

Appendix 5 of BD5104

Water quality assessments

The purpose of this Appendix is to further describe the methods and findings relating to the water quality measures for both plot and stream water samples which are described in Sections 4.2.10.1 and 4.2.10.2 of the main body of the report for project BD5104. The methods summaries, results and discussions are not repeated here but instead the full details of the methods and statistical analyses are given together with detailed information on the smoother function applied to stream water pH samples.

Peat pore water quality assessments

Water samples were collected seasonally (2-4 times per year; see **Table A5.1**) per year from each plot (at both managed monitoring and slope plots) by attaching previously acid washed 50 ml luer-lock syringes to the vertically inserted (10 cm long) peat Rhizon samplers (pore size 0.15 μm , Rhizosphere Research Products B.V., Wageningen, Netherlands). The syringes were held open with a retainer to create a vacuum. All samples were stored in the dark (in cool bags) immediately after collection and kept at 4°C within 24 hours of collection and processed within one month. To identify outliers a Dean and Dixon test (Dean & Dixon, 1951) was used (i.e. the ratio of the outlier gap to the data range, based on a reference value that corresponds to the sample size and confidence level).

Table A5.1 Sampling days for plot water sampling at the three sites (Nidderdale, Mossdale and Whitendale) during the pre- (2012/13) and post-management (2013-2016) period. Onset of management change was in March/April 2013.

Number per year	Nidderdale	Mossdale	Whitendale
1	21/03/2012	15/03/2012	28/03/2012
2	19/07/2012	19/07/2012	19/07/2012
3	26/09/2012	26/09/2012	26/09/2012
4	21/11/2012	21/11/2012	21/11/2012
1	30/04/2013	30/04/2013	30/04/2013
2	13/08/2013	13/08/2013	13/08/2013
3	14/11/2013	14/11/2013	14/11/2013
1	18/02/2014	18/02/2014	18/02/2014
2	17/06/2014	17/06/2014	17/06/2014
3	16/09/2014	16/09/2014	16/09/2014
4	20/10/2014	21/10/2014	20/10/2014
1	05/03/2015	05/03/2015	05/03/2015
2	22/07/2015	22/07/2015	22/07/2015
3	06/10/2015	07/10/2015	07/10/2015
1	09/03/2016	09/03/2016	09/03/2016
2	04/10/2016	04/10/2016	04/10/2016

pH

The pH meter (Thermo Orion Bench-top pH meter, model 420) was manually calibrated at the start of each new working day, using laboratory standard pH buffers at pH 2.0, 4.0 and 7.0. The sample to be measured was briefly mixed using a sample vortex (lab dancer). The electrode was rinsed with ultra-pure deionized water and lightly dried before each measurement. After a measurement had been read the electrode and probe were rinsed twice and placed in ultra-pure deionized water.

UV Absorbance

Water samples (from 2016 onwards) were mixed using a sample vortex (lab dancer), filtered (0.45 µm nitrocellulose filters) and adjusted to room temperature (for about one hour) prior to UV absorbance measurements. The absorbency of samples was measured in a 1 cm wide quartz cell at 254, 400, 465 and 665 nm (Abs_{254} , Abs_{400} , Abs_{465} and Abs_{665}) using an ultra-violet spectrophotometer (Lambda 25 UV-Vis spectrophotometer, PerkinElmer Ltd, Beaconsfield, UK), with a blank (deionised water) reading subtracted from each sample. All samples were analysed in triplicate and the average was used in the analysis (removing any outliers, see Dean and Dixen test above).

Dissolved organic carbon

The dissolved organic carbon (DOC) concentrations were determined using a total carbon analyser (vario TOC cube, Elementar Analysensysteme GmbH, Hanau, Germany) on re-filtered samples (as dirt sometimes entered the sample vials during peat pore water collection with Rhizon samplers) using similar Rhizon samplers. Prior to analysis mixed (lab vortex) high DOC samples (based on observable colour) were diluted by a factor of 2 with deionised water, to ensure DOC concentrations were within the range of standards. The sample vials were then placed on the auto-sampler to begin the run. Samples were acidified with 0.1 ml 10% HCl and purged with oxygen to remove any inorganic carbon. A five-point calibration (5, 10, 20, 50 and 100 ppm) was determined with a sodium carbonate (Na_2CO_3) and potassium hydrogen phthalate (KHP) solution, with standards of 50 ppm regularly analysed throughout a run to account for any machine drift and errors. All samples were analysed in duplicate (but single values were used if a measurement failed or was repeated if it was identified as an outlier, see Dean and Dixen test above) and corrected for any dilution steps during the analysis. Sample vials were acid washed in 10% HCl after each run, to ensure no build-up of residual carbon.

Stream water quality assessments

Stream flow water samples were collected monthly between July 2012 and December 2016 (for dates see **Table A5.2**) from the outflow of the V-notch weirs on all six sub-catchments. Water samples were collected in pre-rinsed and previously acid washed 250 ml polypropylene bottles (Nalgene, Thermo Scientific, Rochester, NY, USA) directly from the outflow of the weirs. All samples were stored in the dark immediately after collection and kept at 4°C within 24 hours of collection and processed within one month. To identify outliers a Dean and Dixon test (Dean & Dixon, 1951) was used (i.e. the ratio of the outlier gap to the data range, based on a reference value that corresponds to the sample size and confidence level).

Table A5.2 Sampling days for stream water sampling at the three sites (Nidderdale, Mossdale and Whitendale) during the pre- (2012/13) and post-management (2013-2016) period. Onset of management change was in March/April 2013.

Number per year	Nidderdale	Mossdale	Whitendale
1	19/07/2012	19/07/2012	19/07/2012
2	28/08/2012	29/08/2012	30/08/2012
3	26/09/2012	26/09/2012	26/09/2012
4	09/10/2012	10/10/2012	12/10/2012
5	21/11/2012	21/11/2012	21/11/2012
6	04/12/2012	05/12/2012	11/12/2012
1	31/01/2013	31/01/2013	31/01/2013
2	19/02/2013	19/02/2013	20/02/2013
3	12/03/2013	12/03/2013	12/03/2013
4	30/04/2013	30/04/2013	30/04/2013
5	21/05/2013	21/05/2013	21/05/2013
6	25/06/2013	26/06/2013	27/06/2013
7	17/07/2013	18/07/2013	19/07/2013
8	13/08/2013	13/08/2013	13/08/2013
9	02/09/2013	03/09/2013	04/09/2013
10	30/09/2013	01/10/2013	02/10/2013
11	14/11/2013	14/11/2013	14/11/2013
12	30/12/2013	30/12/2013	30/12/2013
1	28/01/2014	28/01/2014	23/01/2014
2	19/02/2014	19/02/2014	19/02/2014
3	25/03/2014	25/03/2014	26/03/2014
4	08/04/2014	09/04/2014	10/04/2014
5	19/05/2014	20/05/2014	21/05/2014
6	09/06/2014	11/06/2014	12/06/2014
7	29/07/2014	29/07/2014	29/07/2014
8	04/08/2014	04/08/2014	05/08/2014
9	15/09/2014	16/09/2014	15/09/2014
10	21/10/2014	22/10/2014	22/10/2014
11	10/11/2014	11/11/2014	12/11/2014
12	18/12/2014	18/12/2014	18/12/2014
1	13/01/2015	27/01/2015	27/01/2015
2	20/02/2015	17/02/2015	20/02/2015
3	05/03/2015	05/03/2015	05/03/2015
4	14/04/2015	15/04/2015	16/04/2015
5	10/05/2015	11/05/2015	12/05/2015
6	03/06/2015	04/06/2015	05/06/2015
7	22/07/2015	22/07/2015	22/07/2015
8	18/08/2015	19/08/2015	20/08/2015
9	14/09/2015	15/09/2015	16/09/2015
10	06/10/2015	07/10/2015	07/10/2015
11	12/11/2015	12/11/2015	12/11/2015
12	25/11/2015	01/12/2015	04/12/2015
1	12/01/2016	12/01/2016	12/01/2016
2	23/02/2016	23/02/2016	23/02/2016
3	09/03/2016	09/03/2016	09/03/2016
4	27/04/2016	27/04/2016	27/04/2016
5	03/05/2016	04/05/2016	05/05/2016
6	21/06/2016	22/06/2016	23/06/2016
7	12/07/2016	13/07/2016	14/07/2016
8	25/08/2016	25/08/2016	25/08/2016
9	14/09/2016	15/09/2016	16/09/2016
10	04/10/2016	04/10/2016	04/10/2016
11	24/11/2016	24/11/2016	24/11/2016
12	12/12/2016	12/12/2016	12/12/2016

pH

Flow water samples were subsampled into two 50 ml centrifuge tubes (Polypropylene, Fisherbrand) for measurement duplication (measurements were repeated if measurements differed by more than 10% from each other). The pH meter (Thermo Orion Bench-top pH meter, model 420) was manually calibrated at the start of each new working day, using laboratory standard pH buffers at pH 2.0, 4.0 and 7.0. The sample to be measured was briefly mixed using a sample vortex (lab dancer). The electrode was rinsed with ultra-pure deionized water and lightly dried before each measurement. After each measurement the electrode and probe were rinsed twice and placed in ultra-pure deionized water.

Conductivity

Two 50 ml centrifuge tubes (the same as for pH) were filled with sub samples of the flow water samples for duplication (but single values were used if a measurement was identified as an outlier, see Dean and Dixon test above). The samples were allowed to equilibrate to room temperature and were briefly mixed using a sample vortex (lab dancer) before conductivity measurements were taken. The conductivity meter (Hanna HI9033) was calibrated before each round of measurements using an 84 $\mu\text{S}/\text{cm}$ conductivity standard (Hanna HI7033L), and then the probe was rinsed with ultra-pure deionized water and lightly dried before each measurement took place. After the meter had equilibrated and the conductivity reading had been recorded the probe was rinsed with ultra-pure deionized water and placed in ultra-pure deionized water, ready for the next measurement.

Particulate organic carbon

Particulate organic carbon (POC) was separated from a 50 ml subsample of stream water (after mixing samples on a lab vortex for 1 minute) by filtering under vacuum through a pre-ashed (combusted in a muffle furnace for 2 hours at 550°C) and weighed 0.7 μm glass-fiber filter (Whatman glass microfiber filters, Grade GF/F, 25 mm diameter, Sigma-Aldrich, Dorset, UK); for each stream sample, three such filtrate samples were obtained. Filters were dried overnight in an oven at 105°C, put in a desiccator and re-weighed to determine the amount of particulate matter. Filter papers were combusted in a muffle furnace at 375°C (initial temperature was set to 100 °C, and then increased by 50 °C every 15 minutes to avoid too fierce combustion until 375 °C was reached) for 16 hours following the method by Ball (1964). Blank filter samples filtered with ultrapure water were run to determine any weight loss unrelated to organic matter losses, which was used to correct weight losses containing sample filtrate. Samples were cooled, put in a desiccator and reweighed to determine loss on ignition (LOI) with any values of >100% LOI set to 100% (see Ball, 1964). LOI was then used to calculate organic carbon content (%C_{org}) according to a regression by Ball (1964; $0.458 \cdot \text{LOI} - 0.4$) which was used to derive POC by multiplying %C_{org} with the dry weight of the filtered organic material (i.e. dry material with the ash content subtracted). The mean value of the three filtrate sub-samples (or less if a value was identified as an outlier, see Dean & Dixon test above) was used in the final analysis.

Dissolved organic carbon

Prior to analysis of stream water for DOC concentrations flow samples were briefly mixed on a lab vortex and about 5 ml was filtered through Rhizon samplers (pore size 0.15 μm , Rhizosphere Research Products B.V., Wageningen, Netherlands) in the laboratory to allow comparison of DOC sampled in stream and plot water samples. Samples were then diluted by a factor of 2 with deionised water, to ensure DOC concentrations were within the range of standards (based on colour and test runs).

The DOC concentrations were determined using a total carbon analyser (either LiquiTOC or vario TOC cube, Elementar Analysensysteme GmbH, Hanau, Germany; due to technical reasons, it was not possible to use the same analyser for all samples but results were cross-validated in a comparison between the two analysers for all samples in 2014). Prior to analysis, samples were acidified with 0.1 ml 10% HCl and purged with oxygen to remove any inorganic carbon. A five-point calibration (5, 10, 20, 50 and 100 ppm) was determined with a sodium carbonate (Na_2CO_3) and potassium hydrogen phthalate (KHP) solution, with standards of 50 ppm regularly analysed throughout a run to account for any machine drift and errors. All samples were analysed in triplicate (removing any outliers, see Dean & Dixon test above). Sample vials were acid washed in 10% HCl after each run, to ensure no build-up of residual carbon. Both DOC and POC concentrations were expressed as mg C L^{-1} .

Missing DOC fraction

As DOC flow samples were filtered through 0.15 μm Rhizon samplers (Rhizosphere Research Products B.V., Wageningen, Netherlands) to be comparable to DOC concentrations measured in peat pore plot samples, unfiltered stream water samples in 2015 and 2016 were also passed through 0.45 μm (a common filter size for DOC determination) Whatman nitrocellulose filters using a vacuum pump. This provided two filtrates in addition to the one obtained by filtering flow water for POC using 0.7 μm Whatman glass microfibre filters (GF/F filters). All three filtrate samples were then diluted by a factor of 2 (to be within the range of the analyser) and analysed on the vario TOC cube, Elementar Analysensysteme GmbH, Hanau, Germany using the same method as described above.

The comparison between DOC concentrations (for 2015 and 2016) in the 0.15 μm filtrate samples to either 0.45 μm or 0.7 μm filtrate samples (which are commonly used filter sizes) provided an estimate of the 'missed' DOC fraction in comparison to using a Rhizon sampler for determining stream flow DOC concentrations. The measured average of the missed DOC fraction was applied to all measurements (including to pre 2015). Reported results indicate which DOC concentrations were used (see section 4.2.10.2 and **Table 13** in the main report).

UV Absorbance

For all monthly stream samples the same method as described above for pore water samples was used.

ICP

All flow samples were also analysed by an inductively coupled plasma mass spectrometer (ICP; iCAP 7000 Series ICP spectrometer, Thermo Scientific, Waltham, MA, USA). Stream flow water (about 50 ml of undiluted sample) was first filtered using a 0.7 µm glass-fibre filter (Whatman glass microfiber filters, Grade GF/F, 25 mm diameter, Sigma-Aldrich, Dorset, UK) and transferred into clean centrifuge tubes prior to measurement. Blanks and washes for flow samples were ultrapure deionised water, and two washes were run after every 12 samples. A multi element certified reference material (for flow samples: CertipurR, Merck KGaA, Darmstadt, Germany) was also run between the two washes. Element concentrations were calibrated using 0.5, 1, 2, 5, 10 and 20 ppm concentrations of a multi-element standard (CertipurR, Merck KGaA, Darmstadt, Germany), containing K, Na, Ca, Mg, Fe, Al, Mn, Zn, Cu and Pb, which was made up in a nitric acid matrix. Phosphorus was similarly calibrated using 0.5, 1, 2, 5 and 10 ppm concentrations of a phosphorus standard containing H₃PO₄ (CertipurR, Merck KGaA, Darmstadt, Germany). Argon was used as the carrier gas. Many of the Pb concentrations were below the machine limit of detection, resulting in negative concentrations being recorded. Therefore, Pb was excluded from further analyses.

Data analysis

All statistical analyses were carried out in R version 3.3.1 (R Core Team, 2016). Following Zuur *et al.* (2009), residuals were plotted against fitted values and visually assessed for normality and homogeneity of variance. The critical p-value chosen for significance was 0.05. Linear mixed effects models employing the “lmer” function from the “lmerTest” package (Kuznetsova *et al.*, 2016) were used to test for management and site effects on the pH, elemental concentrations, conductivity, UV spectra (only on stream flow data; see below for details of UV spectra analysis on plot and slope data) and DOC and POC concentrations. Due to producing many small values (i.e. close to 0) and very few large values, DOC, POC, elemental, Hazen, SUVA (i.e. specific ultraviolet absorbance: UV254/DOC) and conductivity data were natural log transformed for analysis. For a statistical output of the fluvial DOC and POC concentrations see **Table A6.4** in Appendix 6.

The managements (the four main managements for plot data - i.e. FI, DN, BR and LB - and the burnt and mown catchments for the slope and stream flow data), sites and time period (either pre-management, i.e. before management implementation, or post-management, i.e. after management implementation) were used as fixed effects, as were the interactions between them. The month in which measurements were made was also included as a fixed effect as were appropriate environmental variables: soil temperature (Tsoil) and the total rainfall from the four weeks before measurements were taken (Rain_4wk) were included in the DOC and POC models, in the various models incorporating UV spectra data from the stream flow samples, the conductivity model and the pH models for the plot and slope data; Rain_4wk was included in the pH model for the stream flow data; and the percentage cover of heather on the plot was also included in the DOC models for the plot and slope data. A random intercept was included for each model, with a nested structure of blocks in sites (to account for spatial heterogeneity) in years (to account for repeated measurements) for the models using plot and slope data. For stream flow data, block was removed from the random structure (one stream per catchment).

Following the 10-step protocol in section 5.10 of Zuur *et al.* (2009), variables were dropped stepwise from each linear mixed effects model and the log-likelihood ratio and AIC value were used to assess whether a variable should be dropped or kept in the model. For the final models, the “Satterthwaite” option was used to calculate the denominator degrees of freedom as the time periods resulted in an unbalanced design (Spilke *et al.*, 2005). Where significant interactions were found, the “glht” function with the “Tukey” option from the “multcomp” package (Hothorn *et al.*, 2008) was used to compare groups within the interaction terms.

As the UV spectra were only used to analyse the plot and slope water samples in 2016, there were only two sets of measurements for these data (March and October 2016), necessitating a different approach. Data were split by month and analysed separately using ANOVAs (function “aov” from the “base” package; R Core Team, 2016), with management/catchment (for plot and slope data, respectively) and site used as fixed effects.

To further investigate the apparent trend within the pH data, a generalised additive model (GAM) was employed using the function “gam” from the “mgcv” package (Wood, 2006). The pH of the stream flow was the independent variable and the number of months elapsed since the start of monitoring (Elapsed) was used the smoothing term to determine the extent to which pH changed over time and the wiggleness of this trend. The average soil temperature during the month of measurement (Tsoil_av) and the total rainfall during the month of measurement (Rain_tot) were also tried as smoothing term.

Results (pH changes over time)

Although the pH of the stream flow samples fluctuated greatly from month to month (**Figure A5.1**), there was an increase of nearly 1 unit over the five years of measurements. Neither Tsoil_av nor Rain_tot had a significant effect on the pH ($p > 0.05$) but the variable Elapsed had a highly significant relationship with pH ($F = 24.4$, $p < 0.0001$). The smoothing term of Elapsed had 7.33 effective degrees of freedom (the effective degrees of freedom is a measure of the wiggleness of the fit) and the model had adjusted R^2 value of 0.39, meaning that 39% of the variation in pH was explained by the smoother.

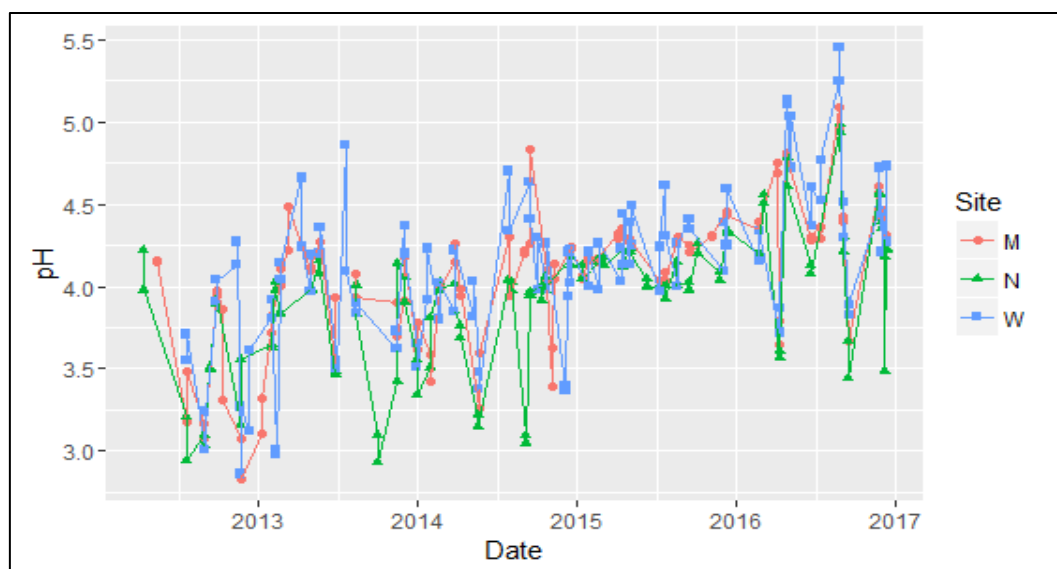


Figure A5.1 Time series of the pH values of the stream flow data for each of the three sites (M = Mossdale, N = Nidderdale and W = Whitendale).

References

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