INSTREAM WOOD AS A POTENTIAL NATURE-BASED SOLUTION TO NUTRIENT POLLUTION

by

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For Alice,

You motivate me to live the life I want to live.

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Abstract

Poor water quality is a ‘wicked problem’ – an uncertain and complex problem with no optimal solution - which poses risks to planetary and public health. Nature-based solutions (NBS) are vital to address water quality challenges on a sufficient spatial and temporal scale to realise long-term water security. The restoration of instream wood has been recognised as a particularly promising NBS to nutrient pollution, one of the most pervasive water quality challenges. Research was conducted to address knowledge gaps about the coupled hydrological and biogeochemical processes that control nutrient removal in the river corridor, and to evaluate the efficacy of instream wood restoration in different environmental settings. A laboratory experiment evaluated protocols of the resazurin-resorufin smart tracer system, which can be used to measure coupled hydrological and biogeochemical processes, showing that concentrations can change by up to 22.5% in 24 hours but in certain conditions samples can be stored for up to 14 days, increasing the geographical and experimental scope in which it can be applied. A microcosm experiment showed that streambed wood can lead to significant increases in microbial metabolic activity, nitrate removal rate and greenhouse has production. This demonstrates the often-neglected contribution of streambed wood to fundamental biogeochemical processes and the impacts on associated ecosystem (dis)services, with consequences for global models of carbon and nitrogen cycles, and for restoration practice. A before-after-control-impact field experiment, using conservative tracer methods coupled with a transport and storage model analysis, investigated the effects of installing instream wood in a lowland sandy stream on transient storage. The results suggest that in a lowland stream wood restoration could decrease transient storage, contrary to what has been observed in upland settings. For the first time, insights from hyporheic zone research are distilled, synthesised, and presented in a framework which is suitable to directly inform river restoration design.

Contents

Acknowledgements iv

Abstract v

List of figures xi

List of tables xvi

List of abbreviations xix

1 Introduction 1-1

1.1 Scientific Rationale 1-1

1.2 Aims and Objectives 1-4

1.3 Thesis Structure 1-4

1.3.1 Chapter 2. Increasing the scope of the resazurin - resorufin smart tracer system in hydrologic and biogeochemical sciences: The effects of storage duration and temperature on preservation. 1-5

1.3.2 Chapter 3. Instream wood as a driver of biogeochemical cycling: Neglected co-benefits and trade-offs in river restoration. 1-6

1.3.3 Chapter 4. A before-after-control-impact approach to assess the effect of instream wood restoration on transient storage in a lowland sandy stream. 1-6

1.3.4 Chapter 5. Restoring the liver of the river: Actionable research insights to guide the restoration of the hyporheic zone for the improvement of water quality. 1-7

1.3.5 Chapter 6. Conclusions and outlook. 1-7

2 Increasing the scope of the resazurin - resorufin smart tracer system in hydrologic and biogeochemical sciences: the effects of storage duration and temperature on preservation 2-9

2.1 Abstract 2-10

2.2 Introduction 2-11

2.3 Materials and Procedures 2-14

2.4 Assessment 2-18

2.4.1 Low concentrations of raz/rru 2-19

2.4.2 High concentration of raz/rru 2-22

2.5 Discussion 2-25

2.5.1 Effect of storage temperature 2-25

2.5.2 Variability of effects in different sample medium 2-26

2.5.3 Implications for experimental designs 2-27

2.5.4 Necessary future research 2-29

2.6 Recommendations 2-29

3 Instream wood as a driver of biogeochemical cycling: Neglected co-benefits and trade-offs in river restoration 3-31
3.1 Abstract 3-32

3.2 Introduction 3-33

3.3 Methodology 3-36

3.3.1 Microcosm set-up 3-36
3.3.2 Sediment collection 3-37
3.3.3 Spiking procedure 3-39
3.3.4 Water sampling and analysis 3-39
3.3.5 Gas sampling and analysis 3-40
3.3.6 Statistical analysis 3-41
3.4 Results 3-42

3.4.1 Carbon concentration and bioavailability 3-42
3.4.2 Dissolved oxygen 3-44
3.4.3 Microbial Metabolic Activity 3-46
3.4.4 Nitrate removal rate 3-47
3.4.5 Greenhouse gas production 3-50
3.5 Discussion 3-52

3.5.1 A fundamental component of river ecosystems 3-52
3.5.2 Explanatory mechanisms 3-52
3.5.3 Effect on ecosystem (dis)services 3-55
3.5.4 Consequences for global models of carbon and nitrogen 3-56
3.5.5 Study limitations 3-57
3.5.6 Implications for policy and practice 3-58

4 A before-after-control-impact approach to assess the effect of instream wood restoration on transient storage in a lowland sandy stream 4-60
4.1 Abstract 4-61

4.2 Introduction 4-62

4.2.1 Nature-based solutions and the hyporheic zone 4-62
4.2.2 Instream wood as a driver of hyporheic exchange 4-63
4.2.3 A mismatch between research and practice 4-64
4.2.4 Shifting the paradigm: from removal to introduction 4-65
4.2.5 Methodological challenges 4-66
4.2.6 Study aims 4-67
4.3 Methodology 4-68

4.3.1 Study site 4-68
4.3.2 Hydrological monitoring 4-69
4.3.3 Characterization of sub-reaches 4-70

4.3.4 Restoration of instream wood 4-72
4.3.5 Solute tracer injections 4-73
4.3.6 Determination of breakthrough curves, data processing and analysis 4-74
4.3.7 Statistical analysis 4-76
4.4 Results 4-78

4.4.1 Breakthrough curve analysis and Transient Storage Model fitting 4-78
4.4.2 BACI effect on transport and storage metrics 4-79
4.4.3 Relationship between discharge and transport and storage metrics 4-84
4.4.4 Comparing experimental designs 4-86
4.5 Discussion 4-88

4.5.1 Challenges in quantifying restoration impacts 4-88
4.5.2 The effect of instream wood restoration on transport and storage 4-90
4.5.3 The effect of discharge on the interaction between instream wood and transient storage 4-92
4.5.4 The experimental approach is important 4-93
4.6 Conclusions 4-94

4.6.1 Does restoring instream wood increase transient storage? 4-94
4.6.2 Limitations of quantifying restoration impacts on transient storage at the field scale 4-95
4.6.3 Recommendations for effective experimental designs to evaluate river restoration 4-95

5 Restoring the liver of the river: actionable research insights to guide the restoration of the hyporheic zone for the improvement of water quality 5-97
5.1 Abstract 5-98

5.2 Introduction 5-99

5.3 Drivers and controls of pollution remediation in the hyporheic zone 5-102

5.3.1 What is the hyporheic zone? 5-102
5.3.2 Properties of hyporheic exchange 5-103
5.3.3 Pollution retention and attenuation in the hyporheic zone 5-103
5.3.4 Designing restoration to target pollution 5-105
5.4 Considerations in planning hyporheic zone restoration 5-106

5.4.1 Sediment hydraulic conductivity 5-107
5.4.2 Gaining or losing conditions 5-107
5.4.3 Stream gradient 5-109
5.4.4 Flow conditions 5-109
5.4.5 Biogeochemical opportunity 5-110
5.4.6 Sediment load 5-112
5.4.6 Sites with the highest restoration potential 5-113
5.5 Designing hyporheic restoration strategies 5-113

5.5.1 Channel-spanning features 5-117
5.5.2 Deflectors 5-119

5.5.3 Channel reconfiguration 5-120
5.5.4 Sediment modifications 5-122
5.5.5 Development 5-124
5.6 Scenarios of hyporheic restoration 5-126

5.6.1 Scenario 1 5-127
5.6.2 Scenario 2 5-130
5.6.3 Scenario 3 5-133
5.6.4 Scenario 4 5-135
5.6.5 Scenario 5 5-138
5.7 Synergies and conflicts 5-140

5.7.1 Natural flood management 5-140
5.7.2 Ecology 5-141
5.7.3 Biogeochemical 5-142
5.8 Discussion 5-143

5.9 Conclusions 5-146

6 Conclusions and Outlook 6-148
6.1 Is instream wood restoration an effective nature-based solution to nutrient pollution? 6-148

6.2 Future research directions 6-150

6.3 Cautions on reductionism and generalisation 6-151

6.4 “This has implications for policy and practice” 6-153

6.5 Accelerating evidence acquisition 6-153

6.6 Integrating planetary and public health 6-155

7 Bibliography 7-156

8 Supporting Information 8-180
8.1 Chpater 2 8-180

8.1.1 Storage temperature ranges 8-180
8.1.2 Using one set of calibration data for every analysis period 8-181
8.1.3 Calibration in different water types 8-183
8.1.4 Percentage change data 8-185
8.1.5 Statistical analysis 8-187
8.2 Chapter 3 8-191

8.2.1 Water temperature 8-191
8.2.2 Grain size distribution 8-191

8.2.3 Loss on Ignition 8-192
8.2.4 pH 8-193
8.2.5 Oxidative redox potential 8-194
8.2.6 Statistical outputs 8-195
8.3 Chapter 4 8-200

8.3.1 Rating curve for calculating discharge 8-200
8.3.2 Wood survey 8-201
8.3.3 Restoration materials 8-202
8.3.4 Tracer injection periods 8-203
8.3.5 Break through curve modelling 8-204
8.3.6 Statistical model fits 8-207
8.3.7 Regression analysis 8-208
8.3.8 Statistical outputs 8-209

List of figures

Figure 2-1. The change in concentration in low starting concentration samples of raz (A-C) and rru (D-F) over time (days) in different mediums: DIW (A, D), GW (B, E) and SW (C, F). Starting concentrations are represented by the dashed black lines. Error bars represent standard deviation; some error bars are not visible because they are smaller than the data symbol. 2-19
Figure 2-2. Percentage change in concentration from low starting concentrations of raz (A-C) and rru (D-F) over time (days) in different mediums - DIW (pink), GW (blue) and SW (green) – and subject to different storage methods: room temperature (A,D), refrigerator (B,E) and freezer (C,F). The dashed lines represent 10% change (positive and negative) from starting concentration. Note the axis break after day 7, represented by the grey shaded area. Note missing data from days 2-7 in the freezer pane..... 2-20
Figure 2-3. The change in concentration in high starting concentration samples of raz (A-C) and rru (D-F) over time (days) in different mediums: DIW (A, D), GW (B, E) and SW (C, F). Starting concentrations are represented by the dashed black lines. Error bars represent standard deviation; some error bars are not visible because they are smaller than the data symbol. 2-23
Figure 3-1. a) River sediment sampling locations with underlying bedrock geology. b) Schematic microcosm design with wood buried by sediment at the bottom and water column and headspace above, with sampling tube and stopcock for gas sampling. c) microcosm treatments where pink = limestone sediment, yellow = sandstone sediment, purple = chalk sediment and grey = no sediment; wood was added to half the treatments and each treatment was replicated 5 times. The location of treatments was randomised. d) Water temperature (^{o}C) before and during the experimental period, where the black line represents temperature at 12 noon, the blue line represents the LOESS (locally weighted smoothing) and the vertical red lines indicate measurement dates. 3-38
Figure 3-2 Measures of carbon quality and quantity in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill. (a) and (b) represent the concentration of non-purgable organic carbon (NPOC) separately at five measurement periods (represented by mean water temperatures) and amalgamated, respectively. (c) represents the concentration of DOC at the end of the study where triplicates of sampling and analysis were conducted to ensure accuracy of results. (d) and (e) represent the specific ultra-violet absorbance at 254 nm and 280 nm, respectively, both at decommission. 3-44
Figure 3-3 Percentage change in dissolved oxygen percent saturation over a 26-hour measurement period in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b). 3-45
Figure 3-4. Production rate of rru per hour normalized for the starting concentration of raz in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill),

presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b). 3-47
Figure 3-5. The rate of nitrate removal per hour in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b). 3-49
Figure 3-6. Positive net production rate of CO2 (a, b), CH4 (c, d) and N_2O (e, f) per hour in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (b, d , f) and amalgamated (a, b, c)..... 3-51
Figure 4-1. Catchment with (a) the location of Wood Brook within the UK and (b) the dominant land use, study reach with control and impact sub-reaches, injection point, tracer measurement locations and Q monitoring point. Scale bar relates to (b) only. Produced using ArcGIS 10.8.1, ESRI. 4-69
Figure 4-2. The Q (m^3/s) throughout the experimental period (April-November 2021) derived from the stage-Q relationship presented in Figure S1. Tracer injection periods are indicated by dashed yellow (before restoration) and purple (after restoration) lines. The solid red line indicates the date of restoration. 4-70
Figure 4-3. Two of the channel-spanning wood bundle features immediately after installation in June 2021...... *4-73*
Figure 4-4. An example (from the tracer injection on 09-06-21) of observed BTC data and the continuous time random walk mobile-immobile model fits at each of the measurement locations, that is at the start of the control reach (after complete mixing of the tracer), at the end of the control reach and start of the impact reach, and at the end of the impact reach. The modelled BTC time is truncated in the plot but actually extended to t_T20. 4-79
Figure 4-5. Timeseries and transient storage metrics plotted against the date of injection. The vertical dashed line represents the date of restoration in the impact reach. Transient storage metrics are the best-fit objective functions from the transient storage model and time series metrics were calculated using modelled BTCs: (a) transient storage index (TSI), (b) skewness, (c) coefficient of variation (CV), (d) holdback, (e) residence time in immobile zones (β), (f) dispersion (D), (g) the rate of exchange of solutes between the mobile zone and immobile zone (λ) (note log10 scale), and (h) velocity. *4-81*
Figure 4-6. Average timeseries metrics and transient storage parameters are plotted separately for the impact (purple) and control (yellow) reach for the before and after periods. Transient storage metrics are the best-fit objective functions from the transient storage model and time series metrics were calculated using modelled BTCs: (a) transient storage index (TSI), (b) skewness, (c) coefficient of variation (CV), (d) holdback, (e) residence time in immobile zones (β), (f) dispersion (D), (g) the rate of exchange of solutes with the mobile zone and immobile zone (λ), and (h) velocity. Error bars represent mean ± standard deviation...... *4-83*
Figure 4-7. Timeseries and transient storage metrics regressed against Q separately for before and after restoration and impact and control reaches. Regression equations and

coefficients of determination are reported in table S4. Transient storage metrics are the best-fit objective functions from the transient storage model and time series metrics were calculated using modelled BTCs: (a) transient storage index (TSI), (b) skewness, (c) coefficient of variation (CV), (d) holdback, (e) residence time in immobile zones (β), (f) dispersion (D), (g) the rate of exchange of solutes with the mobile zone and immobile zone (λ) (note log10 scale), and (h) velocity..... 4-84
Figure 5-1. Hyporheic exchange through a bedform (e.g. sand dune). Thick arrows represent the general movement of water from upstream of the bedform (left) to downstream (right), and do not necessarily represent the pathway of water through the bedform. The colour of the arrow represents the residence time distribution of water in the hyporheic zone. The 'Too short' arrow represents water that returns to the channel before all the pollutants are transformed. The 'Just right' arrow represents water that returns to the channel just as but not before all the pollutants are transformed. The 'Too long' arrow represents water that returns to the channel a long time after all the pollutants are transformed. The purple star represents the point at which all the pollutant has been removed, which is well before the water returns to the channel for the 'too long' flow path. The percentage in the orange cloud represents the remaining percentage of pollutant when the water is returned to the channel, which is still 50% for the 'too short' flow path. 5-105
Figure 5-2. 2(i). Schematics representing the properties of hyporheic exchange (blue/yellow arrows) generated by different restoration techniques and designs. a) Impermeable weir of perfect height in an upland setting. b) Impermeable weir of short height in an upland setting. c) Impermeable weir of tall height in an upland setting. d) Permeable weir of perfect height in an upland setting. e) Impermeable weir of perfect height with a bottom gap in an upland setting. f) Impermeable weir of perfect height with two outflow heights in an upland setting. g) Impermeable weir of perfect height with pool development in an upland setting. h) Impermeable step with fine sediment upstream in a lowland setting. i) Constructed riffle and pool in a lowland setting..... 5-115
Figure 5-3.(ii). Continued from Figure 2(i). j) J-hook and pool in a lowland setting, adapted from Rosgen, 2001. k) Plan view of a J-hook and pool in a lowland setting, adapted from Rosgen, 2001. l) Plan view of a deflector in a lowland setting. m) Plan view of channel reconfiguration (re-meandering) in a lowland setting. n) Plan view of channel reconfiguration (island construction) in a lowland setting. o) High porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. p) Low porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. q) Long low porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. q) Long low porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. r) Low porosity sediment modification coupled with upstream step in a lowland setting, adapted from Bakke, Hrachovec and Lynch, 2020. 5-116
Figure 5-4. Schematic representing scenario 1. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times. 5-127

Figure 5-5. Schematic representing scenario 2. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times. 5-130
Figure 5-6. Schematic representing scenario 3. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times. 5-133
Figure 5-7. Schematic representing scenario 4. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times. 5-135
Figure 5-8. Schematic representing scenario 5. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times. 5-138
Figure 8-1. The air temperature (degrees Celsius) of storage methods room, refrigerator and freezer, over the experimental period. The grey vertical highlighted areas represent sampling days with the widest area in July representing the first week of analysis where samples analysed in 24-hour intervals. The horizontal dashed lines represent the minimum and maximum temperatures, omitting the first 48 hours and final 24 hours..... 8-180
Figure 8-2. The concentration of resazurin and resorufin in low concentration mixtures calculated using calibration information collected on the first day of the experiment (orange circles) and collected on the day of analysis (blue diamonds). 8-182
Figure 8-3. Standard solutions of resazurin (A) and resorufin (B) prepared in different water types, deionised water (dark grey), surface water (light grey) and groundwater (grey). Linear regression equations are presented. 8-184
Figure 8-4. Water column temperature at five measurement periods (represented by mean water temperatures on x axis) in microcosms containing different treatments of sediment and wood (no wood = white infill, wood = grey infill)..... 8-191
Figure 8-5. Grain size distribution of 3 sediment types..... 8-192
Figure 8-6. Loss on ignition at (a) 550 ^{o}C and (b) at 950 ^{o}C of the sediment with wood removed at the end of the experimental period in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill). 8-193
Figure 8-7. pH in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated..... 8-194

Figure 8-8. Oxidation reduction potential (ORP) of the water column at the end of the experimental period in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill). 8-195
Figure 8-9. The stage-discharge relationship established at Wood Brook using observations between 2019 and 2021. Black points represent measured discharge and the black line represents the modelled relationship between stage and discharge. The polynomial equation of the modelled line and the coefficient of determination are presented. 8-200
Figure 8-10. The change in elevation (top) and the distribution of instream wood pieces (red) and accumulations (orange) (bottom) throughout the study reach. The vertical purple dashed line represents the end of the control reach and the start of the impact reach. 8-202
Figure 8-11. An example (observed data and modelled BTCs from the tracer injection on 09-06-21) of the plots were used to assess the identifiability and equifinality of model parameters and the goodness of fit to observed data. The left column includes dotty plots to demonstrate parameter sensitivity where the dots represent parameter values that meet a behavioural error threshold: black dots = top 1%, red dots = top 0.05%. Dotty plots from left to right = Velocity, dispersion (d), residence time in immobile zones (β), and the rate of exchange of solutes with the mobile zone and immobile zone (λ). The middle column of plots shows the model fit in log time vs. log specific conductance, where black dots represent observed data and the black line represents modelled data. The right column of plots shows the model fit in linear time vs. specific conductance, where red dots represent observed data and the black line represents modelled data. Each row represents a different monitoring location, where the 1st row represents the start of the control reach, the 2nd row represents the end of the control reach and the start of the impact reach, and the 3rd row represents the end of the impact reach. Plots produced using adapted MATLAB code from Drummond et al. (2019).. 8-204

List of tables

Table 2-1 Average (n=5) concentrations of non-purgable organic carbon, nitrate, nitrite, ammonium, and phosphate (all presented in mg/L) prior to addition of tracers for 3 mediums: DIW, GW and SW. Standard deviation is reported in brackets. *2-14*
Table 4-1. Physical, geomorphic and instream wood characteristics of the sub-reaches. . *4-72*
*Table 4-2. The significance of different statistical tests is reported for different experimental approaches: BA = before-after (period), CI = control-impact (reach), BACI = before-after-control-impact (period*reach). Yes indicates significant (alpha = 0.05) and No indicates not significant. \ indicates test not conducted. Normality was not achieved for λ, therefore a REML or ANOVA was not performed but instead a Kruskal Wallis analysis. ANOVA = analysis of variance. TSI = transient storage index, CV = coefficient of variation, β = residence time in immobile zones, and λ = the rate of exchange of solutes with the mobile zone and immobile zone.* *4-87*
Table 8-1. Linear regression equations for resazurin and resorufin calculated using 4-standard solution concentrations prepared on the day of analysis. *8-182*
Table 8-2. Mean percentage change (n=3) from starting concentration (day 0) for rru (A) and raz (B) over time (1 – 84 days). DI = deionised, GW = groundwater and SW = surface water. Cells containing "-" indicate where samples were not analysed. *8-185*
Table 8-3. Mean percentage change (n=3) from starting concentration (day 0) for rru (A) and raz (B) over time (1 – 84 days). DI = deionised, GW = groundwater and SW = surface water. Cells containing "-" indicate where samples were not analysed. Cells containing "" indicate where the ULQ was exceeded. *8-186*
Table 8-4. Details of the statistical tests conducted, and associated outputs, to investigate the differences between storage methods. Storage indicates the storage method: R = room temperature, C = cooled, F = frozen. Normal indicates normality of data, tested with Shapiro-Wilk tests. Test indicates the statistical test conducted which was based on the number of storage types compared: KW = Kurskal-Wallis, T = students T-test, WSR = Wilcoxon signed-rank, ANOVA = analysis of variance. Df indicates degrees of freedom. Mean sq. Test stat. indicates the test statistic associated with the statistical test conducted. Sig. indicates whether the statistical test reported statistical significance (yes) or not (no). . *8-187*
Table 8-5. Results of Tukey post-hoc tests for pairwise analysis conducted on significant ANOVA results only. *8-189*
Table 8-6. Results of the Wilcoxon signed-rank tests conducted to identify when tracer concentration was statistically different to starting concentrations. *8-190*
Table 8-7. Outputs of likelihood ratio tests testing the difference in the concentration of non-purgeable organic carbon between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom. *8-196*
Table 8-8. Outputs of likelihood ratio tests testing the difference in the concentration of microbial metabolic activity between microcosms containing wood and those not containing

wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom. *8-196*
Table 8-9 Outputs of likelihood ratio tests testing the difference in the percentage change in the concentration of dissolved oxygen between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom. *8-197*
Table 8-10 Outputs of likelihood ratio tests testing the difference in the nitrate removal rate between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom. *8-197*
Table 8-11. Outputs of likelihood ratio tests testing the difference in the rate of CO_2 production between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom. *8-198*
Table 8-12 Outputs of likelihood ratio tests testing the difference in the rate of CH_4 production between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom. *8-198*
Table 8-13. Outputs of likelihood ratio tests testing the difference in the rate of N_2O production between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom. *8-199*
Table 8-14. Tracer injection dates and masses. *8-203*
Table 8-15. Tables present the model best fit parameters (value) and the standard deviation of the best 0.05% fits (std) for each tracer injection and measurement location. Parameters = velocity, dispersion, the rate of exchange of solutes with the mobile zone and immobile zone (λ), and the residence time in immobile zones (β). Values extracted using adapted MATLAB code from Drummond et al. (2019). *8-205*
Table 8-16. Goodness of fit of each statistical model applied to each transport and transient storage metric, assessed using Akaike information criterion (AIC). REML = Residual maximum likelihood. ANOVA = Analysis of variance. ANCOVA = Analysis of Covariance. TSI = transient storage index. CV = coefficient of variation. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone. *8-207*
Table 8-17. Details of linear regression equations and coefficient of determination (r^2) between transport and transient storage metrics and discharge, the plots of which are presented in Figure 3 of the main text. TSI = transient storage index. CV = coefficient of variation. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone. *8-208*

Table 8-18. Summaries of statistical tests are reported for each transport and storage metric. ANOVA = analysis of variance. ANCOVA = analysis of covariance. Q = discharge. Sum sq. = sum of squares. NumDF = degrees of freedom in the numerator. DenDF = degrees of freedom in the denominator. Std. = standard deviation. SE = standard error. CI = confidence interval. Df = degrees of freedom. Diff = difference in means. Lower = lower confidence interval. Upper = upper confidence interval. TSI = transient storage index. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone. CB = control-before. CA = control-after. IB = impact-before. IA = impact-after. *8-209*
Table 8-19. Summary of statistical tests performed to assess before-after and control-impact experimental designs. Df = degrees of freedom. λ was not normally distributed so a non-parametric Mann-Whitney U test was performed instead of a students T-test. TSI = transient storage index. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone. *8-218*

List of abbreviations

Different abbreviations have been used in each chapter as appropriate for the target audience for which it has been written. Therefore, abbreviations are described in the text at first use, as well as reported below.

% - percent

°C – degrees Celsius

μg – microgram

μl – microlitre

~ – approximately

< – less than

> – greater than

AIC - akaike information criterion

ANOVA – analysis of variance

asl – above sea level

BA – before-after

BACI – before-after-control-impact

BTC – breakthrough curve

C – carbon

CH_4 – methane gas

Chisq - chi squared value

CI – control-impact

CO_2 – carbon dioxide gas

COOLED – stored in a refrigerator

D – dispersion

Df - degrees of freedom

DIW – deionised water

DOC – dissolved organic carbon

DQO – data quality objectives

EC – electrical conductivity

ECD - electron collision detector

FID - flame ionisation detector

FROZEN – stored in a freezer

GHG – greenhouse gas

GW – groundwater

hr – hour

HZ – hyporheic zone

KW - Kruskal Wallis

l – litre

LMEM - linear mixed-effects models

LogLik - log-likelihood value

LOI – loss on ignition

m – meter

m/m – meters/meter (slope)

m/s – meters/second

mg – milligram

min – minute

mm – millimetre

MMA – microbial metabolic activity

N – nitrogen

N_2 – dinitrogen gas

N_2O – nitrous oxide gas

NBS – nature-based solution

NH_3-N – ammonium as nitrogen

NH_4-NH_4 – ammonia as ammonia

nm – nanometre

NO_2-N – nitrite as nitrogen

NO_3 – nitrate

NO_3-N - nitrate as nitrogen

NO_3-NO_3 – nitrate as nitrate

NPOC - non-purgable organic carbon

ORP - oxidative redox potential

P – phosphorus

PO_4-P – phosphate as phosphorus

PO_4-PO_4 – phosphate as phosphate

Q – stream discharge

Raz – resazurin

REML - residual maximum likelihood

RES – river ecosystem service

ROOM – stored at room temperature

Rru – resorufin

SI – supporting information

SW – surface water

t_C - time of maximum tracer concentration

t_{MAX} - the time from the tracer injection to the final sampling time

TPH - tukey post-hoc

TSI - transient storage index

t_T – truncation time

ULQ – upper limit of quantification

V – velocity

WSR - Wilcoxon signed-rank tests

β - residence time therein is defined by the power law residence time distribution of solute within the immobile zone

λ - rate of exchange of water between the mobile zone and immobile zone

1 Introduction

1.1 Scientific Rationale

The sustainable management of water resources amidst increasing environmental and anthropogenic pressures is one of the greatest challenges facing society in the 21st century. Poor water quality is an especially pervasive and persistent component of this challenge, threatening food security and human health, degrading ecosystem health, and ultimately thwarting societal progress (Damania et al., 2019; van Vliet et al., 2021). Despite decades of research, management and regulation, improvements to water quality have been limited in geography and in scope (e.g. the type of the pollutants remediated), and many pollutants persist or worse still continue to increase (Hannah et al., 2022; Whelan et al., 2022). Current approaches to water quality management – typically grey infrastructure like water treatment plants – are insufficient and result in high energy consumption and operating costs (Boano et al., 2020). A more holistic and integrated approach is required to sustainably address contemporary and complex water quality challenges on a sufficient spatial and temporal scale to realise long-term water security.

Nature-based solutions (NBS) are actions to protect, manage and restore ecosystems and ecosystem processes to deliver services that address societal challenges (UNESCO, 2018). NBS in the river corridor could deliver improvements to water quality throughout the river network and reduce the transport of pollutants from rivers to groundwaters, lakes and oceans. Many river pollutants – such as nutrients, heavy metals, pathogens and emerging contaminants – can be retained and transformed to environmentally benign products, primarily in the hyporheic zone where a matrix of physiochemical conditions facilitates a range of biogeochemical reactions (Tesoriero et al., 2013; Boano et al., 2014; Herzog, Higgins and McCray, 2016). The hyporheic zone is the area surrounding the river channel (in

the bed and banks) where surface water and groundwater interact (Ward, 2015). These interactions are driven by energy gradients along the riverbed that arise from hydrostatic and hydrodynamic forces (Sawyer, Cardenas and Buttles, 2011). In many rivers, however, centuries of mismanagement – like straightening, channelization and simplification – has reduced these interactions, degrading the function of the hyporheic zone and limiting its capacity to provide these ecosystem services (Stofleth et al., 2008; Ward et al., 2018).

Understanding of the drivers, controls, and mechanisms underpinning the function of the hyporheic zone are now fairly well understood, and have been comprehensively reviewed by several authors, e.g. Boano et al. (2014), Lewandowski et al. (2019) and Krause et al. (2022). As a fundamental control of water quality in the river corridor, the restoration of the hyporheic zone has received considerable attention in the last decade or so (Hester and Gooseff, 2010; Lewandowski et al., 2019). However, significant uncertainties remain about the effect of different restoration techniques in different environmental settings (Hester, Brooks and Scott, 2018).

The restoration of instream wood has been recognised as a particularly promising technique, which could provide a simple, cost-effective NBS to one of the most persistent water quality challenges, nutrient pollution (Krause et al., 2014; Blaen et al., 2018). Pressures from nutrient pollution have increased since 1940, which include causing ecosystem health decline, contaminating drinking water, and accelerating the production of N_2O, which contributes to climate change (Galloway et al., 2008; Krause et al, 2014, Whelan et al., 2022). I hypothesise that (re)introducing instream wood could increase the rate of transformations of nutrients from polluting forms to environmentally benign forms, by abating two primary limitations of the biogeochemical function of the hyporheic zone: (1) reaction kinetics and (2) physical transport. (1) The decomposition of instream wood could increase the availability of dissolved organic carbon, which often limits biogeochemical reaction rates because it is

necessary as an electron donor in redox reactions and for the microbial metabolic activity that facilitates them (Zarnetske et al., 2012; Quick et al., 2019). (2) Instream wood could drive hyporheic exchange, increasing the delivery of reactants to biogeochemically active zones and the residence time therein (Sawyer and Cardenasm 2012). In reality, these hydrological and biogeochemical process are intrinsically coupled (Findlay, 1995), so the simultaneous abatement of both limitations could prove especially effective.

Despite the aforementioned uncertainties, the success of programmes aiming to improve water quality is not usually limited by scientific understanding (Wohl, Lane and Wilcox, 2015). Instead, success is limited by the capacity to measure and monitor critical ecosystem controls and to evaluate the outcomes of interventions (Friberg et al., 2016; Kirschke et al., 2020). For example, traditional methods for measuring properties of the hyporheic zone struggle to separate metabolically active storage (i.e. that which facilitates high biogeochemical reaction rates) from transient storage in the channel (i.e. dead zones) (Haggerty, Argerich and Martí, 2008). Furthermore, scientific assessments of restoration efficacy often fail to properly isolate the effect of restoration from natural spatial and temporal variability (Christie et al., 2020). Improved methodologies are necessary to accelerate gains in understanding and to enable researchers to rigorously evidence the impact of interventions to directly inform practice and policy. The success of restoration is further limited by the failure to apply research insights to practice (Palmer, Filoso and Fanelli, 2014). For example, hyporheic zone research is seldom applied to river restoration (e.g. in a process-based approach) (Lewandowski et al., 2019). Researchers must commit more seriously to communicating insights from research in a manner which is actionable, which is to say can be used directly to inform decisions.

1.2 Aims and Objectives

The primary aims of the work presented in this thesis are to address knowledge gaps about the impact of (re)introducing instream wood on the function of the hyporheic zone, to evaluate the potential of this technique to provide NBS to water quality challenges and to promote the application of hyporheic zone research in process-based river restoration. To achieve these aims research focussed on the following objectives:

1. To evaluate the techniques and methodologies that are commonly employed to measure hyporheic zone processes and to evaluate the efficacy of river restoration techniques.
2. To determine the effects of wood in river sediments on fundamental ecosystem processes and the consequences for the delivery of ecosystem services and dis-services.
3. To investigate the effect of instream wood restoration on the hydrological properties of the hyporheic zone in the environmental setting in which it is usually used in restoration.
4. To distil and synthesise hyporheic restoration research insights and communicate them in an actionable format so they can directly inform process-based river restoration.

1.3 Thesis Structure

The thesis is structured to systematically address the research objectives outlined in section 1.2. Excluding the introductory and concluding chapters - chapters 1 and 6, respectively - chapters are structured and written in publication format, in general following the structure: abstract, introduction, methodology, results, discussion, conclusions. A combined bibliography and the supporting information for each chapter are presented at the end of the thesis, in sections 7 and 8, respectively. A brief outline of each chapter is presented here, and the research objective(s) which the chapter seeks to address is identified. Where relevant,

information is provided regarding the format in which the chapter is, or is intended to be, published, and the target audience.

1.3.1 Chapter 2. Increasing the scope of the resazurin - resorufin smart tracer system in hydrologic and biogeochemical sciences: The effects of storage duration and temperature on preservation.

The resazurin (raz) – resorufin (rru) smart tracer system is commonly used in hydrologic and biogeochemical applications, for example to estimate hyporheic zone processes such as stream metabolism, nutrient turnover, and the reactivity of groundwater - surface water interactions (González-Pinzón et al., 2015). However, the reliability and comparability of results obtained using this method has been questioned because of a lack of standard protocols. Additonally, its application has been limited in scope and geography by the short duration that it has been assumed samples can be reliably stored before analysis. This chapter aimed to address research objective (1) by evaluating this technique and establishing rigorous and reliable storage protocols to improve its application. For the first time, a laboratory experiment was conducted to systematically test how tracer concentrations change in different waters and the effects of storage temperatures and duration. New evidence-based storage protocols are recommended, which will allow researchers to apply this invaluable system in wider geographical (remote) locations and in more complex experimental designs. Assistance in some of the laboratory work was provided by Qi Su and Abigail Roberts as part of their Master's theses. This work is published in Limnology and Oceanography: Methods as an evaluation of an existing method. doi: 10.1002/lom3.10514 (Howard et al., 2022).

1.3.2 Chapter 3. Instream wood as a driver of biogeochemical cycling: Neglected co-benefits and trade-offs in river restoration.

Research objective (2) is addressed in chapter 3, which investigates the impact of introducing wood to river sediments on microbial metabolic activity (a keystone ecosystem process) and a suite of physiochemical conditions, as well as the consequences for the delivery of associated ecosystem services, benefits to society, (nitrate removal rate) and dis-services, costs to society (greenhouse gas production). A microcosm study including 40 microcosms and 3 sediment types was conducted at Ecolaboratory at the University of Birmingham, and ran for almost 2 years. Insight from chapter 2 informed the protocols adopted in using the raz-rru smart tracer system to measure microbial metabolic activity. The consequences of this work for river restoration practice are discussed. This work is intended to be submitted in the journal of Environmental Science and Technology.

1.3.3 Chapter 4. A before-after-control-impact approach to assess the effect of instream wood restoration on transient storage in a lowland sandy stream.

Chapter 4 addresses research objective (3) by investigating the effect of restoring instream wood on the properties of transient storage (which may include hyporheic exchange) during different flow conditions. A before-after-control-impact field experiment was conducted at the Birmingham Institute of Forest Research field site in Staffordshire, UK. A number of conservative tracer injections were performed resulting in a series of breakthrough curves which were modelled to identify key transport and storage metrics. Modelling work was advised by Dr. Jen Drummond. This chapter also addresses research objective (1) by comparing experimental approaches and considering how different approaches may lead to different, and sometime erroneous, conclusions. Furthermore, the relevance of statistical significance in river restoration evaluations is considered.

1.3.4 Chapter 5. Restoring the liver of the river: Actionable research insights to guide the restoration of the hyporheic zone for the improvement of water quality.

This chapter aims to address research objective (4) by synthesising research on the hyporheic zone and its restoration (including insights from chapters 3 and 4) and distilling it into valuable research insights. These insights are communicated in a format that allows them to be directly applied to decision making, for example in identifying optimal locations and designs of river restoration. To address the paucity of hyporheic restoration demonstration sites, a series of theoretical scenarios are presented which demonstrate how the hyporheic zone may be restored in a range of environmental settings and to abate a range of pressures. This work is intended to be used by researchers, professionals and practitioners to formulate hypotheses about how restoration techniques are likely to perform in different environmental settings. It has been necessary, therefore, to simplify and generalise. It is not intended to include every single relevant reference but instead highlight only the most relevant. This work was in part informed by many discussions and a questionnaire conducted at the River Restoration Centre Annual Network Conference (June 2022), 'Making river restoration mainstream'. It is intended to be published as a book chapter and to inform the guidance published by the River Restoration Centre in the UK. Furthermore, it will be used to guide workshops on restoring the hyporheic zone, the first taking place in October 2022.

1.3.5 Chapter 6. Conclusions and outlook.

In this section, I the author, reflect on the conclusions of the individual chapters and how they interact to form an overall message of this thesis. I identify some key themes that pervade throughout this work or that, whilst only touched upon here, point to some of the grand

challenges we are yet to overcome. The works reported here are contextualised into the wider landscape of water quality challenges, focussing on the relationship between research and practice. I present something of a short manifesto on how I think we - a community of researchers, professionals and policy makers – can accelerate the improvement of policies and practices that seek to deliver water quality improvements.

2 Increasing the scope of the resazurin - resorufin smart tracer system in hydrologic and biogeochemical sciences: the effects of storage duration and temperature on preservation

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2.1 Abstract

The resazurin (raz) - resorufin (rru) smart tracer system has led to significant advances in hydrologic and biogeochemical sciences but its application has been limited in scope and geography by the short duration that it has been assumed samples can be reliably stored before analysis. For the first time, we quantify the effects of sample storage duration and temperature on measured raz and rru concentrations in order to identify robust storage protocols. Raz/rru concentrations equivalent to those typically used in field and laboratory applications were prepared in three different mediums (deionised water, groundwater and surface water). Samples were stored at 20°C, 5°C and -20°C, representing average room, refrigerator and freezer temperatures. Analysis of raz/rru concentration changes were conducted systematically during an 84-day period, with higher frequency of analysis in the first 7 days. For samples stored at room temperature, changes of up to 22.6% were observed over the initial 24 hours. In contrast, for samples stored in the refrigerator and freezer, changes of up to 8.4% and 6.9% were observed in the same period, respectively. Freezing samples provided the best preservation, yielding a maximum of ~10% change after 14 days compared to a maximum of ~30% change when cooled. It is generally recommended that raz/rru samples be stored cooled for up to 48 hours and frozen for up to 14 days. This offers exciting opportunities to broaden the application of the raz-rru system to undertake measurements in wider geographical (remote) locations and at increased sampling frequencies.

2.2 Introduction

The resazurin (raz) - resorufin (rru) smart tracer system has been increasingly adopted in the hydrological sciences since its introduction to the field by Haggerty et al. (2008). It has been applied to a range of experimental designs including hydrological tracer injections (Lemke et al., 2013), flume experiments (Haggerty et al., 2014) and column and batch experiments (Comer-Warner et al., 2018). The adoption of the technique has significantly accelerated scientific advancement in several sub-disciplines and contributed to improving our understanding of important ecosystem controls such as metabolically active groundwater - surface water interactions and system metabolism in rivers (González-Pinzón et al., 2015), lakes (Baranov et al., 2016) and wetlands (Riveros-Iregui et al., 2018). For more information about these applications and contributions to the literature, readers should refer to the recent review by Knapp et al. (2018).

The raz-rru system is capable of providing information about the microbial and biogeochemical conditions and functioning of the environment(s) it is exposed to by its function as a selective binary nano-switch (Knapp et al., 2018). Raz is irreversibly transformed to rru in mildly reducing conditions and mostly in the presence of living microorganisms, but it is effectively conservative in oxic environments such as most surface waters (Haggerty et al., 2008). Both raz and rru are fluorescent, with optimal excitation/emission wavelength pairs at sufficient distance to separate signals. This allows detection on both traditional benchtop fluorometers (such as Varian Cary Eclipse, Agilent Technologies, Inc.) as well as through on-line field fluorometers (such as GGUN FL30, Albillia Sarl, Switzerland) of concentrations < 1 µg/L, depending on the specific instrument employed and the medium (i.e. water type) in which the compounds exist (Lemke et al., 2013; Blaen et al., 2017; Blaen et al., 2018).

Best practice guidelines on how to conduct raz-rru experiments have been developed collaboratively and consecutively by the hydrological community. More recently, these have been refined into standard procedures for both field and laboratory applications (Blaen et al., 2017; Knapp et al., 2018). These guidelines include notes on solute preparation, sample extraction and processing (i.e. filtering and buffering pH), instrument calibration and correction, and data processing (e.g. correcting for signal overlap). Some guidelines exist on sample storage, for example samples should be stored in the dark (Haggerty et al., 2008), at temperatures of around 4 °C and may be frozen (Knapp et al., 2018). However, to date no systematic analysis has been conducted that would explicitly quantify the effects of storage duration, medium and preservation method (i.e. temperature control) on the stability or potential decay of raz and rru. In fact, many applications do not specifically report sample storage time or preservation method which has been identified as a challenge for intercomparison of experimental observations (Lemke et al., 2013).

This lack of standardisation, and paucity of understanding of the impacts of storage duration and preservation method, may lead to overly conservative application which may partly explain the limitation of most previous applications to systems where faster sample analysis is possible, restricting the experimental complexity and geographical scope in which the raz-rru system is applied. Furthermore, not knowing for how long samples can reliably be stored, or by which preservation method, reduces the number of samples which can be collected as most researchers aim to analyse all samples within 24 hours. Increasing the time in which samples can be analysed reliably would allow more samples to be collected and therefore facilitate more complex experimental designs, for example those which aim for both high temporal and spatial sample frequency. Whilst the introduction of on-line fluorometers has facilitated similar designs, these have been mostly restricted to surface water analysis and have been challenged by instrumental error, such as drift and interference (Blaen et al., 2017).

To date, the application of raz/rru in the hydrological sciences is limited almost exclusively to North America and Europe, and then most of these are in easily accessible locations (Knapp et al., 2018). Allowing samples to be analysed following longer storage duration would allow time for samples to be transported to distant labs, therefore facilitating the use of the raz-rru system in remote locations. Furthermore, acceptable long-term storage would allow users to analyse samples from long running or related experiments in the same batch, reducing the significant challenges associated with correcting for instrumental drift or error.

In this study, we investigated the effects of 3 storage temperatures (room temperature, refrigerator, and freezer) and storage duration (holding time) (periodically between 1 day and 84 days) on the concentration of raz and rru in 2 mixtures of different concentrations (low and high) and in 3 mediums (deionised water [DIW], groundwater [GW], and surface water [SW]) representing tracer mixtures and mediums of typical applications. The aim of this work is to provide evidence-based practical guidance on best practice protocols for the storage of raz/rru samples. The critical point at which samples have changed so much as to be deemed unusable is determined by the data quality objectives (DQO) (i.e. tolerable error levels) defined in a research project (Wright, 2004). Projects may propose different DQOs depending on project goals and data application (e.g. for use in decision making). Readers should interpret these results to define their own guidelines (e.g. acceptance criteria or thresholds) and inform necessary judgments with regards to DQOs in individual applications. However, general guidelines are proposed which should avoid unacceptable error in most applications. These guidelines are based on a threshold of 10% change, above which is assumed to be unacceptable, which is similar to thresholds proposed for other analytes, for example for phosphate, nitrate and silicate (Chapman and Mostert, 1990) and dissolved organic carbon (DOC) (Cook et al., 2016).

2.3 Materials and Procedures

Samples were prepared in 3 different mediums, representing a range of potential environmental sample conditions, to analyse potential interference of other water solutes (especially organic carbon) with the stability or decay of raz/rru (Lemke et al., 2014). Ultrapure water (18.2 MΩ·cm) (DIW) was prepared in the lab in a Millipore A10. GW was extracted from a 140 m deep borehole in a permo-triassic sandstone aquifer at Ecolaboratory (University of Birmingham, UK). SW was extracted from the Bournbrook, a small urban river in Birmingham, UK. The GW and SW were filtered on the day of collection using 0.45 μm quantitative ashless filter paper (Whatman – grade no. 42). Concentrations of nutrients (Table 2.1) (NO_3, NO_2, NH_3, PO_4) were analysed on a continuous flow analyser (San++, Skalar, Breda, The Netherlands), with a limit of detection and precision of 0.001 ± 1% mg NO_3-N/L, 0.05 ± 3% mg NO_2-N/L, 0. 0.01 ± 5% mg NH_4-N/L, and 0.05 ± 5% mg PO_4-P mg/L. Concentrations of DOC (Table 2-1) were analysed on a Shimadzu TOC-L analyser (Shimadzu Corporation, Japan) with a limit of detection of 4 μg/L.

Table 2-1 Average (n=5) concentrations of non-purgable organic carbon, nitrate, nitrite, ammonium, and phosphate (all presented in mg/L) prior to addition of tracers for 3 mediums: DIW, GW and SW. Standard deviation is reported in brackets.

All units mg/L	**Deionised water**	**Groundwater**	**Surface water**
Non-purgeable organic carbon (as C)	0.35 (0.10)	1.04 (0.14)	5.23 (0.17)
Nitrate (NO_3-N)	0.01 (0.00)	4.52 (0.04)	2.18 (0.10)
Nitrite (NO_2-N)	0.00 (0.00)	0.00 (0.00)	0.07 (0.00)
Ammonium (NH_3-N)	0.07 (0.01)	0.10 (0.04)	0.18 (0.00)
Phosphate (PO_4-P)	0.15 (0.08)	0.34 (0.17)	0.17 (0.03)

Treatment solutions were prepared using raz sodium salt (Apollo Scientific Ltd., lot: AS456027) and rru sodium salt (Sigma-Aldrich, lot: MKBQ2285V). High concentration

stock solutions were prepared in DIW as suggested by Knapp et al. (2018). 2 mixtures of raz and rru were then prepared in the different mediums, with a low target concentration mixture (30 µg/L raz, 10 µg/L rru) and high target concentration mixture (150 µg/L raz, 50 µg/L rru). These mixtures were selected because they are representative of the concentrations one might expect in hydrological applications, such as field tracer tests (Blaen et al., 2017; Knapp et al., 2018). The actual starting concentrations vary in different mediums (e.g. higher than expected in the GW) which may be explained by variation in background fluorescence which was not accounted for, or by human error in producing the mixtures. For each condition and replicate, 10 ml of sample was transferred to a prelabelled 15ml polypropylene centrifuge tube (Falcon™ 352196).

Samples were transferred to the respective storage method within 2 hours of preparation. In all sample preservation methods, samples were stored in the dark and were undisturbed between analysis periods and temperatures were monitored at 10-minute intervals using a Tinytag Aquatic 2 temperature logger (Gemini Data Loggers Ltd, Chichester, United Kingdom). Samples were stored at three different temperatures: room temperature (mean, 20.49 °C, min 18.43 °C, max 24.37 °C); refrigerator (mean 5.33 °C, min 2.36, max 9.05 °C); freezer (mean -20.40 °C, min -22.3 °C, max -15.85 °C). Averages and ranges were calculated whilst omitting the first 48 hours of the storage period, when loggers were acclimatising. Whilst storage temperatures fluctuated (see Fig. 8-1 in supplemental information (SI)), the average temperatures in each storage method are distinctly different, and represent typical lab storage conditions. Samples stored in the freezer were removed 24 hours ahead of their analysis to defrost in the refrigerator. Therefore, samples stored in the freezer and the refrigerator were analysed at the temperature of the refrigerator. Samples stored at room temperature were analysed at room temperature. The temperature of the sample during

analysis is likely to affect fluorescence intensity (Haggerty et al., 2008; Smart and Laidlaw, 1997) but this is to reflect practicality and normal lab practice.

Samples were analysed on a Varian Cary Eclipse benchtop fluorescence spectrophotometer (Agilent Technologies, Inc.). Emission/excitation wavelength pairs of 600/617.97 nm for raz and 570/586 nm for rru were used, which are within the ranges suggested by Haggerty et al. (2008). The fluorescence spectrophotometer was calibrated with 150 µg/L raz and 30 µg/L rru and a mixture of 30:10 µg/L raz:rru. Signal overlap was corrected using the MATLAB code provided by Knapp et al. (2018). Whilst fresh calibration standard solutions were prepared and analysed at each analysis period, all fluorescence intensities were converted to concentrations using the calibration equations established from standards data acquired on the same day the samples were prepared. This was informed by the comparison of concentrations calculated using calibration equations established for each analysis period with those calculated using one calibration equation, where it was concluded that more error is likely introduced in the preparation of new standards at every period than instrumental error between periods. This comparison is presented in section 8.1.2, including all calibration equations (Table 8-1) and a graph of calculated concentrations (Fig. 8-2). Furthermore, only standards prepared in DIW were used to calibrate the instrument because it is often impractical to calibrate using the sample medium when samples are extracted from different environments (e.g. river water and different depths in the hyporheic zone) and because the focus of this experiment is the effect of storage method and duration. Therefore, in this study we did not consider the implications of different calibration medium on raz/rru concentration changes. More detail is provided in section 8.1.3 of the SI and the effect of calibration standard solution medium is presented in Fig. 8-3. Samples were buffered with 1 M sodium hydroxide (Fisher Scientific, lot: 1991898) to pH >8 to ensure raz was present in its anionic form and therefore exhibits maximum fluorescence intensity (Bueno et al., 2002). Samples

were shaken for 30 seconds before analysis to re-oxygenate waters and ensure dihydroresorufin (a daughter compound of rru) is transformed back to rru (Knapp et al., 2018). Drift standards were included every 6 samples to observe instrument drift, which was insignificant over the analysis periods.

For each sampling interval, triplicates of each medium and each treatment concentration were analysed. Samples were analysed on the day of preparation to confirm the starting concentrations. One set of samples were removed from the freezer as soon as they had fully frozen and were allowed to thaw in the refrigerator before being analysed 24 hours after preparation. This was to examine changes to the spectrophotometric properties of raz/rru in different mediums after the freeze/thaw process, which has resulted in unpredictable changes to fluorescent peak positions and intensities in other analytes like DOC (Spencer et al., 2007; Cook et al., 2016). On days 2, 3, 6 and 7, only samples stored at room temperature and in the refrigerator were analysed as frozen samples would have spent a substantial proportion of the total storage time thawing. Subsequently, samples stored by each method were analysed at 14, 28, 42 and 84 days after preparation.

Four samples were identified as outliers, where measured concentrations differed from the other 2 replicates at least by a factor of 3, and were omitted from analysis. Statistical analysis was conducted to (1) identify the storage duration at which tracer concentrations became significantly different from starting concentrations when subject to different storage methods, and (2) to investigate if the storage method resulted in significantly different recovery of tracers. Analysis was conducted for the low concentration samples only because rru concentrations exceeded the upper limit of quantification (ULQ) in 62% of high concentration samples. Without rru concentrations, raz concentrations could not be calculated, precluding the analysis. Data from low concentration samples of all 3 mediums were amalgamated (for both raz and rru separately) to conduct the statistical analysis on the

effect of storage method and duration, but mediums are plotted and commented on separately in the text. Analysis for (1) was conducted using concentration data in µg/L and for (2) using percentage change from starting concentrations. Shapiro-Wilk tests were used to test for normality. Where data exhibited a normal distribution, a Student's t-test (T) and a one-way analysis of variance (ANOVA) with Tukey post-hoc test (Tukey) were conducted. Where data were not-normally distributed, Wilcoxon signed-rank tests (WSR) and Kruskal-Wallis one-way ANOVA tests (KW) were conducted. Statistical tests were performed in R version 4.1.0 (R Core Team, 2021). The statistical test employed for each result is reported using the abbreviations above and the level of significance is 0.05 for all tests.

2.4 Assessment

Results are presented separately for the low and high concentrations of raz/rru. Samples stored at room temperature = ROOM, samples stored in the refrigerator = COOLED, and samples stored in the freezer = FROZEN. The medium is indicated by DIW for deionised water, GW for groundwater and SW for surface water, which is in subscript following the storage method where appropriate. Relevant results of statistical tests are reported in the text and the output of all statistical tests are reported in section 8.1.5, in tables 8-4, 8-5, 8-6.

2.4.1 *Low concentrations of raz/rru*

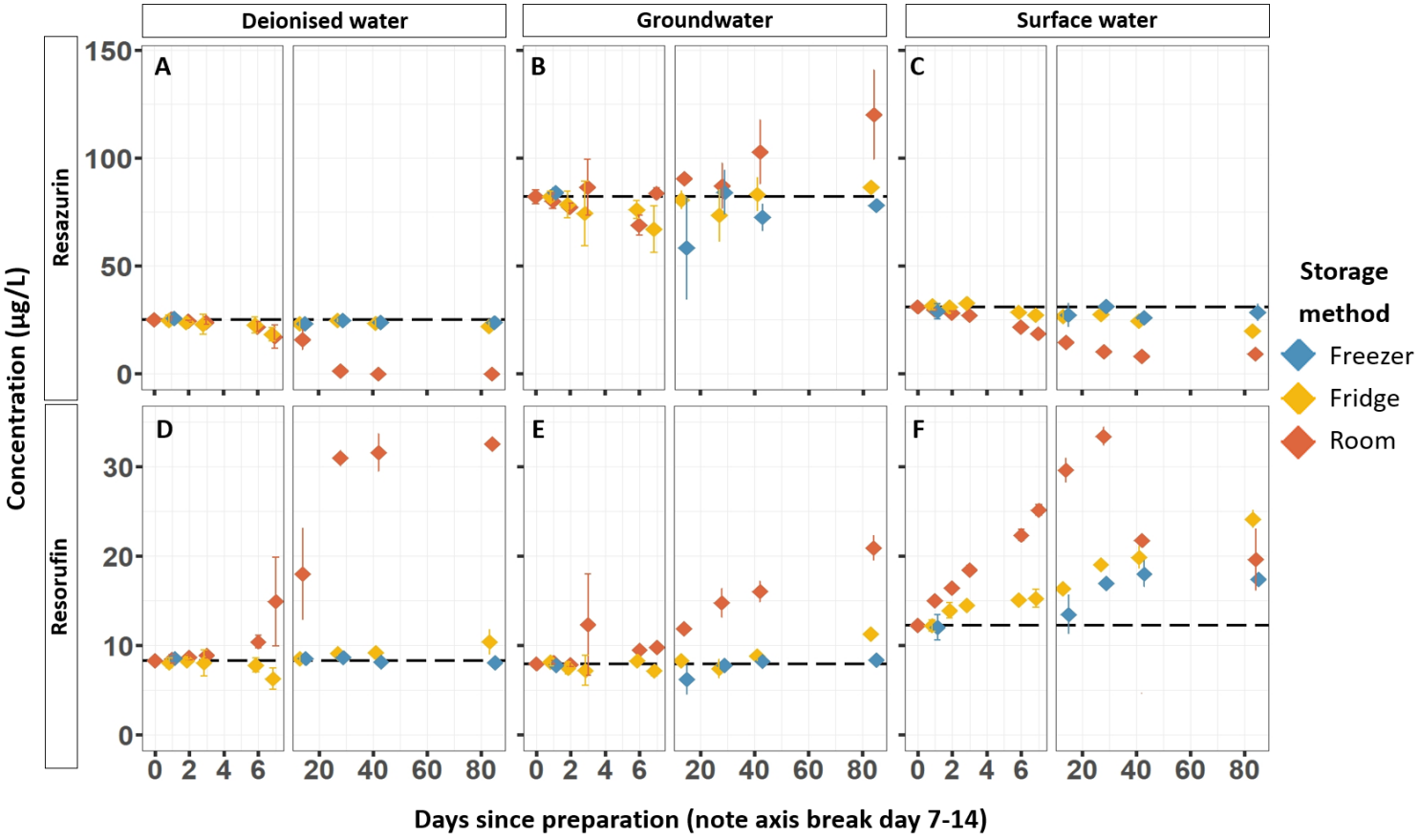


Figure 2-1. The change in concentration in low starting concentration samples of raz (A-C) and rru (D-F) over time (days) in different mediums: DIW (A, D), GW (B, E) and SW (C, F). Starting concentrations are represented by the dashed black lines. Error bars represent standard deviation; some error bars are not visible because they are smaller than the data symbol.

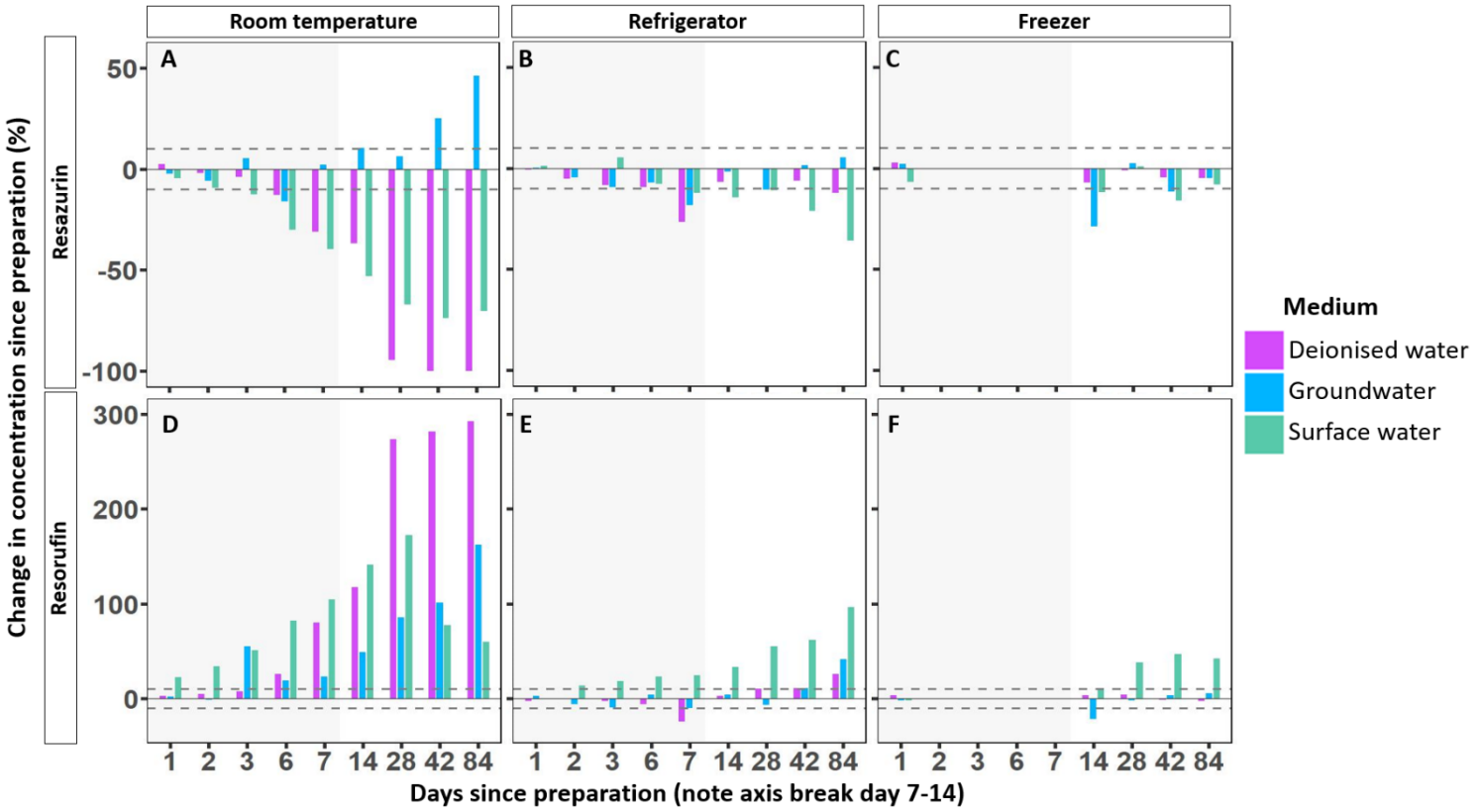


Figure 2-2. Percentage change in concentration from low starting concentrations of raz (A-C) and rru (D-F) over time (days) in different mediums - DIW (pink), GW (blue) and SW (green) – and subject to different storage methods: room temperature (A,D), refrigerator (B,E) and freezer (C,F). The dashed lines represent 10% change (positive and negative) from starting concentration. Note the axis break after day 7, represented by the grey shaded area. Note missing data from days 2-7 in the freezer pane.

In ROOM samples, concentrations of raz mostly remained within the 10% change threshold on days 1, 2 and 3 (Fig. 2-1 A) but on day 6 a significant difference to starting concentration had been realised for the medium amalgamated results (WSR, V=36, P<0.05) (Fig. 2-1 A,B,C). Rru changed more quickly (up to 22.6% after 1 day) and concentrations were significantly different to starting concentrations on day 3 of analysis (WSR, V=0, P<0.01) (Fig. 2-2 D). COOLED samples exhibited a similar pattern but concentrations changed more slowly. A maximum of 1.3% and 2.7% change was observed for raz and rru concentrations, respectively, on day 1 of analysis (Fig. 2-2 B,E). The 10% threshold was first violated on day 7 for raz for all mediums, and day 2 for rru in SW and day 7 for rru in DIW and GW. A significant difference in concentration was first realised on day 6 for raz (WSR, V=33, P<0.05) and day 14 for rru (WSR, V=0, P<0.05) for the medium amalgamated results. FROZEN samples were preserved best (i.e. least deviated from starting concentrations), where a significant difference in concentration was first realised on day 42 for raz (WSR,

V=36, P<0.05), but not at all for rru, even after 84 days (WSR, V=6, P>0.05). Concentrations of raz and rru in FROZEN samples were mostly within the 10% threshold on day 14, except for GW samples.

Rru concentrations were significantly better preserved in COOLED samples than ROOM samples on day 1 (ANOVA, F=4.375, P<0.05; Tukey, P<0.05), but no difference was observed between FROZEN samples and the other storage methods. On day 14 statistical differences between storage methods were also observed (ANOVA, F=21.57, P<0.05), where rru was significantly better preserved in FROZEN samples than in ROOM samples (Tukey, P>0.05), in COOLED samples than ROOM samples (Tukey, P>0.05), but no significant difference between COOLED and FROZEN was observed. The same patterns were observed for the remaining analysis periods (days 28, 42 and 84). Raz concentrations were preserved similarly in all storage methods on days 1, 2 and 3, but on day 6 concentrations were significantly better preserved in COOLED samples than ROOM samples (T, t=2.9923, P<0.05). On day 28 COOLED and FROZEN samples were better preserved than ROOM samples (ANOVA, F=6.37, P<0.01; Tukey, P>0.05), but FROZEN and COOLED storage methods performed similarly.

Raz/rru preservation appeared to be affected by the medium in which samples were prepared, especially for rru. In general, COOLED samples of DIW and GW were still well preserved on day 42 whereas in SW samples changes to the concentration of rru consistently violated the 10% threshold on day 2 and after (see Table 2-2). Similarly, FROZEN samples of DIW and GW were generally well preserved throughout the experimental period (i.e. on day 84) but in SW samples changes to the concentration of rru consistently violated the 10% threshold on day 28 and after. Rru in DIW was better preserved than in the GW and SW samples on day 3 of analysis, exhibiting a 7.7% change compared to a 54.7% change in GW and a 50.6% change in SW (Fig. 2-2 D,E,F). On day 3 and after, however, both tracers

seemed to behave similarly regardless of medium. This is with the exception of raz in GW, which increased on day 6 and after for ROOM samples, with high variance in replicates. This increase in raz, along with the limited increase in rru, may be explained by the transformation of raz, or the decay of rru, to unquantifiable products that fluoresce at a higher intensity but a similar wavelength to raz (Haggerty et al., 2008). The limited increase in rru in the GW (Fig. 2-1 E), as well as the decrease in rru in the $ROOM_{SW}$ samples on day 28 and after (Fig. 2-1 F), may also be explained by the decay of existing rru which is occurring simultaneously to the production of new rru, potentially dampening both signals. In the $ROOM_{SW}$ samples, the concentration of raz decreases quickly, by ~20.9 µg/L (-67.1%) on day 28 where it remained for the duration of the study, suggesting all the raz had been removed (Fig. 2-1 C). The remaining fluorescence signal most likely represents background fluorescence, which has not been corrected for in the calibration, as discussed in materials and methods. $FROZEN_{SW}$ samples exhibited minor decreases in raz but not in an obvious trend, for example decreasing by ~2.1 µg/L (-12.1%) on day 14 and by ~2.5 µg/L (-8.0%) on day 84, which could suggest some unpredictability in how samples respond to storage or otherwise be explained by variance.

2.4.2 High concentration of raz/rru

In general, similar patterns were observed in the high concentration samples. In some samples the concentration of rru quickly increased to exceed the ULQ of the fluorescence spectrophotometer, for example in the high concentration $ROOM_{DIW}$ samples at day 6 (Fig. 2-3 D) and $ROOM_{SW}$ after 1 day (Fig. 2-3 F). With the concentration of rru in the mixtures unmeasurable, the fluorescence signals cannot be corrected for signal overlap and so the concentration of raz cannot be calculated. Therefore, these too have been omitted, which can be seen for the samples mentioned above in the corresponding raz graphs (Fig. 2-3 A,C).

High tracer concentration data are not plotted in relative change graphs (as in Fig. 2-2) because missing data could lead to misleading presentation, but data are presented in table 2-2 in the SI.

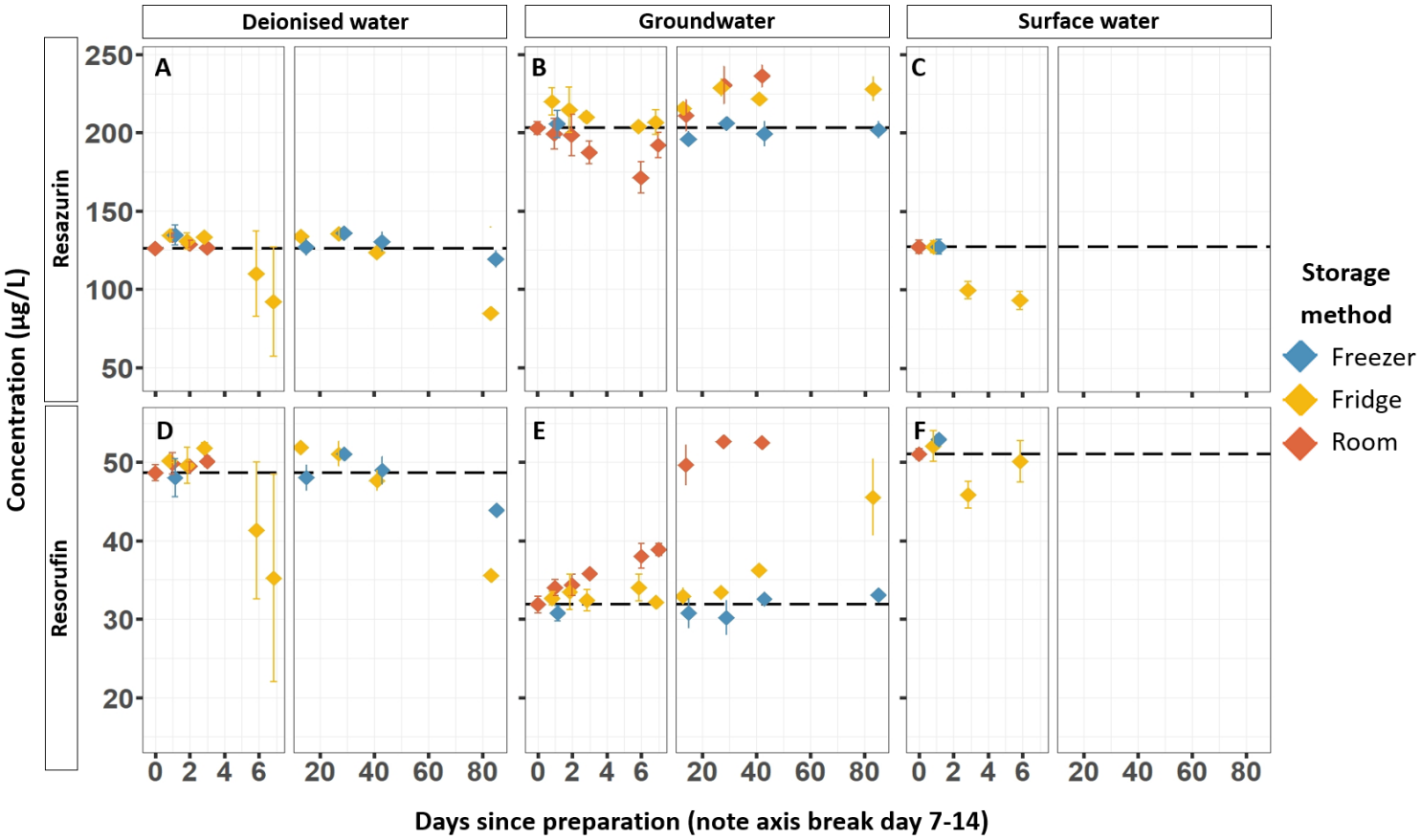


Figure 2-3. The change in concentration in high starting concentration samples of raz (A-C) and rru (D-F) over time (days) in different mediums: DIW (A, D), GW (B, E) and SW (C, F). Starting concentrations are represented by the dashed black lines. Error bars represent standard deviation; some error bars are not visible because they are smaller than the data symbol.

Concentrations of rru had exceeded the ULQ at day 1 in the $ROOM_{SW}$ samples. $COOLED_{SW}$ samples were better preserved with raz/rru concentrations measurable up to day 6. Raz/rru in $FROZEN_{SW}$ samples were not measurable at day 14 but may have been well preserved in intermediate timeframes not analysed here.

Raz and rru in DIW and GW were better preserved than in SW. In $ROOM_{DIW}$, both raz and rru were well preserved on day 3 of analysis, exhibiting <5% concentration change. Large losses of both raz and rru on days 6 and 7 in the $ROOM_{DIW}$ samples are probably not representative of overall patterns and measured concentrations remain within standard deviation of starting concentrations (Fig. 2-3 A,D). In $FROZEN_{DIW}$ samples, both raz and rru were very well preserved on day 84 (the end of the experimental period), exhibiting -5.5% and -9.8% change, respectively.

Lower starting concentrations of raz and rru in the GW samples prevented violation of the ULQ allowing changes to be observed for almost the entire experimental period, excluding only day 84 for $ROOM_{GW}$ samples (Fig. 2-3 B,C). In $ROOM_{GW}$ samples, raz decreased by 15.5% on day 6, before steadily recovering to starting concentrations on day 14 and continuing to increase until the ULQ is violated on day 84. This recovery and increase in raz concentration is as of yet unexplained and remains unpredictable. These patterns could be explained by the transformation or decay of either tracer to some unquantifiable product which fluoresces at a similar wavelength spectrum as to interfere with raz. They could also be attributable to changes in background fluorescence (which was not quantified here), for example due to cycling of DOC. Rru seemed to increase linearly in the first 14 days, increasing by 12.4% on day 3 and 55.8% on day 14. The rate of rru increase slowed after day 14, by which point most of the raz has likely been transformed to rru. $COOLED_{GW}$ samples were better preserved exhibiting a maximum of 6.3% change in raz on day 14, except a spike in concentration on day 1 by 8.4%, which could more likely be explained by some mechanism of fluorescence intensity rather than an actual difference in the absolute concentration of raz. A similar pattern, but of lower magnitude, in the concentration of raz (initial decrease followed by an increase) was also observed in the $COOLED_{GW}$ samples. Rru was also well preserved, changing by <10% at every period until day 42. In $FROZEN_{GW}$

samples, both raz and rru were very well preserved for the entire experimental period, only deviating from the starting concentrations within the range of standard deviation for most samples, exhibiting –0.6% and 3.9% change on day 84, respectively.

2.5 Discussion

Samples containing raz and rru have typically been analysed quickly (within 24 hours of extraction) because of uncertainties about how well sample integrity is preserved. Here, for the first time, the magnitude and rate at which the concentrations of raz and rru change after extraction is quantified. In this discussion, we offer mechanistic explanation of results and outline the implications of these results for experimental designs and the practical analysis of samples.

2.5.1 Effect of storage temperature

The storage method (i.e. temperature) significantly impacted the preservation of concentrations of both raz and rru. Even after 24 hours, samples in some mediums (SW) stored at room temperature had changed by an error level which is unacceptable for most applications. Concentrations in refrigerated samples were preserved much better, which is likely attributable to a decrease in biological activity induced by the lower temperature (Adams et al., 2010). Tracer concentrations changed almost 3 times faster when samples were stored at room temperature compared to in the refrigerator. As such, samples should be cooled as soon as possible - within 10 hours to avoid changes of >10% and much sooner if further storage is required before sample analysis. If possible, samples should be cooled at collection, for example using a portable cooler, especially where air temperature is high.

The temperature in the refrigerator fluctuated significantly, reaching a maximum temperature of >9 °C – high enough to stimulate significant microbial metabolic activity (Adams et al., 2010). Whilst this should not occur in laboratory grade refrigerators, these occurrences are probably not unusual in typical research labs (which are subject to power outages, for example). Refrigeration, therefore, may be a riskier method of storage than freezing, especially for longer durations.

The freezing and thawing process appears to have little or no effect on the measured concentration of raz and rru. Frozen samples of DIW and GW were well preserved (<10% change) for 84 days (the end of the experimental period). SW samples, however, do exhibit notable changes in the concentration of rru after 28 days. It is unlikely that this is explained by the microbiologically facilitated transformation of raz to rru because most microorganisms do not metabolise at temperatures this low (Adams et al., 2010). Furthermore, the increase in the concentration of rru is not reflected in a corresponding decrease in the concentration of raz. This highlights the need to consider other potential mechanisms for the change in concentration or fluorescence intensity. Some of the observed changes in concentration in the frozen samples could be attributed to microbiologically facilitated transformation as the temperature of samples increased during defrosting in the refrigerator for 24 hours. Defrosting samples more quickly, for example in a water bath, may be a suitable solution to reduce this impact, followed by more immediate analysis (Chapman and Mostert, 1990).

2.5.2 Variability of effects in different sample medium

The medium of the sample impacts the fluorescence intensity of raz and rru, and their preservation. Here, medium is characterised by water chemistry (i.e. the concentration of nutrients and DOC) only. Microbiological communities were not characterized but likely

differed in in abundance, composition and diversity in different mediums, which could have affected fluorescence and raz/rru transformations.

This work again highlights the necessity of calibrating fluorometry instruments with standards prepared in the same medium as the samples, as identified in Blaen et al. (2017) and Knapp et al. (2018). However, this is often not practical or possible, for example when samples are extracted from many locations with different waters, like different depths in the hyporheic zone. In this instance, data should be interpreted with care, for example directly comparing concentrations of samples from a similar medium (e.g. just the SW) but not those extracted from different waters (e.g. deep in the hyporheic zone and in the SW). SW samples exhibited the highest changes in concentration of raz and rru, in both magnitude and rate, in all storage methods. This may be explained by the higher concentrations of DOC and nutrients which control microbial metabolism (Brailsford et al., 2019). It may also be explained by SW containing oxygen producing cyanobacteria, which may maintain aerobic conditions in the samples for longer thus facilitating the aerobic respiration of raz. GW samples exhibited unexpected and unpredictable changes in concentration which do not appear to be explained by water chemistry but may be attributable to microorganism community assemblage and dynamics.

2.5.3 Implications for experimental designs

Raz is less stable than rru, exhibiting both faster rates and higher magnitude of change. This may be evidence of the transformation of raz to as of yet unidentified products, i.e. not rru, (O'Brien et al., 2000). However, this may also be explained by the relatively weak fluorescence emission of raz compared to rru, which tends to be characterised by shorter fluorescence lifetimes (Berezin and Achilefu, 2010). In applications where only the

concentration of rru is required – for example in closed system experiments (e.g. Comer-Warner et al., 2019) – the acceptable storage time of samples may be higher. Furthermore, lower concentrations of raz and rru are more suitable for storage. The concentration mixtures used here were selected to represent typical mixtures expected in stream tracer experiments (Blaen et al., 2017), some of which quickly violated the ULQ as concentrations increased. Whilst some fluorometers may be capable of analysing much higher concentrations (Lemke et al., 2013), this work may influence the target concentrations users select, especially if sample storage is required. On top of this, lower variance was observed between repeats of low concentration mixtures compared to high concentration mixtures. Sample dilution may be appropriate, but this should be conducted before storage if possible (i.e. using a dilution factor appropriate for the estimated concentration based on the target concentration), and using the same medium to avoid interfering with fluorescence intensity, for example using stream water from before the tracer injection (which does not contain raz or rru). Otherwise, users may reduce target concentrations to well below the ULQ. Readers are encouraged to first establish the ULQ of their instrument (of tracers in different mediums) in order to inform target concentrations.

For other analytes correction factors have been established to correct for the effect of storage duration and temperature on concentration (e.g. Peacock et al., 2015; Zeng et al., 2020). This data demonstrates that this is not feasible for the raz-rru system in environmental waters due to the unpredictability of concentration change and to the several interacting factors that may influence the rate, magnitude and direction of this change, such as water chemistry, microbiology, and the starting concentration of both tracers. Nor is it possible to use knowledge of the medium and raz/rru composition of samples to reliably predict the amount of change of raz/rru. Furthermore, users must consider that the concentrations of raz and rru may not change similarly in different samples. Therefore, the maximum storage time of all

samples should be limited to the time at which the most volatile samples are expected to change beyond acceptable bounds, most likely SW samples. Users may consider analysing more volatile samples first but this could introduce unacceptable bias.

2.5.4 Necessary future research

The dominant mechanism driving the changes in concentration in raz and rru is the irreversible transformation of the former to the latter. Less attention has been afforded to other potentially significant drivers of concentration change, such as decay and transformation to 'unquantifiable by-products', which has been alluded to but largely ignored (Haggerty et al., 2008). Any changes in the actual concentration of raz and rru in samples containing both are in fact a combination of many processes occurring simultaneously, and sometimes antagonistically, for example the decay of existing rru and the production of new rru. Furthermore, as we have seen here and in other works, the measured concentration of both raz and rru may be affected by a variety of interacting factors which are difficult to separate and correct for. As such, the relationship between the concentrations of raz and rru may be more complex than often assumed. Future work should investigate the mass balance of raz and rru in closed systems of different conditions and seek to identify and characterise alternative decay and by products. As well as implications for sample storage, this work highlights the sensitivity of the raz-rru smart tracer technique to environmental, procedural and practical factors.

2.6 Recommendations

These recommendations are provided to further extend guidelines outlined by other authors (e.g. Blaen et al., 2017 and Knapp et al., 2018). As stated, readers should interpret this work

considering relevant DQOs to identify appropriate storage duration and method for individual applications.

- Samples should be refrigerated or frozen after extraction as soon as possible - within 10 hours to avoid risking >10% change in the concentration of tracers.
- If possible, samples should be analysed within 24 hours, until which they should be stored in a laboratory grade refrigerator.
- If storage duration must exceed 48 hours, samples should be frozen. In this instance, users should expect a change in concentration in raz and rru (from the concentration at the point of freezing) of around 10% after 14 days.
- Samples should be stored at low concentrations of raz and rru - at least half the concentration of the ULQ of the fluorometer in use. This could be achieved by lowering target concentrations or by dilution before storage.

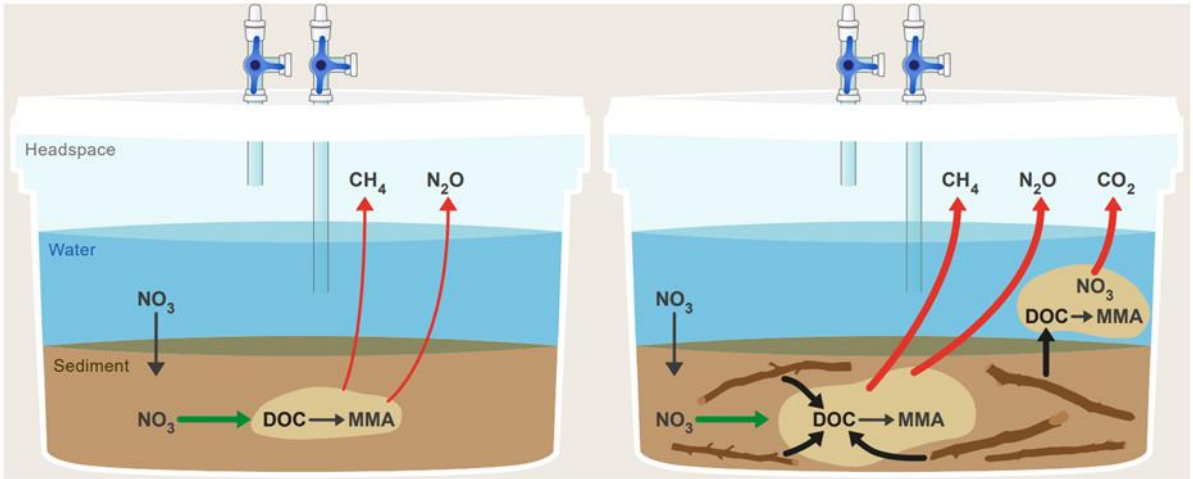
3 Instream wood as a driver of biogeochemical cycling: Neglected co-benefits and trade-offs in river restoration

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3.1 Abstract

The (re)introduction of wood into rivers is increasingly popular but its impact on streambed biogeochemical processing is still poorly understood. It could affect fundamental ecosystem processes through multiple, potentially interacting mechanisms and lead to cascading effects for ecosystem function and the delivery of associated (dis)services. Here, a microcosm study running for approximately 2 years explored the impact of instream wood on key biogeochemical functions for different streambed sediment types throughout a temperature range typical of a temperate climate. The effects on a suite of physiochemical characteristics and microbial metabolic activity (MMA) were measured, as well as on associated ecosystem services (nitrate removal rate) and dis-services (greenhouse gas (GHG) production). Streambed wood significantly increased MMA, a key ecosystem process that underpins stream biogeochemical cycling. This likely explained an increase in the removal rate of nitrate and the production of GHGs. This study demonstrates that instream wood is a key driver of stream biogeochemical cycling. Neglecting to consider streambed wood could lead to potential underestimation of MMA, nutrient spiralling and GHG production, with consequences for instance for global GHG emission estimates. These consequences (increased MMA, nutrient spiralling and potential GHG emission), therefore, should be considered as intentional or non-intentional result of instream wood management practice, notwithstanding of the initial management objectives.

3.2 Introduction

Streams and rivers can deliver societally important ecosystem services but their capacity to do so has been reduced by centuries of mismanagement, like channelization and simplification, e.g. the removal of instream features (Beechie et al., 2010). The realization of the potential of the river corridor to provide nature-based solutions to some of societies most wicked challenges, and the amenability of these systems to restoration, has contributed to the rapid increase in the number and scale of river restoration projects globally (Bernhardt et al., 2007, Roni et al., 2015). One of the most widely encouraged means in which to achieve restoration is the (re)introduction of instream wood (Roni et al., 2015; Wohl, Lane and Wilcox, 2015; Grabowski et al., 2019), which for centuries had been removed for navigation and to reduce localised flooding (Wohl, 2014). This technique is now recommended in policy in the European Union Water Framework Directive (European Commission, 2000) and in national guidelines in the United States (Bureau of Reclamation and U.S. Army Corps of Engineers, 2015). Furthermore, the rate of indirect introduction of wood to river corridors (e.g. from falling trees and branches) is likely to increase as riparian zones are rewooded (Meleason, Gregory and Bolte, 2003), as landscapes are rewilded (Brown et al., 2018), and, in the UK, as beavers (*Castor fiber*) are reintroduced (Larsen, Larsen and Lane, 2021).

The motivation for enhancing instream wood is often vague or unspecific (e.g. renaturalization of the river corridor) or single-objective, such as the creation of habitat for a specific species (e.g. salmonid spawning sites) or to provide natural flood management solutions (Roni et al., 2015; Kaiser, Feld and Stoll, 2020; Neuhaus and Mende, 2021). Wood (re)introduction was perhaps first advocated for ecological restoration (Bernhardt et al., 2005), and more recently has also been adopted to increase the connectivity between hydrological compartments – such as surface water, groundwater, riparian zones and floodplains (Sawyer et al., 2011; Sawyer and Cardenas, 2012; Shelley et al., 2017;

Wilhelmsen et al., 2021) – and to increase river corridor nutrient spiralling (Krause et al., 2014; Blaen et al., 2018). Whilst it may be a suitable strategy to achieve these objectives, there are doubtless ulterior impacts which are seldom considered, and cannot yet be reliably predicted, but could induce cascading effects for ecosystem function and health (Grabowski et al., 2019; Wen Lo, 2021; Schalko and Weitbrecht, 2022). It is, therefore, critical that decision makers are made aware of all the potential impacts of interventions on ecosystem function, intended or ulterior, and the opportunities, risks and conflicts that arise from the co-delivery of ecosystem (dis)services *(*Alves et al., 2019).

A particular paucity exists in the understanding of the function of streams in global biogeochemical cycles, to which streams contribute disproportionately for their relatively small surface area (Downing et al., 2012; Krause et al., 2014; Wohl et al., 2017; Comer-Warner et al., 2018). Wood can compose a significant proportion area of streambed sediments but it has typically been ignored or explicitly omitted in mechanistic studies which have explored streambed biogeochemistry (e.g. Comer-Warner et al., 2018 and Romeijn et al., 2019). There are a number of mechanisms by which streambed wood could theoretically drive and control biogeochemical cycles, such as cycles of carbon(C) and nitrogen(N). It could affect the production, transport and bioavailability of dissolved organic carbon (DOC) (Berggren and Giorgio, 2015; Wohl et al., 2017), a key control of biogeochemical reactions and often a limitation, e.g. for nitrate (NO_3) turnover (Zarnetske et al., 2011). Similarly, its decomposition could introduce nutrients and modify stoichiometric ratios of C:N:Phosphorus(P), which control respiration rates (Hou et al., 2017; Islam et al., 2019). It could also increase streambed hydraulic conductivity, create preferential flow paths and encourage bioturbating aquatic invertebrates, all of which could modify surface water – groundwater interactions which control reaction rates and fluxes (Salehin, Packman and Paradis, 2004; Naganna et al., 2017; Shrivastava et al., 2021). Other potential mechanisms

include providing a surface for microbes and biofilms and creating heterogeneity in the structure of the streambed which can drive hyporheic exchange and facilitate a matrix of redox conditions that are necessary for many step-wise biogeochemical reactions (Boano et al., 2014; Krause et al., 2014; Briggs et al., 2015).

Streams are especially important in regulating global cycles of N and C, controlling the flux of these vital elements from freshwater and terrestrial environments to the atmosphere and marine environments (Hope et al., 1994; Wohl et al., 2017). For example, streams and rivers provide an ecosystem service by retaining and removing 27 Tg N yr^{-1}, >40% of the inputs they receive, although they still contribute 37 Tg N yr-1 to coastal waters (Beusen et al., 2016). Some of this removal is attributable to transformations along the river corridor which can transform polluting aqueous species, such as NO_3, to environmentally benign dinitrogen (N_2) gas or the harmful green-house gas (GHG) nitrous oxide (N_2O) (Seitzinger and Sanders, 1997; Quick et al., 2019). Furthermore, the mineralization of DOC in aerobic respiration and anaerobic respiration leads to the production of carbon dioxide (CO_2) and methane (CH_4), respectively, also harmful GHGs (Romeijn et al., 2019). Streams and rivers provide an ecosystem dis-service, emitting an estimated 1.8 Pg CO_2-C yr^{-1} (Raymond et al., 2013), 26.8 Tg CH_4-C yr^{-1} (Stanley et al., 2015) and 0.68 Tg N_2O-N yr^{-1} (Beaulieu et al., 2010), and these emissions are likely to increase as the temperature of surface waters increase as a result of climate change (Hannah and Garner, 2015; Comer-Warner et al., 2018).

The reactions that result in the provision of these ecosystem (dis)services (e.g. aqueous N removal and GHG production) are facilitated by heterotrophic bacteria which reside mostly in the river sediment where physiochemical conditions remain favourable (Pusch and Schwoerbel, 1994; Nogaro et al., 2013). The microbial metabolic activity (MMA) of these bacteria is a fundamental control of biogeochemical cycling in the river corridor (Findlay, 2010; Knapp et al., 2018). Similar to the keystone species concept, we suggest that MMA

may be understood as a keystone ecosystem process, maintaining ecosystem structure and function (Ganguli et al., 2008). Changes to MMA, as a result of direct intervention or otherwise, could result in changes to biogeochemical cycling and the provision of ecosystem services and dis-services of global significance, the delivery of which often occur simultaneously (Lapierre et al, 2013).

In this paper, a long-term (~2 years) microcosm experiment was conducted to explore the impact of streambed wood on key ecosystem (dis)services – nitrate (NO_3) removal and GHG production – in various sediment types and under a range of temperatures representing a typical annum in a temperate climate. These incubation experiments allow for the systematic analysis of the effect of streambed wood on MMA, NO_3 removal, and GHG production, but comparisons with in-situ measurements remains a challenge (Comer-Warner et al., 2018). It is not the aim to provide absolute and scalable estimates to allow comparison with or extrapolation to in-situ conditions. Instead, the primary aim of this study is to demonstrate the effect of streambed wood on a keystone ecosystem process and to reveal potential trade-offs in the delivery of ecosystem (dis)services that should be considered in river restoration.

3.3 Methodology

3.3.1 Microcosm set-up

Microcosm chambers were constructed from 1.5 l buckets with gas-tight lids, made of polypropylene which blocked out ultraviolet light, and fitted with sampling ports with a three-way stopcock (Fig. 3-1 b). Each of 8 treatments was replicated 5 times, resulting in 40 microcosms in total (Fig. 3-1 c). Microcosms were stored outside at EcoLabratory (University of Birmingham, UK) allowing temperature to fluctuate with environmental conditions (Fig. 1d). Locations were randomised and no significant difference was observed

in temperature between treatments (Fig. 8-4). From assembly in May 2019 until December 2020, microcosms were undisturbed to allow for microbial communities to stabilise and wood to begin to decompose (Comer-Warner et al., 2018). Lids remained closed but sampling ports remained open to allow gas exchange.

3.3.2 Sediment collection

4 sediment treatments were investigated, including no sediment, and sediments overlying sandstone, limestone or chalk geologies (Fig. 3-1 c). Sandstone sediment was collected from wood brook in Staffordshire, UK (52.80, -2.30), from under total tree cover (Fig. 3-1 a). Chalk sediment was collected from the river Lambourn in Berkshire, UK (51.44, -1.38), from under partial tree cover. Limestone sediment was collected from the river Bradford in Derbyshire, UK (53.17, -1.68), from under no tree cover. Sediments were collected from the top 10cm and in the thalweg in February 2019 and stored in a cold room (at approx. 4^{o}C) until microcosm assembly in May 2019, where 500 ml (440 ml in cases where wood was also added) of wet sediment was added to relevant microcosms (Blaen at el., 2018). Large stones and wood (>2cm) were removed before assembly, which would have occupied a disproportionately large volume of the total sediment (Romeijn et al., 2019). After the decommission of the microcosms, grain size analysis and loss on ignition (LOI) (for organic matter and carbonate) were conducted, which are presented in Fig. 8-5 and Fig. 8-6, respectively (Hoogsteen *et al.*, 2015).

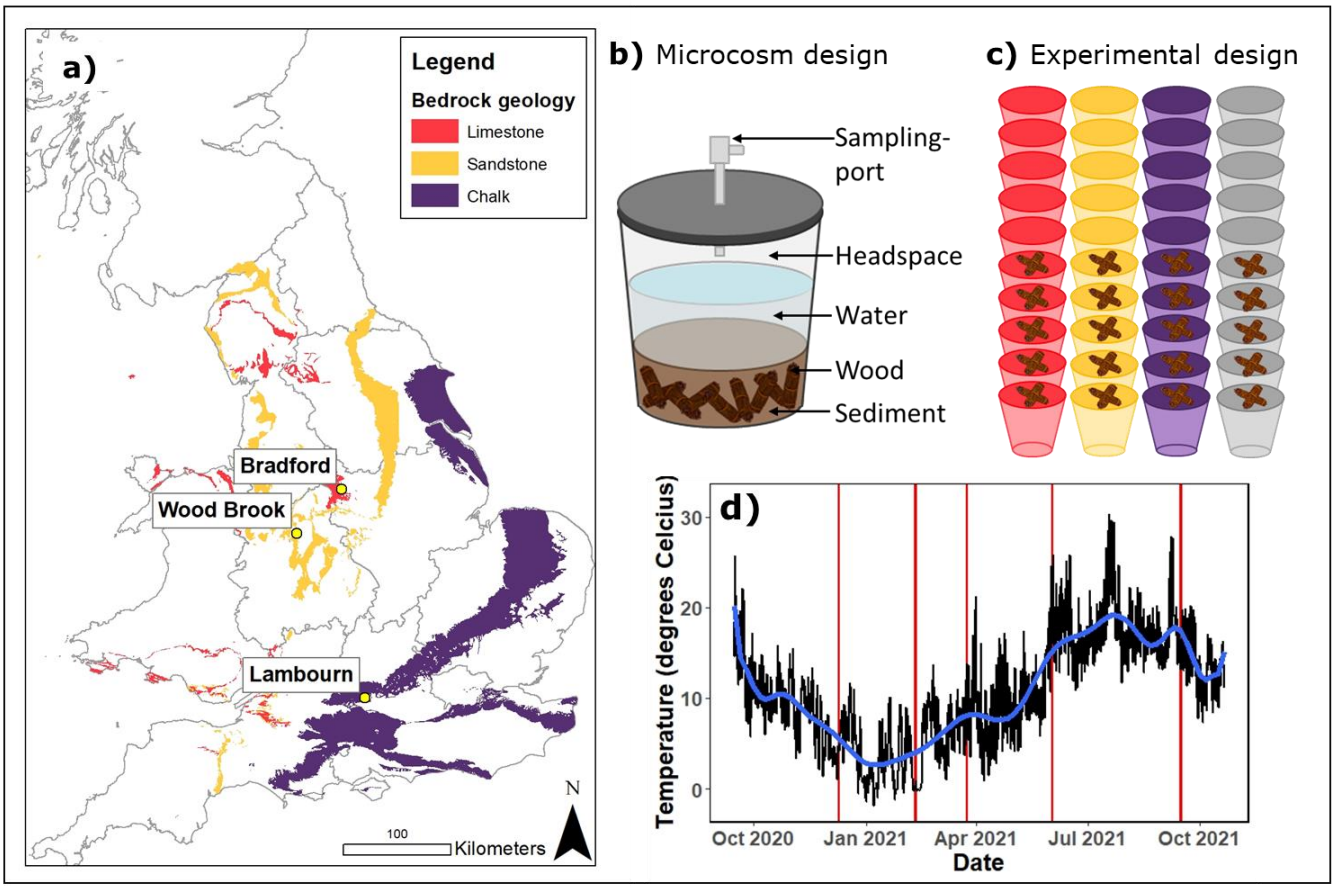


Figure 3-1. a) River sediment sampling locations with underlying bedrock geology. b) Schematic microcosm design with wood buried by sediment at the bottom and water column and headspace above, with sampling tube and stopcock for gas sampling. c) microcosm treatments where pink = limestone sediment, yellow = sandstone sediment, purple = chalk sediment and grey = no sediment; wood was added to half the treatments and each treatment was replicated 5 times. The location of treatments was randomised. d) Water temperature (^{o}C) before and during the experimental period, where the black line represents temperature at 12 noon, the blue line represents the LOESS (locally weighted smoothing) and the vertical red lines indicate measurement dates.

Microcosm composition and construction

Common hazel (*Corylus avellana*) typically surrounds water courses in northern Europe and its wood is used in river restoration, for example in fascines (RRC, 2020). It was introduced to half of the microcosm (with wood treatment), which received 5 x 50mm long sections of large (20mm), medium (10mm) and small (5mm) diameter wood pieces. Wood was added before sediment and so remained buried for the extent of the experiment to simulate buried streambed wood, expect in the treatments without sediment. Artificial stream water – a

nutrient mixture of 10 mg/l NO_3-NO_3, 0.1 mg/l NH_4-NH_4 and 0.1 mg/l PO_4-PO_4 prepared in ultrapure water (18.2 MΩ·cm) – was added to bring the total volume up to 1l, leaving 500 ml headspace.

3.3.3 Spiking procedure

spiking events were conducted throughout the experimental period, scheduled to capture annual temperature variability (Fig. 3-1 d). Microcosms were spiked with $NaNO_3$ and resazurin (raz) (Apollo Scientific Ltd., lot: AS456027) prepared in 12ml volume of ultrapure water 12 hours previously. Raz is a smart tracer which is transformed to its daughter compound, resorufin (rru), in mildly reducing conditions thus providing a proxy for MMA (Haggerty, Argerich and Martí, 2008; Knapp et al., 2018). Concentrations of NO_3 and raz were measured 2 days prior to the preparation of spikes to enable target concentrations to be more accurately achieved. Concentrations of NO_3-NO_3 in the water column were elevated to 50 mg/l, the maximum concentration in safe drinking water (World Health Organization, 2003). Concentrations of raz were elevated to 0.15 mg/l, a typical target concentration which allows high detection but would decay sufficiently between spiking events (Knapp et al., 2018).

3.3.4 Water sampling and analysis

12ml of water was sampled at 0 and 26 hours (after 15 seconds of mixing the column water), after pH (Fig. 8-7) and temperature (Fig. 3-4) were measured with a Hanna probe (HI-98129) and the concentration of dissolved oxygen (DO) was measured with a FireSting Fibreoptic DO probe. The water was replaced with ultrapure water after each measurement period, not after sample at hour 0. Water samples were immediately filtered with 0.45 µm glass fibre

syringe filters (Cole-Parmer) and stored for no more than 48 hours before analysis (Knapp et al., 2018; Howard et al., 2022 [chapter 2]). Concentrations of NO_3 were analysed on a continuous flow analyser (San++, Skalar, Breda, The Netherlands), with a limit of detection and precision of 0.01 ± 5% mg/l NH_4-N and 0.001 ± 1% mg/l NO_3−N, respectively. Concentrations of DOC were analysed on a Shimadzu TOC-L analyser (Shimadzu Corporation, Japan) with a limit of detection of 4 µg/L. Concentrations of raz and rru were measured on a Varian Cary Eclipse benchtop fluorescence spectrophotometer (Agilent Technologies, Inc.). Emission/excitation wavelength pairs of 600/617.97 nm for raz and 570/586 nm for rru were used, which are within the ranges suggested by Haggerty, Argerich and Martí (2008).

3.3.5 Gas sampling and analysis

15 ml of gas was sampled at 0, 2, 6 and 24 hours using a gas tight syringe (SGE Analytical science) leading to negative pressure in the microcosm. The gas samples were immediately transferred to pre-evacuated 12 ml gas vials (Exetainer vial, Labco Ltd.) for storage under positive pressure and at room temperature for no more than 3 months (Sgouridis and Ullah, 2017; Faust and Liebig, 2018). Gas samples were analysed on an Agilent 7890A Gas Chromatograph using a 1 ml sample loop and an over temperature of 60°C. A Flame Ionisation Detector (FID) measured CH_4 and CO_2 (which is methanised with a catalyst before analysis) and an Electron Collision Detector (ECD) measured N_2O. The instrument operated with a hydrogen flow of 48 ml min^{-1}, air flow of 500 ml min^{-1} and a make-up pure nitrogen flow of 2 ml min^{-1}. CH_4 was eluted at 3.5 minutes, CO_2 was eluted at 5.7 minutes, and N_2O eluted at 8 minutes, for a total run time of 9 minutes. Gas concentrations in the headspace were adjusted for standard temperature and pressure and the potential production rates (per microcosm per hour) were calculated between 2 and 6 hours because this represented the

steepest section of the slope when cumulative concentration was plotted against time (White and Reddy, 2003).

3.3.6 Statistical analysis

Q-Q plots, histograms and Shapiro–Wilk tests were performed to test the normality of all data. Linear mixed-effects models (LMEM) were applied with a restricted maximum likelihood estimation using the lme4 package (Bates et al., 2015) in RStudio (RStudio Team, 2021). These tests were first performed on amalgamated data from each of the sediment treatments, excluding the *no sediment* treatment (i.e. sandstone, limestone and chalk), and from every spike event. This isolated the effect of the inclusion of streambed wood from the confounding effects of sediment and temperature. In these models, wood treatment (wood or no wood), sediment type (*sandstone, limestone and chalk*) and temperature (0.8 ^{o}C, 2.6 ^{o}C, 9.6 ^{o}C, 15.1 ^{o}C, 20.7 ^{o}C) were all included as fixed effects. The microcosm number (representing subsamples within one sediment treatment and repeated measures at different temperatures) was included as a random effect to avoid pseudoreplication in treating subsampled observations and repeated measures as independent (Hurlbert, 1984). Following the execution of two LMEMs – one including the fixed term, wood treatment, and one 'null model' not including this term – the model outputs were compared using a likelihood ratio test (Bates et al., 2015). The respective *P* values are reported in text and model outputs of the likelihood ratio test are reported in section 8.2.6 in tables 8-7 – 8-13. The same series of models and tests were performed on subsets of data which included only one sediment type (no sediment, sandstone, limestone and chalk) per model set, where wood treatment and temperature are included as fixed effects and microcosm number is included as a random effect.

3.4 Results

3.4.1 Carbon concentration and bioavailability

Non-purgable organic carbon is further referred to as DOC. Over the entire temperature regime, DOC was not significantly different in microcosms containing wood compared to those without (Fig. 2b,c). However, the concentration of DOC was significantly higher in microcosms containing wood for sediment treatments of *no sediment* ($P<0.05$) and *chalk* ($P<0.05$). This difference was most profound for microcosms containing *no sediment* where an average difference of 475% was observed over the entire temperature regime. For those microcosms containing sediment and no wood, the concentration of DOC was very similar in *sandstone* (15.69 mg/l ± 21.97) and *limestone* (15.65 mg/l ± 18.43) and lower in *chalk* (7.41 mg/l ± 8.23). Substantial concentrations of DOC were also observed in the microcosms containing no wood or sediment (6.23 mg/l ± 8.65) suggesting contamination or autochthonous production.

At the 15.1 ^{o}C measurement period (October 2021) (Fig. 3-2 a), the average concentration of DOC in treatments containing wood was notably higher than at other periods, and highly variable within sediment treatment groups (*no sediment* = 62.79 mg/l ± 71.29; *sandstone* = 49.97 mg/l ± 28.19; *limestone* = 42.17 mg/l ± 50.14; *chalk* = 48.24 mg/l ± 57.96). These spikes in DOC concentration were not observed in microcosms not containing wood, leading to huge percentage differences between microcosms containing wood and those not, of 1460% in *no sediment* treatments, 129% in *sandstone*, 4587% in *limestone* and 4839% in *chalk*. Patterns of similar magnitude were not observed when DOC was rigorously measured at decommission (Fig. 3-2 c).

There was little apparent difference in the $SUVA_{254}$ between microcosms containing wood and those not, expect for the microcosms containing *no sediment* where those containing

wood exhibited higher absorbances indicating a higher aromaticity and, therefore, a lower bioavailability (Fig. 2d) (Weishaar et al., 2003). Similar patterns are observed for $SUVA_{280}$ in the *no sediment*, *sandstone* and *limestone*, but in the *chalk* treatments microcosms containing wood had a higher (by 45%) absorbance than those without, indicating a higher molecular weight of DOC which is associated with lower volatility and bioavailability (Fig. 3-2e) (Navalon, Alvaro and Garcia, 2011). Both $SUVA_{254}$ and $SUVA_{280}$ indicate that *sandstone* sediments provided the lowest quality (i.e. the least bioavailable) DOC, whilst *limestone* and *chalk* provided DOC of similar quality.

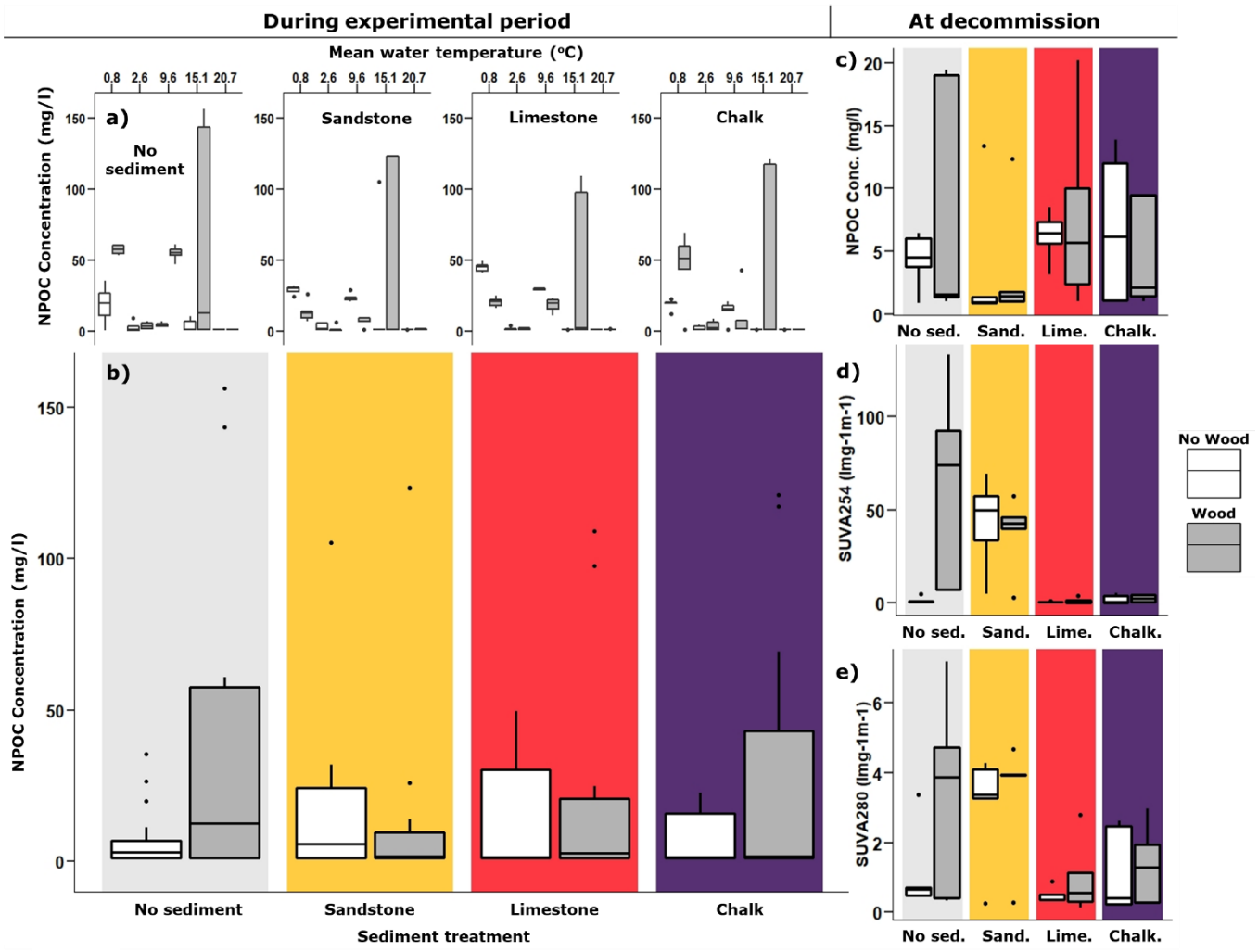


Figure 3-2 Measures of carbon quality and quantity in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill. (a) and (b) represent the concentration of non-purgable organic carbon (NPOC) separately at five measurement periods (represented by mean water temperatures) and amalgamated, respectively. (c) represents the concentration of DOC at the end of the study where triplicates of sampling and analysis were conducted to ensure accuracy of results. (d) and (e) represent the specific ultra-violet absorbance at 254 nm and 280 nm, respectively, both at decommission.

3.4.2 Dissolved oxygen

DO data is presented as the percentage change in the DO % saturation (DO%) over the 26-hour measurement period to account for differences in starting concentrations of DO. Over the entire temperature regime, the percentage change in the DO% was not significantly different in microcosms containing wood compared to those without (Fig. 3-3 b). Only in microcosms of *no sediment* was a significant difference observed ($P<0.05$), with an average -

1.7% change in microcosms containing wood and an average 4.33% change in those not (Fig. 3-3 b). Patterns were somewhat temperature related, especially for microcosms of *chalk* sediment, but the relationships are seemingly complex, probably related to other temporally or seasonally dependant factors (Fig. 3-3 a). Microcosms containing wood exhibited a higher variability within sediment treatments compared to those not, as well as in microcosms of *sandstone* and *chalk* sediments compared to *limestone* or *no sediment* (Fig. 3-3 a).

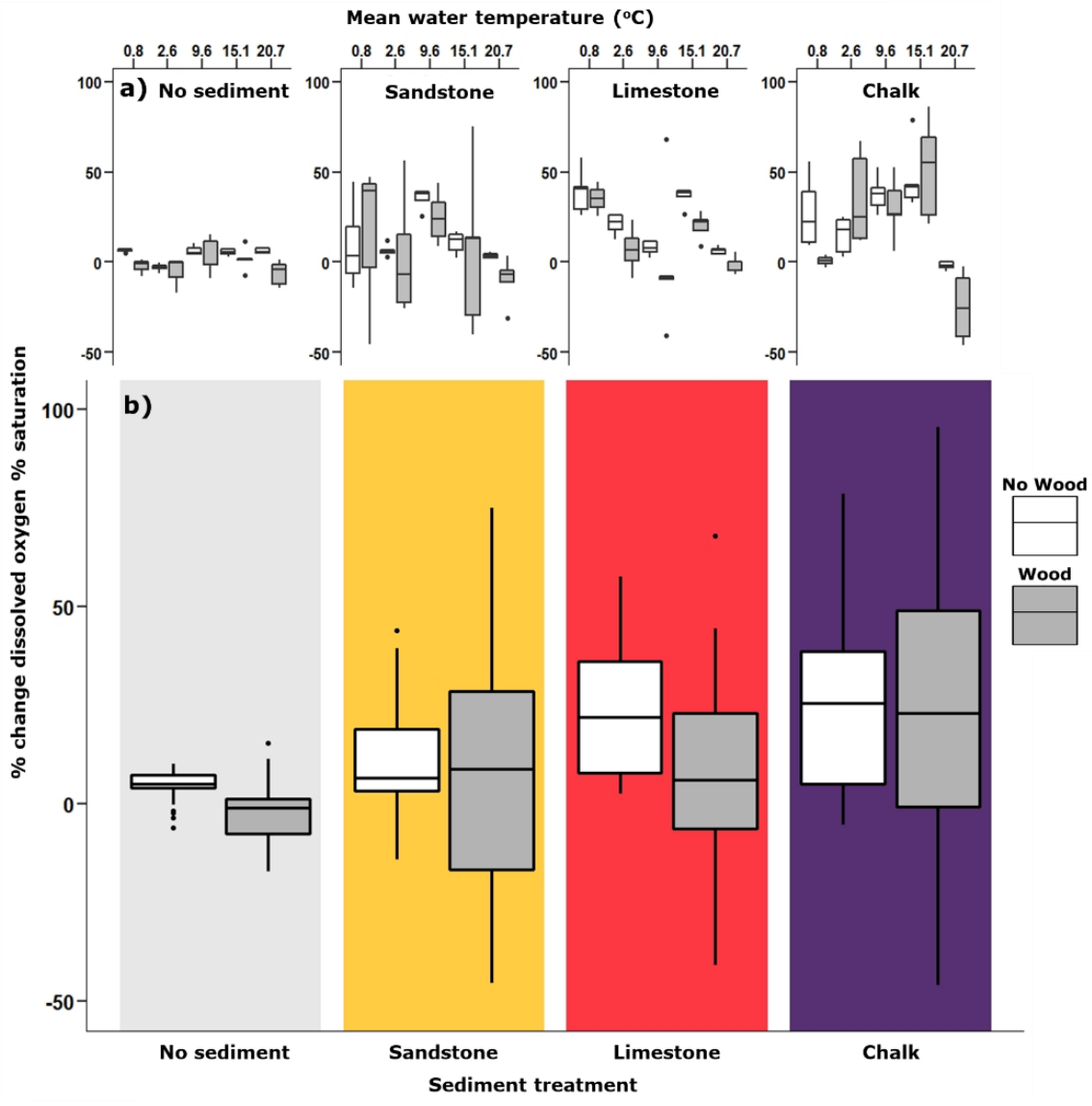


Figure 3-3 Percentage change in dissolved oxygen percent saturation over a 26-hour measurement period in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b).

3.4.3 Microbial Metabolic Activity

MMA results are presented as hourly production of rru (μg) normalised for the starting concentration of raz (μg) at hour 0 (Comer-Warner et al., 2018). Similar MMA was observed to other studies of similar design (e.g. Romeijn et al., 2019). Over the entire temperature regime, MMA was significantly higher in microcosms containing wood compared to those without ($P<0.05$) (Fig. 4b). Within sediment treatments too, MMA was significantly higher in microcosms containing wood for *no sediment* (209.18%, $P<0.05$), *sandstone* (188.16%, $P<0.05$), *limestone* (103.37%, $P<0.05$) and *chalk* (129.86%, $P<0.05$) (Fig. 3-4 b). MMA in the microcosms of *no sediment* and no wood was lower than in other sediment treatments containing no wood but still substantial (0.0023 μg μg^{-1} raz h-1 ± 0.0047). The variability in MMA was higher within groups containing wood compared to those without and the patterns in MMA were positively associated with temperature, though other factors are clearly influential for example at the 0.8 ^{o}C measurement period (December 2020) where a higher than expected MMA is observed (Fig. 3-4 a).

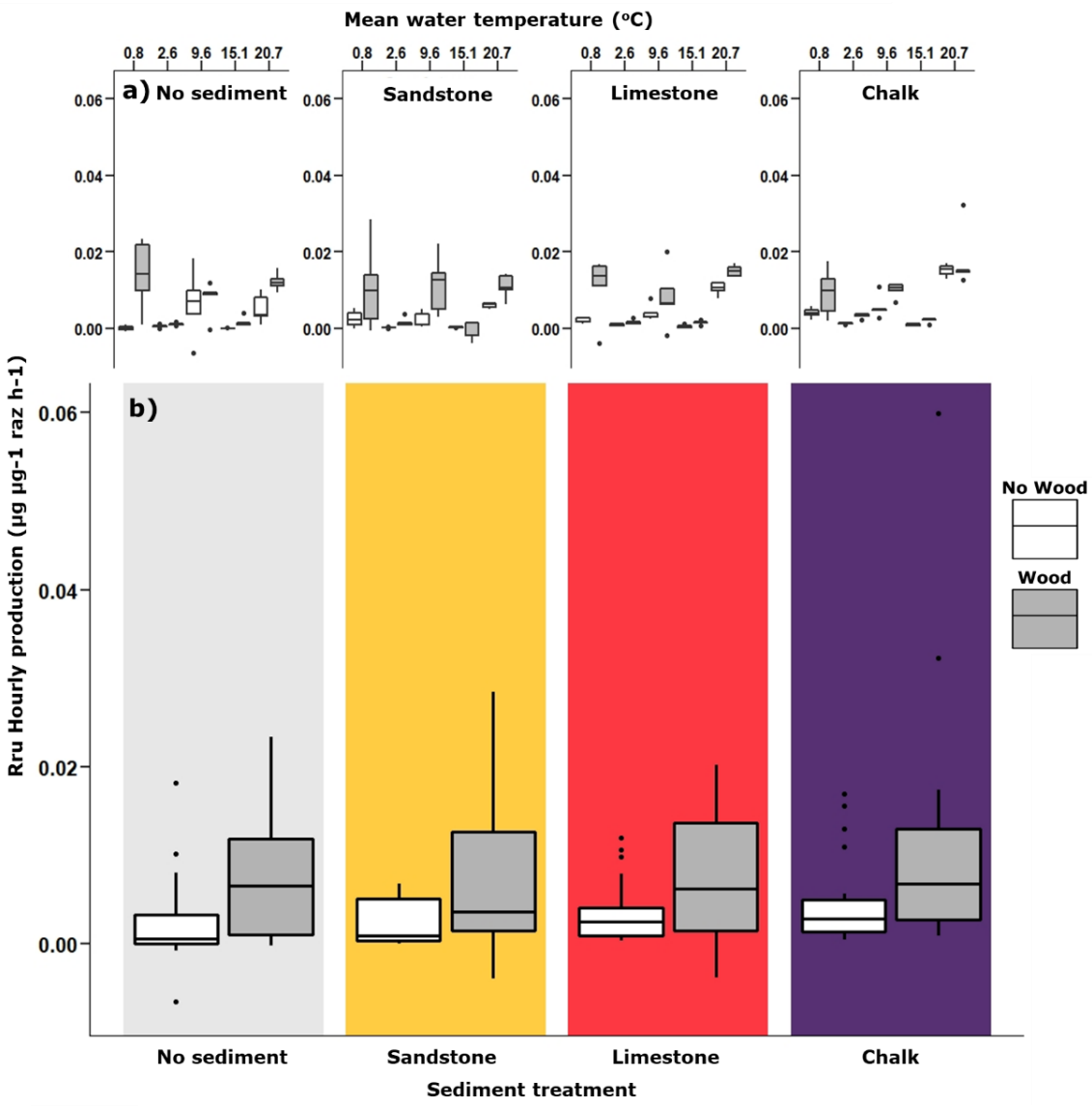


Figure 3-4. Production rate of rru per hour normalized for the starting concentration of raz in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b).

3.4.4 Nitrate removal rate

The rate of NO_3 removal is presented as the percentage reduction in the concentration of NO_3 per hour during the measurement period, to account for the small differences in starting concentration at hour 0 (i.e. a positive value indicates removal). Over the entire temperature regime the NO_3 removal rate was significantly higher in microcosms containing wood

compared to those without ($P<0.05$) (Fig. 3-5 b). However, within sediment treatments NO_3 removal rate was only significantly higher in microcosms containing wood compared to those not for microcosms of *no sediment* ($P<0.05$) or *chalk* sediment ($P<0.05$) (Fig. 3-5 b). The NO_3 removal rate was consistently lower in microcosms of *no sediment* compared to those containing sediment and only small rates were observed in microcosms of *no sediment* and not containing wood (-0.024% ± 0.28). Strong temperature related trends were observed for all treatments, though as with MMA other factors are clearly evident, again at the 0.8 ^{o}C measurement period (December 2020).

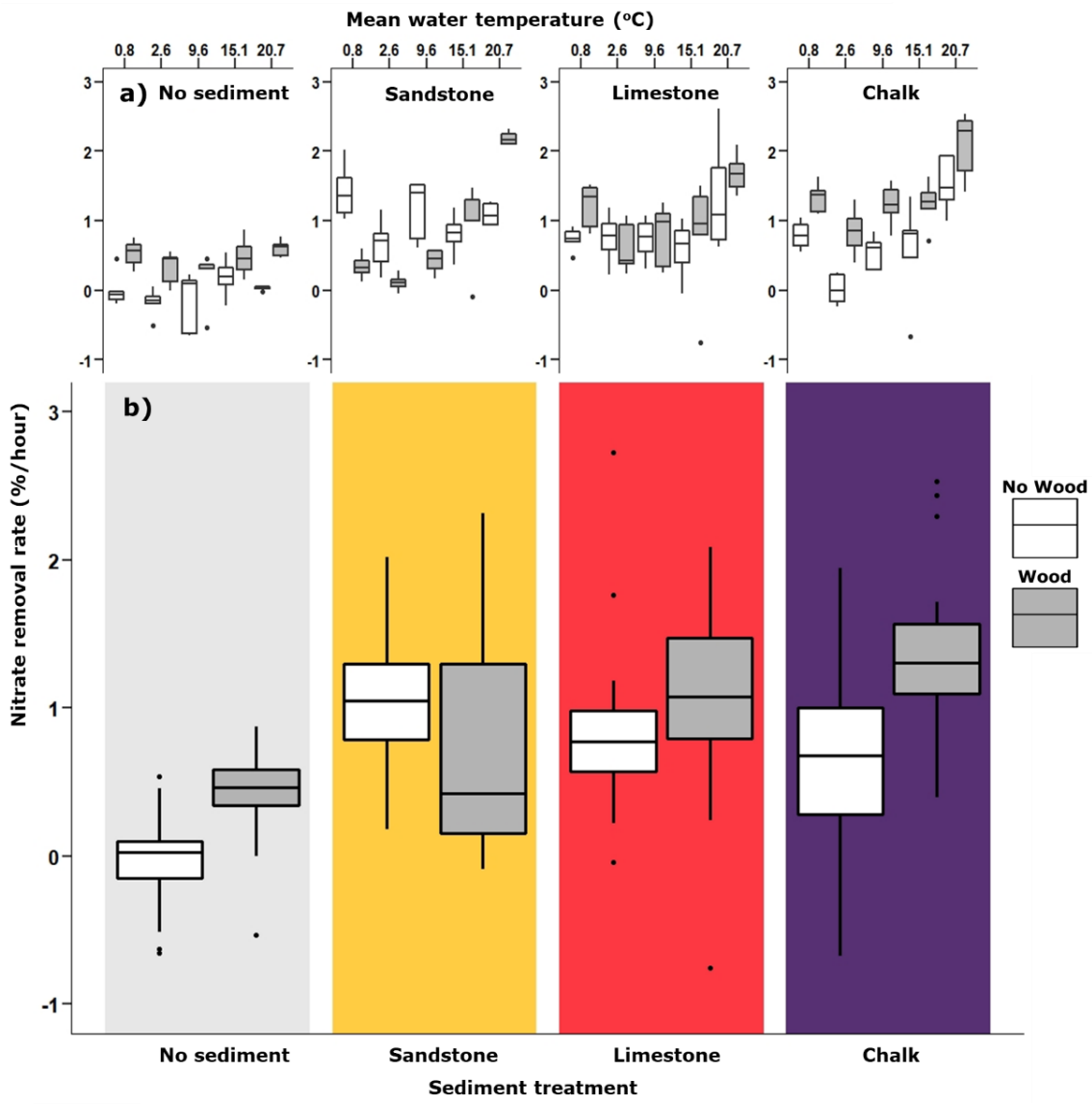


Figure 3-5. The rate of nitrate removal per hour in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b).

3.4.5 Greenhouse gas production

GHG net production rates (where reductions in concentration are equal to 0 production) are presented as μM h-1 and calculated between hours 2 and 6 of the measurement period (White and Reddy, 2003). Over the entire temperature regime, in microcosms containing wood the net CO_2 production rate was significantly higher ($P<0.05$) (Fig. 3-6 a), the net CH_4 production rate was significantly higher ($P<0.05$) (Fig. 6c), and the net N_2O production rate was significantly higher ($P<0.05$) (Fig. 3-5 e). Within sediment treatments, the net CO_2 production rate was significantly higher in microcosms containing wood for *no sediment* ($P< 0.05$), *sandstone* ($P< 0.05$) and *limestone* ($P<0.05$) but not for *chalk* (Fig. 3-6 b). Whilst the net CH_4 production rate appeared to be higher in microcosms containing wood, the difference is only significant for *sandstone* ($P< 0.05$) (Fig. 3-6 c). The net N_2O production rate was significantly higher in microcosms containing wood for *sandstone* ($P< 0.05$), *limestone* ($P< 0.05$) and *chalk* ($P< 0.05$) but not for microcosms containing *no sediment* (Fig. 3-6 e). The net production rate of all GHG was negligible for microcosms of *no sediment* and not containing wood (CO_2 = 10.92 μM h-1 ± 29.64; CH4 = 2.67 μM h-1 ± 12.70; N2O = 0.016 μM h-1 ± 0.041) and of microcosms containing *no sediment* and wood, only CO_2 was produced at notable rates (Fig. 3-6 a). Temperature trends were not explicit (Fig. 3-6 b,d,f).

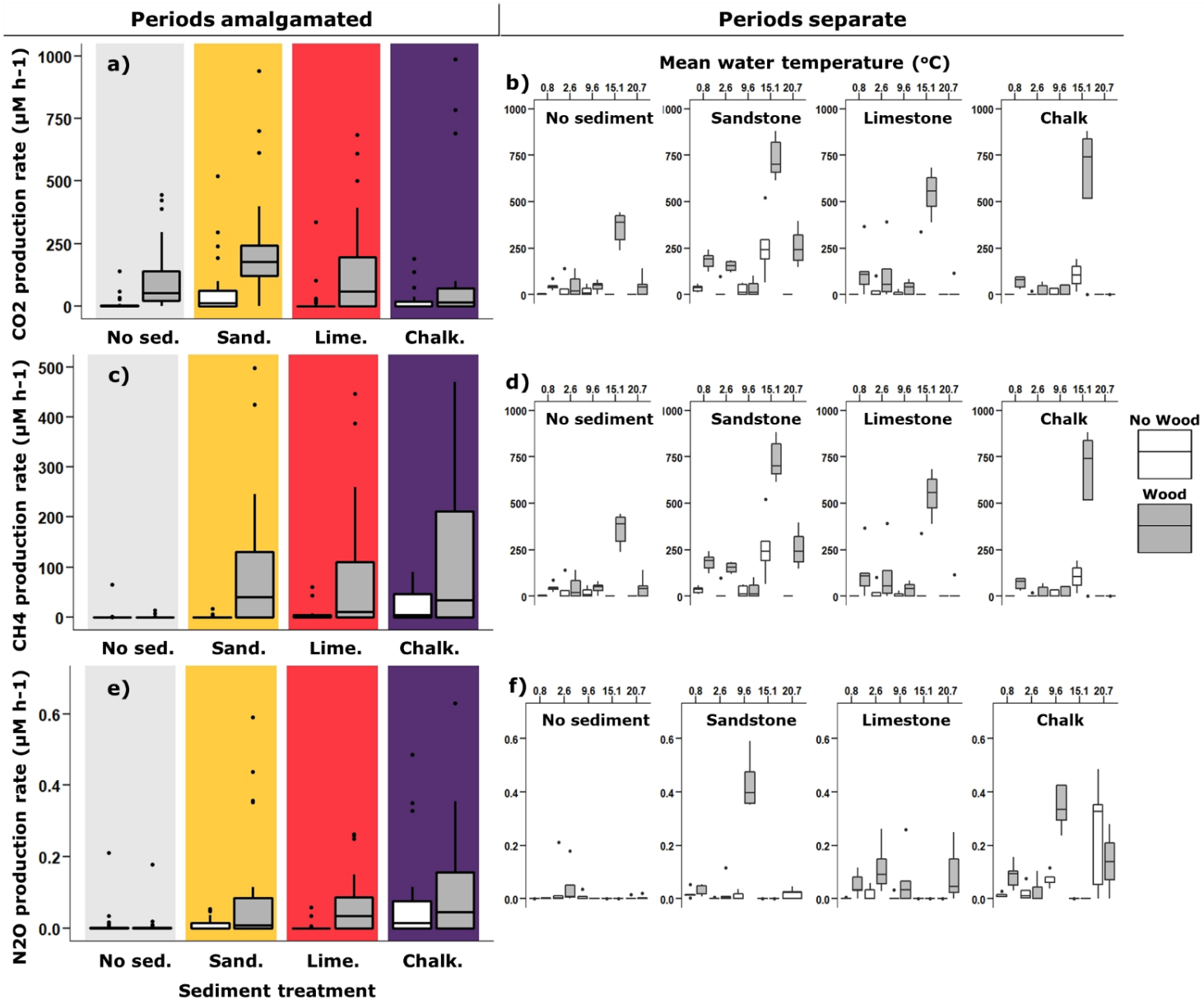


Figure 3-6. Positive net production rate of CO2 (a, b), CH4 (c, d) and N_2O (e, f) per hour in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (b, d , f) and amalgamated (a, b, c).

3.5 Discussion

3.5.1 A fundamental component of river ecosystems

The inclusion of streambed wood generally led to an increase in MMA across a range of temperatures typical of a temperate climate. This demonstrates the importance of wood as a driver of a keystone ecosystem process. Changes to the abundance and distribution of streambed wood, for example by its (re)introduction in river restoration, are therefore likely to lead to changes in MMA, with associated cascading effects for ecological and biogeochemical cycles, well beyond those measured here (Burcher, Valett and Benfiel, 2007; Findlay, 2010). This contributes to the growing body of evidence that suggests wood is a fundamental component of river ecosystems which is vital for their proper function and health (Roni et al., 2015; Grabowski et al., 2019).

3.5.2 Explanatory mechanisms

Several mechanisms by which instream wood could accelerate MMA have been identified, as outlined in the introduction. In this study, only the effect of streambed wood on DOC was explicitly measured, which was overall found to be insignificant for both quantity (i.e. concentration) and quality (i.e. bioavailability). Differences were observed in the *no sediment* and *chalk* treatments, however, which in the absence of wood provided the lowest concentrations of DOC to the water column. This suggests that where sediments contribute little DOC to the water column, the introduction of wood could significantly increase the concentration of DOC. This increase in DOC could have a particularly substantial effect on biogeochemical reaction rates in already DOC limited environments (Zarnetske, et al., 2011).

The absence of an increase in DOC concentration in microcosms containing wood may be explained by the slow rate of wood decomposition in the microcosm environment, in which

decomposition was effectively limited to chemical and biological processes, excluding the physical processes that are the primary driver of decomposition in in-situ conditions (e.g. by causing physical fragmentation) (Jones et al., 2019). Furthermore, smaller pieces of wood decompose more quickly (Jones et al., 2019) and wood can be immobilised in the streambed where it can decompose over long durations (i.e. longer than investigated here), especially in lowland environmental settings (Krause et al., 2014). Wood originating from some tree species may decompose more quickly than the hazel used here - related to lignin content, for example –as will leaves and smaller organic matter, which may also represent suitable additions in river restoration. Therefore, the rate of wood decomposition may be expected be higher in in-situ conditions compared to in the microcosm environment, which could result in a higher contribution of wood to DOC concentrations. On the contrary, DOC could be quickly transported out of sediments in river environments (Krause et al., 2014).

Freshwater carbon dynamics are complex, controlled by a series of biotic and abiotic processes like decomposition, production, mineralization and transport (Wohl et al., 2017; Jones et al., 2019). The relative prevalence of the processes active in the microcosm environment is largely controlled by physiochemical conditions, like temperature and the availability of electron acceptors, e.g. oxygen and N (Berggren et al., 2009). Therefore, the measured concentration and bioavailability of DOC in microcosms at each measurement period was determined by recent reaction histories and antecedent environmental conditions (Berggren et al., 2009). The snapshot of the carbon dynamics afforded by the infrequent sampling limit inference but still allow us to hypothesise the following explanation. High DOC concentrations were observed in all treatments at the first measurement period (0.8 ^{o}C), conducted in December 2020, where microcosms had been left to stabilize for approximately 1 year. Similarly high DOC concentrations were observed at the 15.1 ^{o}C measurement period (September 2021), where a longer time had elapsed since the previous spiking (~3 months)

than for other measurement periods (~1 month). These longer periods without the introduction of additional reactants could have allowed a large DOC stock to accumulate whilst nutrient concentrations (e.g. NO_3) were limited (i.e. severely unbalanced stoichiometric ratios of C:N:P), consequently resulting in a kind of 'luxury uptake' once NO_3 was introduced (Fog, 1988; Pearce et al., 2021).

MMA and the change in the concentration of DO were expected to exhibit similar patterns because they are both measures of aerobic respiration. Such a response was not evident, however, and instead the depletion in DO during measurement periods was not significantly increased in microcosms containing wood whilst MMA was. This may be explained by oxygen production as a product of photosynthesis, but this is unlikely because UV light was excluded. A more probable explanation is an artefact of the experimental procedure, where the DO concentration was measured at hour 0 before mixing (the last disturbance of the water having been ~1 week previous) whereas measurement at hour 26 was after more recent mixing (at hour 0), which could introduce oxygen to the water column. High MMA in microcosms containing neither wood nor sediment may be explained by respiration of autochthonous carbon, which can be extremely bioavailable (Berggren and Giorgio, 2015). The accelerated MMA in microcosms containing wood may be partly explained by the increased concentrations of DOC but it is evident that other mechanisms are also responsible.

To summarise, an increase in the concentration and bioavailability of DOC accounted for some of the changes to MMA, the rate of NO_3 removal, and the production of GHGs. However, it did not explain these responses entirely, and it is evident that other mechanisms are also responsible. Further research is necessary to identify and quantify the mechanisms by which streambed wood leads to a response in biogeochemical functions, for example its capacity to host microbial communities and biofilms and its effect on the structural properties of sediments (e.g. hydraulic conductivity and preferential flow paths).

3.5.3 Effect on ecosystem (dis)services

Managing elevated concentrations of N in freshwaters is one of the biggest challenges facing environmental engineers in the 21st century (National Academies of Sciences, Engineering, and Medicine, 2019). Here we demonstrate that the introduction of instream wood may be a viable technique by which to increase the delivery of ecosystem services, such as to accelerate the removal of N and other pollutants in the hyporheic zone. It could be especially effective in streams where C is already limiting and where wood and DOC are not quickly transported downstream, for example some lowland environmental settings (Zarnetske et al., 2011; Krause et al., 2014). NO_3 removal encompasses a number of N transformation pathways which lead to both temporary removal, such as Dissimilatory Nitrate Reduction to Ammonia (DNRA), and permanent removal from water, such as denitrification (Burgin and Hamilton, 2008; Quick et al., 2019). Whilst it was outside the scope of this study to quantify the relative removal pathway prevalence, it is likely that denitrification was responsible for at least some removal (leading to permanent removal), as evidenced by the accompanied increase in the production of N_2O in microcosms containing wood (Quick et al., 2019). The transformation of NO_3 in denitrification is positive if complete transformation occurs (i.e. to N_2), but incomplete transformation (i.e. to N_2O) merely represents pollution swapping, which is not overall a suitable mitigation solution. A strong relationship between NO_3 removal rate and temperature was evident, as has been reported by other authors (Pfenning and McMahon, 1997), which suggests that NO_3 removal rate in rivers and streams could increase as river water temperature increases (Hannah and Garner, 2015).

The contribution of streams and rivers to global GHG emissions is of global significance, representing an ecosystem dis-service, i.e. a cost to society (Beaulieu et al., 2010; Raymond et al., 2013; Stanley et al., 2015). Some of the key controls of GHG production in river beds are well understood, for example organic matter content and sediment grain size distribution

(Comer-warner et al. 2018; Romeijn et al., 2019; Quick et al., 2019). These controls were evident here, where *sandstone* sediments were the finest (smallest grain size distribution) (Fig. 8-5) and had the highest carbon content (Fig. 8-6), resulting in the highest production rate of CO_2. This response is also evident in the measured oxidative redox potential, which was lower in microcosms containing *sandstone* sediments compared to other sediment types, suggesting reducing conditions (Fig. 8-8). However, this work demonstrates that these controls do represent all of the process that determine GHG production in riverbeds. For example, the presence of wood in the streambed is also an important driver of GHG production. Notably, the presence of wood resulted in larger differences in GHG production than differences between the different sediment types themselves. The increase in GHG prodution should be expected as a result of the (re)introduction of wood in river restoration, which may offset benefits of NO_3 removal essentially representing a movement of pollutants from one system to another. However, GHG production from wood decomposition may occur whether the wood decomposes in the stream environment or terrestrially; it remains unclear if different volumes or species of GHGs are emitted in either scenario. These potential trade-offs require careful consideration by decision makers in a cost-benefit analysis.

3.5.4 Consequences for global models of carbon and nitrogen

Wood can constitute a substantial portion of the total streambed volume but it has typically been ignored or explicitly omitted from studies exploring streambed function, and especially biogeochemical function, to more easily achieve homogeneity or to focus on geological origin (e.g. Romeijn et al., 2019). In this study, we demonstrate that excluding wood from mechanistic experiments may lead to erroneous results and mis-estimations of fundamental ecosystem processes with potentially cascading effects for associated processes. These results (e.g. Beaulieu et al., 2010; Raymond et al., 2013; Stanley et al., 2015) have directly informed

global models and estimates of the flux of elements between streams and rivers and other systems, for example GHG emissions to the atmosphere and the flux of N compounds to marine environments. The representativness of mechanistic studies and, as such, of global models and flux estimates may be improved by considering streambed wood in future studies, for example as an independent or confounding variable. Further research is necessary to identify and investigate potential mechanisms by which streambed wood effects biogeochemistry in the river corridor such as those outlined in the introduction (Krause et al., 2014).

3.5.5 Study limitations

Further limitations of this study are highlighted to prevent improper inference or application of results. Different sediment types have been used here to better understand and represent the range of the potential effects of wood on streambed biogeochemistry. However, sediment treatment labels (i.e. *sandstone, limestone and chalk* and *no sediment*) are not necessarily representative of those geologies. Furthermore, several mechanisms that control the effect of wood in rivers are absent in the microcosm environment, whilst some mechanisms may be exaggerated. For example, the development of microbial assemblages could explain some of the variance observed between microcosms of the same treatment, especially towards the end of the experimental period. The transport of solutes and gases in the microcosm environment is limited to diffusive transport, which could have resulted in the induction of anaerobic conditions more quickly and at shallower depths than in in-situ conditions, where hyporheic exchange may accelerate transport and mixing (Krause et al., 2014). Consequently, the relative production rates of anaerobically produced GHGs (CH_4 and N_2O) may be over-estimated relatively to aerobically produced CO_2. Furthermore, further transformation of gases in the water column or headspace of the microcosm could modify the relative

concentrations of GHGs actually emitted from sediments before sampling, for example oxidation of CH_4 to CO_2 (Miller et al., 2022). Other potential limitations include the irregular spiking to high NO_3 concentrations (atypical of most systems in reality), which could also affect microbial assemblage dynamics, though this could represent high storm-driven high nutrient loading (Pearce et al., 2021). Furthermore, high concentrations of DOC, and associated metrics, in treatments without sediment are not at all representative of instream wood in the surface water column of streams because in these circumstances DOC would be quickly transported (Krause et al., 2014). Whilst these artefacts of the experimental design may prevent reliable inference and scaling of metrics to absolute values, the broad patterns observed here are likely to be somewhat representative.

3.5.6 Implications for policy and practice

This research contributes to the significant body of evidence that supports instream wood as a fundamental driver and control of key ecosystem processes. Changes to the abundance and distribution of instream wood should be expected to lead to significant changes in most facets of ecosystem function, as well as the delivery of both services and dis-services which often occur mutually simultaneously and are, therefore, in conflict. Wood-based restoration could offer suitable nature-based solutions to wicked problems, like high nutrient concentrations, which work with natural processes and deliver multiple co-benefits, as is now widely advocated for in policy (Environment Agency, 2021). Conversely, it could also lead to significant dis-services, such as increased GHG emissions, which managers and planners have a serious responsibility to consider and minimise; also soon to be supported in policy (e.g. in the UK, Environment Agency, 2021).

There are always trade-offs in environmental management, which must be carefully considered by decision makers using appropriate frameworks and sufficient evidence. This work highlights that whilst reductionism can be useful in environmental research, holism is necessary when applying this research to policy and practice. Considering processes and objectives in isolation could generate unintended, ulterior and cascading consequences. We advocate a more holistic perspective to consider the intrinsic interconnectedness of ecosystem processes that determine ecosystem function and the delivery of ecosystem (dis)services.

4 A before-after-control-impact approach to assess the effect of instream wood restoration on transient storage in a lowland sandy stream

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4.1 Abstract

The restoration of instream wood is a promising technique by which to increase transient storage and deliver a suite of nature-based solutions, such as improvements to water quality. However, the influence of instream wood on transient storage in different environmental settings remains uncertain. Whilst most research has focussed on upland settings (typified by steep stream gradients, high hydraulic conductivity sediments, and high stream energy), most restoration using wood occurs in lowland settings. To ensure the transferability of research insight to practice, it is necessary to conduct evaluations of instream wood restoration in the lowland settings in which it is applied. Furthermore, it is necessary to adopt robust methodological approaches which are able to isolate the effect of restoration from confounding and temporally variable factors. In this study, a before-after-control-impact (BACI) experimental approach was used to quantify the effect of restoring instream wood (by introducing 8 channel-spanning wood features) on transient storage in a lowland sandy stream. A series of conservative tracer injections were conducted before and after intervention. The shapes of the resulting breakthrough curves were analysed using temporal metrics and statistical moments to allow a comparison of the dominant transport and retention processes between the control and impacted reaches. Multiple statistical approaches were applied to investigate the effect of restoration in different environmental conditions, namely during different discharge. The BACI experimental approach was not sufficiently sensitive to detect the effect of restoration, but qualitative analysis revealed trends with time and discharge. Transient storage decreased as a result instream wood restoration, which was probably attributable to a decrease in velocity. The magnitude and nature of the effect of instream wood was exaggerated by higher river discharge. Installing channel-spanning features in lowland settings may decrease transient storage, reducing the delivery of

ecosystem services. Therefore, other restoration designs should be considered, such as deflectors.

4.2 Introduction

4.2.1 Nature-based solutions and the hyporheic zone

The hyporheic zone (HZ) has a fundamental impact on most ecosystem functions in the river corridor (Hester and Gooseff, 2010; Lewandowski et al., 2019). As such, it is important for the delivery of many riverine ecosystem services (RES) (Ward et al., 2018). For example, the HZ can facilitate biogeochemical reactions, leading to improved water quality (Boano et al., 2014); buffer temperature extremes, and provide protection and sustenance of biodiversity (Klaar et al., 2020); and buffer hydrological extremes, which can reduce the impact of flood and drought events (Bruno et al., 2020). Therefore, degradation of the HZ is likely to alter ecosystem function and reduce the capacity of the river to provide RES (Stofleth, Shields and Fox, 2008). Many river ecosystems have been historically modified in ways which are likely to have degraded the HZ, for example by straightening, deepening, removal of gravels or addition of fine sediments, local extinction of ecosystem engineers (e.g. the Eurasian beaver, *Castor fiber*, in the UK), and the removal of riparian vegetation and instream wood (sometimes referred to as large woody debris) (Brookes, 1985; Sawyer, Cardenas and Buttles, 2012; Ward et al., 2018; Larsen, Larsen and Lane, 2021). These modifications can lead to changes to the magnitude and properties of hyporheic exchange, such as the rate of exchange of water, solutes and particles between the surface water and the subsurface and the residence time therein. Actions to restore and sustainably manage modified river corridors to improve the function of the HZ could enhance the delivery of RES and provide nature-based solutions to societal challenges, which are essential in helping to abate the global water crisis (Hester

and Gooseff, 2010; Damania et al., 2019). One such way in which this might be achieved is by the restoration of instream wood (Sawyer, Cardenas and Buttles, 2012; Krause et al., 2014).

4.2.2 Instream wood as a driver of hyporheic exchange

Instream wood is an important component of the river corridor which effects many ecosystem functions, including biogeochemical, ecological, geomorphological and hydrological processes (Zimmerman, Goodlett and Comer, 1967; Klaar et al., 2011; Krause et al., 2014). Instream wood similarly plays an important role in modulating hyporheic exchange by driving hydrodynamically and hydrostatically induced pressure gradients across the surface water - sediment interface (Mutz, Kalbus and Meinecke, 2007; Sawyer, Cardenas and Buttles, 2011). This has been demonstrated in a range of environmental settings and by using field, artificial flume and numerical modelling methods. In a field experiment in a temperate lowland sandy river, Klaar et al. (2016) observed increased hyporheic exchange close to instream wood, as well as a longer residence time of water in the HZ. In a similar system, Shelley et al. (2017) observed a higher delivery of surface water into the streambed during high flows when instream wood was present. Ader et al. (2021) showed that the presence of instream wood leads to higher transient storage (which includes hyporheic storage) in a temperate pool-riffle mountain stream. In a 2nd-order semi-arid stream, Lautz, Siegel and Bauer (2006) observed short-term hyporheic interactions associated with wood dams in gaining reaches only. Using flume experiments, Mutz et al. (2007) demonstrated that wood approximately doubled vertical water flux and increased mixing depth, and Sawyer, Cardenas and Buttles (2011, 2012) showed that channel-spanning wood induced downwelling upstream and upwelling downstream, with residence times and flow path lengths greater than some

geomorphic features. Similarly, Wilhelmsen et al. (2021) showed that log jams increased hyporheic exchange by an order of magnitude.

4.2.3 A mismatch between research and practice

A small number of studies have directly investigated the effect of active (re)introduction of instream wood on the HZ, in an effort to understand whether the effects of naturally recruited instream wood can be replicated. The majority of these studies, which are reviewed in section 4.2.2, have been conducted in only one environmental setting: high-order streams with gravel and cobble sediments. However, a large proportion (79% in the UK) of river restoration using instream wood occurs in lowland settings (Cahsman et al., 2018) - typified by low stream gradients and low sediment grain size distribution. The drivers and properties of hyporheic exchange differ distinctly between upland and lowland settings, and therefore the influence of instream wood is also likely differ (Rana, Scott and Hester, 2017; Krause et al., 2022). Whilst lowland settings may have particularly high potential to deliver RES, it is necessary to evaluate the efficacy of restoration techniques in the environmental settings in which they are applied (Green, 2008; Krause et al., 2014; Blaen et al., 2018). This is especially important for HZ restoration because the environmental setting largely determines the function of the HZ (Hester, Brooks and Scott, 2018; Feng et al., 2022). What is more, existing works have largely focussed on (re)introducing wood to systems that were previously devoid of wood. Whilst these circumstances may represent the highest potential for improvement, there are many degraded systems where the wood loading is well below natural, for example those cleared within the last 2 centuries (Stout et al., 2018). It remains unclear whether increasing wood loadings in streams that already contain instream wood can improve the function of the HZ.

4.2.4 Shifting the paradigm: from removal to introduction

Although the importance of wood in river ecosystems is now widely recognised, historically it has been removed from rivers worldwide (Montgomery et al., 2003; Wohl, 2014). On top of this, deforestation of riparian zones has been widespread, limiting the capacity of a natural wood regime to recover by natural recruitment of wood from tree fall and branch fall (Sweeney et al., 2004). Rewooding of riparian zones is of paramount importance to ensure long-term wood recruitment and maintenance of natural wood loadings (Meleason, Gregory and Bolte, 2003). Thankfully riparian zone restoration is increasing, both actively by tree planting and passively by natural regeneration, for example as a result of rewilding (Brown et al., 2018). However, even with a healthy established riparian zone, recovery to natural wood loadings could take more than 250 years (Stout et al., 2018), though this could be accelerated in the presence of beavers (Larsen, Larsen and Lane, 2021). If riparian zones are recently or yet to be established, a further 30 to 100 years can be expected before increases in wood loadings begin (Beechie et al., 2000). Given the scale and immanency of the global water crisis, nature-based solutions must be realised now (Damania et al., 2019). Therefore, actions to accelerate the recovery of instream wood must be investigated.

The active (re)introduction of instream wood is a common technique in river restoration which has been implemented to deliver a range of objectives, such as to reduce bank erosion, improve hydromorphological diversity, improve habitat complexity, and for natural flood management (Roni, Hanson and Beechie, 2008; Roni et al., 2015; River Restoration Centre, 2021). The limited number of studies which have directly investigated the (re)introduction of instream wood have general reported positive outcomes. Sawyer, Cardenas and Buttles (2012) added and then removed channel-spanning instream wood (approx. 1 feature per 10 meters) to a 2nd order mountain stream and reported an increase in hyporheic exchange (to approx. 0.1% of channel discharge) with wood compared to without, which was apparently

limited by losing conditions. Hester, Doyle and Poole (2009) installed experimental weirs (representing log dams) in a 1st order headwater stream and reported negative vertical head gradients (indicating downwelling) upstream of weirs, of up to -0.50 m m^{-1}, and positive vertical head gradients (indicating upwelling) downstream, of up to 0.04 m m^{-1}, during base flow conditions. Clark et al. (2021) investigated the impact of large wood placement on reach-scale water temperature (from which hyporheic exchange can be inferred) in a gravel and cobble-bed stream and reported an insignificant affect, though this was attributed to the poor design of restoration (mostly bank-attached structures). One study, by Rana, Scott and Hester (2017), investigated the impact of installing log dams in a lowland setting, where the channel had flat floodplains on both banks and a slope of 0.066 m/m. In this case, the area of transient storage increased but the rate of exchange between the mobile water and that in transient storage decreased.

4.2.5 Methodological challenges

Discharge (*Q*) is a key control of transport and storage processes (in the channel and the subsurface) but there is contradictory evidence as to the nature and magnitude of this relationship (Ward et al., 2013, 2018; Rana, Scott and Hester, 2017). It is likely that that *Q* controls the effect of instream wood on transport and storage because it effectively defines the relative characteristics of wood in the channel (such as blockage ratio) and determines the hydraulics of water near to the wood, like turbulence and surface water profile (such as hydraulic head over a feature) (Gippel, 1995; Hester, Doyle and Poole, 2009; Sawyer, Cardenas and Buttles, 2012). We hypothesise that instream features exhibit threshold behaviours which are triggered by certain conditions (such as specific *Q*) and change the function of a feature, for example when a feature is first overtopped (blockage ratio ≥ 1).

Typically adopted experimental approaches can fail to separate restoration-induced change from natural variability, like dynamic *Q* (Damgaard, 2019; Seger et al., 2019; Christie et al., 2020). Studies have typically adopted a space-for-time substitution (also known as control-impact [CI]) (Hester, Doyle and Poole, 2009; Rana, Scott and Hester, 2017; Clark et al., 2021) or before-after (BA) (Sawyer, Cardenas and Buttles, 2012) experimental design. An alternative approach is the before-after-control-impact (BACI) methodology, which has been suggested as an ideal experimental design to study the impact of restoration (Stewart-Oaten and Bence, 2001; Ward et al., 2018), and has been adopted for similar investigations on a few occasions (e.g. Santelmann, Harewood and Flitcroft, 2022). The BACI approach can allow the identification of inherent differences between control and restored sites before intervention and so isolate the impact of restoration from temporal or spatial variability (Seger et al., 2019). It is also able to capture the non-stationarity of restoration-induced impact due to confounding temporal variability, such as dynamic *Q* in river systems.

4.2.6 Study aims

The primary aim of this study is to evaluate the efficacy of actively (re)introducing instream wood to enhance reach-scale transient storage in a lowland sandy stream with a reduced wood loading. A BACI approach is adopted to isolate the effect of instream wood restoration on HZ function, and to allow the investigation of the changing influence of instream wood when subject to different *Q* conditions. Further, the influence of the experimental approach (BA or CI compared to BACI) is investigated, to identify approaches that may be (in)appropriate for the environmental impact assessment of river restoration. If the active restoration of instream wood proves to be effective at enhancing transient storage, it could offer a cost-effective and easy implementable technique by which to deliver nature-based

solutions like improving water quality, buffering hydrological extremes, and protecting biodiversity.

4.3 Methodology

4.3.1 Study site

Wood Brook is a second-order stream which flows through the Birmingham Institute of Forest Research (BIFoR) field site in Staffordshire, United Kingdom (52.80268, -2.29855). It drains a catchment area of 3.1km^2 which is dominated by Permo-Triassic red sandstone geology and has an elevation ranging from 90 to 150 m asl, a mean annual temperature of 9^{o}C, and a mean annual precipitation of 690 mm (Blaen et al., 2017; Norby et al., 2016). Land use was historically dominated by intensive agriculture but in the last decade much of the area has been turned over to regenerative agriculture and diverse forest planting. As such, fertilizer application has been reduced but the area is still recognised as a nitrate vulnerable zone under the Nitrates Directive (91/676/EEC). The study reach is primarily covered by riparian woodland of hazel (*Corylus avellana*), alder (*Alnus glutinosa*) and oak (*Quercus petraea*, *Quercus robur*). The stream itself was identified as suitable for restoration having been straightened and deepened, at times flowing through a steep ravine, and cleared of instream wood. This study site represents a common restoration scenario in the UK and for much of northern Europe. It therefore provides a representative evaluation of a common restoration technique that can be suitably, though carefully, extrapoltated to many other systems of similar environmental characteristics.

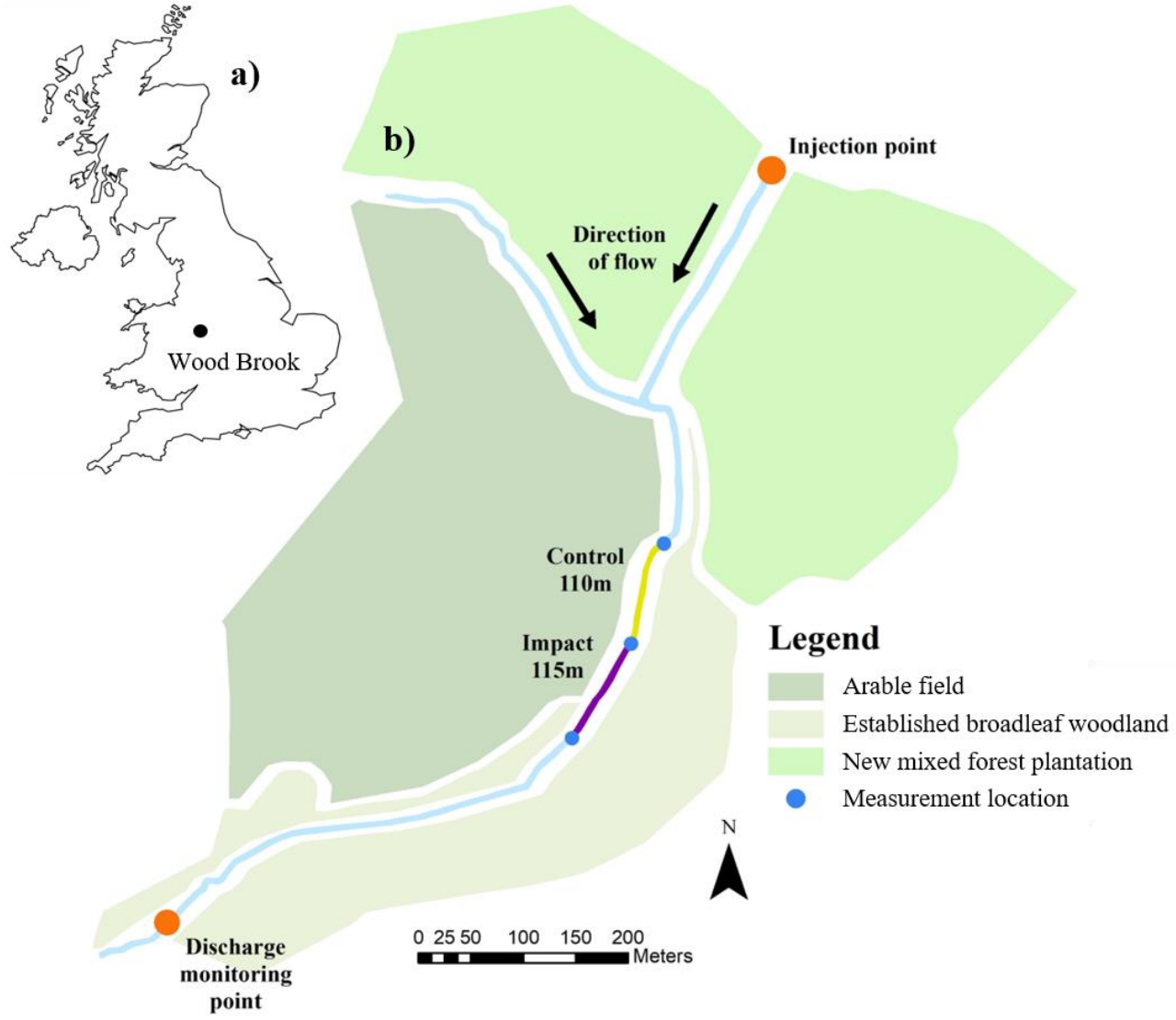


Figure 4-1. Catchment with (a) the location of Wood Brook within the UK and (b) the dominant land use, study reach with control and impact sub-reaches, injection point, tracer measurement locations and Q monitoring point. Scale bar relates to (b) only. Produced using ArcGIS 10.8.1, ESRI.

4.3.2 Hydrological monitoring

Q was monitored 400 m downstream of the study reach throughout the experimental period with a stage-*Q* relationship (Fig. 8-9) established using salt-dilution gauging methods and sensor level recording (Hudson and Fraser, 2005), described in detail in section 8.3.1. Khamis et al. (2021) reported insignificant gains or losses between upstream of the study reach and this point. Furthermore, substantial groundwater inflow or outflow is unlikely with the water

table being several meters below the stream, in large parts disconnected through an underlying layer of clay throughout the study reach (Blaen et al., 2017; Khamis et al., 2021). The average *Q* throughout the period was 0.0079 (m^3/s), with a maximum *Q* of 0.104 (m^3/s) observed in July 2021. The average *Q* for the tracer injections was 0.0049 (m^3/s) before intervention and 0.0056 (m^3/s) after restoration. The *Q* at each injection date is in reported in Table 8-14.

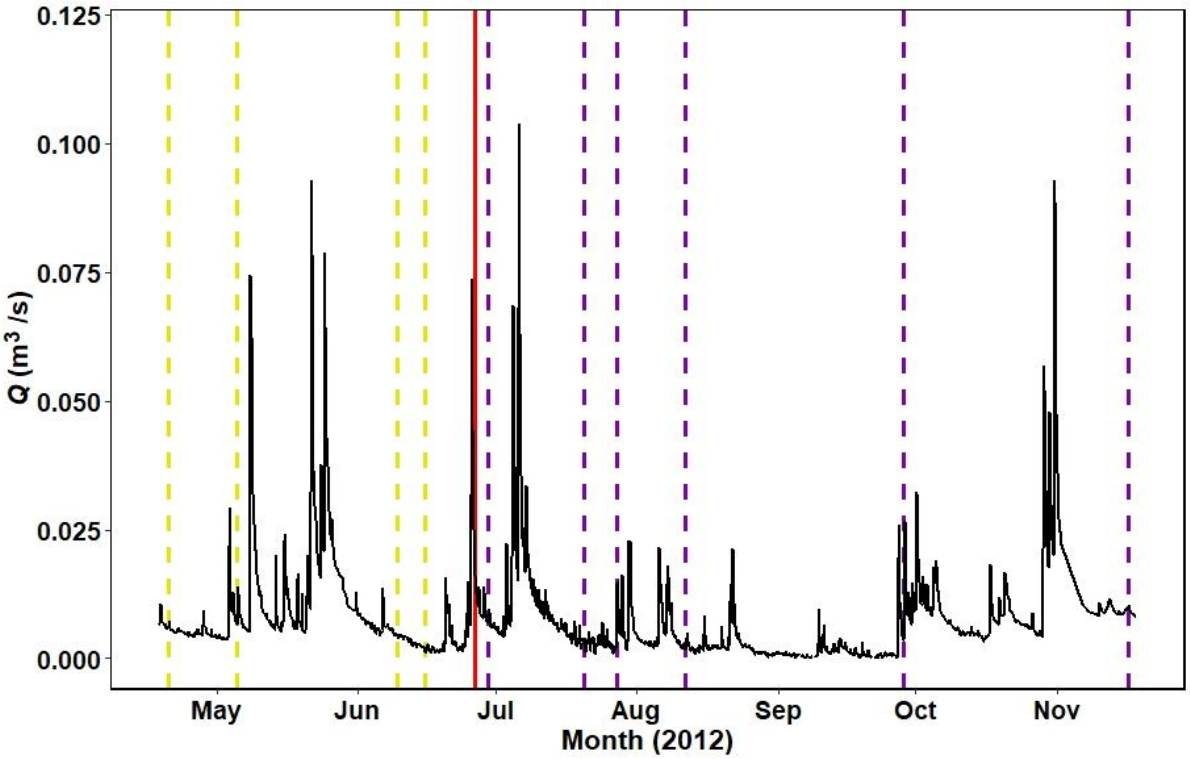


Figure 4-2. *The Q (m^3/s) throughout the experimental period (April-November 2021) derived from the stage-Q relationship presented in Figure S1. Tracer injection periods are indicated by dashed yellow (before restoration) and purple (after restoration) lines. The solid red line indicates the date of restoration.*

4.3.3 Characterization of sub-reaches

Two sub-reaches, control and impact, were identified (yellow and purple lines in Fig. 1) and comprehensively surveyed in 5 m sections for geometry and geomorphic and instream wood

characteristics (Table 4-1). The sub-reaches are similar in all characteristics. The *impact reach* is 5 m longer than the control, but this is unlikely to influence tracer response (Schmadel et al., 2016). Channel width, depth and gradient were measured in March 2021, the latter using an automatic surveying level which was placed in the channel. Sinuosity was calculated by dividing the length of the stream channel by the Euclidian distance between the start and end of the sub-reach. Sediment samples of 500 ml were taken in the thalweg and from the top 10cm of the stream bed in April 2021. Grain size analysis was conducted by mechanical sieving sediment into 5 size fractions (>2 mm, 0.5–2 mm, 0.25–0.5 mm, 0.063–0.25 mm, <0.063 mm) and weighing each component before calculating relative weight contribution (%). A comprehensive instream wood survey was conducted, which is outlined in detail in section 8.3.2 of the SI and plotted in figure 8-10.

Table 4-1. Physical, geomorphic and instream wood characteristics of the sub-reaches.

	Control	Impact
Channel geometry		
Mean width (cm)	213.26	218.04
Mean depth (cm)	22.74	22.52
Mean gradient (%)	-0.44	-0.15
Length (m)	110	115
Sinuosity	1.0185	1.0087
Grain size distribution (%)		
>2 mm	27.86	34.24
0.5–2 mm	11.99	9.05
0.25–0.5 mm	28.29	23.07
0.063–0.25 mm	21.72	22.73
<0.063 mm	10.14	10.91
Wood distribution		
Mean no. large accumulations /m	0.08	0.05
Mean no. medium accumulations /m	0.06	0.07
Mean no. small accumulations /m	0.07	0.08
Mean no. of individual pieces /m	2.17	2.29
Mean % visible organic matter cover	15.57	11.85
Mean no. trees <1m from channel /m	1.08	0.77

4.3.4 Restoration of instream wood

On the 25th June 2021, 8 channel-spanning features were installed in the impact sub-reach with a spacing of ≥10 m, which likely limits feature interaction in this setting (Hester, Brooks and Scott, 2018). Hazel coppice wood bundles with a length similar to the stream width and a diameter of approximately 60 cm were pinned with 5 chestnut (*Aesculus hippocastanum*) stakes (2 upstream and 3 downstream), hammered into the streambed with a fence post

driver, and finally secured with sisal rope. Details of the materials and costs are reported in section 8.3.3. At installation, the features had a high porosity with a wood:air ratio of approximately 50:50. During baseflow, they protruded from the surface water but during spate they were quickly submerged.



Figure 4-3. Two of the channel-spanning wood bundle features immediately after installation in June 2021.

4.3.5 Solute tracer injections

A common method for characterizing hyporheic exchange is the injection of solute tracers and recording of instream breakthrough curves (BTC) (Jones and Mulholland, 2000). This technique yields integrated information about the stream and its transient storage zones, which include both surface (i.e. 'dead zones' in the channel) and subsurface (i.e. hyporheic) zones (Gooseff et al., 2005; González-Pinzón et al., 2015). Information about transport and storage in the stream is acquired by timeseries analysis of BTCs, whilst information about the subsurface is acquired by analysis of transient storage models to calculate metrics which describe the exchange rate of water in the channel with the subsurface, and the residence time therein (Ward et al., 2018; Drummond et al., 2019). Applied in a BACI approach, these techniques can provide a quantitative assessment of reach-scale transport and storage of water

and solutes, capturing temporal and spatial variability and the impact of restoration (e.g. Becker et al., 2013).

A series (n=10) of conservative tracer injections were conducted before (n=4) and after (n=6) restoration, between April and November 2021. Uranine (Fluorescein sodium salt, Fluka Analytical, Code 46950-500G-F) and NaCl (salt) were co-injected almost instantaneously (in a slug injection) 250 m upstream of the study reach (injection point in Fig. 4-1) to allow total mixing of tracers in all flow conditions. Tracer mass was usually decided based on *Q* the day before the injection, although in some cases the injection mass was higher to accommodate associated experiments not reported on here. The injection mass for uranine ranged between 0.75-2 g and for salt between 3 – 26 kg (tracer masses for each injection are reported in Table 8-14). Tracer concentration was measured at the start and end of each sub-reach (measurement locations in Figure 1). Electric conductivity (EC) was measured at 10 second intervals using sensors (Levelogger 5 LTC, Solinst Canada Ltd.) which were calibrated in the field to calculate salt concentration. Grab samples were collected throughout the injection period (mean n = 12) with sampling frequency focussed to target the peak of the BTC. Samples were analysed for uranine concentration within 24 hours of collection on a Varian Cary Eclipse benchtop fluorescence spectrophotometer (Agilent Technologies, Inc.) at an emission/excitation wavelength pair of 494/512 nm. Due to the higher measurement frequency and good sensitivity of EC signal from background, only the EC BTCs were used in modelling and analysis, which should not affect conclusions (Schmadel et al., 2016).

4.3.6 Determination of breakthrough curves, data processing and analysis

NaCl concentration was corrected for background concentration (measured at each location) at injection time zero, and BTCs were normalized for the maximum observed concentration.

Experimental truncation of BTCs occurred in all injections due to cessation of measurement before tracer concentrations had returned to background (Drummond et al., 2012). In order to extend truncated BTCs and to calculate transport and storage metrics, a continuous time random walk mobile-immobile model solute transport model was fitted to each EC BTC, using an adapted version of MATLAB (2021a) source code published with Drummond et al. (2019). In this model the mobile zone (i.e. the channel) is described by instream velocity, V (m s^{-1}), and dispersion, D (m^2 s^{-1}). The rate of exchange of water between the mobile zone and immobile zone (i.e. dead zones and subsurface storage) is described by λ (m^2), and the residence time therein is defined by the power law residence time distribution of solute within the immobile zone, set by the power law slope, β (Drummond et al., 2019). Parameter space was sampled using the Latin Hypercube approach (N = 27,000) using the balanced mean square error equation (reported in Drummond et al., 2018) as the objective function, which was found to provide optimal parameter identifiability. Parameter sensitivity was visualised using dotty plots. Modelled BTCs were truncated to a truncation time, t_T, of 20 (where t_T = (t_{MAX})/t_C; where t_{MAX} = the time from the tracer injection to the final sampling time, and t_C = time of maximum tracer concentration) because this provides sufficient characterization of transport and storage processes (Drummond et al., 2012). BTCs from the first measurement location (at the start of the control sub-reach) were modelled using the slug injection (time = 60 seconds) as the input boundary condition. Subsequent BTCs (i.e. those at the end of the control and impact sub-reaches) were modelled using the BTC of the location immediately upstream as the boundary input condition, as in Blaen et al. (2018). Time series analysis of BTCs was conducted following Ward et al. (2013). Transient storage index (TSI) was calculated as t_T20 - t_{MAX}. It gives an indication of the tracer advective time but because t_T20 was calculated as a function of $t_{MAX,}$ in this instance TSI does not truly represent tailing behaviour as it has elsewhere (Ward et al., 2016). Skewness describes the asymmetry of the

BTC and gives an indication of tailing behaviour relative to the rising limb of the BTC (i.e. the higher the skewness, the more transient storage influenced solute transport) (Nikolakopoulou et al., 2018). The coefficient of variation (*CV*) provides a normalized measure of temporal variance and allows comparison across different advective times (e.g. in different reaches or during different flow conditions) (Nikolakopoulou et al., 2018). Holdback describes the delay in the transport of solutes moving through the system (i.e. deviation from plug flow), where values closer to 1 indicate more holdback (Danckwerts, 1953).

4.3.7 Statistical analysis

The aim of the statistical analysis was to characterise the effect of restoration on the transport and storage metrics described above by comparing period (before-after), reach (control-impact) and the interaction between both (period*reach), i.e. the BACI effect. Further, the effect of experimental design (i.e. BA only, CI only, and BACI) was investigated.

Whilst BACI experimental designs are one of the best ways of quantifying an effect of an environmental change, the outcomes of these studies are particularly sensitive to the applied statistical approaches (Stewart-Oaten and Bence, 2001; Smokorowski and Randall, 2017). Therefore, two statistical approaches were employed which balanced the potential risk of type one error (i.e. spurious rejection of a true null hypothesis) and type two error (i.e. failure to reject a false null hypothesis). Residual maximum likelihood (REML) was used to ensure that statistical bias was not introduced by the unbalanced (non-orthogonal) monitoring periods before and after restoration (Robinson, 1987). The lme4 R package was used to fit the REML model (Bates et al., 2015). The date of tracer injection was included as a random effect which accounts for repeated measures of the same reaches and for the unbalanced

experimental design, whilst allowing a non-singular fit (Barr et al., 2013). Period, reach, and their interaction (period*reach) were included as fixed effects. This approach is appropriate for the experimental design but it may still introduce type one error (but less so than other tests) which results from unspecified random effects (Matuschek et al., 2017). A two-way analysis of variance (ANOVA) was performed (with factors: period, reach, and period*reach), which is one of the most commonly applied approaches to analyse BACI experiments (Smokorowski and Randall, 2017). This approach does not consider the unbalanced monitoring periods (BA) making it less statistically powerful and more sensitive to violate assumptions (Hector et al., 2010). Tukey post-hoc tests (TPH) were applied to ANOVAs to allow pairwise comparison. A two-way analysis of covariance (ANCOVA) (with factors: period, reach, and period*reach, and co-variate: *Q*) was performed to investigate and control for the effect of *Q*. Students T-tests were performed to investigate the effect of restoration in a BA or CI only design.

A level of significance (alpha) of 0.05 was used for all tests but before significance was assessed, P-values of the REML, ANOVA and ANCOVA were corrected using the estimated marginal means method (emmeans R package) which can further account for the unbalanced experimental design (Lenth, 2022). Normality was tested with Shapiro-Wilk normality tests and QQ-plots. Normality in *CV* was achieved by log10 transformation. Only λ could not by transformed to be normally distributed, therefore non-parametric tests were performed: Kruskal Wallis (KW) instead of REML/ANOVA and Mann Whitney U instead of a Student's T-test. All statistical analysis was performed in R version 4.1.0 (R CoreTeam, 2021).

4.4 Results

4.4.1 Breakthrough curve analysis and Transient Storage Model fitting

The continuous time random walk mobile-immobile model well characterized the solute BTCs at every measurement location and provided good parameter identifiability. The goodness of fit was demonstrated in loglog and timeseries plots (an example in Fig. 4-4.), and the parameter identifiability was demonstrated in dotty plots which show values that met a behavioural error threshold (top 1% and top 0.05%) were restricted to a narrow range, indicating that few parameter sets were able to produce a BTC that provided a good fit to observed data (Bottacin-Busolin et al., 2011; Drummond et al., 2019). An example of these plots is presented in figure 8-11 and the parameter best fits and standard deviations are reported in Table 8-15.

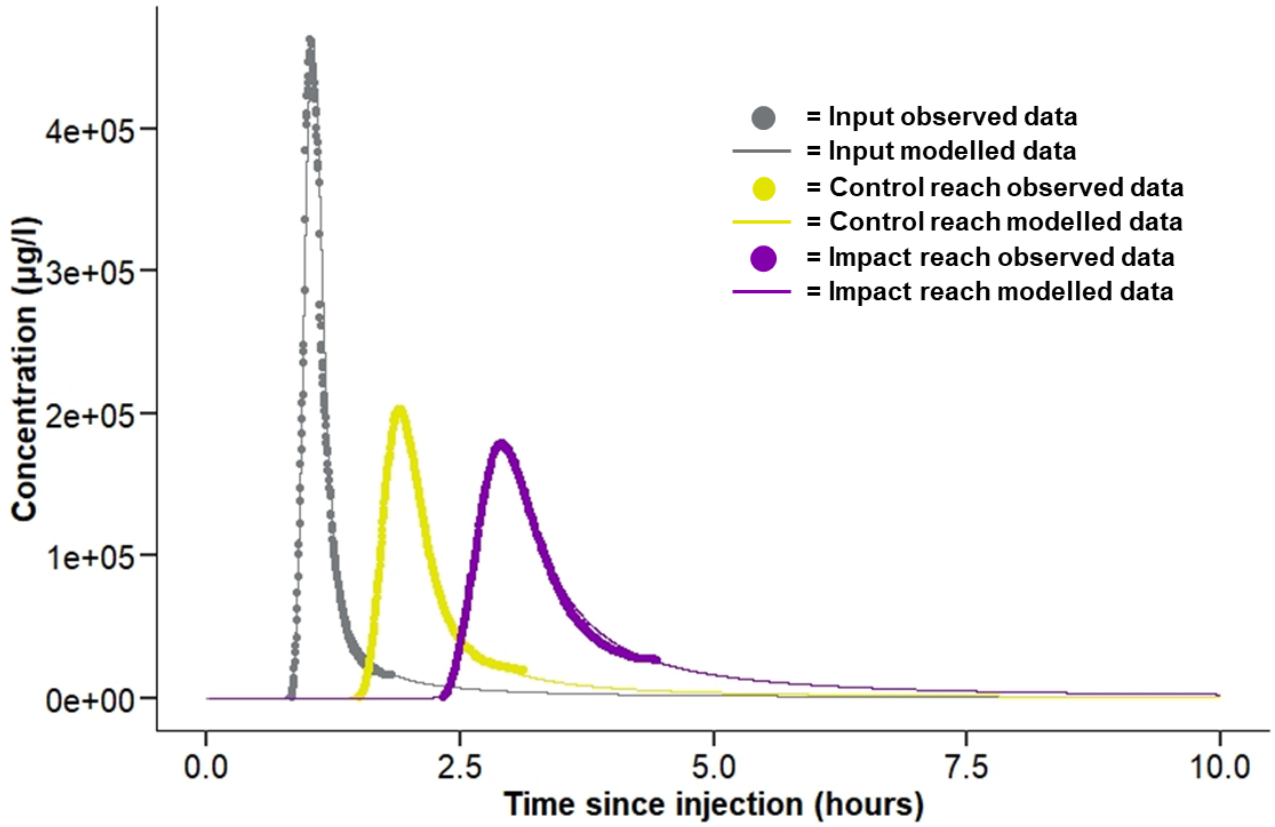


Figure 4-4. An example (from the tracer injection on 09-06-21) of observed BTC data and the continuous time random walk mobile-immobile model fits at each of the measurement locations, that is at the start of the control reach (after complete mixing of the tracer), at the end of the control reach and start of the impact reach, and at the end of the impact reach. The modelled BTC time is truncated in the plot but actually extended to t_T20.

4.4.2 BACI effect on transport and storage metrics

The effect of restoration is difficult to evidence, especially over short-time periods and with few sampling or measurement periods that result in small statistical sampling sizes. No statistically significant BACI effect was observed for any of the transport or storage metrics. Noteworthy trends were observed, however, and we used linear regression analysis to interpret the direction (i.e. positive, negative or neutral) of, and changes to, these trends throughout the experimental period. Regression equations and coefficients of determination are reported in table 8-17. *P* values (including Pr(>F) values) are reported in the text and the statistical test applied is identified (using abbreviations outlined in section 4.3.6). Applying

different statistical tests (i.e. REML, ANOVA or ANCOVA) did not lead to differences in the interpretation of results and for most metrics the REML provided the best fit, as expected. Model fits were assessed with akaike information criterion (AIC), which are presented in table 8-16. Only the results of the REML are reported in text except where extra information was provided by the ANOVA and TPH, for example in the pairwise comparison of the reach*period interaction. A comprehensive overview of model outputs is presented in tables 8-18 and 8-19.

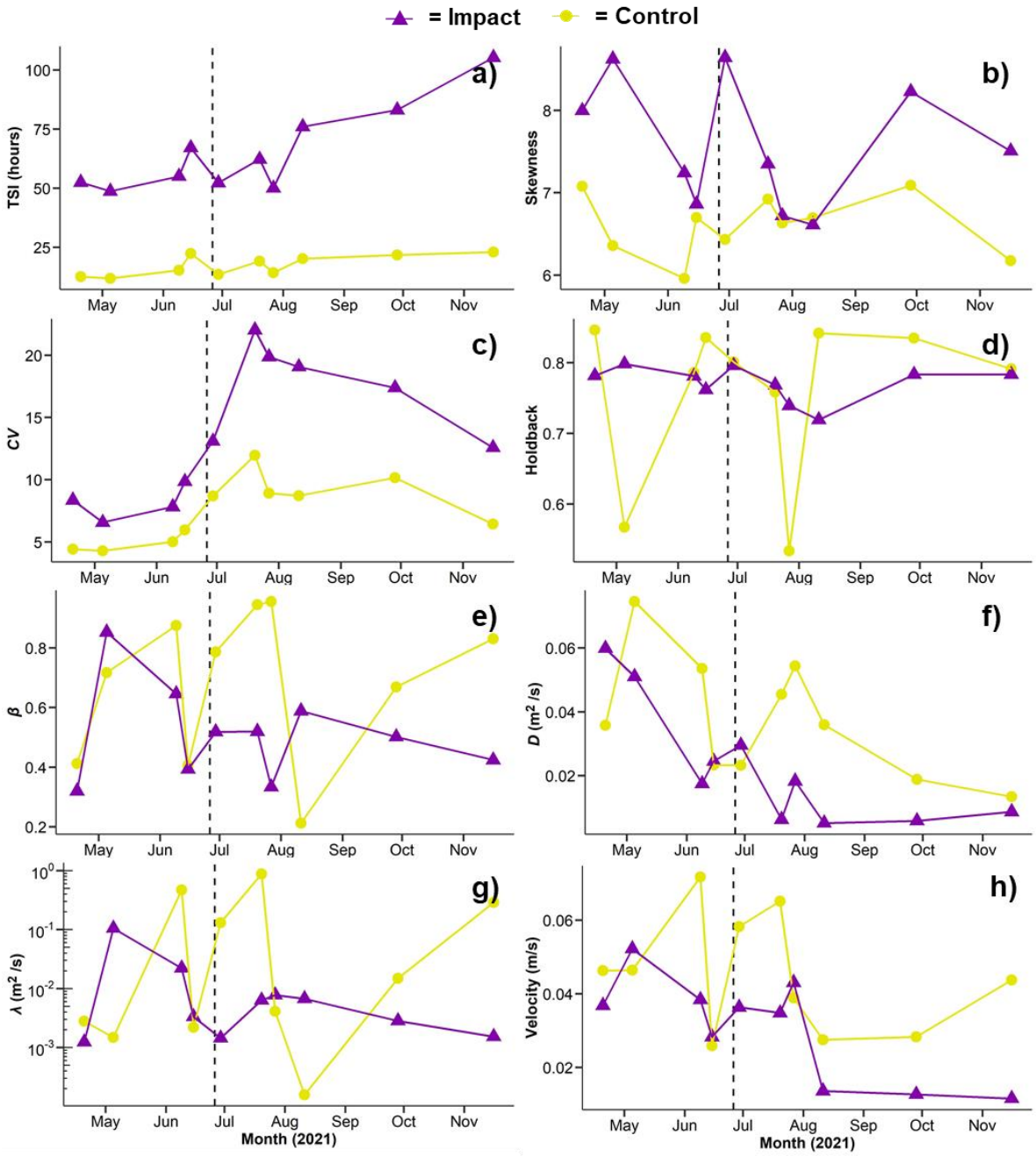


Figure 4-5. Timeseries and transient storage metrics plotted against the date of injection. The vertical dashed line represents the date of restoration in the impact reach. Transient storage metrics are the best-fit objective functions from the transient storage model and time series metrics were calculated using modelled BTCs: (a) transient storage index (TSI), (b) skewness, (c) coefficient of variation (CV), (d) holdback, (e) residence time in immobile zones (β), (f) dispersion (D), (g) the rate of exchange of solutes between the mobile zone and immobile zone (λ) (note log10 scale), and (h) velocity.

TSI differed significantly between reaches (REML, $P<0.05$) both before and after restoration (TPH, $P<0.05$). Before restoration TSI was consistent in both reaches (Fig. 4-5.a) but around

6 weeks after restoration, an increase in TSI is observed in the *impact reach* but not in the *control reach*, which continues until the end of the experimental period - the difference is not significant (TPH, P>0.05) but it is obvious in Fig. 4-6 a. The variance in TSI values also increases in the *impact reach* compared to in the *control*, but this is mostly due one high value observed in November 2021. There is no obvious trend in skewness in either reach (Fig. 4-5 b) but the variance is much higher in the *impact reach* (Fig. 4-6 b). *CV* was significantly higher in the *impact reach* compared to the *control* (REML, P<0.05) and after restoration compared to before in both reaches (REML, P<0.05) (Fig. 4-5 c). The percentage increase from the minimum *CV* was higher in the *impact reach* (235%) compared with the *control* (179%), and remained higher than before restoration (Fig. 6 c). There are no obvious trends in holdback or differences between reaches or periods (Fig. 4-5 d).

More obvious changes were observed between reaches and periods in the transient storage parameters. Similar patterns are observed for β and λ. *B* and λ are similar in magnitude in both reaches at every injection period before restoration (Fig. 4-5.e and g). In the injections immediately after restoration, however, *B* and λ exhibited a substantial decrease in the *impact reach* compared to the *control* which overall persisted until the end of the experimental period – the decreases were not significant (*B*, TPH, P>0.05; λ, KW, P>0.05) but are evident in Fig. 4-6.e and g. *D* was statistically different before and after restoration across both reaches (REML, P<0.05). Before restoration, it generally decreased in both reaches similarly but immediately after restoration it increased in the *control reach* but decreased in the *impact reach*, before becoming more similar again. Fig. 4-6 (f) shows that *D* decreased more in the *impact reach* compared to the control but the difference was not significant (TPH, P>0.05). *V* was significantly higher in the *control reach* than the impact (REML, P<0.05) but not between the periods. After restoration, *V* decreased more in the *impact reach* than the control (Fig. 4-6 h), especially > 6 weeks after restoration.

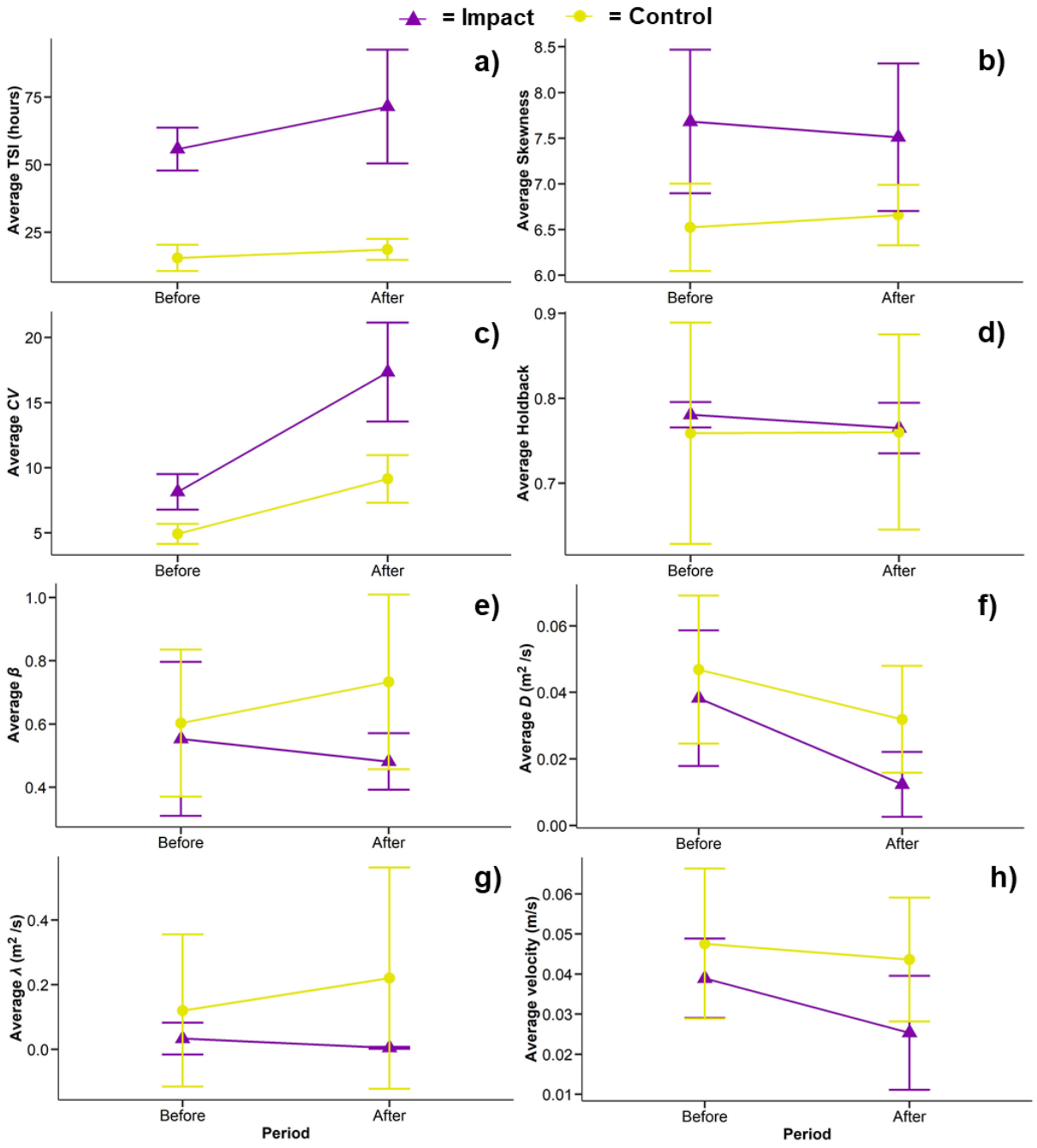


Figure 4-6. Average timeseries metrics and transient storage parameters are plotted separately for the impact (purple) and control (yellow) reach for the before and after periods. Transient storage metrics are the best-fit objective functions from the transient storage model and time series metrics were calculated using modelled BTCs: (a) transient storage index (TSI), (b) skewness, (c) coefficient of variation (CV), (d) holdback, (e) residence time in immobile zones (β), (f) dispersion (D), (g) the rate of exchange of solutes with the mobile zone and immobile zone (λ), and (h) velocity. Error bars represent mean ± standard deviation.

4.4.3 Relationship between discharge and transport and storage metrics

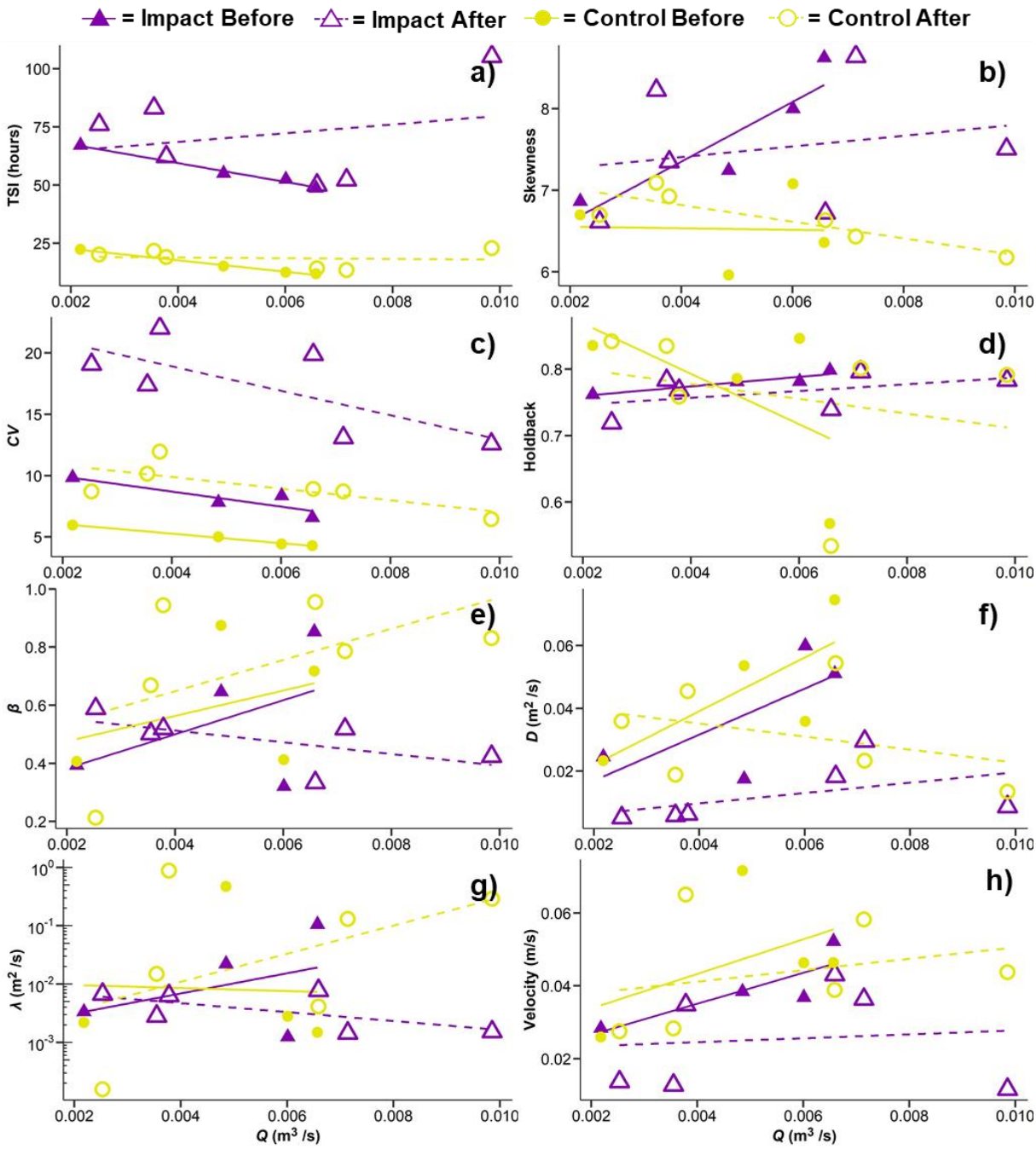


Figure 4-7. Timeseries and transient storage metrics regressed against Q separately for before and after restoration and impact and control reaches. Regression equations and coefficients of determination are reported in table S4. Transient storage metrics are the best-fit objective functions from the transient storage model and time series metrics were calculated using modelled BTCs: (a) transient storage index (TSI), (b) skewness, (c) coefficient of variation (CV), (d) holdback, (e) residence time in immobile zones (β), (f) dispersion (D), (g) the rate of exchange of solutes with the mobile zone and immobile zone (λ) (note log10 scale), and (h) velocity.

TSI was not significantly affected by *Q* (ANCOVA, P>0.05), nor did accounting for it change the effect of reach or period. Before restoration, the relationship between TSI and *Q* was well described ($r^2 = 0.987$) in both reaches by mildly negative linear regressions of similar gradients (Fig. 4-7 a). This relationship was maintained after restoration except for in the *impact reach* for the injection at the highest *Q* (0.00985 m^3/s) which exhibited a much higher TSI of >100 hours (>20% higher than the next highest TSI). *Q* did not significantly affect skewness (ANCOVA, P>0.05) and in general the relationship with *Q* was weak (Fig. 4-7 b). *CV* was significantly affected by *Q* (ANCOVA, P<0.05). Similar, mildly negative, linear regressions well described ($r^2 = 0.78$) this relationship in both reaches before restoration, and the relationship was mostly unchanged in the *control reach* after restoration (Fig. 4-7 c). However, a step change (from intercept 11 to 23) in the *impact reach* was observed after restoration. The relationship between *Q* and holdback was not significant (ANCOVA, P>0.05). It is different in the control and *impact reach* but in both reaches remains mostly unchanged after restoration.

Although the positive relationship between β and *Q* in both reaches before restoration continues in the *control reach*, in the *impact reach* the relationship switches from positive to negative after restoration – the relationship is quite weak ($r^2 = 0.392$), however (Fig. 4-7 e). The effect of *Q* on β is insignificant (ANOCOVA, P>0.05). A very similar pattern is observed in the λ (Fig. 4-7 g). In the *control reach*, the relationship between λ and *Q* is more strongly positive after restoration but poorly described by the linear regression ($r^2 = 0.00115$). Changes to the relationship with *Q* after restoration in both β and λ result in very different dominant transport and storage processes during high *Q*. A similar relationship between *Q* and *D* is observed in the *control* and *impact reach* before restoration (Fig. 4-7 f). After restoration, there is a marked decrease in the intercept of this relationship (from 7.3 to 1.6) in the *impact reach*, though the gradient remains similar. Conversely, in the *control reach*,

whilst the intercept is similar before and after restoration, the direction of the relationship switches from positive to negative, though in both cases the relationship is weak (r^2 = 0.0032 and 0.0049, respectively). *V* in the *impact reach* was positively related to *Q* before restoration (r^2 = 0.708), but after restoration the relationship changed. A linear regression did not well describe the relationship with *Q* (r^2 = 0.011). Instead, *V* was low at low and high *Q*, but similar to before restoration at medium *Q*.

4.4.4 Comparing experimental designs

In 6 out of 8 transport and storage metrics (TSI, skewness, *CV*, holdback, *D*, and *V*) different results were reported in at least 1 statistical test when adopting either a BA or CI approach compared to a BACI approach (Table 4-2). The CI approach was most likely to result in a statistically significant effect of restoration, resulting in 5 significant results that were not observed in the BACI approach. There was no difference in reported significance between REMLs and ANOVAs, but different results were reported by the T-test for skewness, holdback and *D*.

*Table 4-2. The significance of different statistical tests is reported for different experimental approaches: BA = before-after (period), CI = control-impact (reach), BACI = before-after-control-impact (period*reach). Yes indicates significant (alpha = 0.05) and No indicates not significant. \ indicates test not conducted. Normality was not achieved for λ, therefore a REML or ANOVA was not performed but instead a Kruskal Wallis analysis. ANOVA = analysis of variance. TSI = transient storage index, CV = coefficient of variation, β = residence time in immobile zones, and λ = the rate of exchange of solutes with the mobile zone and immobile zone.*

Metric	Test	BA	CI	BACI
TSI	REML	No	Yes	No
	ANOVA	No	Yes	No
	T-test	No	Yes	\
Skewness	REML	No	Yes	No
	ANOVA	No	Yes	No
	T-test	No	No	\
CV	REML	Yes	Yes	No
	ANOVA	Yes	Yes	No
	T-test	Yes	Yes	\
Holdback	REML	No	Yes	No
	ANOVA	No	Yes	No
	T-test	No	No	\
β	REML	No	No	No
	ANOVA	No	No	No
	T-test	No	No	\
D	REML	Yes	No	No
	ANOVA	Yes	No	No
	T-test	No	Yes	\
V	REML	No	Yes	No
	ANOVA	No	Yes	No
	T-test	No	No	\
λ	REML	\	\	\
	ANOVA	\	\	\
	Kruskal Wallis	No	No	No
	Mann Whitney U	No	No	\

4.5 Discussion

The effect of restoring instream wood was not statistically significant in any of the transport and storage metrics we investigated. It is not possible to conclusively determine whether this is because the effect of instream wood restoration on transport and storage is limited, or because the experimental approach was not sensitive enough to detect a real effect. In this discussion we outline the potential likelihood and causes of each possibility, discuss what we can learn by means other than statistical significance, and identify the influence of the experimental approach on the outcomes of river restoration evaluations.

4.5.1 Challenges in quantifying restoration impacts

Quantifying environmental impact following a restoration intervention is essential to evidence its efficiency and success, but it also represents a challenge (Christie et al., 2019). It may be especially challenging to quantify impacts on the hyporheic zone because its properties are particularly variable in space and time. The temporal dynamics of hyporheic exchange change on various temporal scales (from seconds to years), and are probably non-stationary due to anthropogenic and environmental pressures (Milly et al., 2008). The properties of hyporheic exchange also exhibit significant spatial heterogeneity, driven by streambed structural heterogeneity and variability in other biotic and abiotic properties that we are not yet able to characterise or conceptualise (Krause et al., 2022). Furthermore, the drivers and controls of hyporheic exchange exhibit complex interactions that are not yet fully understood, for example how stream discharge and sediment hydraulic conductivity interact to determine the properties of hyporheic exchange. We are also yet to understand the bidirectional feedbacks in these interactions, for example how stream discharge dynamics change the stream hydraulic conductivity over time, which is often considered a fixed property (Rosenberry, Engesgaard and Hatch, 2021).

The challenge of sufficiently capturing and accounting for this spatial and temporal variability is compounded by the practical constraints of research projects, such as financial and time constraints, access to field sites, and the short duration for which projects often run. Practical constraints usually result in sub-optimal experimental designs that further limit the capacity to rigorously and conclusively detect effect or non-effect of a restoration intervention. For example, the experimental design adopted in this study was limited by small effective sample size (and degrees of freedom) (n=10), the unbalanced groups (n=4 before, n=6 after), and the short duration of the experimental period (< 1 year); limitations that are common amongst most environmental impact assessments (Smokorowski and Randall, 2017). The effect of instream wood restoration on transient storage may have been more substantial during conditions not captured here (as in Shelley et al., 2017), or given a longer duration to develop. For example, the porosity of features could have decreased as a result of the capture of sediment and organic matter, and geomorphic features could have developed around features, such as pools upstream and downstream (Fanelli and Lautz, 2008). Though more comprehensive than the majority of similar studies, this experimental design was unlikely to be able to statistically detect the effect of restoration (Smokorowski and Randall, 2017).

However, significance testing is not the only valid method for separating signal (in this case the effect of restoration) from noise, and in fact in some cases it may be inappropriate (Wasserstein, Schirm and Lazar, 2019). There is widespread agreement amongst statisticians that one should not conclude that an effect does not exist only because it was not statistically significant, but instead results should be contextualised in the range of existing knowledge, and in doing so the level of effect that is scientifically or practically meaningful can be determined (Pogrow, 2019; Ziliak, 2019). Following this philosophy, a qualitative assessment of trends was able to reveal useful information about the effect of wood restoration on

transport and storage. Confidence in these trends is low, however, and therefore implications must be applied with caution. Rather than offering predictive insight, these results may guide others in formulating hypotheses which should be more rigorously investigated.

4.5.2 The effect of instream wood restoration on transport and storage

A commonly observed effect of instream wood is to create an obstruction in the channel that causes the impounding of water upstream and induces a head gradient (Shalko and Weitbrecht, 2022). Such response was evident in observed changes to several of the transport metrics in the *impact reach* after restoration, for example reach scale *V* was decreased which was associated with an increase in TSI. This could also explain the changes to *CV* and *D*, which were expected to respond similarly to restoration because they are both measures of the dispersion of tracer from the mean arrival time. However, they responded oppositely: *CV* increased and *D* decreased. The increase in *CV* may be explained by an increase overall dispersal of the tracer as a result of the increased TSI, and decreased *D* may be explained by the reduction in downstream *D* of tracer due to delayed transport. The responses of some metrics do not support the existence of this effect, however. For example, no change was observed to holdback or skewness. An increase in the area of the transient storage zone could explain the TSI, *CV* and *D* results, as has been observed elsewhere (e.g. Rana, Scott and Hester, 2017). However, the area of the transient storage zone was not tested for in this study. Overall, changes to these metrics represent an increase in hydraulic retention time and short-term retention of solutes in the reach (Azinheira et al., 2014). This change itself could provide useful ecosystem services, such as buffering hydrological extremes (e.g. by ‘slowing the flow’) (Bruno et al., 2020) and offering opportunities for improvements to water quality, for example by increasing sunlight irradiation which could increase primary production and facilitate photodegradation of pollutants like plastics (Qian et al., 2021).

Contrary to expectations, λ and β actually decreased after restoration in the *impact reach* compared to the *control reach*. There is poor confidence in this result, however, and the standard deviations of values in both reaches overlap in both periods. A decrease in λ following HZ restoration has been previously observed, for example following fine sediment removal (Ward et al., 2018) and wood introduction (Rana, Scott and Hester, 2017). Rana, Scott and Hester (2017) attributed this decrease at least in part to reduced hydrodynamically driven hyporheic exchange induced by the backwater behind weirs, which decreased *V*. The backwater is also associated with an increase in water level which reduces the relative size of instream wood (blockage ratio) and its effectiveness at inducing scouring, deposition and hydrodynamic forces (Gippel, 1995). Furthermore, reduced *V* can lead to sediment deposition which reduces the sediment hydraulic conductivity (e.g. by colmation), another key control of hyporheic exchange (Kasahara and Hill, 2008), and buries bed forms (Rana, Scott and Hester, 2017). Additionally, the high porosity of the features used here could reduce their capacity to impound water upstream and to create a hydraulic step. *V* here refers to reach-scale velocity which does not necessarily reflect localised velocity, which has been shown to be especially heterogenous around instream wood (Gippel, 1995).

In order for restoration to induce a net increase in λ and β, the reduction in hydrodynamic forces must be superseded by an increase in hydrostatic forces. In general, this may be more easily achieved in upland environmental settings where hydrodynamic forces are maintained by the high stream energy and the steep gradient allows the induction of a large hydraulic step at the weir (Hester, Brooks and Scott, 2018). Conversely, in lowland settings hydrostatic forces may be driven primarily by spatial heterogenous groundwater upwelling, and the opportunity to induce a hydraulic step is limited by the shallow gradient (Krause et al., 2014). Beaver dams may represent an exception to this rule. Whilst they are typically constructed in mid-high order streams and rivers (i.e. in lowland environments), they are able to induce

large volumes of transient storage and hyporheic exchange because they in general generate landscape-level changes, for example reconnection of the channel to the floodplain (Larsen, Larsen and Lane, 2021). This is often an effective strategy and as such artificial replication in beaver dam analogs (BDAs) is become a common restoration technique, though it is not always possible, for example where proximate infrastructure precludes.

4.5.3 The effect of discharge on the interaction between instream wood and transient storage

Q is an important control of transient storage but the nature of its influence is still poorly understood (Ward et al., 2013, 2018). *Q* was expected to control the effect of instream wood on transient storage because it effectively defines the relative characteristics of wood in the channel and therefore, the way in which wood interacts with the flow (Krause et al., 2014). It was hypothesised that instream wood would exhibit threshold behaviours in response to certain *Q* conditions changing the function of a feature, for example when a feature is first overtopped (blockage ratio ≥ 1). The range of *Q* captured in this study was not sufficient to offer insight into this theory, but it could present an interesting route of inquiry in the future. It was also not possible to entirely isolate the effect of *Q* from the effect of time. The effect of wood on transport and storage may change over time as a results of changing background conditions (e.g. groundwater table) and development of features (e.g. a reduction in effective porosity as sediment and organic matter is captured) and local geomorphic setting, which can have a more significant effect on hyporheic exchange than the feature itself and can take >10 years to equilibrate (Fanelli and Lautz, 2008; Wondzell et al., 2009). The duration and timing of the experimental period, and the unbalanced design, resulted in the capture of different *Q* before and after restoration, making it difficult to directly compare the response to *Q* events.

Furthermore, the highest *Q* captured (0.00985 m^3/s) in the study was <10% of the maximum *Q* observed during the experimental period so the observed relationships between *Q* and transport and storage metrics are only representative for the lowest 10% of flow conditions. This does not necessarily exclude the periods of highest hyporheic exchange which may exist during summer baseflow conditions (Azinheira et al., 2014).

Transport and storage metrics seemed to exhibit stronger relationships with either time or *Q*. *V* and TSI decreased and increased respectively after restoration, despite very different *Q* in this period. On the contrary, *CV* and *D* showed step changes in their relationship with *Q* after restoration in the *impact reach*. Whilst the gradient of the trend remained similar to before restoration, the intercept was higher for *CV* and lower for *D*. λ and β increased with *Q* in the *control reach* but decreased with *Q* in the *impact reach* following restoration, the opposite of what was observed by Shelley et al. (2017) and Ward et al. (2018). This may be associated with the relatively higher decrease in *V* at higher *Q*. λ and β were more dissimilar at higher *Q*, suggesting that the impact of wood on transient storage is greater during high *Q*.

4.5.4 The experimental approach is important

BACI experimental designs for environmental impact assessment are widely adopted in some areas of aquatic science, such as riverine and limnological ecology (Smokorowski and Randall, 2017; Seger et al., 2019), but despite previous calls for their adoption to river hydrology there is still poor uptake (Ward et al., 2018). This study reveals the consequences of the failure to adopt the BACI approach, as BA and CI approaches detected effects that were not detected by the BACI approach - the opposite of what Smokorowski and Randall (2017) found in a similar study, though in both studies different experimental approaches led to different conclusions. The failures of BA and CI designs are well recognised, and we posit

that these designs are especially vulnerable to reporting false effects in river hydrology due to the frequent violation of their assumptions. BA designs assume do not account for variability of confounding factors between measurement periods but this is almost never achieved over short-time periods (i.e. intra-annually) as in this study, making it impossible to isolate the effect of restoration from fluctuating background conditions. CI designs assume similarity between paired reaches but rivers are heterogeneous in environmental setting and process dynamics. Properties of the HZ are particularly localised, and large differences may be observed even in small spatial scales. Potential solutions to these challenges are discussed in section 5.3.

4.6 Conclusions

4.6.1 Does restoring instream wood increase transient storage?

With low confidence, the results reported here suggest that restoring instream wood in a lowland sandy stream reduces transient storage during baseflow conditions. This is contrary to what has been observed in upland settings and so highlights the importance of conducting research in the environmental setting in which it is likely to be applied.

Combining insight from the results reported in this study with that which exists in the literature, we hypothesise that the environmental setting defines the relationship between instream wood and *Q*. In lowland settings, installing channel-spanning wood features is likely to decrease transient storage in the short-term, due to the decrease in reach-scale *V* and the reduction of hydrodynamic forcing. On the contrary, in upland settings it is likely to increase transient storgae due to the induction of hydraulics steps and hydrostatic forcing. The magnitude of effect in both scenarios is positively correlated with increased *Q*, where larger *Q* leads to more transient storage in upland sites with instream wood and less transient

storage in lowland sites with instream wood. This understanding may enable improved designs of instream wood features that target/avoid process controls. For example, channel-spanning features of low porosity may be optimal in upland settings (to induce vertical head gradients), whilst alternate deflectors of high porosity may be optimal in lowland settings (to increase localised, feature scale velocity).

4.6.2 Limitations of quantifying restoration impacts on transient storage at the field scale

Understanding the impact of restoration on transient storage, and in particular hyporheic exchange, is essential in predicting its effectiveness for a number of objectives and in improving restoration designs. However, the methods currently widely available to measure transient storage limit our capacity to understand it. Most methods provide data that is limited in space or time, with few exceptions (e.g. distributed temperature sensing) which are usually prohibitively expensive (Briggs et al., 2012). Manual tracer methods result in information-rich data (BTCs) but require significant time input, limiting the number of injections that can be conducted and ultimately resulting in small sample sizes. Automating BTC acquisitions could enable a higher temporal frequency of data to be collected. Furthermore, tracer injections could target specific conditions of interest (such as high Q) as has been applied to other semi-automatic data acquisition in river science. The development of new methodologies to collect semi-continuous data about transient storage is necessary to improve the understanding of dynamic HZs.

4.6.3 Recommendations for effective experimental designs to evaluate river restoration

Though BACI designs are preferable over BA and CI designs, careful consideration is still necessary to ensure experimental power (statistical or otherwise). BACI experiments should

include monitoring durations at least sufficient to capture intra-annual variation (i.e. seasons) in both experimental periods (i.e. before and after), which is to say a total duration of at least 2 years in temperate climates. Furthermore, several control and impact reaches are preferable to capture spatial variability. Under most circumstances this ideal design will not be realistic.

Both CI and BA approaches can be well designed to maximise experimental power, by using balanced designs, considering and measuring confounding factors, and maximising monitoring frequency and duration. Amalgamating the results of many BA and CI experiments, considering the results of individual projects as one group in a larger dataset, could enable the capture of temporal and spatial variability and provide sufficient sample sizes to enable meaningful statistical analysis. This would require standardization of site characterization, experimental design and data collection, as well as thorough data quality auditing and management of long-term data depositories. Boundary organisations (such as the Small Woods Association and the River Restoration Centre in the UK) could help by connecting researchers and practitioners, who are typically possessing complementary skills and resources (Palmer, 2009). For example, practitioners have the expertise, funding and sites to conduct restoration and researchers have the expertise, equipment and time to conduct assessment. Researchers, managers and practitioners must work together to leverage their time and resources to deliver insight of practical benefit, if not statistical significance. River restoration has the potential to deliver crucial nature-based solutions to some of society's most pressing challenges, but this cannot be achieved if we do not continue to critically appraise our efforts.

5 Restoring the liver of the river: actionable research insights to guide the restoration of the hyporheic zone for the improvement of water quality

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5.1 Abstract

Research commonly seeks to inform action but itself, it is not action. In order for research to have real-world impact it must be applied by the professionals and practitioners who make action happen. It is vital, therefore, that research insights are communicated in a manner which is actionable, which is to say can be used directly to inform decisions. Hyporheic zone restoration has been the focus of research for decades, yet it is seldom applied in river restoration design and practice. This may, in part, explain the poor efficacy of river restoration schemes aiming to deliver improvements to water quality. Restoration of the hyporheic zone can offer a nature-based solution to the pervasive and persistent water quality challenges that threaten water security. Here, for the first time, insights from hyporheic zone research are distilled and synthesised, and presented in a framework which is suitable to directly inform restoration design. Important considerations for hyporheic zone restoration are discussed, including elements of the environmental setting and the design of restoration techniques. Insights are contextualised in a series of theoretical scenarios which demonstrate how the hyporheic zone may be restored in a range of environmental settings and by abating a range of pressures. This evidence-based guidance will enable professionals to apply a process-based approach to river restoration for the improvement of water quality.

5.2 Introduction

Society faces significant challenges in sustainably managing water resources to meet increasing demand amidst disrupted supply, leading to increasing water scarcity. The potential of rivers to provide nature-based solutions to some of these challenges is increasingly recognised and, as such, the interest and investment in the restoration of degraded river systems has become substantial (UNESCO, 2018; Logar, Brouwer and Paillex, 2019). Poor water quality is a particularly prevalent and persistent driver of water scarcity, reducing ecosystem health, threatening food production and thwarting human potential (Damania et al., 2019). However, poor water quality in river networks may be particularly amenable to nature-based solutions because, when in good condition, streams and rivers have a significant capacity to retain and process many of the pollutants they receive, for example nutrients, heavy metals, pathogens and emerging contaminants (Tesoriero et al., 2013; Herzog, Higgins and McCray, 2016; UNESCO, 2018). Therefore, the improvement of water quality is one of the most commonly cited objectives of river restoration (Wohl, Lane and Wilcox, 2015). Despite this focus, there is little evidence to support the efficacy of restoration to deliver improvements to water quality, and it remains a pervasive problem (Palmer et al., 2009; Hannah et al., 2022).

The efficacy of restoration schemes is limited by the failure to apply process understanding to their design and implementation (Palmer, 2009; Palmer, Filoso and Fanelli, 2014). Whilst process-based restoration has been adopted to deliver some river restoration objectives, like natural flood management (NFM) and habitat improvement, it is not yet thoroughly applied to realise improvements to water quality (Beechie et al., 2010; Wohl, Lane and Wilcox, 2015). For example, research is unequivocal about the importance of the hyporheic zone for ecosystem function but it is seldom considered in river restoration design (Lewandowski et al., 2019; Hester and Gooseff, 2010; Moren, Worman and Riml, 2017). The hyporheic zone is

particularly important for regulating water quality because it hosts hotspots of the biogeochemical reactions that can remove many pollutants (Boano et al., 2014). It can remediate a range of pollutants including nutrients, heavy metals, and ermerging contaminants, originating from various sources, including point, diffuse and groundwater sources (Krause et al., 2011).

The poor uptake of hyporheic research in river restoration practice may be in part due to the failure of researchers to synthesise research outcomes and frame them saliently to contemporary agendas and within the frameworks and constraints in which practitioners must work. There have been some attempts to summarise hyporheic research (e.g. UK Environment Agency, 2009; Lewandowski et al., 2019; Krause et al., 2022) but in general research outcomes have not been communicated in a manner that is actionable, which is to say offers clear insight that may be used to directly inform decisions (Rose et al., 2020). To communicate insight in a way which is accessible and actionable, it is necessary to summarise, generalise and contextualise evidence, and present it in practical guidelines (Grabowski et al., 2019). For example, insight should be presented in the context of the environmental settings in which it is likely to be applied and the nature of degradation which it may be used to restore.

Some uncertainties still exist about exactly how coupled hydrological and biogeochemical processes in the hyporheic zone determine its effect on water quality (Boano et al., 2014; Ward, 2015). Furthermore, the influence of river restoration on the hyporheic zone may be determined more by the environmental setting in which it occurs, rather than the technique or design of the restoration itself (Krause et al., 2014; Hester, Brooks and Scott, 2018). This certainly limits our ability to exactly predict the response of the hyporheic zone to restoration in different settings. However, most river restoration is conducted with similar uncertainty, and therefore practitioners are experts at working with this uncertainty to maximise the

chance of success. It does not eliminate our ability to offer guidelines of practical benefit that, if applied, will lead to better designs than those that are currently implemented.

The aim of this work is to synthesise and communicate insight from research on restoring hyporheic zone functions in an actionable format, with a focus on river corridor restoration strategies that are familiar to river restoration professionals. It does not intend to comprehensively outline every possible influence and control on the function of the hyporheic zone – readers seeking this should refer to the many existing reviews, such as Boano et al. (2014), Harvey and Gooseff (2015) and Krause et al. (2022). Instead, the most essential considerations have been included which should allow readers to formulate hypotheses about how restoration may influence specific properties of the hyporheic zone (outlined in section 2.2), and the likely implications for water quality objectives. In doing so, readers may identify sites with high restoration potential and improve the design of restoration schemes to enhance the function of the hyporheic zone. This work is aimed at river restoration professionals (practitioners, engineers, managers and planners) and is designed to complement tools that are already used in decision making, along with the values, local knowledge and often-significant expertise of the individual (Dicks, Walsh & Sutherland, 2014).

In section 5.3, we briefly explain the function of the hyporheic zone and how it is driven by processes in the stream and in the streambed, as well as how its properties effect the retention and transformation of pollutants. In section 5.4, we outline the characteristics of the environmental setting that should be considered when planning hyporheic restoration. Section 5.5 sets out in more detail how the architecture and arrangement of restoration strategies and designs effect their influence on the hyporheic zone. Section 5.6 provides a series of scenarios, including environmental setting, pressure on the river system and a solution to restore the hyporheic zone. In section 5.7, potential synergies and conflicts between

restoration of the hyporheic zone for improvements to water quality and for other objectives are briefly discussed. Finally, the discussion (section 5.8) and conclusions (section 5.9) position hyporheic restoration within a larger spatial context (i.e. catchment) and within the context of the current frameworks for river restoration, making recommendations on how to improve river restoration in the future.

5.3 Drivers and controls of pollution remediation in the hyporheic zone

5.3.1 What is the hyporheic zone?

Many definitions of the hyporheic zone are adopted by different disciplines and communities (Ward, 2015), but simply put it may be defined as the area surrounding the river channel (in the bed and banks) where surface water and groundwater interact. Hyporheic exchange can be thought of as a 'parcel' of water moving from the channel into the streambed and back into the channel (Zarnetske et al., 2012). This exchange is driven by energy gradients along the riverbed that arise from hydrostatic and hydrodynamic forces (Sawyer, Cardenas and Buttles, 2011). Hydrostatic forces are usually caused by differences in elevation between two points (like from upstream to downstream of a weir) and the level of the overlying surface water – typically represented by water flowing over something like a weir or a step, or through something like a meander (Boano et al., 2014). Hydrodynamic forces are caused by flow velocity and turbulent energy transferring momentum into the river bed – typically represented by water flow around something (or over something submerged) like a boulder or instream wood. Both hydrostatic and hydrodynamic forces usually contribute to driving hyporheic exchange but one or the other may dominate depending on the environmental setting (Krause et al., 2022).

5.3.2 Properties of hyporheic exchange

The nature of hyporheic exchange can vary and this has important implications for the way the hyporheic zone functions and its effect on water quality. The most important properties of hyporheic exchange with regard to its influence on water quality are its volume and residence time in the hyporheic zone (Ward, 2015). The volume of hyporheic exchange is the volume (or proportion) of the surface water in the channel that enters the hyporheic zone and re-enters the channel in a given reach length. It can also be thought of as the length of reach required for 100% of the water in the channel to enter the hyporheic zone and re-enter the channel (Wondzell, 2011). The residence time is the length of time the surface water remains in the hyporheic zone before it returns to the channel (Boano et al.,2014). Every parcel of water might have a different residence time in the hyporheic zone, but here residence time refers to the distribution of residence times of all the parcels of water (i.e. the average residence time) (see Figure 5-1). Other useful variables include the length of the hyporheic flow paths and the vertical and lateral extent of the hyporheic zone (i.e. distance from the main channel), which can give an indication of the exposure to different environments (i.e. biotic assimilation by plant roots in riparian zones) (Krause et al., 2011). In general, hydrostatic forces induce hyporheic exchange of higher residence times and lower volume of hyporheic exchange, and hydrodynamic forces induce hyporheic exchange of lower residence times and higher volume (Boano et al., 2014).

5.3.3 Pollution retention and attenuation in the hyporheic zone

Pollutants can be retained in the river corridor by sedimentation or by transport into the hyporheic zone where they are deposited (Boano et al., 2014). Some pollutants (e.g. synthetic particles like microplastics) will remain until they are remobilized, often by flood events (Lewandowski et al., 2019; Drummond et al., 2022). Some pollutants may be transformed in

the river corridor, and especially in the hyporheic zone which hosts hotspots of reactions because it contains a matrix of different conditions (Krause et al., 2010). It also hosts the diversity and density of microbes, and high content of organic matter, which are often necessary to facilitate biogeochemical reactions (Zarnetske et al., 2012). Different pollutants are transformed under different conditions and the complete removal of some pollutants requires a series of conditions (Quick et al., 2019). The most important distinction is whether reactions happen in the presence of high dissolved oxygen concentration (aerobic) or low dissolved oxygen concentration (anaerobic). Both of these conditions are found in the hyporheic zone (Zarnetske et al., 2012). Aerobic conditions are generally observed in shallow hyporheic exchange (close to the channel) with short residence times. When water is in the hyporheic zone, oxygen is consumed by aerobic respiration and the concentration of dissolved oxygen decreases leading to anaerobic conditions, which are usually observed in deeper hyporheic exchange with longer residence times (Boano et al., 2014). Sometimes pockets of anaerobic conditions can be found in predominantly aerobic zones, for example within biofilms, which can host anaerobic reactions (Krause et al., 2011).

Reactions take time. As well as determining reaction conditions, the residence time also largely determines how much pollutant is removed from a parcel of water in one visit to the hyporheic zone. This creates a ‘Goldilocks dilemma’: residence times that are too short remove only a small proportion of the total pollutant in the water, but residence times that are too long retain water in the hyporheic zone long after all the pollutant is removed (see Figure 5-1) (Herzog, Higgins and McCray, 2016). The most efficient residence time is one where water re-enters the channel from the hyporheic zone as soon as, but not before, all the pollutant is removed.

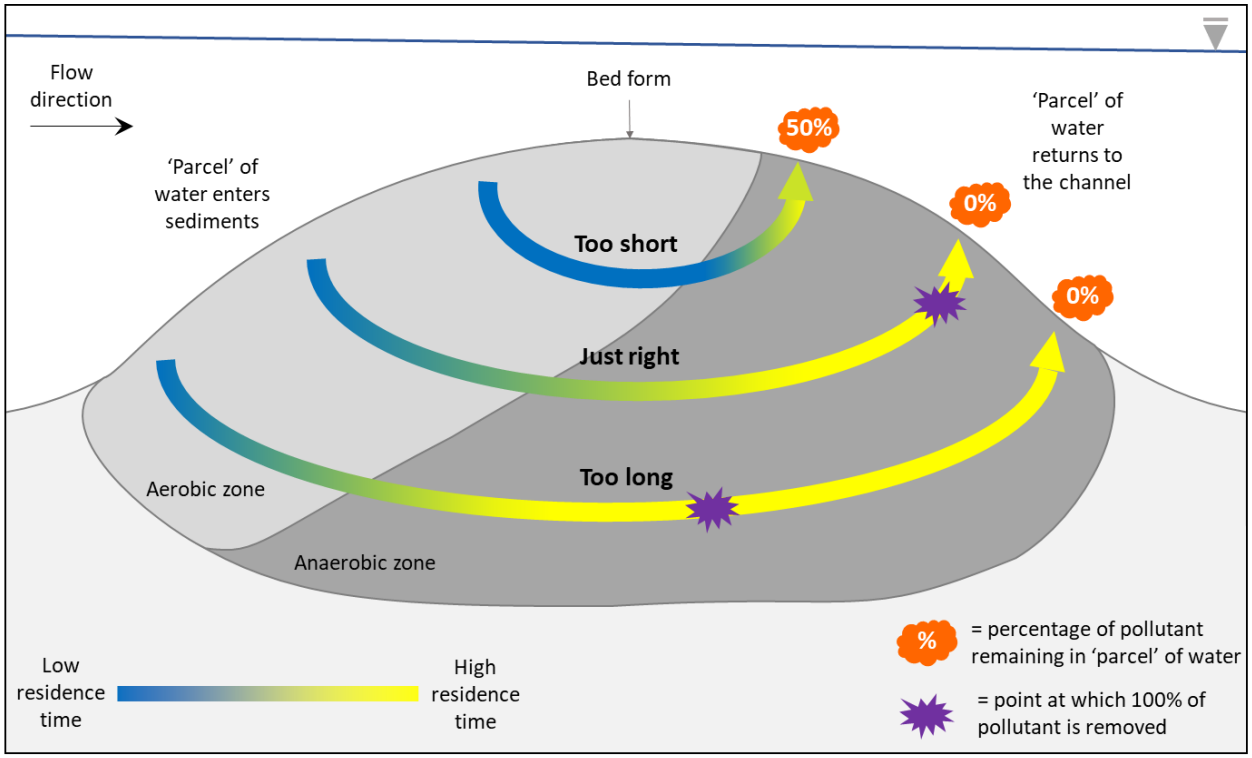


Figure 5-1. Hyporheic exchange through a bedform (e.g. sand dune). Thick arrows represent the general movement of water from upstream of the bedform (left) to downstream (right), and do not necessarily represent the pathway of water through the bedform. The colour of the arrow represents the residence time distribution of water in the hyporheic zone. The 'Too short' arrow represents water that returns to the channel before all the pollutants are transformed. The 'Just right' arrow represents water that returns to the channel just as but not before all the pollutants are transformed. The 'Too long' arrow represents water that returns to the channel a long time after all the pollutants are transformed. The purple star represents the point at which all the pollutant has been removed, which is well before the water returns to the channel for the 'too long' flow path. The percentage in the orange cloud represents the remaining percentage of pollutant when the water is returned to the channel, which is still 50% for the 'too short' flow path.

5.3.4 Designing restoration to target pollution

Restoration may target specific properties of the hyporheic zone in order to induce the necessary reactions that remove a specific pollutant (Ward, Gooseff and Johnson, 2011). For example, where nitrate (NO_3) is identified as a problematic pollutant, restoration may be designed to induce hyporheic exchange of long residence times because the removal of nitrate (usually by denitrification) requires anaerobic conditions (Zarnetske et al., 2012). In systems high in mine-derived pollutants, especially metals such as iron, manganese, copper

and zinc, restoration may be designed to induce a high volume of shallow hyporheic exchange with low residence times, because attenuation is achieved in aerobic conditions (Gandy, Smith and Jarvis, 2007). The removal of many pollutants requires a series of reactions for which a variety of conditions are necessary, for example for chlorinated ethenes (Weatherill et al., 2018). In this case, and where water quality objectives are less specific (e.g. where detailed water quality data is not available), restoration may be designed to induce a variety of hyporheic properties, for example by adopting a range of restoration strategies and designs.

5.4 Considerations in planning hyporheic zone restoration

The environmental setting determines the properties of hyporheic exchange more than the restoration strategy (Hester, Brooks and Scott, 2018). Therefore, identifying the most suitable locations for restoration within the stream network is probably the most important factor in determining outcomes, especially given limited resources which may restrict the scale of restoration. Other works have focussed specifically on identifying suitable sites for hyporheic restoration on a catchment scale, for example Magliozzi et al., (2019) and Kasahara et al. (2009), which is useful for those coordinating restoration in a top-down approach (e.g. regional planners). However, many river restoration schemes are organised in a bottom-up approach, whereby sites are identified based on convenience or opportunity (Koontz and Newig, 2014). In this section, we outline the characteristics of environmental setting and the river corridor that are important controls of hyporheic exchange and that should be considered in restoration planning. This should allow professionals to identify the most suitable locations for restoration within a reach, to identify the causes of degradation that limit the function of the hyporheic zone, and to identify suitable solutions (i.e. restoration strategies).

5.4.1 Sediment hydraulic conductivity

The hydraulic conductivity of the sediment is one of the most important controls on hyporheic exchange (Herzog, Higgins and McCray, 2016; Hester, Hammond and Scott, 2016). The hydraulic conductivity describes the ease at which water moves through the sediment. It is determined by the ratio of sediment grains to pore space; sand has a lower hydraulic conductivity than gravel, for example, because there is less space between sediment grains. High hydraulic conductivity sediments (coarse sediments like cobbles and boulders) are likely to result in a high volume of hyporheic exchange but a low residence time. Low hydraulic conductivity sediments (fine sediments like sands and silts) are likely to result in a low volume of hyporheic exchange but high residence times. Hester, Hammond and Scott (2016) found that a sediment hydraulic conductivity below 10^{-4} m/s (equivalent to clean sand) led to residence times that were too long (i.e. water remained after pollutant was removed) and above 10^{-3} m/s (gravel) led to residence times that were too short (i.e. not all the pollutant was removed before water returned to the channel), though this depends on background conditions (especially slope and discharge). Sediment thickness is also an important control of hyporheic change, and shallow sediments (i.e. short distance to bedrock or inundation layer) will likely limit hyporheic exchange volume and residence time (Toran et al., 2013).

5.4.2 Gaining or losing conditions

Water can flow from the groundwater into the channel (gaining conditions) or from the channel into the groundwater (losing conditions). This is different from hyporheic exchange because it is unidirectional, i.e. the water does not return to its source (Ward, 2015). Strongly gaining conditions can limit or eliminate hyporheic exchange (Cardenas, 2009; Fox, Boano and Arnon, 2014). Losing conditions can either limit or enhance the properties of hyporheic

exchange (Hester, Brooks and Scott, 2018; Krause et al., 2022). Locations where neither strong losing nor gaining conditions exist (neutral conditions) are optimal for hyporheic restoration (Hester, Brooks and Scott, 2018). Strong gaining conditions (as is often observed in chalk streams) may make hyporheic restoration less effective but shallow hyporheic exchange of short residence times may still be generated, which could have a positive effect on water quality. Most commonly, losing conditions are observed in streams with a lower stream gradient and gaining conditions are observed in streams with high gradient – streams of medium gradients are most likely to afford neutral conditions (see section 3.3) (Kasahara and Wondzell, 2003; Hester, Brooks and Scott, 2018). Losing or gaining conditions can be observed for part of the year only, for example when the groundwater table is high after winter (Azinheira et al., 2014). This could result in hyporheic exchange been active for only part of the year, but this can still lead water quality improvements (Hester, Hammond and Scott, 2016).

It is difficult to estimate gaining/losing conditions, which presents a major barrier for professionals in designing hyporheic restoration strategies (Lewandowski et al., 2019). Some fairly simple and affordable tools are available for use in the reach scale, such as mini-piezometers (Rivett et al., 2008) and biological indicators (Graillot et al., 2014), and on a landscape scale, such as statistical methods (Magliozzi et al., 2019). However, more accessible techniques need to be developed to allow professionals to estimate this vital ecosystem control, which would be useful well beyond hyporheic restoration (Krause et al., 2011).

5.4.3 Stream gradient

The stream gradient is the slope or change in elevation in a given distance – slope and stream gradient are used interchangeably here. Upland streams typically have higher gradients and lowland streams and rivers have lower gradients. The stream gradient controls the properties of the hyporheic zone in three main ways. It largely determines the stream energy, including reach scale velocity (Hester, Brooks and Scott, 2018). The river restoration centre (2019) in the UK uses slope as a surrogate for stream energy, where >1% is high energy, 0.125-1% is medium energy and <0.125% is low energy, but a larger range of slopes are observed in more mountainous regions. Stream energy is one of the key controls of hyporheic exchange, especially where the primarily driver is hydrodynamic forces, in which case a higher stream energy leads to a higher volume of hyporheic exchange and longer residence times (Rana, Scott and Hester, 2017; Howard et al., in prep. [chapter 4]). The stream gradient also determines the capacity to induce hydrostatic forces because in a high gradient system, it is easier to create a difference in elevation over short distances, for example from upstream to downstream of a weir. Finally, steam gradient also largely determines the relationship between the water level in the channel and that in the adjacent groundwater, which controls the losing or gaining conditions (Hester, Brooks and Scott, 2018). In general, hyporheic exchange increases but residence time decreases with slope (Herzog, Higgins and McCray, 2016). In most circumstances, restoration should aim to induce hydrostatic forces in high gradient systems and hydrodynamic forces in low gradient systems.

5.4.4 Flow conditions

Hyporheic exchange is controlled by discharge, and its associated hydromorphological conditions like water level and velocity. For example, Hester, Eastes and Widdowson (2019) found that increasing the surface water level from 0.1 m to 1 m (e.g. summer to winter

conditions) increased aerobic reactions by 270% and anaerobic reactions by 78%. In general, higher discharge leads to a higher volume and residence time of hyporheic exchange, but the relationship between discharge, restoration features and hyporheic exchange is not fully understood (Ward et al., 2018). Variations in discharge can change the function of a restoration technique in the channel (Krause et al., 2014). For example, a feature that emerges from the surface water during low flows, primarily driving hydrodynamic hyporheic exchange, may be submerged during high flows, creating a difference in elevation from upstream to downstream and driving hydrostatic hyporheic exchange. It is vital, therefore, to consider the impact of restoration in the full range of flow conditions that the site is subject to (Wohl, Lane and Wilcox, 2015).

It may be possible to design restoration strategies to target specific conditions. For example, if groundwater conditions only allow hyporheic exchange during certain times of the year (e.g. during summer when the groundwater table is low), then restoration may be designed to induce hyporheic exchange during summer baseflow conditions. For example, Hester, Hammond and Scott (2016) used channel-spanning features in an upland setting to achieve 30% nitrogen removal during summer baseflow in a 900 m reach, but the average annual removal was only 3.7%. It seems that whatever the impact of restoration on hyporheic, it is exaggerated by an increase in discharge (Howard et al., in prep. [chapter 4]). The range of flow conditions could also have important practical implications for restoration design, for example in terms of feature stability and longevity.

5.4.5 Biogeochemical opportunity

Biogeochemical opportunity refers to the potential for biogeochemical reactions to occur if transport (i.e. hyporheic exchange) is not limiting, though in most cases both transport and

biogeochemical opportunity control reaction rates in the hyporheic zone (Findlay, 1995). Most reactions occurring in the hyporheic zone that can lead to the improvement of water quality are facilitated by microbes. These microorganisms get their energy by metabolising chemical compounds and in doing so transforming these into different compounds, sometimes from polluting forms to environmentally benign forms (Krause et al., 2010). For example, aqueous nitrate (NO_3), which can cause eutrophication in high concentrations, can be transformed by a series of reactions to gaseous dinitrogen (N_2), which is environmentally benign. Most of the microbes that perform these functions are heterotrophs, meaning that they require a source of dissolved organic carbon for metabolism (Quick et al., 2019). Therefore, the availability and quality (bioavailability) of dissolved organic carbon is a primary control of reaction rates in the hyporheic zone (Zarnetske et al., 2011).

Dissolved organic carbon can be produced in the stream (autochthonously) by primary production, which is easily transported to the hyporheic zone by hyporheic exchange and diffusion. This production is largely dependent on light and nutrient availability, but it can contribute a large part of the total carbon stock and produce carbon which is especially high quality (Berggren and Giorgio, 2015). Dissolved organic carbon may also be introduced to the hyporheic zone by external (allochthonous) sources, which are transported in the stream as particulate organic matter and may have terrestrial (e.g. soils) or aquatic (e.g. dead microbes) origins (Wohl et al., 2017). Furthermore, leaves, wood and fine sediments deposited in the hyporheic zone can decompose and provide a persistent and bioavailable (autochthonous) source of dissolved organic carbon (Krause et al., 2014; Howard et al., in prep [chapter 3]). Overall, a higher availability of dissolved organic carbon can be expected in lowland settings compared to upland because the lower stream energy allows its capture and retention and its sources (e.g. instream wood) are afforded longer times for decomposition (Krause et al., 2014). Furthermore, there is a larger upstream stream length

and catchment area contributing to the introduction of dissolved organic carbon to the stream. The availability of dissolved organic carbon may be increased by introducing wood to the stream, directly or by the enhancement of riparian vegetation, and by the capture of fine sediments (Wohl et al., 2017).

Biogeochemical opportunity is ultimately controlled by the availability of dissolved organic carbon together with the availability of reactive chemical compounds for use in metabolism (such as metals, nutrients, and chlorinated solvents), and the ratio between the two. Therefore, the biogeochemical opportunity may be especially high near to sources of reactive compounds, such as near mine drainage or downstream of waste water treatment plants (Duvert et al., 2019).

5.4.6 Sediment load

The sediment load is the volume of sediment transported or carried by the surface water. Upstream bank erosion or scouring, periodic damn releases, and overland flow (especially over erosion vulnerable land surface, like heavily cultivated fields) can results in substantial sediment load. The deposition of fine sediments like silts and clays can lead to clogging of the spaces between larger sediments (called the interstitial spaces); this is often called siltation or colmation (Kasahara and Hill, 2008). Siltation reduces the sediment hydraulic conductivity which is likely to limit the volume of hyporheic exchange that may be induced. This is especially common upstream of channel-spanning features where the water velocity is decreased leading to the deposition of sediments and consequently a reduction in water depth (Fanelli and Lautz, 2008; Krause et al., 2014). Sedimentation may be the primary reason why hyporheic restoration schemes can become quickly ineffective, even if they are initially effective (Pander, Mueller and Geist, 2014). Where the sediment load is high, restoration

schemes should probably avoid channel-spanning features and sediment modifications, unless they are designed specifically to limit sedimentation, as discussed in sections 5.5.1 and 5.5.4.

5.4.6 Sites with the highest restoration potential

The sites with the greatest potential to improve hyporheic zone function for water quality objectives are those where the groundwater conditions are neutral (i.e. not strongly gaining or losing), usually observed in mid-order streams, and where existing hyporheic exchange is limited by stream degradation (Hester, Brooks and Scott, 2018). These are likely to include streams which have received high fine sediment loads, those which have been incised, straightened or channelized, and those with low geomorphic complexity, for example as result of the removal of wood (Beechie et al., 2010). This is not dissimilar to identifying sites for river restoration with other objectives and therefore practitioners will already possess significant expertise in this area, which is easily transferred to hyporheic restoration.

5.5 Designing hyporheic restoration strategies

Although the environmental setting primarily controls the properties of the hyporheic zone, these properties are amenable to modification by river restoration, particularly at the feature scale. As with all river restoration, it is vital to adopt a technique that is suitable for the environmental setting and that is informed by the probable historic condition of the river and the variability in its conditions in time and space (Wohl, Lane and Wilcox, 2015). It is the interaction between restoration and the environmental setting that determines the properties of the hyporheic zone, so the form of restoration should complement environmental drivers (i.e. hydrostatic and hydrodynamic forces discussed in section 2). A technique that enhances the functioning of the hyporheic zone in one setting could have a negative effect in another.

For example, the occurrence of channel-spanning wood may decrease hyporheic exchange in low-gradient streams (Wondzell et al., 2009; Rana, Scott and Hester, 2017; Howard et al., in prep. [chapter 4]) but increases it in high-gradient streams (Kasahara and Wondzell, 2003).

The restoration techniques discussed here will be familiar to river restoration practitioners as they are commonly employed to deliver many different objectives. However, the design of the selected technique is important and can determine the influence it exerts on the hyporheic zone. The design may refer to the architectural characteristics of the restoration like size, form and material, or its arrangement in the channel, like spacing and orientation. The effect of restoration is likely to differ in different environmental conditions (e.g. discharge) its effect should be considered in the range of conditions it is likely to be subject to (Wohl, lane and Wilcox, 2015). In this section, we briefly outline the mechanisms by which common river restoration techniques are likely to influence the hyporheic zone and how their characteristics are likely to affect their influence. Whilst the efficacy of some techniques to restore the hyporheic zone has received considerable attention, others have hardly been researched at all. Therefore, it has sometimes been necessary to speculate based on related research; these Instances have been clearly highlighted.

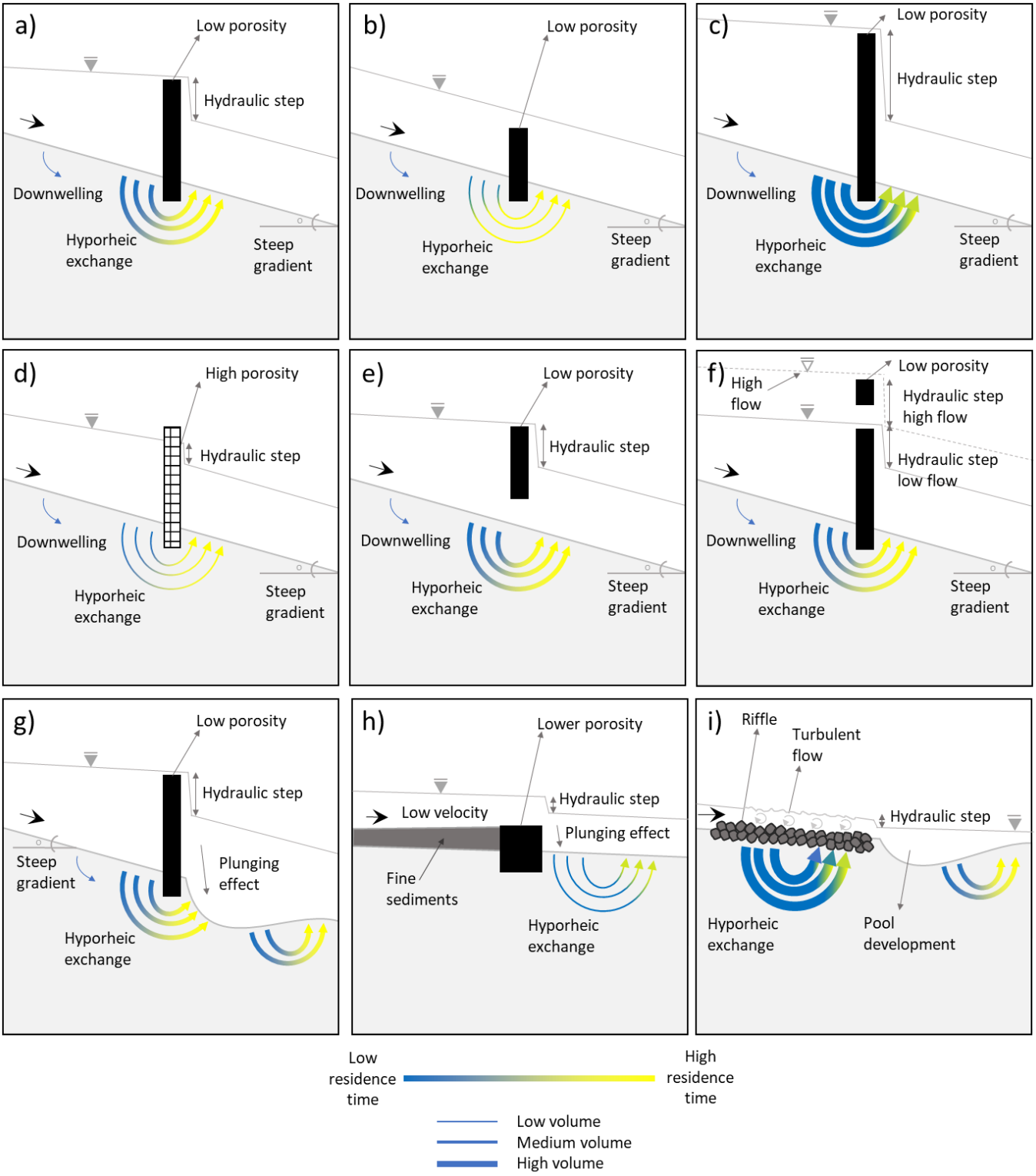


Figure 5-2. 2(i). Schematics representing the properties of hyporheic exchange (blue/yellow arrows) generated by different restoration techniques and designs. a) Impermeable weir of perfect height in an upland setting. b) Impermeable weir of short height in an upland setting. c) Impermeable weir of tall height in an upland setting. d) Permeable weir of perfect height in an upland setting. e) Impermeable weir of perfect height with a bottom gap in an upland setting. f) Impermeable weir of perfect height with two outflow heights in an upland setting. g) Impermeable weir of perfect height with pool development in an upland setting. h) Impermeable step with fine sediment upstream in a lowland setting. i) Constructed riffle and pool in a lowland setting.

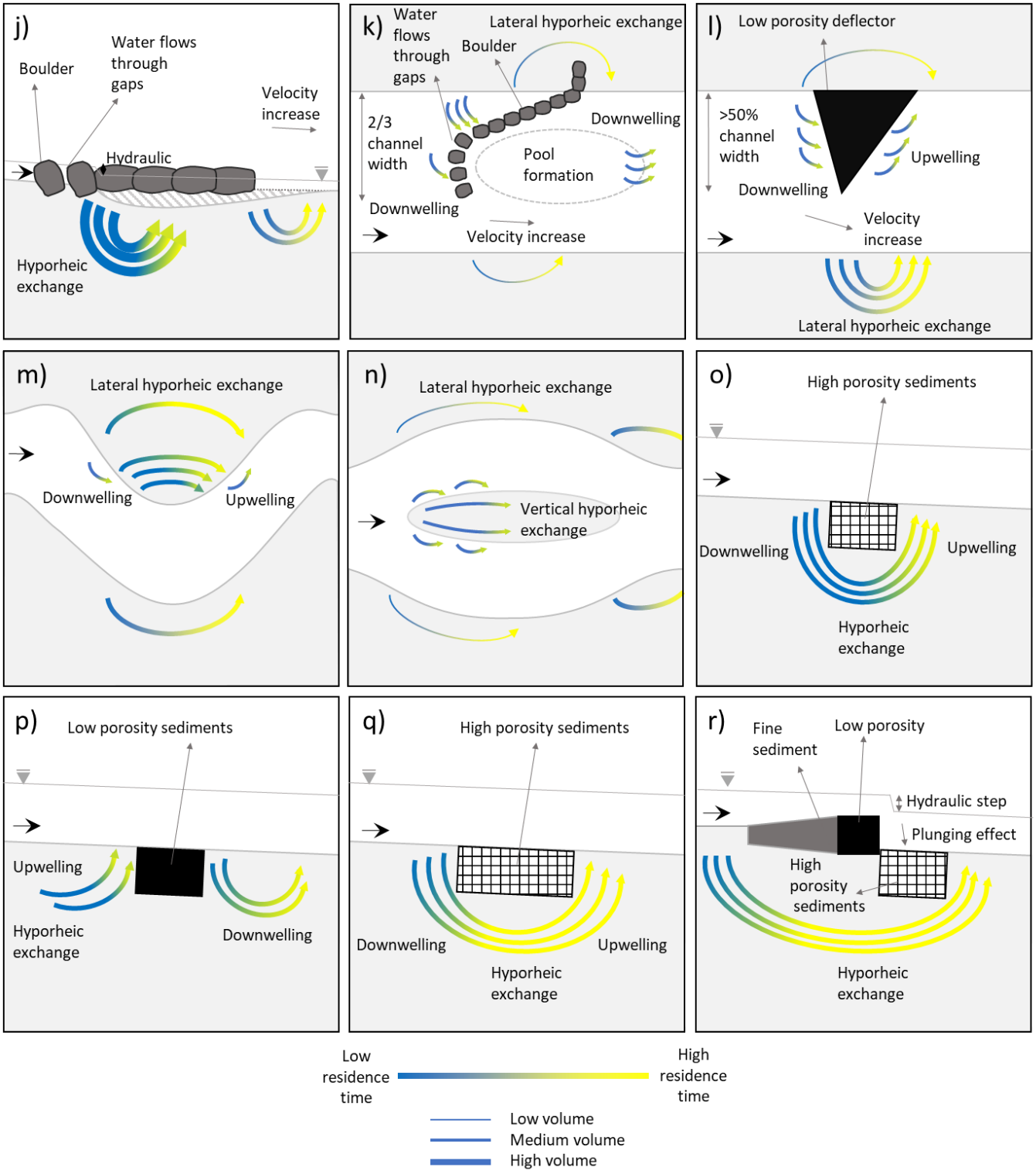


Figure 5-3.(ii). Continued from Figure 2(i). j) J-hook and pool in a lowland setting, adapted from Rosgen, 2001. k) Plan view of a J-hook and pool in a lowland setting, adapted from Rosgen, 2001. l) Plan view of a deflector in a lowland setting. m) Plan view of channel reconfiguration (re-meandering) in a lowland setting. n) Plan view of channel reconfiguration (island construction) in a lowland setting. o) High porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. p) Low porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. q) Long low porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. q) Long low porosity sediment modification in a lowland setting, adapted from Ward, Gooseff and Johnson, 2011. r) Low porosity sediment modification coupled with upstream step in a lowland setting, adapted from Bakke, Hrachovec and Lynch, 2020.

5.5.1 Channel-spanning features

Channel-spanning features may include weirs, steps, dams and in some cases large wood. They are probably the most effective means of inducing hyporheic exchange in upland settings, especially where simplification has occurred such as the removal of instream wood (Hester, Brooks and Scott, 2018). Usually, the aim of a channel-spanning feature is to impound water on the upstream side, creating a difference in elevation in the surface water from upstream to downstream of the feature, often called a hydraulic step (see Figure 5-2a). This generates hydrostatic forces which induce areas of downwelling upstream and upwelling downstream, resulting in hyporheic exchange of high residence times (Hester and Doyle, 2008). The impounding of water upstream also increases the area of the wetted channel which can increase the volume of hyporheic exchange, and the water flowing over the feature may 'plunge' into the downstream streambed, forcing hydrodynamic downwelling and creating pools (Endreny, Lautz and Siegel, 2011).

The characteristics of channel-spanning features that chiefly determine their effect on the hyporheic zone are porosity and height. Porosity refers to the ratio of material to open space, where zero porosity is impermeable. A high porosity (e.g. in some large wood dams) may limit the volume of water that is impounded and the difference in elevation in the surface water profile, leading to a lower volume and residence time of hyporheic exchange (see Figure 5-2d) (Manners, Doyle and Small, 2007). For low porosity features, feature height is the most important characteristic. Taller weirs induce a higher volume of hyporheic exchange but a lower residence time (see Figure 5-2c) (Hester and Doyle, 2008; Feng et al., 2022). The optimal weir height maximises both hyporheic exchange volume and residence time. This optimal height is determined by the environmental setting but for upland sites a height of around 0.5 m has been suggested, which may also lead to the highest anaerobic reaction rates (see Figure 5-2a) (Hester and Doyle, 2008; Liu and Chui, 2020). Similarly, Wade et al.

(2020) observed that beaver dam analogues function with size-dependant behaviours, where small features had little effect but larger features – which created a larger hydraulic step – were able to induce high volumes of hyporheic exchange with sufficient residence time to host anaerobic reactions. Designs that allow water to exit at different heights (for example Figure 5-2f) may maintain a high hyporheic exchange volume and residence time during different discharges.

The most common failure of channel-spanning features is siltation of sediments upstream (Hester, Brooks and Scott, 2018). This could be avoided by introducing a gap at the bottom of the weir (see Figure 5-2e), which would capture less sediment but does not reduce effectiveness (Sawyer, Cardenas and Buttles, 2011), or by using features which concentrate stream power at the centre of the channel (e.g. cross-vanes or W-weirs) thereby flushing sediments through (see Figure 5-2j,k) (Rosgen, 2001).

Features in close proximity can interact, sometimes leading to increased residence times and sometimes to a reduction in the volume of hyporheic exchange (Herzog, Ward and Wondzell, 2019). The interactions of features are complex and so it is unlikely, given current understanding and monitoring techniques, that restoration can be reasonably designed to target positive interactions that increase residence times. Therefore, readers should aim to avoid reducing hyporheic exchange by feature interaction. This reduction in hyporheic exchange is caused by a reduction in the difference in elevation from upstream to downstream of the weir due to the increased water level upstream of the feature immediately downstream (Li et al., 2022). Therefore, features should be spaced a sufficient distance as to not be impacted by the increased water level of the feature immediately downstream. This distance will be determined by discharge, channel width and slope and the feature height, but is typically in the region of 2-5 channel widths (Han, Chu and Endreny, 2015).

In some settings, channel-spanning features can lead to a reduction in hyporheic exchange, at least in the short-term (Rana, Scott and Hester, 2017; Howard et al., in prep. [chapter 4]). This usually occurs in low-gradient streams with sediment of a low hydraulic conductivity. In this case, the reduction in water velocity decreases existing hydrodynamic hyporheic exchange, which is not replaced by hydrostatic hyporheic exchange because of the low stream gradient. This suggests that removing channel-spanning features in lowland streams could increase hyporheic exchange, though to our knowledge this has not been explicitly investigated.

5.5.2 Deflectors

Deflectors may Include vanes, J-hooks, groynes, croys, baffles and in some cases large wood. They are structures that cover part of the channel laterally, usually between one and two thirds, and have typically been adopted to reduce the scour and erosion of banks by redirecting the stream power to the centre of the channel, often creating a deep pool downstream (Rosgen, 2001; Toran et al., 2013). A series of these features can create pool-riffle sequences in certain setting (see Figure 5-2i) (Crispell and Endreny, 2009). Deflectors can generate hydrostatic hyporheic exchange but usually to a lesser extent than channel-spanning features (Hester and Doyle, 2008). Similarly to channel-spanning features, downwelling occurs upstream of the feature and upwelling occurs downstream (see Figure 5-2l) (Crispell and Endreny, 2009; Han, Chu and Endreny, 2015). On top of this though, deflectors can increase the water velocity, stream energy, and turbulence, all of which are likely to induce hydrodynamic hyporheic exchange (Hester and Doyle, 2008; Crispell and Endreny, 2009; Han, Chu and Endreny, 2015). This combination of drivers may provide a good balance between hyporheic exchange volume and residence time, which could prove an effective means of delivering a variety of water quality objectives.

The effect of porosity is in general similar as for channel-spanning features, where a lower porosity is likely to lead to a larger impact, but in some cases gaps in the feature near the centre of the channel can increase velocity and flush out sediments, for example in J-hooks (see Figure 5-2j,k) (Rosgen, 2001). To have the greatest impact, the height of deflectors should exceed the water level during baseflow, and be sufficient to create an elevation difference in the surface water profile from upstream to downstream during flood (Han, Chu and Endreny, 2015). Laterally, the feature should cover at least 50% of the channel but exceeding this is unlikely to have a greater effect on hyporheic exchange (Hester and Doyle, 2008). The angle of the deflector to the flow is likely to be one of the most important design characteristics. Little research has focussed explicitly on the effect of different angles on hyporheic exchange, but reasonable speculation can be made. A larger difference in elevation from upstream to downstream is probably created by features which are orientated perpendicular to the direction of flow (i.e. 90^{o} from the bank) - as indicated in work by Biron et al., (2004) - which will likely induce a higher hyporheic exchange volume and residence time. The pools that can develop as a result of deflector installation may contribute significantly to the total hyporheic exchange in a reach (Crispell and Endreny, 2009). Therefore, features should be spaced sufficiently as to not limit pool development, usually 2-5 times deflector length, but this is determined by stream gradient where closer spacing is suitable in steeper gradients (Rosgen, 2001; Mohammed, Yaser and Shaker, 2016). There are practical considerations in the design of deflectors, such as managing bank erosion, which are outlined in Rosgen (2001).

5.5.3 Channel reconfiguration

Channel reconfiguration is a common restoration technique for rivers that have been channelized, straightened or simplified (e.g. consolidation of many channels into one), all of

which can lead to a reduction in hyporheic exchange (Sparacino et al., 2019). Channel reconfiguration may include restoring meanders and increasing sinuosity, creating islands and braided channels, re-connecting the channel to the floodplain, and re-naturalization of the channel. This broad technique may include many different designs which could affect hyporheic exchange in a variety of ways. However, in general channel reconfiguration results in lower stream gradients, reduced reach scale velocity (though local velocities could be increased), reduced stream energy and deposition of sediments (Bukaveckas, 2007; Becker, Endreny and Robinson, 2013). This will tend to result in a shift in the dominant driver of hyporheic exchange from hydrodynamic to hydrostatic, and subsequently in its properties from high volume and low residence time to lower volume and higher residence time (Mason, McGlynn and Poole, 2012). Channel reconfiguration is also likely to result in the induction of lateral hyporheic exchange (i.e. from one side of a meander to another, called intrameander flow) (see Figure 5-2m), which can have high residence times and lead to high potential for anaerobic removal of pollutants (Kasahara and Hill, 2007; Boano et al., 2014; Krause et al., 2022).

Channel reconfiguration schemes aiming to enhance the function of the hyporheic zone have delivered mixed results; many detected no significant changes to hyporheic exchange or the capacity for pollutant removal, suggesting channel configuration may not be an especially effective means to restore the hyporheic zone (Bukaveckas, 2007; Wohl, Lane and Wilcox, 2015; Martín et al., 2018). Due to the scale of these programmes, they may be especially vulnerable to controls of environmental setting, such as sediment hydraulic conductivity and stream gradient (Kasahara and Hill, 2007; Gabriele, Welti and Hein, 2013). What is clear is that schemes must be well considered and acutely targeted to suit the environmental setting; simply widening incised or restricted channels (sometimes referred to as de-channelization) is probably not sufficient (Martín et al., 2018). Some studies have observed a reduction in the

volume of hyporheic exchange after channel reconfiguration, which has mostly been attributed to the loss of hydrodynamic drivers due to velocity decreases and vertical bed simplification (e.g. loss of bedforms) (e.g. Mason, McGlynn and Poole, 2012). To avoid this, lateral modifications, like meanders and islands, should be coupled with vertical modifications, like steps, deflectors or gravel bars (see Figure 5-2h) (Kasahara and Hill, 2007; Kurth, Weber and Schirmer, 2015).

As a generalization, where the stream gradient is relatively high (>1%), schemes should use large meanders which will create large differences in elevation from upstream to downstream and could generate substantial hydrostatic hyporheic exchange with long residence times (Boano et al., 2014; Krause et al., 2022). Where the stream gradient is low, schemes should instead adopt designs that maintain high velocity (locally and on the reach scale) and generate stream power, such as gravel bars, small islands and steps (see Figure 5-2n) (Kurth, Weber and Schirmer, 2015). Channel reconfiguration is often coupled with floodplain reconnection activities and 'stage zero' concepts, which could prove effective when employed collectively (Wohl, Lane and Wilcox, 2015; Wohl et al., 2021).

5.5.4 Sediment modifications

Modifications to sediments has occurred in many rivers, for example by removal of gravels for construction, dredging of rivers for navigation, or siltation as a result of changes to sediment transport dynamics (Ward et al., 2018). Removal of sediments can lead to a reduction in the sediment depth, and siltation reduces the hydraulic conductivity of the sediment, both of which can limit the function of the hyporheic zone (Fanelli and Lautz, 2008; Toran et al., 2013). Given that the depth and hydraulic conductivity of the river sediments are critically important controls on the function of the hyporheic zone, sediment

modifications offer a promising hyporheic restoration technique. Research has focussed on modifications such as fine sediment removal or gravel cleaning (e.g. Ward et al., 2018), introduction of gravels (e.g. Pander, Mueller and Geist, 2014), and more complex engineered streambeds (e.g. Ward, Gooseff and Johnson, 2011; Herzog, Ward and Wondzell, 2019). Some modifications aim to simply increase the sediment hydraulic conductivity to increase the volume of hyporheic exchange. More complex designs aim to disrupt or divert hyporheic flow paths (the route a parcel of water takes through the hyporheic zone), similarly to natural heterogeneities in the hydraulic conductivity of the sediments, such as peat and clay lenses (Krause et al., 2014). This can generate hydrostatic forcing, manifesting in patches of upwelling and downwelling, and increase residence times, and consequently the potential for anaerobic reactions (Ward, Gooseff and Johnson, 2011).

Sediment modifications may aim to modify sediments across the entire streambed (e.g. Ward, Gooseff and Johnson, 2011; Pander, Mueller and Geist, 2014) or in smaller patches (e.g. Ward et al., 2018; Bakke, Hrachovec and Lynch, 2020). At the streambed scale, Pander, Mueller and Geist (2014) modified 50 m^2 areas of streambed and found that introducing sediments with a higher grain size (i.e. coarser gravel) lead to a higher volume of hyporheic exchange, though probably of short residence times. Sediment depth is probably important, with deeper sediments allowing higher residence times, but this has not been explicitly tested. It may be necessary to dredge fine sediments and remove macrophytes before sediment introduction to avoid siltation (Pander, Mueller and Geist, 2014). Where coarser sediments remain but siltation has occurred, gravel cleaning may be a viable restoration technique, though this has yielded mixed results. Mathers, Robinson and Weber (2021) observed an increase in the volume of hyporheic exchange after fine sediment removal, but Ward et al. (2018) found that fine sediment removal had little effect on transient storage (which includes hyporheic exchange), which was attributed to the associated loss of macrophytes. On the

patch scale, Ward, Gooseff and Johnson, 2011 investigated the effect of installing 3 m x 1.5 m areas of sediment with high or low hydraulic conductivity in the stream bed. High hydraulic conductivity structures created areas of downwelling upstream and upwelling downstream (similar to channel-spanning features) (see Figure 5-2o) whilst low hydraulic conductivity structures created areas of upwelling upstream and downwelling downstream (see Figure 5-2p). High hydraulic conductivity structures positioned adjacent to the stream bed maximised hyporheic exchange, and residence time was increase by increasing feature length (see Figure 5-2q). These kinds of engineered hyporheic zones can lead to significant improvements to many aspects of water quality (Peter et al., 2019).

Some field studies found that the effect of sediment modification on the hyporheic zone did not persist for more than 1 year, primarily due to siltation of sediments (Pander, Mueller and Geist, 2014; Mathers, Robinson and Weber, 2021). To avoid this, sediment modifications could be coupled with vertical steps or weirs which can vastly increase hyporheic exchange (by as much as 89 times) and prevent siltation (see Figure 5-2r) (Bakke, Hrachovec and Lynch, 2020). In general, sediment modifications are probably not suitable in systems with a high sediment load. Caution should be exercised to limit the negative impact on downstream environments caused by the significant mobilisation of fine sediments following sediment modifications (Pander, Mueller and Geist, 2014).

5.5.5 Development

Most instream restoration is likely to induce an evolution of the local geomorphology, and the ecosystem as a whole, to a particular equilibrium state (Wohl, Lane and Wilcox, 2015). The geomorphological state that develops following restoration could have a more substantial effect on the hyporheic zone than the restoration itself. For example, Lautz and Fanelli (2008)

observed the development of a small pool followed by a glide upstream of a channel-spanning feature, and a plunge pool followed by a riffle downstream, generating patchy spatial patterns of hyporheic exchange (see Figure 5-2g). Furthermore, the introduction of deflectors could lead to meandering, which can result in significant lateral hyporheic exchange (Krause et al., 2022). Restoration could be designed to induce a geomorphic condition that generates the desired properties of hyporheic exchange. For example, Kobayashi et al. (2022) designed wood 'training features' to facilitate pond formation during flood events, which probably led to increased hyporheic exchange as indicated by decreased temperatures in the streambed relative to the rest of the channel. Some restoration designs may take more than a decade to reach their maximum effectiveness, and this should be considered in monitoring strategies (Wondzell et al., 2009). Conversely, the evolution of the river system post-intervention could decrease the efficacy of restoration and degrade the function of the hyporheic zone, such as siltation which is discussed in section 4.1.

Much uncertainty exists about how most restoration techniques develop, and in general about how changes to river systems affect the hyporheic zone. For example, the establishment of macrophytes has been shown to effect hyporheic exchange and instream storage both negatively (Romeijn, Hannah and Krause, 2021) and positively (Vereecken et al., 2006). It may be necessary in some cases to design a maintenance plan, for example the removal of silt behind channel spanning features but then this does not meet accepted standards for successful river restoration (Palmer et al., 2005). A network of hyporheic restoration demonstration sites, with long term monitoring programmes, is necessary to improve our understanding of exactly how hyporheic restoration schemes evolve and how their efficacy changes over time, as discussed further in section 7.

5.6 Scenarios of hyporheic restoration

To make research actionable its insights must be directly applicable to the environmental setting in which it will be applied, which as we have seen is the most important control of the function of the hyporheic zone. However, there are few demonstrations of hyporheic restoration that river restoration professionals are able to draw upon to directly inform the designing of hyporheic restoration in a range of environmental settings and to abate a range of pressures. On the contrary, there has been substantial dedicated research into the function of the hyporheic zone in different settings and its interaction with various river restoration techniques, as has been outlined in sections 3 and 4. In this section, this research has been used to inform the design of hyporheic restoration in a series of theoretical scenarios. These scenarios are designed to represent typical situations where hyporheic restoration may be appropriate and possible. They have been constructed to include the considerations that have been made when selecting and designing the restoration technique, and they are structured in the way that the design of hyporheic restoration should be considered: setting, pressure, solution. Pressure refers specifically to the origins of hyporheic zone degradation and not of the stream function in general. It is important to identify and treat the root source or cause of the degradation as well as to mitigate the manifestation of degradation (Kasahara et al., 2009). In some cases restoration designs are speculative, but they still allow readers to formulate sensible hypotheses about the influence of restoration on the hyporheic zone and to select the most suitable designs for restoration.

5.6.1 Scenario 1

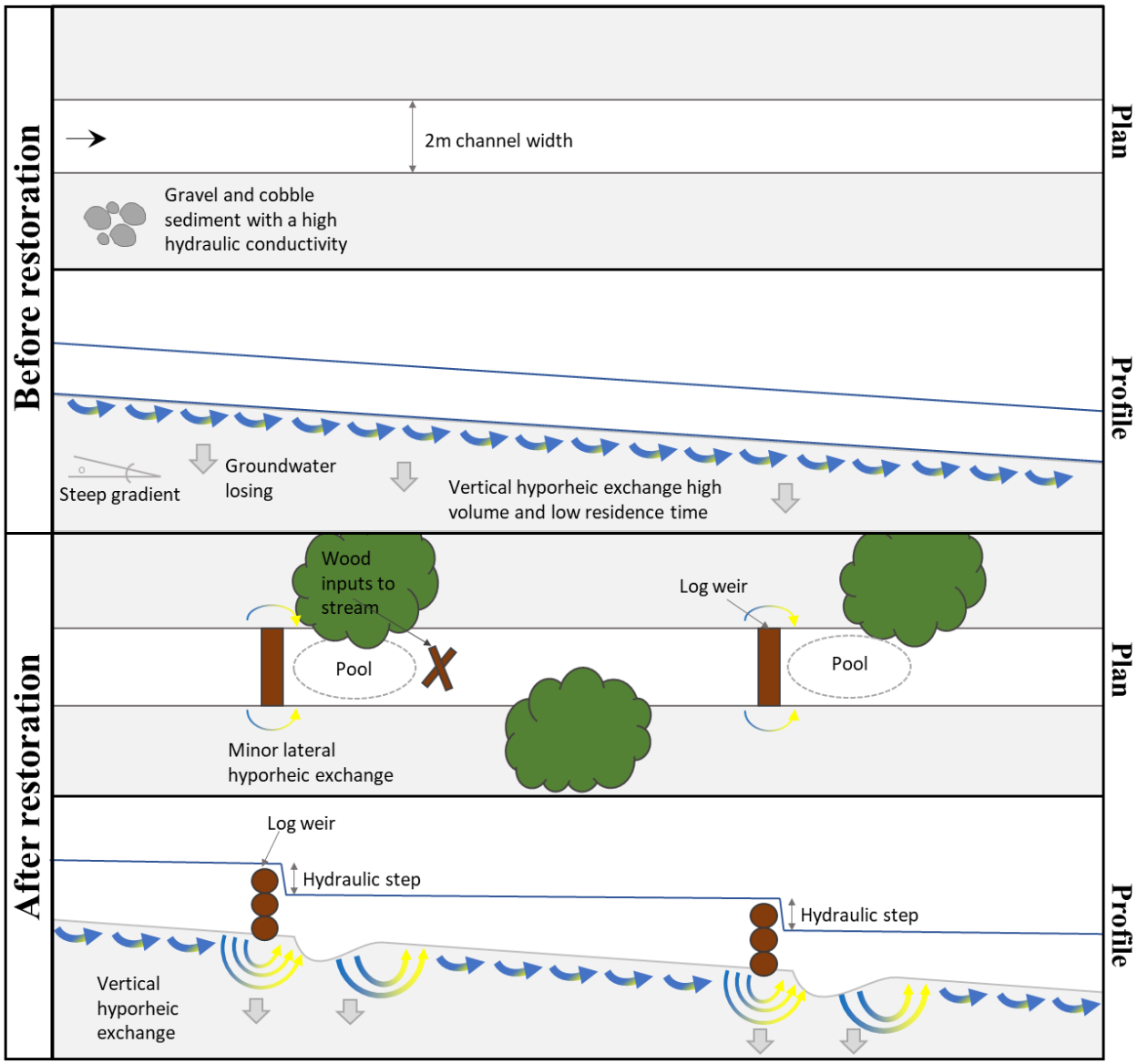


Figure 5-4. Schematic representing scenario 1. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times.

Setting

An upland 2nd order stream with a steep gradient of 5.5%, a width of 2 m, and gravel and cobble sediment. Riparian trees have been removed over a century ago and are prevented from regenerating by intensive sheep grazing. The discharge responds quickly to rainfall and has a summer baseflow of 20 l/s and a winter baseflow of 50 l/s. There are slightly losing conditions throughout the reach, especially in summer. There is little input of sediment into the stream but high input of sheep excrement.

Pressure

The lack of riparian zone has resulted in a low loading of instream wood and ultimately a simplification of the channel. Water travels downstream almost unimpeded and has a high velocity and energy. The flow around and over cobbles creates hydrodynamic forces at the streambed interface and, along with the high hydraulic conductivity of the sediment, which allows a high volume of hyporheic exchange but of only short residence times. The mildly losing conditions leads to some loss of water to the groundwater because there are few areas of low pressure that return water back into the channel. Sheep excrement enters the channel leading to potential pathogen input and moderate nutrient concentrations, but the biogeochemical opportunity is limited by the low carbon availability.

Solution

Sheep should be excluded from the stream and its banks by fencing, allowing riparian vegetation to recover, which may be accelerated by tree planting. This will eventually facilitate the recovery and maintenance of the instream wood loading but it could take several centuries to achieve (Beechie et al., 2000; Stout et al., 2018). To realise hyporheic restoration more quickly, more direct intervention is required. The aim of this restoration should be to increase the residence time of water in the hyporheic zone and reduce the loss to

groundwater. By generating hydrostatic forces, water will be driven deeper into the hyporheic zone by areas of lower pressure and returned to the channel by areas of higher pressure (Sawyer, Cardenas and Buttles, 2011). This is best achieved by introducing channel-spanning features like weirs. The weirs should have a low porosity and a height of 0.5 m. They should be spaced 4-10 m apart and be installed to capitalise on natural stream topography. Some existing boulders can be re-positioned to form weirs, but logs can also be pinned into the bed and the bank and secured with wire or rope. Using logs could also introduce carbon to the system.

5.6.2 Scenario 2

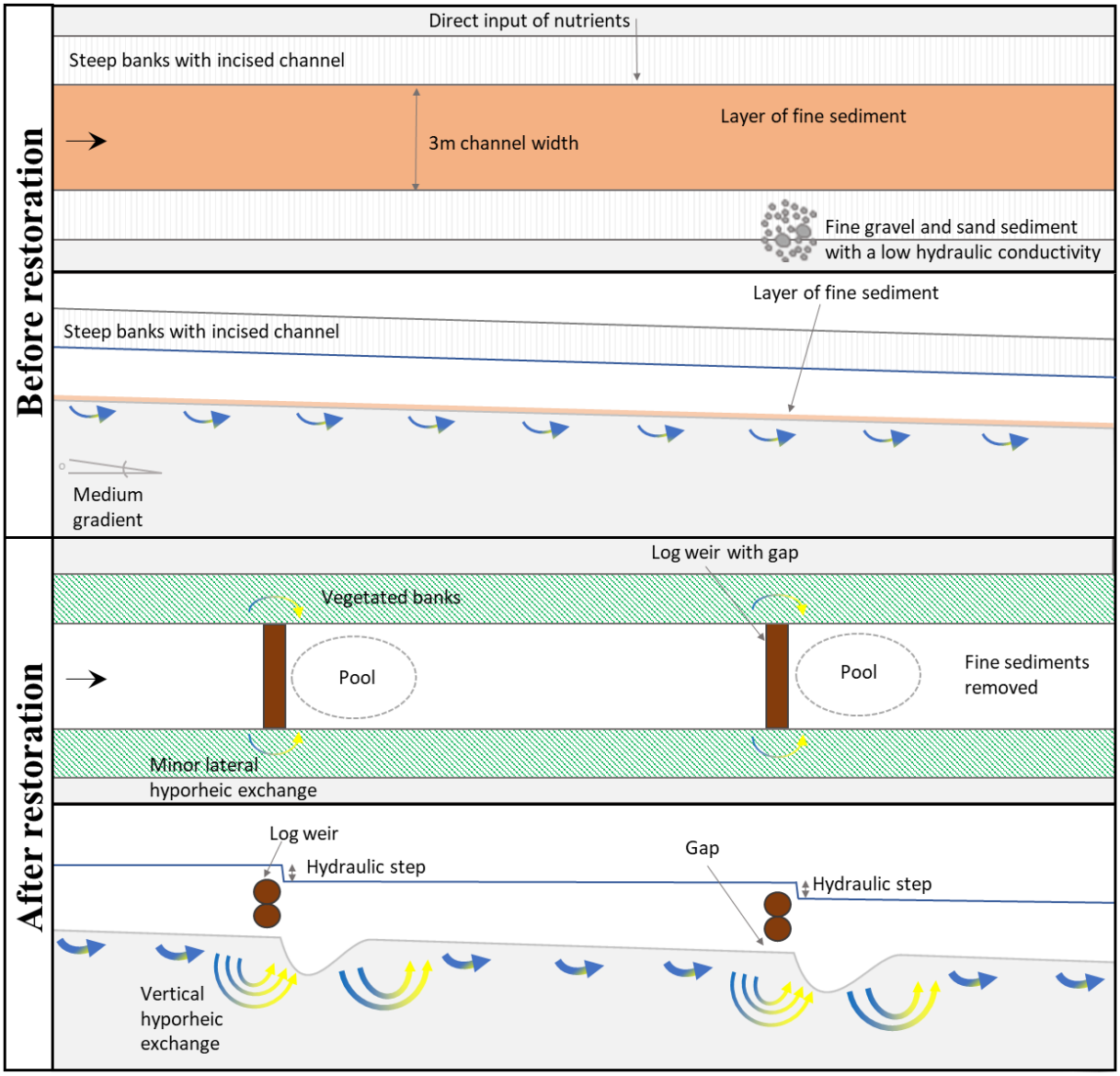


Figure 5-5. Schematic representing scenario 2. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times.

Setting

A 2nd order stream with a medium gradient of 0.9%, a width of 3m, and fine gravel and sand sediment. The groundwater conditions are neutral and the stream discharge responds quickly to rainfall due to extensive overland flow in the catchment. The mean discharge is 10 l/s but maximum flows reach 150 l/s. There is a small storage reservoir upstream which keeps the stream flowing all year round and releases high discharges periodically which transport a high sediment load. The upper catchment consists of deep organic soils and the land use is intensive arable agriculture, which includes high fertilizer addition and heavy cultivation practices.

Pressure

The stream has been straightened, deeply incised (channelized) and cleared of obstacles like wood and boulders. Agricultural activities encroach within 2 meters of the stream. Due to the reservoir releases and sediment entering the stream from overland flow, some gravels have become clogged and the streambed profile is buried in a fine layer of silt, resulting in a smooth surface. As a result, almost no hyporheic exchange occurs. Overland flow directly into the stream, as well as interaction with polluted groundwaters, result in high nutrient concentrations. Organic matter content is also high as a result of the organic soils transported to the stream in overland flow.

Solution

Buffer strips of at least 5 m should be established either side of the stream, containing trees and long grasses, which will intercept runoff and help reduce the direct input of sediment and nutrients to the stream. The challenges of restoration are to avoid inducing bank erosion of soft organic soils and to manage the high sediment load. The medium slope and neutral groundwater conditions allow for the induction of hydrostatic forces. Raised channel-spanning features with a gap at the streambed could generate hyporheic exchange of high

residence times but prevent the deposition of sediment. The medium hydraulic conductivity of the sediment could further facilitate high residence times and the organic matter from organic soils could lead to a high biogeochemical opportunity. Because of the high variability in mean and maximum discharge, the feature could be designed with 2 outlets at different heights, positioned to target baseflow and high flow conditions. Logs could be used to construct the feature, pinned in place at both banks.

5.6.3 Scenario 3

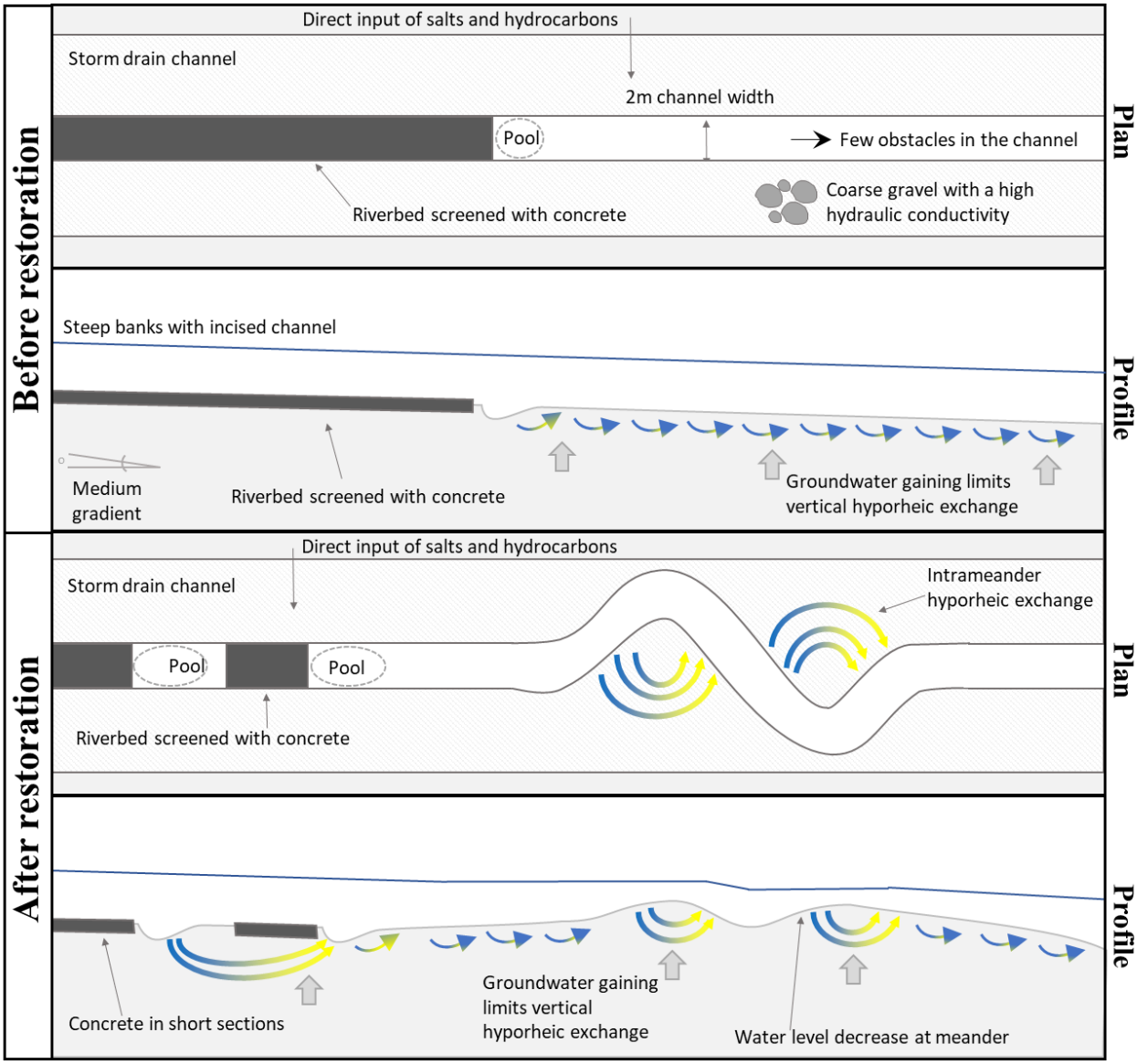


Figure 5-6. Schematic representing scenario 3. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times.

Setting

A 3rd order urban stream with a medium gradient of 0.9% and coarse gravel sediment. The channel width is around 2 m and is enclosed by roads on both sides in an area of around 10 m wide. The groundwater conditions are slightly gaining and the mean annual discharge is 40 l/s. The stream discharge responds quickly to rainfall due to fast overland flow over impermeable surfaces. There is a waste water treatment plant 300 m upstream which releases waste water during peak flows.

Pressure

The channel has been straightened and simplified, and in parts the streambed has been sealed with concrete. Where the streambed is sealed no hyporheic exchange occurs, and elsewhere in the stream hyporheic exchange is limited in volume and residence time by the lack of features and the gaining conditions. The waste water treatment discharges high concentrations of nutrients and carbon into the stream, and other pollutants are introduced to the stream by overland flow.

Solution

Most of the concrete that seals the streambed should be removed, but retaining some sections could induce hyporheic exchange of long residence times and help maintain high water velocity. The channel could be reconfigured to meander throughout the enclosed 10 meters – the medium gradient could allow a hydraulic head across meanders, generating hydrostatically driven hyporheic exchange with long residence times, facilitating the removal of nutrients by anaerobic reactions. As hydrostatic hyporheic exchange may be limited, it is important to maintain stream energy to enhance hydrodynamically driven hyporheic exchange. This may be achieved by features that create a slope in the surface water profile, like steps, or that concentrate the stream energy like j-hooks and w-weirs.

5.6.4 Scenario 4

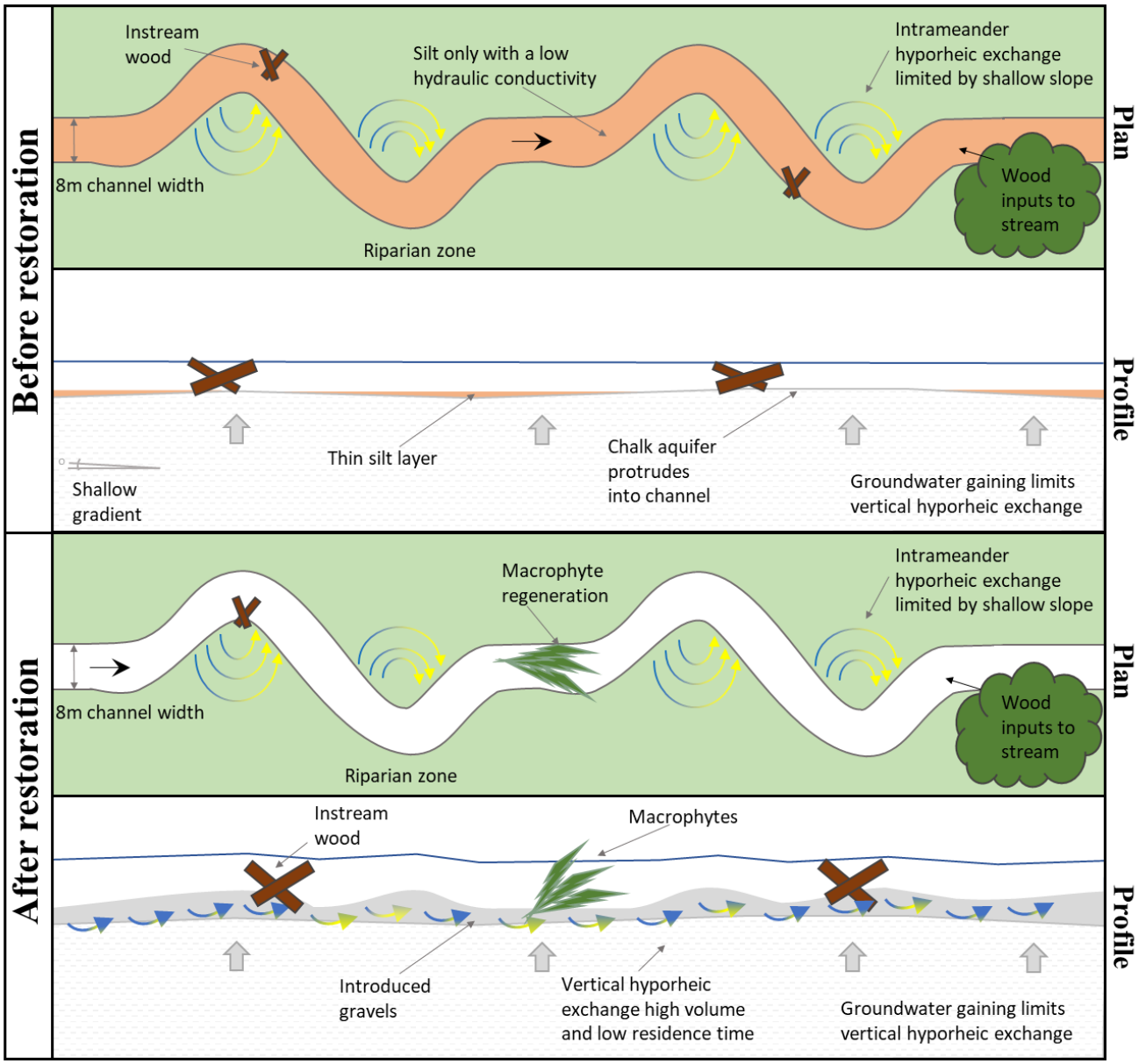


Figure 5-7. Schematic representing scenario 4. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times.

Setting

A 4th order lowland river with a low gradient of 0.1% and thin silty sediment overlying chalk bedrock. The channel is 8 m wide and gently meanders through a lowland meadow, with a thin riparian zone. The groundwater conditions are gaining and the mean discharge is 40 l/s, which responds slowly and mildly to rainfall events. Regenerative farming practices, the high porosity of the underlying chalk aquifer (which limits overland flow), and the maintenance of riparian buffer strips limit sediment inputs to the stream. Groundwaters are polluted with nutrients due to historic organic fertilizer addition which quickly percolated to the porous chalk aquifer. Wood is contributed to the stream by the riparian zone.

Pressure

The river sediments, which were originally fine and coarse gravels, have been removed by the land owner for road construction and have been replaced by a fine layer of silts which directly overlays chalk bedrock, from which groundwater is discharged. The removal of sediments is associated with the loss of instream macrophytes. Together, this has eliminated any opportunity for vertical hyporheic exchange, and only limited hydrostatic hyporheic exchange exists across meander bends because of the shallow stream gradient.

Solution

Sediments should be restored by the reintroduction of chalk gravels of high hydraulic conductivity. A thicker sediment layer is preferable, but this will likely be limited by cost and practicality. Where silt is especially thick it may be necessary to remove it first to prevent siltation of the new gravels, though some remaining silt could maintain high carbon availability and create heterogeneity in the hydraulic conductivity of the sediment, thus increasing hyporheic residence times. Macrophyte regeneration should be encouraged (which may require planting), which can generate instream storage and hyporheic exchange, and trap

fine sediments and introduce carbon, ultimately increasing biogeochemical reaction rates. Furthermore, macrophytes can remove nutrients by direct assimilation (Levi et al., 2015). As a result of this restoration, a higher volume of hyporheic exchange can be expected, but with short residence times.

5.6.5 Scenario 5

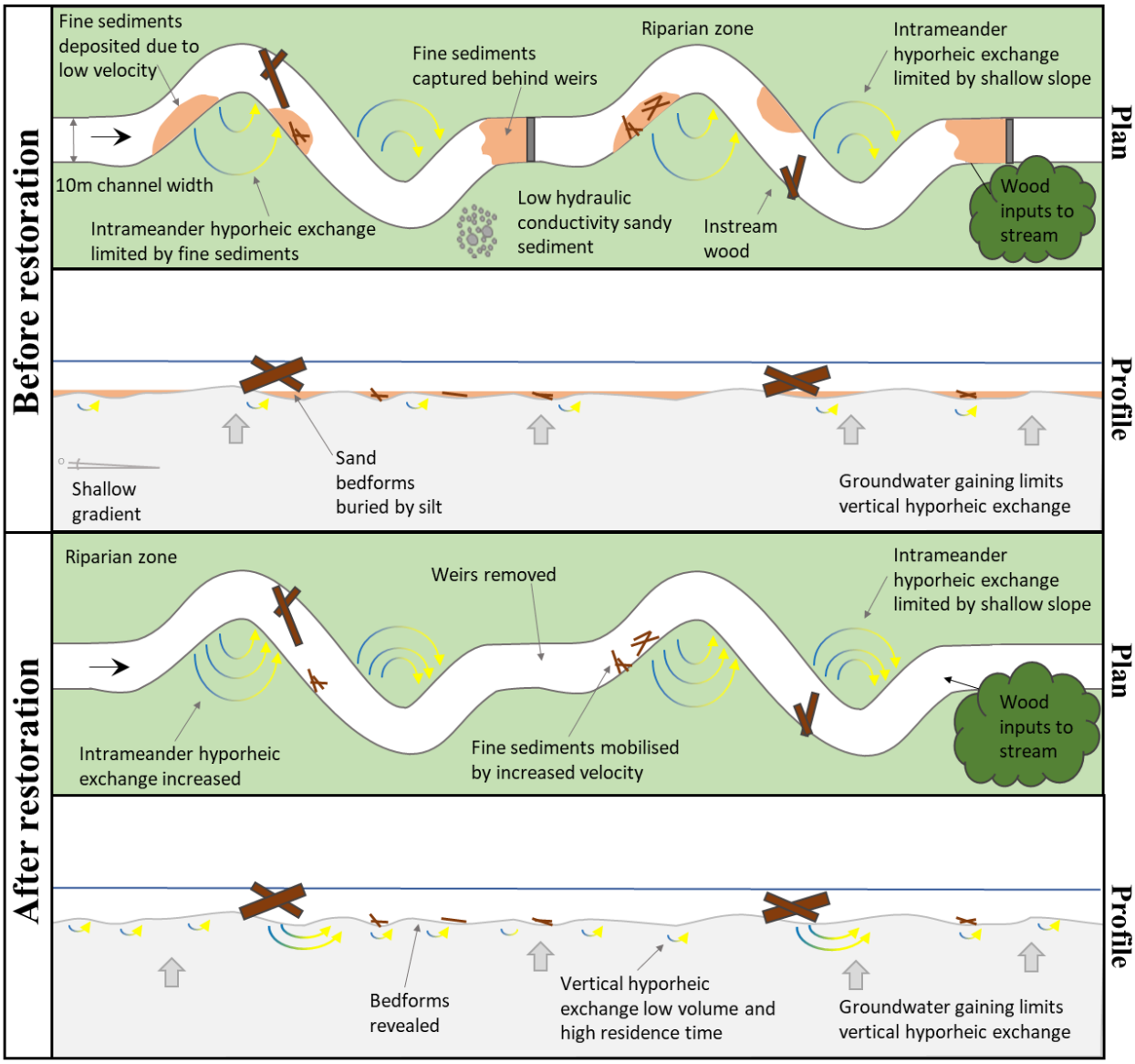


Figure 5-8. Schematic representing scenario 5. Curved arrows represent hyporheic exchange. Arrow thickness represents relative volume (i.e. change from before to after restoration) - thicker arrows represent relatively higher volume. The arrow colour represents residence time distribution, where blue represents shorter times and yellow represents longer times.

Setting

A 4th order lowland river with a low gradient of 0.04% and a sandy streambed. The channel is around 10 m wide and meanders through a wooded floodplain. The groundwater conditions are gaining and the river has a mean discharge of 50 l/s, which responds slowly and mildly to rainfall. Wood has fallen from the surrounding riparian woodland littering the bed with small features which are not long enough to span the channel-width.

Pressure

Several weirs block the channel and impound water upstream. This reduces the reach scale water velocity and the stream energy, dampening hydrodynamic hyporheic exchange. Furthermore, the low velocity encourages deposition of sediments which bury bedforms that readily form in sand streambeds, further limiting hydrodynamic forces. Hyporheic exchange is not induced by hydrostatic forces at the weirs because of the low stream gradient, which prevents the creation of a sizeable hydraulic head from upstream to downstream of the weir, and because of the gaining groundwater conditions. The result is a limited volume of hyporheic exchange.

Solution

The removal of weirs will increase the water velocity, generating hydrodynamic hyporheic exchange around existing wood features and over bedforms. The number and design of wood features may be enhanced by felling bank side trees into the stream and pinning them perpendicular to flow, in a j-hook style design. This could create patches of high velocities, driving hyporheic exchange and mobilising fine sediments. The gaining conditions are likely to limit hyporheic residence times in any case, but water quality objectives could still be realised.

5.7 Synergies and conflicts

River ecosystem processes and intrinsically intertwined and interconnected. Modifications to one process are likely to lead to changes in many aspects of ecosystem function and subsequently the delivery of river ecosystem services and dis-services (Howard et al., in prep. [chapter 3]). This presents the potential for synergies (i.e. co-benefits) and conflicts (i.e. trade-offs) between different objectives and different stakeholders (Hester and Gooseff, 2010; Alves et al., 2019). In other words, hyporheic restoration for water quality could have side-effects that are considered positive or negative by different stakeholder groups. All river restoration presents similar opportunities and risks, which river restoration professionals become adept at considering and managing (Dicks, Walsh & Sutherland, 2014). In this section, some of the more likely and most important side-effects of the restoration of hyporheic zone function are discussed. The discussion is by no means comprehensive and readers should use this section alongside the tools and methods they usually employ to aid in decision making and balancing trade-offs.

5.7.1 Natural flood management

One of the most common motivations of river restoration in the 21st century is NFM, which seeks to restore or enhance catchment processes in order to reduce the frequency and severity of floods (Dadson et al., 2017; Grabowski et al., 2019). Interventions for NFM usually aim to 'slow the flow' (i.e. increase the time it takes for water to travel downstream), which is sometimes achieved by creating instream storage, for example behind weirs and leaky dams. Similar techniques have been suggested here for the restoration of hyporheic zone function (e.g. scenario 1), especially in upland settings where most restoration for NFM is conducted. Therefore, in upland settings techniques employed for either NFM or the improvement of water quality are likely to have co-benefits for both. In lowland settings, restoration for NFM

that reduces water velocity during baseflow conditions could have negative consequences for the function of the hyporheic zone (Rana, Scott and Hester, 2017; Howard et al., in prep. [chapter 4]). However, most NFM measures are designed to only effect flow during flood, as in Figure 5-2e.

5.7.2 Ecology

The hyporheic zone was originally identified by biologists who recognized it as distinct ecotone, hosting organisms that spend some or all of their time there (named hyporheos) (Ward, 2015). Much of the research assessing the effect of river restoration on hyporheic exchange was conducted with the objective of improving this habitat, especially for fish spawning and invertebrates (Hester and Gooseff, 2010). Hyporheic restoration for ecological objectives particularly focuses on vertical hyporheic exchange, often targeting high volume and low residence time, with features like riffles and steps, which deliver high concentrations of dissolved oxygen to the hyporheic zone (Hester and Gooseff, 2010). Most hyporheic restoration measures for the improvement of water quality are likely to also improve hyporheic habitats, as a result of the increased delivery of dissolved oxygen to the subsurface (Noack, Ortlepp and Wieprecht, 2016). Sediment modifications, and other measures that reduce colmation or increase sediment hydraulic conductivity, (e.g. Figure 5-2 I and K, and scenarios 4 and 5) are likely to be especially beneficial for fish habitats. However, hyporheic restoration strategies that target hyporheic exchange of lower volume and higher residence time in replacement of hyporheic exchange of higher volume and lower residence time (e.g. Figure 5-2 m or scenario 1) may be detrimental to some ecological objectives. Furthermore, increasing hyporheic exchange volume and extent can increase the buffering of temperature and hydrological extremes, providing refuge for a range of organisms (Klaar et al., 2020). These areas of refuge may become increasingly important in enabling organisms to adapt to

climate change, for example as surface water temperatures in rivers increase (Thomas, Griffiths and Ormerod, 2015). Using wood in river restoration is likely to offer benefits for both water quality and ecology (Krause et al., 2014). On the contrary, some hyporheic restoration techniques, for example weirs, may reduce longitudinal connectivity which could disrupt ecological pathways and fluxes, for example preventing upstream fish passage.

5.7.3 Biogeochemical

The river corridor can play an important role in biogeochemical cycles, facilitating the exchange of carbon and nutrients between freshwater, terrestrial, atmospheric and marine environments. Carbon can be captured and stored in the river corridor in instream wood, vegetation, aquatic biota, sediments and in particle and dissolved forms (Wohl et al., 2017). The short-term storage of carbon could be increased by restoration measures that induce the deposition and storage of fine sediments, which may include weirs installed for the improvement of water quality (Fanelli and Lautz, 2008). Furthermore, increasing vertical and lateral hyporheic exchange may transport particulate and dissolved organic carbon deeper into river sediments and riparian soils, respectively, increasing the amount and duration of carbon storage (Wohl et al., 2017). Overall, it is likely that hyporheic restoration of the hyporheic zone for improvements to water quality has co-benefits for carbon capture, though in some cases the opposite could be true (e.g. by inducing significant fine sediment mobilisation) (Pander, Mueller and Geist, 2014).

On the contrary, hyporheic restoration for water quality improvements may have associated risks. For example, the increase in biogeochemical reaction rates that hyporheic restoration for water quality improvements seeks to achieve could lead to an increase in the production of greenhouse gases (Romeijn et al., 2019; Howard et al., in prep. [chapter 3]), which

presents a conflict given their contribution to climate change (Wohl, Lane and Wilcox, 2015). Furthermore, biogeochemical reactions in the hyporheic zone can sometimes lead an increase in the concentration of pollutants, for example by the transformation of less polluting forms to more polluting forms, as is the case for the remineralization of nutrients (Krause et al., 2011).

5.8 Discussion

To communicate insight in an actionable format it has been necessary to simplify and generalise. For example, in section 4 restoration techniques have been grouped into broad classifications which have been reviewed in isolation. However, in practice restoration programmes should usually adopt a suite of restoration strategies to create heterogeneity in the properties of hyporheic exchange, facilitating different reactions and consequently allowing the removal of different pollutants, and of pollutants which are removed in a series of reactions (Weatherill et al., 2018). Furthermore, this could allow the activation of different zones of hyporheic exchange during different flow conditions, maintaining the reach scale function of the hyporheic zone (Boano et al., 2014). This strategy could be especially useful in instances where the nature and dynamics of water quality are not well understood, for example where sampling/monitoring frequency is low, because it could maximise the potential of generating the properties of hyporheic exchange that are required to remove unknown pollutants. It is also likely to maximise the delivery of co-benefits (Alves et al., 2019).

Furthermore, restoration strategies have been further reduced to the smallest scale, by typically considering the design of individual features. However, the impact of an individual feature is likely to be negligible and restoration programmes will usually include a series of

features employed in a catchment-based approach whose cumulative effect can realise significant improvements to water quality (Rana, Scott and Hester, 2017). For example, Hester, Brooks and Scott (2018) observed only a 0.28% increase in the rate of denitrification per weir, but achieved a 25% increase using a series of weirs in a 200m reach. The scale of restoration is likely to be a critical determinant of project success. Reach-scale restoration is not usually effective at delivering water quality objectives, and instead basin or catchment scale restoration is necessary (Wohl, Lane and Wilcox, 2015). However, restoration on this scale requires significant coordination and is usually prevented by practicalities like complicated land access and ownership (Kelly and Kusel, 2015). River restoration professionals can apply this insight within practical constraints by linking up several smaller schemes to maximise the scale of restoration. This may require a level of communication between professionals and organisations which is not currently realised. Furthermore, top-down coordination of the typical bottom-up approach may be necessary to deliver larger scale restoration programmes (Koontz and Newig, 2014), but it remains unclear to whom this responsibility might reasonably fall. Boundary organisations like the River Restoration Centre in the UK (https://www.therrc.co.uk/) or the European Centre for River Restoration (https://www.ecrr.org/) in the European Union could play an important role.

The focus of this work has been on river corridor restoration strategies, i.e. those which are conducted directly in, or close to, the channel. However, other restoration techniques have the potential to deliver water quality benefits to the same or a larger extent (Azinheira et al., 2014). Reconnecting channels to their floodplains is a popular technique that has delivered water quality benefits (amongst many others) and may sometimes result in the restoration of the hyporheic zone, for example by increasing lateral hyporheic exchange (Azinheira et al., 2014; Wohl et al., 2021). The rewooding of riparian zones could offer benefits for water quality, hosting hot spots and hot moments of reactions, and increasing hyporheic exchange

(Vidon et al., 2010; Goeller et al., 2019). It could also eventually lead to an increased instream wood loading which is likely to increase hyporheic exchange (Kasahara and Wondzell, 2003). Passive management methods, such as rewilding, are likely to lead to the long-term restoration of the river corridor and the hyporheic zone, although these could take a long time to be realised. Allowing natural dynamics, for example in wood and sediment transport, to shape the evolution of the river corridor is probably the best strategy in delivering long-term, persistent improvements to water quality with minimum input (Krause et al., 2014). For example, the re-introduction of beavers (*Castor fiber*) in the UK could lead to the development of many of the restoration techniques described in this work (Wade et al., 2020; Larsen, Larsen, and Lane, 2021). Ultimately, employing a variety of techniques and approaches to river restoration is the best strategy in maximising the chance of success, especially given the uncertainty surrounding the relative efficacy of different restoration techniques (Palmer et al., 2008).

Nature-based solutions, like restoring the function of the hyporheic zone, are a critical strategy in delivering widespread and long-term improvements to water quality. However, they are only one component of what must be a larger programme of efforts (Haas, Guse and Fohrer, 2017). Water quality objectives will not be achieved without significant reductions in the inputs of pollutants to freshwater bodies, including surface waters and groundwaters (Wohl, Lane and Wilcox, 2015). This may be achieved by tightening regulation to reduce the use and application of potential pollutants, for example inputs of nutrient fertiliser and pesticides to agricultural fields. It may also be achieved by reducing the transport of pollutants to freshwater bodies, for example by increasing retention of fertilizers on fields by using cover crops (Couëdel et al., 2018).

5.9 Conclusions

This work aimed to synthesise and distil the substantial body of research which investigates the restoration of the hyporheic zone for improvements to water quality, communicating its complex insights in an actionable format for the first time. These insights can guide river restoration professionals in delivering more effective restoration programmes that are based on evidence. It is communicated in design considerations and scenarios that are centred around process-based restoration, seeking to re-establishing and enhance the function of the hyporheic zone to harness its capacity to improve water quality. These guidelines allow the application of process-based principles to the improvement of water quality, which until now has been difficult due to the paucity of actionable insight available to professionals.

Despite decades of research and practice, and increasing commitment of resources, there is little evidence to suggest that river restoration is effective at delivering on water quality objectives or worse than this, that it is becoming increasingly effective (Palmer, 2009). The success of restoration is not limited by scientific understanding of ecosystem processes and function (Wohl, Lane and Wilcox, 2015). Instead it is limited by the failure to apply this understanding in practice (Palmer, Filoso and Fanelli, 2014). For example, whilst we understand that effective restoration focusses on processes, which are often invisible (as in hyporheic exchange), a substantial proportion of restoration is still conducted primarily for cosmetic and aesthetic purposes (Beechie et al., 2010; Wohl, Lane and Wilcox, 2015). It is important that researchers continue to improve our understanding of the processes and mechanisms that underpin ecosystem function and the delivery of ecosystem (dis)services. However, if this work is to have real-world impact, it is equally important that researchers commit the same energy, if not more, to ensuring this work is acted upon. This requires researchers to communicate insight from their work in a format that is actionable, or to work closely with those who are able to do so, such as boundary organisations. Similarly, we must

capitalise on the opportunity to learn from the many restoration programmes that are designed and implemented annually. Fatally few of these programmes are currently monitored or evaluated – a missed opportunity which surely contributes to the lack of improvement of river restoration schemes.

Poor water quality remains a persistent and pervasive problem, degrading ecosystems, worsening water scarcity and threatening the success of our global society. Whilst recognition of the importance of water quality is improving, actions and governance to manage water quality are still lacking in comparison to those to manage water quantity, which manifests in more visible events such as floods and droughts (Hannah et al., 2022). The importance of water quality for society must be elevated through education and by increasing public engagement with water quality challenges and solutions. Water quality and water quantity are not separate challenges – they are intrinsically intertwined in the processes that control them. Therefore, the successful management and governance of water resources requires an integrated approach that acknowledges this co-dependency.

6 Conclusions and Outlook

The work presented in this thesis advances our understanding of the effects of instream wood restoration on the coupled hydrological and biogeochemical processes that control the function of the hyporheic zone. This improved understanding has allowed an evaluation of the potential of instream wood restoration to provide a nature-based solution to water quality challenges, in particular nutrient pollution. Furthermore, some of the works can be used to promote the uptake of this and other research into process-based river restoration design and practice, which could elicit an improvement in the efficacy of restoration for water quality objectives.

6.1 Is instream wood restoration an effective nature-based solution to nutrient pollution?

It was hypothesised that instream wood restoration could provide a nature-based solution by enhancing the function of the hyporheic zone, primarily by abating two key limitations: **(**1) reaction kinetics and (2) physical transport. Chapter 3 invetigated limtation (1) using a microcosm experiment to test the effects of introducing instream wood to a range of river sediments and under a range of temperature representative of a typical temperate climate. It showed that wood more than doubled microbial metabolic activity – a keystone ecosystem process - in every sediment type. This change to MMA is likely to influence many biogeochemical cylces and associated ecosystem (dis)services. For example, here nitrate removal rate – an ecosystem service – and green-house gas production – an ecosystem dis-service – were both increased in the presence of wood additions. These results demonstrate

the importance of wood for streambed biogeochmical cyling and show that using wood in river restoration is associated with both risks and opportunities that must be considered, and that omitting or neglecting to consider streambed wood in mechanistsic studies probably leads to less representative global models of carbon and nitrogen.

Chapter 4 was concerned with addressing limitation (2). Although several studies have demonstrated that instream wood restoration can increase transient storage in upland environmental settings, the results reported here suggest that the opposite could be true in lowland environmental settings. Following the introduction of wood features, a reduction in transient storage was observed, accompanied by an increase in the residence time of water in transient storage zones. Furthermore, these patterns were exaggerated by a higher discharge, i.e. an increase in discharge led to an increase in the effects of instream wood on transport and storage. These results suggest that the environmental setting determines the effects of instream wood, which may enable improved designs of restoration to maximise useful properties of transient storage.

Considering the new insight of these works collectively with those in the literature, it can be concluded that only in some circumstances could instream wood restoration provide a nature-based solution to water quality challenges. As with most river restoration, identifying suitable locations for restoration is probably the most important determinant of success. Furthermore, restoration, whether wood-based or otherwise (e.g. geomorphic or grey infrastructure), should be designed to suit the environmental setting and to target existing process controls. River restoration professionals are experts at this process, so with some facilitation – such as accessible published guidance and professional workshops – it is very plausible that hyporheic restoration, using instream wood or otherwise, could be widely adopted into process-based river restoration.

6.2 Future research directions

Chapter 3 demonstrates the impacts of streambed wood on biogeochemical cycling in rivers. However, microcosm experiments are criticised for not sufficiently representing *in-situ* conditions and therefore not providing quantitative results that can be applied to real world applications. For example, in this study diffusion-limited transport processes in the microcosms do not properly represent the delivery of reactants and generation of physiochemical conditions (e.g. anaerobia) that are likely in real streambeds, which are also subject to pressure driven transport. Future research should seek to quantify the effects of streambed wood on biogeochemical cycles in real systems and thus provide quantitative results that can be used in global models of carbon and nitrogen, for example. In section 3.2 several mechanisms are identified by which streambed wood may affect biogeochemical cycles. However, only the contribution of wood to the total carbon stock was investigated here, which probably explained some of the changes but evidence of other mechanisms was evident, for example driving a higher than expected MMA and nitrate removal rate at the lowest temperature (0.8 ^{o}C in December 2020). Future research should seek to identify and quantify the effects of these mechanisms, for example wood-induced changes to the hydraulic conductivity of sedminet as a result of preferential flowpaths, and the contribution of different microbial species from wood additions. Additionally, research should seek to track development and evolution of the dominant microbial communities which may change as a result of streambed wood introduction.

Results reported in chapter 4 suggest that the effect of instream wood is determined by the environmental setting, which is considered in terms of lowland and upland. Some research (e.g. Krause et al., 2022) has begun to identify the organizational principles of the drivers of hyporheic exchange at a landscape scale. However, further research is needed to understand how instream wood, and other features of a similar scale (e.g. geomorphic features), interact

with larger scale drives to determine the properties of transient storage and hyporheic exchange on the reach scale. Furthermore, more work is necessary to understand how the architecture and arrangement of instream wood features determine their effect on transient storage in different settings, which would allow instream wood features to be designed specifically to compliment environmental drivers and controls.

6.3 Cautions on reductionism and generalisation

This work has explicitly aimed to promote the uptake of research into policy and practice, and it has advocated for evidence-based action. However, elements of this work have highlighted some of the risks of the improper application of research which may have occurred in the case of using instream wood in river restoration. These risks can be exacerbated by the approach in which research is conducted or by the way in which it is applied, for example by reductionism and generalisation.

Reductionism in science is a valid and useful method to interpret complex phenomena by reducing them to simpler constituents. For example, the microcosm experiment presented in chapter 3 reduced coupled biogeochemical and hydrological processes that control microbial metabolic activity *in situ* to focus on the biogeochemical processes only - other biogeochemistry studies often reduce processes to even simpler components, for example considering only one phase state or element. The reductionist approach creates an over-simplification that does not fully represent real systems, yet its insights are sometimes used to directly inform environmental policy and management without considering other evidence, for example evidence of potential ulterior impacts. This could lead to the failure to consider important risks and ultimately result in policies that, on balance, do more harm than good. For example, instream wood restoration is now widely encouraged with many schemes citing

ill-defined objectives and delivering few ecosystem services, yet probably contributing to the increased production of GHG in the river corridor. A more reductionist approach probably increases the vulnerability of work to mis-application, i.e. to being cherry-picked to support an argument. Research that is intended to inform policy and/or practice should adopt the least reductionist approach possible (i.e. most holistic) to deliver its objectives within practical constraints, e.g. by measuring potential ulterior impacts other than only the impact of interest. In lieu of measuring all potential ulterior impacts, measuring keystone ecosystem processes (e.g. microbial metabolic activity in river biogeochemistry) may allow researchers, and users of research, to hypothesise about likely ulterior impacts (e.g. GHG production).

Generalisation is similarly useful and necessary in extrapolating research insight beyond its typically limited scope (e.g. reach-scale), affording wider application. However, it can also result in the application of research insights to inappropriate contexts. For example, whilst the majority of research on the impact of instream wood restoration on transient storage has been conducted in upland environmental settings, most instream wood restoration is conducted in lowland environmental settings. As work in chapter 4 revealed, the impact on transient storage is probably opposite in these settings. As a primary control of the capacity of the river corridor to retain and transform pollutants, the mis-application of research insight in this context could lead to negative consequences for water quality. This highlights the necessity to conduct research in the environmental setting in which it is intend to be applied, and to thoroughly characterise and describe this setting. This may be especially important in river systems, where ecosystem processes are often controlled more by the environmental setting than by management interventions.

6.4 “This has implications for policy and practice”

Whilst the primary aim of applied science is the attainment of practical goals, for example by informing practice or policy, applied researchers often commit limited effort to facilitating these aims, and take little responsibility for the way in which their research is applied. This is evident in the extent to which researchers address this in research outputs. Highlighting the implications of research is sometimes a tick-box activity; a sentence bolted on to the end of a research paper: “This has implications for policy and practice”. We must do more. What are the implications exactly, what contexts and settings do they relate to, and how should they be applied? Researchers should address these questions in a short paragraph (like in section 3.5.6), and publishers and journals could request this information in a section similar to the ‘data availability’ or ‘author contributions’ sections that are often included. Furthermore, researchers should consider how research insights can be applied within the legal and practical constraints in which professionals must work. For example, research insight suggests that river restoration should be conducted on a catchment scale, but this is usually not possible. Instead, this insight could be framed to suggest that the scale of river restoration should be maximised by linking up individual projects.

6.5 Accelerating evidence acquisition

Nature-based solutions deployed throughout river networks are a vital strategy in delivering the scale and longevity of improvements to water quality that are necessary for long-term water security. It is essential, however, that the efficacy of potential nature-based solutions is rigorously investigated, and that programmes are designed and deployed based on an objective assessment of sufficient evidence which considers potential risks. Demonstration sites, which provide examples of how nature-based solutions can work in different settings

and to abate a range of pressures, are necessary to evidence efficacy and to be used as templates to inform professionals.

Presently, an enormous opportunity is squandered which could accelerate the acquisition of this evidence base. Thousands of river restoration projects are planned and implemented each year, requiring substantial investment of resources, yet few are monitored to evaluate their outcomes or efficacy. Capitalising upon this opportunity to learn presents the most direct basis by which to acquire the necessary evidence base, but it would require significant coordination on a level not yet realised. Researchers and professionals must collaborate to leverage their complementary skills and resources. Standard methodologies by which to thoroughly evaluate the efficacy of restoration schemes (for various objectives) in specific environmental settings must be developed. In this way, results from evaluations of individual programmes, which by themselves may offer limited insight (e.g. as a result of before-after or control-impact only experimental designs), can be amalgamated into a dataset with real statistical power (as described in section 4.6.3). Some schemes (e.g. EU RiverWIKI, 2022) attempt similar missions but fail because of the lack of method standardisation, quality control and coordination.

To further increase the proportion of river restoration schemes receiving scientifically rigorous evaluations of success, work is necessary to reduce the minimum required 'buy-in' or investment of time and resources. This could be achieved by reconsidering the quality of data which is necessary to achieve the aims of evaluations (such as the DQO in section 2.2), which are often to assess relative efficacy of a restoration technique (i.e. compared to another restoration technique) or relative improvements from a previous condition. To achieve these aims, and provide information of practical benefit, a different standard of data may be required from that which may be required in pure scientific research. This could allow data be

collected via alternative (cheaper) methods, such as citizen science and distributed low-cost sensor networks.

6.6 Integrating planetary and public health

As we have seen, managing complex systems requires dissecting them into smaller constituents that can be more easily considered: planetary or public health, aquatic pollution or atmospheric pollution, water quantity or water quality. However, in doing so we often overlook the interconnectedness and co-dependency of these systems. We fail to consider the inevitable impacts of our actions beyond the system and location we are presently focussed, inexorably pushing problems out of the frying pan and into the fire. As well as water scarcity, numerous significant environmental and anthropogenic challenges threaten our global society. Only by stakeholder and disciplinary integration, which consider planetary and public health holistically, will we overcome these challenges and contribute to a better world for everyone.

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8 Supporting Information

8.1 Chapter 2

8.1.1 Storage temperature ranges

In all sample preservation methods, samples were stored in the dark and were undisturbed between analysis periods and temperatures were monitored at 10-minute intervals using a Tinytag Aquatic 2 temperature logger (Gemini Data Loggers Ltd, Chichester, United Kingdom). Samples were stored at three different temperatures: room temperature (mean, 20.49 °C, min 18.43 °C, max 24.37 °C); refrigerator (mean 5.33 °C, min 2.36, max 9.05 ° C); freezer (mean -20.40 °C, min -22.3 °C, mac -15.85 °C). Averages and ranges were calculated whilst omitting the first and last 48 hours of the storage period, when loggers were acclimatising. Whilst storage temperatures fluctuated, the average temperatures in each storage methods are distinctly different, and represent typical lab storage conditions.

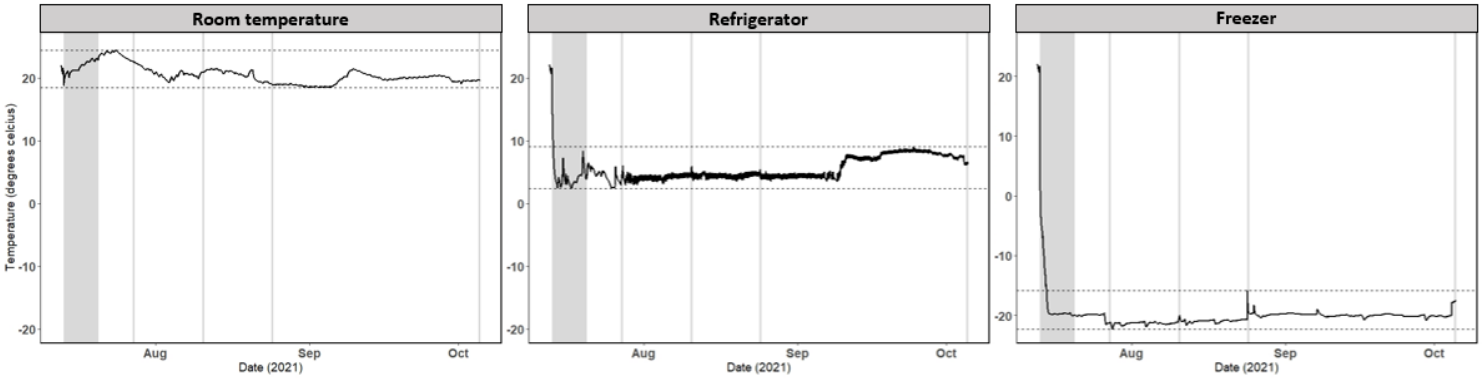


Figure 8-1. The air temperature (degrees Celsius) of storage methods room, refrigerator and freezer, over the experimental period. The grey vertical highlighted areas represent sampling days with the widest area in July representing the first week of analysis where samples analysed in 24-hour intervals. The horizontal dashed lines represent the minimum and maximum temperatures, omitting the first 48 hours and final 24 hours.

8.1.2 Using one set of calibration data for every analysis period

Fluorometry instruments are particularly sensitive to external and internal conditions, such as room temperature and humidity, sensitivity of detectors and the intensity of the light source. This leads to variation in the performance of these instruments depending on these conditions, affecting the measured fluorescence intensity of analytes. As such, it is usually recommended that instruments should be (re)calibrated before every analysis period to reduce instrumental error, and that standard samples should be analysed at regular intervals to account for instrument drift. Whilst this provides improved accuracy for the measurement of analytes in one analysis period, it does not necessarily improve the accuracy between different analysis periods. As resazurin and resorufin are unstable when stored, new standards must be prepared at each analysis period. This introduces significant potential for human error in the preparation of standards. Minor differences in concentration in the same standard at different analysis periods could lead to significant differences in the calibration equations calculated from these standards. In this experiment, calibration procedures were conducted at the start of every analysis period. However, calibration information from only one calibration procedure (on the day the samples were prepared) was used to calculate concentrations from fluorescence signals because it was deduced that the potential for human error in the preparation of standards was higher than the error introduced by instrumental differences.

Table 8-1. Linear regression equations for resazurin and resorufin calculated using 4-standard solution concentrations prepared on the day of analysis.

Analysis period (days)	Resazurin	Resorufin
0	X=(Y-0.8676)/0.8213	X=(Y-12.63)/19.025
1	X=(Y-0.0012)/0.8535	X=(Y-1.8352)/17.913
2	X=(Y-1.8098)/0.7971	X=(Y+0.6145)/19.327
3	X=(Y-1.0709)/0.8453	X=(Y-5.53)/19.723
6	X=(Y+0.6535)/0.7911	X=(Y-4.417)/18.322
7	X=(Y-0.7396)/0.8267	X=(Y-1.7866)/19.352
14	X=(Y-1.1111)/0.7548	X=(Y-1.342)/19.329
28	X=(Y-0.7877)/0.8346	X=(Y-17.229)/17.551
42	X=(Y-0.2336)/0.8263	X=(Y-0.1716)/15.312
84	X=(Y-1.6903)/0.7888	X=(Y-1.9537)/22.01

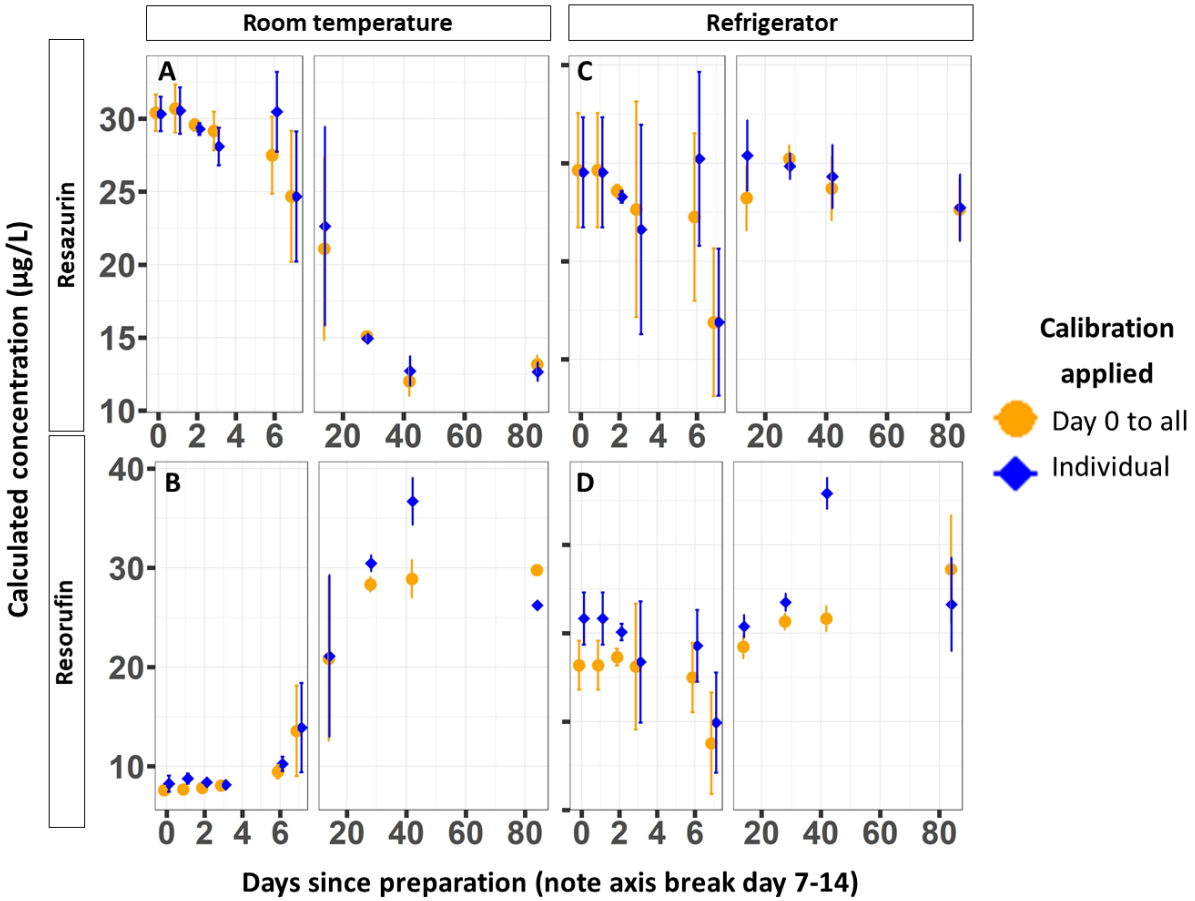


Figure 8-2. The concentration of resazurin and resorufin in low concentration mixtures calculated using calibration information collected on the first day of the experiment (orange circles) and collected on the day of analysis (blue diamonds).

8.1.3 Calibration in different water types

Several characteristics of the matrix (medium) that fluorescent compounds are dissolved in control the intensity at which they fluoresce, for example water chemistry and temperature. It is, therefore, usually recommended that fluorometry instruments are calibrated using standard solutions prepared in the same matrix as the samples that will be analysed and under the same conditions. This ensures that the fluorescence intensity of the compound is the same in the standards and samples and, therefore, that the absolute concentration of the compound in the samples can be calculated. In many hydrologic applications of the resazurin-resorufin technique this is not possible, for example where samples are to be extracted from surface waters and hyporheic waters in the same experiment. This method may present challenges when samples are not analysed immediately, as in this storage experiment, because it would require larger volumes of each water type to be stored, which are also likely to change during the storage period and before standards are prepared. Here, calibrations of resazurin (Fig. 2-5A) and resorufin (Fig. 2-5 B) were conducted using standards prepared in different water types before the samples were prepared. Only minor differences in the fluorescence intensity, and calculated calibration equations, were observed between different waters (Fig. 2-5). Therefore, in this experiment, standards prepared using deionised water only were using in the calibration of the spectrofluorometer.

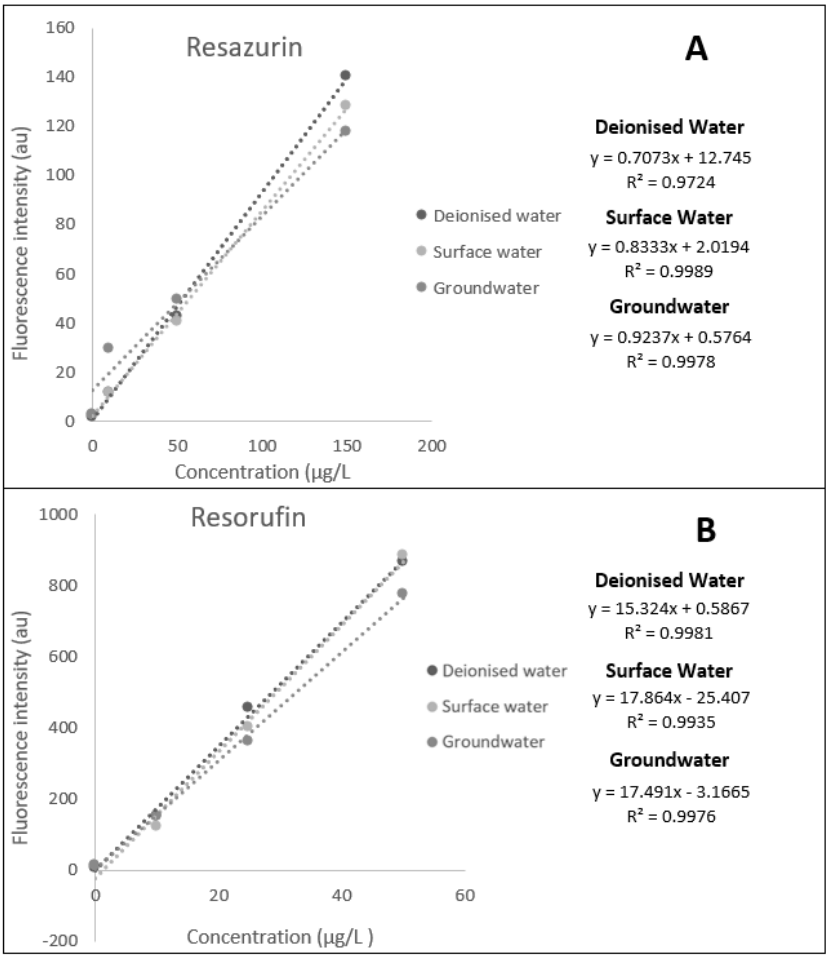


Figure 8-3. Standard solutions of resazurin (A) and resorufin (B) prepared in different water types, deionised water (dark grey), surface water (light grey) and groundwater (grey). Linear regression equations are presented.

8.1.4 Percentage change data

Table 8-2. Mean percentage change (n=3) from starting concentration (day 0) for rru (A) and raz (B) over time (1 – 84 days). DI = deionised, GW = groundwater and SW = surface water. Cells containing "-" indicate where samples were not analysed.

A. Rru

Days	Room temperature			Refrigerator			Freezer		
	DI	**GW**	**SW**	**DI**	**GW**	**SW**	**DI**	**GW**	**SW**
1	2.39	1.99	22.56	-2.74	2.62	-0.30	3.46	-2.09	-1.98
2	4.56	-1.33	34.18	-0.21	-6.22	13.36	-	-	-
3	7.73	54.70	50.59	-2.82	-9.45	18.10	-	-	-
6	25.90	19.09	82.30	-5.97	3.81	23.19	-	-	-
7	80.02	22.96	104.87	-24.38	-10.18	24.56	-	-	-
14	117.38	49.21	141.35	2.90	3.98	33.33	3.36	-22.05	9.88
28	273.79	85.21	172.29	10.04	-6.82	55.15	4.41	-2.08	38.05
42	281.45	101.14	77.45	11.12	10.95	61.72	-1.46	3.36	46.89
84	292.75	162.50	59.90	25.84	41.51	96.73	-2.63	5.35	41.95

B. Raz

Days	Room temperature			Refrigerator			Freezer		
	DI	**GW**	**SW**	**DI**	**GW**	**SW**	**DI**	**GW**	**SW**
1	2.37	-2.42	-4.61	-0.85	0.09	1.27	2.83	2.19	-6.88
2	-2.10	-5.93	-9.49	-5.10	-4.52	-0.51	-	-	-
3	-4.10	5.20	-12.69	-8.27	-9.50	5.20	-	-	-
6	-12.89	-16.09	-30.22	-9.27	-7.28	-7.84	-	-	-
7	-31.13	2.08	-39.66	-26.68	-18.38	-12.26	-	-	-
14	-36.87	10.23	-53.16	-6.93	-1.81	-14.60	-7.26	-28.94	-12.07
28	-94.50	6.15	-67.10	-0.45	-10.59	-11.09	-1.13	2.48	0.84
42	-100.00	25.19	-73.80	-6.24	1.33	-21.14	-4.73	-11.65	-16.09
84	-100.00	46.23	-70.35	-12.37	5.33	-35.86	-5.05	-5.02	-7.99

Table 8-3. Mean percentage change (n=3) from starting concentration (day 0) for rru (A) and raz (B) over time (1 – 84 days). DI = deionised, GW = groundwater and SW = surface water. Cells containing "-" indicate where samples were not analysed. Cells containing "\" indicate where the ULQ was exceeded.

A. Rru

Days	Room temperature			Refrigerator			Freezer		
	DI	**GW**	**SW**	**DI**	**GW**	**SW**	**DI**	**GW**	**SW**
1	2.39	6.73	\	3.11	2.41	2.03	-1.30	-3.48	3.65
2	1.69	7.83	\	1.95	5.05	-	-	-	-
3	2.98	12.44	\	6.49	1.65	-10.14	-	-	-
6	\	19.43	\	-15.09	6.79	-1.83	-	-	-
7	\	22.08	\	-27.57	0.98	\	-	-	-
14	\	55.81	\	6.62	3.29	\	-1.26	-3.48	\
28	\	65.16	\	4.98	4.91	\	4.96	-5.25	\
42	\	64.87	\	-2.03	13.66	\	0.61	2.26	\
84	\	\	\	-26.87	42.95	\	-9.75	3.88	\

B. Raz

Days	Room temperature			Refrigerator			Freezer		
	DI	**GW**	**SW**	**DI**	**GW**	**SW**	**DI**	**GW**	**SW**
1	6.57	-1.80	\	6.59	8.39	-0.07	6.67	1.29	-0.05
2	2.17	-2.17	\	3.67	5.81	-	-	-	-
3	0.35	-7.62	\	5.68	3.54	-21.81	-	-	-
6	\	-15.54	\	-12.84	0.56	-26.90	-	-	-
7	\	-5.30	\	-26.99	1.87	\	-	-	-
14	\	3.96	\	6.17	6.28	\	0.66	-3.40	\
28	\	13.53	\	7.31	12.73	\	7.75	1.48	\
42	\	16.45	\	-1.97	9.20	\	3.39	-1.75	\
84	\	\	\	-32.91	12.33	\	-5.50	-0.61	\

8.1.5 Statistical analysis

Table 8-4. Details of the statistical tests conducted, and associated outputs, to investigate the differences between storage methods. Storage indicates the storage method: R = room temperature, C = cooled, F = frozen. Normal indicates normality of data, tested with Shapiro-Wilk tests. Test indicates the statistical test conducted which was based on the number of storage types compared: KW = Kurskal-Wallis, T = students T-test, WSR = Wilcoxon signed-rank, ANOVA = analysis of variance. Df indicates degrees of freedom. Mean sq. Test stat. indicates the test statistic associated with the statistical test conducted. Sig. indicates whether the statistical test reported statistical significance (yes) or not (no).

A. Rru

Day	Storage	Normal	Test	Df	Mean sq.	Test stat.	Test stat. value	P value	Sig.
1	R,C,F	Yes	ANOVA	2	317.4	F	4.375	0.0258	Yes
2	R,C	Yes	T	11.698	\	t	-1.464	0.1695	No
3	R,C	No	WSR	\	\	V	8	0.02051	Yes
6	R,C	No	WSR	\	\	V	11	0.02813	Yes
7	R,C	Yes	T	11	\	t	-3.6437	0.003863	Yes
14	R,C,F	Yes	ANOVA	2	25391	F	21.57	1.02E-05	Yes
28	R,C,F	Yes	ANOVA	2	57300	F	22.75	5.54E-06	Yes
42	R,C,F	No	KW	2	\	Chi sq.	9.485	0.008717	Yes
84	R,C,F	Yes	ANOVA	2	41384	F	10.92	0.000561	Yes

B. Raz

Day	Storage	Normal	Test	Df	Mean sq.	Test stat.	Test stat. value	P value	Sig.
1	R,C,F	No	KW	2	\	Chi sq.	1.635	0.4415	No
2	R,C	Yes	T	13.997	\	t	1.2696	0.2249	No
3	R,C	No	WSR	\	\	V	39	0.5358	No
6	R,C	Yes	T	13.995	\	t	2.9923	0.0097	Yes
7	R,C	No	WSR	\	\	V	32	1	No
14	R,C,F	No	KW	2	\	Chi sq.	0.6021	0.74	No
28	R,C,F	Yes	ANOVA	2	4818	F	6.37	0.00688	Yes
42	R,C,F	Yes	ANOVA	2	2882	F	2.32	0.123	No
84	R,C,F	Yes	ANOVA	2	1732	F	1.019	0.378	No

Table 8-5. Results of Tukey post-hoc tests for pairwise analysis conducted on significant ANOVA results only.

Compound	Day	Comparison	Diff.	Lower	Upper	P value	Sig.
Raz	28	COOLED-FROZEN	-8.13	42.79	26.54	0.8265	No
		ROOM-FREEZER	-45.98	-80.65	-11.32	0.0083	Yes
		ROOM-COOLED	-37.86	-72.52	-3.20	0.0307	Yes
Rru	1	COOLED-FROZEN	-0.66	-11.39	10.08	0.9870	No
		ROOM-FREEZER	10.57	-0.17	21.30	0.0542	No
		ROOM-COOLED	11.22	0.49	21.96	0.0394	Yes
Rru	14	COOLED-FROZEN	19.21	-24.19	62.61	0.5134	No
		ROOM-FREEZER	110.23	65.31	155.15	0.0000	Yes
		ROOM-COOLED	91.02	46.10	135.94	0.0001	Yes
Rru	28	COOLED-FROZEN	5.39	-57.86	68.64	0.9749	No
		ROOM-FREEZER	149.21	85.96	212.45	0.0000	Yes
		ROOM-COOLED	143.82	80.57	207.06	0.0000	Yes
Rru	84	COOLED-FROZEN	39.66	-37.93	117.24	0.4170	No
		ROOM-FREEZER	139.58	61.99	217.16	0.0005	Yes
		ROOM-COOLED	99.92	22.33	177.50	0.0103	Yes

Table 8-6. Results of the Wilcoxon signed-rank tests conducted to identify when tracer concentration was statistically different to starting concentrations.

Room							
	Raz				**Rru**		
Day	**V**	**P**	**Sig.**	**Day**	**V**	**P**	**Sig.**
1	26	0.3125	No	1	4	0.05469	No
2	36	0.007813	Yes	2	8	0.1953	No
3	23	0.5469	No	3	0	0.007813	Yes
6	36	0.003481	Yes	6	0	0.007813	Yes
7	30	0.1094	No	7	0	0.007813	Yes
14	22	0.2188	No	14	0	0.01563	Yes
28	32	0.05469	No	28	0	0.007813	Yes
42	25	0.3828	No	42	4	0.05469	No
84	15	0.7422	No	84	0	0.007813	Yes
Cooled							
	Raz				**Rru**		
Day	**V**	**P**	**Sig.**	**Day**	**V**	**P**	**Sig.**
1	16	0.8438	No	1	22	0.6406	No
2	26	0.3125	No	2	14	0.6406	No
3	13	0.9375	No	3	7	0.2969	No
6	33	0.03906	Yes	6	5	0.07813	No
7	36	0.007813	Yes	7	17	0.9453	No
14	29	0.1484	No	14	0	0.007813	Yes
28	29	0.1484	No	28	6	0.1094	No
42	28	0.1953	No	42	0	0.007813	Yes
84	26	0.3125	No	84	0	0.007813	Yes
Frozen							
	Raz				**Rru**		
Day	**V**	**P**	**Sig.**	**Day**	**V**	**P**	**Sig.**
1	15	0.7422	No	1	16	0.8438	No
2	\	\	\	2	\	\	\
3	\	\	\	3	\	\	\
6	\	\	\	6	\	\	\
7	\	\	\	7	\	\	\
14	31	0.07813	No	14	17	0.9453	No
28	17	0.9453	No	28	7	0.1484	No
42	36	0.007813	Yes	42	5	0.07813	No
84	33	0.03906	Yes	84	6	0.1094	No

8.2 Chapter 3

8.2.1 Water temperature

Only minor differences were observed in the mean water temperature during the measurement period (measured at the start and the end) between different treatments of sediment and wood. Variability within groups is most likely explained by the minor differences in measurement time (maximum 100 minutes difference) that resulted from practicalities of the experimental design.

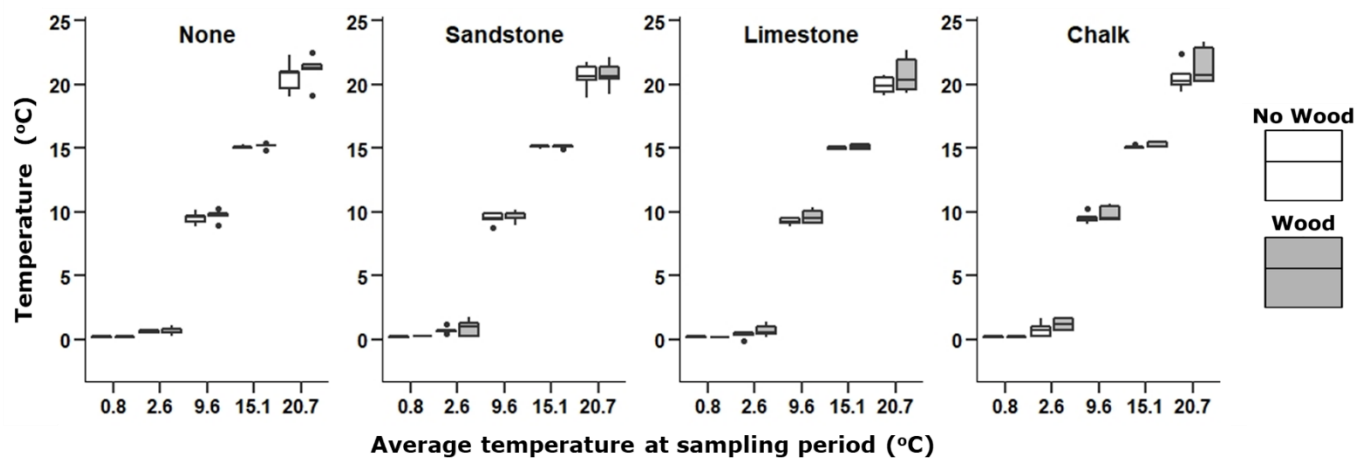


Figure 8-4. Water column temperature at five measurement periods (represented by mean water temperatures on x axis) in microcosms containing different treatments of sediment and wood (no wood = white infill, wood = grey infill).

8.2.2 Grain size distribution

Sieve fractionation was used to determine grain size distribution, using seven size classes from 0.063 mm to >8mm (Day, 1965). Fractionation was conducted after the removal of material >2cm which would have occupied a disproportionally large volume of the microcosms. At decommission of the microcosms, sediments of the same geology were

homogenised and three 500ml samples were analysed by sieve fractionation, before a mean average was calculated. Sandstone sediments are the finest and chalk are coarsest.

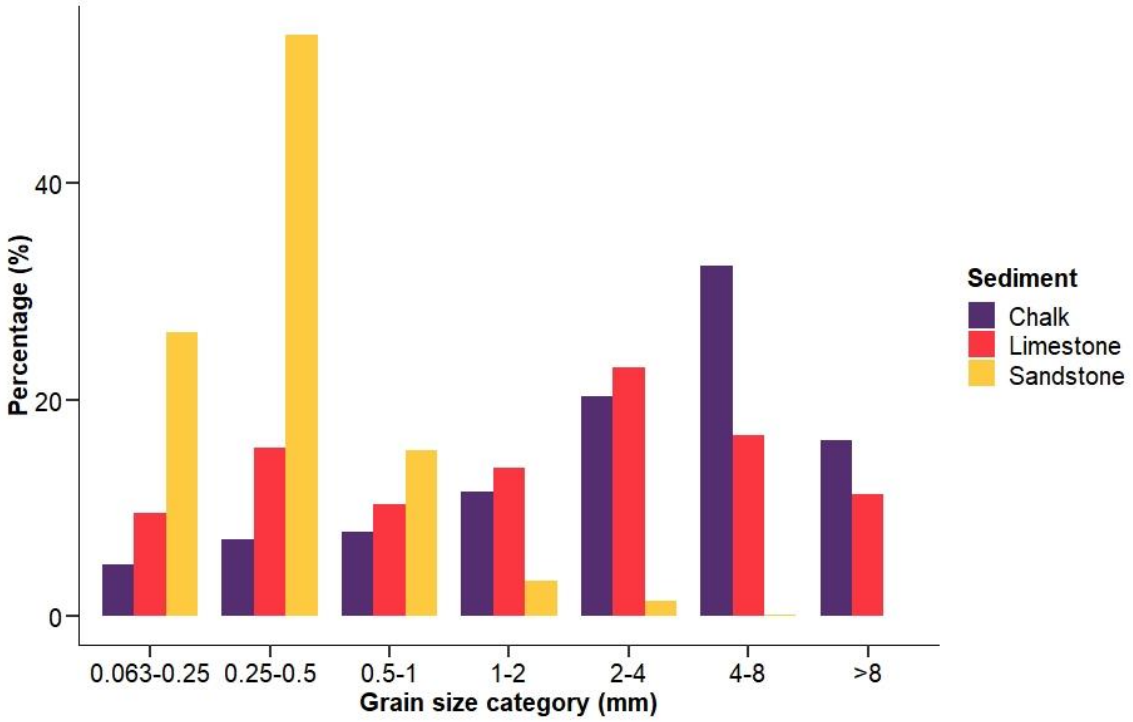


Figure 8-5. Grain size distribution of 3 sediment types.

8.2.3 Loss on Ignition

LOI was conducted following methods by Hoogsteen et al. (2015). Minor differences in LOI 550 ^{o}C and LOI 950 ^{o}C were observed within sediment treatments, suggesting it was not much affected by the prior presence of wood during the experimental period. Sandstone had the highest LOI 550 ^{o}C and the lowest LOI 950 ^{o}C, whilst limestone had the lowest LOI 550 ^{o}C. Limestone and chalk were very similar in LOI 950 ^{o}C.

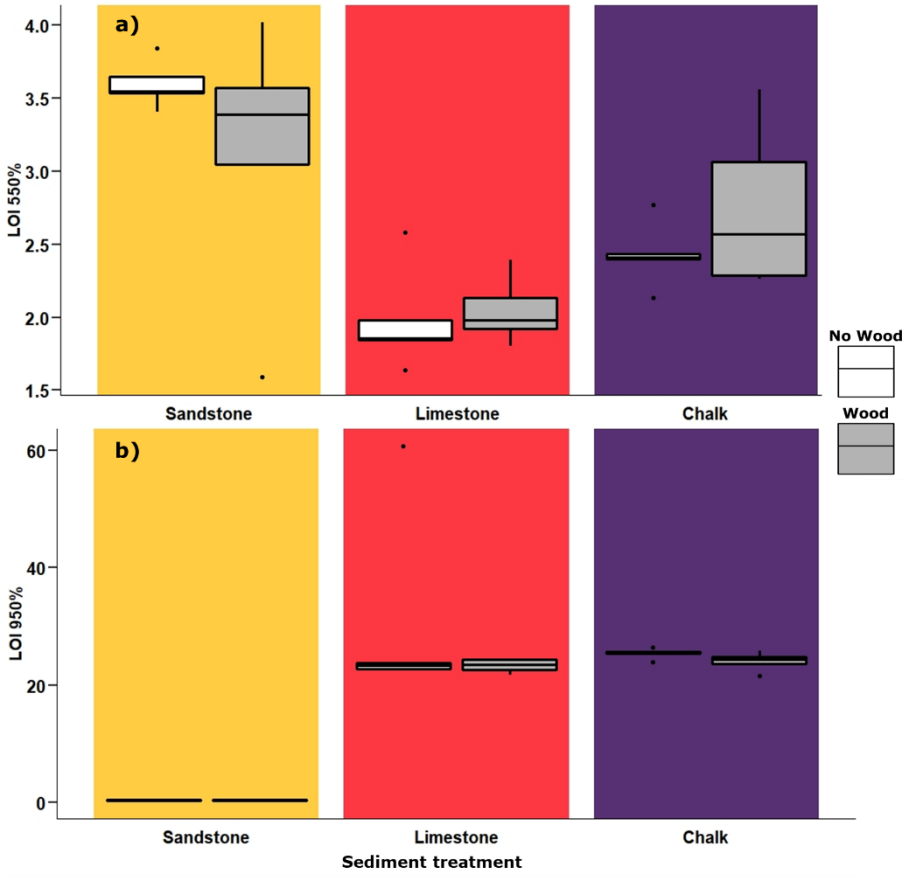


Figure 8-6. Loss on ignition at (a) 550 °C and (b) at 950 °C of the sediment with wood removed at the end of the experimental period in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill).

8.2.4 pH

pH was slightly lower in microcosms containing wood compared to those without for all sediment treatments. The pH is highest in microcosms of *no sediment* and no wood, which also appeared to present the highest variability. pH appeared to be temperature related which may be as a result of poor internal correction of temperature in the pH meter.

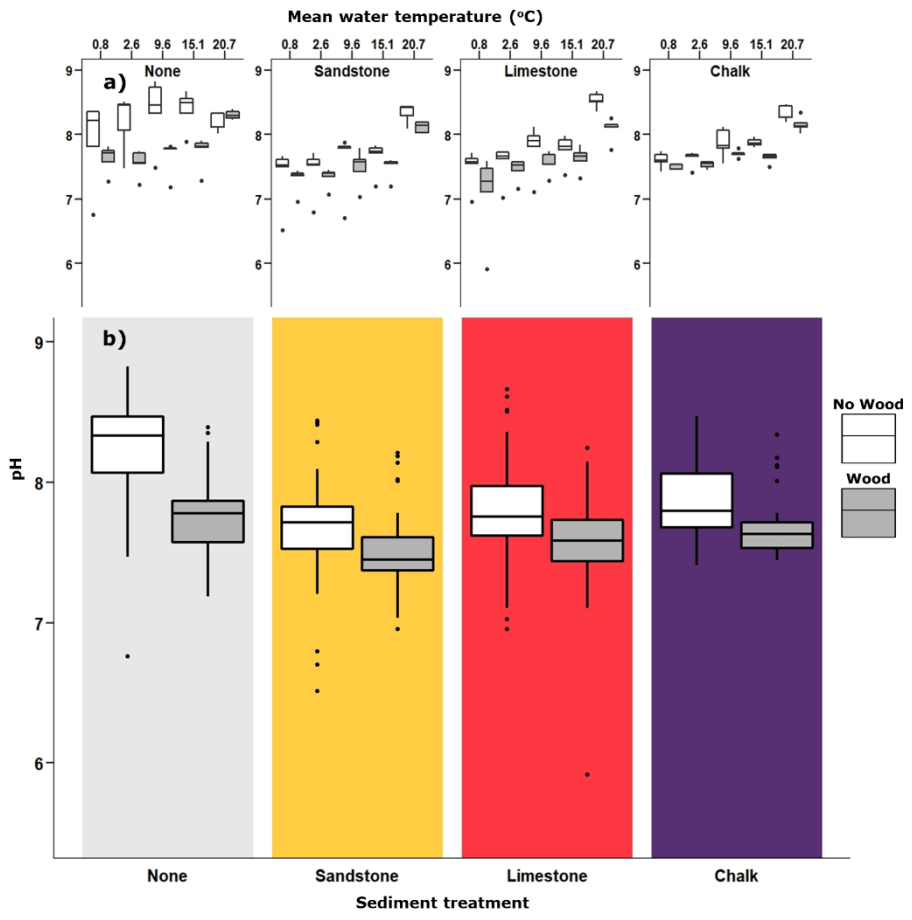


Figure 8-7. pH in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated.

8.2.5 Oxidative redox potential

In microcosms containing sediment, oxidative redox potential (ORP) was lower in the microcosms containing wood compared to those without, but in microcosms containing no sediment ORP was similar for those containing wood and those not. Microcosms of *limestone* sediment and no wood had the highest ORP, whilst microcosms of *sandstone* and wood had the lowest ORP.

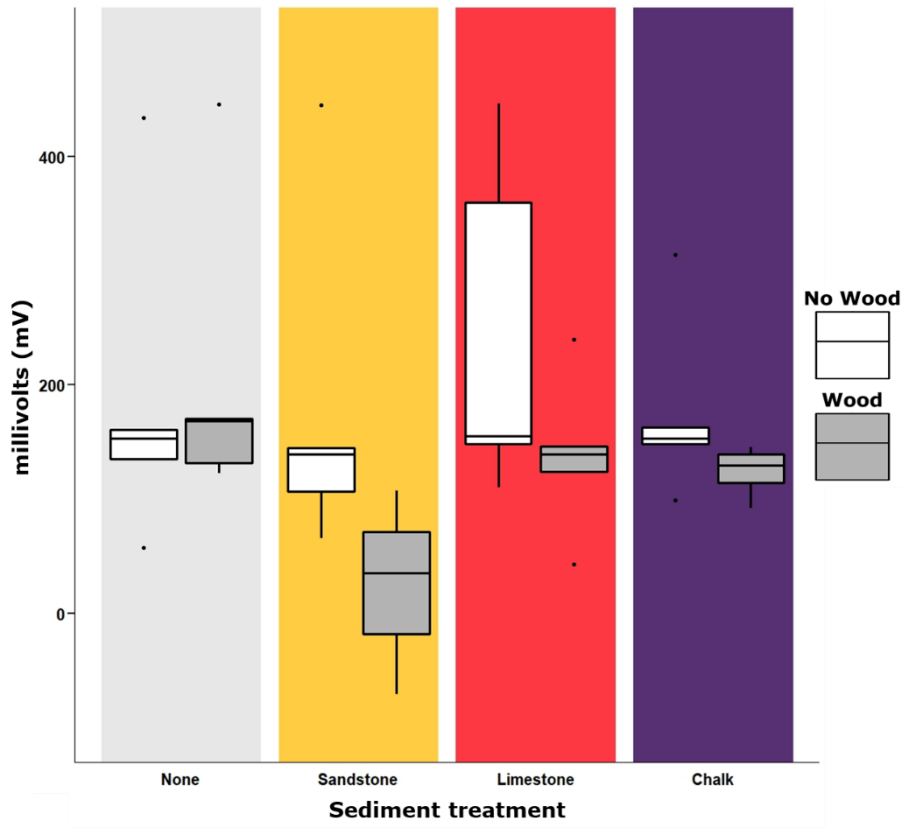


Figure 8-8. Oxidation reduction potential (ORP) of the water column at the end of the experimental period in microcosms containing different treatments of sediment (none = grey, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = grey infill).

8.2.6 Statistical outputs

For each metric of interest, likelihood ratio tests were performed to test the difference between the outputs of a linear mixed-effects model containing the wood treatment as a fixed effect and a linear mixed-effects model without this effect. This allowed the isolation of the effect of streambed wood inclusion. The outputs of the likelihood ratio test are reported in the following tables.

Table 8-7. Outputs of likelihood ratio tests testing the difference in the concentration of non-purgeable organic carbon between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom.

Sediment	Model	AIC	logLik	deviance	Chisq	Df	P.value
All	No Wood	1412.6337	-700.3169	1400.6337	NA	NA	NA
All	Wood	1413.3129	-699.6565	1399.3129	1.3208	1	0.2504
None	No Wood	500.1551	-246.0775	492.1551	NA	NA	NA
None	Wood	492.3181	-241.1590	482.3181	9.8370	1	0.0017
Sandstone	No Wood	482.3512	-237.1756	474.3512	NA	NA	NA
Sandstone	Wood	484.3381	-237.1690	474.3381	0.0131	1	0.9089
Limestone	No Wood	462.0748	-227.0374	454.0748	NA	NA	NA
Limestone	Wood	464.0507	-227.0253	454.0507	0.0242	1	0.8764
Chalk	No Wood	477.0521	-234.5261	469.0521	NA	NA	NA
Chalk	Wood	475.1945	-232.5972	465.1945	3.8577	1	0.0495

Table 8-8. Outputs of likelihood ratio tests testing the difference in the concentration of microbial metabolic activity between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom.

Sediment	Model	AIC	logLik	deviance	Chisq	Df	P.value
All	No Wood	-1024.2265	518.1132	-1036.226	NA	NA	NA
All	Wood	-1037.8649	525.9325	-1051.864	15.6384	1	0.0001
None	No Wood	-341.5120	174.7560	-349.5120	NA	NA	NA
None	Wood	-347.0388	178.5194	-357.0388	7.5267	1	0.0061
Sandstone	No Wood	-360.6791	184.3396	-368.6791	NA	NA	NA
Sandstone	Wood	-365.3035	187.6517	-375.3035	6.6243	1	0.0101
Limestone	No Wood	-368.4429	188.2214	-376.4429	NA	NA	NA
Limestone	Wood	-372.5257	191.2629	-382.5257	6.0829	1	0.0136
Chalk	No Wood	-302.7157	155.3578	-310.7157	NA	NA	NA
Chalk	Wood	-304.8541	157.4270	-314.8541	4.1384	1	0.0419

Table 8-9 Outputs of likelihood ratio tests testing the difference in the percentage change in the concentration of dissolved oxygen between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom.

Sediment	Model	AIC	logLik	deviance	Chisq	Df	P.value
All	No Wood	1606.9055	-797.4528	1594.9055	NA	NA	NA
All	Wood	1607.8680	-796.9340	1593.8680	1.0375	1	0.3084
None	No Wood	319.9046	-155.9523	311.9046	NA	NA	NA
None	Wood	312.9505	-151.4753	302.9505	8.9541	1	0.0028
Sandstone	No Wood	512.5716	-252.2858	504.5716	NA	NA	NA
Sandstone	Wood	514.4297	-252.2149	504.4297	0.1419	1	0.7064
Limestone	No Wood	505.3994	-248.6997	497.3994	NA	NA	NA
Limestone	Wood	507.3759	-248.6880	497.3759	0.0235	1	0.8781
Chalk	No Wood	564.9658	-278.4829	556.9658	NA	NA	NA
Chalk	Wood	566.0771	-278.0385	556.0771	0.8887	1	0.3458

Table 8-10 Outputs of likelihood ratio tests testing the difference in the nitrate removal rate between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom.

Sediment	Model	AIC	logLik	deviance	Chisq	Df	P.value
All	No Wood	251.8705	-119.9352	239.8705	NA	NA	NA
All	Wood	249.6970	-117.8485	235.6970	4.1735	1	0.0411
None	No Wood	64.1284	-28.0642	56.1284	NA	NA	NA
None	Wood	56.9580	-23.4790	46.9580	9.1704	1	0.0025
Sandstone	No Wood	84.3192	-38.1596	76.3192	NA	NA	NA
Sandstone	Wood	83.5891	-36.7945	73.5891	2.7301	1	0.0985
Limestone	No Wood	86.3088	-39.1544	78.3088	NA	NA	NA
Limestone	Wood	87.2126	-38.6063	77.2126	1.0962	1	0.2951
Chalk	No Wood	86.6526	-39.3263	78.6526	NA	NA	NA
Chalk	Wood	72.2914	-31.1457	62.2914	16.3612	1	0.0001

Table 8-11. Outputs of likelihood ratio tests testing the difference in the rate of CO_2 production between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom.

Sediment	Model	AIC	logLik	deviance	Chisq	Df	P.value
All	No Wood	2128.6636	-1058.3318	2116.6636	NA	NA	NA
All	Wood	2114.7920	-1050.3960	2100.7920	15.8716	1	0.0001
None	No Wood	617.5551	-304.7776	609.5551	NA	NA	NA
None	Wood	607.8886	-298.9443	597.8886	11.6665	1	0.0006
Sandstone	No Wood	707.7506	-349.8753	699.7506	NA	NA	NA
Sandstone	Wood	698.3514	-344.1757	688.3514	11.3992	1	0.0007
Limestone	No Wood	705.2383	-348.6192	697.2383	NA	NA	NA
Limestone	Wood	699.9489	-344.9745	689.9489	7.2894	1	0.0069
Chalk	No Wood	724.5080	-358.2540	716.5080	NA	NA	NA
Chalk	Wood	725.1391	-357.5695	715.1391	1.3689	1	0.2420

Table 8-12 *Outputs of likelihood ratio tests testing the difference in the rate of CH_4 production between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom.*

Sediment	Model	AIC	logLik	deviance	Chisq	Df	P.value
All	No Wood	2108.4217	-1048.2108	2096.4217	NA	NA	NA
All	Wood	2096.4341	-1041.2171	2082.4341	13.9876	1	0.0002
None	No Wood	662.3927	-327.1963	654.3927	NA	NA	NA
None	Wood	664.3820	-327.1910	654.3820	0.0107	1	0.9178
Sandstone	No Wood	686.6530	-339.3265	678.6530	NA	NA	NA
Sandstone	Wood	680.3432	-335.1716	670.3432	8.3097	1	0.0039
Limestone	No Wood	707.0152	-349.5076	699.0152	NA	NA	NA
Limestone	Wood	705.7842	-347.8921	695.7842	3.2310	1	0.0723
Chalk	No Wood	722.1812	-357.0906	714.1812	NA	NA	NA
Chalk	Wood	720.4371	-355.2186	710.4371	3.7441	1	0.0530

Table 8-13. Outputs of likelihood ratio tests testing the difference in the rate of N_2O production between microcosms containing wood and those not containing wood for different sediment types. All = all sediment types amalgamated. None = no sediment. AIC = Akaike information criterion. logLik = log-likelihood value. Chisq = Chi squared value. Df = degrees of freedom.

Sediment	Model	AIC	logLik	deviance	Chisq	Df	P.value
All	No Wood	302.8521	-145.4261	290.8521	NA	NA	NA
All	Wood	290.6786	-138.3393	276.6786	14.1735	1	0.0002
None	No Wood	25.0100	-8.5050	17.0100	NA	NA	NA
None	Wood	25.9731	-7.9866	15.9731	1.0369	1	0.3085
Sandstone	No Wood	142.0926	-67.0463	134.0926	NA	NA	NA
Sandstone	Wood	135.4896	-62.7448	125.4896	8.6030	1	0.0034
Limestone	No Wood	-20.6840	14.3420	-28.6840	NA	NA	NA
Limestone	Wood	-24.2150	17.1075	-34.2150	5.5310	1	0.0187
Chalk	No Wood	67.0718	-29.5359	59.0718	NA	NA	NA
Chalk	Wood	64.1434	-27.0717	54.1434	4.9284	1	0.0264

8.3 Chapter 4

8.3.1 Rating curve for calculating discharge

A stage-discharge relationship (R^2 = 0.98) (Figure S1) was established using measured stage (measured every 10 seconds in the monitoring set-up outlined by Blaen et al. (2017)) and a series of salt-dilution gauging measurements (Hudson and Fraser, 2005) conducted between 2019 and 2021. Stage was measured approximately 600 m from the start of the experimental reach but a series of salt dilution gauging measurements conducted by Khamis et al. (2021) demonstrated that discharge at this point well represents discharge throughout the reach, with limited gains or losses.

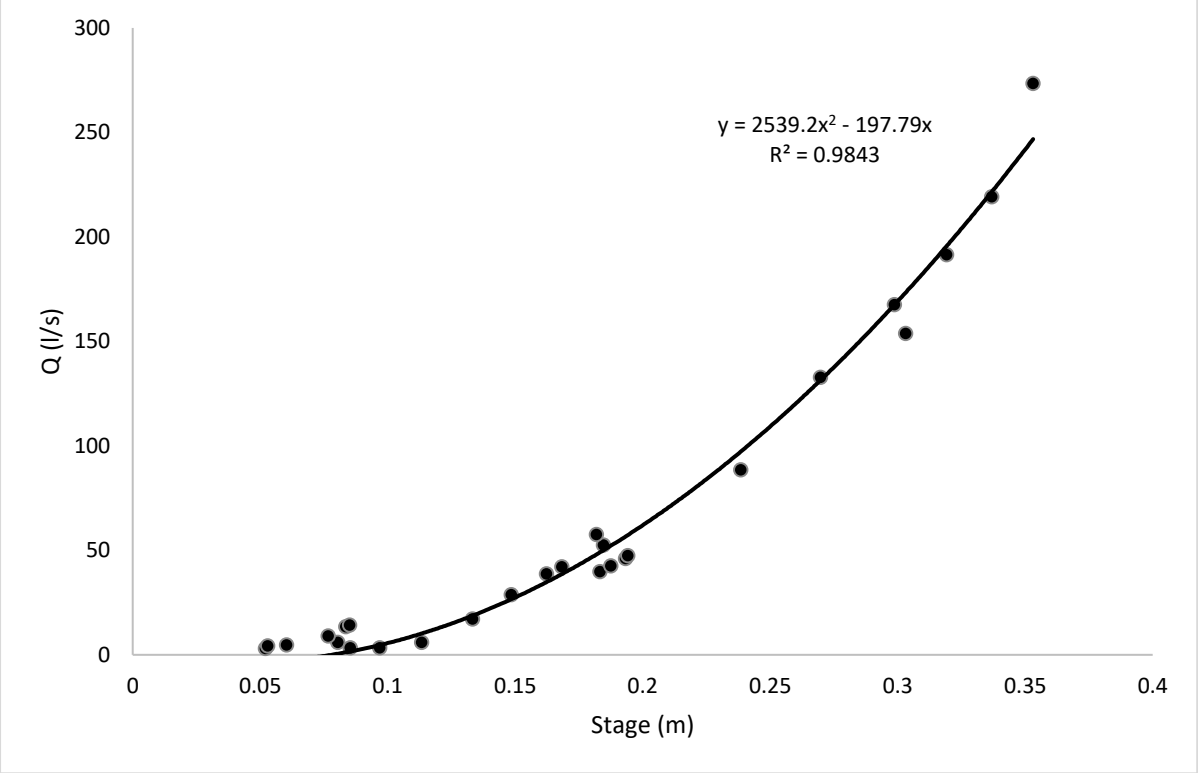


Figure 8-9. The stage-discharge relationship established at Wood Brook using observations between 2019 and 2021. Black points represent measured discharge and the black line represents the modelled relationship between stage and discharge. The polynomial equation of the modelled line and the coefficient of determination are presented.

8.3.2 Wood survey

The survey of wood and wood accumulations was carried out in March 2021. The reach was split into sections of 5 m and surveys were conducted within each section. Single pieces of wood with a visible length of >50cm were counted and size classified, using Vernier callipers to measure mid-diameter width, as in Blaen et al., (2018).). Pieces within 1 m of the channel (defined here as the water level at baseflow) were included as they are expected to interact with the channel during higher discharge. Pieces were classified by mid-diameter: small (20-50 mm), medium (50-100mm) and large (>100mm). Furthermore, the area of the stream bed covered by smaller organic matter (such as smaller wood and leaves) was estimated as percentage area. Accumulations – defined as ≥3 single pieces of wood in contact – were thoroughly characterized and size classified based on quartile ranges (Wooster and Hilton, 2004). Wood accumulations were subject to a more comprehensive survey. Absolute dimensions were measured, both lateral and vertical blockage ratios were estimated, and the ratio of wood:air was estimated. The number of pieces included in wood accumulations was estimated, as well as the size class of its components (using the same classes as for single pieces) by percentage area. The stability was assessed by classifying how the accumulation was retained: part buried, elevated, trapped/pinned, or resting. The visible effect on the flow was assessed as creating a head difference, diverging flow, modifying SW profile or inducing turbulence. Subsequently, accumulations were classified into sizes by their volume (cm^2). Classes were split by quartile ranges of all wood accumulations across all sections: small (>21,000 cm^2), medium (21,001 - 98,250 cm^2) and large (>98,251 cm^2). To further compare the quantity of wood accumulations in each section, an index score was calculated where accumulations are ranked (small=1, medium=2, large=3) and summed. The number of trees within 1m of the channel was counted. The number of tree stems within 1 m of the channel counted as they were expected to interact with the surface water during high flows.

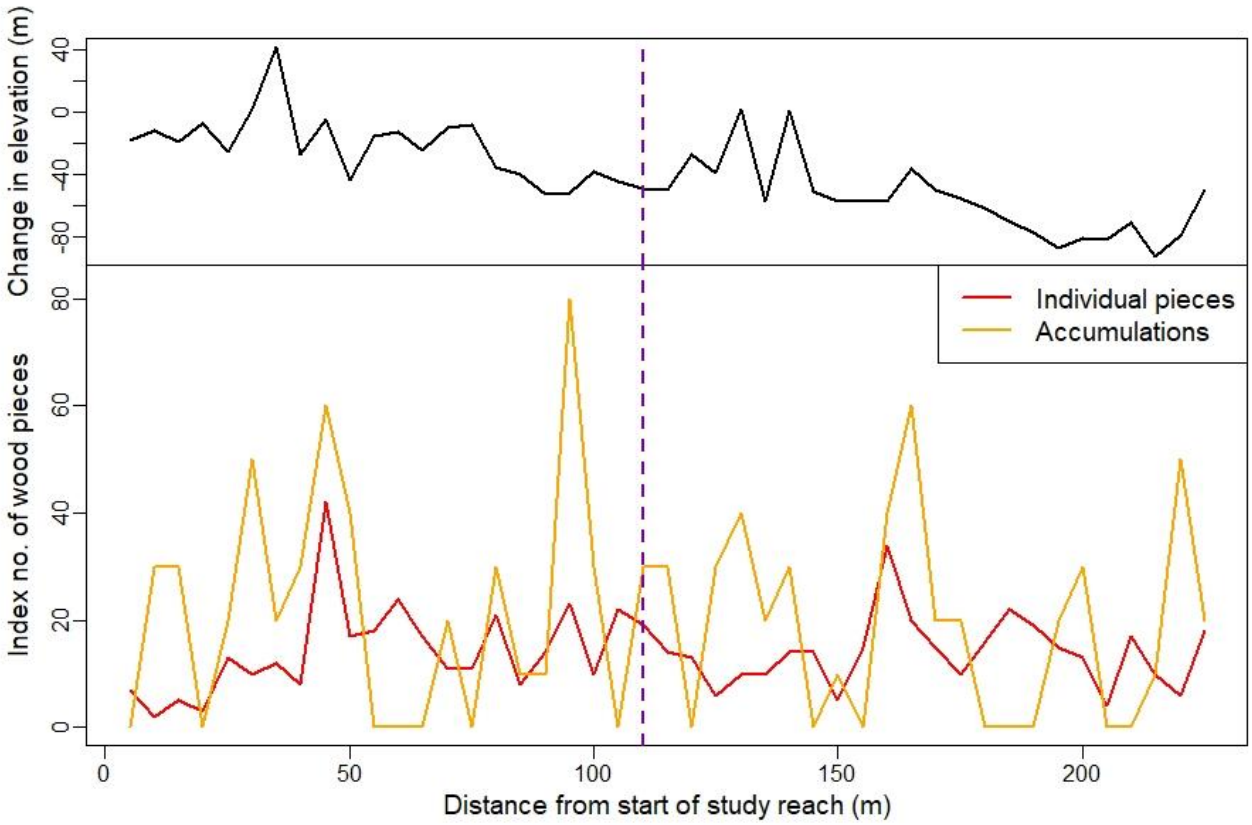


Figure 8-10. The change in elevation (top) and the distribution of instream wood pieces (red) and accumulations (orange) (bottom) throughout the study reach. The vertical purple dashed line represents the end of the control reach and the start of the impact reach.

8.3.3 Restoration materials

Materials were supplied by the Small Woods Association (www.smallwoods.org.uk) and came to a total cost of £25 per feature (supplied May 2021). At the spacing used in this experiment (approximately 1 feature per 15 m), this equates to a cost of ~£170 / 100 m, which is significantly less expensive than most restoration schemes (Logar et al., 2019). 8 features were installed by 2 people in approximately 4 hours.

8.3.4 Tracer injection periods

Table 8-14. Tracer injection dates and masses.

Date	Restoration	Uranine (g)	NaCl (kg)	Q (m^3/s)
20/04/2021	Before	1	3	0.00601
05/05/2021	Before	1.5	3	0.00657
09/06/2021	Before	1	3	0.00485
15/06/2021	Before	0.75	3	0.00218
29/06/2021	After	2	12	0.00714
20/07/2021	After	0.75	26	0.00378
27/07/2021	After	0.75	13	0.00659
11/08/2021	After	0.8	12	0.00253
28/09/2021	After	1.5	15	0.00355
16/11/2021	After	1	10	0.00985

8.3.5 Break through curve modelling

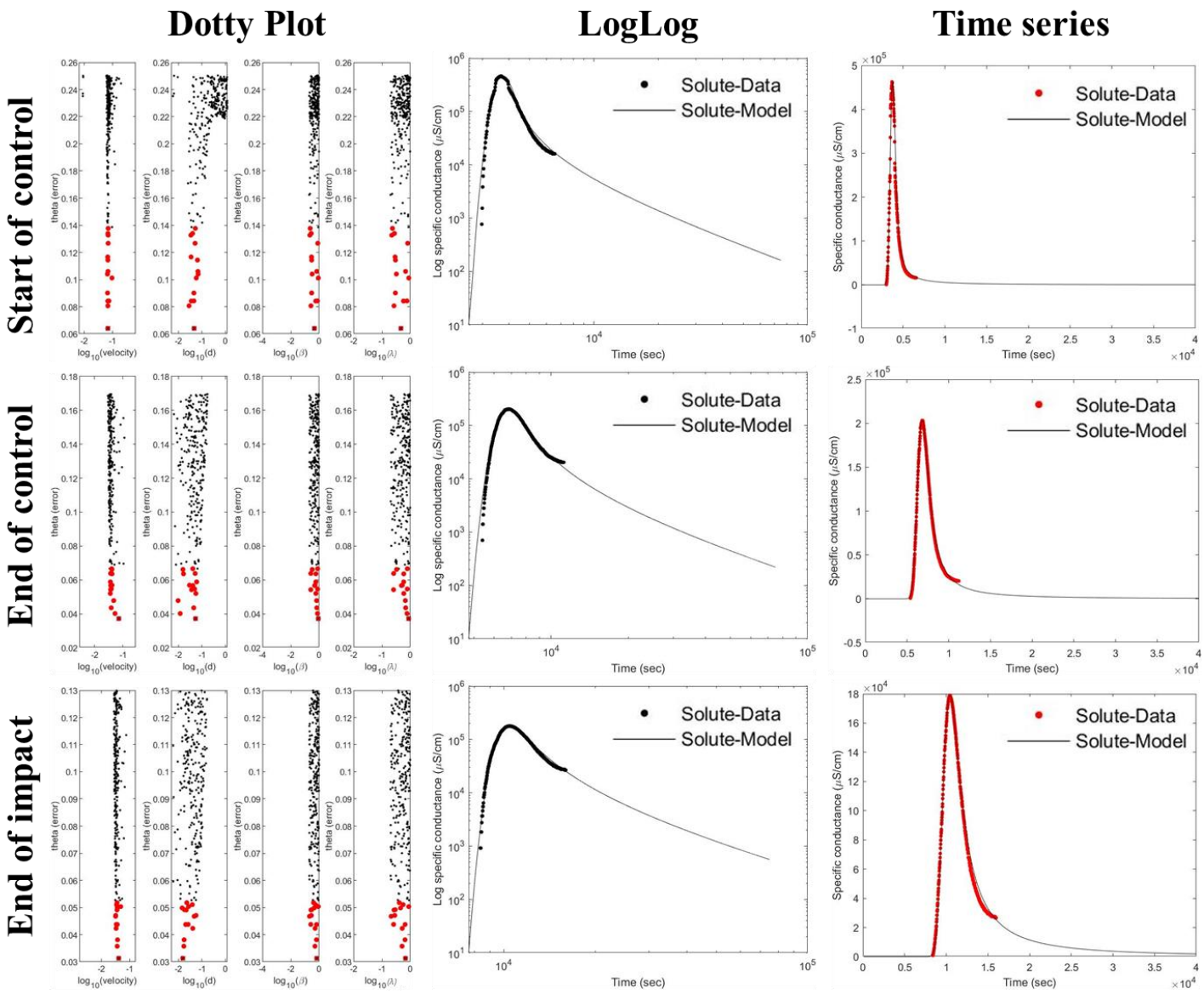


Figure 8-11. An example (observed data and modelled BTCs from the tracer injection on 09-06-21) of the plots were used to assess the identifiability and equifinality of model parameters and the goodness of fit to observed data. The left column includes dotty plots to demonstrate parameter sensitivity where the dots represent parameter values that meet a behavioural error threshold: black dots = top 1%, red dots = top 0.05%. Dotty plots from left to right = Velocity, dispersion (d), residence time in immobile zones (β), and the rate of exchange of solutes with the mobile zone and immobile zone (λ). The middle column of plots shows the model fit in log time vs. log specific conductance, where black dots represent observed data and the black line represents modelled data. The right column of plots shows the model fit in linear time vs. specific conductance, where red dots represent observed data and the black line represents modelled data. Each row represents a different monitoring location, where the 1st row represents the start of the control reach, the 2nd row represents the end of the control reach and the start of the impact reach, and the 3rd row represents the end of the impact reach. Plots produced using adapted MATLAB code from Drummond et al. (2019).

Table 8-15. Tables present the model best fit parameters (value) and the standard deviation of the best 0.05% fits (std) for each tracer injection and measurement location. Parameters = velocity, dispersion, the rate of exchange of solutes with the mobile zone and immobile zone (λ), and the residence time in immobile zones (β). Values extracted using adapted MATLAB code from Drummond et al. (2019).

Start of control reach								
Date of injection	**Velocity**		**Dispersion**		***λ***		***β***	
	value	**std**	**value**	**std**	**value**	**std**	**value**	**std**
20/04/2021	0.058	0.013	0.063	0.022	0.001	0.136	0.304	0.255
05/05/2021	0.066	0.005	0.101	0.047	0.039	0.049	0.805	0.249
09/06/2021	0.068	0.007	0.047	0.013	0.004	0.063	0.466	0.236
15/06/2021	0.065	0.008	0.030	0.011	0.038	0.062	0.764	0.260
29/06/2021	0.063	0.013	0.053	0.033	0.001	0.142	0.276	0.230
20/07/2021	0.063	0.004	0.032	0.018	0.006	0.039	0.466	0.194
27/07/2021	0.070	0.002	0.061	0.016	0.000	0.001	0.742	0.244
11/08/2021	0.051	0.004	0.032	0.016	0.002	0.018	0.274	0.204
28/09/2021	0.041	0.002	0.029	0.012	0.001	0.003	0.320	0.129
16/11/2021	0.027	0.004	0.024	0.005	0.002	0.082	0.421	0.227

End of control reach / start of impact reach								
Date of injection	**Velocity**		**Dispersion**		***λ***		***β***	
	value	**std**	**value**	**std**	**value**	**std**	**value**	**std**
20/04/2021	0.046	0.004	0.036	0.017	0.003	0.009	0.412	0.136
05/05/2021	0.046	0.004	0.075	0.019	0.001	0.050	0.717	0.236
09/06/2021	0.072	0.010	0.054	0.017	0.474	0.124	0.875	0.197
15/06/2021	0.026	0.003	0.023	0.009	0.002	0.035	0.407	0.169
29/06/2021	0.058	0.007	0.023	0.014	0.131	0.127	0.786	0.224
20/07/2021	0.065	0.010	0.045	0.009	0.882	0.235	0.945	0.250
27/07/2021	0.039	0.006	0.054	0.015	0.004	0.055	0.955	0.286
11/08/2021	0.027	0.003	0.036	0.009	0.000	0.063	0.213	0.237
28/09/2021	0.028	0.005	0.019	0.007	0.015	0.134	0.669	0.220
16/11/2021	0.044	0.009	0.013	0.019	0.290	0.113	0.831	0.239

End of impact reach								
Date of injection	Velocity		Dispersion		λ		β	
	value	std	value	std	value	std	value	std
20/04/2021	0.042	0.016	0.048	0.020	0.042	0.265	0.782	0.187
05/05/2021	0.056	0.017	0.057	0.023	0.105	0.218	0.828	0.238
09/06/2021	0.041	0.004	0.016	0.013	0.037	0.057	0.676	0.199
15/06/2021	0.027	0.004	0.037	0.013	0.001	0.036	0.226	0.190
29/06/2021	0.036	0.008	0.031	0.013	0.000	0.177	0.262	0.247
20/07/2021	0.033	0.003	0.011	0.006	0.002	0.042	0.325	0.242
27/07/2021	0.084	0.027	0.018	0.023	0.415	0.324	0.808	0.275
11/08/2021	0.013	0.004	0.006	0.002	0.001	0.205	0.205	0.271
28/09/2021	0.013	0.001	0.005	0.002	0.001	0.003	0.220	0.217
16/11/2021	0.013	0.001	0.006	0.003	0.024	0.034	0.683	0.246

8.3.6 Statistical model fits

Table 8-16. Goodness of fit of each statistical model applied to each transport and transient storage metric, assessed using Akaike information criterion (AIC). REML = Residual maximum likelihood. ANOVA = Analysis of variance. ANCOVA = Analysis of Covariance. TSI = transient storage index. CV = coefficient of variation. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone.

Metric	Test	AIC
TSI	REML	143.62
	ANOVA	163.71
	ANCOVA	165.70
Skewness	REML	48.93
	ANOVA	43.77
	ANCOVA	45.55
CV	REML	-22.19
	ANOVA	-35.90
	ANCOVA	-53.79
Holdback	REML	-41.59
	ANOVA	-70.99
	ANCOVA	-69.22
β	REML	14.90
	ANOVA	1.39
	ANCOVA	1.72
Dispersion	REML	-67.24
	ANOVA	-101.25
	ANCOVA	-100.50
λ	REML	Na
	ANOVA	Na
	ANCOVA	Na
Velocity	REML	-73.0279
	ANOVA	-105.985
	ANCOVA	-105.997

8.3.7 Regression analysis

Table 8-17. Details of linear regression equations and coefficient of determination (r^2) between transport and transient storage metrics and discharge, the plots of which are presented in Figure 3 of the main text. TSI = transient storage index. CV = coefficient of variation. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone.

Metric	Period*Reach	Gradient	Intercept	r^2
	Control Before	-2459	28	0.993
	Impact Before	-4044	76	0.987
	Control After	-151	19	0.0116
TSI	Impact After	1878	61	0.0611
	Control Before	-9.6	6.6	0.00153
	Impact Before	366	5.9	0.824
	Control After	-102	7.2	0.733
Skewness	Impact After	66	7.1	0.0511
	Control Before	-390	6.8	0.994
	Impact Before	-614	11	0.778
	Control After	-476	12	0.52
CV	Impact After	-996	23	0.528
	Control Before	-38	0.94	0.322
	Impact Before	7.1	0.75	0.876
	Control After	-11	0.82	0.0726
Holdback	Impact After	5.1	0.74	0.228
	Control Before	44	0.39	0.135
	Impact Before	59	0.27	0.221
	Control After	54	0.43	0.291
β	Impact After	-20	0.59	0.392
	Control Before	8.5	0.0049	0.561
	Impact Before	7.3	0.0024	0.491
	Control After	-2.1	0.043	0.128
Dispersion	Impact After	1.6	0.0032	0.219
	Control Before	-2.2	0.13	0.000336
	Impact Before	15	-0.039	0.336
	Control After	-4.2	0.24	0.00115
λ	Impact After	-0.55	0.0075	0.284
	Control Before	4.8	0.024	0.245
	Impact Before	4.3	0.018	0.708
	Control After	1.6	0.035	0.0794
Velocity	Impact After	0.54	0.022	0.011

8.3.8 Statistical outputs

Table 8-18. Summaries of statistical tests are reported for each transport and storage metric. ANOVA = analysis of variance. ANCOVA = analysis of covariance. Q = discharge. Sum sq. = sum of squares. NumDF = degrees of freedom in the numerator. DenDF = degrees of freedom in the denominator. Std. = standard deviation. SE = standard error. CI = confidence interval. Df = degrees of freedom. Diff = difference in means. Lower = lower confidence interval. Upper = upper confidence interval. TSI = transient storage index. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone. CB = control-before. CA = control-after. IB = impact-before. IA = impact-after.

TSI

REML

Effect	Sum sq.	NumDF	DenDF	F value	Pr(>F)
Reach	10416.40	1.00	8.00	106.10	6.8E-06
Period	189.00	1.00	8.00	1.93	2.0E-01
Reach*Period	189.40	1.00	8.00	1.93	2.0E-01

Fixed effects	Estimate	Std.	CI 2.5%	CI 97.5%	Pr(>|t|)
(Intercept)	15.50	6.31	3.78	27.22	2.8E-02
Reach (Impact)	40.30	7.01	26.74	53.86	4.3E-04
Period (After)	3.12	8.15	-12.01	18.26	7.1E-01
Reach (Impact)*Period (After)	12.56	9.05	-4.95	30.07	2.0E-01

Random effects	Variance	Std.
Date	61.09	7.82
Residual	98.18	9.91

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	12.60	9.05	8.00	1.39	2.0E-01

ANOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.00	11443.50	21.38	59.22	71.85	2.6E-07
Period	1.00	424.30	-14.15	20.39	2.66	1.2E-01
Reach*Period	1.00	189.40	-11.86	36.99	1.19	2.9E-01
Residuals	16.00	2548.30	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	12.60	11.50	16.00	1.09	2.9E-01

Tukey post-hoc tests	diff	lower	upper	p.value
Impact:Before-Control:Before	40.30	14.77	65.83	1.80E-03
Control:After-Control:Before	3.12	-20.19	26.43	9.80E-01
Impact:After-Control:Before	55.99	32.68	79.29	2.03E-05
Control:After-Impact:Before	-37.18	-60.49	-13.88	1.63E-03
Impact:After-Impact:Before	15.68	-7.62	38.99	2.57E-01
Impact:After-Control:After	52.87	32.02	73.71	1.05E-05

ANCOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.00	11443.50	20.66	59.94	67.38	6.27E-07
Period	1.00	424.30	-14.85	21.20	2.50	1.35E-01
Q	1.00	0.70	-2865.08	2703.27	0.00	9.51E-01
Reach*Period	1.00	189.40	-12.79	37.92	1.12	3.08E-01
Residuals	15.00	2547.70	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	12.60	11.90	15.00	1.06	3.08E-01

Skewness

REML

Effect	Sum sq.	NumDF	DenDF	F value	Pr(>F)
Reach	4.85	1.00	8.00	12.74	7.3E-03
Period	0.00	1.00	8.00	0.00	9.5E-01
Reach*Period	0.11	1.00	8.00	0.30	6.0E-01

Fixed effects	Estimate	Std.	CI 2.5%	CI 97.5%	Pr(>|t|)
(Intercept)	6.52	0.31	5.94	7.10	5.7E-13
Reach (Impact)	1.16	0.44	0.34	1.98	2.9E-02
Period (After)	0.13	0.41	-0.61	0.88	7.5E-01
Reach (Impact)*Period (After)	-0.31	0.56	-1.36	0.75	6.0E-01

Random effects	Variance	Std.
Date	0.01561	0.1249
Residual	0.38053	0.6169

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.307	0.563	8	-0.545	6.01E-01

ANOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1	4.75	0.21	2.10	11.98	3.22E-03
Period	1	0.00	-0.73	1.00	0.00	9.48E-01
Reach*Period	1	0.11	-1.52	0.91	0.29	6.01E-01
Residuals	16	6.34	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.31	0.58	16.00	-0.53	6.01E-01

Tukey post-hoc tests	diff	lower	upper	p.value
Impact:Before-Control:Before	1.16	-0.11	2.43	8.13E-02
Control:After-Control:Before	0.13	-1.03	1.30	9.87E-01
Impact:After-Control:Before	0.99	-0.18	2.15	1.12E-01
Control:After-Impact:Before	-1.02	-2.19	0.14	9.45E-02
Impact:After-Impact:Before	-0.17	-1.33	0.99	9.73E-01
Impact:After-Control:After	0.85	-0.19	1.89	1.30E-01

ANCOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.00	4.75	0.18	2.13	11.36	4.21E-03
Period	1.00	0.00	-0.78	1.01	0.00	9.49E-01
Q	1.00	0.07	-111.16	164.99	0.17	6.84E-01
Reach*Period	1.00	0.11	-1.56	0.95	0.27	6.10E-01
Residuals	6.27	6.27	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.31	0.59	15	-0.52	6.11E-01

Coefficient of variation

REML

Effect	Sum sq.	NumDF	DenDF	F value	Pr(>F)
Reach	0.29	1.00	8.00	178.30	9.46E-07
Period	0.05	1.00	8.00	31.78	4.89E-04
Reach*Period	0.00	1.00	8.00	2.43	1.57E-01

Fixed effects	Estimate	Std.	CI 2.5%	CI 97.5%	Pr(>|t|)
(Intercept)	0.69	0.04	0.61	0.77	1.90E-08
Reach (Impact)	0.22	0.03	0.16	0.27	6.23E-05
Period (After)	0.27	0.06	0.16	0.37	7.37E-04
Reach (Impact)*Period (After)	0.06	0.04	-0.01	0.13	1.57E-01

Random effects	Variance	Std.
Date	0.01	0.08
Residual	0.00	0.04

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	0.06	0.04	8	1.56	1.57E-01

ANOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.00	0.32	0.60	0.78	43.42	6.2E-06
Period	1.00	0.42	0.09	0.35	56.46	1.2E-06
Reach*Period	1.00	0.00	0.15	0.38	0.54	4.7E-01
Residuals	16.00	0.12	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	0.06	0.08	16.00	0.74	4.72E-01

Tukey post-hoc tests	diff	lower	upper	p.value
Impact:Before-Control:Before	0.22	0.04	0.39	1.16E-02
Control:After-Control:Before	0.27	0.11	0.42	1.03E-03
Impact:After-Control:Before	0.54	0.38	0.70	2.00E-07
Control:After-Impact:Before	0.05	-0.11	0.21	8.29E-01
Impact:After-Impact:Before	0.32	0.16	0.48	1.35E-04
Impact:After-Control:After	0.28	0.13	0.42	2.24E-04

ANCOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1	0.32	0.14	0.30	11.36	2.66E-08
Period	1	0.42	0.21	0.36	0.00	4.52E-09
Q	1	0.07	-38.85	-15.80	0.17	1.43E-04
Reach*Period	1	0.00	-0.05	0.16	0.27	2.59E-01
Residuals	15	0.04	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	0.06	0.05	15.00	1.17	2.59E-01

Holdback					
REML					
Effect	Sum sq.	NumDF	DenDF	F value	Pr(>F)
Reach	0.01	1.00	14.00	8.56	1.11E-02
Period	0.00	1.00	14.00	1.36	2.64E-01
Reach*Period	0.00	1.00	14.00	0.00	9.61E-01
Fixed effects	Estimate	Std.	CI 2.5%	CI 97.5%	Pr(>|t|)
(Intercept)	0.82	0.02	0.79	0.85	<2e-16
Reach (Impact)	-0.04	0.02	-0.08	0.00	7.85E-02
Period (After)	-0.02	0.02	-0.06	0.02	4.32E-01
Reach (Impact)*Period (After)	0.00	0.03	-0.05	0.05	9.61E-01
Random effects	Variance	Std.			
Date	0.00	0.00			
Residual	0.00	0.03			
Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	0.00	0.03	7.38	0.05	9.63E-01
ANOVA					
Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1	0.007	-0.089	0.005	8.785	1.03E-02
Period	1	0.001	-0.062	0.028	1.362	2.63E-01
Reach*Period	1	0.000	-0.059	0.062	0.002	9.61E-01
Residuals	14	0.012	\	\	\	\
Estimated marginal means	Estimate	SE	df	t.ratio	p.value	
Reach*Period	0.001	0.028	14.000	0.049	4.72E-01	
Tukey post-hoc tests	diff	lower	upper	p.value		
Impact:Before-Control:Before	-0.042	-0.106	0.022	2.73E-01		
Control:After-Control:Before	-0.017	-0.079	0.044	8.49E-01		
Impact:After-Control:Before	-0.058	-0.117	0.002	5.87E-02		
Control:After-Impact:Before	0.025	-0.032	0.081	5.90E-01		
Impact:After-Impact:Before	-0.016	-0.070	0.039	8.34E-01		
Impact:After-Control:After	-0.041	-0.091	0.010	1.42E-01		
ANCOVA						
Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.000	0.007	-0.092	0.007	8.261	1.30E-02
Period	1.000	0.001	-0.066	0.029	1.281	2.78E-01
Q	1.000	0.000	-5.379	7.872	0.164	6.93E-01
Reach*Period	1.000	0.000	-0.061	0.065	0.004	9.51E-01
Residuals	13.000	0.012	\	\	\	\
Estimated marginal means	Estimate	SE	df	t.ratio	p.value	
Reach*Period	0.002	0.029	13.000	0.063	9.51E-01	

β					
REML					
Effect	Sum sq.	NumDF	DenDF	F value	Pr(>F)
Reach	0.11	1.00	8.00	2.64	1.43E-01
Period	0.00	1.00	8.00	0.08	7.90E-01
Reach*Period	0.05	1.00	8.00	1.19	3.08E-01
Fixed effects	Estimate	Std.	CI 2.5%	CI 97.5%	Pr(>|t|)
(Intercept)	0.603	0.109	0.402	0.804	4.88E-05
Reach (Impact)	-0.050	0.144	-0.327	0.227	7.38E-01
Period (After)	0.130	0.141	-0.129	0.390	3.69E-01
Reach (Impact)*Period (After)	-0.202	0.186	-0.560	0.155	3.08E-01
Random effects	Variance	Std.			
Date	0.006	0.079			
Residual	0.041	0.203			
Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.202	0.186	8.000	-1.090	3.08E-01
ANOVA					
Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.000	0.146	-0.377	0.277	3.076	9.86E-02
Period	1.000	0.004	-0.168	0.429	0.086	7.73E-01
Reach*Period	1.000	0.049	-0.624	0.220	1.032	3.25E-01
Residuals	16.000	0.761	\	\	\	\
Estimated marginal means	Estimate	SE	df	t.ratio	p.value	
Reach*Period	-0.202	0.199	16	-1.016	3.25E-01	
Tukey post-hoc tests	diff	lower	upper	p.value		
Impact:Before-Control:Before	-0.050	-0.491	0.392	9.88E-01		
Control:After-Control:Before	0.130	-0.272	0.533	7.92E-01		
Impact:After-Control:Before	-0.122	-0.524	0.281	8.23E-01		
Control:After-Impact:Before	0.180	-0.223	0.583	5.88E-01		
Impact:After-Impact:Before	-0.072	-0.475	0.331	9.55E-01		
Impact:After-Control:After	-0.252	-0.612	0.108	2.28E-01		
ANCOVA						
Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.000	0.146	-0.375	0.276	3.134	9.70E-02
Period	1.000	0.004	-0.185	0.413	0.088	7.71E-01
Q	1.000	0.061	-21.462	70.867	1.301	2.72E-01
Reach*Period	1.000	0.049	-0.623	0.218	1.051	3.22E-01
Residuals	15.000	0.700	\	\	\	\
Estimated marginal means	Estimate	SE	df	t.ratio	p.value	
Reach*Period	-0.202	0.197	15	-1.025	3.22E-01	

Dispersion

REML

Effect	Sum sq.	NumDF	DenDF	F value	Pr(>F)
Reach	0.001	1.000	8.000	3.952	8.20E-02
Period	0.001	1.000	8.000	6.225	3.72E-02
Reach*Period	0.000	1.000	8.000	0.603	4.60E-01

Fixed effects	Estimate	Std.	CI 2.5%	CI 97.5%	Pr(>|t|)
(Intercept)	0.047	0.008	0.031	0.062	4.41E-05
Reach (Impact)	-0.009	0.011	-0.030	0.013	4.57E-01
Period (After)	-0.015	0.011	-0.035	0.005	1.87E-01
Reach (Impact)*Period (After)	-0.011	0.014	-0.038	0.016	4.60E-01

Random effects	Variance	Std.
Date	4.1E-05	6.4E-03
Residual	2.4E-04	1.6E-02

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.011	0.014	8.000	-0.777	4.60E-01

ANOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1	0.001	-0.034	0.017	4.095	6.00E-02
Period	1	0.002	-0.038	0.008	7.123	1.68E-02
Reach*Period	1	0.000	-0.043	0.021	0.516	4.83E-01
Residuals	16	0.004	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.011	0.0153	16	-0.718	4.83E-01

Tukey post-hoc tests	diff	lower	upper	p.value
Impact:Before-Control:Before	-0.009	-0.042	0.025	8.86E-01
Control:After-Control:Before	-0.015	-0.046	0.016	5.29E-01
Impact:After-Control:Before	-0.034	-0.065	-0.004	2.64E-02
Control:After-Impact:Before	-0.006	-0.037	0.025	9.35E-01
Impact:After-Impact:Before	-0.026	-0.057	0.005	1.18E-01
Impact:After-Control:After	-0.020	-0.047	0.008	2.21E-01

ANCOVA

Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.000	0.001	-0.034	0.017	4.087	6.14E-02
Period	1.000	0.002	-0.039	0.007	7.109	1.76E-02
Q	1.000	0.000	-1.929	5.240	0.969	3.41E-01
Reach*Period	1.000	0.000	-0.044	0.022	0.515	4.84E-01
Residuals	15.000	0.004	\	\	\	\

Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.011	0.0153	15	-0.718	4.84E-01

Velocity					
REML					
Effect	Sum sq.	NumDF	DenDF	F value	Pr(>F)
Reach	0.001	1.000	8.000	7.656	2.44E-02
Period	0.000	1.000	8.000	1.117	3.21E-01
Reach*Period	0.000	1.000	8.000	0.979	3.51E-01
Fixed effects	Estimate	Std.	CI 2.5%	CI 97.5%	Pr(>|t|)
(Intercept)	0.048	0.007	0.034	0.061	2.43E-05
Reach (Impact)	-0.009	0.008	-0.023	0.006	2.84E-01
Period (After)	-0.004	0.010	-0.022	0.014	6.88E-01
Reach (Impact)*Period (After)	-0.010	0.010	-0.028	0.009	3.51E-01
Random effects	Variance	Std.			
Date	0.0001	0.0104			
Residual	0.0001	0.0107			
Estimated marginal means	Estimate	SE	df	t.ratio	p.value
Reach*Period	-0.00963	0.00974	8	-0.989	3.52E-01
ANOVA					
Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.000	0.001	-0.031	0.014	4.696	4.57E-02
Period	1.000	0.000	-0.024	0.016	1.662	2.16E-01
Reach*Period	1.000	0.000	-0.038	0.019	0.502	4.89E-01
Residuals	16.000	0.004	\	\	\	\
Estimated marginal means	Estimate	SE	df	t.ratio	p.value	
Reach*Period	-0.011	0.0153	16	-0.718	4.83E-01	
Tukey post-hoc tests	diff	lower	upper	p.value		
Impact:Before-Control:Before	-0.009	-0.039	0.021	8.43E-01		
Control:After-Control:Before	-0.004	-0.031	0.024	9.76E-01		
Impact:After-Control:Before	-0.022	-0.050	0.005	1.37E-01		
Control:After-Impact:Before	0.005	-0.023	0.032	9.60E-01		
Impact:After-Impact:Before	-0.014	-0.041	0.014	5.10E-01		
Impact:After-Control:After	-0.018	-0.043	0.006	1.87E-01		
ANCOVA						
Effect	Df	Sum sq.	CI 2.5%	CI 97.5%	F value	Pr(>F)
Reach	1.000	0.001	-0.031	0.013	4.869	4.34E-02
Period	1.000	0.000	-0.025	0.015	1.723	2.09E-01
Q	1.000	0.000	-1.278	4.971	1.587	2.27E-01
Reach*Period	1.000	0.000	-0.038	0.019	0.521	4.82E-01
Residuals	15.000	0.003	\	\	\	\
Estimated marginal means	Estimate	SE	df	t.ratio	p.value	
Reach*Period	-0.00963	0.0134	15	-0.721	4.82E-01	

λ			
Kruskal Wallis			
	chi sq.	**df**	**P**
CB, CA, IB, IA	2.2095,	3	0.5301
Bonferroni-Holm			
Comparison	**CB**	**CA**	**IB**
CA	0.91	-	-
IB	0.91	0.91	-
IA	0.91	0.91	0.91

Table 8-19. Summary of statistical tests performed to assess before-after and control-impact experimental designs. Df = degrees of freedom. λ was not normally distributed so a non-parametric Mann-Whitney U test was performed instead of a student's T-test. TSI = transient storage index. B = residence time in immobile zones. λ = rate of exchange of solutes between the mobile zone and immobile zone.

TSI			
T-test	**t**	**df**	**P**
Before-after	1.6572	6.8438	0.1424
Control-impact	-6.0513	5.341	0.00141
CV			
T-test	**t**	**df**	**P**
Before-after	5.4332	6.6935	0.00113
Control-impact	-4.7655	7.1997	0.00189
Skewness			
T-test	**t**	**df**	**P**
Before-after	-0.3365	6.7112	0.7468
Control-impact	-2.3916	6.6273	0.05002
Holdback			
T-test	**t**	**df**	**P**
Before-after	1.106	7.6701	0.3022
Control-impact	2.0901	8.0958	0.0696
β			
T-test	**t**	**df**	**P**
Before-after	-0.5655	3.5438	0.6056
Control-impact	2.1269	6.0333	0.07729
Dispersion			
T-test	**t**	**df**	**P**
Before-after	-2.3672	3.9267	0.07826
Control-impact	2.5539	8.2457	0.03316
λ			
Mann-Whitney U test		**W**	**P**
Before-after		9	0.6095
Control-impact		27	0.1797
Velocity			
T-Test	**t**	**df**	**P**
Before-after	-1.7812	7.929	0.1131
Control-impact	2.1341	9.9313	0.0588