RC1

The reviewer comments are numbered for reference. Each reply is listed below the numbered reviewer comment.

1. Title and text. The term "organic matter quality" seems to be not adequate to describe the measurements here reported. Really, just fluorescence measurements were carried out and interpreted as signatures of some classes of organic components-like markers. I'd suggest the term "organic matter markers" or "organic fluorescent components".

Fluorescence measurements were carried out and interpreted as signatures of organic components. The chemical nature of the fluorescent fraction of the organic matter was surveyed using a fluorescent technique, thus organic matter markers is an appropriate alternative for the title and text. The title has been edited to, "A 21,000 year record of fluorescent organic matter markers in the WAIS Divide ice core". The text has been edited accordingly throughout the manuscript to improve clarity.

2. Line 17 and several other points. Usually, time unit is expressed as "kyr" and not as "kyrs". Please, correct in the text and figures.

All "kyrs" have been corrected to "kyr."

3. Lines 20-22. Here or in the "Results" section, Authors should clarify what They mean with the terms "labile microbial OM", "recalcitrant OM", "bioavailable carbon species" etc. A very short description of these terms could help the reader in better understanding the different biological significance and the different availability in carbon exchange between cryosphere and other ecosystems.

The descriptions of labile (easily altered) and recalcitrant (less easily altered) OM marker descriptions have been added to the abstract and the text.

4. Line 32. Please, cite also Wolff et al., Southern Ocean sea-ice extent, productivity and iron flux over the past eight glacial cycles. Nature, 2006, Vol. 440, 491-496, doi:10.1038/nature04614.

This reference was included upon revision in Lines 36-39.

"Numerous inorganic species trapped in ice has been used to reconstruct past chemical compositions of the atmosphere, its recent change in response to growing human activities as well its past natural variability (Legrand and Mayewski, 1997; Petit et al., 1999; Johnsen et al., 2001; Alley, 2002; Wolff et al., 2006; Jansen et al., 2007; Luthi et al., 2008)."

5. Lines 51 and following. What "OM character" means? Chemical composition? Chemicalspecies or functional groups identification? Authors are requested to clarify their thought.

The text has been edited for clarity, including the usage of OM markers in Lines 54-61. "While still a novel addition to deep ice core research, chemically characterizing the OM markers (i.e.

composition and chemical species) in englacial ice is of particular interest for several reasons: 1) OM markers can be linked to its source (e.g., aquatic, terrestrial) describing different influences of past and present ecosystems, 2) OM markers can serve as a proxy for englacial biological activity from *in situ* production, potentially explaining anomalous concentrations of other gases (e.g., methane, carbon dioxide) in ice core research, and 3) OM could be a pivotal contributor to the global carbon cycle if materials released to surrounding environments are metabolized to greenhouse gases (e.g., carbon dioxide, methane) in a warming climate."

6. Line 55. Even methane formed in anaerobic conditions is a strong forcing factor in the warming climate.

We describe the release of organic material upon melting of polar ice and the potential for it to be metabolized to greenhouse gases, such as carbon dioxide and methane in Lines 54-61. "While still a novel addition to deep ice core research, chemically characterizing the OM markers (i.e. chemical species and composition) in englacial ice is of particular interest for several reasons: 1) OM markers can be linked to its source (e.g., aquatic, terrestrial) describing different influences of past and present ecosystems, 2) OM markers can serve as a proxy for englacial biological activity from *in situ* production, potentially explaining anomalous concentrations of other gases (e.g., methane, carbon dioxide) in ice core research, and 3) OM could be a pivotal contributor to the global carbon cycle if materials released to surrounding environments are metabolized to greenhouse gasses (e.g., carbon dioxide and methane) in a warming climate."

7. Line 72. Since snow density is variable, it is better to express the mean accumulation rate as cm or mm "water equivalent".

This has been edited in the text to an average annual accumulation rate $0.207m_{weq} a^{-1}$ (Banta et al., 2008) in Lines 78-81.

"Snow precipitation at this site is relatively high with an average annual accumulation rate $0.207 m_{weq} a^{-1}$ (Banta et al., 2008)..."

8. Lines 74-76. What means this sentence? Several other ice cores (for instance, Taylor Dome and Talos Dome, in the same Antarctic Sector; Dome C and Dome Fuji, in the inner Antarctica; Dronning Maud Land, in the Atlantic Sector; etc.), even drilled before WD ice core, constitute "equivalent paleoclimate record" to Greenland ice cores. In particular, the EDC, EDML and DF climate records were compared with the climate oscillations recorded along the NGRIP ice core in: EPICA Community Members, Oneto-one coupling of glacial climate variability in Greenland and Antarctica. Nature, 2006, Vol. 444, 195-198, doi:10.1038/nature05301.

The statement was deleted upon revision.

9. Line 80. Please, change "drilling solvent" with "drilling fluid".

"Drilling solvent" was edited to "drilling fluid" in the text.

10. Line 88. Please change "combusted" with "pre-fired".

"Combusted" was be edited to "pre-fired" in the text.

11. Section 2.3. The correction for the absorbance measurements seems to be not clear. Authors are asked to give more information on that. Besides, the absorbance threshold seems to be quite high. If a.u. means, as I think, absorbance unit, the value A = 0.3 corresponds to a percentage transmittance of 50% (A = Log 1/T) that seems to be too low for ice-core melted water at 254 nm. Maybe, some particles were suspended or some gas bubbles were present in the melted samples during the measurements. Authors are requested to clarify this point.

The text was edited to reflect that all of our samples were optically transparent in Lines 100-109. "Prior to fluorescence spectroscopy, absorbance spectra of WD core meltwater samples were collected from 190-1100 nm (UV-Vis spectral range) using a Genesys 10 Series (Thermo-Scientific) Spectrophotometer with a 1 cm path length cuvette and VISIONlite software. Obtaining UV-Vis absorbance spectra are necessary for the post-processing calculations of spectral corrections including primary and secondary inner filter effects (Acree et al., 1991; Tucker et al., 1992). Absorbance values at 254 nm (A₂₅₄) greater than 0.3 absorbance units (a.u.) require dilution prior collecting the UV-Vis absorbance spectra and EEMs. WD core OM samples were optically transparent, with measured A₂₅₄ values well below 0.3 a.u. after blank correction; consequently, no sample dilution prior to UV-Vis absorbance measurements and EEMs was required (Miller and McKnight, 2010; Miller et al., 2010). Spectra were blank corrected against purified water from a Milli-Q system each day. UV-Vis absorbance spectra were subsequently incorporated into the spectral corrections calculations for post-processing the EEMs data."

12. Line 97. Maybe the term "optically dilute" could be changed in "optically transparent" (but I do not think that this term is correct for T% = 50%).

The term "optically transparent" was edited in the text.

13. Section 2.4. Even if a reference is cited, Authors are requested to give some basic information about the PARAFAC multivariate analysis.

Basic information on the setup of our analyses and specific details regarding PARAFAC was added to the manuscript in Lines 123-151.

"EEMs were prepared for multivariate parallel factor (PARAFAC) analysis following a similar procedure previously outlined for sample classification, normalization, and subset selection (Cawley et al., 2012) to model the WD fluorescent OM character. This procedure was selected after failed attempts to validate modeled results of the entire EEMs data set, due to high percentages of outlier removal, noise/scattering interference, normalization effects, and low percentages of data fitted by each component producing high percentages of residual fluorescence. Briefly, EEMs were grouped by fluorescence into separate categories (relatively subjective categorization based on resolved fluorescence patterns; i.e. protein- and humic-like, scattering, etc.), and normalized within each category group to their maximum emission intensities to reduce the compensating effects that occur when normalizing samples over greatly varying fluorescence intensities. Using a randomization selection program, 20 samples were

selected from each group for the representative subset of samples (n=140) for PARAFAC analysis. PARAFAC analysis continues to be widely used to decompose EEMs into individual OM fluorescent chemical components (Bro, 1997; Stedmon et al., 2003; Murphy et al., 2013). A three component PARAFAC model was generated for the subset of samples by drEEM and the N-way toolbox scripts in MATLAB under non-negativity constraints (Stedmon and Bro, 2008; Murphy et al., 2013). The three component model was validated by split half analysis with all of the components in the split models tests finding a match with a Tucker correlation coefficient > 0.95 (Murphy et al., 2013). The core consistency value was 97%, which was within the acceptable range suggested for robust PARAFAC modelling. Two and four component models were attempted, with a validation of the two component model, but a considerably lower core consistency value for the four component model. PARAFAC analysis beyond three component model. Therefore, the three component model was selected to best represent the entire data set and was used for further interpretation of our results.

To investigate how the PARAFAC model components would potentially shift based on climate periods, three separate PARAFAC models (LGM, LD, and Holocene) were also tested, which produced somewhat redundant results (specifically for components one and three; C1 and C3) to our three component PARAFAC model of the entire data set. With large groupings of outliers varying over different climate periods, these separate models were not appropriate tools to analyze statistical changes in all of fluorescing components over time. However, the variation of the fluorescing regions comprising PARAFAC component two (C2) from the LGM to the Holocene were captured by this method, thus those results are presented as qualitative comparative complements to the original model."

14. Section 2.5. Authors should here anticipate why some elements were considered in this paper (e.g., nssCa as crustal marker, ssNa as sea spray indicator, nss-SO4 spikes to identify volcanic deposition signatures, etc.). Besides, more detail is requested in calculating the ss- and nss-fractions of Na, Ca and SO4. Since both Na and Ca can be related to two main sources (sea spray and dust), a four-equation system is necessary to calculate the ss- and nss- fractions (particular attention has to be put in evaluating ssNa during the LGM and nss-Ca during Holocene). Finally, which sea water ratio was used for the calculation of ss-SO4? Have the Authors used the SO4/Na seawater ratio of 0.25 or a lower value?

The crustal marker nssCa was highlighted in the manuscript instead of the three previously reported crustal indicators, and appropriate references were provided. The sea spray indicator (ssNa) and calculation information are referenced already in the text: Bowen 1979, WAIS Divide Project Members 2013. The text has been edited to reflect such changes in Lines 154-162. "Meltwater from the interior section of the ice core was also used for a broad range of elemental analyses (WAIS Divide Project Members, 2013) including calcium (Ca) as an indicator of continental dust. From the CFA system, meltwater was directed through Teflon tubing to two Inductively Coupled Plasma Mass Spectrometers (ICPMS, Element 2 Thermo Scientific) located in an adjacent class 100 clean room for continuous trace element analysis (McConnell et al., 2007). Non-sea salt calcium (nssCa) were calculated following standard procedures from measured total concentrations of Ca using abundances in sea water and mean sediment (Bowen, 1979). Concentrations of sea salt sodium (ssNa) data from the LGM through deglaciation were

previously reported and referenced this work as a sea ice proxy throughout all climate periods (WAIS Divide Project Members, 2013)."

15. Lines 126 and 128. Authors are requested to shortly describe the characteristics of "bioavailable carbon species" and "more recalcitrant species".

The text has been edited to include such definitions in Lines 167-173.

"All samples contained low Ex/Em wavelength (240-270 nm / 300-350 nm) fluorescence characteristic of more easily altered material by microorganisms, representing fluorescent OM markers potentially of proteinaceous (Coble et al., 1990; Coble et al., 1998), polycyclic aromatic hydrocarbon (PAH) (Ferretto et al., 2014), and simple phenol, tannin, or monolignol (Coble, 2014) origin. Fewer samples (2.5%) contained OM fluorescence at higher Ex/Em wavelengths (240-250 nm / 340-530 nm), characteristic of more humic-like markers of terrestrial plant/soil origin (Coble et al., 1990). Examples of low and high Ex/Em wavelength fluorescence can be seen in Supplement Figure 1a-c."

16. Lines 129-131. This early Holocene peak of fluorescent mater is interesting, as well as the larger peak around 21-22 kyr BP. Authors do not discuss these two features in the temporal profile of the WD ice core. I'd like to know the Author interpretation on these large depositions of organic fluorescent compounds, even if as a tentative hypothesis. It should be very interesting to perform some qualitative analysis (e.g., by HPLC-MS measurements) on these samples in order to clarify the nature of the fluorescent compounds.

The fluorescent peaks were discussed prior to the revised manuscript as intensity shifts in each climate period, and do not directly correspond with large depositions of organic fluorescent compounds. Rather, the quantum yields of specific fluorescing material is represented, along with the hypotheses that both fluorescing material and concentration of organic material may be contributing to shifts in fluorescence intensities. Large deposition events of organic material cannot be linked to shifts in fluorescence intensities. Further analyses of these samples is not possible as only 7.5mL of each sample were available and have been used in the present analyses. This figure no longer accurately represents our data set and was removed upon revision.

17. Lines 132 and following. I surely do not want to minimize the contribution of the PARAFAC analysis, but I have to note that the result of its application is quite basic. From Figure S1, the separation of the fluorescent bands at 420 nm Em and 300 nm Em is very clear even without any multi-parametric analysis. The only significant result is the identification of two fluorescent components C1 and C2 at short Em and Ex wavelength. However, the two components are just attributed to two large organic compound classes (amino acid-like fluorescent compounds), without a more specific characterization. Besides, the C1 and C2 fluorescent components are not clearly differentiated in terms of biological origin: C1 is attributed to tyrosine-like fluorescent compounds associated to "microbial processing in aquatic environment", while C2 is described as a fluorescent signature overlapping "between tyrosine- and tryptophan-like" fluorescent compounds. At line 177-178, Authors just report that C2 containing tryptophan-like fluorescence could represent "intact dissolved proteins ….freshly derived from microorganisms". Authors are requested to better organize, in the present section, the discussion on the possible origin of these

components and to enlighten the biological and environmental differences. In conclusion, the PARAFAC analysis seems to be not able to "resolve the representative subset of samples into individual OM fluorescing components", as the Authors assessed at lines 132-133. Even the comparison with the OpenFluor database components did not give significant matches (if I have well understood lines 153-155).

RC1 comment: The separation of the fluorescent bands at 420 nm Em and 300 nm Em is very clear even without any multi-parametric analysis.

Author's reply: This is correct, however Figure S1 highlights examples of different types of fluorescing organic matter, so it was our intention to show notably obvious differences from the WD core. The WD core fluorescent data set comprised a small fraction of material fluorescing in Figure S1 (c), thus we needed to apply a statistical tool, PARAFAC analysis, to decompose the EEMs into individual fluorescing components, even for the overlapping fluorescing material at lower Ex/Em wavelengths. The significant result is that PARAFAC was used as a multiparametric tool to decompose the EEMs data set into three fluorescing components. That information was subsequently categorized to identify the chemical character of the fluorescing organic material characteristic of each climate period. RC1 Comment: "Besides, the C1 and C2 fluorescent components are not clearly differentiated in terms of biological origin: C1 is attributed to tyrosine-like fluorescent compounds associated to "microbial processing in aquatic environment", while C2 is described as a fluorescent signature overlapping "between tyrosine-and tryptophan-like" fluorescent compounds." C1 and C2 fluorescence technique. The specific characterization of C1 has been revised in Lines 180-196 as:

"Three OM PARAFAC components were identified from the WD EEMs (fluorescing regions shown in Figure 2a, and Ex/Em wavelength loading scores shown in Supplemental Figure 2). PARAFAC component one (C1; Figure 2a, top) showed maximum fluorescence in a region analogous to the secondary fluorescence of fluorophore peak B (tyrosine-like, Ex: 240 nm and Em: 300 nm), typically associated with microbial processing in aquatic environments (Coble et al., 1990; Coble et al., 1998). Regions of fluorescence at such Ex/Em wavelengths are commonly referred to as "protein-like" but overlap with fluorescence of other origins (Coble, 2014). However, without the primary region of fluorescence associated with fluorophore peak B (tyrosine-like) at higher Ex/Em wavelengths, the OM fluorescent marker of C1 cannot be determined to be tyrosine-like material of microbial origin by this method. Rather, OM with similar Ex/Em wavelength fluorescence has been documented for simple phenols (e.g., tannins and monolignols) commonly detected in natural waters (Coble, 2014). Simple phenolic OM is characteristically lower in molecular weight, aromaticity, and is considered to be more easily altered in the environment, as compared to more humic-like material (Coble, 2014). Thus, we report the chemical composition of WD OM in C1 to be most similar to monolignol chemical species, ubiquitously found in the environment as the precursors to lignin material detected in vascular plants. Once thought to be generated in the environment from tyrosine, the biosynthesis of monolignols actually originates from phenylalanine via multiple enzymatic reactions, therefore sharing protein-like origin, but ultimately is chemically linked to vascular plants as a fluorescent OM marker (Wang et al., 2013)."

The specific characterization of PARAFAC component two (C2) has been revised in Lines 197-208 to:

"PARAFAC component two (C2; Figure 2a, middle) contained maximum fluorescence at low Ex/Em wavelengths (260-270 nm / 310-320 nm) in regions analogous to the primary fluorescence of fluorophore peak B, and cresol (methylphenol), commonly known as the building blocks of tannins (Kraus et al., 2003), the major components of soil and aquatic humic OM (Tipping, 1986). Secondary fluorescence commonly detected for fluorophore peak B (tyrosine-like) was not observed for C2, and the combination of fluorescence from C1 and C2 do not yield the appropriate primary and secondary fluorescent trends commonly associated with tyrosine-like OM. Therefore, by this method, PARAFAC identified two distinct components, that may have protein-like similarities, but cannot be inherently linked to amino acid-like material and microbial origin. Thus, we determined that C2 fluorescence was characteristic of a combination of protein-like and tannin-like OM markers based on the regions of overlapping fluorescence by this method. Similarly to the chemical species reported for C1, the low Ex/Em wavelength fluorescence of C2 indicates OM markers with lower molecular weights, aromaticity, and chemical species that are more easily degraded in the environment by microorganisms (Coble, 2014)."

The specific characterization of PARAFAC component three (C3) has been revised in Lines 213-226 to:

"Component three (C3) displayed fluorescence commonly associated with more humic-like material. Two humic-like fluorescing regions were identified that comprised C3: fluorescence at 1) Ex/Em: 240-260/380-460 nm, characteristic of fluorophore peak A, and 2) Ex/Em: 300-320/380-460 nm, characteristic of fluorophore peak C, commonly associated with terrestrial plant and/or soil origin (Coble, 1996; Marhaba et al., 2000). Fluorescent OM markers in this region is linked with chemical species having higher molecular weights aromatic nature, and are considered to be less easily altered by biodegradation in the environment as compared to more labile material (Coble et al., 1990; Cory and McKnight, 2005; Murphy et al., 2008; Balcarczyk et al., 2009; Fellman et al., 2010). While commonly referred to as the "more recalcitrant" fraction of fluorescent OM, studies have shown that terrestrial humic-like material is susceptible to photodegradation, therefore should not be considered as an unalterable fraction of OM (Osburn et al., 2001; Stedmon et al., 2007)."

More specific characterizations of each PARAFAC component cannot be determined using this bulk analytical technique, however possible chemical species were suggested. This was clarified in the text. The chemical species associated with PARAFAC C1, C2, and C3 were discussed in the text providing a bulk representation of the organic materials present throughout 21,000 years. This sets the foundation for future work, a point which was also be clarified in the text in Lines 286-288.

"The composition and chemical origins associated with PARAFAC components C1, C2, and C3 provided a bulk level representation of the OM markers present throughout 21.0 kyr and initiated the foundation for future research."

RC1 Comment: "Authors are requested to better organize, in the present section, the discussion on the possible origin of these components and to enlighten the biological and environmental differences." The discussion on the possible origin based on these data is present in the manuscript, highlighting environmental differences over time. RC1 Comment: "In conclusion, the PARAFAC analysis seems to be not able to "resolve the representative subset of samples into individual OM fluorescing components", as the Authors assessed at lines 132-133. Even the comparison with the OpenFluor database components did not give significant matches (if I have well understood lines 153-155)."

The authors disagree. The PARAFAC analysis resolved the representative subset of samples into the only individual OM fluorescing components that were present in the samples. PARAFAC analysis is capable of producing brilliant results of the data set asked of it. With most EEMs resulting in the example provided in Figure S1, it was not surprising to have the low Ex/Em wavelength fluorescent components modeled as two individual components C1 and C2, prior to C3. The order of the modeled components describes the variation in the data set, and was statistically validated with the drEEM program in MATLAB. The OpenFluor database contains various data sets from samples collected around the world. Submission of data to OpenFluor is not a requirement, and is currently still in its growing phases. Scientists are encouraged to upload their PARAFAC datasets upon publication, but it is not required, thus the database it contains does not encompass all possible fluorescent component data. OpenFluor matches with the dataset describe PARAFAC components that have been identified in other ecosystems. A match or no match result describes unique data worth reporting. We felt it was interesting to report that organic material from 6,000 to 27,000 years ago did not match any of the uploaded PARAFAC data currently in the database. Our dataset is the first of its kind from a continuous Antarctic ice core, thus we stress the importance of its upload to OpenFluor upon publication, which in turn will better serve the fluorescent community.

The text has been edited to incorporate our rationale behind the results of the OpenFluor query in Lines 227-233:

"WD ice core OM PARAFAC components were uploaded to the OpenFluor database to compare and contrast C1, C2, and C3 with other environmental OM marker studies, however, no component matches were determined (Murphy et al., 2014). The OpenFluor database is a repository of a selection of samples, and while still growing to encompass a thorough library of fluorescent OM markers from highly variable environments, it is reasonable to expect nonmatching results based on database queries. Our results matched no previously identified PARAFAC components uploaded to the database, which we attribute to the unique scope of this work and the great volume of samples spanning 21.0 kyr from Antarctic ice."

18. Lines 142-143. The terms "red/blue shifted to longer/shorter Em wavelengths" are repetitions. Please, change in "Em-wavelength red/blue shifted" or "shifted to longer/shorter Em wavelengths". Authors should clarify the statistical significance of these shifts (especially from LGM to LD) and anticipate the consequent biological meaning (especially from LGM-LD to Holocene). Besides, which is the meaning of the red or blue shifts? When blue (red) shift occurs, is the C2 component a marker of tyrosine-like (tryptophan-like) fluorescent compounds?

The text has been edited accordingly and descriptions of chemical species associated with the specific shifts are provided in Lines 260-265.

19. Section 3.2. The relationship between glacial cycles and atmospheric deposition of dust in Antarctica is a very relevant and largely discussed topic in ice core studies. Here, the Authors have to take for granted the inverse relationship between site temperature and dust deposition (by citing the most relevant references) and anticipate the discussion on the possible relationships among temperature, dust and biological activity (or OM transport efficiency), as revealed by the

fluorescence temporal profile. At this purpose, Authors should choose the preferred dust indicator among the possible dust markers measured along the WD ice core (nss-Ca, Mn and Sr), also basing on the correlations between the elements (lines 165-166).

The authors agree and highlight our speculative relationships to better infer the influences of continental dust loading on OM markers. nssCa was selected to be our preferred dust indicator, highlighting that the concentrations agree with other ice core records, and then discuss the proxy in terms of OM influence over time (Lines 316-323).

We incorporated these statements as:

"Concentrations of nssCa has been shown to be a valuable proxy for terrestrial crustal dust in paleoclimate ice core records (McConnell et al., 2007; Gornitz, 2009; Lambert et al., 2012). As such, it is plausible to envisage a link between the concentrations of nssCa and other transported materials influenced by aeolian deposition, (e.g., OM concentration and character, microbial biomass, and pollen grains). The relationship between glacial cycles and atmospheric deposition of dust in Antarctica is largely discussed in ice core studies. We applied an assumption that common transport processes of dust and OM markers together could be hypothesized only if dust and OM originated from the same continental areas. Therefore, in this work, we merely speculate on the influence of dust concentrations and OM composition measurable by fluorescence spectroscopy."

20. Lines 174 and 176. Maybe, "throughout time" is better than "throughout history".

The text has been edited to reflect this addition throughout the manuscript.

21. Line 198-200. Common transport processes of dust and OM could be hypothesized only if dust and OM originated from the same continental areas. In LGM, Southern South America was supposed to be the major dust source area for Antarctica. In LD and, especially, Holocene, even Australia could have played a significant role. Therefore, Authors implicitly suppose that OM was originated in these continental regions. For OM originated by marine sectors (C1, C2?, part of C3), the relationship with dust transport processes cannot be considered significant because they can follow very different pathways (e.g., implying different meridional or zonal atmospheric circulation modes).

Common transport processes of OM were only hypothesized, and we inferred a local South American major dust source region for Antarctica. The text was edited throughout the discussion sections to reflect these revisions.

22. Lines 200-201. Authors here refer on relationships between dissolved organic carbon and dust markers. I suppose DOC measurements were not performed as part of this paper (see following sentence in the text). Authors should give more information on that or cite some reference.

DOC concentrations were not performed as part of this work. The supplemental information section outlining these relationships were removed upon revision.

23. Line 204. I think Authors refer to Figure 4.

Indeed, we did. Thank you. The manuscript has been edited.

24. Lines 205-212. This part has to be completely revised. The complex relationship between dust deposition in Antarctic ice cores and climatic cycles cannot be discussed in this form in this paper and, how I have already pointed out, has been (and will be) the topic for several specific papers. Authors are requested to report the major literature references about LGM-LD-Holocene dust/climate pattern and focus the discussion on the relationship among climate, dust (possibly) and OM fluorescent markers. Besides, I have to note that the detail in the discussion on the behavior of OM data and dust profiles along the WD ice core is not so high to appreciate specific differences in nss-Ca, Mn and Sr profiles. Therefore, since the three dust-marker profiles were not singularly discussed and differentiated, I'd suggest to replot Figure 4 with just one dust marker (maybe, nss-Ca).

One dust marker (nssCa) is now presented and only discussed in terms of the fluctuations of dust concentrations with the different OM chemical species present in different climate periods. This section was completely revised to reflect the changes and a new Figure 4 is now incorporated (see below with caption).



Figure 4. Trace element concentration of (top) non-sea salt calcium (nssCa; ppb), and (bottom) the δ 18O (per mil) temperature record (Marcott et al., 2014) from the West Antarctic Ice Sheet Divide ice core as a function of time (kyr before present 1950), dating from the Last Glacial Maximum (LGM), through the last deglaciation (LD), to the mid-Holocene.

25. Section 4.3. Even this section has to be largely revised. Authors assume a series of speculations to correlate changes of OM fluorescent markers to changes in climatic and environmental conditions, as evaluated by changes in sea-ice coverage (by ss-Na – Authors could add the ss-Na profile in figure 4), dust production and transport (by dust markers) and volcanic eruption frequency (by nss-SO4 spikes) in the LGM, LD and Holocene. However, no reliable comparison among the different time profiles is shown. In particular, while dust and sea ice markers show a progressive decreasing during the LD, the OM fluorescent profile shows an abrupt change (at about 18.5 kyr BP) from high LGM values and very low LD and Holocene levels. All the discussion is too elemental and also the changes in C1 and C2 relative contributions are not clearly interpreted. From the data here reported, I can just see that OM fluorescent markers are high in the LGM, when dust and sea spray are high. However, there is not experimental evidence on which climatic or environmental factors (more efficient meridional or zonal atmospheric transport, larger sea ice coverage, higher input from continental areas, larger emissions from marine biota, etc.) could have driven the OM deposition at the WD site. Finally, the relationship between volcanic activity (as recorded by the nss-SO4 spikes along the WD ice core) and OM fluorescent markers is, in my opinion, really unsustainable. Volcanic signatures in Antarctic ice core are mainly related to long range atmospheric (especially stratospheric) transport of SO2 emitted during eruptions occurred at hemispheric scale and it is really difficult to correlate changes in WD OM to sporadic, short-time and widespread volcanic emissions without a strong experimental evidence.

The authors agree. The co-registered geochemical WD dataset were used to speculate on the origin of the OM characterized by fluorescence spectroscopy. No direct comparisons were reported because none were available for this project; that was beyond the scope of this work. The manuscript has been edited to reflect this information in Lines275-278. "It is important to note no direct comparisons between dust concentrations and OM qualitative markers or concentrations can be made with these data, as that was beyond the scope of this work. Rather, this information was subsequently utilized as discussion points to infer more information regarding the OM marker origin detected in the WD ice core."

The PARAFAC components C1 and C2 relative contributions were discussed in terms of percentages relative to the other components. See above responses outlining our PARAFAC component discussion section in further detail. The sections outlining the volcanic signatures is highly speculative and was removed upon revision.

26. Lines 225-226. What this sentence means? What is compared to the open ocean?

The authors meant to state a comparison of more to less sea-ice extent. This was edited in Lines 350-351.

27. Lines 233-234. Authors are requested to better discuss the red shift of the C2 component, explaining which amino acid-like components increases its contribution to fluorescent OM and at which biological source can be attributed. What "external environments" means?

The red shift clarification was added with chemical species clarifications as stated above. "External environments" was deleted upon revision of the manuscript.

28. Lines 237-243. The pattern of the OM fluorescent markers during the ACR is not visible in Figure 3 (neither in Figure 2). This part is merely speculative and not supported by experimental evidences.

Correct. The dust record was used in Figure 4 as a discussion point to speculate on the variation in OM character during the ACR, specifically for the variability in PARAFAC C2 transitioning from LGM, through the LD, to the Holocene. The text was edited to reflect these revisions in Lines 367-372.

"The years between 13.0-11.5 kyr BP, at the end of the LD, defined as the Antarctic cold reversal, incorporate a depression of temperature, just prior to the early Holocene, where reports of Ca and dust concentrations increase (Delmonte et al., 2002). Also measured in the WD ice core (increases in nssCa; Figure 4), we speculate that these environmental fluctuations during the Antarctic cold reversal, may also explain the fluorescent OM variation in the LD (Figure 3a-c)."

29. Lines 244-250. How can the Authors explain the very low levels of OM fluorescent markers during the Holocene, when climatic conditions should promote higher terrestrial and marine biological productivity? Which could be the significance of the large spike in OM fluorescent profile (Figure 2) at about 10 kyr BP?

This section was completely revised and Figure 2 was removed upon revision. We cannot discount that carbon productivity is reportedly higher in the Holocene, however, that does not ensure efficient transport of materials to Antarctica. We thus reported our findings and discuss these ideas with the need for future investigations that could answer such questions.

30. Line 258. Please, change "Concentrations of nss-sulfur..." with "Spikes in nss-SO4 concentrations..."

The sections outlining volcanic activity discussed in terms of OM character were removed.

31. Lines 261-262. Authors are requested to clarify how volcanic activity can stimulate OM production. How is calculated the percentage of the fluorescent OM attributed to the volcanic activity? The relationship between volcanic activity and OM deposition at WD site is, in my opinion, not plausible and not supported by experimental data (at least, by experimental data here reported). Have the Authors measured OM fluorescent peaks in ice core sections with volcanic depositions? In absence of experimental support, the discussion about the volcanic activity and OM fluorescent markers should be removed from the manuscript.

Indeed, we do not have experimental support, merely just speculations on this topic. Volcanic activity discussion sections were removed upon revision.

32. Conclusions section. This part should be changed accordingly to the changes suggested along the different manuscript sections.

This section was revised accordingly based on all the reviewer's comments. RC2

The reviewer comments are numbered for reference. Each reply is listed below the numbered reviewer comment.

1. You state that "Ice core studies rely on the paradigm that atmospheric deposition is the sole mechanism for specific gases and materials to become trapped in the ice" (Lines 47- 48) yet it is unclear if you apply this paradigm to your work. If you do not allow even a remote chance for insitu production of this organic matter, then please explicitly state so in your work.

We apply this conservative approach for organic matter preservation in ice cores, however, do so acknowledge the possibility for *in situ* production of organic matter. This acknowledgement is addressed in Lines 54-61.

"While still a novel addition to deep ice core research, chemically characterizing the OM markers (i.e. composition and chemical species) in englacial ice is of particular interest for several reasons: 1) OM markers can be linked to its source (e.g., aquatic, terrestrial) describing different influences of past and present ecosystems, 2) OM markers can serve as a proxy for englacial biological activity from *in situ* production, potentially explaining anomalous concentrations of other gases (e.g., methane, carbon dioxide) in ice core research, and 3) OM could be a pivotal contributor to the global carbon cycle if materials released to surrounding environments are metabolized to greenhouse gases (e.g., carbon dioxide, methane) in a warming climate."

We can only speculate on the possibility of *in situ* organic matter production due to methodological limitations and thus, that clarification is edited in the text in Lines 67-70. "For this study, all possible sources of OM markers detected within the West Antarctic Ice Sheet (WAIS) Divide ice cores were considered, however we can only speculate on the possibility of *in situ* OM production due to methodological limitations."

2. In lines 179-183 you mention the possibility of in situ OM processing but then do not discuss if such transformation could affect the samples in this work.

The detection of tyrosine-like and tryptophan-like organic material would mark the signs of microbially derived OM produced in the WD ice core. However, it would also mark the signs of microbially derived transported material to the WD ice core. Due to methodological limitations and the lack of protein-like resolved fluorophores, we can only mentioned the possibility of in situ processing in the WD ice core. Future work in deep ice will have to be conducted to specifically test for such occurring transformations/identification of microbially produced OM. This section was edited accordingly in Lines 294-305:

"We continue to acknowledge the fluorescence overlap at low Ex/Em wavelengths (C1 and C2) with regions of fluorescence that describe "protein-like" material characteristic of microbial processes (*in situ* OM production and transformation), however cannot confirm OM microbial origin by fluorescence spectroscopy alone. Miteva et al. (2016) reported that the presence of microorganisms in deep ice cores also suggests the possibility of *in situ* OM processing, which could have important implications for gaseous climate records (Rhodes et al., 2013; Miteva et al., 2016; Rhodes et al., 2016). Including *in situ* biological OM transformations in ice core research was recently proposed as an alternate mechanism for CH₄ production in ice from firn layers of the WD ice core (Rhodes et al., 2016). At this juncture, our bulk level fluorescent results cannot

argue that *in situ* OM production is a major contribution to the OM markers detected by EEMs in the WD ice core due to the lack of resolved tyrosine- and tryptophan-like fluorescing components."

3. You mention that tryptophan-like florescence in C2 may derive from microorganisms, and then mention that the presence of microorganisms may result in in situ OM processing, but step back from linking the two aspects.

This section was completely revised and no longer should include a discussion of the linkage between C2 and in situ production.

4. In the following paragraph you then mention that Holocene terrestrial plants and soils are the likely source of the C3 OM yet do not mention if in situ processes may affect this material or if you ascribe this material to be solely brought in via atmospheric transport. Please clarify your stance on the source and possible post-depositional processes affecting the samples as both aspects are essential to your interpretations of the data.

Upon clarification of the points mentioned above from the previous comment, this section was edited accordingly.

5. Please check that all figures are cited in the text. In lines 128-144 you mention Supplemental Figures 1a-b. You do not refer to Figure 2 in the text. As you refer to the Supplemental Figures but not Figure 2, then perhaps their roles should be reversed with the current Figure 2 included in the Supplementary Information and vice versa.

Figure 2 was cited in the old manuscript Line 129. All figures are cited appropriately in the revised manuscript.

6. The left bars and corresponding explanation in the caption of Figure 3 are confusing. In the article text you explicitly state that C3 only occurs during the Holocene. As most readers will likely first look at the figures and captions before reading the article, it bears mentioning in the caption that C3 is specific to the Holocene. Demonstrating the variation in C2 by various time periods (LGM, LD and Holocene) is useful but then makes the reader immediately wonder what is the variation in C1 between climate periods. If there is no substantial variation between time periods for C1, please mention this fact in the caption.

New figures 2 and 3 are incorporated into the revised manuscript. Supplement Figures describing the separate PARAFAC model are also included to highlight that variation in C2 was the only component shifting with climate. These figures were discussed appropriately in the text. Figure 2 and caption:



Figure 2. PARAFAC analysis results for West Antarctic Ice Sheet Divide ice core organic matter showing a) components one, two, and three (C1, C2, and C3), and b) the fluorescence percentage of each component contributing to the overall fluorescence signature over the Last Glacial Maximum (LGM), last deglaciation (LD), and Holocene climate periods as a function of time (kyr before present 1950). Average fluorescence percentages (gray dashed lines) are provided for each component, separately calculated for each climate period. Fluorescent data were reported in Raman Units. Note: C3 average fluorescence percentages are considerably lower in the LGM and LD, and did not correspond to resolved fluorophores.



Figure 3. West Antarctic Ice Sheet organic matter supplemental climate PARAFAC analysis combination results of component two (C2) variation with climate periods a) Last Glacial Maximum (LGM), b) last deglaciation (LD), and c) Holocene. Component one (C1) identified in each climate PARAFAC model showed no variability over time, and component three (C3) was only identified in the Holocene (Supplement Figure 3).

7. This sentence is confusing (Lines 227-229): "During the LGM, tundra ecosystems covered more expansive areas of the Earth (Ciais et al., 2012) and while C was cycling, productivity in the environment differed from warmer climates (Ciais et al., 2012 and references within)". Do you mean due to the colder temperatures and increased ice cover and tundra during the LGM, that net C productivity was less than in the other warmer times periods of this study?

Yes, that was the intended meaning of that sentence. The text was revised to reflect the differences between the Holocene and the LGM C productivity measurements in Lines 399-402.

8. The final conclusion overstates the results of the study. To state that labile, microbially derived OM "were the greatest contributors to Earth's atmospheric composition throughout history" is not correct. Labile OM may have been the greatest contributor of total OM in the atmosphere over the time periods covered in this paper, but this situation may not be the case before the LGM. In addition, in this sentence it is not clear what aspect of the "Earth's atmospheric composition" that you mean.

Correct, the sentence was an overstatement. The conclusion section was revised accordingly with all changes made in the manuscript.

9. Line 16 = Define PARAFAC as this is the first time that you use this acronym.

A definition of multivariate parallel factor analysis (PARAFAC) was added to the abstract.

10. Line 48: Place "idea" after "that" in "Extending that to include".

The word "idea" was included.

11. Line 222: Remove the comma after "LGM".

This sentence was deleted upon revision.

RC3

The reviewer comments are numbered for reference. Each reply is listed below the numbered reviewer comment.

1. The first weakness of the manuscript is the use of poorly defined wording rendering difficult (sometimes obscure) the reading of the manuscript. For instance, I guess that, when saying "OM quality", you mean "fluorescent signal of the OM"? Also what is a recalcitrant OM?

Fluorescence measurements were carried out and interpreted as signatures of organic components. The chemical nature of the fluorescent fraction of the organic matter was surveyed using a fluorescent technique, thus organic matter markers is an appropriate alternative for the title and text, as recommended by Reviewer #1. In the organic matter community, the words/phrases quality, composition, and chemical nature are interchangeably used to infer the same meaning from fluorescent measurements. Definitions of the terms have been edited appropriately upon revision to clarify any confusion and improve compatibility with both the ice core and organic matter characterization communities.

2. Some abbreviates appear in the text without definition. For instance, what is the PARAFAC model that is already mentioned in the abstract, also please indicate what is the basic of this kind of model?

A definition of multivariate parallel factor (PARAFAC) analysis was edited in the abstract (Lines 16-21) and basic information was provided about the analysis in the methods section (Section 2.4).

Abstract: "Multivariate parallel factor (PARAFAC) analysis is widely used to isolate the chemical components that best describe the observed variation across three-dimensional fluorescence spectroscopy (excitation emission matrices; EEMs). Fluorescent OM markers, identified by PARAFAC modellingfrom the LGM (27.0-18,0 kyr BP; before present 1950), through the last deglaciation (LD; 18.0-11.5 kyr BP), to the early to mid-Holocene (11.5-6.0 kyr BP) provided evidence of different types of fluorescent OM chemical species in the WD ice core over 21.0 kyr."

3. The abbreviates C1, C2 and C3: I guess that they refer to component 1 etc (and not to C1 carbone chain etc).

Correct. The annotation of the abbreviation is set first in Line 21-25, and was also edited in the abstract for clarity.

"Low excitation/emission wavelength fluorescent PARAFAC component one (C1), associated with fluorescence characteristic of lignin phenols was the greatest contributor throughout the ice core, suggesting a strong signature of terrestrial OM in all climate periods. The component two (C2) OM marker, encompassed distinct variability in the LD describing chemical species similar to tannin- and phenylalanine-like material. Component three (C3), associated with humic-like terrestrial material further resistant to biodegradation, was only characteristic of the Holocene, suggesting that more complex organic polymers such as lignins or tannins may be an ecological marker of warmer climates."

4. In section 2.3, please define A254 and re-define EEMs here.

A definition of A_{254} (absorbance at 254nm) was provided in Lines 100-109. The acronym "EEMs" is defined in the Introduction section in Line 64. Section 2.3 is now edited as the following:

"Prior to fluorescence spectroscopy, absorbance spectra of WD core meltwater samples were collected from 190-1100 nm (UV-Vis spectral range) using a Genesys 10 Series (Thermo-Scientific) Spectrophotometer with a 1 cm path length cuvette and VISIONlite software. Obtaining UV-Vis absorbance spectra are necessary for the post-processing calculations of spectral corrections including primary and secondary inner filter effects (Acree et al., 1991; Tucker et al., 1992). Absorbance values at 254 nm (A₂₅₄) greater than 0.3 a.u. require dilution prior collecting the UV-Vis absorbance spectra and EEMs. WD core OM samples were optically transparent, with measured A₂₅₄ values well below 0.3 a.u. after blank correction; consequently, no sample dilution prior to UV-Vis absorbance measurements and EEMs was required (Miller and McKnight, 2010; Miller et al., 2010). Spectra were blank corrected against purified water from a Milli-Q system each day. UV-Vis absorbance spectra were subsequently incorporated into the spectral corrections calculations for post-processing the EEMs data."

5. Section 2.4: I don't understand the following sentence "A three component PARAFAC model was generated for the subset of samples by drEEM and the N-way toolbox scripts » : what is « drEEM » and N-way ?, please define.

The section has been edited appropriately in Lines 123-151.

"EEMs were prepared for multivariate parallel factor (PARAFAC) analysis following a similar procedure previously outlined for sample classification, normalization, and subset selection (Cawley et al., 2012) to model the WD fluorescent OM character. This procedure was selected after failed attempts to validate modeled results of the entire EEMs data set, due to high percentages of outlier removal, noise/scattering interference, normalization effects, and low percentages of data fitted by each component producing high percentages of residual fluorescence. Briefly, EEMs were grouped by fluorescence into separate categories (relatively subjective categorization based on resolved fluorescence patterns; i.e. protein- and humic-like, scattering, etc.), and normalized within each category group to their maximum emission intensities to reduce the compensating effects that occur when normalizing samples over greatly varying fluorescence intensities. Using a randomization selection program, 20 samples were selected from each group for the representative subset of samples (n=140) for PARAFAC analysis. PARAFAC analysis continues to be widely used to decompose EEMs into individual OM fluorescent chemical components (Bro, 1997; Stedmon et al., 2003; Murphy et al., 2013). A three component PARAFAC model was generated for the subset of samples by drEEM and the N-way toolbox scripts in MATLAB under non-negativity constraints (Stedmon and Bro, 2008; Murphy et al., 2013). The three component model was validated by split half analysis with all of the components in the split models tests finding a match with a Tucker correlation coefficient > 0.95 (Murphy et al., 2013). The core consistency value was 97%, which was within the acceptable range suggested for robust PARAFAC modelling. Two and four component models were attempted, with a validation of the two component model, but a considerably lower core consistency value for the four component model. PARAFAC analysis beyond three components produced additional modelled results of noise, thus we were unable to validate a four component model. Therefore, the three component model was selected to best represent the entire data set and was used for further interpretation of our results.

To investigate how the PARAFAC model components would potentially shift based on climate periods, three separate PARAFAC models (LGM, LD, and Holocene) were also tested, which produced somewhat redundant results (specifically for components one and three; C1 and C3) to our three component PARAFAC model of the entire data set. With large groupings of outliers varying over different climate periods, these separate models were not appropriate tools to analyze statistical changes in all of fluorescing components over time. However, the variation of the fluorescing regions comprising PARAFAC component two (C2) from the LGM to the Holocene were captured by this method, thus those results are presented as qualitative comparative complements to the original model."

6. Concerning units: Line 98 : what is au ?

The unit description was added to the text in Lines 105-107.

"WD ice core OM samples were optically transparent, with measured A₂₅₄ values well below 0.3 absorbance units (a.u.) after blank correction; consequently, no sample dilution prior to UV-Vis absorbance measurements and EEMs was required (Miller and McKnight, 2010; Miller et al., 2010)."

7. I will avoid the use of RU for Raman unit (RU is sometimes used for relative unit). Also I am not sure that the readers of CP, specially those working on ice cores, are familiar with this Raman unit ? A few words on that would help (see also my comment on Figure 2).

Raman Units (R.U.) are the technical unit from the fluorescence instrument and are appropriate for this work. More information is provided on how this information is scaled to the intensity under the peak for MQ Water in Lines 116-119.

"Data were normalized by the area under the Raman peak of a Milli-Q water sample each day at Ex = 350 nm and Em = 365-450 nm (e.g., maximum value 3.33877×10^5 counts per second at 396 nm) based on previously reported ranges (Lawaetz and Stedmon, 2009)."

8. Introduction, first paragraph (lines 31-446): This paragraph can be improved significantly, for both the wording and the cited references. Two of your co-authors have a nice expertise on the chemistry of ice cores, they certainly can also help here. From my side I would suggest to start with an overall sentence: "In addition to its water stable isotope content that provides a proxy record of past temperature (see Dansgaard et al. (1993), for instance), ice archives atmospheric information on trace gases like CO2 and CH4 encapsulated in air bubbles and chemical species trapped in the ice lattice. Numerous inorganic species trapped in ice has been used to reconstruct

past chemical composition of the atmosphere, its recent change in response to growing human activities as well its past natural variability (see Legrand and Mayewski for a review)."

We appreciate the suggested wording changes and have revised the text accordingly.

9. I here agree with another reviewer of the manuscript that the Nature paper from Wolff and coworkers (2006) is an excellent example that you have to mention of what was done on deep Antarctic ice cores in terms of changing sea-ice dust emission and marine biological productivity over the 8 climatic cycles. Then focus on what was done on organics saying "In contrast, as reviewed by Legrand et al. (2013), information on the load and composition of the organic matter archived in ice are still very limited."

Indeed, this reference and suggested text was incorporated into the revised manuscript.

10. I think you can find in this review paper relevant references that can be useful for your introduction. In particular, I suggest to report the work from Amanda Grannas made of the nature of OM in polar ice and those done on the HULIS like content of ice.

Grannas' work involved sampling snow events to test OM photoreactivity, therefore, modern events, but at some point in time, so were the WD ice core samples. Our focus is on the organic matter signatures in deep ice, thus this particular reference was not included as a separate discussion point.

11. Section 2.1.: line 75: WD is not at all the first Antarctic ice record available for comparison with Greenland records. Please modify the text.

This sentence was deleted upon revision.

12. Section 2.2: line 86: what is the difference between cracks and fractures?

The text has been revised to include such definitions in Lines 90-92. "The quality of the ice cores was excellent, well below the brittle ice zone without cracks (a section of ice containing a break in continuity, and fractures (a section broken completely)."

13. Section 2.5: Please write a few sentences explaining why your choice was to show these inorganic species. Note that, as far as I know (and checking your fig 4), I see no reason to use three species (Mn, Sr, and Ca) for dust (except if you have in mind to discuss the ratio between the 3 in view to eventually highlight the source region, which seems not to be the case).

The manuscript has been revised to report nssCa as our dust marker for this work.

14. Figure 2: Are there any possibility of estimate from the Raman values how much is the concentration of OM? Indeed, given the scarcity of data on organics, even an order of magnitude would be welcome here. From that and using a typical conversion factor OM/C you can estimate the TOC or DOC content of ice. Also I am surprised that the spikes shown in the fluorescence intensity during the LGM are not more commented in the text.

Regarding the Holocene peak, this information was removed upon revision of the manuscript. An estimation of concentration based on the Raman values would be considerably guessing based on our raw data without filtration and therefore was not included in this work.

15. Line 184-195: I assume that "Humic-like fluorescent OM" corresponds to Humic like substances observed in the atmosphere of many regions. If correct, did you consider these species as primary emitted (with soil particles for instance) or secondary produced from oxidation of gaseous organic precursors emitted by the continental biosphere (vegetation)?

Unfortunately, our methodological limitations prevent us from differentiating the two categories, but an acknowledgement to that point was added in the text in Lines 220-223: "Humic-like material encompasses both OM produced by soils and from the oxidation of gaseous organic precursors emitted by the continental biosphere (signatures of vegetation) (Coble, 1996). Unfortunately, we cannot determine the absolute origin by fluorescence spectroscopy, thus can only consider the source of C3 to be a terrestrial soil/plant signature."

16. Section 4.2: Your discussion on change of dust tracers is quite oversimplified and I would recommend you to revisit previous works done on this topic.

This section will undergo considerable revisions to highlight the usage of the dust tracers to supplement the results of the WD OM characterization work by fluorescence spectroscopy. We used this information to better understand the different environmental changes over the three climate periods.

17. Lines 255-265: This discussion is from my point of view rather confusing. It is incorrect to say that nssS concentrations are used to trace back volcanic eruptions. Only the narrow peaks of nssS are related to volcanic eruptions whereas the background nssS level in Antarctica originates in marine biogenic emissions (please revisit here the paper from Wolff et al., 2006 for instance). Also, I don't think that the wording of the following sentence makes sense "Therefore, volcanic eruptions increase the potential for particles and chemicals to be transported to polar regions and deposited onto ice- sheets." Please modify.

This section was purely speculative and without any experimental results connecting the OM character with volcanic activity, this section was removed from the manuscript upon revision.

18. Supplementary material: Following your line 201 on a correlation between DOC and nssCa, I checked the S2 figure (extracted below) that strongly bothers me. Indeed, if the DOC unit you report is correct, DOC levels of this Antarctic ice are as high as 200 μ M. If I am right that means 12*200 μ g L-1 i.e. 2400 ppbC. If correct, please comment with respect to the review of Legrand et al. (CP, 2013). It is very likely that you have a large DOC contamination in this shallow WD core. Also, sorry but I don't see a good correlation in this figure between dust and DOC!!! Please comment.

Correct. The shallow WD core DOC concentration numbers are a cause for concern. We cannot validate how this data could have resulted from contamination or is a reflection of surface DOC

concentrations and processes. Therefore, this section was removed the Supplemental Information. Upon revising the manuscript, using only nssCa for a discussion on the dust concentration changes with climate, these Supplemental figures are no longer relevant.

SC1

The short comments are numbered for reference. Each reply is listed below the numbered comment.

1. Line 88. When I have investigated the use of 'septa sealed vials', I find a contaminant fluorescent signal coming from the septa, which in my tests has always been fluorescent. Can the authors confirm that their septa sealed amber glass vials produced zero fluorescence blanks?

This is a good point. In response, we designed a laboratory blank experiment with our glass amber vials, MQ Water, and punctured septa lids, all stored in the refrigerator in the dark at 4 °C for 6 weeks. The fluorescence spectra for our daily blank water (freshly dispensed MQ Water) and the laboratory blanks were collected to investigate any contaminating fluorescence. The EEMs figure is provided below for your convenience as an example of the blank experiment fluorescent results (Fluorescence Intensity is provided on the z-axis in Raman Units):



While relatively low fluorescence was detected, we still wanted to query our dataset and subsequent PARAFAC modeling to make sure this signature was not affecting our modeled results and further interpretation of the WAIS Divide ice core OM. Fluorescence from this laboratory blank was subtracted from all EEMs prior to all PARAFAC models presented in the revised work. Two important aspects were determined from this experiment: a) The fluorescent signal in the EEM above were not determined to be consistent across all WD samples. We obtained plenty of EEMs without fluorescence in this region, thus cannot determine if the septa-lids produce consistent systematic fluorescent signatures for our project. The inconsistent nature of this potential contaminant fluorescence signal most probably contributed to the fact that it was not modelled as a separate component in our original PARAFAC modeling efforts. Extra care was taken for the fluorescent categorization of the separate bins for the original model, including this fluorescent "contaminant" feature, but was subsequently not modeled in the results. Evidence of the robustness of the models is seen not only in Figure 2, but also in Supplement Figure 3, where the clearly resolved fluorophores share no overlapping regions of the contaminant signature.

b) Even with the contaminant fluorescence subtracted from our dataset, our results did not change, strengthening the robust nature of all our PARAFAC models capturing organic matter fluorescence from our ice core samples only. We very confidently present our revised PARAFAC models in Figures 2 and 3, and also Supplement Figure 3.

We also revised our Supplement Figure 1 to incorporate the raw EEMs examples with the new blank subtraction from the septa-lid blank experiment, and provided examples of fluorescent OM from every climate period. New Supplement Figure 1 and caption:



Figure 1: Examples of West Antarctic Ice Sheet Divide ice core Excitation Emission Matrices (EEMs) showing low excitation/emission wavelength organic matter (OM) fluorescence from a) the Last Glacial Maximum ice (26.221 kyr BP; before present 1950) and b) the deglaciation ice (12.650 kyr BP 1950), and c) both lower and higher excitation/emission wavelength fluorescent OM from the Holocene (9.401 kyr BP 1950; dating scale WDC06A-7) (WAIS Divide Project Members, 2013)). Fluorescence intensities are reported on the z axis in Raman Units (R.U.). Note: All examples were post-processed for septa-lid blank subtraction, and Raman and Rayleigh-Tyndall scattering effects.

Lastly, we edited the text to clarify Teflon septa sealed amber glass vials, and methodological blank subtraction procedures to incorporate this result.

2. Line 89-90. Following on from my previous comment, were the blanks run just on the melting system, or the melting system and amber glass vials? It is not clear at present.

Blanks were run through the melting system. Blanks were not collected into the discrete sample vials individually, however a representative EEM is presented above that tests blank MQ Water in our sealed combusted amber vials. The clarification on the melting system can be found in Lines 92-97.

"Meltwater (7.5 mL for each sample) from the inner most section of the ice cores was directed to a discrete sample collector (Gilson 223), and dispensed into pre-fired (425 °C for 4 h) Teflon lined septa sealed amber glass vials, maintained at 4 °C to minimize volatilization and atmospheric exposure. Deionized water blanks were routinely analyzed through the melter head to ensure that the melting system remained contaminant free. A Milli-Q water blank was also collected in the sealed amber glass vials for fluorescence analysis due to potential septa lid contamination. Any resulting fluorescence was subtracted from all EEMs prior to further analyses."

Blanks were also run through the melting system into a targeted ultraviolet biological sensor (TUBS) spectrofluorometer, which uses an excitation wavelength of 224nm and collects emission from 280-400nm. All readings of blanks through this unit showed no fluorescence within the 280-400nm emission range.

3. Line 97. What was the actual absorbance values? These should be plotted as a time series, as A254 is used as a surrogate for DOC in terrestrial systems. It would be interesting for the reader to see this data and for the authors to compare values to other terrestrial systems (e.g. rivers, groundwaters).

All absorbance values measured at 254nm were measured below the MQ Water blank run on each day, therefore no values can be used to interrogate the quantity of DOC.

4. Line 106-107. Were the data also processed to remove Rayleigh-Tyndall scatter? How were the Raman and Rayleigh-Tyndall scatter lines processed? Were they replaced by zeros, by NaN (not a number) or was data interpolated? All of these effects can have subtle influence on the resultant PARAFAC model, so it is good to report them.

The EEMS were post-processed to remove the Rayleigh-Tyndall scattering using a MATLAB script of smootheem.m in drEEM version 0.3.0; Murphy et al. 2014. The text was edited and a reference was added to provide more information on smoothing technique used to remove each scattering effect in Lines 119-122.

"Post-processing of the fluorescence data was completed in MATLAB to generate 3D EEMs, which included sample corrections for our specific septa-lid/vial blank subtraction, and Raman and Rayleigh-Tyndall scattering following the smoothing procedures outlined in drEEM (Decomposition routines for Excitation Emission Matrices; v. 0.3.0) (Murphy et al., 2013)."

5. Lines 108-110. The authors must specify what they did for sample classification, normalisation and subset selection. It will be different from Cawley et al (2012), which is just one fluorescence case study, and on pulp mills, so not really very relevant to this research.

Lines 123-134 were revised to include further details on the procedure for sample classification, normalization, and subset selection prior to PARAFAC modeling.

"EEMs were prepared for multivariate parallel factor (PARAFAC) analysis following a similar procedure previously outlined for sample classification, normalization, and subset selection (Cawley et al., 2012) to model the WD fluorescent OM character. This procedure was selected after failed attempts to validate modeled results of the entire EEMs data set, due to high percentages of outlier removal, noise/scattering interference, normalization effects, and low percentages of data fitted by each component producing high percentages of residual fluorescence. Briefly, EEMs were grouped by fluorescence into separate categories (relatively subjective categorization based on resolved fluorescence patterns; i.e. protein- and humic-like, scattering, etc.), and normalized within each category group to their maximum emission

intensities to reduce the compensating effects that occur when normalizing samples over greatly varying fluorescence intensities. Using a randomization selection program, 20 samples were selected from each group for the representative subset of samples (n=140) for PARAFAC analysis. PARAFAC analysis continues to be widely used to decompose EEMs into individual OM fluorescent chemical components (Bro, 1997; Stedmon et al., 2003; Murphy et al., 2013)."

6. Lines 108-110. Somewhere in this section the authors must quote the value of the standard(s) that they were using. This could be the Raman intensity of Milli-Q water at a specific wavelength, or the intensity of quinine sulphate standards run using the same instrument configuration, or an International Humic Substances Standard, or a tryptophan or tyrosine standard.

The text has been revised at Lines 116-119:

"Data were normalized by the area under the Raman peak of a Milli-Q water sample each day at Ex = 350 nm and Em = 365-450 nm (e.g., maximum value 3.33877×10^5 counts per second at 396 nm) based on previously reported ranges (Lawaetz and Stedmon, 2009)."

7. Lines 110-111. More detail is needed on the PARAFAC model, to allow the reader to assess its strength in modelling the data. It is crucial in this paper, as the PARAFAC model is the crux of the whole analysis and interpretation. 1. One would expect to see the core consistency value given. A 'passable' model could be considered have a value of >90%, and a good model a score of >99%. 2. It would be very informative to know why the authors chose a 3 component model over a 2 or 4 component model – did the 4 component model try to model noise, for example? Or did it model a plausible 4th component, but with a low core consistency. 3. The percentage of the data fitted by each component is very valuable information, especially if compared with that from a two and four component model. 4. And finally, a split-half analysis is very useful, especially if the authors perform a split half analysis using randomly split datasets and a split half analysis fails on the latter test, then it tells you that the LGM and Holocene need different PARAFAC models.

The text has been edited to reflect all pertinent information supporting our three component PARAFAC model in Lines134-151.

"A three component PARAFAC model was generated for the subset of samples by drEEM and the N-way toolbox scripts in MATLAB under non-negativity constraints (Stedmon and Bro, 2008; Murphy et al., 2013). The three component model was validated by split half analysis with all of the components in the split models tests finding a match with a Tucker correlation coefficient > 0.95 (Murphy et al., 2013). The core consistency value was 97%, which was within the acceptable range suggested for robust PARAFAC modelling. Two and four component models were attempted, with a validation of the two component model, but a considerably lower core consistency value for the four component model. PARAFAC analysis beyond three components produced additional modelled results of noise, thus we were unable to validate a four component model. Therefore, the three component model was selected to best represent the entire data set and was used for further interpretation of our results.

To investigate how the PARAFAC model components would potentially shift based on climate periods, three separate PARAFAC models (LGM, LD, and Holocene) were also tested,

which produced somewhat redundant results (specifically for components one and three; C1 and C3) to our three component PARAFAC model of the entire data set. With large groupings of outliers varying over different climate periods, these separate models were not appropriate tools to analyze statistical changes in all of fluorescing components over time. However, the variation of the fluorescing regions comprising PARAFAC component two (C2) from the LGM to the Holocene were captured by this method, thus those results are presented as qualitative comparative complements to the original model."

8. Line 126-127. Amino-acid like fluorescence is too general. Only tryptophan and tyrosine have aromatic groups which fluoresce, and even then, without independent amino acid analyses to confirm their presence, one can never be sure that these compounds are responsible for the fluorescence. If the fluorescence is from an amino acids source, then C1 and C2 look most like a 'tryrosine-like' compound. Tyrosine would excite at both ~225 nm and ~275 nm and emit at about 310 nm. But the molecular structure is such that you must observe both the 225 and 275 nm excitation of the 310 nm emission, not just one or the other, as you show in Figure 3. Supplemental Figure 1 confirms the absence of a ~275 nm excitation peak. Therefore C1 and C2 are not 'tyrosine-like' or 'tryptophan-like'. Model compounds and contaminants that exhibit a single peak in this general region include simple phenols such as cresol (see Aiken, 2014 in Coble et al. (eds) Aquatic Organic Matter Fluorescence), PAHs such as fluorene (Ferretto et al 2014, DOI:10.1016/j.chemosphere.2013.12.087) and aviation fuel (see Baker et al. 2014, Encyc. Anal. Chem. DOI: 10.1002/9780470027318.a9412).

Lines 167-173 have been edited in the text to reflect our reinterpretations and these suggestions. "All samples contained low Ex/Em wavelength (240-270 nm / 300-350 nm) fluorescence characteristic of more easily altered material by microorganisms, representing fluorescent OM markers potentially of proteinaceous (Coble et al., 1990; Coble et al., 1998), polycyclic aromatic hydrocarbons (PAHs) (Ferretto et al., 2014), and simple phenols, tannins, or monolignol (Coble, 2014) origin. Fewer samples (2.5%) contained OM fluorescence at higher Ex/Em wavelengths (240-250 nm / 340-530 nm), characteristic of more humic-like, markers of terrestrial plant/soil origin (Coble et al., 1990). Examples of low and high Ex/Em wavelength fluorescence can be seen in Supplement Figure 1a-b." Further discussion of these materials can be found in subsequent text and in the Discussion section.

9. Line 128. The reference to 'recalcitrant species' is speculative. It would be better to specify the excitation and emission wavelengths of this peak or peaks. I am not aware of fluorescence in this region being recalcitrant – instead bio- and photo- degradation studies show that it is degradable (for example, Osburn et al and Stedmon and Cory, both in Coble et al 2014).

The text has been edited accordingly in Lines 223-226.

10. Line 129 and Figure 2. There is almost no meaning in 'total OM fluorescence intensities'. Each fluorophore has a different fluorescence efficiency. For example, in this study, you identify three fluorescent components, but each will have a different amount of emitted fluorescence per g C present. So, summing the three is meaningless. It is particularly relevant as low molecular weight compounds such as tryptophan-like and tyrosine-like compounds (argued to be C1 and C2 here) have less chance of their emitted fluorescence being reabsorbed within the molecule,

and they therefore have relatively high fluorescence efficiency. In contract, fulvic-like compounds (arguably C3 here) can reabsorb their emitted fluorescence, resulting in a much lower fluorescence efficiency. Figure 2 is therefore just meaningless and instead each PARAFAC component score (C1, C2, C3) needs to be presented.

This is a good point, however, this figure was created initially to provide a complete record of OM information that tracks relative fluorescent changes of the samples with depth. Removing this figure removes a complete record of all our samples. Using a subset of the samples to build a PARAFAC model created a limitation in the way we can present the depth profile. With our current PARAFAC model, samples were selected as a representative OM character subset of the entire record, not specifically organized to balance how many samples were included in each climate period. It was more informative to categorize the OM chemical fluorescence into specific groups prior to modeling, rather than to group the climate periods, however both methods were tested. With statistical outlier testing, it was very challenging to keep a balanced data set in each climate period, thus the most informative results were produced from the categorized subset PARAFAC model. To investigate how the PARAFAC model would shift based on climate periods, three separate supplemental PARAFAC models were generated, which produced somewhat redundant results to our original PARAFAC model, and again had large groupings of outliers in some climate periods. However, the changes in PARAFAC component 2 over time were captured using this method, thus added to this work in Figure 3. The supplemental PARAFAC models and loading scores are presented in the Supplement section as Figures 3 and 4. The results of the original and supplemental models were revised and two new figures (Figures 2 and 3) in the main text were generated to more accurately represent our data set and interpretation.

Edited text regarding fluorescent component one (C1) in Lines 180-196:

"Three OM PARAFAC components were identified from the WD EEMs (fluorescing regions shown in Figure 2a, and Ex/Em wavelength loading scores shown in Supplemental Figure 2). PARAFAC component one (C1; Figure 2a, top) showed maximum fluorescence in a region analogous to the secondary fluorescence of fluorophore peak B (tyrosine-like, Ex: 240 nm and Em: 300 nm), typically associated with microbial processing in aquatic environments (Coble et al., 1990; Coble et al., 1998). Regions of fluorescence at such Ex/Em wavelengths are commonly referred to as "protein-like" but overlap with fluorescence of other origins (Coble, 2014). However, without the primary region of fluorescence associated with fluorophore peak B (tyrosine-like) at higher Ex/Em wavelengths, the OM fluorescent marker of C1 cannot be determined to be tyrosine-like material of microbial origin by this method. Rather, OM with similar Ex/Em wavelength fluorescence has been documented for simple phenols (e.g., tannins and monolignols) commonly detected in natural waters (Coble, 2014). Simple phenolic OM is characteristically lower in molecular weight, aromaticity, and is considered to be more easily altered in the environment, as compared to more humic-like material (Coble, 2014). Thus, we report the chemical composition of WD OM in C1 to be most similar to monolignol chemical species, ubiquitously found in the environment as the precursors to lignin material detected in vascular plants. Once thought to be generated in the environment from tyrosine, the biosynthesis of monolignols actually originates from phenylalanine via multiple enzymatic reactions, therefore sharing protein-like origin, but ultimately is chemically linked to vascular plants as a fluorescent OM marker (Wang et al., 2013)."

Edited text regarding PARAFAC component two (C2) in Lines 197-212:

"PARAFAC component two (C2; Figure 2a, middle) contained maximum fluorescence at low Ex/Em wavelengths (260-270 nm / 310-320 nm) in regions analogous to the primary fluorescence of fluorophore peak B, and cresol (methylphenol), commonly known as the building blocks of tannins (Kraus et al., 2003), the major components of soil and aquatic humic OM (Tipping, 1986). Secondary fluorescence commonly detected for fluorophore peak B (tyrosine-like) was not observed for C2, and the combination of fluorescence from C1 and C2 do not yield the appropriate primary and secondary fluorescent trends commonly associated with tyrosine-like OM. Therefore, by this method, PARAFAC identified two distinct components, that may have protein-like similarities, but cannot be inherently linked to amino acid-like material and microbial origin. Thus, we determined that C2 fluorescence was characteristic of a combination of protein-like and tannin-like OM markers based on the regions of overlapping fluorescence by this method. Similarly to the chemical species reported for C1, the low Ex/Em wavelength fluorescence of C2 indicates OM markers with lower molecular weights, aromaticity, and chemical species that are more easily degraded in the environment by microorganisms (Coble, 2014)."

The specific characterization of PARAFAC component three (C3) has been revised in Lines 213-226 to:

"Component three (C3) displayed fluorescence commonly associated with more humic-like material. Two humic-like fluorescing regions were identified that comprised C3: fluorescence at 1) Ex/Em: 240-260/380-460 nm, characteristic of fluorophore peak A, and 2) Ex/Em: 300-320/380-460 nm, characteristic of fluorophore peak C, commonly associated with terrestrial plant and/or soil origin (Coble, 1996; Marhaba et al., 2000). Fluorescent OM markers in this region is linked with chemical species having higher molecular weights aromatic nature, and are considered to be less easily altered by biodegradation in the environment as compared to more labile material (Coble et al., 1990; Cory and McKnight, 2005; Murphy et al., 2008; Balcarczyk et al., 2009; Fellman et al., 2010). While commonly referred to as the "more recalcitrant" fraction of fluorescent OM, studies have shown that terrestrial humic-like material is susceptible to photodegradation, therefore should not be considered as an unalterable fraction of OM (Osburn et al., 2001; Stedmon et al., 2007)."

11. Line 134 and Figure 3. The PARAFAC scores for C1, C2 and C3 need to be presented in Figure 3. At the moment, no raw data from the PARAFAC model is presented in the paper, yet this is the main focus. The reader has no way of seeing the data and judging its nature e.g. variability over time. Just drawing some PARAFAC model EEMs over an x-y plot would be unacceptable to the fluorescent organic matter research community.

The model scores are presented in Supplement Figure 2. New figure and caption:



Figure S2: PARAFAC loading scores for excitation (ex) and emission (em) wavelength fluorescence for the three component model of the 21.0 kry record of West Antarctic Ice Sheet Divide organic matter. Results of the PARAFAC model are displayed as a function of the individual fluorescing components, a) component one (C1), b) component two (C2), and c) component three (C3), for the six split half categories annotated in the legend.

Supplemental PARAFAC models (three models depicting fluorescence in different climate periods) and loadings are also presented as Supplement Figures 3 and 4.

12. Line 134-136. This observation needs quantification (see comment above).

This information is now included as Figure 2a-b. New Figure and caption:



Figure 2. PARAFAC analysis results for West Antarctic Ice Sheet Divide ice core organic matter showing a) components one, two, and three (C1, C2, and C3), and b) the fluorescence percentage of each component contributing to the overall fluorescence signature over the Last Glacial Maximum (LGM), last deglaciation (LD), and Holocene climate periods as a function of time (kyr before present 1950). Average fluorescence percentages (gray dashed lines) are provided for each component, separately calculated for each climate period. Fluorescent data were reported in Raman Units. Note: C3 average fluorescence percentages are considerably lower in the LGM and LD, and did not correspond to resolved fluorophores.

13. Line 137-139. As in my earlier comment, you cannot have just one of the two excitation peaks that 'tyrosine-like' compounds excite at, and then call it 'tyrosine-like'.

This was addressed in the new fluorescence results section (see above revised text, responses, and line numbers for your reference).

14. Line 139-145. There is most fluorescence at 310 nm, so this is not 'tryptophan-like' at all, as this would also have a peak at 350 nm. More fundamentally, there is a line through the EEM at 310nm which cannot be real. Is this an artefact of the design process of Figure 2, or is it in the actual PARAFAC model? If the latter, it means the model is not correctly modelling the data. Is there anything instrumental e.g. physical filters that change over at 310 nm that could be the cause of this artefact? Is it still present in the 2 component model?

See above sections for our revisions regarding protein-like fluorescence. Yes, the feature was present in the 2 component model and can be clearly seen as a result of the data set in the loading scores (Supplement Figure 2 for the emission spectra). The best explanation of this feature is that PARAFAC is doing a great job modelling the EEMs it was given. We acknowledge that less resolved fluorophores or more "challenging" qualitative fluorescence data also is modeled in PARAFAC, if the samples were not reported as outliers. As it appears to be a "line through the component" for PARAFAC C2, we attempted to remove the emission scans at 310nm for all excitation wavelengths, and subsequently smooth the data. However, this feature was not characteristic of one emission wavelength across all excitation wavelengths, and removing more emission wavelengths to compensate will compromise our results. Thus we reported the PARAFAC modelled component as it exists from our analysis and discuss it accordingly. We report this "unusual" feature in the results section (Lines 208-212) and discuss the potential sources for the variation in further detail in the subsequent discussion section (Lines 364-373).

15. Line 142. If you performed a single PARAFAC model, then the location of the modelled fluorescence can't change over time. So how can the location of the peak 'move' from LGM to Holocene? Is this from extra PARAFAC analyses that the reader doesn't know about? Or is it a subjective analysis of the original EEMs?

An extra PARAFAC analysis was performed and the manuscript was revised to include this information (see methods, results, and discussion sections, along with Supplement Figures 3 and 4).

16. Line 145-150. I would disagree with this interpretation. This fluorescence is typical of 'peak A' and 'peak C' compounds. A peak 'M' fluorescence would be blue shifted compared to 'peak A', and in your component C3 there are two peaks and they both have the same emission wavelengths.

The manuscript was revised to correct for the correct interpretation of peaks A and C, result reporting, and discussion of Holocene OM chemical fluorescence.

17. Lines 153-155. The fact that no one else has reported your fluorescence peaks is either very exciting or very worrying. It would suggest that what you are seeing is not anything that has been reported before e.g. you are not seeing 'tyrosine-like' fluorescence, and by implication, you can't definitively interpret it as a microbial signal.

Of the data available in the OpenFluor database, a repository of a selection of samples (not every fluorescent study completed), our results showed no matches with other PARAFAC components. This is reasonable given the scope of the project and the great volume of samples spanning 6,000

to 27,000 years ago from ice. Yes, we agree that what we are seeing is not anything that has been reported before to this database. We also agree that your suggestion as the correct interpretation of the PARAFAC components would not distinctly be tyrosine-like or tryptophan-like, thus the interpretation of a microbial signal is not definitive. The text was edited accordingly to reflect these suggestions in Lines 227-233:

"WD ice core OM PARAFAC components were uploaded to the OpenFluor database to compare and contrast C1, C2, and C3 with other environmental OM marker studies, s, however, no component matches were determined (Murphy et al., 2014). The OpenFluor database is a repository of a selection of samples, and while still growing to encompass a thorough library of fluorescent OM markers from highly variable environments, it is reasonable to expect nonmatching results based on database queries. Our results matched no previously identified PARAFAC components uploaded to the database, which we attribute to the unique scope of this work and the great volume of samples spanning 21.0 kyr from Antarctic ice."

18. Line 160 and Figure 4. The authors state that Figure 4 shows the 'PARAFAC components', but there is just one line. What is this? Is it C1, or C2, or C3? All three components must be shown individually, here and in Figure 3.

The PARAFAC components determined in each climate period are no longer linked to this figure visually. The new Figure 4 and caption have been included in the revised manuscript:



Figure 4. Trace element concentration of (top) non-sea salt calcium (nssCa; ppb), and (bottom) the δ 18O (per mil) temperature record (Marcott et al., 2014) from the West Antarctic Ice Sheet Divide ice core as a function of time (kyr before present 1950), dating from the Last Glacial Maximum (LGM), through the last deglaciation (LD), to the mid-Holocene.

19. Line 175. C1 and C2 PARAFAC model scores need to be plotted in Figure 3. Line 184. C3 PARAFAC model scores need to be plotted in Figure 3.

Model scores are now provided in Supplement Figure 2.

20. Line 189-191. This observation is unremarkable, as all humic and fulvic substances standards have a higher fluorescence intensity at the short excitation wavelength (see examples in Aiken (2014)).

Correct. This was deleted upon revision.

21. Line 191. It sounds like you are saying that there are plants and soil in the ice? I'm sure you don't mean that?

Yes, thank you. This was an error in phrasing and was deleted upon revision.

22. Line 214. No fluorescence data over time is presented (except for the total fluorescence, which is not meaningful). So this section is speculative.

Correct. This section was revised based on the contributions of each component over time (Figure 2).

A 21,000 year record of <u>fluorescent</u> organic matter <u>markersquality</u> in the WAIS Divide ice core

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Abstract. Englacial ice contains a significant reservoir of organic material (OM), preserving a chronological record of materials from Earth's past. Here, we investigate if OM <u>composition quality</u> surveys in ice core research can provide paleoecological information on the dynamic nature of our Earth through time. Temporal trends in OM <u>compositionquality</u> from the early Holocene extending back to the Last Glacial Maximum (LGM) of the West

- 15 compositionquality from the early Holocene extending back to the Last Glacial Maximum (LGM) of the West Antarctic Ice Sheet Divide (WD) ice core were measured by fluorescence spectroscopy. <u>Multivariate parallel factor</u> (PARAFAC) analysis is widely used to isolate the chemical components that best describe the observed variation across three-dimensional fluorescence spectroscopy (excitation emission matrices; EEMs) assays. Fluorescent OM markers, identified by intensity fluctuations and PARAFAC modelling -of the EEMs of fluorescent OM from the
- 20 LGM (27.0-18,0 kyrs BP; before present 1950), through the last deglaciation (LD; 18.0-11.5 kyrs BP), to the early to-mid-Holocene (11.5-6.0 kyrs BP) provided evidence of different types of fluorescent_OM composition and originchemical species in the WD ice core over 21.0 kyrs. Low excitation/emission wavelength fluorescent_Two proteinaceous-PARAFAC components one (C1)(C1 and C2), associated with chemical species similar to simple lignin phenols was the greatest contributorere- throughout the ice core, characteristic of fluorescent_OM prevailing in
- 25 all climate periods, suggesting a strong signature of <u>terrestrial labile microbial-OM in all climate periods</u>. <u>The</u> component two (C2) OM marker, encompassed distinct variability in the LD describing chemical species similar to <u>tannin- and phenylalanine-like material</u>. <u>Component three A humic-like component (C3)</u>, <u>associated with</u> <u>characteristic of humic-like terrestrial material further resistant to biodegradationterrestrial and marine OM</u> <u>fluorescence</u>, was only characteristic observed during of the Holocene, suggesting that more complex organic
- 30 polymers such as lignins or tannins recalcitrant OM-may be an ecological marker of warmer climates. Fluctuations in WD ice core OM fluorescence over 21.0 kyrs BP may be driven by environmental changes at the source, and potentially its interaction with the atmosphere. We suggest that fluorescent OM <u>markers signatures</u> observed during the LGM were the result of greater continental dust loading of <u>microbially derived proteinaceouslignin precursor</u> (monolignol)-material in a drier climate, with lower marine influences when sea ice extent was higher, and
- 35 continents had more expansive tundra cover. As the climate warmed, the <u>record of OM markers quality record</u> in the WD ice core changed, reflecting shifts in carbon productivity as a result of global ecosystem response.

1 Introduction

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In addition to its water stable isotope content that provides a proxy record of past temperatures (Dansgaard et al.,

- 40 1993), ice archives atmospheric information on trace gases like CO₂ and CH₄ encapsulated in air bubbles and chemical species trapped in the ice lattice. Numerous inorganic species trapped in ice has been used to reconstruct past chemical compositions of the atmosphere, its recent change in response to growing human activities as well its past natural variabilityIce cores are a powerful tool for reconstructing the timing and extent of past changes in Earth's climate, providing records of biological, chemical, and physical constituents over time (Legrand and
- 45 Mayewski, 1997; Petit et al., 1999; Johnsen et al., 2001; Alley, 2002; Wolff et al., 2006; Jansen et al., 2007; Luthi et al., 2008). Detailed paleorecords of temperature, precipitation, dissolved chemicals, and gas composition of the lower atmosphere, volcanic eruptions, solar variability, sea surface productivity, anthropogenic emissions, and a variety of other climate and biogeochemical indicators can all be determined from ice core studies, providing an invaluable record of our past with implications for the future. In contrast, as reviewed by Legrand et al. (2013).
- 50 <u>information on the load and composition of the organic matter (OM) archived in deep ice are still very limited</u> (Legrand et al., 2013).

In addition to the extraordinary range of materials catalogued within ice cores, ice-Ice also provides a unique environment for preserving microbes and other biological material (i.e. plant fragments, seeds, pollen grains, fungal spores, and organic matter [OM]) (Priscu et al., 2007; Miteva et al., 2009; Barletta et al., 2012; WAIS Divide

55 Project Members, 2015). OM, comprised of biomolecules from living and decaying organisms, and also input from surrounding environments, plays a significant role in aquatic ecosystems affecting many biogeochemical processes that, in turn, influence its contribution to the global carbon cycle (Battin et al., 2008). Englacial OM may also contribute to global carbon dynamics upon its decomposition after being released from the ice lattice by melting and retreat (Priscu and Christner, 2004; Priscu et al., 2008). Therefore, as a reservoir of <u>chronologically preserved</u> OM and a potential source of carbon and biological material, determining ice core OM <u>composition quality (chemical character)</u> and reactive nature is essential to understanding past carbon signatures that could impact our future.

Ice core studies rely on the paradigm that atmospheric deposition is the sole mechanism for specific gases and materials to become trapped in the ice. Extending that <u>idea</u> to include OM, the trapped material becomes a catalog of preserved paleoecological markers of Earth's <u>history</u>, <u>thathistory</u>, <u>which</u> can be used to better understand biogeochemical processing, carbon stocks, and cycling events. While still a novel addition to deep ice core research, chemically characterizing the OM <u>markers (i.e. composition and chemical species) constituents inof</u> englacial ice is of particular interest for several reasons: 1) OM <u>markers character</u> can be linked to its source (e.g., aquatic, terrestrial) describing different influences of past and present ecosystems, 2) OM <u>markers character</u> can serve as a proxy for englacial biological activity from *in situ* production, potentially explaining anomalous concentrations of

70 other gases (e.g., methane, carbon dioxide) in ice core research, and 3) OM could be a pivotal contributor to the global carbon cycle if materials released to surrounding environments are metabolized to CO_2 -greenhouse gasses (e.g., carbon dioxide and methane) in a warming climate.

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We hypothesize that Antarctic englacial ice contains a chronological record of OM<u>markers</u> that reflects changes following the Last Glacial Maximum (LGM). To test this hypothesis we used fluorescence spectroscopy to generate Excitation Emission Matrices (EEMs), a bulk analytical method commonly used to probe OM source and

nature in aquatic ecosystems. Fluorescence spectroscopy is advantageous to employ for a large sample set with low sample volumes due to rapid data acquisition and the wealth of information generated describing OM fluorescing <u>componentsmarkers</u>, chemical character, and source influences (Coble et al., 1990; Coble, 1996; Stedmon and Cory, 2014). For this study, all possible sources of OM <u>markers</u> detected within the West Antarctic Ice Sheet (WAIS)

80 Divide ice cores were considered, however we can only speculate on the possibility of *in situ* OM production due to methodological limitations. A total of 1,191 meltwater samples of limited volume (~7.5 mL) were examined from 1400 m of deep ice, corresponding to 21.0 kyrs, extending from the LGM, through the last deglaciation (LD), to the mid-Holocene. This is the first high temporal resolution analysis of englacial OM markers by fluorescence spectroscopy from the Antarctic ice sheet.

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2 Methods

2.1 WAIS Divide sample site description

Ice cores were collected as a part of the multidisciplinary WAISCORES project at 79.467 °S and 112.085 °W, Antarctica (Figure 1)₇ (WAIS Divide Project Members, 2013, 2015). Snow precipitation at this site is relatively high with an average annual accumulation rate 0.207m_{weg} a⁻¹ (Banta et al., 2008) (-22 cm y⁻¹), compared to other Antarctic locations, resulting in ice cores containing a high resolution record of trapped gases, chemicals, and biotic and abiotic constituents over the last 65,000 years (dating scale WDC06A-7 (WAIS Divide Project Members, 2013))₂. The WAIS Divide (WD) ice core represents the first continuous Southern Hemisphere equivalent paleoclimate record to the Greenland ice core GISP2, GRIP, and North GRIP projects.

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2.2 Ice core collection, preparation, and melting

Ice core drilling and recovery was completed in 2012 to a depth of 3405 m, using a hydrocarbon-based drilling solvent-fluid (Isopar-K; ExxonMobil Chemicals). Ice cores were transported to the National Ice Core Laboratory (NICL) in Denver, Colorado, for ice core processing. For this project, 1400 m of ice cores (depths: 1300 – 2700 m below the surface) dating from 6.0 to 27.0 kyrs BP [before present 1950] by the WDC06A-7 timescale (WAIS Divide Project Members, 2013)), were cut into 3x3x100 cm long sections and transferred to the Desert Research Institute (DRI) in Reno, Nevada, for continuous melting (4.5 mL min⁻¹) in a closed continuous flow analysis (CFA) system (McConnell et al., 2002; McConnell et al., 2007; McConnell et al., 2014). The quality of the ice cores was excellent, well below the brittle ice zone without cracks (a section of ice containing a break in continuity, but still intact), and fractures (a section broken completely). eracks or fractures.

Meltwater (7.5 mL for each sample) from the inner most section of the ice cores was directed to a discrete sample collector (Gilson 223), and dispensed into <u>combusted-pre-fired</u> (425 °C for 4 h) <u>Teflon lined</u> septa sealed amber glass vials, maintained at 4 °C to minimize volatilization and atmospheric exposure. -Deionized water blanks were routinely analyzed <u>through the CFA system</u> to ensure <u>a that the melting system remained</u> contaminant free

110 <u>environment</u>. -<u>A Milli-Q water blank was also collected in the sealed amber glass vials for fluorescence analysis due to potential septa lid contamination. Any resulting fluorescence was subtracted from all EEMs prior to further analyses.</u>

115 **2.3 Ice core OM absorbance**

Prior to fluorescence spectroscopy, absorbance spectra of WD core meltwater samples were collected from 190-1100 nm (UV-Vis spectral range) using a Genesys 10 Series (Thermo-Scientific) Spectrophotometer with a 1 cm path length cuvette and VISIONlite software. Obtaining UV-Vis absorbance spectra areis necessary for the postprocessing calculations of to calculate the spectral corrections including of primary and secondary inner filter effects

- 120 when post processing EEMs data (Acree et al., 1991; Tucker et al., 1992). Absorbance values at 254 nm (A₂₅₄) greater than 0.3 a.u. require dilution prior collecting the UV-Vis absorbance spectra and EEMs. WD ice core OM samples were optically transparentdilute, with measured A₂₅₄ values well below 0.3 absorbance units (a.u.) after blank correction; consequently, no sample dilution prior to UV-Vis absorbance measurements and EEMs was required (Miller and McKnight, 2010; Miller et al., 2010). Spectra were blank corrected against purified water from
- 125 a Milli-Q system each day. <u>UV-Vis absorbance spectra were subsequently incorporated into the spectral corrections</u> <u>calculations for post-processing the EEMs data.</u>

2.4 Fluorescence spectroscopy

- EEMs were generated on a Horiba Jobin Yvon Fluoromax-4 Spectrofluorometer equipped with a Xenon lamp light
 source and a 1 cm path length quartz cuvette. Excitation (Ex) wavelengths were scanned from 240-450 nm in 10 nm intervals and emission (Em) was recorded between 300-560 nm in 2 nm increments. Data integration time was 0.25 s and data acquisition was carried out in signal/reference mode using a 5 nm bandpass on both Ex and Em monochromators, normalizing the fluorescence Em signal with the Ex light intensity. Data were normalized by the area under the Raman peak of a Milli-Q water sample each day at Ex = 350 nm and Em = 365-450 nm (e.g.,
- 135 <u>maximum value 3.33877 x 10⁵ counts per second at 396 nm</u>) based on previously reported ranges (Lawaetz and Stedmon, 2009). Post_processing of the fluorescence data was completed in MATLAB to generate 3D EEMs, which included sample corrections for <u>our specific septa-lid/vial blank subtraction</u>, and Raman and Rayleigh-Tyndall scattering following the smoothing procedures outlined in drEEM (Decomposition routines for Excitation Emission Matrices; v. 0.3.0) (Murphy et al., 2013) and blank water subtraction.
- 140 EEMs were prepared for <u>multivariate</u> parallel factor <u>(PARAFAC)</u> <u>multivariate</u> analysis (PARAFAC) following a <u>similar</u> procedure previously outlined for sample classification, normalization, and subset selection (Cawley et al., 2012) to model the WD fluorescent OM character. <u>This procedure was selected after failed attempts</u> to validate modeled results of the entire EEMs data set, due to high percentages of outlier removal, noise/scattering interference, normalization effects, and low percentages of data fitted by each component producing high
- 145 percentages of residual fluorescence. Briefly, EEMs were grouped by fluorescence into separate categories (relatively subjective categorization based on resolved fluorescence patterns; i.e. protein- and humic-like, scattering, etc.), and normalized within each category group to their maximum Em intensities to reduce the compensating effects that occur when normalizing samples over greatly varying fluorescence intensities. Using a randomization selection program, 20 samples were selected from each group for the representative subset of samples (n=140) for

- 150 PARAFAC analysis. PARAFAC analysis continues to be widely used to decompose EEMs into individual OM fluorescent chemical components (Bro, 1997; Stedmon et al., 2003; Murphy et al., 2013). A three component PARAFAC model was generated_for the subset of samples by drEEM and the N-way toolbox scripts in MATLAB under non-negativity constraints (Stedmon and Bro, 2008; Murphy et al., 2013). The three component model was validated by split half analysis with all of the components in the split model tests finding a match with a Tucker
- 155 correlation coefficient > 0.95 (Murphy et al., 2013). The core consistency value was 97%, which was within the acceptable range suggested for robust PARAFAC modelling. Two and four component models were attempted, with a validation of the two component model, but a considerably lower core consistency value for the four component model. PARAFAC analysis beyond three components produced additional modelled results of noise, thus we were unable to validate a four component model. Therefore, the three component model was selected to best represent the entire data set and was used for further interpretation of our results.
 - To investigate how the PARAFAC model components would potentially shift based on climate periods, three separate PARAFAC models (LGM, LD, and Holocene) were also tested, which produced somewhat redundant results (specifically for components one and three; C1 and C3) to our three component PARAFAC model of the entire data set. With large groupings of outliers varying over different climate periods, these separate models were
- 165 not appropriate tools to analyze statistical changes in all of fluorescing components over time. However, the variation of the fluorescing regions comprising PARAFAC component two (C2) from the LGM to the Holocene were captured by this method, thus those results are presented as qualitative comparative complements to the original model.

170 2.5 Elemental Analysis

Meltwater from the interior section of the ice core was also used for a broad range of elemental analyses (WAIS Divide Project Members, 2013)_including sodium (Na), sulfur (S), calcium (Ca) as an indicator of continental dust, manganese (Mn), and strontium (Sr) as co-registered datasets. From the CFA system, meltwater was directed through Teflon tubing to two Inductively Coupled Plasma Mass Spectrometers (ICPMS, Element 2 Thermo

- Scientific) located in an adjacent class 100 clean room for continuous trace element analysis (McConnell et al., 2007). Sea salt sodium (ssNa) as well as non sea salt sulfur (nssS) and Non-sea salt calcium (nssCa) concentrations were calculated following standard procedures from measured total concentrations of Na, S, and Ca using abundances in sea water and mean sediment (Bowen, 1979). Concentrations of sea salt sodium (ssNa) data from the LGM through deglaciation were previously reported and referenced in this work as a sea ice proxy throughout all
- 180 <u>climate periods (WAIS Divide Project Members, 2013).</u>

3 Results

3.1 Fluorescent OM markers in the WD core

WD EEMs (1,191 samples covering 1400 m of ice core) dating from the LGM (27.0 – 18.0 kyrs ago BP) to the mid
 Holocene (11.5 – 6.0 kyrs BP) were analyzed to characterize the OM fluorescing components in ice. All samples contained low Ex/Em wavelength (240-270 nm / 300-350 nm) amino acid-like-fluorescence characteristic of more

<u>easily altered material by microorganisms, representing bioavailable fluorescent OM markers potentially of</u> <u>proteinaceous</u> (Coble et al., 1990; Coble et al., 1998), <u>polycyclic aromatic hydrocarbon (PAH)</u> (Ferretto et al., 2014), and simple phenol, tannin, or monolignol (Coble, 2014) <u>origin</u>. earbon species at low Ex/Em wavelengths

- 190 (240-270 nm / 300-350 nm), while fFewer samples (2.5%) contained OM fluorescence at higher Ex/Em wavelengths (240-250 nm / 340-530 nm².5%), characteristic of more humic-like recalcitrant-markers of terrestrial plant/soil origin (Coble et al., 1990). Examples of low and high Ex/Em wavelength fluorescence can be seen species (examples in Supplement Figure 1a-bc). The LGM was characterized by relatively high total OM fluorescence intensities (Raman Units; Figure 2), however the largest increase occurred in the early Holocene, spanning 37
- 195 discrete EEMs samples that corresponded to 0.318 kyrs between 9.738-10.056 kyrs ago BP. Since OM is a complex mixture containing a broad range of molecules inand potentially overlapping fluorescent regions, the application of PARAFAC analysis was used to resolve the representative subset of samples into individual OM fluorescing components characterized by their Ex/Em maxima. The PARAFAC fluorescing components were analyzed to describe the chemical composition of the OM fluorescent markers, and the modelled results were then further
- 200 <u>interrogated to identify the contributions of each fluorescing component in-over the three</u> different climate periods. Three WD OM PARAFAC components were identified (Figure 3a). Ice from the LGM and LD time periods contained fluorescence characterized by PARAFAC components one and two (C1 and C2), while the Holocene was characterized by fluorescence of all three components.
- 205 <u>Figure 23a, and Ex/Em wavelength loading scores shown in Supplemental Figure 2).</u> PARAFAC component <u>one</u> (C1; Figure 2a, top) showed maximum fluorescence in a region analogous to the secondary fluorescence of fluorophore peak B (tyrosine-like, Ex: 240 nm and Em: 300 nm), <u>which is</u> typically associated with microbial processing in aquatic environments (Coble et al., 1990; Coble et al., 1998). <u>Regions of fluorescence at such Ex/Em</u> wavelengths are commonly referred to as "protein-like" but overlap with fluorescence of other origins (Coble,
- 210 2014). However, without the primary region of fluorescence associated with fluorophore peak B (tyrosine-like) at higher Ex/Em wavelengths, the OM fluorescent marker of C1 cannot be determined to be tyrosine-like material of microbial origin by this method. Rather, OM with similar Ex/Em wavelength fluorescence has been documented for simple phenols (e.g., tannins and monolignols) commonly detected in natural waters (Coble, 2014). Simple phenolic OM is characteristically lower in molecular weight, aromaticity, and is considered to be more easily altered in the
- 215 <u>environment, as compared to more humic-like material (Coble, 2014). Thus, we report the chemical composition of WD OM in C1 to be most similar to monolignol chemical species, ubiquitously found in the environment as the precursors to lignin material detected in vascular plants. Once thought to be generated in the environment from tyrosine, the biosynthesis of monolignols actually originates from phenylalanine via multiple enzymatic reactions, therefore sharing protein-like origin, but ultimately is chemically linked to vascular plants as a fluorescent OM</u>
- 220 <u>marker (Wang et al., 2013).</u> <u>PARAFAC component two (C2; Figure 2a, middle) contained maximum fluorescence at low Ex/Em</u> wavelengths (260-270 nm / 310-320 nm) in regions analogous to the primary fluorescence of fluorophore peak B, and cresol (methylphenol), commonly known as the building blocks of tannins (Kraus et al., 2003) and the major

components of soil and aquatic humic OM (Tipping, 1986). Secondary fluorescence commonly detected for 225 fluorophore peak B (tyrosine-like) was not observed for C2, and the combination of fluorescence from C1 and C2 do not yield the appropriate primary and secondary fluorescent trends commonly associated with tyrosine-like OM. Therefore, by this method, PARAFAC identified two distinct components, that may have protein-like similarities, but cannot be inherently linked to amino acid-like material and microbial origin. Thus, we determined that C2 fluorescence was characteristic of a combination of protein-like and tannin-like OM markers based on the regions of 230 overlapping fluorescence by this method. Similarly to the chemical species reported for C1, the low Ex/Em wavelength fluorescence of C2 indicates OM markers with lower molecular weights, aromaticity, and chemical species that are more easily degraded in the environment by microorganisms (Coble, 2014). -also corresponded to amino acid like fluorescence, however this component displayed overlap between the tyrosine and tryptophan like fluorescent regions. Because of this overlap, C2 was further evaluated to investigate its variation as a function of 235 climate (Figure 3). Component C2 was found to be red shifted to longer Em wavelengths from the LGM to the LD, and blue shifted to shorter Em wavelengths from the LD to the Holocene ice (Figure 3b); thus explaining the overlapping fluorescence in C2 in the overall PARAFAC model (Figure 3a). We acknowledge the relatively sharp shift in fluorescence intensity ~Em 310 nm across all Ex wavelengths (Supplement Figure 2b), however could not remove this feature (Em spanning > 10nm) without compromising the integrity of the component in this model. 240 Further evaluation of the variability potentially represented in this component is discussed in Section 3.2 as a function of climate. Component three (C3; Figure 2a, bottom) displayed fluorescence commonly associated with more humiclike material. Two humic-like fluorescing regions were identified that comprised C3: fluorescence at 1) Ex/Em: 240-260/380-460 nm, characteristic of fluorophore peak A, commonly associated with terrestrial plant and/or soil origin, 245 (Coble, 1996; Marhaba et al., 2000), and 2) Ex/Em: 300-320/380-460 nm, characteristic of fluorophore peak MC, commonly associated with terrestrial plant and/or soil origin (Coble, 1996; Marhaba et al., 2000)commonly associated with marine environments (Coble, 1996). Fluorescent OM markers in this region are and also linked with chemical species having higher molecular weights, aromatic nature, and are considered to be less easily altered by biodegradation in the environment as compared to more labile material reduced aromatic chemical species 250 potentially exposed to microbial processing in aquatic environments (Coble et al., 1990; Cory and McKnight, 2005; Murphy et al., 2008; Balcarczyk et al., 2009; Fellman et al., 2010). Humic-like material encompasses both OM produced by soils and from the oxidation of gaseous organic precursors emitted by the continental biosphere (signatures of vegetation) (Coble, 1996). Unfortunately, we cannot determine the absolute soil versus plant origin by fluorescence spectroscopy, thus can only consider the source of C3 to be a terrestrial soil/plant signature. While 255 commonly referred to as the "more recalcitrant" fraction of fluorescent OM, studies have shown that terrestrial humic-like material is susceptible to photodegradation, therefore should not be considered as an unalterable fraction of OM (Osburn et al., 2001; Stedmon et al., 2007). The C3-maximum fluorescence intensities fluctuated mildly in the Holocene, but intensities were higher at lower Ex wavelengths (fluorophore peak A region). WD ice core OM PARAFAC components were uploaded to the OpenFluor database to compare and 260 contrast C1, C2, and C3 with other environmental OM marker studies, however, no component matches were

determined (Murphy et al., 2014). <u>The OpenFluor database is a repository of a selection of samples, and while still</u> growing to encompass a thorough library of fluorescent OM markers from highly variable environments, it is reasonable to expect non-matching results based on database queries. Our results matched no previously identified <u>PARAFAC components uploaded to the database, which we attribute to the unique scope of this work and the great</u> volume of samples spanning 21.0 kyr from Antarctic ice.

3.2 Fluorescent OM marker contributions as a function of climate

- The fluorescent intensity percentages of the components (OM marker contributions) varied not only by composition
 but also throughout the 21.0 kyr record (Figure 2b). Average percentages of fluorescence intensities for each component are represented as a function of climate (LGM, LD, and Holocene) as grey dashed lines in Figure 2b. C1 depicts the only OM marker with the greatest contributions for the LGM comparatively, describing a dominance of monolignol OM chemical species over time. In contrast, C2 and C3 OM marker contributions were lowest for the LGM, describing chemical species associated with these fluorescent regions being more prevalent in the
- 275 environment or more effectively transported to WD in warmer climates. Similar average percentages were reported for C2 in the LD and the Holocene (23.52 and 22.09 %), however the standard deviations for both climate periods were highly variable (17.45 and 21.49), as a result of the varying fluorescent intensities, thus no discernable trend based on the average contributions was deduced. C3 contributions were the lowest throughout all climate periods and it is important to note that even though average contributions are presented for the LGM and LD, no observed
 280 resolved fluorophores were detected, i.e. OM markers characteristic of fluorophore peaks A and C were
 - representative of the Holocene only.

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<u>Three separate PARAFAC models, categorized by climate period, were subsequently generated to identify</u> <u>imperceptible component variation potentially masked by the original model (Supplement Figures 3 and 4). For</u> these models C1 was found to be identical for all climate periods using this technique, and since C3 was only

- 285 <u>characteristic of the Holocene, comparing these models in this manner elucidated the variation in C2 over time</u> (Figure 3a-c; LGM-C2, LD-C2, and Holocene-C2). While all C2 fluorescence was situated in the same low Ex/Em wavelength region (250-300 nm / 300-350nm) for all climate periods, the breadth and maxima fluorescent regions shift, potentially describing different types of fluorescing material over time. LGM-C2 maximum fluorescence is detected at shorter Em wavelengths for the LGM (Figure 3a), compared to the LD (Figure 3b), corresponding to a
- 290 shift to OM chemical species with higher molecular weights and aromaticity, comparatively. Although quite similar, LGM-C2 and LD-C2 resulted from different fluorescing composition, suggesting each climate period contains unique material preserved in ice cores. Following the LD, Holocene-C2 is noticeably unique compared to the LD (Figure 3c), and shared overlapping regions of fluorescence with phenylalanine (Teale and Weber, 1957). A shift to shorter Em wavelengths (blue shift) is commonly associated with the opposite trend of a red shift, i.e. indicating chemical species at lower molecular weights and aromaticity. Thus our results indicate that C2 OM markers of the
- original PARAFAC model (Figure 2a) and the supplementary PARAFAC models (C2 OM markers across all

climate periods combined in Figure 3) describe chemical species of tannin-like and protein-like -origin, with more protein-like influence in the youngest ice.

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3.23 Trace element concentrations

The extent of terrestrial dust contributions to the <u>fluorescent</u> OM <u>markers</u> in the WD ice core was explored by analyzing the concentrations of elemental nssCa, <u>Mn, and Sr</u>, commonly used to reconstruct past atmospheric composition in paleoclimate research. Figure 4 shows the concentrations of nssCa, <u>Mn, and Sr</u> together with the WD ice core <u>OM PARAFAC components and the</u>-co-registered the δ¹⁸O temperature record for reference (Marcott et al., 2014). The highest concentrations of nssCa, <u>Mn, and Sr</u> were observed during the LGM (Figure 4), indicating greater dust loads to Antarctica in the older ice. The transition between the LGM and the LD was characterized by a decrease in nssCa, <u>Mn, and Sr</u> concentrations over 2.0 kyrs, followed by concentrations that, on average, remain considerably lower than reported for the LGM, results that were consistent with other Arctic and Antarctic paleodust

- 310 records from ice cores, (Albani et al., 2016). A strong positive correlation between nssCa and Sr was found throughout the WD ice core (R²=0.894), with nssCa and Mn, and Sr and Mn less strongly correlated (R²=0.516 and R²=0.478). It is important to note no direct comparisons between dust concentrations and OM qualitative markers or concentrations can be made with these data, as that was beyond the scope of this work. Rather, this information was subsequently utilized as discussion points to infer more information regarding the OM marker
 arisin datasted in the WD ice core
- 315 <u>origin detected in the WD ice core.</u>

4 Discussion

4.1 Deep englacial OM nature and origin

The worlds glaciers and ice sheets are believed to hold nearly six petagrams of carbon (Hood et al., 2015),

- 320 representing a significant component of the global carbon cycle. Ice environments function as sinks of allochthonous OM by atmospheric deposition and aeolian transport (Stubbins et al., 2012), yet our understanding of the OM source and its reactivity in these reservoirs, especially in deep ice, is in its infancy. We applied fluorescence spectroscopy to determine the climate specific differences in OM <u>markers source material</u> and reactive nature throughout time<u>history</u>. The composition and chemical origins associated with PARAFAC components C1, C2, and C3
- 325 provided a bulk level representation of the terrestrial OM markers preserved throughout 21.0 kyr and initiated the foundation for future research. The contributions of fluorescent OM markersfluorescence represented by PARAFAC components C1 were dominant throughout the LGM, LD, and the Holocene compared to C2 and C3, indicating and C2 was present in all climate periods in the WD ice core, providing a consistent record of OM with chemical species similar to monolignols over timemicrobial OM signatures throughout history. As precursors to lignin-like polymers,
- C1 OM markers in the WD ice core represent the continual presence of terrestrially produced material effectively transported to West Antarctica. Decreasing contributions of C1 OM markers with increasing contributions of C2 and C3 suggest that more complex terrestrially derived OM is a function of ecosystem changes in a warming climate. WeOf continue to acknowledge the fluorescence overlap at low Ex/Em wavelengths (C1 and C2) with regions of

fluorescence that describe "protein-like" material characteristic of microbial processes (in situ OM production and

- transformation), however cannot confirm OM microbial origin by fluorescence spectroscopy alone. the two components, OM containing tryptophan like fluorescence (C2) has been shown to represent intact dissolved proteins, considered to be freshly derived from microorganisms ._Miteva et al. (2016) reported that the presence of microorganisms in deep ice cores also suggests the possibility of *in situ* OM processing, which could have important implications for gaseous climate records (Rhodes et al., 2013; Miteva et al., 2016; Rhodes et al., 2016). Including *in*
- 340 situ biological OM transformations in ice core research was recently proposed as an alternate mechanism for CH₄ production in ice from firn layers of the WD ice core (Rhodes et al., 2016). -<u>At this juncture, our bulk level fluorescent results cannot argue that *in situ* OM production is a major contribution to the OM markers detected by EEMs in the WD ice core due to the lack of resolved tyrosine- and tryptophan-like fluorescing components. To test specifically for such signatures, further research linking microbial metabolism to available OM energy substrates in</u>
- 345 <u>ice cores is required.</u>

<u>The Hhumic-like fluorescent OM marker contributions were considerably higher in the youngest ice, was</u> not detected in the older ice from the LD and LGM periods, suggesting lower abundances produced <u>and/or</u> ineffectively interacting with the atmosphere/transport mechanisms to Antarctica in colder climates.in external environments or lack of transport to Antarctica during colder periods. Resolved humic-like fluorophores (peaks A

- 350 and C) were not detected in the older ice from the LD and LGM periods. The first appearance of resolved humic-like OM fluorophores fluorescence was reported at 11.061 kyrs old BP, 500 years after the Holocene began. The two regions of fluorescence maxima ascribed to PARAFAC component C3 describe a pairing of different types of more recalcitrant OM species produced from terrestrial and aqueous environments. Throughout the Holocene, the fluorescent maximum at Ex/Em: 240 260/380 460 nm consistently had higher fluorescent intensities than its pair at
- 355 Ex/Em: 300 320/380 460 nm, describing more fluorescent chemical species characteristic of OM derived from terrestrial plants and/or soils in the younger ice. Considering the overall characterization of OM markers with terrestrial linkages in the WD ice core With no humic like signatures detected for the LGM or the LD, we hypothesize that increasing warmer temperatures beyond the LGMduring the Holocene were associated with more expansive vegetation cover and increased production and degradation of complex_OM in terrestrial, freshwater, and marine environments.

4.2 Continental dust in the WD ice core as an indicator of OM transport

Concentrations of nssCa, Sr, and Mn hasve been shown to be a valuable proxy proxies offor terrestrial crustal dust in paleoclimate ice core records (McConnell et al., 2007; Gornitz, 2009; Lambert et al., 2012). -As such, it is plausible
 to envisage a link between the concentrations of nssCathese dust tracers and other transported materials influenced by aeolian deposition, (e.g., OM concentration and charactercomposition, microbial biomass, and pollen grains). The relationship between glacial cycles and atmospheric deposition of dust in Antarctica is largely discussed in ice core studies. We applied an assumption that common transport processes of dust and OM markers together could be hypothesized only if dust and OM originated from the same continental areas. Therefore, in this work, we merely

370 <u>speculate on the influence of dust concentrations and OM composition measurable by fluorescence spectroscopy.</u>

WD shallow ice core data showed a positive relationship between the concentrations of dissolved organic C and both nssCa and Sr (Supplement Figure 2). While organic C concentrations were not available for this study, we present the fluorescent chemical character of the OM <u>markers</u> measured concurrently with nssCa, <u>Mn</u>, and <u>Sr</u> concentrations to <u>estimate the potential strength and continental locale of classify</u> various transport mechanisms <u>as a</u>

375 <u>discussion point potentially responsible for the -of-specific types of fluorescing OM throughout history-(Figure 3)</u>.

The LGM contained the highest concentrations of nssCa, Mn, and Sr, when annual snowfall deposition was low (WAIS Divide Project Members, 2013). We speculate that the larger nssCa, Mn, and Sr concentrations measured during the LGM likely originated from increased local continental dust loading (of South America origin) as well as more efficient atmospheric transport. At the end of the LGM, <u>nssCa</u> concentrations of <u>nssCa</u>, Sr, and Mn declined around 18.0 – 16.0 kyrs BP, near the beginning of the LD, reflecting a decrease in continental dust loading as the climate warmed. Throughout the LD and the Holocene, abrupt increases or spikes in nssCa_, and Sr, and Mn concentrations were observed over shorter time scales, <u>potentially representing other continental contributors (e.g.,</u> <u>Australia) that may play a significant role</u>. -These increases in the LD and Holocene may be linked to other atmospheric events, emphasizing the plethora of mechanisms by which OM can be transported to Antarctica.

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4.3 OM marker source fluctuations over time

- Fluctuations in ice core OM fluorescent markers quality may be driven by a multitude of variables, including: ecosystem productivity, changes in precipitation and accumulation due to temperature shifts, sea ice extent, wind 390 patterns, fires, and volcanic activity, most of which are in some way governed by the relative climate conditions. Sea ice extent was determined from the concentration of ssNa in the WD ice core (WAIS Divide Project Members, 2013)., the results of which were used in this study as a proxy to evaluate marine influences on OM quality from the LGM to the early Holocene. Higher concentrations of ssNa wereas associated with more extensive sea ice cover in colder climates, whereas decreased ssNa concentrations coincideding with $\delta^{18}O$ enrichment, and implied less sea ice 395 extent during warmer climates (WAIS Divide Project Members, 2013). Higher OM fluorescence intensities in the LGM, may indicate higher concentrations of transported OM at WD. Higher concentrations of crustal dust (of South American origin) were predicted to also be a result of greater sea ice extent in the LGM, compared to lower transport when sea ice extent retreats (WAIS Divide Project Members, 2013). Changes in atmospheric circulation could also affect OM transport to WD and has been reported as a possible explanation for the decrease in ssNa 400 concentrations at the end of the LGM (WAIS Divide Project Members, 2013). Concentrations of ssNa can thus serve
- as a proxy for multiple reconstructions, and may also be used to indicate more or less marine influences on the WD ice core OM markers. We speculate that extensive sea ice cover would reduce is associated with less the effect of marine influences on the WD ice core OM, compared to when the ocean is less covered.open ocean.
- 405 <u>As our WD OM marker record represents the first continuous ice core data set, we cannot directly compare</u> 405 <u>our record with other ice core OM records to better understand C cycling of our past. Instead, we employ marine</u> <u>sediment records that described C production and cycling on the same relative dating scales (LGM to mid</u> <u>Holocene). During the LGM, tundra ecosystems covered more expansive areas of the Earth (Ciais et al., 2012) and</u>

while C was cycling, productivity in the environment differed from warmer climates (Ciais et al., 2012, and references within). We attribute the qualitative fluorescent OM marker contributions changes in the WD ice core

- 410 fluorescent OM-record to be a function of changing environmental influences throughout over time. Three OM markers were identified to track the relative contributions of different types of fluorescing material in the LGM, LD, and the Holocene. The largest contributions of C1 were present in the LGM, indicating that C materials similar to monolignol species, potentially originating from South America, were prevalent signatures of a more ice covered Earth. Lower contributions of OM markers similar to tannin- and humic-like material were observed in the LGM,
- 415 describing lower abundances in the environment or less effective interaction with the atmosphere. At the onset of warming, the years in between the LGM and the Holocene (6.0 kyrs) were a transitional period, encompassing a climate with rising temperatures and decreasing sea ice extent. The contributions of all three PARAFAC components shift from the LGM to the LD, towards decreased monolignol OM and greater influence of more tannin- and humic-like material. Intriguingly, PARAFAC components C1 and C2 were present in both the LD and
- 420 the LGM (Figure 3), despite relatively rapid changes in climate. OM markers (LGM-, LD-, and Holocene-C2) represented by the supplementary PARAFAC models (Figure 3a-c)from the LD alone, after the LGM, capturedshowed C2 variation during the relatively rapid changes in climate. All three components identified as C2 had different Ex/Em maxima, indicating shifts to different OM markers characteristic of this transition time. a slight red shift in C2 (Figure 3b), suggesting more freshly produced fluorescent OM from external environments. The
- 425 years between 13.0-11.5 kyr BP, at the end of the LD, defined as the Antarctic cold reversal, incorporate a depression of temperature, just prior to the early Holocene, where reports of Ca and dust concentrations increase (Delmonte et al., 2002). Also measured in the WD ice core (increases in nssCa between 13.0 and 11.5 kry BP; Figure 4), we speculate that these environmental fluctuations during the Antarctic cold reversal may also explain the fluorescent OM variation in the LD (Figure 3a-c). We submit that the fluctuations in C2 OM markers, fluorescent
- 430 <u>OM red shift, observed in the LD, wereis a result of a multitude of environmental changes reflecting different C</u> dynamics <u>(i.e. more marine, terrestrial, and microbial influences) occurring in occurring after the LGM the transitory</u> period between the LGM and the Holocene. Decreased continental dust loads and the potential for more marine inputs may be important factors contributing to differences in OM character between the LD and LGM.

The years between 13.0 11.5 kyrs BP, at the end of the LD, defined as the Antarctic cold reversal, incorporate a depression of temperature, just prior to the early Holocene, where reports of Ca and dust concentrations increase (Delmonte et al., 2002). Also measured in the WD ice core (Figure 4), these environmental fluctuations during the Antarctic cold reversal, may also explain the fluorescent OM variation in the LD (Figure 3b). We submit that the C2 fluorescent OM red shift, observed in the LD, is a result of a multitude of environmental ehanges (i.e. more marine, terrestrial, and microbial influences) occurring in the transitory period between the LGM

440 and the Holocene.

The Holocene <u>marked was</u> the only period <u>containing shown to contain</u> evidence of a more humic-like OM PARAFAC component (C3), <u>albeit with relatively low percent contributions</u>. <u>Based on marine sediment records</u>, <u>T</u>the Holocene is characterized by higher levels of C productivity and vegetation cover (Ciais et al., 2012), with atmospheric temperatures and the potential for marine influences at their highest. With gross terrestrial C production

- estimated to be double that of the LGM (Ciais et al., 2012), it is reasonable to predict that the Holocene would contain the most heterogeneous mixture of OM, from marine, freshwater, and terrestrial environments, comparatively. As such, the Holocene contains both bioavailable C species of microbial origin and more recalcitrant humic like OM (Figure 3a). With temperatures rising rapidly, Earth's atmosphere changed drastically in the Holocene, and atmospheric concentrations of CO₂ and CH₄ increased substantially (WAIS Divide Project Members,
- 450 2013, 2015) from natural processes. Reports of higher concentrations of CO₂ and CH₄ also suggests evidence of increased levels of C utilization and production in the Holocene, which agrees with the more-humic-like and potentially more degraded types of fluorescing OM markers observed in the youngest ice. Overall, the OM present in the WD ice core was dominated by materials fluorescing at low Ex/Em wavelengths, describing more easily altered chemical species upon exposure to surrounding environments if released from the ice lattice. Further
- 455 research is necessary to measure the patterns of potential OM transformation pathways upon melting and retreat. Specifically, measuring the susceptibility of ice core OM to photodegradation and also microbial metabolism (respiration rates, CO₂ and/or CH₄ concentration accumulation), is a necessary next step to project how this material could impact atmospheric concentrations of greenhouse gases in a warming climate.
- Volcanic eruptions inject particles, aerosols, and gases into the atmosphere (e.g., H₂O, SO₂, CO₂, H₂S, HCl,
 and HF) regionally and globally depending on the height of the volcanic plume. Therefore, volcanic eruptions increase the potential for particles and chemicals to be transported to Polar regions and deposited onto ice sheets. Concentrations of non sea salt sulfur (nssS) are commonly used to trace volcanic signatures in polar ice cores and to reconstruct past volcanic activity on Earth. The direct effects of volcanic activity on OM quality and quantity were beyond the scope of this study, however, the nssS data from the WD ice core is referenced in this work to highlight volcanic activity as a possible mechanism for stimulating OM production. Volcanic eruptions detected in the WD ice core, using the nssS calculated volcanic detection threshold, accounted for 4.5% of all OM characterized by EEMs (nssS concentrations shown in Supplement Figure 3). Although only a small percentage, volcanic activity was detected in all climate periods, suggesting another possible mechanism for the fluorescent OM character changes since the LGM.
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5 Conclusions

OM is a complex mixture of heterogeneous, polydisperse, and polycyclic molecules, the nature of which may result from multiple sources. Analyse is of the WD OM <u>markers character</u> by fluorescence spectroscopy allowed for the development of a series of interrelated climate and chemical records focused on understanding changes of \underline{C}

- 475 <u>dynamics in atmospheric-global systems spanning 21.0 kyrs of Earth's history. PARAFAC modelling of the WD ice core fluorescent OM markers identified components used to better understand ecological influences in a changing climate, providing more information on the types of C produced than is currently reported in marine sediment records. -Simple phenolic Proteinaceous fluorescent OM markers ce-were dominant features in all WD samples over time, suggesting a strong microbial-terrestrial OM control of Earth's past ecosystems. More humic-like OM
 480 fluorescence, characteristic of more degraded recalcitrant material was only detected in the Holocene, at a time when
 </u>
 - temperatures were warmer, precipitation and accumulation was greater, C productivity was higher, and tundra
 - 47

ecosystems were less expansive, advancing the probability for <u>effective interaction of more</u>-organic materials_to <u>interact</u>-with the atmosphere. OM fluorescent <u>character-markers</u> detected in the WD ice core within different climates may have fluctuated as a result of the diverse variables introduced as the atmosphere and <u>microbial</u> <u>communitiesC</u> processing dynamics shifted over time. Taken together, <u>proteinaceous-the</u> fluorescent OM markers

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signatures in the WD ice core suggests that simple phenolic chemical species such as monolignols and cresols (precursors to lignins and tannins), labile, microbially derived OM prevailed in all climate periods, and were the greatest contributors to Earth's atmospheric compositions throughout history.

490 Author contribution

C. Foreman, J. D'Andrilli, J. Priscu, and J. McConnell designed the experiments and J. D'Andrilli and M. Sigl carried them out. Both J. D'Andrilli and M. Sigl were a part of the ice core melting team. J. McConnell and M. Sigl calculated the dating scale for all samples. J. D'Andrilli prepared the manuscript with contributions from all coauthors.

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Competing interests

The authors declare that they have no conflicts of interest.

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References

Acree, W. E., Tucker, S. A., and Fetzer, J. C.: Fluorescence Emission Properties of Polycyclic Aromatic Compounds in Review, Polycycl Aromat Comp, 2, 75-105, 10.1080/10406639108048933doi, 1991.

- 520 Albani, S., Mahowald, N. M., Murphy, L. N., Raiswell, R., Moore, J. K., Anderson, R. F., McGee, D., Bradtmiller, L. I., Delmonte, B., Hesse, P. P., and Mayewski, P. A.: Paleodust variability since the Last Glacial Maximum and implications for iron inputs to the ocean, Geophysical Research Letters, 43, 3944-3954, 10.1002/2016GL067911doi, 2016.
- Alley, R. B.: The Two-Mile Time Machine: Ice Cores, Abrupt Climate Change, and Our Future, Princeton
 525 University Press, Princeton, New Jersey, 2002.
 Balcarczyk, K. L., Jones, J. B., Jaffe, R., and Maie, N.: Stream dissolved organic matter bioavailability and

Balcarczyk, K. L., Jones, J. B., Jaffe, R., and Male, N.: Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost, Biogeochemistry, 94, 255-270, 10.1007/s10533-009-9324-xdoi, 2009.

Banta, J. R., McConnell, J. R., Frey, M. M., Bales, R. C., and Taylor, K.: Spatial and temporal variability in snow accumulation at the West Antarctic Ice Sheet Divide over recent centuries, J Geophys Res-Atmos, 113, Doi 10.1029/2008jd010235doi, 2008.
Barletta, R. E., Priscu, J. C., Mader, H. M., Jones, W. L., and Roe, C. H.: Chemical analysis of ice vein microenvironments: II. Analysis of glacial samples from Greenland and Antarctica, Journal of Glaciology, 58, 1109-1118, DOI 10.3189/2012JoG12J112doi, 2012.

- Battin, T. J., Kaplan, L. A., Findlay, S., Hopkinson, C. S., Marti, E., Packman, A. I., Newbold, J. D., and Sabater, F.: Biophysical controls on organic carbon fluxes in fluvial networks, Nature Geoscience, 1, 95-100, DOI 10.1038/ngeo101doi, 2008.
 Bro, R.: PARAFAC. Tutorial and applications, Chemometr Intell Lab, 38, 149-171, Doi 10.1016/S0169-7439(97)00032-4doi, 1997.
- Cawley, K. M., Butler, K. D., Aiken, G. R., Larsen, L. G., Huntington, T. G., and McKnight, D. M.: Identifying fluorescent pulp mill effluent in the Gulf of Maine and its watershed, Marine Pollution Bulletin, 64, 1678-1687, DOI 10.1016/j.marpolbul.2012.05.040doi, 2012.
 Ciais, P., Tagliabue, A., Cuntz, M., Bopp, L., Scholze, M., Hoffmann, G., Lourantou, A., Harrison, S. P., Prentice, I.

Ciais, P., Tagliabue, A., Cuntz, M., Bopp, L., Scholze, M., Hoffmann, G., Lourantou, A., Harrison, S. P., Prentice, I.
C., Kelley, D. I., Koven, C., and Piao, S. L.: Large inert carbon pool in the terrestrial biosphere during the Last
Glacial Maximum, Nature Geoscience, 5, 74-79, 10.1038/Ngeo1324doi, 2012.

- 545 Glacial Maximum, Nature Geoscience, 5, 74-79, 10.1038/Ngeo1324doi, 2012.
 Coble, P. G., Green, S. A., Blough, N. V., and Gagosian, R. B.: Characterization of Dissolved Organic-Matter in the Black-Sea by Fluorescence Spectroscopy, Nature, 348, 432-435, DOI 10.1038/348432a0doi, 1990.
 Coble, P. G.: Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy, Marine Chemistry, 51, 325-346, Doi 10.1016/0304-4203(95)00062-3doi, 1996.
- 550 Coble, P. G., Del Castillo, C. E., and Avril, B.: Distribution and optical properties of CDOM in the Arabian Sea during the 1995 Southwest Monsoon, Deep-Sea Res Pt Ii, 45, 2195-2223, Doi 10.1016/S0967-0645(98)00068-Xdoi, 1998.

Coble, P. G., Lead, J., Baker, A., Reynolds, D. M., Spencer, R. G. M. (eds): Aquatic Organic Matter Fluorescence, Cambridge University Press, Cambridge, 2014.

Cory, R. M., and McKnight, D. M.: Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter, Environmental Science & Technology, 39, 8142-8149, DOI 10.1021/es0506962doi, 2005.
 Dansgaard, W., Johnsen, S. J., Clausen, H. B., Dahljensen, D., Gundestrup, N. S., Hammer, C. U., Hvidberg, C. S.,

Steffensen, J. P., Sveinbjornsdottir, A. E., Jouzel, J., and Bond, G.: Evidence for General Instability of Past Climate
 from a 250-Kyr Ice-Core Record, Nature, 364, 218-220, DOI 10.1038/364218a0doi, 1993.

- Delmonte, B., Petit, J. R., and Maggi, V.: Glacial to Holocene implications of the new 27000-year dust record from the EPICA Dome C (East Antarctica) ice core, Clim Dynam, 18, 647-660, 10.1007/s00382-001-0193-9doi, 2002. Fellman, J. B., Hood, E., and Spencer, R. G. M.: Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review, Limnology and Oceanography, 55, 2452-2462, 10.4319/lo.2010.55.6.2452doi, 2010.
- Ferretto, N., Tedetti, M., Guigue, C., Mounier, S., Redon, R., and Goutx, M.: Identification and quantification of known polycyclic aromatic hydrocarbons and pesticides in complex mixtures using fluorescence excitation-emission matrices and parallel factor analysis, Chemosphere, 107, 344-353, 10.1016/j.chemosphere.2013.12.087doi, 2014.
 Gornitz, V.: Mineral Indicators Of Past Climates, in: Encyclopedia of Paleoclimatology and Ancient Environments,
- edited by: Gornitz, V., Encyclopedia of Earth Sciences Series, Springer Netherlands, 573-583, 2009.
 Hood, E., Battin, T. J., Fellman, J., O'Neel, S., and Spencer, R. G. M.: Storage and release of organic carbon from glaciers and ice sheets, Nature Geoscience, 8, 91-96, 10.1038/Ngeo2331doi, 2015.
 Jansen, E., Overpeck, J., Briffa, K. R., Duplessy, J.-C., Joos, F., Masson-Delmotte, V., Olago, D., Otto-Bliesner, O., Peltrier, W. R., Rahmstorf, S., Ramesh, R., Raynaud, D., Rind, D., Solomina, O., Villalba, R., and Zhang, D.:
- 575 Paleoclimate, in: IPCC 2007: Climate Change 2007 The Physical Science Basis, edited by: Solomon, S., Qin, D.,

Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., Cambridge University Press, Cambridge, 2007.

Johnsen, S. J., DahlJensen, D., Gundestrup, N., Steffensen, J. P., Clausen, H. B., Miller, H., Masson-Delmotte, V., Sveinbjornsdottir, A. E., and White, J.: Oxygen isotope and palaeotemperature records from six Greenland ice-core

580 stations: Camp Century, Dye-3, GRIP, GISP2, Renland and NorthGRIP, J Quaternary Sci, 16, 299-307, DOI 10.1002/jqs.622doi, 2001.
Kruss, T. F. C. Xu, Z. Proston, C. M. Dahlgran, P. A. and Zacaski, P. Li Linking Chamical Possibility and

Kraus, T. E. C., Yu, Z., Preston, C. M., Dahlgren, R. A., and Zasoski, R. J.: Linking Chemical Reactivity and Protein Precipitation to Structural Characteristics of Foliar Tannins, Journal of Chemical Ecology, 29, 703-730, 10.1023/A:1022876804925doi, 2003.

- 585 Lambert, F., Bigler, M., Steffensen, J. P., Hutterli, M., and Fischer, H.: Centennial mineral dust variability in highresolution ice core data from Dome C, Antarctica, Clim Past, 8, 609-623, 10.5194/cp-8-609-2012doi, 2012. Lawaetz, A. J., and Stedmon, C. A.: Fluorescence Intensity Calibration Using the Raman Scatter Peak of Water, Appl Spectrosc, 63, 936-940, doi, 2009.
- Legrand, M., and Mayewski, P.: Glaciochemistry of polar ice cores: A review, Rev Geophys, 35, 219-243, Doi 10.1029/96rg03527doi, 1997.
- Legrand, M., Preunkert, S., Jourdain, B., Guilhermet, J., Fain, X., Alekhina, I., and Petit, J. R.: Water-soluble organic carbon in snow and ice deposited at Alpine, Greenland, and Antarctic sites: a critical review of available data and their atmospheric relevance, Clim Past, 9, 2195-2211, 10.5194/cp-9-2195-2013doi, 2013. Luthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J. M., Siegenthaler, U., Raynaud, D., Jouzel, J., Fischer,
- H., Kawamura, K., and Stocker, T. F.: High-resolution carbon dioxide concentration record 650,000-800,000 years before present, Nature, 453, 379-382, 10.1038/nature06949doi, 2008.
 Marcott, S. A., Bauska, T. K., Buizert, C., Steig, E. J., Rosen, J. L., Cuffey, K. M., Fudge, T. J., Severinghaus, J. P., Ahn, J., Kalk, M. L., McConnell, J. R., Sowers, T., Taylor, K. C., White, J. W., and Brook, E. J.: Centennial-scale changes in the global carbon cycle during the last deglaciation, Nature, 514, 616-619, 10.1038/nature13799doi,
- 2014.
 Marhaba, T. F., Van, D., and Lippincott, L.: Rapid identification of dissolved organic matter fractions in water by spectral fluorescent signatures, Water Research, 34, 3543-3550, Doi 10.1016/S0043-1354(00)00090-7doi, 2000.
 McConnell, J. R., Lamorey, G. W., Lamber, S. W., and Taylor, K. C.: Continuous ice-core chemical analyses using inductively Coupled Plasma Mass Spectrometry, Environmental Science & Technology, 36, 7-11,
- 10.1021/es011088zdoi, 2002.
 McConnell, J. R., Aristarain, A. J., Banta, J. R., Edwards, P. R., and Simoes, J. C.: 20th-Century doubling in dust archived in an Antarctic Peninsula ice core parallels climate change and desertification in South America, P Natl Acad Sci USA, 104, 5743-5748, 10.1073/pnas.0607657104doi, 2007.
- McConnell, J. R., Maselli, O. J., Sigl, M., Vallelonga, P., Neumann, T., Anschutz, H., Bales, R. C., Curran, M. A. J.,
 Das, S. B., Edwards, R., Kipfstuhl, S., Layman, L., and Thomas, E. R.: Antarctic-wide array of high-resolution ice core records reveals pervasive lead pollution began in 1889 and persists today, Sci Rep-Uk, 4, 10.1038/srep05848doi, 2014.
 Miller, M. P., and McKnight, D. M.: Comparison of seasonal changes in fluorescent dissolved organic matter among aquatic lake and stream sites in the Green Lakes Valley, J Geophys Res-Biogeo, 115, 10.1029/2009jg000985doi,
- 615 2010.
 Miller, M. P., Simone, B. E., McKnight, D. M., Cory, R. M., Williams, M. W., and Boyer, E. W.: New light on a dark subject: comment, Aquat Sci, 72, 269-275, 10.1007/s00027-010-0130-2doi, 2010.
 Miteva, V., Teacher, C., Sowers, T., and Brenchley, J.: Comparison of the microbial diversity at different depths of the GISP2 Greenland ice core in relationship to deposition climates, Environ Microbiol, 11, 640-656, DOI
- 10.1111/j.1462-2920.2008.01835.xdoi, 2009.
 Miteva, V., Sowers, T., Schüpbach, S., Fischer, H., and Brenchley, J.: Geochemical and Microbiological Studies of Nitrous Oxide Variations within the New NEEM Greenland Ice Core during the Last Glacial Period, Geomicrobiology Journal, 33, 647-660, 10.1080/01490451.2015.1074321doi, 2016.
- Murphy, K. R., Stedmon, C. A., Waite, T. D., and Ruiz, G. M.: Distinguishing between terrestrial and
 autochthonous organic matter sources in marine environments using fluorescence spectroscopy, Marine Chemistry, 108, 40-58, 10.1016/j.marchem.2007.10.003doi, 2008.
 Murphy, K. R., Stedmon, C. A., Graeber, D., and Bro, R.: Fluorescence spectroscopy and multi-way techniques. PARAFAC, Anal Methods-Uk, 5, 6557-6566, 10.1039/c3ay41160edoi, 2013.
 Murphy, K. R., Stedmon, C. A., Wenig, P., and Bro, R.: OpenFluor- an online spectral library of auto-fluorescence
- by organic compounds in the environment, Anal Methods-Uk, 6, 658-661, 10.1039/c3ay41935edoi, 2014.

Osburn, C. L., Morris, D. P., Thorn, K. A., and Moeller, R. E.: Chemical and optical changes in freshwater dissolved organic matter exposed to solar radiation, Biogeochemistry, 54, 251-278, 10.1023/A:1010657428418doi, 2001. Petit, J. R., Jouzel, J., Raynaud, D., Barkov, N. I., Barnola, J. M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V. M., Legrand, M., Lipenkov, V. Y., Lorius, C., Pepin, L., Ritz, C.,

- Saltzman, E., and Stievenard, M.: Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica, Nature, 399, 429-436, Doi 10.1038/20859doi, 1999.
 Priscu, J. C., and Christner, B. C.: Earth's Icy Biosphere, in: Microbial Diversity and Bioprospecting, edited by: Bull, A. T., American Society for Microbiology Press, Washington D.C., 130-145, 2004.
 Priscu, J. C., Christner, B. C., Foreman, C. M., and Royston-Bishop, G.: Biological Material in Ice Cores in:
- 640 Encyclopedia of Quaternary Sciences, Elsevier B.V., UK, 2007. Priscu, J. C., Tulaczyk, S., Studinger, M., Kennicutt, M. C., Christner, B. C., and Foreman, C. M.: Antarctic Subglacial Water: Origin, Evolution, and Ecology., in: Polar Lakes and Rivers, edited by: Vincent, W., and Laybourn-Parry, J., Oxford Press, 119-136, 2008.
- Rhodes, R. H., Fain, X., Stowasser, C., Blunier, T., Chappellaz, J., McConnell, J. R., Romanini, D., Mitchell, L. E., and Brook, E. J.: Continuous methane measurements from a late Holocene Greenland ice core: Atmospheric and insitu signals, Earth Planet Sc Lett, 368, 9-19, 10.1016/j.epsl.2013.02.034doi, 2013.
 Rhodes, R. H., Fain, X., Brook, E. J., McConnell, J. R., Maselli, O. J., Sigl, M., Edwards, J., Buizert, C., Blunier, T., Chappellaz, J., and Freitag, J.: Local artifacts in ice core methane records caused by layered bubble trapping and in
- situ production: a multi-site investigation, Clim Past, 12, 1061-1077, 10.5194/cp-12-1061-2016doi, 2016.
 Stedmon, C. A., Markager, S., and Bro, R.: Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy, Marine Chemistry, 82, 239-254, 10.1016/s0304-4203(03)00072-0doi, 2003. Stedmon, C. A., Markager, S., Tranvik, L., Kronberg, L., Slätis, T., and Martinsen, W.: Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea, Marine Chemistry, 104, 227-240, http://dx.doi.org/10.1016/j.marchem.2006.11.005doi, 2007.
- Stedmon, C. A., and Bro, R.: Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial, Limnol Oceanogr-Meth, 6, 572-579, doi, 2008.
 Stedmon, C. A., and Cory, R. M.: Biological Origins and Fate of Fluorescent Dissolved Organic Matter in Aquatic Environments, Camb Env Ch, 278-299, Book_Doi 10.1017/Cbo9781139045452doi, 2014.
 Stubbins, A., Hood, E., Raymond, P. A., Aiken, G. R., Sleighter, R. L., Hernes, P. J., Butman, D., Hatcher, P. G.,
- Striegl, R. G., Schuster, P., Abdulla, H. A. N., Vermilyea, A. W., Scott, D. T., and Spencer, R. G. M.: Anthropogenic aerosols as a source of ancient dissolved organic matter in glaciers, Nature Geoscience, 5, 198-201, Doi 10.1038/Ngeo1403doi, 2012. Teale, F. W. J., and Weber, G.: Ultraviolet fluorescence of the aromatic amino acids, Biochemical Journal, 65, 476-

Teale, F. W. J., and Weber, G.: Ultraviolet fluorescence of the aromatic amino acids, Biochemical Journal, 65, 476-482, doi, 1957.

665 Tipping, E.: Humic substances in soil, sediment and water: Geochemistry, isolation and characterization edited by G. R. Aiken, D. M. McKnight, R. L. Wershaw and P. MacCarthy, Wiley, 1985. No. of pages: 692. Price: £61.35 (hardback), Geological Journal, 21, 213-214, 10.1002/gj.3350210213doi, 1986. Tucker, S. A., Amszi, V. L., and Acree, W. E.: Primary and Secondary Inner Filtering - Effect of K2cr2o7 on Fluorescence Emission Intensities of Ouinine Sulfate, J Chem Educ, 69, A8-&, doi, 1992.

 WAIS Divide Ice Core Project Science Coordination Office, WAIS Divide website: <u>http://www.waisdivide.unh.edu/</u>.
 WAIS Divide Project Members: Onset of deglacial warming in West Antarctica driven by local orbital forcing, Nature, 500, 440-444, 10.1038/nature12376doi, 2013.
 WAIS Divide Project Members: Precise interpolar phasing of abrupt climate change during the last ice age, Nature,

520, 661-U169, 10.1038/nature14401doi, 2015.
Wang, Y., Chantreau, M., Sibout, R., and Hawkins, S.: Plant cell wall lignification and monolignol metabolism, Front Plant Sci, 4, ARTN 220 10.3389/fpls.2013.00220doi, 2013.

Wolff, E. W., Fischer, H., Fundel, F., Ruth, U., Twarloh, B., Littot, G. C., Mulvaney, R., Röthlisberger, R., de
 Angelis, M., Boutron, C. F., Hansson, M., Jonsell, U., Hutterli, M. A., Lambert, F., Kaufmann, P., Stauffer, B., Stocker, T. F., Steffensen, J. P., Bigler, M., Siggaard-Andersen, M. L., Udisti, R., Becagli, S., Castellano, E., Severi, M., Wagenbach, D., Barbante, C., Gabrielli, P., and Gaspari, V.: Southern Ocean sea-ice extent, productivity and iron flux over the past eight glacial cycles, Nature, 440, 491-496, http://www.nature.com/nature/journal/v440/n7083/suppinfo/nature04614 S1.htmldoi, 2006.



Figure 1: Location of the West Antarctic Ice Sheet (WAIS) Divide in western Antarctica, with elevation contour lines: 112.085°W Longitude, 79.467°S Latitude, and 1,766m surface elevation

700 (http://www.waisdivide.unh.edu/).



Figure 2: Changes in West Antarctic Ice Sheet Divide ice core organic matter total fluorescence intensity,
 provided in Raman Units (R.U.), over time (kyrs before present 1950). Climate periods are labeled in shaded gray bars to describe the Last Glacial Maximum (LGM), the last deglaciation (LD), and the early to mid-Holocene.



Figure 2. PARAFAC analysis results for West Antarctic Ice Sheet Divide ice core organic matter showing a)
 components one, two, and three (C1, C2, and C3), and b) the fluorescence percentage of each component
 contributing to the overall fluorescence signature over the Last Glacial Maximum (LGM), last deglaciation
 (LD), and Holocene climate periods as a function of time (kyr before present 1950). Average fluorescence
 percentages (gray dashed lines) are provided for each component, separately calculated for each climate
 period. Fluorescent data were reported in Raman Units. Note: C3 average fluorescence percentages are
 considerably lower in the LGM and LD, and did not correspond to resolved fluorophores.



Figure 3: PARAFAC components identified for West Antarctic Ice Sheet Divide ice core organic matter showing a) component one (C1), component two (C2), and component three (C3) and the various overlapping features for the Last Glacial Maximum (LGM), last deglaciation (LD), and the Holocene climate periods. PARAFAC component variation for C2 is depicted in (b) for all climate periods.





Figure 4: Trace element concentrations (ppb) from the West Antarctic Ice Sheet Divide ice core, dating from the Last Glacial Maximum (LGM), through the last deglaciation (LD), to the mid-Holocene for manganese (Mn), strontium (Sr), and non-sea salt calcium (nssCa), with the δ^{48} O (per mil) temperature record, and the contributing OM PARAFAC components as a function of time (kyrs before present 1950).



Figure 4. Trace element concentration of (top) non-sea salt calcium (nssCa; ppb), and (bottom) the δ18O (per mil) temperature record (Marcott et al., 2014) from the West Antarctic Ice Sheet Divide ice core as a function of time (kyr before present 1950), dating from the Last Glacial Maximum (LGM), through the last deglaciation (LD), to the mid-Holocene.







Figure 1: Examples of West Antarctic Ice Sheet Divide ice core Excitation Emission Matrices (EEMs)showing low excitation/emission wavelength organic matter (OM) fluorescence from a) the Last GlacialMaximum ice (26.221 kyr BP; before present 1950) and b) the deglaciation ice (12.650 kyr BP 1950), and c)both lower and higher excitation/emission wavelength fluorescent OM from the Holocene (9.401 kyr BP 1950;dating scale WDC06A-7) (WAIS Divide Project Members, 2013)). Fluorescence intensities are reported on thez axis in Raman Units (R.U.). Note: All examples were post-processed for septa-lid blank subtraction, andRaman and Rayleigh-Tyndall scattering effects.



Figure 2: PARAFAC loading scores for excitation (ex) and emission (em) wavelength fluorescence for the three component model of the 21.0 kry record of West Antarctic Ice Sheet Divide organic matter. Results of the PARAFAC model are displayed as a function of the individual fluorescing components, a) component one (C1), b) component two (C2), and c) component three (C3), for the six split half categories annotated in the legend.





Figure 3. Three climate categorized supplementary PARAFAC models of the West Antarctic Ice Sheet Divide ice core fluorescent organic matter showing components identified for a) the Last Glacial Maximum (27.0-18.0 kyrs BP 1950), b) last deglaciation (18.0-11.5 kyrs BP 1950), and c) the Holocene (11.5-6.0 kyrs bP 1950; dating scale WDC06A-7) (WAIS Divide Project Members, 2013).





fluorescing components one, two, and three (C1, C2, and C3) for a) the Last Glacial Maximum (27.0-18.0 kyrs BP 1950), b) last deglaciation (18.0-11.5 kyrs BP 1950), and c) the Holocene (11.5-6.0 kyrs bP 1950; dating scale WDC06A-7) (WAIS Divide Project Members, 2013).

815 <u>References</u>

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WAIS Divide Project Members: Onset of deglacial warming in West Antarctica driven by local orbital forcing, Nature, 500, 440-444, 10.1038/nature12376doi, 2013.