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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 molecular composition. 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 assessed DOM composition with absorbance and fluorescence measurements, as well as parallel factor analysis (PARAFAC). We also calculated DOC and DON loads based on daily discharge measurements, as well as measured precipitation and air temperature. In the catchments in Uruguay, the fluvial DOM was characterized by higher temporal variability of DOC and DON loads which were clearly related to a higher temporal variability of precipitation and a DOM composition with rather plant-like character relative to the Danish catchments. Moreover, we consistently found a higher temporal variability of DOC an DON loads in the intensive farming catchments than in the extensive farming catchments, with the highest temporal variability in the Uruguayan intensive farming catchment. Moreover, the composition of DOM exported from the intensive farming catchments was always complex and related to microbial processing in both Denmark and Uruguay. This was indicated by low C : N ratios, several spectroscopic DOM composition indexes and the PARAFAC fluorescence components. 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 agriculture, 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). 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; Prairie, 2008). Changes in DOC and DON concentrations and loads may affect ecosystem functions of freshwater ecosystems (Stanley et al., 2012; van Kessel et al., 2009).

Climate, soil and topography variables are usually strong predictors of DOM in streams, as these often control the terrestrial storage of organic matter and the hydrologic connection between catchments and streams (Stanley et al., 2012). 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 in the catchments, which has a range of consequences, including plant cover, catchment hydrology, soil character and nutrient export (Stanley et al., 2012). Recent studies in northern temperate climate have found that the intensity of agriculture strongly affects 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 the 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 anthro-

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

Similar 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. However, all studies 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). 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 concentrations. 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 representative, detailed understanding of the composition of DOM (e.g. Fellman et al., 2010; Helms et al., 2008), especially when combined with 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, high humification, low contribution of protein-like fluorophores, and is likely released from microbial sources (Wilson and Xenopoulos, 2009; Williams et al., 2010; Graeber et al., 2012b; Fellman et al., 2011). 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). However, most of this information results from 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 methods, including spectroscopic measurements with other measurements of DOM composition (e.g. with SEC) will allow a more accurate interpretation of the spectroscopic measurements and a better understanding of DOM composition patterns (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. We used this technique to compare the quantity and variability of DOC and DON concentrations, loads and molecular composition for catchments with extensive and intensive farming in temperate (Denmark) and subtropical (Uruguay) climates. We hypothesized that (i) the higher and more variable precipitation in Uruguay will result in higher and more variable DOC and DON loads from catchments to streams, that (ii) the warmer climate in Uruguay will strongly affect DOM composition by higher microbial activities in soils and streams, that (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 composition is affected similarly 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 pastures (extensive farming) or arable farming (intensive farming, Table 1). The Danish intensive farming catchment was characterized by subsurface tile drainage.

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

2.2 Field sampling and laboratory measurements

Precipitation and air temperature were measured at the sample sites within the catchments in Uruguay on the site with instruments (Rain-o-matic precipitation sensor, Pronamic, Ringkøbing, Denmark, November 2009–September 2012) or were extracted from country-wide data from the Danish Meteorological Institute (DMI) in Denmark (February 2010–May 2012). Both Danish catchments have the same temperature values, since these were in the same temperature grid of the DMI data. 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 for 2011, since only for this year simultaneous, continuous precipitation measurements exist for all four catchments.

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Water samples were collected on average every fortnight from 2 April 2010 to 14 March 2012 from the outflows of the two catchments in Denmark and from 2 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 ~ 2 with hydrochloric acid to stabilize the 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 and within the range of the final pH values, no effects of acidification on fluorescence measurements (Patel-Sorrentino et al., 2002) or SEC measurements (Huber et al., 2011) of DOM composition is expected. 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, residue DOM from acidification, neutralization and additional filtration was checked against filter and acidification blanks.

Absorbance was measured on a UV-2401 UV/Vis spectrophotometer (Shimadzu, Duisburg, Germany), using the same 1 cm quartz glass cuvettes to correspond with the fluorescence measurements, as well as with 5 cm quartz glass cuvettes for calculation of absorbance-based indexes. Absorbance was measured between 190–800 nm. Be-

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fore 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 $nm\,s^{-1}$, using a LS-50B fluorescence spectrometer (Perkin-Elmer, Rodgau, Germany). Samples exhibiting an absorbance > 0.3 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 unnecessary (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 SEC system (see Supplement for a plot of a typical chromatogram from SEC, Graeber et al., 2012a). SEC 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 = HS_C, nitrogen = HS_N) with higher aromaticity based on UV measurements at 254 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, 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

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(Graeber et al., 2012a). The quantification limit of SEC for DOC and DON in each fraction was 0.01 $mg\,L^{-1}$ and values below the quantification limit were set to 0.005 $mg\,L^{-1}$. Specific UV absorbance at 254 nm was determined for the HS fraction ($SUVA_{HS}$) and for all DOM fractions ($SUVA_{bulk}$) as $L\,mg^{-1}\,m^{-1}$. 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 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 nm and according to the processing used in the drEEM toolbox. The resulting 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 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 decreases 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. This approach was compared to other potential approaches in Kauppila and Koskiaho (2003) and was found to provide most reliable estimates of nutrient loads from discontinuous concentration data. 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, 2013). All following statistics assume independent temporal replicates, as neither of the DOC and DON concentrations or DOM composition variables were temporally autocorrelated in any of the catchments (acf function R Core Team, 2013). 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 the DOC concentrations were correlated to the discharge values, Spearman rank correlations were used for each of the catchments separately (cor.test function, R Core Team, 2013).

To assess changes in the 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 rho and here, a high Spearman's rho 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 rho indicates a low sensitivity.

To investigate the changes of DOM composition with country and type of farming and to assess 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, $HMWS_C$, $HMWS_N$ 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).

Based on the same variables as for PCA, the effects of country and type of farming on DOM composition were tested using multivariate statistics: To assess if differences in DOM composition across the catchments were significant, permutative multivariate analyses of variance (PERMANOVA) were used (adonis function, vegan package, Oksanen et al., 2013) and to assess changes 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 statistics.

3 Results

3.1 Climate and discharge

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.

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A clear difference in the temporal variability of the precipitation was observed between countries ($p < 0.001$, Levene's test), 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. 1a). As can be seen from these numbers and the plot (Fig. 1a), the precipitation pattern was similar for the catchments in Uruguay and not even distinguishable from each other for the two catchments in Denmark ($p > 0.53$ for both Denmark and Uruguay, Levene's test).

A larger difference between catchments within countries was observed for the discharge, 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, Fig. 1b). In detail, 80 % of the discharge occurred in 9 and 20 % of the sampled period in the intensive farming and extensive farming catchment in Uruguay, respectively (Fig. 1b). Moreover, 80 % of the discharge occurred in 43 and 73 % of the sampled period in the intensive farming and extensive farming catchment in Denmark, respectively (Fig. 1b). The observed higher temporal variability of the discharge in intensive farming than in extensive farming was highly significant for both Denmark and Uruguay ($p < 0.001$, Levene's test).

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 (±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 (±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 (±SD = 6.1 °C, min = 2.4 °C, max = 30.5 °C).

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, Figure 2). Intensive farming resulted in higher DON con-

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centrations than extensive farming in both countries (Fig. 2a and b), while the effect of intensive farming on DOC was only significant in Denmark (Fig. 2a).

The concentrations of DOC and DON were always positively correlated to discharge in Denmark (Spearman rank correlation, Spearman's rho > 0.65, $p < 0.001$), but never in Uruguay (Spearman's rho < 0.17, $p > 0.05$).

Loads of DOC and DON in the catchments with intensive farming catchments were more temporally variable during the sampling period than in the catchments with extensive farming and were more variable in Uruguay than in Denmark (Fig. 2c and 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 sampling period (Fig. 2c and d). In contrast, 80 % of the total DOC and DON load in the Danish extensive farming catchment was exported during 60 % of the sampling period (Fig. 2c and d).

Country had a significant effect on the temporal variability of DOC and DON loads ($p < 0.001$, Levene's test). Moreover, in Denmark, the land use type also had a significant effect on the temporal variability of DOC and DON loads ($p < 0.001$), while in Uruguay land use type only affected the temporal variability of DOC loads ($p = 0.022$) but not DON loads ($p = 0.094$).

Loads of DOC and DON were highly sensitive to changes in discharge (Spearman's rho > 0.92) in all but the extensive farming catchment in Denmark (Spearman's rho = 0.53). In contrast, the sensitivity of DOC and DON loads to changes in either DOC or DON concentration were low (Spearman's rho < 0.31), again except from the Danish extensive farming catchment (DOC: Spearman's rho = 0.74, DON: Spearman's rho = 0.80).

The annual DOC and DON load for 2011 was comparable between the study catchments within the same order of magnitude and no effect of land use or country could be observed (Table 2). The highest annual DOC load was found in the Danish extensive farming catchment, whereas the highest annual DON load was found in the Uruguayan intensive farming catchment (Table 2). The median daily loads of DOC and DOC ex-

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hibited a different pattern than 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) and land use ($R^2 = 0.13$, $p < 0.001$) had a significant effect on DOM composition and a significant interaction effect between country ($R^2 = 0.03$, $p < 0.001$) and land use was found. Furthermore, the effects of land use type were significant within each country ($p < 0.001$, $R^2_{\text{Denmark}} = 0.24$, $R^2_{\text{Uruguay}} = 0.14$, PERMANOVA).

Country had a significant effect on the temporal variability of the DOM composition ($p < 0.001$, PERMDISP). The land use 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. Together, these axes explained 73 % of the total variance. The first and third PCA axes were positively correlated to the scores of the Danish catchments and negatively to the scores of the Uruguayan catchments (Fig. 3a and b). The second PCA axis separated land use types and was positively correlated to scores of the two catchments with with extensive farming (Fig. 3a). The fourth PCA axis was neither correlated to country or land use type (3b.). The first PCA axis was positively correlated $E_2 : E_3$, $C2$ and freshness index and negatively correlated to $C3$, $\text{SUVA}_{\text{bulk}}$ and SUVA_{HS} (Fig. 3a). The second PCA axis was positively correlated to C:N_{HS}, C:N_{bulk}, $C1$ and negatively correlated to HMWS_{C} and freshness index (Fig. 3a). The third PCA axis was positively correlated to $S_{350–400}$ and negatively correlated to $S_{275–295}$ and S_R (Fig. 3b). The fourth PCA axis was positively correlated to HS_{C} and HS_{N} and negatively correlated to HMWS_{N} and HMWS_{C} (Fig. 3b).

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To get a better understanding of the changes in DOM composition, we exemplary investigated the absolute values of some DOM composition variables (Fig. 4). In all four catchments, DOM consisted mainly of humic substances and no clear significant effect of country or farming type was found here (Fig. 4a and b). Contrarily, intensive farming resulted in lower $C:N_{bulk}$, a higher fluorescence index and a lower $C1$ in both Denmark and Urgugay (Fig. 4c and d). Moreover, S_R was significantly lower in Denmark than in Uruguay (Fig. 4e).

4 Discussion

In this study, we could 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 prove our first hypothesis, since the DOC and DON loads were more temporally variable in Uruguay than in Denmark. However, the annual as well as the median daily loads were not higher in Uruguay than in Denmark. Moreover, our second hypothesis of a strong effect of climate on DOM composition could be confirmed. However, in opposition to the idea of higher microbial processing in Uruguay, several DOM composition indices pointed to a rather plant-derived DOM in Uruguay and a rather microbial-derived DOM in Denmark. Concerning our third hypothesis, only DON but not DOC concentrations were generally higher in the two intensive farming catchments. In contrast, we found a higher temporal variability of DOC and DON loads in the intensive farming catchments. Thus, our third hypothesis could only partly be supported by the data. Finally, we could clearly prove our fourth hypothesis that DOM composition is 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) were the source of these changes.

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4.1 Differences of climate and discharge between the catchments

Distinct climate 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 the transport of water through shallow groundwater pathways or even overland flow. In contrast, the Danish catchments had a higher buffer capacity for water from precipitation and the water likely followed deeper groundwater flowpaths through the catchment to the stream.

Within the two countries, the temporal variability of discharges was much more different between intensive and extensive farming than could be expected solely based on the precipitation patterns. Here, the difference in Denmark was more extreme 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 the tile drainage of the Danish intensive farming catchment. This resulted in a hydrological shortcut and a much faster and stronger reaction of the 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 stronger reaction of the discharge to those events.

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4.2 Effects of climate on fluvial DOM quantity

The positive relationship between DOC and DON concentrations and discharge values was likely the main reason for the high loads in Denmark relative to the catchments in Uruguay. Due to that, DOC and DON concentrations were higher at higher discharges and were further increasing the DOC and DON loads. In contrast, the DOC and DON concentrations were independent from discharge in Uruguay and thus were not increasing the loads.

The temporal variability of the DOC and DON loads was high in Uruguay due to high variability of discharges, as we found with a sensitivity analysis of the load calculation. 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 which in which the temporal variability of the loads was not 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 increased the temporal variability of DOC and DON loads.

4.3 Effects of climate on fluvial DOM composition

In accordance to our second hypothesis, we could show that climate has 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 likely derived from plant sources and not as microbially processed as in Denmark. This implies a lower 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, allowing 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 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, global studies of the catchment sources would greatly advance the understanding of the mechanisms behind DOM export from catchments in different climates.

4.4 Effects of farming intensity on fluvial DOM quantity

The partly missing effect of intensive arable farming on DOC concentrations fits to the idea 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 opposes the notion of a general effect of intensive agriculture on DOC concentrations for other regions than the northern temperate climate zone (Graeber et al., 2012b).

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 whole-catchment studies on DON concentrations in streams (Willett et al., 2004; Stanley and Maxted, 2008). The disparity of results from soil and whole-catchment studies on the effects of agriculture on DON concentrations may be a result of DON measurement problems, which were 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). We propose that the differences in results between soil and stream studies is an artefact of the indirect determination of DON. The novel direct measurement techniques used in this study (Graeber et al., 2012a) or new treatments to remove nitrate and ammonium before the indirect measurement of DON (Lee and Westerhoff, 2005; Vandenbruwane et al., 2007; Chon et al., 2013; Graeber et al., 2012c) should be used in more 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

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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 likely also affected 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 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 midwestern USA (Dalzell et al., 2007; Royer and David, 2005). However, this effect was never before shown for DON loads. 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 composition

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. 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 sources in deeper soil layers.

The other variables of DOM composition also support the idea of microbially produced DOM: in the PCA, the intensive farming catchments and $HMWS_C$ were positively correlated which indicates rather microbial sources, since HMWS were found to be released by extracellular polymeric substances of biofilms (Stewart et al., 2013). Fluorescence index and freshness index were also higher in the intensive farming catchments, indicating a relatively recent, rather 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 rather 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) 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 climate zones.

Our results strongly imply microbial processing in deeper soil layers as being the source for the DOM in intensive farming. Several typical parts of agricultural management may either solely or in interaction be responsible for this pattern. Soil tillage destroys soil organic matter (SOM) aggregates and can result in strong microbial processing of the SOM within such aggregates (Ewing et al., 2006). This should also 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 US American agricultural catchments (Sickman et al., 2010). Furthermore nitrogen and phosphorus fertilizer addition to soils in intensive farming may be the source of higher microbial activities and with that result in higher release of DOM from SOM. However, extrapolations of the effects of intensive farming on DOM composition in other intensive farming catchments, as well as implications for responsible mechanisms remain speculative and need to be tested by additional studies.

5 Conclusions

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.

We could prove 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 may act in the two investigated regions. Moreover, intensive

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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 fit to recent findings from other studies in temperate climate and imply general mechanisms, by which intensive farming shapes the composition of DOM in streams. Based 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 globally affect DOM in aquatic ecosystems, as well as linked ecosystem processes and biogeochemical cycles.

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 spectroscopic DOM composition. Future DOM monitoring programs need to include similar techniques, if the effects human activities on DOM should 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 Fig. 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.

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Table 1. Name, position (WGS 84) and land use of the investigated catchments. DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.

Catchment	Coordinates	Catchment size (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 (∼ 70); arable farming (∼ 30)
UY, intensive	33°54′13″ S 56°00′23″ W	8.4	Arable farming and dairy farms (90); extensive pasture (7); urban (3)

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Table 2. Annual 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)

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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, ubiquitous 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 and 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)

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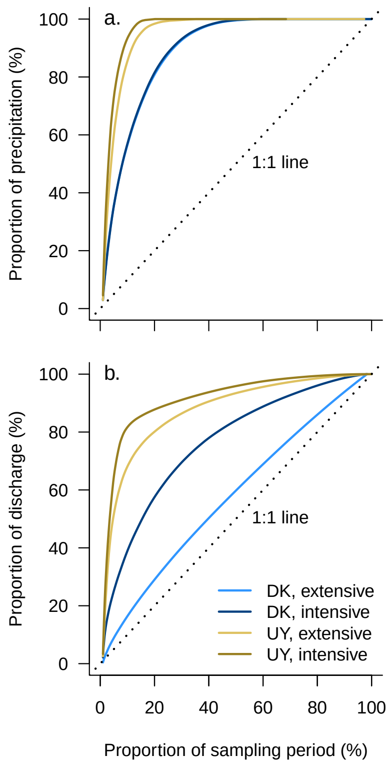

Figure 1. Ranked precipitation **(a)** and discharge values **(b)** vs. the proportion of the sampling period. Precipitation data for both the intensive and extensive catchment in Denmark is included but very similar **(a)**. The 1 : 1 line represents a completely equal precipitation **(a)** or discharge **(b)** across the whole sampling period. Plot style adapted from Dalzell et al. (2007).

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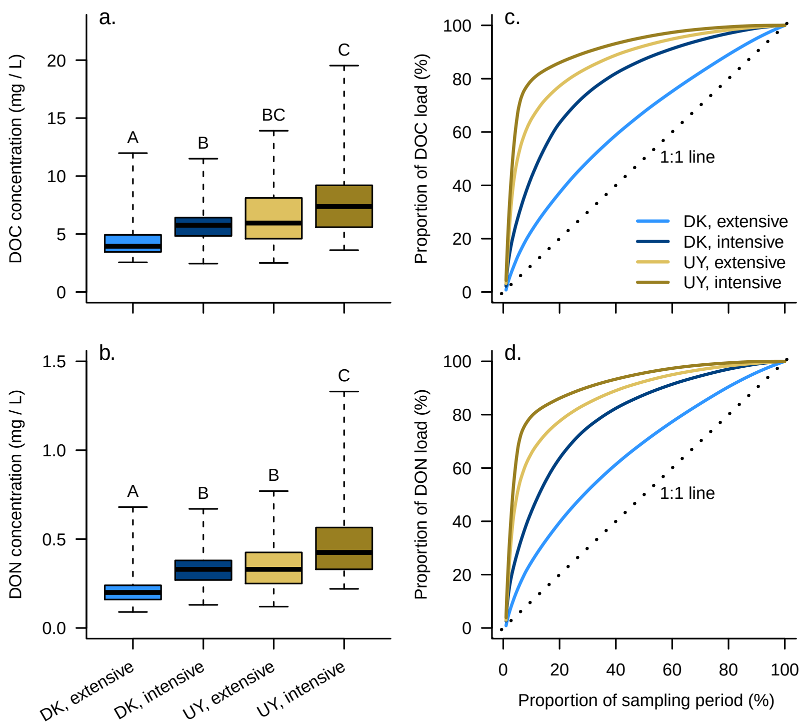

Figure 2. Concentrations (panels **(a)** and **(b)**) and ranked daily load vs. the proportion of the sampling period (panels **(c)** and **(d)**, plot style adapted from Dalzell et al., 2007) for dissolved organic carbon (DOC) and dissolved organic nitrogen (DON). The 1 : 1 line in the panels **(c)** and **(d)** represents an equal DOC and DON load across the whole sampling period. Capital letters indicate significantly different groups ($p < 0.05$, Nemenyi pairwise test). DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.

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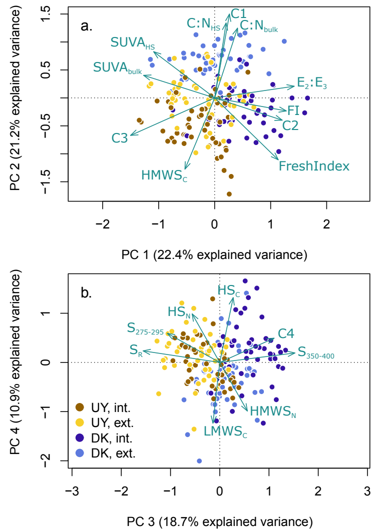

Figure 3. Principal component analysis (PCA) of dissolved organic matter (DOM) composition. The first four axes (PCA axis 1 and 2: panel **(a)**, PCA axis 3 and 4: panel **(b)**) of the PCA explain 73 % of the variance. Only those DOM composition variables are shown, which can be interpreted with high 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; $S_{275–295}$, $S_{350–400}$ and S_R: slope of absorbance at 275–295 nm, 350–400 nm and the ratio (R) of these two slopes; $SUVA_{HS}$ and $SUVA_{bulk}$: absorbance at 254 nm, normalised by dissolved organic carbon concentration, for humic substances (HS) and all DOM fractions, respectively; $C:N_{HS}$ and $C:N_{bulk}$: molar carbon to nitrogen ratio for HS and all DOM fractions, respectively; HS_C and HS_N, $HMWS_C$ and $HMWS_N$ 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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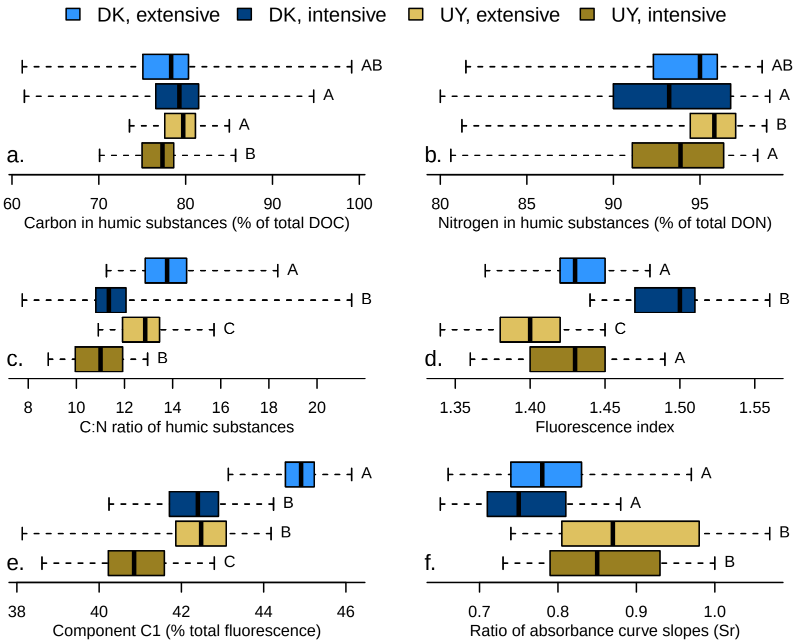

Figure 4. Selected variables of dissolved organic matter composition (DOM). Capital letters indicate significantly different groups ($p < 0.05$, Nemenyi pairwise test). DK = Denmark, UY = Uruguay, extensive = extensive farming, intensive = intensive farming.

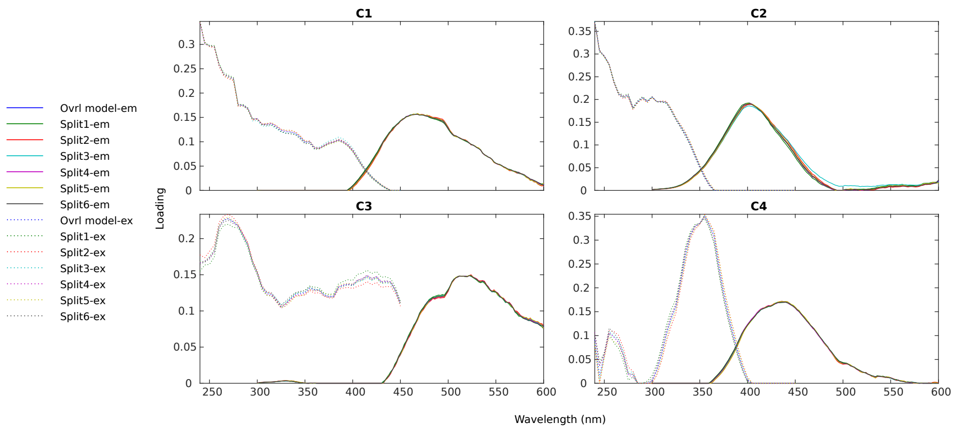

Figure A1. Split-half validation of the PARAFAC model. The models for six halfs generated by the standard method described in Murphy et al. (2013) are shown. When the fits of the splits are similar to each other and the entire model, a high stability of the model and low randomness of the fluorophores is given.

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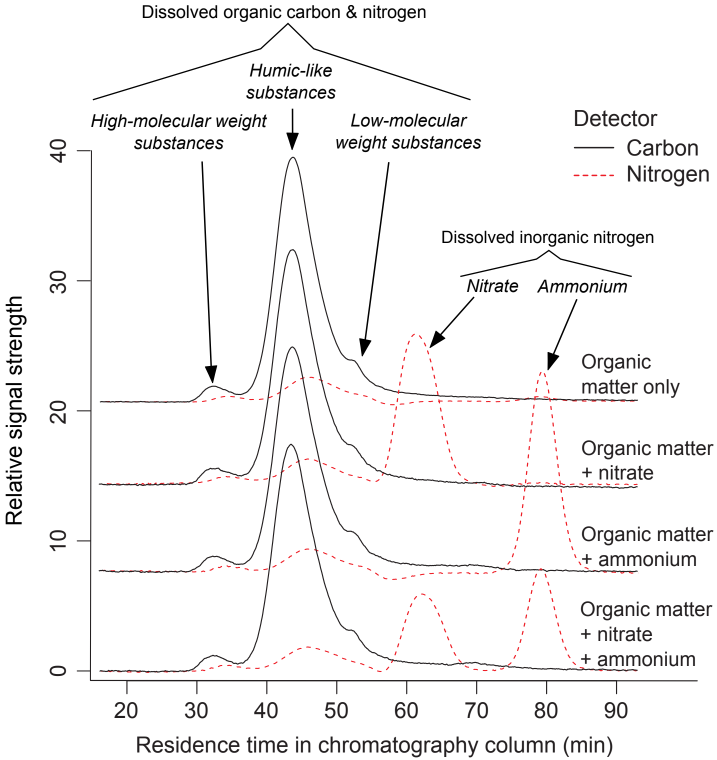

Figure B1. Typical chromatogram of size-exclusion chromatography to show the distribution of DOM fractions with and without added nitrate and ammonium. The sample for this chromatogram was taken at a wetland outflow in Brandenburg, Germany.