Dear editor,

Please find resubmitted the manuscript entitled “Long-chain diols in rivers: distribution and potential biological sources” (bg-2018-116). We thank you, and the reviewers, for your positive comments and we have used these to revise and improve our manuscript. The reviewers and you requested minor changes and we took these into account. Below we have listed our responses (in bold) to the reviewers concerns and suggested changes.

We hope that with this revision we have addressed all issues and that the revised manuscript is suitable for publication in BG.

On behalf of the co-authors,

Sincerely,

Julie Lattaud

We are pleased to inform you that both reviewers suggested that the manuscript should be published subjected to minor revisions. Please respond to the reviewers' comments and submit a final version of your manuscript for potential publication in BG.

Dear editor, we thank you for the positive feedback and addressed the reviewers' comments below and in the revised manuscript.

Reviewer 1

We thank the anonymous reviewer 1 for her/his helpful comments on our manuscript. Below follows our reply to the main comments and, where applicable, how we changed the manuscript.

General comments

> *"The critical problem is the conclusion that LCDs in fast flowing parts of rivers are not coming from in situ living plankton but from stagnant waters of these river systems such as lakes or side ponds. The authors should sample SPM and surface sediments in these lakes or side ponds."*
>
> **We agree with the reviewer that lakes and side ponds are the logical subject of a follow-up study. It is known from the literature that LCDs occur in most lakes investigated (e.g. Rampen et al., 2014). We are currently sampling several lakes that are part of a river system to prove the hypothesis developed in our manuscript. The results of this sampling campaign will be reported in a future manuscript.**

Specific comments

> -1 "*Page 11, lines 5-9, the authors should confirm it by sampling the SPM at different depth in the water column"*
> **A depth profile has been analyzed for the Godavari River. Three depths (0, 4, and 8 m below the river surface) were sampled close to the river mouth in both wet and dry seasons (W10 and G10, respectively, see supplement and as illustrated in Figure 1). No consistent difference was found in diol distributions between the different depths. We added on page 3 line 18 "The SPM was sampled at the surface of the river except for two depth profiles (0, 4 and 8 m deep) which were sampled during the wet season and during the dry season, respectively".**
>
> -2 *"The authors analyzed the GDGTs, except the BIT index what other information could you get from the GDGTs?"*
> **The current manuscript is focused on the LCDs and their ability to trace fluvial input into the marine environment. Similarly, the BIT index can be used to trace soil and riverine OC transported to the marine environment by the same river, which makes it a straightforward parameter for comparison. The occurrence and distribution of GDGTs, as well as their ability to transfer environmental signals from the catchment to the marine sedimentary archive have been discussed extensively in Freymond et al. (2017) and Freymond et al. (2018) for the Danube River. The data for the Godavari River are subject of another, still ongoing study in this area on soil OC transport.**
>
> -3 *"What is the relationship between temperature, precipitation and LCDs?"*
> **There is no relation between temperature and LCD as is written in lines 2-6, page 13. There might be an indirect link between precipitation and LCDs but it is not clear from our data. For example, in the Godavari system the concentration of LCDs in the riverbed sediment is lower during the wet season than during the dry season, in contrast to the SPM, where LCD concentrations are lower during the dry season.**

Reviewer 2

We thank Dr. Plancq for his helpful comments on our manuscript. Below follows our reply to the main comments and, where applicable, how we changed the manuscript.

General comments

-*"However, the current manuscript can be improved before publication. The authors discuss their finding in context of seasonality but sampling was only done during two separate months per year, which makes difficult to make inferences about the seasonality. It would be also nice to show the non-correlation between temperature and LCD fractional abundances of different isomers in the data section and not as a minor mentioning within the discussion. This aspect could have been discussed a bit more in the context of stronger temperature gradients between lakes and air temperature versus rivers and air temperature. Those two points become even more relevant since the abstract suggests a focus on LCD in relation to season, precipitation and temperature."*

We agree with the reviewer that only two months of sampling does not reflect a whole year of primary production. However, the climate of the Godavari River system is characterized by only two distinct seasons, i.e a wet and a dry season. The sampling months have been chosen to reflect these two seasons. Samples from the Rhine River have been collected in spring and autumn, and thus does not reflect all seasons of the area. This is specified in the revised version of the manuscript (page 1, line 20, we removed the mention of seasonality). We added page 13 lines 10-11 "Potentially, this difference could be due to the fact that we are using mean annual air temperature and not in situ river temperatures".

-*"It is also unclear why GDGTs (BIT index) and Chlorophyll a are relevant for the outlined questions or are helpful in understanding spatial distributions of LCD and their source organisms. Although Chlorophyll a is sometimes used as indicator of primary productivity, it has been shown that there are more suitable parameters (Lyngsgaard et al., 2017). The result description could also be a bit more concise and part of the results shown in tables. Why did the authors not take SPM samples integrated over a greater part of the water column? Villanueva et al. (2014) showed maximum LCD concentration a few meters below the surface, and even though this is based on a lake, stagnant parts of the river systems could have a similar vertical distribution."*

We have used the BIT index as an indicator for marine input in river systems, especially in the lower part of the river. We have used chlorophyll a as indicator for primary production, as the data was readily available. Indeed, Lynsgaard et al. (2017) suggest that chlorophyll a failed to reflect the summer primary productivity in the Baltic Sea and that phytoplankton carbon biomass concentration is a better proxy. Unfortunately, we did not fix the phytoplankton and did not perform any biovolume counts. Therefore, we cannot obtain these phytoplankton biomass concentration values and chose to use chlorophyll a instead.

The SPM from the water column were taken at the surface (0 m), at the middle of the water column (2 or 4 m) and as close as the bottom as possible (8 m) to cover as much as possible the water column. We added these details on page 3 lines 18-19 "The SPM was sampled at the surface of the river except for two depth profiles (0, 4 and 8 m deep) which were sampled during the wet season and during the dry season, respectively".

Specific comments

-Page 1, line 20, 26 "*Not only SPM but also sediment samples were investigated; "…in relation with season, precipitation, temperature, and source catchments" may be misunderstood as you making statements in all three rivers about seasonality even though SPMs were only sampled once*

during spring and once during autumn per location. Stating in the abstract that the relationship between LCD and temperature/precipitation was investigated and then only mentioning no correlation in the discussion may be perceived as misleading."
For the Godavari River, the two months sampled are reflecting the only two seasons existing in this area, i.e. the wet and the dry season. For the Rhine River we agree that sampling in spring and autumn does not reflect all seasons of the area, and this will be nuanced in a revised version of the manuscript. The Danube samples covers a large temperature gradient where we could have detected a temperature/diols relationship. The relationship between temperature and LCDs distribution is only briefly mentioned as no correlation found but we will clarify and somewhat extend that discussion in the revised manuscript (page 13 lines 10-11). The relationship between precipitation and LCDs distribution was especially investigated in the Godavari River system as the two months of sampling differ mainly in precipitation, and is discussed on page 9 lines 8-18 and pages 10-11 lines 31-32 and 1-3 of the initial manuscript.

-Page 2, line 6 *"It would be clearer if you write that those culture experiments have been made on marine, lacustrine, soil and in snow living species."*
We added page 2 lines 6-7 "(isolated from snow, soil, marine and freshwater environments)"

-Page 2, line 7 *"Please be more specific. I think what you mean is that LCD signature of marine core top samples differ significantly from those of marine and lacustrine eustigmatophyte algae cultures. It would be good to state here that in marine versus lacustrine environments the C32 1,15 is less abundant that the C30 1,15-diol."*
We clarified the sentence and specify the difference between the LCD signature in marine and lacustrine sediments page 2 lines 8-9 "i.e. cultures of eustigmatophytes produce mainly the C_{32} 1,15-diol while in marine sediments the C_{30} 1,15-diol is generally dominant".

-*Page 2, line 19 "See comment page 2, line 6"*
We think that our sentence has made it clear that the cultures studied were lacustrine only. Page 2, lines 19-20 "Rampen et al. (2014b) studied the LCD distribution of several freshwater eustigmatophyte cultures"

-*Page 3, line 16 "SPM of which water depth interval?"*
We added page 3 line 18 "The SPM was sampled at the surface of the river except for two depth profiles (0, 4 and 8 m deep) which were sampled during the wet season and during the dry season, respectively".

-*Page 5, lines 1-2 "Why were the filters of the Rhine and the incubation experiment base hydrolyzed (Page 5, Lines 1-2) and not the other samples? It would be worth explaining the reason why in the methods."*
The samples for the Godavari and Danube systems have been primarily studied for GDGTs and have been treated as such. The standard protocol for LCDs extraction includes a base hydrolysis step to allow enhance recovery. However, a recent study (Reiche et al., in preparation) showed that base hydrolysis does not yield much more LCDs than direct extraction. Hence there will be little difference in the results between the two work-up procedures.

-*Page 5, lines 19-20 "It is said that the C22 7,16-diol was used as internal standard, while in Page 6, Lines 23-24, the C22 5,17-diol is indicated as internal standard. Is it a typo?"*
This is a typo, it should be the C_{22} 7,16-diol, it is corrected accordingly. Page 6 line 26 "C_{22} 7,16-diol"

-Page 7, line 28 "Please use the names of the primers provided by Stoeck et al. (2010). Why has this primer been used when it only yielded low quality V4 reads in the original paper? Organism-specific abundances may be biased by the quality of primer annealing to the template. It should be mentioned that denovo sequencing has been done. These constraints should be discussed.
More specific primers could probably be used in future work instead of the universal eukaryotic primer. Since so far LCD producers all belong to the heterokonts, a primer specific to this algae group adapted to NGS would potentially yield better results (Coolen et al., 2004, Bittner et al., 2012)."

The names of the primers are TAReuk454FWD1 and TAReuk454REV3 (Stoeck et al., 2010), they have been added on page 7, lines 31 and 32. The original paper compares V4 sequencing and V9 sequencing. While V9 primers seem indeed to capture the highest level of diversity, the V4 primers do not underperform at all (see Fig. 4 on Stoeck paper). These primers (Tareuk) have been extensively used for many years after the original publication and they are considered among the best primers for targeting microbial eukaryotes in the environment. Furthermore, the V4 region is a longer fragment than the V9, this allows a higher resolution in discriminating species from the same genus or clades within the same species. More specific primers can be used, like in Villanueva et al. (2014) where they used specific eustigmatophyte primers. However, we wanted, in this study, to look at all potential diol producers that includes organisms outside of the heterokont group (like *Azolla filliculoides*, Jetter et al., 2009).

-Page 9, line 8 "9 Why writing the unit with a dot ("ng.L-1")? More commonly written without the dot."

We have removed all dots from units.

-Page 9, line 9 "Since figure 1c is referred to before 1b, I would change the numbering of figures to match the order of their mentioning."

We changed figure 1 accordingly.

-Page 9, lines 26-28 "The LCDs from Black Sea sediments have been quantified but only the fractional abundances are discussed in the text and shown in Figure 2c. All the other data (from the Basin, Reservoir and Delta) are absolute quantifications. Why?"

The Black Sea sediments have only been used to identify the marine end member in the ternary plots which is based on fractional abundances. We do have the absolute concentrations of the LCDs in the Black Sea, and they have been added on page 10 lines 1-2 "In the Black sea sediment the main LCD was the C_{30} 1,15-diol (6500±9000 ngg^{-1} sed) followed by the C_{30} 1,14-diol (1100±1600 ngg^{-1} sed)".

-Page 10, line 15 "Why not providing a cumulative column diagram for the different groups found in the DNA analysis?"

We added a cumulative diagram as supplement 3 with the DNA sequencing results.

-Page 12, lines 11, 28 "Discrepancies between text and ternary plot labels. Plot suggests all C28, C30 and C32 diols but in the text it is written as C30 1,15 diols, C32, 1,15 diols…"

The plots legend of figure 4 is correct, we corrected the text, page 12, lines 29-30 "This diagram uses the fractional abundances of the C_{28} 1,13-diol, C_{32} 1,15-diol and the sum of the C_{30} 1,13 and C_{30} 1,15-diols".

-Page 13, line 11 "Why does the DNA work in lake Challa by Villanueva et al. (2014) suggest a role of novel uncultivated eustigmatophytes in LCD production in riverine ecosystems?"
Sequences of novel uncultivated eustigmatophytes were found in Lake Challa and their gene copies distribution fit with the LCD distribution, suggesting they may be a source for LCDs.

-Page 14, line 2 "It is known that different algae have different chlorophyll signatures and chlorophyll a is very common. It was therefore unlikely that a relationship could have been found. Additionally, chlorophyll a is not necessary the best indicator of primary productivity."
We agree with the comments, unfortunately we do not have the total chlorophyll data available.

-Page 14, line 7 "Did the authors also do incubation experiments on waters in dead arms? 13C may have been unsuccessful because LCD may have been produced in situ during blooms and incubation experiments may have been done on post-blooming waters. Please include those aspects in discussion."

We did not perform any incubations in dead arms of rivers. We agree with the reviewer t that the incubation could have failed because of the timing of the experiment (post-bloom) rather than because of the absence of the producers although it should be noted that we did detect incorporation in other algal biomarkers. We added on page 14, lines 13-15 "suggesting that the incubation time may be too short for LCD producers to take up the ^{13}C or that the LCDs are not synthesized *in situ* during the time of sampling".

Note on Figures

-1a "It would be good to extent the white frame of the overview map further so that the labels are all within white background. Since reading the actual elevations is irrelevant and within the work area also not changing, I would reduce the labels on the scale to 0 to 4500 m."

We made the white frame bigger and reduce the elevation scale. See figure 1 page 20.

-1b,c "Since fig b and c use the same x scale, it would be better to put them closer together. Please write in the caption what those error bars represent. Standard error? 95%CI? Variability? (Same for Figures 1 and 3)."

We put the figures closer together to make the reading clearer. We added to the figure legend that the error bars represent the standard deviation. Page 19, line 4 and page 20, figure 1.

-2a "Scale as discussed in 1a."
We extended the white frame of the overview map to make the label more visible.

-2b,c "Why BIT index is shown far away from marine influence? I guess there are no error bars for the Reservoir samples because there was only one sample. This should be specified in the figure caption."
The BIT values for the part removed from the sea are only to show that the riverine end member is very different from the marine end member. We specified that there is only one sample for the reservoir, page 19 line 6 "(n=1 for the reservoir)".

-3a, b "Please write if the samples are from sediment or SPM. Why no error bars here? Why BIT index?"
We added that the samples are from SPM, page 19, line 9-10 "concentration of LCDs and BIT values (September and March) in the Rhine SPM.". The BIT index here allows us to compare

with the Danube River to clearly show the difference between marine and riverine environments.

-4a, b "This Figure could be improved by using different symbols, for example in 4b different symbols could be used to distinguish more easily the culture samples from the SPM samples. The description of Figure 4a in Page 12, Lines 11-12 is different from the figure caption. The same applies for Figure 4b (Page 12, Lines 28-29). Please clarify. As the 1,14-diols were excluded, it would be appropriate to specify on the Figure "C30 1,13+1,15-diols" instead of only "C30 diols""
We changed the symbols for cultures samples for figure 4b. The figure caption is correct, the description should be C_{32} 1,15-diol; C_{30} 1,13 + 1,15-diols and C_{28} 1,13-diol. We therefore corrected it in the text (page 12, lines 29-30), and we made a more detailed caption.

-5a, b, c "Please harmonise axis labels concerning diol concentrations. Why do gene copies have sometimes error bars and diols concentrations never have them? Why is chlorophyll a concentration shown without error bars? Why does the LCD concentration sometimes have error bars and sometimes not?"

Diol concentrations are only reflecting one point and one measurement, in contrast to the gene copies which are from triplicate analysis. Only one replica has been done to measure the chlorophyll concentration, which is why there are no error bars. LCDs concentrations have error bars when they represent the average of a river section (for the Godavari and Danube systems). The LCD concentrations for the Rhine reflect only one site. We specify this in the figure caption, page 19 line 9 "n=1 per site".

Long-chain diols in rivers: Distribution and potential biological sources

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Abstract. Long chain diols (LCDs) occur widespread in marine environments and also in lakes and rivers. Transport of LCDs from rivers may impact the distribution of LCDs in coastal environments, however relatively little is known about the distribution and biological sources of LCDs in river systems. In this study, we investigated the distribution of LCDs in suspended particulate matter (SPM) of three river systems (Godavari, Danube, and Rhine) in relation with precipitation, temperature, and source catchments. The dominant long-chain diol is the C_{32} 1,15-diol followed by the C_{30} 1,15-diol in all studied river systems. In regions influenced by marine waters, such as delta systems, the fractional abundance of the C_{30} 1,15-diol is substantially higher than in the river itself, suggesting different LCD producers in marine and freshwater environments. A change in the LCD distribution along the downstream transects of the rivers studied was not observed. However, an effect of river flow is observed, i.e. the concentration of the C_{32} 1,15-diol is higher in stagnant waters, such as reservoirs and during seasons with river low stands. A seasonal change in the LCD distribution was observed in the Rhine, likely due to a change in the producers. Eukaryotic diversity analysis by 18S rRNA gene sequencing of SPM from the Rhine showed extremely low abundances of sequences (i.e. <0.32% of total reads) related to known algal LCD producers. Furthermore, incubation of the river water with ^{13}C-labelled bicarbonate did not result in ^{13}C incorporation into LCDs. This indicates that the LCDs present are mainly of fossil origin in the fast flowing part of the Rhine. Overall, our results suggest that the LCD-producers in rivers predominantly reside in lakes or side ponds that are part of the river system.

1 Introduction

Long-chain diols (LCDs) occur widespread in marine environments and have been shown to mainly consist of C_{28} and C_{30} 1,13-diols, C_{30} and C_{32} 1,15-diols (Versteegh et al., 1997, 2000; Gogou and Stephanou 2004; Smith et al., 2013; Schmidt et al., 2010; Rampen et al., 2012, 2014a) and C_{28} and C_{30} 1,14-diols (Sinninghe Damsté et al., 2003; Rampen et al., 2011, 2014a). Culture studies showed that eustigmatophyte algae (isolated from snow, soil, marine and freshwater environments) produce 1,13- and 1,15-diols (Volkman et al., 1999; Rampen et al., 2007, 2014b), but with a distribution different than encountered in marine sediments, i.e. cultures of eustigmatophytes produce mainly the C_{32}

1,15-diol, while in marine sediments the C_{30} 1,15-diol is generally dominant. Furthermore, eustigmatophyte algae are rarely reported in marine environments, indicating that the cultivated marine eustigmatophytes are likely not the main producers of 1,13- and 1,15-diols in marine environments (Volkman et al., 1999). Culture studies of *Proboscia* diatoms show that they produce mainly 1,14-diols and minor amounts of 1,13-diols (Sinninghe Damsté et al., 2003; Rampen et al., 2007), while the marine dictyochophycean alga *Apedinella radians* (Rampen et al., 2011) also produces 1,14-diols. *Proboscia* diatoms are mainly present in upwelling areas and are likely the main source of 1,14-diols in nutrient-rich marine environments (Rampen et al., 2008; Willmott et al., 2010; Gal et al., 2018).

LCDs also occur in freshwater environments, i.e. in lakes (Shimokawara et al., 2010; Castañeda et al., 2011; Zhang et al., 2011; Romero-Viana et al., 2012; Rampen et al., 2014b; Villanueva et al., 2014) and in rivers (De Bar et al., 2016; Lattaud et al., 2017a). Shimokawara et al. (2010) showed that the LCD distribution in Lake Baikal was similar to that of cultivated freshwater eustigmatophytes, indicating that they may be a source of LCDs in freshwater environments. In addition, Villanueva et al. (2014) observed a correlation between the LCD concentration in the water column of Lake Chala and the abundance of 18S rRNA gene copies of uncultivated eustigmatophytes. Rampen et al. (2014b) studied the LCD distribution of several freshwater eustigmatophyte cultures, showing that for the Goniochloridaceae and Monodopsidaceae families the main LCD is the C_{32} 1,15-diol, while the LCDs of members of the Eustigmataceae family are dominated by a mix of C_{28} 1,13-, C_{30} 1,15- and C_{32} 1,15-diols. Interestingly, an increase in the temperature at which these algae were cultivated resulted in an increase of the fractional abundance of the C_{32} 1,15-diol (Rampen et al., 2014b). Apart from lakes, LCDs have recently also been reported to occur also in rivers (De Bar et al., 2016; Lattaud et al., 2017a) with the C_{32} 1,15-diol and, to a lesser extent, the C_{30} 1,15-diol as the most abundant LCDs. In contrast to marine and lake systems, however, the spatial occurrence and sources of LCDs in river systems have not been studied in detail.

In this study we investigated three river systems, i.e. the Rhine, Godavari, and Danube rivers, to constrain the impact of river characteristics on the distribution of LCDs. Furthermore, we analyzed the algal community composition using 18S rRNA gene sequencing and quantitative PCR (qPCR) analysis and performed labelling studies in the Rhine to constrain potential biological sources of LCDs.

2. Material and methods

2.1. Material

2.1.1. Godavari

The Godavari is the largest river of India not draining the Himalayas with a catchment area of $310x10^3$ km^2 (Balakrishna and Probst, 2005) and a length (of the main river) of 1465 km (Ramasubramanian et al., 2006). The principal tributaries of the river are the Pranhita, Waiganga and Wardha forming a subcatchment (called Pranhita) in the North and the Indravati and Sabari draining the Eastern Ghats (called Indravati). In addition to these subcatchments, the main stem of the Godavari River can be divided into the Upper Godavari (from the source to Sironcha), the Middle Godavari (from Sironcha to the Dowleswaram reservoir) and the lower Godavari (downstream of the reservoir). The climate over the basin is semi-arid to monsoonal (10 to 45°C, Biksham and Subramanian, 1988) and rainfall increases along a west-east gradient. Maximal rainfall is experienced during the Southwest monsoon over India (annual rainfall is 1185 mm with 84% falling during monsoonal months, June to September, Biksham and Subramanian, 1988; Pradhan et al., 2014). Ca. 98 % of the total suspended sediment load of the Godavari River is transported during the monsoon period (Rao et al., 2015).

A total of 62 (20 in dry and 42 in wet season) SPM and 65 (34 in dry and 30 in wet season) riverbed sediments were collected as described by Usman et al. (2018) during the dry (February/March) and the wet (July/August) seasons in 2015 (Fig. 1a). The SPM was generally sampled at the surface of the river except for two location where multiple depth profiles (0, 4 and 8 m deep) were obtained during the wet and dry seasons. For SPM sampling, 5-50 L of river water was filtered over a pre-ashed glass fiber filter (Whatman GF-F, 0.7 µm, 142 mm diameter). Riverbed sediments were collected from the middle of the stream from bridges, using a van Veen grab sampler.

2.1.2. Danube and Black Sea

The Danube is the second largest river of Europe with a catchment area of $800x10^3$ km² and a length of 2850 km (Freymond et al., 2017). Its catchment can be divided into four sub-regions: the upper Danube (from the source to the Gate of Devin), the Middle Danube (from the Gate of Devin to the Iron Gates reservoir) and the lower Danube (downstream of the Iron Gates reservoir to the delta) and the delta. The Danube flows directly into the Black Sea through three main branches of its delta: Chilia, Sulina, and Sf. Gheorghe. In the Black Sea, south of the Crimea peninsula, surface water flows westward onto the northwestern shelf. On the shelf, the surface current turns southward along the coast (Tolmazin, 1985). The climate in the Danube catchment is diverse, with an oceanic influence on the western part of the upper basin, a Mediterranean influence in the south and central part of the middle basin and a continental climate influence in the other parts. The annual precipitation varies from 2000 $mm.y^{-1}$ in the mountain area to 500 $mm.y^{-1}$ in the plains (Rimbu et al., 2002).

46 riverbed sediments (main river and tributaries, Fig. 2a) were collected as described by Freymond et al. (2018) in spring 2013 and 2014. The riverbed sediments were wet sieved with milliQ water over a 63 µm sieve on a shaking table and the fine fraction (<63 µm) was studied. In total 14 surface sediments from the Black Sea were included in this study. Of these, 10 surface sediments (Fig. 2a) were obtained as described by Kusch et al. (2010, 2016; called the Meteor/Poseidon surface sediments), one was collected in 2016 by the R/V Pelagia during the cruise 64PE408, and three were collected in 2017 with the R/V Pelagia during the cruise 64PE418 (collectively called the Pelagia surface sediments).

2.1.3. Rhine

The Rhine is the third largest river of Europe with a catchment area of $185x10^3$ km² and a length of 1320 km (Hoffmann et al., 2007). It can be divided into three hydrological areas: the upper Rhine (the Alpine region), the Middle Rhine (German and French Rhine) and the lower Rhine (the delta region). The upper Rhine receives up to 2000 mm of precipitation per year, the middle Rhine is characterized by a temperate oceanic climate, with annual rainfall ranging from 570 to 1100 mm and the lower Rhine receives an average of 800 mm of rain per year and has a temperate oceanic climate influenced by the North Sea and the Atlantic Ocean (Pfister et al., 2004).

Five sites along the middle Rhine were sampled (Fig. 3a): Karlsruhe (station of the Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg), Mainz (station of the Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht), Koblenz (station of Landesamt für Umwelt Rheinland-Pfalz), Cologne (Ecological Rhine-Station of the University of Cologne) and Kleve-Bimmen (International monitoring station). 62 L of river water was collected manually with a bucket at the side of the river at each station in March and September 2016. Of this 20 L was used for LCD analysis, 1 L for DNA analysis and 500 mL for chlorophyll analysis and 40 L was used for stable carbon isotope incubations.

2.2. Methods

2.2.1. ^{13}C-labelling study

For the incubation experiments, two 20 L Nalgene bottles were filled with Rhine water and incubated in presence of light at ambient air temperature during 52 h (day/night cycle) with 100 mg ^{13}C-labelled bicarbonate (Cambridge Isotope Laboratories, Inc., USA). The bottles were shaken at the start of the incubation, then once a day to avoid particles sinking to the bottom of the bottle. The bottles were not sealed so gas exchange with the atmosphere was possible. The water was filtered using pre-ashed glass fiber filters

(Whatman GF-F, 0.7 µm, 142 mm diameter) using a peristaltic pump (WTS, McLane Labs, Falmouth, MA). All samples were kept frozen at -20°C.

2.2.2. Lipid extraction

Filters of the Rhine and the incubation experiment were base hydrolyzed with 12 mL of 1 N KOH in methanol (MeOH) solution by refluxing for 1 h. Afterwards the pH was adjusted to 4 with 2 N HCl : MeOH (1 : 1, v/v) and the extract was transferred into a separatory funnel. The residues were further extracted once with MeOH : H_2O (1 : 1, v/v), twice with MeOH, and three times with dichloromethane (DCM). The extracts were combined in the separatory funnel and bidistilled water (6 mL) was added. The combined solutions were mixed, shaken and separated into a MeOH : H_2O and a DCM phase, after which the DCM phase was removed and collected into a centrifuge tube. The MeOH : H_2O layer was re-extracted twice with 3 mL DCM. The pooled DCM layers were dried over a Na_2SO_4 column and the DCM was evaporated under a stream of nitrogen. The extract was then acid hydrolyzed with 2 mL of 1.5 N HCl in MeOH solution under reflux for 2 h. The pH was adjusted to 4 by adding 2 N KOH : MeOH. 2 mL of DCM and 2 mL of bidistilled water were added to the hydrolyzed extract, mixed and shaken and, after phase separation, the DCM layer was transferred into another centrifuge tube. The remaining aqueous layer was washed twice with 2 mL DCM. The combined DCM layers were dried over a Na_2SO_4 column and the DCM was evaporated under a stream of nitrogen.

The SPM filters and riverbed sediments from the Godavari were freeze-dried, and river sediments were homogenized by milling. Both filters and riverbed sediments were extracted as described by Usman et al. (2018). Briefly, extraction was performed (3x) using an Accelerated Solvent Extractor (ASE 350, Dionex, Thermo-Scientific, Sunnyvale, CA, USA) with 9 : 1 (v/v) DCM : MeOH at 100°C and 7.6×10^6 Pa. The extracts were dried under N_2 and an 80% aliquot was further processed for analysis. The samples from the Danube were extracted as described by Freymond et al. (2018) using microwave–extraction (MARS) with 9 : 1 DCM : MeOH (v/v, 25 min at 100°C). The Meteor/Poseidon Black Sea surface sediments were extracted three times ultrasonically with a 9 : 1 DCM : MeOH (v/v) solvent mixture after addition of 1.96 µg of C_{22} 7,16-diol as internal standard. The four Pelagia Black Sea surface sediments were extracted using an ASE with a DCM : MeOH mixture 9 : 1 (v/v) and a pressure of 7.6×10^6 Pa at 100°C.

2.2.3. Separation of the lipid extract

To the total lipid extracts of the Rhine SPM, the incubation experiment and the Pelagia Black Sea surface sediments an internal standard was added (C_{22} 7,16-diol). They were subsequently separated into 3 fractions on an Al_2O_3 (activated for 3 h at 150°C) column. The apolar fraction was eluted with 4 column volumes of 9 : 1 (v/v) hexane (hex) : DCM, the ketone fraction with 3 column volumes of 1 : 1 (v/v) hex : DCM and the polar fraction (containing the diols) with 3 column volumes of 1 : 1 (v/v) DCM : MeOH.

For all other samples, the C_{46} glycerol trialkyl glycerol tetraether (GDGT, Huguet et al., 2006) was added as an internal standard.

The Godavari SPM from the wet season and Danube total extracts were saponified with KOH in MeOH (0.5 M, 2 h at 70°C). 5 mL of MilliQ water with NaCl was added and the neutral phase was back extracted with hexane and further separated into an apolar and polar fractions on a SiO_2 column with hex : DCM (9 : 1, v/v) and DCM : MeOH (1 : 1, v/v), respectively. For the Godavari river sediments and SPM from the dry season, the total extracts were saponified with KOH in MeOH (0.5 M, 2 h at 70°C) and subsequently separated on a SiO_2 column with hex : DCM (9 : 1, v/v) and DCM : MeOH (1 : 1, v/v), respectively. The Meteor/Poseidon surface sediments total extracts were saponified with KOH in MeOH at 80°C for 2 hours.

The neutral fraction was recovered in hexane and separated into an apolar and a polar fraction by silica gel column chromatography using DCM : hex (2 : 1, v/v) for the apolar and DCM : MeOH (1 : 1, v/v) for the polar fraction.

2.2.4. Diol analysis

The polar fractions were transferred into GC vials and silylated with N,O-Bis(trimethylsilyl)trifluoro-acetamide (BSTFA) and pyridine (10 µL each) and heated at 60°C for 30 min, after which ethyl acetate was added. All diols except the Meteor/Poseidon Black Sea surface sediments were analyzed by gas chromatography (Agilent 7990B GC) coupled to mass spectrometry (Agilent 5977A MSD) (GC-MS) equipped with a fused silica capillary column (Agilent CP Sil-5, length 25 m; diameter 320 µm; film thickness 0.12 µm). The temperature program for the oven was as follows: starting at 70°C, increased to 130°C at 20°C/min, increased to 320°C at 4°C/min, held at 320°C during 25 min. Flow was held constant at 2 mL/min. The MS source is held at 250°C and the quadrupole at 150°C. The electron impact ionization energy of the source was 70 eV. The diols of the Meteor/Poseidon Black Sea surface sediment were analyzed by GC-MS using an Agilent 6850 GC coupled to an Agilent 5975C MSD equipped with a fused silica capillary column (Restek Rxi-1ms, length 30 m; diameter 250 µm; film thickness 0.25 µm). The temperature program for the oven was as follows: held at 60°C for 3 min, increased to 150°C at 20°C/min, increased to 320°C at 4°C/min, held at 320°C during 15 min. Flow was held constant at 1.2 mL/min. The MS source is held at 230°C and the quadrupole at 150°C. The electron impact ionization energy of the source was 70 eV.

The diols were identified and quantified via SIM (Single Ion Monitoring) of the m/z= 299.3 (C_{28} 1,14-diol), 313.3 (C_{28} 1,13-diol, C_{30} 1,15-diol), 327.3 (C_{30} 1,14-diol) and 341.3 (C_{30} 1,13-diol, C_{32} 1,15-diol) ions (Versteegh et al., 1997; Rampen et al., 2012).

Absolute concentrations were calculated using the C_{22} 7,16-diol as internal standard for the Rhine SPM, Meteor/Poseidon and Pelagia surface sediments and the C_{46}GTGT as internal standard for the Godavari sediments and SPM and Danube SPM.

2.2.5. ^{13}C analysis of LCDs

LCDs in the polar fractions of the extracts of the SPM of the ^{13}C incubation experiments were isolated using semi-preparative normal phase HPLC. Prior to injection the polar fraction was dissolved in 750 µL hex : isopropanol (99 : 1, v/v) and filtered over a polytetrafluoroethylene (PTFE) filter (0.45 µm pore size). 3x 250 µL was injected on an 1260 infinity LC system (Hewlett Packard, Palo Alto, CA, USA) equipped with a thermostated autoinjector, column oven, and a Foxy R1 fraction collector (Teledyne Isco, Lincoln, NE, USA) as described in De Bar et al. (2016). Briefly, the different diol isomers were separated over a normal phase semi preparative Alltech Econosphere silica column (250 mm x 10 mm; 10 µm) at room temperature. After 35 min at 14% A (hex :isopropanol, 9 : 1, v/v) and 86% B (hexane) the mobile phase was adjusted to 100 % A in 1 min. It was then held at 100% A between 35 and 55 min. Finally, the column was reconditioned with 14% A in hex at 3 mL/min. The fractions were collected from 15 to 40 min every 30 s and analyzed by GC-MS as described above. The LCDs of interest eluted between 22.5 and 27.5 min and were collected in 3 pools. Pool 1 from 22.5 to 24.5 min (containing 100% C_{32} 1,15-diol, 95% C_{30} 1,15-diol), pool 2 from 25 to 26 min (containing 83% C_{28} 1,14-diol and 5% C_{30} 1,15-diol) and pool 3 from 26.5 to 27.5 min (containing 100% C_{28} 1,13-diol, 17 % C_{28} 1,14-diol and 100% C_{30} 1,13-diol). These pools were analyzed using gas chromatography–isotope ratio mass spectrometry (GC irMS, ThermoFinningan Delta^{PLUS} isotope ratio monitoring mass spectrometer coupled to an Agilent 6890 GC via a Combustion III interface). The gas chromatograph was equipped with a fused silica capillary column (25 m x 320 µm) coated with CP Sil-5 (film thickness = 0.12 µm) with helium as carrier gas (2 mL/min). The LCDs were silylated as described above using BSTFA with a known δ^{13}C value of -32.2 ± 0.5‰. Subsequently, the LCDs were injected

splitless at an oven temperature of 70 °C (injector temperature was 250°C), then the oven was programmed to 130°C at 20°C/min, and then at 20°C/min to 320°C/min at which it was held for isothermal (10 min). The $\delta^{13}C$ values were calculated by integrating the masses 44, 45, and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and that of CO_2-peaks produced by the CO_2 reference gas with a known ^{13}C-content at the beginning and end of the analytical run. All samples were analyzed in triplicate and the average is reported.

2.2.6. Glycerol Dialkyl Glycerol Tetraether (GDGT) analysis

GDGTs were analyzed from the polar fractions of the Rhine and Pelagia Black Sea surface sediments. Prior to GDGT analysis an aliquot of the polar fractions was filtered through a 0.45 µm PTFE membrane filter using hex : isopropanol (99 : 1, v/v). Analysis were performed using Agilent 1260 UHPLC coupled to a 6130 quadrupole MSD in selected ion monitoring mode following the method described by Hopmans et al. (2016). The Branched *versus* Isoprenoid Tetraether (BIT) index was calculated according to Hopmans et al. (2004). This proxy reflects soil and river input into marine environments but is also affected by in-situ marine production of brGDGT (De Jonge et al., 2014; Sinninghe Damsté et al., 2016).

2.2.7. 18S rRNA gene sequencing analysis

18S rRNA gene sequencing analysis was performed exclusively on DNA extracted from the Rhine water. To this end, 1/8 of the "DNA" filter was extracted using the PowerSoil kit (QIAGEN, Valencia, CA) following manufacturer's instructions. To amplify the eukaryotic V4 region of the 18S rRNA gene, we used the universal forward primer TAReuk454FWD1, V4F (5'-CCA GCA SCY GCG GTA ATT CC-3', *S. cerevisiae* position 565-584) and a reverse primer TAReuk454REV3, V4R (5′-ACTTTCGTTCTTGAT(C/T)(A/G)A-3′, *S. cerevisiae* position 964-981) from Stoeck et al. (2010). PCR reactions were performed on 5 replicates for each sample and each reaction included about 6 ng DNA template, 1.75 µL of each primer, 25 µL MasterMix phusion, 1.5 µL of DMSO and 19.25 µL deionised nuclease-free water for a total volume of 50 µL. Specifically, PCR consisted of an initial denaturation at 98 °C for 30 s, 11 x [98°C for 10 s, 53°C for 30 s, 72°C for 30 s]; 17 x [98°C for 10 s, 48°C for 30 s, 7°C for 30 s] as described in Logares et al. (2012). The PCR products were stained with SYBR® Safe (Life Technologies, the Netherlands) and visualised on a 1% agarose gel. Bands were excised with a sterile scalpel and purified with Qiaquick Gel Extraction Kit (QIAGEN, Valencia, CA) following the manufacturer's instructions. Equimolar concentrations of the barcoded PCR products were pooled and sequenced on GS FLX Titanium platform (454 Life Sciences) by Macrogen Inc., South Korea.

To estimate the concentration of total 18S rRNA genes of the Rhine SPM we carried out quantitative PCR (qPCR) using the same primers and the same cycling conditions as described above. qPCR analysis was performed on a Biorad CFX96TM Real-Time System/C1000 Thermal cycler equipped with CFX ManagerTM Software. Each reaction contained 12.5 µL MasterMix phusion, 8.25 µL deionised nuclease-free water, 0.75 µL DMSO, 1 µL from each primer and 0.5 µL Sybr green and 1 µL of DNA template. Reactions were performed on an iCycler iQTM 96-well plates (Bio-Rad). A mixture of V4 18S rRNA gene amplicons obtained as described above was used to prepare standard solutions. All qPCR reactions were performed in triplicate with standard curves from 640 to 6.4 x 10^8 V4 18S rRNA molecules per microliter. Specificity of the qPCR was verified with melting curve analyses (50°C to 95°C).

2.2.8. Bioinformatic analyses

Bioinformatic analyses of the sequencing results were carried out using the bioinformatic pipeline Quantitative Insight Into Microbial Ecology (QIIME) (Caporaso et al. 2010). 121 232 raw sequencing reads

were cleaned and demultiplexed, then chimeras and singletons were removed as described previously (Balzano et al. 2015) for a final dataset consisting of 58 480 good quality reads. Sequences were clustered into operational taxonomic units (OTUs) based on 97 % sequence identity. The dataset was then normalized by multiplying the percentage of reads with the concentration of V4 copies measured by qPCR. Relationships between LCDs and microbial eukaryotes were inferred by Spearman correlation analyses using the QIIME script observation_metadata_correlation.py and p-values were corrected for false discovery rate (Benjamini and Hochberg 1995).

2.2.9. Chlorophyll analysis

Rhine River water filters for pigment analysis were extracted following Holm-Hansen et al. (1965) and Arar and Collins (1997). Briefly, 20 mL cold acetone was added to the filters and stored in the fridge overnight. Subsequently, they were sonicated for 2 min in an ice bath to avoid chlorophyll degradation and 10 mL was transferred into a centrifuge tube and centrifuged for 10 min at 4000 rpm. 3 mL of the extract was then transferred into the observatory cuvette. The chlorophyll measurement was realized using a Fluorescence Spectrophotometer (Hitachi f-2500) calibrated with two standards containing 50 µg/L and 100 µg/L of chlorophyll a in acetone. Samples were measured one time to obtain Rb (fluorescence before acidification) and another time when 2 drops of a solution of 10% hydrochloric acid was added to obtain Ra (fluorescence after acidification). Chlorophyll concentration was then calculated as followed:

$$\boldsymbol{Chl-a} = \frac{(\boldsymbol{Rb}-\boldsymbol{Ra})\times\frac{\boldsymbol{A}}{\boldsymbol{B}}\times\boldsymbol{V_{extracted}}}{\boldsymbol{V_{filtrated}}} \quad (1)$$

Where Chl-a is the chlorophyll a concentration, A and B are constants obtained by measuring the standards ($\boldsymbol{A} = \frac{\boldsymbol{Chl-a_{standard}}}{\boldsymbol{Rb_{standard}}}$ and $\boldsymbol{B} = \boldsymbol{1} - \frac{\boldsymbol{Ra_{standard}}}{\boldsymbol{Rb_{standard}}}$).

3. Results

3.1. LCDs in the Godavari

The most abundant LCD in the SPM collected during the dry season is the C_{30} 1,15-diol (average 500±520 ng L^{-1}, n=18) followed by the C_{32} 1,15-diol (200±170 ng L^{-1}, Fig. 1b). The C_{30} 1,13- and C_{30} 1,14-diols occur in substantially lower concentrations (30±30 ng L^{-1} and 30±20 ng L^{-1}, respectively), whilst the C_{28} 1,13 and C_{28} 1,14-diols only occur in even lower concentrations (4±5 ng L^{-1} and 4±6 ng L^{-1} respectively, Fig. 1b). In the SPM collected during the wet season, the concentration of total LCDs is significantly higher than during the dry season (t-test, $p<0.05$). The C_{32} 1,15-diol is the most abundant of the LCDs in the wet season SPM (Fig. 1b, 740±710 ng L^{-1}, n=41), followed by the C_{30} 1,15-diol (500±530 ngL^{-1}), with much lower concentrations of C_{30} 1,13- and C_{30} 1,14-diol (20±10 and 30±20 ng L^{-1}, respectively).

In the Godavari riverbed sediments, the C_{32} 1,15-diol and C_{30} 1,15-diol are the most abundant LCDs. The C_{32} 1,15-diol is higher in abundance in the sediments collected during the dry season than in the wet season sediments (330±370 ng g^{-1}, n=30; 160±240 ng g^{-1}, n=34, respectively; Fig. 1c) except for the wet season sediments from the Dowleswaram reservoir where highest abundances are found (500±80 ng g^{-1}, n=2).

3.2. LCDs in the Danube and Black Sea

In the Danube sediments the main LCD is also the C_{32} 1,15-diol (3600±1300 ng g^{-1}, n=51), followed by the C_{30} 1,15-diol and the C_{30} 1,13-diol (1500±1000 ng g^{-1} and 500±100 ng g^{-1} respectively). Furthermore, sediment from the Iron Gates reservoir shows the highest concentration (t-test, $p<0.001$) of C_{32} 1,15-diol

(5400 ng g^{-1}, n=1, Fig 2b) in comparison with any of the other parts of the Danube River system (1500±3200 ng g^{-1}, n=22 in the Upper Danube; 3800±2400 ng g^{-1}, n=14 in the Middle Danube; 4400±3400 ng g^{-1}, n=10 in the Lower Danube; and 3000±1400 ng g^{-1}, n=4 in the delta).

For the Black Sea sediments (n=14) the C_{30} 1,15-diol is the main LCD, with the two sediments from sites located closest to the river mouth (P128 and P177, Fig. 2a) having a lower fractional abundance of this diol (0.57) than all other sediments (0.80 of all LCDs). In the Black sea sediments the main LCDs were the C_{30} 1,15-diol (6500±9000 ng g^{-1}) followed by the C_{30} 1,14-diol (1100±1600 ng g^{-1}).

3.3. LCDs in the Rhine

The main LCD in the SPM (n=5 in March; n=5 in September) of the Rhine is the C_{32} 1,15-diol (2.7±1.2 ng L^{-1} in March; 4.6±2.5 ng L^{-1} in September) followed by the C_{30} 1,15-diol (0.7±0.2 ng L^{-1} in March; 2.5±0.9 ng L^{-1} in September). The C_{30} 1,13-diol (0.5±0.2 ng L^{-1} in March; 0.6±0.2 ng L^{-1} in September) and C_{28} 1,13-diol (0.3±0.1 ng L^{-1} in March; 1.2±0.3 ng L^{-1} in September) are also present, while the C_{30} 1,14-diol (0.1±0 ng L^{-1} in March; 0.4±0.2 ng L^{-1} in September) and C_{28} 1,14-diol (0.1±0 ng L^{-1} in March and 0.2±0.1 ng L^{-1} in September) are only minor compounds. The concentrations of the C_{32} 1,15-diol was the highest at the sampling location in Karlsruhe in September with 9.1 ng L^{-1} (see Fig. 3b) and varies from 1.6 - 9.1 ng L^{-1} for all sites, while the C_{30} 1,15-diol varies from 1.0 - 7.6 ng L^{-1}. The LCD concentration is significantly higher in September than in March (sum of all diols is 9.4±3.8 ng L^{-1} and 4.3±1.5 ng L^{-1}, respectively, $p<0.001$). The BIT index varies from 0.64 to 0.93 (Fig. 3b), and is higher in September than in March (average 0.92±0.01 and 0.79±0.02, respectively). In March there is an increase of the BIT index downstream (0.64 in Karlsruhe to 0.86 in Kleve) but it remains constant in September. The chlorophyll concentrations vary from 1 to 6 µg L^{-1} and peak in September at most locations (4±2 and 3±1 µg L^{-1} in September and March, respectively) indicating a small seasonal trend.

We sequenced the 18S rRNA gene from the Rhine SPM using universal eukaryote primers. Overall the libraries were dominated by reads affiliated to Opisthokonta (31 %), Stramenopiles (28 %), Hacrobia (24 %) and Alveolata (10 %) (see supplemental Figure 2). All the LCD-producing phytoplankton known to date (Eustigmatophyceae, *Proboscia spp*., and *Apedinella radians*) are affiliated to the Stramenopile supergroup, and the Stramenopiles found here mostly include diatoms and Chrysophyceae. However the diatom operational taxonomic units (OTUs) most closely related to *Proboscia* belong to the genera *Melosira*, *Aulacoseira*, and *Actinocyclus* which have never been reported to contain LCDs. The presence of LCDs within Chrysophyceae has also never been determined.

One eustigmatophyceaen OTU (denovo161 *Monodus guttula*, Supplement 3) represented by two reads (one in Koblenz and one in Kleve in September) and five 18S rRNA gene reads (one in Cologne in March, three in Mainz, one in Koblenz and two in Kleve in September) associated with two OTUs from Pedinellales (denovo18 unidentified Pedinellales and denovo338 *Pseudopedinella sp*., Supplement 3) were found in the Rhine SPM. The concentration of 18S rRNA genes varied between 2.7±0.1x10^7 and 1.0±0.1x10^8 copiesL^{-1} in March and 1.6±0.1x10^7 and 4.7±0.7x10^7copiesL^{-1} in September (Fig. 5c and supplement 1). Spearman rank correlation analyses performed using QIIME indicate that none of the OTU found here exhibit significant correlation with LCDs (data not shown).

4. Discussion

4.1. Where are LCDs produced in rivers?

For the different river systems a link between water conditions and LCD concentrations can often be observed. In particular, for the Godavari SPM there is a higher concentration of LCDs in the SPM from the wet season compared to that of the dry season (1900±1000 ng L^{-1} vs. 570±260 ng L^{-1}, respectively). The

Godavari sediments collected during the dry season have higher concentration in LCDs (t-test, $p < 0.05$) than the riverbed sediments from the wet season, opposite to what is observed in the SPM. During the wet season, the Godavari is more turbid and has a higher flow velocity. This high turbidity of the river water (Balakrishna and Probst, 2005; Syvitsky and Saito, 2007) may reduce LCD production as this limits light availability and, therefore, algal productivity. However, the higher concentration of LCDs observed in the SPM during the wet season indicates that this explanation is likely not valid. Alternatively, the low concentration of LCDs in the wet season sediments could be due to the high flow velocity of the river water that prevents the LCDs formed in the rivers to be deposited in the river bed sediments.

During the wet season, the C_{32} 1,15-diol is present in significantly (t-test, $p<0.001$) higher quantities in the sediments of the Dowleswaram reservoir (500±80 ng g^{-1}) compared to other parts of the river system, indicating that either the C_{32} 1,15-diol production is enhanced in the reservoir or that the C_{32} 1,15-diol is transported from upstream and accumulates within the reservoir. In the reservoir, both SPM and sediments have a higher organic carbon content compared to the rest of the river, whereas the suspended particle load is only slightly lower (Usman et al., 2018), which could be explained by contribution of primary produced organic carbon. This suggests that increased production of the C_{32} 1,15-diol is facilitated by the calm stagnant conditions in the reservoir. Similarly, in the Danube system the highest concentration of the C_{32} 1,15-diol is also found in a calm and stagnant water area: the Iron Gates reservoir (5500 ng g^{-1} vs. 3200±1100 ng g^{-1} on average for the rest of the catchment, Fig. 2b).

Pradhan et al. (2014) reported a major input (i.e. 40-45%) of OM from freshwater algae in sediments from the Upper Godavari, based on the C/N ratio and $\delta^{13}C$ values of the sedimentary organic carbon, suggesting optimal conditions for aquatic production in impoundments in this driest part of the river basin. Indeed, significantly (t-test, $p<0.05$) higher concentrations of the C_{32} 1,15-diol are found in the Upper Godavari sediments collected during the dry season compared to the rest of the catchment, indicating that low flow, calm and stagnant water conditions are optimal for C_{32} 1,15-diol production.

Collectively, our results suggests that LCDs and especially the C_{32} 1,15-diol are preferentially produced in relatively calm and stagnant areas of river systems.

4.2. River versus marine LCDs

During the dry season, the SPM in the delta of the Godavari exhibits a LCD distribution significantly (t-test, $p<0.05$) deviating from the general distribution in river SPM and sediments, i.e. the LCDs are dominated by the C_{30} 1,15-diol (fractional abundance of 0.74±0.14 for the delta *vs.* 0.47±0.16 for the rest of the river in the dry season) rather than the C_{32} 1,15-diol. This dominance of the C_{30} 1,15-diol is different from LCD distributions usually found in rivers (De Bar et al., 2016; Lattaud et al., 2017a, this study), but similar to that observed in tropical marine sediments (Rampen et al., 2014a; Lattaud et al., 2017a). This suggests a marine influence on the LCDs in the delta during the dry season. Indeed, the electrical conductivity of the delta river water in this season is typical for brackish water (Gupta et al., 1997; Sarma et al., 2009, 2010). The electrical conductivity decreases land inwards, indicating that the influence of marine waters is substantially reduced upstream.

The LCD distributions in the Black Sea sediments are also dominated by the C_{30} 1,15-diol (fractional abundance >0.9, Fig. 2c), whereas the C_{32} 1,15-diol is the most abundant diol in the sediments of the Danube. The fractional abundance of the C_{32} 1,15-diol decreases with increasing distance from the river mouth as do the values for the BIT index (Fig. 2c). This decrease in C_{32} 1,15-diol abundance is similar to that observed in the delta of the Godavari River during the dry season, but can now be followed along a much larger gradient; the Black Sea sediment has a clear marine signal (BIT=0.06±0.03, n=12, this study; Kusch et al., 2016) with a dominant C_{30} 1,15-diol, whereas the Danube River is dominated by the C_{32} 1,15-diol and has an average BIT index value of 0.91±0.04 (n=43, Freymond et al., 2017).

To visualize the differences between marine and river LCD distributions, a ternary plot was generated with the poles representing the different fractional abundances of the LCDs: C_{30} 1,15-diol, C_{32} 1,15-diol and the sum of C_{30} 1,13 and C_{28} 1,13-diols (Fig. 4a). The 1,14-diols were excluded from this plot as they likely have a different biological source (Sinninghe Damsté et al., 2003; Rampen et al., 20011, 2014b). Data from the river SPM from this study have been included as well as river SPM and sediments from Lattaud et al. (2017a) and lake sediments from Rampen et al. (2014b). For the marine dataset, the marine sediments from the studies of Lattaud et al. (2017b), Rampen et al. (2012) and De Bar et al. (2016) were used. This ternary diagram shows that river SPM and sediments, as well as the lake sediments contain a higher proportion of C_{32} 1,15-diol than open marine surface sediments, where their fractional abundance nearly always is <10%. This major difference in the distribution of LCDs in marine and freshwater environments suggests that LCDs are likely produced by different organisms in freshwater and marine systems (cf. Lattaud et al., 2017a). This difference is useful to differentiate river influenced sediments and marine sediment (cf. Lattaud et al., 2017b).

4.3. Who is producing LCDs in river systems?

4.3.1. Comparison with culture data

In all of the three river systems investigated here the C_{32} 1,15-diol is the major diol (average fractional abundance of 0.47±0.17 for Danube, Rhine, and Godavari), followed by the C_{30} 1,15-diol (0.31±0.21) (Fig. 1-3). To constrain potential biological producers of the LCDs, the LCD distributions in river SPM from this study and those of Lattaud et al. (2017a) and De Bar et al. (2016) were plotted in another ternary diagram (Fig. 4b), along with the LCD distribution of cultured eustigmatophyte algae (data from Rampen et al., 2014b). This diagram uses the fractional abundances of the C_{28} 1,13-diol, C_{32} 1,15-diol and the sum of the C_{30} 1,13- and C_{30} 1,15-diols. SPM from delta regions with a clear marine contribution were excluded, i.e. SPM with low BIT values (BIT<0.3).

Most of the river LCD distributions are similar to the LCD distribution of *Goniochloris sculpta* from the Goniochloridaceae family, especially for the Rhine (this study), Danube (this study) and Tagus (data from De Bar et al., 2016). However, LCD distributions in rivers from a tropical region such as the Godavari and Amazon do not plot close to this species (Rampen et al., 2014b). This observation may point at a role of temperature in the distribution of LCDs, or at different producers in tropical freshwater systems. However, there is no significant correlation between the fractional abundance (excluding 1,14-diols) of the C_{32} 1,15-diol, or other diols, in rivers and mean annual air temperature of the river catchment ($r^2 = 0.002$, $p = 0.6$). This is in contrast to the study of Rampen et al. (2014b), where a positive relation between the growth temperature of cultures of eustigmatophyte families and the fractional abundance of C_{32} 1,15-diol was observed. Potentially, this difference could be due to the fact that we are using mean annual air temperature and not in situ river temperatures.

Villanueva et al. (2014) determined the diversity and abundance of specific eustigmatophyte algae using 18S rRNA gene sequences of the SPM at different water depths in Lake Chala, tropical East Africa. Villanueva et al. (2014) found 214 eustigmatophycean sequences affiliated to 5 distinct phylogenetic clades one of which affiliated to the Goniochloridaceae family, and four novel groups, two of which closely related to the Monodopsidaceae and Eustigmatophyceae families. This suggests a role of novel uncultured Eustigmatophyceae in LCD production in riverine ecosystems. Furthermore, they quantified LCDs in monthly sediment trap material from the middle of the lake and observed that LCD distributions varied on a seasonal basis. The proportion of C_{32} 1,15-diol was highest in February and June, while the C_{30} 1,15-diol dominated in April, indicating separate blooms of different LCD producers. Our results suggest that there are potentially unknown eustigmatophycean LCD producers in river systems or that there may be multiple LCD producers, depending on the season and the location.

4.3.2. 18S rRNA gene sequencing analysis of SPM in the Rhine

An alternative approach to comparing culture data to lipid distribution for identifying producers of LCDs is to analyze the DNA composition of river water in which LCDs are detected (cf. Villanueva et al., 2014). To characterize the producers of the 1,13- and 1,15-diols, we sequenced V4 region of the 18S RNA gene in the SPM from the Rhine using 454 sequencing. This sequencing effort yielded ca. 60 000 reads for the pooled samples (see Supplement 3) but we only detected 3 OTUs and 9 reads associated with potential LCD producers. Indeed, the near absence of eustigmatophyte reads in the Rhine SPM (Fig. 5a) suggests that they are not the major producers of LCDs. Also dichtyochophytes were not detected in all the SPM in contrast to LCDs. There is no correlation between the OTUs found in the Rhine River and the concentration of 1,13- and 1,15-diols.

To pinpoint the producers of the 1,14-diols, we investigated the diatom distribution in the Rhine water. *Proboscia*, the only currently known diatom genus producing 1,14-diols (Sinninghe Damsté et al., 2003; Rampen et al., 2014b), contains marine species (Moita et al., 2003; Lassiter et al., 2006; Takahashi et al., 2011) and, consistently, was not detected in our libraries. Other genera from the same group as *Proboscia* (radial centric diatoms) were found at all sites in March, and are also found in Karlsruhe and Mainz in September (Fig. 5b). To establish whether these diatoms represent a potential source of 1,14-diols, we estimated their abundance by quantifying the concentration of total 18S rRNA gene copies multiplied with the percentage of each OTU of the total reads. However, there is no correlation between the concentration of 1,14-diols and the number of gene copies per liter of radial centric diatoms ($r^2 = 0.08$; p value = 0.4).

4.3.3. Are LCDs coming from dead OM or *in situ* living organisms?

Interestingly, there is no significant correlation ($r^2=0.2$, $p=0.2$) between the concentration of chlorophyll a (Fig. 5d) and the concentration of total LCDs, 1,14-diols , or 1,13- plus 1,15-diols (Fig. 5a, b), i.e. there is no apparent link between primary production and LCD production. The lack of correlation between LCDs and both OTUs and Chl-a suggests that the LCDs in the Rhine are either not produced *in situ* or are derived from unknown organisms (Villanueva et al., 2014).

To distinguish if the LCDs are a part of dead organic matter, we performed an incubation experiment using ^{13}C-labelled bicarbonate. After 52 h, ^{13}C incorporation was detected in lipids such as β-sitosterol ($\Delta\delta$ = +120‰) indicating uptake by phytoplankton. However, at the same time, we did not detect any incorporation of ^{13}C in LCDs, suggesting that the incubation time may be too short for LCD producers to take up the ^{13}C or that the LCDs are not synthesized *in situ* during the time of sampling. If LCD producers are photosynthetic eukaryotes as indicated by culture studies (Volkman et al., 1999; Rampen et al., 2007, 2014b) then the incubation time used in the experiment should be sufficient for them to take up the ^{13}C-bicarbonate dissolved in the water. This suggests that LCDs are likely not synthesized at any of the sampling locations of the Rhine.

The absence of *in situ* LCD production could be due to the high flow velocity at these sampling sites. As also observed in the Godavari and Danube, the high flow velocity flow areas show a lower abundance of LCDs, while low flow areas show a higher abundance of LCDs. It is likely that LCDs in the Rhine would be produced in more stagnant waters like in lakes, or dead river branches, and that they would be more abundant in these areas. Thus, LCDs, which are likely degraded more slowly than DNA, reflect a fossil signal, while the DNA reflects an *in situ* signal. Similarly, Villanuaneva et al. (2014) showed that, while LCDs were abundantly present in the surface water of Lake Chala, the DNA of eustigmatophytes could not be detected.

5. Conclusion

We studied three river systems to determine where LCDs are produced in rivers, if their distribution is different from that of marine LCDs, and to constrain their producers. Confirming previous results, riverine LCDs show a striking difference in distribution from marine LCDs as they are characterized by a high fractional abundance of the C_{32} 1,15-diol (>40%), while marine LCDs have generally more of the C_{30} 1,15-diol (>50%). The C_{32} 1,15-diol is more abundant in calm stagnant waters than in fast flowing parts of the rivers, indicating that they are likely produced in calmer water. Comparison of LCD distributions of Eustigmatophyceae cultures with those in rivers indicate that *Goniochloris* species might be an important 1,13- and 1,15-diols producer in some river systems. 18S rRNA gene analysis of one of these rivers, the Rhine, did, however, not lead to any identification of this species, nor did a labelling study using bicarbonate lead to labelling of LCDs. This might indicate that LCDs in fast flowing parts of rivers are not derived from *in situ* living plankton but from plankton residing in stagnant waters of these river systems such as lakes or side ponds.

Competing interests.

The authors declare that they have no conflict of interest.

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References

Arar, E.J. and Collins, G.B.: In Vitro Determination of Chlorophyll-a and Pheophytin-a in Marine and Freshwater Algae by Fluorescence, Environmental Protection Agency (EPA), Method 445.0, 1997.

Balakrishna, K. and Probst, J.L.: Organic carbon transport and C/N ratio variations in a large tropical river: Godavari as a case study, India, Biogeochem., 73, 457-473, doi.org/10.1007/s10533-004-0879-2, 2005.

Balzano, S., Abs, E. and Leterme, S.C.: Protist diversity along a salinity gradient in a coastal lagoon. Aquatic Microbial Ecology 74, 263-277, doi.org/10.3354/ame01740, 2015.

Benjamini, Y. and Hochberg, Y.: Controlling the false discovery rate. A practical and powerful approach to multiple testing. . Journal of the Royal Statistical Society Series B-Methodological 57, 289-300, 1995. jstor.org/stable/2346101.

Biksham, G. and Subramanian, V.: Sediment transport of the Godavari River basin and its controlling factors. J. Hydrol., 101, 275-290, doi.org/10.1016/0022-1694(88)90040-6, 1988.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J. and Knight, R.: QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335-336, 10.1038/nmeth.f.303, 2010.

Castañeda, I.S., Werne, J.P., Johnson, T.C. and Powers, L.A.: Organic geochemical records from Lake Malawi (East Africa) of the last 700 years, part II: Biomarker evidence for recent changes in primary productivity. Palaeogeogr. Paleoclim. Palaeocl., 303, 140-154, doi.org/10.1016/j.palaeo.2010.01.006, 2011.

De Bar, M., Dorhout, D.J. C., Hopmans, E.C., Sinninghe Damsté, J.S. and Schouten, S.: Constraints on the application of long chain diol proxies in the Iberian Atlantic margin, Org. Geochem., 184-195, doi.org/10.1016/j.orggeochem.2016.09.005, 2016.

De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A. and Sinninghe Damsté, J.S.: *In situ* produced branched glycerol dialkyl glycerol tetraethers in suspended particulate matter from the Yenisei River, Eastern Siberia. Geochim. Cosmochim. Acta 125, 476–491, doi.org/10.1016/j.gca.2013.10.031, 2014.

Freymond, C.V., Peterse, F., Fischer, L.V., Filip, F., Giosan, L. and Eglinton, T.I.: Branched GDGT signals in fluvial sediments of the Danube River basin: Method comparison and longitudinal evolution, Org. Geochem., 103, 88-96, doi.org/10.1016/j.orggeochem.2016.11.002, 2017.

Freymond, C.V., Kundig,N., Stark, C, Peterse, F., Buggle, B., Lupker, M., Platze, M., Blattmann, T.M., Filip, F., Giosan, L., and Eglinton, T.I.: Evolution of biomolecular loadings along a major river system, Geochim. Cosmochim. Act., 223, 389-404, 10.1016/j.gca.2017.12.010, 2018.

Gal, J.-K., Kim, J.-H., and Shin, K.-H.: Distribution of long chain alkyl diols along a south-north transect of the northwestern Pacific region: Insights into a paleo sea surface nutrient proxy. Org. Geochem., *in press*, doi.org/10.1016/j.orggeochem.2018.01.010, 2018.

Gogou, A., and Stephanou, E.G.: Marine organic geochemistry of the Eastern Mediterranean: 2. Polar biomarkers in Cretan Sea surficial sediments. Mar. Chem. 85, 1-25, doi.org/10.1016/j.marchem.2003.08.005, 2004.

Gupta, L.P., Subramanian, V. and Ittekkot, V.: Biogeochemistry of particulate organic matter transported by the Godavari River, India. Biogeochim. 38, 103-128, 10.1023/A:1005732519216DO, 1997.

Hoffmann, T., Erkens, G., Gerlach, R., Klostermann, J. and Lang, A.: Trends and controls of Holocene floodplain sedimentation in the Rhine catchment, CATENA, 77, 96-106, doi.org /10.1016/j.catena.2008.09.002, 2007.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W. and Strickland, J.D.H.: Fluorometric determination of chlorophyll, ICES J. Mar. Sci., 30, 3-15, doi-org/10.1093/icesjms/30.1.3, 1965.

Hopmans, E.C., Weijers, J.W.H., Schefuß, E., Herfort, L., Sinninghe Damsté, J.S. and Schouten, S.: A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids, Earth Planet. Sc. Lett., 224, 107-116, doi:10.1016/j.epsl.2004.05.012, 2004.

Hopmans, E.C., Schouten, S. and Sinninghe Damsté, J.S.: The effect of improved chromatography on GDGT-based palaeoproxies, Org. Geochem., 93, 1-6, doi.org/10.1016/j.orggeochem.2015.12.006, 2016.

Huguet, C., Hopmans, E.C., Febo-Ayala, W., Thompson, D.H., Sinninghe Damsté, J.S. and Schouten, S.: An improved method to determine the absolute abundance of glycerol dibiphytanyl glycerol tetraether lipids, Org. Geochem., 37, 1036-1041, doi.org/10.1016/j.orggeochem.2006.05.008, 2006.

Kusch, S., Rethemeyer, J., Schefuß, E. and Mollenhauer, G.: Controls on the age of vascular plant biomarkers in Black Sea sediments, Geochim. Cosmochim. Act., 74, 7031-7047, 10.1016/j.gca.2010.09.005, 2010.

Kusch, S., Rethemeyer, J., Hopmans, E.C., Wacker, L. and Mollenhauser, G.: Factors influencing ^{14}C concentrations of algal and archaeal lipids and their associated sea surface temperature proxies in the Black Sea, Geochim. Cosmochim. Ac., 188, 35-57, doi.org/10.1016/j.gca.2016.05.025, 2016.

Lassiter, A.M., Wilkerson, F.P., Dugdale, R.C. and Hogue, V.E.: Phytoplankton assemblages in the CoOP-WEST coastal upwelling area. Deep-Sea Research Part II-Topical Studies in Oceanography 53, 3063-3077, 10.1016/j.dsr2.2006.07.013, 2006.

Lattaud, J., Kim, J.-H., De Jonge, C., Zell, C., Sinninghe Damsté, J.S. and Schouten, S.: The C_{32} alkane-1,15-diol as a tracer for riverine input in coastal seas, Geochim. Cosmochim. Ac., 202, 146-158, doi.org/10.1016/j.gca.2016.12.030, 2017, 2017a.

Lattaud, J., Dorhout, D., Schulz, H., Castañeda, I.C., Schefuss, E., Sinninghe Damsté, J.S. and Schouten, S.: The C_{32} alkane-1,15-diol as a proxy for late Quaternary riverine input in coastal margin, Clim. Past, 13, 1049-1061, doi.org/10.5194/cp-13-1049-2017, 2017b.

Logares, R., Audic, S., Santini, S., Pernice, M.C., de Vargas, C. and Massana, R.: Diversity patterns and activity of uncultured marine heterotrophic flagellates unveiled with pyrosequencing, ISME J., 6, 1823-1833, doi: 10.1038/ismej.2012.36, 2012.

Moita, M.T., Oliveira, P.B., Mendes, J.C. and Palma, A.S.: Distribution of chlorophyll a and *Gymnodinium catenatum* associated with coastal upwelling plumes off central Portugal. Acta Oecologica-International Journal of Ecology 24, S125-S132, 10.1016/S1146-609X(03)00011-0, 2003.

Pfister, L., Kwadijk, J., Musy, A., Bronstert, A. and Hoffmann, L.: Climate change, land use change and runoff prediction in the Rhine-Meuse basins. River Res. Applic., 20, 229-241, doi:10.1002/rra.775, 2004.

Pradhan, U.K., Wu, Y., Shirodkar, P.V., Zhang, J. and Zhang, G.: Multi-proxy evidence for compositional change of organic matter in the largest tropical (peninsular) river basin of India, J. Hydrol., 519, 999-1009, doi.org/10.1016/j.jhydrol.2014.08.018, 2014.

Ramasubramanian, R., Gnanappazham, L., Ravishankar, L. and Navamuniyammal, M.: Mangroves of Godavari – Analysis Through Remote Sensing Approach, Wetl. Ecol. Manag., 14, 29-37, doi: 10.1007/s11273-005-2175-x, 2006.

Rampen, S.W, Schouten, S., Wakeham, S.G.and Sinninghe Damsté, J.S.: Seasonal and spatial variation in the sources and fluxes of long chain diols and mid-chain hydroxy methyl alkanoates in the Arabian Sea, Org. Geochem., 38, 165–179, doi.org/10.1016/j.orggeochem.2006.10.008, 2007.

Rampen, S.W., Schouten, S. and Sinninghe Damsté, J.S.: Occurrence of long chain 1,14-diols in *Apedinella radians*, Org. Geochem., 42, 572-574, doi:10.1016/j.orggeochem.2011.03.009, 2011.

Rampen, S.W., Willmott, V., Kim, J.-H., Uliana, E., Mollenhauer, G., Schefuß, E., Sinninghe Damsté, J.S. and Schouten, S.: Long chain 1,13- and 1,15-diols as a potential proxy for palaeotemperature reconstruction, Geochim. Cosmochim. Acta. 84, 204–216, doi.org/10.1016/j.gca.2012.01.024, 2012.

Rampen, S.W., Willmott, V., Kim, J-H., Rodrigo-Gamiz, M., Uliana, E., Mollenhauer, G., Schefuss, E., Sinninghe Damsté, J.S. and Schouten, S.: Evaluation of long chain 1,14-alkyl diols in marine sediments as indicators for upwelling and temperature, Org. Geochem, 76, 39-47, doi.org/10.1016/j.orggeochem.2014.07.012, 2014a.

Rampen, S.W., Datema, M., Rodrigo-Gámiza, M., Schouten, S., Reichart, G.-J. and Sinninghe Damsté, J.S.: Sources and proxy potential of long chain alkyl diols in lacustrine environments, Geochimi. Cosmochimi. Acta, 144, 59-71, doi.org/10.1016/j.gca.2014.08.033, 2014b.

Rao, K.N., Saito, Y., Nagakumar, K.C.V., Demudu, G., Rajawat, A.S., Kubo, S. and Li, Z.: Palaeogeography and evolution of the Godavari delta, east coast of India during the Holocene: An example of wave-dominated and fan-delta settings. Palaeogeogr.Paleoclim. Palaeocl., 440, 213-233, doi.org/10.1016/j.palaeo.2015.09.006, 2015.

Rimbu, N., Boroneant, C., Buta, C. and Dima, M.: Decadal variability of the Danube River flow in the lower basin and its relation with the North Atlantic oscillation. Int. J. Climatol., 22, 1169-1179, doi: 10.1002/joc.788, 2002.

Romero-Viana, L., Kienel, U. and Sachse, D.: Lipid biomarker signatures in a hypersaline lake on Isabel Island (Eastern Pacific) as a proxy for past rainfall anomaly (1942–2006 AD). Palaeogeogr. Paleoclim. Palaeocl., 350–352, 49–61, doi:10.1016/j.palaeo.2012.06.011, 2012.

Sarma, V.V.S.S., Gupta, S.N.M., Babu, P.V.R., Acharya, T., Harikrishnachari, N., Vishnuvardhan, K., Rao, N.S., Reddy, N.P.C., Sarma, V.V., Sadhuram, Y., Murty, T.V.R. and Kumar M.D.: Influence of river discharge on plankton metabolic rates in the tropical monsoon driven Godavari estuary, India. Estuar. Coast. Shelf Sci. 85, 515-524, 10.1016/j.ecss.2009.09.003, 2009.

Sarma, V.V.S.S., Prasad, V.R., Kumar, B.S.K., Rajeev, K., Devi, B.M.M., Redyy, N.P.C., Sarma, V.V. and Kumar, M.D.: Intra-annual variability in nutrients in the Godavari estuary, India. Cont. Shelf Res., 30, 2005-2014, 10.1016/j.csr.2010.10.001, 2010.

Schmidt, F., Hinrichs, K.-U. and Elvert, M.: Sources, transport, and partitioning of organic matter at a highly dynamic continental margin. Mar. Chem. 118 37-55, doi:10.1016/j.marchem.2009.10.003, 2010. 10.1016/j.orggeochem.2010.05.013.

Shimokawara, M., Nishimura, M., Matsuda, T., Akiyama, N. and Kawai, T.: Bound forms, compositional features, major sources and diagenesis of long-chain alkyl mid-chain diols in Lake Baikal sediments over the past 28,000 years. Org. Geochim., 41, 753-766, 2010.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Abba, B., Muyzer, G. and Schouten, S.: A diatomaceous origin for long-chain diols and mid-chain hydroxy methyl alkanoates widely occurring in Quaternary marine sediments: indicators for high nutrient conditions. Geochim. Cosmochim. Acta, 67, 1339–1348, doi:10.1016/S0016-7037(02)01225-5, 2003.

Sinninghe Damsté, J.S.: Spatial heterogeneity of sources of branched tetraethers in shelf systems: The geochemistry of tetraethers in the Berau River delta (Kalimantan, Indonesia). Geochim. Cosmochim. Acta, 186, 13-31, doi.org/10.1016/j.gca.2016.04.033, 2016.

Smith, M., De Deckker, P., Rogers, J., Brocks, J., Hope, J., Schmidt, S., Lopes dos Santos, R. and Schouten, S.: Comparison of Uk'37, TEXH86 and LDI temperature proxies for reconstruction of south-east Australian ocean temperatures. Org. Geochim., 64, 94.104, 10.1016/j.orggeochem.2013.08.015, 2013.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.-W. and Richards, T.A.: Multiple marker parallel tag envvironmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol., 19, 21-31, 10.1111/j.1365-294X.2009.04480.x, 2010.

Syvitsky, J.P.M. and Saito, Y.: Morphodynamics of deltas under the influence of humans, Global Planet. Change, 57, 261-282, doi.org/10.1016/j.gloplacha.2006.12.001, 2007.

Takahashi, K., Jordans, R. and Priddle, J.: The Diatom genus Proboscia in subartic waters, Diatoms Research, 9, 411-428, 10.1080/0269249X.1994.9705317.

Tolmazin, D.: Changing Coastal oceanography of the Black Sea. I: Northwestern Shelf, Prog. Oceano., 15, 217-276, doi.org/10.1016/0079-6611(85)90038-2, 1985.

Usman, M.O., Kirkels, F.M.S.A., Zwart, H.M., Basu, S., Ponton, C., Blattmann, T.M., Ploetze, M., Haghipour, N., McIntyre, C., Peterse, F., Lupker, M., Giosan, L. and Eglinton T.I.: Reconciling drainage and receiving basin signatures of the Godavari River system. Biogeosc. 15, 3357-3375, doi.org10.5194/bg-15-3357-2018.

Versteegh, G.J.M., Bosch, H.J. and de Leeuw, J.W.: Potential palaeoenvironmental information of C_{24} to C_{36} mid-chain diols, keto-ols and mid-chain hydroxy fatty acids; a critical review, Org. Geochem., 27, 1–13, doi.org/10.1016/S0146-6380(97)00063-6, 1997.

Versteegh, G.J.M., Jansen, J.H.F., de Leeuw, J.W. and Schneider, R.R.: Mid-chain diols and keto-ols in SE Atlantic sediments: a new tool for tracing past sea surface water masses?, Geochim. Cosmochim. Act. 64, 1879–1892, doi.org/10.1016/S0016-7037(99)00398-1, 2000.

Villanueva, L., Besseling, M., Rodrigo-Gámiz, M., Rampen, S.W., Verschuren, D. and Sinninghe Damsté, J.S.: Potential biological sources of long chain alkyl diols in a lacustrine system, Org. Geochem., 68, 27–30, doi.org/10.1016/j.orggeochem.2014.01.001, 2014.

Volkman, J.K., Barett, S.M. and Blackburn, S.I.: Eustigmatophyte microalgae are potential sources of C_{29} sterols, C_{22}–C_{28}n-alcohols and C_{28}–C_{32}n-alkyl diols in freshwater environments, Org. Geochem.,30, 307–318, doi.org/10.1016/S0146-6380(99)00009-1, 1999.

Willmott, V., Rampen, S. W., Domack, E., Canals, M., Sinninghe Damsté, J. S. and Schouten, S.: Holocene changes in Proboscia diatom productivity in shelf waters of the northwestern Antarctic Peninsula. Antarct. Sci. 22, 3–10, 10.1017/S095410200999037X, 2010.

Zhang, Z., Metzger, P. and Sachs, J.P.: Co-occurrence of long chain diols, keto-ols, hydroxy acids and keto acids in recent sediments of Lake El Junco Galapagos Islands. Org. Geochem. 42, 823–837, doi:10.1016/j.orggeochem.2011.04.012, 2011.

Legend

Figure 1: a. Location of the Godavari samples (Usman et al., 2018) with enhanced view of the delta region, b. concentrations of LCDs in SPM (collected during wet and dry seasons) and c. concentrations of LCDs in sediments (collected during wet and dry seasons). Grey bars indicate standard deviation for each area.

Figure 2: a. Location of the Danube samples with enhanced view of the Danube river mouth samples, b. concentration of LCDs (this study) and BIT values (from Freymond et al., 2017) in the Danube sediments (n=1 for the reservoir) and c. fractional abundance of the LCDs in the Danube (average of the Upper, Middle and Lower Danube), Danube delta, Danube river mouth (stations P128 and P177) and Black Sea (12 stations). Grey bars indicate standard deviation for each area.

Figure 3: a. Location of the Rhine samples (n=1 per site) and b. concentration of LCDs and BIT values (September and March) in the Rhine SPM.

Figure 4: Ternary plot (C_{28} 1,13-diol; C_{30} 1,13- and C_{30} 1,15-diols; C_{32} 1,15-diol) of a. marine sediments (from Rampen et al., 2012, De Bar et al., 2016, Lattaud et al., 2017b), river sediments (this study, Lattaud et al., 2017a), lake sediment (from Rampen et al., 2014b) and river SPM (this study, Lattaud et al., 2017a) and b. cultivated algae (Rampen et al., 2014b) and river SPM (this study, Lattaud et al., 2017a).

Figure 5: Results of the 18S rRNA analysis of the Rhine water, a. gene copy per liter of eustigmatophytes and dictyochophytes and concentration of 1,13- and 1,15-diols as well as concentration of the C_{32} 1,15-diol, b. gene copy number per liter of radial centric diatoms and concentration of 1,14-diols, c. total gene copy per liter and d. chlorophyll a concentration.

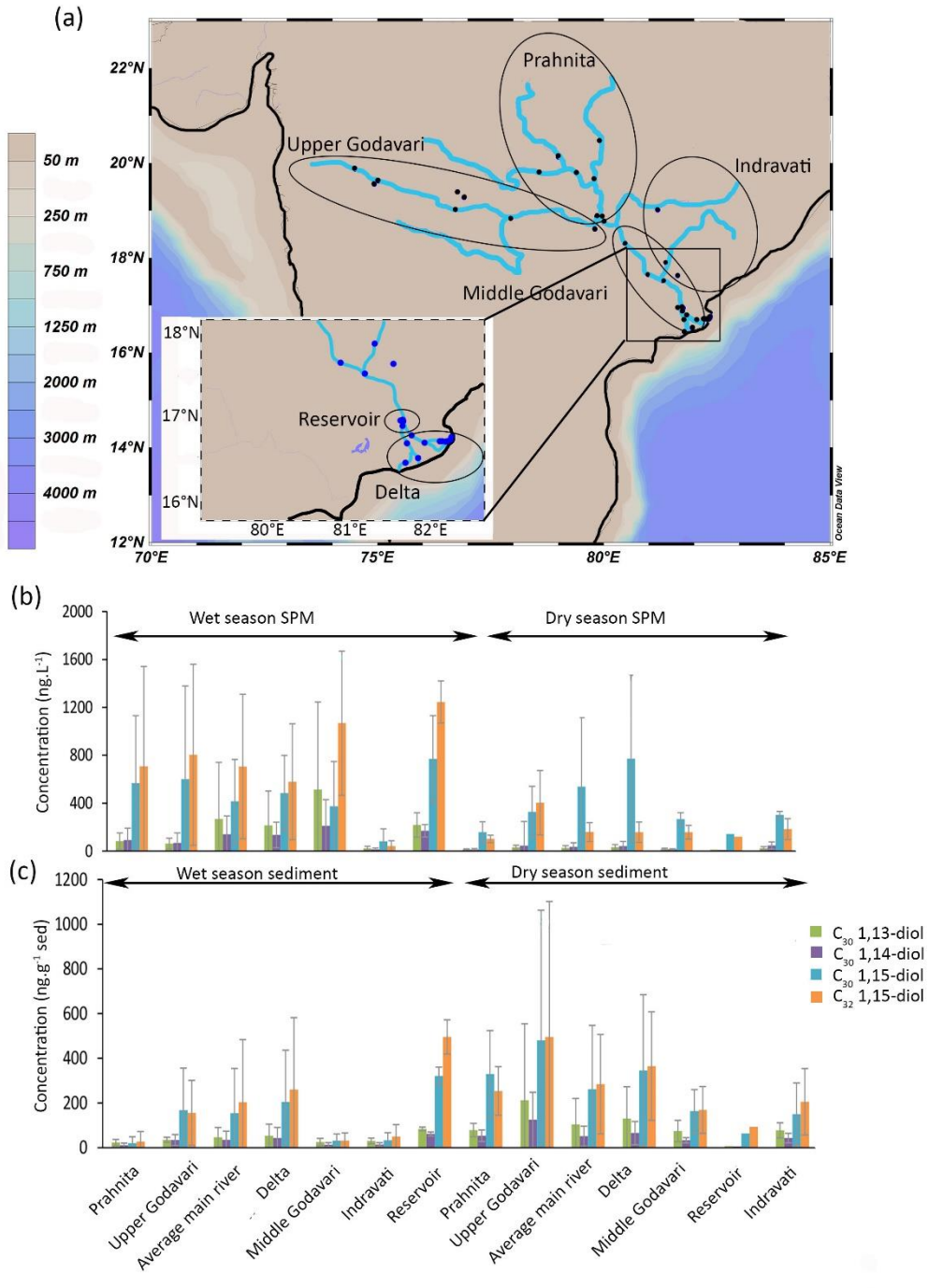
(a)
Prahnita
Upper Godavari
Indravati
Middle Godavari
Reservoir
Delta
50 m
250 m
750 m
1250 m
2000 m
3000 m
4000 m
22°N
20°N
18°N
16°N
14°N
12°N
70°E
75°E
80°E
85°E
18°N
17°N
16°N
80°E
81°E
82°E
Ocean Data View
(b)
Wet season SPM
Dry season SPM
Concentration (ng.L-1)
2000
1600
1200
800
400
0
(c)
Wet season sediment
Dry season sediment
Concentration (ng.g-1 sed)
1200
1000
800
600
400
200
0
C30 1,13-diol
C30 1,14-diol
C30 1,15-diol
C32 1,15-diol
Prahnita
Upper Godavari
Average main river
Delta
Middle Godavari
Indravati
Reservoir
Prahnita
Upper Godavari
Average main river
Delta
Middle Godavari
Reservoir
Indravati

Figure 1

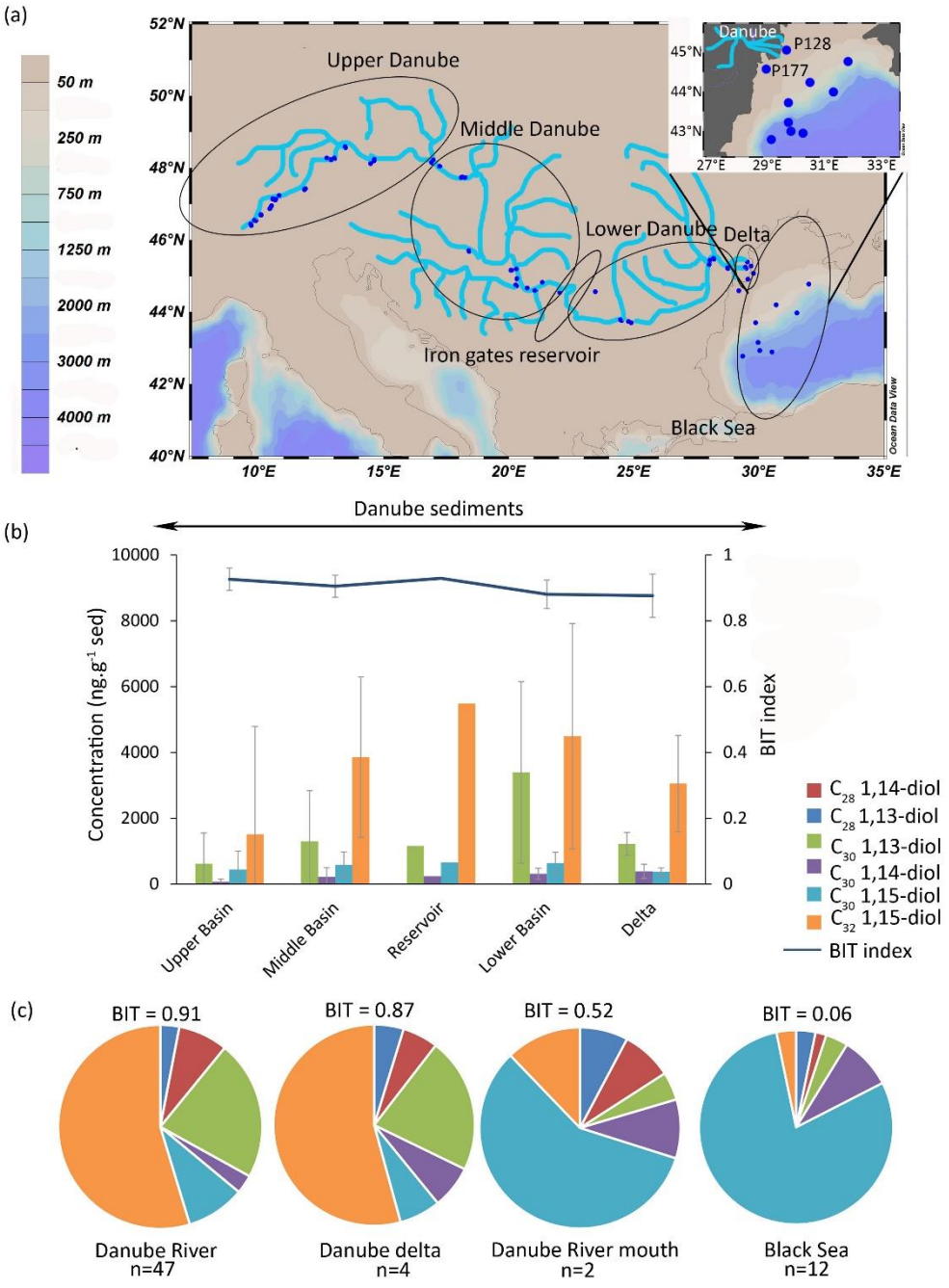
(a)
50 m
250 m
750 m
1250 m
2000 m
3000 m
4000 m
Upper Danube
Middle Danube
Lower Danube
Delta
Iron gates reservoir
Black Sea
Danube
P128
P177
52°N
50°N
48°N
46°N
44°N
42°N
40°N
10°E
15°E
20°E
25°E
30°E
35°E
45°N
44°N
43°N
27°E
29°E
31°E
33°E
Ocean Data View
(b)
Danube sediments
Concentration (ng.g-1 sed)
BIT index
10000
8000
6000
4000
2000
0
1
0.8
0.6
0.4
0.2
0
Upper Basin
Middle Basin
Reservoir
Lower Basin
Delta
C28 1,14-diol
C28 1,13-diol
C30 1,13-diol
C30 1,14-diol
C30 1,15-diol
C32 1,15-diol
BIT index
(c)
BIT = 0.91
BIT = 0.87
BIT = 0.52
BIT = 0.06
Danube River
n=47
Danube delta
n=4
Danube River mouth
n=2
Black Sea
n=12

Figure 2

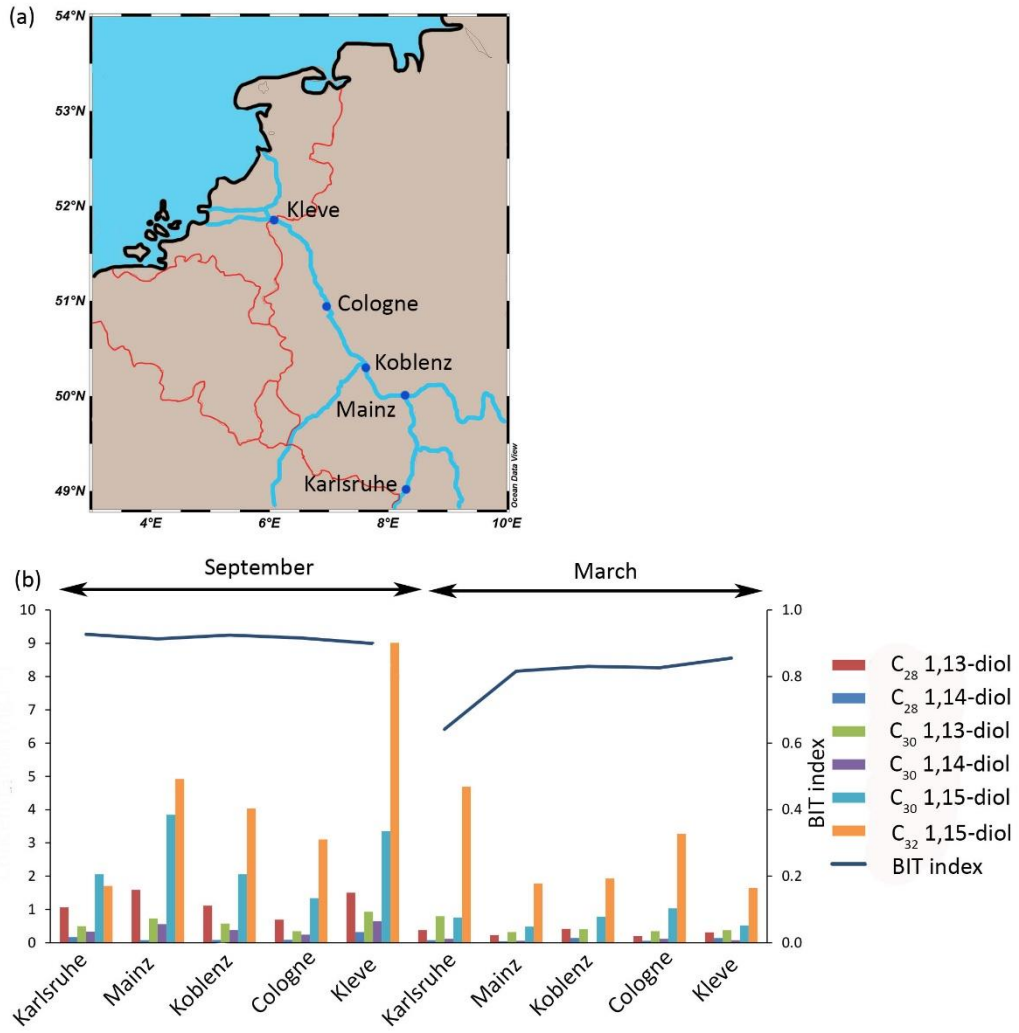
(a)
54°N
53°N
52°N
51°N
50°N
49°N
4°E
6°E
8°E
10°E
Kleve
Cologne
Koblenz
Mainz
Karlsruhe
Ocean Data View
(b)
September
March
10
9
8
7
6
5
4
3
2
1
0
1.0
0.8
0.6
0.4
0.2
0.0
BIT index
C28 1,13-diol
C28 1,14-diol
C30 1,13-diol
C30 1,14-diol
C30 1,15-diol
C32 1,15-diol
BIT index
Karlsruhe
Mainz
Koblenz
Cologne
Kleve
Karlsruhe
Mainz
Koblenz
Cologne
Kleve

Figure 3

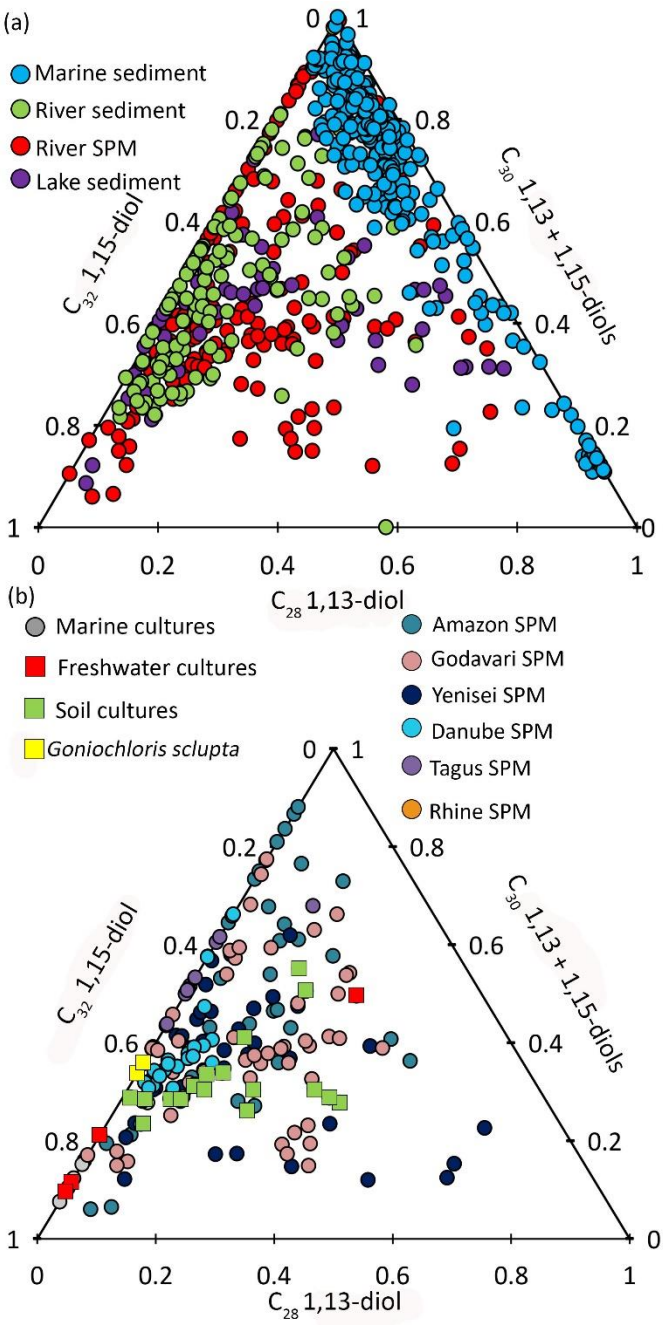
(a)
Marine sediment
River sediment
River SPM
Lake sediment
C32 1,15-diol
C30 1,13 + 1,15-diols
C28 1,13-diol
(b)
Marine cultures
Freshwater cultures
Soil cultures
Goniochloris sclupta
Amazon SPM
Godavari SPM
Yenisei SPM
Danube SPM
Tagus SPM
Rhine SPM
C32 1,15-diol
C30 1,13 + 1,15-diols
C28 1,13-diol

Figure 4

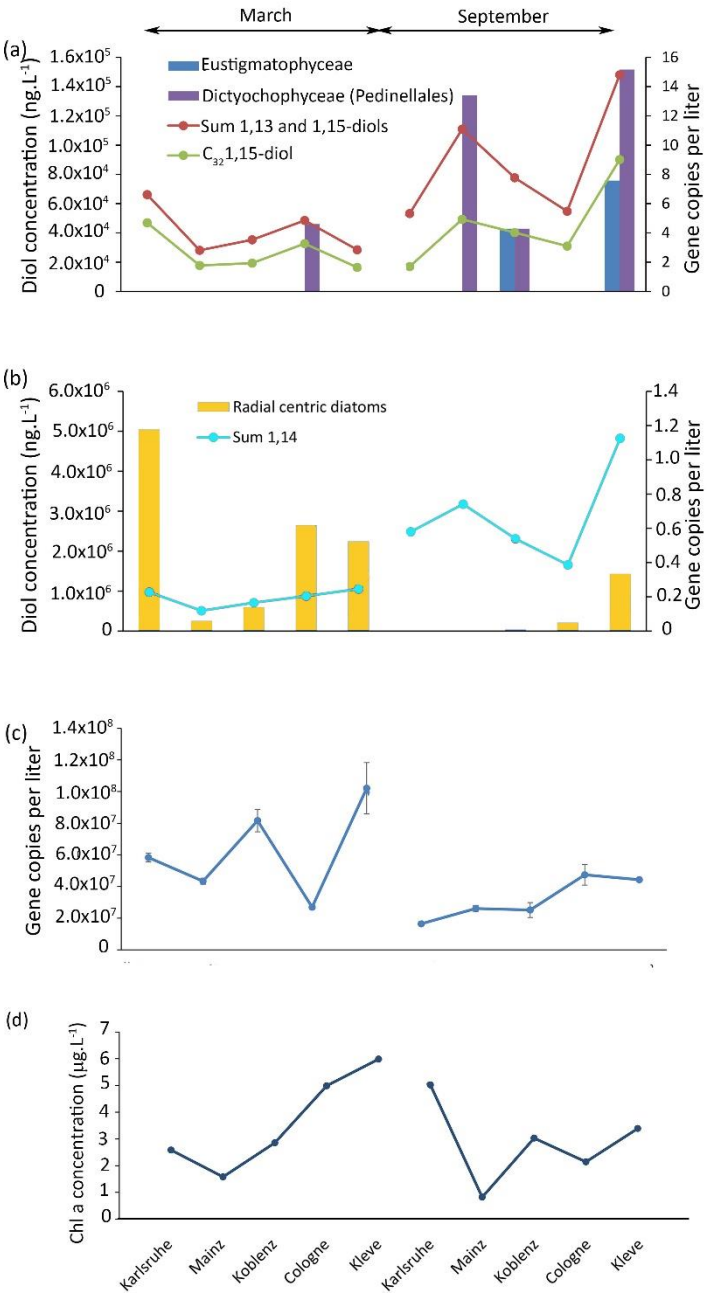
March
September
(a)
Eustigmatophyceae
Dictyochophyceae (Pedinellales)
Sum 1,13 and 1,15-diols
C32 1,15-diol
Diol concentration (ng.L-1)
Gene copies per liter
(b)
Radial centric diatoms
Sum 1,14
Diol concentration (ng.L-1)
Gene copies per liter
(c)
Gene copies per liter
(d)
Chl a concentration (µg.L-1)
Karlsruhe
Mainz
Koblenz
Cologne
Kleve
Karlsruhe
Mainz
Koblenz
Cologne
Kleve

Figure 5