Article (refereed)

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Title: Effects of storm events on mobilisation and in-stream processing of dissolved organic matter (DOM) in a Welsh peatland catchment

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Abstract: Peatlands are important contributors of dissolved organic matter (DOM) to downstream aquatic systems. We investigated the effects of storm events on dissolved organic carbon (DOC) concentrations and DOM quality in a stream draining a Welsh peatland catchment. Intensive stream samples were collected and analysed for pH, DOC, dissolved organic nitrogen (DON), absorbance and fluorescence. Soil water samples and samples of sphagnum pore water were also collected, and a simple end-member mixing model was applied to account for changes occurring during the events. Fluorescence data were interpreted using parallel factor analysis (PARAFAC). DOC concentrations increased and pH decreased during the storm events. The soil water data and the mixing model indicated that this was due to a change of flow paths and draining of the DOC-rich acrotelm. Absorbance indices and the DOC/DON ratio suggested that the DOM released during events was less degraded. There was a striking, inversely related diurnal pattern in absorbance and fluorescence after the discharge peak. The diurnal pattern and a lack of fit with the mixing model suggested that fluorescing DOM was mainly produced in-stream. Fluorescence has been found to peak in the morning and decline during day-time due to photo-bleaching. We hypothesise that the input of additional DOM during events causes a change in the diurnal pattern, giving a peak at mid-day, when the processing of the additional DOM is highest.

Response to Reviewers: Dear editor/reviewer

The manuscript "Effects of storm events on mobilisation and in-stream processing of dissolved organic matter (DOM) in a Welsh peatland catchment" has been revised according to the reviewer's comments.

The main issue addressed in the review was the potential iron interference of the spectrophotometric analyses. We have now explored this issue thoroughly, and our conclusion is that that there may have been some iron interference, but that this does not appear to affect the interpretation - our main focus was temporal patterns throughout the events, not absolute levels, and these patterns were hardly affected by the iron correction. The issue has been adressed in the methods (lines 173-195 and results (lines 351-364+394-401) sections. For absorbance and sUVa, Fe corrected values were included in the revised paper, based on a published equation (Weishaar et al 2003). The fluorescence estimate was based on a similar regression, but it was more crude as we had to produce the equation ourselves

based on the data in the paper by Ohno et al. (2008). Hence, we did not feel it would be appropriate to present these data, but the effect was much the same as that for absorbance, and again did not alter the interpretation. Having accounted for Fe interference in our revised analysis, described this in the methods, and found that it did not alter the interpretation, we did not feel there was a need to alter or expand the discussion and conclusions further on this subject (cf specific comment 11). However, further detail could be included if requested by the editor.

Specific comments:
2. The filters were rinsed with both water and sample before proper filtering, to exclude the possibility of filter bleed. A description of this has been included in the text (lines 155-156 and 160).
3. The two different filters were used for two different aliquots of the sample. This has now been made clearer in the text (lines 157-158). Due to risk of contamination, samples to be analysed for fluorescence had to be filtered at CEH Wallingford according to their procedures. The different filter size was also according to their procedures. 1.2 μm is sufficient to remove substituents that can cause scattering. There is hardly any difference in fluorescence between 0.45 and 1.2 μm, as the important region is around 0.1 μm, where microbial and cellular material is removed from the solution.
4. sUVa and E2/E3 are not specific measures of particular properties of DOM, but are correlated with some properties. This is thoroughly explained at the beginning of the discussion on DOM quality. In our opinion it would be confusing and too complicated to include this information in the methods section. The interpretation of absorbance or fluorescence analyses are not explained either, for the same reason. It is merely stated in the introduction that spectrophotometric analysis gives information on DOM quality (lines 103-107).
5. The fluorescence exhibited by two of the sphagnum samples may indicate some protein-like material. The excitation wavelength fits with that of tyrosine or especially tryptophan, but the emission wavelength was somewhat lower. However, as explained (lines 284-285) this was not a significantly strong feature across all samples for a component in this region to be validated. PARAFAC extracts components in all regions of the EEM measured, so if this was a strong feature, it would have been expressed in a valid component. As stated in lines 371-374 some overlap with the fluorescence caused by contamination in some of the samples may have reduced the chances of validating a component in this region, but most likely not, as it was only a slight overlap between the regions of true and contaminant fluorescence. Hence, as a true component in this region was not validated, we did not include a thorough discussion of what this component, if validated, would have represented, even though it was most likely some protein-like material.
6. This has already been addressed.
7. Yes, we did measure blank samples from these tubes. This has now been stated in lines 370-371.
8. The point of the mixing model was merely to give an indication of the shape of the end-member variation through the event, not the exact proportions. The mixing model also highlights the strong deviation of fluorescence from anything resembling conservative mixing. We consequently think the mixing model serves as a good illustration, and would like to keep it. A couple of lines (439-441) on the reliability of the different models were included.
9. True, this is not really a good way to express this. The main reason to include E2/E3 is that it is a different type of parameter, and when both parameters indicate the same change in properties, this strengthens the conclusion. E2/E3 is in fact also a more robust parameter, because there is less risk of contamination when only using spectrophotometric analysis (a line on this has been included in the text, line 457). However, more readers will be familiar with the sUVa index, so it would not be a good alternative to use E2/E3 only. As explained above, there is no reason to believe that iron interference would have a strong impact on E2/E3.
10. The mechanism is not clear here, as it cannot be drawn from the data themselves. However, it is not likely to be related to sorption in the mineral soil as the catchment is strongly peat-dominated. Sorption to organic soil is more likely, but we have no evidence to support such a mechanism.
11. The discussion has been shortened wherever possible. The main changes have been made to the first section, Effects of events on DOC concentration. Here the last two paragraphs have been removed.

We found this discussion interesting, but removing them makes the manuscript more focused. Accordingly, some details have been removed from the results chapter, as these were mainly a basis for this discussion. No complete paragraphs have been removed from the last two sections of the discussion, as these are the most important parts. The third section has been left as it was, while the second section has been modified to make the discussion shorter and clearer.

Figures: Figure 7 is mainly an example, and can be removed. We would like to keep the rest of the figures, as they give important background, or serve as useful illustrations to the reader. The figure numbers are thus changed for figures 8-11, to 7-10. Some cosmetic changes have been made to figures 3-5+7.

Yours sincerely
Kari Austnes

Effects of storm events on mobilisation and in-stream processing of dissolved organic matter (DOM) in a Welsh peatland catchment

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Abstract

Peatlands are important contributors of dissolved organic matter (DOM) to downstream aquatic systems. We investigated the effects of storm events on dissolved organic carbon (DOC) concentrations and DOM quality in a stream draining a Welsh peatland catchment. Intensive stream samples were collected and analysed for pH, DOC, dissolved organic nitrogen (DON), absorbance and fluorescence. Soil water samples and samples of sphagnum pore water were also collected, and a simple end-member mixing model was applied to account for changes occurring during the events. Fluorescence data were interpreted using parallel factor analysis (PARAFAC). DOC concentrations increased and pH decreased during the storm events. The soil water data and the mixing model indicated that this was due to a change of flow paths and draining of the DOC-rich acrotelm. Absorbance indices and the DOC/DON ratio suggested that the DOM released during events was less degraded. There was a striking, inversely related diurnal pattern in absorbance and fluorescence after the discharge peak. The diurnal pattern and a lack of fit with the mixing model suggested that fluorescing DOM was mainly produced in-stream. Fluorescence has been found to peak in the morning and decline during day-time due to photo-bleaching. We hypothesise that the input of additional DOM during events causes a change in the diurnal pattern, giving a peak at mid-day, when the processing of the additional DOM is highest.

Introduction

Peatlands are important contributors of dissolved organic matter (DOM) to downstream aquatic systems. Mean dissolved organic carbon (DOC) fluxes in rivers draining peat dominated areas are higher than in rivers draining most other landscape types, in the order of 50-100 kg ha^{-1} yr^{-1} (Aitkenhead and McDowell 2000). Percentage peat cover is found to be a good predictor of DOC concentration across large spatial scales (Aitkenhead et al. 1999).

The concentration of DOC is commonly found to correlate positively with discharge in streams and rivers in temperate and boreal catchments (e.g. Thurman 1985, p. 50; Hope et al. 1994; Soulsby et al. 2003). Likewise, DOC concentrations have frequently been found to increase during storm events (e.g. Hinton et al. 1997; Buffam et al. 2001). However, most studies of discharge-DOC concentration relationships have been conducted in catchments with organo-mineral or mixed soils (Clark et al. 2007a). Given the significant contribution of peat systems to DOC concentrations and fluxes, it is important to investigate the relationship between discharge and not only DOC (as a measure of DOM quantity) but also DOM quality in these systems. Studying these relationships is especially important in a climate change perspective, given that annual precipitation, as well as extremes of daily precipitation, are likely to increase in northern Europe (Christensen et al. 2007).

In streams draining organo-mineral soils, the increased DOC concentration at high discharge is commonly explained by changes in flow paths towards increased lateral flow through the upper horizons, where DOC concentrations are higher (e.g. McDowell and Likens 1988; Boyer et al. 1997; Hinton et al. 1998; Inamdar et al. 2006). Change in flow path can be an important control on DOC concentration in peat

streams as well, but seems to depend upon the hydrological connectivity between the peat and the underlying bedrock/mineral soil (Clark et al. 2007a, b). Peat soils consist of an upper horizon (the acrotelm) with roots and decomposing plant material, and a lower horizon (the catotelm) with dense peat (Evans et al. 1999). The stored carbon in the catotelm is hydrologically disconnected from the stream (Billett et al. 2006). Where there is hydrological connectivity between the peat and the mineral soil, base flow is characterised by alkaline DOC poor groundwater (Worrall et al. 2002; Clark et al. 2007a). As the water table rises during an event, resulting in subsurface flow in the acrotelm, stream DOC concentration increases due to the input of DOC rich soil water (Evans et al. 1999; Worrall et al. 2002; Soulsby et al. 2003; Clark et al. 2007a, b). However, further progress of the event may introduce low DOC, rain-like water (Clark et al. 2007b), due to exhaustion of the acrotelm or the occurrence of saturated overland flow or macropore flow (Evans et al. 1999; Worrall et al. 2002). Hence, stream concentration can be described by mixing of water from three different source areas, so-called end-members (Worrall et al. 2002). In other peat catchments, base flow is chemically similar to the acidic DOC rich soil water in the acrotelm (Clark et al. 2007b). In these systems stream water during events derives from two end-members only: soil water and the rain-like water (Clark et al. 2007a). This causes a decrease in stream DOC concentration during events (Clark et al. 2007a; Eimers et al. 2008).

Literature on the effects of storm events on DOM quality is sparse. If storm events cause changes of flow paths and not merely dilution, one would assume a change in the quality of the DOM released to the stream (Buffam et al. 2001). $DO^{14}C$ data from peat moorland in the upper Conwy catchment showed that base flow releases old, soil-derived DOC, whereas high flow releases younger DOC, probably

derived from recent plant material (Evans et al. 2007). Probably, a difference in age also implies different degree of degradation, which in turn affects DOM quality (Qualls and Haines 1992; Kalbitz et al. 2003; Saadi et al. 2006). Spectrophotometric measurement of absorbance and fluorescence can give information on DOM quality in terms of chemical characteristics of the organic material and its bioavailability (e.g. Senesi 1990; Peuravuori and Pihlaja 1997; Croué et al. 1999; McKnight et al. 2001; Kalbitz et al. 2003; Fellman et al. 2008). To our knowledge fluorescence scanning of high frequency event samples has not previously been conducted.

In the present study high frequency measurement of chemical and spectrophotometric characteristics of high frequency stream water samples was conducted in a peatland catchment in Wales. The objectives of the study were: 1) To investigate the effect of storm events on DOC concentration, 2) to investigate the effects of storm events on DOM quality, and 3) to explore the usefulness of fluorescence analyses to assess short term changes in DOM quality.

Methods

Field site

The study site is a small (3.2 km^2) peatland catchment in north Wales drained by the stream Afon Ddu (Fig. 1). The catchment is a sub-catchment of the upper Conwy catchment, and it is part of the large Migneint blanket peatland area. Catchment altitude ranges from 440 to 540 m.a.s.l., with the main part being below 500 m.a.s.l.. Average temperature is 8°C and mean annual rainfall is 2300 mm. Hydrological response to rainfall events is rapid (Billett et al. 2007). The bedrock is volcanic, mixed

rhyolitic and basaltic, of Ordovician origin. Peat soils (histosols) dominate, but at elevated sites podzols occur. The soil depth is usually around 1-2 m, but may be up to 5 m. The vegetation is dominated by heather (*Calluna vulgaris* L.) and *Sphagnum* spp. mosses, with scattered grasses and sedges. There is some drainage ditching in the catchment dating from the 1930s-1960s.

Water sampling

Intensive high-flow event sampling in the Afon Ddu was conducted in the autumn of 2007 (sampling point at Ordnance Survey coordinates 277520,344920). Samples were collected during autumn because DOC concentrations were expected to be high (Clark et al. 2007b). Samples were collected during three events (September 17^{th}-18^{th}, October 3^{rd}-4^{th}, October 26^{th}-30^{th}), hereafter referred to as events 1, 2 and 3. During events 1 and 2, samples were collected for 24 hours (event 1 two-hourly, event 2 hourly), while during the larger event 3 samples were collected two-hourly for 96 hours. Samples were collected using a Xian 1000 autosampler (Hach Lange, Germany).

Samples were collected from a number of nearby locations during each event, to provide information on potential end-members. These were sphagnum pore water samples collected by manual squeezing, and three different types of soil water samples collected from blanket peat: bulked soil water collected at 5 cm using micro-rhizon suction samplers and soil water collected at 5 and 10 cm using zero-tension lysimeters.

151 *Analyses*

152

153 All samples were split in three for different analyses. The aliquot that was to be
154 analysed for carbon and nitrogen was filtered immediately (within maximum 2 days)
155 through Whatman sterile 0.45µm cellulose nitrate filters. The filters were rinsed with
156 deionised water and an aliquot of sample. All samples were stored at <3°C.

157 The event sample aliquots that were to be analysed for fluorescence and
158 absorbance were brought to CEH Wallingford as soon as possible (within 0-5 days).
159 Prior to analysis all samples were filtered through Whatman 1.2 µm glass microfibre
160 GFC filters, after rinsing with water and an aliquot of sample. All equipment used was
161 acid washed. The water used was purified with a NANOpure DIamond Analytical and
162 UV Systems from Barnstead. One cm quartz cells were used. Fluorescence analyses
163 were performed on a Varian Cary Eclipse instrument. Several blanks were run, to
164 ensure that the equipment used had no residual fluorescence. The samples were
165 scanned for emission from 280 to 500 nm at excitation 200 to 400 nm. Absorbance
166 was measured on a Varian Cary 50 instrument, measuring the spectrum from 200 to
167 800 nm. Whenever the absorbance was above 0.3 cm^{-1}, the samples were diluted to
168 below this level, and both analyses repeated, as inner-filtering correction (see below)
169 has been found to be insufficient above this level (Ohno 2002). The sUVa index was
170 calculated as absorbance measured at 254 nm divided by DOC (mg l^{-1}) (Vogt et al.
171 2004) and the E2/E3 index as absorbance measured at 250 nm divided by absorbance
172 measured at 365 nm (Peuravuori and Pihlaja 1997).

173 Both absorbance and fluorescence may be affected by iron interference.
174 Absorbance may be overestimated, as Fe ions absorb light (Weishaar et al. 2003).
175 Fluorescence may be underestimated, due to quenching of the fluorescence signal

176 caused by Fe-DOM complexation (Zepp et al. 2004; Ohno et al. 2008). To investigate
177 possible iron intereference, data from biweekly sampling (November 2006 to January
178 2008) at the same spot were used, as Fe concentration was not analysed in the event
179 samples. Fe in the biweekly samples were analysed by ICP-OES. Preliminary
180 analyses showed that the Fe concentration was positively linearly related to DOC
181 concentration ($R^2 = 0.59$). This is in line with Neal et al. (2008), who observed strong
182 correlation between Fe and DOC concentration across a range of UK sites. However,
183 there was also a negative linear relationship with log(flow) (Environment Agency
184 discharge data for the River Conwy at Cwm Llanerch
185 (http://www.nwl.ac.uk/ih/nrfa/station_summaries/066/011.html) ($R^2 = 0.30$). Hence,
186 Fe concentration could be reasonably well explained ($R^2 = 0.78$) using multiple linear
187 regression with DOC concentration and log(flow) as explanatory variables. A linear
188 relationship ($R^2 = 0.82$) was established between Cwm Llanerch flow and Afon Ddu
189 stage (data from August to November 2007). Fe concentration in the event samples
190 was estimated using the multiple linear regression equation with event sample DOC
191 concentration and Cwm Llanerch flow (calculated from Afon Ddu stage) as inputs.
192 The contribution of Fe to absorbance at 254 nm was estimated using the equation for a
193 pure Fe^{3+} solution in Weishaar et al. (2003). Weishaar et al. showed that the
194 absorbances of Fe and DOM are additive. A crude estimate of Fe quenching of
195 fluorescence was done based on data in Ohno et al. (2008).

196 The remaining analyses on event samples were conducted at CEH Bangor.
197 Unfiltered samples were analysed for pH using a Metrohm SM 702 Titrino. Filtered
198 samples were analysed for total dissolved nitrogen (TDN) and non purgeable organic
199 carbon (NPOC) by elemental analysis using a Thermalox TOC/TN Analyser. The
200 purging prior to total carbon analysis removes inorganic carbon as CO_2 (by addition

of 11 µl of 1M HCl and purging with oxygen for 90 sec), so that only dissolved organic carbon (DOC) is determined. Nitrate-N (NO_3-N) and ammonium-N (NH_4-N) were analysed by autoanalyser (Skalar SA-40). NO_3-N was analysed by the sulphanilamide/NEDA/Cd/Cu reduction method, with extinction at 540nm, and NH_4-N by the Indol-phenol blue method, with extinction at 660nm. Dissolved organic nitrogen (DON) was calculated by subtracting NO_3-N and NH_4-N from TDN.

Discharge

Discharge at the Afon Ddu lower site was measured by a Starflow Ultrasonic Doppler instrument with an integrated micrologger (Unidata, Western Australia). Due to instrument failure, the velocity measurements were not correct, so accurate discharges could not be calculated. However stage data were reliable, and provided an effective proxy for the discharge changes associated with each event.

Rain data used were from the Snowdon Environmental Change Network site, about 20 km north-west of the Afon Ddu catchment (Countryside Council for Wales 2008).

Data analysis

The event data are described quantitatively using different approaches. Base flow composition was defined as the average stream water composition of samples taken when there was minimal change in stream stage (the first 3, 5 and 11 samples in event 1, 2 and 3, respectively; average for pH calculated via H^+). In event 1 the stable stage level did not represent a true base flow level, as it closely followed an earlier event.

To account for changes in the different parameters during the events, the percentage change from the base flow to a maximum or minimum was calculated. Base flow and average soil water (the micro-rhizon and the two zero-tension lysimeter samples) was compared for each event using two sample t-test. Results for sphagnum pore water could not be compared statistically to base flow or soil water, as there was only one sample per event. Correlation of parameters was done using Pearson's r. Results were considered significant for $P < 0.05$. Minitab Release 14 was used as statistical software. Regression analyses for the Fe-estimation were performed with JMP 7.0.1.

A simple two end-member mixing model was applied to each event and parameter (H^+ and DOC concentration, absorbance at 254 nm and fluorescence intensity), using the average base flow as one end-member and average soil water as the other end-member. This corresponds to the end-member model described by Worrall et al. (2002) but omits the rain end-member (dilution). The model calculates the proportion of each of the two end-members contributing to stream water, starting from the first non-base flow sample. It is a crude model, as there were probably other end-members; base flow is not a true end-member; and none of the parameters are conservative. The intention was simply to assess whether the proposed two end-member system could consistently explain observed variations in different parameters, i.e. that the contribution of the two end-members was consistent for different parameters throughout the event.

Fluorescence data were analysed using parallel factor analysis (PARAFAC) in MATLAB (version R2007b), according to the procedure described by Stedmon and Bro (2008). PARAFAC decomposes the complex set of sample emission-excitation matrices (EEMs) and extracts specific components (Stedmon et al. 2003),

representing groups of fluorophores with similar fluorescence characteristics (Stedmon and Markager 2005). The model is defined as

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{ijk}$$

$$i = 1,...,I; \qquad j = 1,...,J; \qquad k = 1,...,K \tag{1}$$

where x_{ijk} is the fluorescence intensity of the ith sample at emission wavelength j and excitation wavelength k. a_{if} is directly proportional to the concentration of the fth component in sample i. b_{jf} is linearly related to the quantum efficiency (fraction of absorbed energy emitted as fluorescence) of the fth component at emission wavelength j. c_{kf} is linearly proportional to the specific absorption coefficient of the fth component at excitation wavelength k. The residual matrix ε_{ijk} represents the variability not accounted for by the model, and the model is found by minimising the sum of squared residuals (Stedmon et al. 2003).

The raw data were corrected for inner-filtering effects according to Parker and Barnes (1957). Inner filtering reduces the fluorescence intensity due to the absorption of the excitation beam and of emitted light after excitation (Lakowicz 1983, p. 44). The correction suggested by Lakowicz (1983, p. 44) and applied by e.g. McKnight et al. (2001) and Ohno (2002) gave nearly identical results as the Parker and Barnes (1957) correction, but the latter correction was selected because it takes account of the dimensions of the excitation and emission beams. The matrices were then corrected for instrument bias, i.e. the effects of lamp output and instrument sensitivity/response. A blank, measured on a sealed cell of Milli-Q water, was subtracted to remove/reduce the Raman line (Hudson et al. 2007). Finally a mask was applied to the data matrix, setting all data in the regions without fluorescence (excitation wavelength exceeds emission wavelength) to zero, and replacing all data in the regions greatly influenced

by the two Rayleigh-Tyndall lines (Hudson et al. 2007) with missing values (NaN in MATLAB).

A series of PARAFAC models with three to seven components were fitted to the data. As some of the components gave negative excitation or emission loadings, non-negativity constraints were applied. The PARAFAC model could be split half validated (i.e. separate modelling of two halves of the samples gave statistically identical results, cf. Stedmon, C. A. and Bro, R. 2008) for up to three components. Initial exploration revealed that the sphagnum pore water samples from event 1 and 2 showed a high residual fluorescence at low excitation and emission wavelength. Some other samples, especially soil water samples, also exhibited some fluorescence in this region. However, overall this feature was present in too few samples for a model including a component in this region to be validated. Thus, the two sphagnum pore water samples were considered as outliers, and removed prior to the final modelling. The final model was the three component model giving the smallest residual error out of ten models fitted following random initialisation of the model. The maximum fluorescence intensity of the different components (Fmax) for individual samples was corrected for dilution by multiplying by the dilution factor.

Results

Hydrology

The rainfall during event 1, 2 and 3 was 16, 17 and 114 mm, respectively (Fig. 2). Event 1 was the second in a series of events following an 11 day dry period. There was a 26 mm event just prior to event 1, and the stage was 198 mm at the onset of the

event. During the event, stage rose to 334 mm. Event 2 was preceded by 7 dry days, with a series of large events before that (total precipitation 347 mm). The stream stage was 138 mm at the onset of the event and maximum stage during the event was 211 mm. Event 3 was preceded by a 10 day dry period. The stream stage was at 131 mm at the onset. During the event, stage rose to 1045 mm. Event 3 was clearly the largest of the three events, and one of the largest of the whole autumn.

pH and DOC

The pH decreased during all events (Fig. 3). The largest decrease (2.3 pH units from base flow levels) occurred in event 3, compared to 0.9 and 1.1 pH units in events 1 and 2, respectively. Base flow pH was significantly higher than that of soil water in all events. pH of sphagnum pore water was at a similar level as that of soil water.

DOC concentrations increased during all events (Fig. 3), by 16%, 44% and 89% relative to base flow in events 1, 2 and 3, respectively. The DOC concentration was significantly and negatively correlated with pH (r^2 0.59, 0.81 and 0.86 for event 1, 2 and 3, respectively). Soil water DOC concentrations were higher than those of base flow, but the difference was only significant for event 2 and 3. DOC concentration in sphagnum pore water was higher than in soil water in event 1 and 2, and at the same level as in soil water in event 3.

DOC/DON ratio

The DOC/DON ratio data (Fig. 4) were noisy, but there was an upward trend in all events. Maximum values were 58, 57 and 103% higher than base flow values in event

1, 2 and 3, respectively. There was no significant difference between soil water and base flow DOC/DON ratios in any of the events. The DOC/DON ratios of the sphagnum samples were far lower than those of both base flow and soil water samples.

Absorbance

Absorbance at 254 nm increased during events 2 and 3 (Fig. 5), with 30 and 53% maximum increases from base flow, respectively. In these events, absorbance was significantly and positively correlated with DOC concentration (r^2 0.88 and 0.92, respectively). In event 3 there was a striking diurnal pattern in absorbance after the initial peak, with consistent maxima at 7 am and minima at 1 pm. Soil water absorbance was significantly higher than that of base flow in events 2 and 3. Absorbance of sphagnum pore water was generally lower than that of both base flow and soil water.

The sUVa index (Fig. 5) decreased from base flow levels during event 1 (16% change), and more clearly during event 3 (28% change). There was no apparent pattern in event 2. The four deviating samples in event 2 with relatively low sUVa values correspond to the four samples with relatively low DOC, and appears to be caused by a DOC measurement error, as this deviation was not observed for absorbance. The reason for this error could not be established. Base flow sUVa was significantly higher than that of soil water in all events. sUVa in sphagnum pore water was far lower than sUVa in both base flow and soil water.

There was a rising trend in E2/E3 (Fig. 5) for all events, with a change from base flow to maximum levels of 5, 3 and 27% for events 1, 2 and 3, respectively. Soil

water E2/E3 was significantly higher than that of base flow in all events. E2/E3 in sphagnum pore water was at a similar level as in soil water.

The average estimated Fe concentration was 2.2, 1.6 and 1.5 mg l^{-1} for events 1, 2 and 3, respectively, i.e. above the level (0.5 mg l^{-1}) where iron interference should be considered (Weishaar et al. 2003). The estimated Fe concentration followed closely the DOC concentration, and was only slightly modified by flow. Fig. 5 shows the effect of correcting for Fe absorbance on absorbance and sUVa. The effect is pronounced, and the resulting sUVa values are more realistic (cf. Weishaar et al. 2003, table 1). However, the temporal patterns are preserved to a high degree. As the following discussion is mainly focused on the relative changes and temporal patterns, the issue of iron interference will not be further discussed, but it should be kept in mind that the absorbance and sUVa values are overestimated. E2/E3 is probably not strongly affected by iron interference. Weishaar et al. (2003) observed similar iron interference at 280 nm as 254 nm, so the interference at 365 nm is not believed to be very different. Moreover, the E2/E3 values obtained are realistic (cf. Peuravuori and Pihlaja 1997, table 2).

Fluorescence

Component 3 from the PARAFAC analysis was considered to represent contamination, as it only appeared in the samples where a certain plastic tube was used for dilution. Blank samples using the same plastic tube exhibited the same feature. This component is consequently not discussed in the following. However, this component overlapped with the region that was poorly modelled for the sphagnum

373 samples, and the presence of this contamination component may have added to the
374 difficulty of validating a model with a true component in this region.

375 The two remaining components are shown in Fig. 6. Both components were
376 double-peaked. Component 1 (C1) had maximum excitation at <250 nm with a
377 secondary peak at 310 nm, and maximum emission at 440 nm. Component 2 (C2) had
378 a maximum at excitation 260 nm with a secondary peak at 360 nm, and maximum
379 emission at 474 nm. The temporal variation in the fluorescence intensity (Fig. 7) did
380 not seem to coincide with the changes in stream stage. Throughout the sampling
381 periods the intensity levels were either stable or decreasing. C1 had the clearest
382 decreasing trend. This resulted in a decrease in the C1/C2 ratio in all events, with a
383 levelling off (event 1 and 2) or increase (event 3) towards the end of the events (Fig.
384 7). The decrease from base flow levels to minimum levels was 7, 18, and 17% in
385 events 1, 2 and 3, respectively. C1 was higher in base flow than soil water, but this
386 was only significant for event 2. C2 was higher in soil water, and this was significant
387 for event 2 and 3. Both C1 and C2 were lower in sphagnum pore water than in base
388 flow and soil water. For C1/C2, base flow was significantly higher than soil water in
389 all events. C1/C2 of sphagnum pore water was at the same level as for base flow.

390 In event 3, there was a clear diurnal pattern in C1 and C2, especially observed
391 after the stage peak. The fluorescence peaked at 1 pm and was lowest during the night
392 (9 pm to 7 am). The pattern coincided with the in situ temperature variation, while it
393 was inversely related to the pattern observed for absorbance (Fig. 8).

394 Ohno et al. (2008) investigated Fe quenching of PARAFAC-derived
395 components. Their results could be used as basis for a crude estimate of fluorescence
396 quenching, as their components 1 and 2 corresponded well with C1 and C2,
397 respectively. The effect on overall levels was pronounced, but the temporal patterns

were preserved to a high degree. Ohno et al. (2008) showed that component 1 was more strongly quenched than component 2. However, the estimated effect of quenching on C1/C2 was minor. Hence, Fe quenching seems unimportant to the temporal patterns in fluorescence, and will not be further discussed.

Mixing model

Mixing models for event 1 and 2 did not show any consistent patterns between parameters (data not shown). For event 3 (Fig. 9), there was a consistent, although not identical, pattern in H^+ concentration, DOC concentration and absorbance at 254 nm. The curves showed a decrease in the proportion of base flow-type water at the start of the event and a subsequent increase coinciding with the decrease in stream stage. The models based on H^+ and absorbance indicated lower and higher contribution of soil water at peak flow compared to the model based on DOC concentration, respectively. Mixing models for event 3 based on C1 and C2 were not consistent with those of the other three parameters (Fig. 9).

Discussion

Effects of events on DOC concentration

The higher pH and lower DOC concentration in base flow compared to soil water indicates that the peat system in Afon Ddu is not like the system described by Clark et al. (2007a, b), where base flow was chemically similar to soil water. Like DOC concentration, absorbance at 254 nm was also lower in base flow than in soil water.

Absorbance is generally known to be closely correlated with DOC concentration (e.g. Dobbs et al. 1972; Brandstetter et al. 1996; Korshin et al. 1997). Relatively high baseflow DOC concentration indicates a significant input of drainage from the peat, whilst the high baseflow pH demonstrates some hydrological connectivity with underlying base-rich bedrock and/or mineral soil patches within the catchment.

The increased DOC concentration, increased absorbance and decreased pH at high flow can be explained by an increased contribution of soil water, due to a rising water table within the acrotelm and subsurface flow (Evans et al. 1999; Worrall et al. 2002). A higher similarity between soil water and stream water at high flow with respect to DOC concentrations was also observed by Billett et al. (2006). The change in stream chemistry could not solely be caused by an increased input of water from the sphagnum layer, as this could not have explained the trend in absorbance. There was little evidence of dilution at peak flow. The process could thus be reasonably well described for event 3 by the applied two end-member mixing model, rather than a three end-member model as suggested in Worrall et al. (2002). The discrepancies observed between the DOC concentration, H^+ concentration and absorbance at 254 nm were most likely due to the lack of conservative mixing. The most reliable model is probably the one for DOC, as H^+ concentration is unlikely to be conservative, and absorbance behaves abnormally at peak flow. The inconsistency between parameters in the models for event 1 and 2 can probably be explained by the limited size of the events and small changes in stream chemistry, giving too much noise in such a crude model.

Effects of events on DOM quality

448 The sUVa index provides a measure of the change in DOM quality expressed by
449 deviation from the correlation between DOC concentration and absorbance. sUVa has
450 been shown to be positively correlated with aromaticity and molecular weight (Croué
451 et al. 1999; Weishaar et al. 2003; Hood et al. 2005). Both sUVa and aromaticity
452 increase upon biodegradation (Kalbitz et al. 2003; Saadi et al. 2006). A high sUVa is
453 also associated with lower bioavailability (Fellman et al. 2008). Thus, the decrease in
454 sUVa at high flow indicates that the DOM released at high flow is less degraded and
455 aromatic and of lower molecular weight. This is supported by the increase in E2/E3,
456 as E2/E3 is negatively correlated to aromaticity and molecular weight (Peuravuori and
457 Pihlaja 1997). E2/E3 is a more robust parameter than sUVa, giving a smoother trend.
458 DOC/DON data from this study are noisy, but clearly show increases in all
459 three events. Increasing DOC/DON may be indicative of increasing aromaticity
460 (McKnight et al. 1997; Hood et al. 2005), because algal and microbial material has
461 low DOC/DON (McKnight et al. 1994) and low aromaticity (McKnight et al. 2001).
462 However, even the lowest DOC/DON values observed are much higher than those
463 associated with algal or microbial material (McKnight et al. 1994). Moreover, the
464 highest DOC/DON values observed correspond to the lower range of values observed
465 for different litter leachates (Magill and Aber 2000). Hence, in this case it is more
466 likely that increasing DOC/DON indicates decreasing degree of degradation (Melillo
467 et al. 1982; Qualls and Haines 1992; Currie et al. 1996; Yano et al. 2004). Thus both
468 absorbance and DOC/DON indicate that base flow is characterised by older and
469 probably more degraded DOM, while soil water comprises younger, less degraded
470 DOM (Evans et al. 2007).
471 With increased absorbance at high flow, one might expect to see a similar
472 response in fluorescence. However, unlike absorbance, fluorescence does not

473 invariably correlate well with DOC, and fluorescence relative to absorbance may vary
474 between different sources (Baker et al. 2008). In addition, fluorescing structures
475 constitute only a minor part of humic molecules (Miano et al. 1988; Senesi et al.
476 1991). Thus, it is possible for fluorescence to decrease even if absorbance, as a bulk
477 parameter, increases.

478 The mixing model for event 3 indicates that stream fluorescence during the
479 event was not a result of mixing of soil water and base flow water, as it was for
480 absorbance. For C1, soil water fluorescence was not sufficiently low to explain the
481 decrease, and C2 remained approximately at base flow levels throughout the event
482 despite the higher fluorescence intensity in soil water. The effect could not be
483 explained by increased contribution of low fluorescence sphagnum pore water, as this
484 does not fit with the observed changes in absorbance, DOC/DON and C1/C2 during
485 the events. Rather, the lack of consistency between fluorescence parameters and the
486 other parameters in the mixing models, the diurnal variation in fluorescence, and the
487 co-variation in fluorescence and in situ stream temperature observed in event 3,
488 collectively suggest that stream fluorescence may be governed by in-stream microbial
489 processes.

490 In-stream processing of DOM has been found to be an important control on
491 stream DOC in a similar catchment (Dawson et al. 2001). The microbial processing
492 probably occurs in biofilms, as it is generally believed that this is where most
493 microorganisms in natural systems exist (Sutherland 2001). Biofilms on the stream
494 bed, rather than suspended aggregates in the water column tend to dominate
495 ecosystems with high downstream transport and sediment-surface-area to water-
496 volume ratio, as in headwaters (Battin et al. 2008). Lock and Hynes (1976) showed
497 that the stream bottom, not the water itself is responsible for the major part of DOC

498 removal, and biofilms on the stream bed have been shown to remove a substantial
499 amount of DOC (Fiebig et al. 1990; Fiebig and Lock 1991). In the Afon Ddu there
500 were no indications of in-stream processing of bulk DOC. This either confirms that
501 only a minor part of the DOM released is subject to such processing, or that the
502 spectrophotometric methods, introducing less uncertainty than the DOC analysis, are
503 better for detecting short-term variation in DOM. The in-stream processing will be
504 further covered in the next section.

505 Microbial processing of DOM is limited by microbial capacities, i.e. reaction
506 rates (Battin et al. 2008). Hence, one can expect increased flow to give dilution of
507 stream fluorescence. The trends in C1 and C2 can thus be explained by dilution as the
508 main control, and only some compensation by fluorescing material from the soil. This
509 indicates that highly fluorescing DOM in soil water is not as mobile as less
510 fluorescing DOM. The compensation by soil water was highest for C2, where the
511 levels were higher in soil water than in base flow. The C1/C2 ratio would not be
512 affected by dilution, so the downwards trend was due to the small contribution of
513 fluorescing material from the soil.

514 A decreased C1/C2 represents a shift in peak position to higher wavelengths,
515 and this has been associated with higher density of aromatic rings, higher degree of
516 aromatic substitution and conjugation, higher molecular weight, and higher
517 hydrophobicity (Senesi 1990; Coble et al. 1998; Sharma and Schulman 1999, p. 20-
518 21; Wu et al. 2003). A peak shift to higher wavelengths may also signify an increase
519 in the importance of humic compared to fulvic acids (Senesi 1990). The C1/C2 ratio
520 thus suggests that the aromaticity and molecular weight of DOM was higher in soil
521 water compared to base flow, which is opposite to the conclusion based sUVa, E2/E3
522 and DOC/DON. However, this fits in well with the idea that the fluorescing material

is mainly produced in-stream, which would make base flow fluorescing material relatively younger than that deriving from soil water, as opposed to what is the case for the bulk material. The overall conclusion is still that DOM released at high flow is generally less degraded, as the fluorescing part constitutes only a minor part of the DOM.

Effects of events on in-stream processing of DOM

As discussed above, the diurnal pattern in fluorescence observed in event 3 appears to reflect diurnal variation in in-stream production of fluorescing DOM. Both temperature and light are environmental factors that vary on a daily timescale. Temperature has a positive effect on fluorescence, as the activity of the DOM-producing microorganisms is stimulated by increasing temperature (Christ and David 1996; Gödde et al. 1996; Freeman et al. 2001). UV light is known to cause a decrease in fluorescence, through so-called photo-bleaching (e.g. Skoog et al. 1996; Moran et al. 2000; Patel-Sorrentino et al. 2004), and can be expected to co-vary with stream temperature, peaking at mid-day.

To our knowledge a diurnal pattern in fluorescence has only been observed by Spencer et al. (2007). They measured in situ fluorescence during base flow by means of a fluorometer measuring at 370 nm excitation and over a broad emission band centred at 460 nm. However, whereas fluorescence in event 3 peaked at mid-day and was positively related to temperature, the diurnal pattern reported by Spencer et al. peaked in the early morning (around 9 am), had a minimum in late afternoon (around 6 pm), and was negatively related to temperature. According to Spencer et al., the diurnal pattern was related to day-time photo-bleaching of the DOM. This is

548 confirmed by base flow data from similar in situ measurements (excitation 330 nm,
549 emission 450 nm) from Afon Ddu (data not shown) and another stream (Nant y
550 Brwyn), 2 km away on the same area of blanket peat (Fig. 10), which showed a
551 similar diurnal pattern.

552 We hypothesise that during an event the diurnal fluorescence pattern is shifted,
553 due to the extra input of soil water DOM represented by absorbing material. Nieto-
554 Cid et al. (2006) showed that fluorescent humic substances could be produced by
555 microbial degradation of DOM on a short (<1 day) timescale, and that the magnitude
556 of production was related to microbial activity. If even a small proportion of the DOM
557 transported from the soil can be used by biofilm microbial communities, this could
558 explain the rapid, diurnal production of fluorescent DOM. Degradation would be
559 expected to peak at mid-day due to the higher temperatures, which could explain the
560 shift in the diurnal cycle, and also the negative relationship between fluorescence and
561 absorbance in event 3. This positive effect of temperature apparently surpasses any
562 negative effect of UV light at mid-day, perhaps because the cloud cover in the period
563 during and after the storm event was high, and UV intensity consequently low.
564 Alternatively, or additionally, given the high input of DOM, photo-bleaching could
565 have had a positive impact on production of fluorescing DOM, as UV radiation is
566 found to increase the substrate quality, and thus the degradability, of terrestrially
567 derived DOM (Moran et al. 2000; Tranvik and Bertilsson 2001; Anesio et al. 2005).

568 The hypothesis appears to be supported by event data from the Nant y Brwyn
569 (Fig. 10). At base flow the in situ fluorescence minimum was at about 4-7 pm and
570 peaked in the early morning (6-8 am). In the first day of the event shown (June 18th),
571 the pattern was shifted, and after the peak in discharge (June 19th) the in situ
572 fluorescence remained high all day until a minimum at 8 pm. In the following few

days in situ fluorescence continued to stay high throughout a longer part of the day, with a delayed minimum compared to base flow, until June 24th, when the maximum was again at 8 am and the minimum at 4 pm. There was more noise in these data than the event data for the Afon Ddu, as the PARAFAC model involves smoothing. To compare, the intensities at the wavelength measured at Nant y Brwyn were extracted from the event 3 EEMs (data not shown), and although they exhibited more noise, the diurnal pattern could still be distinguished. If the hypothesis is correct, then at least part of the absorbing, humic DOM released during events must be rapidly processed close to where it was released. However, more data and a more quantitative approach are needed to confirm this. The hypothesis implies that the production of fluorescing DOM not only peaks at a different time of the day during and after an event, but that there is a general increase in fluorescence intensity. Dilution may explain why this was not observed.

Conclusions

In peatlands where base flow is mainly high pH/low DOC ground water, events result in increased DOC concentration due to flushing of the shallow acrotelm. The increase in DOC is accompanied by decreased pH. The DOM released during events appears to be less degraded, less aromatic and more bioavailable. An increased input of this material is believed to have caused stimulation of in-stream microbial communities, producing fluorescing metabolites.

Absorbance proved to be a good indicator of changes in DOM quality and an identification of the shift of flow paths and sources throughout an event. Fluorescence

598 was less useful in that respect, due to the apparent dominance of in-stream production.
599 However, absorbance and fluorescence data combined gave indications of short term
600 changes in in-stream processing of DOM during events which have not previously
601 been observed.
602
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611
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Figure captions

Fig. 1 Location of the field site (left) and map of the Afon Ddu catchment (right).

Fig. 2 Stream stage and precipitation for the whole autumn (top) and for single events (bottom). Markers in top panel indicate time of sampling. Note different time scale for event 3.

Fig. 3 pH and dissolved organic carbon (DOC) concentration in stream samples, sphagnum pore water and soil water from the three events. BF = average base flow; Sph = sphagnum pore water; MR = soil water collected by micro-rhizons; ZT5 = soil water collected by zero-tension lysimeter at 5 cm; ZT10 = soil water collected by zero-tension lysimeter at 10 cm. Note different time scale for event 3.

Fig. 4 Dissolved organic carbon/dissolved organic nitrogen (DOC/DON) ratio in stream samples, sphagnum pore water and soil water from the three events. BF = average base flow; Sph = sphagnum pore water; MR = soil water collected by micro-rhizons; ZT5 = soil water collected by zero-tension lysimeter at 5 cm; ZT10 = soil water collected by zero-tension lysimeter at 10 cm. Note different time scale for event 3.

Fig. 5 Absorbance at 254 nm, sUVa (absorbance at 254 nm/DOC concentration) and E2/E3 (absorbance at 250 nm/absorbance at 365 nm) in stream samples, sphagnum pore water and soil water from the three events. BF = average base flow; Sph = sphagnum pore water; MR = soil water collected by micro-rhizons; ZT5 = soil water collected by zero-tension lysimeter at 5 cm; ZT10 = soil water collected by zero-tension lysimeter at 10 cm. Note different time scale for event 3.

Fig. 6 Excitation-emission matrices of the two parallel factor analysis (PARAFAC) generated components C1 (top) and C2 (bottom). The scale is emission loading*excitation loading (i.e. $b_j c_k$ in equation 1).

Fig. 7 Maximum fluorescence intensity (Fmax) of component C1 and C2 and the ratio of the two in stream samples, sphagnum pore water and soil water from the three events. BF = average base flow; Sph = sphagnum pore water; MR = soil water collected by micro-rhizons; ZT5 = soil water collected by zero-tension lysimeter at 5 cm; ZT10 = soil water collected by zero-tension lysimeter at 10 cm. Note different time scale for event 3.

Fig. 8 Maximum fluorescence intensity (Fmax) of component C1 and C2 in event 3 plotted with in situ temperature (top) and absorbance at 254 nm (bottom).

Fig. 9 Two end-member mixing model for event three, showing the proportion of base flow type water needed to explain stream concentration when the other end-member is soil water (average of the micro-rhizon sample and the two zero-tension lysimeter samples). Mixing model excluding (left) and including (right) C1 and C2.

Fig. 10 Fluorescence measured in situ (excitation 330 nm, emission 450 nm) and discharge in Nant y Brwyn.

Figure 1

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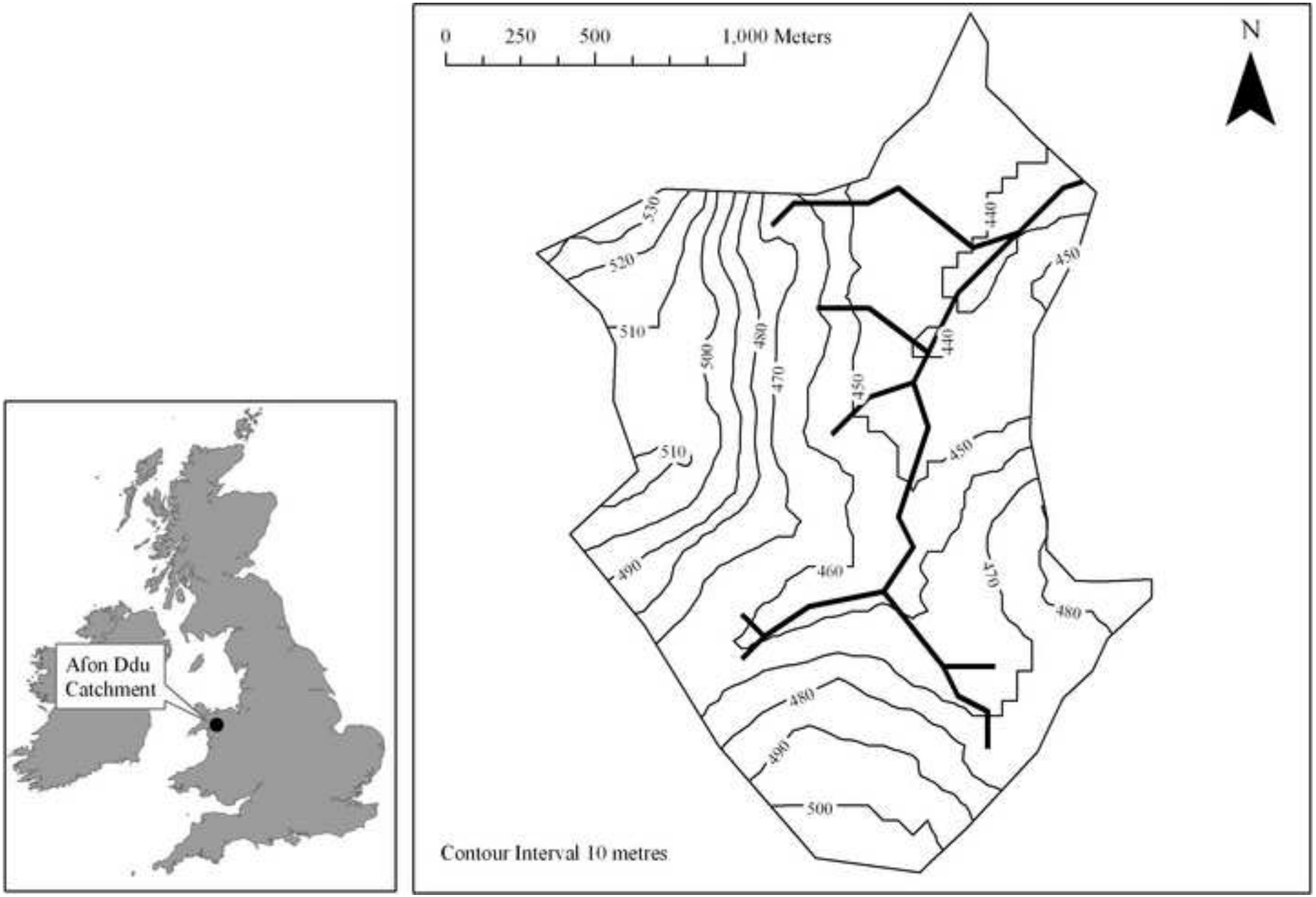

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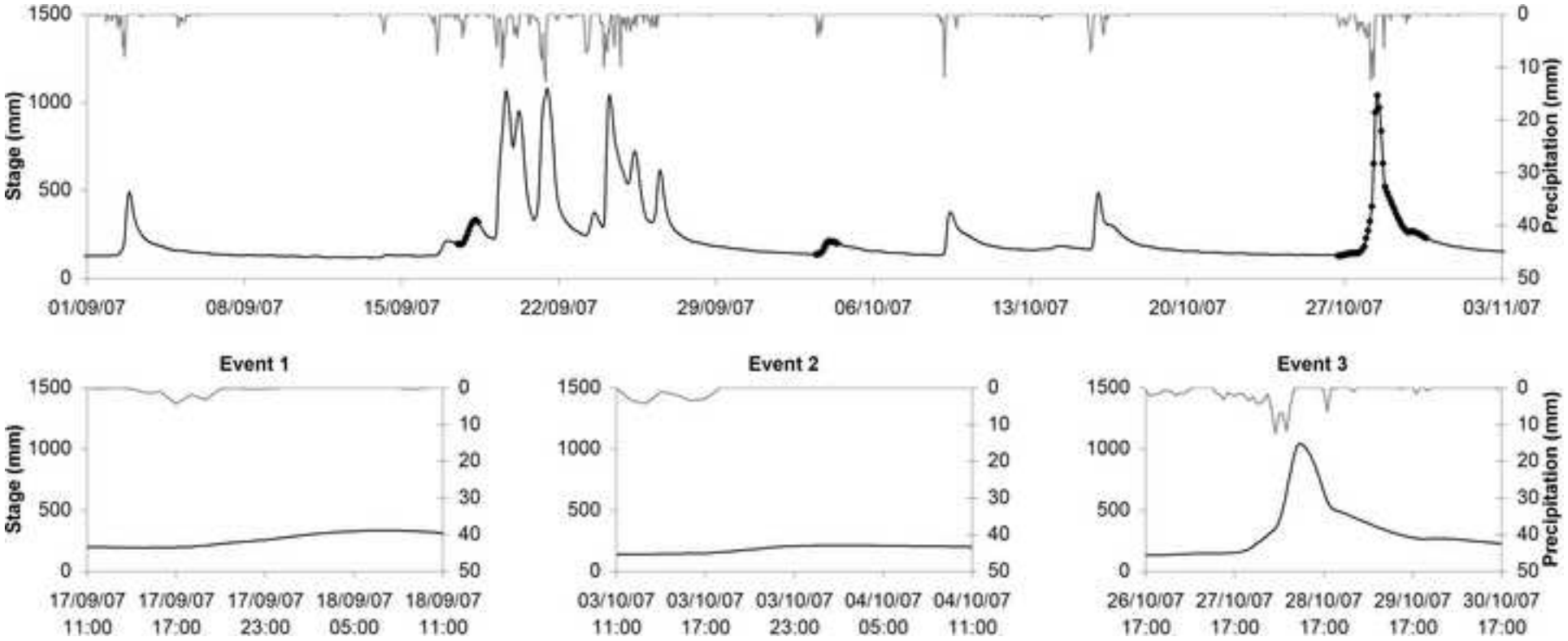

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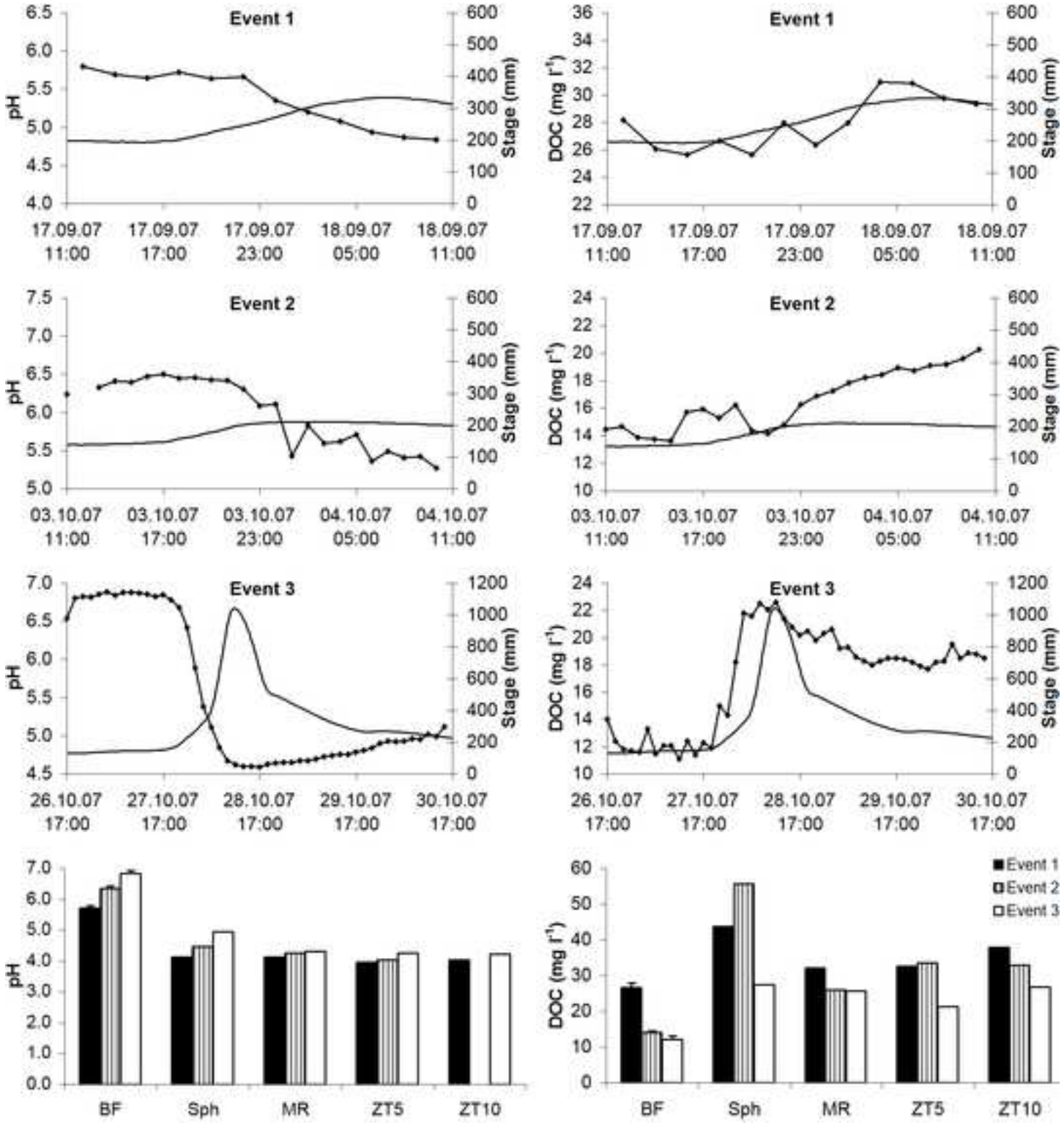

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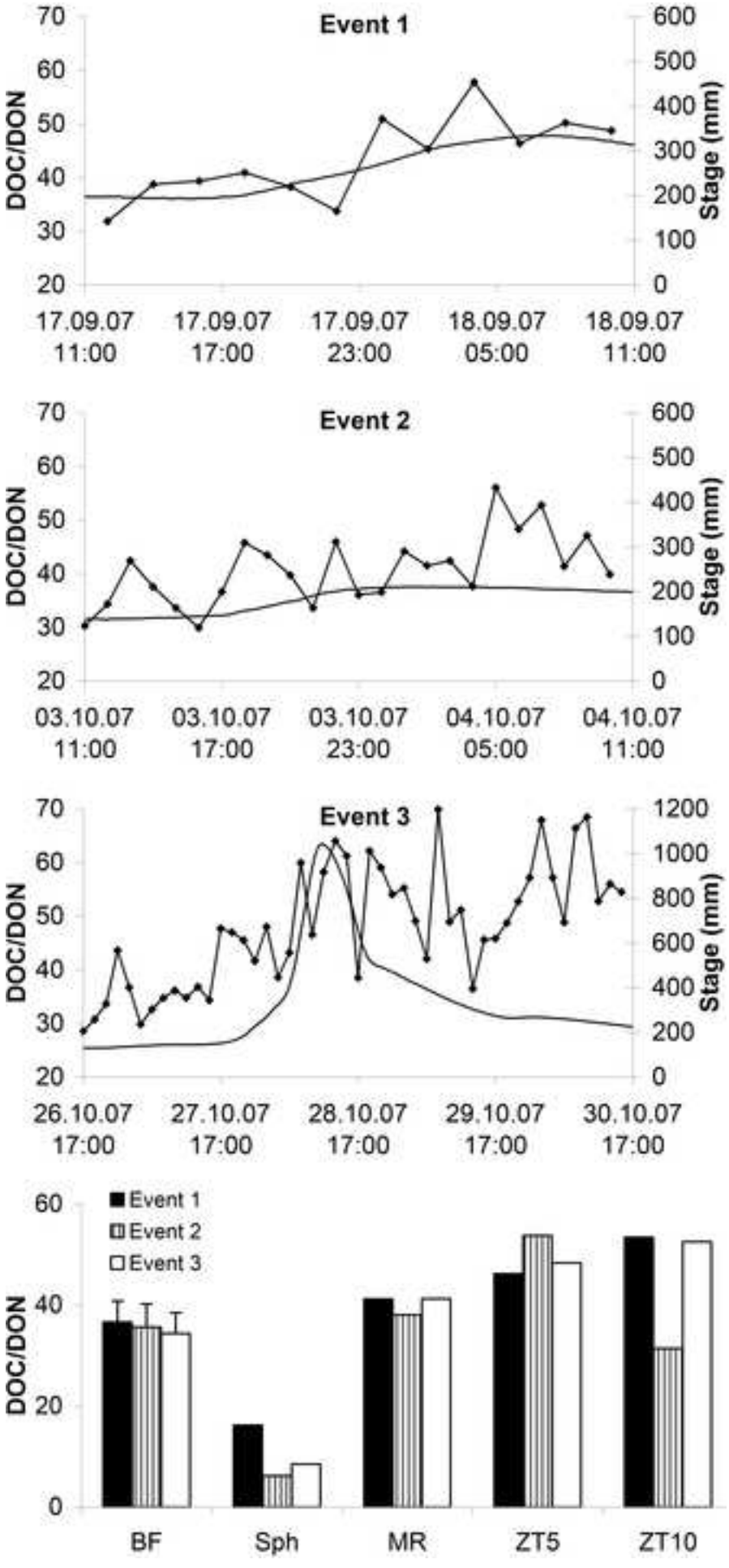

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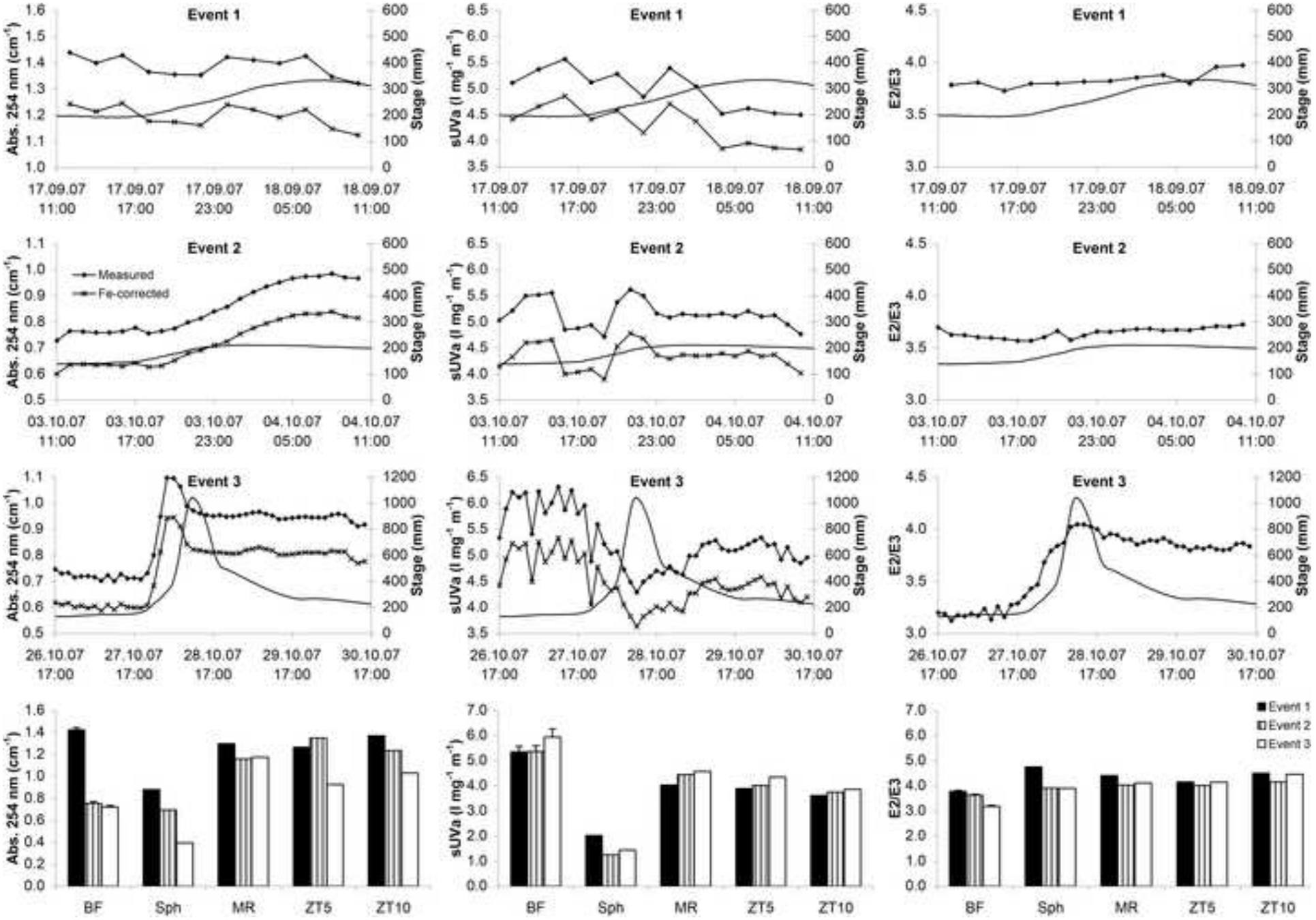

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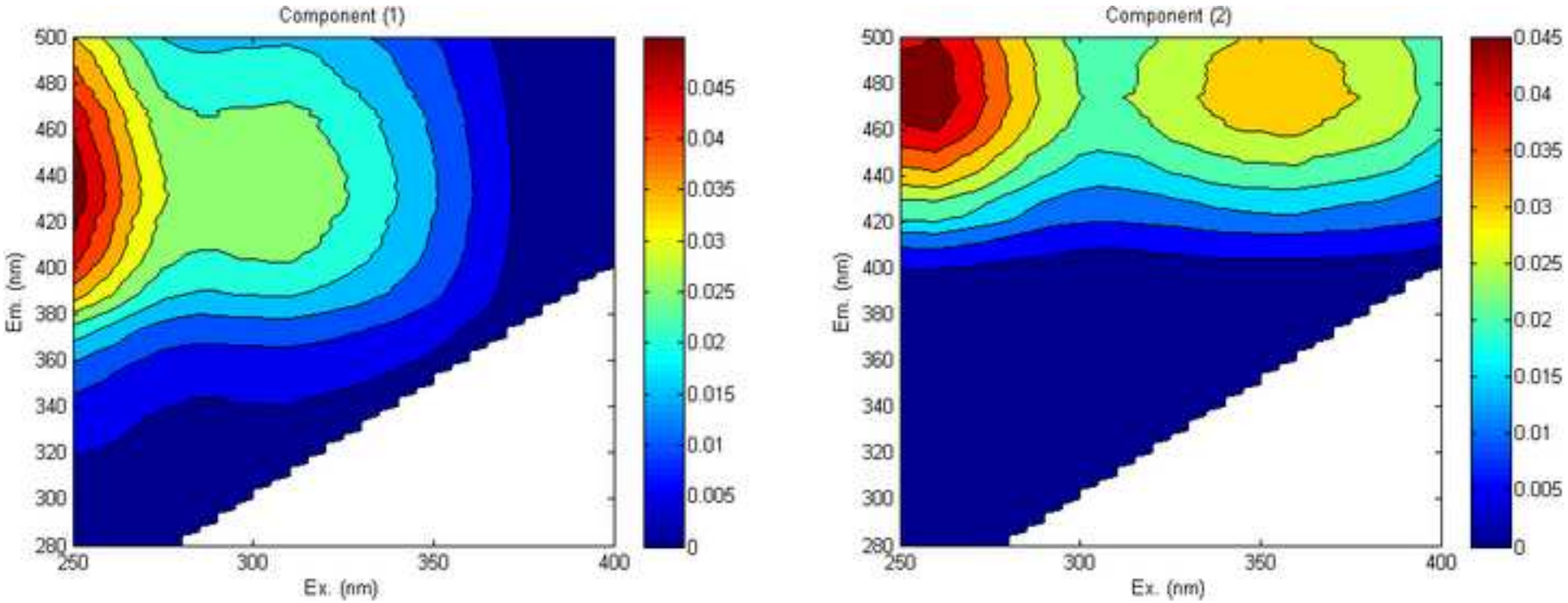

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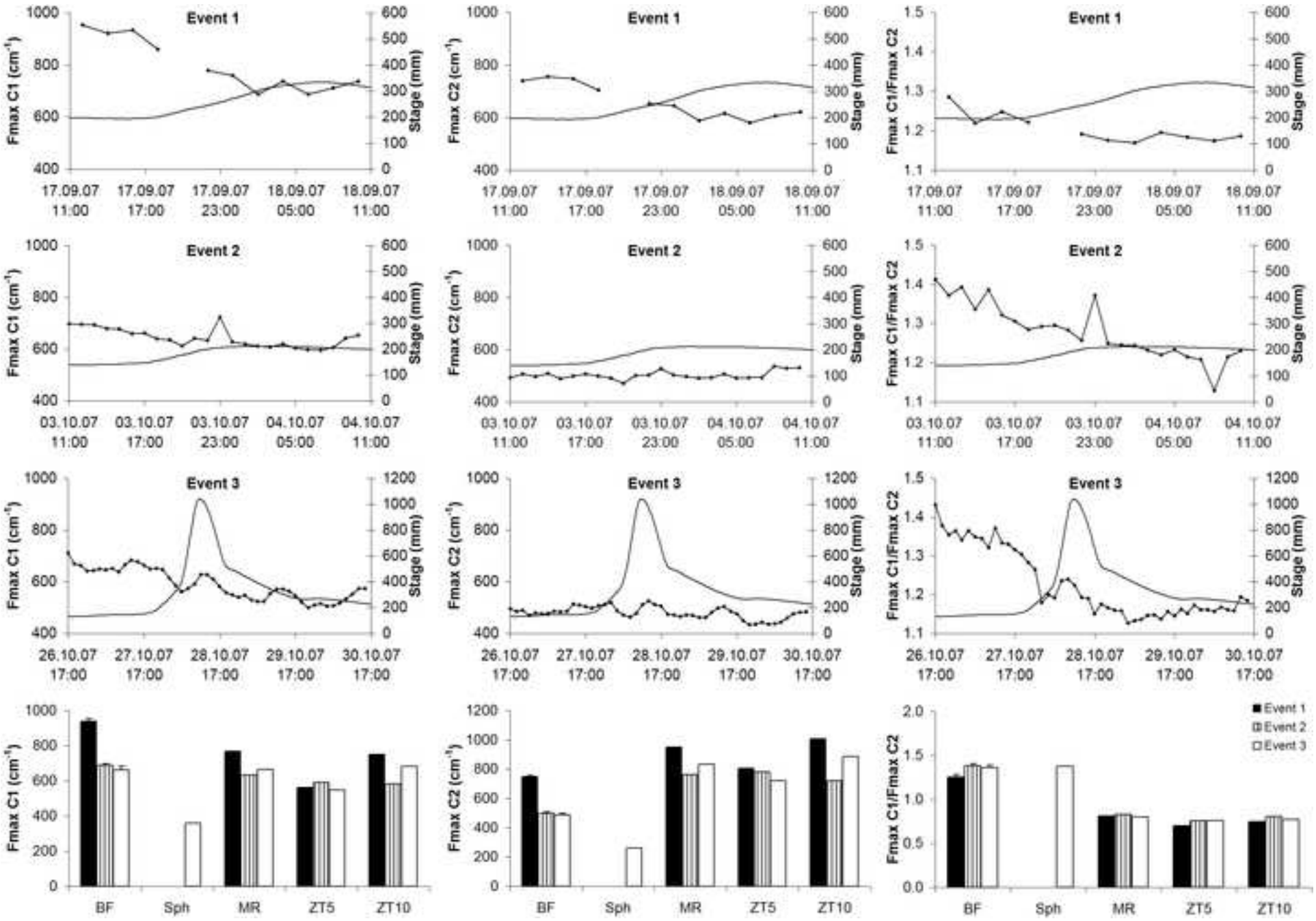

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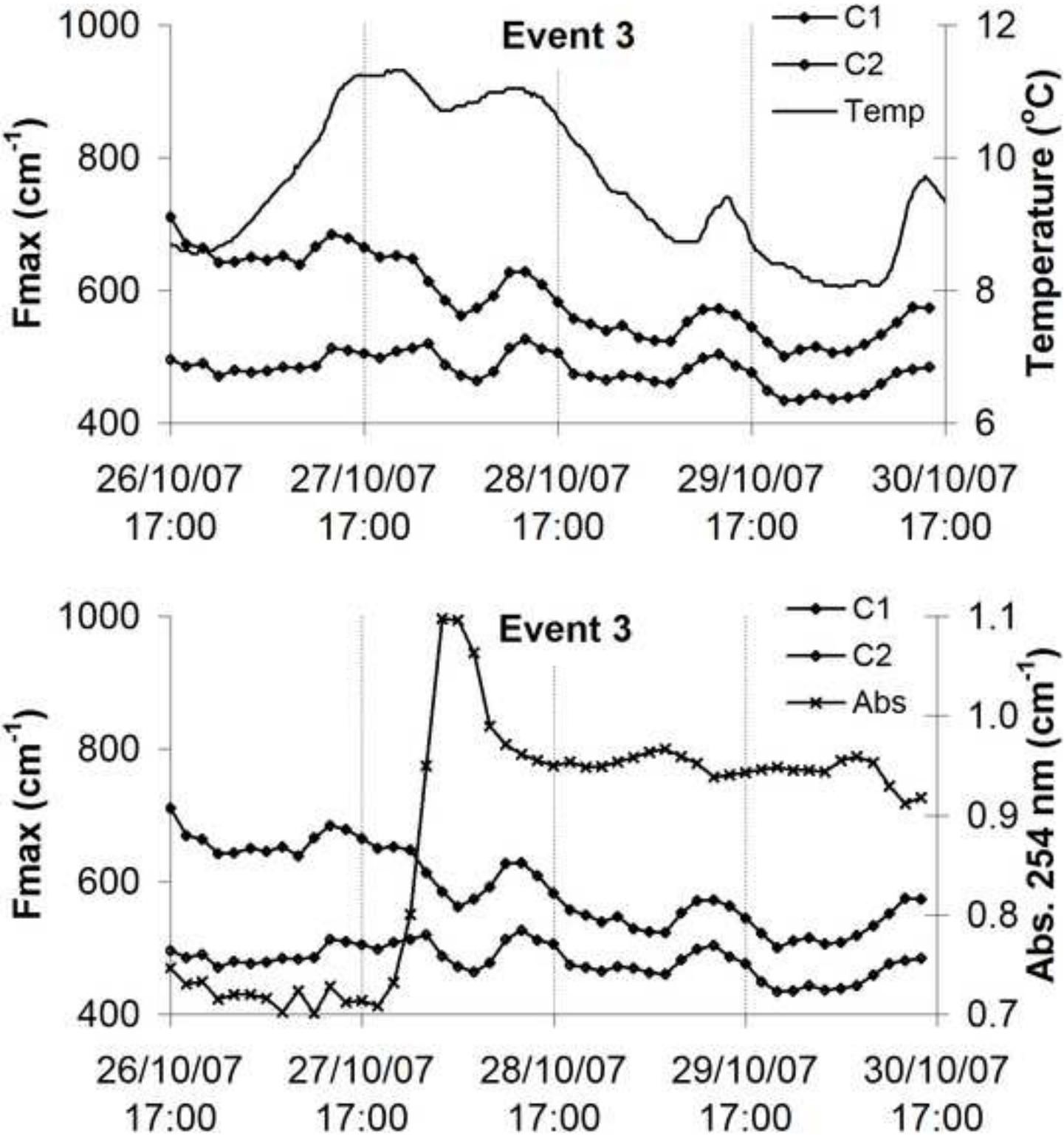

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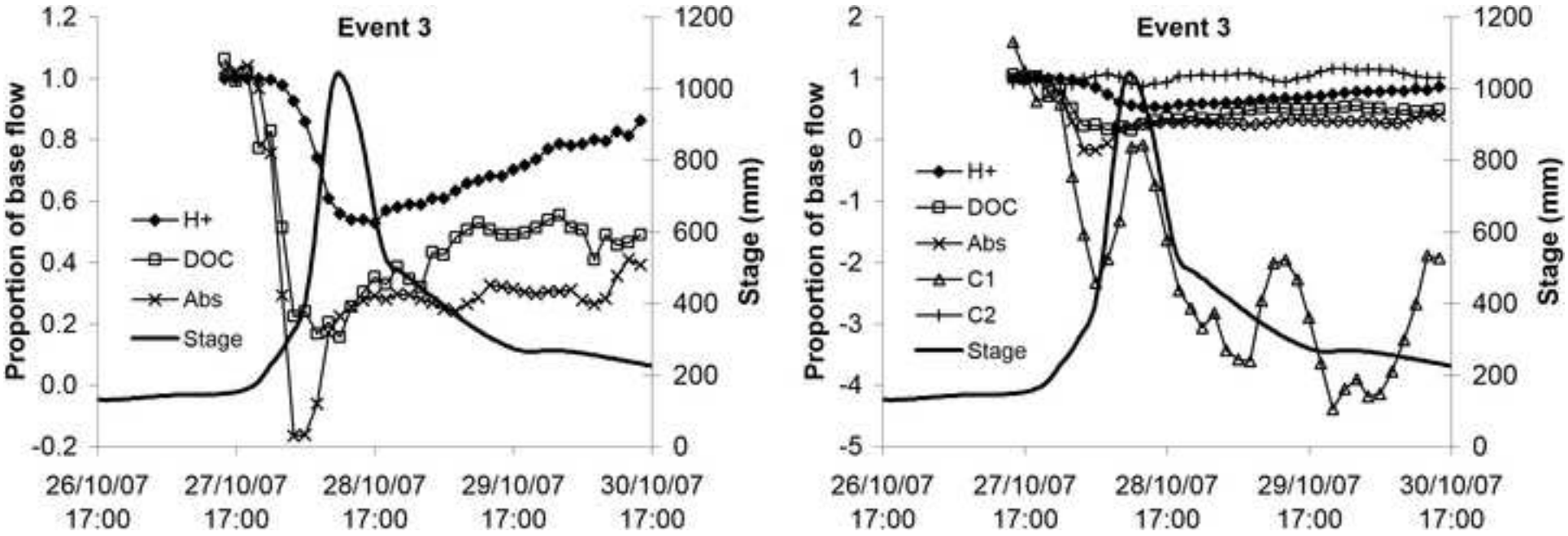

Figure 10

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