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Manuscript Draft

Manuscript Number: STOTEN-D-20-04550R1

Title: The impact of rainfall events, catchment characteristics and estuarine processes on the export of dissolved organic matter from two lowland rivers and their shared estuary

Article Type: Research Paper

Keywords: DOC; DON; eutrophication; river flow; estuary; Christchurch Harbour

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Order of Authors: Anouska Panton; Fay Couceiro; Gary R Fones; Duncan Purdie

Abstract: Terrestrially-derived dissolved organic carbon (DOC) and nitrogen (DON) transported by rivers have been recognised as contributors to aquatic nutrient burdens, and can be of importance in rivers and estuaries already impacted by anthropogenic inorganic nutrient discharges. The concentration of DOC and DON and the flux of both to the estuary and ultimately the coastal zone is dependent upon many factors including rainfall, catchment land use, and biological processes. DOC and DON concentrations together with nitrate plus nitrite and ammonium concentrations were measured in the anthropogenically-impacted estuary Christchurch Harbour (UK) and at sites in the lower reaches of its two source rivers, the Hampshire Avon and the Stour, at weekly intervals for a year during which time several extreme rainfall events occurred. A series of transects along the estuary were also performed after weekly sampling was completed. DOC concentrations were correlated between both rivers and the estuary and were positively related to increases in river flow, but DON concentrations revealed a more complicated picture. Peak instantaneous fluxes of DOC and DON exceeded 60000 kg C d-1 and 7000 kg N d-1 respectively both in the Stour and the estuary during high flow periods. The sources of both and routes by which they enter the aquatic system may account for the differences in dynamics, with flushing of superficial soils being a key source of DOC and point sources such as sewage treatment works being proposed as sources of DON. Removal processes within the estuary were also of importance for DON concentrations while DOC behaved more conservatively with some evidence of local production within the estuary. Estimated annual loads of DON and DOC to the coastal zone from Christchurch Harbour were 118 kg N km-2 y-1 and 2296 kg C km-2 y-1.

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3 March 2020

***Dear Editor,***

We would like to submit our manuscript entitled **"The impact of rainfall events, catchment characteristics and estuarine processes on the export of dissolved organic matter from two lowland rivers and their shared estuary"** to be considered for publication in *Science of the Total Environment*.

In this manuscript, we describe an investigation of the effect of seasonal changes in river flow and rainfall events on the flux of dissolved inorganic and organic nutrients at the lowest gauging stations of two UK rivers, the Hampshire Avon and the Stour, as well as from their shared estuary Christchurch Harbour. The two rivers differ in their catchment geology and therefore have different groundwater components to their total river discharge. These sites are also subject to high dissolved inorganic nitrogen loads and are at particular risk of complications such as eutrophication and hypoxia. We believe that readers of *Science of the Total Environment* will find this paper particularly valuable because it presents new high-frequency measurements of dissolved organic nutrients in rivers and an estuary subject to greatly elevated dissolved inorganic nitrogen loads. This is in contrast to the majority of publications where the rivers are typically much lower in their dissolved inorganic nitrogen concentrations, and hence adds an important insight to the carbon and nitrogen cycles within these systems as well as to the methodological complications potentially encountered in high-nutrient waters. The paper also considers the role of climate, catchment geology, sediment biogeochemistry and groundwater sources in the dynamics and export of both DOC and DON to coastal waters.

We hope that you will find it suitable for publication on your journal.
The material included in our manuscript constitutes an original research that is not under consideration by any other publication.

Yours sincerely,

**Anouska Panton**
Corresponding author
School of Ocean and Earth Science, University of Southampton, National Oceanography Centre, European Way, Southampton, UK SO14 3ZH
E-mail: Anouska.Panton@noc.soton.ac.uk

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*Title Page

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Our apologies. We combined two figures just before submission and failed to update the legends. This has now been rectified.

## Abstract

Terrestrially-derived dissolved organic carbon (DOC) and nitrogen (DON) transported by rivers have been recognised as contributors to aquatic nutrient burdens, and can be of importance in rivers and estuaries already impacted by anthropogenic inorganic nutrient discharges. The concentration of DOC and DON and the flux of both to the estuary and ultimately the coastal zone is dependent upon many factors including rainfall, catchment land use, and biological processes. DOC and DON concentrations together with nitrate plus nitrite and ammonium concentrations were measured in the anthropogenically-impacted estuary Christchurch Harbour (UK) and at sites in the lower reaches of its two source rivers, the Hampshire Avon and the Stour, at weekly intervals for a year during which time several extreme rainfall events occurred. A series of transects along the estuary were also performed after weekly sampling was completed. DOC concentrations were correlated between both rivers and the estuary and were positively related to increases in river flow, but DON concentrations revealed a more complicated picture. Peak instantaneous fluxes of DOC and DON exceeded 60000 kg C $d^{-1}$ and 7000 kg N $d^{-1}$ respectively both in the Stour and the estuary during high flow periods. The sources of both and routes by which they enter the aquatic system may account for the differences in dynamics, with flushing of superficial soils being a key source of DOC and point sources such as sewage treatment works being proposed as sources of DON. Removal processes within the estuary were also of importance for DON concentrations while DOC behaved more conservatively with some evidence of local production within the estuary. Estimated annual loads of DON and DOC to the coastal zone from Christchurch Harbour were 118 kg N $km^{-2}$ $y^{-1}$ and 2296 kg C $km^{-2}$ $y^{-1}$.


Keywords: DOC, DON, eutrophication, river flow, estuary, Christchurch Harbour

## 1. Introduction

Dissolved organic matter (DOM) is an important source of carbon and nitrogen to aquatic ecosystems and a great deal has been learnt about the role of DOM in global biogeochemical cycles over the last few decades (e.g. Yamanaka & Tajika 1997, Hansell & Carlson 2014 and refs within). Human activity, in particular the type of land use, has been shown to determine both the source and the composition of DOM, and new processes such as atmospheric deposition have been recognised as important inputs of both carbon and nitrogen to nutrient cycles (e.g. Cornell et al. 2003, Muller et al. 2008). The role of rivers in the transport of DOM to estuaries and coastal seas is also becoming increasingly better understood (e.g. Raymond et al. 2016, Casas-Ruiz et al. 2017, Drake et al. 2018). Once considered simply to be passive transporters of terrestrially-derived DOM from soils to the sea, rivers are now recognised as dynamic systems where both production and loss processes can potentially alter both the concentration and the composition of DOM during transport (Battin et al. 2008, Bertuzzo et al. 2017, Graeber et al. 2018, Harris et al. 2018). The balance of such processes can differ, however, even between reaches of the same river and hence the flux of both dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) is hard to predict (Wymore et al. 2018). The development of models and management tools to predict the composition of DOM entering rivers as well as to quantify the flux of carbon and nitrogen from riverine systems into estuaries has therefore been limited despite recent advances (e.g. Anderson et al. 2019, Yates et al. 2019). As it has also been demonstrated that terrestrial DOM is more bioavailable than previously believed (Autio et al. 2016; Wiegner et al. 2006), both DOC and DON are implicated as potential contributing factors to problems such as eutrophication and hypoxia within estuaries (Seitzinger and Sanders 1997; Paerl et al. 1998; Wiegner et al. 2009).

Stochastic rainfall events that lead to rapid and sustained increased river flow rates are expected to have a disproportionate impact on these estuarine nutrient burdens. For example, much of the export of DOM from soils to streams occurs during brief periods of high river flows following intense rainfall events (Inamdar & Mitchell 2007; Morel et al. 2009; Hitchcock & Mitrovic 2013). As these events are expected to increase in temperate latitudes over the coming century and beyond (IPCC

2014), it is important to understand now how they may impact upon concentrations of DOC and DON both within rivers and downstream in estuaries under different flow conditions. Much work has been done on the impact of rainstorms on DOC and DON concentrations in rivers and streams with various different types of watershed characteristics (e.g. Buffam et al. 2001; Inamdar & Mitchell 2007; Morel et al. 2009). The majority of these studies, however, have focussed on systems that have low inorganic nutrient loads (nitrogen typically <100 μM) with few studies investigating the role DOC and DON will play in rivers and estuaries already burdened by high inorganic nutrient loads and at imminent risk of eutrophication.

The Christchurch Harbour Macronutrients Project was designed to investigate the impact of stochastic rainfall events on the transport and biogeochemical cycling of macronutrients in two temperate UK south coast rivers, the Hampshire Avon and the Stour, as well as in their shared estuary, Christchurch Harbour (UK). The Hampshire Avon has been previously identified as a river of national importance due to its predominantly agricultural watershed and elevated inorganic nutrient loadings (mean nitrate concentration ≈ 400 μM; Jarvie et al. 2005). We present here a unique data set of frequent observations from the lowest river gauging stations and estuary impacted by high nitrate concentrations. The goals of our study were to ascertain the annual variability of DON and DOC in the context of high inorganic nitrogen loads, to examine the impact of rainfall events leading to rapid but sustained increases in river flow on the potential fate of DOC and DON, and to investigate the role of the shared estuary in determining the organic and inorganic nutrient flux into coastal waters.

## 2. Materials and methods

### 2.1. Study area

Christchurch Harbour is a shallow microtidal estuary on the south coast of England with a single outflow into the English Channel (Fig. 1). The mean tidal range during spring tides is 1.2 m, and the mean water depth outside of the main channel is approximately 0.5 m (Huggett et al. 2020). Two rivers, the Hampshire Avon (hereafter referred to simply as the Avon) and the Stour, drain into the estuary with a total catchment area of 2779 $km^2$. The mean flow of the Avon and Stour at the lowest

gauging stations on each river is 19.5 $m^3$ $s^{-1}$ and 13.8 $m^3$ $s^{-1}$ respectively (Centre for Ecology and Hydrology (CEH), 2008). A third small river, the Mude, drains into the estuary near the outlet (Fig. 1) but only has a mean flow of 0.1 $m^3$ $s^{-1}$ (CEH, 2008) and was not included in this study. The predominant land use types in the catchments of these two rivers are similar with over 75% of each catchment being a mixture of grassland and arable/horticultural land, but also with some woodland and small areas (1-2%) of heathland and urban areas (Table 1., CEH 2008). A catchment map with land use classification is available in the Supplementary Information (Supplementary Fig. 1). The geology between the two catchments does differ however with the Avon draining from predominantly chalk and the Stour draining from a mixture of chalk (50%) and clay (30%). These geological differences are reflected in their Baseflow Indices (BFI) – the Avon has a BFI of 0.90 indicating a high groundwater component in river discharge, and the Stour has a BFI of 0.65 suggesting that a lower proportion of river discharge originates from stored catchment sources (CEH 2008).

**2.2. Sampling regime**

Water samples were collected from Environment Agency gauging stations at Knapp Mill (50.744 N, -1.782 W) on the Avon and Throop (50.764 N, -1.842 W) on the Stour, as well as at Mudeford Quay (50.724 N, -1.7409 W) at the mouth of the Christchurch Harbour estuary at 5-8 day intervals between May 2013 and April 2014. The two river flow gauging stations were the closest to the estuary on each of the respective rivers located 12.7 km (Throop) and 6.2 km (Knapp Mill) upstream from the mouth of the estuary. Surface water was collected using a clean bucket and immediately decanted into acid-cleaned HDPE bottles for later inorganic nutrient analysis or into combusted (450°C for a minimum of 4 hours) glass bottles for total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) analysis. At each site surface water temperature and conductivity (salinity at the estuarine site) were measured *in situ* using an EXO2 multi-parameter sonde (Xylem, UK). At the estuary site, water temperature and salinity were also measured at depth just above the sediment. Samples from the estuary mouth were collected at low tide.

Boat transects were carried out at high tide in Christchurch Harbour fortnightly between 27$^{th}$ May 2014 and 4$^{th}$ September 2014 at 6 sites along a salinity gradient from the mouth of the estuary to an upstream site within the Stour (Fig. 1). A YSI 6600 sonde (Xylem, UK) was used to measure salinity

and temperature profiles and the depth of highest chlorophyll fluorescence was sampled using a 5 L Niskin bottle except on 27th May 2014 when surface water was sampled using a clean bucket.

On return to the lab, water samples for later nitrate ($NO_3^-$) plus nitrite ($NO_2^-$) analysis were filtered through a 25mm diameter GF/F filter using an inline syringe unit, preserved with 0.015M $HgCl_2$ (100 µL per 20mL), and stored in the dark at room temperature (Kirkwood 1996). Samples for DOC and TDN analysis were filtered through a combusted 47mm diameter Whatman GF/F filter (nominal pore size 0.7 µm) on an acid-washed glass filter rig under low vacuum (< 10 mmHg) and 20mL of filtrate stored in combusted glass vials with acid-washed Teflon septa. Phosphoric acid (60 µL of 50% (v/v)) was added to each sample vial before storing at 4°C (Badr et al. 2003). Samples for ammonium and urea analyses were also collected during this filtration and were pipetted into 25 mL push top glass vials. Reagents for ammonium ($NH_4^+$) and urea analyses were added immediately and vials were incubated at room temperature in the dark for up to 24 hours (ammonium) and for between 3 to 5 days (urea). Urea samples were not collected during the boat transects.

**2.3. Analytical methods**

Concentrations of nitrate plus nitrite were determined at the University of Portsmouth on a QuAAtro segmented flow nutrient analyser (SEAL Analytical, UK). Ammonium and urea concentrations were measured according to the method of Holmes et al. (1999) and Goeyens (1998) respectively at all sites from late August 2013. Dissolved inorganic nitrogen (DIN) concentrations were calculated by adding nitrate plus nitrite and ammonium concentrations. Concentrations of DOC and TDN were measured with a TOC-$V_{CPN}$ analyser (Shimadzu, Japan) calibrated with a mixed standard of potassium hydrogen phthalate and glycine. Certified Reference Materials (DSR from University of Miami, USA) were used to validate results by comparing against the certified concentrations for DOC (41-44 µM; analytical mean 45.8 µM) and TDN (31-33 µM; analytical mean 30.5 µM). Concentrations of dissolved organic nitrogen (DON) were quantified by subtracting DIN concentration from TDN concentration. DON data from the first 5 sampling dates are not available.

**2.4. Additional data and calculations**

Daily mean river flow data for both river sites was provided by the Environment Agency with data for the study period as well as historic flow data from the period 2000 - 2010 inclusive from which an

11-year daily mean flow was calculated for each river. Limited data for DOC and DIN concentration during the sampling period were also available from the Environment Agency for the Knapp Mill site only and are presented for comparison purposes. Rainfall data was acquired from the Meteorological Office station at Bisterne situated 6.3 kilometres north of Knapp Mill on the Avon. The flushing time of the estuary was calculated using a simple tidal prism method as described by Huggett et al. (2020). Instantaneous fluxes for the river sites were calculated by multiplying the measured concentration of each nutrient by the daily mean flow for the same day at that site. For the estuary, the daily mean flow for both rivers were summed together to determine the total daily mean flow and then multiplied by the nutrient concentrations measured at the estuary mouth. Annual fluxes were calculated by linearly interpolating between known concentration data points to obtain daily concentrations and then calculating flux as above before summing all daily values for each site. Annual fluxes were divided by catchment size to allow direct comparison of annual yield to other rivers and estuaries. The baseflow contribution to daily river flow was estimated using the smoothed minima technique of Gustard et al. (1992) as detailed in Jordan et al. (1997).

Nitrate and river flow data from the Avon weekly sampling campaign have previously been published in Pirani et al. (2016) where the data were combined with water quality and phosphate data to develop a model to determine past nutrient fluxes based on historical river flows.

## 3. Results

### 3.1. River flow and estuarine flushing time

Water temperature in both rivers and the estuary followed a seasonal cycle with minimum temperatures between 5.7 °C and 6.2 °C observed in winter and maximum summer temperatures reaching between 22.8 °C and 23 °C in July 2013 (data not shown).

Daily mean flow in both rivers decreased from the start of sampling (April 2013) to a summer low flow state equal to the estimated baseflow for each river by around mid-July 2013 and remained low until a sharp increase towards the end of October 2013 (Fig. 2a, b). This period of elevated flow lasted for approximately 3 weeks before flow decreased again during a dry period from mid-November to

mid-December 2013, although it remained above the summer low flow values. A second period of elevated flow started with sharp increases in flow in both rivers on the 16th December 2013 with sustained flows above both the 11-year mean and the highest flow values from the earlier period of elevated flow until the sampling finished on 10th April 2014 (total duration 150 d). Both periods of elevated flow were associated with increased rainfall locally (Fig. 2a). Flow data was unavailable at Knapp Mill for several days in early January 2014 after the gauging station was struck by lightning, but river flow rates both immediately before and after the loss of data were substantially elevated from background and so the flow was assumed to remain elevated across the period of missing data. Mean flow rates were more variable in the Stour than in the Avon, but mean flow during low flow periods was typically higher in the Avon. Daily mean flow rates for both rivers as well as the summed daily river flow and daily rainfall for the period of the estuarine transect sampling in summer 2014 also show an increase in river flow after rainfall events (Fig. 2c).

In each river, the daily mean flow during the study period was significantly different (Mann-Whitney U test, Avon $U$ 60449, Stour $U$ 58501, both $p < 0.05$) to the historic daily mean river flow from 11 years of Environment Agency data (Fig. 2a, b). These differences are particularly evident in the second elevated flow period where daily mean flows were up to 3 times greater in the Avon and 7 times higher in the Stour than the 11-year daily mean. Low rainfall in early December 2013 also lead to a period where mean daily flow was as low as 12% (Stour) or 28% (Avon) of the 11-year daily mean.

Flushing times within the estuary ranged from 0.1 days with combined river flows of 100 $m^3 s^{-1}$ to 1.5 days with minimum summer combined flows of 10 $m^3 s^{-1}$. The flushing time was consistently less than 1 day over the second elevated flow period from the 17th December 2013 until the end of sampling in April 2014.

### 3.2. Inorganic and organic nutrient concentrations

Concentrations of nitrate plus nitrite throughout the sampling period were higher in the Stour (mean 502 µM) than in the Avon (mean 381 µM) and at the estuary mouth (mean 328 µM; Fig. 3a-c). The highest concentrations at all sites were seen during the periods of decreasing flow following each of the elevated flow periods. The impact of low nutrient coastal waters can be seen in the estuary at

188 Mudeford over the periods of low river flow when nitrate plus nitrite concentrations are lower than in
189 either river (Fig. 3c). Spearman correlations revealed a significant relationship between DIN
190 concentrations in both rivers as well as between each river and the DIN concentration in the estuary (ρ
191 0.533 – 0.606, $p < 0.001$ for all). Ammonium concentrations ranged from < 1 µM to 9.8 µM and were
192 lower on a weekly basis in the Avon (mean 2.4 µM) than in the Stour (mean 3.8 µM) or the estuary
193 (mean 3.8 µM). Concentrations of ammonium were always < 2.5% of DIN in the estuary and < 1.8%
194 of DIN in the rivers.
195 DOC concentrations ranged from 167 – 486 µM (mean 249 µM) in the Avon, 156 – 1119 µM
196 (mean 353 µM) in the Stour, and 162 – 676 µM (mean 273 µM) in the estuary (Fig. 3d-f). A general
197 pattern of relatively low DOC concentrations (< 300 µM) was observed at all three sites (Fig. 3d-f)
198 between May and October 2013 before the first period of elevated river flow, and subsequently
199 concentrations at all sites increased during elevated river flow and later decreased as flow declined.
200 Again, Spearman correlations revealed a significant relationship between DOC concentrations in both
201 rivers as well as between each river and the DOC concentration in the estuary (ρ 0.680 – 0.769, $p <$
202 0.001 for all).
203 DON concentrations ranged from 0 – 83 µM (mean 32 µM) in the Avon, 0 – 155 µM (mean 40
204 µM) in the Stour, and 0 – 54 µM (mean 17 µM) in the estuary (Fig. 3g-i). There was no clear pattern
205 in DON concentration at any one of the sites and the response to elevated river flow events differed
206 between sites and between events. Concentrations of DON increased in both the Avon and the Stour
207 just prior to the start of the first elevated flow period, but this increase was not observed in the
208 estuary. Over the course of this first elevated flow period DON concentrations in both rivers
209 decreased and then increased steadily again, but in the estuary concentrations remained low. The
210 greatest concentrations in the estuary and in the Avon occurred on the same date (7$^{th}$ March 2014)
211 during the second elevated flow period, but the highest concentration of DON in the Stour was
212 observed during the lower flow conditions between the two elevated flow periods in early December
213 2013 (Fig 3h). Spearman correlation revealed a significant relationship between DON concentration
214 in both rivers (ρ 0.504, $p < 0.005$), but there was no relationship between either of the rivers and the

DON concentration in the estuary. The proportion of DON in TDN ranged from 0 – 26% across the three sites with a mean proportion of 5% in the estuary, 8% in the Avon, and 7% in the Stour.

Urea concentrations ranged from < 1 µM to 3.6 µM but there was no clear pattern between sites. Mean urea concentration was 1.2 µM in the Avon, 1.2 µM in the estuary, and 1.4 µM in the Stour.

### 3.3. Relationships with river flow

Overall there was a pattern of increasing DIN with river flow in both rivers up to a flow of approximately 25 $m^3 s^{-1}$ in the Stour and 35 $m^3 s^{-1}$ in the Avon, after which DIN concentration decreased as river flow increased further (Fig. 4a, b). DOC concentrations in both rivers increased to a peak as river flow increased at the lower range of river flows (up to approximately 25 $m^3 s^{-1}$ in the Avon and up to approximately 50 $m^3 s^{-1}$ in the Stour; Fig. 4c, d). Above these river flows there appears to be a positive relationship between river flow and DOC concentration. In the Avon the relationship between DON and river flow is complicated with one peak in DON concentration below 20 $m^3 s^{-1}$ and another peak at approximately 70 $m^3 s^{-1}$ (Fig. 4e). DON concentration in the Stour was highest at low river flow but there was a smaller second peak at approximately 60 $m^3 s^{-1}$ (Fig. 4f).

During dry periods the calculated baseflow is equal to or very close to the measured river flow, but rainfall events can decrease the proportion of measured flow contributed by baseflow (see Fig. 2). When DIN, DOC and DON are plotted against the proportion that baseflow contributes to measured flow then some relationships become clearer (Fig. 5). The concentration of DIN in both rivers appears to increase when the proportion of baseflow increases (Fig. 5a, b), whilst the opposite is true for DOC with the highest concentrations observed when baseflow is contributing less to river flow (Fig. 5c, d). Again, the variability in DON concentrations does not appear to have a clear relationship with baseflow contribution (Fig. 5e, f).

### 3.4. Estuarine results

In the estuary it is clear that periods of elevated river flow restricted the inflow of more saline coastal waters (Fig. 6). At a combined river flow of 40 $m^3 s^{-1}$ or greater the salinity at the mouth of the estuary (both surface and bottom) was typically less than 5 and frequently less than 1, resulting in Christchurch Harbour becoming essentially a freshwater lake under very high river flow conditions.

These total river flows correspond to all dates sampled from 17th December 2013 until the end of the weekly sampling programme and so reflect the second elevated flow period in its entirety.

Surface salinity within the estuary decreased from the mouth to the upstream sites. There is a clear conservative relationship between nitrate plus nitrite concentration and salinity in both the weekly sampled data at Mudeford (crosses; Fig. 7a) and the estuarine transects (coloured circles). Ammonium concentration is relatively low (< 3 μM) at a salinity of 20 or greater, but there is more variability at lower salinities (Fig. 7b). The dominance of nitrate and nitrite in this system is evident in the clear relationship between DIN and salinity (Fig. 7c). The relationships between DOC and salinity (Fig. 8a) and between DON and salinity (Fig .8b), however, are not as clear. In the weekly DOC samples and some of the estuarine transect samples there is the suggestion of a conservative relationship, but there are other estuarine samples with high concentrations of DOC at each end of the salinity range (e.g. 7th August 2014) or with peaks in the mid-salinity range (e.g. 10th July 2014). Interpretation of the DON data is complicated by the large number of values at concentrations below the limit of detection, but a general relationship between salinity and DON concentration appears to be present with lower DON at higher salinities (Fig. 8b, coloured circles).

### 3.5. DOC: nitrate ratios

Ratios of DOC: nitrate were less than 2.5 at all sites throughout the weekly sampling and the highest ratios (1.5 to 2) were observed during high flow periods in late October and in December/ January (Fig. 9a). DOC: nitrate ratios were also low across the estuarine transect sampling study with a mean ratio of 1.87 (Fig. 9b). Only 4 samples had a DOC: nitrate ratio > 3 and 3 of these samples occurred in the mid to low estuary on the same date (7th August 2014) when DOC concentrations were high and DIN concentrations were amongst the lowest observed. The maximum ratio observed on this date was 18.8 at a mid-estuary site.

### 3.6. Fluxes of DIN, DOC, and DON

When the instantaneous flux of DIN, DOC, or DON is calculated the importance of increased flow events becomes evident (Fig. 10, Supplemental Fig. 2). The maximum DIN fluxes of up to 80000 kg N $d^{-1}$ are observed in the estuary during the second elevated flow period, whereas the maximum values observed in the rivers at the same time reach only 45000 or 47000 kg N $d^{-1}$ respectively in the

Avon and the Stour. Peak fluxes of DOC (over 60000 kg C $d^{-1}$) are seen in both the Stour and the estuary over January-February 2014. Instantaneous DOC fluxes in the Avon occur around the same time but only reach half of these values at approximately 27000 kg C $d^{-1}$. Peak DON fluxes in the Avon reach 5400 kg N $d^{-1}$ in January 2014, but peaks in the Stour and the estuary both exceed 7000 kg N $d^{-1}$ in late February and early March 2014. Annual yields are shown in Table 2.

## 4. Discussion

Two periods of sustained rainfall with associated elevations in river flow were captured during the year of sampling with an initial wetting up period in late October 2013 being followed by a prolonged period of several months duration between December 2013 and March 2014. These events allow the dynamics of DIN, DOC and DON concentrations within the two rivers and the estuary to be examined under a range of hydrological conditions.

### 4.1. DIN dynamics and sources

Concentration of DIN in both rivers was consistently high (> 290 µM) throughout the study period, reinforcing the status of these rivers and the shared estuary as an impacted system. A substantial proportion of each catchment (> 35%) is used for arable or horticultural purposes, and thus a major source of DIN is likely to be agricultural in nature. Both Heppell et al. (2017) and Yates et al. (2019) report a positive relationship between the percentage of arable land use and nitrate or total nitrogen concentrations in the upper reaches of the Hampshire Avon catchment. Rainfall events should result in an increase in the flux of this diffuse-source DIN from land into the rivers (Withers & Lord 2002). The finding that DIN concentrations are high when river flow is dominated by baseflow, however, implies that there are also point sources contributing to the DIN load (e.g. effluent from sewage treatment works), or that the groundwater may also be high in DIN, and finally that in-stream processes such as macrophyte and microalgal uptake at these relatively downstream sites use only a small portion of the total DIN. Jarvie et al. (2005) calculated that the effluent load of nitrate to the Avon at Knapp Mill was around 11% of river load and also identified that groundwater is a major source of nitrate in the Avon. Approximately 75% of nitrate in U.K. groundwater is believed to be

297 from agricultural sources, although other sources such as atmospheric deposition, discharges or leaks
298 from septic tanks and sewers, and the spreading of sewage sludge on land may also contribute (Rivett
299 et al. 2007). In the Hampshire Chalk aquifer, isotopic analysis has shown that denitrification is an
300 insignificant process within the unsaturated zone and, as a result, the nitrate concentrations in
301 groundwater have been increasing since the 1970s (Rivett et al. 2007). Little has been published on
302 the water chemistry of the Stour but, as the Stour has a lower BFI and is less dependent on
303 groundwater contributions, the majority of the DIN is suspected to be from agricultural or sewage
304 treatment sources. The conservative relationship between DIN and salinity again suggests that
305 biological processing within the estuary has minimal impact on total DIN concentration with the
306 result that Christchurch Harbour exports the majority of the DIN to the coastal seas of the English
307 Channel.

308 **4.2. DOC dynamics and sources**

309 Whilst daily flow in the two rivers behaved differently with the Stour displaying greater variability
310 and more rapid fluctuations in flow than the Avon, the DOC concentrations in both rivers behaved in
311 a similar manner over the year as demonstrated by a significant Spearman correlation over time. Little
312 variability in concentration was observed during the lowest flow summer months, potentially
313 reflecting that instream production-loss processes at these downstream sites on each river
314 counterbalance any terrestrial inputs of fresh DOC (Creed et al. 2015). This proposed excess in DOC
315 was also observed in the estuarine concentrations over the same period with constant concentrations
316 throughout the summer baseflow period being transported to the coastal zone.

317 The DOC dynamics under elevated flow conditions throughout the system, however, show a
318 different pattern. Rapid increases in DOC concentration in both rivers as river flows increased and
319 during subsequent peaks within the longer period of prolonged high flows reflect flushing of
320 terrestrial organic matter, which is transported rapidly downstream by the increased flows,
321 subsequently escaping any significant upstream biogeochemical processing. The maximum DOC
322 concentration measured during these pulses in the Stour was more than double that measured in the
323 Avon, reflecting the different catchment characteristics of the two rivers. The Avon is groundwater-
324 dominated in a predominantly chalk catchment (Jarvie et al. 2005, Yates et al. 2016) whilst the Stour

has less permeable clay soils where surface runoff can result in rapid transport of DOC into the river. In contrast, groundwater DOC concentration in chalk aquifers is typically low. Rivett et al. (2007) reported a mean DOC concentration of 60.8 ± 19.2 µM from 1725 groundwater samples across the major Cretaceous Chalk aquifer in England, and Stuart and Lapworth (2016) determined the mean baseline concentration of groundwater DOC in Hampshire chalk at 67.8 µM. Point sources such as sewage treatment works (STWs) are present in both catchments with over 140 STWs and 30 fish farm discharges in the Avon catchment alone (Jarvie et al. 2005), and sources such as these are likely to have contributed to the rapid pulses in DOC observed. Other sources such as groundwater seepage and ditch drainage occur on longer timescales and may have contributed to the continued elevation of DOC concentrations during prolonged high river flow events (Morel et al. 2009). Atmospheric deposition from rainwater could also contribute to the DOC concentration, with studies reporting mean DOC concentrations in rainwater ranging from approximately 8 µM in mid-Wales (Wilkinson et al. 1997) to 50 µM in Greece (Pantelaki et al. 2018) and up to120 µM in the coastal United States (Willey et al. 2000). When DOC concentrations are plotted against the number of days since rainfall last occurred, it can be seen that concentrations are high within the first two days then stabilise at around 300 µM or lower at all sites (Fig. 11). This is further evidence that a major source of DOC to these rivers during rainfall events was the flushing of superficial soils. This is commonly seen in wetland systems rich in organic soils (Inamdar & Mitchell 2007; Worrall et al. 2012) but has also been observed in more agricultural catchments to the one studied here (Royer and David 2005; Morel et al. 2009).

Within the estuary there is some evidence of DOC production during the estuarine transect sampling, especially on 10$^{th}$ July 2014 where one mid-estuary sample stands out as higher than the surrounding samples. The chlorophyll *a* concentration on the same day at this site was 93 µg L$^{-1}$ and a dinoflagellate bloom was later confirmed using inverted microscopy. These production processes appear to remain relatively localised within the estuary, however, as concentrations both upstream and downstream of the site were over 100 µM lower. On the 7$^{th}$ August 2014 DOC concentrations were elevated throughout the estuary (Fig. 8a, green circles) but the lowest concentration was observed at mid-salinity. There was no associated increase in either chlorophyll *a* or river flow (data not shown).

353 Ammonium concentration was also relatively high on this date, especially at salinities between 10 and
354 15 which correspond to the upper estuary sites on that date. Discharge from Holdenhurst sewage
355 treatment works on the lower Stour (Fig. 1) could be the cause of this elevated DOC and ammonium
356 before mixing with the higher salinity waters (e.g. Maier et al. 2012). There may be evidence of
357 localised DOC production near the mouth of the estuary on this date also, as there is an increase in
358 DOC at a salinity of 32. There are shallow sand banks between sites 1 and 2 at the mouth of the
359 estuary. Sand is a highly permeable sediment and at depths of 1-2 m benthic production processes
360 could be tightly coupled to the water column (Huettel et al. 2014). The source of this DOC peak could
361 therefore be the sediments rather than water column processes with the incoming tide or waves
362 driving pore-water exchange and flushing the DOC into the water column (Huettel et al. 2014).
363 The mean estuarine flushing time estimated for low summer flows is around 1.5 days which may
364 be too short a period for significant uptake of DOC relative to the total concentration to occur within
365 the estuary before the DOC is exported to the coastal zone. Annual DOC yield was at the higher end
366 of ranges reported for global rivers and estuaries excluding the Nushagak River (Table 2), and is
367 comparable to the range of export values estimated by Worrall et al. (2012) and Jarvie et al. (2017) for
368 rivers in the United Kingdom based on land use characteristics and predominant soil types.
369 **4.3. DOC: nitrate relationship**
370 As the accumulation of nitrate in aquatic ecosystems has been identified as a major environmental
371 concern, the importance of carbon as an essential nutrient coupled to the microbial processing of
372 nitrate has become more evident (Taylor & Townsend 2010). The molar ratio of DOC: nitrate is being
373 increasingly used as an indicator for the potential fate of nitrate within a system (e.g. Sandford et al.
374 2013, Wymore et al. 2016, Heppell et al. 2017), with heterotrophic nitrogen assimilation proposed to
375 be carbon-limited at a DOC: nitrate threshold ratio of 3.5 across all systems (Taylor & Townsend
376 2010). During the weekly sampling the DOC: nitrate ratio was < 2.5 at all times and at all sites, and
377 the ratio only increased above 3.5 in the estuarine transects on 2 sampling dates (25$^{th}$ June 2014 and
378 7$^{th}$ August 2014). This would imply that in-stream processing of nitrate by heterotrophic organisms is
379 carbon limited throughout the majority of the year, and is further evidence that control of
380 anthropogenic nitrogen inputs to this system is needed. Interestingly the highest DOC: nitrate ratios

during the weekly sampling were observed during the high flow events over the winter period, implying that the increased DOC delivery to the river during rainfall events may have the potential to relieve some of the carbon limitation if nitrate concentrations were decreased. Localised areas of DOC production within the estuary in summer months, such as algal blooms, are enough to raise the ratio and may result in areas of nitrate drawdown.

**4.4. DON dynamics and sources**

In contrast to the DOC dynamics, DON concentrations showed greater variability between sites with a significant relationship observed between the two rivers but no relationship between either of the rivers and estuarine DON concentrations. Concentration-discharge plots (Fig. 4) for each river are suggestive of a point source, such as a sewage treatment works, acting as the major source of DON in the rivers with concentration generally decreasing with increased flow. As flow in each river increases past ~ 40 $m^3 s^{-1}$, however, concentrations increase, and this was particularly obvious in the Avon. This may reflect increased transport of DON from 'new' sources such as septic tanks connected via localised flooding. The highest concentration of DON in the Stour occurred at low river flow rates in early winter between the two high flow periods and at a time when relatively low water temperatures (7.4 °C) would be expected to limit biological production processes within the river. This is further evidence for an external source contributing to DON concentrations within the Stour.

DON concentrations were lower in the estuary over the dry summer months during the weekly sampling than in either river, reflecting the mixing of higher salinity low nutrient waters but also perhaps some DON removal in the lower reaches of the rivers and the estuary. The concentration range for DON in the estuary was higher during the summer boat transect work than during the weekly sampling campaign (Fig. 8b, coloured circles), and whilst the overall pattern appears to be one of decreasing concentration as salinity increases, there are certain dates that show interesting patterns. For example, the samples on 12th June and 25th June 2014 (Fig. 8b, white and red circles) both appear to have higher DON concentrations in the upper estuary at the 3 sites in lower salinity waters than would be expected in a simple conservative mixing relationship. This would imply that there is removal of DON within the estuary between sampling sites 4 and 3 (Figure 1). There are large areas of sand banks between these two sites and at the time combined river flows were relatively low at

around 20 to 25 $m^3s^{-1}$ which would result in an increase in the estuarine flushing time. The estuary is shallow and so, in addition to water column processes such as phytoplankton uptake, bacterial respiration, and photo-oxidation (Seitzinger & Sanders 1997; Wiegner et al. 2006; Badr et al. 2008), it is possible that these sandy sediments were acting as a sink for DON. Hopkinson et al. (1999) observed sediments, including sands, acting as a sink for DON in an estuary in Massachusetts with a degree of both temporal and spatial heterogeneity across a seasonal cycle, and Agedah et al. (2009) also report uptake of DON by sediments in the anthropogenically-impacted Colne estuary (UK). The rates reported by Agedah et al. (2009), however, were slow in relation to the residence time of the estuary and thus they proposed that the majority of DON in the system was exported to the coastal zone. In contrast Burdige and Zheng (1998) reported estuarine sediments in Chesapeake Bay to act as a source of DON to the water column. As DON concentrations were substantially lower than DOC or DIN concentrations throughout this study the impact of any potential removal or production process is more likely to be seen reflected in the total DON concentration at the estuary mouth. Unfortunately the data presented here is not sufficient to fully resolve the role of the sediments in the processing of DON in Christchurch Harbour, but further work is ongoing on sediment-water exchanges in this system.

A key feature of the two rivers studied was the very high concentrations of nitrate plus nitrite (> 290 µM) throughout the year. As the concentration of DON is derived by subtracting the DIN concentration from the corresponding total dissolved nitrogen concentration, it is possible to get values that are negative or below the limit of detection when DON concentrations are low relative to DIN. Vandenbruwane et al. (2007) found the likelihood of this occurring to be of particular importance in samples where the proportion of DON to TDN is ≤ 15%. The mean DON: TDN ratios in both rivers and the estuary were below 10% which may explain in part the number of DON samples that were found to be below the limits of detection in this study. This in turn may explain the lack of relationship observed between DON concentrations and flow. Yates et al. (2019) determined the proportion of DON to TDN in intensively farmed arable catchments underlain by chalk, such as our Avon catchment in particular, to be < 10%. However, the magnitude of the river flows during rainfall events still result in thousands of kilograms of nitrogen being transported to the estuary and

beyond on a daily basis as DON. While this flux was an order of magnitude lower than the flux of DIN in this system it is still a considerable yield of nitrogen when compared to many other global estuaries (Table 2).

There was no clear relationship found between DOC and DON at any of the sites or across all of the sites. The potential reasons for this are twofold – either the factors controlling DOC and DON dynamics in these systems are different, or the components of the DOM pool are utilised by the microbial community at different rates (Wiegner et al. 2006, 2009). Several studies have shown that the dynamics of DON and DOC within the same river can differ with DON being cycled faster within rivers than DOC (Stepanauskas et al. 2000; Solinger et al. 2001; Wiegner et al. 2006; Inamdar & Mitchell 2007). In addition the ultimate fate of the organic carbon and nitrogen can differ as it can be either incorporated into bacterial biomass or oxidised and excreted (Hopkinson et al. 1999).

### 4.5. The impact of rainfall events

The resolution of sampling in this study was not high enough to fully resolve the behaviour of DOC or DON within the Rivers Avon or Stour or within Christchurch Harbour during storms using hysteresis curves (e.g. Lloyd et al. 2016), but weekly sampling over the course of a year has demonstrated that local increases in rainfall result in sudden increases in both river flow and riverine DOC concentration and thus flux. Elevated river flows also increase the DIN flux to the coastal zone (Supplemental Fig. 2). DON dynamics are more complicated to resolve possibly due to the interplay of different sources as well as currently unidentified removal processes within the estuary. Whilst the river flows observed during the year of study could be considered atypical in comparison to the 11-year mean flows for each river, they are certainly relevant when considering the estimated fluxes of each river under future climate change conditions of drier summers and more frequent stochastic storm events (IPCC, 2014).

## 5. Conclusion

There are few studies detailing the yields of dissolved organic nutrients in rivers and estuaries that are already known to be anthropogenically-impacted with elevated inorganic nutrient loads, despite

their potential contribution to causing problems such as eutrophication and hypoxia. Annual yields of dissolved inorganic and organic nutrients at the lowest gauging stations of the Hampshire Avon and the Stour, as well as in their shared estuary of Christchurch Harbour, are comparable to and often greater than yields documented from other riverine and estuarine systems both within the UK and globally. Whilst the yield of DON was typically an order of magnitude smaller than the corresponding yield of nitrate plus nitrite at each site, the range of 118 -198 kg N $km^{-2}$ $y^{-1}$ is still an ecologically important load of nitrogen potentially available to the aquatic microbial community. The processes controlling the dynamics of DOC and DON differed in both the rivers and the estuary, highlighting the importance of considering the component parts of DOM when investigating the role of DOM in aquatic systems.

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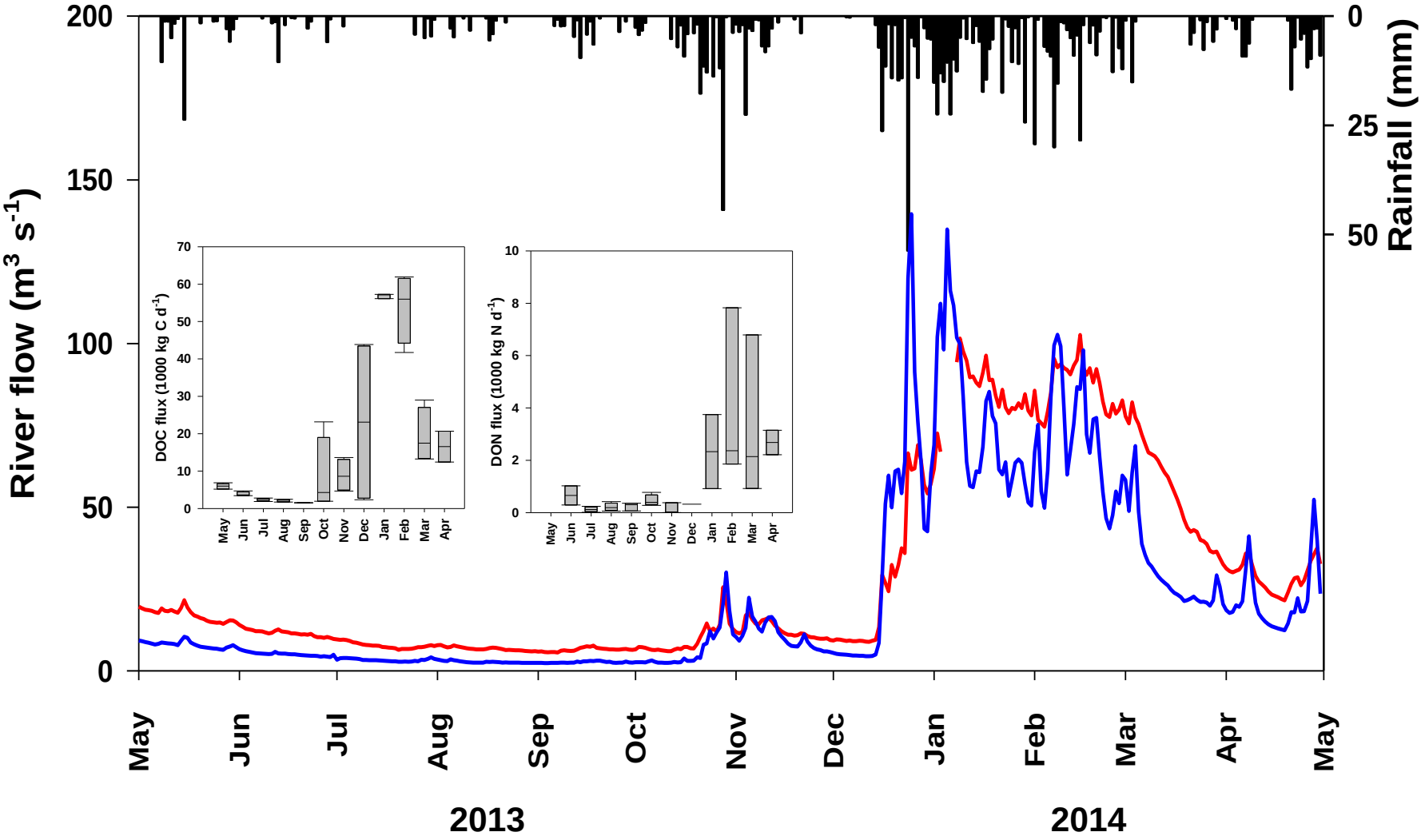
*Graphical Abstract



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

## Highlights

- Rapid transport of DOC from soils to river after rainfall events
- DOC and DON processing within estuary uncoupled
- DOC:nitrate < 3 in both rivers impacts microbial nitrate processing

## Abstract

Terrestrially-derived dissolved organic carbon (DOC) and nitrogen (DON) transported by rivers have been recognised as contributors to aquatic nutrient burdens, and can be of importance in rivers and estuaries already impacted by anthropogenic inorganic nutrient discharges. The concentration of DOC and DON and the flux of both to the estuary and ultimately the coastal zone is dependent upon many factors including rainfall, catchment land use, and biological processes. DOC and DON concentrations together with nitrate plus nitrite and ammonium concentrations were measured in the anthropogenically-impacted estuary Christchurch Harbour (UK) and at sites in the lower reaches of its two source rivers, the Hampshire Avon and the Stour, at weekly intervals for a year during which time several extreme rainfall events occurred. A series of transects along the estuary were also performed after weekly sampling was completed. DOC concentrations were correlated between both rivers and the estuary and were positively related to increases in river flow, but DON concentrations revealed a more complicated picture. Peak instantaneous fluxes of DOC and DON exceeded 60000 kg C $d^{-1}$ and 7000 kg N $d^{-1}$ respectively both in the Stour and the estuary during high flow periods. The sources of both and routes by which they enter the aquatic system may account for the differences in dynamics, with flushing of superficial soils being a key source of DOC and point sources such as sewage treatment works being proposed as sources of DON. Removal processes within the estuary were also of importance for DON concentrations while DOC behaved more conservatively with some evidence of local production within the estuary. Estimated annual loads of DON and DOC to the coastal zone from Christchurch Harbour were 118 kg N $km^{-2}$ $y^{-1}$ and 2296 kg C $km^{-2}$ $y^{-1}$.

Keywords: DOC, DON, eutrophication, river flow, estuary, Christchurch Harbour

## 1. Introduction

Dissolved organic matter (DOM) is an important source of carbon and nitrogen to aquatic ecosystems and a great deal has been learnt about the role of DOM in global biogeochemical cycles over the last few decades (e.g. Yamanaka & Tajika 1997, Hansell & Carlson 2014 and refs within). Human activity, in particular the type of land use, has been shown to determine both the source and the composition of DOM, and new processes such as atmospheric deposition have been recognised as important inputs of both carbon and nitrogen to nutrient cycles (e.g. Cornell et al. 2003, Muller et al. 2008). The role of rivers in the transport of DOM to estuaries and coastal seas is also becoming increasingly better understood (e.g. Raymond et al. 2016, Casas-Ruiz et al. 2017, Drake et al. 2018). Once considered simply to be passive transporters of terrestrially-derived DOM from soils to the sea, rivers are now recognised as dynamic systems where both production and loss processes can potentially alter both the concentration and the composition of DOM during transport (Battin et al. 2008, Bertuzzo et al. 2017, Graeber et al. 2018, Harris et al. 2018). The balance of such processes can differ, however, even between reaches of the same river and hence the flux of both dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) is hard to predict (Wymore et al. 2018). The development of models and management tools to predict the composition of DOM entering rivers as well as to quantify the flux of carbon and nitrogen from riverine systems into estuaries has therefore been limited despite recent advances (e.g. Anderson et al. 2019, Yates et al. 2019). As it has also been demonstrated that terrestrial DOM is more bioavailable than previously believed (Autio et al. 2016; Wiegner et al. 2006), both DOC and DON are implicated as potential contributing factors to problems such as eutrophication and hypoxia within estuaries (Seitzinger and Sanders 1997; Paerl et al. 1998; Wiegner et al. 2009).

Stochastic rainfall events that lead to rapid and sustained increased river flow rates are expected to have a disproportionate impact on these estuarine nutrient burdens. For example, much of the export of DOM from soils to streams occurs during brief periods of high river flows following intense rainfall events (Inamdar & Mitchell 2007; Morel et al. 2009; Hitchcock & Mitrovic 2013). As these events are expected to increase in temperate latitudes over the coming century and beyond (IPCC

2014), it is important to understand now how they may impact upon concentrations of DOC and DON both within rivers and downstream in estuaries under different flow conditions. Much work has been done on the impact of rainstorms on DOC and DON concentrations in rivers and streams with various different types of watershed characteristics (e.g. Buffam et al. 2001; Inamdar & Mitchell 2007; Morel et al. 2009). The majority of these studies, however, have focussed on systems that have low inorganic nutrient loads (nitrogen typically <100 μM) with few studies investigating the role DOC and DON will play in rivers and estuaries already burdened by high inorganic nutrient loads and at imminent risk of eutrophication.

The Christchurch Harbour Macronutrients Project was designed to investigate the impact of stochastic rainfall events on the transport and biogeochemical cycling of macronutrients in two temperate UK south coast rivers, the Hampshire Avon and the Stour, as well as in their shared estuary, Christchurch Harbour (UK). The Hampshire Avon has been previously identified as a river of national importance due to its predominantly agricultural watershed and elevated inorganic nutrient loadings (mean nitrate concentration ≈ 400 μM; Jarvie et al. 2005). We present here a unique data set of frequent observations from the lowest river gauging stations and estuary impacted by high nitrate concentrations. The goals of our study were to ascertain the annual variability of DON and DOC in the context of high inorganic nitrogen loads, to examine the impact of rainfall events leading to rapid but sustained increases in river flow on the potential fate of DOC and DON, and to investigate the role of the shared estuary in determining the organic and inorganic nutrient flux into coastal waters.

## 2. Materials and methods

### 2.1. Study area

Christchurch Harbour is a shallow microtidal estuary on the south coast of England with a single outflow into the English Channel (Fig. 1). The mean tidal range during spring tides is 1.2 m, and the mean water depth outside of the main channel is approximately 0.5 m (Huggett et al. 2020). Two rivers, the Hampshire Avon (hereafter referred to simply as the Avon) and the Stour, drain into the estuary with a total catchment area of 2779 $km^2$. The mean flow of the Avon and Stour at the lowest

77 gauging stations on each river is 19.5 $m^3 s^{-1}$ and 13.8 $m^3 s^{-1}$ respectively (Centre for Ecology and
78 Hydrology (CEH), 2008). A third small river, the Mude, drains into the estuary near the outlet (Fig. 1)
79 but only has a mean flow of 0.1 $m^3 s^{-1}$ (CEH, 2008) and was not included in this study. The
80 predominant land use types in the catchments of these two rivers are similar with over 75% of each
81 catchment being a mixture of grassland and arable/horticultural land, but also with some woodland
82 and small areas (1-2%) of heathland and urban areas (Table 1., CEH 2008). A catchment map with
83 land use classification is available in the Supplementary Information (Supplementary Fig. 1). The
84 geology between the two catchments does differ however with the Avon draining from predominantly
85 chalk and the Stour draining from a mixture of chalk (50%) and clay (30%). These geological
86 differences are reflected in their Baseflow Indices (BFI) – the Avon has a BFI of 0.90 indicating a
87 high groundwater component in river discharge, and the Stour has a BFI of 0.65 suggesting that a
88 lower proportion of river discharge originates from stored catchment sources (CEH 2008).

89 **2.2. Sampling regime**

90 Water samples were collected from Environment Agency gauging stations at Knapp Mill (50.744
91 N, -1.782 W) on the Avon and Throop (50.764 N, -1.842 W) on the Stour, as well as at Mudeford
92 Quay (50.724 N, -1.7409 W) at the mouth of the Christchurch Harbour estuary at 5-8 day intervals
93 between May 2013 and April 2014. The two river flow gauging stations were the closest to the
94 estuary on each of the respective rivers located 12.7 km (Throop) and 6.2 km (Knapp Mill) upstream
95 from the mouth of the estuary. Surface water was collected using a clean bucket and immediately
96 decanted into acid-cleaned HDPE bottles for later inorganic nutrient analysis or into combusted
97 (450°C for a minimum of 4 hours) glass bottles for total dissolved nitrogen (TDN) and dissolved
98 organic carbon (DOC) analysis. At each site surface water temperature and conductivity (salinity at
99 the estuarine site) were measured *in situ* using an EXO2 multi-parameter sonde (Xylem, UK). At the
100 estuary site, water temperature and salinity were also measured at depth just above the sediment.
101 Samples from the estuary mouth were collected at low tide.

102 Boat transects were carried out at high tide in Christchurch Harbour fortnightly between 27$^{th}$ May
103 2014 and 4$^{th}$ September 2014 at 6 sites along a salinity gradient from the mouth of the estuary to an
104 upstream site within the Stour (Fig. 1). A YSI 6600 sonde (Xylem, UK) was used to measure salinity

and temperature profiles and the depth of highest chlorophyll fluorescence was sampled using a 5 L Niskin bottle except on 27th May 2014 when surface water was sampled using a clean bucket.

On return to the lab, water samples for later nitrate ($NO_3^-$) plus nitrite ($NO_2^-$) analysis were filtered through a 25mm diameter GF/F filter using an inline syringe unit, preserved with 0.015M $HgCl_2$ (100 µL per 20mL), and stored in the dark at room temperature (Kirkwood 1996). Samples for DOC and TDN analysis were filtered through a combusted 47mm diameter Whatman GF/F filter (nominal pore size 0.7 µm) on an acid-washed glass filter rig under low vacuum (< 10 mmHg) and 20mL of filtrate stored in combusted glass vials with acid-washed Teflon septa. Phosphoric acid (60 µL of 50% (v/v)) was added to each sample vial before storing at 4°C (Badr et al. 2003). Samples for ammonium and urea analyses were also collected during this filtration and were pipetted into 25 mL push top glass vials. Reagents for ammonium ($NH_4^+$) and urea analyses were added immediately and vials were incubated at room temperature in the dark for up to 24 hours (ammonium) and for between 3 to 5 days (urea). Urea samples were not collected during the boat transects.

**2.3. Analytical methods**

Concentrations of nitrate plus nitrite were determined at the University of Portsmouth on a QuAAtro segmented flow nutrient analyser (SEAL Analytical, UK). Ammonium and urea concentrations were measured according to the method of Holmes et al. (1999) and Goeyens (1998) respectively at all sites from late August 2013. Dissolved inorganic nitrogen (DIN) concentrations were calculated by adding nitrate plus nitrite and ammonium concentrations. Concentrations of DOC and TDN were measured with a TOC-$V_{CPN}$ analyser (Shimadzu, Japan) calibrated with a mixed standard of potassium hydrogen phthalate and glycine. Certified Reference Materials (DSR from University of Miami, USA) were used to validate results by comparing against the certified concentrations for DOC (41-44 µM; analytical mean 45.8 µM) and TDN (31-33 µM; analytical mean 30.5 µM). Concentrations of dissolved organic nitrogen (DON) were quantified by subtracting DIN concentration from TDN concentration. DON data from the first 5 sampling dates are not available.

**2.4. Additional data and calculations**

Daily mean river flow data for both river sites was provided by the Environment Agency with data for the study period as well as historic flow data from the period 2000 - 2010 inclusive from which an

11-year daily mean flow was calculated for each river. Limited data for DOC and DIN concentration during the sampling period were also available from the Environment Agency for the Knapp Mill site only and are presented for comparison purposes. Rainfall data was acquired from the Meteorological Office station at Bisterne situated 6.3 kilometres north of Knapp Mill on the Avon. The flushing time of the estuary was calculated using a simple tidal prism method as described by Huggett et al. (2020). Instantaneous fluxes for the river sites were calculated by multiplying the measured concentration of each nutrient by the daily mean flow for the same day at that site. For the estuary, the daily mean flow for both rivers were summed together to determine the total daily mean flow and then multiplied by the nutrient concentrations measured at the estuary mouth. Annual fluxes were calculated by linearly interpolating between known concentration data points to obtain daily concentrations and then calculating flux as above before summing all daily values for each site. Annual fluxes were divided by catchment size to allow direct comparison of annual yield to other rivers and estuaries. The baseflow contribution to daily river flow was estimated using the smoothed minima technique of Gustard et al. (1992) as detailed in Jordan et al. (1997).

Nitrate and river flow data from the Avon weekly sampling campaign have previously been published in Pirani et al. (2016) where the data were combined with water quality and phosphate data to develop a model to determine past nutrient fluxes based on historical river flows.

## 3. Results

### 3.1. River flow and estuarine flushing time

Water temperature in both rivers and the estuary followed a seasonal cycle with minimum temperatures between 5.7 °C and 6.2 °C observed in winter and maximum summer temperatures reaching between 22.8 °C and 23 °C in July 2013 (data not shown).

Daily mean flow in both rivers decreased from the start of sampling (April 2013) to a summer low flow state equal to the estimated baseflow for each river by around mid-July 2013 and remained low until a sharp increase towards the end of October 2013 (Fig. 2a, b). This period of elevated flow lasted for approximately 3 weeks before flow decreased again during a dry period from mid-November to

mid-December 2013, although it remained above the summer low flow values. A second period of elevated flow started with sharp increases in flow in both rivers on the 16th December 2013 with sustained flows above both the 11-year mean and the highest flow values from the earlier period of elevated flow until the sampling finished on 10th April 2014 (total duration 150 d). Both periods of elevated flow were associated with increased rainfall locally (Fig. 2a). Flow data was unavailable at Knapp Mill for several days in early January 2014 after the gauging station was struck by lightning, but river flow rates both immediately before and after the loss of data were substantially elevated from background and so the flow was assumed to remain elevated across the period of missing data. Mean flow rates were more variable in the Stour than in the Avon, but mean flow during low flow periods was typically higher in the Avon. Daily mean flow rates for both rivers as well as the summed daily river flow and daily rainfall for the period of the estuarine transect sampling in summer 2014 also show an increase in river flow after rainfall events (Fig. 2c).

In each river, the daily mean flow during the study period was significantly different (Mann-Whitney U test, Avon $U$ 60449, Stour $U$ 58501, both $p < 0.05$) to the historic daily mean river flow from 11 years of Environment Agency data (Fig. 2a, b). These differences are particularly evident in the second elevated flow period where daily mean flows were up to 3 times greater in the Avon and 7 times higher in the Stour than the 11-year daily mean. Low rainfall in early December 2013 also lead to a period where mean daily flow was as low as 12% (Stour) or 28% (Avon) of the 11-year daily mean.

Flushing times within the estuary ranged from 0.1 days with combined river flows of 100 $m^3 s^{-1}$ to 1.5 days with minimum summer combined flows of 10 $m^3 s^{-1}$. The flushing time was consistently less than 1 day over the second elevated flow period from the 17th December 2013 until the end of sampling in April 2014.

### 3.2. Inorganic and organic nutrient concentrations

Concentrations of nitrate plus nitrite throughout the sampling period were higher in the Stour (mean 502 µM) than in the Avon (mean 381 µM) and at the estuary mouth (mean 328 µM; Fig. 3a-c). The highest concentrations at all sites were seen during the periods of decreasing flow following each of the elevated flow periods. The impact of low nutrient coastal waters can be seen in the estuary at

188 Mudeford over the periods of low river flow when nitrate plus nitrite concentrations are lower than in
189 either river (Fig. 3c). Spearman correlations revealed a significant relationship between DIN
190 concentrations in both rivers as well as between each river and the DIN concentration in the estuary (ρ
191 0.533 – 0.606, $p < 0.001$ for all). Ammonium concentrations ranged from < 1 µM to 9.8 µM and were
192 lower on a weekly basis in the Avon (mean 2.4 µM) than in the Stour (mean 3.8 µM) or the estuary
193 (mean 3.8 µM). Concentrations of ammonium were always < 2.5% of DIN in the estuary and < 1.8%
194 of DIN in the rivers.
195 DOC concentrations ranged from 167 – 486 µM (mean 249 µM) in the Avon, 156 – 1119 µM
196 (mean 353 µM) in the Stour, and 162 – 676 µM (mean 273 µM) in the estuary (Fig. 3d-f). A general
197 pattern of relatively low DOC concentrations (< 300 µM) was observed at all three sites (Fig. 3d-f)
198 between May and October 2013 before the first period of elevated river flow, and subsequently
199 concentrations at all sites increased during elevated river flow and later decreased as flow declined.
200 Again, Spearman correlations revealed a significant relationship between DOC concentrations in both
201 rivers as well as between each river and the DOC concentration in the estuary (ρ 0.680 – 0.769, $p <$
202 0.001 for all).
203 DON concentrations ranged from 0 – 83 µM (mean 32 µM) in the Avon, 0 – 155 µM (mean 40
204 µM) in the Stour, and 0 – 54 µM (mean 17 µM) in the estuary (Fig. 3g-i). There was no clear pattern
205 in DON concentration at any one of the sites and the response to elevated river flow events differed
206 between sites and between events. Concentrations of DON increased in both the Avon and the Stour
207 just prior to the start of the first elevated flow period, but this increase was not observed in the
208 estuary. Over the course of this first elevated flow period DON concentrations in both rivers
209 decreased and then increased steadily again, but in the estuary concentrations remained low. The
210 greatest concentrations in the estuary and in the Avon occurred on the same date (7$^{th}$ March 2014)
211 during the second elevated flow period, but the highest concentration of DON in the Stour was
212 observed during the lower flow conditions between the two elevated flow periods in early December
213 2013 (Fig 3h). Spearman correlation revealed a significant relationship between DON concentration
214 in both rivers (ρ 0.504, $p < 0.005$), but there was no relationship between either of the rivers and the

DON concentration in the estuary. The proportion of DON in TDN ranged from 0 – 26% across the three sites with a mean proportion of 5% in the estuary, 8% in the Avon, and 7% in the Stour.

Urea concentrations ranged from < 1 µM to 3.6 µM but there was no clear pattern between sites. Mean urea concentration was 1.2 µM in the Avon, 1.2 µM in the estuary, and 1.4 µM in the Stour.

### 3.3. Relationships with river flow

Overall there was a pattern of increasing DIN with river flow in both rivers up to a flow of approximately 25 $m^3 s^{-1}$ in the Stour and 35 $m^3 s^{-1}$ in the Avon, after which DIN concentration decreased as river flow increased further (Fig. 4a, b). DOC concentrations in both rivers increased to a peak as river flow increased at the lower range of river flows (up to approximately 25 $m^3 s^{-1}$ in the Avon and up to approximately 50 $m^3 s^{-1}$ in the Stour; Fig. 4c, d). Above these river flows there appears to be a positive relationship between river flow and DOC concentration. In the Avon the relationship between DON and river flow is complicated with one peak in DON concentration below 20 $m^3 s^{-1}$ and another peak at approximately 70 $m^3 s^{-1}$ (Fig. 4e). DON concentration in the Stour was highest at low river flow but there was a smaller second peak at approximately 60 $m^3 s^{-1}$ (Fig. 4f).

During dry periods the calculated baseflow is equal to or very close to the measured river flow, but rainfall events can decrease the proportion of measured flow contributed by baseflow (see Fig. 2). When DIN, DOC and DON are plotted against the proportion that baseflow contributes to measured flow then some relationships become clearer (Fig. 5). The concentration of DIN in both rivers appears to increase when the proportion of baseflow increases (Fig. 5a, b), whilst the opposite is true for DOC with the highest concentrations observed when baseflow is contributing less to river flow (Fig. 5c, d). Again, the variability in DON concentrations does not appear to have a clear relationship with baseflow contribution (Fig. 5e, f).

### 3.4. Estuarine results

In the estuary it is clear that periods of elevated river flow restricted the inflow of more saline coastal waters (Fig. 6). At a combined river flow of 40 $m^3 s^{-1}$ or greater the salinity at the mouth of the estuary (both surface and bottom) was typically less than 5 and frequently less than 1, resulting in Christchurch Harbour becoming essentially a freshwater lake under very high river flow conditions.

These total river flows correspond to all dates sampled from 17th December 2013 until the end of the weekly sampling programme and so reflect the second elevated flow period in its entirety.

Surface salinity within the estuary decreased from the mouth to the upstream sites. There is a clear conservative relationship between nitrate plus nitrite concentration and salinity in both the weekly sampled data at Mudeford (crosses; Fig. 7a) and the estuarine transects (coloured circles). Ammonium concentration is relatively low (< 3 μM) at a salinity of 20 or greater, but there is more variability at lower salinities (Fig. 7b). The dominance of nitrate and nitrite in this system is evident in the clear relationship between DIN and salinity (Fig. 7c). The relationships between DOC and salinity (Fig. 8a) and between DON and salinity (Fig .8b), however, are not as clear. In the weekly DOC samples and some of the estuarine transect samples there is the suggestion of a conservative relationship, but there are other estuarine samples with high concentrations of DOC at each end of the salinity range (e.g. 7th August 2014) or with peaks in the mid-salinity range (e.g. 10th July 2014). Interpretation of the DON data is complicated by the large number of values at concentrations below the limit of detection, but a general relationship between salinity and DON concentration appears to be present with lower DON at higher salinities (Fig. 8b, coloured circles).

### 3.5. DOC: nitrate ratios

Ratios of DOC: nitrate were less than 2.5 at all sites throughout the weekly sampling and the highest ratios (1.5 to 2) were observed during high flow periods in late October and in December/ January (Fig. 9a). DOC: nitrate ratios were also low across the estuarine transect sampling study with a mean ratio of 1.87 (Fig. 9b). Only 4 samples had a DOC: nitrate ratio > 3 and 3 of these samples occurred in the mid to low estuary on the same date (7th August 2014) when DOC concentrations were high and DIN concentrations were amongst the lowest observed. The maximum ratio observed on this date was 18.8 at a mid-estuary site.

### 3.6. Fluxes of DIN, DOC, and DON

When the instantaneous flux of DIN, DOC, or DON is calculated the importance of increased flow events becomes evident (Fig. 10, Supplemental Fig. 2). The maximum DIN fluxes of up to 80000 kg N $d^{-1}$ are observed in the estuary during the second elevated flow period, whereas the maximum values observed in the rivers at the same time reach only 45000 or 47000 kg N $d^{-1}$ respectively in the

Avon and the Stour. Peak fluxes of DOC (over 60000 kg C $d^{-1}$) are seen in both the Stour and the estuary over January-February 2014. Instantaneous DOC fluxes in the Avon occur around the same time but only reach half of these values at approximately 27000 kg C $d^{-1}$. Peak DON fluxes in the Avon reach 5400 kg N $d^{-1}$ in January 2014, but peaks in the Stour and the estuary both exceed 7000 kg N $d^{-1}$ in late February and early March 2014. Annual yields are shown in Table 2.

## 4. Discussion

Two periods of sustained rainfall with associated elevations in river flow were captured during the year of sampling with an initial wetting up period in late October 2013 being followed by a prolonged period of several months duration between December 2013 and March 2014. These events allow the dynamics of DIN, DOC and DON concentrations within the two rivers and the estuary to be examined under a range of hydrological conditions.

### 4.1. DIN dynamics and sources

Concentration of DIN in both rivers was consistently high (> 290 µM) throughout the study period, reinforcing the status of these rivers and the shared estuary as an impacted system. A substantial proportion of each catchment (> 35%) is used for arable or horticultural purposes, and thus a major source of DIN is likely to be agricultural in nature. Both Heppell et al. (2017) and Yates et al. (2019) report a positive relationship between the percentage of arable land use and nitrate or total nitrogen concentrations in the upper reaches of the Hampshire Avon catchment. Rainfall events should result in an increase in the flux of this diffuse-source DIN from land into the rivers (Withers & Lord 2002). The finding that DIN concentrations are high when river flow is dominated by baseflow, however, implies that there are also point sources contributing to the DIN load (e.g. effluent from sewage treatment works), or that the groundwater may also be high in DIN, and finally that in-stream processes such as macrophyte and microalgal uptake at these relatively downstream sites use only a small portion of the total DIN. Jarvie et al. (2005) calculated that the effluent load of nitrate to the Avon at Knapp Mill was around 11% of river load and also identified that groundwater is a major source of nitrate in the Avon. Approximately 75% of nitrate in U.K. groundwater is believed to be

297 from agricultural sources, although other sources such as atmospheric deposition, discharges or leaks
298 from septic tanks and sewers, and the spreading of sewage sludge on land may also contribute (Rivett
299 et al. 2007). In the Hampshire Chalk aquifer, isotopic analysis has shown that denitrification is an
300 insignificant process within the unsaturated zone and, as a result, the nitrate concentrations in
301 groundwater have been increasing since the 1970s (Rivett et al. 2007). Little has been published on
302 the water chemistry of the Stour but, as the Stour has a lower BFI and is less dependent on
303 groundwater contributions, the majority of the DIN is suspected to be from agricultural or sewage
304 treatment sources. The conservative relationship between DIN and salinity again suggests that
305 biological processing within the estuary has minimal impact on total DIN concentration with the
306 result that Christchurch Harbour exports the majority of the DIN to the coastal seas of the English
307 Channel.

308 **4.2. DOC dynamics and sources**

309 Whilst daily flow in the two rivers behaved differently with the Stour displaying greater variability
310 and more rapid fluctuations in flow than the Avon, the DOC concentrations in both rivers behaved in
311 a similar manner over the year as demonstrated by a significant Spearman correlation over time. Little
312 variability in concentration was observed during the lowest flow summer months, potentially
313 reflecting that instream production-loss processes at these downstream sites on each river
314 counterbalance any terrestrial inputs of fresh DOC (Creed et al. 2015). This proposed excess in DOC
315 was also observed in the estuarine concentrations over the same period with constant concentrations
316 throughout the summer baseflow period being transported to the coastal zone.

317 The DOC dynamics under elevated flow conditions throughout the system, however, show a
318 different pattern. Rapid increases in DOC concentration in both rivers as river flows increased and
319 during subsequent peaks within the longer period of prolonged high flows reflect flushing of
320 terrestrial organic matter, which is transported rapidly downstream by the increased flows,
321 subsequently escaping any significant upstream biogeochemical processing. The maximum DOC
322 concentration measured during these pulses in the Stour was more than double that measured in the
323 Avon, reflecting the different catchment characteristics of the two rivers. The Avon is groundwater-
324 dominated in a predominantly chalk catchment (Jarvie et al. 2005, Yates et al. 2016) whilst the Stour

has less permeable clay soils where surface runoff can result in rapid transport of DOC into the river. In contrast, groundwater DOC concentration in chalk aquifers is typically low. Rivett et al. (2007) reported a mean DOC concentration of 60.8 ± 19.2 µM from 1725 groundwater samples across the major Cretaceous Chalk aquifer in England, and Stuart and Lapworth (2016) determined the mean baseline concentration of groundwater DOC in Hampshire chalk at 67.8 µM. Point sources such as sewage treatment works (STWs) are present in both catchments with over 140 STWs and 30 fish farm discharges in the Avon catchment alone (Jarvie et al. 2005), and sources such as these are likely to have contributed to the rapid pulses in DOC observed. Other sources such as groundwater seepage and ditch drainage occur on longer timescales and may have contributed to the continued elevation of DOC concentrations during prolonged high river flow events (Morel et al. 2009). Atmospheric deposition from rainwater could also contribute to the DOC concentration, with studies reporting mean DOC concentrations in rainwater ranging from approximately 8 µM in mid-Wales (Wilkinson et al. 1997) to 50 µM in Greece (Pantelaki et al. 2018) and up to120 µM in the coastal United States (Willey et al. 2000). When DOC concentrations are plotted against the number of days since rainfall last occurred, it can be seen that concentrations are high within the first two days then stabilise at around 300 µM or lower at all sites (Fig. 11). This is further evidence that a major source of DOC to these rivers during rainfall events was the flushing of superficial soils. This is commonly seen in wetland systems rich in organic soils (Inamdar & Mitchell 2007; Worrall et al. 2012) but has also been observed in more agricultural catchments to the one studied here (Royer and David 2005; Morel et al. 2009).

Within the estuary there is some evidence of DOC production during the estuarine transect sampling, especially on 10$^{th}$ July 2014 where one mid-estuary sample stands out as higher than the surrounding samples. The chlorophyll *a* concentration on the same day at this site was 93 µg $L^{-1}$ and a dinoflagellate bloom was later confirmed using inverted microscopy. These production processes appear to remain relatively localised within the estuary, however, as concentrations both upstream and downstream of the site were over 100 µM lower. On the 7$^{th}$ August 2014 DOC concentrations were elevated throughout the estuary (Fig. 8a, green circles) but the lowest concentration was observed at mid-salinity. There was no associated increase in either chlorophyll *a* or river flow (data not shown).

353 Ammonium concentration was also relatively high on this date, especially at salinities between 10 and
354 15 which correspond to the upper estuary sites on that date. Discharge from Holdenhurst sewage
355 treatment works on the lower Stour (Fig. 1) could be the cause of this elevated DOC and ammonium
356 before mixing with the higher salinity waters (e.g. Maier et al. 2012). There may be evidence of
357 localised DOC production near the mouth of the estuary on this date also, as there is an increase in
358 DOC at a salinity of 32. There are shallow sand banks between sites 1 and 2 at the mouth of the
359 estuary. Sand is a highly permeable sediment and at depths of 1-2 m benthic production processes
360 could be tightly coupled to the water column (Huettel et al. 2014). The source of this DOC peak could
361 therefore be the sediments rather than water column processes with the incoming tide or waves
362 driving pore-water exchange and flushing the DOC into the water column (Huettel et al. 2014).
363 The mean estuarine flushing time estimated for low summer flows is around 1.5 days which may
364 be too short a period for significant uptake of DOC relative to the total concentration to occur within
365 the estuary before the DOC is exported to the coastal zone. Annual DOC yield was at the higher end
366 of ranges reported for global rivers and estuaries excluding the Nushagak River (Table 2), and is
367 comparable to the range of export values estimated by Worrall et al. (2012) and Jarvie et al. (2017) for
368 rivers in the United Kingdom based on land use characteristics and predominant soil types.
369 **4.3. DOC: nitrate relationship**
370 As the accumulation of nitrate in aquatic ecosystems has been identified as a major environmental
371 concern, the importance of carbon as an essential nutrient coupled to the microbial processing of
372 nitrate has become more evident (Taylor & Townsend 2010). The molar ratio of DOC: nitrate is being
373 increasingly used as an indicator for the potential fate of nitrate within a system (e.g. Sandford et al.
374 2013, Wymore et al. 2016, Heppell et al. 2017), with heterotrophic nitrogen assimilation proposed to
375 be carbon-limited at a DOC: nitrate threshold ratio of 3.5 across all systems (Taylor & Townsend
376 2010). During the weekly sampling the DOC: nitrate ratio was < 2.5 at all times and at all sites, and
377 the ratio only increased above 3.5 in the estuarine transects on 2 sampling dates (25$^{th}$ June 2014 and
378 7$^{th}$ August 2014). This would imply that in-stream processing of nitrate by heterotrophic organisms is
379 carbon limited throughout the majority of the year, and is further evidence that control of
380 anthropogenic nitrogen inputs to this system is needed. Interestingly the highest DOC: nitrate ratios

during the weekly sampling were observed during the high flow events over the winter period, implying that the increased DOC delivery to the river during rainfall events may have the potential to relieve some of the carbon limitation if nitrate concentrations were decreased. Localised areas of DOC production within the estuary in summer months, such as algal blooms, are enough to raise the ratio and may result in areas of nitrate drawdown.

**4.4. DON dynamics and sources**

In contrast to the DOC dynamics, DON concentrations showed greater variability between sites with a significant relationship observed between the two rivers but no relationship between either of the rivers and estuarine DON concentrations. Concentration-discharge plots (Fig. 4) for each river are suggestive of a point source, such as a sewage treatment works, acting as the major source of DON in the rivers with concentration generally decreasing with increased flow. As flow in each river increases past ~ 40 $m^3 s^{-1}$, however, concentrations increase, and this was particularly obvious in the Avon. This may reflect increased transport of DON from 'new' sources such as septic tanks connected via localised flooding. The highest concentration of DON in the Stour occurred at low river flow rates in early winter between the two high flow periods and at a time when relatively low water temperatures (7.4 °C) would be expected to limit biological production processes within the river. This is further evidence for an external source contributing to DON concentrations within the Stour.

DON concentrations were lower in the estuary over the dry summer months during the weekly sampling than in either river, reflecting the mixing of higher salinity low nutrient waters but also perhaps some DON removal in the lower reaches of the rivers and the estuary. The concentration range for DON in the estuary was higher during the summer boat transect work than during the weekly sampling campaign (Fig. 8b, coloured circles), and whilst the overall pattern appears to be one of decreasing concentration as salinity increases, there are certain dates that show interesting patterns. For example, the samples on 12$^{th}$ June and 25$^{th}$ June 2014 (Fig. 8b, white and red circles) both appear to have higher DON concentrations in the upper estuary at the 3 sites in lower salinity waters than would be expected in a simple conservative mixing relationship. This would imply that there is removal of DON within the estuary between sampling sites 4 and 3 (Figure 1). There are large areas of sand banks between these two sites and at the time combined river flows were relatively low at

around 20 to 25 $m^3s^{-1}$ which would result in an increase in the estuarine flushing time. The estuary is shallow and so, in addition to water column processes such as phytoplankton uptake, bacterial respiration, and photo-oxidation (Seitzinger & Sanders 1997; Wiegner et al. 2006; Badr et al. 2008), it is possible that these sandy sediments were acting as a sink for DON. Hopkinson et al. (1999) observed sediments, including sands, acting as a sink for DON in an estuary in Massachusetts with a degree of both temporal and spatial heterogeneity across a seasonal cycle, and Agedah et al. (2009) also report uptake of DON by sediments in the anthropogenically-impacted Colne estuary (UK). The rates reported by Agedah et al. (2009), however, were slow in relation to the residence time of the estuary and thus they proposed that the majority of DON in the system was exported to the coastal zone. In contrast Burdige and Zheng (1998) reported estuarine sediments in Chesapeake Bay to act as a source of DON to the water column. As DON concentrations were substantially lower than DOC or DIN concentrations throughout this study the impact of any potential removal or production process is more likely to be seen reflected in the total DON concentration at the estuary mouth. Unfortunately the data presented here is not sufficient to fully resolve the role of the sediments in the processing of DON in Christchurch Harbour, but further work is ongoing on sediment-water exchanges in this system.

A key feature of the two rivers studied was the very high concentrations of nitrate plus nitrite (> 290 µM) throughout the year. As the concentration of DON is derived by subtracting the DIN concentration from the corresponding total dissolved nitrogen concentration, it is possible to get values that are negative or below the limit of detection when DON concentrations are low relative to DIN. Vandenbruwane et al. (2007) found the likelihood of this occurring to be of particular importance in samples where the proportion of DON to TDN is ≤ 15%. The mean DON: TDN ratios in both rivers and the estuary were below 10% which may explain in part the number of DON samples that were found to be below the limits of detection in this study. This in turn may explain the lack of relationship observed between DON concentrations and flow. Yates et al. (2019) determined the proportion of DON to TDN in intensively farmed arable catchments underlain by chalk, such as our Avon catchment in particular, to be < 10%. However, the magnitude of the river flows during rainfall events still result in thousands of kilograms of nitrogen being transported to the estuary and

beyond on a daily basis as DON. While this flux was an order of magnitude lower than the flux of DIN in this system it is still a considerable yield of nitrogen when compared to many other global estuaries (Table 2).

There was no clear relationship found between DOC and DON at any of the sites or across all of the sites. The potential reasons for this are twofold – either the factors controlling DOC and DON dynamics in these systems are different, or the components of the DOM pool are utilised by the microbial community at different rates (Wiegner et al. 2006, 2009). Several studies have shown that the dynamics of DON and DOC within the same river can differ with DON being cycled faster within rivers than DOC (Stepanauskas et al. 2000; Solinger et al. 2001; Wiegner et al. 2006; Inamdar & Mitchell 2007). In addition the ultimate fate of the organic carbon and nitrogen can differ as it can be either incorporated into bacterial biomass or oxidised and excreted (Hopkinson et al. 1999).

### 4.5. The impact of rainfall events

The resolution of sampling in this study was not high enough to fully resolve the behaviour of DOC or DON within the Rivers Avon or Stour or within Christchurch Harbour during storms using hysteresis curves (e.g. Lloyd et al. 2016), but weekly sampling over the course of a year has demonstrated that local increases in rainfall result in sudden increases in both river flow and riverine DOC concentration and thus flux. Elevated river flows also increase the DIN flux to the coastal zone (Supplemental Fig. 2). DON dynamics are more complicated to resolve possibly due to the interplay of different sources as well as currently unidentified removal processes within the estuary. Whilst the river flows observed during the year of study could be considered atypical in comparison to the 11-year mean flows for each river, they are certainly relevant when considering the estimated fluxes of each river under future climate change conditions of drier summers and more frequent stochastic storm events (IPCC, 2014).

## 5. Conclusion

There are few studies detailing the yields of dissolved organic nutrients in rivers and estuaries that are already known to be anthropogenically-impacted with elevated inorganic nutrient loads, despite

their potential contribution to causing problems such as eutrophication and hypoxia. Annual yields of dissolved inorganic and organic nutrients at the lowest gauging stations of the Hampshire Avon and the Stour, as well as in their shared estuary of Christchurch Harbour, are comparable to and often greater than yields documented from other riverine and estuarine systems both within the UK and globally. Whilst the yield of DON was typically an order of magnitude smaller than the corresponding yield of nitrate plus nitrite at each site, the range of 118 -198 kg N $km^{-2}$ $y^{-1}$ is still an ecologically important load of nitrogen potentially available to the aquatic microbial community. The processes controlling the dynamics of DOC and DON differed in both the rivers and the estuary, highlighting the importance of considering the component parts of DOM when investigating the role of DOM in aquatic systems.

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**Table**



**Table 1** Catchment characteristics of the Hampshire Avon and the Stour (CEH, 2008)

| Catchment characteristics | Stour at Throop | Hampshire Avon at Knapp Mill |
|---|---|---|
| Catchment area ($km^2$) | 1073.0 | 1706.0 |
| Baseflow index | 0.65 | 0.90 |
| Woodland (%) | 9 | 13 |
| Arable/horticultural (%) | 46 | 37 |
| Grassland (%) | 36 | 40 |
| Urban extent (%) | 2 | 2 |
| Geology | Mixed<br>Chalk ~50%, clay ~30%, limestone, Upper Greensand | Mixed<br>Predominantly chalk, lower catchment sands, gravels, clays |

**Table 2** Annual yields of dissolve inorganic nitrogen (DIN), dissolved organic nitrogen (DON), and dissolved organic carbon (DOC) from study sites and a selection of streams and rivers across the UK, Europe, and the world. Units are kg C or N $km^{-2}$ $y^{-1}$

| Site | DIN (yield in kg C or N $km^{-2}$ $y^{-1}$) | DON (yield in kg C or N $km^{-2}$ $y^{-1}$) | DOC (yield in kg C or N $km^{-2}$ $y^{-1}$) | Catchment area ($km^2$) | Reference |
|---|---|---|---|---|---|
| Hampshire Avon | 2735 | 198 | 1752 | 1706 | This study |
| Stour | 4282 | 189 | 3340 | 1073 | |
| Christchurch Harbour[a] | 2882 | 118 | 2296 | 2779 | |
| ***United Kingdom*** | | | | | |
| Thames | - | - | 1400 | 9948 | Jarvie et al 2017 |
| Severn | - | - | 3300 | 9895 | |
| Tay | - | - | 5000 | 4587 | |
| Tamar | 1032-3477 | - | - | 917 | Tappin et al. 2013 |
| Plym [a] | - | 110 | - | 170 | Badr et al. 2008 |
| Mersey[a] | 17000 | - | - | 3400 | Nedwell et al. 2002 |
| UK flux[a] | 1570[b] | - | 2300-5200[c] | 244000[c] | Nedwell et al. 2002, Worrall et al. 2012 |
| ***Europe*** | | | | | |
| Danube | - | - | 1200 | 817000 | Worrall et al. 2012 |
| Po | - | - | 3000 | 70000 | |
| Rhine | - | - | 1400 | 164500 | |
| Seine | - | - | 900 | 7390 | |
| ***World*** | | | | | |
| Lena | 14 | 56 | 2338 | 2460000 | Holmes et al. 2012 |
| Yukon | 31 | 57 | 1771 | 830000 | |
| Mackenzie | 16 | 18 | 820 | 1780000 | |
| Yellow | - | - | 42.5 | 752443 | Wang et al. 2012 |
| Changjiang | - | - | 814.4 | 1940000 | |
| Nushagak | | | 6900 | 25000 | Worrall et al. 2012 |
| Wailuku | 9-11[d] | 24-38 | 829-1282 | 659 | Wiegner et al. 2009 |

[a] full catchment including estuary

[b] Nedwell et al. 2002

[c] Worrall et al. 2012

[d] Nitrate only

Figure
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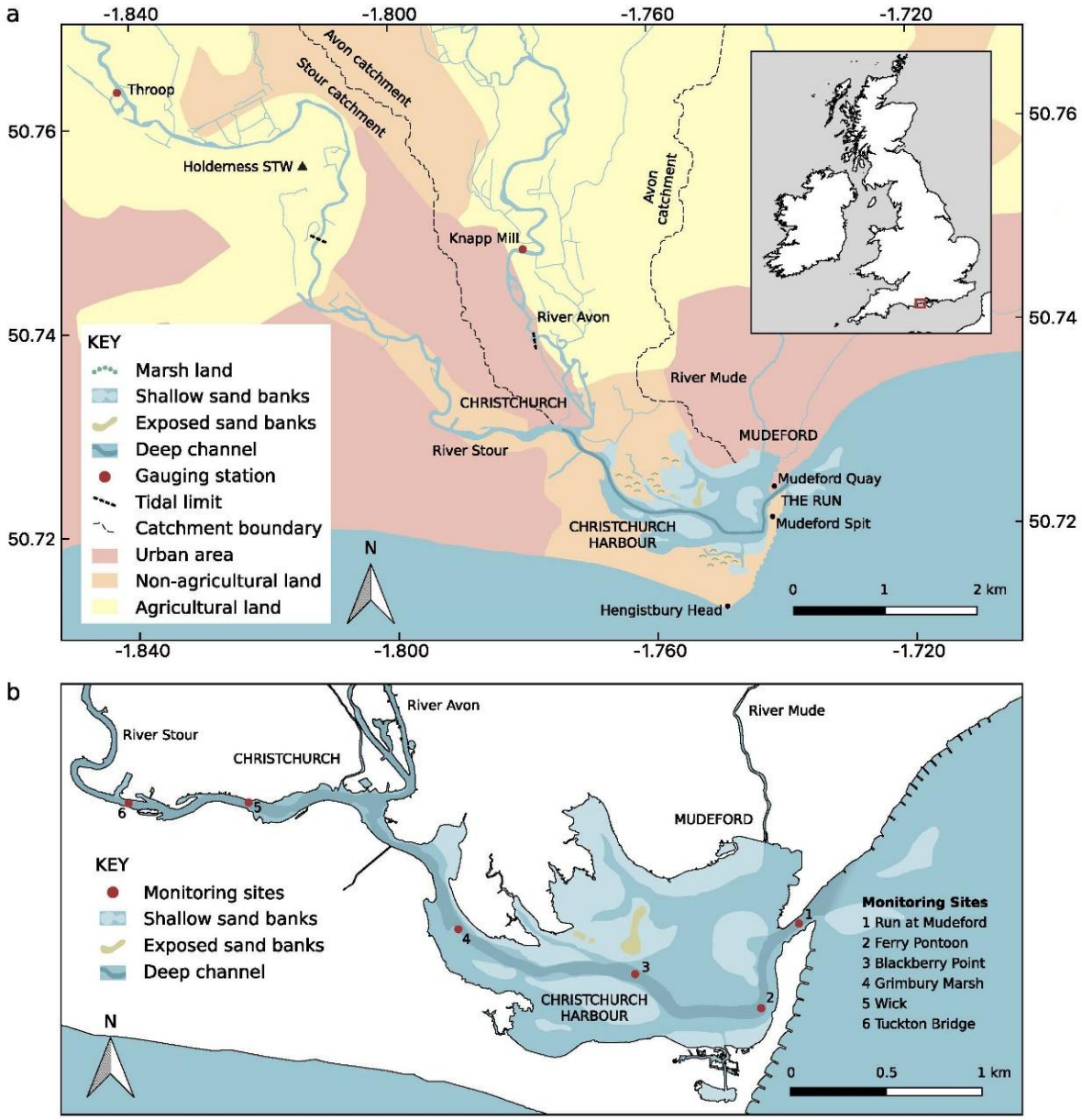


**Fig. 1** Map of sample sites used in this study (a) showing sites of weekly sampling with the tidal limit of each river, local sewage treatment works (STW) and land use, and (b) depicting the estuarine sampling sites in Christchurch Harbour.

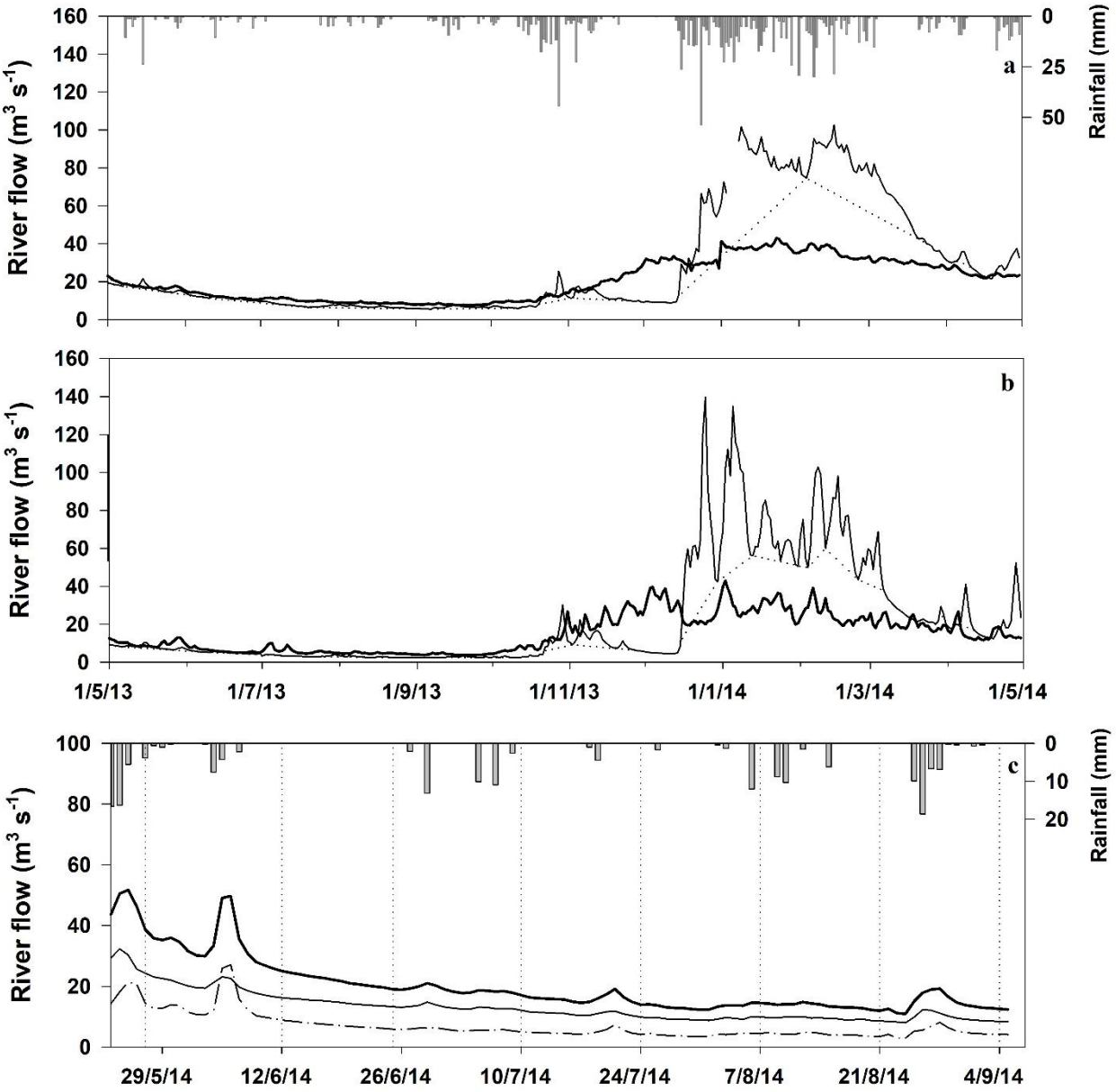


**Fig. 2** Daily mean river flow ($m^3 s^{-1}$) during weekly sampling campaign measured at (a) Knapp Mill and (b) Throop. Bold line indicates river flow over 2013-2014 sampling period, fine line indicates 11-year daily average (2000-2010 inclusive) at each station, and dotted line indicates calculated baseflow contribution (see text for method). (c) Daily mean river flow ($m^3 s^{-1}$) measured at Knapp Mill (thin solid line) and Throop (dashed line) during estuarine transect sampling with total summed daily river flow (bold line). Dotted vertical lines indicate estuarine sampling dates. Daily rainfall (mm) from Bisterne is presented as grey bars in (a) and (c)

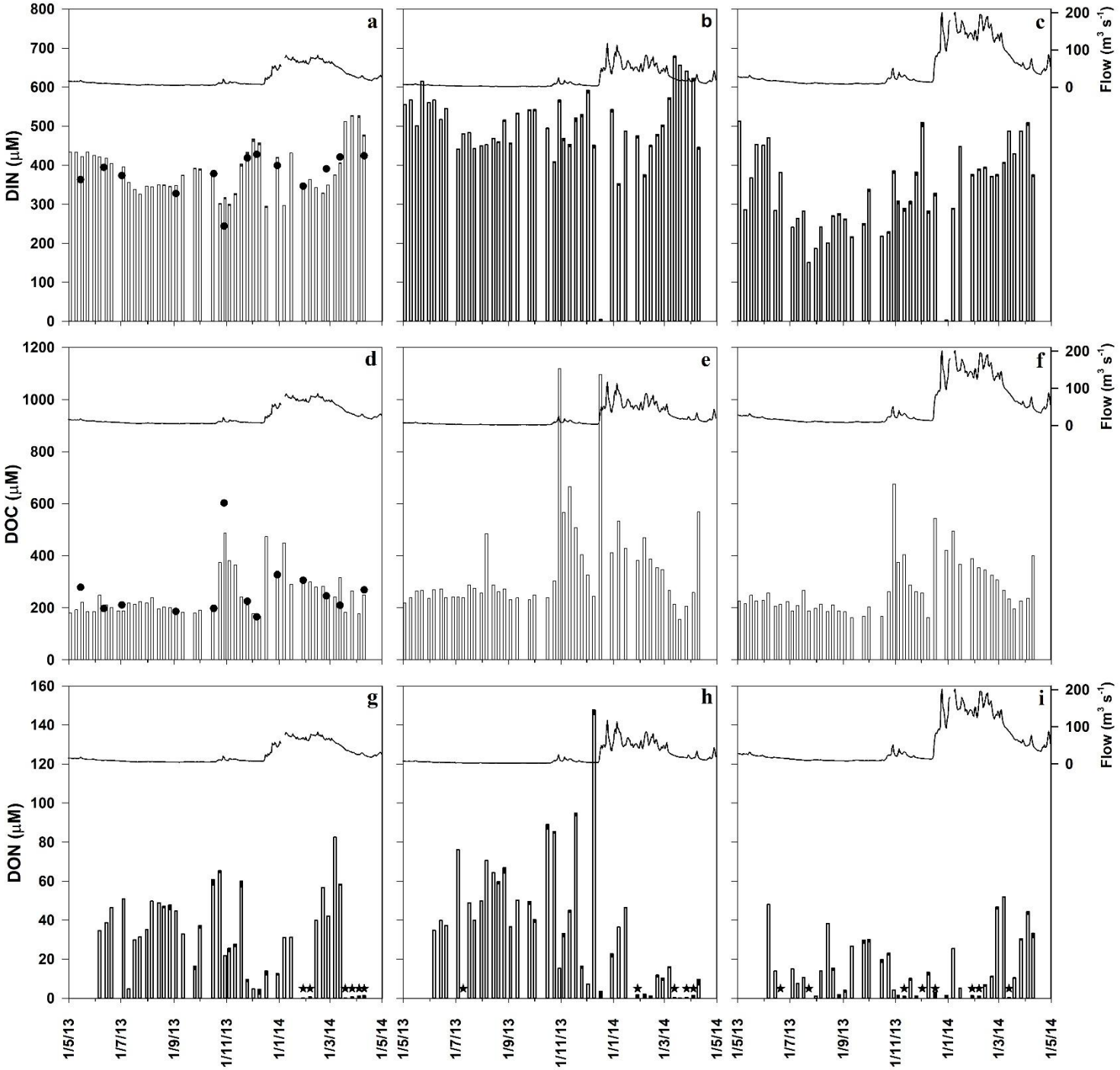


**Fig. 3** (a-c) Dissolved inorganic nitrogen (DIN, μM) concentration at each site (white bar is nitrate + nitrite, black bar ammonium), (e-f) dissolved organic carbon (DOC, μM), and (g-i) dissolved organic nitrogen (DON, μM, white bar) and urea (black bar). Stars represent samples below DON detection limit. Sites are from left to right: Avon, Stour, and the estuary mouth at Mudeford. Solid line in each plot is river discharge for that site ($m^3$ $s^{-1}$, scale on right axis). Filled circles in (a) and (d) represent Environment Agency data from the Avon site

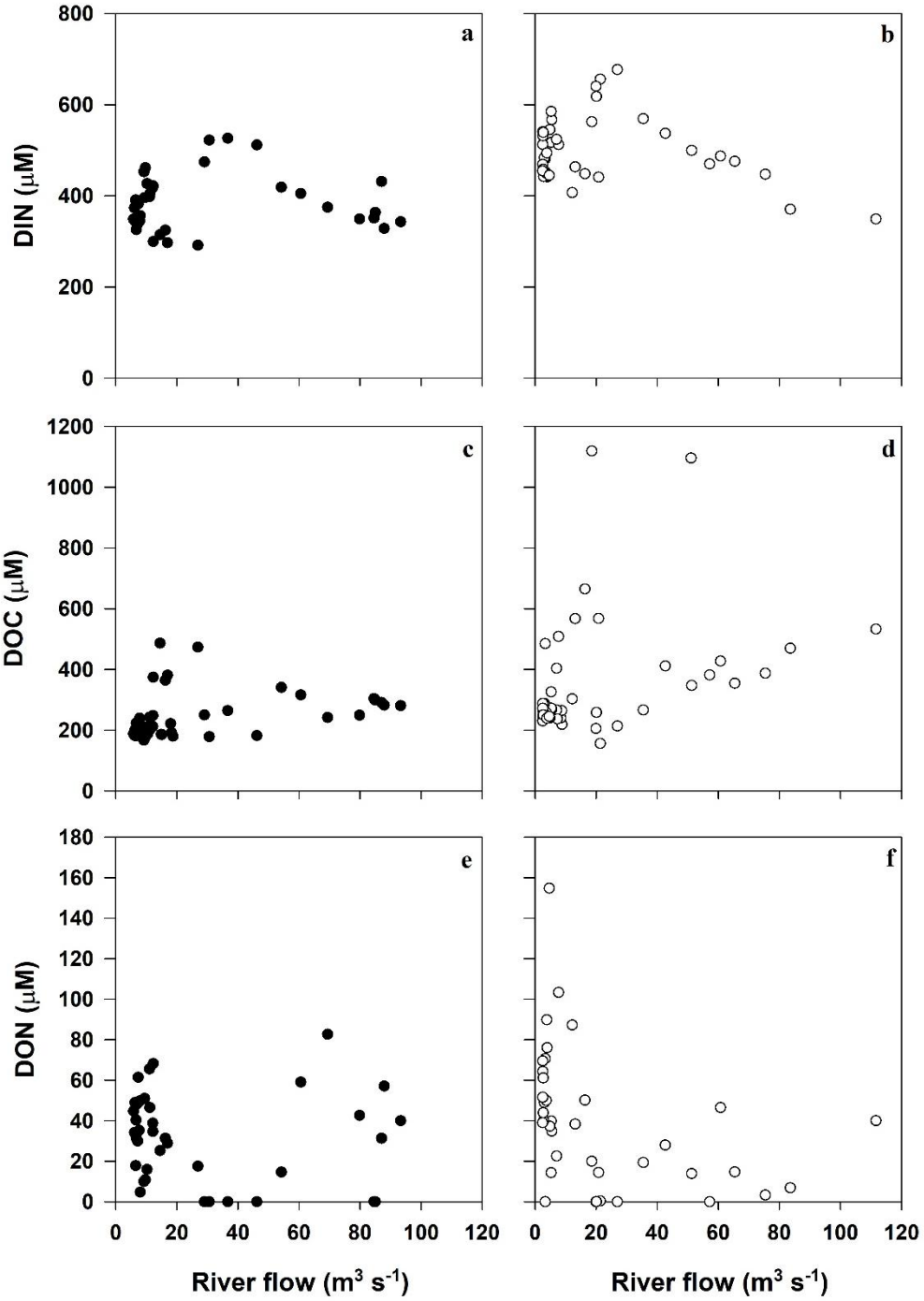


**Fig. 4** Relationship between DIN (µM; a,b), DOC (µM; c,d), and DON (µM; e,f) and daily mean river flow ($m^3$ $s^{-1}$) in the Avon (left column) and the Stour (right column)

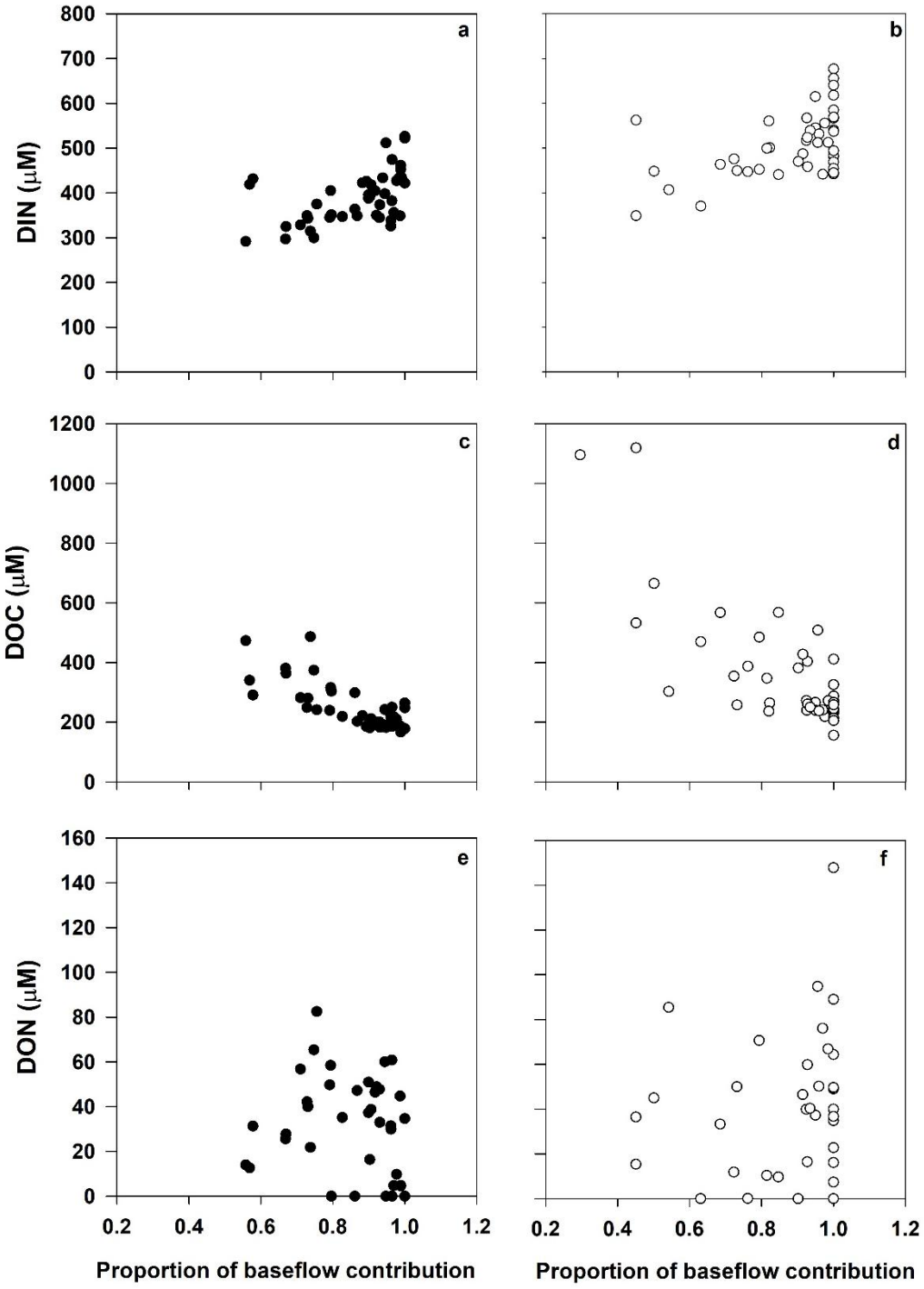


**Fig. 5** Relationship between DIN (µM; a,b), DOC (µM; c,d), and DON (µM; e,f) and proportion of estimated baseflow contribution to total river flow in the Avon (left column) and the Stour (right column)

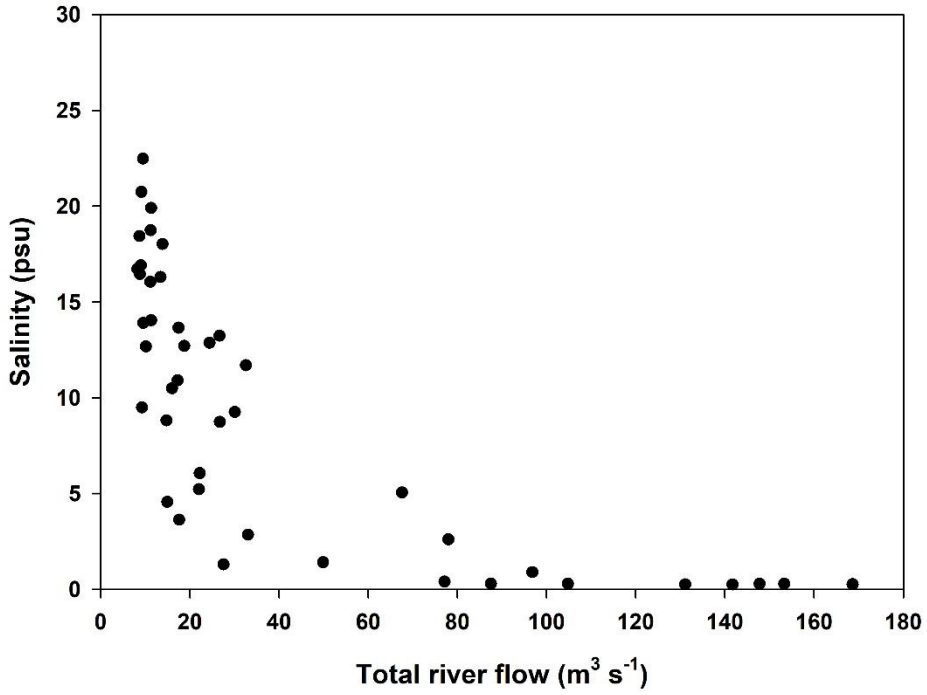


**Fig. 6** Relationship between total river flow ($m^3 s^{-1}$) and salinity (psu) at the mouth of the estuary at low tide

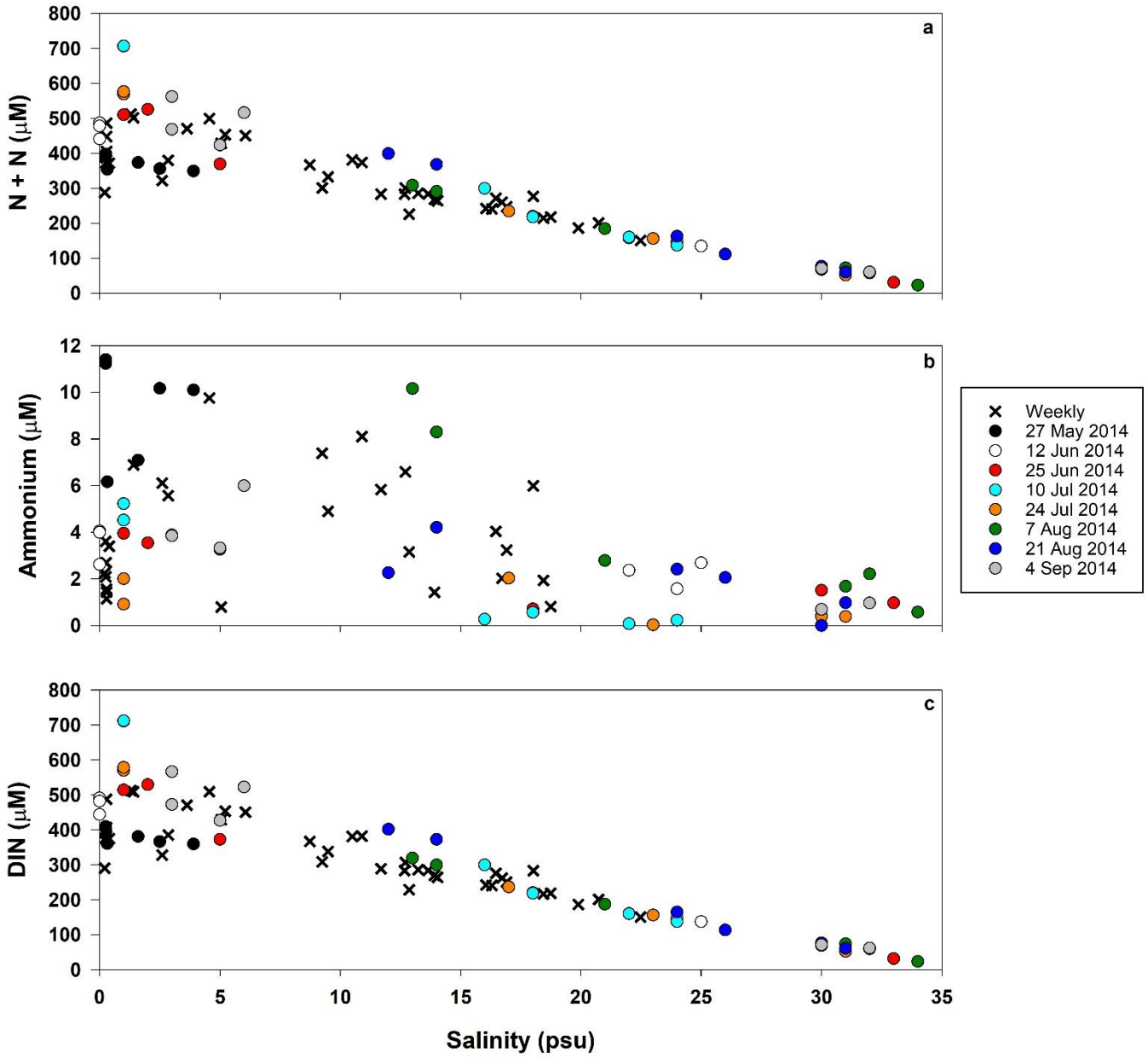


**Fig. 7** Relationship between salinity (psu) in the estuary and concentrations of (a) nitrate plus nitrite (µM), (b) ammonium (µM), and (c) DIN (µM). Black crosses indicate weekly sampling data from the estuary mouth at Mudeford and coloured circles indicate estuarine sampling data

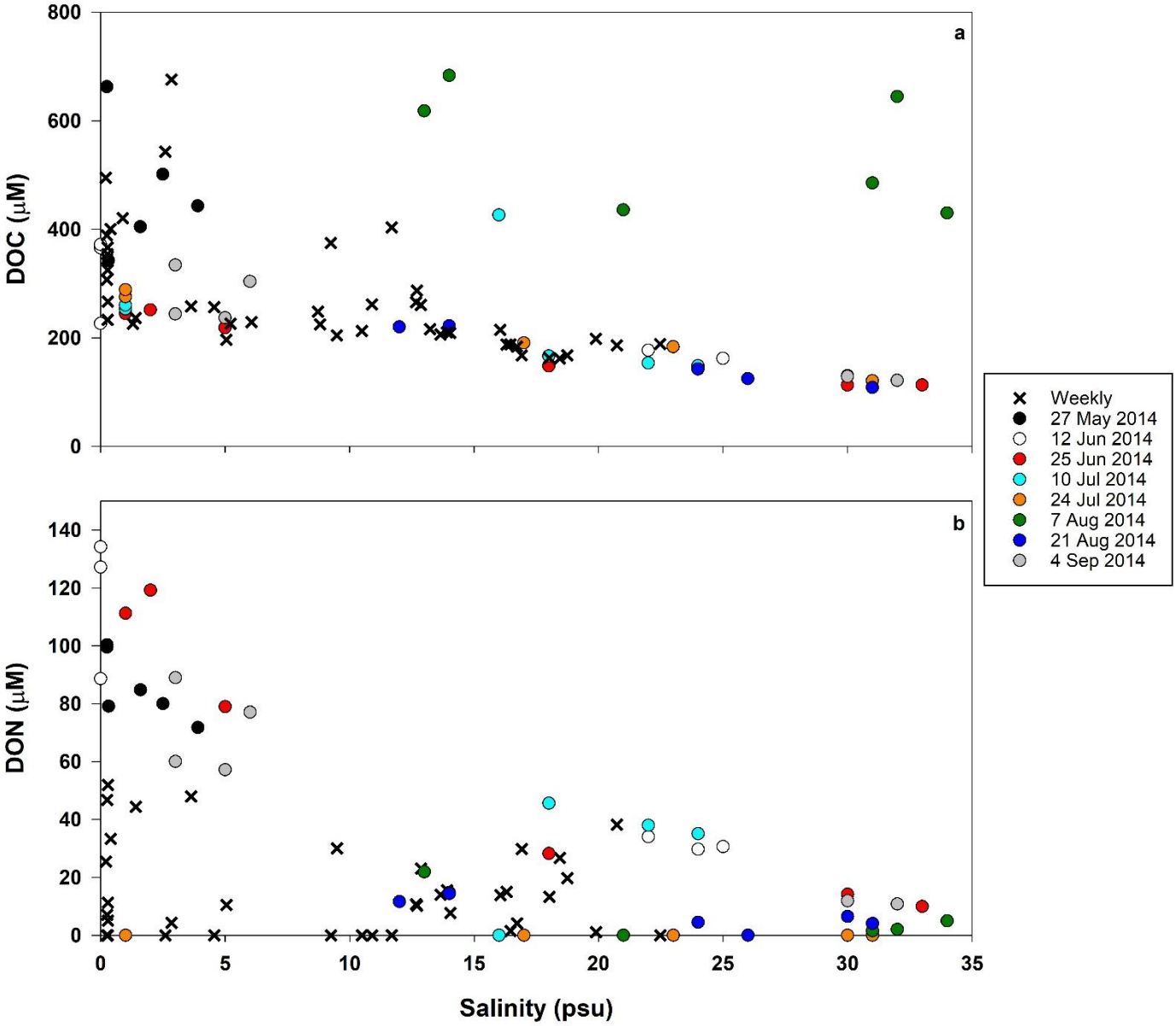


**Fig. 8** Relationship between salinity (psu) in the estuary and concentrations of (a) DOC (µM), and (b) DON (µM). Black crosses indicate data from weekly sampling from the estuary mouth at Mudeford and coloured circles indicate estuarine sampling data (dates in key)

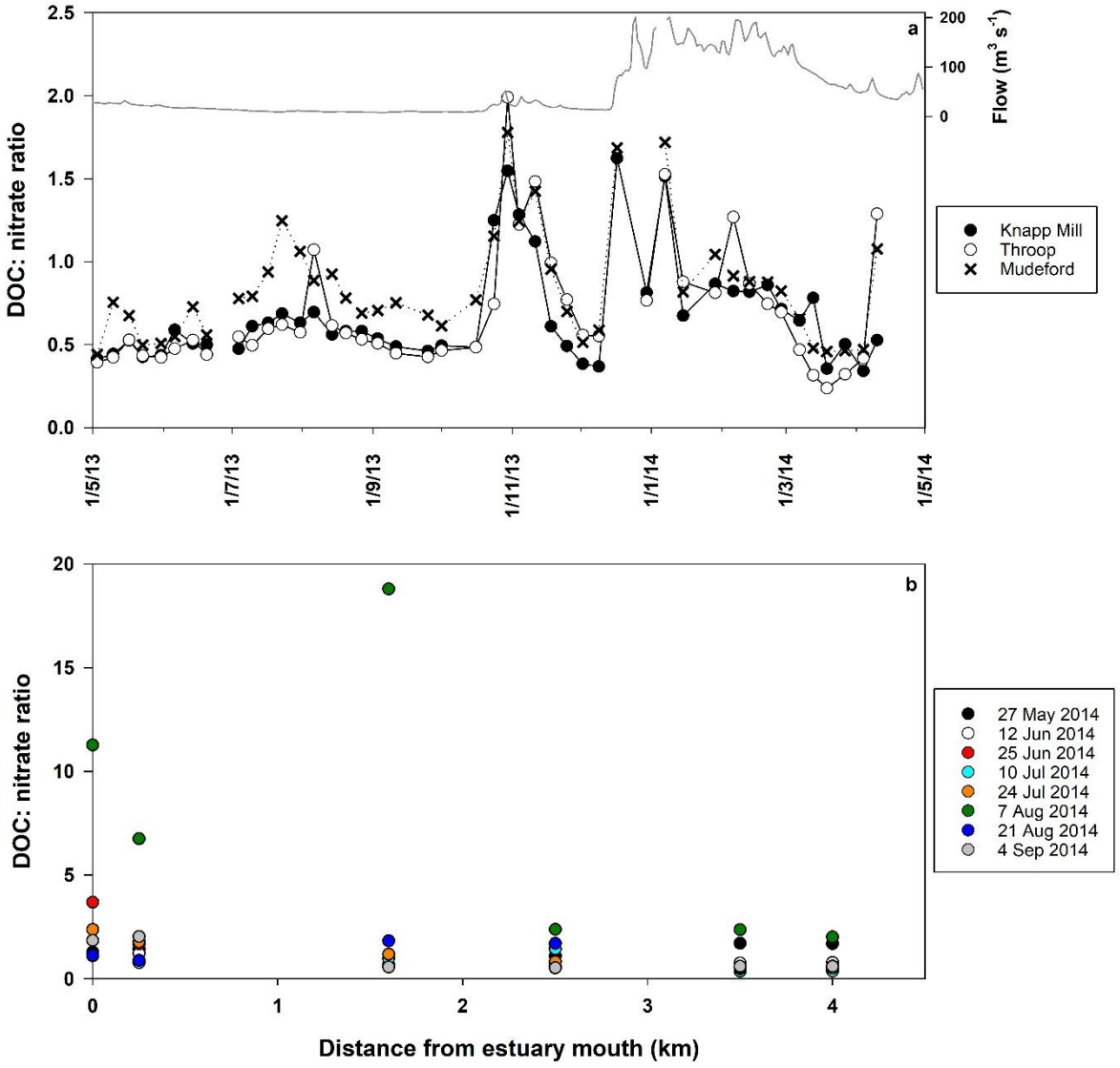


**Fig. 9** DOC: nitrate ratio from (a) weekly sampling sites (Avon filled circles, Stour open circles, estuary mouth at Mudeford crosses) and from (b) estuarine transects (key indicates sampling date). Solid line in (a) denotes total river flow ($m^3$ $s^{-1}$) for reference.

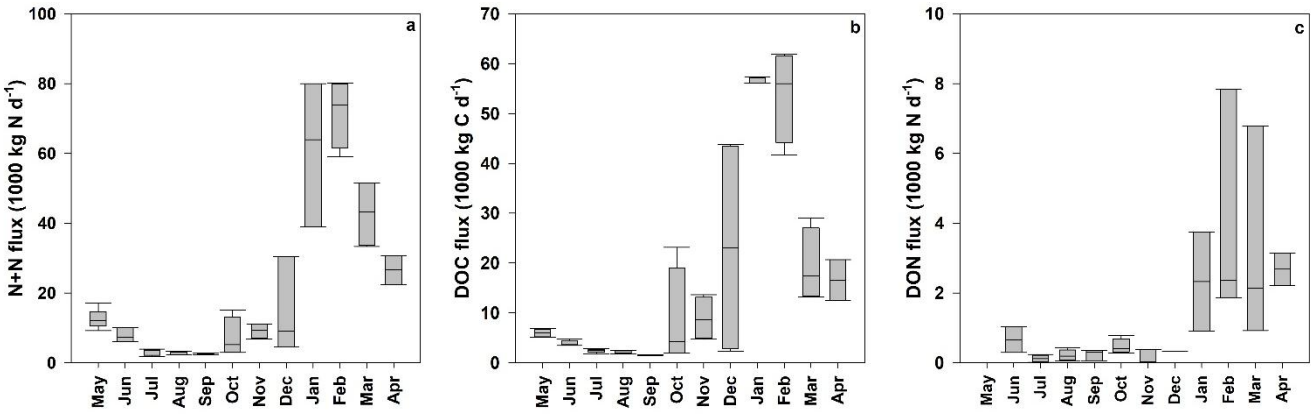


**Fig. 10** Boxplots of instantaneous estuarine fluxes of (a) nitrate plus nitrite (1000 kg N $d^{-1}$), (b) DOC (1000 kg C $d^{-1}$), and (c) DON (1000 kg N $d^{-1}$) from weekly sampling data (May 2013 – Apr 2014)

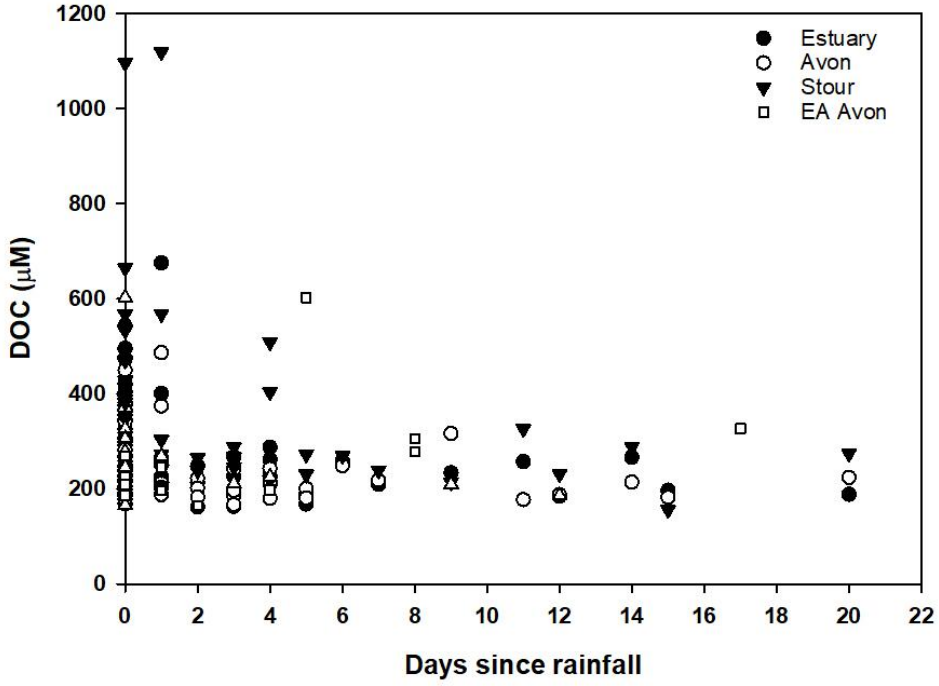


**Fig. 11** Concentration of DOC (µM) plotted against the number of days since rainfall for each sample. Triangles are samples from the Stour, filled circles are from the estuary mouth at Mudeford, and open circles are from the Avon. Open squares are Environment Agency data from the Avon

**Supplementary material for on-line publication only**

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Author contributions**

AP: Methodology, Investigation, Writing – Original Draft, Writing – Review & Editing; FC: Conceptualization, Investigation; GF: Funding acquisition; DP: Conceptualization, Writing – Original Draft, Writing – Review & Editing, Supervision, Funding acquisition