Going with the flow: planktonic processing of dissolved organic carbon in streams

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Abstract

A large part of the organic carbon in streams is transported by pulses of terrestrial dissolved organic carbon (tDOC) during hydrological events, which is more pronounced in agricultural catchments due to their hydrological flashiness. The majority of the literature considers stationary benthic biofilms and hyporheic biofilms to dominate uptake and processing of tDOC. Here, we argue for expanding this viewpoint to planktonic bacteria, which are transported downstream together with tDOC pulses, and thus perceive them as a less variable resource relative to stationary benthic bacteria. We show that pulse DOC can contribute significantly to the annual DOC export of streams and that planktonic bacteria take up considerable labile tDOC from such pulses in a short time frame, with the DOC uptake being as high as that of benthic biofilm bacteria. Furthermore, we show that planktonic bacteria efficiently take up labile tDOC which strongly increases planktonic bacterial production and abundance. We found that the response of planktonic bacteria to tDOC pulses was stronger in smaller streams than in larger streams, which may be related to bacterial metacommunity dynamics. Furthermore, the response of planktonic bacterial abundance was influenced by soluble reactive phosphorus concentration, pointing to phosphorus limitation. Our data suggest that planktonic bacteria can efficiently utilize tDOC pulses and likely determine tDOC fate during downstream transport, influencing aquatic food webs and related biochemical cycles.

Keywords: terrestrial DOC; agricultural catchment; flood pulse; hydrology; bacteria

Highlights

- Dissolved organic carbon (DOC) largely transported in pulses
- Limited access of benthic and hyporheic bacteria to DOC pulses
- Long access time and strong reaction of planktonic bacteria to DOC pulses
- Strong response of planktonic bacteria
- Planktonic DOC uptake in lowland streams likely underestimated
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1. Introduction

Pulses of terrestrial dissolved organic carbon (tDOC) are responsible for a significant contribution to the DOC budget of fluvial networks, and these pulses often contain DOC of higher concentration and different molecular composition relative to DOC at base flow (Raymond et al., 2016 and references therein). Terrestrial DOC is processed during the transport in freshwaters and exported as greenhouse gases to the atmosphere or deposited as particulate organic carbon in aquatic sediments (Battin et al., 2008, 2009; Raymond and Spencer, 2015; Ward et al., 2013, 2017). Agriculture substantially increases the fraction of tDOC transported in pulses to receiving waters (Dalzell et al., 2007; Graeber et al., 2012b, 2015; Heinz et al., 2015).

Microorganisms are likely responsible for a large fraction of tDOC removal from hydrologic pulses (Raymond et al., 2016). Such pulses are perceived differently by stationary bacteria of the benthic and hyporheic zone than by free-living, planktonic bacteria subjected to concomitant downstream transport (Fig. 1). Bacteria in the benthic and hyporheic zone will perceive a tDOC pulse as such, as they can access the pulse only during the limited time it passes by. Conversely, planktonic bacteria in the stream water column will be transported downstream with a DOC pulse and should perceive the pulse as a rather constant DOC source, slowly diminishing downstream in concentration and quality. The longer availability of the pulsed DOC will give the planktonic bacteria more time to metabolize it. Furthermore, biogeochemical processes in the hyporheic zone of small streams can substantially influence the DOC load, but these processes become hydrologically constrained at high discharges (Boano et al., 2014; Wondzell, 2011).

The recently developed pulse-shunt concept predicts that processing and biological retention of tDOC will be shifted from small streams to higher-order streams and rivers, as large pulses of DOC may bypass retention in small streams and should be mainly processed in larger rivers and coastal systems (Raymond et al., 2016). In this concept, DOC retention is conceptualized according

65 to the nutrient spiraling concept, which assumes that DOC is mainly retained by stationary benthic
66 or hyporheic biofilms (Newbold et al., 1981). The authors follow the current paradigm that biofilms
67 within the stream benthic and hyporheic zone are considered to be the main contributors for DOC
68 processing (Battin et al., 2016; Wiegner et al., 2005) as these zones "extend the residence time of
69 organic carbon during downstream transport" (Battin et al., 2008).

70 In contrast to the assumed dominance of stationary biofilms in the processing of DOC, free-
71 living bacteria were recognized as well adapted to changing "feast and famine" conditions with a
72 fast response to "unannounced and irregular windfalls of food" (Koch, 1971). This case was made
73 for *Escherichia coli* within human intestines (Koch, 1971). Similar "feast and famine" conditions
74 exist for free-living planktonic bacteria in running waters due to the unstable, pulsed nature of
75 tDOC inputs from the terrestrial environment (Dalzell et al., 2007; Graeber et al., 2012b, 2015;
76 Heinz et al., 2015; Raymond et al., 2016).

77 Due to their adaptation to the irregular availability of tDOC, planktonic bacteria should be
78 able to respond quickly to pulsed inputs of tDOC. Considerable planktonic DOC uptake is
79 commonly observed in bioavailability experiments with bacteria in solution (Fellman et al., 2009;
80 Fischer et al., 2002; Qualls and Haines, 1992; Wickland et al., 2012; Wiegner and Seitzinger, 2004)
81 and high planktonic DOC uptake was observed in a flume experiment (Kamjunke et al., 2017).
82 However, it has not been experimentally investigated to date, how the response of planktonic
83 compares to that of benthic biofilm bacteria during short pulses of labile tDOC, and if this depends
84 on stream or catchment characteristics.

85 We hypothesize that temporally limited pulses of tDOC often dominate DOC export in
86 streams and that planktonic bacteria and benthic bacterial biofilms can be equally important for
87 processing of such tDOC pulses. We furthermore hypothesize that planktonic bacteria can compete
88 with benthic bacteria in the uptake of tDOC from pulses because they adapt their bacterial
89 abundance, bacterial production, and DOC uptake more rapidly to the new source than benthic

biofilm bacteria and have a longer contact time with the tDOC in a pulse (Fig. 1). To test the importance of DOC pulses for DOC export, we reanalyzed literature data. To assess the contribution of benthic and planktonic bacteria to short-term tDOC processing typical for DOC pulses, we combined a short-term laboratory experiment with an extrapolation to stream-stretch level. Within this laboratory experiment, we tested how fast the DOC uptake and bacterial production of planktonic bacteria and benthic biofilm bacteria adapt to a short-term tDOC pulse.

2. Methods

2.1 Sites and experiment procedures

We selected eight lowland streams with a gradient in catchment size (0.1 – 46.4 km²) and agricultural land use (0 – 92% arable land) in central Jutland in Denmark (latitude 55.9° – 56.3° N, longitude 9.3° – 9.9° E). Please see Table A1 in Appendix A for further details on the sites.

We sampled stream water from the middle of the water column in the main current and gravel from the benthic zone midstream of the eight streams on three dates in October 2015.

Senescent alder (*Alnus glutinosa* L.) leaves were collected from trees in the autumn before the experiment to produce leaf leachate. Then the leaves were dried in a greenhouse with frequent intermixing to improve evaporation. We produced the leaf leachate by incubation of 10 g DW alder leaves in 1600 mL deionized (DI) water for 24 h in the dark at 15°C directly before each of the three start dates of the experiment. Before combining the leaf leachate with stream water, we filtered the leaf leachate through pre-rinsed 0.2 µm membranes (Advantec, mixed-cellulose ester) to remove bacteria. The filters were rinsed with 1 L of DI water before filtration to remove residual DOC.

From each of the eight streams, we sampled 2 L of water and filtered 600 mL of this stream water through pre-rinsed 0.2 µm membranes to remove bacteria. We mixed 300 mL of unfiltered stream water and 300 mL of 0.2 µm filtered stream water with 200 mL of leaf leachate solution.

Subsequently, four different treatments were prepared and then incubated for three days: 10 mL gravel in 60 mL of bacteria-free water, i) either with the leaf-leachate solution as described before (n=5 per stream site) or ii) without leachate (control, n=3 per stream site) or unfiltered stream water (70 mL) without gravel, iii) either mixed with the leaf-leachate solution (n=5 per stream site) or iv) without leachate (control, n=3 per stream site). Treatments i) and ii) comprised the benthic zone treatments and treatments iii) and iv) comprised the water-column treatments. The number of technical replicates was lower in the control since we expected less variability than for the treatments with leaf leachate.

The target DOC concentration of the treatments with leaf leachate was 50 mg DOC L^{-1}. However, we got slightly deviating concentrations in the treatments due to different stream water background DOC concentration and because we produced a new leaf leachate for each of the three experiment starting days (50.5 ± 3.6 mg L^{-1}; mean ± 1SD). We considered this effect in the calculation of the effect of leaf leachate on DOC uptake, as we measured and used the site-specific target DOC concentrations in later calculations. We chose a concentration of 50 mg DOC L^{-1} to avoid the limitation of labile tDOC during the time frame of the experiment.

The treatments were incubated for three days at 15°C in the dark in 100 mL brown glass vials with a cap lightly screwed on. During the incubation, we used a benchtop shaker to shake the vials at 100 RPM. The movement of the shaker was sufficient to mix the water in the vials but did not move the gravel, hence abrasion of biofilms was unlikely.

2.2 Measurements

We measured stream width and average stream depth at each of the sampling sites. To measure stream width, we used depth measurements along lateral transects from one stream bank to the other at intervals of 10 cm in narrower streams (with a width < 2 m) and 20 cm in wider streams.

Before and after the incubation, we measured bacterial production (BP). The BP of planktonic bacteria was measured (Simon and Azam, 1989) using the leucine incorporation technique as described in detail in Kamjunke et al. (2015). With ^{14}Cleucine (12.2 MBq µmol^{-1}, Sigma, 50 nM final concentration, we spiked triplicate 5 ml aliquots, and one formalin-treated control (3.7 %, final concentration) on the sampling day. Samples were incubated in the laboratory at *in situ* temperature for one hour in the dark on a shaker. We stopped the incorporation with formalin and added 0.6 ml 50% trichloracetic acid (TCA). Proteins were extracted for 15 min and filtered onto 0.2 µm cellulose ester membrane filters (Advantec, Toyo Roshi Kaisha Ltd., Japan). Filters were rinsed twice with 1 ml 5% TCA and once with 80% ethanol. After dissolving the filters in 0.5 ml Soluene (Packard) and adding 2.5 ml Hionic Fluor (Packard) to each scintillation vial, radioactivity was measured using a Liquid Scintillation Analyzer (2300 TR, Packard). For quenching, we used the external standard ratio method. Carbon production was calculated using the equations of Simon and Azam (1989).

For the bacteria within benthic biofilms, we also estimated BP by leucine incorporation. Here, a pebble of about 1 cm length was transferred to scintillation vials and covered with 4 ml sterile-filtered stream water. We spiked triplicate aliquots and one formalin-treated control (3.7 %, final concentration) with ^{14}Cleucine (5 mM final concentration). After incubation for one hour under continuous shaking and extraction with TCA on ice, biofilms were removed from gravel by ultrasonication for 1 min (20%; Ultrasonic Homogenizer, 4710 Series, Cole-Parmer Instrument Co., Chicago, Illinois). Gravel was removed and rinsed, and the supernatant was filtered and measured as described above. We estimated the surface area of each pebble by wrapping it in tin foil, weighing this foil and relating this to the weight of one cm^2 foil.

The abundance of suspended bacteria was estimated from formalin-fixed samples (3.7% final concentration) after staining with acridine orange and counting using an epifluorescence microscope (Axioskop2, Zeiss) as according to Kamjunke *et al.* (2015). We fixed the biofilm

bacteria with formalin in sterile-filtered stream water and counted the biofilm bacteria after their detachment from the pebbles (about 1 cm in length) by ultrasonication. We conducted staining and counting as described above.

We measured the composition of stream water DOC for each site with fluorescence spectroscopy and size-exclusion chromatography (SEC, LC-OCD-OND, DOC Labor Dr. Huber, Karlsruhe, Germany). Fluorescence was measured from 240 to 450 nm excitation and from 300 to 600 nm emission (Aqualog, Horiba, Oberursel, Germany). Based on fluorescence measurements, we calculated the fluorescence index, humification index, and freshness index. Further details on the fluorescence measurements, the calculation and interpretation of the indexes, and the SEC methodology have been provided previously (Heinz et al., 2015; Huber et al., 2011). See Appendix A for the results of DOM composition.

We measured nitrate concentration of stream water by ion chromatography (Dionex, Thermo Fisher Scientific, Hvidovre, Denmark) and ammonium concentration by the indophenol blue method. We used high-temperature catalytic oxidation to measure DOC and total dissolved nitrogen concentration (TOC-L, Shimadzu Europe, Duisburg, Germany). Concentrations of soluble reactive phosphorus (SRP) for the streams were obtained from the Danish National Environmental Monitoring program (Kronvang et al., 2005) or other studies (Goyenola et al., 2015; Hille et al., 2014).

2.3 Reanalysis of literature data on DOC loads

Based on discharge and DOC load data (DOC load = DOC concentration x discharge)from an earlier study (Graeber et al., 2015), we calculated pulse statistics for four streams with different intensity of agriculture (refer to Table 1 for the results). Here, we defined a pulse as a discharge that exceeded the 25th percentile of a flow duration curve (FDC). The FDCs were calculated separately for each of the four streams, and the 25th percentile was a reliable threshold to distinguish between hydrologic pulses and base flow (see the plot of hydrographs and 25th percentile threshold in

188 Appendix B). The DOC load was summed up for the periods defined as pulses, i.e., days on which
189 the discharge exceeded the 25th percentile of the FDC to obtain the DOC in the pulses. Analogously,
190 the DOC load not contained in pulses was the sum of the DOC load of all days on which the
191 discharge did not exceed or was equal to the 25th percentile of the FDC.

192 ## 2.4 Calculations

193 We calculated DOC uptake of added leaf leachate during the incubation for each
194 experimental vial as:

195 (equation 1)

196 where DOC_{uptake} is the change of the DOC concentration in mg L^{-1} h^{-1} per experimental vial
197 for each stream and treatment (benthic zone or water-column treatments). $startDOC_{LL}$ and
198 $endDOC_{LL}$ are the start and end concentration of each stream and treatment with leaf leachate;
199 $startDOC_{control}$ and $endDOC_{control}$ are the start and end concentration of each stream and treatment in
200 the control without leaf leachate, and $time_{incubation}$ is the length of incubation in hours.

201 To investigate the potential impact of the water column and the active benthic zone on DOC
202 uptake at the stream scale, we estimated DOC uptake for each stream reach. To achieve this, we
203 considered active sediment layers of 0 to 1, 0 to 3 and 0 to 7 cm in which microbial DOC uptake
204 may happen (Fischer et al., 2002). We scaled the DOC uptake to the stream-stretch scale as follows:

205 (equation 2)

206 $DOC_{extrapolated\ uptake}$ is the extrapolated change in DOC concentration per hour for the stretch.
207 For the sediment, $V_{active\ stretch}$ was the estimated active sediment volume per meter stream length
208 (active sediment depth of 0.01, 0.03, or 0.07 m x stream width in m x 1 m stream length). For the
209 water column, we used data from the cross-sectional measurements, and calculated $V_{active\ stretch}$ as
210 average stream depth in m x stream width in m x 1 m stream length. Furthermore, we calculated
211 $DOC_{specific\ uptake}$ in mg C $L_{active\ volume}^{-1}$ h^{-1} as DOC_{uptake} in the experimental vials (mg C L^{-1} h^{-1}) multiplied

by the water volume (L) of the experimental vials and divided by the active water or sediment volume in the experimental vials ($L_{active\ volume}$). Here, the active volume in the vials is defined by the volume of water within the incubation vials for the water-column treatment (0.07 L) and by the volume of gravel within the incubation vials for the benthic-zone treatment (0.01 L).

We assessed differences between benthic and planktonic DOC uptake separately for each stream, as a ratio of planktonic to benthic DOC uptake. For this ratio, we used the mean values of the planktonic and benthic $DOC_{extrapolated\ uptake}$ of each stream.

We calculated the response ratios of planktonic and benthic BP by dividing the mean planktonic BP of each stream by the mean benthic BP of the same stream. We did the same to calculate the planktonic and benthic response ratios of bacterial abundance.

2.5 Statistics

We conducted all statistics in the R statistical package (version 3.4.1) (R Core Team, 2017). For each stream site, we compared DOC uptake between the treatments with Kruskal-Wallis tests (kruskal.test function, stats package), since normal distribution was not given for the untransformed data. We tested with a t-test (t.test function), whether the ratio of DOC uptake in the water column and benthic zone differed from 1, with 1 meaning that the DOC uptake in the water column and the benthic zone is equal. The DOC uptake ratio was normal distributed.

We compared bacterial abundance and BP with and without added leaf leachate for each stream site with Kruskal-Wallis tests, as we did for DOC uptake. With paired t-tests (paired by stream site, t.test function, stats package), we compared the response ratio of planktonic and benthic BP with paired t-tests. We did the same to compare the benthic and planktonic response ratios of bacterial abundance. The differences of the response ratios of BP were normal distributed. The differences of the response ratios of bacterial abundance were ln-transformed to achieve normal distribution.

We used linear regressions (lm function) to relate the response ratio of BP and bacterial abundance to changes in inorganic nutrient concentrations (total dissolved nitrogen, ammonium, nitrate + nitrite, soluble reactive phosphorus), DOC concentration and/or composition (fluorescence index, freshness index, humification index, specific-UV absorbance at 254 nm, size-exclusion chromatography carbon and nitrogen fractions and their C:N ratios), land use (percentage of agricultural land use), catchment size, stream width and stream length upstream of each sampling site. If several variables significantly explained BP or bacterial abundance, we checked whether linear models with multiple explanatory variables (combined models) would increase the explanatory power over separate linear models with single explanatory variables (lm function in combination with anova function in R). Here, we started with the explanatory variable with the highest R^2 and subsequently added variables with less explanatory power. We $\log_{10}$-transformed the response ratios and the explanatory variables to achieve normal distribution and homoscedasticity of residuals prior to analysis.

3. Results

3.1 DOC pulses

In our reanalysis of the literature data from Graeber et al. (2015) on DOC loads, we found that 41 – 87% of the total annual DOC load was transported with hydrologic pulses (Table 1). The contribution of the pulses was higher in the subtropical region than in the temperate region and, within the two regions, higher in the catchments with arable farming. The DOC was transported within many (22 – 41 pulses) short pulses (median length 4-5 days, Table 1). See also the hydrographs in Appendix B for a more detailed representation of the pulses.

3.2 DOC uptake

The planktonic DOC uptake differed from the benthic DOC uptake in four of the eight streams ($p < 0.05$, Kruskal-Wallis test, Fig. 2A). The mean ratios between planktonic and benthic DOC uptake were not different between treatments (t-test, $p > 0.05$, Fig. 2B).

In our extrapolation of DOC uptake for a stream stretch, the contribution of water column DOC uptake to total stream DOC uptake exhibited a positive relationship with the cross-sectional water volume of the sites (Fig. 2C). Relative uptake of DOC in the water column was negatively related to active sediment depth with the highest contribution of the water column found for streams with large water volume and 1 cm active sediment depth (81%, Fig. 2C) and the lowest for streams with small water volume and 7 cm active sediment depth (7%, Fig. 2C).

3.3 Bacterial production and abundance

There was a clear planktonic response to the leaf leachate DOC addition in all streams; i.e., the planktonic BP to the leaf leachate DOC addition was higher than that in control treatments in all streams (Fig. 3A). For the benthic BP, this was the case only for two of the eight streams (Fig. 3B). The response ratio of the BP was higher (paired t-test, $p = 0.018$) for planktonic bacteria (response ratios of 5 – 17, Fig. 3C) than for benthic bacteria (response ratios of 1 – 4, Fig. 3D).

The bacterial abundance reacted in a similar manner to the leaf leachate addition, as we found for BP. However, we found a much higher variability among replicates (Fig. 4). The planktonic bacterial abundances were higher with leaf leachate in four of the eight streams (Fig. 4A), and the benthic bacterial abundances were higher in only two of the eight streams (Fig. 4B). The planktonic and benthic response ratios of bacterial abundance differed markedly (paired t-test, $p = 0.045$), with planktonic response ratios of 1 – 126 (Fig. 4C) and benthic response ratios of 1 – 4 (Fig. 4D).

The response ratio of the planktonic BP was best correlated with stream length in the catchment upstream of the sampling site (linear model, adj. $r^2 = 0.68$, $p = 0.007$, Fig. 5A) but also positively correlated with stream width (adj. $r^2 = 0.60$, $p = 0.015$) and negatively correlated with ammonium concentration (adj. $r^2 = 0.60$, $p = 0.015$). However, stream width positively correlated (adj. $r^2 = 0.51$, $p = 0.029$) with stream length and, hence, its influence on the response ratio of planktonic BP was neglected. Ammonium concentration was only found to vary between 8 and 40 µg N L^{-1} (mean = 22 µg N L^{-1}), which was much lower than the nitrate concentration, varying between 23 and 4245 µg N L^{-1} (mean = 1944 µg N L^{-1}). In contrast to ammonium concentration, nitrate concentration was not correlated to the response ratio of the planktonic BP (adj. $r^2 < 0.01$, $p = 0.43$). The much higher concentration of nitrate and the missing correlation of nitrate with the BP response ratios makes N limitation as controlling factor unlikely.

To further test whether ammonium concentration explained variation not explained by stream length in the catchment, we compared a linear model with the response ratio of the planktonic BP as dependent variable and stream length as an independent variable to a model with stream length and ammonium concentration as independent variables. We found that the combined model was not significantly better at explaining the response ratio of planktonic BP than the model with stream length only (ANOVA, $F = 0.77$, $p = 0.42$).

The response ratio of the planktonic bacterial abundance was also best correlated with stream length (linear model, adj. $r^2 = 0.79$, $p = 0.002$, Fig. 5B). It was significantly negatively correlated with SRP concentration (adj. $r^2 = 0.67$, $p = 0.008$, Fig. 5C), positively correlated with catchment size (adj. $r^2 = 0.58$, $p = 0.017$) and negatively correlated with ammonium concentration (adj. $r^2 = 0.50$, $p = 0.031$). Catchment size was highly positively correlated with stream length in the catchment and, therefore, was neglected as an explanatory variable (adj. $R^2 = 0.88$, $p < 0.001$). We discarded ammonium as an important variable for the same reasons as for the response ratio of planktonic BP. As for the response ratio of planktonic BP, a combined model with stream length

and ammonium concentration did not improve the explanation of the response ratio of planktonic bacterial abundance significantly, compared to a model with stream length only (ANOVA, $F = 0.12$, $p = 0.746$).

Concentration of SRP was not correlated with stream length (adj. $r^2 = 0.25$, $p = 0.116$) and including SRP concentration in the linear model with stream length improved the model significantly (ANOVA, $F = 20.45$, $p = 0.006$). Here, the negative correlation of the response ratio of the planktonic bacterial abundance with SRP concentration was largely a result of a positive correlation between the bacterial abundance in the control and SRP concentration (linear model, adj. $r^2 = 0.49$, $p = 0.03$) besides the missing correlation between the planktonic bacterial abundance in the treatment of leaf leachate and SRP concentration (adj. $r^2 = 0$, $p = 0.94$).

4. Discussion

4.1 DOC pulses

For three of the four catchments in our literature reanalysis, we found that most of the annual DOC load was transported in pulses. This supports similar findings in the literature (Raymond et al., 2016 and references therein) and supports the notion that intensive agriculture results in a higher unevenness of DOC export (Dalzell et al., 2007; Graeber et al., 2012a; Heinz et al., 2015).

It has been reported in some studies that DOC concentration changes with discharge (Dalzell et al., 2007; Stanley et al., 2012), while this was not supported in other studies (Graeber et al., 2012a; Heinz et al., 2015). The change in DOC concentration with discharge in agricultural systems has been reported to also change DOM composition towards high-molecular and colloidial DOC indicating terrestrial plant sources in the catchment, which may be activated during a hydrologic pulse (Dalzell et al., 2007, 2011). A shift towards humic-like, complex terrestrial DOC with increasing DOC concentration has also been reported in another study of small streams (Graeber et

al., 2012a) but could not be supported for mid-western US agricultural ditches (Warrner et al., 2009). Altogether, change of either DOC concentration or composition seems to depend mostly on the catchment configuration and history, but we also deem it likely that it depends on previous pulses and hence of the state of the catchment DOC pools and whether these were emptied or reduced recently.

During any pulse event, hydrological and biogeochemical conditions change in addition to DOC load. Our concept proposes that planktonic bacteria may have an important role in DOC processing during such events due to their contact time with the pulse and the constrained hydrological exchange with the hyporheic zone (Wondzell, 2011; Boano et al., 2014) (Fig. 1). However, planktonic bacteria can only have the proposed important role if they are able to take up considerable amounts of DOC.

4.2 DOC uptake

In our experiment, DOC uptake in the water column and benthic zone was comparable. Based on our extrapolation, we estimated that bacteria in the water column may account for 5 – 80% of the total DOC uptake. Here, the contribution of the water column was negatively affected by active sediment depth and positively by the water volume in the cross section. For our lowland streams, we assumed that the active sediment depth was rather shallow (≤ 7 cm), which is supported by both bacterial activity (Fischer et al., 2002) and chemical gradient (Hartwig and Borchardt, 2015) measurements in lowland streams and rivers. Based on the positive correlation of water column DOC uptake to cross-sectional water volume and because our streams was rather small (< 6 m stream width), we assume an even larger contribution of the water column to DOC uptake for larger streams and rivers. However, our extrapolation does not consider the high probability of limited DOC transport from the water column to the sediment (Wondzell, 2011), which may further reduce the contribution of benthic and hyporheic DOC uptake.

353 Our results suggest that DOC uptake in the water column of fluvial systems can represent a
354 considerable proportion of DOC uptake and, analogous to lakes (Tranvik, 1992), needs to be
355 considered as a place where tDOC uptake can potentially happen. However, we must treat the
356 outcome of this extrapolation carefully, as it relies on laboratory data, measured in vials on a shaker
357 in the dark, hence not taking unidirectional flow, primary production and photodegradation into
358 account (Mineau et al., 2016). The few studies in the literature that presented estimates of DOC
359 uptake scaled across different stream orders lack specific uptake rates for landscape functional units
360 within and across aquatic ecosystems (Raymond et al., 2016). This situation would be improved by
361 *in situ* DOM uptake experiments assessing the contribution of the main compartments of stream and
362 river ecosystems in the field (water column, benthic zone, hyporheic zone, but also floodplains and
363 backwaters) in streams and rivers, as has been shown for *in situ* nutrient uptake experiments based
364 on DOC concentration enrichments (e.g. Johnson et al., 2015). Alternatively, using DOC enriched
365 with the heavy stable isotope of carbon would be an option, however, this likely would be very
366 expensive to execute for hydrological pulses. A trade-off between vial and in-stream experiments
367 could be flume experiments (Kamjunke et al., 2017), which are easier to control and less expensive
368 than in-stream experiments, and more realistic than vials.

369 In our laboratory setup we assume that benthic and planktonic bacteria receive a similar kind
370 of DOC in terms of concentration and composition during a period of three days. This fits to the
371 median pulse length of 4-5 days we calculated in Table 1. However, this is a simplification of the *in*
372 *situ* situation and further investigations in flumes and/ or streams would be needed to validate our
373 results.

374 ## 4.3 Bacterial production and abundance

375 Bacterial production and bacterial abundance of planktonic bacteria reacted much stronger
376 to the labile tDOC (alder leaf-litter leachate) than those of benthic bacteria. This is in accordance to
377 an *in situ* investigation, in which planktonic but not benthic bacteria were responsive to DOC

quantity and composition (Kamjunke et al., 2015) and may have several reasons. First, benthic bacterial communities are probably not subjected to the same "feast and famine" conditions as planktonic bacteria (Freeman and Lock, 1995) because benthic biofilms develop a polysaccharide matrix to store and process different sources of organic carbon, and that acts as a buffer against variable DOC supply, limiting the need for benthic bacteria to rapidly respond to pulses of potentially labile DOC from terrestrial environments (Fischer et al., 2002; Freeman and Lock, 1995; Kaplan and Newbold, 2000). Benthic bacterial communities are usually less connected to the water column than planktonic bacteria due to lower convection in the boundary layer and diffusion limitation, which is especially true for DOC, for which the diffusion coefficient is less than 50% compared to inorganic nutrients (Stewart, 2003). Assuming that the biofilms on the gravel we studied were mature, the likely already high density of benthic bacteria may have prevented further fast biofilm growth (e.g. Besemer et al., 2007). It is likely that many of the same factors apply to hyporheic biofilms, which also should build-up a polysaccharide matrix and should even be less connected to the surface water.

The negative relationship of the response ratios of planktonic bacterial production and abundance with upstream stream length may be explained by differences in the bacterial communities among sites. Recent studies demonstrated that bacterial communities in the headwaters of fluvial networks are more diverse than downstream communities, due to their close connection to the terrestrial environments allowing a more complex mix of aquatic and terrestrial bacteria to co-exist, which is followed by environmental sorting of the bacterial community along the fluvial network (Besemer et al., 2013; Niño-García et al., 2016). Furthermore, bacterial communities from different freshwater and estuarine environments are adapted to utilize different fractions of the DOM pool (Amaral et al., 2016; Logue et al., 2016). The less diverse planktonic bacterial communities of streams with longer upstream flow paths should be less reactive to variable DOM composition than the diverse bacterial community of headwater streams with shorter flow paths, that have a larger relative interface with their terrestrial surroundings. This different

reactivity is what we found for the response of BP and bacterial abundance to the labile tDOC source in our study. However, without further information on the bacterial community diversity this remains only empirical evidence and discussions on potential mechanisms remain speculation.

Interestingly, we found a strong negative correlation between SRP concentration and the bacterial abundance response ratio of the planktonic bacteria. This resulted from the missing correlation between SRP concentration and planktonic bacterial abundance in the treatments with leaf leachate, combined with a positive correlation between the SRP concentration and the planktonic bacterial abundance in the control without leaf leachate (data not shown). Since the bacterial abundance response ratio was calculated by the division of bacterial abundance in the treatments with leaf leachate treatment and the control, this resulted in a negative correlation of the response ratio of the planktonic bacterial abundance to SRP concentration. The bacteria may have experienced P-limited conditions in the control, and likely insufficient P was provided by the leaf leachate (data not available). We speculate that a combination of DOC and SRP might stimulate DOC uptake by the planktonic bacteria even more than observed in our experiments, as it will reduce the stoichiometric imbalance between the DOC source and the bacteria (Cross et al., 2005).

5. Conclusions

According to the current paradigm of DOC uptake, the benthic zone is viewed as the primary biologically reactive component of low-order streams, while the water column mainly performs conservative transport (Battin et al., 2016; Newbold et al., 1981).

We conclude that the water column is essential for DOC processing in low-order streams based on two lines of evidence: i. The high importance of pulsed DOC transport according to the literature (Dalzell et al., 2007; Graeber et al., 2012b; Heinz et al., 2015; Raymond et al., 2016) and our reanalysis of a literature dataset (Table 1) combined with the fact that planktonic bacteria are transported with the pulse and should perceive it as a rather constant concentration (Fig. 1). ii. Our

experimental findings that small-stream planktonic bacteria are highly responsive to labile tDOC in the water column (Fig. 3, 4), which results in high planktonic DOC uptake in the laboratory and potentially high uptake in the field as shown by the extrapolation to the stream stretch (Fig. 2).

The role of planktonic bacteria in the processing of fluvial DOC is likely more important than currently acknowledged and a considerable part of carbon taken up by planktonic bacteria from DOC pulses may move through and affect stream and semi-aquatic food webs. Consequently, the fate of DOC taken up by planktonic bacteria must be considered in models of biogeochemical cycles related to streams and the contribution of those cycles to the global carbon cycle.

6. Acknowledgements

We thank M. Skjærbæk, K. Jensen and U. Link for their assistance in the laboratory. D. Graeber and J.R. Poulsen were funded by a grant from DCE - Danish Centre for Environment and Energy, Aarhus University. B. Kronvang was supported by the Danish Council for Strategic Research [BufferTech project, grant no. 1305–00017B]. B. Gücker was supported by the Brazilian National Council for Scientific and Technological Development [CNPq, grant no. 302280/2015-4] and the Foundation for Research Support of the Federal State of Minas Gerais [FAPEMIG, grant no. CRA PPM 00596-16]. J. Rasmussen was funded by a grant from the Carlsberg Foundation [grant no. 2013_01_0833]. N. Kamjunke was funded by the German Federal Ministry of Education and Research [BMBF, grant no. 02WT1290A]. M. Heinz and D. Zak were funded by the German Research Foundation (DFG) projects [grant numbers PU 136/7-1 and ZA 742/2-1, respectively].

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Tables

Table 1: Characteristics and effects of hydrological pulses on dissolved organic carbon (DOC) load in catchments with arable farming and pasture within subtropical (Uruguay) and temperate climate (Denmark). The calculation is based on daily discharge measurements and DOC load data for more than two years, and pulses were defined as discharges larger than the discharge at the 25th percentile of a flow-duration curve. Reanalysis of data from Graeber et al. (2015).

Site	DOC load in pulses (% of total DOC load)	Total pulse duration (% of sampling period)	Number of pulses	Median (min - max) length per pulse (days)
Subtropical, arable farming	87	30	40	5 (2-15)
Subtropical, pasture	80	28	31	4 (2-34)
Temperate, arable farming	69	28	22	5 (2-30)
Temperate, pasture	41	30	41	4 (2-26)

Figure captions

Fig. 1: Perspectives on a dissolved organic carbon (DOC) pulse within a fluvial system. For stationary bacteria of the benthic and hyporheic zone, the DOC pulse must appear as such, but they will not receive the full pulse due to transport limitation into the sediment. For free-living planktonic bacteria, the pulse will be perceived as a slowly decreasing load, because they are transported downstream together with the pulse of which the bacteria process a part, reducing the DOC concentration. The labile part of the DOC pulse will be removed within the fluvial system and the recalcitrant part within the time frame of fluvial transport will be exported.

Fig. 2: Dissolved organic carbon (DOC) uptake by planktonic and benthic bacteria for each of the eight streams (A, $n_{control}$=3, $n_{leaf\ leachate}$ = 5), mean DOC uptake ratio (B, n_{stream} = 8) and estimated contribution of the water column to total stream DOC uptake (C, n_{stream} = 8). The DOC uptake in panel B shows the DOC uptake per day for the benthic-zone treatment (60 mL bacteria-free filtered stream water + 10 mL gravel + leaf-leachate DOC) and the water-column treatment (70 mL unfiltered stream water + leaf-leachate DOC). The estimated contribution in panel B shows the estimated contribution of the water column to total DOC uptake for each stream site and three hypothetical biologically active sediment depths (1, 3 and 7 cm).

Fig. 3: Bacterial production (BP) by the planktonic (A) and benthic bacteria (B) with (n = 5) and without leaf leachate (control, n = 3). Panels C and D show the planktonic and benthic response ratio of BP, respectively (n_{stream} = 8).

Fig. 4: Bacterial abundance (BA) of planktonic (A) and benthic bacteria (B) with (n = 5) and without leaf leachate (control, n = 3). Panels C and D show the planktonic and benthic response ratio of the bacterial abundance, respectively (n_{stream} = 8).

Fig. 5 Relationships of the bacterial production (BP) and bacterial abundance (BA) response ratios with total stream length in the catchment (panels A, B) and of the BA response ratios with

617 soluble reactive phosphorus concentration (SRP, panel C). BP and BA response ratios were
618 calculated as BP or BA in treatments with leaf leachate divided by BP or BA in treatments without
619 leaf leachate addition. **p = 0.01-0.001.

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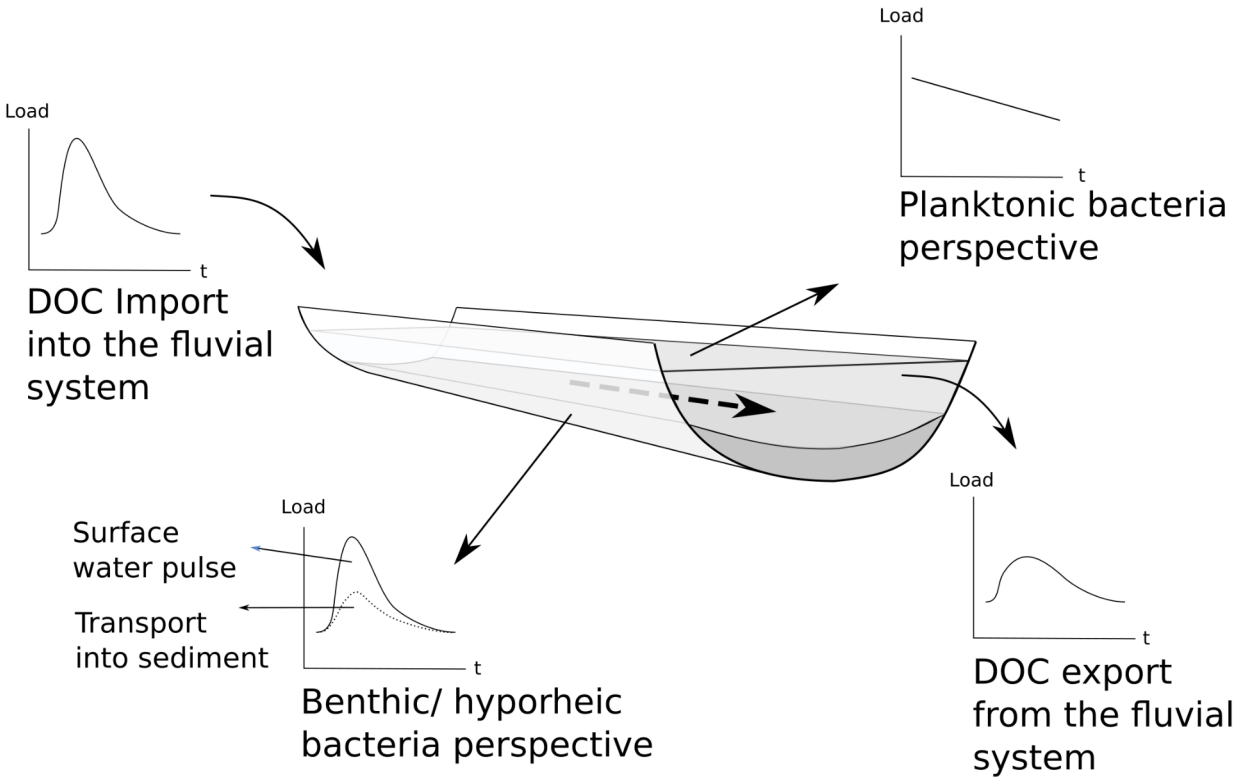

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622 Figure 1

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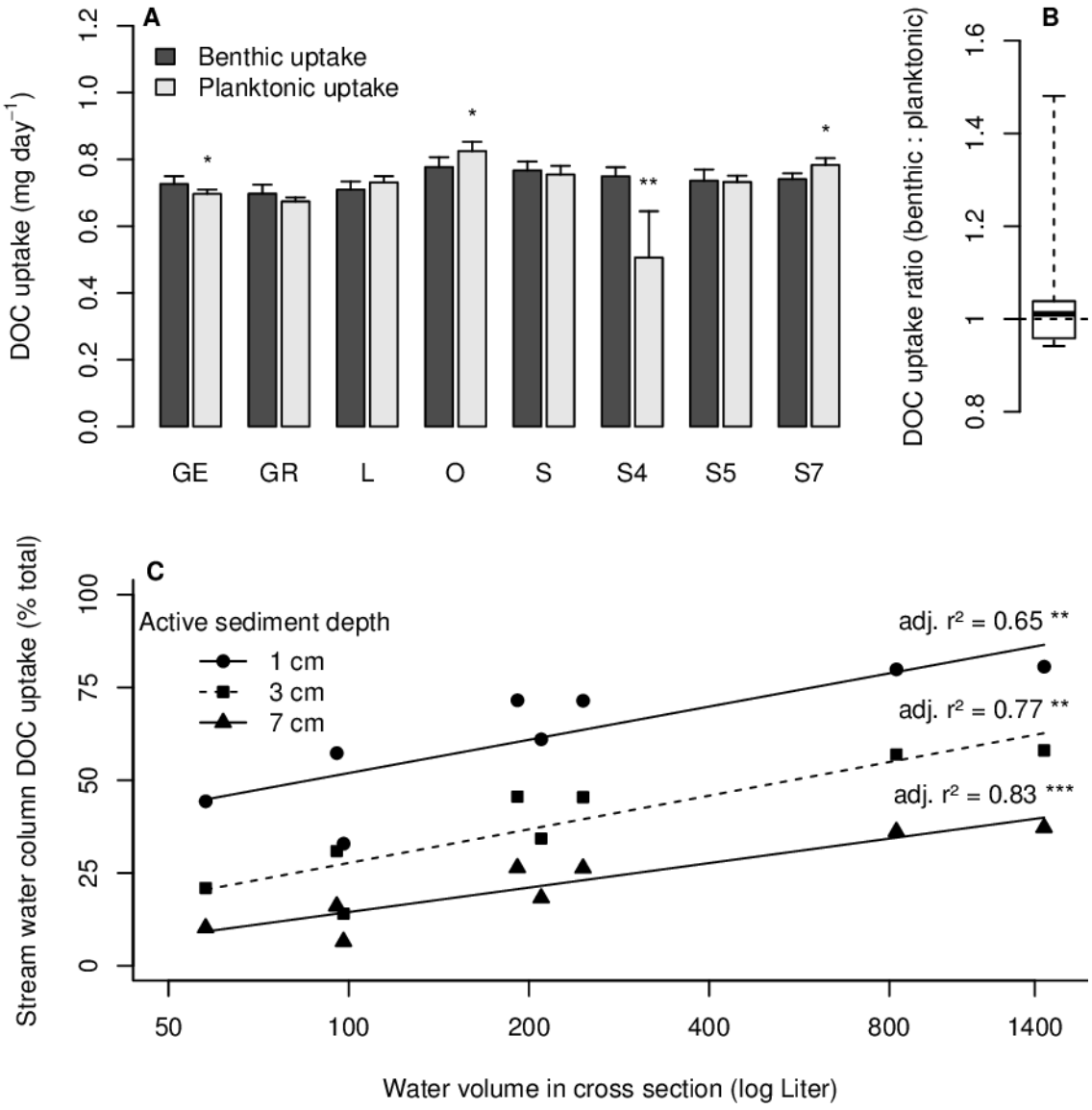

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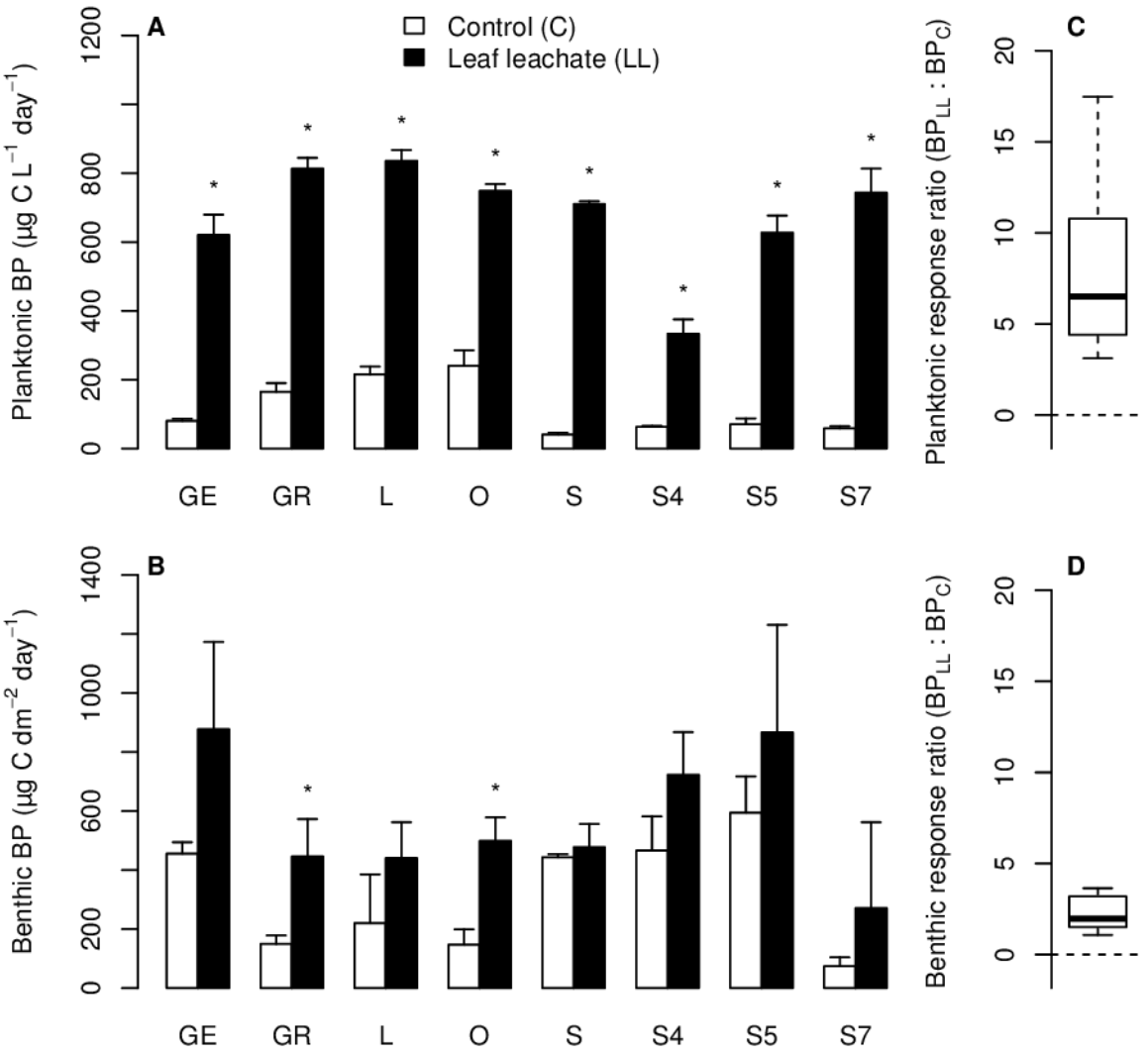
626 Figure 2



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628 Figure 3

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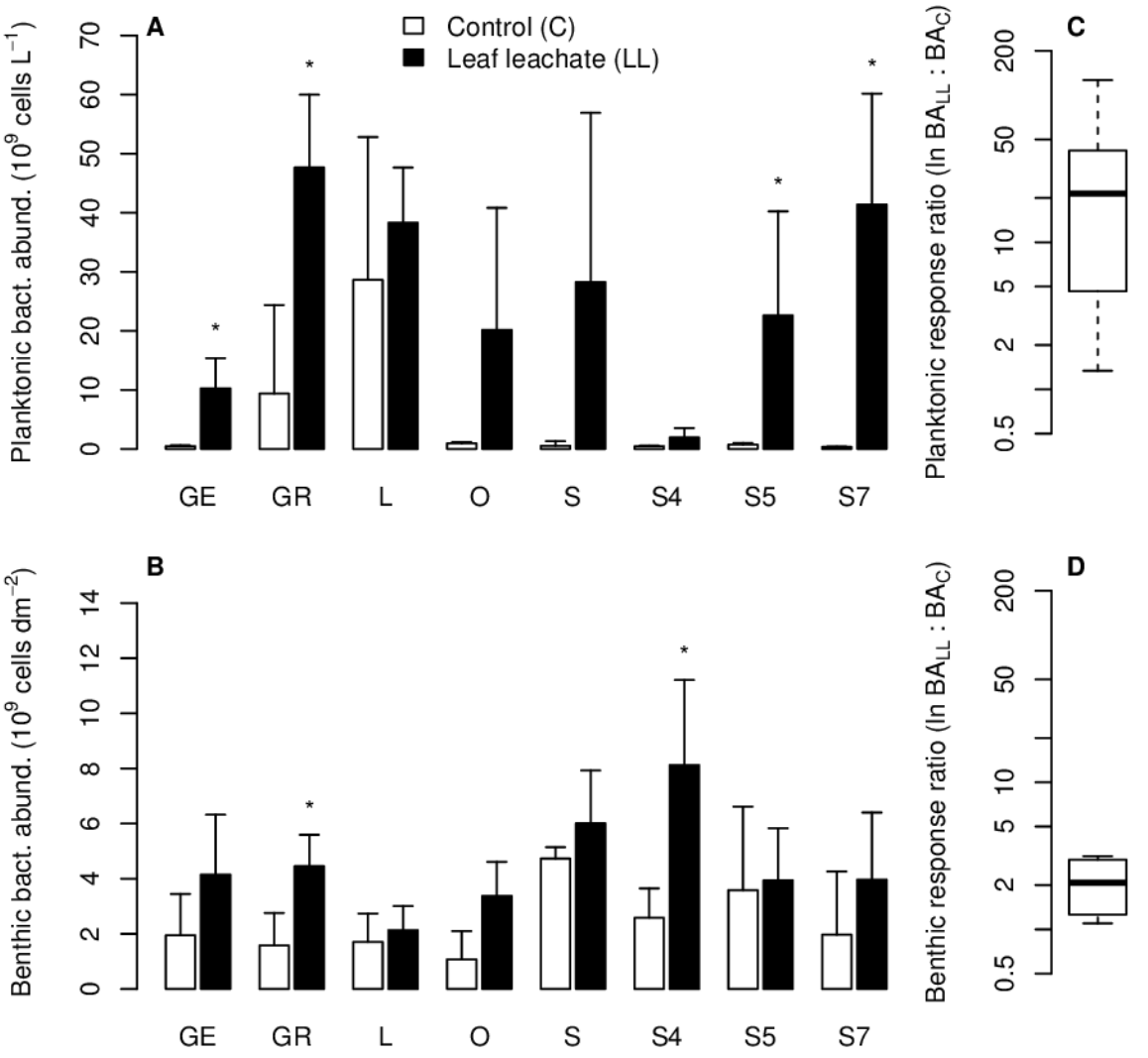

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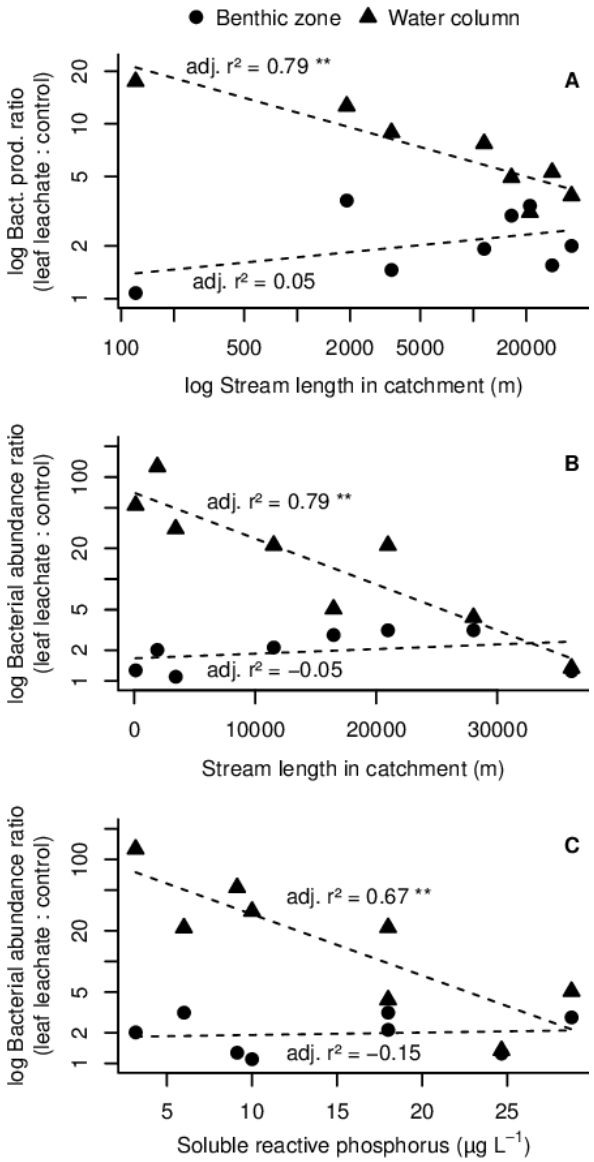
632 Figure 4



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634 Figure 5

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Appendix A

Table A1. Position and catchment characteristics of the investigated streams. Catchment size represents the surface catchment size, based on topographic maps. Land use contributions were calculated based on the Areal Informations Systemet – AIS (Nielsen et al. 2000).

Stream	Latitude (dec.°)	Longitude (dec.°)	Catchment size (km²)	Total stream length (km)	Arable (%)	Forested (%)	Pasture/ Grassland (%)	Other land use (%)
GE - Gelbæk	56.2253	9.8116	11.8	11.5	92	2	0	6
GR - Granslev	56.2849	9.8980	7.4	16.5	29	59	7	5
L - Lemming Å	56.2448	9.5301	57.0	36.1	82	7	1	10
O - Odderbæk	55.9230	9.2895	27.6	20.9	68	31	0	1
S - Sandemands-bækken	56.1565	9.4948	0.1	0.1	0	100	0	0
S4 - Javngyde Bæk	56.1073	9.8232	46.4	28.0	87	4	0	9
S5 - Ellerup Bæk	56.2275	9.7624	3.9	3.4	93	3	0	4
S7 - Skærbæk	56.0826	9.4224	4.6	1.9	26	71	0	3

Table A2. Dissolved organic carbon (DOC) concentration and dissolved organic matter (DOM) characteristics of the investigated streams, based on fluorescence, absorbance and size-exclusion chromatography measurements (SEC). HIX = humification index, FI = fluorescence index, BA = beta:alpha/ freshness index, DOC = DOC concentration, measured by size-exclusion chromatography (SEC; mg L^{-1}), DOC_{HMWS} = DOC concentration of the high-molecular weight SEC fraction (mg L^{-1}), DOC_{HS} = DOC concentration of the humic-substance like weight SEC fraction (mg L^{-1}), DON_{HMWS} = dissolved organic nitrogen (DON) concentration of the high-molecular weight SEC fraction (mg L^{-1}), DON_{HS} = DON concentration of the humic-substance like weight SEC fraction (mg L^{-1}), SUVA = Specific absorbance of bulk DOM at 254 nm (L mg^{-1} m^{-1}) , $SUVA_{HS}$ = Specific absorbance of the humic-substance like SEC DOM fraction at 254 nm (L mg^{-1} m^{-1}).

Stream	HIX	FI	BA	DOC	DOC_{HMWS}	DOC_{HS}	DOC_{LMWS}	DON_{HMWS}	DON_{HS}	SUVA	$SUVA_{HS}$
GE - Gelbæk	0.95	1.63	0.63	5.18	0.08	4.56	0.54	0.009	0.61	3.3	4.3
GR - Granslev	0.92	1.63	0.64	1.68	0.07	1.26	0.35	0.008	0.07	2.5	3.1
L - Lemming Å	0.94	1.57	0.58	3.29	0.07	2.78	0.43	0.005	0.15	3.3	4.9
O - Odderbæk	0.95	1.52	0.56	5.46	0.05	4.84	0.56	0.005	0.28	3.9	4.8
S - Sandemands-bækken	0.88	1.62	0.63	0.58	0.01	0.41	0.15	< 0.005	< 0.005	3.1	3.7
S4 - Javngyde	0.94	1.62	0.63	4.86	0.11	4.09	0.67	0.005	0.24	3.2	4.6

Bæk S5 - Ellerup Bæk	0.96	1.59	0.6	6.09	0.08	5.39	0.62	0.009	0.33	3.7	4.7
S7 - Skærbæk	0.92	1.55	0.55	1.67	0.01	1.28	0.37	0.005	0.05	3.4	5

651

652 # Appendix references

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Appendix B

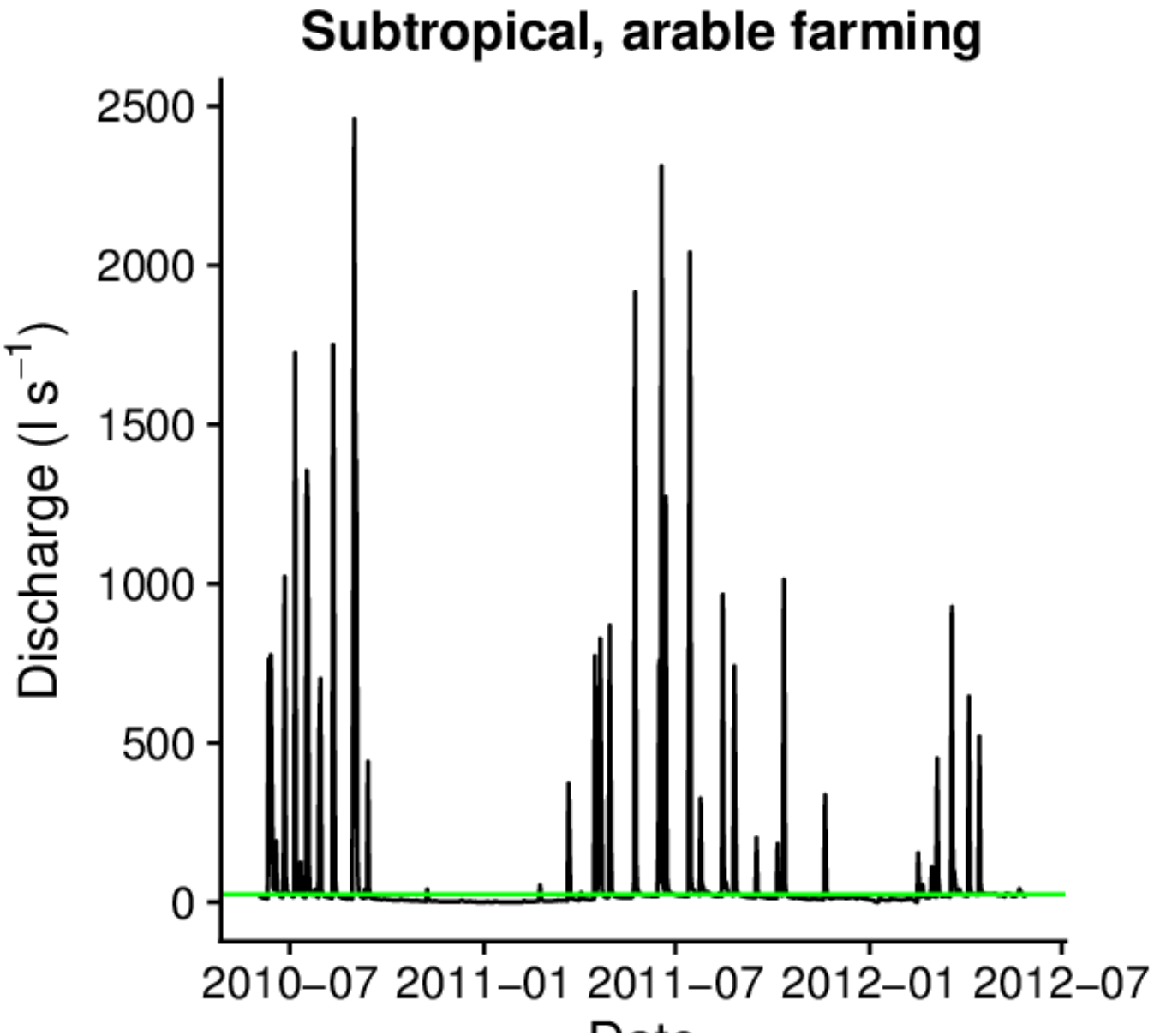

Subtropical, arable farming
2500
2000
1500
1000
500
0
Discharge (l s^{-1})
2010–07
2011–01
2011–07
2012–01
2012–07

Fig. B1: Hydrograph of the subtropical stream with an arable-farming dominated catchment. The green line indicates the pulse threshold (the 75th percentile of a flow-duration curve), hence discharges above this threshold were classified as hydrologic pulses.

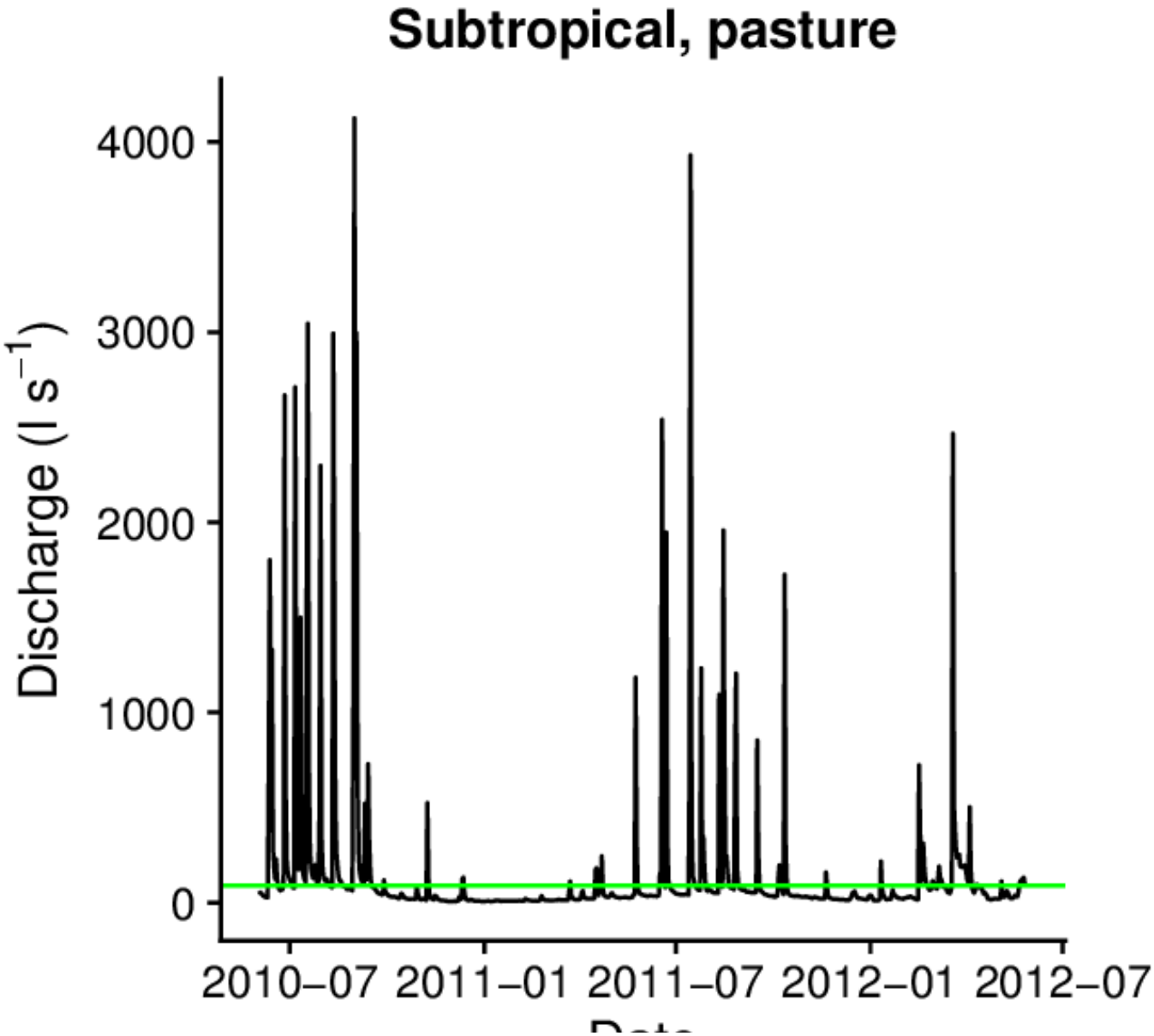
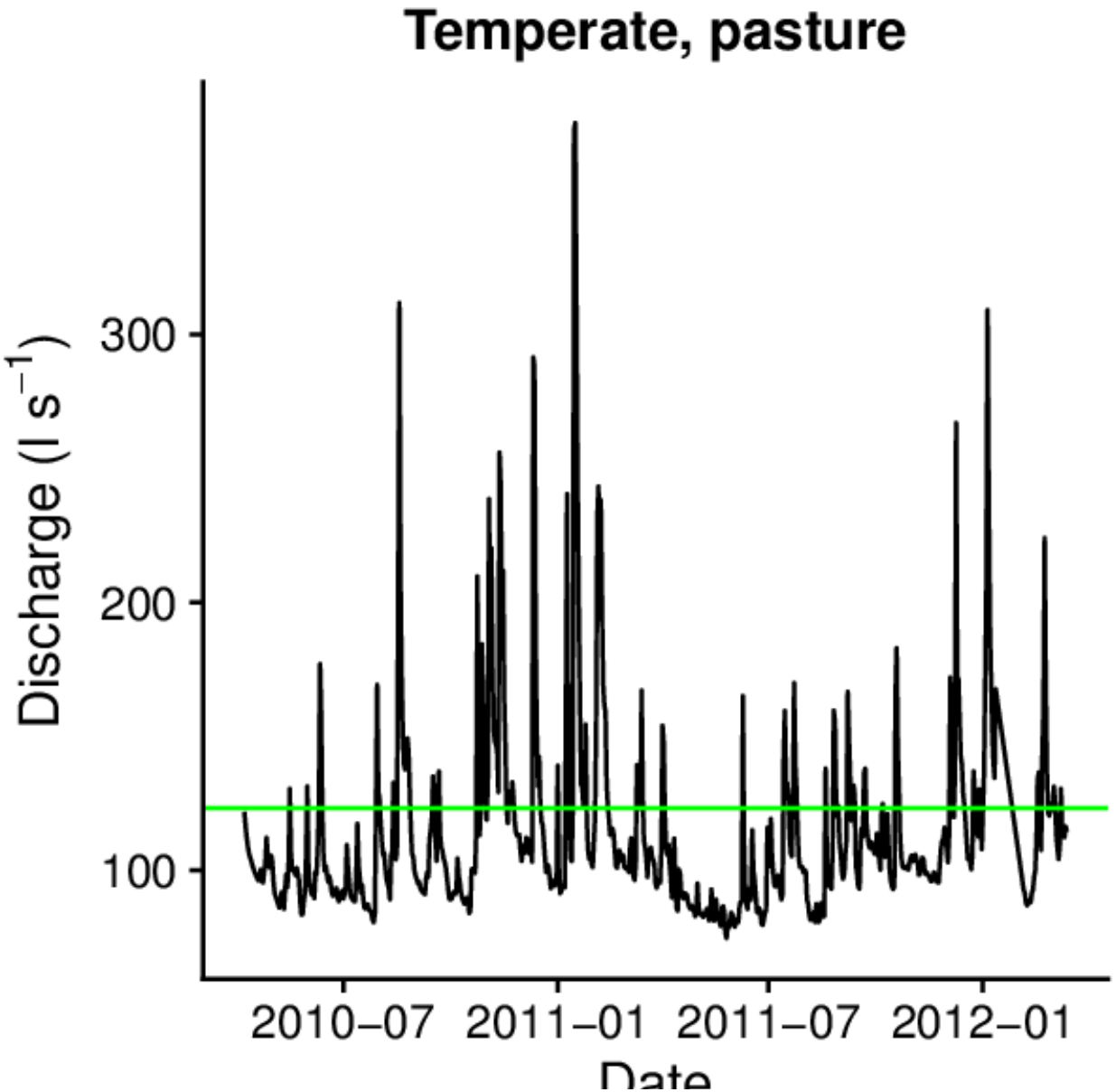

Subtropical, pasture
4000
3000
2000
1000
0
Discharge (l s^{-1})
2010–07
2011–01
2011–07
2012–01
2012–07

Fig. B2: Hydrograph of the subtropical stream with a pasture-dominated catchment. The green line indicates the pulse threshold (the 75th percentile of a flow-duration curve) and discharges above this threshold were classified as hydrologic pulses.


Temperate, pasture
Discharge (l s^{-1})
300
200
100
2010–07
2011–01
2011–07
2012–01
Date

672 Fig. B3: Hydrograph of the temperate stream with an arable-farming dominated catchment. The green line indicates the pulse threshold (the 75th
673 percentile of a flow-duration curve) and discharges above this threshold were classified as hydrologic pulses.

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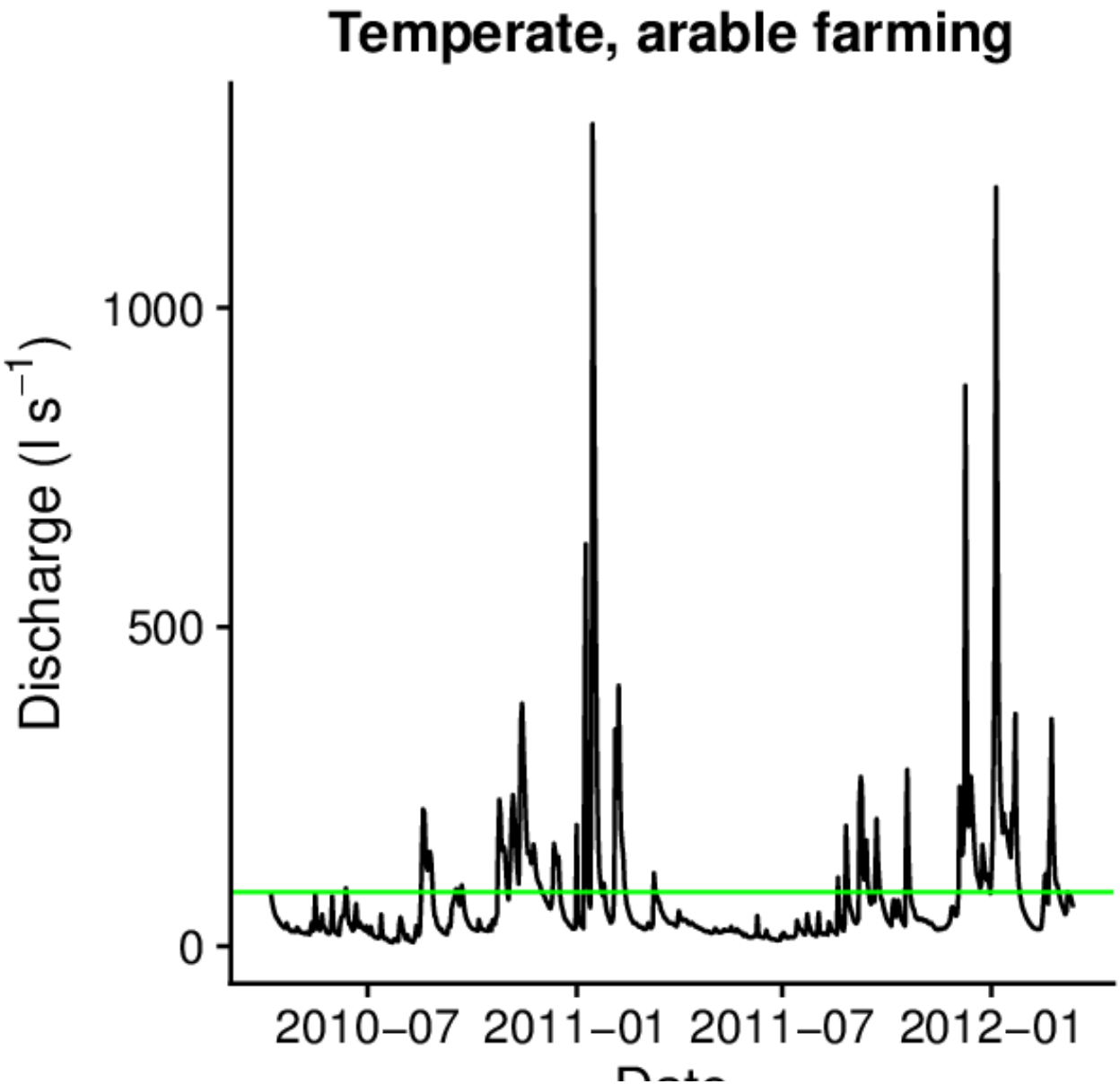
Temperate, arable farming
Discharge (l s^{-1})
1000
500
0
2010–07
2011–01
2011–07
2012–01

Fig. B4: Hydrograph of the temperate stream with a pasture-dominated catchment. The green line indicates the pulse threshold (the 75th percentile of a flow-duration curve) and discharges above this threshold were treated as hydrologic pulses.