Manuscript with markups and author's responses

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1 Procedure

This document contains the manuscript with markups to show the changes and the author response for two revisions. Changes for the first revision are marked in blue and changes for the second revision are marked in red. Please see the respective author responses below for details

2 Manuscript with markups

Interacting effects of climate and agriculture on fluvial DOM in temperate and subtropical catchments

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Abstract. Dissolved organic matter (DOM) is an important factor in aquatic ecosystems, which is involved in a large variety of biogeochemical and ecological processes and recent literature suggests that it could be strongly affected by agriculture in different climates. Based on novel monitoring techniques, we investigated the interaction of climate and agriculture effects on DOM quantity and

5   quality. To examine this, we took water samples over two years in two paired intensive and extensive farming catchments in each Denmark (temperate climate) and Uruguay (subtropical climate). We measured dissolved organic carbon (DOC) and nitrogen (DON) concentrations and DOC and DON molecular fractions with size-exclusion chromatography. Moreover, we characterised DOM quality with absorbance and fluorescence measurements, as well as parallel factor analysis (PARAFAC).

10   We also calculated the DOC and DON loads based on daily discharge measurements, as well as measured precipitation and air temperature. The fluvial DOM in the catchments in Uruguay was characterized by higher temporal variability of DOC and DON loads which were clearly to a higher temporal variability of precipitation and a DOM composition with rather plant-like character relative to the Danish catchments. Moreover, we found a consistently higher temporal variability of

15   DOC an DON loads in the intensive farming catchments than in the extensive farming catchments, with highest temporal variability in the Uruguayan intensive farming catchment. Furthermore, the composition of DOM exported from the intensive farming catchments was consistently complex and always related to microbial processing in both Denmark and Uruguay. This was indicated by low C:N ratios, several spectroscopic DOM composition indexes and PARAFAC fluorescence com-

20   ponents. We propose that the consistent effect of intensive farming on DOM composition and the temporal variability of DOC and DON loads is related to similarities in the management of agri-

culture, which may have wide-scale implications for fluvial DOM composition, as well as related
ecological processes and biogeochemical cycles.

1 Introduction

Dissolved organic matter (DOM) is an important biogeochemical component in aquatic ecosystems, which is involved in a large variety of ecological processes (Prairie, 2008; Fellman et al., 2010; Berman and Bronk, 2003). Amongst other elements, DOM contains carbon (dissolved organic carbon, DOC) and nitrogen (dissolved organic nitrogen, DON): DOC can be an important source for aquatic microbial respiration and DON can be an important source of nitrogen to aquatic ecosystems (Berman and Bronk, 2003; Prairie, 2008). ~~The largest biogeochemically reactive fractions of DOM are dissolved organic carbon (DOC) and nitrogen (DON): DOC is an important source for aquatic microbial respiration and DON can be an important source of nitrogen to aquatic ecosystems (Berman and Bronk, 2003; Berman and Bronk, 2008).~~[1] Therefore, changes in DOC and DON concentrations and loads may affect ecosystem functions of freshwater ecosystems (Stanley et al., 2012; van Kessel et al., 2009).

Climatic, soil and topographic variables are usually strong predictors of DOM in streams, as these often control the terrestrial storage of organic matter and the hydrological connectivity between catchments and streams (Stanley2012). For example, a large portion of the global variability of DOC concentrations is explained by soil C:N ratios (Aitkenhead and McDowell, 2000) and annual runoff predicts catchment DOC export across climates (Mulholland, 1997). However, the effects of landscape and climate are strongly altered by land use, which has a range of consequences for vegetation cover, catchment hydrology, soil properties and nutrient export (Stanley et al., 2012). Recent studies in northern temperate climate have found that the intensity of agricultural management strongly affects the molecular composition and seasonality of fluvial DOM (e.g. Dalzell et al., 2007; Williams et al., 2010; Graeber et al., 2012b; Stanley et al., 2012). However, it is still unclear, if similar effects of agriculture on fluvial DOM are also found in other climates.

Contradictory effects of agriculture on DOM quantity in terms of DOC concentrations have been reported (Stanley et al., 2012; Graeber et al., 2012b). These different effects could be a result of differences in catchment size, climate, land use history, sampling strategy and agricultural management (Stanley et al., 2012). We propose that in small catchments, intensive agriculture results in increased DOC concentrations and loads in the draining freshwater systems, since increased microbial activity and anthropogenic soil disturbance by tilling can release previously inert DOC from the soil matrix (Balesdent et al., 2000; Sickman et al., 2010; Ewing et al., 2006). However, most studies to date were undertaken in larger catchments or in catchments with a mix of catchment sizes, where this effect may be obscured by in-stream processing of agricultural DOC (Graeber et al., 2012b).

In contrast to DOC concentration, the temporal variability of DOC loads from catchments with intensive agriculture to temperate freshwater systems was found to be consistently high due to discharge fluctuations during short-term, high-discharge events (Dalzell et al., 2007; Royer and David,

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2005; Graeber et al., 2012b). Thus, it is likely that intensive agriculture will have a similar effect on the temporal variability of DOC loads in other climates.

Similarly to DOC, contradictory effects of agriculture on DON concentrations have been found (Stanley and Maxted, 2008; van Kessel et al., 2009; Siemens and Kaupenjohann, 2002; Williams et al., 2005; Willett et al., 2004; Petrone, 2010), largely due to the same factors as for DOC. However, all but one study (Heinz et al., 2015) of DON in agricultural environments to date have been based on indirect calculation of DON as the difference between total dissolved nitrogen and dissolved inorganic nitrogen, potentially leading to high uncertainty in the calculated DON concentrations (Lee and Westerhoff, 2005; Graeber et al., 2012a). Therefore, size-exclusion chromatography (SEC) represents a novel, direct measurement alternative to assess DOC and DON concentrations and molecular composition that is sufficiently fast to be used in monitoring programs (Graeber et al., 2012a; Huber et al., 2011). By using this novel approach it would be possible to test the existing opinions on the role of agriculture for DON export. We propose that the same factors as for DOC (higher microbial activity, soil disturbance) should affect DON, thus increasing the DON concentrations in the export from small, intensive agricultural catchments.

Fluorescence and absorbance spectroscopy have been the methods of choice for the assessment of DOM composition to date. These measurements allow a detailed understanding of DOM composition (e.g. Fellman et al., 2010; Helms et al., 2008), especially when combined to parallel factor analysis (PARAFAC, Murphy et al., 2013; Stubbins et al., 2014). Spectroscopic measurements of DOM composition revealed that DOM from catchments with intensive agriculture is usually dominated by complex, humic fluorophores, is characterised by high humification and low contribution of protein-like fluorophores, and is likely to be released from microbial sources (Wilson and Xenopoulos, 2009; Williams et al., 2010; Graeber et al., 2012b; Fellman et al., 2011; Heinz et al., 2015)~~(Wilson and Xenopoulos, 2009; Wilson and Xenopoulos, 2010; Wilson and Xenopoulos, 2012b; Wilson and Xenopoulos, 20~~ Moreover, existing time series indicate a stable composition of DOM exported from agricultural catchments across seasons, most likely linked to stable catchment DOM sources (Graeber et al., 2012b; Heinz et al., 2015)~~(Graeber et al., 2012b)~~[3]. However, these studies focused on agricultural catchments in temperate climate and time series of spectroscopic DOM composition were limited to one year or less. Therefore, a more complete understanding of the effects of agriculture on DOM composition and its temporal variability in different climates over extended time periods is required. Moreover, a combination of techniques, including spectroscopic measurements with other analytical methods (e.g. with SEC) will allow a more accurate interpretation of spectroscopic measurements and a better understanding of DOM composition (Stubbins et al., 2014).

The combination of SEC and spectroscopic measurements constitutes a novel monitoring technique, which will allow greater insight into the effects of agriculture on DOM in freshwater systems.

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[3]change-5

We used this technique to compare the quantity and variability of DOC and DON concentrations, loads and DOM quality for catchments with extensive and intensive farming in temperate (Denmark) and subtropical (Uruguay) climates. Intensive farming was characterized by intensive crop production with- or without tile drainage, depending on the soil, whereas extensive farming was characterized by non-fertilized, extensively used pastures.[4] We hypothesized that i) the higher and more variable precipitation in Uruguay will result in higher and more variable DOC and DON loads in streams, ii) the warmer climate in Uruguay will strongly affect DOM quality through higher microbial activity in soils and streams, iii) within a similar climate, the higher anthropogenic soil disturbance and higher variability of runoff from intensive farming catchments results in higher DOC and DON concentrations and higher temporal variability of DOC and DON loads, and that iv) DOM quality will be similarly affected by intensive farming relative to extensive farming across climates, due to similar agricultural management practices (fertilization, soil tillage).

2 Methods

2.1 Study sites

Two catchments in Denmark (temperate climate) and two catchments in Uruguay (subtropical climate) were chosen for this study. The catchments were characterized by either pasture (extensive farming) or arable farming (intensive farming, Table 1). The Danish intensive farming catchment was characterized by subsurface tile drainage. Both, the extensive catchment in Denmark and the extensive catchment in Uruguay contained a large area of the catchment with extensive land use. Since no larger forests exist naturally in Uruguay, extensively used pasture is the most natural land use in Uruguay. Please see Goyenola et al. (2015) for further details on the land use in the catchments.[5]

In Denmark, the soils in the intensive farming catchment were dominated by gleyic Luvisols, while in the extensive farming catchment the soils were dominated by haplic Luvisols (World Reference Soil Database classification, European comission and European Soil Bureau Network, 2004). In Uruguay, the intensive farming catchment was dominated by luvic Phaeozem and eutric Vertisols, while the Uruguayan extensive farming catchment was dominated by eutric Regosols (SOTERLAC database, ISRIC foundation, www.isric.org).

2.2 Field sampling and laboratory measurements

In Uruguay, precipitation and air temperature were measured at the sample sites with instruments (Rain-o-matic precipitation sensor, Pronamic, Ringkøbing, Denmark, November 2009 – September 2012), while in Denmark data were extracted from country-wide data set provided by the Dan-

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[5]change-3

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ish Meteorological Institute (DMI, February 2010 – May 2012). Both Danish catchments had the same temperature values, since they were in the same temperature grid of the DMI data set. In both countries, discharge was measured every 10 min by a pressure transducer in combination with a depth-discharge relationship (Hymer software, version 3.0.11, Orbicon, Roskilde, Denmark) and summed up to daily values for further analysis. Annual precipitation could only be compared between the catchments only for 2011, since only for this year simultaneous, continuous precipitation measurements exist for all four catchments.

Water samples were collected on average every fortnight from 02. April 2010 to 14. March 2012 from the outflows of the two catchments in Denmark and from 02. June 2010 to 29. May 2012 from the outflows of the two catchments in Uruguay. At each sampling date, a water sample was taken, filtered with pre-rinsed (1 L MilliQ water) GF/C filters (Whatman, GE Healthcare Europe, Brondby, Denmark) in Uruguay or 0.45 µm filters (Frisenette, MontaMil, mixed cellulose ester, Knebel, Denmark) in Denmark and acidified to pH $\sim$ 2 with hydrochloric acid to allow to stabilize DOM during storage. Subsequently, the samples were frozen for later analysis of DOM concentration and molecular composition. The samples were acidified and frozen, since they had to be sent to a laboratory in Germany to be measured between February and April 2012 and in October 2012, which resulted in long storage times, for which filtration and cooling is not sufficient (Hudson et al., 2009).

Before the laboratory measurements, all samples were brought to the same target pH of 7.5 ± 0.5. A final mean pH of 7.52 (SD = 0.16, min = 7.2, max = 7.9) was reached by neutralization of the samples with sodium hydroxide. Changes in DOM fluorescence by acidification can be fully reversed by neutralization of the samples with no effects of acidification on fluorescence measurements (Patel-Sorrentino et al., 2002) or SEC measurements (Huber et al., 2011) of DOM composition expected in the range of the final pH values. Moreover, the Uruguayan samples have been re-filtered with pre-rinsed (with 150 mL MilliQ water) 0.45 µm filters (Minisart, cellulose-acetate, Sartorius Göttingen, Germany) to correspond with the Danish samples. However, according to a recent study, different filter sizes or types do not strongly affect measurements of DOM composition (Nimptsch et al., 2014). Moreover, no residue DOM from acidification, neutralization and additional filtration could be found when checking with filter and acidification blanks.

Absorbance was measured on a UV-2401 UV/Vis spectrophotometer (Shimadzu, Duisburg, Germany), using 1 cm quartz glass cuvettes to correspond with fluorescence measurements, as well as with 5 cm quartz glass cuvettes for calculation of absorbance-based indexes. Absorbance was measured between 190 – 800 nm. Before calculating the absorbance-based indexes, the mean absorbance between 600 – 800 nm was subtracted from single absorbance values to correct for instrument baseline offset (Green and Blough, 1994).

Excitation was measured between 240 – 450 nm in 5 nm steps and emission was measured between 300 – 600 nm in 2 nm steps. Both were measured with a bandwidth of 5 nm and a speed of $1000\,\mathrm{nm\,s^{-1}}$, using a LS-50B fluorescence spectrometer (Perkin-Elmer, Rodgau, Germany). Sam-

ples exhibiting an absorbance $> 0.3\,\mathrm{cm}^{-1}$ were diluted to a lower fluorescence to allow precise correction of the inner-filter effect (Ohno, 2002), although a recent study deemed such dilution un-
165 necessary (Kothawala et al., 2013). All samples were measured at room temperature.

Size-exclusion chromatography (SEC) was used for the analysis of the molecular-size composition of DOC and DON. The sum of DOC and DON molecular-size fractions represents DOC and DON concentrations. The system used in this study was developed by Huber et al. (2011) and the direct measurement of DON with high accuracy was demonstrated in freshwater systems for this
170 SEC system (see Fig. B1 in the appendix for a typical chromatogram from SEC, Graeber et al., 2012a)[6]. The SEC system uses a combination of ultraviolett (UV)- and infrared-organic carbon detection and UV-organic nitrogen detection (Graeber et al., 2012a; Huber et al., 2011). This procedure detects non-humic high molecular weight substances (carbon = $HMWS_C$, nitrogen = $HMWS_N$) of hydrophilic character (polysaccharides, proteins, amino sugars), humic-like substances (carbon
175 = HS_C, nitrogen = HS_N) with higher aromaticity based on UV measurements at $254\,\mathrm{nm}$, and low-molecular weight acids and circumneutral substances which were combined as low-molecular weight substances in this study (carbon = $LMWS_C$, Graeber et al., 2012a; Huber et al., 2011). These LMWS refer to neutral, hydrophilic to amphiphillic substances (alcoholes, aldehydes, ketones, sugars, amino acids, Huber et al., 2011). Nitrogen could not be determined for the LMWS fraction,
180 since it cannot accurately be separated from nitrate (Huber et al., 2011). Unlike wastewaters (Chon et al., 2013) this fraction contains very little DON and therefore does not contribute significantly to DON determination in freshwaters, when using SEC (Graeber et al., 2012a). The quantification limit of SEC for DOC and DON in each fraction was $0.01\,\mathrm{mg\,L}^{-1}$ and values below the quantification limit were set to $0.005\,\mathrm{mg\,L}^{-1}$. Specific UV absorbance at $254\,\mathrm{nm}$ was determined for the HS
185 fraction ($SUVA_{HS}$) and for all DOM fractions ($SUVA_{bulk}$) as $\mathrm{L\,mg}^{-1}\,\mathrm{m}^{-1}$. $SUVA$ is positively correlated to the aromaticity of DOM (Weishaar et al., 2003).

2.3 Treatment of spectroscopic and chromatographic data

The drEEM toolbox was used to standardise all measured excitation-emission-matrixes (EEMs, Murphy et al., 2013): Spectral correction was based on instrument-specific values for excitation and using
190 a correction kit for emission (BAM fluorescence calibration kit, Pfeifer et al., 2006). Inner-filter effect correction was based on absorbance measurements and using the processing proposed in the drEEM toolbox, which accurately removes the inner-filter effect (Kothawala et al., 2013). All samples were Raman-normalized, based on measurements of the Raman peak at $350\,\mathrm{nm}$ and according to the processing used in the drEEM toolbox and described in Murphy et al. (2013). The resulting
195 Raman units are well comparable between instruments and studies (Lawaetz and Stedmon, 2009).

Using the drEEM toolbox, a parallel factor analysis (PARAFAC) model with four components ($C1 - C4$) was validated using residual and sum-of-squared-error investigation, as well as split-half

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validation (see supplement for plots of split-half validation) and random initialisation (Murphy et al., 2013). For interpretation, the PARAFAC components were compared with datasets in the OpenFluor database (www.openfluor.org, Murphy et al., 2014) and with published literature.

Based on fluorescence measurements, three indices were calculated: i) the fluorescence index, which indicates more a microbial (~ 1.9) or a terrestrial higher plant (~ 1.4) origin of the DOM (Cory and McKnight, 2005), ii) the freshness index, with values > 1 representing DOM recently released from microbial organisms, and values of $0.6 - 0.8$ representing older or plant DOM (Parlanti et al., 2000) and iii) the humification index for which higher values indicate more humified DOM (Ohno and Bro, 2006). Based on absorbance measurements, four indexes were calculated: i) $E_2 : E_3$, which is negatively correlated to the relative size of the DOM molecules (Helms et al., 2008; Peuravuori and Pihlaja, 2004), and three absorbance slope indices (Helms et al., 2008): ii) $S_{275-295}$ and S_R, which are positively related to irradiation and decrease during incubation experiments and iii) $S_{350-400}$, which is negatively related to irradiation and increases during incubation experiments. Moreover, all three slope indices are negatively related to the molecular weight of DOM (Helms et al., 2008).

Instead of using absolute concentrations or Raman units, the fraction concentrations from SEC ($HMWS_C, HMWS_N, HS_C, HS_N, LMWS_C$) and the PARAFAC components ($C1 - C4$) were converted to percentages, either as a proportion of the total concentration of SEC fractions or of the total fluorescence of the sample (PARAFAC), in order to investigate changes in DOM composition independently from changes in DOM quantity. For both DOC and DON, all SEC fractions were summed to estimate total DOM quantity, hereinafter, these sums will be referred to as DOC and DON concentrations.

Based on SEC, molar C:N ratios were calculated for HS ($C : N_{HS}$) and all SEC fractions ($C : N_{bulk}$). Molar C:N ratios were not calculated for HMWS, since HMWS nitrogen concentrations were partly below the quantification limit, which resulted in unreliable C:N ratios.

2.4 Calculation of DOC and DON daily and annual loads

DOC and DON concentrations were linearly interpolated between sampling occasions and loads were calculated for each day with load calculated as discharge times interpolated DOC or DON concentration (Kauppila and Koskiaho, 2003). To calculate the annual load, all daily loads of one year were summed up and normalized by the catchment area (Table 1). This approach was compared to other potential approaches in Kauppila and Koskiaho (2003) and was found to provide the most reliable estimates of nutrient loads from discontinuous concentration data. However, since considerable bias of the interpolated data is possible and cannot be ruled out, we only discuss strong patterns related to the calculated DOC and DON loads. Annual loads could only be compared between all study catchments in 2011, as simultaneous continuous time series of DOC concentration, DON concentration and discharge were only available for all catchments during this period.

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2.5 Statistical analyses

All statistical analyses were conducted in R (R Core Team, 2015). All following statistics assume independent temporal replicates, as neither DOC and DON concentrations or DOM composition variables were temporally autocorrelated in any of the catchments (acf function R Core Team, 2015). All permutation tests and resampling procedures were conducted with 9999 iterations.

To assess the effects of country and farming type within countries on DOC and DON concentrations, permutative one-way tests were used (oneway_test function, coin package, Hothorn et al., 2006). Moreover, to assess pairwise differences between the sampled catchments, Nemenyi tests were used (adapted oneway_test function, coin package, Hollander et al., 2013). To investigate, if DOC concentration was correlated with discharge, a Spearman rank correlation was used for each of the catchments independently (cor.test function, R Core Team, 2015).

To assess changes in temporal variability of precipitation, discharge, DOC loads and DON loads between countries and farming types within the countries, Levene's test based on medians (leveneTest function, car package, Fox and Weisberg, 2011) was used. To assess, whether the temporal variability of DOC and DON loads was dependent on discharge or on DOC and DON concentrations, a sensitivity analysis of the load calculations was conducted for each catchment separately. This was done as described in Pouillot and Delignette-Muller (2010), but based on bootstrap resampling of the DOC and DON concentrations and discharge values. The output of this analysis is Spearman's ρ and here, a high Spearman's ρ indicates a high sensitivity of the temporal variability of the loads on the temporal variability of the respective input variable (either concentration or discharge) and a low Spearman's ρ indicates a low sensitivity.

To investigate the changes of DOM composition with country and type of farming and to examine the relationships between DOM composition variables, a principal component analysis was conducted for all 20 variables of DOM composition: $HMWS_C$, $HMWS_N$, HS_C, HS_N, $LMWS_C$, $SUVA_{HS}$, $SUVA_{bulk}$, $C:N_{bulk}$, $C:N_{HS}$, $C1 - C4$, fluorescence index, freshness index, humification index, $E_2:E_3$, $S_{275-295}$, $S_{350-400}$ and S_R. To reach normal distribution of the DOM composition variables with fixed limits, $HMWS_C$, $HMWS_N$ and HS_N were logit-transformed. Moreover, $C:N_{bulk}$ was log-transformed. ~~To reach normal distribution of the DOM composition variables, $HMWS_C$, $HMWS_N$ and $C:N_{bulk}$ needed to be log-transformed. Moreover, HS_N needed to be reflected and log-transformed.~~ Based on the approach described in Borcard et al. (2011), only variables that could be interpreted with high confidence were included in the interpretation of the PCA. We used the Scree test and Kaiser criterion to define the optimal number of PCA axes (Gotelli and Ellison, 2004). Prior to the PCA, all variables were centered and scaled (autoscaled) get comparable scale levels (scale parameter, rda function, vegan package, (R Core Team, 2015)).[7]

Based on the same variables as for PCA, the effects of country and farming type on DOM composition were tested using multivariate statistics: To assess if differences in DOM composition be-

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[7]change-15;description of auto-scaling, PCA

tween catchments were significant, permutative multivariate analyses of variance (PERMANOVA) were used (adonis function, vegan package, Oksanen et al., 2013) and to assess differences in their variability, permutative multivariate dispersal tests (PERMDISP) were used (betadisper and permutest.betadisper function, vegan package). Multivariate tests were based on Euclidean distances with independence of the replicates as the only assumption (Anderson, 2001). However, variable transformations from the PCA were kept to maximize comparability between the PCA plot and the statistical analyses. Prior to the PERMANOVAs/ PERMDISPs, all variables were centered and scaled (autoscaled) get comparable scale levels (scale function, stats package, (R Core Team, 2015)).[8]

To assist in the interpretation of the effects of country and farming type on the DOM composition, Nemenyi tests (adapted oneway_test function, coin package, Hollander et al., 2013) of the effect of the sampled site on carbon or nitrogen in humic substances, C:N ratio of humic substances, fluorescence index, PARAFAC component C1 and ratio of absorbance curve slopes (Sr) were conducted.[9]

3 Results

3.1 Climate and discharge

The Danish catchments were characterized by a colder climate than the Uruguayan catchments. The mean air temperature in the Danish catchments was 7.4 °C ($\pm$SD = 6.9 °C, min = -11.8 °C, max = 22.8 °C). In the Uruguayan extensive farming catchment, the mean air temperature was 17.2 °C ($\pm$SD = 6.5 °C, min = 1.1 °C, max = 32.1 °C). In the Uruguayan intensive farming catchment, the mean air temperature was 16.5 °C ($\pm$SD = 6.1 °C, min = 2.4 °C, max = 30.5 °C).

Different precipitation patterns were observed in the different countries. In Denmark, the annual precipitation in 2011 was 735 mm for the extensive farming catchment and 745 mm for the intensive farming catchment. In Uruguay, the annual precipitation in 2011 was 901 mm for the extensive farming catchment and 1127 mm for the intensive farming catchment.

A clear difference in the temporal variability of the precipitation was observed between countries ($p < 0.001$, Levene's test, $n_{Denmark} = 1640$, $n_{Uruguay} = 1991$[10]), as 80% of the precipitation occurred in 6% and 8% of the sampled period in the intensive farming and extensive farming catchment in Uruguay, respectively. In contrast, 80% of the precipitation occurred in 20% of the sampled period in both, the intensive farming and extensive farming catchment in Denmark (Fig. 1 a.). As can be seen from these results and the plot (Fig. 1 a.), the precipitation pattern was not significantly different between the intensive and extensive farming catchments in either Denmark or Uruguaythe precipitation pattern was similar for the catchments in Uruguay and not even distinguishable from

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[8]change-16;description of auto-scaling, PERMANOVA/PERMDISP
[9]Change-1
[10]added sample sizes

each other for the two catchments in Denmark[11] ($p > 0.53$ for both Denmark and Uruguay, Levene's test, depending on the stream n was 820–996[12]).

Discharge differed significantly between catchments within countries, with the catchments in Uruguay exporting a larger volume of water within a shorter period of time than the catchments in Denmark ($p < 0.001$, Levene's test, , $n_{Denmark} = 1554$, $n_{Uruguay} = 1990$, Fig. 1 b.). In detail, 80% of discharge occurred in 9% and 20% of the sampled period in the intensive and extensive farming catchment in Uruguay, respectively (Fig. 1 b.). Moreover, 80% of discharge occurred in 43% and 73% of the sampled period in the intensive and extensive farming catchment in Denmark, respectively (Fig. 1 b.). The temporal variability of discharge was higher in the intensive than in the extensive farming catchments, both in Denmark and in Uruguay ($p < 0.001$, Levene's test, $n = 777$ for each of the Danish streams and $n = 995$ for each of the Uruguayan streams[13]).

3.2 DOC and DON concentrations and loads

In Uruguay, DOC and DON concentrations were higher than in Denmark ($p < 0.001$, permutative one-way tests, $n_{Denmark} = 98$, $n_{Uruguay} = 95$[14], Figure 2). The effect of intensive farming on DOC was only significant in Denmark ($p < 0.05$, Figure 2a.), while intensive farming resulted in higher DON concentrations than extensive farming in both countries ($p < 0.05$, Nemenyi pairwise tests, n was 47–49, depending on the site[15], Figure 2b.),

The concentrations of DOC and DON were positively correlated with discharge for both Danish catchments (Spearman rank correlation, $\rho > 0.63$, $p < 0.001$, $n = 48$), but not for the Uruguayan catchments ($\rho < 0.11$, $p > 0.44$, $n_{intensive} = 48$, $n_{extensive} = 46$[16]).

Loads of DOC and DON in the catchments with intensive farming were more temporally variable during the sampling period than in the catchments with extensive farming and were more variable in Uruguay than in Denmark (Figure 2c., d.). The highest temporal variability was found in the intensive farming catchment in Uruguay, in which more than 80% of the total DOC and DON load was exported in less than 10% of the sampled period (Figure 2c., d.). In contrast, 80% of the total DOC and DON load in the Danish extensive farming catchment, was exported during 60% of the sampled period (Figure 2c., d.).

The temporal variability of DOC and DON loads was significantly different between countries ($p < 0.001$, Levene's test, $n_{Denmark} = 1414$, $n_{Uruguay} = 1455$[17]). Moreover, in Denmark, the farming type also had a significant effect on the temporal variability of DOC and DON loads ($p < 0.001$,

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[11]change-18
[12]added sample sizes
[13]added sample sizes
[14]added sample sizes
[15]added sample sizes
[16]added sample sizes
[17]added sample sizes

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$n = 707$[18]), while in Uruguay farming type only affected the temporal variability of DOC loads ($p = 0.022$, $n_{intensive} = 728$, $n_{extensive} = 727$[19]) but not DON loads ($p = 0.094$).

Loads of DOC and DON were highly sensitive to changes in discharge (Spearman rank correlation, $\rho > 0.92$, n was 707–728, depending on the site[20]) and to a lesser extend for the extensive farming catchment in Denmark ($\rho = 0.53$). In contrast, the sensitivity of DOC and DON loads to changes in either DOC or DON concentration were low ($\rho < 0.31$), again except from the Danish extensive farming catchment (DOC: $\rho = 0.74$, DON: $\rho = 0.80$).

The annual DOC and DON load in 2011 was comparable between the study catchments, within the same order of magnitude and no effect of farming type or country could be observed (Table 2). The highest DOC load was found in the Danish extensive farming catchment, whereas the highest DON export was found in the Uruguayan intensive farming catchment (Table 2). The median daily loads of DOC and DON exhibited a different pattern as the annual loads for 2011, with the highest median DOC and DON loads always in the extensive farming catchment in Denmark (Table 2). Moreover, the range of DOC and DON loads was highest in the intensive farming catchment in Uruguay and lowest in the extensive farming catchment in Denmark (Table 2).

3.3 Molecular DOM composition

Table 3 shows the characteristics and interpretation of the PARAFAC components.

Country ($R^2 = 0.17$, p < 0.001, PERMANOVA, n was 47–49, depending on the site[21]) and farming type ($R^2 = 0.13$, p < 0.001) had a significant effect on DOM composition and a significant interaction effect between country and farming type was found ($R^2 = 0.03$, p < 0.001). Furthermore, the effects of farming type were significant within each country (p < 0.001, $R^2_{Denmark} = 0.24$, $R^2_{Uruguay} = 0.14$, PERMANOVA).

Country had a significant effect on the temporal variability of DOM composition (p < 0.001, PERMDISP, n was 47–49, depending on the site[22]). The farming type within the countries had no significant effect on the temporal variability of DOM composition (p > 0.05).

Four PCA axes were selected to be optimally representing DOM composition ($n = 193$, number of variables $= 20$[23]). Together, these axes explained 73% of the total variance. The first and third PCA axes were positively correlated with the scores of the Danish catchments and negatively with the scores of the Uruguayan catchments (Figure 3a., b.). The second PCA axis separates farming types and was positively correlated with scores of the two catchments with extensive farming (Figure 3a.). The fourth PCA axis was neither correlated with country or farming type (3b.). The first PCA axis was positively correlated with $E_2 : E_3$, $C2$ and freshness index and negatively correlated

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[18] added sample sizes
[19] added sample sizes
[20] added sample sizes
[21] added sample sizes
[22] added sample sizes
[23] added sample sizes

with $C3$, $SUVA_{bulk}$ and $SUVA_{HS}$ (Figure 3a.). The second PCA axis was positively correlated with $C:N_{HS}$, $C:N_{bulk}$, $C1$ and negatively correlated with $HMWS_C$ and freshness index (Figure 3a.). The third PCA axis was positively correlated with $S_{350-400}$ and negatively correlated with $S_{275-295}$ and S_R (Figure 3b.). The fourth PCA axis was positively correlated with HS_C and HS_N and negatively correlated with $HMWS_N$ and $HMWS_C$ (Figure 3b.).

To get a better understanding of the changes in DOM composition, we ~~exemplary~~[24] investigated the absolute values of some DOM composition variables (Figure 4). In all four catchments, DOC and DON consisted mainly of humic substances and no clear significant effect of country or farming type could be found here (Nemenyi test, $p > 0.05$, n was 47–49, depending on the site[25], Figure 4a., b.). However, intensive farming resulted in lower $C:N_{bulk}$ ($p < 0.05$), a higher fluorescence index and a lower $C1$ in both Denmark and Urgugay (Figure 4c., d.). Moreover, S_R was significantly lower in Denmark than in Uruguay (Figure 4e.).

4 Discussion

In this study, we show that the combination of SEC and spectroscopic measurements allows great insight into the effects of agriculture on DOM export to freshwater systems. We could partly support~~prove~~[26] our first hypothesis, since variability of DOC and DON loads was higher in Uruguay than in Denmark, but the size of annual DOC and DON loads was comparable between both countries. Our second hypothesis of a strong effect of climate on DOM composition was confirmed. However, contrary to the idea of higher microbial processing in Uruguay, several DOM composition indices pointed to a predominantly plant-derived DOM in Uruguay and a predominantly microbial-derived DOM in Denmark. Our third hypothesis of higher DOC and DON concentrations and higher temporal variability of DOC and DON loads in intensive farming could only partly be confirmed. In the two intensive farming catchments, DON, but not DOC concentrations were higher than in the extensive farming catchments within the same country. In contrast, we found a higher temporal variability of DOC and DON loads in the intensive farming catchments in both countries. Finally, we could clearly support~~prove~~[27] our fourth hypothesis that DOM composition was affected similarly by intensive farming relative to extensive farming across climates and the direction of the changes in DOM composition strongly suggests that the management practices in intensive farming (fertilization, soil tillage) could be responsible for these changes.

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[24]change-19
[25]added sample sizes
[26]change-19
[27]change-20

4.1 Differences of climate and discharge between the catchments

Distinct climatic patterns differentiated Denmark from Uruguay. Uruguay was characterized by higher temperatures, as well as higher and more temporally variable precipitation. Within the two countries, temperature and precipitation, as well as its temporal variability only varied to a small degree between catchments.

Discharge was significantly more temporally variable in Uruguay than in Denmark. In addition to the higher temporal variability of precipitation, this reflects a lower buffer capacity for precipitation events in the Uruguayan catchments. The reasons are likely linked to the transport of water through shallow groundwater pathways or overland flow. In contrast, the Danish catchments had a higher buffering capacity for water from precipitation and were likely more dominated by groundwater discharge.

In both countries, the temporal variability of discharges was significantly different between intensive and extensive farming than could be expected solely on the basis of precipitation patterns. Furthermore, the difference was more pronounced in Denmark than in Uruguay. The reason for the higher difference of the temporal variability of discharges between the Danish catchments than the Uruguayan catchments was likely due to the tile drainage in the Danish intensive farming catchment. This resulted in a hydrological shortcut and a much faster and stronger response of discharge to precipitation events in the Danish intensive farming catchment than in the Danish extensive farming catchment (Dalzell et al., 2007). The Uruguayan intensive farming catchment was not artificially drained and the reasons for its higher temporal variability of discharge relative to the Uruguayan extensive farming catchment remain unclear. However, it is likely that the removal of buffer zones along the streams in the intensive agricultural areas in the Uruguayan intensive farming catchment lowered the buffer capacity of the soils for water from precipitation events and resulted in a faster response of discharge to rainfall events.

4.2 Effects of climate on fluvial DOM quantity

Discharge was an important driver of DOC and DON concentrations and loads in the Danish catchments. In contrast, DOC and DON loads but not concentrations were dependent on discharge in Uruguay. Moreover, according to the sensitivity analysis of the load calculations, the temporal variability of DOC and DON loads of both catchments in Uruguay well correlated with discharge variability, but not with temporal variability of DOC or DON concentrations. We also found a high sensitivity of the DOC and DON loads to the temporal variability of the discharge in the Danish intensive farming catchment. However, the temporal variability of DOC and DON loads was lower in this catchment than in the Uruguayan catchments, since the temporal variability of discharges was lower. The only catchment, in which which in which[28] the temporal variability of the loads was not

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[28]change-21

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primarily affected by the temporal variability of discharges was the Danish extensive farming catchment, for which the DOC and DON concentration had a stronger effect on DOC and DON loads than the discharge. The reason is likely the low discharge variability in this catchment, which resulted in larger importance of DOC and DON concentrations for DOC and DON loads.

Different patterns of precipitation were the ultimate driver for the differences of the temporal variability of DOC and DON loads across climates. The more variable precipitation in Uruguay resulted in a more variable discharge and with that, more variable DOC and DON loads. In addition the more variable discharge also affected the DOC and DON concentrations in Denmark, which further increased the temporal variability of DOC and DON loads.

4.3 Effects of climate on fluvial DOM quality

In accordance with our second hypothesis, we found that climate had a strong effect on DOM composition. However, based on the PCA and in contrast to our hypothesis, the Uruguayan catchments were characterized by rather plant-derived DOM relative to the Danish catchments: This notion was implied by higher percentages of $C3$ and lower percentages of $C2$ and $C4$, together indicating DOM of plant origin (Søndergaard et al., 2003; Cory and McKnight, 2005), higher $SUVA_{bulk}$ and $SUVA_{HS}$, both indicating higher aromaticity (Weishaar et al., 2003), lower $E_2 : E_3$, indicating higher molecular weight (Peuravuori and Pihlaja, 2004), as well as higher $S_{275-295}$, S_r and lower $S_{350-400}$, together indicating DOM not yet processed by microbial organisms (Helms et al., 2008).

Altogether, fluvial DOM in Uruguay was to be likely derived from plant sources and was probably less microbially processed than in Denmark. This implies a lower soil and/or stream microbial activity in the Uruguayan than in the Danish catchments, which is surprising due to the higher temperatures in Uruguay. One explanation could be that in Uruguay the microbial processing of DOM from agricultural catchments is still limited by nutrient levels, whereas in Denmark, the long history of nutrient pollution (Kronvang et al., 2005) resulted in higher overall nutrient levels in the environment, facilitating higher levels of microbial processing. Another explanation could be the high temporal variability of precipitation and discharge in the Uruguayan catchments. Here, plant-derived organic matter which was stored in the upper soil layers could have been degraded during the long periods without precipitation and could be flushed out during high flow events. Based on this mechanism, one would expect a more variable DOM composition in Uruguay than in Denmark, since the DOM in Uruguay should be dominated by microbial sources during low flow and plant sources during high flow. In fact, a larger multivariate dispersal of DOM composition was found for the Uruguayan catchments in comparison to the Danish catchments. However, based only on in-stream measurements, we cannot infer the mechanisms behind the differences of DOM composition in the two climates. Here, additional comparative studies of the catchment sources in different climate zones would greatly advance the understanding of the mechanisms behind DOM export from catchments in different climates.

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4.4 Effects of farming intensity on fluvial DOM quantity

The lack of clear effects of intensive arable farming on DOC concentration suggests that the effects of agriculture are depending on the history of land use and the current status of soil organic matter in the catchment (Stanley et al., 2012) and contradicts the notion of a general effect of intensive agriculture on DOC concentrations for regions outside the northern temperate climate zone (Graeber et al., 2012b; Heinz et al., 2015)[29].

In contrast, the clear effect of intensive farming on DON concentrations in both countries could indicate a general effect of intensive agriculture, and is supported by studies in agricultural soils (van Kessel et al., 2009). However, no such clear effect of agriculture was found in the past in catchment-scale studies on DON concentrations in streams (Willett et al., 2004; Stanley and Maxted, 2008). The disparity of results between soil and catchment-scale studies on the effects of agriculture on DON concentrations may be a result of DON measurement problems, as was clearly stated in some soil DON studies (Siemens and Kaupenjohann, 2002; Solinger et al., 2001). In detail, the indirect determination of DON as the difference between total dissolved nitrogen and dissolved inorganic nitrogen can result in severe miscalculations of DON concentrations in high-nitrate environments (Graeber et al., 2012a; Lee and Westerhoff, 2005; Vandenbruwane et al., 2007). Therefore, we propose that the differences in results between soil and stream studies are an artefact of the indirect determination of DON. This is supported by the strong increase of fluvial DON concentrations in catchments with intensive agriculture, which was found in a recent study when using the direct measurement of DON concentrations with SEC (Heinz et al., 2015).[30] We recommend the use of the novel direct measurement technique shown in this and other studies (Graeber et al., 2012a; Heinz et al., 2015)~~The novel direct measurement techniques used in this study (Graeber et al., 2012a)~~[31] or treatments to remove nitrate and ammonium before indirect determination of DON (Lee and Westerhoff, 2005; Vandenbruwane et al., 2007; Chon et al., 2013; Graeber et al., 2012c) should be used in future studies to re-assess the effects of agriculture on DON concentrations in soils and in streams.

In contrast to the differences in the temporal variability of DOC and DON loads between the countries, precipitation was not the dominant driver of the differences in DOC and DON loads between intensive and extensive farming catchments within Denmark and Uruguay. The reason is that the precipitation patterns were highly similar within countries and even completely overlapping for the Danish catchments, whereas the discharges and with that DOC and DON loads mostly showed significant differences between the catchments within a country. Here, factors which were affecting discharges were also likely to affect DOC and DON loads: As described above, the subsurface tile drainage in the Danish intensive farming catchment (Dalzell et al., 2007) and the removal of buffer

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[29]change-6

[30]change-7

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zones for intensive farming in Uruguay may have been responsible for the higher temporal variability of discharges which then resulted in higher temporal variability of DOC and DON loads.

The high temporal variability of DOC and DON loads in intensive farming catchments is in accordance to earlier studies on DOC loads, which were conducted in the mid-western USA (Dalzell et al., 2007; Royer and David, 2005). However, this effect has only once before been shown for DON loads in temperate catchments (Heinz et al., 2015). A higher temporal variability of DOC and DON loads has effects on the biogeochemistry of the downstream aquatic ecosystems, where the different availability of DOC and DON over time could affect the variability of connected ecosystem functions such as primary production, respiration and denitrification (Prairie, 2008; Berman and Bronk, 2003).

4.5 Effects of farming intensity on fluvial DOM quality

In our study, DOM composition was strongly and similarily affected by the type of farming intensity in the two countries, which strongly supports our fourth hypothesis. We found an interaction effect between country and type of farming, however, this effect explained much less variance than the effect of farming type.

The similarity of the effect of farming type across countries is supported by the PCA, which revealed on the second axis that in Uruguay and Denmark the effects of farming type resulted in a similar shift in DOM composition. This shift was characterized by lower $C:N_{bulk}$, lower $C:N_{HS}$, lower $C1$, higher $HMWS_C$ and a higher freshness index for the intensive farming catchments and was slightly more pronounced for the Danish than for the Uruguayan catchments.

Low DOM C:N ratios have been related to higher DOM bioavailability and microbial sources of DOM (C:N ratio around 5–10, Sun et al., 1997; Petrone et al., 2009) and thus DOM from intensive farming catchments with median C:N ratios of 11 could indicate a shift in soil or in-stream DOM sources and could be of higher biogeochemical activity than DOM from extensive farming catchments(Heinz et al., 2015)[32]. Interestingly, the C:N ratios of the relatively complex humic substances ($C:N_{HS}$) were also lower in the intensive farming catchments. In soils, DOM C:N ratios as in our study are only found in deeper layers as a result of heavy microbial processing (Kaiser and Kalbitz, 2012) and high DOM complexity similar to ours is typically found for DOM released from soil organic matter (Schmidt et al., 2011). Thus, the complex, humic fluvial DOM with low C:N ratios in catchments with intensive farming is a strong indication of microbial sources in deeper soil layers (Heinz et al., 2015)of sources in deeper soil layers[33].

The other variables of DOM composition also support the notion of microbially produced DOM: in the PCA, the intensive farming catchments and $HMWS_C$ were positively correlated, which indicates rather microbial sources. High-molecular weight substances were found to be released by

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[32]change-9

[33]change-10

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530   extracellular polymeric substances of biofilms (Stewart et al., 2013)and in another study of temperate catchments, the same effect of intensive agriculture on fluvial $HMWS_C$ was found (Heinz et al., 2015).[34]Fluorescence index and freshness index were also higher in the intensive farming catchments, indicating a relatively recent, more microbial source of the humic fraction of DOM (Cory and McKnight, 2005; Parlanti et al., 2000), and the PARAFAC component $C1$ was also positively correlated to intensive farming, which indicates higher oxygen usage and microbial production according to studies in marine waters (Stedmon and Markager, 2005b; Kowalczuk et al., 2013). In conclusion, fluvial DOM from intensive farming catchments is relatively complex, but of more microbial origin compared to fluvial DOM from extensive farming catchments in both Denmark and Uruguay. Similar effects of intensive farming on DOM in streams were shown for temperate agricultural catchments (Williams et al., 2010; Wilson and Xenopoulos, 2009; Graeber et al., 2012b)(Williams et al., 2010; Williams et al., 2009; Williams et al., 2012b)[35] but never before in a comparison between different climates. Moreover, the high similarity of the effect of intensive farming on DOM in Denmark and Uruguay implies that the same mechanism is responsible for the changes in fluvial DOM composition in intensive farming catchments in different climatic zones.

545   Our results strongly imply microbial processing in deeper soil layers as being the source for the DOM in intensive farming. Several typical agricultural management practices may have been responsible for this pattern, either solely or in interaction. Soil tillage breaks up soil organic matter (SOM) aggregates and can result in strong microbial processing of SOM within these aggregates (Ewing et al., 2006). This may result in release of aged DOM previously bound to such SOM to freshwater ecosystems and, in fact, a high age of fluvial DOC was found in a study of U.S. agricultural catchments (Sickman et al., 2010). Furthermore nitrogen and phosphorus fertilizer addition to soils in intensive farming may promote higher microbial activity and result in higher release of DOM from SOM. However, extrapolation of the effects of intensive farming on DOM composition in other intensively farmed catchments, as well as the understanding of the mechanisms responsible for these effects remain speculative and should be tested by additional studies.

5   Conclusions

This study found distinct effects of climate on fluvial DOM, thus supporting earlier findings that climate is the main driver of DOM export from catchments. However, this is the first study to test the effect of climate on DOM quality. We found strong differences in DOM quality between the catchments in the two investigated climatic zones but cannot clearly attribute this to either climatic or soil factors. Further studies of DOM sources in the catchments are needed to get a clearer picture of why these differences between different climatic regions are found.

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[34]change-11

[35]change-12

We have shown that fluvial DOM from intensive farming is complex and of microbial origin and that effects of intensive farming on DOM composition superimpose effects of climate or soil which

565 may act in the two investigated regions. Moreover, intensive farming is strongly linked to a high temporal variability of the export of DOC and DON to freshwater ecosystems, which may affect the predicability of ecosystem processes fuelled by DOC and DON. These effects of intensive farming on DOM composition agree with recent findings from other studies in temperate climate and imply general mechanisms, by which intensive farming impacts the composition of DOM in streams. Based

570 on the composition of fluvial DOM, we find it likely that this mechanism is linked to the management of agricultural soils and that intensive farming may affect DOM in aquatic ecosystems, as well as linked ecosystem processes and biogeochemical cycles globally.

The effects of agriculture on DOM could only accurately be assessed by a combination of novel monitoring techniques, which combine direct measurements of DOC and DON with an analysis of

575 spectroscopic DOM composition. Future DOM monitoring programs should include similar techniques, if the effects human activities on DOM are to be accurately evaluated.

Appendix A: Split-half validation of the PARAFAC model

The split-half validation proved that the number of components is stable even for subsets of the dataset (Fig. A1). This is one of the main criteria when validating the number of components for a data-set (please see Murphy et al., 2013, for further details on the validation steps).

Appendix B: Typical chromatogram with the fractions of DOC and DON

In Figure B1 a typical chromatogram of a DOM sample is shown. Several treatments with and without nitrate and ammonium are shown to give an idea of the separation of DON, nitrate and ammonium, which allows the direct DON measurement. Please see Graeber et al. (2012b) for further comparisons of the indirect and direct determination of DON.[36]

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[36]change-2a

References

590

Aitkenhead, J. and McDowell, W.: Soil C: N ratio as a predictor of annual riverine DOC flux at local and global scales, Global Biogeochem. Cy., 14, 127–138, doi:10.1029/1999GB900083, http://onlinelibrary.wiley.com/doi/10.1029/1999GB900083/full, 2000.

Anderson, M.: A new method for non-parametric multivariate analysis of variance, Austral Ecology, 26,

595
32–46, doi:10.1111/j.1442-9993.2001.01070.pp.x, http://onlinelibrary.wiley.com/doi/10.1111/j.1442-9993.2001.01070.pp.x/full, 2001.

Balesdent, J., Chenu, C., and Balabane, M.: Relationship of soil organic matter dynamics to physical protection and tillage, Soil and Tillage Research, 53, 215–230, doi:10.1016/S0167-1987(99)00107-5, http://www.sciencedirect.com/science/article/pii/S0167198799001075, 2000.

600
Berman, T. and Bronk, D.: Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems, Aquat. Microb. Ecol., 31, 279–305, doi:10.3354/ame031279, http://www.int-res.com/abstracts/ame/v31/n3/p279-305/, 2003.

Borcard, D., Gillet, F., and Legendre, P.: Numerical ecology with R, Springer Verlag, Heidelberg, 2011.

Chon, K., Lee, Y., Traber, J., and von Gunten, U.: Quantification and characterization of dissolved organic nitro-

605
gen in wastewater effluents by electrodialysis treatment followed by size-exclusion chromatography with nitrogen detection, Water Res., 47, 5381 – 5391, doi:10.1016/j.watres.2013.06.019, http://www.sciencedirect.com/science/article/pii/S0043135413005101, 2013.

Cory, R. and McKnight, D.: Fluorescence Spectroscopy Reveals Ubiquitous Presence of Oxidized and Reduced Quinones in Dissolved Organic Matter, Environ. Sci. Technol., 39, 8142–8149, doi:10.1021/es0506962, http:

610
//pubs.acs.org/doi/abs/10.1021/es0506962, 2005.

Dalzell, B. J., Filley, T. R., and Harbor, J. M.: The role of hydrology in annual organic carbon loads and terrestrial organic matter export from a midwestern agricultural watershed, Geochim. Cosmochim. Ac., 71, 1448–1462, doi:doi:10.1016/j.gca.2006.12.009, http://www.sciencedirect.com/science/article/pii/S0016703706022563, 2007.

615
European comission and European Soil Bureau Network: The European Soil Database distribution version 2.0, Tech. rep., European comission, http://eusoils.jrc.ec.europa.eu/ESDB_Archive/ESDB_Data_Distribution/ESDB_data.html, 2004.

Ewing, S. A., Sanderman, J., Baisden, W., Wang, Y., and Amundson, R.: Role of large-scale soil structure in organic carbon turnover: evidence from California grassland soils, J. Geophys. Res.-Biogeo., 111,

620
G03 012–G03 012, doi:10.1029/2006JG000174, http://onlinelibrary.wiley.com/doi/10.1029/2006JG000174/abstract, 2006.

Fellman, J., Hood, E., and Spencer, R.: Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review, Limnol. Oceanogr., 55, 2452–2462, doi:10.4319/lo.2010.55.6.2452, http://www.aslo.org/lo/toc/vol_55/issue_6/2452.html, 2010.

625
Fellman, J. B., Petrone, K. C., and Grierson, P. F.: Source, biogeochemical cycling, and fluorescence characteristics of dissolved organic matter in an agro-urban estuary RID B-1345-2011, Limnol. Oceanogr., 56, 243–256, doi:10.4319/lo.2011.56.1.0243, http://www.aslo.org/lo/toc/vol_56/issue_1/0243.html, 2011.

Fox, J. and Weisberg, S.: An R Companion to Applied Regression, Sage, Thousand Oaks CA, second edn., http://socserv.socsci.mcmaster.ca/jfox/Books/Companion, 2011.

21

630 Gotelli, N. J. and Ellison, A. M.: A primer of ecological statistics, vol. 1, Palgrave Macmillan, Hants, United Kingdom, 2004.

Goyenola, G., Meerhoff, M., Teixeira-de Mello, F., González-Bergonzoni, I., Graeber, D., Fosalba, C., Vidal, N., Mazzeo, N., Ovesen, N., Jeppesen, E., et al.: Phosphorus dynamics in lowland streams as a response to climatic, hydrological and agricultural land use gradients, Hydrology and Earth System Sciences Discus-

635 sions, 12, 3349–3390, doi:10.5194/hessd-12-3349-2015, 2015.

Graeber, D., Gelbrecht, J., Kronvang, B., Gücker, B., Pusch, M. T., and Zwirnmann, E.: Technical Note: Comparison between a direct and the standard, indirect method for dissolved organic nitrogen determination in freshwater environments with high dissolved inorganic nitrogen concentrations, Biogeosciences, 9, 4873–4884, doi:10.5194/bg-9-4873-2012, http://www.biogeosciences.net/9/4873/2012/bg-9-4873-2012.

640 html, 2012a.

Graeber, D., Gelbrecht, J., Pusch, M., Anlanger, C., and von Schiller, D.: Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams, Sci. Total Environ., 438, 435–446, doi:doi:10.1016/j.scitotenv.2012.08.087, http://www.sciencedirect.com/science/article/pii/S0048969712011813, 2012b.

645 Graeber, D., Gücker, B., Zwirnmann, E., Kronvang, B., Weih, C., and Gelbrecht, J.: Dialysis is superior to anion exchange for removal of dissolved inorganic nitrogen from freshwater samples prior to dissolved organic nitrogen determination, Environ. Chem., 9, 529–536, doi:10.1071/EN11170, http://www.publish.csiro.au/paper/EN11170.htm, 2012c.

Green, S. and Blough, N.: Optical absorption and fluorescence properties of chromophoric dissolved organic

650 matter in natural waters, Limnol. Oceanogr., 39, 1903–1916, doi:10.4319/lo.1994.39.8.1903, 1994.

Heinz, M., Graeber, D., Zak, D., Zwirnmann, E., Gelbrecht, J., and Pusch, M.: Comparison of organic matter composition in agricultural versus forest affected headwaters with special emphasis on organic nitrogen, Environ. Sci. Technol., 49, 2081–2090, doi:10.1021/es505146h, 2015.

Helms, J., Stubbins, A., Ritchie, J., Minor, E., Kieber, D., and Mopper, K.: Absorption spectral slopes and slope

655 ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter, Limnol. Oceanogr., 53, 955–969, doi:10.4319/lo.2008.53.3.0955, http://www.aslo.org/lo/toc/vol_53/issue_3/0955.html, 2008.

Hollander, M., Wolfe, D. A., and Chicken, E.: Nonparametric statistical methods, vol. 751, John Wiley & Sons, Weinheim, 2013.

660 Hothorn, T., Hornik, K., Van De Wiel, M. A., and Zeileis, A.: A lego system for conditional inference, The American Statistician, 60, 257–263, doi:10.1198/000313006X118430, http://amstat.tandfonline.com/doi/full/10.1198/000313006X118430, 2006.

Huber, S., Balz, A., Abert, M., and Pronk, W.: Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography - organic carbon detection - organic nitrogen detection (LC-OCD-OND), Wa-

665 ter Res., 45, 879–885, doi:doi:10.1016/j.watres.2010.09.023, http://www.sciencedirect.com/science/article/pii/S0043135410006779, 2011.

Hudson, N., Baker, A., Reynolds, D., Carliell-Marquet, C., and Ward, D.: Changes in freshwater organic matter fluorescence intensity with freezing/ thawing and dehydration/ rehydration., J. Geophys. Res.-Biogeo., 114,

G00F08–G00F08, doi:10.1029/2008JG000915, http://onlinelibrary.wiley.com/doi/10.1029/2008JG000915/
abstract, 2009.

Kaiser, K. and Kalbitz, K.: Cycling downwards–dissolved organic matter in soils, Soil Biol.
Biochem., 52, 29–32, doi:10.1016/j.soilbio.2012.04.002, http://www.sciencedirect.com/science/article/pii/
S0038071712001381, 2012.

Kauppila, P. and Koskiaho, J.: Evaluation of annual loads of nutrients and suspended solids in Baltic rivers,
Nord. Hydrol., 34, 203–220, doi:10.2166/nh.2003.013, http://www.iwaponline.com/nh/034/nh0340203.htm,
2003.

Kothawala, D. N., Murphy, K. R., Stedmon, C. A., Weyhenmeyer, G. A., and Tranvik, L. J.: In-
ner filter correction of dissolved organic matter fluorescence, Limnol. Oceanogr-Meth., 11, 616–630,
doi:10.4319/lom.2013.11.616, http://www.aslo.org/lomethods/free/2013/0616.html, 2013.

Kowalczuk, P., Durako, M. J., Young, H., Kahn, A. E., Cooper, W. J., and Gonsior, M.: Characterization of
dissolved organic matter fluorescence in the South Atlantic Bight with use of PARAFAC model: Interannual
variability, Mar. Chem., 113, 182–196, doi:10.1016/j.marchem.2009.01.015, http://www.sciencedirect.com/
science/article/pii/S0304420309000139, 2009.

Kowalczuk, P., Tilstone, G. H., Zabłocka, M., Röttgers, R., and Thomas, R.: Composition of dissolved organic
matter along an Atlantic Meridional Transect from fluorescence spectroscopy and Parallel Factor Analy-
sis, Mar. Chem., 157, 170–184, doi:10.1016/j.marchem.2013.10.004, http://www.sciencedirect.com/science/
article/pii/S0304420313001783, 2013.

Kronvang, B., Jeppesen, E., Conley, D., Sondergaard, M., Larsen, S., Ovesen, N., and Carstensen, J.: Nu-
trient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and
coastal waters, J. Hydrol., 304, 274–288, doi:doi:10.1016/j.jhydrol.2004.07.035, http://www.sciencedirect.
com/science/article/pii/S0022169404004901, 2005.

Lawaetz, A. and Stedmon, C.: Fluorescence intensity calibration using the Raman scatter peak of
water, Appl. Spectrosc., 63, 936–940, doi:https://www.s-a-s.org/journal/viewer/abstract/597/, 10.1366/
000370209788964548., 2009.

Lee, W. and Westerhoff, P.: Dissolved organic nitrogen measurement using dialysis pretreatment, Environ. Sci.
Technol., 39, 879–884, doi:10.1021/es048818y, http://pubs.acs.org/doi/abs/10.1021/es048818y, 2005.

Milori, D. M., Martin-Neto, L., Bayer, C., Mielniczuk, J., and Bagnato, V. S.: Humification degree of soil humic
acids determined by fluorescence spectroscopy, Soil Sci., 167, 739–749, doi:10.1097/00010694-200211000-
00004, http://journals.lww.com/soilsci/Abstract/2002/11000/Humification_Degree_of_Soil_Humic_Acids_
Determined.4.aspx, 2002.

Mulholland, P.: Dissolved organic matter concentration and flux in streams, J. N. Am. Benthol. Soc., 16, 131–
141, doi:10.2307/1468246, http://www.jstor.org/stable/1468246, 1997.

Murphy, K. R., Hambly, A., Singh, S., Henderson, R. K., Baker, A., Stuetz, R., and Khan, S. J.: Organic mat-
ter fluorescence in municipal water recycling schemes: toward a unified PARAFAC model, Environ. Sci.
Technol., 45, 2909–2916, doi:10.1021/es103015e, http://pubs.acs.org/doi/abs/10.1021/es103015e, 2011.

Murphy, K. R., Stedmon, C. A., Graeber, D., and Bro, R.: Fluorescence spectroscopy and multi-way
techniques: PARAFAC, Anal. Methods, 5, 6557–6566, doi:10.1039/C3AY41160E, http://pubs.rsc.org/en/
Content/ArticleLanding/2013/AY/c3ay41160e, 2013.

23

Murphy, K. R., Stedmon, C. A., Wenig, P., and Bro, R.: OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment, Analytical Methods, 6, 658–661, doi:10.1039/C3AY41935E, http://pubs.rsc.org/en/Content/ArticleLanding/2014/AY/c3ay41935e, 2014.

Nimptsch, J., Woelfl, S., Kronvang, B., Giesecke, R., González, H. E., Caputo, L., Gelbrecht, J. G., von Tuempling, W., and Graeber, D.: Does filter type and pore size influence spectroscopic analysis of freshwater chromophoric DOM composition?, Limnologica - Ecology and Management of Inland Waters, 48, 57 – 64, doi:http://dx.doi.org/10.1016/j.limno.2014.06.003, http://www.sciencedirect.com/science/article/pii/S0075951114000401, 2014.

Ohno, T.: Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter, Environ. Sci. Technol., 36, 742–746, doi:10.1021/es0155276, http://pubs.acs.org/doi/abs/10.1021/es0155276, 2002.

Ohno, T. and Bro, R.: Dissolved organic matter characterization using multiway spectral decomposition of fluorescence landscapes, Soil Sci. Soc. Am. J., 70, 2028–2037, doi:10.2136/sssaj2006.0005, https://www.soils.org/publications/sssaj/abstracts/70/6/2028, 2006.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H.: vegan: Community Ecology Package, http://CRAN.R-project.org/package=vegan, r package version 2.0-7, 2013.

Parlanti, E., Worz, K., Geoffroy, L., and Lamotte, M.: Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs, Org. Geochem., 31, 1765–1781, doi:10.1016/S0146-6380(00)00124-8, http://www.sciencedirect.com/science/article/pii/S0146638000001248, 2000.

Patel-Sorrentino, N., Mounier, S., and Benaim, J.: Excitation-emission fluorescence matrix to study pH influence on organic matter fluorescence in the Amazon basin rivers, Water Res., 36, 2571–2581, doi:10.1016/S0043-1354(01)00469-9, http://www.sciencedirect.com/science/article/pii/S0043135401004699, 2002.

Petrone, K.: Catchment export of carbon, nitrogen, and phosphorus across an agro-urban land use gradient, Swan-Canning River system, southwestern Australia, J. Geophys. Res.-Biogeo., 115, G01016, doi:10.1007/s10533-008-9238-z, http://onlinelibrary.wiley.com/doi/10.1029/2009JG001051/abstract, 2010.

Petrone, K., Richards, J., and Grierson, P.: Bioavailability and composition of dissolved organic carbon and nitrogen in a near coastal catchment of south-western Australia, Biogeochemistry, 92, 27–40, doi:10.1007/s10533-008-9238-z, http://link.springer.com/article/10.1007%2Fs10533-008-9238-z, 2009.

Peuravuori, J. and Pihlaja, K.: Preliminary study of lake dissolved organic matter in light of nanoscale supramolecular assembly, Environ. Sci. Technol., 38, 5958–5967, doi:10.1021/es040041l, http://pubs.acs.org/doi/abs/10.1021/es040041l, 2004.

Pfeifer, D., Hoffmann, K., Hoffmann, A., Monte, C., and Resch-Genger, U.: The kit spectral fluorescence standards - a simple calibration tool for the standardization of the spectral characteristics of fluorescence instruments, J. Fluoresc., 16, 581–587, doi:10.1007/s10895-006-0086-8, http://link.springer.com/article/10.1007/s10895-006-0086-8, 2006.

Pouillot, R. and Delignette-Muller, M. L.: Evaluating variability and uncertainty separately in microbial quantitative risk assessment using two R packages, Int. J. Food Microbiol., 142,

24

330–340, doi:10.1016/j.ijfoodmicro.2010.07.011, http://www.sciencedirect.com/science/article/pii/
S0168160510003843, 2010.

Prairie, Y. T.: Carbocentric limnology: Looking back, looking forward, Can. J. Fish. Aquat. Sci., 65, 543–548,
doi:10.1139/f08-011, http://www.nrcresearchpress.com/doi/abs/10.1139/f08-011, 2008.

R Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Com-
puting, Vienna, Austria, http://www.R-project.org/, ISBN 3-900051-07-0, 2015.

Royer, T. and David, M.: Export of dissolved organic carbon from agricultural streams in Illinois,
USA, Aquat. Sci., 67, 465–471, doi:10.1007/s00027-005-0781-6, http://link.springer.com/article/10.1007/
s00027-005-0781-6, 2005.

Schmidt, M., Torn, M., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I., Kleber, M., Kögel-Knabner, I.,
Lehmann, J., Manning, D., et al.: Persistence of soil organic matter as an ecosystem property, Nature, 478,
49–56, doi:10.1038/nature10386, http://www.nature.com/nature/journal/v478/n7367/abs/nature10386.html,
2011.

Sickman, J., DiGiorgio, C., Davisson, M., Lucero, D., and Bergamaschi, B.: Identifying sources of dissolved
organic carbon in agriculturally dominated rivers using radiocarbon age dating: Sacramento-San Joaquin
River Basin, California, Biogeochemistry, 99, 79–96, 2010.

Siemens, J. and Kaupenjohann, M.: Contribution of dissolved organic nitrogen to N leaching from four German
agricultural soils, J. Plant Nutr. Soil Sci., 165, 675–681, doi:10.1002/jpln.200290002, http://onlinelibrary.
wiley.com/doi/10.1002/jpln.200290002/abstract, 2002.

Solinger, S., Kalbitz, K., and Matzner, E.: Controls on the dynamics of dissolved organic carbon and nitrogen
in a Central European deciduous forest, Biogeochemistry, 55, 327–349, http://www.jstor.org/stable/1469963,
2001.

Søndergaard, M., Stedmon, C., and Borch, N.: Fate of terrigenous dissolved organic matter (DOM) in estuaries:
Aggregation and bioavailability, Ophelia, 57, 161–176, doi:10.1080/00785236.2003.10409512, http://www.
tandfonline.com/doi/abs/10.1080/00785236.2003.10409512, 2003.

Stanley, E. and Maxted, J.: Changes in the dissolved nitrogen pool across land cover gradients in Wisconsin
streams, Ecol. Appl., 18, 1579–1590, doi:10.1890/07-1379.1, http://www.esajournals.org/doi/abs/10.1890/
07-1379.1, 2008.

Stanley, E. H., Powers, S. M., Lottig, N. R., Buffam, I., and Crawford, J. T.: Contemporary changes in dissolved
organic carbon (DOC) in human-dominated rivers: is there a role for DOC management?, Freshwater Biol.,
57, 26–42, doi:10.1111/j.1365-2427.2011.02613.x, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2427.
2011.02613.x/abstract, 2012.

Stedmon, C. and Markager, S.: Resolving the variability in dissolved organic matter fluorescence in
a temperate estuary and its catchment using PARAFAC analysis, Limnol. Oceanogr., 50, 686–697,
doi:10.4319/lo.2005.50.2.0686, http://www.aslo.org/lo/toc/vol_50/issue_2/0686.html, 2005a.

Stedmon, C. and Markager, S.: Tracing the production and degradation of autochthonous fractions of dissolved
organic matter by fluorescence analysis, Limnol. Oceanogr., 50, 1415–1426, doi:10.4319/lo.2005.50.5.1415,
http://www.aslo.org/lo/toc/vol_50/issue_5/1415.html, 2005b.

Stedmon, C., Thomas, D., Granskog, M., Kaartokallio, H., Papadimitriou, S., and Kuosa, H.: Characteristics of dissolved organic matter in Baltic coastal sea ice: Allochthonous or autochthonous origins?, Environ. Sci. Technol., 41, 7273–7279, doi:10.1021/es071210f, http://pubs.acs.org/doi/abs/10.1021/es071210f, 2007.

790   Stewart, T. J., Traber, J., Kroll, A., Behra, R., and Sigg, L.: Characterization of extracellular polymeric substances (EPS) from periphyton using liquid chromatography-organic carbon detection–organic nitrogen detection (LC-OCD-OND), Environ. Sci. Pollut. R., 20, 3214–3223, doi:10.1007/s11356-012-1228-y, http://link.springer.com/article/10.1007%2Fs11356-012-1228-y, 2013.

Stubbins, A., Lapierre, J., Berggren, M., and Dittmar, T.and del Giorgio, P.: What's in an EEM? Molecular
795   Signatures Associated with Dissolved Organic Fluorescence in Boreal Canada, Environ. Sci. Technol., 48, 10 598–10 606, doi:10.1021/es502086e, http://pubs.acs.org/doi/abs/10.1021/es502086e, 2014.

Sun, L., Perdue, E., Meyer, J., and Weis, J.: Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river, Limnol. Oceanogr., 42, 714–721, doi:10.4319/lo.1997.42.4.0714, http://avto.aslo.info/lo/toc/vol_42/issue_4/, 1997.

800   van Kessel, C., Clough, T., and van Groenigen, J.: Dissolved organic nitrogen: an overlooked pathway of nitrogen loss from agricultural systems?, J. Environ. Qual., 38, 393–401, doi:10.2134/jeq2008.0277, https://dl.sciencesocieties.org/publications/jeq/abstracts/38/2/393, 2009.

Vandenbruwane, J., De Neve, S., Qualls, R., Salomez, J., and Hofman, G.: Optimization of dissolved organic nitrogen (DON) measurements in aqueous samples with high inorganic nitrogen concentrations, Sci. Total
805   Environ., 386, 103–113, doi:10.1016/j.scitotenv.2007.06.025, http://www.sciencedirect.com/science/article/pii/S0048969707007000, 2007.

Weishaar, J., Aiken, G., Bergamaschi, B., Fram, M., Fujii, R., and Mopper, K.: Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon, Environ. Sci. Technol., 37, 4702–4708, doi:10.1021/es030360x, http://pubs.acs.org/doi/abs/10.1021/es030360x,
810   2003.

Willett, V., Reynolds, B., Stevens, P., Ormerod, S., and Jones, D.: Dissolved organic nitrogen regulation in freshwaters, J. Environ. Qual., 33, 201–209, doi:10.2134/jeq2004.2010, https://dl.sciencesocieties.org/publications/jeq/abstracts/33/1/201, 2004.

Williams, C., Yamashita, Y., Wilson, H., Jaffe, R., and Xenopoulos, M.: Unraveling the role of land use and mi-
815   crobial activity in shaping dissolved organic matter characteristics in stream ecosystems, Limnol. Oceanogr., 55, 1159–1171, doi:10.4319/lo.2010.55.3.1159, http://www.aslo.org/lo/toc/vol_55/issue_3/1159.html, 2010.

Williams, M., Hopkinson, C., Rastetter, E., Vallino, J., and Claessens, L.: Relationships of land use and stream solute concentrations in the Ipswich River basin, northeastern Massachusetts, Water Air Soil Poll., 161, 55–74, doi:10.1007/s11270-005-2830-0, http://link.springer.com/article/10.1007/s11270-005-2830-0, 2005.

820   Wilson, H. and Xenopoulos, M.: Effects of agricultural land use on the composition of fluvial dissolved organic matter, Nat. Geosci., 2, 37–41, doi:10.1038/ngeo391, http://www.nature.com/ngeo/journal/v2/n1/abs/ngeo391.html, 2009.

Yamashita, Y., Maie, N., Briceno, H., and Jaffé, R.: Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela, J. Geophys. Res.-Biogeo., 115, G00F10,
825   doi:10.1029/2009JG000987, http://onlinelibrary.wiley.com/doi/10.1029/2009JG000987/full, 2010a.

Yamashita, Y., Scinto, L. J., Maie, N., and Jaffé, R.: Dissolved Organic Matter Characteristics Across a Subtropical Wetland's Landscape: Application of Optical Properties in the Assessment of Environmental Dynamics, Ecosystems, 13, 1006–1019, doi:10.1007/s10021-010-9370-1, 2010b.
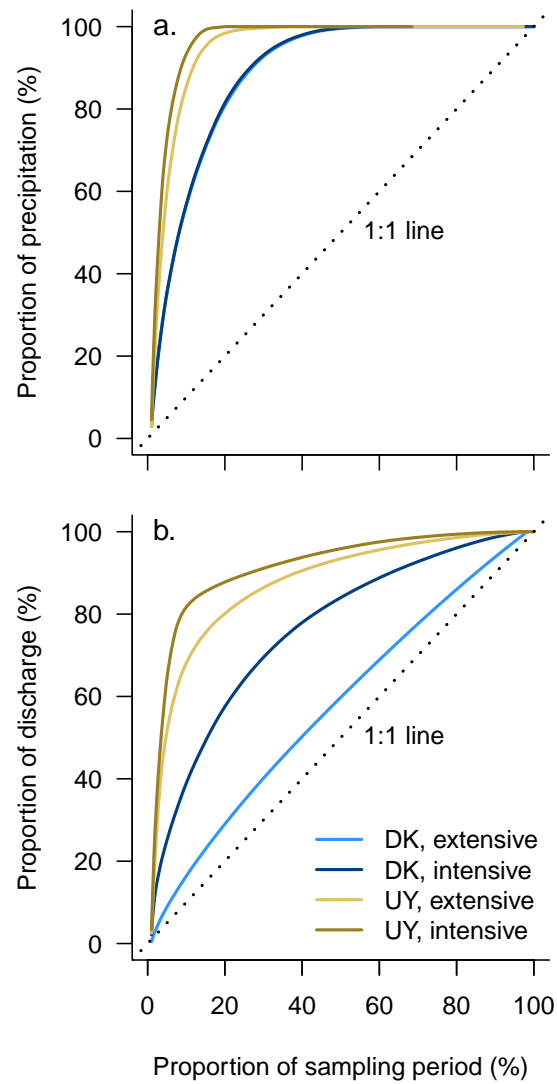
Figure 1. Ranked precipitation (a.) and discharge values (b.) versus the proportion of the sampling period. Precipitation data for both the intensive and extensive catchment in Denmark is included but very similar (a.). The 1:1 line represents a completely equal precipitation (a.) or discharge (b.) across the whole sampling period. Plot style adapted from Dalzell et al. (2007).
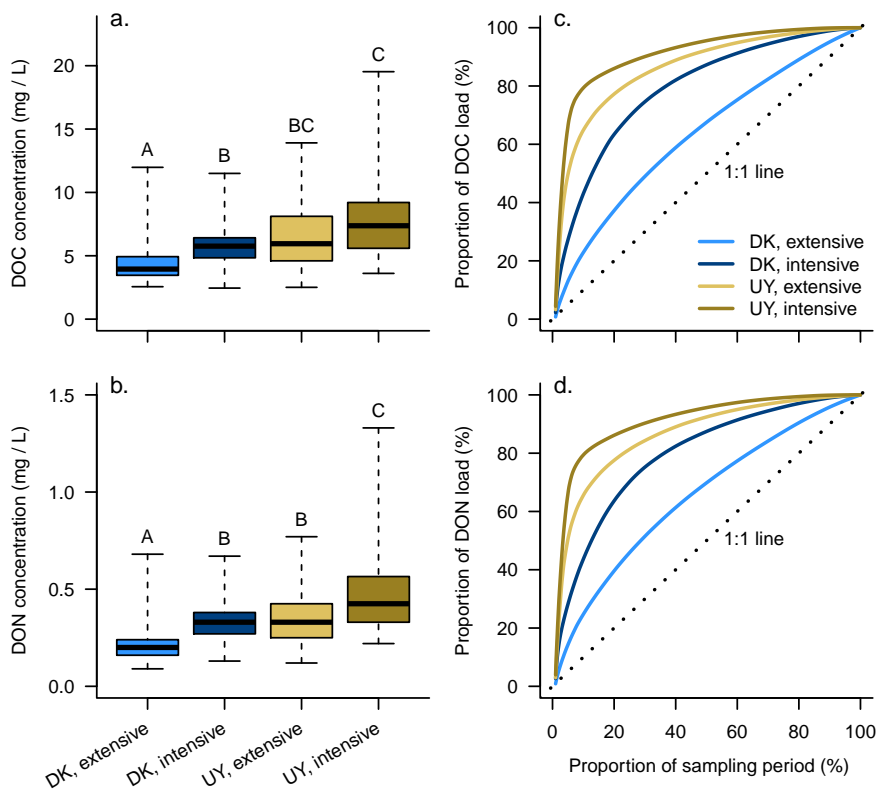
Figure 2. Concentrations (panels a. and b.) and ranked daily load versus the proportion of the sampling period (panels c. and d., plot style adapted from Dalzell et al. (2007)) for dissolved organic carbon (DOC) and dissolved organic nitrogen (DON). The 1:1 line in the panels c. and d. represents a completely equal DOC and DON load across the whole sampling period. Capital letters indicate significantly different groups ($p < 0.05$, Nemenyi pairwise test, n was 47–49, depending on the stream). DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.
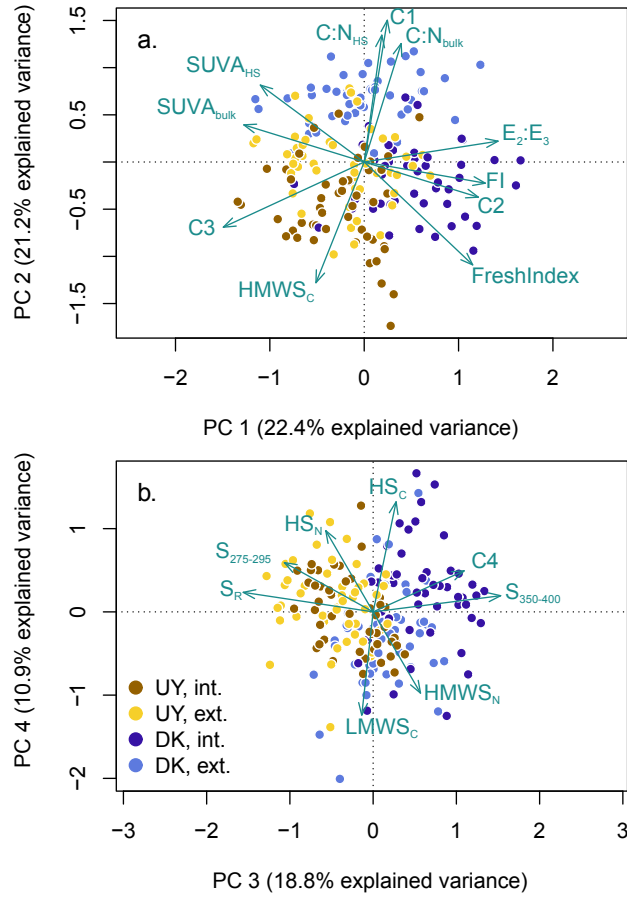
Figure 3. Principal component analysis (PCA) of dissolved organic matter (DOM) composition. The first four axes (PCA axis 1 & 2: panel a., PCA axis 3 & 4: panel b.) of the PCA explain 73% of the variance. Only those DOM composition variables are shown, which can be interpreted with high confidence (Borcard et al., 2011). $C1 - C4$: Fluorescence components 1 to 4 based on parallel factor analysis (see also Table 3); FI: fluorescence index; FreshIndex: freshness index; $E_2 : E_3$: Ratio of absorbance at 250 nm to absorbance at 365 nm; $S_{275-295}$, $S_{350-400}$ & S_R: Slope of absorbance at 275-285 nm, 350-400 nm and the ratio (R) of these two slopes; $SUVA_{HS}$ & $SUVA_{bulk}$: absorbance at 254 nm, normalised by dissolved organic carbon concentration, for humic substances (HS) and all DOM fractions, respectively; $C : N_{HS}$ & $C : N_{bulk}$: molar carbon to nitrogen ratio for HS and all DOM fractions, respectively; HS_C & HS_N, $HMWS_C$ & $HMWS_N$ or $LMWS_C$: carbon (C) and nitrogen (N) in the humic substance (HS), high-molecular weight substance (HMWS) or low-molecular weight substance fraction (LMWS) based on size-exclusion chromatography. No values for $LMWS_N$ exist, because N in LMWS is indistinguishable from N in nitrate. DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.

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Figure 4. Selected variables of dissolved organic matter composition (DOM). Capital letters indicate significantly different groups ($p < 0.05$, Nemenyi pairwise test, n was 47–49, depending on the stream). DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.

Table 1. Names, position of the sampled catchment (WGS 84) and farming type of the investigated catchments. DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.

Catchment	Coordinates	Catchment area (km^2)	Land use, percentages of catchment area (%)
DK, extensive	56°17'2" N 9°53'51" E	7.4	Forest (59); arable farming (29); pasture/meadow (7); other (5)
DK, intensive	56°13'29" N 9°48'41" E	11.8	Arable farming (92); forest (2); urban (1); other (5)
UY, extensive	33°49'31" S 56°16'55" W	18.8	Extensive pasture ($\sim$70); arable farming ($\sim$30)
UY, intensive	33°54'13" S 56°00'23" W	8.4	Arable farming and dairy farms (90); extensive pasture (7); urban (3)

Table 2. Loads of DOC and DON for the sampled catchments in 2011 and the median (range) daily loads for the whole sampling period. DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.

Catchment	Annual DOC load	Annual DON load	Daily DOC load	Daily DON load
	$kg\,km^{-2}\,yr^{-1}$		Median (range) $kg\,km^{-2}\,d^{-1}$	
DK, extensive	2077.8	99.4	5.0 (31.0)	0.24 (1.8)
DK, intensive	1267.9	75.2	1.6 (57.7)	0.10 (4.0)
UY, extensive	1019.7	53.3	1.2 (93.7)	0.06 (4.6)
UY, intensive	1824.5	105.2	1.1 (176.3)	0.07 (9.6)

Table 3. Excitation maxima (Ex., secondary maxima in brackets), emission maximum (Em.) and tentative interpretation of fluorescence components based on parallel factor analysis (PARAFAC).

Component	Definition (nm)	Tentative interpretation
C1	Ex.: <240 (385) Em.: 468	Terrestrial humic-like, found in freshwater environments (Murphy et al., 2011; Yamashita et al., 2010b; Kowalczuk et al., 2009); relates to oxygen usage, microbial production and humification in marine systems (Stedmon and Markager, 2005b; Kowalczuk et al., 2013); removed by UV and visible light (Stedmon and Markager, 2005b)
C2	Ex.: <240 (300) Em.: 402	microbial, humic-like, found in freshwater environments (Murphy et al., 2011; Fellman et al., 2010; Stedmon and Markager, 2005a) potentially related to algal, autothochthonous sources (Søndergaard et al., 2003); related to terrestrial sources in marine systems (Stedmon and Markager, 2005a; Stedmon et al., 2007); removed by UV light (Stedmon et al., 2007)
C3	Ex.: 270 (415) Em.: 512	Fulvic-acid like, complex, ubiquituous fluorophore (Yamashita et al., 2010b, a; Stedmon et al., 2007; Stedmon and Markager, 2005a); plant/ soil-derived semi-quinone like radical according to combined electron-spin resonance and fluorescence measurements (Milori et al., 2002; Cory and McKnight, 2005); similar component exported from wetlands and arable farming (Graeber et al., 2012b)
C4	Ex.: 355 (255) Em.: 440	Humic-like, reduced-semiquinone character (Cory & McKnight 2005); positively related to bacterial production (C4 in Williams et al 2010); similar component exported from arable farming catchments (Graeber et al 2012), susceptible to chlorination (oxidation, Murphy et al., 2011)
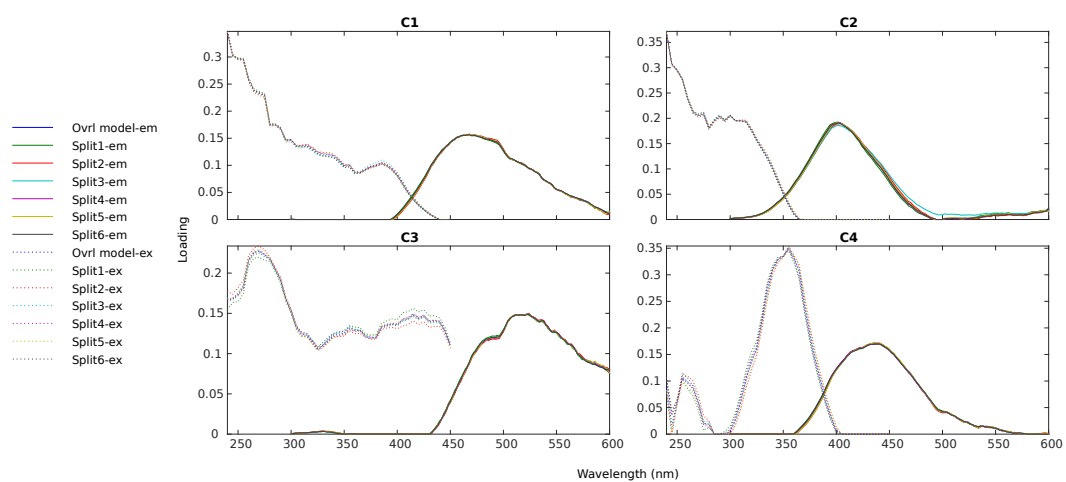
Figure A1. Split-half validation of the PARAFAC model. The models for six halfs generated by the standard method described in Murphy et al. (2013) are shown. When the fits of the splits are similar to each other and the entire model, a high stability of the model and low randomness of the fluorophores is given.
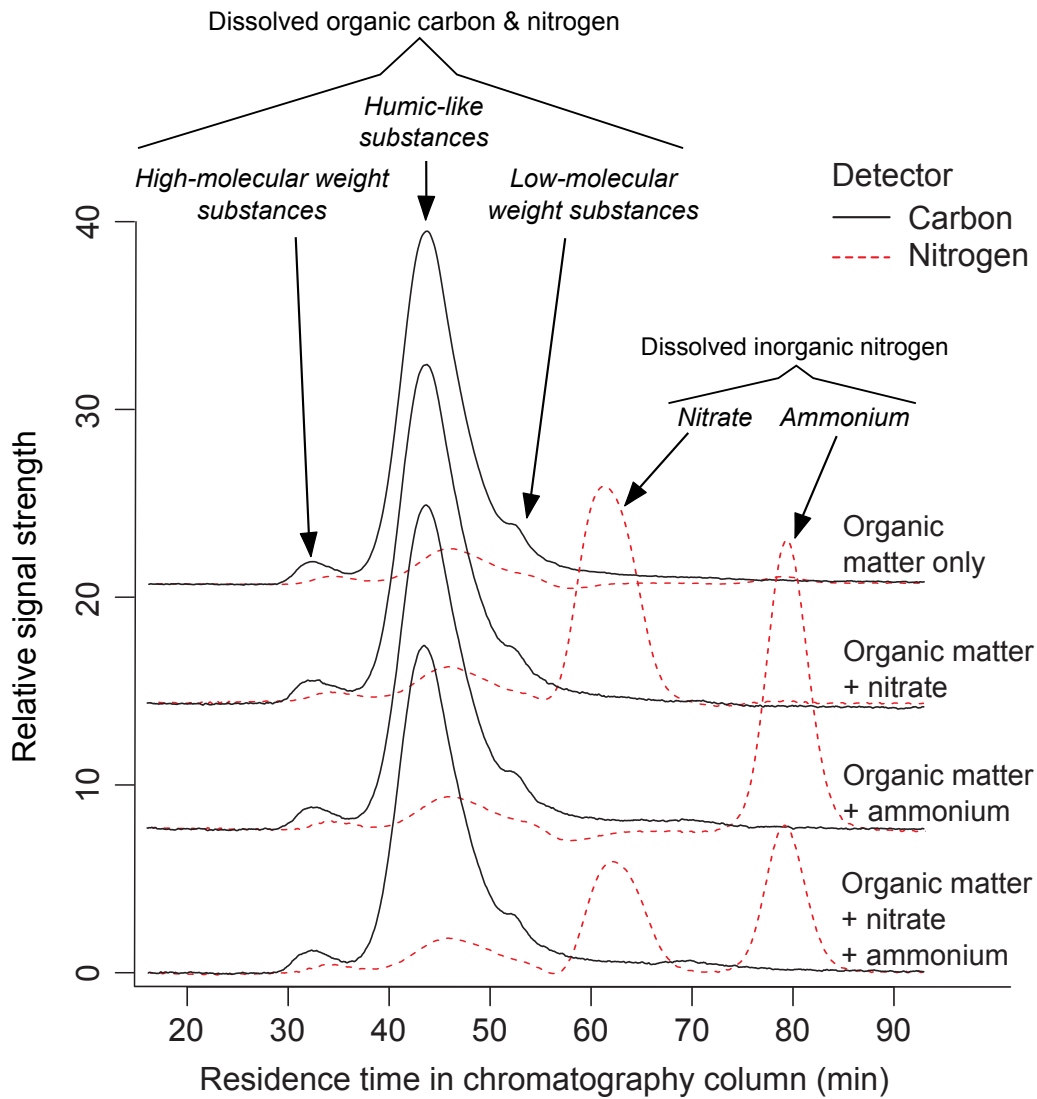
Figure B1. Typical chromatogram of size-exclusion chromatography to show the distribution of DOM fractions with and without added nitrate and ammonium. The sample for this chromatogram was taken at a wetland outflow in Brandenburg, Germany.

3 First revision

First revision: Author's response to referee comments

May 5, 2015

1 Description of procedure

All changes in result of the referee comments are marked in the manuscript in blue. These are called "change-1..x" and are referred to within the appropriate sections in the reply below. All referee comments are marked in bold and the replies always start with "Reply:".

2 English correction

The English language of the revised version of the manuscript was checked by a native speaker to further improve the text. These changes were not marked in the text since only the language was improved but not the content was changed. However, when content was changed, this was marked.

3 General remarks

According to the comments by the editor reviewers we changed the manuscript with special emphasis on:

- **the background of the data sets**
  We further now further explain the background of the datasets in the methods and in the reply to referee 2.

- the **differences between the study areas**
  We now further explain the differences between the study areas in the study site description and refer to another study for more details.

- **the representativeness of the study areas and the results**
  We carefully checked our interpretation concerning representativeness and adjusted it when necessary. We moreover explained our motivation for the choice of sites to referee 2 in-depth.

- **clarification and motivation of the used statistical methods**
  We extended the statistical analyses section in the revised manuscript to better clarify our statistics and improved the explanation of the motivation where necessary. We also discuss the background of our statistics in the reply to referee 1.

4 Reply to the comments by referee 1

1) Indicate sample sizes for statistical tests. No sample sizes are mentioned anywhere in the paper.

Reply: We thank the reviewer for raising our attention to the missing sample sizes. Please see the end of this document for an overview of the sample sizes. We will include the sample sizes at the appropriate positions of the revised manuscript (see markups called "added sample sizes" in the manuscript text).

2) It seems to me that after converting some data to ratios, the statistical analysis has proceeded in much the same way as it would have for the raw dataset. Some, but not all, variables were transformed to improve their distributions (relative to the assumption of normality), then ANOVA, PCA were performed. I However, methods for statistical analysis of compositional data /ratios are special due to the constraint that the data sum to one (closed data/constant sum constraint). There is a whole field of multivariate statistical analysis devoted to the analysis of compositional data, e.g. in the field of geology. There is an R package called composition, and several other packages, specifically directed at analysing compositional data (incl. imputing missing data). The logratio transformation is often used prior to linear modelling. See papers by J. Aitchison starting in the 1980s. Also a very readable R tutorial about the problem with ratios at http://advan.physiology.org/content/37/3/213.

Reply: The reviewer is right that the data from the PARAFAC and SEC were converted to ratios. However, no classical ANOVAs or linear models were applied in this study. Please see the points below for a description of the used test types and their relationship to ratio data:

1. We performed permutative MANOVAs (PERMANOVAs, often called ANOSIM or analysis of similarity) , a non-parametric alternative to MANOVAs and permutative multivariate tests of dispersion (PERMDISP), a multivariate non-parametric alternative to Levenes test (described in lines 238–247 of the submitted manuscript). According to the literature (Anderson 2001, see submitted manuscript for reference), no special treatment is needed for compositional data when using PERMANOVAs, since the data is converted into a dissimilarity matrix before applying the statistic (in our case Euclidean distances). Since the data for PERMDISPs is also translated into a dissimilarity matrix before the statistic is done, the

same applies to PERMDISP. Both statistics can be based on ratio data or presence-absence data and on different dissimilarity indexes. We only transformed some of the data, to keep the same transformations as in the PCA and to make the PERMANOVAs / PERMDISPs representative for the data depicted in the PCA.

2. As stated in the first part of the reply, a part of the data for the PCA and PERMANOVAs / PERMDISPs was transformed prior to its application, to allow the application of the linear relationships on which a PCA is based. We agree with the reviewer that the commonly used log transformation is problematic with ratios with fixed limits (0..1). Thus, we used logit transformations ($logit(x) = -log(1/x - 1)$)for the three variables which were ratios with fixed limits and which had to be transformed to reach normal distribution: $HMWS_N$, $HMWS_C$, and HS_N and repeated the PERMANOVAs and PCA. No difference could be detected for the PERMANOVAs or PERMDISPs. Only very small differences could be detected for the PCA, most notably a change from 18.7 to 18.8% explained variance for the 4th PCA axis. To prove that no significant changes occurred, we show the PCA results from the submitted manuscript version (Fig.1) and the PCA results with the revised transformation (Fig.2) below. We included the PCA based on the revised data transformations in the revised manuscript (Fig.3 of the revised manuscript). Moreover, we changed the description of the transformation in the text (change-17, section on statistical analyses in methods).

3. No linear models were applied in this study. Instead, we used Spearman rank correlations, but never for ratio data with fixed limits.

4. Differences in univariate data were assessed with permutative one-way statistics (often called Monte-Carlo tests). Hence, neither normal distribution nor homoscedacity was assumed for the data. This test works well with ratio data, since it has no assumption on the data probability distribution.

5. Levenes test based on the median was used to assess the variability of univariate data, but never for ratio data with fixed limits.

3) Currently, some data used in the PCA are bounded by (0..1) and some are not (e.g. fluorescence index) , but overall the dataset does not sum to 100% (as it would in a typical compositional dataset). This does not sound like a good situation for starting a PCA. A simple approach would be to autoscale the raw (not compositional) data prior to PCA (transformation of some variables might still be advisable), which takes care of differences in scale between different variables, produces readily interpretable plots, and has other useful properties as described by Bro and Smilde (2014) in their recent PCA tutorial. The autoscaling will allow the PCA to reveal compositional
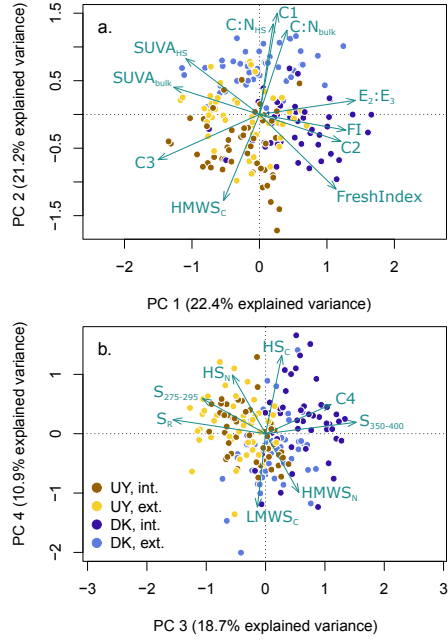
Figure 1: **Principal component analysis (PCA) of dissolved organic matter (DOM) composition, included in submitted version of the manuscript.** The first four axes (PCA axis 1 & 2: panel a., PCA axis 3 & 4: panel b.) of the PCA explain 73% of the variance. Only those DOM composition variables are shown, which can be interpreted with high confidence. $C1 - C4$: Fluorescence components 1 to 4 based on parallel factor analysis; FI: fluorescence index; FreshIndex: freshness index; $E_2 : E_3$: Ratio of absorbance at 250 nm to absorbance at 365 nm; $S_{275-295}$, $S_{350-400}$ & S_R: Slope of absorbance at 275-285 nm, 350-400 nm and the ratio (R) of these two slopes; $SUVA_{HS}$ & $SUVA_{bulk}$: absorbance at 254 nm, normalised by dissolved organic carbon concentration, for humic substances (HS) and all DOM fractions, respectively; $C : N_{HS}$ & $C : N_{bulk}$: molar carbon to nitrogen ratio for HS and all DOM fractions, respectively; HS_C & HS_N, $HMWS_C$ & $HMWS_N$ or $LMWS_C$: carbon (C) and nitrogen (N) in the humic substance (HS), high-molecular weight substance (HMWS) or low-molecular weight substance fraction (LMWS) based on size-exclusion chromatography. No values for $LMWS_N$ exist, because N in LMWS is indistinguishable from N in nitrate. DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.
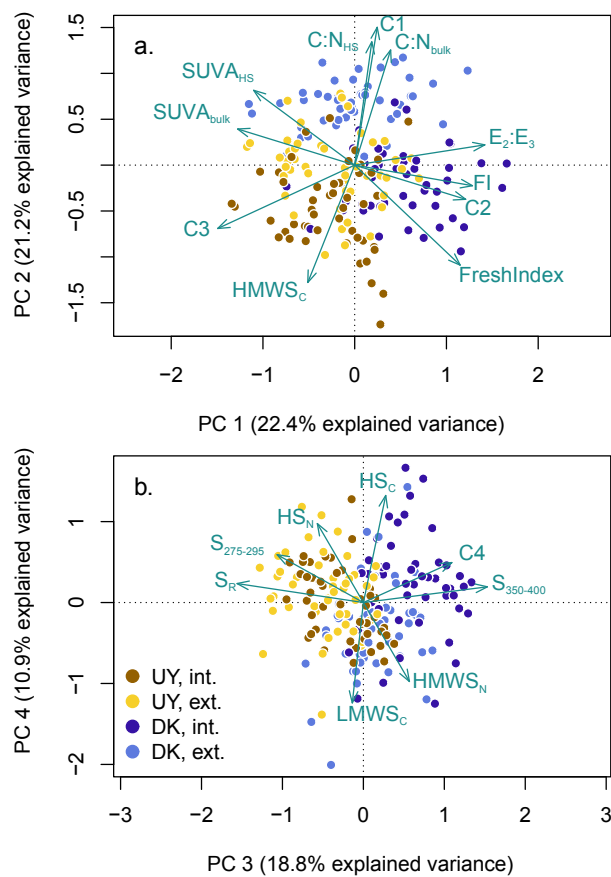
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Figure 2: **Principal component analysis (PCA) of dissolved organic matter (DOM) composition, with revised data transformations (see reply to Referee comment 1, item 2).** The details of the figure are explained in the figure caption of Figure 1.

differences between samples, which was the motivation for generating ratio data.

Reply: We agree that the data is on different scales and auto-scaling needs to be applied. In fact, in the submitted version of the manuscript, auto-scaling was applied to the data before making the PCA. For this, we used the parameter scale of the rda() function. Similarly, we auto-scaled the data by using the scale() function before applying the PERMANOVAs/ PERMDISPs. In both cases, the function was mentioned in the submitted version of the manuscript, but we forgot to mention the auto-scaling. We thank the reviewer for raising our attention to the missing description, which was included in the revised version of the manuscript (PCA: change-15; Last sentence, 4th paragraph of the Statistical Analyses section; PERMANOVAs/ PERMDISPs: change-16; Last sentence, 5th paragraph of the Statistical Analyses section).

4) Consider also the underlying assumptions of ANOVA, box and whisker plots and other statistical representations in the analysis of ratio data. When comparing ratio/percentages, it is common to arcsin transform the data first or use a chi-squared test.

Reply: We considered all statistics / plots. No further adjustments in addition to the ones explained in reply to comments 1-3 need to be done. We used logit transformation instead of arcsin transformation (see above for details).

5 Reply to the comments by referee 2

5.1 General comments

After reviewing the manuscript, I have a few concerns and questions regarding the manuscript that I would like the authors to address. First, the land use groupings used in the manuscript were not fully agricultural differences. One watershed in Denmark had forests as its dominant land use. This watershed seemed to have very different DOM and discharge properties than the other watersheds. I think these differences in land use need to be discussed and acknowledged. Second, I am not certain that the sample size of the study and observed results show strong climate and land use influences on the DOM. There was a lot of overlap for sampling events between rivers. Climate likely has an important influence and this manuscript shows clear evidence of that but I think the evidence is not as strong as the discussion surrounding them implies. Finally, I wondered if data were available to compare the SEC-DON method with the subtraction method in your study. If so adding these comparisons might further strengthen the interesting discussion regarding the SEC-DON method.

Reply: At first, we want to thank the referee for his/ her in-depth review of

our manuscript, which surely must have been time consuming. This helped us to detect weak points and to improve the study. Please see the following replies to the general comments:

- Concerning the land use:

  Both, the extensive catchment in Denmark and the extensive catchment in Uruguay contained a large area of the catchment with extensive land use. Since no forests exist in the prairie of Uruguay, these would also not appear in even completely pristine catchments. In fact, the only larger forests in Uruguay are artificial Eucalyptus plantations, thus, in this case, a forest does not necessarily mean that the land use is pristine. Extensive pasture is the most natural land use in Uruguay, as are commercially used forests in Denmark. We included a short explanation on the pristine/ near-pristine land-use/ catchment vegetation in both countries in the study sites section within the methods of the revised manuscript (see change-3 in the revised version of the manuscript).

- Concerning the sample size:

  It is clear to the authors, that four catchments are not a large sample size in terms of spatial sampling, but we had a very large sample size in terms of temporal sampling. To achieve both with a large sample number was difficult to reach due to constraints in time, financial circumstances and manpower. We acknowledge that the low sample size in terms of catchments would limit our conclusions, if this would have been the only study on this topic, but there are many studies with a large spatial dataset (for a discussion of these studies, please see the sections "Effects of farming intensity on fluvial DOM quantity" and "Effects of farming intensity on fluvial DOM composition" of the revised manuscript, and for DOC concentrations especially the cited review of Stanley et al 2012) and, especially for DOM composition measurements, a lack of investigations of temporal variability. Most studies on agricultural catchment effects are limited to one vegetation season (as we write in the introduction), some to one year (Graeber et al. 2012 and the recently published Heinz et al. 2015, EST, DOI: 10.1021/es505146h) and, apart from our manuscript, none with two years of data and two different climate regions. The publication by Heinz et al. strongly supports our findings for temperate catchments and is now cited in the introduction and discussion of the revised manuscript (change-4 to change-12).

- Concerning the assessment of climate effects:

  We think that our evidence on the presence or absence of climate effects is very strong for the investigated catchments. For the DOM amount, we built a very clear line of evidence, starting with measurements of precipitation, discharge, DOC and DON concentrations and loads (please see discussion of the submitted manuscript, section 4.2). Furthermore, we found highly significant effects of country (and hence climate region) on

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DOM composition (PERMANOVA with p ¡ 0.001, results section of the submitted manuscript) and of country on the temporal variability on DOM composition (PERMDISP with p ¡ 0.001, results section of the submitted manuscript). Based on these results the country had a strong effect on DOM composition, but, as we write at the end of the discussion of climate effects on DOM composition (section 4.3): "based only on in-stream measurements, we cannot infer the mechanisms behind the differences of DOM composition in the two climates."

- Concerning the further interpretation of climate effects:

We do not want to make final suggestions for the whole climate region or on the acting mechanisms. In fact, we write in the conclusion of the submitted manuscript: "Distinct effects of climate on fluvial DOM have been found in this study and support earlier findings that climate is the main driver of DOM export from catchments. However, never before this has been tested for the molecular composition of DOM. We found strong effect between the catchments in the two investigated climate zones but cannot clearly attribute this to one climate or soil factor. Further studies of the DOM sources in the catchments are needed to get a clearer picture why these differences between different climate regions are found." However, we will carefully check the discussion section for any implication that would suggest that we want to make final conclusions for the whole climate region.

- Concerning the direct measurement of DON with SEC:

An in-depth comparison of direct (SEC) and indirect DON measurements (by subtraction of DIN from TDN) is available in Graeber et al., 2012a, (Biogeosciences,9,4873-4884). This study is cited several times within the submitted manuscript and one intention to publish the Biogeosciences study was actually to make further laborious comparisons between the direct and indirect measurements in studies with applied SEC for DON measurements unnecessary. The advantages of the direct measurement of DON by SEC are mentioned repeatedly in the submitted manuscript: In the introduction to introduce SEC as a better alternative to the indirect method; in the methods, where it is described in detail and it is even mentioned that "the direct measurement of DON with high accuracy was demonstrated in freshwater systems for this SEC system (Graeber et al., 2012a)". Moreover, its mentioned in the discussion, where we discuss the outcome on different measurement types for DON concentrations in the comparison of different literature sources. Finally, in the supplement a typical chromatogram of the SEC is shown to explain its mechanism (Figure B1, this is not cited yet in the methods section, but a citation will be included in the revision of the manuscript, see change-2 in the revised version of the manuscript). In the explanation of the appendix, we also will cite the Biogeosciences study (see change-2a in the revised version of the manuscript). Thus, the advantages of the direct measurement over

the indirect measurement of DON are extensively discussed in the submitted manuscript. We believe that further inclusion of methodological data and further methodological discussion is out of the scope of this monitoring study and strongly recommend reading the Biogeosciences publication instead.

5.2 Specific comments

5.2.1 Title

Title: The statistical tests focused on main effects. I am not certain that "interaction" properly describes the study design. When the interaction was tested, the evidence for an interaction was significant but weak in magnitude.

Reply: The study design was made to study interaction effects, since we tried to find similar catchments in different climates. We therefore think that it is justified to write "Interacting".

5.2.2 Introduction

p137.L3-8 - I found this description of DOC and DON more complex than perhaps it needs to be. Consider simplifying that statement to say that DOM contains N and C and among other elements and then conclude as written with the ecosystem implications for DON and DOC

Reply: Text was changed accordingly (change-13).

I think the reader needs more information regarding farming practices in the introduction. It was not immediately clear to me what the difference between intensive and extensive farming practices are. I also wonder how representative pasture lands are when being used as the lone extensive farming practice. These differences might only be related to terminology, but I feel some clarification is needed for the reader to understand your studys framework. I think adding this information will help the read understand the fourth hypothesis (p139.L20).

Reply: We added a sentence, in which we state the difference between intensive and extensive farming as we understand it (change-14). Moreover, we now mention another publication in which further details on the catchments are stated (change-14).

5.2.3 Methods

How do SEC DOC and DON concentrations compare to DOC measured by a TOC analyzer and DON as TDN - DIN? I think some information on this will help assure the reader that your values are

comparable to those of other studies. If this work has been completed elsewhere than perhaps included a statement that tells the reader where they can find this information would be useful. Moreover, the study is introduced suggesting that SEC based measures of DON, especially, might provide a better estimate of DON than the common subtraction based approach. I am curious how DON in your study would differ if you used subtraction as opposed to SEC. Hence, I felt the paper never revealed if the insights and results gained from SEC-DON were more insightful than the subtraction method. If these ideas are covered elsewhere than less focus on these methods in the introduction might allow you to focus more on agricultural practices and climate. If these comparisons are novel then I think they should be included in the results and discussed.

Reply: A study on this has been done and was cited in the submitted manuscript. Please see the reply to the general comments for details.

In general, I found the statistical description clear and understandable with the correct level of detail needed for a reader to run these tests using their own data. I was curious how the difference in variance rather than normality of each variable influenced the PCA and MANOVA. I general scale (center = T, scale = T) after normalizing the data. This sets the data range to similar units between variables, which I find helps the multivariate data fit better with reduced dimensions. Variables with large ranges can at times disproportionately influence the multivariate analysis over variables with relatively small ranges like the freshness index and FI. I would suggest re-running the PCA and MANOVA using scaled data. If the non-scaled and scaled data are similar then the report analysis are good and you might consider noting that this did not influence the data ordination. If the results differ greatly between scaled and non-scaled, I recommend using the scaled data.

Reply: The data was already scaled in the submitted manuscript, but we forgot to describe it in the methods. This is now included in the revised manuscript. Please see change-16 and change-17 in the manuscript text (section on statistical analyses), as well as the reply to referee 1 for further details.

5.2.4 Results

Table 1 - I am concerned that the streams understudy do not match fully the study design (County*Land Use). The UY watersheds fit fairly well into agricultural groups but the DK watersheds are a forest with farming vs arable farming. This might explain why DK-Extensive was markedly different based on DOC and DON loads. I think these land use distinctions and possible other underlying differences like soil type should be more clearly stated in the methods

and discussed more fully. **In other words, I would like more discussion geared to convince the reader that your observations are due to climate and farming practice differences rather than differences in background nutrient levels, hydrology, geology, watershed slope, and the contribution of other land uses.**

Reply: Please see the response to your general comments for an explanation (paragraphs "Concerning the land use" and "Concerning the assessment of climate effects").

Table 3 & Figure A1 - I find it surprising that your PARAFAC model does not contain a protein-like peak. Typically, 3 or 4 component models have UV humic-like peak, Vis humic-like peak, and protein-like peak. Upon visual inspection of Figure A1, it looks like the excitation spectra is pretty broad for each component. This might suggest that component number, though reproducible, is not correct for your data. Perhaps adding a few residual and corrected EEMs would be useful for the reader to better understand the PARAFAC output. I am curious if the model systematically misses the protein like peak, which would be evident in the residual EEMs or to see that these samples dont have a protein-like peak. I think some discussion might be needed in order to interpret the model for the reader.

Reply: We agree that it is quite unusual not to have protein-like PARAFAC components but we also could not find any when we used more components. Moreover, the model was generated based on the standards described in Murphy et al. 2013 (see manuscript text, section on the "Treatment of spectroscopic and chromatographic data"). Thus, the models were thoroughly checked for systematic residuals, unusual spectra shapes, split-half validity and maximum explanatory power. As we have a large number of samples and since we followed the best-possible practice to generate the PARAFAC model, we do not see the necessity to show modelled and residual EEMs.

For PC3 - If the three spectral slope indicators are interpreted the same with respect to size why do they show up as opposites? This would suggest that both directions are small and large sized DOM. It wasnt clear to me how this pattern would relate to light exposure differences between watersheds. Perhaps some clarification is need because the smaller sized and lower C:N patterns seem important based on the manuscripts conclusions.

Reply: The referee is right, that it looks weird that all three are supposed to be negatively related to molecular weight (according to Helms et al 2008, see manuscript for reference), although they appear as opposites in the PCA. The best explanation is that molecular weight is not affecting these three indicators in our study, but that it is microbial availability or previous irradiation, which are also related to these indicators (Helms et al 2008. In fact, $S_{275-295}$ and S_R are positively related to irradiation and decrease during incubation experiments and $S_{350-400}$ is negatively related to irradiation and increases during incubation

experiments. Since the spectral indicators are related to country, we postulated in the discussion of the submitted manuscript that the DOM from the Danish catchments is not yet microbially processed (high potential microbial availability relative to Uruguayan catchments, see first paragraph of "Effects of climate on fluvial DOM composition section" in the discussion).

p150.L1-7 & Figure 4 - For individual DOM assessments, I found it unclear why only 6 of the 20 indicates were displayed and presented. Perhaps adding a little more detail regarding why only these variables were selected over others might be useful. Do these factors highlight different components of the PCA? They seem to highlight some of the PCA axes but not all.

Reply: As we described in the methods (section on "Statistical analyses") and the figure caption of the PCA (Fig. 3), we only chose to show the variables with can be interpreted with high confidence according to a rule set by Borchard et al 2011 (see manuscript for reference). This was done to simplify the plot and allow easier interpretation.

5.2.5 Discussion

I am concerned that the data set might be too focus to resolve strong climate and land use patterns. The N for the study is 4 watersheds (two climate zones and two land use categories). The catchments could just be different and influenced by the observed precipitation patterns rather than broad climatic differences. Differences in climate between Denmark and Uruguay are much greater than the observed differences in DOM quality and quantity. I am uncertain that broad climate generalities in DOM with land use can be drawn from such a small set of watersheds. I agree that the data supports the idea that land use, especially intensive agriculture, has strong affects on DOM and these are seen in two watersheds from countries with very different climate. I am not certain if these results can be generalized. I dont wish to discount the findings but I dont think the evidence was as strong as presented for the hypothesis. There was some evidence that DOM shared similarities between intensive farm systems but the DOM of these watersheds also shared many similarities overall. My interpretation would be that the study found some evidence, rather than strong evidence, in support of the hypothesis that raises important questions and ideas.

Reply: Please refer to the discussion of the general comments for a detailed reply.

p151.L7 & 8 - I did not understand how Denmark could have a higher water buffering capacity if it is extensively tile drained. Wouldnt this be swamped out by the faster movement of water sug-

gested in the next paragraph?

Reply: The intensive agricultural catchment in Denmark was tile drained (not both catchments), which reduces the water buffer capacity, but it still seems to be higher than for the Uruguayan catchments. This pattern is clearly visible from Fig. 1, where the precipitation was less different between Denmark and Uruguay than the discharges. Moreover, in Fig. 1b, the difference of the discharge between the intensive and extensive agriculture catchment is stronger for Denmark than for Uruguay, which is best explained by the tile drainage of the intensive agriculture catchment in Denmark.

p152.L14-19 - Could this be due to the fact that this catchment was mostly forested and not as human impacted? This might allow the system to have more stability in discharge and DON/DOC inputs might be controlled by seasonal cycles in litter production and groundwater vs surface water contributions to stream flow.

Reply: Interesting thought! However, the fact of being forested cannot explain the apparent stronger microbial influence on DOM composition in the Danish catchments. Moreover, this is in contrast to what we expected and cannot be explained by "...seasonal cycles in litter production and groundwater vs surface water contributions to stream flow" as the referee suggests, since this was also the case for the intensive agriculture catchment (which was not forested). We tried to give some explanations for these patterns but cannot be sure. Thus, we already wrote in the submitted manuscript at the end of this paragraph: "However, based only on in-stream measurements, we cannot infer the mechanisms behind the differences of DOM composition in the two climates."

p154.L9-11 - Given that DON-agriculture effect was so large, why wasnt this also evident through DOM optical measures? PARAFAC did not identify a protein-like peak, which would suggest there was little DON at least of that type. I feel discussion is needed to explain why these are consistent patterns.

Reply: That the protein-like peak is related to DON content is a common but wrong assumption. The N content of this peak is totally unknown. In fact, it is a peak of non-humified DOM whose spectrum is similar to tyrosine or tryptophan, a characterization which is not linked to C:N ratio or DON concentration. In fact, when you leach fresh leaf material, you will get similar peaks which often dominate the EEM (even when you measure the fluorescence of freshly cooked coffee or tea) and there is no reason why these substances should have a low C:N ratio or high DON content. My personal oppinion is that this peak should be re-named to avoid this kind of confusions (something like non-humified DOM). Moreover, in our study most DON ($¿$ 80%) was bound to higher-molecular humic-substance like DOM (please see Fig. 4). Finally, the low protein-like fluorescence of fluvial DOM in agricultural catchments is supported by other studies from the temperate region (Graeber et al. 2012 STOTEN, Heinz et al. 2015, EST). We mention that we find complex DOM

with low C:N ratios and discuss the potential reasons for that intensively in the manuscript. We believe that we sufficiently discuss the character of the DOM and DON and that we do not need a longer discussion.

p154.L19-26 - Are you able to check this statement by comparing the subtraction method to the SEC-DON method using your study. If you measured TDN and DIN, this type of discussion would help strengthen the argument for the novel approach used in the your manuscript.

Reply: We believe that we sufficiently prove the reliability of the direct DON measurement in the Biogeosciences study mentioned in the reply to the general comments. Please see this reply for further details.

p155.L5-10 - This could be true and is a likely mechanisms. How might the presence of a high percentage of forested land in the Denmark catchment influences these patterns?

Reply: Forest by itself does not result in a less variable discharge than any other vegetation form. In contrast, the tile drainage and removal of buffer zones can increase the variability of discharges, as we write in the manuscript text, to which the referee refers to.

5.2.6 TECHNICAL CORRECTIONS

p144.L15 - Do you mean "LMWS" rather than "HMWS"?

Reply: No, HMWS is meant.

p147.L7 - Consider simplifying this statement. I think similar works for all catchments

Reply: The sentence was simplified. Please see change-18 in the manuscript text.

p150.L1 - what is meant by "exemplary"? Consider clarifying this statement

Reply: We deleted "exemplary".

Figure 3 - I find the dots a little small on these figures. Perhaps changing the shape and color would make it easier to see light blue from blue dots.

Reply: In the finally produced pdf, the figure will be larger and better visible. Moreover, we would like to keep the color consistent between the plots. Therefore, we decided to keep the current design.

P150.L9 & 21 - Did you mean "support" rather than "prove"

We replaced "prove" by "support" in both cases (change-19 and change-20).

P152/L14 - Did you mean "in which" rather than "which in which"?
We replaced "which in which" by "in which" (change-21).

Figure 2 - I found it somewhat confusing that this figure shows concentrations paired with percentages for loads. Consider pairing load values with load % and concentration values with concentration %
Reply: We wanted to show the percentage of the loads, since the load per time is more informative than the absolute loads. In contrast, it is not logical to calculate concentration per time (x concentration was exported in y time), thus we stick to the absolute concentrations.

6 Sample sizes for the statistics

DK = Denmark, UY = Uruguay

6.1 Levene's tests

6.1.1 DOC and DON loads for each country and for land use within country

Statistic described in the third paragraph of the Statistical analyses section of the revised manuscript.

DK = 1414, UY = 1455

Catchment	Number of samples
Intensive UY	728
Extensive UY	727
Intensive DK	707
Extensive DK	707

The high sample numbers were the result of the interpolation of the DOC and DON loads for each day between the sampling occasions (as described in the Calculation of DOC and DON daily and annual loads section of the revised manuscript).

6.1.2 Precipitation

Effect of country or land-use type within country. Whole time period is included. Statistic described in the third paragraph of the Statistical analyses section of the revised manuscript.

DK = 1640, UY = 1991

Catchment	Number of samples
Intensive UY	996
Extensive UY	995
Intensive DK	820
Extensive DK	920

6.1.3 Discharge

Effect of country or land-use type within country. Whole time period is included. Statistic described in the third paragraph of the Statistical analyses section of the revised manuscript.

DK = 1554, UY = 1990

Catchment	Number of samples
Intensive UY	995
Extensive UY	995
Intensive DK	777
Extensive DK	777

6.2 Permutative one-way tests

6.2.1 Effect of country on DOC and DON concentrations

Statistic described in the second paragraph of the Statistical analyses section of the revised manuscript.

DK = 98 samples, UY = 95 samples

6.3 Nemenyi pairwise tests

6.3.1 Effect of the sampled catchment on DOC and DON concentrations

Statistic described in the second paragraph of the Statistical analyses section of the revised manuscript.

Catchment	Number of samples
Intensive UY	48
Extensive UY	47
Intensive DK	49
Extensive DK	49

6.3.2 Effect of the sampled catchment on carbon or nitrogen in humic substances, C:N ratio of humic substances, fluorescence index, PARAFAC component C1 and ratio of absorbance curve slopes (Sr)

Statistic shown in Figure 4 of the revised manuscript and described in the 6th paragraph of the Statistical analyses section of the revised manuscript (see Change-1 in the manuscript).

Catchment	Number of samples
Intensive UY	48
Extensive UY	47
Intensive DK	49
Extensive DK	49

6.4 Spearman rank correlations

6.4.1 Correlation between DOC or DON concentrations and discharge values

Statistic described in the second paragraph of the Statistical analyses section of the revised manuscript.

Catchment	Number of samples
Intensive UY	48
Extensive UY	46
Intensive DK	48
Extensive DK	48

The slightly lower number of samples is a result of the fact that not for all sampling dates discharge values were available.

Due to an error in the data preparation, less samples were included in the Spearman correlations of the submitted manuscript. This was corrected, and the Spearman rank correlations were done again with the sample numbers given above. No significant changes in the results occurred and the slightly changed rho and p values were included in the revised version of the manuscript (second paragraph in the DOC and DON concentrations and loads section of the results).

6.5 Sensitivity analysis of the load calculations

Statistic described in the third paragraph of the Statistical analyses section of the revised manuscript.

Catchment	Number of samples
Intensive UY	728
Extensive UY	727
Intensive DK	707
Extensive DK	707

The high sample numbers were the result of the interpolation of the DOC and DON loads for each day between the sampling occasions (as described in lines 209-211 of the submitted manuscript).

6.6 Principal component analysis

Statistic described in the 4th paragraph of the Statistical analyses section of the revised manuscript.

For the PCA, 193 samples and 20 variables were used, resulting in a sample to variable ratio of 9.65.

6.7 Permutative multivariate analysis of variance, permutative multivariate dispersal tests

Statistic described in the 5th paragraph of the Statistical analyses section of the revised manuscript. Same number of samples as in section 6.3.2

4 Second revision

Second revision: Author's response to editor comments

May 5, 2015

1   Description of procedure

All changes in result of the editor comments are marked in the manuscript in red and the sections are referred to in the response. All editor comments are marked in bold and the replies always start with "Reply:".

2   Editor comments

1. Before accepting the paper I like to see a justification of the calculated loads. In 2.4 it is stated: 'DOC and DON concentrations were linearly interpolated between sampling occasions' (line 220 in the revised manuscript). This seems illogical because the concentration data don't show autocorrelation (lines 230-232). If concentrations are really uncorrelated, it might be better to use the mean value. However, I guess that although you didn't find autocorrelation in the data (fortnightly), autocorrelation does exists at a smaller time scale. At a small time scale the concentrations is expected to be continuous. This might justify the interpolation. If concentrations and the discharge are correlated (lines 309 - 310) the interpolation might induce bias.

Reply: We thank the editor for his detailed comments but have to partly disagree concerning his arguments on use of interpolation for calculating loads for non-autocorrelated DOC and DON concentrations. The missing auto-correlation revealed that the DOC or DON concentration for one sampling occasion is not correlated to the value at the sampling occasion before but, nonetheless, it must still be assumed that the concentration has changed over time to reach its value at the second sampling occasion. The assumption we make here is that this change was linear. However, when using means, the bias would likely be higher, since we would have to assume a constant concentration for a certain period of time (usually half of the period between two sampling occasions) with an abrupt change to the new value.

We agree with the editor on the higher likelihood of auto-correlation at shorter time steps. But due to lacking data we cannot test, if the auto-correlation would exist at shorter time steps, but it is much more likely than for a fornightly sampling scheme.

Concerning the correlation of concentration to discharge: Any kind of interpolation will introduce bias, since the to-be interpolated values are unknown. We were and are aware of that and thus only discussed strong patterns/ differences of DOC and DON loads between the sites and are not discussing weak trends. Moreover, to point the potential bias of the interpolation out to the readers, we now include the following statement in the manuscript (Methods, Calculation of DOC and DON daily and annual loads): "This (..the interpolation..., author comment) approach was compared to other potential approaches in Kauppila and Koskiaho (2003) and was found to provide the most reliable estimates of nutrient loads from discontinuous concentration data. However, since considerable bias of the interpolated data is possible and cannot be ruled out, we only discuss strong patterns related to the calculated DOC and DON loads."

2. Technical correction: Maj should be May (line 122)

Reply: We corrected this word (Methods, Field sampling and laboratory measurements).