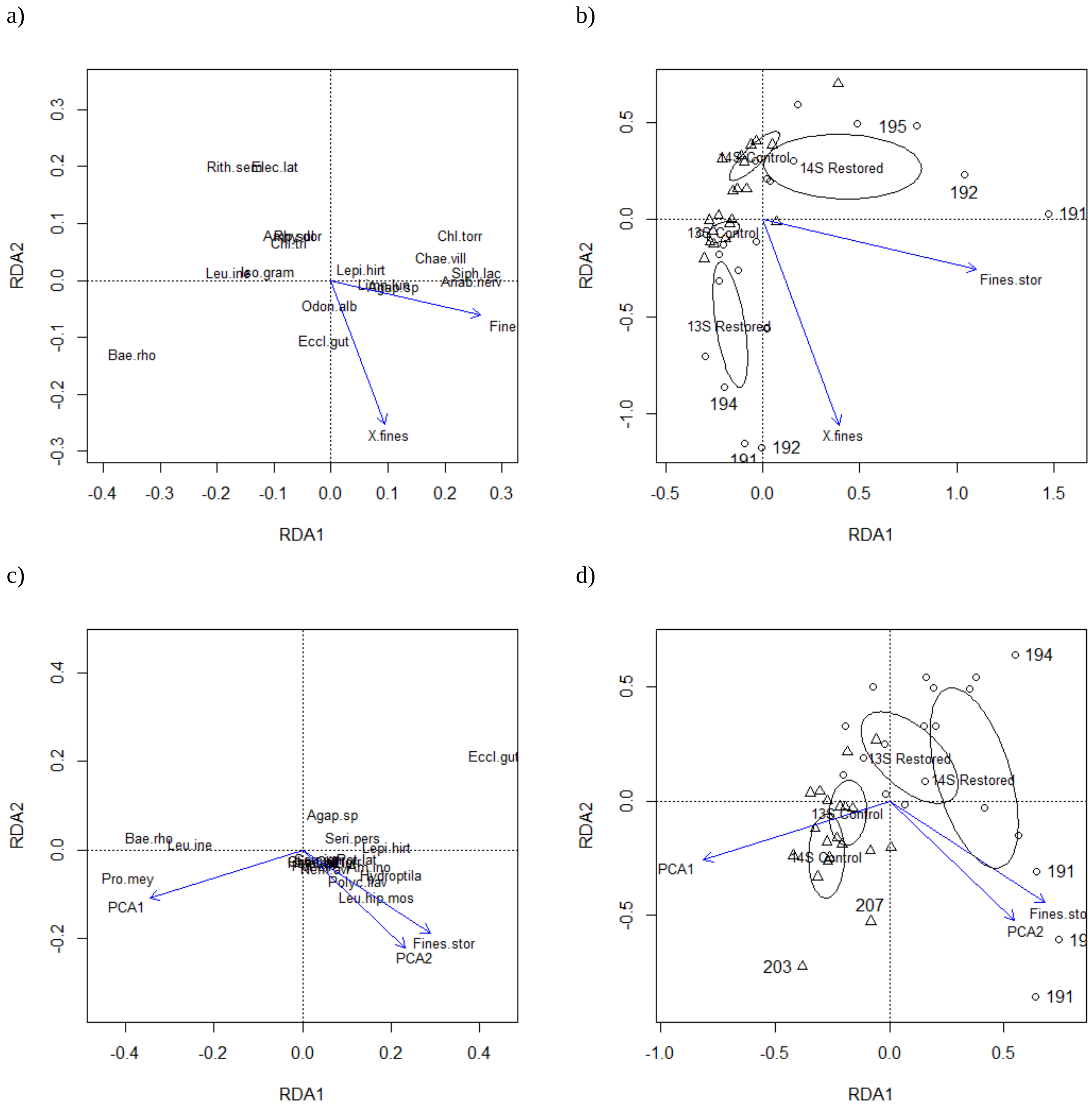


Fig 2.14 Biplots of RDA results, species abundance explained by axes over a threshold amount (15% spring, 30% autumn), sample locations, and significant environmental gradients for a) and b) spring, and c) and d) autumn samples. A key to species abbreviations is given in Appendix 2.4

Riverfly species composition was more strongly explained by the measured variables in autumn, compared to spring (Adjusted $R^2 = 31.9$ and 18.3 respectively, Table 2.10), and many species abundance explained over 30% (Fig 2.14). Species composition was best explained by surface percentage fines and interstitial fine sediments in spring, but a much wider range of sediment and channel characteristics summarised by the PCA axes were significant in autumn (Table 2.10).

Table 2.10 Summary of results from the model selection process. Global and reduced model R^2. Indicating where variables were selected from their groups (G) or retained in the final reduced models (R).

	Spring	coefficients	Autumn	coefficients
Adjusted Global R2	13.7		34	
Mean pebble size	G		G	
% Fines	G, R	A1:-0.004 A2:-0.015	G	
Fines stored	G, R	A1:0.043 A2:0.016	G, R	A1:0.013 A2:-0.024
% CO			G	
PCA 1	G		G, R	A1: -0.044 A2:-0.058
PCA 2			G, R	A1:0.006 A2:-0.056
Reduced model R2	18.3		31.9	

In spring channel treatments had distinct communities from each other explained by the gradient of fine sediment (Fig 2.14 b), with subtly different significant variables each year (Percentage fines 2013, Fines stored 2014). These condition were most extreme in sites 192 and 191 (see Fig 2.2a for location) which had much greater amounts of fines and pool like conditions. Within the restored reach there was an among sample species gradient along fine sediment. Species indicative of the finer sediment habitat in the restored channel were a number of cased caddis*:* *Anabolia nervosa*, *Chaetopteryx villosa villosa* and *Ecclisopteryx guttulata*, in addition to the mayfly *Siphlonurus lacustris*, and stonefly *Chloroperla torrentium* (Fig 2.14a, Table 2.11)

In autumn, control and restored communities were strongly divided on the gradient defined by PCA1, relating to the amounts of large and immobile embedded particles (Fig 2.14 d). Variation among restored samples was driven by a gradient of surface sand cover and interstitial fines. Over time, the restored and control communities became more distant on the PCA1 gradient. Species indicative of the large and immobile sediments (Table 2.11) were the stoneflies *Protonemoura meyeri* and *Leuctra inermis*, and the mayfly *Beatis rhodani*. Cased caddisfly *Ecclisopteryx guttulata* was strongly associated with negative PCA1 scores indicating small sediments, but at intermediate levels of % sand and interstitial fines. A mixture of taxa were related to high PCA2 values (% sand cover), and interstitial fines: *Leuctra hipporus/moselyi*., *Ecdyonurus venosus gp*, the uncased caddis, *Polycentropus flavomaculatus flavomaculatus* and the small cased caddis *Hydroptila sp* (Table 2.11).

Table 2.11 Invertebrates that were well explained by the RDA axes, listed according to their sites, season and environmental gradient associations.

Season	Reach	Driving Variables	Associated Species
Spring	Control	Average fines, and few fines	*Rithirogena semicolorata* *Electragena lateralis* *Leuctra inermis*
	Restored	High percentage fines	*Amalitus inopinatus* *Ecclisopteryx guttulata*
	Restored	High interstitial fines	*Anabolia nervosa* *Siphlonurus lacustris* *Chloroperla torrentium* *Chaetopteryx villosa villosa*
Autumn	Control	Larger sediment, embedded	*Baetis rhodani* *Leuctra inermis* *Protonemoura meyeri*
	Restored	Sandy and interstitial fines	*Leuctra hipporus/moselyi* *Ecdyonurus venosus* *Polycentropus flavomaculatus* *Hydroptila sp*
	Restored	Small sediments, pebble and gravel	*Ecclisopteryx guttulata*

2.4.6 Correlation with environmental variables

For the dataset as a whole there was very poor correlation between the biotic indices and the measured environmental variables, with few significant correlations (Table 2.12). Riverfly species richness and abundance were most strongly correlated to the physical variables. Both were positively correlated with channel width, % earth and % pebble. Species richness was negatively correlated to RHS1, PCA1 and 2, and many of the large sediment measures. Correlation between biotic measure and environment were stronger when each season and channel treatment were considered separately (Table 2.13). Biodiversity indices of the restored samples were more strongly correlated to more environmental variables, however the combination of biotic response and significant environmental driver was inconsistent.

In spring, species richness was negatively correlated to % boulders in the control channel. In the restored channel species richness, abundance and diversity were negatively correlated to RHS2 (absence of in channel vegetation and shading from more complex bank vegetation), and positively to % pebble or an estimation of mean sediment size. Additionally, abundance was negatively correlated to PCA2 relating to increasing amounts of sand. In autumn, control channel richness and Shannon diversity was negatively correlated to increasing sediment size, whereas abundance conversely, was negative related to increasing sand cover. Restored richness, and diversity was positively related to % fines and sand, whereas again, abundance was negatively correlated to % fines, sands and gravel cover, but positively to % pebble. Interestingly rarefied richness, not species density was significantly correlated in autumn. Negatively correlated with sediment size in control channel contrasting with a positive relationship to fine sediment in the restored channel.

Table 2.12 Significant Spearman rank correlations between biotic indices and environmental variables using the full dataset, (red = negative, green= positive relationship).

Measure	Mean.peb	D50	Max.peb	D85	% Embed	% Fines	Width	% BO	% PE	% GR	% EA	RHS1	RHS2	PCA1	PCA2
Richness	-0.29	-0.28	-0.34	-0.30	-0.27	0.23	0.36	-0.38	0.34	0.24	0.22	-0.32	-	-0.29	-0.30
Rarefied Richness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abundance	-	-	-	-	-	-	0.31	-	0.27	-	0.22	-	-	-	-0.45
Shannon Diversity	-	-	-0.22	-	-	-	-	-	-	-	-	-	-0.23	-	-
Simpson Diversity	-	-	-	-	-	-	-	-	-	-	-	-	-0.23	-	-

Table 2.13 Significant Spearman rank correlations between riverfly biotic indices and environmental variables using separate season and treatment datasets (red = negative, green= positive relationship).

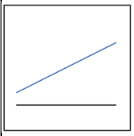
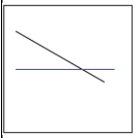
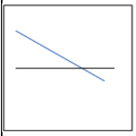
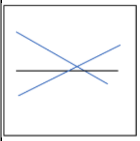
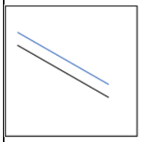
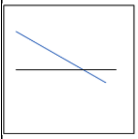
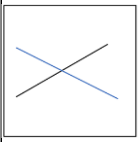
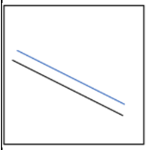
Season	Treatment	Measure	Mean.peb	D50	Max.peb	% Fines	Fines.stor	Width	% BO	% PE	% GR	% SA	RHS1	RHS2	PCA2
Spring	Control	Richness	-	-	-	-	-	-	-0.52	-	-	-	-	-0.54	-
Spring	Control	Shannon	-	-	-	-	-	-	-	-	-	-	-	-0.46	-
Spring	Restored	Richness	-	-	-	-	-	-	-	0.49	-	-	-	-0.68	-
Spring	Restored	Abundance	-	0.45	-	-	-	0.54	-	-	-	-	0.62	-0.72	-0.61
Spring	Restored	Shannon	-	-	-	-	-	-	-	0.45	-	-	-	-0.51	-
Spring	Restored	Simpson	0.46	-	-	-	-	-	-	-	-	-	-	-0.47	-
Autumn	Control	Rar.Rich	-	-	-0.52	-	-	-	-	-	-	-	-0.57	-	-
Autumn	Control	Abundance	-	-	-	-	-	-	-	-	-	-0.60	-	-	-
Autumn	Control	Shannon	-	-	-	-	-	-	-	-	-	-	-0.54	-	-
Autumn	Restored	Rar.Rich	-	-	-	-	0.55	-	-	-	-	0.47	-	-	-
Autumn	Restored	Abundance	-	-	-	-0.45	-0.46	-	-	0.65	-0.55	-	-	-	-0.5
Autumn	Restored	Shannon	-	-	-	-	0.50	-	-	-	-	0.5	-	-	-
Autumn	Restored	Simpson	-	-	-	-	-	-	-	-	-	0.5	-	-	-

2.4.7 Modelling biotic indices

In many cases biotic measures of neither treatment reach showed a relationship to the measured environmental gradient (Type A – Fig 2.5, n=85). The majority of biotic measures for the control reach showed no relationship to any environmental gradient, whereas the restored channel measures did (Type E, n= 45), both positive and negative. In a small number of models, metrics of control samples responded to the gradient uniquely, i.e. where restored indices did not (Type F, n=7). Many indices measured in autumn showed a strong difference in values due to river treatment rather than environmental gradient (Type B, n =25), a few models had both significant slope and interaction, either for both channel treatments (Type D, n=2), or just in the restored (Type C, n =4)

Some biotic indices and seasons were more sensitive than others. No models for rarefied richness had any significant parameters. Spring abundance and species richness were the most sensitive to environmental change. Spring diversity responded to few gradients and, autumn diversity was the least responsive. Overall autumn biodiversity was not well characterised by the variables measured. River effects were strongest for autumn abundance and richness models, confirming trend seen in 2.4.1, with the restored channel having higher species richness regardless of environmental gradient. Generalised trends of biotic response to environmental gradients are summarised in Table 2.14, and explored further in the discussion.

Table 2.14 Summary of significant model, stating environmental predictor and biotic response, season, treatment and direction of relationship. Control channel relationships are indicated by italics.

Variable	Trend	Relationship type
Large sediment (D85/D50)	Positive effect in spring for abundance, and diversity	
Intermediate sediment (mean pebble, D50, XPE)	Positive effect on spring richness *Negative- Autumn effect diversity*	 
Fine sediments (Fines stored/% fines, SA) Stored fines % Sand cover	Negative effect on spring abundance, richness diversity Positive effect on autumn diversity, but negative on autumn abundance (the best models for autumn diversity) *Negative effect on Control (and restored) abundance in autumn*	  
RHS 2 (Algae or Mosses) and PCA 2 (Channel width and amount of sand)	Negative effect on spring richness and diversity (but not abundance) Negative effect on autumn abundance	
RHS 1 (Few channel features, sediment size) and PCA 1 (Sediment size and large sediments)	Positive effect on spring richness and diversity *Negative- autumn effect diversity*	
Channel Width	Positive effect spring abundance *Positive effect spring and autumn abundance*	

2.5 Discussion

By determining the major environmental and biological differences between restored and unrestored reaches and their causes, this study has identified a number of potential opportunities to improve the accuracy and sensitivity of future monitoring of hydromorphological restoration. Poor results from monitoring of recent river restoration projects could be due to any number of methodological shortcomings in assessment techniques and not necessarily a true failure of the restoration project.

2.5.1 Detecting hydromorphological impacts using biodiversity indices.

By the time of the final sampling period, restoration treatments differed based on the majority of metrics, with the restored channel exhibiting higher riverfly species richness and diversity. However, biodiversity indices present a limited explanation of the mechanisms of ecological changes resulting from the restoration measures. The use of diversity indices is an extreme simplification, reducing multiple species information to a single synthetic value in comparison to multivariate analysis (Dray et al. 2012), and as a result there is a corresponding loss of statistical power traded for a simple and intuitive concept.

Abundance and Richness

Abundance values are known to be highly variable at small scales (Heino et al. 2004), and Miller et al. (2010) doubt their usefulness in contrasting restored and unrestored sites. Indeed, this study also found abundance to be variable and of limited use, however it was shown to emphasise the heterogeneity within treatments, disturbance effects of the restoration process, and was useful to evaluate and standardise sampling effort. Species richness is known to be strongly driven by sample abundance (Gotelli and Chao 2013). In this study, by using a more robust assessment that accounted for abundance differences and estimated for missing or rare species such as Chao estimator (Chao et al. 2005), apparent differences between channel treatments lessened. This suggests that the use of a rarefied richness value or other estimate that standardises for the difference in abundance between samples should strictly be used.

Controlling for abundance effects of sampling effort in species richness estimation is not routinely done in studies evaluating river restoration, and species density may give misleading results (Li et al. 2001), especially early in the recovery phase. Bady et al. (2005) and Barnes, Vaughan & Ormerod (2013) found species richness estimates to be strongly dependent on sampling effort. Species richness is also limited, as it does not account for difference in taxa abundance, nor their biological or ecological function in the community. The power of richness estimators is also limited in cases where small sample sizes may result in the rejection of sample replicates with too low abundance, in order to maintain a high enough minimum number of individuals. Estimators also lack accuracy where rare species are frequent, and continue to accumulate with additional sampling effort (Melo 2005). Non parametric richness estimators however, proved useful in this case, where rare species were present but not dominant.

Alpha diversity

Mean sample alpha diversity increased in the restored channel over time, however only Shannon diversity was significantly different between treatments. Evaluating restored channels, Haase et al. 2013, also found Shannon's, but not Simpsons’ diversity to be higher in restored channels, however Leps et al. (2015) and Erba et al. (2006) found diversity indices in general to be poor indicators. Average sample richness, and alpha diversity are just one scale of species diversity, and these values are dependent on the scale of the sampling unit (Barton et al. 2013). Other measure such as gamma and beta diversity were more informative than mean sample alpha, particularly in spring during the early stages of channel adjustment.

Beta Diversity

Beta diversity was significantly higher in restored reaches compared to control in spring and more pronounced when using the Chao distance, indicating that rare species accounted for differences between samples within reaches. Partitioning revealed the increased beta diversity in the restored channel to be due to higher, and gradually increasing over time, proportions of species turnover compared to the control channel. This increase in turnover is likely to be from two contributing processes. Firstly the gradual addition of new species, which was confirmed by IndVal results, showing that over time the restored channel was characterised by a larger number of indicator taxa, representing a colonisation gradient post restoration (Winking et al. 2014). Secondly, a greater amount of environmental sorting of existing species into more distinct habitats, that occur in separate samples, reflecting the greater habitat heterogeneity available. Measures of beta diversity have proved useful in other studies of restoration impacts on biodiversity. Jähnig & Lorenz (2008) found no difference in alpha diversity between restored and unrestored sites, but that different substrates hosted different assemblages and since there was a greater diversity of substrates in restored channels this might result in higher beta diversity. Community turnover due to microhabitat availability was also found to be more sensitive to changes in land use (Carlson et al. 2013). However, neither beta diversity nor turnover were mentioned in the reviews of restoration project success by Haase et al. (2013), Palmer et al. (2014), Leps et al. (2015) or Verdonschot et al. (2015).

Gamma Diversity

By aggregating all the samples within a reach to calculate richness and diversity, sampling covered a much larger extent and number of individuals, and so was better able to discriminate between the channel treatments. Due to higher beta diversity, each sample in the restoration site collectively contributed to increase reach gamma diversity. Unfortunately, explicit consideration of the scales of diversity created by restoration projects is rare, due to the high costs of sampling and processing.

2.5.2 Environmental drivers of biodiversity.

Use of linear and generalised linear models to quantify biotic response to a range of environmental variables is growing in popularity (Barnes et al. 2013, Sundermann et al. 2013, Leps et al. 2015), and is of practical importance, enabling researchers to quantify and predict biotic response to specific variables that are often targeted during restoration. In this study however, most model combinations were not significant. This can be attributed to the inability of models to indicate change locally that is influenced by multiple pressures acting at larger scales, and eliciting species specific responses (Downes 2010). For example, Heino et al. (2007), found that diversity indices did not respond to local disturbance, only to catchment type, catchment area and water chemistry. Related to the complicating effects of unmeasured variables, there has been criticism of approaches using a measure of central tendency to model species abundances in relation to single or multiple explanatory variables. When there are likely to be many other variables acting at a range of scales, and so causing scatter in a model, Lancaster & Belyea (2006) recommend using quantile regression. This method models the limits; maximum and minimum of biota response, which can be considered more ecologically meaningful.

Biotic metrics measured within the restored channel respond most to environmental gradients, either through correlation, or regression with a number of local environmental drivers. This indicates a renewed role of local environmental control due to the restoration, where biodiversity within the channel is driven by differences in local habitat quality and availability. Global correlations were mostly found between species density and abundance, which increased with channel width, % pebble and % earth, indicating sample sites with an adjusting channel. Species richness reduced with increasing sediment size suggesting a selection of specialist taxa associated with larger sediment sizes, and more rapid flow velocities.

Overall relationships identified were highly variable. Inconsistencies between correlations and models, indicate caution is required when interpreting correlations, as this is not causation (Downes 2010). Relationships should also be considered scale and technique specific. For example, autumn rarefied richness was significantly correlated with large and fine sediments in both channels, however when modelled, rarefied richness showed no significant relationship to any environmental gradient. In contrast species richness was most often significantly related to local environment, but not in a way that could be easily interpreted due to its strong dependence on abundance. Diversity indices generally responded little to environmental drivers. Interestingly only Simpson diversity showed significant relationships (negatively correlated to increasing sediment size) in the control channel, whereas Shannon diversity was not sensitive to any environmental drivers. This highlights the strength of using a range of diversity measures initially for impact detection, some of which may be better suited to certain environmental conditions, e.g. a reduced level of environmental heterogeneity in the control channel, until we can be more specific about the predicted effects of hydromorphological restoration.

There was a strong seasonality to the significant models, with autumn measurements of biodiversity in the control channel more strongly related to environmental factors, whereas spring diversity was most responsive in the restored channel. The presence of interstitial fines, and surface sand also has a seasonal pattern, consistently relating to lower abundances and diversity in spring, but higher diversity in autumn. This is partly in agreement with Verdonschot et al. (2015), where restoration had positive effects on riverfly richness and diversity, attributable to the distinct communities that can assemble in sandy habitats. The addition of a seasonal aspect to this study indicates that fines or the low velocity refuges that fines may be indirectly indicating, were seasonally variable limiting factors.

2.5.3 Changes in the riverfly community.

Community composition is often less studied due to sampling and identification limitations, and the less communicable nature of results in comparison to more simple diversity indices. Past successes of using the community matrix to quantify difference in community similarity has been mixed, from no clear patterns identified using NMDS in Lepori et al. (2005), to Carlson et al. (2013) concluding that taxonomic composition was a better indicator of disturbance than diversity metrics. The results often depend on the data that is used, and the specific comparisons being made. One benefit of using a site x community matrix is that the species identity is retained, compared to measures of dissimilarity. This allows investigation of the species that contribute most to local diversity (Legendre and De Cáceres 2013). Looking at which individuals change may be more elucidating than comparing richness and diversity or pressure specific indices such as PSI or ASPT due to the growing knowledge about taxa autecology (Schmidt-Kloiber and Hering 2015), and the local behaviour of taxa due to context specific community interactions. The Rottal riverfly communities did indeed differ between restoration treatments, most strongly in autumn, however in spring, differences were more pronounced in beta diversity. The restored community became more diverse and distinct overtime as more species colonised the channel, compared to the control community which consisted of wide spread and ubiquitous taxa. The addition of further orders of the invertebrate community may not have been valuable, since studies of the effects of restoration using the full invertebrate, and EPT richness or diversity, indicated an identical response between the two measures (Verdonschot et al. 2015). However, the application of the high spatial and temporal resolution sampling scheme used in this study has rarely been applied to full community datasets to quantify measures such as beta diversity or temporal turnover.

Environmental drivers of community change

Environmental drivers of community assembly were season specific. In spring restored and control communities diverged based on % fines and interstitial fines storage gradients. However, in autumn communities differed due to a gradient of large sediment (PCA1). Samples within the restored channel varied due to gradients of interstitial fines and sand cover. Both types of fine sediment measure, sand cover and interstitial fines, were consistently significant, indicating the availability of a wider range of mobile sediment niches in the restored compared to control. The novel measure of interstitial fines was a significant driver of riverfly composition, and appears useful to indicate the quality of gravels available, however, it is not routinely used since it is labour intensive to measure. Fine sediments are generally well known to impact on the invertebrate community (Jones et al. 2012), this study confirms that the presence and types of fines sediment available was important and more precise measurement may be worthwhile the effort.

Direct comparisons of significant environmental drivers of diversity indices and community composition models were difficult to find in the literature due to several reasons. Firstly, drivers from the local mesoscale are often superseded by wider scale factors when these are included in the spatial extent of the study. For example Johnson et al. (2007), conclude that wider catchment land use and chemistry drivers were significant for invertebrate community composition. Secondly biotic communities are commonly related to artificially constructed gradients, such as hydromorphological degradation (Johnson and Hering 2009). Or finally that the role of environmental drivers is inferred through the change in community traits, rather than directly modelled (Januschke et al. 2014).

Species responses

IndVal and ordination analysis both confirmed that the restored riverfly community becomes more unique over time. This can be attributed to the increasing presence of Trichoptera species, which indicate the maturing restored channel. The arrival of both cased and non-cased species, indicated a greater availability of case building material, the presence of low energy habitats and increasing availability of prey taxa. Similarly, Petts & Greenwood (1985), describe channel adjustment and community change in an upland stream after an end of flow regulation, the recovering channel was colonised by Trichoptera and predatory taxa.

In both seasons many species showed a response to a gradient of fine sediment, with certain species characterising fines dominated sites in the restored channel. In spring this included the cased caddis: *Anabolia nervosa, Chaetopteryx villosa villosa* and *Ecclisopteryx guttulata,* the mayfly *Siphlonurus lacustris,* and stonefly *Chloroperla torrentium*. However different species were associated with fine and small sediments in autumn, specifically the stonefly *Leuctra hipporus/moselyi,* mayfly *Ecdyonurus venosus gp*, cased and uncased caddis *Polycentropus flavomaculatus,* and *Hydroptila sp,* perhaps reflecting a use of low energy refugia during higher autumn flows or passive deposition as described in Lancaster (2000).

Many of the species seen occurring in restored samples, have been classed as good quality indicators for small mountain streams in Germans studies (Lorenz et al. 2004). High quality indicator species were *Ecclisopteryx guttulata, Lepidostoma hirtum, Sericostoma personatum, Ecdyonurus venosus gp* and *Chaetopteryx villosa villosa,* However the presence *of Polycentropus flavomaculatus* would indicate poorer quality. The value of an indicator depends on its sensitivity to the factor of interest, transferability across regions and applicability to a range of sampling and study scales. The presence of taxa that indicate good quality habitat, or resources that were previously lacking e.g. small mobile sediment, are a useful evaluation tool for hydromorphological change compared to perhaps less appropriate indicators such as conservation status (Haase et al. 2013). Although it must be appreciated that community change is dependent on a taxa's wider availability and ability to arrive from the catchment species pool (Spänhoff and Arle 2007, Verdonschot et al. 2015). Riverfly taxa are known to indicated good ecological quality and their proportion of species richness and abundance in relation to the full invertebrate community would potentially have indicated changes in habitat quality in this case.

2.5.4 Sampling effects: season, sampling intensity and taxonomic resolution.

The high intensity sampling approach taken in this study allowed the identification of strong seasonal patterns of riverfly community composition, species richness and diversity, and biota environment relationships. Underestimations of the species pool and complex species specific responses, were also identified as possible limitations to impact detection.

Sampling effort

Rarefaction should be used to standardised samples before comparing sample species richness. Bady et al. (2005), found richness estimates to be strongly dependent on sampling effort, and 10 replicated Petersen grab samples were found to underestimate actual richness, however this varied between rivers. Li et al. (2001), also found an initial rapid increase in species richness with 4-8 Surber samples, approximating 500-1000 individuals, however new taxa continued to be added after 50 samples. Similarly, this study confirms that even as many as 10 x 3-minute kick samples could underestimate gamma diversity depending on the distribution of diversity within the reach. Although the additional effort required for small increases in richness would be practically prohibitive and possibly not ecologically meaningful. This should be assessed on a case by case basis, since species abundance will depend on the microhabitats included in the sample, and different sampling effort will be needed for different habitats and stream types. Direct assessment and quantification of study sampling effort for example using rarefaction or statistical estimates will add to this knowledge and qualify uncertain or conflicting results.

Seasonal Patterns

This study uncovered strong differences between riverfly communities of each season, in terms of the performance of species environment models, and specific environmental gradients driving riverfly response. Autumn sample alpha richness, species diversity and community composition differentiated better between channel treatments due to the presence of rare species occurring in the restored channel. Despite this strong difference between treatments, autumn biodiversity indices were not well characterised by the environmental variables measured. Rarefied richness was significantly correlated to measured variables in autumn, but not so when modelled directly. However, in general models indicated a consistent autumn pattern that was true for both treatments, of high species richness but reduced abundance in samples with more small sediments. Abundance, species richness, and diversity of spring samples responded most strongly to environmental gradients and resulted in beta diversity differences between channel treatments. The strong influence of RHS2 (moss and algae in the channel) suggests the possible role of seasonal growth of vegetation structure as per Armitage et al. (2001). In studies assessing restoration, sampling in autumn is less common, possibly due to difficulties of sampling during wetter periods with higher flow. Studies contrasting spring and summer seasons, omitting an autumn sample, have found little difference between treatments for these seasonal communities (Jähnig et al. 2010). However, where seasonal difference are considered explicitly, Göthe, Angeler & Sandin (2013) found a complex community organisation that varied between season. Bady et al. (2005), also found much variation between faunal metrics with season and location, and suggest that functional metrics that do not rely on species identity such as richness , ASPT or number of rheophilic taxa (Álvarez-Cabria et al. 2010), can be more stable between time periods, making them better suited to generalising and comparing between locations. However, autumn and spring seasons illustrate unique responses to seasonal high and low flows that should be considered jointly, and contrasted to determine the overall dynamics of a restored community change.

Taxonomic resolution and rare species

Indicator Value analysis revealed that different species from within the same family indicated different channel treatments, suggesting that family level identification would be insensitive. The level of identification required can depend on the aims of the particular study. However, this work illustrated that species level identification was required; particularly when attempting to discriminate between nearby reaches, which are more likely to contain similar and related families. Even closely related and coexisting species of freshwater invertebrate can have very different autecology e.g. feeding behaviours, microhabitat use, mobility (Schmidt-Kloiber and Nijboer 2004), meaning that only higher resolution taxon identification would be sensitive to subtle community changes. This study agrees with Jones (2008) that the taxonomic level should be determined by project aims, and in this case the local community changes brought about by morphological changes in a relatively natural setting are indeed likely to be subtle and family level metrics to be insensitive. Rare species characterised the restored channel. This study agrees with the recommendations of Nijboer & Schmidt-Kloiber (2004), that the power of rare taxa to indicate natural circumstances is essential and should be included in ecological assessments. Rarer more specialist taxa, colonising in limited numbers gradually, are likely to indicate the novel habitats and patchiness created by restoration, and should be assessed as a measure of success.

2.5.5 Evaluating restoration project outcomes

It was clear that the restored channel was distinct from the upstream control in both physical structure and riverfly community. Unlike other criticisms of river restoration projects (Palmer et al. 2010), a significant difference in habitat heterogeneity and community assembly was found after a relatively short period. This could be due to the high dynamic potential of hydrological and sediment processes, and an absence of significant larger scale impairment within the catchment (Miller et al. 2010, Smith et al. 2014a). This meant there was a range of nearby sources of colonising taxa that were able to arrive rapidly, and were well suited to the newly created habitat structures and dynamic sediment regime.

The restored reach contained a number of features that indicated the presence of longitudinal and lateral sediment dynamic processes: mobile sediments, increased width, reduced embeddedness and gradual bank undermining, that Clarke, Bruce-Burgess & Wharton (2003) consider fundamental characters of fluvial systems to be restored. This is in contrast to the limited improvements in biodiversity found by Friberg et al. (2014) after a decade, where restoration did not fully reinstate the dynamic river morphological processes that would create habitat, particularly the lack of lateral movement of water and interaction with banks and floodplain that would generate sediment supply.

The absence of characteristic bank and channel features in the restored channel was an indication of its geomorphic newness. Sear, Briggs & Brookes (1998) identify the early stages of channel adjustment after restoration similarly to be a growth of in-channel sediment stores, bank exposure by erosion and changing the geometry. The initial spring period in 2013 can be seen as a time of significant morphological adjustment (Day 2013, Braid 2014) that presented challenging conditions to the invertebrate community. As a result of this short term habitat instability, the initial sampling period shows reduced richness and biodiversity in the restored channel, however over the timeframe of the study these values increased to surpass those of the control. The community that developed was more responsive to the increased range of environmental gradients, particularly of smaller and more mobile sediments. This suggests that the project has indeed targeted habitats and processes that were locally limited (Lepori et al. 2005, Verdonschot et al. 2015) and ecologically relevant to the target taxa group. Biological response times of restoration projects are highly variable (Miller et al. 2010, Kail et al. 2015). In this study, significant differences in biodiversity indices were maintained after 2 years, a relatively short time period. Further observation would be needed to confirm these changes are sustained, as the data suggested the community assembly was still changing.

2.6 Conclusion

Restoration measures should be seen as a short term disturbance to both physical habitat and biological communities. Although a simplified measure, higher average sample riverfly species richness or Shannon diversity could distinguish restoration effects, which were strongest in autumn. Over time the restored channel differentiate further from control. A more enlightening comparison however, was that of beta and gamma diversity of the reach as a whole, since a larger sample size or extent could detect the addition of rare colonising species only present in the restored channel. Beta diversity was comparatively much higher in restored channels during spring seasons, reflecting the addition of unique species and a range of Trichoptera, increasing the amount of species turnover between samples relative to nestedness. Riverfly community assembly also different in the restored channel, most clearly seen in autumn. Several species present indicated high environmental quality.

There were strong differences in sediment properties and habitat type between restored and control samples that remained over the course of the study. The restored channels were characterised by a lack of channel features indicating active sediment transport processes. Restored samples contained a wider range of sediment composition, made of smaller particles, sands and gravels and reduced embeddedness. Physical conditions and habitat features remained stable over time. The presence of interstitial fines and sand cover indicate good hydromorphological function and also proved to be biologically important. Overall there was little evidence of local environmental filtering exerted on riverfly biodiversity metrics generally. However significant relationships to a number of environmental variables suggest that biodiversity within the restored channel was driven by differences in local habitat quality and availability. Riverfly community assembly could be explained by environmental variables, most strongly for autumn communities. Specific environmental drivers of both community and diversity were different and season specific, indicating biotic response to different set of seasonal stressors. A number of relationships were species specific.

The strongest detection of restoration impacts relies on an investment in spatial replication of sampling for project evaluation. The rewards are a greatly increased understanding of the mechanisms of invertebrate community response to human intervention. Further recommendations for future monitoring are that a method of standardisation is used, either through a statistical estimate, rarefaction, or species accumulation curves to account for difference in sampling effort, evaluate coverage of the species pool, or ensure sampling sufficiency. Riverfly sampling should cover both spring and autumn seasons. Environmental properties and habitat features need to be measured and quantified to prove changes occur. Particular physical indicators of restoration proved to be measures of fine and mobile sediments, so these should be the target of evaluation monitoring. To monitor biological changes, measures of beta diversity capture the spatial diversity improved in restoration, and should also be targeted. Species level identification for riverfly taxa is essential, as are the inclusion of infrequently occurring species. Extending monitoring to including the full invertebrate community has the potential to identify further indicators of hydromorphological quality.

Chapter 3 - Defining post-restoration recovery within a regional gradient of catchment morphology, biology and natural variability.

3.1 Introduction

Due to the recognised importance of functioning freshwater systems (Geist 2011) there has been a marked increases in the number of river restoration projects aiming to reverse historic damage and mitigate current pressures on rivers (Feld et al. 2011, Palmer et al. 2014). This has been accompanied by a growing body of literature documenting their success or otherwise (Matthews et al. 2010, Smith et al. 2014b, Kail et al. 2015, Verdonschot et al. 2015). A large proportion of the literature documents that river restoration projects, whilst achieving physical habitat goals, fail to achieve the expected improvements in biodiversity or ecological function, particularly with reference to changes in aquatic macroinvertebrates. Among the numerous potential reasons for these failings, three are the subject of this chapter. Firstly, a problem of low quality and quantity of pre- and post-project monitoring (Miller et al. 2010). How monitoring is planned and designed determines the ability of a study to discriminate between natural variation and the effect of treatment. Secondly, physical limitations imposed from the catchment or wider scale factors such as surrounding land use, and quality of the upstream area, that indicate overall environmental quality and the availability of potential colonisers (Matthews et al. 2010). Finally, restoration goals are often poorly specified with regards to ecological recovery, additionally many researchers question the value of a predetermined end point, especially within the timescales of post project monitoring (Spänhoff and Arle 2007, Harris and Heathwaite 2012).

3.1.1 Study design for impact assessment.

Smiley Jr. et al. (2009) emphasise the inappropriateness of using routine monitoring techniques to answer questions related to impact assessment. These techniques will not be effective in identifying environmental factors responsible for impairment, since this is not what they were designed to do. Impact assessment requires samples or observations taken from impacted and control locations, both before and after intervention takes place. Sampling during the before period provides an estimate of background variation, and control sites are needed to isolate the effect of the intervention from natural changes. Impacts on patterns of natural variation in space and time are also possible, and will only be detected through monitoring over a number of repeated cycles or scales of the pattern of variation e.g. over a range of seasons and replication of seasonal samples (Downes et al. 2002). The ideal sample design therefore is the Before-After-Control-Impact (BACI) model, or in highly variable natural systems multiple-BACI (MBACI) with replicated time periods or locations. A BACI approach is, however, rare to find in assessment of response to hydromorphological restoration (Geist and Hawkins 2016). In a review by Kail et al. (2015) of 316 studies on restoring hydromorphology in rivers, only 30 met full BACI requirements. Due to the large investment needed to collect and especially, to process large amounts of biological samples, this intensity of sampling is not undertaken, or high resolution data are not available, because studies make use of pre-existing monitoring data.

A typical evaluation approach involves evaluating restoration using upstream impacted sites as surrogates for before data in a paired space-for-time substitution approach (Jähnig et al. 2010, Friberg et al. 2011, Verdonschot et al. 2015). Occasionally natural, or unimpacted sites can also be included as controls (Muotka et al. 2002, Lepori et al. 2005), but sampling over more than one season or year is less common. Any deviation from the ideal monitoring set up means compromising the inferential power of the study (Downes et al. 2002). Upstream and downstream sites are also not statistically independent locations, as they are connected by a certain distance. Downes et al. (2002), therefore encourage the use of controls on separate non impacted rivers, as this removes the problem of autocorrelation i.e. the influence of one sample on another due to their proximity. Absence of temporal or spatial replication also means there is no assessment of natural variability for each stream type.

3.1.2 Biological and hydromorphological potential.

An MBACI design explicitly places the experimental stream in the landscape context. This recognises the widely held view of fluvial landscapes as multi-scaled nested hierarchies of interacting terrestrial and aquatic elements (Poole 2002), consisting of heterogeneous and evolving patches within a wider constraining landscape. Even though hydromorphological river restoration often focuses at the reach scale, reach behaviour is strongly governed by structures and processes acting at larger scales (Lorenz and Feld 2013, Polvi et al. 2014, Stoll et al. 2016), such as surrounding land use or valley geology.

Sampling is often a snapshot of conditions within a year or season, that presumes that the sample is representative of a channel type or pressure response (i.e. what would be recorded by sampling over longer time scales). Without temporal replication baseline stability is assumed rather than measured. The temporal stability of biodiversity response measurements depends on the stability of the environmental conditions, the particular biotic measure used and their relationships to each other. Therefore temporal and seasonal variation is an intrinsic part of river systems (Clarke et al. 2002, Thoms 2006), which cannot be captured by a space for time substitution design (Leps et al. 2016).

Casting a wider eye over nearby rivers allows an appreciation of regional structural complexity (Leps et al. 2016) when initially considering the appropriateness of restoration measures and also defining natural reference conditions (Dufour and Piegay 2009, Leps et al. 2015). Locations will be constrained by their geomorphic potential (Polvi et al. 2014), governed by surrounding land use and extent of geological processes. For example, a confined valley with low gradient hillslopes will have little sediment input, and therefore low potential complexity derived from sediment bed forms and boulders (Polvi et al. 2014). Including more streams in a study allows for the identification of a natural local hydromorphological gradient (Feld et al. 2014), that will have partly driven the evolution of the local and regional species pool. This will determine if there are likely to be taxa present that are adapted to the desired conditions post-restoration and available to recolonise. The lack of a suitable regional species pool is often cited as the cause of restoration project failure. For example, Stoll et al. (2016), found that even when local habitat quality was good, colonization was limited by the low regional gamma diversity. Wider appreciation of the range of taxa present within a study region will illustrate the limits of an area's species pool. This is a reflection of regional habitat availability, including historic catchment processes or pressures shaping the community by selecting for traits such as tolerance to widespread pollution or adaptation to the local hydrological disturbance regime. Not only is regional diversity important, but it is also necessary to consider if species will be able to reach newly available locations through dispersal mechanisms (Spänhoff and Arle 2007). The definition of nearby, and accessible, is variable and is determined by the individual taxa and their traits.

3.1.3 Habitat heterogeneity-beta diversity relationship and restoration success

The idea that biodiversity increases with increased habitat heterogeneity, and that the creation of this heterogeneity will lead to a biotic improvement, is a key tenet of restoration theory. This is reflected in the majority of aims of restoration projects, (Palmer et al. 2014) where specific goals relate to improving biodiversity and instream habitat, through methods of channel and instream hydromorphological enhancements. Such goals appear logical since the major effect of modern and historical pressures has been the simplification of channel structure and alteration of biotic communities towards resistant generalist species e.g. urban systems and the Urban Stream Syndrome (USS; Walsh et al. 2005).

Heterogeneity is well defined as a general concept, as the "variability in a process or pattern over space and time" (Palmer and Poff 1997). In the context of the stream environment this refers to the presence of particular habitat types, their variety and arrangement within the channel or stream network, and their persistence through time. The elements of habitat heterogeneity are considered strong drivers of species richness (Beisel et al. 2000) diversity, and overall community composition, where each unique habitat selects for certain species (Giller and Malmqvist 1998). Each component of habitat structure; type, size, diversity and spatial arrangement, can be important to varying degrees depending on the specific study. For example Barnes, Vaughan & Ormerod (2013), found that a particular habitat type had its own characteristic complexity, and it was the presence of more complex habitat types that determined biological diversity and abundance, rather than simply the diversity of habitat types present. The creation of habitat heterogeneity in restoration projects occurs in a number of ways (Palmer et al. 2010), and there are numerous ways to measure and quantify habitat characteristics, more than 50 worldwide (Fernandez et al. 2011), making comparisons between results difficult. These methods range from calculating the Shannon diversity of recorded habitat features, (Jähnig et al. 2010), using ordination axis scores derived from multivariate data, to forming specialised habitat diversity indices e.g. the Spatial Diversity Index (Verdonschot et al. 2015).

Measures of habitat and biological diversity are scale dependent (Barton et al. 2013), and the scales used to measure either biodiversity and environmental heterogeneity often vary between studies. Heino et al. (2015) found that the level of environmental control on beta diversity was scale dependent, and also related to invertebrate dispersal traits. A study's particular scale of habitat heterogeneity or beta diversity measurement is not an usually an explicit consideration, until it is discovered to be unexpectedly unimportant (Palmer et al. 2010), possibly due to measurement at the wrong scale for the target taxa. There is clearly scope for further explicit consideration of the scaling of the beta diversity-habitat heterogeneity relationship. Which may go some way to explain why the biotic response to habitat heterogeneity has been variable. There is also the potential to better characterise beta diversity patterns e.g. by using partitioning into their constituent parts of turnover and nestedness (Baselga 2013, Rolls et al. 2016).

3.1.4 Restoration goals and trajectory.

The direct reversal of restored sites to a historical state is unlikely. There is a debate in restoration ecology of whether this backward looking approach is either desirable, or even possible due to the dynamic nature of ecosystems (Hobbs and Harris 2001), and the irreversibility of some changes e.g. species extinctions or altered levels of atmospheric chemicals. The regional context of a particular restored site remains different from its historical version, subject to continued human pressures and altered wider scale land use and hydrological regime.

Restoration theories are moving away from the ideal of static reference states to process-based functioning reference conditions (Dufour and Piegay 2009). McDonald et al. (2004) build on the keystone approach of Palmer et al. (1997) of restoring desirable ecological structure i.e. communities, which will generate appropriate ecological function, to include restoring equivalent geomorphic structures and function. The wider goal of ecological function may still rely on general biodiversity, as we continue to build knowledge on which species matter most to ecosystem function (Palmer et al 1997). Placing a restoration project on a "path" of development towards more natural structure and function can only be achieved if one knows the wider spatial and temporal context from a BACI type study design. This allows an appreciation of the potential physical forms and biota-habitat associations found in an area with similar geology, hydrology and land use. Longer term studies are required to determine if the site is moving away from a local poor quality example, and towards a contemporary good quality example. Hence post project monitoring beyond 5 years (Matthews et al. 2010) or longer (Muotka et al. 2002) is to be encouraged to understand succession of communities and the evolution of ecological processes.

3.2 Aims

Biodiversity patterns are often non-linear, or have abrupt thresholds of change (Hobbs and Harris 2001), therefore surveying along a restricted or incomplete environmental gradient may be inadequate for comparing biological change between locations or determining general trends. Also well appreciated is the inherent natural dynamic nature of river systems, which could result in the instability of many well used biodiversity metrics or community assembly over a small spatial extent, short environmental gradient, or timeframe. This calls into question whether a short term space for time substitution study design, comparing upstream with downstream, is adequate to assess the effects and success of hydromorphological restoration?

This chapter aims to answer this question by comparing restored and control channels from chapter 2, with a wider range of nearby study sites. Setting the restored and control rivers within a wider landscape context aims to counter the limitation of restricted ecological or environmental gradients, and quantify the degree of natural variability found in this system. A set of representative natural and historically straightened streams were studied alongside the restored channel, to test the following hypotheses:

1. Conclusions based the Rottal restoration evaluation in chapter 2, using an upstream downstream comparison, are upheld even when further investments are made to quantify natural variability.
2. The upstream channel is a suitable comparison channel and representative of straightened channels.

3. Biological and environmental gradients are extended by including more sites from within the region.
4. Biodiversity metrics and community assemblages are stable over time and among rivers within river type.
5. Biodiversity metrics and community assemblage gradients exist along a range of quantified environmental gradients or categorical channel type from natural to impaired.

3.3 Methods

3.3.1 Study sites

In total 7 reaches (Fig 3.1) were selected in the Glen Clova area of the South Esk catchment surrounding the Rottal Burn restoration project (2.3.1). The Glen Clova catchment lies on low permeability Dalradian metamorphic geology and has an annual average rainfall of 1400mm. Average long term flow (1991-2015) at Gella Bridge on the South Esk (elevation 232m, catchment area 130km^2), which marks the southern limit of the study site, was 5.48m^{3}s^{-1}. This is a characteristic upland landscape (median elevation, 560m) and flows are significantly influenced by spring snow melt. Heath and blanket bog vegetation dominates the valley sides with rough grazing or semi-improved pasture on the valley. Reaches were selected based on their similarity in width, sediment supply, draining catchment size and slope, to initial restoration project sites.

Additional study reaches consisted of two straightened modified sections flowing through rough grazing: March (MA), a small straightened stream (Fig 3.2c), and a tributary of the South Esk and Glenmoye Downstream (GD), situate below a road bridge and the upstream Glenmoye reach (Fig 3.2 b). Three natural channel planform sites were selected, Moulzie Burn (MO, Fig 3.2 e), an upper tributary to the South Esk, flowing through rough grassland, Glenmoye Upstream (GU, Fig 3.2 a), and Prosen (PR, Fig 3.2d), both bordered by rough grazing. Classification to channel type was based on channel planform, and within type sites were ranked on a rough hydromorphological gradient base on a subjective scale of naturalness considering channel planform, evidence of morphological processes, channel and riparian vegetation. However, some inconsistencies in site categorisation occurred, in that straightened reaches tended to be narrower due to embankments created through periodic dredging which constrained channel movement further. Some meanders in MO, at first thought to be un-modified, had a constrained channel form which restricted geomorphological processes locally in the channel. The sites represent a gradient of local conditions approximating the pre and post condition of the restored channel.

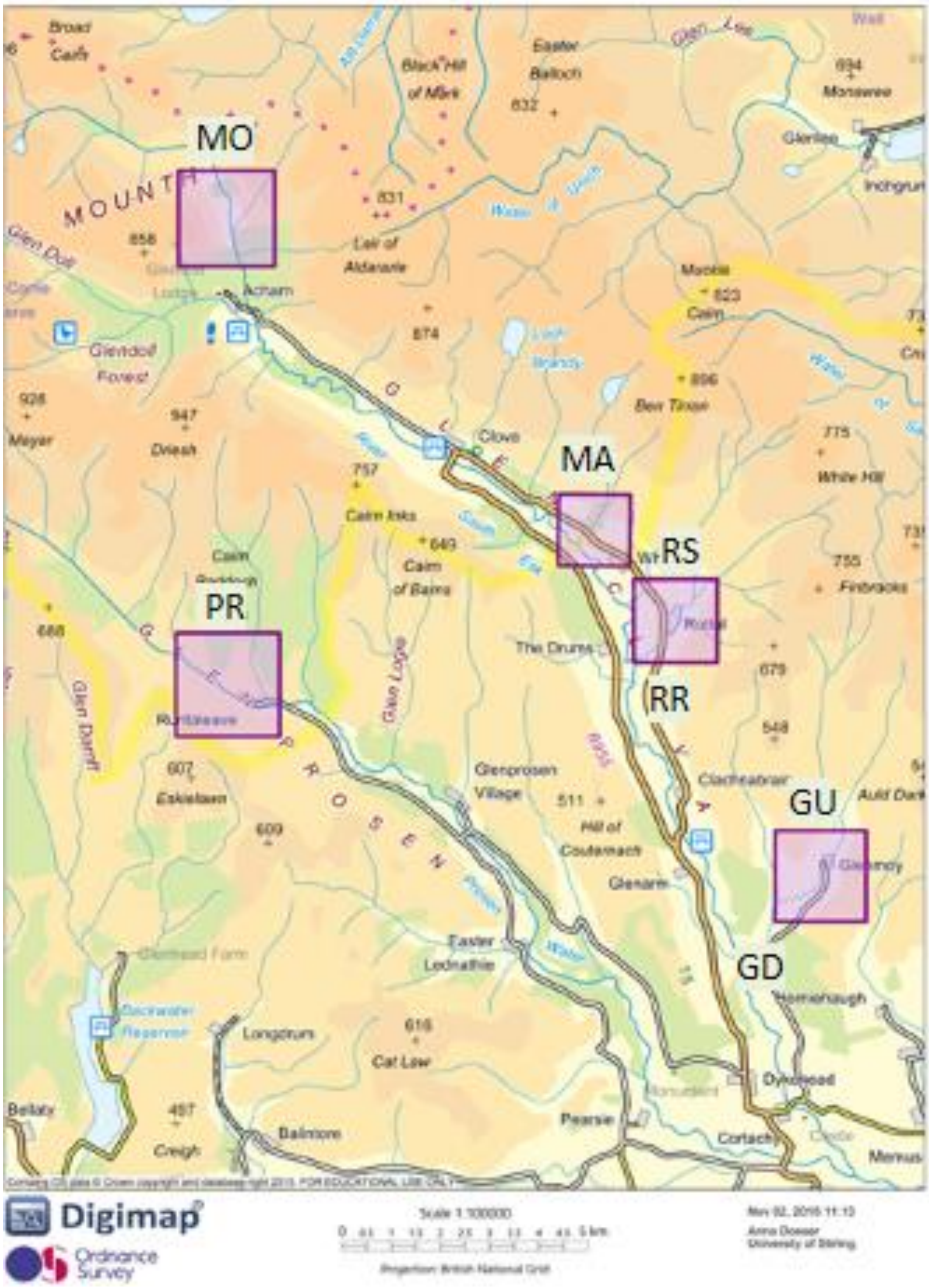


Fig 3.1 Location map of sample rivers in the South Esk Catchment. Straight channel Glenmoye Downstream (GD) and March Burn (MA). Natural channels: Prosen (PR), Moulzie (MO) and Glenmoye Upstream (GU), and restoration and control channel sites from Chapter 2: Rottal Restored (RR) and Rottal Straight (RS).

Fig 3.2 Site photos of sample rivers in the South Esk Catchment a) Glenmoye upstream (GU)., b) Glenmoye downstream (GD) c) March Burn (MA), d) Prosen (PR), and e) Moulzie (MO).

3.3.2. Sampling

All sites were sampled for benthic macroinvertebrates in May and November of 2013 and 2014 using the methods described in 2.3.3. Habitat surveys were carried out in August and September of 2013 and 2014 using the methods described in 2.3.4. Ten evenly spaced reaches were sampled in each river (Appendix 3.1), except in short channels MA and GD where 6 samples were taken.

3.3.3. Statistical analysis

3.3.3.1 Biodiversity indices

Riverfly biodiversity metrics of abundance, species richness, rarefied richness and diversity indices (Shannon and Simpson's) were calculated for every sample, and mean values were determined for each river and sampling period (year and season). Firstly, the stability over time of each metric was assessed using a non-parametric Mann-Whitney U test of difference. Testing the significance of the difference between 2013 and 2014, for each river and season individually. The size, significance and direction of change was calculated by simple subtraction, and was illustrated in plots using an arrow to show the change of mean value from one time period to the other.

To examine the change in metrics along a gradient of channel type and observe the variation within and between each type, mean values and standard errors were plotted for each river and season individually. Metrics were also assessed using aggregated values for each season and river type. Rivers were arranged by channel type, plotting in order from most impacted to least (l-r): straightened, control, restored and natural, to aid comparisons between and within channel types.

3.3.3.2 Defining the regional species pool and geomorphic potential

The extension of biological and environmental gradients was illustrated using unconstrained ordination of the community data, multivariate environmental measurements and habitat features. Correspondence Analysis (CA) was carried out on the full community data set for each season, to determine the full extent of the regional riverfly community assembly. The position of each river and channel type along the community gradient was examined, including placing of the Rottal restored and control channels within the regional community.

To test the similarity of riverfly communities among rivers and river types, both Bray Curtis and Chao distances were calculated between all pairs of samples for each time period individually (n=4). Since previous analysis of indices (2.4.1) indicated strong variability between each year for both spring and autumn, seasons were considered separately. The significance of the difference between average river community composition (centroid location) was tested using PERMANOVA, and the power (R^2) of river identity or river type to explain community composition differences was compared.

The species that drove the differences between rivers and river types was investigated using Indicator Values (IndVal), and also by considering the species contribution to the ordination solution. The top 25 contributing species were considered as indicators for that channel in each season. Likewise, environmental gradient extension was investigated using Principle Components Analysis (PCA) for continuous measurements and its factorial analogue Multiple Correspondence Analysis (MCA) for the categorical habitat features. The extension of the gradient and the location of each river and channel type on the gradient of environment is described.

The stability of riverfly community composition and channel habitat conditions over time was assessed for each river individually. The average community for each river over the 4 sampling periods is illustrated with a 95% confidence ellipse. The power (R^2) and significance (p values) of year, to explain variation in community composition was tested using PERMANOVA, with both the Bray Curtis and Chao community dissimilarity measures. Environmental gradients that correlated significantly with sample community composition at the 5% levels are also shown, suggesting physical changes that could drive the community change. Change in the composition of habitat features and sediment character over time was not significant and are not shown for brevity.

3.3.3.3 Beta diversity

Using the total variance in a site by species matrix as a measure of beta diversity is gaining in popularity (Legendre and De Cáceres 2013). To compare variation of beta diversity between rivers and within river types, the invertebrate community data was separated into each of the 4 sampling seasons. Bray Curtis and Chao distances were calculated pairwise between all samples within each time period and Principle Co-Ordinate Analysis (PCoA) carried out using the resulting distance matrix. The median distance from each sample to the river group centroid (average community) was calculated and compared between rivers. The significance of the difference in distance to centroid for each river and channel type was tested using PERMANOVA, to identify which rivers differed in beta diversity. The stability of beta diversity over time was analysed by comparing mean distance to centroid over all time periods within each river.

Additionally beta diversity was partitioned into contributions from abundance based equivalents of turnover and nestedness (Baselga 2013), to uncover evidence of a potential gradient of turnover or nestedness dominance between rivers and river types. The consistency of the proportions of turnover and nestedness were compared within channel types and the Rottal restored and control channels were related to this pattern. The direct relationship between habitat heterogeneity and beta diversity was assessed combining mean group dispersion of multivariate analysis of both environmental measurements and community data. Environmental mean group dispersion for each river was determined using a PCoA with Euclidean distance. Beta diversity was quantified as median distance to river centroid (average) using both the Bray Curtis and Chao distances. Each distance pair was plotted on a scatter plot for each of the 4 sample periods and biological distance measures. A linear relationship was hypothesised between habitat heterogeneity and beta diversity to evaluate the location of each river on this possible relationship.

3.3.3.4 Drivers of community composition

Redundancy Analysis (RDA) was used to assess the environmental gradients driving the turnover of riverfly community composition across the region. This method of constrained ordination is an extension of regression for modelling multivariate species response to environmental predictors. Each sampling period was analysed separately using all the full set of samples (n=62). This enabled the assessment of regional predictors, their role within each river and between types, as well as consistency of gradients over time and seasons. A global model using all possible environmental predictors was used, if significant, reduction of the predictors was carried out using forward selection methods, with global model R^2 as a second stopping criteria (Blanchet et al. 2008a). Final reduced models for each sampling period were compared using the adjusted R^2 and the significant environmental variables selected. Biplots for each sampling period show the location of each sample, and species that were well explained by the model. Relationships between species and rivers to significant environmental vectors were examined

3.3.3.5 Modelling biodiversity

Initial exploration of the relationship between each environmental variable and biotic measure through a scatter plot, indicated possible distinct trends differed between rivers, river types and also between season. As a result of this data for each season was considered separately with both years combined. The model structure most appropriate for the properties of each dataset was used. Species richness and rounded rarefied richness were analysed using a generalised linear model mixed effects model (glmm) with Poisson distribution. The distribution of abundance values indicated overdispersion so a negative binomial glmm was used. Diversity indices Shannon and Simpson were used untransformed in a linear mixed effects model (lme).

Each biotic metric was modelled using each environmental variable including the river type (natural, restored and straight) as an interaction term allowing for different relationships to exist between different river types. To account for the non-independence of samples from within each river a random intercept term was included in each model of river identity. Predictor variables were scaled to enable direct comparisons of effect size between models. 160 models were generated. Each model was evaluated for river type specific slopes, interactions, and type effects on the model intercept. Slope estimates and confidence intervals (CIs) for each river type were extracted from the model covariance matrix. Where the CIs did not extend across 0, this slope was significant and illustrates the direction and magnitude of the relationship (Anderson et al. 2000). Unlike in Chapter 2 model R^2 or deviance were not reported, since its clear meaning as a goodness of fit of the models is not extended from lm and glm to mixed effects models (Bolker 2015). However, the river effect i.e. the difference in intercept from the restored to natural or straightened river types (-ive indicates restored has higher intercept), and significance of the interaction between river types was noted. PCA analysis and agglomerative hierarchical clustering of models using Euclidean distance and the Ward method was used to identify models with particular properties, allowing general model types to be identified (Fig 2.5).

All statistical analyses and graphics were produced using the R environment (R Core Team 2016) in R Studio version 0.99.902 (RStudio Team 2015) with the use of the following packages: vegan (Oksanen et al. 2016), Sciplot (Morales and Murdoch 2012), Labdsv (Roberts 2016). betapart (Baselga et al. 2013) FactoMinerR (Le et al. 2008), Packfor (Dray et al. 2016) , Lme4 (Bates et al. 2015), glmmADMB (Fournier et al. 2012), and cluster (Maechler et al. 2015).

3.4 Results

A total of 71 riverfly species were found in the 7 sites in the South Esk catchment over the sampling period. 20 of which were the 5 most abundant species occurring in each treatment reach and season (Table 3.1).

Table 3.1. Summary of biological samples, riverfly abundance, richness, diversity, and singly occurring species (mean, maximum and minimum). Mean abundance and standard deviation of 5 most abundant species per season, year and treatment (S- straight, R-restored, N-Natural) combination.

River	March (MA)	Glenmoye Downstream (GD)	Rottal Straight (RS)	Rottal Restored (RR)	Glenmoye Upstream (GU)	Moulzie (MO)	Prosen (PR)
Treatment	S	S	S	R	N	N	N
No. Samples	6	6	10	10	10	10	10
Spring Abundance	400.2 (126-671)	455.0 (156-1237)	444.3 (134-793)	288.4 (24-1058)	589.7 (125-1225)	276.05 (36-513)	358.45 (66-726)
Autumn Abundance	492 (196-763)	374.8 (160-560)	165.9 (47-351)	417.4 (145-1131)	482.7 (209-727)	312.75 (47-576)	322.1 (115-616)
Spring Richness	22.08 (14-26)	21.75 (13-31)	17.2 (12-21)	15.95 (5-25)	21.45 (12-30)	18 (9-24)	20.45 (15-28)
Autimn Richness	25.58 (21-30)	21.7 (16-26)	16.05 (11-22)	23.85 (15-31)	18.4 (9-24)	22.7 (11-31)	20.45 (12-25)
Spring Shannon	2.17 (1.81-5.54)	2.12 (1.34-2.46)	1.93 (1.65-2.36)	1.88 (1.11-2.48)	2.13 (1.54-2.52)	2.11 (1.80-2.40)	2.14 (1.63-2.61)
Autumn Shannon	2.31 (2.16-2.53)	2.02 (1.75-2.42)	2.00 (1.50-2.42)	2.14 (1.58-2.59)	1.72 (1.02-2.11)	2.16 (1.40-2.64)	1.91 (1.33-2.57)
Spring Simpson	0.82 (0.72-0.90)	0.81 (0.58-0.87)	0.78 (0.71-0.87)	0.77 (0.61-0.86)	0.82 (0.69-0.89)	0.83 (0.73-0.89)	0.81 (0.64-0.90)
Autumn Simpson	0.83 (0.78-0.88)	0.78 (0.70-0.87)	0.79 (0.63-0.87)	0.80 (0.65-0.89)	0.71 (0.44-0.83)	0.80 (0.54-0.90)	0.74 (0.58-0.89)
Spring Singletons	5.1 (0-9)	5.3 (2-9)	3.4 (1-7)	4.3 (1-7)	5.0 (0-8)	4.3 (1-8)	4.5 (1-8)
Autumn Singletons	5.5 (2-10)	4.5 (1-8)	4.3 (0-11)	6.9 (1-13)	3.9 (0-7)	6.7 (2-12)	5.3 (2-10)
Ameletus inopinatus				S 15 (21)			
Amphinemura sulcicollis	A 166 (61), S 119 (67)					S 55 (49), A 30 (23)	S 31 (25)

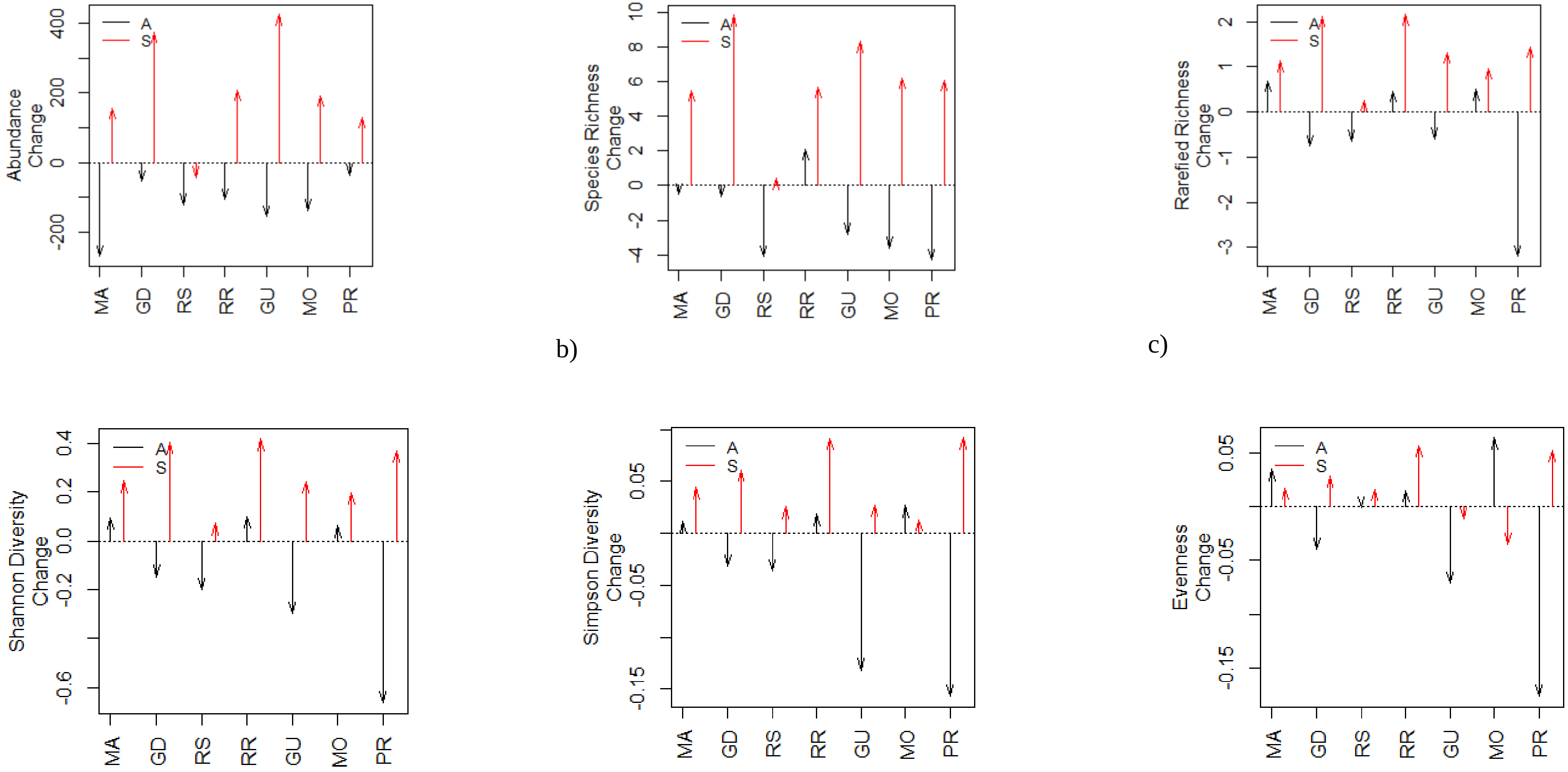
Baetis muticus		S 61 (37)			S 92 (54)		S 28 (24)
Baetis rhodani	S 31 (15)	S 151 (112), A 46 (19)	S 173 (105), A 26 (27)	S 82 (82), A 37 (29)	S 157 (101), A 65 (40)	S 40 (34), A 21 (20)	S 110 (78), A 50 (26)
Ecclisopteryx guttulata				A 77 (76)			
Ecdyonurus venosus		A 30 (16)	A 8 (5)	A 22 (14)	A 37 (29)		
Electrogena lateralis			S 21 (13)				
Glossosoma conformis							A 14 (20)
Hydropsyche instabilis		A 12 (9)					
Hydropsyche siltalai					A 16 (35)		
Hydroptila sp						S 33 (55), A 104 (105)	
Isoperla grammatica	S 16 (5)						
Leuctra hipporus/moselyi				A 21(17)			
Leuctra inermis	S 102 (58)	S 30 (30)	S 95 (51), A 13 (11)	S 68 (72)	S 42 (19)	S 26 (21)	A 12 (17)
Limnophilidea (small)	A 38 (16)						
Polycentropus flavomaculatus						A 26 (19)	
Protonemoura meyeri	A 77 (41)	A 90 (70)	A 27 (26)		A 86 (63)		A 85 (88)
Rithrogena semicolorata		S 60 (51), A 118 (56)	S 37 (25), A 53 (37)	S 32 (43), A 133 (108)	S 103 (56), A 201 (68)	S 35 (37), A 28 (23)	S 52 (36), A 94 (60)
Serratella ignita		S 29 (40)			S 43 (88)		
Siphonoperla torrentium	S 18 (17)		S 24 (16)	S 31 (38)			

3.4.1 Biodiversity indice stability, over time, between and within river types.

3.4.1.1 Biodiversity metric stability over time.

The direction and degree of change over time for all metrics in each season was relatively consistent across all channel types, suggesting not a gradient of biodiversity stability in space, but a strong change over time. Most metric values significantly decrease from 2013-2014 in autumn (Table 3.2 and Fig 3.3), and increase in spring. There were small amounts of variation within each channel type which tended to occur in autumn. Rarefied richness and Shannon diversity of MA samples uniquely increased in autumn. Notably large decreases in autumn rarefied richness and diversity occurred for PR, however an absence of change marks out MO. In autumn metrics did not change in the RR contrary to other reaches. In spring the restored (RR) channel follows the same trend as the other reaches. Conversely in autumn metrics in RS respond similarly to other channels, however in spring RS metrics did not change over time.

In summary there were significant changes in biotic metric from one year to another, with a consistent direction of change within each season for most rivers. This indicates the instability of even undisturbed sites in the short term. River specific changes were more variable in autumn. Within each channel type, metrics of straight channels were most variable. However, MA and MO break with the overall catchment pattern, indicating river specific causes for this deviance. Both the restored and control channels show atypical changes for one season, whilst responding similarly to surrounding channels for the other.



a)

b)

c)

d)

e)

f)

Fig 3.3 Stability plots, showing the direction and magnitude of change of mean value from over time (2013 to 2014), for spring (S - Red) and autumn (A - Black) samples of riverfly a) Abundance, b) Species richness, c) Rarefied Species Richness, d) Shannon diversity, e) Simpson diversity, f) Evenness.

Table 3.2 Summary table of biotic metric stability; direction (increase +/ decrease -) and significance of change over time (2013-2014) using a Wilcoxon 2 sample test of year, for each season. ns = Not significant.

	Season	S	A	S	A	S	A	S	A	S	A	S	A
Type	River	Richness		Rarefied Richness		Abundance		Shannon		Simpson		Evenness	
Straight	MA	+	ns	+	+	+	-	+	+	+	ns	ns	ns
	GD	+	ns	+	-	+	ns	+	-	+	ns	ns	ns
	RS	ns	-	ns	-	ns	-	ns	-	ns	-	ns	ns
Restored	RR	+	ns	+	ns	+	ns	+	ns	+	ns	+	ns
Natural	GU	+	-	+	-	+	-	+	-	ns	-	ns	-
	MO	+	-	+	ns	+	-	+	ns	ns	ns	ns	+
	PR	+	-	+	-	+	ns	+	-	+	-	+	-

3.4.1.2 Between river and river type comparisons: abundance, richness and rarefied richness

There were only small differences in abundance and richness between channel types (Fig 3.4b, 3.5 b, 3.6 b), overall straight channels had higher values than natural ones, however there was considerable variation between individual rivers and seasons (Fig 3.4a, 3.5a, 3.6a). Standardising for sample effort using rarefied richness (Fig 3.6) reduced the difference between the river types, however difference in richness values were very small (e.g. 1 taxa). Variability in abundance and richness between rivers of each type depended on season, channel type and the metric considered. Straight rivers were most variable in autumn, mainly due to higher abundance and richness values in MA samples. In natural rivers abundance varied more in spring, however richness and rarefied richness varied more in autumn due to very low values in samples from GU and PR. A response gradient of each metric within the natural channels could be identified, decreasing from GU-PR-MO, in abundance, but increasing in richness.

Restored Rottal (RR) had abundance values similar to other natural channels and intermediate richness and rarefied richness values, but lower than that for straightened rivers, since these have the highest values. During the initial spring season RR samples had low metric values that increased in the second year, however this trend was also seen in all others channel types. RR autumn species richness appeared consistently high compared to other rivers, since other channels experienced a reduction richness from 2013-2014.

The Rottal control channel (RS) had low abundance and richness compared to other stream types. RS samples had above average spring abundance, but this did not increase over time as seen in all other rivers. Low species richness was a result of reduced sample size in autumn, which increased after rarefaction, however values remained lower in spring. These atypical values inflate the difference between control and restored, to accentuate improvements in autumn but less so in spring.

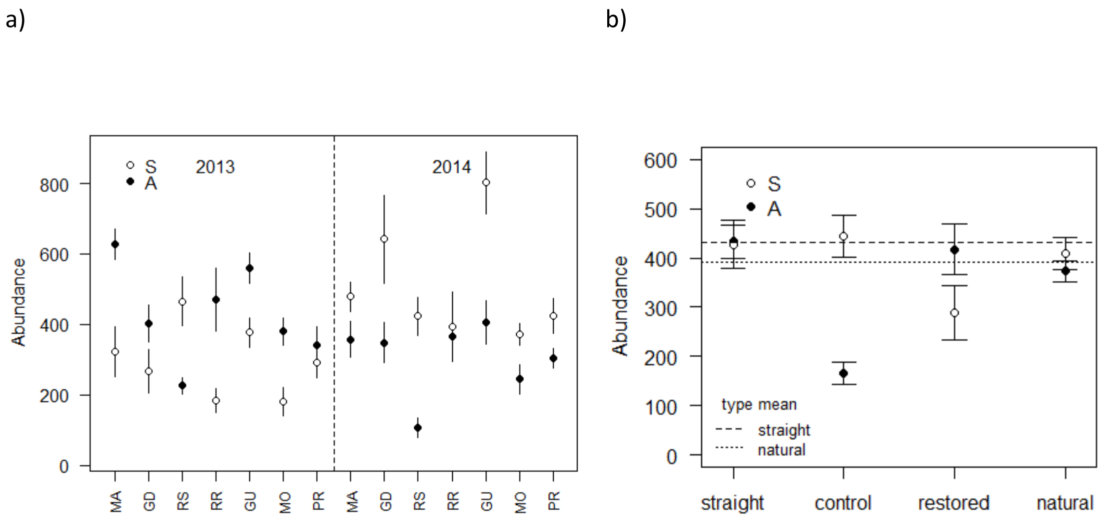


Fig 3.4 Sampling occasion mean abundance value and 95% confidence intervals for a) Each river and sampling occasion and b) Each channel treatment type and season, (years combined). (S: Spring, A: Autumn).

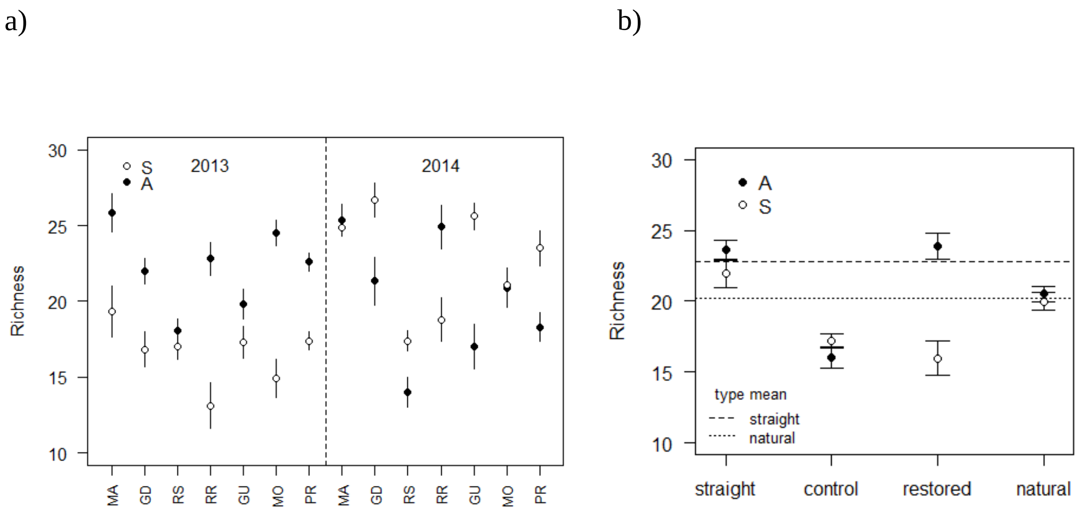


Fig 3.5 Sampling occasion mean riverfly richness value and 95% confidence intervals for a) Each river and sampling occasion and b) Each channel treatment type and season (years combined). (S: Spring, A: Autumn)

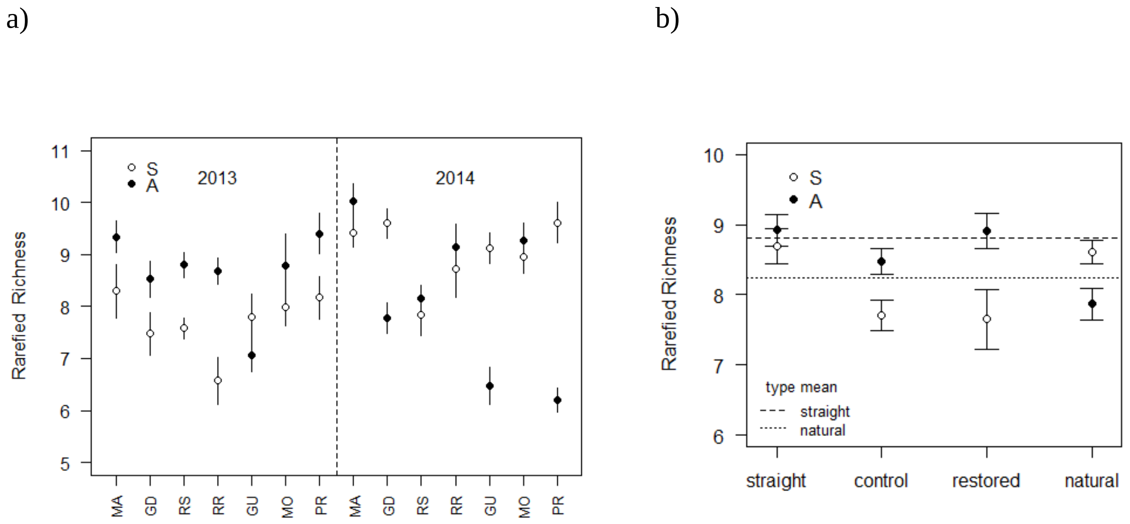


Fig 3.6 Sampling occasion mean rarefied riverfly richness value and 95% confidence intervals for a) Each river and sampling occasion and b) Each channel treatment type and season (years combined), (S: Spring, A: Autumn).

3.4.1.3 Between river and river type comparisons: Diversity Indices

Shannon and Simpson diversity indices, though Simpson's to lesser degree were higher in straightened channels compared to natural ones (Fig 3.7 and Fig 3.8). However, a significant contributor to this difference was the reduction of diversity in autumn 2014 in GU and PR. Diversity values among rivers were most consistent in spring. In autumn there was higher variety for both straightened and natural channels. Samples in MA were more diverse than GD, and diversity in GU and PR was significantly reduced compared to MO. Importantly, RR would be evaluated very differently depending on the season considered and the river it was compared to. In autumn RR had as high diversity as straight channels, in spring however RR was the least diverse. RS diversity indices were generally low compared to all other channel types.

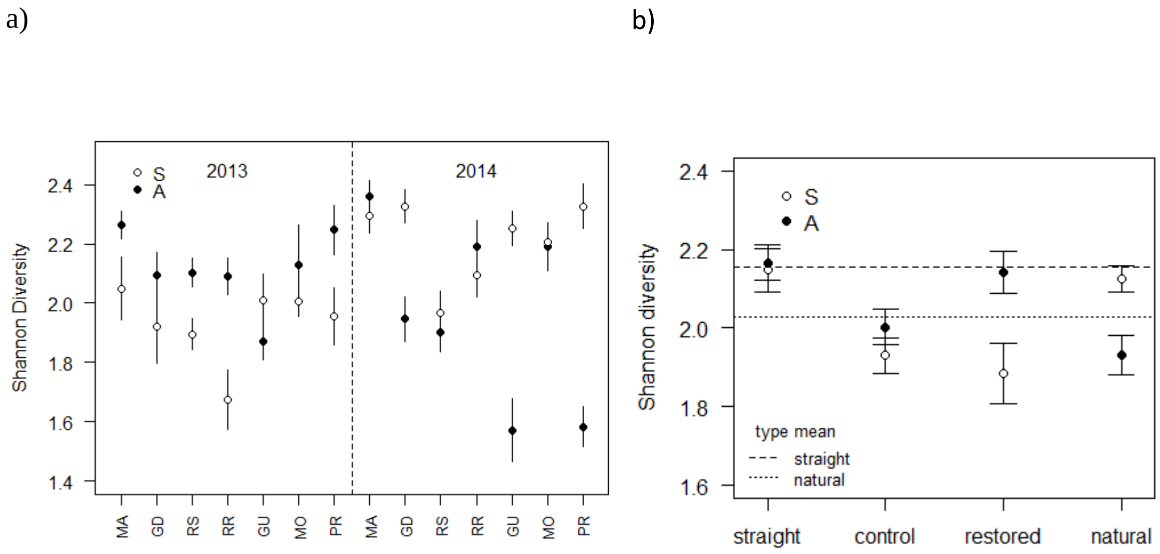


Fig 3.7 Sampling occasion mean Shannon Diversity value and 95% confidence intervals for a) Each river and sampling occasion and b) Each channel treatment type and season (years combined). (S: Spring, A: Autumn).

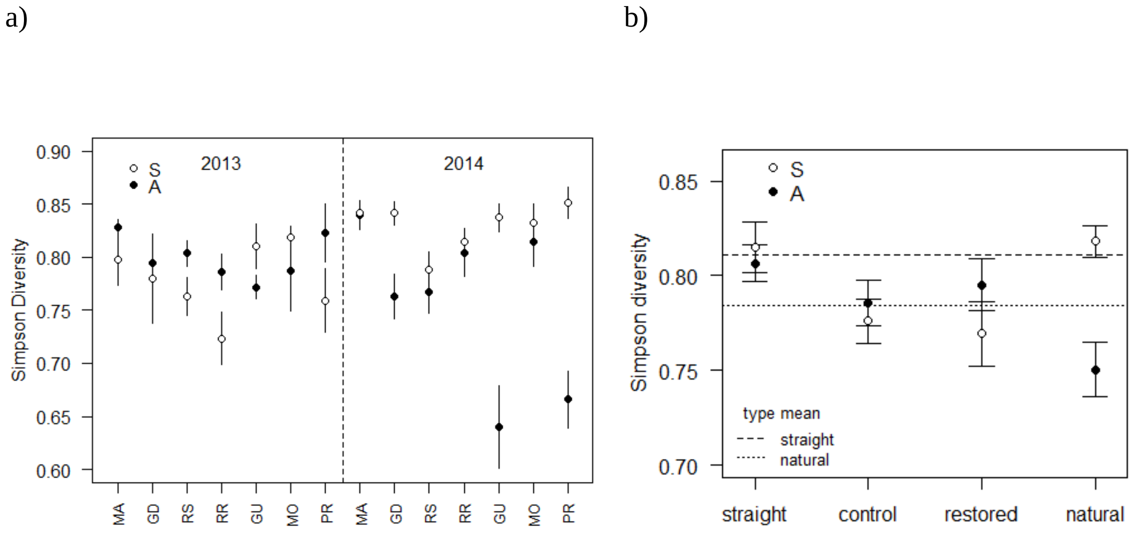


Fig 3.8 Sampling occasion mean Simpson Diversity value and 95% confidence intervals for a) Each river and sampling occasion and b) Each channel treatment type and season (years combined). (S: Spring, A: Autumn).

3.4.2 Regional species pool and geomorphic potential

3.4.2.1 Species pool and river contributions

All rivers and types contributed to the extension of the riverfly community in some way. Tests showed that both river identity ($p< 0.001$) and channel type ($p< 0.05$) were significant to explain variation in catchment community composition for both Bray-Curtis and Chao distances. For all sampling occasions (Table 3.3) there was always at least one of the 7 rivers or 4 channel types (Straight, Control, Restored, Natural) that was different from one of the others.

Table 3.3 Summary of significance tests of community composition difference, using river identity and river type as explanatory variables, R^2 and model significance.

Sample Period	Distance measure	R^2 - River	p- River	R^2 - Type	p- Type
Spring	Bray-Curtis	0.33	0.001		
Autumn	Bray-Curtis	0.46	0.001		
2013 S	Bray-Curtis	0.44	0.001	0.20	0.001
2013 A	Bray-Curtis	0.59	0.001	0.19	0.001
2014 S	Bray-Curtis	0.46	0.001	0.16	0.001
2014 A	Bray-Curtis	0.57	0.001	0.29	0.001
Spring	Chao	0.57	0.001		
Autumn	Chao	0.86	0.001		
2013 S	Chao	0.73	0.001	0.28	0.001
2013 A	Chao	0.92	0.001	0.17	0.024
2014 S	Chao	0.72	0.001	0.32	0.001
2014 A	Chao	0.89	0.001	0.22	0.008

Spring communities and highly contributing species are shown in Fig 3.9. Rivers MO and MA extend the community composition along Axis 1 and 2. Glenmoye samples, both GD and GU, extended negatively on A1 and positively on A2. PR channels position at the centre of the plot

(Fig 3.9 a), containing an average community for the region. RR samples covered a wide range of the community gradient, sharing assemblage properties with other natural channels MO and PR. RS conversely covered a narrow community gradient nested within the restored channel.

In autumn regional communities of MO and MA strongly drove the extension of the community assembly gradient, and in contrasting ways (3.10a, inset). Although MA communities were unique to that river, there was little variation among samples, indicating less community differentiation between samples. MO samples however covered a larger extent of community space. Contrasting with spring, autumn RS channel samples, in addition to resembling RR also resembled communities in PR. RR communities overlapped significantly with RS and PR sites, however a few samples showed similarities to those of MO (3.10 a. inset). River identity was more significant than channel type in defining composition (higher R^2 Table 3.3), with straight channel communities consisting of a subset of adjacent natural communities. Channels of the same type often occupied disparate species space. The community ordination space for both seasons was much extended beyond the Rottal channels. Community differentiation between channels was clearer in spring (Fig 3.9), but more significant in autumn, likely due to the influence of MA and MO (Fig 3.10).

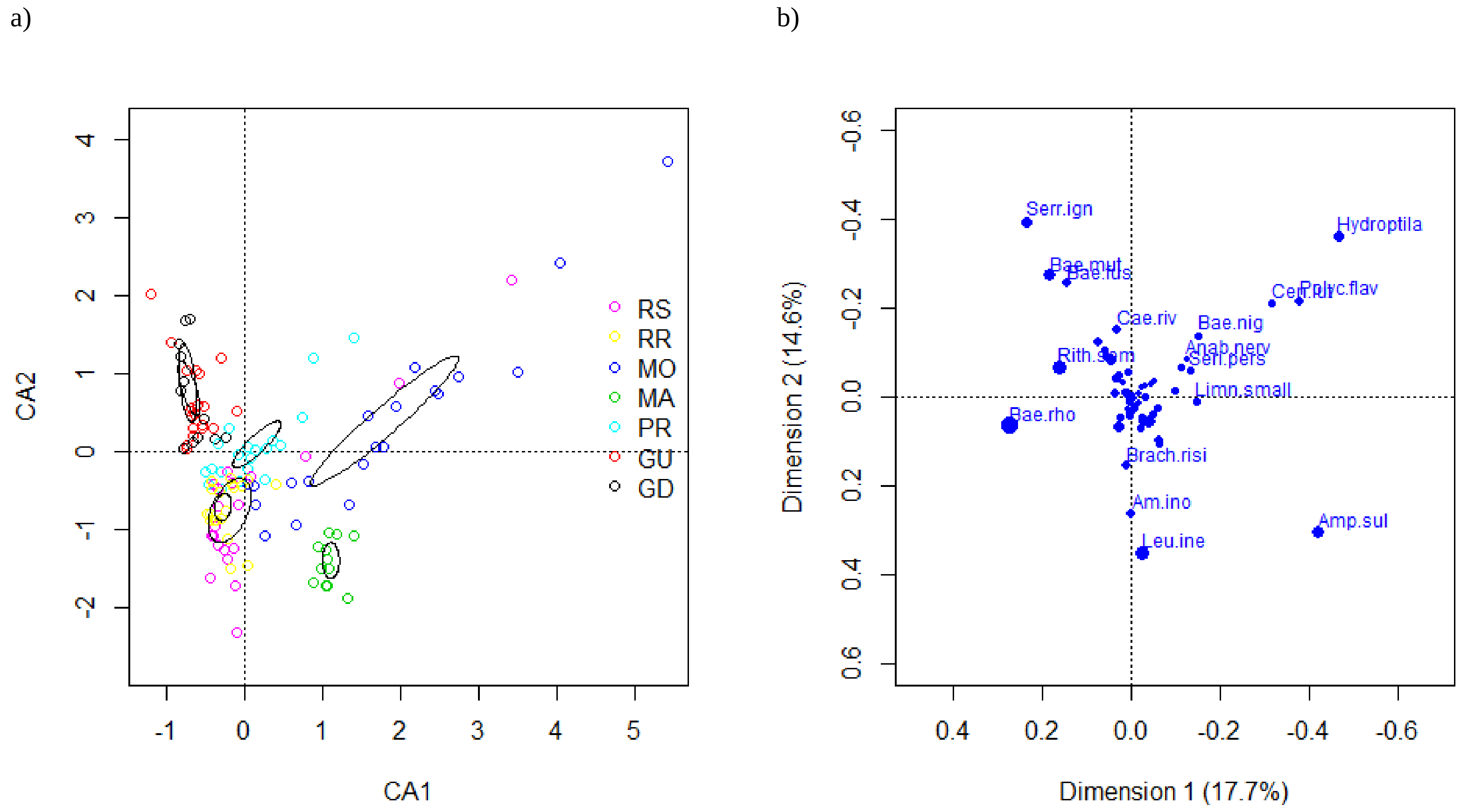


Fig 3.9 Correspondence analysis (CA) biplot for Spring South Esk communities, years (2013-2014) combined a) sample sites, coloured by river identity (7) with 95% confidence ellipses around each river, b) Contribution biplot of riverfly species with a contribution > 1%. A key to species abbreviations is given in Appendix 2.4

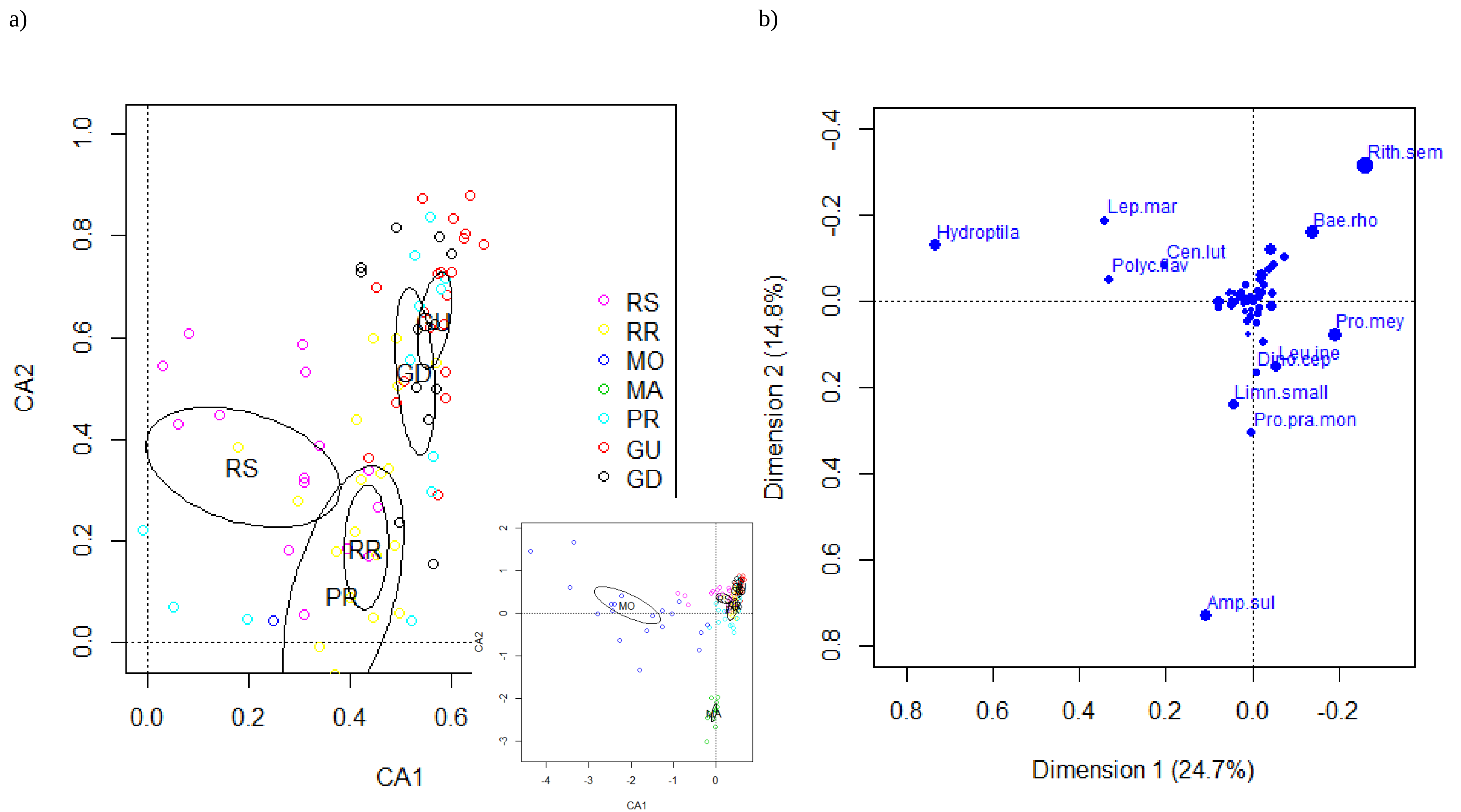


Fig 3.10 Correspondence analysis (CA) biplot for Autumn South Esk communities, years 2013 and 2014 combined a) sample sites coloured by river identity (7) with 95% confidence ellipses around each river, inset: Extended CA axis showing locations of MO left of axis and MA below axis b) Contribution biplot of riverfly species with a contribution > 1%. A key to species abbreviations is given in Appendix 2.4

3.4.2.2 Species identity and indicators

Indicator Value analysis identified a large number of riverfly taxa that were significant indicators of each river (Table 3.4). Many of these were in agreement with the highly contributing species to the Correspondence Analysis (Fig 3.9b and Fig 3.10b). The highest number of indicator taxa per river in spring was for GU (10) and MA (9). For autumn the most indicators were found in MA and RS (both 8). Within some streams were seasonal patterns of congeneric indicators in the Baetidae, Leptophlebiidae and Nemouridae families (Table 3.4).

MO and MA contained the most distinct communities with many species that were present all year round (Fig 3.9b, and Fig 3.10b). MA was characterised by stoneflies and cased caddis *Potamophylax cingulatus*, *Halesus digitatus* and *H. radiatus*. MO contained the non cased caddis species *Polycentropus flavomaculatus*, *Hydropsyche pellucidula*, and the small cased *Hydroptila.sp* throughout the year, and the cased *Sericostoma personatum* in spring. The Rottal restored (RR) community was more distinct from other rivers in comparison to the control channel, containing 8 indicators taxa in autumn, whereas the control samples contained none. In both seasons RR was characterised by the cased caddis *Ecclisopteryx guttulata*, additionally indicated in autumn by the caddis *Lepidostoma hirtum*, upland mayfly *Ameletus inopinatus*, and large stoneflies *Perlodes mortoni* and *Diura bicaudata.* PR had few characteristics species, with only *Glossosoma conformis* present all year round. Glenmoye communities were indicated by a number of mayflies and non cased caddis. GU, the natural planform channel was characterised by *Hydropsyche siltalai*, and Heptageniids *Ecdyonurus venosus* and *Rhithrogena semicolorata*. While *Hydropsyche instabilis* and *Paraleptophlebia submarginata*, indicated the downstream straightened section, GD. In summary, there was high riverfly diversity within the regional species pool, a larger gradient of species was achieved by surveying further rivers in the region. There appeared to be stronger community similarities between sites within the same river (Glenmoye and Rottal) compared to communities from each river type.

Table 3.4 Summary of IndVal results, showing the season (Spring – S, Autumn – A or both – B, C = significant contribution from taxa to Correspondence Analysis) each species significantly indicated.

Order	Family	Genus/ Species River	MA	GD	RS	RR	GU	MO	PR
E	Ameletidae	*Ameletus inopinatus*			S	A			
	Baetidae	*Baetis fuscatus*		S					
		Baetis muticus		A			S		
		Baetis niger						S-C	
		Baetis rhodani			S-C		A		
		Centroptilum luteolum						B-C	
	Caenidae	*Caenis rivulorum*					S		
	Ephemerellidae	*Serratella ignita*					S		
	Heptageniidae	*Ecdyonurus venosus*					B		
		Electrogena lateralis			S	A			
		Rhithrogena semicolorata					B		
	Leptophlemiidae	*Leptophlebia marginata*		S				A	
		Paraleptophlebia submarginata		A			S		
	Siphlonuridae	*Siphlonurus lacustris*						S-C	
P	Chloroperlidae	*Chloroperla tripunctata*			S				
		Siphonoperla torrentium				A			
		Leuctra hippopus/moselyi	S			A			
		Leuctra inermis	B						
	Nemouridae	*Amphinemura sulcicollis*	B						

		Nemoura avicularis						A-C	
		Protonemura meyeri	S						A-C
		Protonemura montana/ praecox	A						
	Perlidae	*Dinocras cephalotes*	B						
		Perla bipunctata							S
	Perlodidae	*Diura bicaudata*				A			
		Isoperla grammatica				A-C			
		Perlodes mortoni				A			
	Taeniopterygidae	*Brachyptera risi*	A		S-C				
T	Brachycentridae	*Brachycentrus subnubilus*						A-C	
	Glossosomatidae	*Agapetus sp*		A		S			
		Agapetus fuscipes		S					
		Glossosoma conformis							B
	Goeridae	*Silo pallipes*							S
	Hydropsychidae	*Hydropsyche instabilis*		A			S		
		Hydropsyche pellucidula						B	
		Hydropsyche siltalai					B		
	Hydroptilidae	*Hydroptila sp*						B	
		Oxyethira sp						A	
	Lepidostomatidae	*Lepidostoma hirtum*				A			
	Limnephilidae	*Limnophilidea (small)*	B						
		Ecclisopteryx guttulata				B			
		Halesus digitatus digitatus	S						
		Halesus radiatus	S					A-C	

	Potamophylax cingulatus	B					
	Potamophylax latipennis	B					
Odontoceridae	*Odontocerum albicorne*		A				
Philopotomidae	*Philopotamus montanus*					B	
Polycentropodidae	*Polycentropus flavomaculatus*						B
Rhyacophilidae	*Rhyacophila dorsalis dorsalis*					S	
	Rhyacophila munda		S				
Sericostomatidae	*Sericostoma personatum*						S

3.4.2.3 Environmental gradients and river characteristics

Observing the sample locations and river identities in Fig 3.11, the ordination shows the environmental gradient was extended beyond the Rottal channels (RR and RS), mainly in the positive direction of Axis 1 (A1). The first 3 PCA axes accounted for a total of 65.4% of the variation in the environmental character of the sample sites. The strongest environmental gradient described 44.8% of the variation between sites, and combined many of the sediment variables recorded in the pebble count, as well as the percentage boulder from the visual assessment (Fig 3.11). Positive scores on A1 indicate large and embedded sediment particles, and fewer finer mobile particles of sand or gravel. Axis 2 (A2) was less strongly correlated to any single variable, however it was most strongly derived from percentage sand from the visual assessment, percent fines from the pebble count and the measure interstitial fines storage. Axis 3 (8.8%) was related to percentage silt and cobble but did not differentiate between any of the rivers or types.

There was significant overlap on A1 by rivers GU and GD, PR, MA and RS. New samples add little to the A2 gradient other than the range that was already covered by Rottal restored. Straight and natural channel types had similar environmental properties, with both Glenmoye reaches being more similar within river than to type. RS was similar to the small straight MA, whereas the RR resembled the wider meandering MO.

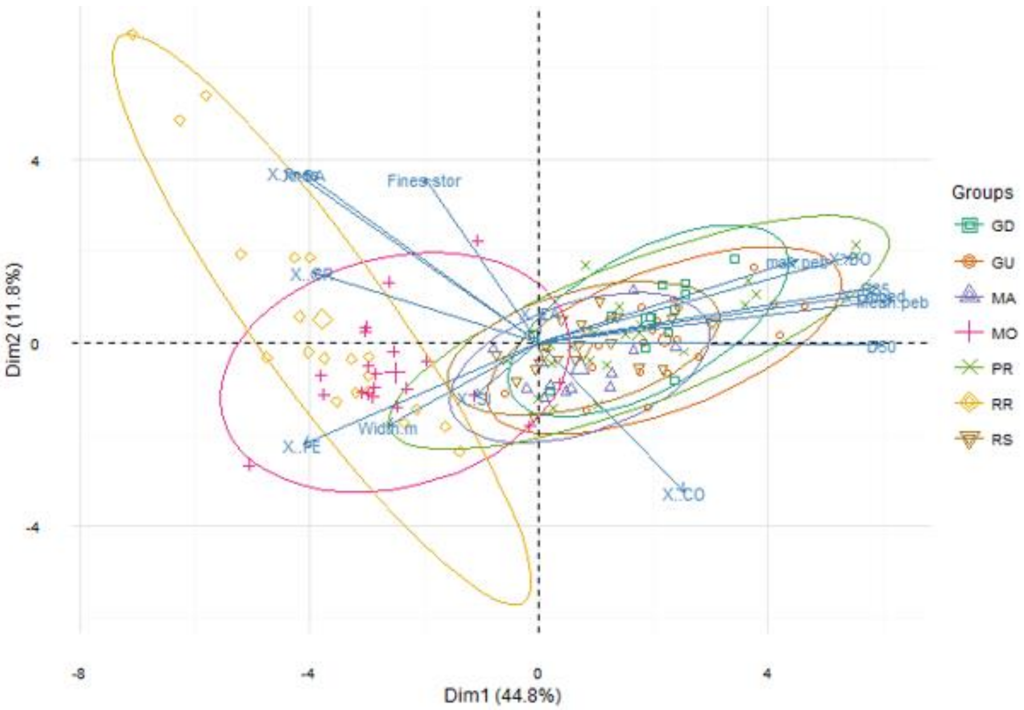


Fig 3.11. Samples measured sediment variables plotted in PCA axis 1 and 2, for the 7 rivers. Large point and circles shows group centroid and 95% confidence ellipses.

Rottal Control (RS) and MA sites position on the positive side of A1,and span only a small range of A2. These sites were characterised by a range of larger substrate sizes, more embedded particles and fewer fine sediments. GU, GD and PR reaches contained on average larger substrate sizes, but contained a wider range of both of large and small sediment sizes. RR and MO position on the negative side of A1, characterised by a range of smaller sediments and a lower percentage of embedded particles. Only the restored channel, RR, contained samples with a large variability on A2, this was mainly due to samples 191 and 192 near the confluence with the South Esk (Fig 2.2a), which plotted in top left corner. These sites were characterised by significantly more fines and sand in the channel, a character not found in the other reaches.

The distribution of habitat features among river showed a different pattern from that of measured sediment variables (Fig 3.12). Each MCA axis explained a small amount of variance compared to the PCA. Axes 1-3 explained just 17.3% of the total regional habitat variance. Values were small since there were a very large number of variables used to construct the axis, and high dimensionality in the habitat composition.

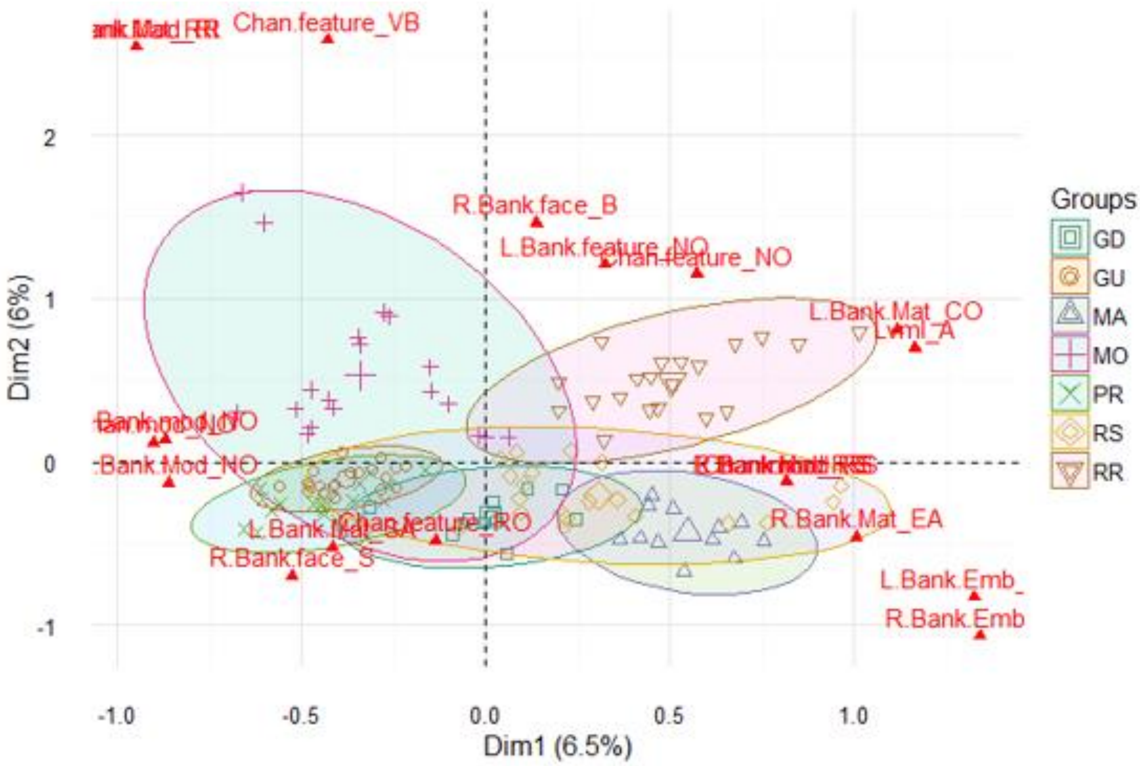


Fig 3.12 Samples and RHS variables plotted in MCA axis 1 and 2. Point and circle shows group centroid and 95% confidence ellipses. The variables shown are the 20 that contribute most to the MCA solution.

Axis 1 (A1) categorised samples based on the presence of bank modifications (sectioning and embankments), and the amount of mossy vegetation in the channel. This axis best discriminated between the rivers, and a grouping based on channel type was also observed. Natural channels appeared on the negative side of A1 and straight channels on positive A1. Axis 2 (A2) described the presence or absence of channel features, particularly large exposed rocks and also the type of bank vegetation, and some features particular to MO: reinforced banks using large boulders as rip-rap and vegetated bars.

Rottal control (RS) sites shared many habitat features with the two other straightened channels MA and GD, although those 2 rivers appeared distinct from each other. The occurrence of embankments in MA and some RS samples (201 and 209, Fig 2.2a) positioned these rivers higher on A1 compared to GD. All straight channels had simple bank vegetation, in-channel rock features and mossy vegetation. Natural channels PR and GU, shared features of banks without modification or sectioning, the presence of rock features and simple bank vegetation. MO was also unmodified by embankments or resectioning, however a number of sites had bank reinforcement through the placement of riprap. Reinforcement, the lack of rock features, and bare bank vegetation distinguishes MO from the other unmodified channels. Samples from the

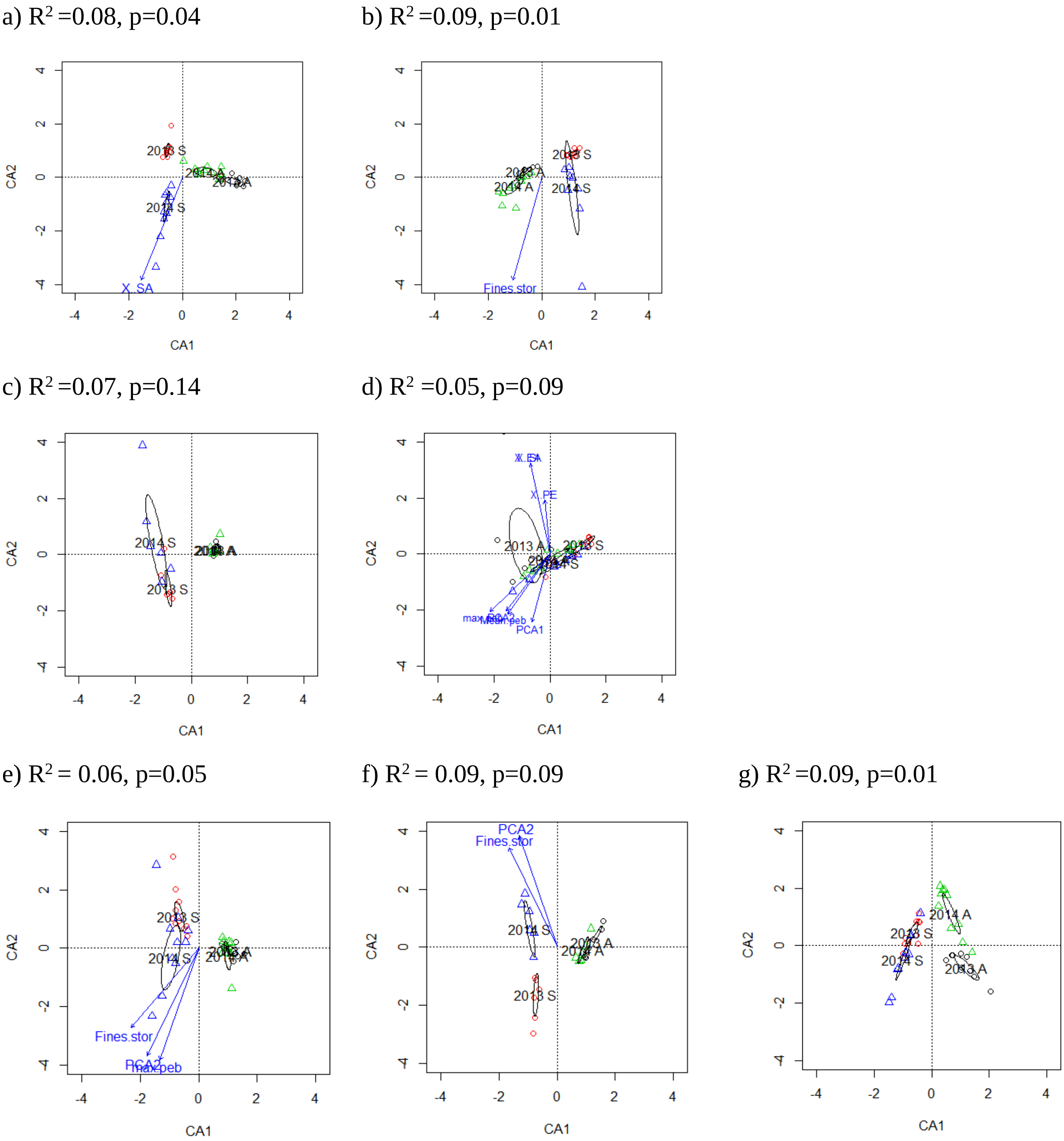
Restored channel (RR) sites locate on the biplot with positive values of both A1 and A2. They were characterised by an absence of channel and bank features, little moss cover and few rock features or embankments. They were categorised as resectioned, since their current planform was the result of recent man made disturbance, otherwise their habitat features would have equated more closely to the natural channel MO.

3.4.2.4 Community stability over time

Riverfly communities were generally stable from 2013-2014. Only in a few cases did communities significantly different, in either spring or autumn or both (Fig 3.13). The amount of variation explained by sample year was also small (6%- 13%). Communities in natural channels, PR and GU and RR changed significantly. Straight channel communities were more temporally variable in spring. This change was not explained by any environmental drivers for MA, but for GD, communities in spring 14 correlated with higher interstitial fines and PCA2, which also relates to high fine sediments.

The temporal consistency of invertebrate communities in natural channels differs seasonally from that in straight channels. In spring, communities were stable in MO and PR. However, some autumn communities in MO 2013 were atypical, these samples correlated with higher amounts of earth and sand. However, in PR significant differences between autumn communities were not correlated to any measured variables. In GU conversely communities were stable in autumn, but not spring. Changes correlated to higher PCA2 scores relating to fine sediment, and higher maximum pebble size in spring 2014. Change in GU was more similar to GD, i.e. within the same river channel, rather than channel type.

RS communities were similar to other straight channels, changing from one spring to the next correlated to an increase in sand cover in 2014. The RR communities also changed most over time in spring samples, correlating with higher amounts of interstitial fine sediment present in 2014. Highest temporal instability of community assembly occurred for spring samples, frequently correlated with higher amounts of fine sediments, % sand or interstitial fines in 2014. However, this trend is depended on channel type, as 2/3 natural channels had stable communities in spring.


a) R^2 =0.08, p=0.04

b) R^2 =0.09, p=0.01

c) R^2 =0.07, p=0.14

d) R^2 =0.05, p=0.09

e) R^2 = 0.06, p=0.05

f) R^2 = 0.09, p=0.09

g) R^2 =0.09, p=0.01

Fig 3.13 Results of the CA, showing community composition change over time within each river a) RR, b) RS, c) MA, d) MO, e) GU, f) GD, g) PR, over the 4 sample periods, Spring 2013, Autumn 2013, Spring 2014 and Autumn 2014. Where significant correlations to environmental variables were found these gradients arrows are plotted, R^2 and the significance of a PCoA model of community composition with year as an explanatory factor are reported above..

3.4.3. Beta diversity

3.4.3.1 Gradient of beta diversity among rivers and between types

Beta diversity was significantly different when tested with river identity for in 6/8 sampling periods. Fig 3.14 illustrates boxplots of median of distance to group centroid using both the Chao and Bray Curtis distances as measures of beta diversity. Pairwise comparisons show that only one or two rivers were responsible for this difference. Overall there was no gradient in beta diversity between river types, however there was often a difference between rivers within the natural type (7/8) and only once in straight channels (Fig 3.14 c) MA>GD, Autumn 2013 B-C distance. Within the natural channels it was frequently MO which had significantly higher beta diversity. Beta diversity in the restored channel was high compared to most other channels across the time periods, most closely resembling diversity patterns in MO. RS had similar beta diversity values to other straight channels, although beta diversity was comparatively high in autumn 2014.

3.4.3.2 Stability of beta diversity over time or season

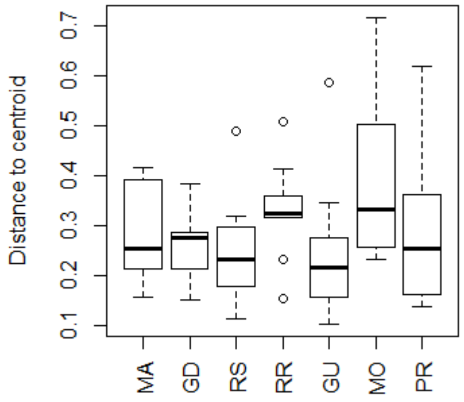
Values of beta diversity within each river were consistent over time when using the Bray Curtis distance measure. Beta diversity values were more variable however in straight channels, when using Chao distance. Table 3.5 shows the results of a permutation test between years and season, indicating significance at the 95% level. Only in MA was this significant, where Bray Curtis distance was seasonally structure in 2013, whereas Chao beta diversity increased over time. For the GD and RS, Chao distance was also significantly different, but the effect of season or year varied in each case.

Table 3.5 Results of tests of group dispersion (beta diversity) change over time. Sig 0.05 *, 0.001 **. And contrast tests indicating which sample period had higher beta diversity.

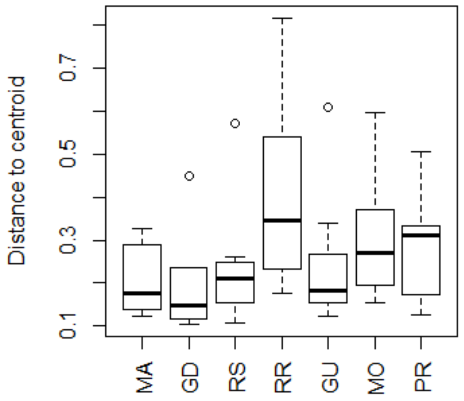
Distance	Bray-Curtis		Chao	
River	Beta	Contrast	Beta	Contrast
MA	*	S13>A13	*	2013>2014
GD	ns	-	**	S14>A14
RS	ns	-	*	A14>S14
RR	ns	-	ns	-
GU	ns	-	ns	-
MO	ns	-	ns	-
PR	ns	-	ns	-

.

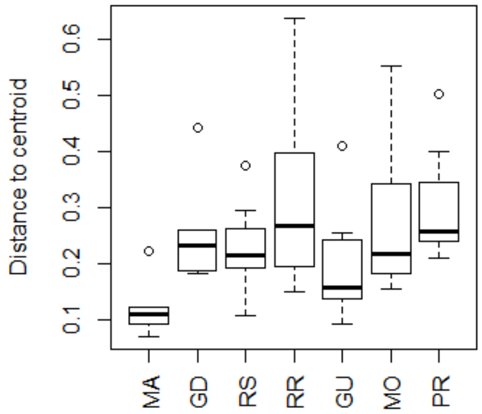
a) p=0.163



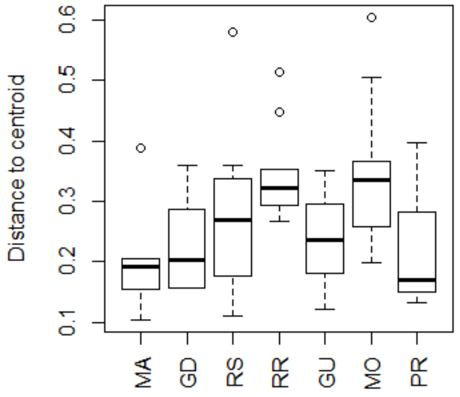
b) p=0.073



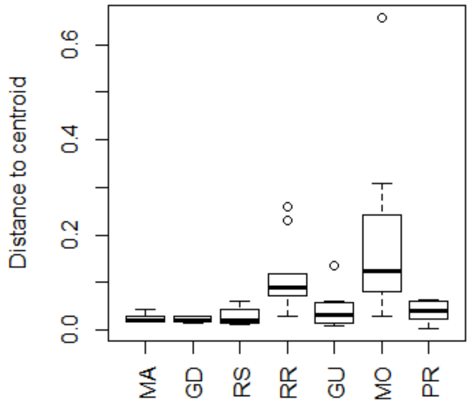
c) p=0.009



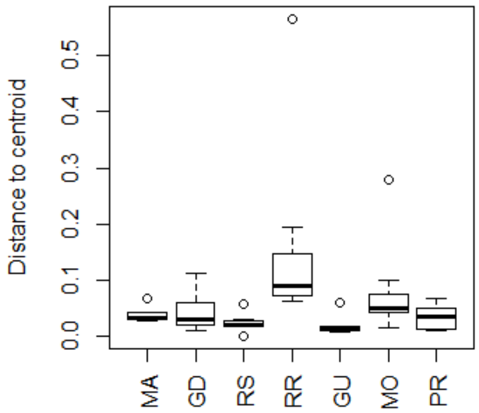
d) p=0.016

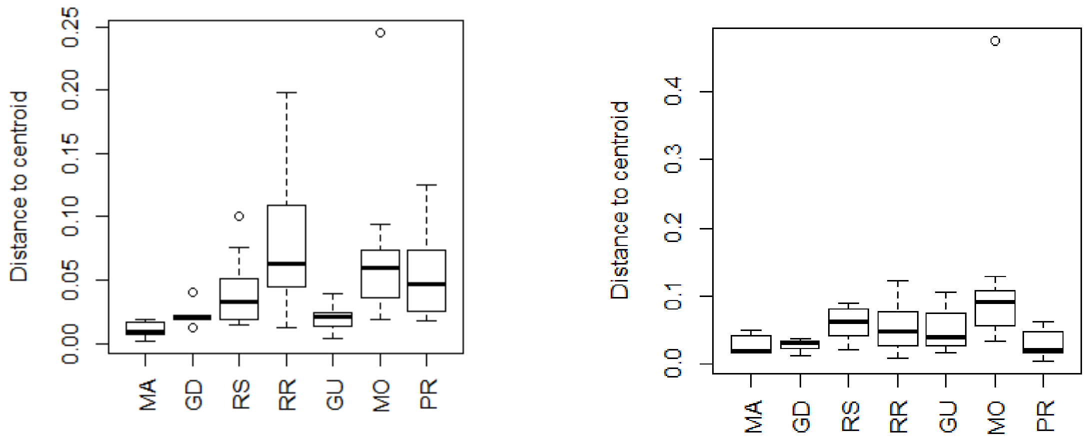


e) p=0.001



f) p=0.001




g) p=0.002

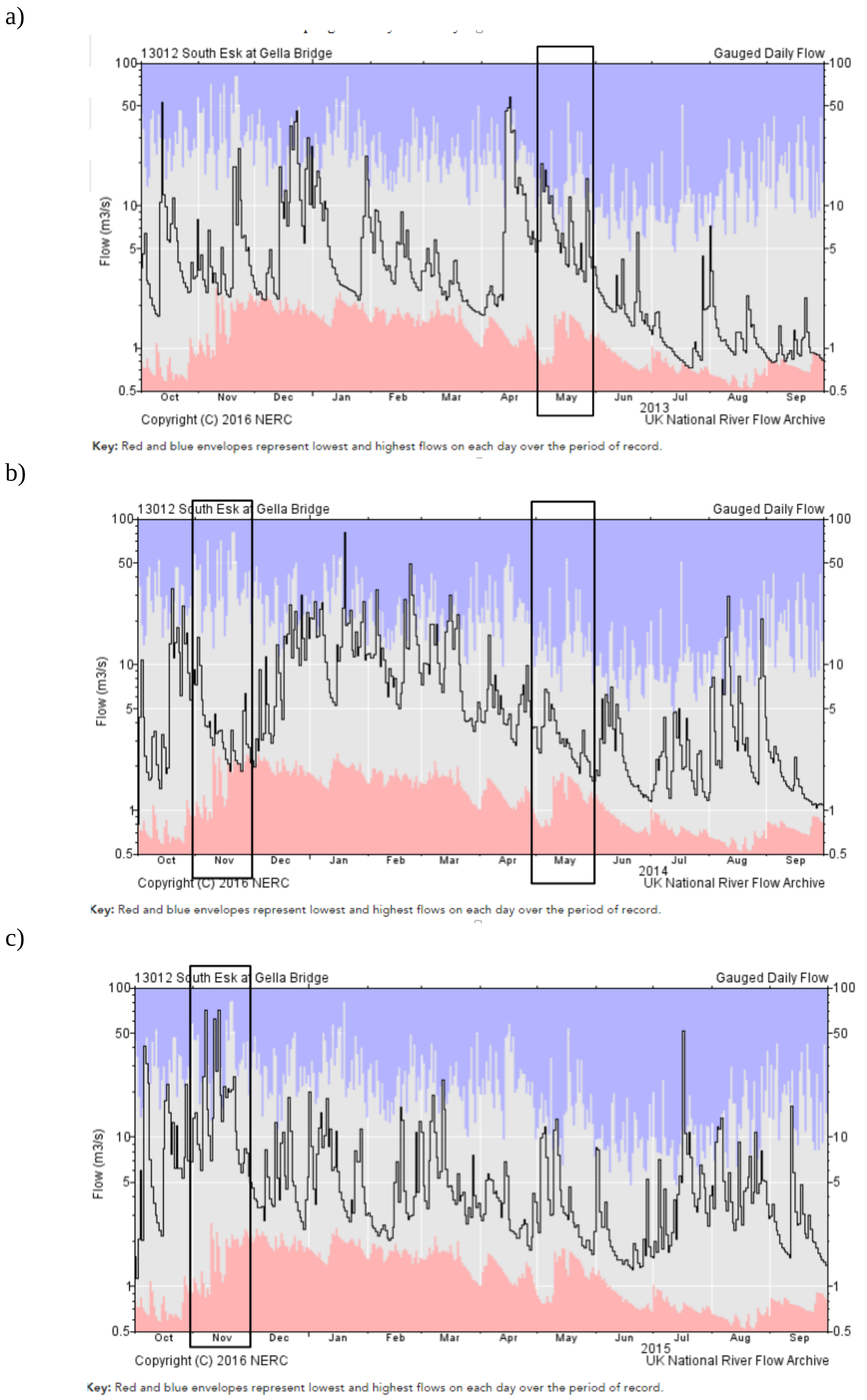
h) p=0.016

Fig 3.14 Median distance to group centroid of community (beta diversity). Result of the significance of river identity (p value), using a) Bray Curtis distance in Spring 2013, b) Spring 2014, c) Autumn 2013, d) Autumn 2014, and e) Chao distance in Spring 2013, f) Spring 2014, g) Autumn 2013, h) Autumn 2014.

3.4.3.3 Beta diversity partitions of nestedness and turnover

Species turnover refers to the replacement of one species by another from one sample to the next. Nestedness is the loss of species from one sample to another, resulting in the second sample consisting of a subset of the first. The relative contributions of abundance based "nestedness" and "turnover" to the Bray-Curtis dissimilarity for each river and sampling period are shown below the mean value in Fig 3.16 a-d. Turnover tended to have a higher contribution in natural channels, however this was river specific and changed between sampling periods. There was a strong temporal pattern in beta diversity across the rivers, which prompted an inspection of the antecedent flow regime for the South Esk catchment. Over the time period of the study the flow regime highlights significant differences between spring and autumn flow conditions from one year to the next (Fig 3.15). Spring sampling in 2013 followed a significant period of higher rainfall compared to 2014 and could be considered to have experienced high flow stress. Conversely autumn sampling in 2013 followed a relatively dry period compared to the following year, experiencing low flow stress. This high flow pattern corresponds to patterns in beta diversity where spring 2013 and autumn 2014 (wetter periods) were dominated by nestedness, or the component contributed equally. However in spring 2014 and autumn 2013 (dry periods) for certain rivers (MO, PR and MA), beta diversity was dominated by turnover.

In natural channels a greater contribution from turnover to overall beta diversity was seen consistently in MO, and occasionally in PR. In straight rivers, nestedness dominated and this trend did not change with time period. However in dry periods in either spring or autumn, turnover could contribute much more (MA in Fig 3.16b, and GD in Fig 3.16c). Despite having different planforms, both Glenmoye channels shared similar beta diversity patterns to neighbouring channels rather than to channels of the same type. In RR 2014 samples showed contribution patterns more like those in natural channels. The steady increase in turnover contribution over time was the most dominant trend. Beta diversity components in RS were most often in the similar to straight channels, showing a consistent seasonal pattern of higher nestedness in spring.



Key: Red and blue envelopes represent lowest and highest flows on each day over the period of record.

Key: Red and blue envelopes represent lowest and highest flows on each day over the period of record.

Key: Red and blue envelopes represent lowest and highest flows on each day over the period of record.

Fig 3.15. Daily gauged flow data from Gella Bridge (Fig 2.2a GS) for the duration of the project. May 2013-November 2014. A) October 2012- 2013, b) October 2013 - 2014, and c) October 2014-2015. Sampling windows outlined in black box.

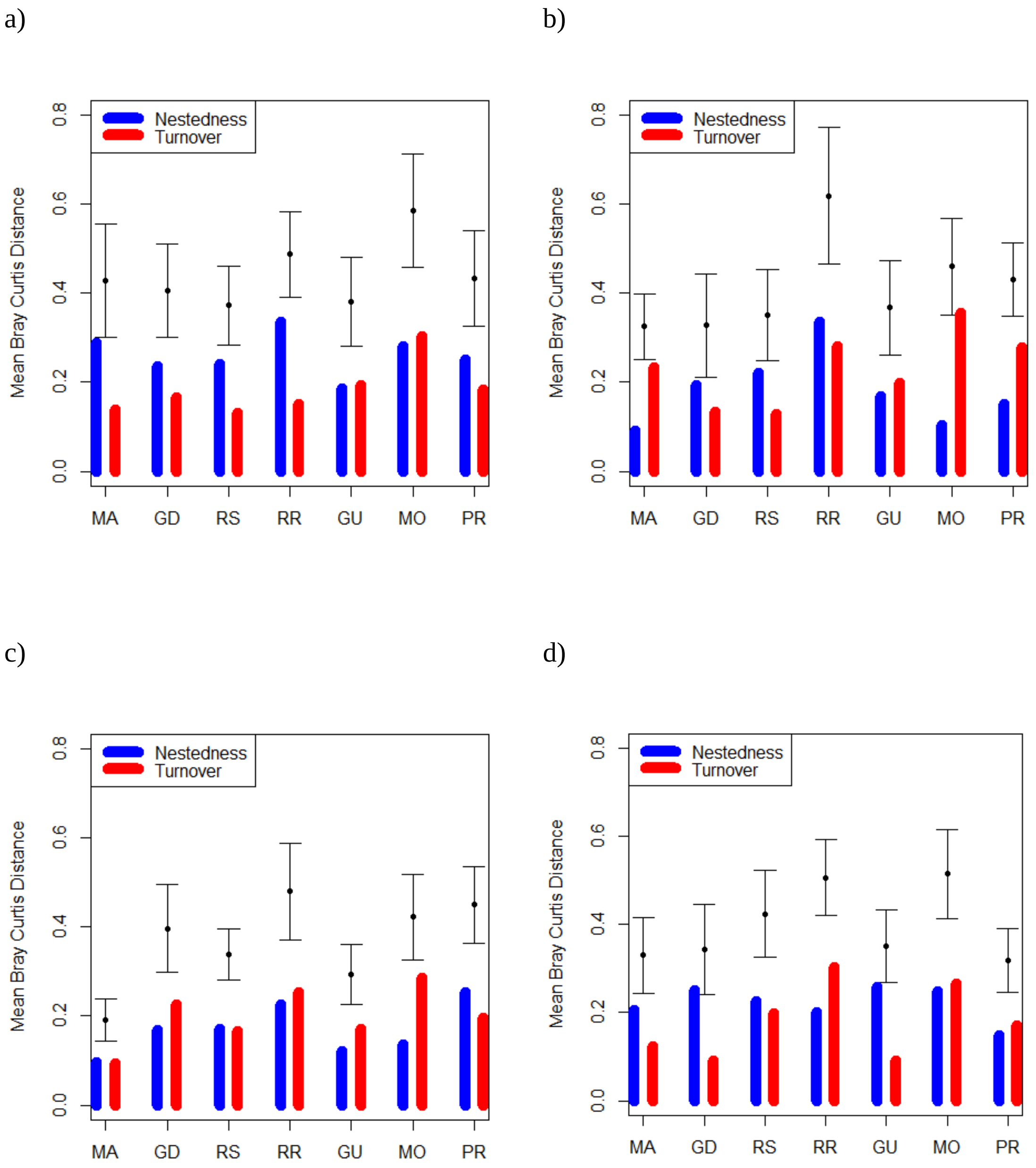


Fig 3.16 Within sampling group mean Bray-Curtis dissimilarity and standard error (dot and whisker), and the partition of the dissimilarity into nestedness and turnover components for the 7 sample rivers for a) Spring 13 - *wet*, b) Spring 14 – *dry*, c) Autumn 13 -*dry*, and d) Autumn 14 *wet*

3.4.3.4 Beta diversity and habitat heterogeneity relationship

The relationship between habitat heterogeneity and beta diversity for all rivers and sample periods is shown in figure 3.17. Rivers that plot above the regression line indicate lower than average riverfly beta diversity for the given habitat heterogeneity, and vice versa if below. Generally, the natural channel types had both higher habitat and beta diversity, conversely for straight channels these were both low or no relationship was evident. Occasional differences in patterns between distance measure (Chao or Bray Curtis) indicate whether the diversity had an important rare species component. Temporal and seasonal variability can be seen in the habitat heterogeneity beta diversity relationships, which may be related to antecedent flow conditions. In autumn 2013 and spring 2014, both dry occasions, most rivers plot close to the regression line, indicating a strong adherence to the linear relationship. In comparison during the other wetter sampling periods rivers were more distant from the line, suggesting a weaker adherence.

Natural channels frequently have both high habitat heterogeneity and beta diversity for both distance measures and seasons, and so position in the upper or middle ranges of the plot (Fig 3.17). MO consistently exhibited high diversity for both habitat and riverfly community, but PR showed limited habitat diversity. In autumn 2014 MO, the relationship changed depending on the distance measure used (Fig 3.17 e+f), indicating limited beta diversity when using the Chao distance. Straight channels consistently lie at the lower range of both habitat and beta diversity, however with some exceptions. In autumn 2013 MA had relatively high beta diversity for a limited habitat heterogeneity, this is shown only for Bray Curtis distance indicating its diversity was driven by the distribution of common or abundant species. The restored RR had consistently high habitat heterogeneity and beta diversity, resembling most closely the patterns exhibited by the natural MO. RS sites conversely had low values similar to the other straight channels.

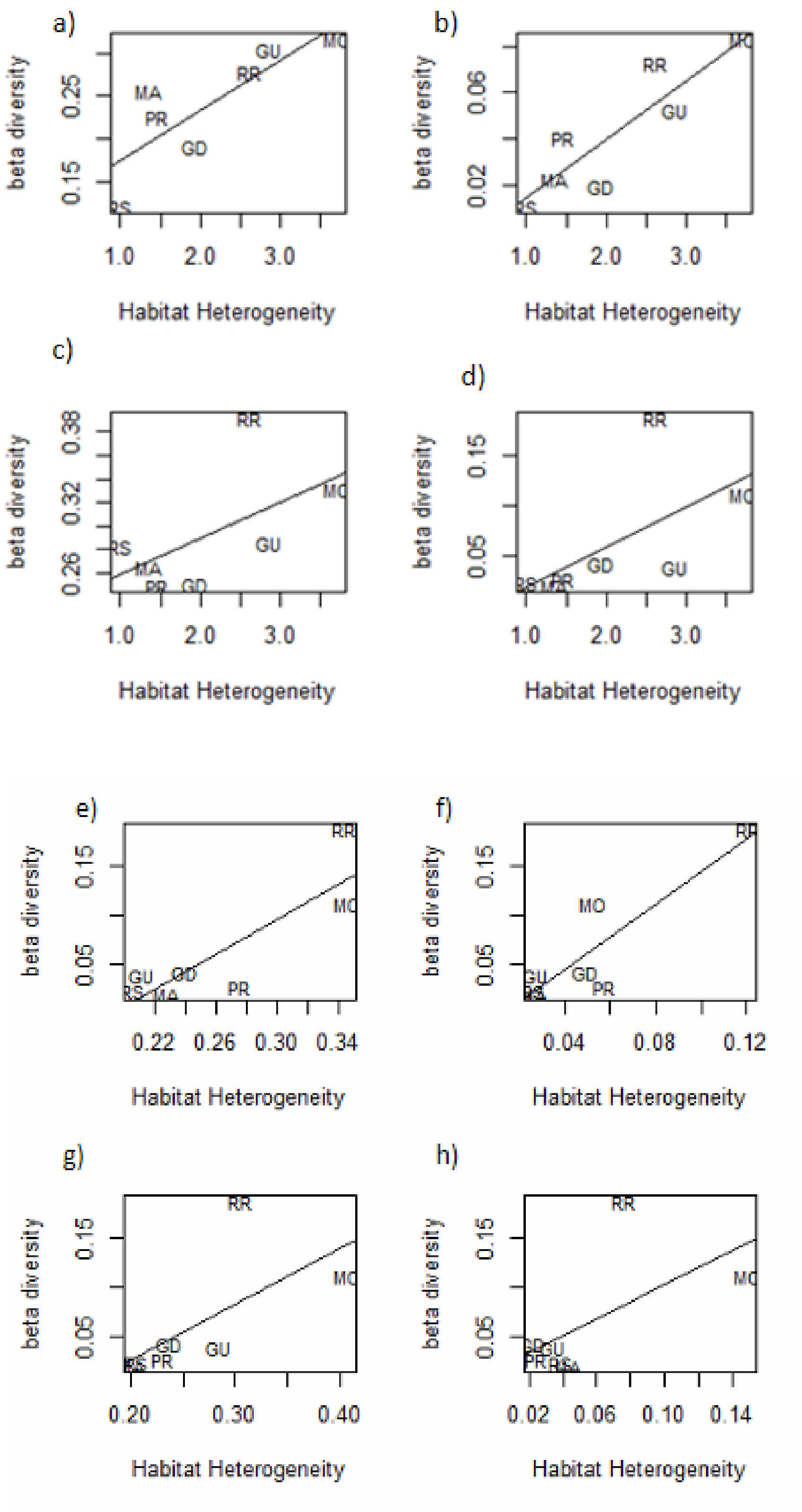


Fig 3.17 Habitat heterogeneity (mean distance to group centroid of PCA) plotted against Beta diversity (Median distance to group centroid PCoA using Bray Curtis and Chao dissimilarities). Line is a linear model based on the 7 data points in the sampling period. a) A 2013 Bray, b A 2013 Chao, c) S 2013 Bray, d) S 2013 Chao, e) A 2014 Bray, f) A 2014 Chao g) S 2014 Bray, h) S 2014 Chao. (S -Spring, A – Autumn).

3.4.4 Drivers of community composition

The regional gradient of riverfly assemblage and response to local environment was expanded beyond those found in the Rottal (Chapter 2) by including more rivers. This is indicated by samples extending the plot area of the constrained ordination (RDA) shown in Fig 3.18 and Fig 3.19. The extension beyond the community of the Rottal channels was provided mostly by natural channels MO, PR and GU, and also MA, whereas GD community responded similarly to those in the Rottal. Models using the environmental variables explained between 19-32% of variation in community data (Table 3.6). The strongest response of riverfly composition to environmental gradients was found in autumn 2013.

Many variables were significant in both seasons and study years. Consistently strong drivers were those related to fine sediments, mean and large sediment sizes, channel features, channel dimensions and degree of modification (Table 3.6). RHS1 consistently drove axis 1 separating natural channel communities from straightened ones. RHS2 structured a gradient of communities within the natural channels, with MO and MA consistently both containing communities corresponding to uniform, featureless channels described by high RHS2 (Fig 3.18a and b, Fig 3.19 a and b). Fine sediment related variables were significant for all sampling periods except the wet autumn in 2014. The role of the specific driver, be it % sands, % fines or interstitial fines, varied for each case. For example, stored fines structured the riverfly community within natural rivers in the dry spring 2014, however for samples from the wet spring in 2013, stored fines described the community change between different rivers, and it was the mean sediment size and boulder cover that structured communities within rivers. Certain sample communities in RS, GD and RR were specifically associated with higher amounts of interstitial fines. PCA2 also described fine sediment properties and was a significant driver of riverfly community composition during the low flow stress sampling periods. Percentage embedded particles was consistently a significant gradient in autumn.

Table 3.6 Summary of significant environmental variables (with plotting code used in Fig 3.18-19) selected by RDA for each sampling period, global and reduced model R^2.

Sampling period	Spring 2013	Spring 2014	Autumn 2013	Autumn 2014
Global model R^2	29.4	22.8	32.5	23.9
Mean Pebble (mean.peb)	X			
Percentage embedded particles (% Embed)			X	X
Percentage boulder (%.BO)	X		X	
Percentage particles < 2mm (% Fines)	X			
Interstitial fine sediments (Fines.stor)	X	X	X	
Percentage sand (% SA)	X			
Width	X	X	X	
RHS 1	X	X	X	X
RHS 2	X	X	X	
PCA 2		X	X	
Reduced model R^2	30	23.7	32.5	19.2

Natural rivers and restored communities covered a larger plot area compared to other straightened types (Fig 3.19 and 3.19), indicating a stronger and longer environmentally driven gradient of community assembly within each river. On most sampling occasions, natural rivers overlapped minimally on one of the axes, indicating a strong role of river identity in driving the community. However, straight MA and GD shared ordination space with MA and RR, illustrating similarities between types in community response to habitat. Community assembly in straight channels was driven by the same gradients as natural ones, however these variables appeared to drive change in communities between rivers rather than between samples within rivers. Communities in restored channels appeared similar to straight rivers (GD and RS) however, community assembly responded to a long environmental gradient. Control (RS) community variation explained by environmental drivers was small, but to the same extent as for other straight channels.

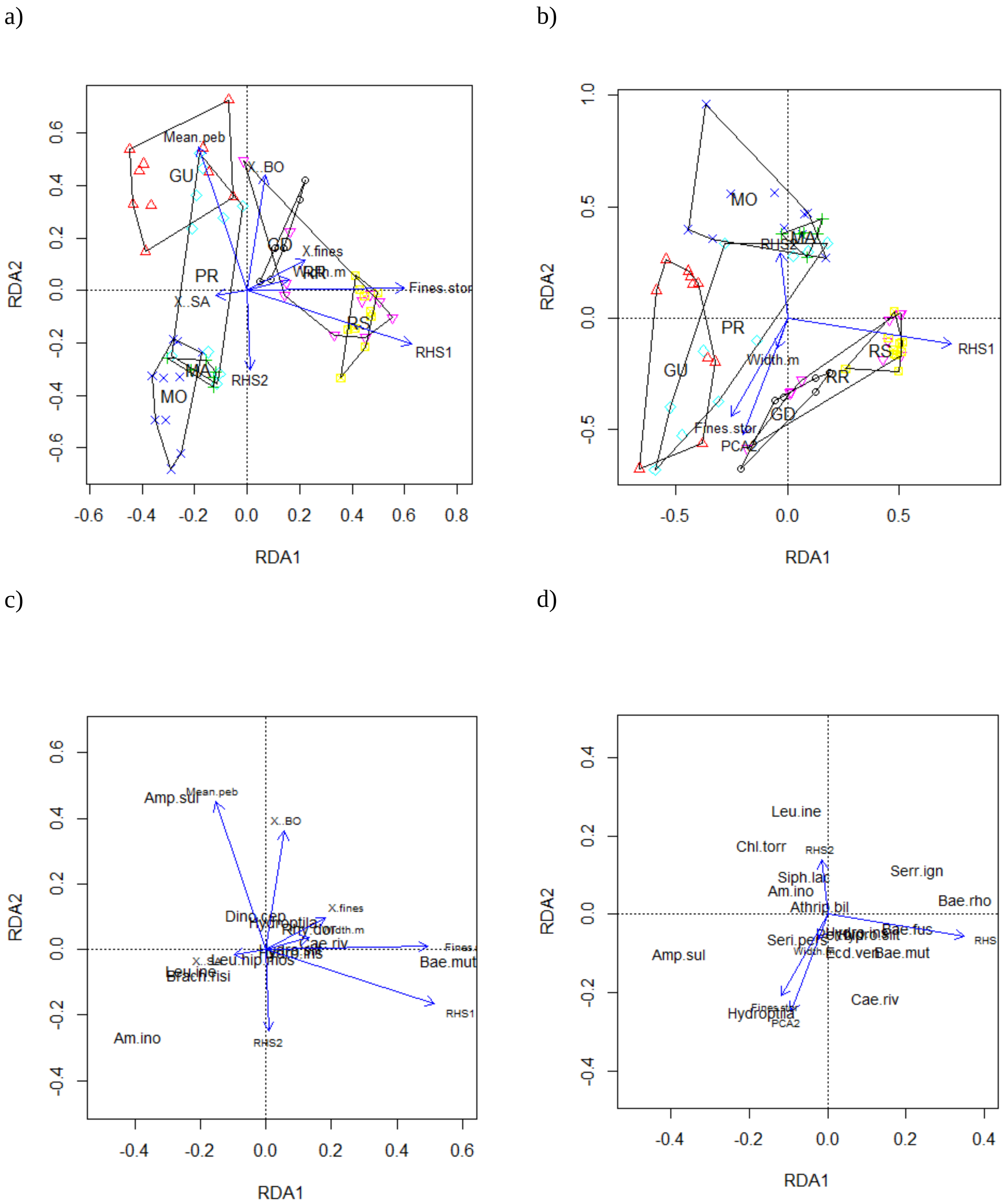


Fig 3.18 Biplots of RDA results showing species and sample locations, and significant environmental gradients for Spring samples in a) 2013 samples and rivers outlined b) 2014 samples and rivers outlined, and c) 2013 species explained by RDA over 35% and d) 2014 species explained by RDA over 30%. A key to species abbreviations is given in Appendix 2.4.

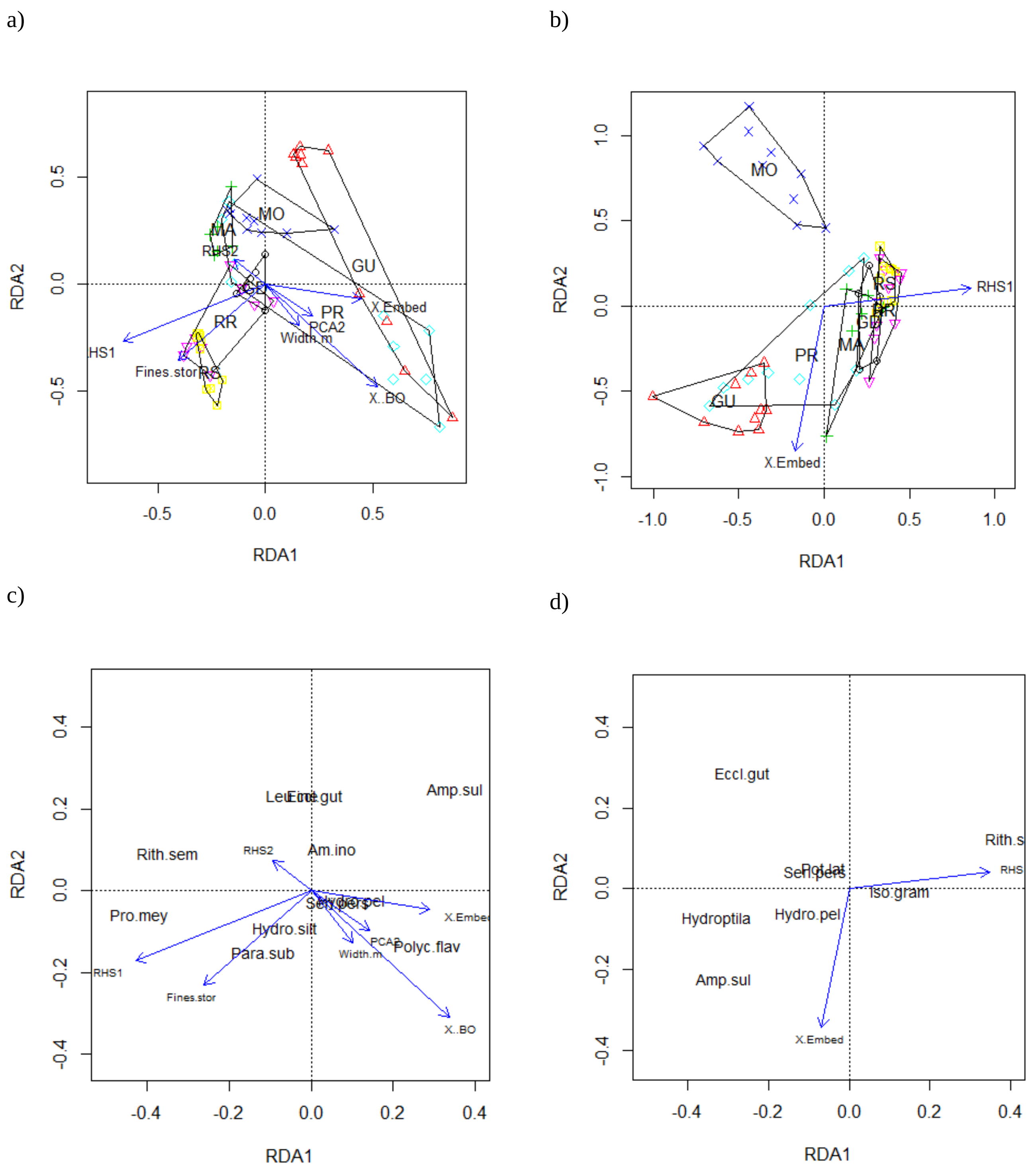


Fig 3.19 Biplots of RDA results showing species and sample locations, and significant environmental gradients for Autumn samples in a) 2013 samples and rivers outlined b) 2014 samples and rivers outlined, and c) 2013 species explained by RDA over 35% and d) 2014 species explained by RDA over 25%. A key to species abbreviations is given in Appendix 2.4.

3.4.5 Modelling biodiversity

3.4.5.1. Wider catchment biotic relationships to local environment

There were very few significant relationships between biotic indices and environmental gradients, 106 models had no significant slopes of any river type. In natural channels there were 18 significant model slopes (10 negative and 8 positive), and for straightened channels only 7 (5 negative and 2 positive), these are summarised in Table 3.7. This was a very small number in comparison to the restored channel, where 41 (17 positive and 24 negative) relationships were found (2.4.7). Of the few significant models, these were mostly between spring abundance or richness, and a range of sediment gradients. Rarefied richness was better modelled in autumn, as were diversity indices (Table 3.7).

Table 3.7 Summary of significant models between biodiversity indices and single environmental variables, bold are those that **agree** with restored channel relationships (n=5), underlined = opposite to restored, neither indication = unique relationships. (S – Spring, A - Autumn).

Negative relationships	Positive relationships
Natural n= 10	Natural n= 8
S Abundance ~ % SA	S Abundance ~ Stored fines
S Abundance~ RHS2	**S Abundance ~ D50**
S Richness ~ % Sand	**S Abundance ~ PCA1**
A Rarefied richness~D85	**S Abundance ~ Mean pebble size**
A Rarefied richness ~ % Exposed boulder	S Species density ~ Stored fines
A Rarefied richness ~ Mean pebble size	A Abundance ~ D50
A Rarefied richness ~ PCA2	S Shannon Diversity~ Stored fines
A Shannon Diversity ~ Stored fines	A Simpson Diversity ~ % gravel
A Simpson Diversity~ Stored fines	
S Simpson Diversity ~ Maximum pebble	
Straight n= 5	Straight n =2
A Richness ~ Width	S Richness ~ % Sand
S Shannon Diversity ~ Width	S Richness ~ Stored fines
A Shannon Diversity ~ Maximum pebble size	
A Simpson Diversity ~ D50	

3.4.5.2. Uniqueness of restored channel relationships

In only 5 of the models was there concordance between significant relationships present in the restored channel with relationships found for other channel types. 7 models were actually converse to the relationships found for the restored channel (Table 3.7). Natural channels had more significant biodiversity environment relationships that straight. Many sediment gradients (D50, mean pebble size and PCA1) were positively related to abundance, in agreement with the restored channel. Agreement was also seen for the negative relationship between spring abundance and richness with RHS2 and % sand gradients. The most frequent model disagreement between restored and natural channels however was for diversity responses to stored fines, which differed between types for both Shannon and Simpson diversity, and in both seasons. In straight channels, all negative relationships were unique to that channel type. Of the 2 positive relationships, spring richness ~ % sand, was in opposition to both restored and natural channel relationships. However the relationship of spring richness ~ stored fines, agreed with that of natural channels, but contrasted to restored channel models. There was no concordance between any models found for straight channels with those in restored.

3.5 Discussion

By using a more rigorous study design this assessment has placed the Rottal Burn restoration project in the context of the wider physical and biological landscape. This has confirmed with more confidence some of the conclusions made about the impact of the restoration measures made in the previous chapter, but others have been brought into question.

3.5.1 Quantifying ecological and hydromorphological potential

Sites sampled in this study exhibited a greater range of habitats and riverfly communities than initially considered in the simple upstream-downstream restoration assessment detailed in chapter 2. The gradient of community assembly was successfully extended, by including further reaches to widen the species pool. Community assembly was however, more strongly related to river identity, or location within the catchment rather than habitat quality or artificial channel classification type. The gradient of habitat conditions was extended to a lesser degree, with significant redundancy among rivers. Sediment properties varied more strongly within rivers, and habitat features characterised each channel identity rather than type. Even apparent gross differences in habitat conditions can present a similar substrate environment for invertebrates.

3.5.1.1 Type specific riverfly biodiversity and communities

Rivers in this study were grouped into natural or straightened channel forms. Despite appearing unnatural and degraded in their hydromorphology, samples from straight channels on average exhibited higher values of biodiversity metrics than natural channels. This is converse to expectations, or our perceptions of good "quality" habitat or even the detrimental effects of the presence of embankments or channelization. March Burn could probably be considered the least natural site; straight, narrow, embanked in places, with simple riparian vegetation. However, it had the highest species richness of all sites. These findings question the value of comparing average sample (alpha) diversity across river types to assess habitat quality for a number of reasons. Firstly, richness, or more specifically, species density has a strong reliance on abundance, which can be highly variable and affected by short term disturbances. Secondly, species diversity, richness and abundance values often relate to patch type (Barnes et al. 2013), and so logically increasing heterogeneity of patch types will introduce some low richness patches which will reduce average richness. In this case comparing richness between equivalent habitat types could be more appropriate (Lorenz et al. 2009). Thirdly , it is possible here that diversity indices are responding to channel stability, with higher abundances (and thus richness) being found in the modified but more stable channels (Louhi et al. 2011).

In this study, richness and diversity metrics were not necessarily sensitive to the perceived hydromorphological quality gradient, and this brings into question conclusions based on the recovery of the restored channel relative to the control channel in chapter 2. This raises key issues about assessing habitat quality; firstly that we cannot rely on our perception of environmental quality or judge what conditions are equivalent (Pedersen et al. 2007, Violin et al. 2011) and secondly that we should aim to quantify and measure physical conditions (Brierley et al. 2010). There was also a strong effect of channel identity on community composition; communities of straightened channels were nested within their adjacent natural sections (Rottal Burn and Glenmoye Burn). Although GD was a straight channel compared to GU's more meandering and active planform, there was very little difference in the average riverfly community composition or diversity of the sample assemblages, despite the difference in channel form. Restored channel communities however, showed a departure from controls in community assembly, a positive indication for the success of the restoration project. Nevertheless, the situation for Glenmoye, confirms the strong role that upstream or adjacent channels have on community composition (Brooks et al. 2002), being a major source of colonisers (Hughes 2007), despite the very different physical appearance of the sample reaches.

Many species present across all channel types were indicators of good quality upland streams according to Lorenz et al. (2004). Even the modified March Burn contained *Potamophylax cingulatus*, *Halesus digitatus* and *H. radiatus* which are all indicative of the highest quality. Many of the species were significant as indicators of individual channels, suggesting that distinct communities exist in each river distributed across the catchment, despite the close proximity of the rivers to each other (mostly within 10km).

3.5.1.2 Environmental drivers of catchment biodiversity and community

Overall there were very few significant relationships between biodiversity indices and measured environmental variables, suggesting that environmental filtering had little effect. This is in contrast to a similar study by Schmera et al. (2013), where communities among near pristine upland catchments were strongly driven by local conditions. In this study, more models were significant for natural channel riverfly abundances and diversity, compared to straight channels. This can be attributed to the availability of a longer environmental gradient present in the natural channels, and suggests that the degree of environmental filtering detected can be specific to the range of available environmental conditions. In the absence of an environmental gradient response, other metacommunity processes are likely to drive community composition or diversity. On the few occasions where environmental factors were significant, they mostly related to habitat descriptors RHS2 and the quantities of large or fine sediment.

River Habitat Survey features strongly discriminated between river identity, and maybe this was why RHS variables were highly significant in the models. The particular significance of the fine sediment variable (interstitial or surface deposits) varied with sample period (possibly related to high or low flow stress – see 3.5.2), where the presence or absence of fines, indicated either habitable or flow stressed conditions. This indicates a complex relationship, or co-occurrence of species richness with fine sediments, that may not be as simple as an immediate and local use of the sediment resource, or the actions of processes that fine sediment can indicate.

3.5.1.3 Identifying the resources that are limiting

Considering the relatively short gradient of conditions within each channel type, the model results suggest that even small changes in a limiting variable can structure communities when these conditions are approaching a threshold value. Interpreting relationships around threshold values can lead to confusion, since the presence of apparently contradictory relationships between sites may indicate that they lie at different locations along a broader, non-monotonic relationship to the environmental gradient. Alternative methods, such as quantile regression (Lancaster and Belyea 2006), are worth pursuing to uncover these relationships more clearly. Determining which factors are limiting the development of natural or higher quality biotic communities is key to efficient environmental management. Identifying parts of the environmental gradient where there are significant changes in diversity or community identity are a way to do this. The high degree of variability between significant biotic x environment relationships identified here, suggests this will be difficult, and that relationship are likely to be specific to the site and scale of the survey measurements.

3.5.1.4 Habitat heterogeneity and beta diversity

One of the fundamental assumptions of river restoration is that degradation leads to reduced habitat heterogeneity, and in turn, reduced beta diversity. Restoration projects commonly aim to reverse both of these impacts. In this study, channelized rivers had reduced habitat diversity and possessed habitat features that indicated channel stability; presence of bank vegetation, large immobile bed material accumulating larger mosses and high velocity habitats. Stream power was probably high enough to enable active sediment transport but due to the absence of sediment inputs from stable banks there was little opportunity for this. As a result, straight channels were characterised by large and immobile sediments, with few finer particles or associated depositional features. In comparison natural channels consisted of a wider range of bed sediments, however PR and GU contained fewer mobile sediments than Rottal restored and MO, indicating a diversity of conditions within the natural typology. MO represented a higher energy stream lacking bank or channel vegetation and with actively eroding banks. Ordination based on all physical variables showed natural reaches to be more diverse than channelized reaches.

There was a clear relationship between habitat heterogeneity and the biota across the catchment, which was only observable by surveying reach scale beta diversity. The beta diversity relationship to habitat heterogeneity was river, not type specific, as physical diversity varied within the natural channels. The habitat heterogeneity-beta diversity relationship was strongest in natural channels, where the proportion of beta diversity represented by species turnover was also high. This is an appropriate scale and method to assess beta diversity and its relationship habitat heterogeneity, similar to that described by Heino, Melo, and Bini (2015). This is informative way in which to contrast mechanisms of community assembly between stream types, but requires high intensity sampling within multiple discrete reaches to uncover such relationships.

3.5.2 Variability and stability of biodiversity and community metrics

Aggregating samples and sites into logical groupings based on proximity (river) or perceived habitat quality (type) is a commonly used approach to increase the statistical power of data analysis and help develop general rules linking biological response to environmental change. However, this study indicates a strong river specific pattern of environment, habitat and community composition drivers, that can only partly be attributed to artificial groupings. The high amount of context dependence is a problem for planning and management of monitoring resources, but provides insights into the underlying mechanisms driving biological or environmental patterns.

Not only was river identity, more so than perceived naturalness important, but seasonal patterns and rankings between rivers changed over time. A strong inter annual difference in antecedent flow conditions immediately prior to sampling (Fig 3.15) made temporal grouping into year or seasons less valuable. In effect, this resulted in 4 distinct sets of environmental conditions to which the invertebrate community responded, superimposed on the influence of variation in local habitat, and thus negating replication in the study design. The role of flow disturbance on invertebrate communities is briefly discussed followed by an assessment of the temporal stability of community composition and biodiversity measures and their relation to the local environment during the different sample periods.

3.5.2.1 Variation in flow regime

Fluctuation in riverine communities over time is to be expected due to the dynamic physical nature of river systems (Thoms 2006), a large amount of this variation stemming from the annual hydrological regime. In the Cairngorms area, the hydrological regime is strongly influenced by spring snowmelt, with discharge during the rest of the year reflecting precipitation and highly responsive overland flow due to extensive impervious geology (Soulsby et al. 2001).

The patterns of biological instability observed in this study approximates one of short periods of high flow stress or stability prior to sampling. Although not quantified directly, interpretation of flow data and consistent responses among rivers in each time period suggests this explanation has merit. Consistently rivers show highest riverfly abundances, richness and diversity occurring after a short period of stable conditions in spring 2014 or autumn 2013. This resulted in a reversal of seasonal patterns in biodiversity indices from 2013-2014, over-riding any more general seasonal patterns. To uncover true seasonal patterns would require longer term data, since there was a lack of strict seasonal replication.

Flow variation is recognised as one of the main factors structuring stream invertebrate communities (Clausen and Biggs 1997). The effects of high flow stress can be locally catastrophic, although over wider scales and longer time periods stream invertebrate communities are generally resilient (Gibbins et al. 2001). For example, Gibbins et al. (2010) observed a rapid and dramatic loss of mayflies from patches of fine and relatively unstable sediment during periods of increased discharge. Greenwood & Booker (2015) too, found aquatic invertebrate diversity, abundance and many aspects of community composition to be affected by time since flood, though community response to flood events was also variable between sites.

3.5.2.2 Biodiversity stability

Significant changes in biotic metrics occurred from one year to another, with a consistent direction of change within each season for most of the rivers studied. This highlights the intrinsic instability of even undisturbed sites in the short term, against which to compare local restoration projects. Beta diversity was more stable over time, however straight channels were most vulnerable to temporal fluctuations. This can be attributed to the lack of channel diversity and absence of sheltered low flow areas during high flow events, which thus constrains beta diversity, in addition to a lack of development of diverse habitat patches during stable periods. Change in beta diversity over time in straight channels was strongest using the Chao distance, suggesting that most species differences were from rarer, opportunistic species regularly arriving in samples, but not persisting though periods of high flow stress. Whether these rare species will persist longer and could be consisted permanent members of the community can only be determined through longer term monitoring. The regular arrival of new species indicates high connectivity with potential species from the regional species pool, a positive indication that a diverse community would be able to colonise newly created or alternative habitat if created. Richness and diversity patterns between rivers and over time revealed how the different channel features and short term disturbance interact to produce diversity patterns; the dynamic aspect of such patterns is often neglected.

3.5.2.3 Community stability

Greater differentiation between riverfly communities occured in spring, with more overlap of river community identity in autumn. This finding is in contrast to chapter 2, which found that Rottal restored and control communities differentiated most in autumn (2.4.2). However, it is in agreement with community patterns in a nearby area studied by Soulsby et al. (2001), and is consistent with the general principle that stream communities in autumn are more similar as they are made up of those adapted to the increased sediment transport and erosion processes that typically occur in that season (Wood et al. 2016).

Average community change over time was small compared to season and river differences, indicating either strong persistence within river or high rates of species replacement from nearby sites. Considering the high dispersal ability of riverfly adults within the range of this study, this is not surprising. Only natural channels had significant between-year differences in spring communities; this was not always correlated to an in-channel environmental change, but was occasionally due to changes in the amounts of fines sediments, so could be direct consequence of flow disturbance. However, direct environmental models of community change were highly variable over time, and lacked any clear seasonal or disturbance pattern. An approach using functional measures, linked to taxa identity may imply more stability over time (Bady et al. 2005, Culp et al. 2011), as well as better describing the mechanisms for assemblage stability. The limitation of biological sampling to the riverfly community may skew an assessment of community persistence, which may show different patterns using alternative orders such as Coleoptera or Diptera which have different lifecycle requirements. Altermatt et al. (2013) even advocate for EPT taxa to be considered separately due to unique response to environmental factors shown by each order.

3.5.2.4 Habitat heterogeneity-biodiversity relationships

The proportion of beta diversity caused by turnover of species from one site to the next varied over the sample periods. Turnover increased in certain channels when there were drier stable antecedent conditions, regardless of the sampling season. This was true for rivers in all channel types. Patterns in the natural and restored channels can be explained by relatively high habitat and flow diversity, allowing patch differentiation and sorting of invertebrates into discrete patches, rather than being homogenised by flow through a uniform channel.

As a result of the community response to disturbance, during low flow stress periods the habitat heterogeneity beta diversity relationship was stronger and more stable along the diversity gradient, compared to wetter more disturbed periods. During periods of high flow stress, natural channels had lower than expected beta diversity for the given habitat heterogeneity, indicating that factors other than the environmental heterogeneity were limiting beta diversity. Habitat heterogeneity-beta diversity patterns appear temporally variable and affected by disturbance regime, in agreement with Heino et al. (2013), who conclude that an absence of the habitat heterogeneity - beta diversity relationship might be related to recurring flood disturbances, leading to temporary local extinctions and more random biological patterns. Brown (2007) also shows that severe disturbance events can constitute a large scale environmental factor, swamping the influence of local habitat heterogeneity, that would typically promote community stability. This study also illustrates how the availability of habitat heterogeneity at certain sites, restored and natural, reduces the disturbance effects of the high flow stress, suggesting an interaction between habitat heterogeneity and flow disturbance, increasing threshold below which the community is maintained. This agrees with the conclusions of Brown (2003), that maximizing heterogeneity will promote temporally stable and diverse communities, a situation which appears to have now been achieved in the restoration of the Rottal Burn.

3.5.3 Assessing restoration in a wider context.

A large number of studies evaluating hydromorphological restoration use upstream - downstream site pairs, or a space for time substitution sampling design. This is understandable given the limited resources often devoted to monitoring. This study shows that river identity, not channel type was a strong driver of habitat availability, sediment composition, riverfly community composition and biodiversity. The fact that upstream-downstream sites were more similar in their community than to channels of the same type is a weakness of this approach and emphasises the need for setting multiple criteria for selecting control sites (Smiley Jr. et al. 2009). Admittedly, channels used in this case were very close, and sites separated by a greater length could be less similar. However, in highlighting the importance of river identity the results emphasise that the choice of river to act as the control site is likely to have an important bearing on whether a restoration is judged successful.

3.5.3.1 Appearance of the restored and control channels

Both the restored and control channels display an atypical response in biodiversity indices for one of the four sampling periods compared to surrounding channels. For certain sample periods the control channels often had the lowest species diversity or richness compared to the catchment mean. Meanwhile the equivalent measurements in the restored channels were high, serving to inflate the comparative difference between restored and control. However, due to different responses to the varied hydrological conditions experienced in each season, the perceived "quality" of restored relative to control sites varied over time. Restored channel biodiversity values were generally average across the catchment, but increased over time. A longer term assessment is required to appreciate the full trajectory of change at this site and data collection according to the same design has continued in 2015 and 2016.

Seasonal biodiversity patterns that may be present in the restored channel were conflated by the earliest monitoring period (spring 2013) representing a time of rapid adjustment to recent disturbance, and the high flow conditions present prior to sampling. Riverfly richness and diversity increased in the second spring, as was the case for all other channels. Making it difficult to distinguish if this was due to channel recovery, or release from flow stresses prior to sampling. The high flow conditions experienced soon after channel construction, physically aided the restored channel geomorphic processes to initiate, and structures to form. Restored channels take time to recover from the disturbance of the restoration itself (Tullos et al. 2009); the intermediate biological and habitat conditions and high dynamism in comparison to other channels indicates continuing evolution.

Beta diversity in the restored channel was resilient to autumn flow stress. Persistence of beta diversity through high flow periods was a rare property in the catchment shared only by Moulzie. This resilience came from both the type of species and channel structures present, which afforded a diversity of habitats, including areas of low energy that may act as flow refugia. The habitat types present in the restored channel were intermediate to natural and straightened channel types. The natural planform was the result of man-made re -sectioning, and its evolution beyond what was measure in the wider catchment is limited by its location in the landscape (Polvi et al. 2014).

The riverfly communities present in the restored site and biodiversity of samples were more structured by the local environmental conditions. This strong environmental control mechanism present in the restored channel described in 2.5.2, was unique in the catchment among the streams studied. This is possibly a response to disturbance and colonisation rather than a permanent property of a naturally meandering channels, since there were few models significant in other natural channels. Some model significance for natural channel types also suggests this relationship could be driven by the presence of persistent geomorphic processes. Many biodiversity-environment relationships in the restored channel were new to the region. And so it would not have been possible to predict these important drivers by measuring responses at other sites across the catchment as proxies. This demonstrates the importance of manipulation experiments (Downes 2010) and the need to better utilise planned disturbance events, such as restoration or engineering, as natural experiments.

Control channel richness and diversity was dissimilar from other straightened channels, although this does not necessarily imply that the control site fails to accurately represent "before" conditions. Strong abundance controls on species richness were found in the control channel, highlighting the need to standardise richness measured across comparison sites, especially in an impaired system which may have lower abundance due to its stressed nature. Habitat and environmental conditions present were similar to other examples of straightened channels in the catchment, but the community was a nested subset of species from the restored channel, indicating the interaction of channel structure and channel identity.

3.5.3.2 Space is no substitution for place or time

This study identifies strong site specific patterns of riverfly diversity and community assembly, that vary over time and in unique ways depending on river habitat character. Restricting attention to pairs of sites (Jähnig et al. 2010, Haase et al. 2013, Verdonschot et al. 2015), a chronosequence of post restoration sites (Muotka et al. 2002), degraded control and natural reference site, (Lepori et al. 2005), or even aggregating effects over many projects (Leps et al., 2016) will not be able to quantify the degree of local spatial variability. This strong, site-specific temporal variability also has implications for assessing the effect of restoration age (Haase et al. 2013, Leps et al. 2016). By using only a single sampling period the temporal patterns of biota and their response to physical conditions are overlooked, yet may have a strong bearing on judgements of the degree of project success.

Attempts to improve restoration monitoring, e.g. by including water quality, quantifying hydrological degradation and sampling a wider range of taxa (Paillex et al. 2017), are indeed useful additions. However, limiting to upstream versus downstream comparisons within single time periods of sampling, makes the assumption that biodiversity metrics are stable, within a possibly highly dynamic study area. The ability of the chosen metrics to distinguish “good” and "poor" conditions from background variation is therefore likely to be unpredictable.

This study shows that no single nearby site would have provided a stable benchmark against which to assess the success of restoration over this short time period. However, this was only made evident by studying more than one time period, with replicate streams, and replicate samples within streams, to distinguish treatment patterns from natural temporal and spatial variation. Sampling limitations are occasionally recognised in restoration appraisal reviews (Matthews et al. 2010, Leps et al. 2016). For example, Matthews et al. (2010), acknowledges that the inherent natural variability of invertebrates in space and time is not well captured by standard monitoring techniques. However, more attention is given to wider scale influences such as landuse of the upstream area. Invertebrate response to specific variables is often noisy and therefore regarded with low confidence. Thus, Haase et al. (2013) found invertebrate communities to be different between hydromorphologically restored and unrestored sites, yet it was deemed that this pattern could not be distinguished from the overall patchiness of the riverine community. However their study made no attempt to quantify this variability. Considering the investment of time and resources required to obtain macroinvertebrate data, improved sampling designs that specifically, and thus efficiently, target the scale of changes made to habitat type and heterogeneity by restoration projects, are long overdue.

3.6 Conclusions

When compared with further nearby rivers the restored reach indicated a trajectory toward natural habitat and riverfly community, and was still in a state of change. However, depending on any single comparison to certain rivers, or by using a single season, restoration success would be evaluated differently. A strong influence of river identity was found making upstream downstream pairs more similar to each other than to impact type. This highlights the limits of using paired evaluation studies, without quantification of the natural background variation between sites or over time, from which to distinguish restoration effect. Upstream Rottal control sites in comparison to nearby straightened channel had relatively low riverfly abundance, richness and diversity, which served to inflate differences to restored samples. This bias was lessened by standardising for sampling effort. Some impacted straight channels actually contained diverse communities that indicate good ecological quality, emphasising a weakness of using an impact typology compared to setting multiple criteria for selecting control sites. The maintenance of high beta diversity and strong response of biodiversity metrics to local environmental conditions was a unique feature of the restored channel, even in comparison to natural rivers.

The river community species pool was well extended by including further reaches. A high number of low abundance and infrequent taxa suggest there is a high connectivity and flow of species throughout the region. Additional sediment conditions were not encountered by surveying further sites with high redundancy of samples. Habitat features however were characteristic of river identity and impact type to some extent. There was strong temporal variability of biodiversity metrics across all site sites. Beta diversity was more stable over time, however straight channels were most vulnerable to temporal fluctuations. A suggested explanation for strong temporal variability in metrics was a response to short term antecedent flow conditions. Annual variation in flow negated temporal replication of seasons in the study design, meaning true seasonal patterns would require longer-term monitoring data to evaluate. Riverfly community composition showed expected seasonal difference due to insect lifecycle, unlike diversity, communities were mostly consistent over time.

Local environmental conditions had little role in determining biodiversity indices, this was most evident in straightened channels. Some aspects of natural channel biodiversity were well modelled, but considerably less so than in restored channels. Suggesting that environmental filtering of species by local habitat conditions can only occur if a wide gradient of conditions is present. Riverfly community assembly was better explained by environmental variables compared to biodiversity, and autumn communities most well explained. There was an absence of consistent patterns in models for community composition, but the amount of fine sediments, maximum pebble size and qualitative habitat features were frequently significant. A relationship between habitat heterogeneity and beta diversity was observed across the catchment, facilitated by the nested study design. Beta diversity was higher in natural channels, and turnover of species between samples contributed more than nestedness. The proportion of the turnover contribution was also increased when sampling followed dryer conditions.

In addition to the recommendations for improving restoration monitoring made in chapter 2, further refinements can be made regarding study design. The use of subjectively defined impact typologies was simplistic and misleading. Channel status should be quantified by measuring the physical parameters suspected to be degraded or improved such as sediment dynamics. A multiple BACI, rather than the use of upstream-downstream design is recommended, using control channel selected based on multiple criteria and located set on a different river from impact. This will enable the quantification of natural variability in space and time against which to compare restoration effects.

Chapter 4 - The riverfly assemblage of a small urban stream and its response to flood defence engineering.

4.1 Introduction

4.1.1 The value and importance of urban rivers.

Human communities have historically focussed development around rivers and water resources due to the key services they can provide, from transportation, waste disposal, defence and sources of energy. In Scotland rivers have been significantly modified over the last 200 years (Werritty and Hoey 2004), and presently in lowland areas the encroachment of urban development and transport infrastructure onto farmland has the potential to affect baseline hydrology, geomorphology and water quality (Gilvear et al. 2002). The European Union Water Framework Directive (WFD) (2000/60/EC) requires EU Member States to protect and improve all surface waters, preventing deterioration of aquatic ecosystems, and where possible, restoring them to good ecological quality. In the Scottish river basin district, 30% of river water bodies that are at risk of failing to meet the environmental objectives of the WFD are impacted by morphological change from urban development or historical engineering, with 644km of Scottish rivers affected (SEPA 2007).

4.1.2 Characteristics of urban channels

Changes occurring to streams from urban development have been appreciated for a long time. Wolman (1967, cited in Gurnell, Lee & Souch 2007) observed alterations to patterns of water flow and sediment transport that affect river form and process, consisting of a cycle of aggradation during the construction phase, followed by bed scour and bank erosion once the urban area has been developed. More recently the characteristics of urban streams have been summarised under the concept of the "Urban Stream Syndrome" (USS) (Walsh et al. 2005), that includes changes to river hydrology, geomorphology and water chemistry that ultimately lead to altered stream ecology.

The origin of many of the changes to urban channels comes from the increased area of impervious surface, which increases surface runoff and the frequency and magnitude of high flows, resulting in a characteristically flashy flow regime (Gurnell et al. 2007) . The altered flow regime can directly result in significant changes to channel geomorphology, in addition to direct impacts from construction activities and a constriction of the channel bed and banks using artificial materials, which can themselves accentuate the symptoms of the USS. Historic industrial production can still leave a legacy of contamination by heavy metal pollution, and modern day contaminants of salt, combustion and petroleum products and other organic pollutants (Brown et al. 2009) act to alter water chemistry.

As a result of these significant modifications to the stream ecosystem, biotic communities in urban streams are commonly characterised by reduced species richness and the dominance or presence of more tolerant species (Paul and Meyer 2001, Walsh et al. 2005). Stream biota can be sensitive at even low levels of impervious surface cover (Miltner et al. 2004), and benthic macroinvertebrates often show the highest sensitivity (Brown et al. 2009), although different taxa groups may respond to different stressors uniquely (Wenger et al. 2009). Improvements in the condition of stream invertebrate faunas in urban areas of the UK in recent decades have also been attributed to a reduction of major point pollution sources (Vaughan and Ormerod 2012).

Most urban stream studies contrast urban with rural or forest reference reaches, or consider a gradient of urban impacts (Paul and Meyer 2001), rarely on the properties within the urban channel itself. Many aspects of urban ecological understanding remain weak, for example trophic and other type of species interactions, and subtle or non-lethal effects on taxa in response to urbanisation (Wenger et al. 2009), while even less is known about consequences for ecosystem function (Meyer et al. 2005).

4.1.3. Engineering pressures

The amount of impervious land cover is often used as an indicator of urban stress. This measure shows significant relationships to the symptoms of the USS (Paul and Meyer 2001). However, distinct episodes of environmental disturbance, planned or unplanned, continue to impact on urban stream function after urban development has finished. Periodic construction work is required for flood management or urban infrastructure such as roads and services. Construction work and dredging occurring directly within the channel often require the use of heavy machinery, and explicitly aim to simplify channel morphology to increase conveyance. Despite concerns that gravel extraction from rivers damages the aquatic habitat, few studies have quantified these effects on benthic invertebrates. Rempel & Church, (2009) found the effects of a gravel mining event in a large alluvial river to have a short-lived impact on community composition. A longer lasting effect can be that of reducing habitat heterogeneity, which is also associated with loss of refugia and food sources (Harvey and Wallerstein 2009). Fine sediment mobilisation and transport from within the channel and also generated through associated construction activities, may have wider reaching effects.

Construction activities in Scotland undertaken near or in water courses must be authorised under the Water Environment (Controlled Activities) (Scotland) Regulations 2011 (“CAR”) (SEPA 2011). Good practice states that ecological harm should be minimised by limiting runoff from the site through the employment of structures such as diversion ditches, silt traps or surface drainage protection. Due to the high levels of rainfall in Scotland the risk of pollution of this nature is high (SEPA 2009). More widely, erosion around construction sites is ubiquitous and is identified as one of the most obvious sources of sediment inputs to urban streams (Wenger et al. 2009).

4.1.4. Effects of fine sediments on benthic macroinvertebrates

There has been a great deal of research into the effect of fine sediment loading on benthic macroinvertebrates due to its widespread and damaging nature (Larsen et al. 2011). The major impact on invertebrates is due to the deposition of fines on the river bed (Jones et al. 2012), which can impact directly and indirectly through a number of mechanisms (Fig 4.1).

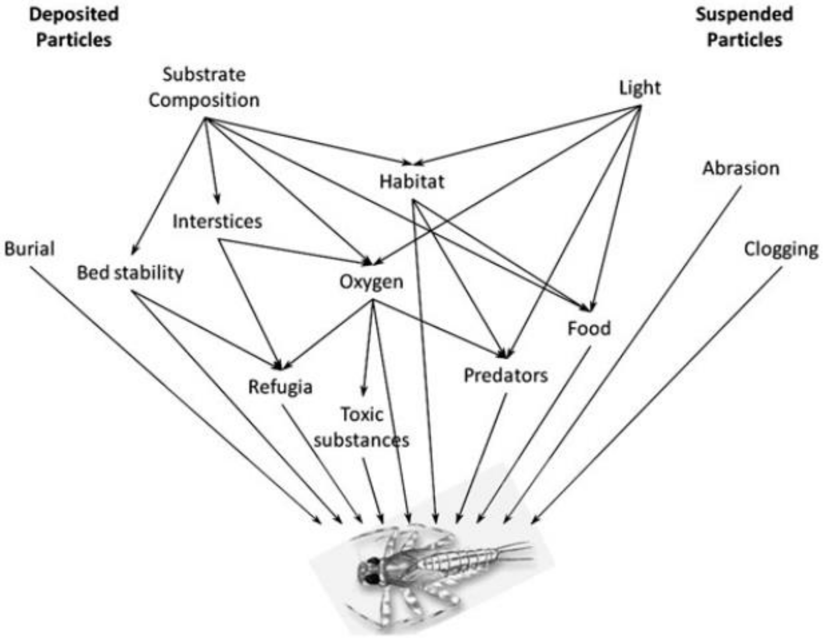


Fig 4.1. Effects of fine sediment, deposited and suspended particles, on benthic macroinvertebrates. Reproduced from (Jones et al. 2012).

Studies have investigated the effects of a number of these impacts on the benthic community, e.g. the instability of smaller and more mobile bed sediments creates unstable habitats dominated by chironomids (Paul and Meyer 2001), while toxic substances (Von Bertrab et al. 2013) bind to organic sediments, and lead to high mortality due to toxicity exposure or ingestion. The amount of fine sediment covering the bed is a common way to quantify sedimentation stress (Glendell et al. 2014) and has been linked to a loss of sensitive species and reductions in abundance. The species that remain are often resistant to the effects of fine sediment stress through traits such as polivoltinism, short life cycles, small body size (Buendia et al. 2013), which makes monitoring changes in these traits a potential biotic metric (Alonso et al. 2013).

Urban stream studies show that fines are actually selectively removed through the process described in the USS of flashy flow and reduced sediment supply from artificial banks, (Finkenbine et al. 2000, Pizzuto et al. 2000), indicating that urban streams may in fact be fine-sediment limited, despite periodic high fine sediment loading. Since many researchers consider taxa in urban channels to be adapted to the high stress conditions of the USS, their response to episodic fine sediments inputs could be variable.

4.1.5 Limitations of existing habitat and diversity monitoring

Harris & Heathwaite (2012) criticise the predominant ecological paradigms used in assessing ecological outcomes emphasising the need to use higher resolution sampling and analysis of both, spatial scales and temporal frequencies to uncover new insights into the complexity of ecological pattern and process. A mismatch between the spatial scales of significant environmental gradients and that of sampling procedures in relation to the target taxa can produce noisy data and weak correlations (Durance et al. 2006, Downes and Reich 2008, Naura et al. 2011). The mesoscale is a common focus of scientific study due to the flexibility to scale up to the catchment scale or down to the microscale. However few field studies consider the microscale, despite the small scale nature of macroinvertebrates and their dependence on conditions at the boundary layer (Harvey and Clifford 2009).

There have been few studies in river ecology or hydrology that consider the river habitat in a spatially explicit manner at the microscale of centimetre or meter. Considerably more studies do partition and survey small patches of hydraulic habitat or biotope, but then remove the spatial context of that patch by pooling invertebrate samples when analysing and interpreting the relationships uncovered (Tickner et al. 2000). Studies that survey substrate specific patches, such as Robson & Chester (1999), Jahnig, Lorenz & Hering, (2009), Milner et al. (2015) commonly find type specific communities. When a microscale approach was taken by Pedersen & Friberg (2007) to record physical and biotic community data, it was revealed that differences in hydraulics and sediment stability resulted in significant differences in macroinvertebrate metrics within similar looking riffles.

In summary, despite a rapidly urbanising world, we know comparatively little about urban stream ecology and need to better understand the mechanisms through which the symptoms of the USS act on stream ecosystems. Periodic in-channel and riparian construction for flood defences has the potential to damage stream biota directly or indirectly though the mobilisation of fine sediments. The possible fine scale alterations to bed heterogeneity and sediment composition on small and short-lived benthic macroinvertebrates would be best studied using a fine scale sampling approach, maintaining a spatial reference and concordance between biological and environmental samples.

4.2 Aims

This study aims to survey an urban river case study, in a way that will sufficiently capture the potentially subtle changes caused by engineering to stream hydrology, geomorphology and ecology, at a scale that is relevant and meaningful to benthic invertebrate communities. By monitoring before and after a planned channel disturbance, this work will address the following research hypotheses.

1. Physical disturbance from engineering and its associated fine sediment release constitute a significant disturbance to channel habitat that can be detected at the fine scale.
2. Urban stream habitat quality and availability is simplified and does not vary within or between study sites and treatments.
3. Urban riverfly communities constitute tolerant taxa that are insensitive to disturbance and so do not exhibit change in biodiversity or community composition after impact.

4.3 Methods

The Brox Burn presents a suitable case study to investigate microhabitat and macroinvertebrate community convergence and their response to human induced disturbance. It is a small, easily accessible stream, with a planned relatively small and localised engineering action occurring during the study period. The action of dredging was predicted to remove small scale bed form heterogeneity, something that can only be assessed using fine scale survey methods. The type of modification is very typical of that imposed generally on urban streams for flood relief purposes.

4.3.1 Geographical setting

The Brox Burn is a small base-rich lowland stream (SEPA 2010) with a 34km^2 catchment in central Scotland (Fig 4.2 a). The upstream land is principally agricultural, a mixture of arable (28%), grassland (33%) and some woodland (12%). Urban landuse accounts for 12% of the catchment (Fig 4.2 b). The river flows eastward through the town of Broxburn finally discharging into the river Almond (Halcrow 2009). Entering the urbanised area at the suburb of Uphall the channel is limited to a narrow riparian margin and backed by residential gardens and occasional fields. Along its route the channel is closely constrained by residential gardens, walls and crossed by a number of foot and road bridges as well as services running under and over the stream (Halcrow 2009).

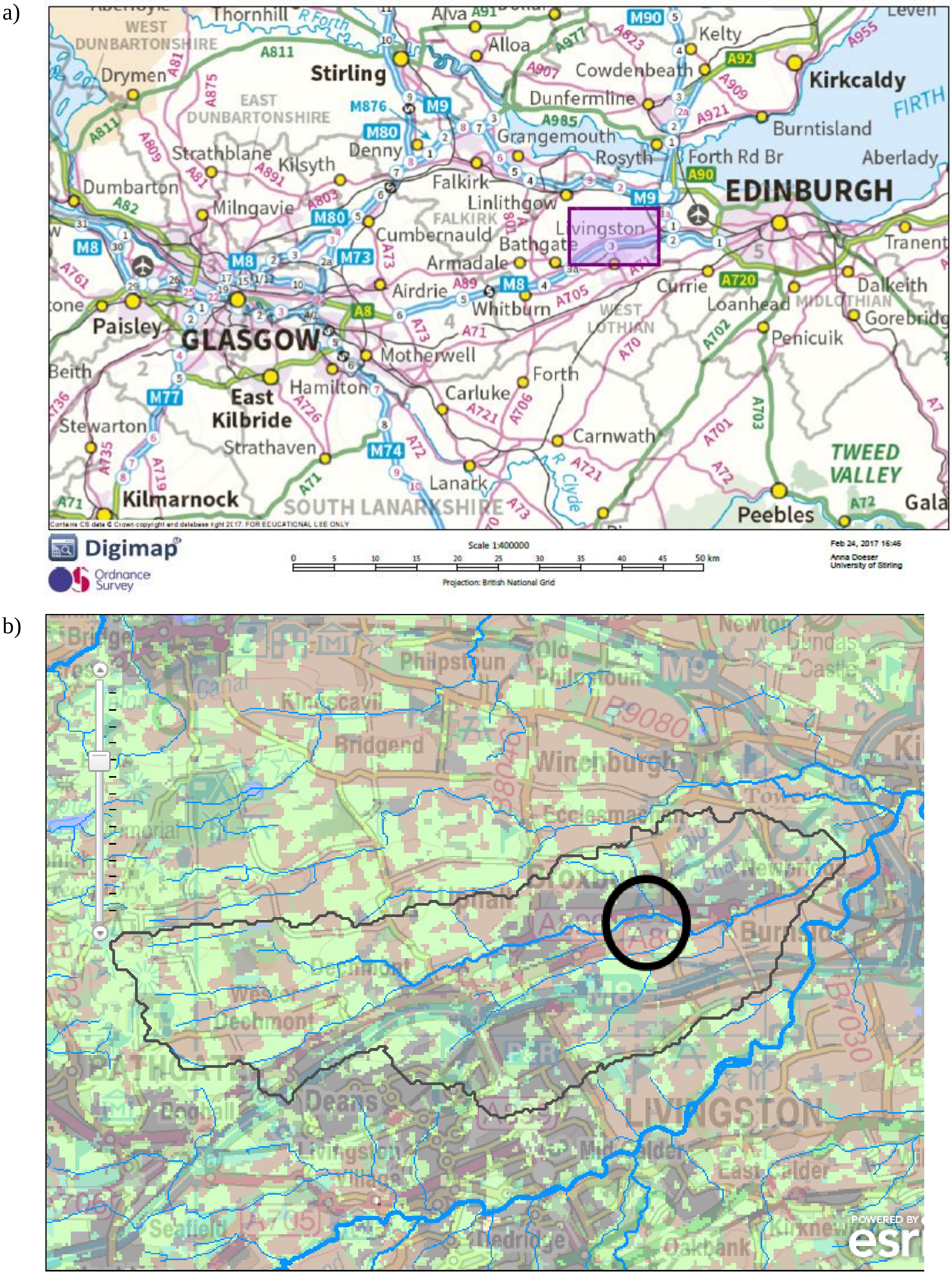


Fig 4.2 a) Brox Burn geographic location within Scotland b) catchment outline with land use. Urban= grey, arable and horticulture = red, woodland and grassland = green (NRFA 2016), position of study sites (circled).

The Brox Burn was classified in 2008 as having poor ecological status, albeit with low confidence (SEPA 2010). Key pressures identified on this water body are barriers to fish migration and point source pollution from manufacturing. Specifically, benthic invertebrates were classed as moderate status with high confidence and morphology was rated good with medium confidence.

The Brox Burn has experienced flooding on a number of occasions in recent years, including a significant event in August 2008, which caused an estimated £2.5 million in damages and clean-up costs (Halcrow 2009). As a result of flood risk analysis, a flood prevention scheme targeting high risk areas was initiated, consisting of the building of flood walls and embankments and dredging to improve conveyance in key areas. As part of the flood prevention scheme the Brox Burn study area was subject to two types of disturbance during the period July 2013 to September 2013. Firstly by direct channel modification through dredging of the channel bed to remove larger sediments and to increase conveyance capacity. This also disturbed an un-dredged area downstream that was drained and used for access. Secondly (and unintentionally), downstream areas were impacted by the transport and deposition of fine sediment mobilised from the upstream bed and banks, during the timeframe of the works (Fig 4.3a and b). Due to the destructive nature of direct dredging, impacts these were not measured at the time of work, but were measured immediately before construction work commenced, and sampling repeated the following year.

Fig 4.3 Photo of a) failed sediment stopping net and b) in channel and riparian engineering work upstream of IMP1.

4.3.2 Study design

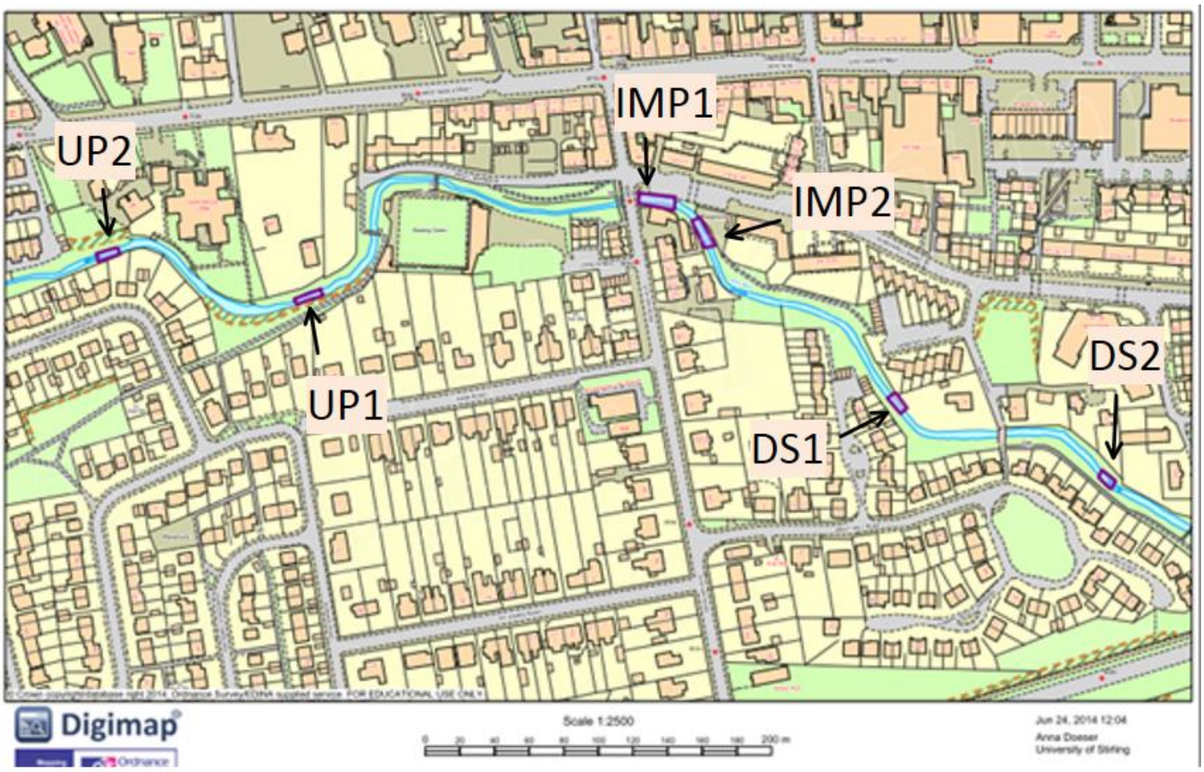


Fig 4.4 Location map of the 6 sampled reaches (purple boxes) and site names (Upstream: UP2, UP1, Impacted: IMP1, IMP2, Downstream: DS1, DS2) on the Brox Burn.

The Brox Burn study site (Fig 4.4) is a small river (3m wide) in an urban setting. The bed is predominantly cobble and rubble. The channel is affected by old flood walls, bridges and embankments and more contemporary gabions, the older stonework structures having contributed coarse substrate to the channel.

Two impacted reaches were selected within the extent of the direct influence of the planned dredging and construction works (IMP1 and IMP2, Fig 4.4, Fig 4.5a and b). IMP1 was a section from the road bridge to approximately 50m downstream, over which the river bed was due to be re-graded from a slope of 0.4 % – 2 % to a more uniform 1 % -1.8 % incline. IMP2, downstream of IMP1, was also drained for access but no sediment was removed. In July and August 2013 and 2014, each of these impact sites were sampled for a length of 20m, however due to the time intensive nature of the survey method, reaches up and downstream were surveyed using a shorter section length of 10m. Two reaches up and downstream of impacted sites were surveyed (UP2, UP1, DS1, DS2, Fig 4.4, 4.5 c-f). Sites were selected based on their similarity to the impact reaches in terms of channel planform, shading, slope and bank material.

Fig 4.5 Site photos of the 6 study reaches: a) and b) Upstream: UP2, UP1, c) and d) Impacted: IMP1, IMP2, e) and f) Downstream: DS1, DS2.

The sampling approach used a high resolution habitat mapping at a scale of 1m by 0.5m grid cells, drawing on previous channel mapping work by Wright et al. (1981), and Pedersen & Friberg (2007). Invertebrates + subsampled from a number of cells within a standardised grid overlay. Habitat variables were recorded at the cell (micro) scale, based on data recorded during the River Habitat Survey (Environment Agency 2003) spot check.

4.3.3 Quantifying fine sediment pollution

Fine sediment deposition was quantified using AstroTurf matting secured on the stream bed by metal pegs and retrieved weekly. Astroturf mats have been used successfully in studies of seed dispersal along river banks (Gurnell et al. 2006, Cockel 2010), and also specifically to record in channel sediment deposition (Von Bertrab et al. 2013). Other methods such as nets, would have been more difficult to deploy in sufficient number and in an urban location are more likely to be subject to human disturbance.

AstroTurf square mats (30cm by 30cm) were deployed in the 2 downstream sites, with the 2 upper most sites acting as controls (Fig 4.6). Impacted sites (IMP1 and 2) were not monitored due to the physical disturbance directly to the channel made by engineering works during this time. The mats were placed in 2 in 'fast' flows and 2 in 'slow' areas, identified visually. Mat coverage was evenly spaced to cover the full width of the channel along the 10m reach. The 4 reaches were sampled each week for 9 weeks (16th August -14th October 2013), replacing the mats each time with newly cut ones or washed mats. Sediment deposition was monitored until a succession of high flows had flushed the fine sediment through the study reaches. Construction work in the early part of the engineering scheme to divert the channel and create an alternative crossing point may have caused considerable sediment disruption, so it was possible that significant fine sediment impacts had also occurred locally prior to surveys commencing.

Fig 4.6 Locating a sediment collection mat on the stream bed.

During mat retrieval an open-ended plastic trug was used to enclose the mat to reduce flow during collection and to minimise the loss of sediment. Individual mats were bagged and labelled to be washed and analysed in the lab. Mats were washed to separate out the sediment, the wash water was poured through a 90 µm sieve and the solid material transferred to a foil tray for drying at 105°C for 24 hours. However, since this was a very time consuming process it was untenable to complete the processing of all mats this way. Consequently 48 of the 143 mats were processed by drying the whole contents of the sample bag and subtracting the average weight of a dry mat (mean = 119g, standard deviation = 9g).

4.3.4 Environmental data collection

The reach was divided into 1m x 0.5m grid cells using a tape measure and quadrat (Fig 4.7a). Working systematically from one side of the channel to the other a number of environmental variables were measured for each cell: depth and flow velocity at 3 locations at 0.6 depth (Fig .4.7b). Habitat features of the surface water flow type, percentage cover of all substrate sizes present, amount of organic matter as the % surface covered with Coarse Particulate Organic Matter (CPOM) or Large Woody Debris (LWD), total % cover of cell by vegetation, % occupied by each vegetation type. The presence of channel features such as exposed large sediments, either cobbles or boulders, and the presence of trash was also noted.

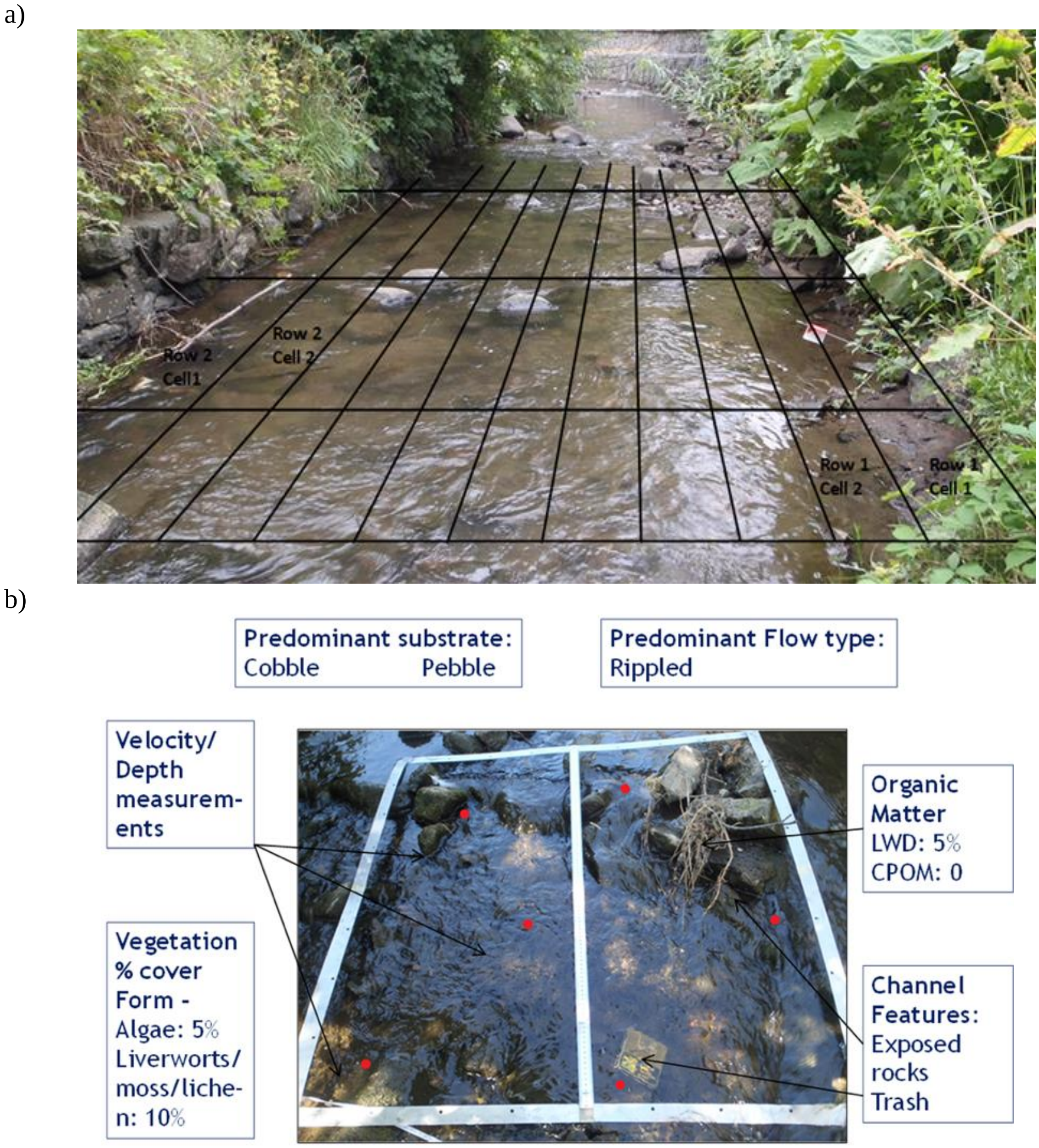


Fig 4.7. a) diagram outlining reach scale survey design, delineating sample cells, b) sampling features within a cell (2 in each quadrat), red dots indicate location systematic sampling of depth and velocity measurements.

4.3.5 Biological data collection

To maintain parity with a standard 3 minute kick sample, the sampling effort for invertebrates was aimed at 3 minutes total sample collection time, to be spread over a 10m long section with 15 second sampling periods in each discrete patch. Alternate cells in 2 adjacent rows were sampled with a break of 3 rows between sampling rows, this typically resulted in 6-7 samples per 5m length (Fig 4.8). This method was followed to ensure good coverage of the whole range of habitat conditions while reducing disturbance to adjoining cells from which samples would be collected in the future. The invertebrate sampling was done by placing 2 kick nets at the downstream end of a cell. The channel bed within the cell was then raked over with a garden hand fork, with all habitats disturbed, lifting cobbles and scraping mosses/algae for 15 seconds. Material from both nets was placed in a labelled bag, with material from discrete cells remaining separated. Invertebrates were preserved using Industrial Methylated Spirits (IMS) and refrigerated until processing. All riverfly individuals were then extracted, identified to species and enumerated.

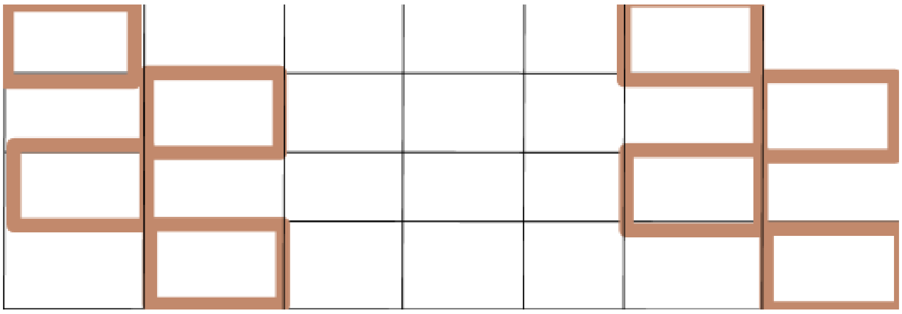


Fig 4.8 Outline of invertebrate sampling scheme, river flows from l-r. Samples taken from cells outlines in brown rectangles, alternate cells for 2 rows with a break of 3 non sampled rows.

4.3.6 Statistical analyses

Describing and comparing habitat

Environmental data were filtered to cells only with complete records for all variables under consideration, resulting in 568 data points in 2013, and 514 in 2014. To determine the significant environmental gradients, firstly describing samples across all sites in the pre-impact periods Principal Components Analysis (PCA) was carried out on scaled variables, using site identity as a supplementary variable. To test the uniqueness of reach habitat conditions across the study area, the significance of reach identity was tested using PERMANOVA, using scaled and centred data and Euclidean distance. Habitat diversity within sites was compared using median distance to reach centroid (the average conditions). Pairwise post hoc tests, with a Bonferroni correction, determined which reaches had significantly different habitat diversity. Change of habitat composition and diversity over time from 2013 to 2014 was assessed using PERMANOVA, testing the effect of year for each reach individually. A PCA within each reach indicated the habitat variables that separate the year groups, and describe habitat change over time.

Comparing biodiversity indices

Within each sampling period differences between reach values for each riverfly biodiversity index; average cell abundance, species richness, Shannon diversity and Simpson diversity, and reach scale species richness, were plotted and tested. Non-parametric Wilcoxon-Mann Whitney tests were used to compare change over time (2 groups). Kruskal -Wallis tests compared between sites and impact groups within each year. Significant results were followed up with post hoc pairwise tests corrected for multiple comparisons. Reach level species richness was calculated as the reach species pool using the Chao estimator (Chao and Chiu 2016), with value confidence intervals calculated by permutation tests. The PSI score,-Proportion of Sediment Sensitive (Extence et al. 2013), is a pressure specific macroinvertebrate Index indicating fine sediment pollution. This score was calculated manually for each sample, using highest resolution (species or family) sensitivity data available. The score is based on the abundance of taxa with different sensitivity scores (1-4) present in the sample. Result is a final score indicating river bed condition from 0 - heavily sedimented to 100 - unsedimented.

Community composition differences and change over time.

Unconstrained ordination by Correspondence Analysis (CA) (Borcard et al. 2011), was used to compare riverfly community assembly between reaches within each year, and also assess changes over time for each reach individually. The significance of the distinctiveness of the reach community was tested between sites using reach identity, or over time using year, as explanatory variables using PERMANOVA. Differences in beta diversity between reaches or changes over time, were assessed by comparing the median sample distance to group centroid (average community) in a PCoA, using untransformed species abundance data and a Bray-Curtis distance matrix. Important riverfly species changes over time identified in CA, were illustrated using contribution biplots, which reduce the apparent domination of plots by rare species (Greenacre 2013). Species showing affinity and fidelity to a particular year, and may indicate normal or impacted conditions within each reach. These were investigated using Indicator Value (IndVal) analysis (Dufrene and Legendre 1997). CA biplots, combined with IndVal results were interpreted to determine species-specific differences between sites and change over time. Riverfly species characteristic of a reach and the reaches contribution to the overall Brox Burn community diversity was described.

All statistical analyses and graphics were produces using the R environment (R Core Team 2016) in R Studio version 0.99.902 (RStudio Team 2015) with the use of the following packages: vegan (Oksanen et al. 2016), Sciplot (Morales and Murdoch 2012), Labdsv (Roberts 2016). FactoMinerR (Le et al. 2008) and ca (Nenadic and Greenacre 2007).

4.4 Results

4.4.1 Quantification of fine sediment deposition

The weekly record of sediment deposition in 2 upstream and 2 downstream sites is shown in Fig 4.9. This plot illustrates that during weeks 4 to 7 (6th September to 7th October 2013) of recording, significant amounts of fine sediment entered the channel below the control sites, were mobilised through the channel, and deposited in downstream reaches resulting in a peak of sediment moving downstream over time. Considered over the sampling period, sediment loading downstream increased by almost an order of magnitude.

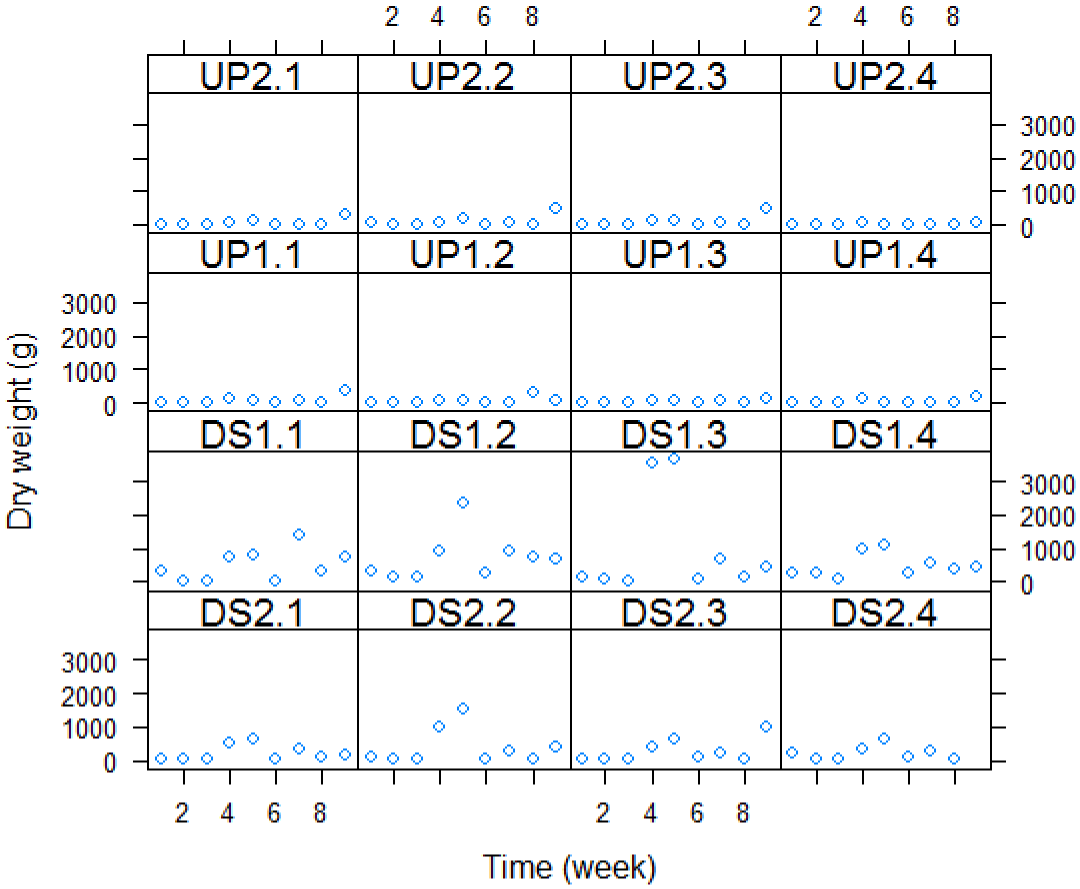


Fig. 4.9 Dry weight (g) of sediment deposited plotted over the 9 weekly time collection periods, separated by each mat location (e.g. DS2.1-2.4 - where 2.1 and 2.4 are high water velocity locations, 2 and 3 slow) and reach from most upstream to most downstream (UP2, UP1, DS1, DS2)

4.4.1.1 *Parts of the stream affected and magnitude of impact*

Even fast flowing parts of the downstream channels experienced an average sediment deposition of 348 g/mat, 3.34kg/m^2 more fine sediment compared to upstream controls (total load~3.9kg/m^2) during the highest sediment stress period (Table 4.1). Slow, depositing areas received on average 591g/mat, 507g more than upstream controls equating to 5.6kg more sediment per m^2 (total load~ 6.6kg/m^2). The impact of altering sediment recording methods, to retain and then subtract the mat weight, would be small considering the mean amount of sediment load over the recording period (Table 4.1) and the standard deviation of mat weight estimates (9g). The sediment delivered was lessened slightly by distance from the source of the disturbance. For instance DS2 samples experienced lower maximum sediment values compared to DS1, indicating that sediment concentrations and likely impacts were diluted downstream.

Table 4.1. Average sediment deposition by channel and velocity group, during the 4-week highest impact period (6th September 2013 to 7th October 2013).

	Mean (g/mat)	Min	Max
US fast	43.0	5.5	176.2
US slow	83.5	6.7	418.3
DS fast	348.6	51.1	918.6
DS slow	591.4	57.9	2137.8

4.4.1.2 Timing and duration of the sediment event

The 4 weeks of greatest sediment impact and general disturbance occurred during the annual low flow period (Fig 4.10). The presence of sedimentation due to disturbance appears to end by week 8 (Fig 4.9), after which it increased across all sites, indicating the start of natural movement of smaller sediment particles within the channel due to seasonally increased flow, rather than an increased supply from the banks of the channel. Peak deposition occurred during weeks 4-7, resulting in a continuous 4-week period of sediment stress.

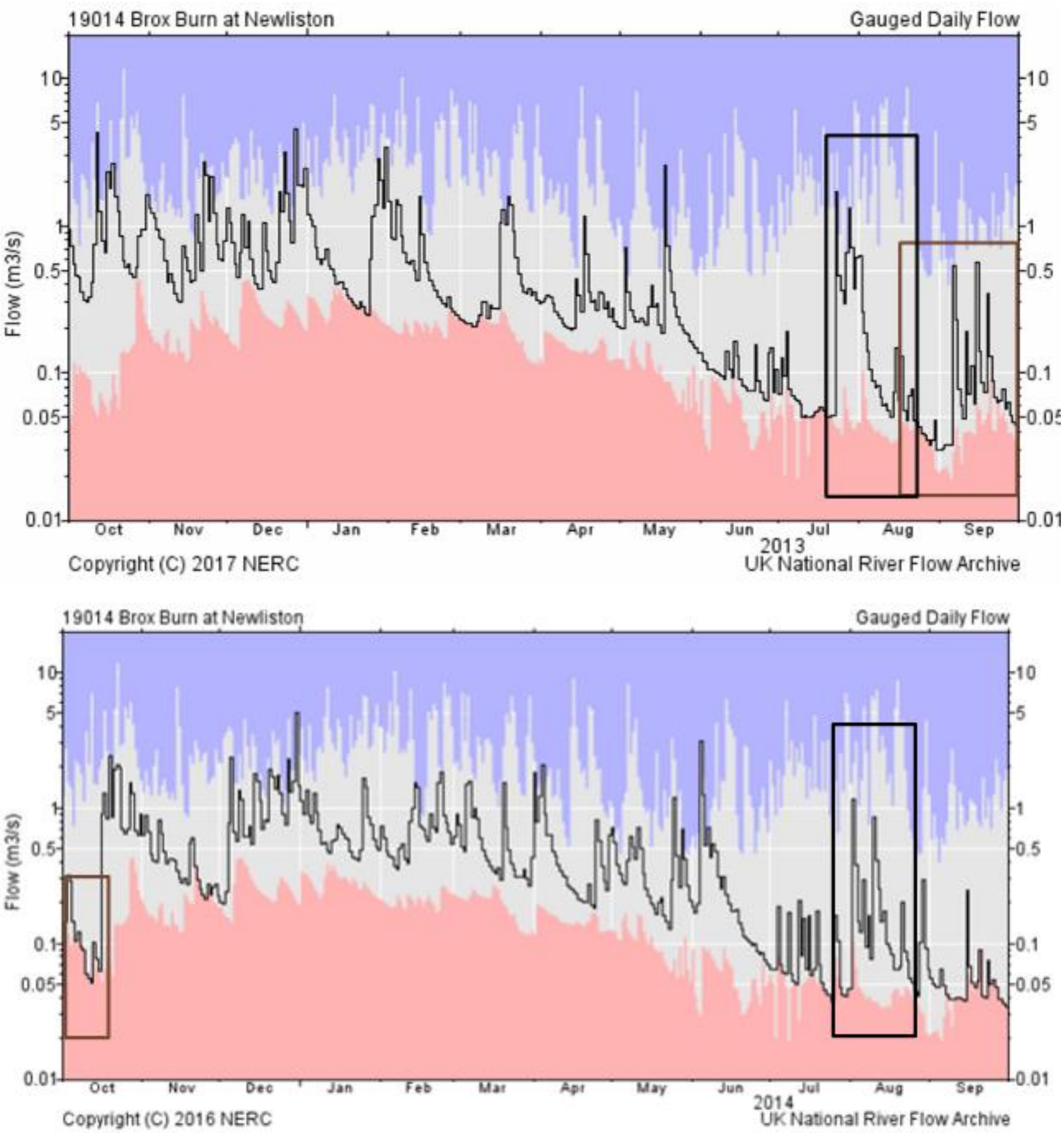


Fig 4.10 Gauged Daily flow at Newliston (m^3/s), a) October 2012-October 2013, and b) October 2013-October 2014. Black box indicates study habitat mapping and invertebrate sampling period, brown box indicated fine sediment sampling period (NRFA 2016).

In summary, the engineering work upstream resulted in a short period of high fine sediment release and deposition in downstream sites. The volume deposited was considerable when viewed in the light of experimental studies and occurred at a sensitive time (low water levels and warm water), and could therefore be expected to exert a detrimental effect on the stream biota. In subsequent analysis the two downstream sites DS1 and DS2, will be considered fine sediment impacted, where DS2 is affected less due to its distance downstream.

4.4.2 Reach habitat characteristics and diversity

4.4.2.1 Habitat gradients and site characteristics

Reaches within the Brox Burn substantially share habitat characteristics (Fig 4.11 and 4.12). Ordination showed that the major environmental gradient (PCA1) in the habitat samples was related to hydrological variables of flow, depth and flow variability (Table 4.2). High scores on A1 were unique to a number of samples in IMP1 and IMP2 (Fig 4.11 and 4.12) describing higher values of flow velocity, depth and variability, compared to the up and downstream sites. The second axis described a gradient of sediment size variables; overall grain size and % Pebble, distinguishing between samples within the reach. Reaches become more similar in habitat conditions in the second year. Upstream and downstream sites, had similar hydraulic conditions, but arrange on a gradient of sediment size, beginning with predominantly small sediment size in UP1, increasing in size to DS1, UP2 and finally DS2. Pairwise permanova tests indicated that in 2013 all sites were significantly different in their habitat composition.

Habitat composition within all reaches changed significantly from 2013-2014, however the amount of variation described by year was very low (R^2 of year = 1.9-6.3). Environmental gradients describing change over time in each reach are summarised in Table 4.3. Post impact, IMP2 sites still had the largest range of sample habitat characteristics, including most sites with higher water velocities and flow ranges. IMP1 however no longer contained samples in the high flow and depth range, and became more similar to other reaches in terms of flow and sediment characteristics due to a loss of sites with larger grain size.

Table 4.2. Summary of the PCA outputs describing habitat gradients between samples in 2013, and 2014. R^2 of each axis, and of reach as an explanatory variable for each axis, and variables that describe axis 1 and 2.

	Axis 1	Axis2
2013	20.83	13.89
reach R^2	16.4%	6.4%
	Flow range Flow standard deviation Flow velocity depth	Pebble Grain size
2014	21.17	14.2
reach R^2	20.1%	7%
	Flow standard deviation Flow range Flow velocity	Pebble Grain size

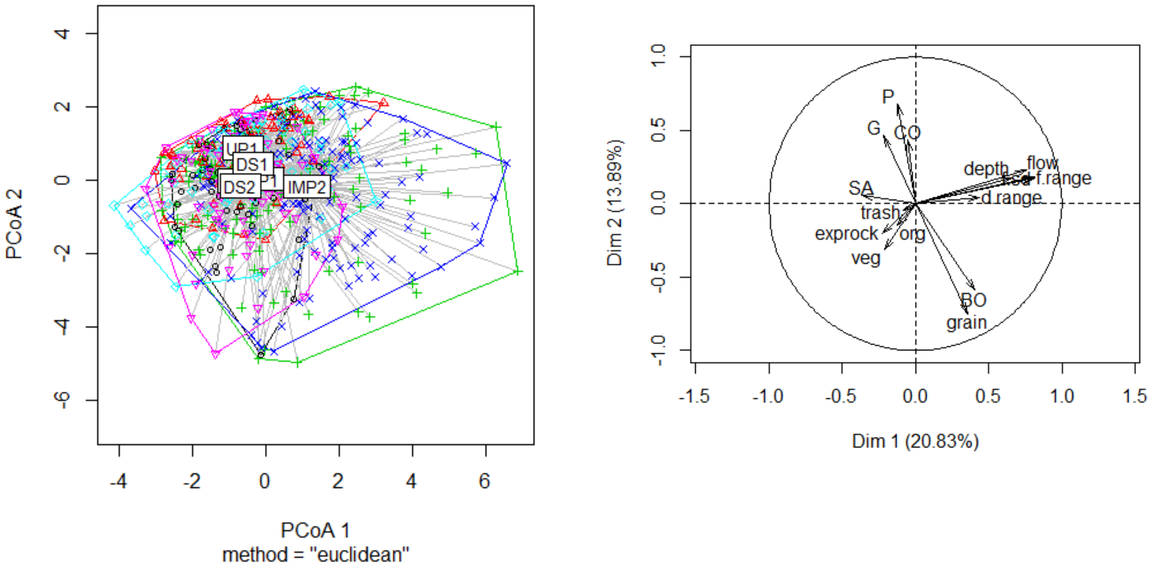


Fig 4.11 a) PCA Ordination biplot showing distribution of samples with hulls delineating reach groups, b) environmental drivers of main PCA gradients across samples in all sites in the Brox Burn

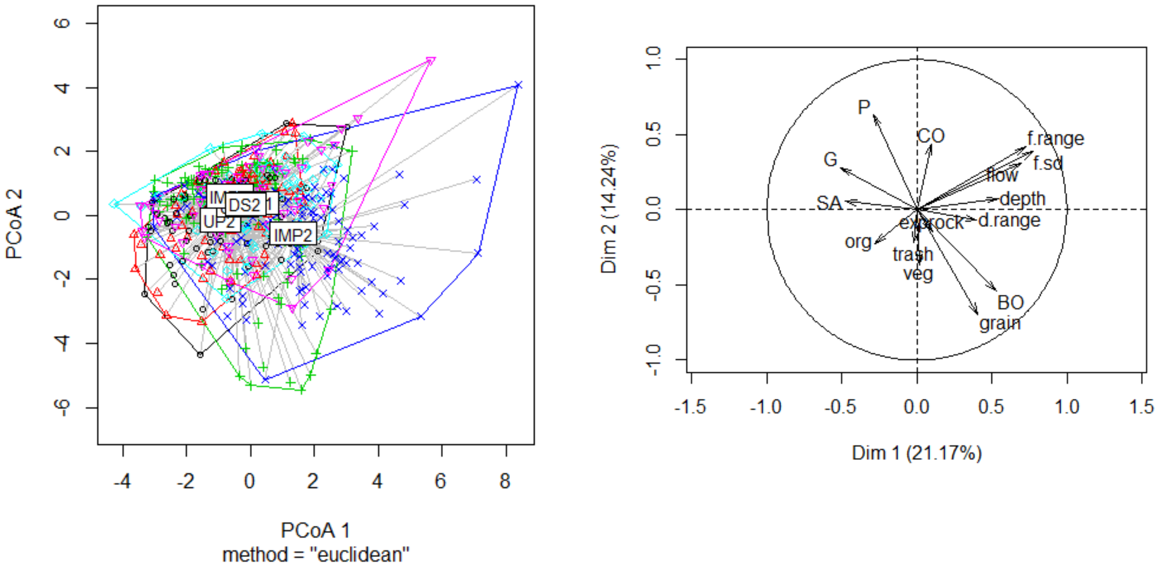


Fig 4.12 a) PCA Ordination biplot showing distribution of 2013 samples with hulls delineating reach groups, b) environmental drivers of main PCA gradients across samples in all sites in the Brox Burn

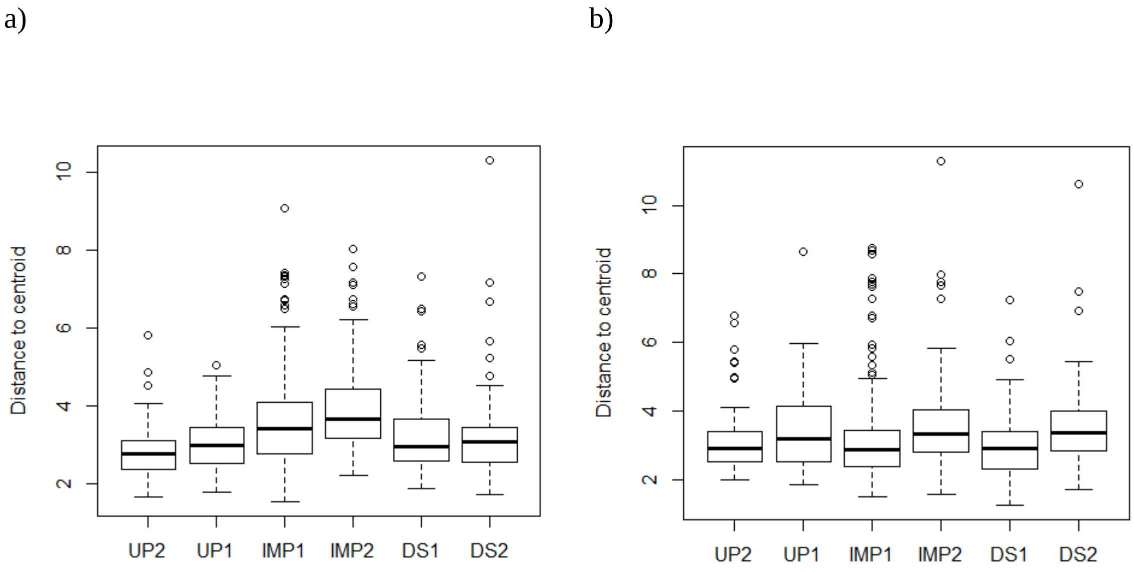


Fig 4.13 Median distance to group centre for sample reaches, based on PCoA of habitat data using Euclidean distance – a measure of habitat heterogeneity, for a) 2013 and b) 2014.

4.4.2.2. Habitat diversity

Initially habitat diversity was greatest in IMP1 and IMP2, significantly higher than for other reaches (Fig 4.13). These sites also covered a larger spatial extent, exhibiting a possible area x diversity relationship. UP2 was the least diverse, being significantly lower than all sites except UP1. Sampling after the disturbance showed habitat diversity was significantly reduced in IMP1 and IMP2 (Table 4.3, Fig 4.13b), but not in the other reaches. This change over time alters the relative habitat diversity of the reaches in 2014. The greatest habitat diversity was in IMP2, DS2 and UP1, with no consistent trend within or between treatment groups.

In summary, significant variables that discriminated between reaches related to hydraulic factors and sediment size. Impacted reaches initially had greater habitat diversity, due to the presence of faster flowing deeper sites with larger sediment sizes. However, these habitat types were removed by the engineering work in IMP1, but remained in IMP2, despite reduced diversity. For sediment impacted reaches any signal of fines and % surface sand has not persisted into year 2, nor impacted on habitat diversity. Upstream control reaches varied in their annual regime of fine sediment distribution, suggesting a natural spatial and temporal variability.

Table 4.3 Summary of physical habitat changes within sites over time, R^2 of model of multivariate habitat with year as an explanatory variable. Significance of permanova test of habitat heterogeneity difference over time and direction of change from 2013-2014 (< 0.001 ***, < 0.01 **, < 0.05 *).

Site	R^2 of year	2013-2014 change environmental gradient
UP2	4.3	Increase in sand and organic material reduced flow and range
UP1	5.6	Reduction in sand and gravel, increase in grain size, lower flow velocity, Increase organic material and vegetation cover
IMP1	3.7 *** reduction	Fewer high flow, and deep Reduces flow range Smaller grain size Less vegetation
IMP2	1.9 * reduction	Increases in flow and range, less gravel, more boulder cover
DS1	6.3	Reduced sand cover Greater depth higher flow velocities and flow ranges
DS2	5.6	Reduction in grain size, Higher flow velocities and depth

4.4.3 Channel biodiversity and change over time

A total of 30 riverfly species were found across the Brox Burn sample reaches over the sampling period, 7 of which accounted for the 5 most abundant species within each individual reach (Table 4.4). Identification of the full community to a mixed level: species (Coleoptera), genus and family (Diptera), tribe (Chironomid), family (Mollusca) order (other taxa) was made for 2 sites (IMP1 and US1) in 2013. Riverflies made up 25% of both the total species richness and abundance.

Table 4.4 Summary of biological samples, riverfly abundance, richness, diversity, and singly occurring species (mean, maximum and minimum). Mean abundance and standard deviation of 5 most abundant species per season, year and treatment combination.

Site	UP2	UP1	IMP1	IMP2	DS1	DS2
Treatment	Control		Dredged	Disturbed	Fine sediment impact	
Reach length	10m	10m	20m	20m	10m	10m
No. Samples	28	29	55	50	27	26
Abundance	115.5 (1-336)	109.5 (2-346)	211.5 (1-868)	82.5 (3-348)	131.5 (1-581)	80.5 (3-266)
Richness	7.9 (1-13)	8.2 (1-12)	7.5 (1-14)	7.0 (3-12)	6.5 (1-12)	7.4 (3-12)
Shannon	1.4 (0-2)	1.5 (0-2)	1.3 (0-1.9)	1.3 (0.4-1.9)	1.3 (0-1.9)	1.5 (0.6-1.9)
Simpson	0.6 (0-0.8)	0.6 (0-0.8)	0.6 (0-0.8)	0.6 (0.1-0.8)	0.6 (0-0.8)	0.7 (0.3-0.8)
Baetis rhodani	41.3 (47.6)	50.4 (59.4)	96.3 (114.5)	36.7 (41.8)	50 (63.4)	11.5 (12.3)
Ecdyonurus venosu	12.8 (11.6)	8.4 (16.9)	25.1 (33.6)	8.8 (14.5)	8.7 (12.3)	4 (4.9)
Hydropsyche siltalai	4.4 (10.4)	5.5 (13.6)	12.3 (29.4)	0.5 (1.3)	7 (26.5)	0.8 (2.6)
Leauctra fusca	11.5 (16.6)	9.8 (11.6)	22.6 (22.8)	8.2 (10.4)	8 (7.3)	6.8 (6.6)
Serratella ignita	7.5 (9.2)	14.2 (13.7)	37.6 (30.6)	15.7 (24.2)	31.3 (47.6)	12.7 (16)
baetis scambus	4.8 (8.3)	4.5 (7.1)	2.8 (6.1)	5 (5.4)	11.2 (11.9)	8.8 (9.9)
Hydroptila sp	25.4 (31.2)	9 (9.8)	5.5 (7.7)	4.5 (5.8)	11.7 (18.3)	31.4 (43.3)

4.4.3.1. Invertebrate abundance and species richness

Pre-impact, in 2013 the highest riverfly abundances were found in IMP1, slightly higher than in upstream sites, however there were few statistically significant differences (Table 4.5). Samples in IMP1 had significantly greater abundance compared to both IMP2 and DS1. No significant differences were found between average cell richness, or the calculated reach scale richness in 2013 (Fig 4.14a and c). Post impact in all sites abundance and species richness increased in the second year, significantly for all reaches, except UP2 abundance. Boxplots (Fig 4.14 b and d) show that a notable change over time can be seen in the range of abundances and richness in cells within sites, which is greater in the impacted sites in 2014. This indicates the presence of an increasingly small scale patchy distribution of individuals within the channel. In comparison, for downstream sites, particularly DS1, richness sample values were more uniform across the reach. Reach scale species richness (Fig. 4.14e), increased significantly over time for upstream and downstream reaches but not for those directly impacted by engineering. Average cell species richness and abundance was not significantly different between reaches in 2014, except for between IMP1 and IMP2 (Table 4.5).

Overall in both years there was a similar abundance and richness of riverfly species across reaches. There was no indication of impact on relative abundance or richness compared to nearby sites, since all sites changed in the same direction and to a similar degree. However, changes in the range of values in certain treatment types were evident.

Table 4.5 Results of Mann-Whitney tests of difference of biological metrics between sites, Kruskal - Wallis tests of difference of biological metrics between treatment groups. Significantly different pairs of sites or treatments are indicated (< 0.001 ***, < 0.01 **, < 0.05 *).

Year	Variables	Site	Treatment
2013	Abundance	IMP1 > DS1, IMP1 > IMP2 ***	Impact>Down *
2013	Richness		
2013	Shannon’s		
2013	Simpson’s		
2014	Abundance	IMP1- IMP2 *	
2014	Richness		
2014	Shannon’s		Impact>Down *
2014	Simpson’s		Impact>Down *

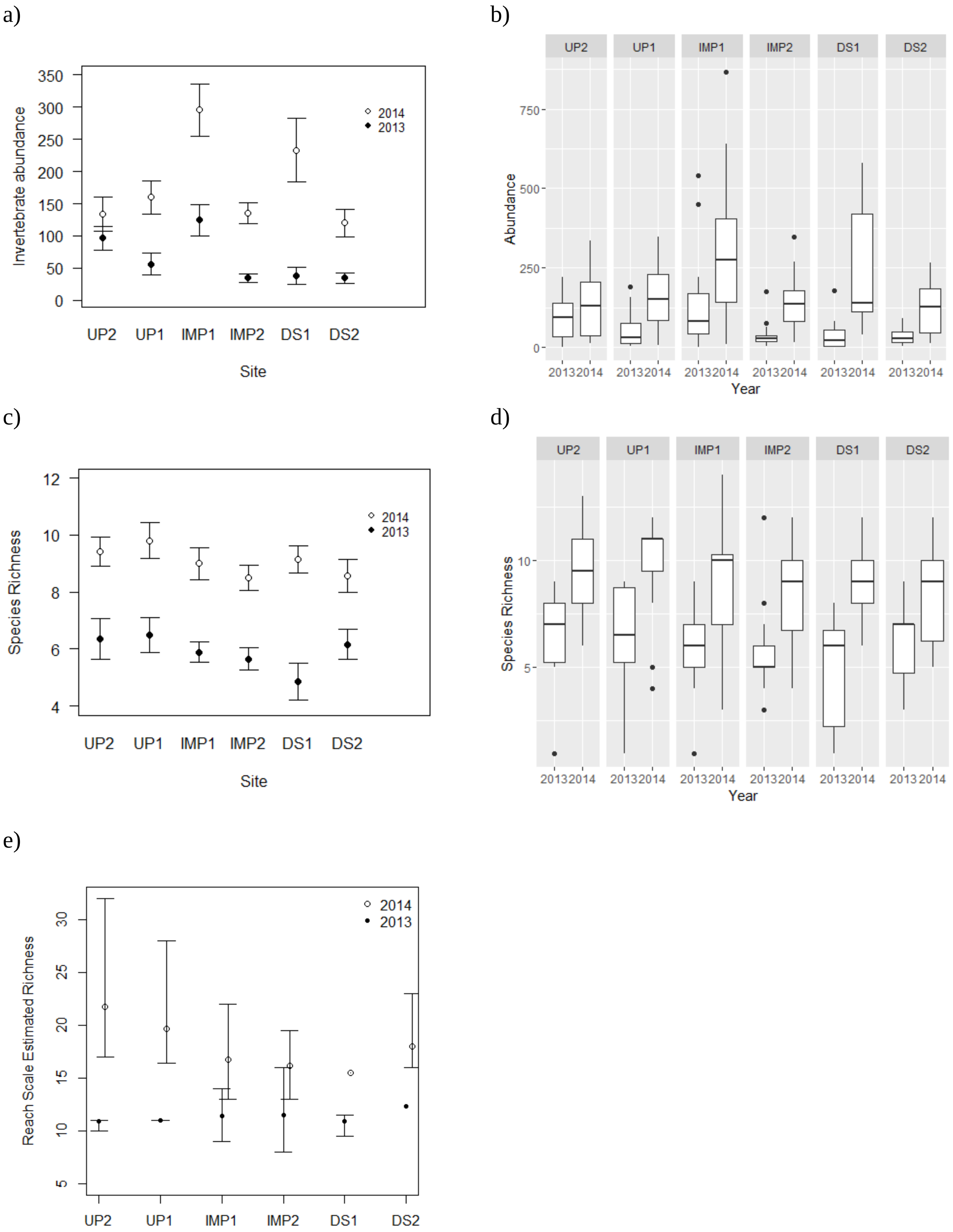


Fig 4.14 Sample occasion mean value and 95% confidence intervals in each site and year for a) riverfly abundance, c) species richness, e) reach scale estimated species richness. Box and Whisker plots, showing median and spread of data in each site and year for, b) riverfly abundance and, d) species richness.

4.4.3.2. Species diversity

For both diversity indices there was no difference between any reaches in either year (Fig 4.15a and c). Post impact, diversity increased in certain sites (Shannon: UP2, IMP1, IMP2, DS1, Simpson: DS1), but these differences were not consistent within treatment groups. Simpson's index was less sensitive to any changes that may have occurred. A change in the distribution of values within each reach was seen however (Fig 4.15b and d), with a reduced range of within reach cell level species diversities in downstream sites in 2014, illustrating more sample cells consistently with higher diversity

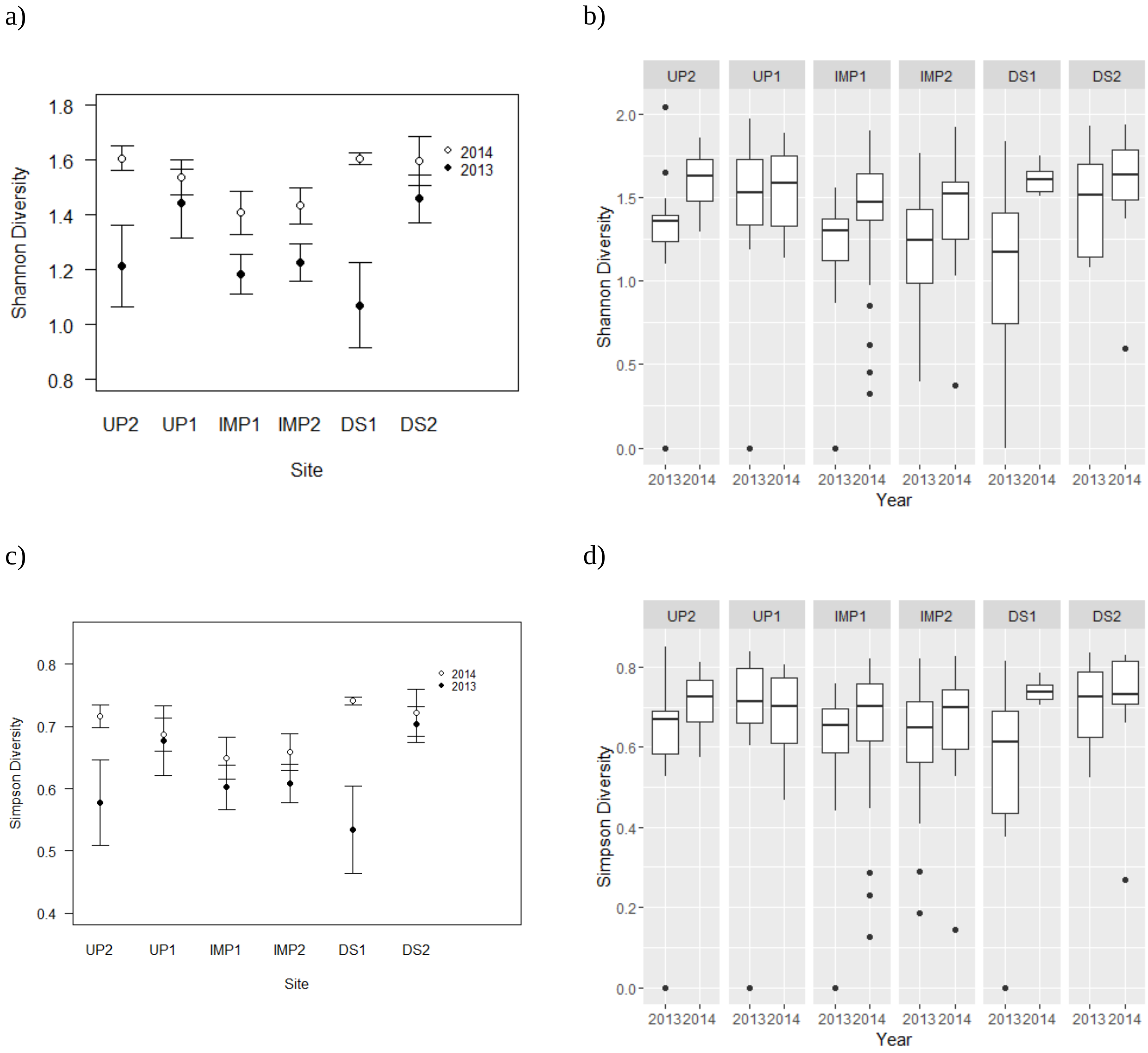


Fig 4.15 Sample occasion mean value and 95% confidence intervals in each site and year for a) riverfly Simpson's diversity and, .c) riverfly Shannon diversity. Box and Whisker plots, showing median and spread of data in each site and year for b) riverfly Simpson's diversity and d) riverfly Shannon diversity

4.4.3.3. Changes in Sediment sensitive taxa

PSI scores for all sample cells were almost all 100, indicating samples unaffected by sediment impacts. This may have been affected by restricting the community to the riverfly taxa, of which most taxa were in group A i.e. the most sensitive in the PSI classification. However, it would be expected that due to diversity within this group, fewer sediment sensitive riverfly could have been present. Only 7 samples from 215, contained fewer sensitive riverfly and had PSI scores of less than 100 (Table 4.6). All but one were from 2014, and from both upstream and downstream sites. This was due to the presence of a small number of certain taxa in these sites: *Caenis rivulorum, Habrophlebi*a *fusca,* and *Leptoceridae sp,* most probably *Adicella reducta* and *Mystacides longicorni*s or *M.azurea*, which all occur in this part of Scotland, but were too small to identify to species with confidence.

Table 4.6 Summary of samples with PSI scores below 100, indicating sample location, year and sample ID number, and identity of less sensitive taxa.

PSI score	Sample, year, ID number	Species present
93.3	IMP1 2013 (17.4)	*Habrophlebia fusca*
88.2	UP1 2014 (10.8)	*Leptoceridae sp*
88.2	DS1 2014 (4.1)	*Leptoceridae.sp*
89.47	DS2 2014 (4.5)	*Leptoceridae.sp*
71	DS2 2014 (4.7)	*Leptoceridae.sp*
91.3	UP2 2014 (9.7)	*Leptoceridae.sp*
95.8	UP2 2014 (10.6)	*Caenis rivulorum*

4.4.4 Community composition and change over time.

4.4.4.1 Community composition and river beta diversity within time periods

Generally, geographically nearby sites contained similar riverfly communities, and so located close to each other on the ordination biplot (Fig 4.16a and c). In 2013 UP2 and IMP1 reaches appeared separate from the other reaches, indicating distinctive communities. IndVal showed that UP2 was characterised by the presence of 3 caddis species: *Hydroptilla.sp* and the rarer *Potamophylax latipennis* and *Sericostoma personatum,* this was also confirmed by CA (4.16b). The IMP1 community was unique due to high concentrations of the mayflies *Serratella ignata* and *Ecdyonurus venosus gp* and stoneflies *Euleuctra geniculata* and *Leuctra fusca***.** Other reaches were broadly similar, however contained one or two indicator species. UP1 was characterised by the presence of the caseless caddis *Polycentropus flavomaculatus***.** DS1 appeared to have higher abundances of *Baetis scambus* and *B. rhodani*. IMP2 and DS2 located at the centre of the ordination, containing average communities. Significance tests showed that site identity was significant in determining species composition (R^2 = 20.7, p= 0.001). Post hoc tests revealed that IMP1 was significantly different from all other reaches and that IMP2 was significantly different from both of the upstream reaches. Beta diversity did not differ between any of the sites.

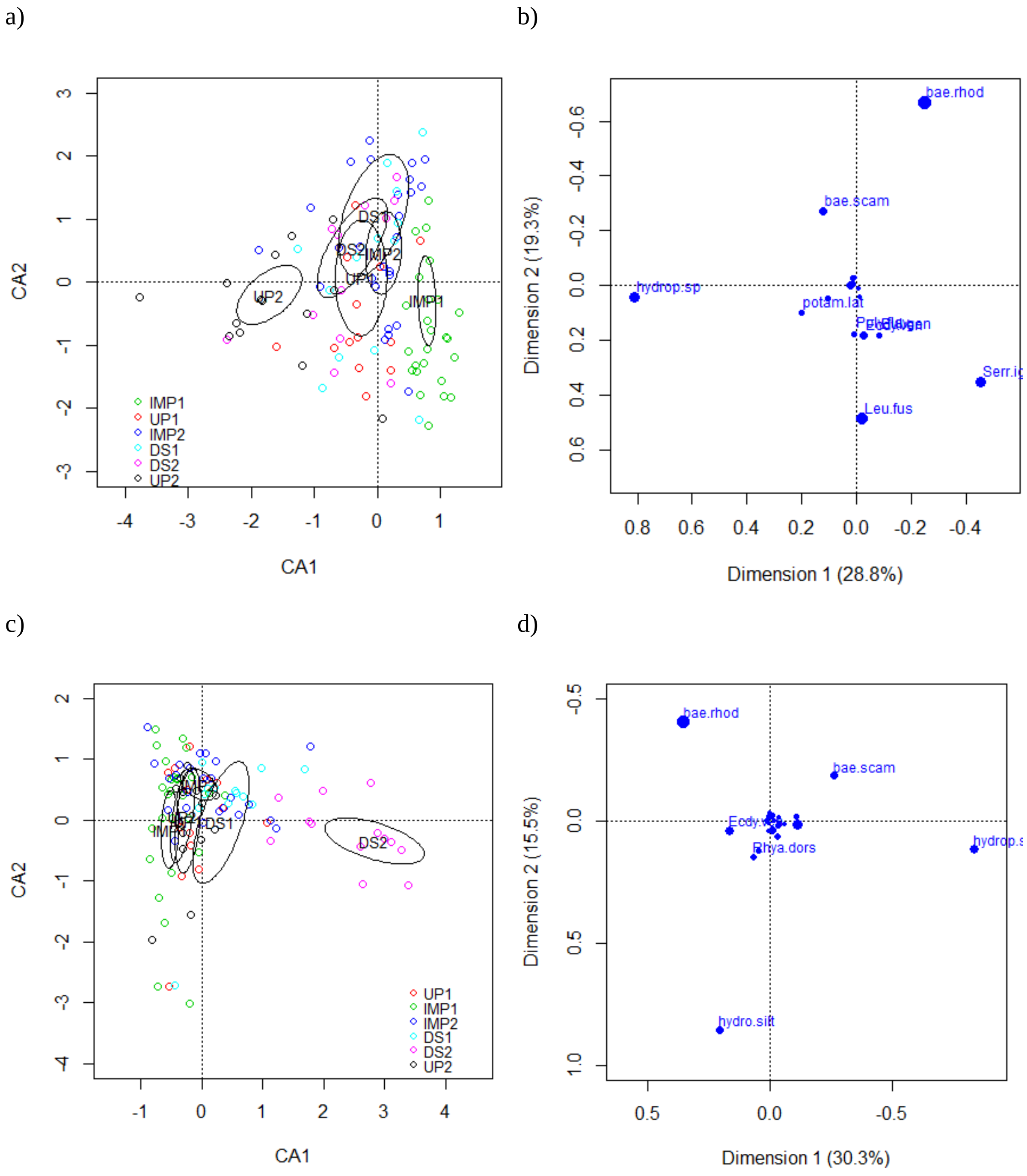


Fig 4.16. Correspondence Analysis results for 2013 a) 2013 and 2014 c) location of samples and reach groups based on species composition , and b) 2013, and 2014 d) Contribution biplot showing species that contribute over 1% to the solution, location indicates sample/reach associations and size of dot indicate relative abundance in total communty. A key to species abbreviations is given in Appendix 2.4.

In 2014 tests showed that site identity was again a significant determinant of species assemblage (R^2 = 18, p=0.001.) Post hoc tests indicated that the DS2 reach was community significantly different from all others, where it previously had an average community. Impacted (IMP1 and IMP2) sites were significantly different from each other, but more similar to the river community in general. Beta diversity did not differ between any of the sites. DS2 communities were distinct due to the presence of the small cased caddis *Hydroptilla sp*, previously most abundant in the upstream sites, became highly abundant here. A number of indicator taxa changed the reach which they indicated from one year to the next. *Euleuctra geniculata* was also found with highest abundance in DS2, previously this species was characteristic of IMP1. *Serratella ignata*, which previously characterised IMP1, became most common in DS1, along with *Baetis scamubus* (consistent from 2013). However, *Ecdyonurus venosus gp* and *Leuctra fusca* remained significant in parts of IMP1 composition, with the addition of *Alainis muticus* and *Baetis rhodani*. Upstream site communities were more similar to each other, and to the overall Brox Burn riverfly composition compared to 2013.

Sites were combined and considered as impact groups (Upstream, Impact and Downstream, Table 4.7), based on their similarity in appearance and exposure to conditions, rather than on the basis of site identity. In 2013 treatment was a significant driver of community composition, however this was driven by certain individual sites UP2 and IMP1, and the species within those sites. In the 2014 downstream sites were significantly different from the other groups, however upstream and impacted sites were not. Impacted communities become more like those in upstream controls, whereas downstream samples become more unique, particularly due to the community in DS2. Beta diversity did not differ between any of the treatment groups.

Table 4.7 Summary of PERMANOVA results, testing difference between treatment group community composition, R^2 = variance explained, and significance of model using treatment (< 0.001 ***, < 0.01 **, < 0.05 *).

Year	Pairs	F. Model	R^2
2013	Imp vs UP	6.077292	0.07 **
2013	Imp vs DS	4.504266	0.06 **
2013	UP vs DS	2.625335	0.05
2014	UP vs Imp	2.101175	0.03
2014	UP vs DS	6.361891	0.11 **
2014	Imp vs DS	7.978664	0.09 **

Community composition was highly variable from one year to the next across all reaches. For DS1 there was a significant reduction beta diversity as measured by group dispersion. In concordance with earlier results (4.4.3.1), showing higher species richness in 2014 samples, IndVal also indicated that 2014 samples were characterised by a wider range of species. Upstream control communities were not consistent over time. They experience the same general increase in diversity as all other sites, with some specific changes (Fig 4.17a and b). Site UP2, showed a reduction in two species of cased caddis that had previously made this site unique from others nearby. There was a complete loss however of *Potamophylax latipennis* and a reduction in *Hydroptilla.sp* abundance. However, the continued presence of *Sericostoma personatum* remained indicative of this site. UP1 gained *P. submarginata,* which was also present in adjacent IMP1 and IMP2, and occurred at low abundance in both DS sites, but not UP2.

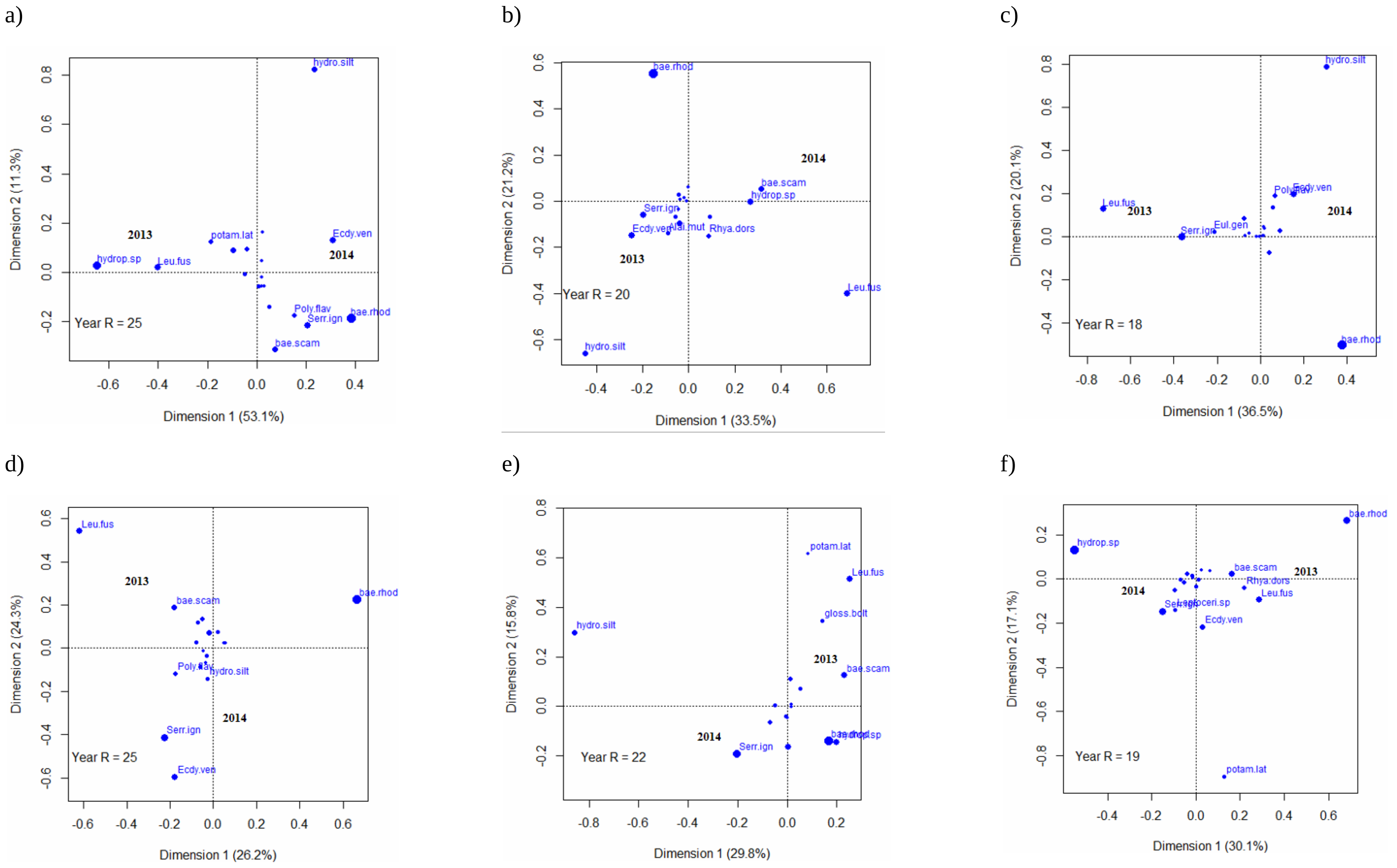


Fig 4.17 Contribution biplot of CA solution within site over time, showing species that contribute over 1% to the solution, points scaled by abundance for 6 sites in the Brox Burn, with approximate year group centroid location (2013 and 2014), Year R^2 = percentage community change explained by year only from PCoA. A key to species abbreviations is given in Appendix 2.4

4.4.4.2. Effects of disturbance on community composition

Compared to upstream control reaches, the 4 impacted sites downstream experienced the addition of many species common across all downstream sites, specifically a marked increase in the small cased caddis *Hydroptila.sp* (Fig 4.17). Direct sediment removal through dredging in IMP1 resulted in the loss of a number of taxa that had made this site distinct, and replacement by a set of ubiquitous species. Specific taxa were lost, *Glossosoma boltoni* was present in small numbers throughout the reach initially, but absent in 2014. The single addition to IMP1 was of *Alainis muticus*, which has high affinity to IMP1, being found only there and in sparse numbers at the neighbouring UP1. *A. muticus* was abundant in UP1 in 2013, forming a likely source population. General construction disturbance, without direct dredging experienced by IMP2, did not result in a reduced community, but taxa previously found IMP1, *S. ignata* and *E. genuiculata*, evidently found refuge there. In downstream sediment impacted sites, in addition to the overall increase in species richness over time, these sites experienced specific community changes, resulting in contrasting communities within the downstream group. DS1 gained the caddis and mayfly species *Hydropsyche siltalia* and *Baetis scambus*, which were uncommon in DS2. However, DS2 was distinguished post impact by the highest occurrence of *Euleuctra geniculata*, and a loss of the previously common, and elsewhere widespread *Rhyacophila dorsali*s.

In summary, in both survey years certain individual reaches had distinct communities, even in this small spatial extent and urban setting. All sites increased in diversity in the second year, however the particular disturbance treatment a reach experienced determined the taxa gained or lost. Control site communities were not consistent in their community composition over time. High natural inter-annual variation poses a problem for impact detection using purported stable controls as comparisons. Beta diversity across all sites was stable, with only DS1 showing reduced community turnover between samples in 2014.

4.5 Discussion

4.5.1 Habitat and biodiversity in the Brox Burn

There has been little characterisation of urban stream communities or their habitat diversity, beyond simple monitoring for pollutants or quantifying the effects of the urbanisation process. This is despite the high amenity value for nearby populations and numerous ecosystem services provided by urban river systems (Francis 2012).

Small scale variation in water depth and velocity were strong drivers of local habitat diversity. At small spatial sampling scales and short timescales, hydraulic variables become most significant, since compared to other variables that are constrained by channel resources, they can provide the most heterogeneity. Hydraulic roughness is generated by the morphology of the bed substrate, and so this is typically measured or manipulated as a proxy for habitat diversity (Reid et al. 2010). The emergence of hydraulic roughness as a strong environmental gradient is due to the shallow nature of the study stream (Padmore 1998).

Within the extent of the study area, a range of flow and sediment habitat conditions were available over a small area. This might be a reason for the high total river diversity of 16 and 27 EPT species in 2013 and 2014 respectively. These values are high compared to reported riverfly richness in other urban studies: 4 in Wenn (2008), 3 in Violin et al. (2011), and 10 in Wood et al. (2016), which are more comparable to the mean cell ($0.5m^2$) richness of 5 and 9 species in the present study. However, urban stream species richness could be much higher than currently appreciated. By sampling a wide range of habitats, locations and spatial extent, a large number of individuals were sampled (n=27, 850) , while the absence of chemical pollutants (SEPA 2010) may also contribute to the observed high richness.

Sample reaches varied in their sediment composition, with UP1 and DS1 containing higher amounts of riffle quality gravel and pebble cover, however the riverfly communities present were not particularly distinct from other sites. The strongest pattern of community similarity appeared to be driven by proximity, with nearby reaches containing similar communities. This is perhaps not surprising since good quality upstream reaches are a significant source of colonisers (Lorenz and Feld 2013, Winking et al. 2014) and the flashy hydrological regime likely enhances longitudinal connectivity. A lack of small scale habitat diversity does not preclude an area from contributing to the wider habitat diversity and species pool. There is still much uncertainty surrounding the assumption of a habitat diversity and biodiversity relationship at a range of spatial scales (Palmer et al. 2010). For instance, reach UP2 had low habitat diversity, but a distinct community consisting of the cased caddisfly species *Hydroptilla.sp* and the rarer *Potamophylax latipennis* and *Sericostoma personatum.* Conversely, IMP2 had the most diverse and distinct habitat type with greatest depth and velocity and variability, but community composition was similar to other parts of the system. These findings are consistent with many other studies that find local community is only partially controlled by local environmental habitat (Lepori et al. 2005), and will be explored more in the next chapter.

4.5.2 Physical effects of dredging, disturbance and sedimentation

Habitat diversity in dredged and disturbed reaches was reduced, though not to levels significantly below that in control reaches. Dredging at IMP1 result in habitat simplification, and closer similarity to surrounding reaches, by removing high flow and depth ranges, and also larger substrates that were relatively rare in the local area. This is in agreement with Harvey & Wallerstein (2009) who assessed the impact of similar flood defence work. The disturbed but not directly dredged IMP2, still provided the largest range of habitats, indicating that the temporary disturbance caused by construction access and flow diversion, did not alter habitat strongly. Evidently this specific type of impact has less long lasting consequences for physical habitat.

Fine sediment was released by the engineering activities and distributed downstream, recorded by the sediment mats as a pulses of sediment input. Even fast flowing parts of downstream reaches received significant amounts. Sampling in varied and repeated locations illustrates the high temporal and spatial variability and patchiness of fines deposited, as noted by other authors (Larsen et al. 2009, Von Bertrab et al. 2013). Sediment loads delivered to the downstream channels were high, similar to the maximum of the experimental loads used by Wood et al. (2016) of 1,3 and 5 kg/m^2, as initial sediment treatments. Over the course of their experiment however, these amounts were reduced by flow, resulting in a summer average sediment load of 131g/m^2 retained in the net during invertebrate sampling. Sediment loads measured, were also at the upper end of the range of those encountered by Von Bertrab et al. (2013) (1-6kg/m^2), who used a similar turf mat collection method. The amount of sediment received was lessened slightly by distance from the source, with concentrations and impacts being diluted downstream. Therefore, the severity of sediment stress at a particular location will depend on a combination of the severity of flushing, cover of retaining microhabitats present, as well as proximity to the sediment source.

The 4 weeks of greatest sediment impact and general disturbance occurred during the annual low flow period. Wood & Armitage (1997) note that base flow period is the peak dispersing time for benthic invertebrates. Considering the timing of the peak sediment episode and of invertebrate resampling, it is possible that towards the end of summer, breeding adults of sensitive species may have departed the area by drift or local extinctions due to sediment stress. Over the 4 weeks of high sediment exposure, physical stresses comprised increases in turbidity, bed smothering and possible chemical contamination due to the past industrial nature of the area (Jones et al. 2012). However, urban communities are usually resilient to short term loads of suspended and benthic sediment, due to adaptation to the natural fluctuations in sediment movement (Wood and Armitage 1997). So could 3-4 weeks be considered short term in this case? Larsen et al. (2011) use a 3-week period of treatment for experimental sediment baskets, finding significant changes to the community in this short time period, for abundance and richness. They found that a nested community emerged, favouring taxa with certain life history traits. Affected species were both mobile swimmers and attached immobile taxa, and those using gill respiration. Due to the long time elapsed between the sediment episode and resampling, it is possible that significant recovery has already occurred, despite the high sediment loads. Additionally, the use of a set of restricted taxa, the riverfly, which have broadly similar biological traits and sediment sensitivity (Extence et al. 2013), will limit the range of effects that can be observed compared to more varied invertebrate taxa including Diptera and Molluscs, or the relative proportion of EPT.

4.5.3 Consistency of control sites over time

Even in the absence of man-made disturbance there was variation between the control sites, and over time, in the pattern of fine sediment deposition, suggesting natural spatial and temporal variability of patches due to stochastic movement of small sediments. The fluvial and sediment regime in urban streams often acts to selectively remove sand and pebble sized particles leaving a coarse armoured layer (Pizzuto et al. 2000), although where bed sediments are mobile, removal of fines can improve intra-gravel oxygen levels (Finkenbine et al. 2000). This was supported by the river community PSI values, in both control and impacted sites, indicating that a number of fine sediment-tolerant taxa were present in small localised patches, even though overall sand cover reduced in 2014 (4.4.2.1). The change in species in the control sites over time, amounts to a spatial rearrangement of nearby common species (i.e. they converge towards the centre of the ordination).

4.5.4 Strong inter-annual community change

Habitat composition and riverfly community composition changed over time in all sites from 2013 to 2014. Abundance, species richness and diversity uniformly increased in 2014 regardless of treatment. The change from one year to another in all measures of habitat and biota indicates a strong signal of annual variability driven by flow regime (Fig 4.10), which confounds the effect of disturbance by dredging or fine sediment stress. The communities in each year reflect those responding to recent antecedent hydrological conditions in combination with local morphological stress. Few studies on stream biodiversity explicitly consider the temporal variability of communities, or the role of antecedent flow regime, even less so in assessments of urban communities, although flow regime is known to cause significant changes to community metrics and assembly. However insights can be drawn from studies of non-urban streams where temporal coherence of the community is disrupted by drought or high flow stress (Brown 2007, Greenwood and Booker 2015), though often with a rapid subsequent recovery (Huttunen et al. 2012). Generally, periods of flow stability tend to improve coherence of community assembly between time periods and mitigate urban pressures of sediment or chemical pollution. For example, Walsh & Webb (2016), found that low discharge streams or dry conditions lead to a reduced effect of the amount of impervious land cover, a common proxy for urbanisation on measures of stream biotic integrity. Considering the characteristically flashy nature of urban streams, of which the

Brox Burn is typical (Fig 4.10) it is likely that temporal variability of communities and biodiversity metrics will be high, and may be magnified further by the fine spatial scale of sampling used in this study.

4.5.5 Biotic response to dredging and channel disturbance

Despite significant changes in habitat composition, habitat diversity, riverfly abundance, richness or diversity, including beta diversity, did not differ from control sites before or after impact. This study thus suggests that richness and diversity indices may be insensitive to small scale habitat changes, or putative effects of a range of man-made channel stressors, in the face of stronger naturally-induced inter-annual variation. Although diversity and richness can often distinguish between urban and rural stream types (Paul and Meyer 2001), urban settings present an environment where the species pool is likely to have already been reduced to insensitive species. Typically an urban hydrological regime, urban and impervious land cover, and laterally restricted channel, select for species poor assemblages comprising taxa that are stress tolerant (Walsh et al. 2005). Since urban stream communities are often then pre-adapted to regular fluvial disturbance, with limited spatial refugia, perhaps the communities are impervious to the additional man-made stress of engineering works.

There was, however, a change to the distribution of sample riverfly richness within each impacted site. The distribution of species was more patchy and heterogeneous in 2014, indicated by an increase in the spread of values. However, this was not accompanied by an increase in beta diversity, which indicates the presence of nested communities, or perhaps because beta diversity does not vary at this small scale in urban streams. Violin et al. (2011) considered that change in channel habitat complexity was a strong predictor of sensitive taxa richness and biotic index, when channel complexity was defined as the number of transitions between habitat types at the larger scale (riffle- pool-run - debris dam) per 100m.

This study observed a general movement of taxa in a downstream direction. For example, the increase in abundance of *Hydroptila* sp and *E. geniculata* from US to DS sites, possibly due to drift behaviour. Drift is an avoidance response to short term stress periods (Larsen and Ormerod 2010) associated with rising discharge (Giller and Malmqvist 1998), possibly leading to the denuding of upstream areas, unless compensated by adult dispersal (Malmqvist 2002). Urban streams experience increased longitudinal connectivity (Gurnell et al. 2007) and increases in discharge variability, with the addition of occasional episodes of chemical or sediment pollution, which suggest that drift should be a strong driver of community assembly in urban streams. Correspondingly, Arango, James & Hatch (2015) found rapid early colonisation of a restored urban reach via disturbance-tolerant taxa from the drift. Before impact, species composition appeared related to location on the river, driven by the proximity to nearby up or downstream sites colonised by drift or active movement. This pattern was disrupted by anthropogenic impacts in both IMP and DS pairs, which, although closest geographically, differ significantly in their community composition. This trend agrees with the idea of streams as a patchy discontinuum, consisting of a broad scale pattern of community arranged with occasional interruptions (Poole 2002, Reid et al. 2010) at the segment scale. Dredging and sediment disturbance impose an additional filter on the community, beyond the background processes of drift, niche filtering and biotic interactions.

Though there are many studies comparing conditions of urban streams to nearby rural counterparts (Meyer et al. 2005, Tullos et al. 2009, Laub et al. 2012) or studying the early stages of adjustment to recent urbanisation (Chin 2006), there are few studies on biodiversity patterns within urban streams, how they respond to an altered flow and sediment regime, or habitat heterogeneity. Typically habitat degradation is often overlooked as a driver for urban communities, compared to changes to flow and sediment regime either because riparian corridors maintain physical habitat, or because the habitat is considered uniformly degraded within urban sites (Allan 2004). Indeed some urban channels can be complex, even in comparison to nearby rural channels (Laub et al. 2012).

More pronounced effects of channel disturbance were seen when looking at the reach scale species richness. At this scale, richness increases from 2013-14 observed across reaches, were reduced at IMP1 and IMP2 compared to surrounding sites. This indicates a slower recovery after dredging of coarser scale species richness, suggesting limited recolonization. Perhaps this is due to a lack of suitable food resources present, and limited dispersal ability of potential colonists from upstream sources (Winking et al. 2015), that would encourage drifting communities to settle within the time period of the study. Arango et al. (2015), observed that drifting taxa only stayed when their food resources were available, beginning with Chironomids, followed by scraper feeders, *Baetid* and *Leptophlebiid* mayflies, and late colonisers were predators such as *Rhyacophila* and *Perlodid* stoneflies. Indeed, the impact of dredging resulted in loss of the unique community found initially in IMP1, of grazing- and gatherer-feeding taxa (*Glossosoma boltoni, Serratella ignita* and *Euleuctra geniculata*). IMP1 became more similar to upstream and DS1 communities but not the nearby IMP2. The similarity of dredged sites to the river community in general, indicates that species re-populated from nearby sites, most notably colonisation by *Baetis mutitucs* from upstream sites.

Though the IMP1 site experienced habitat simplification through a loss of high velocity and hydraulically variable patches caused by the loss of small scale topographical complexity, this was not accompanied by a corresponding reduction in beta diversity. This suggests that factors other than local habitat, such as spatially aggregated distributions resulting from community interactions or behaviour, may act to maintain community beta diversity. However, the lack of habitat heterogeneity may have resulted in greater community instability due to the loss of flow refugia or structures contributing to retention of food resources (Brown 2007).

4.5.6 Biotic response to fine sediment deposition

There was little long term physical change to sediment impacted habitats. Evidence of fine sediment or % surface sand did not persist into 2014, nor did it impact on habitat diversity or biodiversity, although a change in the distribution of diversity among patches could be seen. The presence of mostly sediment sensitive riverfly taxa (PSI - category A; Extence et al. 2013) in almost all samples suggest that sediment stress did not change over time nor was it concentrated downstream of the disturbance.

In 2014 downstream samples had consistently high diversity diverse, i.e. there was less variation in diversity between cells. This was supported by the significant reduction in beta diversity in DS1, albeit to a level no less than any other channel in that year. This suggests a loss of low diversity, or low habitat quality cells, perhaps caused by higher flushing flows preceding surveying.

After impact the two downstream communities become more different from each other, some taxa were lost or gained that reflected the high rate of flow mediated movement over small extents. DS2 communities stand out due to the high abundance of *Hydroptilla sp* and movement of *E. geniculata*. Studies of the short term effects of fines indicate that *E. geniculata* and *Hydroptilla sp* are sensitive to fine sediment additions (Larsen et al. 2011, Wood et al. 2016). *Hydroptila* sp are associated with patches with low shear stress and Froude number (Mérigoux et al. 2004), having poor resistance to flow disturbance, they have been found to accumulate in refugia (Lancaster 2000). Past sediment impacts are unlikely to be influencing the 2014 community composition, which can be considered the result of passive transport by the current. *Rhyacophila dorsali*s characterised DS2 in the first year, however its abundance was much reduced in the second year. This taxa may be a victim of the sediment disturbance the year before, although Wood et al. (2016), found that *R. dorsalis* was unaffected by fines, and its mobility and respiration traits suggest it would be resistant (Schmidt-Kloiber and Hering 2015). A number of reasons could account for its absence, for example longer term impacts of the sediment exposure on breeding success, lack of suitable prey or summer emergence before sampling.

4.6 Conclusions

Determining if the direct and indirect physical impacts on the urban channel through engineering and associated disturbances, also damage stream biodiversity and ecosystem function is important for construction site management and regulation. Dredging resulted in a reduction in habitat diversity, particularly the removal of deep and fast flowing areas that were rare in the channel. Engineering associated disturbance for access had little lasting effect on in-channel habitat, however there was a significant release of fine sediment temporarily deposited in downstream sites. Habitat character was broadly similar across all study reaches, but hydraulic diversity created by channel bed roughness was a source of physically diverse niches for riverfly larvae.

Riverfly species diversity was high, with sediment sensitive taxa present throughout the study period. Species composition was not strongly dependent on environmental conditions or quality. Although fine sediment pollution was experienced at a sensitive time, there was rapid biotic recovery by the time of resampling. Dredging resulted in a change in spatial arrangement of species patches rather than a reduction in overall biodiversity. Species richness and diversity were highly resilient to these short-term disturbances. A strong reach identify effect was observed. Disturbed sites were rapidly recolonised by drift from the diverse taxa present in adjacent and highly connected parts of the stream. However, the disturbance disrupts the broad scale drift pattern by adding an additional environmental filter. River wide changes from one year to the next in species assemblage and biodiversity metrics, suggests that urban channels have complex small scale spatial and temporal ecological dynamics driven by naturally occurring annual hydraulic and sediment disturbance regimes, and biotic interactions between highly dynamic generalist taxa. High annual dynamism, even in control channels was a strong signal that impeded impact detection and ability to generalise from these finding. This suggests that urban streams can be more temporally variable than their rural counterparts, and caution should be used when interpreting comparisons based on short term studies.

By restricting the community data to riverfly taxa limits the range of biota life history traits; integrating behavioural strategies that other invertebrate orders employ may have revealed a stronger response to the impacts studied. To better understand and quantify riverfly community composition and diversity this study recommends covering a larger sampling extent since distribution of taxa can be patchy. Assessment of habitat type and quality alone do not form good guides as to gauging biological quality. Due to constraints on natural channel processes, maintaining small scale diversity of bed structure and hydraulic diversity at both the microscale and river segment scale will benefit urban stream biodiversity.

Chapter 5 - Application of spatial techniques to uncover mechanisms of biodiversity structure in an urban stream.

5.1. Introduction

5.1.1 The spatial nature of rivers

The spatial nature of rivers has long been appreciated (Vannote et al. 1980, Frissell et al. 1986) and ecologists have incorporated this into bio-assessment in a number of ways to account for, or quantify directly, heterogeneity at a number of hierarchically arranged spatial scales (Cooper et al. 1997, Hering et al. 2003, Thorp et al. 2008). Observing spatial patterns of habitat and environmental variables are of interest because macroinvertebrate communities are structured by forces that have spatial components. The spatial patterns of species assemblages themselves can suggest the mechanisms that control species distributions (Legendre and De Cáceres 2013). This has long been appreciated at larger scales e.g. river zones for fish (Huet 1954) and for riverfly richness as a function of latitude (Vinson & Hawkins 2003, review in Heino 2009). Smaller scale spatial patterns have been successfully used to explain community richness and composition of benthic macroinvertebrates. For example, Beisel et al. (2000), found that mesohabitat heterogeneity increased benthic invertebrate richness, and the surrounding habitat mosaic was also important. Including a measure of habitat structure in addition to type can improve models of abundance (Palmer et al. 2000) and community composition (Cortes et al. 2011).

5.1.2 Spatial autocorrelation, a problem or opportunity

Bio-assessment relies on biological communities indicating, and integrating local environmental conditions and being responsive to known pressures (Birk et al. 2012). An ongoing area of stream ecology research is to quantify the extent to which environmental variables influence invertebrate communities at a range of spatial scales (Feld and Hering 2007, Lorenz and Feld 2013). However, Lennon (2000) warned that conclusions made about the relationship between environmental factors and biotic response can be overestimated, particularly if those environmental factors express strong spatial autocorrelation.

Spatial autocorrelation (SAC) is where values at pairs of locations a certain distance apart are more similar (positive autocorrelation) than can be expected for a randomly associated pair of observations (Legendre 1993). Spatial structure is a ubiquitous problem for ecologists, because all ecological data must come from a particular physical location. There are two types of SAC in biotic data originating from different processes; (i) *induced spatial dependence*, which can be attributed to unmeasured environmental factors that themselves have a spatial structure; (ii) *true spatial autocorrelation*, relating to purely endogenous population and community processes such as ecological drift (the random reproduction and survival of individuals due to competition or predation) or random dispersal (Legendre and Legendre 2012).

The presence of induced spatial dependence is problematic, because it violates a number of model assumptions, particularly the independence of samples and error structures. SAC occurring in either, or both, environmental and species data can inflate type 1 errors resulting in erroneous interpretation of species-environment relationships (Peres-Neto and Legendre 2010, Dray et al. 2012). Despite the known problems about non-independence of data this aspect is not often considered in studies of species-environment relationships, unless spatial structure is of explicit interest to the study. Even though doing so can improve the reliability of models (Dormann 2007). SAC is dismissed by many as a statistical inconvenience, relying on the strength of underlying trends to counter its biases (Lennon 2000), and sadly its ecological usefulness as a tool for understanding mechanisms of community assembly, are often overlooked. When studies do consider the spatial nature of stream data, clearer conclusions can be drawn about the role of each factor driving community assembly at a range of spatial scales. For example, Grenouillet et al. (2008), revealed a patchy spatial pattern of invertebrates on a French riverbed, indicating biotic interactions with fish, whereas fish and diatoms showed longitudinal patterns driven by habitat patch size. On a larger scale, encompassing the Mediterranean, Bonada et al. (2012) found that benthic macroinvertebrate taxon richness was not affected by SAC, but that community composition was.

5.1.3 Scale dependency of ecological phenomenal

In addition to spatial autocorrelation ecological relationships are also scale dependent, changing the importance and influence of predictors depending on the grain size of measurement. Many aspects of environment and biota relationships in streams have been found to be scale dependent; habitat heterogeneity (Cooper et al. 1997), impacts of fine sediments (Larsen et al. 2009, Jones et al. 2012), influence of land use and geography (Johnson et al. 2007), flow effects (Hart and Finelli 1999), and significantly beta diversity (Barton et al. 2013, Heino, Melo, Siqueira, et al. 2015). When ecological phenomena are interpreted, this is with respect to the specific scale of sampling (Cooper et al. 1998). Spatial elements of sampling design are summarised by Legendre & Legendre (2012), as i.) *grain size*, the size of the sampling units, or duration of measurements, ii) *sampling interval*, the distance between neighbouring sampling units (average) or temporal lag, and iii) *extent*, the total length, area or volume included in the study.

We already know that stream habitats are patchy, heterogeneous systems (Poole 2002), however, the perceived extent and patterns of these patches varies with the grain and extent of the investigation (Cooper et al 1998). Legendre & Legendre (2012) state that with a certain sampling regime one cannot see structures smaller than the grain size or larger than extent used. As such a sampling design should follow what is already known about the scale of the pattern or process. River systems encompass many spatial and temporal scales of physical structure and process, while biota too, vary between and within taxonomic orders in their perception and experience of the environment. The potential mismatch of sampling scale between physical predictors and biological response is considered to be a source of error, and responsible for weak, or confounding relationships (Parsons et al. 2003, Larsen et al. 2009, Friberg 2014).

Scale dependence is well accepted, and a certain scale of sampling grain and extent are implicit in studies, determined by the research question and study organism (Barton et al. 2013). Sampling grain size and extent for stream and river research is highly variable, often dependent on practical considerations in terms of time constraints restricting sampling at high resolution, making interpretation and generalisation about the underlying processes difficult (Seymour et al. 2016). The use of a nested sampling strategy (e.g. in Lamouroux et al. 2004) to account for the hierarchical organisation of physical and biological properties of river systems is common and can allow an explicit consideration of scale dependence.

In order to account for SAC, the spatial location of the sample in relation to others is needed, i.e. spatial data. The combination of small, mesoscale sampling combined with the retention of spatial location has been used to relate community characteristics to local habitat quality and heterogeneity at a range of scales (Thomson et al. 2004, Pedersen and Friberg 2007, Wallis et al. 2012). However, these types of study overlook the potential confounding effects of spatial dependence, or the opportunity to explicitly describe spatial patterns of stream invertebrate assembly.

5.1.4 Using biological traits to discover mechanisms of pattern formation

Scale dependence of relationships is also affected by the biological properties of the organisms considered. Contrasting taxa requirements and dispersal capabilities between and within orders of, for example, bacteria, macroinvertebrates, plants and fish are used to unpick the different mechanisms and scales of community assembly (Johnson et al. 2007, Grenouillet et al. 2008), with low concordance of assemblage patterns observed between disparate taxa at small spatial scales. At broad spatial scales, (e.g. catchment or region), interspecific variation in dispersal ability among macroinvertebrates is often taken into consideration to understand community distribution across the environment. Where dispersal ability determines the rate of exchange between patches of varying quality across the stream landscape, this mediates the relative roles of spatial factors and local environmental conditions in determining local community structure (Heino 2013). Heino et al. (2015b), for example, illustrate how species sorting mechanisms are more significant for intermediate dispersers, and similarly Grönroos et al. (2013) illustrate that active dispersers show a much stronger relationship to the local environment conditions than passive. Other traits such as body size, trophic group or life form can also be considered (Heino, Soininen, et al. 2015).

Therefore, it is logical to presume that an organism’s means and level of mobility, will determine its distribution at the small scale. Contrasting short term resource requirements, community interactions and habitat tolerances with its rate of exchange between patches (Cooper et al. 1998). For example, Cooper et al. (1997) contrast 2 grazers, the fast moving mayfly *Baetis* with the slow moving caddisfly *Glossosoma* through ranges of algal patchiness; these taxa were found to have different tracking abilities to reach resources based on their mobility.

5.1.5. Developments in spatial analysis

A recent development in spatial analysis is the advancement of spatial eigenvector techniques (Dray et al. 2006, 2012, Griffith and Peres-Neto 2006). These methods allow the identification of SAC in data, either to remove it by spatial detrending (Peres-Neto and Legendre 2010), or to isolate and investigate spatial patterns explicitly. These techniques use the spatial coordinates of sample points and a user defined network between points, to generate a set of potential spatial predictors that can be tested against univariate or multivariate response variables of environmental measurements or applied to community data. Unidirectional patterns can be modelled with Moran's Eigenvector Maps (MEMs), and these techniques have been extended to consider asymmetrical spatial patterns such as those driven by wind or flowing water, termed Asymmetric Eigenvector Maps (AEMS) (Blanchet et al. 2008b).

Spatial eigenvectors possess many properties that make them well suited to analysis of ecological data. They are more flexible than autoregressive models and trend surface analysis techniques (Dray et al. 2006), and more adequate to detect patterns at multiple scales and in fine detail (Blanchet et al. 2011). Combined with variance partitioning (Borcard et al. 1992), they make a flexible tool to describe and account for SAC in environmental and community data. As a result these techniques have recently been applied to stream research in a number of ways. These include comparisons of how macroinvertebrates perceive types of distance in a glacial stream network (Cauvy-Fraunie et al. 2015) or analysis of how dams and flow stress affect diatom distribution (Liu et al. 2013).

5.1.6 The spatial structure of urban streams

As described in Chapter 4, very little attention has been paid to urban stream community patterns, biotic response to environmental change or natural dynamics. It is assumed that habitat diversity is limited, as a history of direct modification and the urban stream syndrome, create uniform habitats or unnatural spatial structures. Allan (2004), for example, considered habitat degradation to have reduced influence on degraded urban biota, either because riparian corridors maintain physical habitat quality but water quality is degraded, or because the habitat is uniformly degraded within urban sites. Laub et al. (2012) however, found that some urban streams have high channel complexity for some aspects, in comparison to forested reference streams. Given that physical structures, habitat type and heterogeneity may be limiting in urbans streams it is possible that stream invertebrates may respond to spatial structure and arrangement of patches. An interpretation of this spatial structure could improve our understanding of responses to disturbance, or identify potential insights for habitat improvement.

5.2 Aims

This study aims to make use of recent analytical approaches that explicitly consider spatial elements of riverfly community data, to reveal mechanisms of community organisation within the urban stream environment. This chapter aims to uncover how disturbance affects community spatial patterns, and the scale at which the local environment structures the community, specifically addressing the following hypotheses:

1. Riverfly communities show a distinct spatial patterns of community organisation including those related to directional nature of streamflow.
2. Disturbance through engineering pressures removes or changes the nature of spatial patterns.
3. Community composition is driven by environmental gradients, and this relationship is variable
    (i) naturally over time and between locations,
    (ii) in response to human pressures of dredging and fine sediment pollution,
    (iii) due to changing sampling grain size of environmental variables,
    (iv) for different components of the invertebrate community based on their locomotion method.

4. Spatial autocorrelation of environmental variables confounds their direct importance as drivers for species composition, and this environmental spatial structuring is altered by disturbance

5.3 Methods

The data used in this study were fine scale habitat measurements and macroinvertebrate samples from a small urban stream. The study site, the Brox Burn, is located in the West Lothian region of central Scotland, 18km west of Edinburgh. Six sample reaches, described in 4.3.2, were surveyed using a 1 x 0.5m grid structure, in summer 2013 and 2014, using the protocol described in 4.3.4 for physical measurements and 4.3.5 for benthic macroinvertebrates. The spatial location of each cell in the sampling grid was used to relate cell-specific biological and environmental measurements to each other using spatial analysis, as detailed below. All analyses presented here were restricted to the component of the invertebrate community represented by the riverflies (Ephemeroptera, Plecoptera and Trichoptera).

5.3.1 Spatial Eigenvector generation and testing

Spatial patterns of invertebrate communities were analysed using spatial eigenvector analysis: Moran's Eigenvector Maps (MEM) and Asymmetric Eigenvector Maps (AEM) (Dray et al. 2006, Blanchet et al. 2008b). This method produces N-1 numbered eigenvectors, that describe broad scale (low numbers) to fine scale (high numbers) spatial patterns emergent from the dataset, including both positive and negative correlation.

Analysis was completed following the steps in (Borcard et al. 2011). Firstly, a connection matrix was constructed between all sampling points using Delaney triangulation, with unwanted links manually removed (Fig 5.1 a). For directional vectors (AEMs) an origin point upstream was specified to indicate the direction of flow (Fig 5.1b), and reciprocal lateral connections were included between samples. In both symmetric and asymmetric methods an inverse distance weighting was applied to the links, ensuring that nearby sites influenced each other more strongly than distant ones.

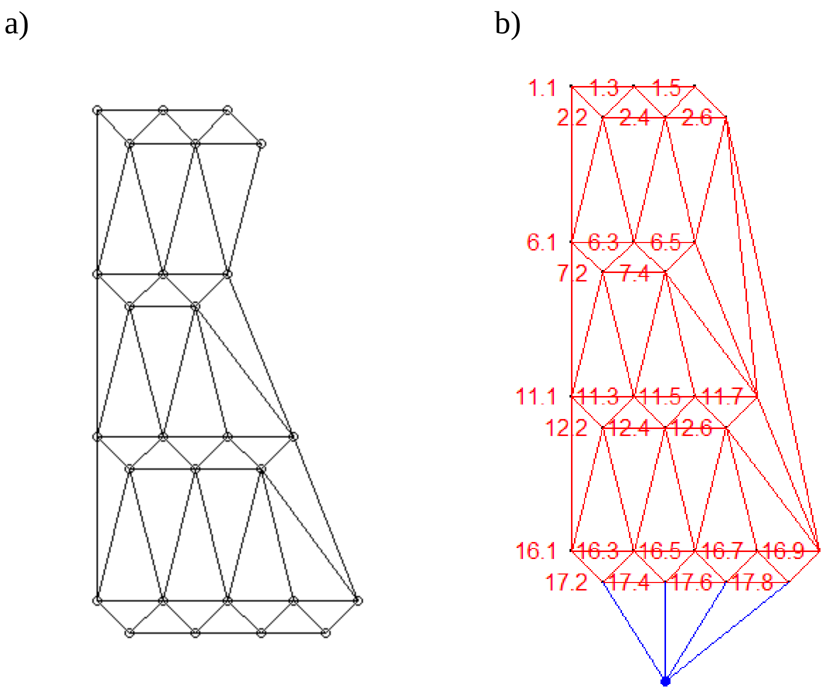


Fig 5.1 Example connections matrix for a site used in eigenvector analysis, a) for MEMs, and b) for AEMs with origin of directionality (flow) at the bottom of the map.

To determine the significant spatial patterns that explain the community assembly Redundancy Analysis (RDA) was used. This method of constrained ordination is an extension of regression for modelling multivariate species response to environmental predictors. Every reach and time period dataset was modelled separately, since spatial models generated are specific to each set of spatially connected sample points. Species abundance data was firstly Hellinger transformed to fit the assumptions of linear regression methods (Legendre and Gallagher 2001). As per the standard methodology for MEMs the species data was first detrended using the sample point coordinates, x and y prior to analysis.

The spatial eigenvectors significant in explaining the invertebrate community distribution were selected within the MEM function using AIC, or using RDA and forward selection methods. To ensure correct type I error rates, forward selection used the R^2 of the full model containing all positive eigenvectors as a second stopping criteria (Blanchet et al. 2008a). Vectors representing both positive and negative correlation were included in both selection procedures. Multivariate species composition was modelled with all significant eigenvectors (MEM and AEM separately) using RDA. Significant RDA axes were extracted and sample scores plotted on a plan outline of the stream reach to visually examine the type of spatial patterns uncovered. To summarise what scale of eigenvectors were significant, a score was generated ranging between 0-1 indicating broad to fine scale patterns. The average eigenvector number which represents its scale, was standardised by the number of samples in each site (since the number of eigenvectors is N-1).

5.3.2 Determining species locomotion traits

Using data on species locomotion from the online database of Schmidt-Kloiber & Hering (2015) freshwaterecology.info, and Tachet et al. (2010) species were allocated to one of 3 mobility groups: fast, mid and slow. The number of taxa and a brief description is given in Table 5.1, full taxa allocation is in Appendix 5.1. To investigate the interaction between taxa mobility traits and their spatial patterns within the stream site, each spatial eigenvector analysis was repeated using each separate trait group.

Table 5.1 Number of taxa assigned to each locomotion trait group and description of movement method.

Group	Number of taxa	Description and invertebrate order
Fast	8	swimming and walking - mayflies and some stoneflies
Mid	15	Walking and burrowing- mobile caddis and stoneflies
Slow	7	semi sessile - mostly cased caddis

5.3.3 Environmental drivers of community composition

Riverfly community composition was analysed using constrained ordination analysis (RDA) to identify significant environmental variables driving differences between sample community composition for the following data sets:

i) between the 6 reaches within each year,
ii) within each reach over time
iii) between cell samples within each reach and sampling period

Species data was firstly transformed using the Hellinger transformation (Legendre & Gallagher, 2001). For each dataset the community was modelled using the full set of environmental variables initially, then a forward selection procedure with the global model R^2 as the second stopping criteria (Blanchet et al. 2008a), reduced the model to the significant terms. The significance of each model, axis and model term, was tested using permutation tests. The independent effect of each environmental term was obtained using the "by margin" method. For reach, and sample period specific data sets, significant variables were first selected from a reduced group (sediment, hydrology and channel features) due to the relatively low number of samples within each dataset. Variables selected from each group were then passed to a global model which was further reduced using the methods described above.

5.3.4 Changing the spatial sampling grain using focal statistics

To address the effect of the spatial grain of sampling, on the ability of each environmental variable to explain community variation, cell measurements were aggregated over a range of spatial extents using focal statistics. Four zones of spatial grain were considered. The smallest extent was the initial sampling cell only, increasing in size to include the 6 cells directly upstream (Fig 5.2). This design assumes that an organism's main direction of movement, and that of resources and structuring forces, will follow the direction of flow.

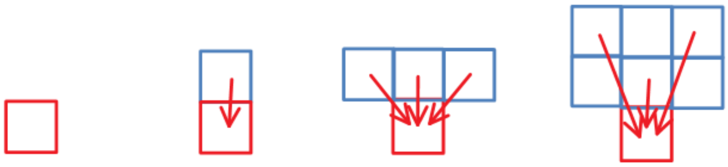


Fig 5.2 The shape and extent of the 4 sampling grain sizes used termed F0-F3 (left to right), increasing number of upstream cell (blue) in relation to the focal cell (red), arrows indicate assumed direction of influence.

Mean and sum functions were used to summarise values of the environmental variables within the focal extent. For all variables this function was the mean value, e.g. mean velocity, depth, except for the presence/absence of trash and bolder features, which used the sum of the cells where the feature was present.

To look at the change in relationship between riverfly community composition and environmental predictors at different spatial extents, each environmental variable and scale combination was used individually as a predictor to model the community. Site community data were aggregated by treatment category into Upstream, Impact and Downstream for each sampling period (see Chapter 4 for details). Prior to analysis species data were spatially detrended using reach specific MEMs calculated as described above, in order to control for spatial autocorrelation. Residuals from each RDA model with significant MEMs were then used as the community response variable. Peres-Neto & Legendre (2010) found MEMs to adequately control for spatial autocorrelation, better than polynomial regression with x and y. RDAs were then conducted with the detrended and non detrended species data to examine the amount of spatially autocorrelated variation described by each variable, at each focal zone.

5.3.5 Trait specific response to habitat and scale

The influence of species mobility traits on the importance of specific environmental variables and their interaction with spatial grain was investigated using the following analysis. Abundances for all species within each trait group were pooled to create three pseudo species: fast, mid and slow (defined in Table 5.1) for each sample. Pooled abundances were Hellinger transformed to meet linear model assumptions. Site community data were again grouped by treatment category (Upstream, Impact and Downstream) for each sampling period. Forward selection of environmental variables in RDA was carried out using the full set of environmental variables with the pseudo species as the response, at each spatial grain (F0-F3). The proportion of variance in each individual pseudo species (fast, mid, slow) abundance explained by the model, was extracted and compared between spatial grains and treatments. The environmental gradient associations with each trait group at each focal zone were investigated.

5.3.6 Variance partitioning

Significant environmental and spatial variables selected in earlier methods for individual reach and sample period were passed to the variance partitioning analysis. MEMs were recalculated without prior detrending since the sample x and y coordinates were included in the partitioning to quantify linear trends. Environmental variables used were those that were significant in the RDA at the finest spatial grain only (F0). The significance of each unique fraction was tested using a permutation test. The shared fractions between any spatial variable (MEM, AEM and xy co-ordinates) and environmental variables were calculated, and also the relative unique and shared contributions of MEMs and AEMs.

All statistical analyses and graphics were produced using the R environment (R Core Team 2016) in R Studio version 0.99.902 (RStudio Team 2015) with the use of the following packages: vegan (Oksanen et al. 2016), Packfor (Dray et al. 2016), sapacemakeR (Dray 2013), AEM (Blanchet et al. 2014) and raster (Hijmans 2016).

5.4 Results

5.4.1 Significance, scale and patterns of spatial variables

The Brox Burn riverfly community assembly showed significant spatial patterns. Unidirectional spatial variables (MEMs) were significant for all sites, and could account for a large proportion (between 4 % and 35%) of the riverfly community variation (Figure 5.3a). Before impact, the most significant role of spatial structure in 2013 was seen in downstream reaches. The change in importance of spatial structuring overtime varies among reaches, though for sediment impacted reaches there was a significant decrease in R^2 over time. In 2014 upstream sites had the greatest amount of spatial structure. Significant MEMs ranged from broad to fine scales. Over time there was an overall change to broader scale patterns in impacted reaches (Fig 5.3b), except for DS1, where community patterns became more fine scale, or patchy. Directional spatial variables (AEMs) were significant for all reaches (Appendix 5.2), but only during a single sampling year, which varied between sites. Where significant, directional spatial variables could account for up to 28% of the variation in invertebrate community. Most AEMs occurred at a broad scale, around 0.2.

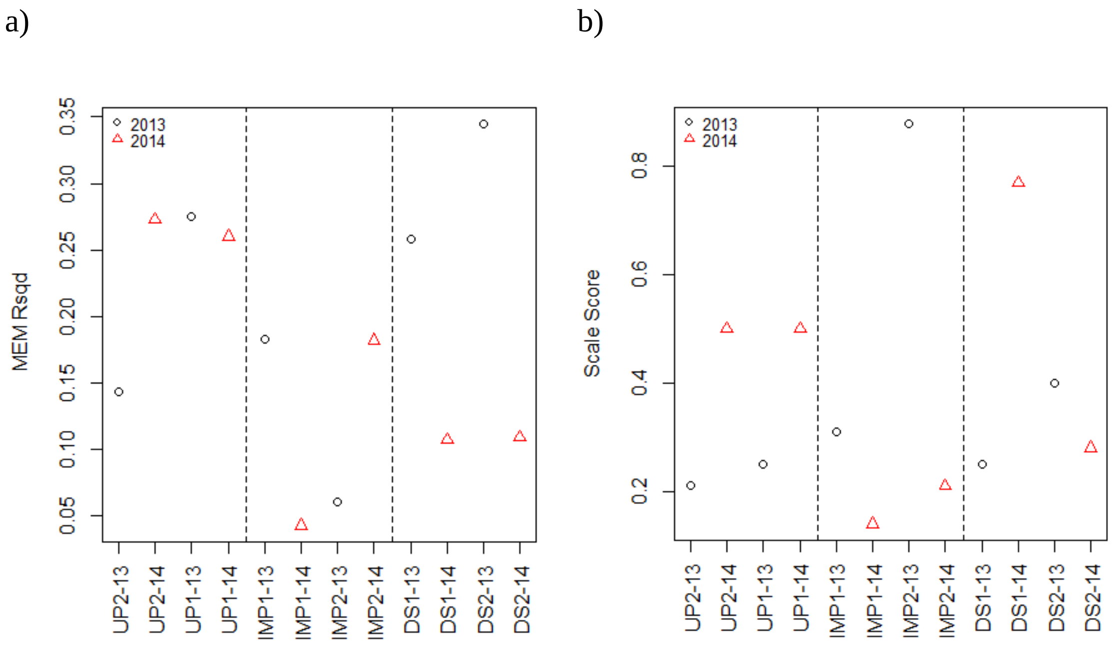
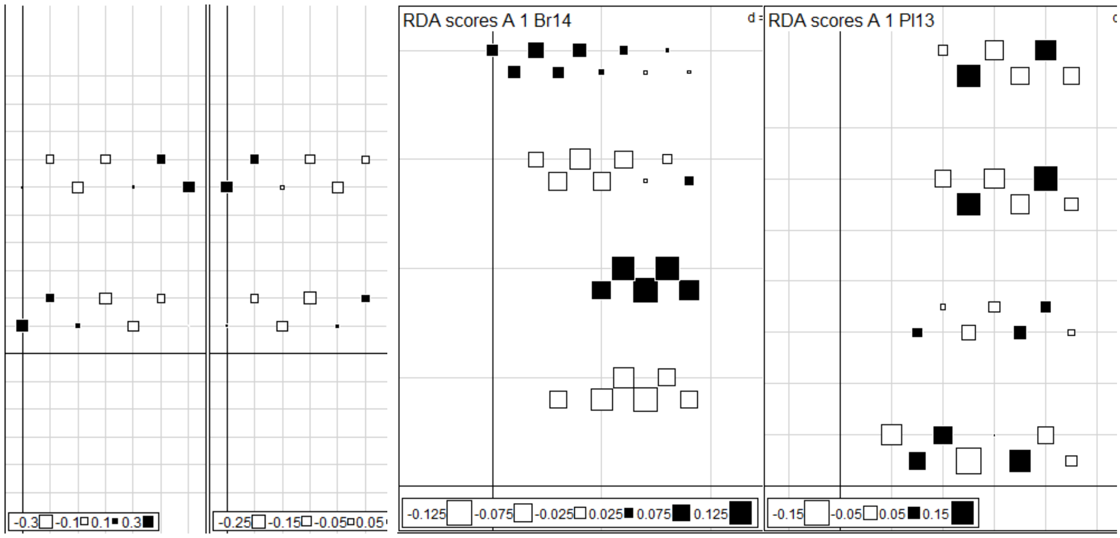


Fig 5.3 Output of from RDA model comparing importance of spatial structuring of the invertebrate fauna using MEMs at six sites, pre and post impact, a) model R^2, and b) average spatial scale score (0.2= broad scale, 0.8 = fine scale).

The ordination score of each sample represents the similarity of communities as explained by the spatial variables. Similar community assemblies group together on the ordination axes, and when these scores are plotted on a map, this shows the spatial location of similar communities. Distinct patterns of community arrangement emerge from the analysis for MEMs and AEMs (Fig 5.4) and can be used to interpret the mechanisms that structure the spatial arrangement of taxa. The type of patterns and which reaches they occurring in can be summarised as the following:

1. Edge and centre – MEM (2013: US1, US2, DS1, DS2 and 2014: US1, US2, DS2), AEM (DS2, UP1)
2. Longitudinal – MEM (2013: IMP1, 2014: IMP2, IMP1) AEM (IMP1, IMP2)
3. Small scale patches – MEM (2013: IMP2 2014: DS1), AEM (DS1, UP2)



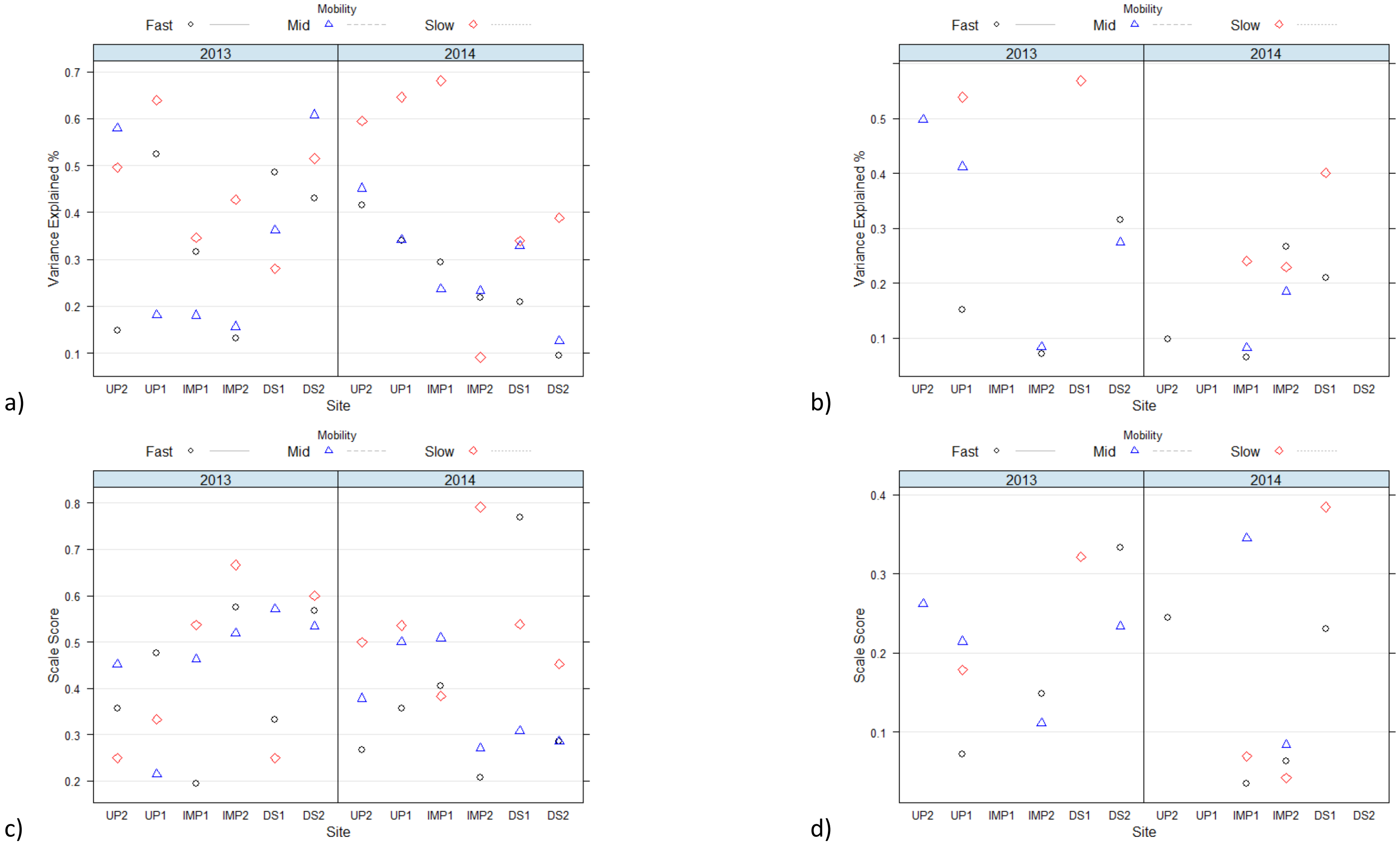
a) DS1 2013 b) IMP1 2014 c) IMP2 2013

Fig 5.4 Example patterns of riverfly community response to spatial variables. Plots represent a plan view of the river bed, water flows from top to bottom. Each square is a sample scaled by its score on an RDA axis (usually Axis1), of a model with the significant MEMs as explanatory variables (a) edge and centre, b) Longitudinal, c) small scale patches).

Many channel-specific MEM spatial patterns remained consistent over time. However, for IMP2 the change from fine scale patches to a broad scale longitudinal patterns was illustrated as was the change in DS1 from a distinct edge and centre (Fig 5.4a) broad pattern, to finer scale patches.

Splitting the riverfly community into separated mobility traits was expected to illustrate how fast or slow moving taxa might respond at contrasting spatial patterns or scales. The role of spatial structure in each mobility group was highly variable among sites in each year, and particularly for medium mobility taxa, however some distinct trends were seen for fast and slow taxa. On almost all occasions MEMs, and AEMs better explain variation in abundance of the slow mobility group (higher R^2) - up to 70% of community variation, compared to fast taxa (Fig 5.5 a). A downstream trend in amount of spatial structuring emerges in 2014 for both fast and slow taxa. Slow species were explained increasingly by spatial distribution up to site IMP2, where there was a large decrease, while fast taxa were consistently less spatially structured from upstream to down.

Fig 5.5 Output of R^2, from RDA model comparing importance of spatial structuring of the pooled trait group community, based on significant a) MEMs and b) AEMS. Average spatial scale score of significant eigenvectors for c) MEMs and d) AEMs.



Much fewer AEMs were significant for trait groups, compared to MEMs, however in several reaches certain trait groups were spatial structured where the community as a whole was not (IMP2 13 fast and mid, DS1 13 slow, UP2 14 fast) suggesting that only some parts of the riverfly community were affected by flow patterns. Variance explained by AEMs was reduced in 2014 compared to the previous year. Significant AEMs were mostly at the broad scale (5.5d), as for whole community (Appendix 5.2b). The relationships between trait groups and scales of MEMs was highly variable across all reaches. However, in 2014 scales of patterns of fast taxa were consistently broader than those for slow taxa. Except for sediment impacted DS1 2014, where fast taxa were structured by fine scale patterns (5.5c).

Spatial patterns of the different taxa groups appear using MEMs. Fast taxa had strong channel edge and centre patterns (5.6a), slow taxa more fine scale patches across the whole channel width (5.6c), and medium taxa showed intermediate patterns often following a longitudinal pattern (Fig 5.6b). Interpreting spatial patterns of AEMs was hindered by the reduction in significant models, and did not appear to follow the same trend as for MEMs and is not discussed further.

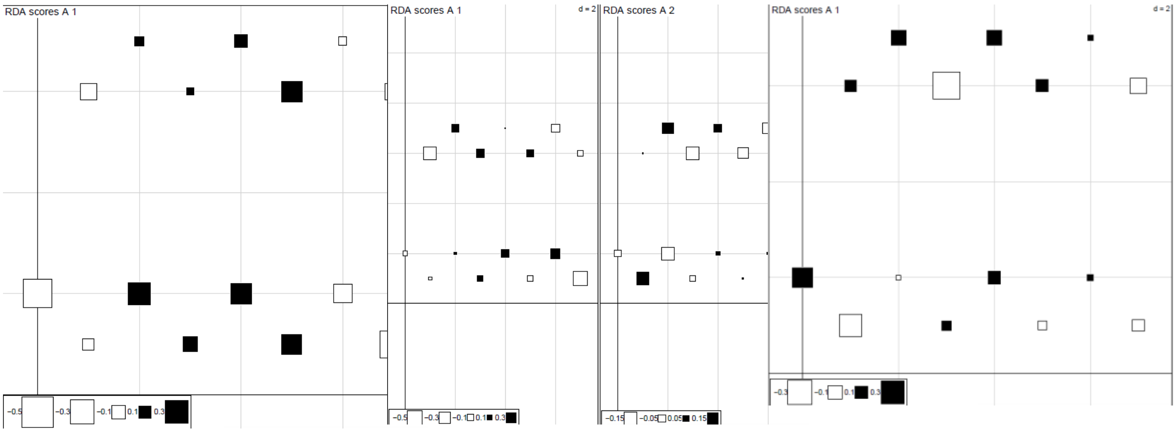

a) Fast b) Mid c) Slow

Fig 5.6 Example of trait community spatial patterns in site DS2 2013. Plots represent a plan view of the river bed, water flows from top to bottom showing each sample scaled by its score on an RDA axis (usually Axis1), of a model with the significant MEMs as explanatory variables for a) fast, b) mid and c) slow riverfly communities.

5.4.2 Gradients of community change between and within reaches, and change over time

Environmental variables described a gradient of species occurring within all reaches rather than between reaches, illustrated by a significant amount of overlap of community composition between the sites (Fig 5.7). The number of significant environmental variables and the strength of environmental structuring increased from 2013 to 2014, to include more flow and sediment variables (Table 5.2). Water depth was consistently the strongest driving variable. Impact reaches covered a wider gradient of conditions, with a relatively larger area of the plot (high within site diversity) covered by IMP1 and IMP2 before impact. In IMP1 communities were different from the overall river, being associated with the higher amounts of vegetation and represented by higher abundance of S*erratella ignata* (Fig 5.7a and c). Upstream communities were nested within those of other sites in both years and changed little in relation to other sites.

Table 5.2 Significant environmental variables from RDA analysis of all reaches in each year, variance explained by each variable and its significance (Veg- vegetation, PE- pebble, GR- gravel, SA- sand, CO – cobble, Org- organic material).

Year	Variable	Variance	P value
2013	Total	11.6	0.001
	Depth	3.7	0.001
	% Veg	3.2	0.004
	% PE	3.2	0.002
	% GR	3.1	0.003
2014	Total	24.4	0.001
	Depth	6.7	0.001
	% SA	2.7	0.005
	Flow	2.6	0.006
	Grain	2.5	0.004
	% Veg	1.9	0.024
	% Org	1.8	0.022
	Flow range	1.6	0.043
	% GR	1.5	0.05
	% CO	1.3	0.087

After the anthropogenic disturbance, differences can be seen in the communities from IMP1 and DS1 and 2. The extent of environmental gradient driving species composition in IMP1 was reduced, communities became more like the upstream sites (Fig 5.7 e). Downstream communities differentiated more from other sites, particularly DS2, consisting of high abundances of *Hydroptila* sp. and *Baetis scambus* associated with deeper water and smaller sediment sizes.

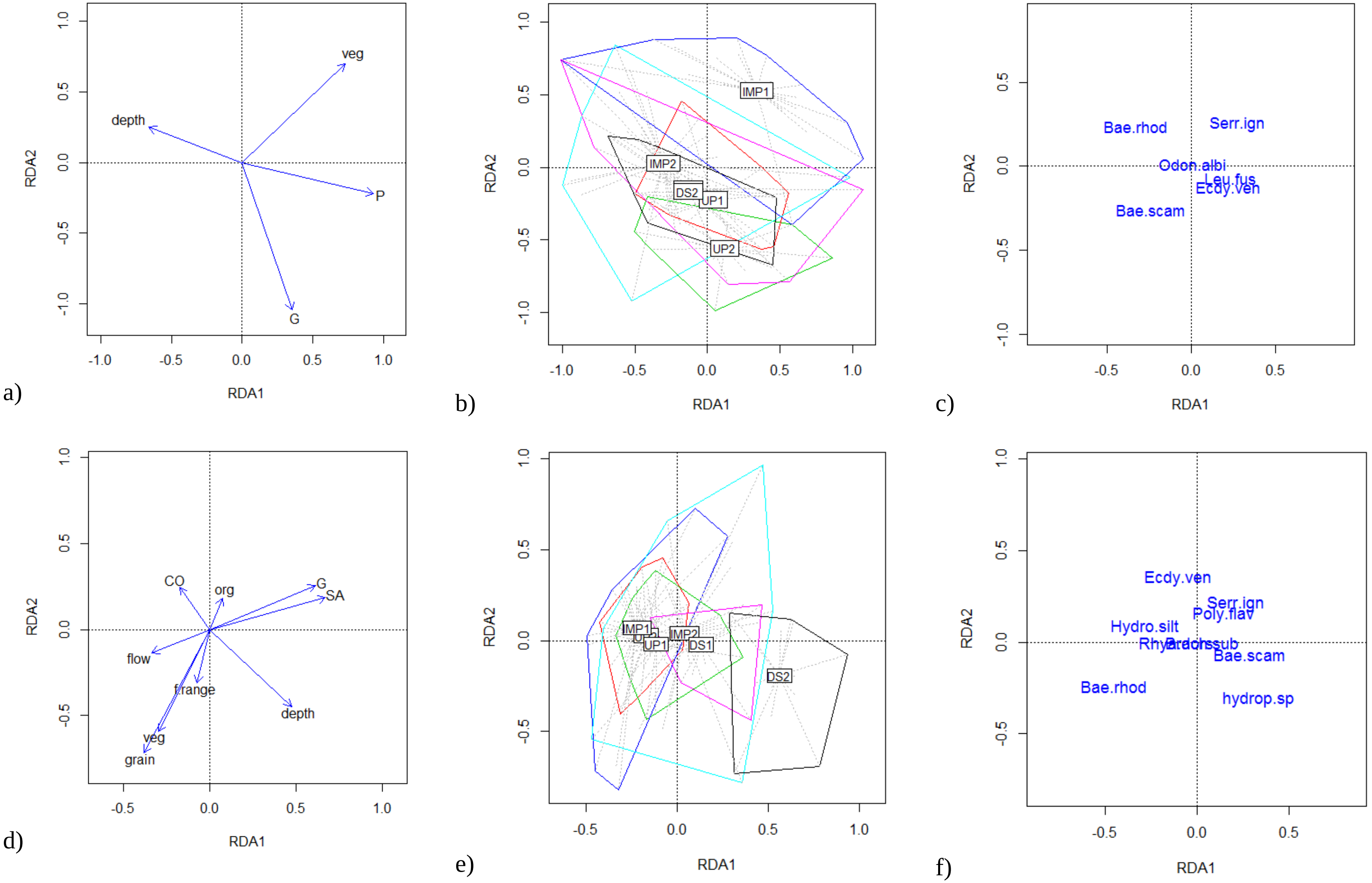


Fig 5.7 Summary of RDA solution. Showing the biplot for a) significant environmental vectors, b) location and hull of reach group and c) species locations, where abundance was explained by over 10% (2013) and 25% (2014) by the RDA for 2013. Repeated in d), e) and f) for 2014. A key to species abbreviations is given in Appendix 2.4. .

The change in riverfly community composition from one year to another was significant in all reaches (Table 5.3). Most significant changes over time occurred in US2 and IMP1, and a marked reduction in community diversity in DS1 (Fig 5.8e) Increases in sand cover explained the community change for many reaches (Fig 5.8 a and e), favouring *Leuctra. fusca* and *Hydroptila sp* in US2 2013, and *Baetis rhodani*, *Beatis scambus*, *Seratella ignata* and *Hydropsyche siltalii* in DS1. In some cases community change, could not be well described by changing habitat conditions e.g. in DS2 (Appendix 5.3) *E. geniculata* arrived in this section in 2014, but was not strongly related to any of the measured variables, nor was the loss of *R. dorsalis*. A change in grain size best explained community change over time in IMP1. Species *Leuctra fusca*, *Seratella ignata* and *Rhyacophila dorsalis* were associated with larger sediment and greater cobble cover occurring in 2013. There was little change in community composition in IMP2 over time.

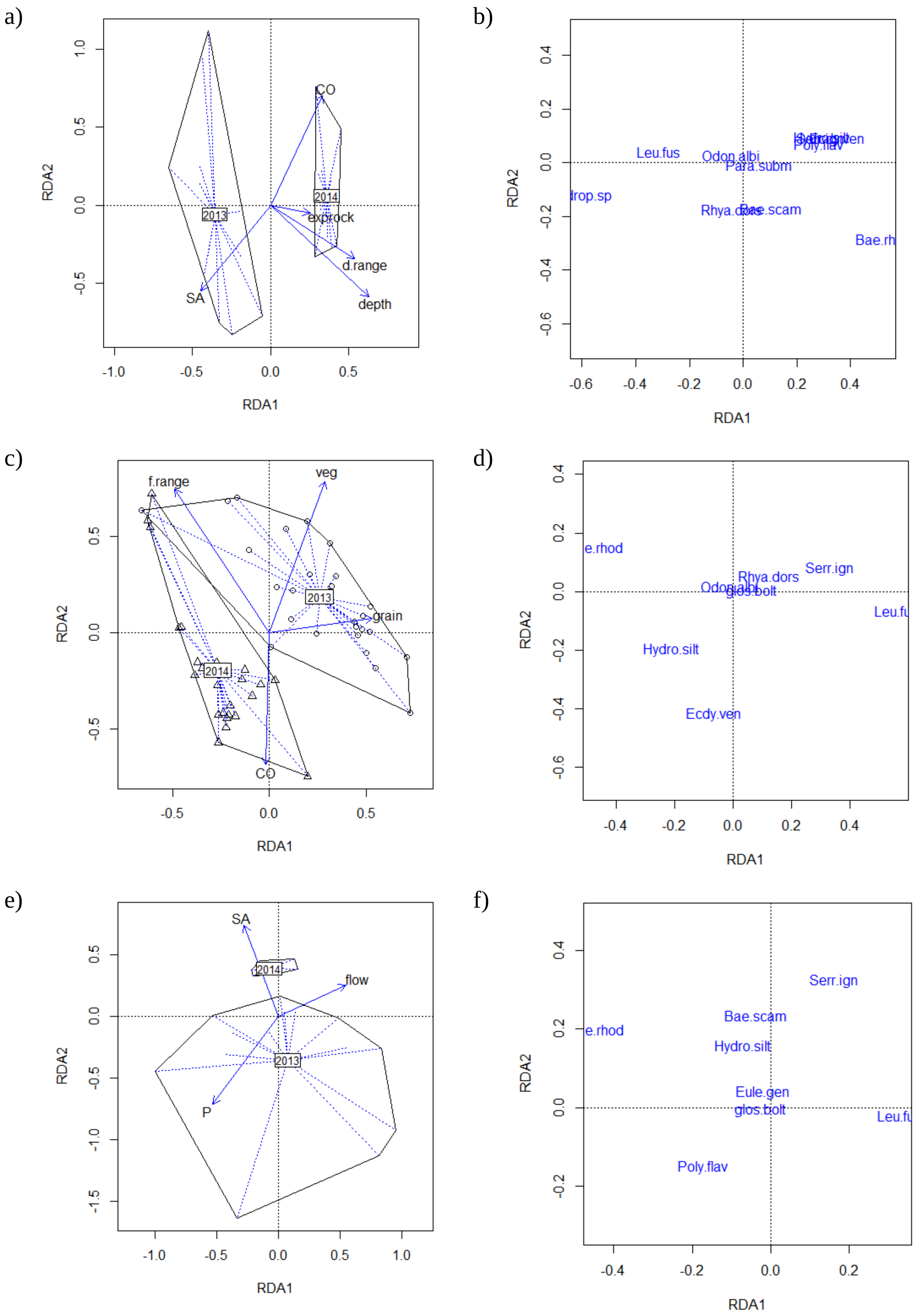


Fig 5.8 Biplots of RDA analysis within each site for both 2013 and 2014 illustrating change over time of species composition of samples, and arrows indicate the environmental gradients for a) US2, c) IMP1, d) DS1, and species associations to sites and gradients, for b) US2, d) IMP1 and f) DS2. A key to species abbreviations is given in Appendix 2.4.

When reaches and sample periods were considered separately, the environment drivers of within site community change, i.e. from one cell to another, were highly variable (Table 5.3). Communities within upstream sites were structured by local hydraulic factors and organic materials, impacted and downstream sites by sediment and hydraulic factors. Communities sampled in the second year were better explained by local environment compared to 2013. The exception to this was DS1 in 2014, when only water velocity was significant, not sediment or channel features as for the other downstream site. Due to the high variability of significant variables between reaches and over time, attributing a change in structing local habitat conditions to engineering impact would be uncertain.

Table 5.3. Variables selected by the within site RDA forward selection, year selected (1=2013, 2=2014) and adjusted R^2 for the reduced model. Variables underlined were significant in more than two reaches in one year. Importance of year to explain composition (R^2).

Site/Variable	US2	US1	IMP1	IMP2	DS1	DS2
R^2: 2013 (1)	9.5	11.4	17.1	10.6	35.1	11.7
R^2: 2014 (2)	35	19.5	43.6	47.8	13	30.5
Year (R^2)	47	18	27	13	16	18
Boulder			2			2
Cobble		1				
Pebble				2	1	2
Gravel						2
Sand			1,2	2		
Grain size				1		
Depth	1			2		
Depth range		2	1			1
Flow velocity					2	
velocity range	2	2	2			
velocity variability				2		
Vegetation	2					
Organic	2					
Exposed rock					1	

Table 5.4 A summary of the species environment relationships that explained species abundance within sites or over time, and the site and year (13/14) in which they are observed, if no year is stated this relationship is maintained in both sample years. (F.range- velocity variation, Veg- % vegetation cover, Grain -grain size, P- % pebble, SA - % sand, BO - % boulder, CO - % cobble).

Species	BO +	Flow+	F.range +	P+	SA +	SA -	Grain +	Veg +	Veg-	CO+	Depth+	Depth -
Baetis rhodani	DS2 13 IMP1 14	IMP2	IMP2	IMP2 DS1	DS1 14	UP114		UP1			UP2	IMP2
Hydroptila.sp	IMP1	DS1	UP113	IMP1	DS1 14 UP2 13							IMP2
Seratella ignata		DS1			DS1 14	UP114	IMP1 13	UP1 14		UP2 14 IMP1 13	IMP2	
Leuctra fusca		DS1 13 IMP2	IMP 2 UP1 13 DS1 13		UP1 13 UP2 13	DS1 13	IMP1 13	IMP1 13		IMP1 13	IMP2	UP2 13
Polycentropus flavomaculatus				DS1 13		DS1			UP1 13	UP2 14		
Hydropsyche siltalai			IMP1 13		DS1 14	UP1 14		UP1		UP2 14		
Baetis scambus			IMP1 13		DS1 14 UP2			UP1			UP2	
Ecdyonurus venosus		DS1	IMP1 13		DS1 14			UP1		UP2 14	IMP2	
Rhyacophila dorsalis		IMP2 DS1	IMP1 UP1 14		UP2 13 UP1 13			IMP1		IMP1		
Baetis muticus						UP1		UP1				

5.4.3 Spatial grain and autocorrelation

There was a clear pattern of increasing or decreasing significance with a change in grain size for several variables, particularly the smaller sediments, sand, gravel and pebble (Fig 5.9-Fig 5.11). Most variables that were significant at larger grains were also significant at the smallest. However, vegetation cover, trash and exposed rocks were only significant when their presence was evaluated over a larger upstream area.

Most of the single environmental variable models were insignificant to explain riverfly community composition, especially after the SAC was removed from the response data. More environmental variables were significant in 2014 for all six study sites, and communities in impacted sites could be best explained by local habitat. Certain habitat structures were less affected by SAC however, for example percentage cobble was not reduced by spatial detrending, compared to the smaller sediments, pebble, gravel and sand coverage. Hydraulic variables were also strongly affected by SAC, whereas organic material and vegetation were only slightly reduced.

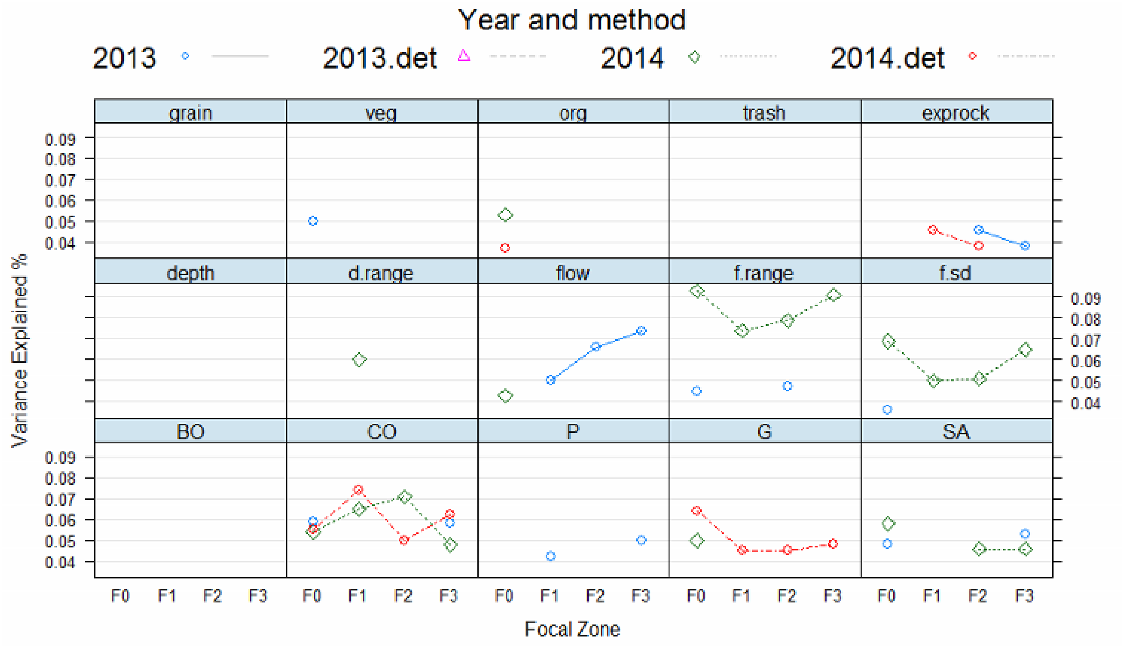


Fig 5.9 Plot of model fit - R^2 from RDA of Upstream communities with each environmental predictor (T, using spatially detrended (2013.det, 2014.det) or non detrended data at each focal extent.

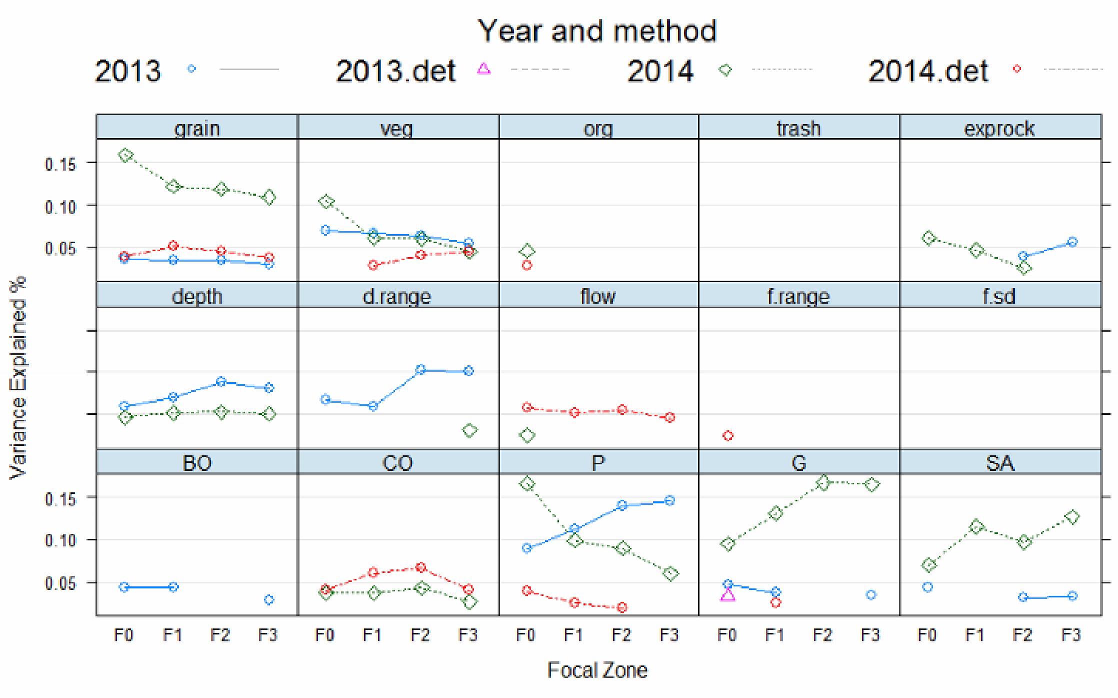


Fig 5.10 Plot of R^2 from RDA of Impacted communities with each environmental predictor, using spatially detrended (2013.det, 2014.det) or non detrended data at each focal extent.

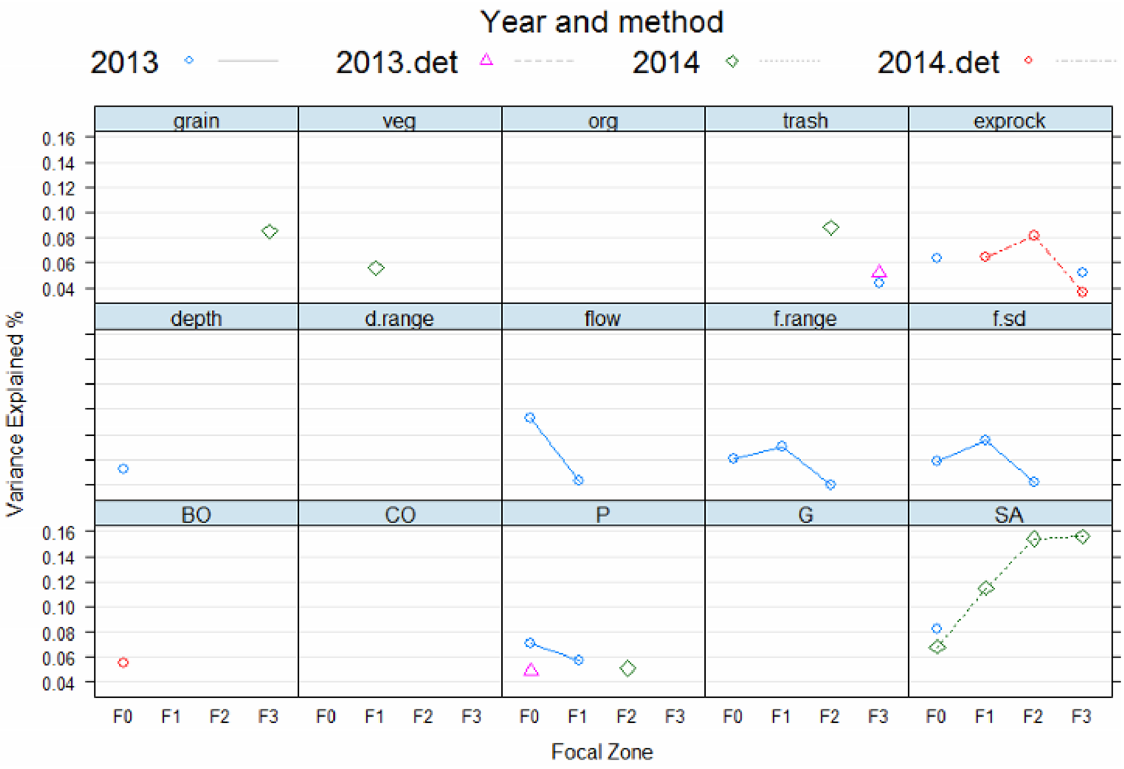


Fig 5.11 Plot of R^2 from RDA of Downstream communities with each environmental predictor, using spatially detrended (2013.det, 2014.det) or non detrended data at each focal extent.

5.4.4 The interaction of sample grain with species locomotion traits

The pooled psuedo-species (fast, medium, slow moving taxa) varied in their relationships to local environmental gradients. Additionally, the relationship type and strength for each mobility group changed depending on the spatial grain of measurement, impact site and survey year (Appendix 5.4). There was however an effect of habitat disturbance in the impacted sites, which resulted in a change in the best sample grain from large to small for all mobility groups. Despite these highly variable and context dependent findings some general relationships between fast and slow groups and environmental gradients could be observed, and are summarised in Table 5.5.

Table 5.5 A summary of the presence and direction of relationship between environmental variables and pooled mobility trait category at each spatial scale observed from RDA biplots. (F= Fast and S= Slow).

Sample Zone	F0		F1		F2		F3	
Upstream	2013	2014	2013	2014	2013	2014	2013	2014
Sand	S-	F+/S-	F+				F+/S+	
Flow velocity		F+/S-					F+/S-	
Flow range	F+		F+/S-		F+/S-			
Vegetation					F+/S-			
Impact								
Grain size			F+/S-		F+		F+	
Vegetation							F+	
Downstream								
Sand	F+/S-			F-		F-	F-/S+	
Flow velocity	F-/S+							
Grain size								F+

Percentage sand and vegetation cover, flow velocity and grain size were more consistently significant at a range of sample grains. Fast taxa were positively associated with sand at all sample grains, except in downstream sites where their abundance reduced in samples with larger sand patches. Slow taxa had low abundance with high sand cover at small sample grains but higher abundances when sand was present over a larger extent. Fast taxa abundance was high only at larger extents of high flow velocities, but showed a variable relationship to local flow velocity. Slow taxa were negatively associated with high velocity patches in upstream sites, but positively associated in downstream sites. Fast taxa had higher abundance with larger and highly vegetated patches. Fast taxa were also more abundant in patches of all extents with larger grain sizes.

5.4.5 Variance partitioning of spatial and local environmental contributions

In many reaches and sample years, more than half of the significant environmental structuring can be attributed solely to its spatial arrangement (Fig 5.12, significant variables summarised in Table 5.3). However, the degree of spatial autocorrelation of environmental variables was not consistent within year or reach, for example UP2 2013 and DS2 2014, had no shared fraction, indicating that these models explaining community composition had no underlying spatial structure. However, for UP1 2013, and IMP1 2013/4, and DS2 2013 after accounting for SAC the environmental component was no longer significant.

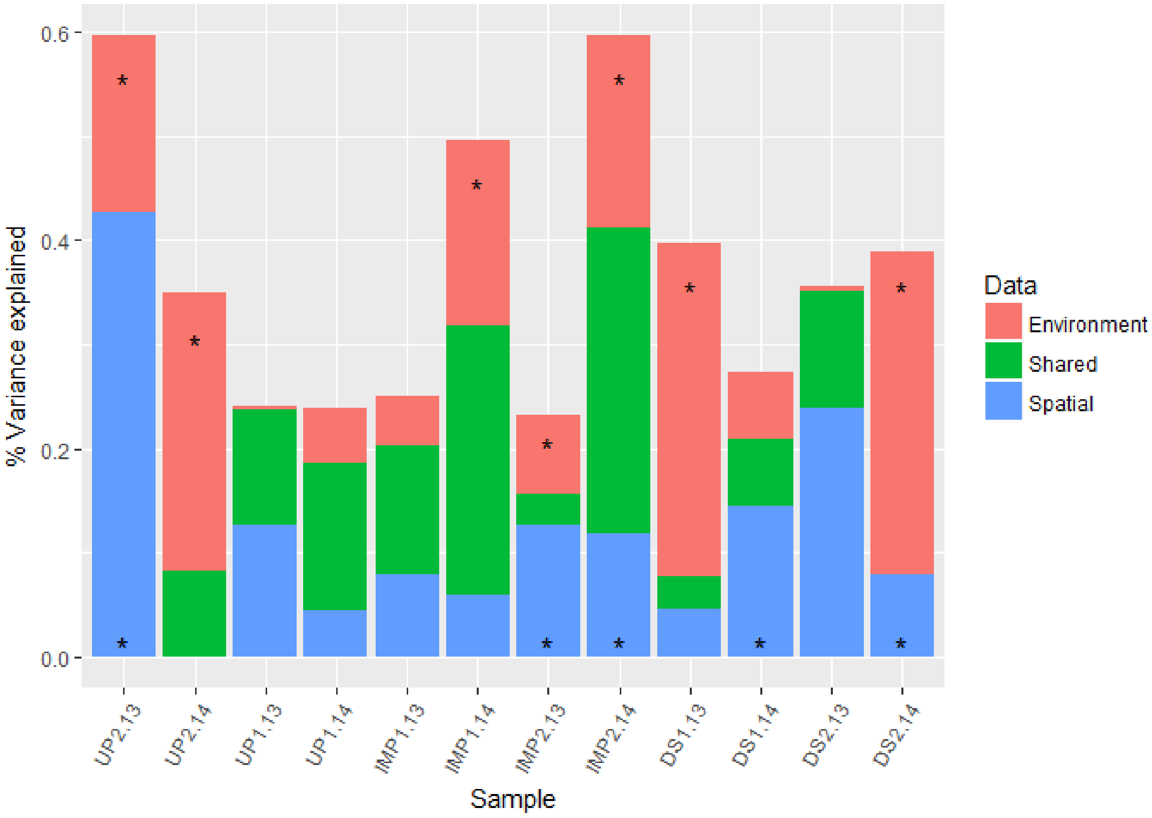


Fig 5.12. Selected fractions from variance partitioning. Amount of variance in community assembly explained by unique environmental, unique spatial (MEM, AEM and XY summed) and any shared fraction between environmental and all spatial. * indicates significance of unique fraction of 0.05 or higher.

Across most sites in the second sample year the shared spatial and environmental portion increased, meaning the local community arrangement although generally better explained by environmental variables, a significant amount of this was due to the spatial structure of the habitat. Models including small sediment variables, pebble and sand, and hydrology were the most highly spatially structured. Variables that were least affected by SAC were vegetation cover and organic material, indicated by a small shared fraction.

Despite the universal presence of flowing water, in many cases directional spatial variables were not significant in determining the riverfly community composition within the reach (Fig 5.13). In half the cases where they occur the unique AEMs fraction was not significant. The contribution of directional and non-directional spatial variables to community composition was highly variable over time and across sites, and was not clearly related to sampling year or impact. Significant unique fractions of flow created spatial structure were only found on 3 occasions in UP2 2013, IMP1 2014 and DS1 2014.

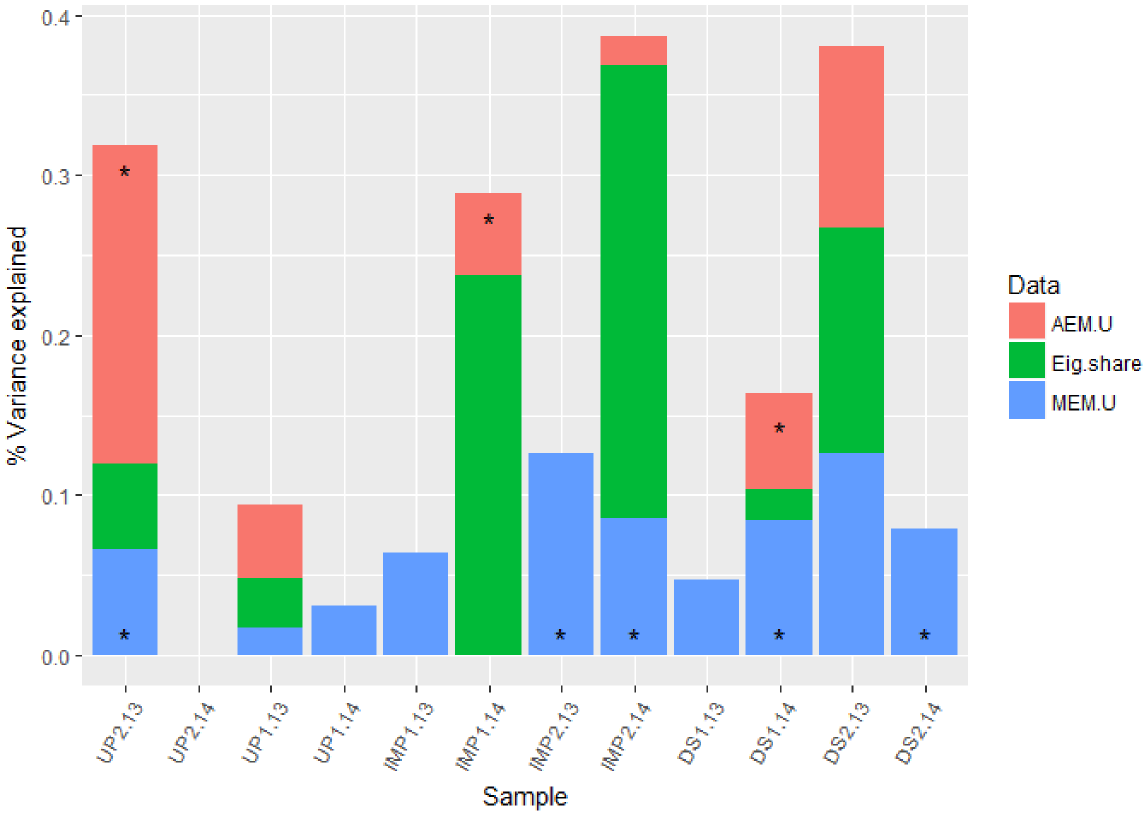


Fig 5.13. Selected fractions from the variance partitioning. Amount of variance in community assembly explained by unique types of spatial variables: MEMs and AEMs and any shared fraction. * indicates significance of unique fraction of 0.05 or higher.

The presence of a significant unique AEM fraction did not appear to coincide with the presence of significant hydraulic variables. Although there were occasions where AEMS were significant and variables of depth range and flow range were selected as environmental predictors, other cases also had significant hydraulic variables but no significant AEMS.

5.5 Discussion

5.5.1 Urban stream spatial patterns

This study confirmed what several others have previously shown (Wallis et al. 2012, Liu et al. 2013), namely that spatial patterns, MEMs more so than AEMs, were significant explanatory factors of stream invertebrate community assembly. Within certain reaches riverfly communities exhibited clear patterns of arrangement into locations within the channel. This premise forms the basis of the physical biotope approach, or functional habitat (Padmore 1997), as well as the multi-habitat sampling approach used by many (Hering et al. 2003). In the present case, differentiation of communities into edge and centre, longitudinal or small scale patches was confirmed using a bottom-up approach, with patterns being derived from the community data, removing potential bias caused by surveyor interpretations which result in scale or pattern mismatches.

Unique spatial structure can originate from two sources described as, (i) exogenous, relating to unmeasured environmental variables that have a spatial structure, and (ii) true spatial autocorrelation relating to purely endogenous processes (Fortin and Dale 2011, Bonada et al. 2012), such as within community movement (Lancaster and Briers 2008), changing lifecycle requirements or territory. This study showed there was a significant amount of within community unique spatial structure, typically highest in all sites in 2013.

Pattern, scale and significance of spatial structure was variable between sites and overtime, suggesting a high dynamism of small scale patterns. Dredged and sediment impacted sites showed reduced significance of spatial patterning of riverfly communities, and a change to broad scales. This could be a result of the removal of habitat diversity and homogenisation in IMP1, which experienced a loss of range of depth and velocities and large grain sizes (4.4.2.1). In the absence of fine scale patterns of bed features, the major driving force that provided structure to invertebrates was flow, supported by the presence of significant AEMs in 2014. In upstream undisturbed reaches, patterns changed over time to finer scales, but maintained a consistent edge and centre arrangement. Corresponding habitat changes in these sites did not offer a clear explanation for natural pattern and scale fluctuations, but short term variability of antecedent flow conditions (Fig 4.10) could facilitate the development of community driven spatial structure.

Though this interpretation is limited by using a short term snapshot of community arrangement, and would require replicated temporal sampling to confirm.

Three types of spatial pattern were observed in the community composition: edge and centre, longitudinal and fine scale patches. Over time, distinct channel sections establish, made of geomorphic units occupied by a spatial pattern of biota (Petts 2000). These units and patterns vary within a stream, and are removed, and re-establish through the interaction of physical channel structures and fluvial geomorphic processes over time. The structures identified through spatial analysis of the community indicate the possible geomorphic mechanisms or conditions that cause community assembly. Edge and centre patterns, or edge effects can be caused by the influence of a strong central current or significant riparian structures that interact with the channel, such as trailing or overhanging vegetation or eroding banks. This study supports findings by Armitage et al. (2001), that bank margins are diverse entities where complex changes in flow, vegetation, and bank morphology create a mosaic of habitat patches. This could be specifically significant in urban, chemically and physically disturbed streams, compared to adjacent riffles which Roy et al. (2003), showed to exhibit decreased richness and density. However, edge sites are often excluded from studies (Furse et al. 2006). This could lead to a significant underestimation of diversity in disturbed environments such as urban streams where fluctuating flow regimes imply that transitional habitats may be extensive.

Directional patterns, AEMs, are expected to be found in systems where there is an evident directional driver, such as flowing water (Blanchet et al. 2011). It is thus recommended to use AEMs wherever this could be expected. However, these results showed that directional spatial patterns were less frequently observed in the community assembly than MEMs, and where present, they were rarely uniquely significant. Variance partitioning showed there were only three cases where AEMS were uniquely significant (US2 2013, IMP1 14, DS1 14). Thus, AEMs shared a large amount of explanatory power with non-directional MEMs. Conversely, and albeit at a larger spatial scale, Cauvy-Fraunie et al. (2015) found a strong directional influence present in benthic macroinvertebrate communities in glacial streams, due to glacial meltwater. Interrogation of the type of spatial patterns should be encouraged to better understand the underlying forces of community assembly.

The lack of strong directional patterns in the present study could indicate a number of things. Firstly, that small scale flow-mediated invertebrate movement, was less significant compared to active movement. Secondly, that in-channel resources and habitats that cause community arrangement are themselves less structured by flow, for example due to a high frequency of disturbance or immobility. Although AEMs were not often significant, when present they had high explanatory power. The variable nature of AEMs on a local scale, between sites and within sites over time, can be attributed to the high temporal variability of reach scale hydraulics, especially at the spatial scales considered here. By using a single snapshot sample, the measured importance of local hydraulics in reach scale stream patterns could vary strongly with stage of the hydrograph at the time of sampling. The presence of significant AEMs did not appear to be correlated to the direct measurements of cell level hydraulic variables, indicating that AEMs described a broader scale community organising force, distinct from cell scale hydraulics. This was confirmed by the occurrence of AEMs consistently at broad scales. This combination of significant reach scale and cell level hydraulic variables, supports findings by Lamouroux et al. (2004), Pedersen & Friberg (2007) and Cortes et al. (2011) that flow hydraulics can be important at a range of spatial scales to describe stream bed invertebrate community assembly.

A limitation of the focal statistics spatial extent design, assumes that a taxon's main direction of movement is downstream. However, this may not always be the case (Lancaster et al. 2006), and is further called into questioned by the low overall significance of AEM patterns. This suggests that a range of focal shapes could be explored, and targeted at certain groups of taxa, e.g. filter feeding or predatory, that may exhibit a specialist type of spatial arrangement. Overall this study found a complicated and variable pattern of significance of unique and joint spatial effects which are often overlooked at this small spatial extent, i.e. within the reach scale. Further exploration of these patterns through mapping of ordination scores (Borcard and Legendre 1994), overlain by physical habitat measurements may be helpful. Considering empirical data on the scales and rates of invertebrate movement, which can be substantial (Townsend & Hildrew (1976) - 'continuous redistribution'), in studies of species-environment relationships may be especially worthwhile, since our understanding of how movement behaviour produces patterns is poor (Lancaster and Briers 2008).

Considering taxa mobility traits helps to understand and explain variation in spatial patterns by suggesting that mechanisms underlying species sorting could be related to expected rates of movement at a local scale. Species belonging to different trait groups respond differently because they require resources that have different spatial arrangements, e.g. algal grazers verses predators (Cooper et al. 1998), and therefore perceive distance and connectivity differently. Admittedly, the relationship between spatial patterns and mobility trait groups was highly variable in its importance, type and scale for both directional and non-directional patterns. However, a few interesting trends were uncovered and are discussed below.

Slow moving taxa, rather than fast, were the best explained by MEM and AEMs, indicating a stronger spatial structure, particularly of fine scale patches across the whole channel width. Composition of fast-moving taxa was generally poorly explained by spatial variables, but did show strong channel edge and centre patterns, indicating differentiation into areas of fast/deep centre and slower shallower edges. Poor performance of spatial variables might be due to high rates of movement of more mobile taxa between sample patches, that are not well captured using small scale short term sampling. Fast moving taxa might also use inferior patches as stepping stones for movement. Spatial pattern and scale results were highly context dependent, with both fine (0.8) and broad (0.2) scale patterns identified in different sites for an identical community (DS1 2014 and IMP2 2014). This suggests a complex interaction between the full suite of biological and ecological traits each species possesses, which act to trade-off against each other (Resh et al. 1994), with the multiple environmental factors that determine niche occupancy.

5.5.2 Scale dependency of community turnover and local control

Environmental drivers of community turnover at three spatial scales were considered in this study; between reaches within the river, between samples within each reach and within a reach over time. Using each arrangement of data was an explicit consideration of how spatial or temporal grain and extent of samples might affect observed patterns of beta diversity (Barton et al. 2013). The identity and significance of environmental variables changed depending on the extent considered, but increased in the second year, when species richness across all sites was greater. Urban stream taxa are characterised by their high stress tolerance in comparison to non-urban streams (Walsh and Webb 2016), which might suggest low responsiveness to those environmental gradients present. Contrary to this, a number of taxa were found to be sensitive to the small scale changes in physical habitat conditions within and between sites.

Local hydraulics were consistently an important factor in shaping the community, confirming the identification of small scale hydraulic variability maintained by bed roughness as an important habitat feature discussed in chapter 4. Many communities within each sample reach and time period, responded similarly along this gradient of local hydraulics. This confirms findings of Lamouroux et al. (2004), that high and low flow variables, measure by Froude (Fr) number, are important at the microscale. In dredged and sediment impacted sites community changes were explained by the physical habitat properties that had been altered by the impact. After dredging site IMP1 experienced a loss of taxa associated with deep waters and slow flows, reflecting the loss of channel structure and loss of variation in bed slope. Within site analysis showed that, although reduced, hydraulic heterogeneity remained important in structuring the community. Downstream sediment impacted reaches differed from the others more strongly in the second year, being characterised by communities of deeper, slow flowing, habitats with sands and gravels. Change over time, indicated that the reduction in beta diversity experienced in DS1 in 2014 could be explained by a sand gradient. Sand effects on diversity are commonly reported, due to the substrate itself and its unstable nature (Paul and Meyer 2001), selecting for a reduced set of sand tolerant taxa.

Identifying how a variable limits species abundance or community composition can be complex. The habitat differences within sites from one year to the next (Table 4.3) were not necessarily the drivers of the change in community identified here by direct gradient analysis. Similarly, a change in community composition could not always be attributable to an environmental gradient or even any of the single variables measured, highlighting the need to model community changes directly to identify causation not correlation. As an example, community turnover, and diversity reductions from 2013 to 2014 in site DS1 were strongly linked to communities associated with high amounts of sand, whereas the PCA (Table 4.3) indicated that amounts of sand in this reach had actually reduced over time. This suggests that sand was a limiting resource and strongly influenced the communities more than other more widely available resources or habitats. This contradiction can also be seen in the species response to environmental predictor changes over time, with conflicting associations between taxa and certain conditions in different reaches (Table 5.4). These include both positive and negative associations with sand cover, depending on presence in upstream or downstream locations. The results of variable reduction using ordination analysis are only relevant to the specific dataset used in each analysis, rather than absolute and easily interpretable values. Although it can be illuminating to compare and contrast between spatial extents, analyses using each data set is asking a specific question about relationships between those specific communities and environmental conditions that may not be transferable to other situations (Heino et al. 2012). Model fit and the significance of local environmental sorting increased across all sites in the second year. This may show how low species richness limits model power, and the ability to infer habitat quality from community properties and turnover. Ordination analysis are useful as exploratory preliminaries to testing more specific species-environment relationships (Downes 2010) using non-central tendency measures such as quantile regression (Lancaster and Belyea 2006) that can better describe upper and lower ranges of limiting variables, or manipulation experiments.

5.5.3 Effects of sampling grain and spatial autocorrelation

Although rivers are often described as patchy systems (Thorp et al. 2008), there has been little empirical quantification of what "patchiness" actually is (Cooper et al. 1997). The high resolution sampling combined with focal statistics in this study proved a useful method to investigate patch size and its importance for stream biota. This study illustrated the changing importance of environmental variables based on the patch character of the variable, and how the patch might be used or perceived by the stream biota based on its rate of movement. This supports the use of a sliding window of sampling (Fortin and Dale 2011), even within studies at the microscale, appreciating that factors outside the immediate sample area can influence observations.

The importance of some variables changed clearly with a change in sample grain; sand, pebble and gravel for example, however the significance of the effect on biota community composition was often removed once SAC was removed. Vegetation cover and the presence of trash and exposed rock features were only significant when considered at larger sampling grains. Large rock features in streams can be an important habitat, for example boulders create a local diversity of flow and sediment distributions as well as providing refuge areas during high flows (Townsend 1989). Additionally, they are importance as emergent features for oviposition and emerging insects. In urban streams, large rock features commonly derive from unnatural collapsed stonework but could exceed their importance in more natural water courses, and so care must be taken to record their presence at potentially larger scales than those of biotic samples.

Many studies implicitly survey environmental variables at a range of nested sampling grains either for practical or arbitrary reasons. The significance of certain sample grain sizes, indicates that certain biological and environmental mechanisms are important. Firstly, significance could be driven either by patterns and processes exhibited by members of the invertebrate community, for example having specific requirements at a certain scale, e.g. territory (Sjostrom 1985). Secondly the scale of the environment could indirectly indicate the presence of another important or unmeasured features e.g. the presence of coarse particulate matter, twigs, leaves etc. indicates the availability of riparian trees and vegetation with links to the channel. Thirdly the size of a sampling unit alters the chance that the important habitat feature is recorded, depending on its size or arrangement. For instance, does sampling cover boundary habitats, the full extent of patch sizes, or sufficient extent to capture the occurrence of infrequent features? This study suggests that presence of easily observable features such as trash pieces, exposed large sediments and estimates of vegetation cover, are significant and could easily be incorporated in surveys when occurring within the vicinity of a biological sample, in a similar way to the "sweep up" of the RHS (Raven et al. 1998).

This study found that large amounts community assembly explained by the physical variables modelled at all grain sizes individually, was due to spatial autocorrelation (SAC) among the community sampled. This was also confirmed in the variance partitioning of the most adequate forward selected models, where more than half of the environmental structuring could be attributed to its spatial arrangement, thereby making many predictors insignificant. This meant that much of the species composition was determined by spatial location, or underlying spatial patterns rather than a result of response local environmental conditions, termed environmental filtering.

The importance of SAC is well appreciated (Lennon 2000, Dormann 2007, Legendre and Legendre 2012), as is its potential to bias community-environment models. These results are in agreement with Bonada et al. (2012), who also found strong spatial structure in community composition in Mediterranean streams. Conversely, at larger spatial scales, for example whole river basins, Grönroos et al. (2013) found spatial structuring was generally negligible, and that active dispersers with terrestrial adults showed strong environmental control. In combination with these findings, this research suggest that amount of SAC is dependent on the scale and system under study. Furthermore, spatial structure can vary within a scale and study system. Lloyd et al (2005), warn that one cannot assume that even similar and geographically close rivers will have the same degree of spatial patterning of environmental factors. This poses a difficulty for ecological understanding and its application to restoration. However, Dormann (2007) argues that though spatial models may not be transferrable in space, since the SAC correction is based on the specific local neighbourhood, the spatial bias present in assessment of environmental filtering must be addressed. Commonly used non spatial models, are also biased by this spatial configuration, however it remains un parameterised, and a potentially high source of error.

Disturbance has been suggested as a cause of SAC (Dormann 2007, Grenouillet et al. 2008), however in this study impacted (IMP1 and IMP2) communities showed less influence of SAC for individual variable models, compared to other sites. This contrasts with results using the best site-specific models in variance partitioning. In this second case, although impacted sites did show an increase in unique environmental significance, the joint spatial component also increased. This means that there was a corresponding increase in the importance of the spatial structure of environmental control on the community distribution. This contradiction highlights the bias that accompanies modelling of species- environment relationships before accounting for SAC, which by default favours variables that are highly spatial autocorrelated (Lennon 2000). Depending on the aims of the study it can be enlightening to spatially detrend community data before analysis, for example to determine unique factors of environmental control. Uncovering afterwards that significant portions of environmental drivers are important because of their spatial pattern, addresses issues of habitat spatial arrangement and structure. Some environmental factors were more affected by SAC than others. Cobble, organic matter and vegetation showed low amounts of SAC, compared to initially significant hydraulic and small sediments, which were no longer significant after detrending. A discussion on the significance or causes of the joint environmental and spatial component in variance partitioning, or induced spatial dependence is lacking in the literature. Patterns of individual variables are neglected, in favour of multivariate habitat indices or measures of dissimilarity (Lloyd et al. 2005).

The unique, non-spatial environmental fraction, represents the species–environment relationships associated with local environmental conditions (Borcard et al. 2011) or the environmental filtering model based on the niche of species in the community. The presence of a significant unique environmental fraction depends on both the habitat variable considered and the properties of the community. For example, a significant unique environmental fraction was found when community dispersal rates were intermediate (Heino, Melo, Bini, et al. 2015), otherwise other mechanisms (mass effects and dispersal limitation) tended to structure community. Considering the spatial extent used in this study, all locations are likely to be accessible by most individuals, and subsequently occurrence in a sample is more likely related to short term movement and use of small patches. This study shows that at these small scales, environmental variables were significant drivers of riverfly community and niche processes acted to sort taxa into discrete habitats.

5.5.4 Sampling grain community trait interaction

The combination of taxa trait information with multiple spatial and scale related patterns and processes is a growing field of ecology termed *Metacommunity Phylogenetics* (Peres-Neto et al. 2012, Tornwall et al. 2015, Seymour et al. 2016). This study found relationships between taxa movement rates and habitat variables at certain scales, that has implications for understanding the impacts of modification of in-channel habitat and patterns in stream ecology more generally. If an analogy is made between the larger scale process of dispersal, where weak disperser are better explained by local scale environmental factors than high dispersers (Grenouillet et al. 2008), we might suppose that slow, immobile taxa would show greater environmental control. Yet, this was not supported. The sampling grain of each trait group strongest model with environmental variables varied highly with site, sometimes with fast taxa best explained at the smallest grain size. There was however an effect of habitat disturbance in the impacted sites, which resulted in a change in the best sample grain from large to small for all mobility groups. This possibly reflects a loss of habitat diversity, whereby larger more complex habitat patches have not developed, or as a signal of early community succession, where resident taxa have dropped out from the drift due to the presence of specific resources (Arango et al. 2015). A number of relationships between fast and slow taxa groups between the amount and arrangement of sand present at small or large extents, indicates the importance of these small mobile particles. However, the absence of a clear relationship between mobility trait group and spatial scales of environmental variables is likely to be a trade off with other processes and biological properties, suggesting that a multiple trait approach might be more lucrative to understand the role of small scale habitat controls.

5.6 Conclusions

Spatial patterns were present in the riverfly communities at a range of broad and fine scales. Flow mediated directional patterns were less important, highlighting a flaw in the assumption that spatial patterns in streams will have a downstream direction. Three types of spatial patterns were identified; edge and centre, longitudinal and fine scale patches, which indicate the possible physical processes that generate these patterns. The importance of edges was significant, as marginal areas are often overlooked by many in small channels, however banks and their interaction with the channel may be a source of habitat and biological diversity. In some cases, flow patterns were significant as were broad scale longitudinal patterns and directly measured hydrological variables, indicating that flow hydraulics can be important at a range of spatial scales to describe stream bed riverfly community assembly. The impact of dredging and sediment pollution was to alter spatial patterns from fine to broad, indicating biological effects of habitat homogenisation.

Environmental variables were significant drivers of community composition, mainly differentiating between samples within reaches rather than between reaches. Riverfly communities in all reaches were temporally unstable, changing significantly from one year to the next. Dredging and sediment pollution resulted in the loss of community and habitat diversity, and a change in community identity. Across all sites the presence of sand was a strong driver of community composition. The importance of individual environmental variables changed based on the patch size, which supports the use of a sliding window approach to sampling, depending on the target variable. Vegetation cover and the presence of trash or exposed rocks would be best surveyed at larger sampling grains. It was not shown that fast moving taxa were determined by factors at broad scales, or slow taxa by fine scales, which might be expected if considering locomotion as a small-scale version of dispersal. However, both fast and slow taxa groups were related to presence and extent of fine sediment patches depending on whether the resource was present at amounts that were either limiting or stress inducing. This highlights a general caveat of ordination findings, that significant variables and species-environment relationships uncovered, are dependent on the question posed and the gradients present in the data set used, and must be treated with caution if applying to other sites.

Spatial autocorrelation was a significant problem that rendered many single environmental variables insignificant as predicted of community composition. This problem causes the overestimation of environmental filtering in studies which do not account for SAC. Hydraulic variables and small sediments were most affected by SAC. Although a problem in modelling which assumes independence of sampling, the SAC patterns of habitat and biota are an interesting aspect that is not well studied or understood. The explicit consideration of spatial location to either remove SAC or investigate its patterns directly is an area of stream research that has much potential for further exploitation. Eigenvector analysis detects multiscale spatial patterns of community assembly, which integrate many relevant physical and biological factors and relationships of interest to stream ecologists. From a monitoring perspective, the optimum scales and pattern of survey design for accurate understanding of invertebrate community dynamics remain elusive (Lancaster and Briers 2008); a small scale and nested sampling approach will be most useful to uncover these. There is great potential to combine new spatial techniques with multiple trait data and wider taxa groups beyond riverfly, that possess more trait variety, to look at spatial arrangement and resource use by invertebrates more generally, and how this changes over time or due to disturbance.

Chapter 6 – Conclusions and general discussion

Based on a long history of research via observation and manipulative experiments, there is a broad level understanding of the associations between invertebrates and variables such as velocity, depth, substrate and flow regime in streams. It is therefore unsettling that we now seem ill-equipped to predict the outcome of planned large scale changes to channel hydrology and morphology on stream macroinvertebrates. This comes at a time when rivers and their catchments are under mounting pressure from direct modification, land use intensification and from a legacy of decades of channelisation. Recent legislation, in the form of the EU Water Framework Directive, places a greater emphasis on the management of water bodies as holistic systems, situated within the wider landscape consisting of natural structures (in their broadest sense) and sustaining natural processes. This thesis addresses some of the key potential shortcomings in recent bio-assessment that others have alluded to (Miller et al. 2010; Friberg 2010; Haase et al. 2013), but which have rarely been explored in the context of direct channel manipulations.

A high spatial resolution sampling approach applied to the Rottal Burn channel realignment project in Chapter 2, revealed that measures of average sample richness were ineffective in uncovering differences between the straightened homogeneous channel and restored meandering channel. However, reach scale beta diversity and turnover of species from one sample to another successfully reflected the increase in habitat diversity brought about by the restoration. In depth investigation of species composition, retaining both species identity and low abundance taxa, showed how reach scale species richness gradually increased, through the arrival of rare and specialist taxa into novel habitats.

Chapter 3 set the upstream versus downstream comparison approach as a basis for evaluating restoration projects, within the wider constraints of the catchment geomorphic and biological potential. It is only at this scale, when replicate streams were considered, over the same time period and over two seasons, that an interaction between year and season becomes apparent, negating temporal replication. The large variation in traditional diversity indices between channels unaffected by man-made disturbance also becomes apparent. Replicate streams of varying planform and habitat diversity, showed how more heterogeneous channels could maintain high gamma diversity over time, during possible periods of high antecedent flow stress by providing a number of low flow refuges along their length. Streams in this study exhibited riverfly community and diversity properties that were not related to any *a priori* defined channel type but related more strongly to channel identity. When placed against this catchment background the restoration trajectory of the Rottal Burn is favourable. However, any single stream would have provided an unstable benchmark for comparisons and the choice of a comparison stream would have affected the judgement as to the level of success of the restoration. The geographic setting in an area of naturally dynamic hydromorphology and associated biota, has assisted in rapid establishment of functioning sediment transport and biodiversity patterns through the mechanism of species sorting.

A surprisingly high diversity of riverfly was uncovered in the high spatial resolution study of the urban Brox Burn in Chapter 4. Small scale hydraulic heterogeneity driven by bed roughness created a range of microhabitats that were highly temporally variable due to the flashy flow regime typical of urban water courses. Riverfly community responses to direct channel dredging could not be detected by measurements of average sample richness and diversity, however distinct changes were seen in gamma diversity, the identity of community members and their arrangement among sample patches. Although sediment release due to engineering activities was considerable, this was relatively short-lived and apparently had little detrimental impact on biodiversity, despite periods of high sediment loading. Indeed, the paucity of fine sediment may have been limiting to several taxa, as there was limited sediment supply from artificially protected banks, in line with many urban streams.

Chapter 5 took a more exploratory approach, looking at recent developments in spatial methods, and novel applications that can be used to unpick the direct role of physical habitat, from spatial autocorrelation and spatial dependency. By using riverfly mobility traits, some mechanisms were suggested for spatial patterns and scale dependency that were related to a species rate and mode of movement through the streambed landscape. This chapter confirmed the strong role of local hydraulics in community composition, however due to the discovery of strong spatial patterns and a high amount of spatial autocorrelation, especially in hydraulic variables, their significance warrants closer scrutiny. Several interesting spatial patterns were discovered, with mobile and less mobile taxa displaying distinct spatial arrangements. However, directional (flow-related) forces were less significant than expected, possibly due to the short-lived nature of flow mediated patterns when sampling at fine scales, or active re-distribution of biota.

Three key themes run through this body of work. Firstly, that simple biodiversity indices of abundance, richness and diversity are likely to be insufficient to capture the subtle changes brought about by alterations to hydromorphological structure or function. Secondly, that data collected through routine monitoring or using sample designs limited in their spatial or temporal replication, are unable to separate the signal of hydromorphological alterations from natural background noise. And finally, there is a problem of scale and context dependency in the relationships uncovered, meaning that general rules cannot easily be extracted from many studies.

The use of two seemingly disparate case studies of realignment in a natural upland catchment, and flood protection engineering in an urban stream, illustrate how these issues affect any study in stream ecology. In practice, from the invertebrate point of view, restoration and engineering both constitute an unexpected disturbance (Tullos et al. 2009), and findings from both degradation and restoration unite under the same topic (Wolter et al. 2013) of community resilience and resistance to hydromorphological change.

6.1 Describing biodiversity

Richness and diversity measures may be sufficient for routine monitoring and to detect long term biotic changes due to widespread pressures such as eutrophication or acidification that result in strong species filtering. However, the nature of hydromorphological disturbance is strongly physical and spatial. Turnover of species between sample locations at a range of scales is of more use for detecting these types of subtle changes. Combined with direct measures of habitat heterogeneity this provides an approximation of observed and expected concordance to species sorting, that, when compared to natural metacommunity processes for the study system, can indicate departure from natural processes and identify an impacted ecosystem. There are now many options to make full use of community count data to further understand patterns of beta diversity (Baselga 2013, Legendre and De Cáceres 2013). We are only limited by its interpretation (Anderson et al. 2011) and application at the correct scale (Barton et al. 2013).

6.2 Separating signal from the noise - sound sample design for impact detection

The strength of MBACI survey designs is well acknowledged, however they are rarely used in assessments of stream restoration projects (Kail et al. 2015), due to their highly resources intensive nature. Despite the long-standing appreciation of streams as highly dynamic systems across a range of spatial and temporal scales, replication of sampling in space is rare, while longer term monitoring of the same location over a number of time periods is also commonly neglected, in favour of space for time substitution approaches. Although time and resource intensive, the use of a range of regional comparators in this study provided an effective means of placing target sites in perspective, based on the natural dynamics and wider range of conditions to which the regional species pool is adapted. Sampling season was shown to be key in determining if derived metrics were sensitive to the target stress. Greater attention to seasonal diversity patterns, and persistence of patterns from one year to the next will improve our understanding of the sensitivity of certain indices. The recommendations of Downes (2010) regarding the employment of rigorous sampling design, and explicit consideration of scales relevant to the target taxa and physical system are strongly supported, so that resources for monitoring are not wasted.

All ecological data is inherently spatial, coming from a certain location set within the context of a wider riverscape. Yet this spatial context is either overlooked, or is lost through the physical pooling of subsamples. Additionally, scale mismatches between ecological and environmental data is a weakness when determining the drivers of community change. Data with strong spatial and temporal fidelity, at a range of spatial scales, may be needed at the outset until a greater understanding allows the sampling window to be adjusted to a manageable but adequate scale. New spatial techniques and multi-trait approaches are now at the disposal of ecologists to undertake this. Additionally, much data is already spatially referenced; it only takes careful application of these approaches to incorporate spatial aspects, whether to remove the ‘nuisance’ or reveal patterns that can then be interpreted, according to their environmental or biological context.

6.3 Dealing with context

Many cases of context dependence were uncovered in this study, leading to difficulty in defining general rules for riverfly community assembly, and contributing to what Lawton (1999) termed as the "mess" of community ecology. A number of methodological approaches have been employed in these chapters that help to define and quantify that context, and minimise noise that is due to true sampling error. Firstly, direct quantification of temporal variability in systems that are intrinsically highly variable, even in control or reference streams that appear outwardly similar, is required. This is consistent with the view of Downes (2010), that reaches are not representative. Secondly, robust statistical methods should be used that standardise measurements for sampling effort when comparing across habitats. Thirdly, treatment sites are better positioned on a gradient of their properties based on direct measurement rather than being assigned *a priori* to a descriptive class (e.g. control and impact). At the very least it is important to quantify the natural variability between members of that class.

There is no shortage of opportunity to advance our knowledge of the mechanisms that drive community response under a range of conditions by using monitoring of restoration operations, or indeed engineering impacts (Vaughan et al. 2009 and Feld et al. 2011), including negative results (Geist 2015). Exploiting these opportunities more systematically can only improve our ability to reconstruct past impacts, and to forecast the ecological effects of planned changes to the hydromorphology of rivers across a range of contexts.

References

Allan, J. D., 2004. Landscapes and Riverscapes : The Influence of Land Use on Stream Ecosystems. *Annual Review of Ecology and Systematics*, 35, 257–284.

Allan, J. D. and Castillo, M. M., 2007. *The foundations of stream ecology*. Stream Ecology: Structure and function of running waters. Dordrecht: Springer Netherlands.

Alonso, C., Aroviita, J., Baattrup-Pedersen, A., Bulletti, B., Brabec, K., Bogestrand, J., Catalina Perez, M., Dudley, B., Ecke, F., Friberg, N., Gothe, E., Greene, S., Gunn, I. D. M., Hajek, O., Hendriks, D., Jones, J. I., Kairi, K., Kalivodova, M., Komprodova, K., Kohut, L., Kraml, J., Laize, C., Larsen, S. E., Lorenz, A. W., Lebiedzinski, K., Mader, H., Mayr, P., Murphy, J. F., McDonald, C., Nemethova, S., Noble, R. A., O'Hare, M., Raapysjarvi, J., Segersten, J., Turunen, J., Verdonschot, P. F. M., and Vink, J., 2013. *Impacts of hydromorphological degradation and disturbed sediment dynamics on ecological status*. Available: http://www.reformrivers.eu/news/207 [Accessed: 2nd Oct 2016].

Altermatt, F., Seymour, M., and Martinez, N., 2013. River network properties shape a-diversity and community similarity patterns of aquatic insect communities across major drainage basins. *Journal of Biogeography*, 40 (12), 2249–2260.

Álvarez-Cabria, M., Barquín, J., and Antonio Juanes, J., 2010. Spatial and seasonal variability of macroinvertebrate metrics: Do macroinvertebrate communities track river health? *Ecological Indicators*, 10 (2), 370–379.

Anderson, D., Burnham, K., and Thompson, W., 2000. Null Hypothesis Testing : Problems , Prevalence , and an Alternative. *The Journal of Wildlife Management*, 64 (4), 912–923.

Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., Sanders, N. J., Cornell, H. V., Comita, L. S., Davies, K. F., Harrison, S. P., Kraft, N. J. B., Stegen, J. C., and Swenson, N. G., 2011. Navigating the multiple meanings of beta diversity: A roadmap for the practicing ecologist. *Ecology Letters*, 14, 19–28.

Anderson, M. J. and Walsh, D. C. I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, 83 (4), 557–574.

Arango, C. P., James, P. W., and Hatch, K. B., 2015. Rapid ecosystem response to restoration in an urban stream. *Hydrobiologia*, 749 (1), 197–211.

Armitage, P. D., Lattmann, K., Kneebone, N., and Harris, I., 2001. Bank profile and structure as determinants of macroinvertebrate assemblages-seasonal changes and management. *Regulated Rivers: Research & Management*, 17 (4–5), 543–556.

Bady, P., Doledec, S., Fesl, C., Gayraud, S., Bacchi, M., and Scholl, F., 2005. Use of invertebrate traits for the biomonitoring of European large rivers: the effects of sampling effort on genus richness and functional diversity. *Freshwater Biology*, 50 (1), 159–173.

Barnes, J. B., Vaughan, I. P., and Ormerod, S. J., 2013. Reappraising the effects of habitat structure on river macroinvertebrates. *Freshwater Biology*, 58 (10), 2154–2167.

Barton, P. S., Cunningham, S. a., Manning, A. D., Gibb, H., Lindenmayer, D. B., and Didham, R. K., 2013. The spatial scaling of beta diversity. *Global Ecology and Biogeography*, 22, 639–647.

Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19 (1), 134–143.

Baselga, A., 2013. Separating the two components of abundance-based dissimilarity: Balanced changes in abundance vs. abundance gradients. *Methods in Ecology and Evolution*, 4 (6), 552–557.

Baselga, A., Orme, D., Villéger, S., De Bortoli, J., and Leprieur, F., 2013. betapart: Partitioning beta diversity into turnover and nestedness components. Version 3.1. Available: https://cran.r-project.org/package=betapart [Accessed 3rd March 2014].

Bates, D. M., Machler, M., Bolker, B., and Walker, S., 2015. Fitting Linear Mixed-Effects Models Using {lme4}. *Journal of Statistical Software*, 67 (1), 1–48.

Beisel, J., Usseglio-Polatera, P., and Moreteau, J., 2000. The spatial heterogeneity of a river bottom : a key factor determining macroinvertebrate communities. *Hydrobiologia*, 422/423, 163–171.

Bernhardt, E. S., Palmer, M. A., Allan, J. D., Alexander, G., Barnas, K., Brooks, S., Carr, J., Clayton, S., Dahm, C., Galat, D., Gloss, S., Goodwin, P., Hart, D., Hassett, B., Jenkinson, R., Katz, S., Kondolf, G. M., Lake, P. S., Lave, R., Meyer, J. L., Donnell, T. K. O., Pagano, L., Powell, B., and Sudduth, E., 2005. Synthesizing U.S. River Restoration Efforts. *Science*, 308 (April), 636–637.

Von Bertrab, M. G., Krein, A., Stendera, S., Thielen, F., and Hering, D., 2013. Is fine sediment deposition a main driver for the composition of benthic macroinvertebrate assemblages? *Ecological Indicators*, 24, 589–598.

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., van de Bund, W., Zampoukas, N., and Hering, D., 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. *Ecological Indicators*, 18, 31–41.

Blanchet, F. G., Legendre, P., and Borcard, D., 2008a. Forward selection of explanatory variables. *Ecology*, 89 (9), 2623–2632.

Blanchet, F. G., Legendre, P., and Borcard, D., 2008b. Modelling directional spatial processes in ecological data. *Ecological Modelling*, 215 (4), 325–336.

Blanchet, F. G., Legendre, P., and Gauthier, O., 2014. AEM: Tools to construct Asymmetric eigenvector maps (AEM) spatial variables. R package version 0.5-2/r119. Available: https://R-Forge.R-project.org/projects/sedar/ [Accessed 8 March 2015].

Blanchet, F. G., Legendre, P., Maranger, R., Monti, D., and Pepin, P., 2011. Modelling the effect of directional spatial ecological processes at different scales. *Oecologia*, 166 (2), 357–368.

Bolker, B., 2015. Linear and generalized linear mixed models. *In*: Fox, G. A., Negrete-Yankelevich, S., and Sosa, Vinicio, J., eds. *Ecological Statistics: Contemporary theory and application*. Oxford, UK: Oxford University Press, 309–333.

Bonada, N., Dolédec, S., and Statzner, B., 2012. Spatial autocorrelation patterns of stream invertebrates: exogenous and endogenous factors. *Journal of Biogeography*, 39 (1), 56–68.

Borcard, D., Gillet, F., and Legendre, P., 2011. *Numerical Ecology with R*. New York, NY: Springer New York.

Borcard, D. and Legendre, P., 1994. Environmental control and spatial structure in ecological communities: an example using oribatid mites (Acari, Oribatei). *Environmental and Ecological Statistics*.

Borcard, D., Legendre, P., and Drapeau, P., 1992. Partialling out the Spatial Component of Ecological Variation. *Ecology*, 73 (3), 1045–1055.

ter Braak, C. J. F. and Šmilauer, P., 2014. Topics in constrained and unconstrained ordination. *Plant Ecology*.

Bradley, D. C., Cadman, D., Nigel, J., Mersey, H., and Forbes, J., 2012. *Ecological indicators of the effects of abstraction and flow regulation ; and optimisation of flow releases from water storage reservoirs*. Edinburgh. Sniffer . Available from https://www.wfduk.org/resources [Accessed: 29 November 2012].

Braid, A., 2014. Hydro-Morphological Assessment of the Rottal Burn Restoration Project. MSc, Abertay University.

Brierley, G., Reid, H., Fryirs, K., and Trahan, N., 2010. What are we monitoring and why? Using geomorphic principles to frame eco-hydrological assessments of river condition. *The Science of the total environment*, 408 (9), 2025–33.

Brooks, S. S. and Lake, P. S., 2007. River restoration in Victoria, Australia: Change is in the wind, and none too soon. *Restoration Ecology*, 15 (3), 584–591.

Brooks, S. S., Palmer, M. a, Cardinale, B. J., Swan, C. M., and Ribblett, S., 2002. Assessing Stream Ecosystem Rehabilitation: Limitations of Community Structure Data. *Restoration Ecology*, 10 (1), 156–168.

Brown, B. L., 2003. Spatial heterogeneity reduces temporal variability in stream insect communities. *Ecology Letters*, 6 (4), 316–325.

Brown, B. L., 2007. Habitat heterogeneity and disturbance influence patterns of community temporal variability in a small temperate stream. *Hydrobiologia*, 586 (1), 93–106.

Brown, L. R., Cuffney, T. F., Coles, J. F., Fitzpatrick, F., McMahon, G., Steuer, J., Bell, A. H., and May, J. T., 2009. Urban streams across the USA: lessons learned from studies in 9 metropolitan areas. *Journal of the North American Benthological Society*, 28 (4), 1051–1069.

Buendia, C., Gibbins, C. N., Vericat, D., Batalla, R. J., and Douglas, A., 2013. Detecting the structural and functional impacts of fine sediment on stream invertebrates. *Ecological Indicators*, 25, 184–196.

Carlson, P. E., Johnson, R. K., and McKie, B. G., 2013. Optimizing stream bioassessment: habitat, season, and the impacts of land use on benthic macroinvertebrates. *Hydrobiologia*, 704 (1), 363–373.

Cauvy-Fraunie, S., Espinosa, R., Andino, P., Jacobsen, D., and Dangles, O., 2015. Invertebrate metacommunity structure and dynamics in an Andean glacial stream network facing climate change. *PLoS ONE*, 10 (8), 1–19.

Chao, A., Chazdon, R. L., and Shen, T. J., 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecology Letters*, 8 (2), 148–159.

Chao, A. and Chiu, C.-H., 2016. Nonparametric Estimation and Comparison of Species Richness. *eLS*, 1–11.

Church, M., 2015. Channel Stability: Morphodynamics and the Morphology of Rivers. *In*: Rowinski, P. and Radecki-Pawlik, A., eds. *Rivers-Physical, Fluvial and Environmental Processess*. Spinger, 255–277.

Clarke, R. T., Furse, M. T., Gunn, R. J. ., Winder, J. ., and WRIGHT, J. F., 2002. Sampling variation in macroinvertebrate data and implications for river quality indices. *Freshwater Biology*, 47, 1735–1751.

Clarke, S. J., Bruce-Burgess, L., and Wharton, G., 2003. Linking form and function: towards an eco-hydromorphic approach to sustainable river restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 13 (5), 439–450.

Clausen, B. and Biggs, B. J. F., 1997. Relationships between benthic biota and hydrological indices in New Zealand streams. *Freshwater Biology*, 38 (2), 327–342.

Cockel, C. P., 2010. Alien and Native Plants of Urban River Corridors : A Study of Riparian Plant Propagule Dynamics along the River Brent , Greater London by. PhD, Queen Mary, Universoty of London.

Cooper, S. D., Barmuta, L., Sarnelle, O., Kratz, K. I. M., Diehl, S., Journal, S., American, N., Society, B., Mar, N., and Kratz, K. I. M., 1997. Quantifying Spatial Heterogeneity in Streams. *Journal of the North American Benthological Society*, 16 (1), 174–188.

Cooper, S. D., Diehl, S., Kratz, K. I. M., and Sarnelle, O., 1998. Implications of scale for patterns and processes in stream ecology. *Australian Journal of Ecology*, 23 (1), 27–40.

Cortes, R. M. V., Varandas, S., Teixeira, A., Hughes, S., Magalh, M., Barquín, J., Alvarez-cabria, M., and Fernandez, D., 2011. Effects of landscape metrics and land-use variables on macroinvertebrate communities and habitat characteristics. *Limnetica*, 30 (2), 347–362.

Covich, A. P., Austen, M. C., Bärlocher, F., Chauvet, E., Cardinale, B. J., Biles, C. L., Inchausti, P., Dangles, O., Solan, M., Gessner, M. O., Statzner, B., and Moss, B., 2004. The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. *BioScience*, 54 (8), 767–775.

Culp, J. M., Armanini, D. G., Dunbar, M. J., Orlofske, J. M., Poff, N. L., Pollard, A. I., Yates, A. G., and Hose, G. C., 2011. Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. *Integrated environmental assessment and management*, 7 (2), 187–97.

Day, N., 2013. River Engineering and Restoration Methods: an analysis of the impact and benefits of an intervention project. MSc, University of Abertay Dundee.

Dormann, C. F., 2007. Effects of incorporating spatial autocorrelation into the analysis of species distribution data. *Global Ecology and Biogeography*, 16 (2), 129–138.

Downes, B. J., 2010. Back to the future: little-used tools and principles of scientific inference can help disentangle effects of multiple stressors on freshwater ecosystems. *Freshwater Biology*, 55, 60–79.

Downes, B. J., Barmuta, L., Fairweather, P. G., Faith, D. P., Keough, M. J., Lake, P. ., Mapstone, B. D., and Quinn, G. P., 2002. *Monitoring Ecological Impacts. Concepts and practice in flowing waters*. Cambridge, UK: Cambridge University Press.

Downes, B. J. and Reich, P., 2008. What is the Spatial Structure of Stream Insect Populations? Dispersal Behaiour at different Life-history Stages. *In*: Lancaster, J. and Briers, R. ., eds. *Aquatic Insects: Challenges to populations*. Wallingford, UK: CAB International, 184–203.

Dray, A. S., Pélissier, R., Couteron, P., Fortin, M., Legendre, P., Bivand, R., Blanchet, F. G., Cáceres, M. De, Dufour, A., Heegaard, E., Jombart, T., Oksanen, J., Thioulouse, J., Wagner, H. H., Monographs, S. E., August, N., Dray, S., Pélissier, R., Couteron, P., Fortin, M., Legendre, P., and Bellier, E., 2012. Community ecology in the age of multivariate multiscale spatial analysis. *Ecological Monographs*, 82 (3), 257–275.

Dray, S., 2013. spacemakeR: Spatial modelling. R package version 0.0-5/r113. Available: https://r-forge.r-project.org/projects/sedar [Accessed 15 November 2014].

Dray, S., Legendre, P., and Blanchet, F. G., 2016. packfor: Forward Selection with permutation (Canoco p.46). Version 0.0-8/r136. Available: https://r-forge.r-project.org/projects/sedar/ [Accessed: 20 November 2016].

Dray, S., Legendre, P., and Peres-Neto, P. R., 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling*, 196 (3–4), 483–493.

Dudgeon, D., 2010. Prospects for sustaining freshwater biodiversity in the 21st century: linking ecosystem structure and function. *Current Opinion in Environmental Sustainability*, 2 (5–6), 422–430.

Duerdoth, C. P., Arnold, A., Murphy, J. F., Naden, P. S., Scarlett, P., Collins, A. L., Sear, D. A., and Jones, J. I., 2015. Assessment of a rapid method for quantitative reach-scale estimates of deposited fine sediment in rivers. *Geomorphology*, 230, 37–50.

Dufour, S. and Piegay, H., 2009. From the myth of a lost paradise to targeted river restoration: forget natural references and focus on human benefits. *River Research and Applications*, 25, 568–581.

Dufrene, M. and Legendre, P., 1997. Species Assemblages and Indicator Species : The Need for a Flexible Asymmetrical Approach. *Ecological Monographs*, 67 (3), 345–366.

Durance, I., Lepichon, C., and Ormerod, S. J., 2006. Recognising the importance of scale in the ecology and management of riverine fish. *River Research and Applications*, 22, 1143–1152.

EnviroCentre, 2014. *Rottal Burn Restoration Project, Glen Clova Annual Monitoring: Year One*. Esk Rivers & Fisheries Trust. Available:www.erft.org.uk.

Environment Agency, 2003a. *River Habitat Survey in Britain and Ireland Field Survey Guidance Manual : 2003 Version PART FOUR – PHOTOGRAPH GALLERY*. Environement Agecny.

Environment Agency, 2003b. *River Habitat Survey in Britain and Ireland Field Survey Guidance Manual : 2003 Version*.

Erba, S., Buffagni, A., Holmes, N., O'Hare, M., Scarlett, P., and Stenico, A., 2006. Preliminary testing of River Habitat Survey features for the aims of the WFD hydro-morphological assessment: an overview from the STAR Project. *Hydrobiologia*, 566 (1), 281–296.

Ernst, A. G., Warren, D. R., and Baldigo, B. P., 2012. Natural-Channel-Design Restorations That Changed Geomorphology Have Little Effect On Macroinvertebrate Communities In Headwater Streams. *Restoration Ecology*, 20 (4), 532–540.

Esk Rivers and Fisheries Trust, 2012. *Report on the implementation of the trust' s fisheries management plan*. Esk Rivers & Fisheries Trust. Available:www.erft.org.uk.

Everard, M., 2008. Selection of taxa as indicators of river and freshwater wetland quality in the UK. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18, 1052–1061.

Extence, C. A., Chadd, R. P., England, J., Dunbar, M. J., Wood, P. J., and Taylor, E. D., 2013. The Assessment of Fine Sediment Accumulation in Rivers using Macro-Invertebrate Community Response. *River Research and Applications*, 29, 17–55.

Feld, C. K., 2013. Response of three lotic assemblages to riparian and catchment-scale land use: implications for designing catchment monitoring programmes. *Freshwater Biology*, 58 (4), 715–729.

Feld, C. K., de Bello, F., and Dolédec, S., 2014. Biodiversity of traits and species both show weak responses to hydromorphological alteration in lowland river macroinvertebrates. *Freshwater Biology*, 59, 233–248.

Feld, C. K., Birk, S., Bradley, D. C., Hering, D., Kail, J., Marzin, A., Melcher, A., Nemitz, D., Pedersen, M. L., Pletterbauer, F., Pont, D., Verdonschot, P. F. M., and Friberg, N., 2011. From Natural to Degraded Rivers and Back Again : A Test of Restoration Ecology Theory and Practice. *Advances in Ecological Research*, 44, 119–209.

Feld, C. K. and Hering, D., 2007. Community structure or function: effects of environmental stress on benthic macroinvertebrates at different spatial scales. *Freshwater Biology*, 52 (7), 1380–1399.

Fernandez, D., Barquin, J., and Raven, P. J., 2011. A review of river habitat characterisation methods : indices vs . characterisation protocols. *Limnetica*, 30 (2), 217–234.

Finkenbine, J. K., Atwater, J. W., and Mavinic, D. S., 2000. Stream health after urbanisation. *Journal of the American Water Resources Association*, 36 (5), 1149–1160.

Fortin, M.-J. and Dale, M. R. ., 2011. *Spatial Analysis A Guide for Ecologists*. Cambridge, UK: Cambridge University Press.

Fournier, D., Skaug, H., J, A., Ianelli, J., A, M., and Maunder, M., 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim. Methods Softw*, 27, 233–249.

Francis, R. a., 2012. Positioning urban rivers within urban ecology. *Urban Ecosystems*, 15 (2), 285–291.

Friberg, N., 2009. Ecological Consequences of River Channel Management. *In*: Ferrier, R. C. and Allan, J., eds. *Handbook of Catchment Management*. WILEY-BLACKWELL, 556.

Friberg, N., 2010. Pressure-response relationships in stream ecology: introduction and synthesis. *Freshwater Biology*, 55 (7), 1367–1381.

Friberg, N., 2014. Impacts and indicators of change in lotic ecosystems. *Wiley Interdisciplinary Reviews: Water*, 1 (6), 513–531.

Friberg, N., Baattrup-Pedersen, A., Kristensen, E. A., Kronvang, B., Larsen, S. E., Pedersen, M. L., Skriver, J., Thodsen, H., and Wiberg-Larsen, P., 2014. The River Gels?? restoration revisited: Habitat specific assemblages and persistence of the macroinvertebrate community over an 11-year period. *Ecological Engineering*, 66, 150–157.

Friberg, N., Bonada, N., Bradley, D. C., Dunbar, M. J., Edwards, K., Grey, J., Hayes, R. B., Hildrew, A. G., Lamouroux, N., Bonada, R. I. A., Edwards, F. K., and Trimmer, M., 2011. Biomonitoring of Human Impacts in Freshwater Ecosystems : The Good , the Bad and the Ugly. *Advances in Ecological Research*, 44, 1–69.

Friberg, N., Sandin, L., and Pedersen, M. L., 2009. Assessing the effects of hydromorphological degradation on macroinvertebrate indicators in rivers: examples, constraints, and outlook. *Integrated Environmental Assessment and Management*, 5 (1), 86–96.

Frissell, C. a., Liss, W. J., Warren, C. E., and Hurley, M. D., 1986. A hierarchical framework for stream habitat classification: Viewing streams in a watershed context. *Environmental Management*, 10 (2), 199–214.

Furse, M., Hering, D., Moog, O., Verdonschot, P., Johnson, R. K., Brabec, K., Gritzalis, K., Buffagni, A., Pinto, P., Friberg, N., Murray-Bligh, J., Kokes, J., Alber, R., Usseglio-Polatera, P., Haase, P., Sweeting, R., Bis, B., Szoszkiewicz, K., Soszka, H., Springe, G., Sporka, F., and Krno, I., 2006. The STAR project: context, objectives and approaches. *Hydrobiologia*, 566 (1), 3–29.

Gardener, M., 2014. *Community Ecology. Analytical Methods Using R and Excel*. Exeter: Pelagic Publishing.

Geist, J., 2011. Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators*, 11 (6), 1507–1516.

Geist, J., 2015. Seven steps towards improving freshwater conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25 (4), 447–453.

Geist, J. and Hawkins, S. J., 2016. Habitat recovery and restoration in aquatic ecosystems: current progress and future challenges. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26, 942–962.

Gibbins, C., Batalla, R. J., and Vericat, D., 2010. Invertebrate drift and benthic exhaustion during disturbance: response of mayflies (Ephemeroptera) to increasing shear stress and river-bed instability. *River Research and Applications*, 26, 499–511.

Gibbins, C. N., Dilks, C. F., Malcolm, R., Soulsby, C., and Juggins, S., 2001. Invertebrate communities and hydrological variation in Cairngorm mountain streams. *Hydrobiologia*, 462, 205–219.

Giller, P. S. and Malmqvist, B., 1998. *The Biology of Streams and Rivers*. Oxford, UK: Oxford University Press.

Gilvear, D. J., Heal, K. V, and Stephen, a, 2002. Hydrology and the ecological quality of Scottish river ecosystems. *The Science of the total environment*, 294 (1–3), 131–59.

Glendell, M., Extence, C., Chadd, R., and Brazier, R. E., 2014. Testing the pressure-specific invertebrate index (PSI) as a tool for determining ecologically relevant targets for reducing sedimentation in streams. *Freshwater Biology*, 59 (2), 353–367.

Gotelli, N. J. and Chao, A., 2013. Measuring and estimating species richness, species diversity and biotic similarity from sampling data. *Encyclopedia of Biodiversity*, 5 (February), 608–625.

Gotelli, N. J. and Colwell, R. K., 2011. Estimating species richness. *In*: Magurran, A. E. and McGill, B., eds. *Biological Diversity. Frontiers in Measurement and Asessment*. Oxford, UK: Oxford University Press, 39–54.

Göthe, E., Angeler, D. G., and Sandin, L., 2013. Metacommunity structure in a small boreal stream network. *Journal of Animal Ecology*, 82 (Hanski 1999), 449–458.

Greenacre, M., 2006. Tying up the loose ends in simple, multiple and joint corresponsence analysis. *In*: Rizzi A., V. M. (eds), ed. *Compstat 2006 - Proceedings in Computational Statistics*. Physica-Verlag HD, 136–185.

Greenacre, M., 2013. Contribution Biplots. *Journal of Computational and Graphical Statistics*, 22 (1), 107–122.

Greenacre, M. and Primicerio, R., 2013. *Multivariate Analysis of Ecological Data*. Bilbao: Fundacion BBVA.

Greenwood, M. J. and Booker, D. J., 2015. The influence of antecedent floods on aquatic invertebrate diversity, abundance and community composition. *Ecohydrology*, 8 (2), 188–203.

Grenouillet, G., Brosse, S., Tudesque, L., Lek, S., Baraillé, Y., and Loot, G., 2008. Concordance among stream assemblages and spatial autocorrelation along a fragmented gradient. *Diversity and Distributions*, 14 (4), 592–603.

Griffith, D. A. and Peres-Neto, P. R., 2006. Spatial Modeling in Ecology : The Flexibility of Eigenfunction Spatial Analyses. *Ecology*, 87 (10), 2603–2613.

Grönroos, M., Heino, J., Siqueira, T., Landeiro, V. L., Kotanen, J., and Bini, L. M., 2013. Metacommunity structuring in stream networks: Roles of dispersal mode, distance type, and regional environmental context. *Ecology and Evolution*, 3, 4473–4487.

Gurnell, A., Lee, M., and Souch, C., 2007. Urban Rivers: Hydrology, Geomorphology, Ecology and Opportunities for Change. *Geography Compass*, 1 (5), 1118–1137.

Gurnell, a. M., van Oosterhout, M. P., de Vlieger, B., and Goodson, J. M., 2006. Reach-scale interactions between aquatic plants and physical habitat: River Frome, Dorset. *River Research and Applications*, 22 (6), 667–680.

Haase, P., Hering, D., Jähnig, S. C., Lorenz, a. W., and Sundermann, A., 2013. The impact of hydromorphological restoration on river ecological status: a comparison of fish, benthic invertebrates, and macrophytes. *Hydrobiologia*, 704 (1), 475–488.

Halcrow, 2009. *West Lothian Council Broxburn Flood Prevention Scheme Review of August 2008 flood incident March 2009 Halcrow Group Limited*.

Harris, G. P. and Heathwaite, a. L., 2012. Why is achieving good ecological outcomes in rivers so difficult? *Freshwater Biology*, 57, 91–107.

Hart, D. D. and Finelli, C. M., 1999. Physical-Biological coupling in streams : The Pervasive Effects of Flow on Benthic Organisms. *Annual Review of Ecology and Systematics*, 30, 363–395.

Harvey, G. L. and Clifford, N. J., 2009. Microscale hydrodynamics and coherent flow structure on rivers: Implications for the characterization of physical habitat. *River Research and Applications*, 25, 160–180.

Harvey, G. L. and Wallerstein, N. P., 2009. Exploring the interactions between flood defence maintenance works and river habitats : the use of River Habitat Survey data. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 702 (May), 689–702.

Heino, J., 2009. Biodiversity of aquatic insects : spatial gradients and environmental correlates of assemblage-level measures at large scales. *Freshwater Reviews*, 2, 1–29.

Heino, J., 2013. The importance of metacommunity ecology for environmental assessment research in the freshwater realm. *Biological Reviews*, 88, 166–178.

Heino, J., Grönroos, M., Ilmonen, J., Karhu, T., Niva, M., and Paasivirta, L., 2013. Environmental heterogeneity and β diversity of stream macroinvertebrate communities at intermediate spatial scales. *Freshwater Science*, 32 (1), 142–154.

Heino, J., Grönroos, M., Soininen, J., Virtanen, R., and Muotka, T., 2012. Context dependency and metacommunity structuring in boreal headwater streams. *Oikos*, 121 (4), 537–544.

Heino, J., Louhi, P., and Muotka, T., 2004. Identifying the scales of variability in stream macroinvertebrate abundance, functional composition and assemblage structure. *Freshwater Biology*, 49 (9), 1230–1239.

Heino, J., Melo, A. S., and Bini, L. M., 2015. Reconceptualising the beta diversity-environmental heterogeneity relationship in running water systems. *Freshwater Biology*, 223–235.

Heino, J., Melo, A. S., Bini, L. M., Altermatt, F., Al-Shami, S. A., Angeler, D. G., Bonada, N., Brand, C., Callisto, M., Cottenie, K., Dangles, O., and Dudgeon, D., 2015. A comparative analysis reveals weak relationships between ecological factos and beta diversity of stream insect metacommunities at two spatial levels. *Ecology and Evolution*, 5 (6), 1235–1248.

Heino, J., Melo, A. S., Siqueira, T., Soininen, J., Valanko, S., and Bini, L. M., 2015.

Metacommunity organisation, spatial extent and dispersal in aquatic systems: Patterns, processes and prospects. *Freshwater Biology*, 60 (5), 845–869.

Heino, J., Mykrä, H., Hämäläinen, H., Aroviita, J., and Muotka, T., 2007. Responses of taxonomic distinctness and species diversity indices to anthropogenic impacts and natural environmental gradients in stream macroinvertebrates. *Freshwater Biology*, 52 (9), 1846–1861.

Heino, J., Soininen, J., Alahuhta, J., Lappalainen, J., and Virtanen, R., 2015. A comparative analysis of metacommunity types in the freshwater realm. *Ecology and Evolution*, 5 (7), 1525–1537.

Hering, D., Buffagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I., Feld, C., Johnson, R., Pinto, P., Skoulikidis, N., Verdonschot, P., and Zahrádková, S., 2003. The Development of a System to Assess the Ecological Quality of Streams Based on Macroinvertebrates – Design of the Sampling Programme within the AQEM Project. *International review of hydrobiology*, 88 (3–4), 345–361.

Hijmans, R. J., 2016. raster: Geographic Data Analysis and Modeling.

Hobbs, R. J. and Harris, J. a, 2001. Restoration Ecology : Repairing the Earth ' s Ecosystems in the New Millennium. *Restoration Ecology*, 9 (2), 239–246.

Huet, M., 1954. Biologie, profils en long et en travers des eaux courantes. *Bull. Fr. Piscic.*, (175), 41–53.

Hughes, J. M., 2007. Constraints on recovery: Using molecular methods to study connectivity of aquatic biota in rivers and streams. *Freshwater Biology*, 52 (4), 616–631.

Huttunen, K.-L. A., Mykra, H., Muotka, T., Mykrä, H., Muotka, T., Mykra, H., and Muotka, T., 2012. Temporal variability in taxonomic completeness of stream macroinvertebrate assemblages. *Freshwater Science*, 31 (2), 423–441.

Jacobs, 2011. *Physical restoration options for priority catchments – phase 1 River South Esk catchment restoration scoping study Project funders Research contractor*. Edinburgh. Sniffer . Available from https://www.wfduk.org/resources [Accessed: 29 November 2012].

Jähnig, S. C., Brabec, K., Buffagni, A., Erba, S., Lorenz, A. W., Ofenböck, T., Verdonschot, P. F. M., and Hering, D., 2010. A comparative analysis of restoration measures and their effects on hydromorphology and benthic invertebrates in 26 central and southern European rivers. *Journal of Applied Ecology*, 47 (3), 671–680.

Jähnig, S. C. and Lorenz, A. W., 2008. Substrate-specific macroinvertebrate diversity patterns following stream restoration. *Aquatic Sciences*, 70 (3), 292–303.

Jahnig, S., Lorenz, A. W., and Hering, D., 2009. Restoration effort , habitat mosaics , and macroinvertebrates - does channel form determine community composition ? *Aquatic*

Conservation: Marine and Freshwater Ecosystems, 169 (November 2008), 157–169.

Januschke, K., Jähnig, S. C., Lorenz, A. W., and Hering, D., 2014. Mountain river restoration measures and their success(ion): Effects on river morphology, local species pool, and functional composition of three organism groups. *Ecological Indicators*, 38, 243–255.

Johnson, R. K., Furse, M. T., Hering, D., and Sandin, L., 2007. Ecological relationships between stream communities and spatial scale: implications for designing catchment-level monitoring programmes. *Freshwater Biology*, 52 (5), 939–958.

Johnson, R. K. and Hering, D., 2009. Response of taxonomic groups in streams to gradients in resource and habitat characteristics. *Journal of Applied Ecology*, 46, 175–186.

Jones, F. C., 2008. Taxonomic sufficiency : The influence of taxonomic resolution on freshwater bioassessments using benthic macroinvertebrates. *Environmental Reviews*, 16, 45–69.

Jones, J. I., Murphy, J. F., Collins, A. L., Sear, D. A., Naden, P. S., and Armitage, P. D., 2012. The impact of fine sediment on macro-invertebrates. *River Research and Applications*, 1071 (May 2011), 1055–1071.

Jost, L., Chao, A., and Chazdon, R. L., 2011. Compositional similarity and beta diversity. *In*: Magurran, A. E. and McGill, B. J., eds. *Biological Diversity: Frontiers in Measurement*. Oxford, UK: Oxford University Press.

Kail, J., Brabec, K., Poppe, M., and Januschke, K., 2015. The effect of river restoration on fish, macroinvertebrates and aquatic macrophytes: A meta-analysis. *Ecological Indicators*, 58, 311–321.

Lambert, C. P. and Walling, D. E., 1988. Measurement of channel storage of suspended sediment in a gravel-bed river. *Catena*, 15 (1), 65–80.

Lamouroux, N., Doledec, S., and Gayraud, S., 2004. Biological traits of stream macroinvertebrate communities: effects of microhabitat, reach, and basin filters. *Journal of the North American Benthological Society*, 23, 449–466.

Lancaster, J., 2000. Geometric scaling of microhabitat patches and their efficacy as refugia during disturbance. *Journal of Animal Ecology*, 69, 442–457.

Lancaster, J. and Belyea, L. R., 2006. Defining the limits to local density: alternative views of abundance-environment relationships. *Freshwater Biology*, 51 (4), 783–796.

Lancaster, J. and Briers, R. A., 2008. *Aquatic Insects: Challenges to Populations: proceedings of the Royal Entomological Society's 24th symposium. University of Edinburgh, 2007*. Wallingford, UK: CAB International.

Lancaster, J., Buffin-Bélanger, T., Reid, I., and Rice, S., 2006. Flow- and substratum-mediated movement by a stream insect. *Freshwater Biology*, 51 (6), 1053–1069.

Larsen, S. and Ormerod, S. J., 2010. Low-level effects of inert sediments on temperate stream invertebrates. *Freshwater Biology*, 55 (2), 476–486.

Larsen, S., Pace, G., and Ormerod, S. J., 2011. Experimental effects of sediment depoistion on the structure and function of macroinvertebrate asemblages in temperate streams. *River Research and Applications*, 27, 257–267.

Larsen, S., Vaughan, I. P., and Ormerod, S. J., 2009. Scale-dependent effects of fine sediments on temperate headwater invertebrates. *Freshwater Biology*, 54 (1), 203–219.

Laub, B. G., Baker, D. W., Bledsoe, B. P., and Palmer, M. A., 2012. Range of variability of channel complexity in urban, restored and forested reference streams. *Freshwater Biology*, 57 (5), 1076–1095.

Lawton, J. H., 1999. Are There General Laws in Ecology ? *Oikos*, 84 (2), 177–192.

Le, S., Josse, J., and Husson, F., 2008. FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, 25 (1), 1–18.

Legendre, P., 1993. Spatial autocorrelation: trouble or new paradigm? *Ecology*, 74 (6), 1659–1673.

Legendre, P. and De Cáceres, M., 2013. Beta diversity as the variance of community data: Dissimilarity coefficients and partitioning. Appendix S3. *Ecology Letters*, 1–16.

Legendre, P. and Gallagher, E. D., 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129 (2), 271–280.

Legendre, P. and Legendre, L., 1998. Ordination in reduced space. *In*: *Numerical Ecology*. Amsterdam: Elsevier Ltd, 387–480.

Legendre, P. and Legendre, L., 2012. Spatial analysis. *In*: *Numerical Ecology*. Amsterdam: Elsevier Ltd.

Lennon, J. J., 2000. Red-shifts and red herrings in geographical ecology. *Ecography*, 23 (1), 101–113.

Lepori, F., Palm, D., Brännäs, E., and Malmqvist, B., 2005. Does Restoration of Structural Heterogeneity in Streams Enhance Fish and Macroinvertebrate Diversity ? *Ecological Applications*, 15 (6), 2060–2071.

Leps, M., Sundermann, A., Tonkin, J. D., Lorenz, A. W., and Haase, P., 2016. Time is no healer: increasing restoration age does not lead to improved benthic invertebrate communities in restored river reaches. *Science of The Total Environment*, 557–558, 722–732.

Leps, M., Tonkin, J. D., Dahm, V., Haase, P., and Sundermann, A., 2015. Disentangling environmental drivers of benthic invertebrate assemblages: The role of spatial scale and riverscape heterogeneity in a multiple stressor environment. *Science of the Total Environment*, 536, 546–556.

Li, J., Herlihy, A., Gerth, W., Kaufmann, P., Gregory, S., Urquhart, S., and Larsen, D. P., 2001.

Variability in stream macroinvertebrates at multiple spatial scales. *Freshwater Biology*, 46, 87–97.

Liu, J., Soininen, J., Han, B. P., and Declerck, S. A. J., 2013. Effects of connectivity, dispersal directionality and functional traits on the metacommunity structure of river benthic diatoms. *Journal of Biogeography*, 40 (12), 2238–2248.

Lloyd, N. J., Mac Nally, R., and Lake, P. S., 2005. Spatial autocorrelation of assemblages of benthic invertebrates and its relationship to environmental factors in two upland rivers in southeastern Australia. *Diversity and Distributions*, 11 (5), 375–386.

Lorenz, A., Hering, D., Feld, C. K., and Rolauffs, P., 2004. A new method for assessing the impact of hydromorphological degradation on the macroinvertebrate fauna of five German stream types. *Hydrobiologia*, 516 (1–3), 107–127.

Lorenz, A. W. and Feld, C. K., 2013. Upstream river morphology and riparian land use overrule local restoration effects on ecological status assessment. *Hydrobiologia*, 704 (1), 489–501.

Lorenz, A. W., Jähnig, S. C., and Hering, D., 2009. Re-meandering german lowland streams: Qualitative and quantitative effects of restoration measures on hydromorphology and macroinvertebrates. *Environmental Management*, 44 (4), 745–754.

Louhi, P., Mykrä, H., Paavola, R., Huusko, A., Vehanen, T., Mäki-Petäys, A., and Muotka, T., 2011. Twenty years of stream restoration in Finland: little response by benthic macroinvertebrate communities. *Ecological applications : a publication of the Ecological Society of America*, 21 (6), 1950–61.

MacDougall, K., 2012. *Rottal Burn Restoration Glen Clova (presentation)*. Esk Rivers & Fisheries Trust. Available:www.erft.org.uk.

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., and Hornik, K., 2015. cluster: Cluster Analysis Basics and Extensions. R package version 2.0.3, Available: http://cran.r-project.org/web/packages/cluster/index.html [Accessed 12 Sept 2015].

Malmqvist, B., 2002. Aquatic invertebrates in riverine landscapes. *Freshwater biology*, 47, 679–694.

Malmqvist, B. and Rundle, S., 2002. Threats to the running water ecosystems of the world. *Environmental Conservation*, 29 (2), 134–153.

Matthews, J., Reeze, B., Feld, C. K., and Hendriks, a. J., 2010. Lessons from practice: assessing early progress and success in river rehabilitation. *Hydrobiologia*, 655 (1), 1–14.

McDonald, A., Lane, S. N., Haycock, N. E., and Chalk, E. A., 2004. Rivers of dreams : on the gulf between theoretical and practical aspects of an upland river restoration. *Transactions of the institute of British Geographers*, 29, 257–281.

Melo, A. S., 2005. A critique of the use of jackknife and related non-parametric techniques to estimate species richness. *Community Ecology*, 5 (2), 149–157.

Mérigoux, S., Dolédec, S., Merigoux, S., and Doledec, S., 2004. Hydraulic requirements of stream communities: A case study on invertebrates. *Freshwater Biology*, 49 (5), 600–613.

Meyer, J. L., Paul, M. J., and Taulbee, W. K., 2005. Stream Ecosystem Function in Urbanizing Landscapes. *Journal of the North American Benthological Society*, 24 (3), 602–612.

Miller, S. W., Budy, P., and Schmidt, J. C., 2010. Quantifying macroinvertebrate responses to in-stream habitat restoration: Applications of meta-analysis to river restoration. *Restoration Ecology*, 18 (1), 8–19.

Milner, V. S., Willby, N. J., Gilvear, D. J., and Perfect, C., 2015. Linkages between reach-scale physical habitat and invertebrate assemblages in upland streams. *Marine and Freshwater Research*, 66 (5), 438–448.

Miltner, R. J., White, D., and Yoder, C., 2004. The biotic integrity of streams in urban and suburbanizing landscapes. *Landscape and Urban Planning*, 69 (1), 87–100.

Morales, M. and Murdoch, D., 2012. sciplot: Scientific Graphing Functions for Factorial Designs. Version 1.1-0. Available: https://cran.r-project.org/package=sciplot [Accessed 14 Sept 2015].

Moss, B., 2000. Biodiversity in fresh waters – an issue of species preservation or system functioning? *Environmental Conservation*, 27 (1), 1–4.

Moss, B., 2015. Mammals, freshwater reference states, and the mitigation of climate change. *Freshwater Biology,* 60, 1964–1976.

Muhar, S., Januschke, K., Kail, J., Poppe, M., Schmutz, S., Hering, D., and Buijse, a. D., 2016. Evaluating good-practice cases for river restoration across Europe: context, methodological framework, selected results and recommendations. *Hydrobiologia,* 769 (1), 3–19.

Muotka, T., Paavola, R., Haapala, A., Novikmec, M., and Laasonen, P., 2002. Long-term recovery of stream habitat structure and benthic invertebrate communities from in-stream restoration. *Biological Conservation*, 105, 243–253.

National Biodiversity Network, 2014. NBN gateway. *Biological Records Centre*.

Natural Scotland, 2015. *The river basin management plan for the Scotland river basin district 2009–2015*.

Naura, M., Sear, D., Alvarez, M., Pe, F., and Fern, D., 2011. Integrating monitoring , expert knowledge and habitat management within conservation organisations for the delivery of the water framework directive : A proposed approach Barqu ´ The Water Framework Directive requires Eu-. *Limnetica,* 30 (2), 427–446.

Nenadic, O. and Greenacre, M., 2007. Correspondence Analysis in R, with two- and three-dimensional graphics: The ca package. *Journal of Statistical Software*, 20 (3), 1–13.

Nijboer, R. C. and Schmidt-Kloiber, A., 2004. The effect of excluding taxa with low abundances or taxa with small distribution ranges on ecological assessment. *Hydrobiologia*, 516, 347–363.

Nilsson, C., Polvi, L. E., Gardeström, J., Hasselquist, E. M., Lind, L., and Sarneel, J. M., 2015. Riparian and in-stream restoration of boreal streams and rivers: Success or failure? *Ecohydrology*, 8 (5), 753–764.

O'Hara, R. B., 2005. Species richness estimators: How many species can dance on the head of a pin? *Journal of Animal Ecology*, 74 (2), 375–386.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G., Solymos, P., Stevens, M. H. H., and Wagner, H., 2016. vegan: Community Ecology Package.

Padmore, C., 1998. The role of physical biotopes in determining the conservation status and flow requirements of British rivers. *Aquatic Ecosystem Health and Management*, 1 (1), 25–35.

Padmore, C. L., 1997. Biotopes and their hydraulics: a method for defining the physical component of freshwater quality. *In*: Boon, P. J. and Howell, D. ., eds. *Freshwater Quality: Defining the Indefinable*. Edinburgh: The Stationary Office, 251–257.

Paillex, A., Schuwirth, N., Lorenz, A. W., Januschke, K., Peter, A., and Reichert, P., 2017. Integrating and extending ecological river assessment: Concept and test with two restoration projects. *Ecological Indicators*, 72, 131–141.

Palmer, M. A., Ambrose, R. F., and Poff, N. L., 1997. Ecological Theory and Community Restoration Ecology. *Restoration Ecology*, 5 (4), 291–300.

Palmer, M. A., Hondula, K., and Koch, B. J., 2014. Ecological resotration of streams and rivers: shifting strategies and shifting goals. *Annual Review of Ecology, Evolution, and Systematics*, 45, 247–271.

Palmer, M. A., Menninger, H. L., and Bernhardt, E. S., 2010. River restoration, habitat heterogeneity and biodiversity: a failure of theory or practice? *Freshwater Biology*, 55, 205–222.

Palmer, M. A. and Poff, N. L., 1997. The Influence of Environmental Heterogeneity on Patterns and Processes in Streams. *Journal of the North American Benthological Society*, 16 (1), 169–173.

Palmer, M. A., Swan, C. M., Nelson, K., Silver, P., and Alvestad, R., 2000. Streambed landscapes : evidence that stream invertebrates respond to the type and spatial arrangement of patches. *Landscape Ecology*, 15, 563–576.

Parsons, M., Thoms, M. C., and Norris., R. H., 2003. Scales of macroinvertebrate distribution in relation to the hierarchical organization of river system. *Journal of the North American*

Benthological Society, 22 (1), 105–122.

Paul, M. J. and Meyer, J. L., 2001. Streams in the Urban Landscape. *Annual Review of Ecology and Systematics*, 32, 333–365.

Pedersen, M. L. and Friberg, N., 2007. Two lowland stream riffles – linkages between physical habitats and macroinvertebrates across multiple spatial scales. *Aquatic Ecology,* 41 (3), 475–490.

Pedersen, M. L., Friberg, N., Skriver, J., Baattrup-Pedersen, A., and Larsen, S. E., 2007. Restoration of Skjern River and its valley-Short-term effects on river habitats, macrophytes and macroinvertebrates. *Ecological Engineering*, 30 (2 SPEC. ISS.), 145–156.

Peres-Neto, P. R. and Legendre, P., 2010. Estimating and controlling for spatial structure in the study of ecological communities. *Global Ecology and Biogeography*, 19 (2), 174–184.

Peres-Neto, P. R., Leibold, M. A., and Dray, S., 2012. Assessing the effects of spatial contingency and environmental filtering on metacommunity phylogenetics. *Ecology*, 93 (8), S14–S30.

Petts, G. E., 2000. A perspective on the abiotic processes sustaining the ecological integrity of running waters. *Hydrobiologia*, 422/423, 15–27.

Petts, G. E. and Amoros, C., 1996. *Fluvial Hydrosystems*. London: Chapman & Hall.

Petts, G. E. and Greenwood, M., 1985. Channel changes and invertebrate faunas below Nant-Y-Moch dam, River Rheidol, Wales, UK. *Hydrobiologia*, 122 (1), 65–80.

Pizzuto, J. E., Hession, W. C., and McBride, M., 2000. Comparing gravel-bed rivers in paired urban and rural catchments of southeastern Pennsylvania. *Geology*, 28 (1), 79–82.

Podani, J. and Csanyi, B., 2010. Detecting indicator species: Some extensions of the IndVal measure. *Ecological Indicators*, 10 (6), 1119–1124.

Polvi, L. E., Nilsson, C., and Hasselquist, E. M., 2014. Potential and actual geomorphic complexity of restored headwater streams in northern Sweden. *Geomorphology*, 210, 98–118.

Poole, G. C., 2002. Fluvial landscape ecology: addressing uniqueness within the river discontinuum. *Freshwater Biology*, 47 (4), 641–660.

R Core Team, 2016. R: A Language and Environment for Statistical Computing.

Raven, P. J., Dawson, F. H., and Everard, M., 1998. Quality assessment using River Habitat Survey data. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 499 (April), 477–499.

Reece, P. F., Reynoldson, T. B., Richardson, J. S., and Rosenberg, D. M., 2001. Implications of seasonal variation for biomonitoring with predictive models in the Fraser River catchment, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, 58 (7), 1411–1417.

Reid, H. E., Brierley, G. J., and Boothroyd, I. K. G., 2010. Influence of bed heterogeneity and habitat type on macroinvertebrate uptake in peri-urban streams. *International Journal of Sediment Research*, 25 (3), 203–220.

Rempel, L. L. and Church, M., 2009. Physical and ecological response to disturbance by gravel mining in a large alluvial river. *Canadian Journal of Fisheries and Aquatic Sciences*, 66 (1), 52–71.

Resh, V. H., Hildrew, A. G., Statzner, B., and Townsend, C. R., 1994. Theoretical habitat templets, species traits, and species richness: a synthesis of long-term ecological research on the Upper Rhone River in the context of concurrently developed ecological theory. *Freshwater Biology*, 31, 539–554.

Roberts, D. W., 2016. labdsv: Ordination and Multivariate Analysis for Ecology. Version: 1.8-0, Available: https://cran.r-project.org/package=labdsv [Accessed 1 Jan 2016].

Robson, B. J. and Chester, E. T., 1999. Spatial patterns of invertebrate species richness in a river: the relationship between riffles and microhabitats. *Australian Journal of Ecology*, 24, 599–607.

Rolls, R. J., Heino, J., and Chessman, B. C., 2016. Unravelling the joint effects of flow regime, climatic variability and dispersal mode on beta diversity of riverine communities. *Freshwater Biology*, 61 (8), 1350–1364.

Roni, P., Hanson, K., and Beechie, T., 2008. Global Review of the Physical and Biological Effectiveness of Stream Habitat Rehabilitation Techniques. *North American Journal of Fisheries Management*, 28 (3), 856–890.

Rossi, J.-P., 2011. rich: An R Package to Analyse Species Richness. *Diversity*, 3 (4), 112–120.

Roy, A. H., Rosemond, A. D., Paul, M. J., Leigh, D. S., and Wallace, J. B., 2003. Stream macroinvertebrate response to catchment urbanization (Georgia, U.S.A.). *Freshwater Biology*, 48 (2), 329–346.

RStudio Team, 2015. RStudio: Integrated Development Environment for R.

Schmera, D., Erős, T., and Heino, J., 2013. Habitat filtering determines spatial variation of macroinvertebrate community traits in northern headwater streams. *Community Ecology*, 14 (1), 77–88.

Schmidt-Kloiber, A. and Hering, D., 2015. www.freshwaterecology.info – An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. *Ecological Indicators*, 53, 271–282.

Schmidt-Kloiber, A. and Nijboer, R. C., 2004. The effect of taxonomic resolution on the assessment of ecological water quality classes. *Hydrobiologia*, 516 (1–3), 269–283.

Sear, D. A., Briggs, A., and Brookes, A., 1998. A preliminary analysis of the morphological adjustment within and downstream of a lowland river subject to river restoration. *Aquatic*

Conservation: Marine and Freshwater Ecosystems, 183 (October 1996), 167–183.

Sear, D. A., Newson, M. D., and Thorne, C. R., 2003. *Guidebook of applied fluvial geomorphology*.

SEPA, 2007. *Significant water management issues in the Scotland river basin district*. Edinburgh. SEPA. Available: https://www.sepa.org.uk.

SEPA, 2009. *Temporary Construction Methods*. Edinburgh. SEPA. Available: https://www.sepa.org.uk.

SEPA, 2010. *Broxburn RBMP Water body information sheet*. Edinburgh. SEPA. Available: https://www.sepa.org.uk.

SEPA, 2011. *The Water Environment (Controlled Activities) A Practical Guide*. Edinburgh. SEPA. Available: https://www.sepa.org.uk.

Seymour, M., Deiner, K., and Altermatt, F., 2016. Scale and scope matter when explaining varying patterns of community diversity in riverine metacommunities. *Basic and Applied Ecology*, 17 (2), 134–144.

Silva, D. R. O., Ligeiro, R., Hughes, R. M., and Callisto, M., 2014. Visually determined stream mesohabitats influence benthic macroinvertebrate assessments in headwater streams. *Environmental monitoring and assessment*, 186 (9), 5479–88.

Sjostrom, P., 1985. Territoriality in nymphs of Dinocras cephalotes (Plecoptera). *Oikos*, 45 (3), 353–357.

Smiley Jr., P. C., Shields Jr., F. D., and Knight, S. S., 2009. Designing Impact Assessments for Evaluating Ecological Effects of Agricultural Conservation Practices on Streams. *JAWRA Journal of the American Water Resources Association*, 45 (4), 867–878.

Smith, B., Clifford, N. J., and Mant, J., 2014a. Analysis of UK river restoration using broad-scale data sets. *Water and Environment Journal*, 28 (4), 490–501.

Smith, B., Clifford, N. J., and Mant, J., 2014b. The changing nature of river restoration. *Wiley Interdisciplinary Reviews: Water*, 1 (3), 249–261.

Soulsby, C., Malcolm, R., Gibbins, C., and Dilks, C., 2001. Seasonality, water quality trends and biological responses in four streams in the Cairngorm Mountains, Scotland. *Hydrology and Earth System Sciences*, 5 (3), 433–450.

Spänhoff, B. and Arle, J., 2007. Setting attainable goals of stream habitat restoration from a macroinvertebrate view. *Restoration Ecology*, 15 (2), 317.

Stoll, S., Breyer, P., Tonkin, J. D., Früh, D., and Haase, P., 2016. Scale-dependent effects of river habitat quality on benthic invertebrate communities - Implications for stream restoration practice. *Science of the Total Environment*, 553, 495–503.

Strayer, D. L., 2006. Challenges for freshwater invertebrate conservation. *Journal of the North*

American Benthological Society, 25 (2), 271–287.

Strayer, D. L. and Dudgeon, D., 2010. Freshwater biodiversity conservation: recent progress and future challenges. *BioOne*, 29 (1), 344–358.

Sundermann, A., Gerhardt, M., Kappes, H., and Haase, P., 2013. Stressor prioritisation in riverine ecosystems: Which environmental factors shape benthic invertebrate assemblage metrics? *Ecological Indicators*, 27, 83–96.

Sundermann, A., Stoll, S., and Haase, P., 2011. River restoration success depends on the species pool of the immediate surroundings. *Ecological Applications*, 21 (6), 1962–1971.

Tachet, H., Richoux, P., Bournaud, M., and Usseglio-Polatera, P., 2010. *Invertebrs d'eau douce: systematique, biologie, ecologie*. 3rd edn. Paris: CNRS Editions.

Thoms, M. C., 2006. Variability in riverine ecosystems. *River Research and Applications*, 22 (2), 115–121.

Thomson, J. R., Taylor, M. P., and Brierley, G. J., 2004. Are River Styles ecologically meaningful? A test of the ecological significance of a geomorphic river characterization scheme. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 14 (1), 25–48.

Thorp, J. H., Thoms, M. C., and Delong, M. D., 2008. *The Riverine Ecosystem Synthesis*. London: Elsevier.

Tickner, D., Armitage, P. D., Bickerton, M. A., and Hall, K., 2000. Assessing stream quality using information on mesohabitat distribution and character. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 10, 179–196.

Tornwall, B., Sokol, E., Skelton, J., and Brown, B. L., 2015. Trends in stream biodiversity research since the river continuum concept. *Diversity*, 7 (1), 16–35.

Townsend, C. R., 1989. The patch dynamics concept of stream community ecology. *Journal of the North American Benthological Society*, 8 (1), 36–50.

Townsend, C. R. and Hildrew, A. G., 1976. Field Experiments on the Drifting , Colonization and Continuous Redistribution of Stream Benthos. *Journal of Animal Ecology*, 45 (3), 759–772.

Townsend, C. R. and Hildrew, A. G., 1994. Species traits in relation to a habitat templet for river systems. *Freshwater Biology*, 31, 265–275.

Townsend, C. R., Uhlmann, S. S., and Matthaei, C. D., 2008. Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology*, 45 (6), 1810–1819.

Tullos, D. D., Penrose, D. L., Jennings, G. D., and Cope, W. G., 2009. Analysis of functional traits in reconfigured channels: implications for the bioassessment and disturbance of river restoration. *Journal of the North American Benthological Society*, 28 (1), 80–92.

Underwood, A. J., 1991. Beyond BACI : Experimental Designs for Detecting Human Environmental Impacts on Temporal Variations in Natural Populations. *Australian Journal* *of Marine and Freshwater Research1*, 42, 569–587.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., and Cushing, C. E., 1980. The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37 (1), 130–137.

Vaughan, I. P., Diamond, M., Gurnell, A. M., Hall, K. A., Jenkins, A., Milner, N. J., Naylor, L. A., Sear, D. A., Woodward, G., and Ormerod, S. J., 2009. Integrating ecology with hydromorphology : a priority for river science and management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 125 (October 2008), 113–125.

Vaughan, I. P. and Ormerod, S. J., 2010. Linking ecological and hydromorphological data: approaches, challenges and future prospects for riverine science. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 20 (S1), S125–S130.

Vaughan, I. P. and Ormerod, S. J., 2012. A 20-Year View of Monitoring Ecological Quality in English and Welsh Rivers. *In*: Boon, P. J. and Raven, P. J., eds. *River Conservation and Management*. John Wiley & Sons, Ltd.

Venables, W. . and Ripley, B. D., 2002. *Modern Applied Statistics with S*. Fourth. New York: Springer.

Verdonschot, R. C. M., Kail, J., McKie, B. G., and Verdonschot, P. F. M., 2015. The role of benthic microhabitats in determining the effects of hydromorphological river restoration on macroinvertebrates. *Hydrobiologia*, 769 (55), 1–12.

Vinson, M. R. and Hawkins, C. P., 1998. BIODIVERSITY OF STREAM INSECTS : Variation at Local , Basin , and Regional Scales. *Annual Review of Entomology*, 43, 271–293.

Vinson, M. R. and Hawkins, C. P., 2003. Broad-scale geographical patterns in local stream insect genera richness. *Ecography*, 26 (6), 751–767.

Violin, C. R., Cada, P., Sudduth, E. B., Hassett, B. A., Penrose, D. L., and Bernhardt, E. S., 2011. Effects of urbanization and urban stream restoration on the physical and biological structure of stream ecosystems. *Ecological Applications*, 21 (6), 1932–1949.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, a, Green, P., Glidden, S., Bunn, S. E., Sullivan, C. a, Liermann, C. R., and Davies, P. M., 2010. Global threats to human water security and river biodiversity. *Nature*, 467 (7315), 555–561.

Wallis, C., Maddock, I., Visser, F., and Acreman, M., 2012. A framework for evaluating the spatial configuration and temporal dynamics of hydraulic patches. *River Research and Applications*, 28, 585–593.

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., and Ii, R. P. M., 2005. The urban stream syndrome : current knowledge and the search for a cure Source : Journal of the North American Benthological Society , Vol . 24 , No . 3 (Sep ., 2005), Published by : The University of Chicago Press on behalf of the Society for Freshwate. *Journal of the North American Benthological Society*, 24 (3), 706–723.

Walsh, C. J. and Webb, J. A., 2016. Interactive effects of urban stormwater drainage, land clearance, and flow regime on stream macroinvertebrate assemblages across a large metropolitan region. *Freshwater Science*, 35 (1), 324–339.

Ward, J. V., 1998. Riverine landscapes: Biodiversity patterns, disturbance regimes, and aquatic conservation. *Biological Conservation*, 83 (3), 269–278.

Wenger, S. J., Roy, A. H., Jackson, C. R., Bernhardt, E. S., Carter, T. L., Filoso, S., Gibson, C. a., Hession, W. C., Kaushal, S. S., Martí, E., Meyer, J. L., Palmer, M. a., Paul, M. J., Purcell, A. H., Ramírez, A., Rosemond, A. D., Schofield, K. a., Sudduth, E. B., and Walsh, C. J., 2009. Twenty-six key research questions in urban stream ecology: an assessment of the state of the science. *Journal of the North American Benthological Society*, 28 (4), 1080–1098.

Wenn, C. L., 2008. Do freshwater macroinvertebrates reflect water quality improvements following the removal of point source pollution from Spen Beck , West Yorkshire ? *Earth & E-nvironment*, 3, 369–406.

Werritty, A. and Hoey, T., 2004. *Geomorphological changes and trends in Scotland : river channels and processes.* Scottish Natural Heritage Commissioned Report No.053.

Winking, C., Lorenz, A. W., Sures, B., and Hering, D., 2014. Recolonisation patterns of benthic invertebrates: A field investigation of restored former sewage channels. *Freshwater Biology*, 59 (9), 1932–1944.

Winking, C., Lorenz, A. W., Sures, B., and Hering, D., 2015. Start at zero: succession of benthic invertebrate assemblages in restored former sewage channels. *Aquatic Sciences*, 78 (4), 683–694.

Wolter, C., Lorenz, S., Scheunig, S., Lehmann, N., Schomaker, C., Nastase, A., and García De Jalón, D., 2013. *Review on ecological response to hydromorphological degradation and restoration*. REFORM. Available: http://www.reformrivers.eu [Accessed 8th June 2015].

Wood, P. and Armitage, P., 1997. Biological Effects of Fine Sediment in the Lotic Environment. *Environmental management*, 21 (2), 203–17.

Wood, P. J., Armitage, P. D., Hill, M. J., Mathers, K. L., and Millett, J., 2016. Faunal response to fine sediment deposition in urban rivers. *In*: Gilvear, D. J., Greenwood, M. T., Thoms, M. C., and Wood, P. J., eds. *River Science: Research and Management for the 21st Century*. John Wiley & Sons, Ltd.

Wright, J. F., Hiley, P. D., Ham, S. F., and Berrie, a. D., 1981. Comparison of three mapping procedures developed for river macrophytes. *Freshwater Biology*, 11 (4), 369–379.

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M., 2009. GLM and GAM for count Data. *In*: *Mixed Effects Model and Extensions in Ecology with R*. New York: Springer, 579.

Appendices

Appendix 2.1

Fine sediment calculation/calibration equation

Total water volume = volume of a cylinder (v= π* r^2 *h) - (units = cm cubed)

where

Radius = 2/Width = 38/2=19 cm

Width = average width at middle of sampler = 38cm

H=Depth = that recorded on the data sheet (cm)

Sample volume = 2 litres or 2000cm cubed

Sediment g/cm cubed = 2000/weight

Where

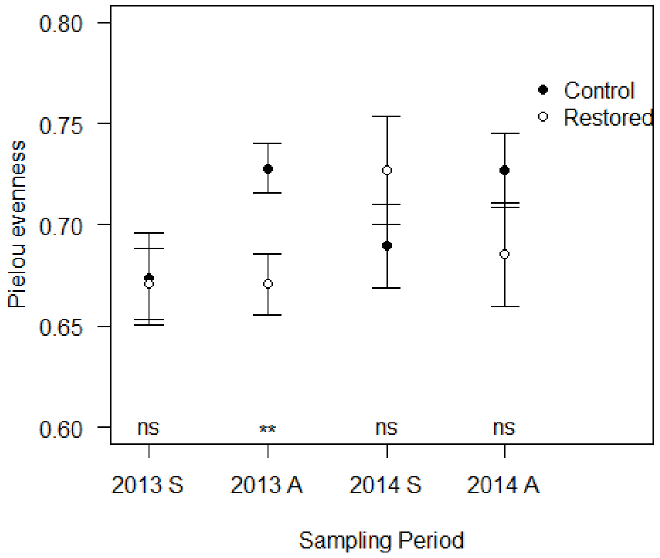
Weight = dried sediment weight (g)

Total weight = Sediment per cm cubed * Total water volume

Total weight is for a fixed sampling area of the trug base diameter = 35cm therefore area = 962cm2, or 0.1m2

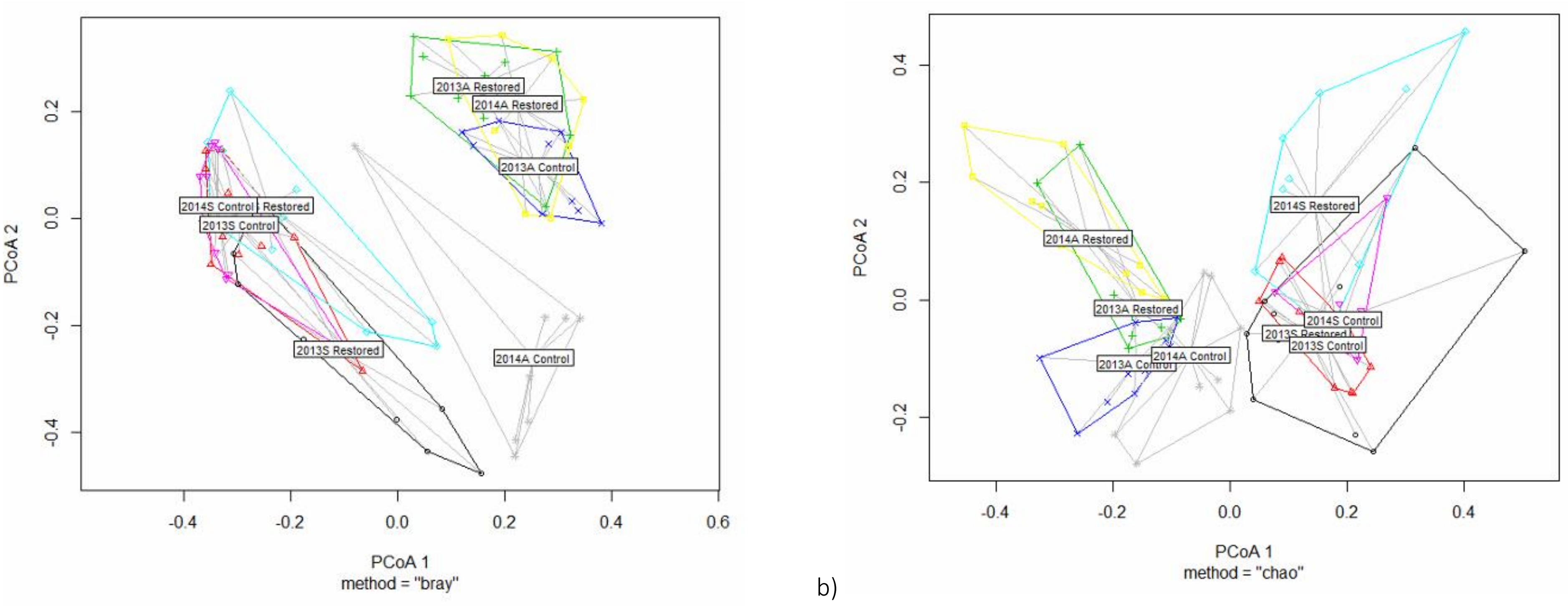
Appendix 2.2

Mean sample Pielou's Evenness with standard error, for each sample period and stream treatment.



Appendix 2.3

PCoA result showing the location of sample x site x year groups of all global analysis with all samples in the Rottal study using a) Bray-Curtis distance and b) Chao distance.



a)

b)

Appendix 2.4

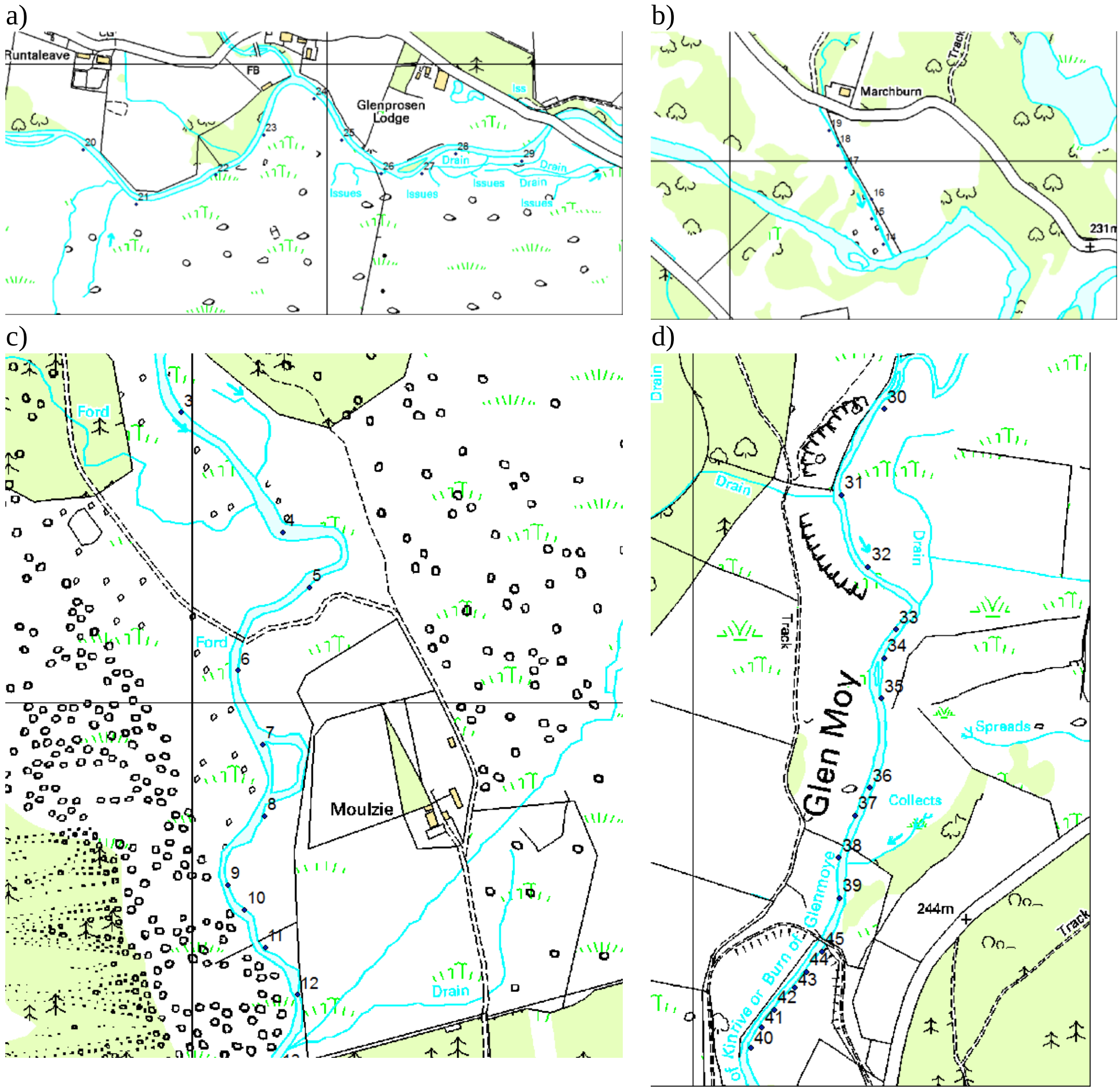
Inventory of species and codes used in ordination biplots

Species Abbreviation	Species Name	Species Abbreviation	Species Name
Adic.red	*Adicella reducta*	Hydroptila	*Hydroptila sp*
Agap.del	*Agapetus delicatulus*	Iso.gram	*Isoperla grammatica*
Agap.fus	*Agapetus fuscipes*	Lep.mar	*Leptophlebia marginata*
Agap.sp	*Agapetus sp*	Leptoc.sp	*Leptoceridae sp*
All.aur	*Allogamus auricollis*	Lepi.hirt	*Lepidostoma hirtum*
Am.ino	*Ameletus inopinatus*	Leu.fus	*Leauctra fusca*
Amp.sul	*Amphinemura sulcicollis*	Leu.hip.mos	*Leuctra hippopus/moselyi*
Anab.nerv	*Anabolia nervosa*	Leu.ine	*Leuctra inermis*
Athrip.bil	*Athripsodes bilineatus*	Leu.nig	*Leuctra nigra*
Bae.fus	*Baetis fuscatus*	Limn.fusc	*Linmephilus fuscicornis*
Bae.mut/Alai.mut	*Baetis muticus*	Limn.lun	*Limnephilus lunatus*
Bae.nig	*Baetis niger*	Limn.small	*Limnophilidea (small)*
Bae.rho	*Baetis rhodani*	Myst.long	*mystacides longicornis*
Bae.scam	*baetis scambus*	Nem.avi	*Nemoura avicularis*
Bera.pul	*Beraea pullata*	Nem.cam	*Nemoura cambrica*
Bera.mau	*Beraea maurus*	Nem.pic	*Nemurella picteti*
Brach.risi	*Brachyptera risi*	Odon.alb	*Odontocerum albicorne*
Brach.sub	*Brachycentrus subnubilus*	Oxyeth	*Oxyethira sp*
Cae.riv	*Caenis rivulorum*	Para.sub	*Paraleptophlebia submarginata*
Cap.bif	*Capnida bifrons*	Per.bip	*Perla bipunctata*
Cen.lut	*Centroptilum luteolum*	Per.mor	*Perlodes mortoni*
Chae.vill	*Chaetopteryx villosa villosa*	Phil.mon	*Philopotamus montanus*
Chl.torr	*Siphonoperla torrentium*	Plect.con	*Plectrocnemia conspersa*
Chl.tri	*Chloroperla tripunctata*	Plect.gen	*Plectrocnemia geniculata*
Dino.cep	*Dinocras cephalotes*	Polyc.flav	*Polycentropus flavomaculatus*
Diu.bic	*Diura bicaudata*	Pot.cin	*Potamophylax cingulatus*
Dru.ann	*Drusus annulatus*	Pot.lat	*Potamophylax latipennis*
Eccl.gut	*Ecclisopteryx guttulata*	Pro.mey	*Protonemura meyeri*
Ecd.ven	*Ecdyonurus venosus*	Pro.pra.mon	*Protonemura montana/ praecox*

Elec.lat	*Electrogena lateralis*	Proc.penn	*Procloeon pennulatum*
Eule.gen	*Euleuctra geniculata*	Psych.pus	*Psychomyia pusilla*
Gloss.con	*Glossosoma conformis*	Rhy.dor	*Rhyacophila dorsalis dorsalis*
glos.bolt	*Glossosoma boltoni*	Rhy.mun	*Rhyacophila munda*
Habr.fusc	*Habrophlebia fusca*	Rith.sem	*Rhithrogena semicolorata*
Hale.dig	*Halesus digitatus digitatus*	Seri.pers	*Sericostoma personatum*
Hale.rad	*Halesus radiatus*	Serr.ign	*Serratella ignita*
Hydro.ins	*Hydropsyche instabilis*	Silo.pall	*Silo pallipes*
Hydro.pel	*Hydropsyche pellucidula*	Siph.lac	*Siphlonurus lacustris*
Hydro.silt	*Hydropsyche siltalai*	Taen.neb	*Taeniopteryx nebulosa*
		Tino.waer	*Tinodes waernii*

Appendix 3.1

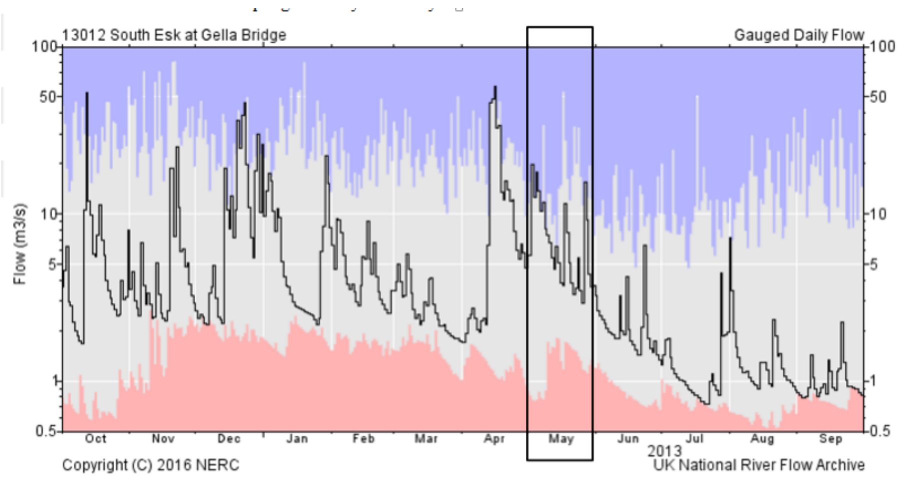
Sample location maps for the 5 additional sites in the South Esk Catchment for a) Prosen (PR), b) March Burn (MA), c) Moulzie (MO) and d) Glenmoye upstream (GU) and Glenmoye downstream (GD).



Appendix 3.2

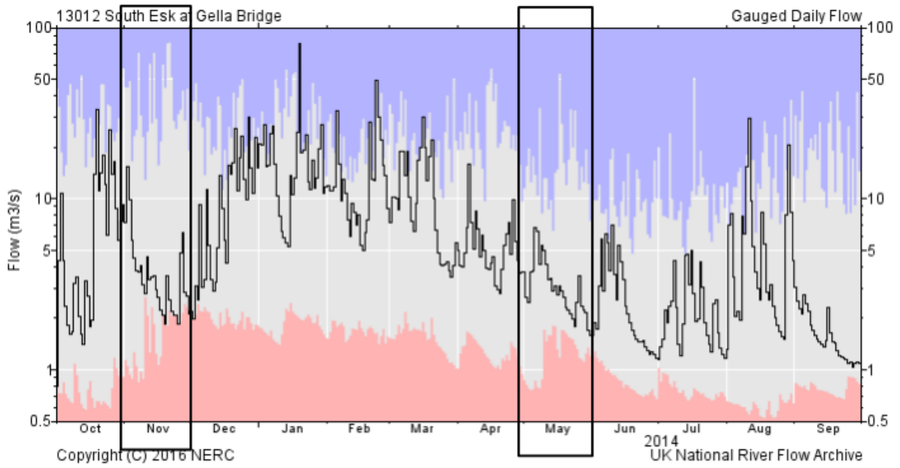
Daily gauged flow data from Gella Bridge (Fig 2.2) for the duration of the project. May 2013-November 2014. A) October 2012- 2013, b) October 2013 - 2014, and c) October 2014-2015

a)



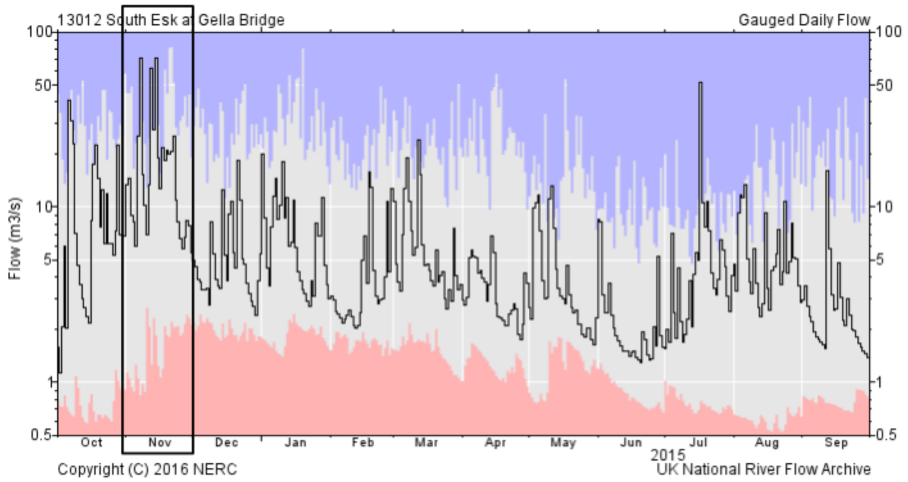
Key: Red and blue envelopes represent lowest and highest flows on each day over the period of record.

b)



Key: Red and blue envelopes represent lowest and highest flows on each day over the period of record.

c)



Key: Red and blue envelopes represent lowest and highest flows on each day over the period of record.

Appendix 5.1

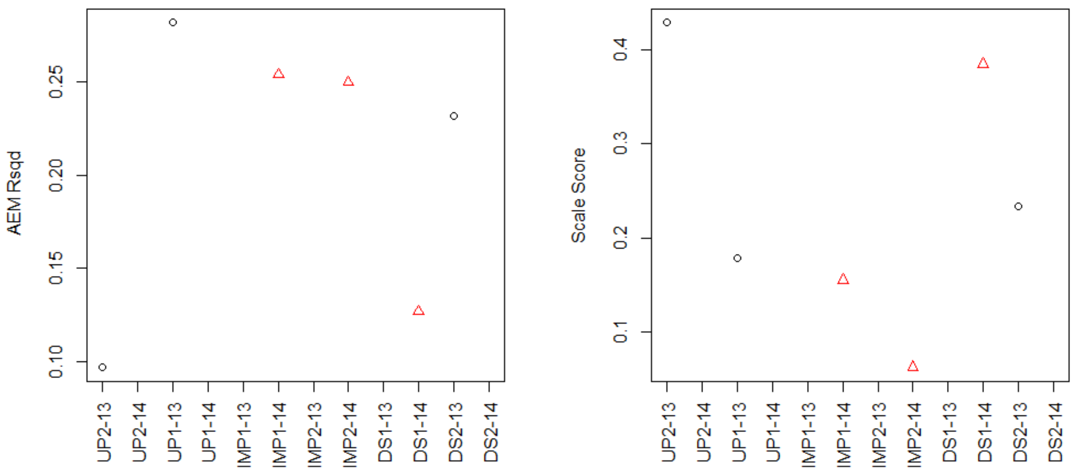
Categorisation of the Brox Burn EPT taxa into mobility groups based on trait data from Schmidt-Kloiber & Hering (2015) and Tachet et al. (2010)

	Mobility	Freshwaterecology.info	Tachet	overall
Lectra.fusca	5 burrowing	5 walking	5 walking, 2burrowing, 1 interstitial	mid
Euleuctra.geniculata	10 burrowing		same as Lectra	mid
Nemoura.cam.err	5 walking	5 other	5 walking	fast
Isoperla.gramatica	no data		4 walking 1 interstitial	mid
Serratella.ignita	8 walking	2 swimming	1 swimming 5 walking	fast
Ecdyornus.dis	4 swimming	6 other	1 swimming 5 walking	fast
Baetis.rhodani	6 swimming	4 walking	3 swimming 4 walking 1 interstitial	fast
Baetis.scambus	6 swimming	4 walking	same as Baetis	fast
Alainites.muticus	5 burrowing	5 walking	same as Baetis	med
Habrophlebia.fusca	2 swimming	8 walking	2 swim, 4 walking	fast
Rithrogena.semicolorata	10 walking		1 swim, 5 walking, 1 interstitial	mid
Paraleptophlebia.submarginata	3 burrowing	7 walking	1 swim, 4 walking, 1 burrowing	mid
Rhyacophila.dorsalis	10 walking		2 swim, 3 walk, 1 temp fixed	fast
hydroptilla sp	7 walking 3 other	mostly found in cases…	3 walking 1 temp fixed	mid
glossosoma.boltoni	no data		1 walking, 3 temp fixed	slow
Sericostoma.per	10 walking		4 walking, 1 burrowing, 1 interstitial	med
Polycentropus.flavomaculatus	10 semi sessile		1 swim in column, 1 walk, 3 temp fixed	slow
Polycentropus.irroratus	10 semi sessile		as Polycentropus	slow
Odontocerum.albicorn	8 walking	2 semi sessile	3 walking, 2 burrowing	mid
Tinodes.waernii	10 walking		2 walking, 3 semi sessile, 1 perm fixed	slow
Hydropsyche.siltalii	no data		2 walking 3 semi sessile	slow

Hydrophyche.pel	3 walking	7 semi sessile	as Hydrophyche	slow
Hydropsyche.cont	5 walking	4 semi, 1 other	as Hydrophyche	slow
Leptoceridae.sp	5 swimming	5 walking	1 swim in column, 3 walking	fast

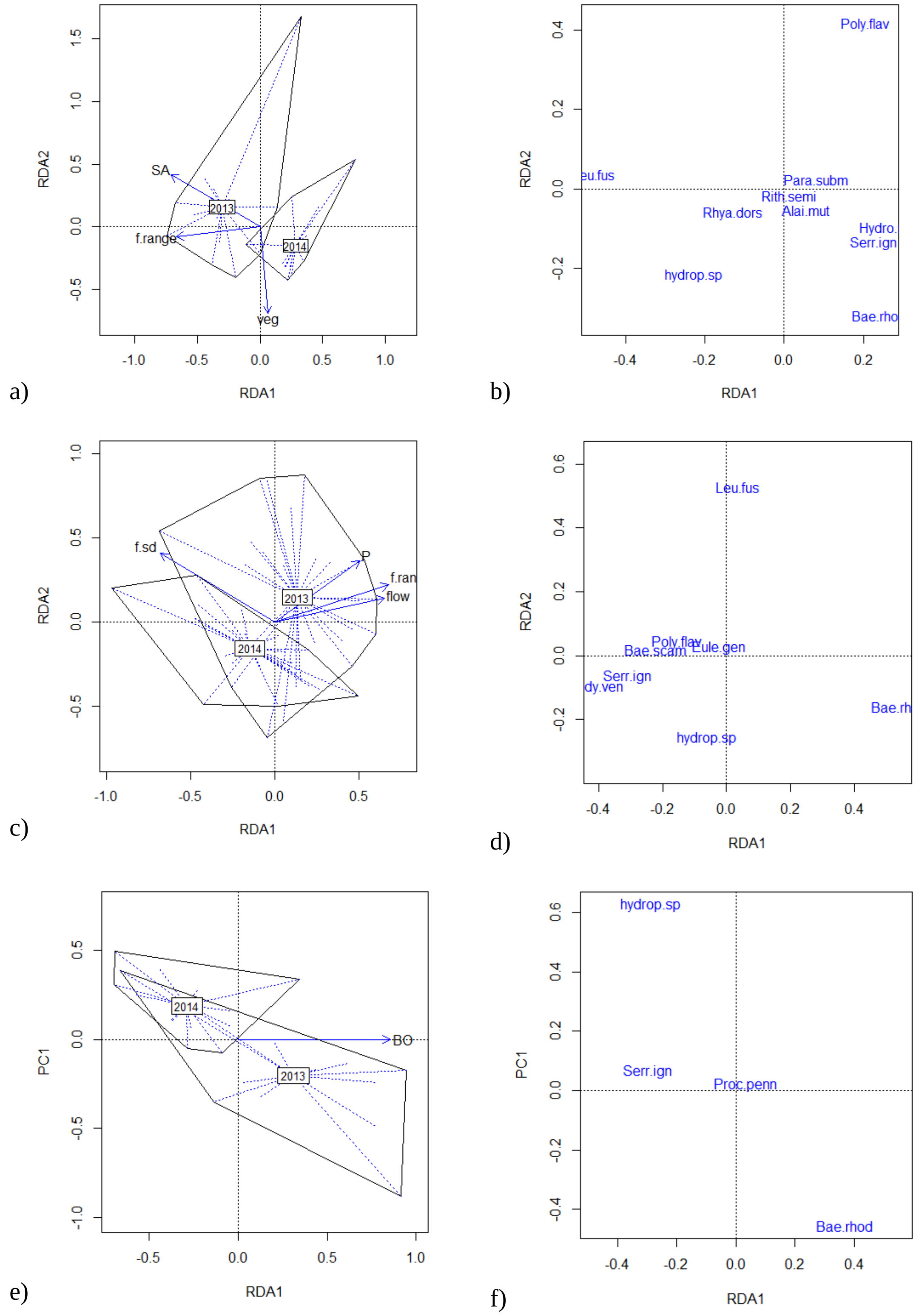
Appendix 5.2

Output of from RDA model comparing importance of spatial structuring of the invertebrate fauna using AEMs at six sites, pre and post impact, a) model R^2, and b) average spatial scale score (0.2= broad scale, 0.8 = fine scale).


a) b)

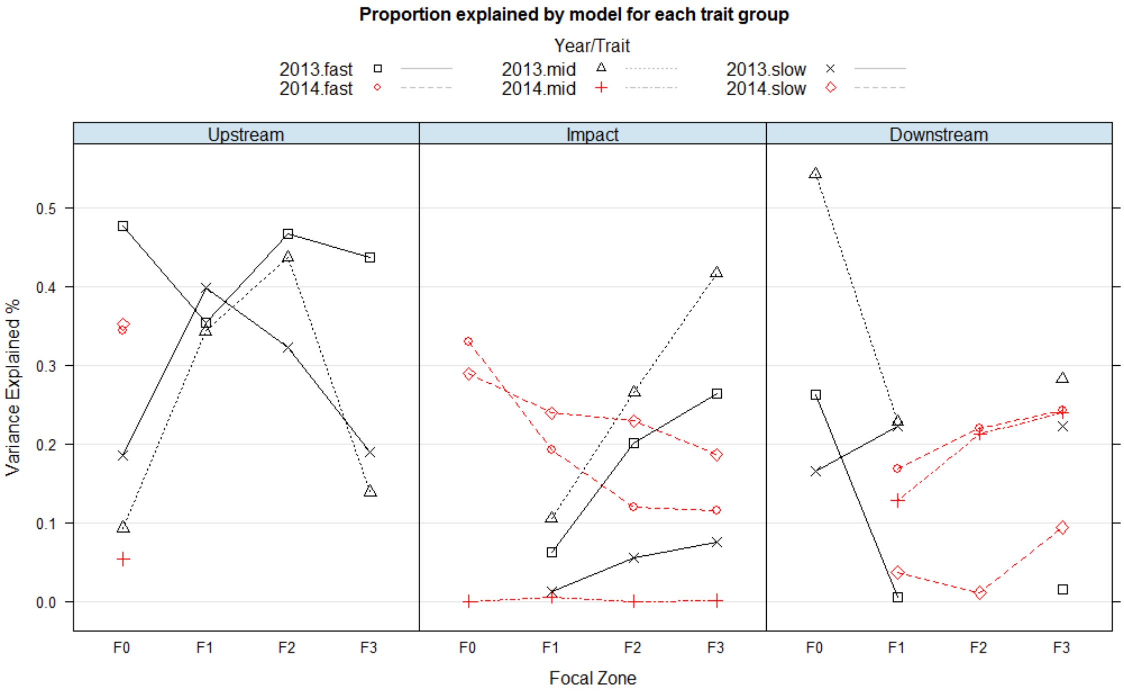
Appendix 5.3

Biplots of RDA analysis within each site for 2013 and 2014, left plots: points show samples and arrows indicate the environmental gradients, right plots: species locations, for a) US2, b) US1, c) IMP1, d) IMP2, e) DS1, f) DS2.



Appendix 5.4

Summary of individual pseudo species trait group R^2 for best RDA model using forward selected variables for each site treatment category (Upstream, Impact and Downstream) in each year.



Summary of individual pseudo species trait group R^2 for best RDA model using forward selected variables for each site treatment category (Upstream, Impact and Downstream) in each year.