Dear Editor,

We thank you for your comments and the opportunity to resubmit a revised version of the manuscript. We agree with you and the two anonymous referees that an expansion of discussion of the palmitic acid δD data will highly benefit the manuscript. Accordingly, we put the focus of our revision on the discussion of the palmitic acid isotope data. We also addressed your concerns regarding potential contribution by riverine derived palmitic acid. Furthermore, we made substantial changes to the discussion of the alkenone δD data in response to the concerns of anonymous referee #2 regarding the correlation between the high concentration alkenone δD data and the water δD data.

The point by point responses to both anonymous referees can be found below, along with the annotated version of the revised manuscript. In the revised manuscript, additions are marked in blue, while deleted parts are stroke out and marked in red.

Yours sincerely,

Christoph Häggi, on behalf of the co-authors

Reply to anonymous Referee #1

We thank anonymous Referee #1 for the thoughtful review. Especially the comments on turnover rates will help to improve the manuscript (see below). Detailed responses to the different issues raised in the review can be found below. To facilitate the revision, the comments from Referee #1 are written in blue whereas our responses are in black.

Anonymous Referee #1: As mentioned above, I think I would put the emphasis a little different. The authors very delicately suggest the option of overprint of the signal by advection of allochthonous alkenones especially at low alkenone concentrations. I think this is most likely the main reason for the lack of temperature and δD correlations between measured and alkenone derived values when the low concentration samples are included. Alkenones are less susceptible to degradation resulting in a relatively large fraction old or "fossil" alkenones in every individual SPM sample and this fraction is probably larger at low concentrations. The turnover rate of fatty acids is higher than that of alkenones and a large fraction of the C16 FAs will have been produced in the water mass they were obtained from, resulting in a better correlation between the C16 FA δD and water mass properties.

Response: We agree that alkenones are less susceptible to degradation than palmitic acid and that the δD values recorded in palmitic acid are therefore less susceptible to overprint by pre-formed compounds. Accordingly, we expanded section *4.3.2.Palmitic acid δD* of the manuscript to that regard. Our hesitation to give this line of argument more room in our discussion mainly stems from the observation that temperature reconstructions based on alkenones also show large deviations. Unlike salinity values in the Amazon continental margin that are subject to fast and large changes, temperature values are fairly stable and there is no nearby water mass that would provide fossil alkenones with a temperature signal as low as the one reconstructed (see page 10, lines 8-12 of the original version of the manuscript). In our opinion, lower haptophyte growth rates due to light limitation and low salinity are an elegant way to explain this bias, since they potentially account for both the δD and temperature deviations.

Anonymous Referee #1: SPM samples represent a snapshot in time and space which makes it really easy to miss an algal bloom or the production season of specific biomarker lipids such as alkenones. In that sense it would have been nice to compare the presented results with cell counts or molecular technique based community composition estimates. There are for instance alkenone producing haptophytes that thrive in low salinity environments, but their production season might be different from the more open ocean species (assuming that the authors did catch the open ocean alkenone production season). The "fossil" alkenones that affect the UK and δD correlations at low concentrations might be derived from other water masses, but also from different time intervals, possibly re-suspended from the (shelf) sediment and transported by the Amazon outflow. This is exactly why the authors suggest to analyze both C16 FAs (or another more general lipid) and alkenones and I think that is a good suggestion.

Response: Having sampled the study area during one single cruise (Mulitza et al., 2013) we might indeed have missed the main algal bloom season of certain alkenone producing species. We also agree that cell counts and/or other molecular techniques would have been a nice addition to our study. However, this was beyond the scope of the study and we relied on the C_{37}/C_{38} ratio to assess different haptophyte sources (e.g. Conte et al., 1998). Our results based on the C_{37}/C_{38} ratio do not suggest a

large scale shift to coastal alkenone producers (see page 4, lines 4-17 of the original version of the manuscript).

Anonymous Referee #1: However, the C16 FA has its own potential biases. It has become clear that the hydrogen isotopic composition of lipids from photoautotrophic organisms are correlated with salinity and/or reflect the δD of the water and photoautotrophic organisms fractionate to a similar extend. However, heterotrophic organisms fractionate very differently and might show no or a different relationship with salinity. The C16 FA can be derived from many different organisms and different contributions from organisms with different metabolisms could potentially affect the hydrogen isotopic composition of FAs. Fortunately, it seems that in many of these open ocean water column ecosystems photoautotrophic microorganisms are the dominant contributors to the C16 FA pool. The high turnover rate of the fatty acids also make them less interesting for paleo reconstructions on longer time scales.

Response: We agree that heterotrophic organisms could lead to changes in palmitic acid δD in sedimentary records (see page 14, lines 21-23 of the original version of the manuscript). The question is whether the heterotrophic contribution in the water column and in the sediment is large enough to significantly overprint the original phototrophic signal. There are some reassuring studies showing that the influence of heterotrophic organisms is not large enough to alter the signal of phototrophic organisms (Huang et al. 2004, Li et al. 2009) which also seems to be the case in the Amazon Plume. Besides the potential influence by heterotrophs, there are also variations in palmitic acid isotopic fractionation factor for different species of phototrophic organisms (e.g. haptophyte algae) (Chivall et al. 2014). In our study, these variations appear to have no influence on the isotopic fractionation on an ecosystem level. To rule out these effects, we propose to test the consistency of a down core δD signal among multiple lipids (see page 14, lines 24-25 of the original version of the manuscript).

Anonymous Referee #1: I think the authors should emphasize the difference in turnover rates between FAs and alkenones a bit more and the perhaps put less emphasis on less alkenone production at low salinities.

Response: We agree that turnover rates between alkenones and palmitic acids are different and expanded on the role of turnover rates in section *4.3.2.Palmitic acid δD* of the manuscript. We do, however, maintain that turnover rates alone are insufficient to explain the observed patterns. Lower turnover rates alone would not explain the salinity relationship of alkenone concentration that is clearly visible in our data (see Figure 2c of the original version of the manuscript) and is also insufficient to explain the large temperature deviation in our data (see Figures2b and 4b of the original version of the manuscript).

Page 2; line 14 to 19: I don't think it is necessarily true that alkenone production is low at low salinity, light limitation is something different. With sampling SPM during a cruise it is relatively easy to miss the main "production" season. Haptophyte community composition analysis might help answer these questions in the future.

Response: In the studied area, light limitation and low salinity are coinciding, since Amazon derived freshwater is extremely suspension rich (Smith and Demaster 1996). Hence, the two effects work in concert and are generally hard to disentangle. The abstract of the manuscript was amended to clarify

this issue. In terms of haptophyte composition analysis, we fully agree that community analysis would have been interesting, but was unfortunately beyond the scope of this study.

Page 4; line 1 to 3: This is not what Kasper et al. 2015 have suggested. They suggested that there is no clear glacial interglacial δD alkenone shift because during the glacial the core location was closer to the coast due to low sea level, resulting in more freshwater influence (low salinity and δD water) and more negative δD alkenone values than "normally" found during glacials. On top of that there might be a small species effect. Species variability did not make salinity reconstructions impossible, they suggest that salinity might not have changed that much.

Response: Page 4, lines 1-3 were corrected. The sentence now reads: "However, in some cases, factors like species variability complicated δD based salinity reconstructions."

Page 10; line 19: Schouten et al., 2006 does not discuss coastal haptophytes. This reference belongs to the first half of this sentence.

Response: The Schouten et al. (2006) reference was moved to the first half of the sentence.

Page 12; line 17 to 19: I agree that at low alkenone concentrations the fraction "fossil" might be large and affecting α, for instance, but could it be possible the authors missed the haptophyte bloom and/or main alkenone production season?

Response: It is possible that we missed the main haptophyte bloom, which would have made advection more likely. As outlined above, we do not think that advection is the dominant factor responsible for the deviations in δD.

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Reply to anonymous Referee #2

We thank anonymous Referee #2 for the helpful comments, which will lead to a substantial improvement of our manuscript. In the following we like to address the different points raised by the referee. To facilitate the revision, the comments from Referee #2 are written in blue whereas our responses are in black.

Anonymous Referee #2: The observation that palmitic acid, a compound produced by all organisms on Earth, display nearly constant hydrogen isotopic fractionation across large environmental and salinity gradient should be discussed further. There are all kinds of different algae and bacteria living in these water masses with different water chemistry. This observation would mean palmitic acid hydrogen isotopic fractionation relative to environmental water is highly conserved in various organisms. A couple of previous papers also support this conclusion. For example, Li et al (2009, GCA, 73, 4803) shows that palmitic acid hydrogen isotopic values are constant in the sediment core when dD values of other compounds show large variations: the most probable explanation for the constant palmitic acid dD values is that various organisms living in the water column display the same hydrogen isotopic fractionation relative to the sea water. Although Li et al., argue, based branched fatty acid dD values, heterotrophic bacteria have different hydrogen isotopic fractionation values, the fact that the resulting sedimentary combined PA dD values show constant values (hence faithfully recording sea water isotopic ratios) indicate either the contribution of heteorotrophic bacteria is small (even in the sediments), or the suggested difference between phototrophs and heterotrophic bacteria is not manifested in the real natural system. The sediment data are particularly important for supplementing the evidence presented in this paper, because sediment will have, undoubtedly, large heterotrophic bacteria input. One possibility is to consider if the newly produced PA from a heteorotrophic bacteria during biodegradation may actually have the same hydrogen isotopic values as the PA in the decomposing organic matter. It is not impossible to consider a scenario that, because PA exists in all organisms, the enzymes leading to produce this compound may share such a great deal of similarity, and hence the hydrogen isotopic fractionation relative to source water is all constant across different organisms. For heteorotrophs some of the hydrogen on PA would come from food rather than water, but perhaps that proportion is relatively small when all heteorotrophs are considered.

Response: We agree that the discussion about the palmitic acid δD could be expanded and thank Referee #2 for his suggestions. Accordingly, we expanded section *4.3.2. Palmitic acid δD* of our manuscript along the lines suggested by the referee. We especially emphasized the consistency of isotopic fractionation along the large variety of environments and palmitic acid producers.

Anonymous Referee #2: To say that when alkenone concentrations are higher than 10 ng/L, its hydrogen isotopic ratios are correlated to water dD and salinity is an overstatement. P values are too high, and if residual is plotted, it is too large across the salinity gradient.

Response: We agree that the p value of 0.05 for the δD_{H2O} - δD_{C37} correlation of high C_{37} concentration samples is high. We included a short discussion about the p value in the revised manuscript and also modified the abstract to that regard.

Anonymous Referee #2: If alkenone advection is the culprit, surely PA will also be affected. If the argument is the PA gets degraded faster hence the influence on hydrogen isotopic ratios is smaller than alkenones (which is more recalcitrant), one has to explain why regenerated PA from decomposers would not have been messed up for its H isotopic signal. I think overall alkenones simply do not track water hydrogen isotopic ratios or salinity trends. The main reason is probably the species effect and do not think the C37/C38 ratio is a reliable indicator of species (the ratio changes at different grow rate and salinity, and in particular different strains of the same species). E Hux has much greater hydrogen isotopic fractionation that the coastal species I Galbana, and any water isotopic signal (about 40 per mil in modern Amazon plume) is simply overwhelmed by the species effect. Clearly, the percentage of galbana and E hux does not change linearly across the salinity gradient, otherwise one could still expect to see some kind of linear relationship between alkenone dD and salinity. This corroborate with the results from Chesapeake Bay where species effect basically cancels the salinity effect.

Response: We agree that alkenone advection is unlikely the dominant factor responsible for the lack of correlation between δD_{C37} and δD_{H2O}, since this explanation is insufficient to explain the deviations observed for the temperature reconstruction (see page 10, lines 8-12 of the original version of the manuscript). However, we also doubt that the species effect is the dominant factor. If species variability would be the dominating factor, the C_{37} concentration in the low salinity outflow plume would not be necessarily low. Furthermore, it would be expected that the fractionation factor would increase in proximity to the coast, which is not the case. Although the C_{37} /C_{38} ratio has its limitations, a strong species variation would arguably lead to large variations in the C_{37}/C_{38} ratio (M´Boule et al. 2014, Conte et al. 1998), which we do not observe. We have expanded the discussion in Section *4.3.1 Alkenone δD* by further stressing why we doubt that advection is the dominant controlling factor of δD in C_{37}.

Anonymous Referee #2: I do not think paired measurement of dD values of PA and alkenones will improve paleosalinity reconstruction based on the results from this study: the chances are that more confusion will be generated when two disagrees.

Response: Indeed, we do not claim that paired δD_{PA} and δDC_{37} analyses would necessarily improve salinity reconstructions. We rather suggest that the paired use could give indications if one of the proxies is biased due to one of the many potential issues discussed in the manuscript (see page 14, lines 24-26 of the original version of the manuscript). Conversely, a good match between the δD of two independent biomarkers would add additional confidence to the validity of a salinity reconstruction. We clarified this in the section *5. Conclusions* of the revised manuscript version.

Anonymous Referee #2: However, I would suggest in future get the core top sediments across this salinity gradient, and measure the hydrogen isotopic ratios of PA and alkenones. Sediment would integrate all input sources, include heterotrophic bacteria, and can serve as a better test or calibration of paleosalinity reconstruction using PA dD values.

Response: We absolutely agree that the analysis of core top sediments would be an important addition to the study of δD in biomarkers from suspended particles. In the Amazon Plume, the study of core top sediments is however somewhat complicated by the marked temporal and spatial variations in the salinity gradient. Furthermore, there is little to no modern sedimentation in some areas on the continental slope ocean wards of the Amazon Plume.

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Testing the D/H ratio of alkenones and palmitic acid as salinity proxies in the Amazon Plume

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Abstract

The stable hydrogen isotope composition of lipid biomarkers, such as alkenones, is a promising new tool for the improvement of paleosalinity reconstructions. Laboratory studies confirmed the correlation between lipid biomarker δD composition (δD_{Lipid}), water δD composition (δD_{H_2O}) and salinity. Yet, there is limited insight into the applicability of this proxy in oceanic environments. To fill this gap, we test the use of the δD composition of alkenones (δD_{C37}) and palmitic acid (δD_{PA}) as salinity proxies using samples of surface suspended material along the distinct salinity gradient induced by the Amazon Plume. Our results indicate a positive correlation between salinity and δD_{H_2O}, while the relationship between δD_{H_2O} and δD_{Lipid} is more complex: δD_{PA} correlates strongly with δD_{H_2O} (r^2=0.81) and shows a salinity dependent isotopic fractionation factor. δD_{C37} only correlates with δD_{H_2O} in a small number (n=8) of samples with alkenone concentrations >10 ng L^{-1} ~~(r^2=0.51)~~, while there is no correlation if all samples are taken into account. These findings are mirrored by alkenone based temperature reconstructions, which are inaccurate for samples with low alkenone concentrations ~~< 10 ng L^{-1}~~. Deviations in δD_{C37} and temperature are likely to be caused by limited haptophyte algae growth due to low salinity and light limitation imposed by the Amazon Plume. Our study confirms the applicability of δD_{Lipid} as a salinity proxy in oceanic environments. But it raises a note of caution concerning regions where low alkenone production can be expected due to ~~very~~ low salinity ~~conditions~~ and light limitation, for instance, under strong riverine discharge ~~To circumvent these limitations, we suggest the complementary use of δD_{C37} and δD_{PA}~~.

1 Introduction

The precise reconstruction of past ocean salinity is still a pending issue in paleoclimatology (Rohling, 2007). Until recently, most paleosalinity studies have relied on foraminifera based reconstructions of the stable oxygen isotope composition of seawater, which correlates with salinity (Epstein and Mayeda, 1953). However, temperature also controls the oxygen isotope composition of foraminifera, making corrections in the estimation of paleosalinity necessary (Lea et al., 2000;Rostek et al., 1993). The imprecision associated with this approach has led to the search for ~~other~~ alternative salinity proxies. The use of the hydrogen isotopic composition of algal lipids (δD_{Lipid}) for the reconstruction of the stable hydrogen composition of water (δD_{H_2O}) is one of such recent developments (Sessions et al., 1999;Schouten et al., 2006). As outlined in a theoretical framework by Rohling (2007), this method has the potential to lead to more precise reconstructions of surface water salinity in combination with foraminifera based $\delta^{18}O$.

So far, efforts to apply δD_{Lipid} as a salinity proxy have mainly involved the use of long-chain alkenones. Long-chain alkenones have the advantage of being exclusively produced by specific haptophyte algae, and of showing good preservation over geologic timescales (Marlowe et al., 1984;Marlowe et al., 1990). Laboratory studies have confirmed the correlation of the D/H ratio of the C_{37} alkenones (δD_{C37}) with δD_{H_2O} (Englebrecht and Sachs, 2005;Schouten et al., 2006). Furthermore, the D/H fractionation factor between alkenones and water ($\alpha_{C_{37}}$)

$$\alpha_{C37} = \frac{\delta D_{C37} + 1000}{\delta D_{H_2O} + 1000} \quad (1)$$

was found to be salinity dependent, leading to a potentially twofold way to reconstruct salinity (Schouten et al., 2006). There are, however, potential factors that may compromise the use of δD_{C37} and $\alpha_{C_{37}}$ as salinity proxies. $\alpha_{C_{37}}$ is, for instance, inconsistent among different haptophyte algae species. Species preferring shelf environments have a higher $\alpha_{C_{37}}$ than species favoring open marine habitats (M'Boule et al., 2014). In some situations $\alpha_{C_{37}}$ has shown a small temperature dependency (Zhang and Sachs, 2007). Furthermore, $\alpha_{C_{37}}$ is also dependent on algal growth phase and rate (Schouten et al., 2006;Wolhowe et al., 2009;Chivall et al., 2014b). All these factors potentially exceed the effects of salinity and may impede the use of δD_{C37} as a paleosalinity proxy. Nevertheless, paleoclimate studies have made

successful use of δD_{C37} as a paleosalinity proxy (van der Meer et al., 2008;Giosan et al., 2012;Schmidt et al., 2014;Pahnke et al., 2007;van der Meer et al., 2007). However, in some cases, factors like species variability ~~have made~~ complicated δD_{C37} based salinity reconstructions ~~impossible~~ (Kasper et al., 2015).

Apart from alkenones there is a variety of other algal lipids which feature a distinct $\delta D_{H2O} - \delta D_{Lipid}$ relationship (Zhang et al., 2009;Sauer et al., 2001;Nelson and Sachs, 2014). Among these less frequently used compounds is palmitic acid. Palmitic acid is a saturated fatty acid, which is highly abundant in most aquatic environments. The infrequent use of palmitic acid is mainly due to its ubiquitous occurrence, which does not allow linkage to a single group of producing species. Furthermore, palmitic acid is less resistant to degradation than alkenones (Sun and Wakeham, 1994). Nevertheless, δD of palmitic acid (δD_{PA}) has been successfully used as a paleoclimate indicator in several studies (Huang et al., 2002;Smittenberg et al., 2011;Shuman et al., 2006).

Although there are numerous laboratory and paleoclimate studies confirming the applicability of δD_{Lipid} to reconstruct the past isotopic composition of water, there have been only few calibration studies in oceanic environments (Schwab and Sachs, 2011;Schwab and Sachs, 2009;Wolhowe et al., 2015). To fill this gap, we analyzed δD_{C37} and δD_{PA} of suspended particle samples along the salinity gradient induced by the Amazon freshwater plume and tested their applicability as salinity proxies (Fig. 1). Along with the hydrogen isotope analyses, we also tested the accuracy of the $U^{k'}_{37}$ temperature proxy (Müller et al., 1998) under the influence of the Amazon Plume. Potential impact of haptophyte species variability was monitored using the C_{37}/C_{38} ratio (Rosell-Mele et al., 1994), as defined below.

$$C_{37}/C_{38} = \frac{C_{37:3}Me + C_{37:2}Me}{C_{38:3}Et + C_{38:3}Me + C_{38:2}Et + C_{38:2}Me} \quad (2)$$

2 Methods

2.1 Study area

The study area is situated offshore northern Brazil and French Guyana close to the Amazon estuary (Fig 1). A large portion of the research area is influenced by freshwater outflow from the Amazon River, which induces a steep salinity gradient (Lentz and Limeburner, 1995). The freshwater plume is generally transported northwestwards by the North Brazil Current along

the coastline of northern Brazil and French Guyana, while areas to the southeast of the Amazon River Estuary are largely unaffected by the Amazon freshwater discharge (Geyer et al., 1996). The geometry and transport of the freshwater ~~Plume~~ plume are subject to large seasonal variations. The plume reaches its maximum extent during peak Amazon discharge in boreal summer (Molleri et al., 2010), while its northwestward transport is controlled by wind-stress along the shelf (Geyer et al., 1996).

2.2 Sampling

Sampling was conducted during the RV *Maria S. Merian* cruise MSM20/3 from February 21th to March 9th 2012 (Mulitza et al.). Samples of suspended particles were collected along a southeast to northwest transect off northeastern South America across the Amazon Plume (Fig. 1). Samples were taken via the ships seawater inlet at about 6 meters below sea level operated by a diaphragm pump. Between 100 and 500 litres of water were filtered over a period of 30 to 150 minutes on pre-combusted GFF filters. After sampling, filters were wrapped in pre-combusted aluminium foil and stored at -20°C. Along with the suspended particle samples, water samples were collected at the beginning and at the end of each filtering period. Water samples were sealed with wax and stored at 4°C before analysis. On-board salinity and temperature measurements were conducted in one second intervals by a SeaBird Electronics SBE 45 Micro thermosalinograph (accuracy 0.002°C and 0.005 psu).

2.3 Stable isotope analysis of water

The stable hydrogen isotope composition of seawater samples was determined at MARUM – Center for Marine Environmental Sciences, University of Bremen, with a Thermal-Conversion/Elemental-Analyser operated at 1400°C coupled to a ThermoFisher Scientific MAT 253 mass-spectrometer. Measurements were repeated ten times for each seawater sample. Four in-house water standards used for calibration were calibrated against IAEA standards VSMOW, GISP and SLAP. The maximum deviation from the calibration slope was 1.6 ‰ vs. VSMOW and the average deviation was 0.7 ‰ vs. VSMOW.

2.4 Lipid analysis

Suspended particle samples were freeze-dried in a Christ Alpha 1-4 freeze-dryer. Lipids were extracted in a DIONEX Accelerated Solvent Extractor (ASE 200) using a dichloromethane

(DCM): methanol (MeOH) 9 : 1 solution at 1000 psi and 100 °C for three cycles lasting 5 minutes each. Prior to extraction 2-Nonadecanone and erucic acid were added as internal standards for the ketone and acid fractions, respectively. After extraction, samples were dried in a Heidolph ROTOVAP system. The extracts were saponified using 0.1 M KOH in MeOH, yielding neutral and acid fractions. The neutral fraction was separated in three fractions using activated silica gel chromatography (1% H_2O). The first fraction was eluted with hexane, yielding saturated and unsaturated hydrocarbons. The second fraction was eluted with (DCM), yielding ketones, including alkenones. The third fraction was eluted with DCM:MeOH 1:1, yielding polar compounds. The acid fraction was methylized with MeOH of known isotopic composition (-156 ± 2 ‰ vs. VSMOW), yielding the corresponding fatty acid methyl esters (FAMEs). The FAMEs were subsequently cleaned over pipet columns containing two centimeters of silica. In order to remove unsaturated compounds, further cleaning over columns of two centimeters of $AgNO_3$ was conducted. Ketones and FAMEs were analyzed using a ThermoFisher Scientific Focus gas chromatograph equipped with an Rxi-5ms 30x column (30 m, 0.25 mm, 0.25 μm) and a flame ionization detector. Compounds were quantified by comparing the integrated peak areas of the compounds to external standard solutions. Precision of compound quantification is about 5% and precision of $U_{37}^{k'}$ reconstructions is 0.38°C based on multiple standard analyses. Compound-specific isotope analyses was carried out on a ThermoFisher Scientific MAT 253 Isotope Ratio Mass Spectrometer coupled via a GC Isolink operated at 1420°C to a ThermoFisher Scientific Trace GC equipped with a HP-5ms column (30 m, 0.25 mm, 1 μm). For each sample duplicate injections of C_{37} and palmitic acid were conducted. Measurement accuracy was controlled by *n*-alkane standards of known isotopic composition every six measurements and by the daily determination of the H^+_3 factor using H_2 as reference gas. H^+_3 factors varied between 5.6 and 6.2, while the mean absolute deviation of external standards was 2.2‰. In order to prevent a bias introduced by variable alkenone distribution, the δD of alkenones was analyzed for $C_{37:2}$ and $C_{37:3}$ together rather than separately (van der Meer et al., 2013). δD values for palmitic acid were corrected for the methyl group added during methylation.

3 Results

Onboard sea surface temperature measurements resulted in uniform values of 28.5 ± 0.5 °C, while salinity varied between 10 and 36 psu (Fig. 1; Table 1). The hydrogen isotope analyses of seawater samples yielded δD values between 6 and -15 ‰ (all isotope values are given vs.

VSMOW). The values correlated linearly with sea surface salinity (Fig. 2a). The suspended particle samples yielded C_{37} alkenone concentrations between 0.2-65.3 ng L^{-1} (Table 1). Samples with a salinity >25 psu showed variable concentrations (0.2-65.3 ng L^{-1}), while samples with a salinity <25 psu had concentrations consistently lower than 10 ng L^{-1}. There were little to no alkenones (concentration <1 ng L^{-1}) in filter samples with a salinity <15 psu (Fig. 2c, Table 1). The fatty acid analysis yielded almost exclusively short chain compounds, of which palmitic acid had concentrations between 1.4 and 27 μg L^{-1} (Fig. 2d). Variations in palmitic acid concentrations showed a weak inverse correlation with salinity (Fig. 2d). For samples with alkenone concentrations >10 ng L^{-1}, sea surface temperature reconstructions agreed within the calibration error of 1.5°C with onboard temperature measurements (Fig. 2b, Table 1). Samples with a concentration <10 ng L^{-1} featured a larger scatter with deviations from onboard measurements of up to 10°C (Fig. 2b). The ratio of the C_{37}/C_{38} alkenones resulted in values between 0.9 and 1.7 (Table 1), indicating the prevalence of open ocean haptophyte contribution throughout the transect (Rosell-Mele et al., 1994). The $C_{37:4}$ alkenone, sometimes used as a salinity proxy, was not present in our samples.

Due to the absence of alkenones in the low salinity samples, isotope analysis of the C_{37} alkenone was only possible in samples with a salinity > 15 psu. For these samples, δD_{C37} varied between -176 ‰ and -205 ‰ (Fig. 3a, Table 1). When all samples are taken into account, δD_{C37} and δD_{H_2O} do not correlate (Fig. 3a). If only the samples with an alkenone concentration >10 ng L^{-1} were considered, linear regression yielded a correlation between δD_{C37} and δD_{H2O} with a slope of 1.36 ‰ δD_{C37} per 1‰ δD_{H2O} ($r^2 = 0.51$, $p < 0.05$; Fig. 3a). $\alpha_{C_{37}}$ varied between 0.79 and 0.84 and showed no significant salinity dependence (Fig. 3c). In contrast to δD_{C37}, δD_{PA} strongly correlates with δD_{H_2O}, regardless of lipid concentration ($r^2 = 0.81$, $p < 10^{-7}$; Fig. 3b). The slope of the linear regression is 1.72 ‰ δD_{PA} per 1 ‰ $\delta D_{H2O.}$ The fractionation factor between palmitic acid and water (α_{PA}) yielded values between 0.79 and 0.83, featuring a significant salinity dependency with an increase of 0.001 per salinity unit (Fig. 3d).

4 Discussion

4.1 Lipid sources

4.1.1 Alkenone sources

The C_{37}/C_{38} ratio was used for the assessment of the dominant alkenone source (Conte et al., 1998). Open marine species like *Emiliana huxleyi* and *Gephyrocapsa oceanica* produce alkenones with a C_{37}/C_{38} between 0.5 and 1.5 (Conte et al., 1998). Coastal species like *Isochrysis galbana* and *Chrysotila lamellosa* produce alkenones with a C_{37}/C_{38} ratio >2, sometimes even >10 (M'Boule et al., 2014;Prahl et al., 1988;Marlowe et al., 1984). The C_{37}/C_{38} ratio of the samples from the Amazon Plume varied between 0.9 and 1.7 and alkenone production was therefore likely dominated by open marine species (Conte et al., 1998). Since some of the samples feature values at the upper limit for open marine species, some (probably small) contribution by coastal haptophytes cannot be ruled out (Kasper et al., 2015). Alternatively, the small variations in the C_{37}/C_{38} ratio could also be the effect of species variability within open marine haptophytes (Conte et al., 1998). In contrast to previous laboratory and field studies (Ono et al., 2009;Chu et al., 2005), we do not find a correlation between salinity and the C_{37}/C_{38} ratio (not shown here).

4.1.2 Palmitic acid sources

Palmitic acids are not exclusively produced by aqueous organisms and are also synthesized by terrestrial plants and bacteria (Eglinton and Eglinton, 2008). Unlike aqueous organisms, terrestrial plants also synthesize long-chain fatty acids (Eglinton and Hamilton, 1967), which were not present in the filter samples. This indicates that the palmitic acids found in the Amazon Plume are exclusively produced by aquatic organisms. Also, the fast turnover rates of palmitic acid makes a contribution by riverine compounds unlikely. Furthermore, previous studies have generally confirmed that palmitic acids in marine environments are predominantly produced by marine algae (Pearson et al., 2001).

4.2 Temperature reconstruction

Oceanic temperature reconstructions based on alkenones are a widely used tool in paleoclimatology (Bard et al., 1997;Rühlemann et al., 1999). The global calibrations in use are based on open marine haptophyte species (Prahl and Wakeham, 1987;Müller et al., 1998).

Our reconstructed temperatures show deviations of up to 10°C from instrumentally measured temperature for samples with alkenone concentration <10 ng L^{-1} (Fig. 2b). These anomalous, generally lower than expected values, could be caused by different processes. First, coastal species bear a temperature-$U_{37}^{k'}$ relationship with a markedly lower slope than open marine species (Sun et al., 2007;Versteegh et al., 2001). Hence, a larger alkenone contribution by coastal haptophyte species would lead to the observed lower temperatures. Second, lower salinity is reported to cause metabolic stress in alkenone producers leading to anomalous reconstructed temperatures (Harada et al., 2003). Third, variations in haptophyte growth rate due to nutrient or light limitation could also lead to variations in reconstructed temperatures (Epstein et al., 1998;Versteegh et al., 2001). The latter two points would also lead to lower alkenone concentrations and thus enhance the possibility of overprint by advection of allochthonous alkenones.

Variations in haptophyte algae composition recorded by changes in the C_{37}/C_{38} ratio do not show a correlation with the residue

$$T_{residue} = T_{measured} - T_{reconstructed} \quad (3)$$

of the temperature reconstruction (not shown here). Hence, variations in species composition are likely insufficient to account for the $T_{residue}$. Conversely, there is a correlation between $T_{residue}$ and salinity (Fig. 4a). Salinity might therefore be an important cause for the large $T_{residue}$ (Harada et al., 2003). The riverine waters of the Amazon Plume are generally nutrient rich (Santos et al., 2008), which makes a scenario of nutrient limitation unlikely to impact temperature control of $U_{37}^{k'}$ in our study area. The high sediment load delivered by the Amazon River, however, leads to light limitation in the study area (Smith and Demaster, 1996). Light limitation is indeed reported to lower reconstructed $U_{37}^{k'}$ temperatures by up to 7°C (Versteegh et al., 2001). Since diminished alkenone production due to low salinity and light limitation would lead to smaller alkenone concentrations, this would also explain why high concentration samples feature no temperature deviation (Fig. 4b). The advection of allochthonous alkenones biasing temperature reconstructions has been suggested in other studies (Rühlemann and Butzin, 2006;Benthien and Müller, 2000). In our samples, $U_{37}^{k'}$ overprint by advected alkenones can be considered less likely, since there are no nearby areas where alkenones with a lower temperature signal could originate from.

In conclusion, there are multiple potential factors influencing the $U^{k'}_{37}$ deviation in the Amazon Plume. Given that low alkenone concentrations are consistently associated with large negative temperature deviations, reduced alkenone production due to low salinity and light limitation in the Amazon Plume might be the most important factor for the temperature deviations (Fig. 4a, b) (Versteegh et al., 2001;Harada et al., 2003).

4.3 Stable hydrogen isotope signals

4.3.1 Alkenone δD

If all samples are considered, there is no correlation between δD_{C37} and δD_{H_2O} (Fig. 3a). Given the relationship between C_{37} concentration, $T_{residue}$ and salinity (Fig. 4a, b), we also tested whether there would be a better fit between δD_{C37} and δD_{H_2O} for high C_{37} concentration samples. There is indeed ~~There is only~~ a correlation between δD_{C37} and δD_{H2O} for samples with a C_{37} concentration >10 ng L^{-1} (Fig. 3a). However, with a p-value of 0.05 and a low sample number of n=8, this relationship has to be viewed with caution. Nevertheless, we consider it to be an important information to study the potential factors leading to the deviation between δD_{C37} and δD_{H_2O}. Especially, since this relation reflects a generally constant α_{C37} of 0.81 and agrees with results obtained for open marine species cultured at different salinities (M'Boule et al., 2014). For a potential impact on δD_{C37}~~Again~~, factors similar to those considered for the temperature deviations have to be scrutinized ~~for a potential impact on δD_{C37}~~: synthesis by coastal haptophyte species (M'Boule et al., 2014;Schouten et al., 2006), changes in growth rate and phase (Schouten et al., 2006;Wolhowe et al., 2009), overprint by advected material and variations in salinity (Schouten et al., 2006). Since temperature is more or less uniform over the entire study area, a temperature effect as reported by Zhang and Sachs (2007) is not expected to play a role.

As previously mentioned, variations in the C_{37}/C_{38} ratio imply only limited variation in haptophyte species composition. Moreover, the values of α_{C37} are between 0.795 and 0.835 and are only slightly higher than observed in laboratory experiments studying open marine haptophytes (Schouten et al., 2006), but are markedly lower than observed for coastal haptophytes (M'Boule et al., 2014). This again suggests that the studied alkenones are predominantly of open marine haptophyte origin. Although there are no signs for a full scale change from open marine to coastal haptophytes, the variability in habitat preference may still

be sufficient to have a significant influence on α_{C37}. The C_{37}/C_{38} variability found in a sediment core collected offshore Mozambique by Kasper et al. (2015) was similar to the one found in our samples and the associated species variability was likely large enough to significantly influence δD_{C37}. In our samples, the C_{37}/C_{38} ratio does however not correlate with α_{C37} and species variations alone are therefore unlikely to be the dominant cause for the absent correlation between δD_{C37} and δD_{H2O} in low salinity samples. In contrast to laboratory studies (Schouten et al., 2006), we find no clear relationship between salinity and fractionation factor (Fig. 3c). The absence of a salinity-α_{C37} relationship was also reported in a field study by Schwab and Sachs (2011) who explained their findings by the presence of additional factors such as species variability and temperature, which may have counteracted the effects of salinity. If the relation between δD_{C37} and δD_{H2O} for high concentration samples is used to calculate the residue for each sample,

$$\delta D_{res\ C37} = \delta D_{C37} - \left(1.358 \times \delta D_{H_2O} - 194.558\right) \quad (4)$$

it becomes apparent that low concentration samples have higher residuals (Fig. 4d). Furthermore, $\delta D_{res\ C37}$ correlates with salinity, which indicates that $\delta D_{res\ C37}$ is largely influenced by the input of low salinity Amazon freshwater (Fig. 4c). This observation would also fit with the assumption that the lower C_{37} concentration in those samples were a result of lower growth rate, because lower growth rate leads to a higher fractionation factor (M'Boule et al., 2014;Schouten et al., 2006;Sachse and Sachs, 2008). Since the steep salinity gradient of the Amazon Plume leads to a wide range of surface water isotopic composition over a short geographic distance, we cannot exclude some influence of advected alkenones in samples with low or absent in situ alkenone production. As this effect is insufficient to explain the large $T_{residue}$, advection is likely not the main factor responsible for the absence of a correlation between δD_{C37} and δD_{H_2O}. Although the deviation in δD_{C37} cannot be tied to a single factor, low alkenone production associated with the low salinity, suspension rich Amazon waters is likely the most important factor (Wolhowe et al., 2015). Thus, the temperature- and δD_{C37} deviations are likely caused by similar effects (Fig. 4a-d).

4.3.2 Palmitic acid δD

In contrast to δD_{C37}, δD_{PA} correlates well with δD_{H2O} (Fig. 3b). Furthermore, α_{PA} correlates with salinity (Fig. 3d) and thus confirms the relationship between salinity and α observed in various laboratory and field studies for palmitic acid and other lipids (Schouten et al.,

2006;M'Boule et al., 2014;Chivall et al., 2014a). Our findings imply that the limiting factors potentially leading to variations in α_{C37} do not influence α_{PA}. The factors that could potentially influence δD_{PA} are largely similar to those influencing δD_{C37} (Chivall et al., 2014a). Unlike for alkenones there is, however, no clear evidence for a growth rate dependence of α_{PA} (Zhang et al. 2009).

One striking difference between palmitic acid and alkenones in our samples is the different abundance of the two compounds. Palmitic acid concentrations were about three orders of magnitude higher than alkenone concentrations (Fig. 2–c, d). This is unsurprising, since palmitic acid is typically very abundant in marine environments (Pearson et al., 2001). In further contrast to the C_{37} concentration, the palmitic acid concentration was not lower in low salinity samples, but featured a trend towards higher concentrations. This indicates that palmitic acid producing organisms were not negatively affected by the low salinity, sediment rich Amazon input like haptophyte algae, but rather benefited from the high nutrient supply by the Amazon (Santos et al., 2008). This marked difference supports the notion that low alkenone production rates in parts of the study area were responsible for the α_{C37} deviations. Furthermore, the high palmitic acid concentrations also limit the influence of a possible overprint of the in situ signal by allochthonous compounds. Apart from that, the high turnover rate of palmitic acid may further impede the influence of allochthonous compounds. This is also in contrast to alkenones, which are comparably stable towards degradation (Sun and Wakeham 1994). Therefore, the lower turnover rate of alkenones renders these compounds more susceptible to overprint by older, allochthonous compounds.

Our study shows that α_{PA} remains relatively stable over a range of varying environmental conditions. This finding is similar to one reached by studies along a lake transect from Southern Canada to Florida, which found a good agreement between δD_{PA} and δD_{H2O} over a variety of ecological environments (Huang et al., 2004, 2002). The α_{PA} of 0.82 observed in those studies is also in the range of α_{PA} observed in the Amazon Plume (0.79-0.83). This further indicates that species composition and other factors are not influencing α_{PA} to a large extent on an ecosystem level. Potential variations of α_{PA} from different contributors are either small or levelled out by integration over ecosystems. A surprising constancy in δD_{PA} has also been observed in a sediment core from the Santa Barbara Basin (Li et al., 2009). There, the δD_{PA} remained constant even in the presence of heterotrophic palmitic acid producers. This could indicate that the constancy in α_{PA} is not only limited to phototrophic organisms as

observed here and by Huang et al. (2004), but also extends to heterotrophic organisms. The constancy could be caused by the very similar biosynthetic pathway for palmitic acid in bacteria and eukaryotes (Li et al., 2009).

Although there are multiple lacustrine studies successfully applying δD_{PA} as paleoenvironmental proxy (Smittenberg et al., 2011;Shuman et al., 2006) and δD_{PA} faithfully records δD_{H2O} in our study, there are still multiple factors that could overprint a surface δD_{PA} signal. Especially in open oceanic environments, palmitic acid production deeper in the water column could alter the signal recorded at the surface. After deposition, bacterial activity in the sediment could also overprint the original upper water column signal (Perry et al., 1979).

5 Conclusions

Our study shows that δD_{PA} in suspended particle samples from the Amazon Plume salinity gradient records variations in salinity. For δD_{C37}, this correlation is only present in samples above a C_{37} concentration of 10 ng L^{-1}. The low alkenone concentrations are likely caused by the sediment-rich freshwater input of the Amazon River impeding haptophyte growth and affecting α_{C37}. Hence, the ubiquitous nature of palmitic acid proved to be highly beneficial in the study area. Moreover, palmitic acid bears the advantage of easier isotopic measurement and a high availability in most environments. The use of δD_{PA} as a standalone salinity proxy has to be considered with caution. Potential disadvantages of palmitic acid include post depositional degradation, ~~and~~ compound synthesis deeper in the water column, which may not record surface conditions and the bacterial overprint in the sediment. A possible way to circumvent these limitations, as well as the problems encountered for δD_{C37}, could be the alongside use of δD_{PA} and δD_{37}. δD_{PA} is not sensitive to the low concentration issues encountered in this study, while δD_{C37} is only produced in surface waters and not susceptible to synthesis or degradation deeper in the water column or sediments. Therefore, the combined study of compound-specific hydrogen isotope composition of more than one compound could yield important information on influences in δD_{Lipid} other than salinity.

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Table 1. Average geographic position, average measured sea surface temperature (SST), average sea surface salinity (SSS), C_{37} concentration, palmitic acid (PA) concentration, $U^{k'}_{37}$, C_{37}/C_{38} ratio, δD of water (δD_{H_2O}), δD of C_{37} (δD_{C37}) and δD of palmitic acid (δD_{PA})for each sample. Values for salinity and temperature are the average of onboard measurements taken in one second intervals during each filtering period. Errors represent the standard deviation of these measurements. δD values of water represent the mean of two samples taken at the beginning and the end of each filtering period, each sample represents the mean of ten replicate injections. Errors represent the propagated standard deviation of these measurements. δD values of C_{37} and palmitic acid are the means of duplicate measurements. Errors represent the range between the duplicate measurements.

Sample	Lat	Long	SST (C°)	SSS (psu)	Conc. C_{37} (ng L^{-1})	Conc. PA (µg L^{-1})	$U^{K'}_{37}$	C_{37}/C_{38}	δD_{H_2O}	δD C_{37}	δD PA
PP10	1.9035	-48.4169	28.37 ± 0.03	36.2 ± 0.09	47.7	1.3	0.98	1.46	4.8 ± 0.9	-190.1 ± 0.5	-170.8 ± 1
PP11	1.7587	-48.2568	28.99 ± 0.04	34.72 ± 0.51	54.2	N/A	0.96	1.56	6.6 ± 1.2	-189.2 ± 3.7	N/A
PP12	1.7123	-48.2975	29.28 ± 0.05	31.65 ± 1.1	65.3	6	0.95	1.45	2.3 ± 1.1	-185.4 ± 2.2	-183.5 ± 0.8
PP13	1.6655	-48.3388	29.31 ± 0.18	28.06 ± 1.2	20.6	16.6	0.96	1.47	-2.6 ± 1.6	-200.8 ± 1.9	-193.2 ± 1.7
PP14	1.6197	-48.3791	29.17 ± 0.03	25.79 ± 0.51	5.7	12.3	0.94	1.42	-4.1 ± 1.1	-206.3 ± 1.3	-197.5 ± 0.4
PP15	1.5724	-48.421	29.28 ± 0.05	22.86 ± 0.47	8.6	19.4	0.95	1.44	-6.7 ± 1	a	-205.4 ± 0.9
PP16	1.5676	-48.4632	29.23 ± 0.05	20.91 ± 0.47	1.4	13.9	0.89	1.33	-9.2 ± 0.9	a	-209.7 ± 0.6
PP17	1.6199	-48.5119	29.02 ± 0.07	20.55 ± 0.41	1.5	8.7	0.89	1.19	-11.8 ± 1.4	-176.9 ± 0.3	-205.9 ± 0
PP19	2.0306	-48.759	28.67 ± 0.02	17.84 ± 0.55	3.8	N/A	0.71	2.52	-14.5 ± 1.3	a	N/A
PP20	2.0858	-48.7282	28.73 ± 0.03	21.15 ± 1.38	2.6	N/A	0.81	1.08	N/A	a	N/A
PP21	2.1431	-48.6728	28.82 ± 0.02	26.22 ± 1.63	1.3	N/A	0.79	1.12	N/A	a	N/A
PP22	2.1815	-48.6369	28.82 ± 0.05	30.76 ± 1.2	2.8	N/A	0.91	1.44	N/A	a	N/A
PP23	2.2205	-48.6038	28.9 ± 0.02	33.25 ± 0.5	2.8	N/A	0.95	1.43	N/A	a	N/A
PP24	2.259	-48.6055	28.93 ± 0.02	33.89 ± 0.11	4.9	N/A	0.97	0.99	3.8 ± 0.9	-191.8 ± 1.9	N/A
PP25	2.3389	-48.7336	28.84 ± 0.04	27.45 ± 1.27	5.1	N/A	0.87	0.92	N/A	a	N/A
PP26	2.2984	-48.7711	28.82 ± 0.03	23.96 ± 1.09	0.4	N/A	0.87	1.25	N/A	a	N/A
PP27	2.2674	-48.7995	28.71 ± 0.04	20.8 ± 0.71	0.4	N/A	0.65	0.98	N/A	a	N/A
PP33	2.0652	-48.5919	28.6 ± 0.04	17.44 ± 0.24	1.1	N/A	0.68	1.01	N/A	a	N/A
PP34	1.9301	-48.5528	28.63 ± 0.04	16.02 ± 0.12	6.6	N/A	0.78	1.27	N/A	a	N/A
PP35	1.7071	-48.4395	28.45 ± 0.04	18.21 ± 0.39	0.8	N/A	0.76	1.03	N/A	a	N/A
PP36	1.6196	-48.4013	28.55 ± 0.06	24.34 ± 0.4	2.2	16.5	0.85	1.17	-9.1 ± 1.2	a	-204.3 ± 0.2
PP37	1.7662	-48.4925	28.37 ± 0.03	17.63 ± 1.27	0.6	N/A	0.76	1.2	N/A	a	N/A
PP38	2.0088	-48.6108	28.35 ± 0.05	14.14 ± 0.76	0.7	N/A	0.64	1.02	-17.4 ± 0.9	a	N/A
PP40	2.8827	-49.4089	28.73 ± 0.03	33.54 ± 0.06	4.0	N/A	0.81	0.99	N/A	a	N/A
PP41	2.8566	-49.3425	29.08 ± 0.06	29.34 ± 1.32	0.2	2.1	0.81	1.8	0.2 ± 0.9	a	-188 ± 1.1
PP42	2.8342	-49.3151	29.04 ± 0.03	26.65 ± 1.52	0.2	2.0	0.86	1.25	-2.2 ± 1.1	a	-197.1 ± 0.7
PP43	3.1391	-49.3335	28.46 ± 0.04	36.16 ± 0.11	16.7	5.5	0.97	1.55	5.9 ± 1.3	-180.3 ± 0.6	-183.4 ± 0.8
PP44	3.0999	-49.3064	28.23 ± 0.03	34.89 ± 0.45	59.1	N/A	0.98	1.54	6.3 ± 1.1	-189 ± 1.4	N/A
PP45	3.0627	-49.4272	28.51 ± 0.02	32.83 ± 0.8	33.3	N/A	0.98	1.63	4.1 ± 0.9	-190.8 ± 0.4	N/A
PP46	3.0911	-49.4337	28.68 ± 0.04	33.1 ± 0.65	9.2	N/A	0.96	1.42	N/A	a	N/A
PP47	3.0554	-49.4321	28.49 ± 0.01	29.2 ± 0.08	6.1	16.4	0.96	1.29	0 ± 0.9	-177.2 ± 1.4	-201.6 ± 0.7
PP48	2.915	-49.3347	28.03 ± 0.02	23.42 ± 0.27	7.7	7.2	0.88	1.14	-9.2 ± 1.4	-197.9 ± 0.5	-202.3 ± 1.6
PP49	2.8972	-49.4713	28.07 ± 0.03	21.86 ± 0.46	1.3	16.2	0.89	1.23	-8.4 ± 1	a	-211.7 ± 0.3
PP51	3.1025	-49.7931	28.3 ± 0.06	18.31 ± 0.21	2.2	N/A	0.74	1.04	N/A	a	N/A
PP52	3.098	-49.6761	28.68 ± 0.03	24.91 ± 0.16	0.6	27.0	0.86	1.23	-10 ± 1.3	a	-204.9 ± 1.6
PP53	3.5031	-50.1667	28.25 ± 0.08	20.33 ± 1.93	1.0	N/A	0.85	1.38	N/A	a	N/A
PP54	3.5576	-50.3623	28.2 ± 0.1	18.63 ± 0.6	0.3	11.9	0.82	1.05	N/A	a	N/A
PP55	3.9688	-50.5373	28.27 ± 0.16	16.94 ± 1.38	0.7	N/A	0.75	1.04	-16 ± 0.8	a	N/A
PP57	4.4874	-51.2401	28.04 ± 0.05	15.88 ± 0.09	0.1	17.7	0.82	b	-18.2 ± 0.7	a	-220.3 ± 0.8
PP60	6.1499	-51.2679	28.09 ± 0.03	36.16 ± 0.01	2.0	2.7	0.99	b	5.8 ± 0.8	-183.2 ± 1.2	-182.4 ± 0.6
PP61	5.5698	-51.8561	27.93 ± 0.09	32.19 ± 1.28	23.4	N/A	0.98	1.11	2.1 ± 1.3	-191.1 ± 2.7	N/A
PP62	5.3201	-51.9255	27.9 ± 0.04	22.72 ± 1.32	3.4	23.2	0.97	1.1	-8.3 ± 0.9	-192 ± 5.4	-209.7 ± 1.4
PP65	4.766	-51.5166	27.55 ± 0.08	17.58 ± 4.51	1.1	20.2	0.97	1.05	N/A	a	N/A
PP66	6.658	-52.8391	28.09 ± 0	36.06 ± 0	7.1	4.01	0.96	1.2	6.2 ± 0.7	-195.5 ± 0.1	-188.9 ± 0.5
PP67	5.9423	-52.6319	27.91 ± 0.07	25.25 ± 1.1	9.2	13.4	0.97	1.32	-4.9 ± 1.2	-183.7 ± 2	-206.7 ± 0
PP68	5.79	-52.7484	27.53 ± 0.06	23.4 ± 0.17	4.6	N/A	0.96	1.16	-7.1 ± 1.2	-192.5 ± 0.4	N/A
PP69	6.0839	-53.601	27.47 ± 0.03	22.69 ± 0.24	2.5	N/A	0.8	1.45	N/A	a	N/A
PP70	6.2821	-53.1561	27.64 ± 0.03	24.96 ± 0.74	2.4	N/A	0.96	1.03	N/A	a	N/A

[N/A] No measurements conducted

[a] C_{37} yield was not high enough for isotope analysis

[b] No clear peak distinction for C_{38}

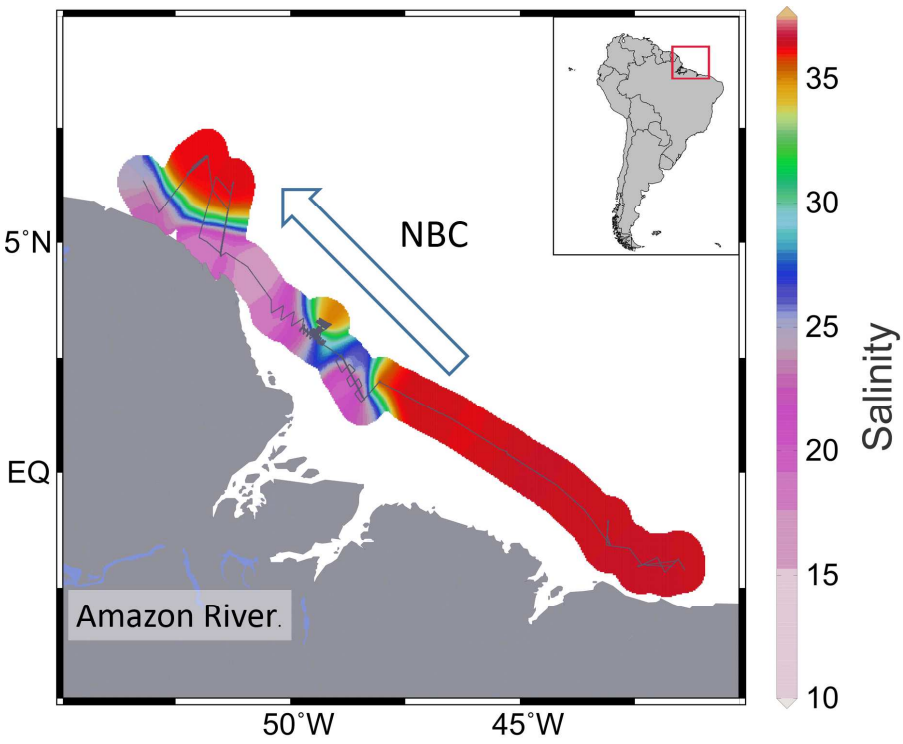


Figure 1. Map of the low salinity plume of the Amazon River outflow derived from the interpolation of onboard salinity measurements. The grey line shows RV Maria S. Merian cruise track MSM20/3 (Mulitza et al., 2013). The blue arrow depicts the North Brazil Current (NBC).

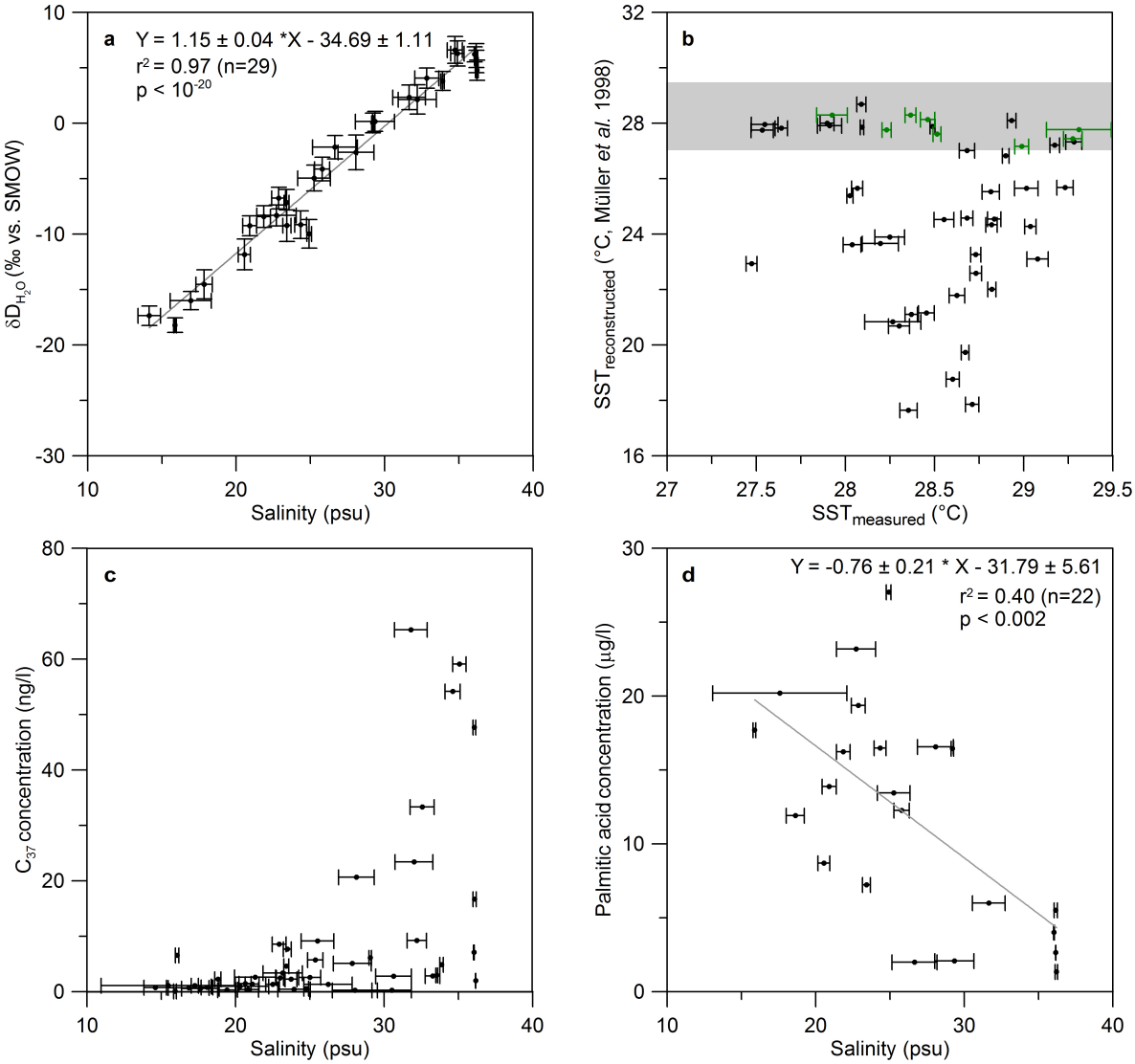


Figure 2. a) δD_{H_2O} plotted against salinity; b) $U_{37}^{k'}$ based sea surface temperature (SST) reconstruction using the calibration by Müller et al. (1998) plotted against measured temperature. Green data points represent samples with a C_{37} concentration > 10 ng L^{-1}. The grey bar indicates the range of measured SST; c) Concentration of the C_{37} alkenones plotted against salinity; d) Palmitic acid concentration plotted against salinity.

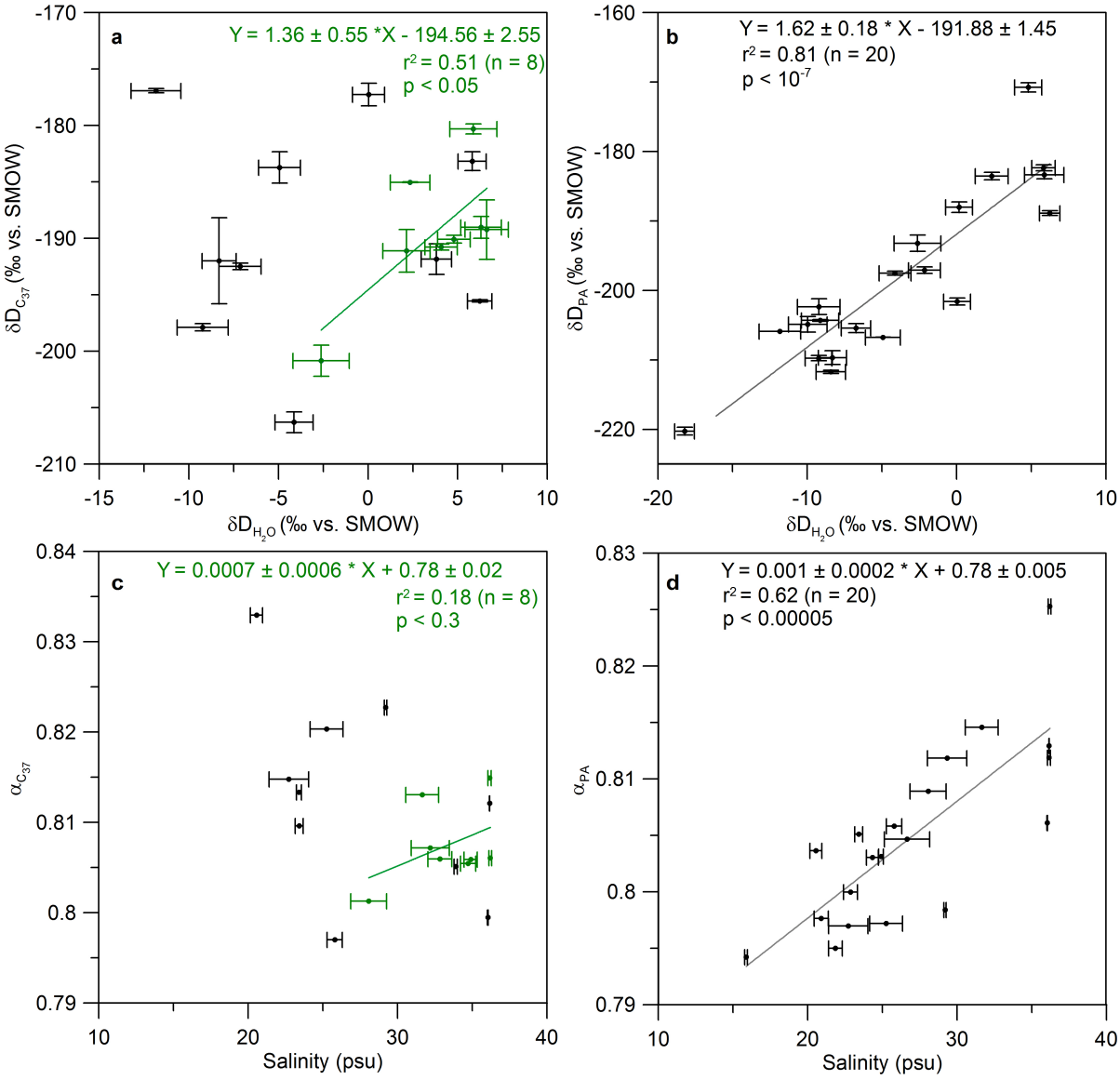
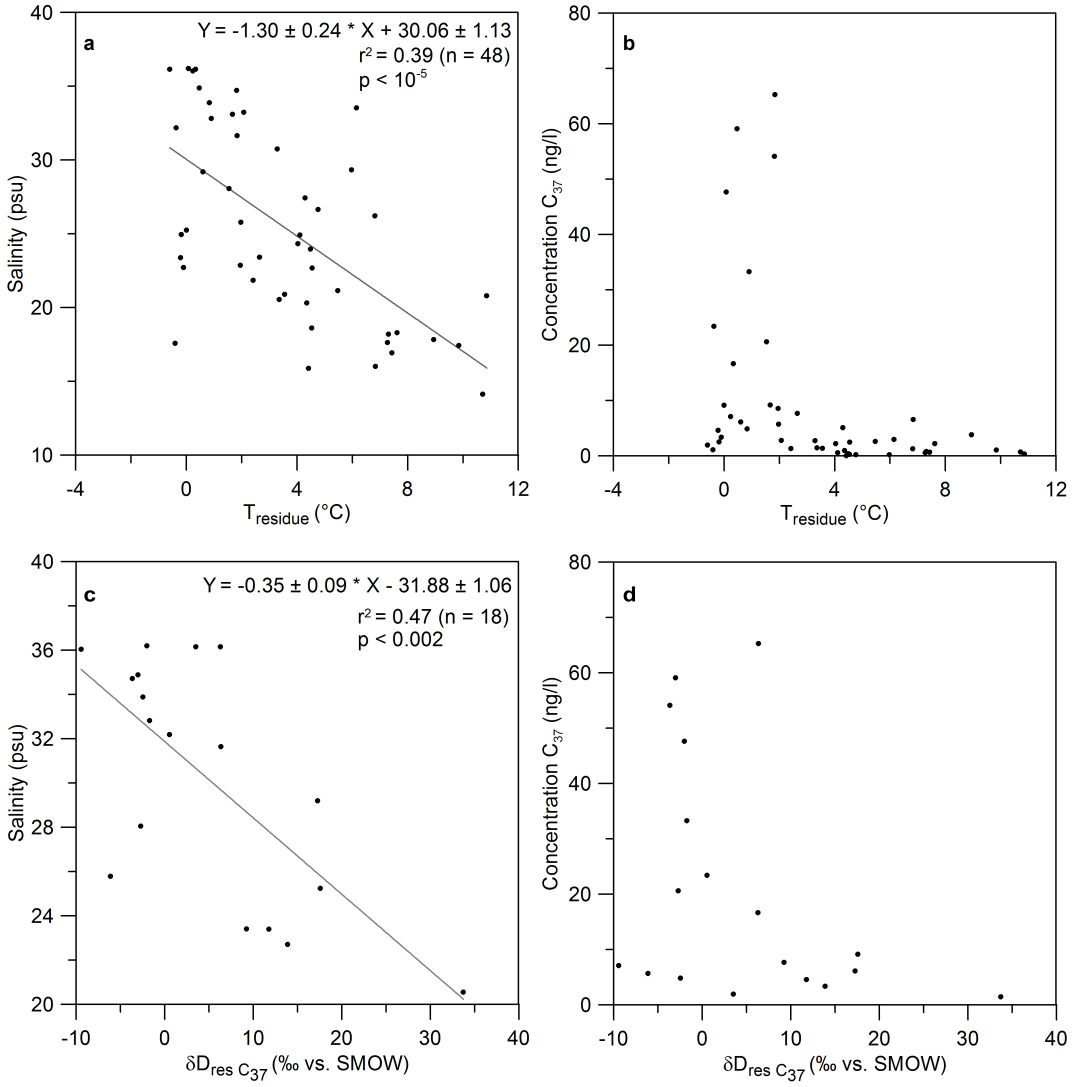


Figure 3. Results of the δD_{lipid} analysis. a) $\delta D_{C_{37}}$ against δD_{H_2O}. Green data points represent samples with a C_{37} concentration > 10 ng L^{-1}; b) δD_{PA} against δD_{H_2O}; c) $\alpha_{C_{37}}$ against salinity. Green data points represent samples with a C_{37} concentration > 10 ng L^{-1}; d) α_{PA} against salinity.



1

2 Figure 4. Residues of the $U_{37}^{k'}$ based SST reconstruction plotted against salinity (a) and C_{37}
3 concentration (b). Residues of the $\delta D_{C_{37}}$ measurement plotted against salinity (c) and C_{37}
4 concentration (d).