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Climate driven carbon and microbial signatures through the last ice age

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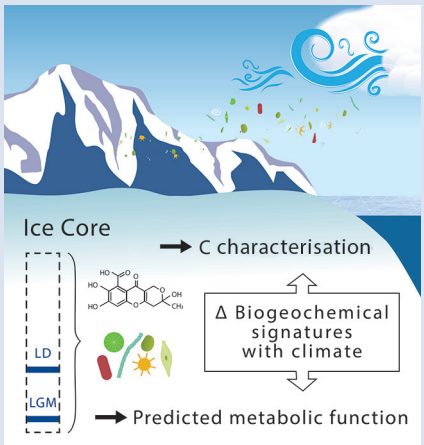
Abstract



Ice cores preserve diverse materials as millennial-scale proxies for Earth's history. While major ions and elemental analyses are commonly investigated in palaeoclimate reconstructions, the integration of biological measurements is rapidly developing. Although the limited number of data herein impose constraints on broader generalisations, we show that microbial assemblages and organic matter (OM) composition from Byrd Station and West Antarctic Ice Sheet Divide ice cores may serve as palaeoecological markers from the Last Glacial Maximum (LGM; section ~20.5 ka BP) and last deglaciation periods (LD; section ~14.5 ka BP), reflecting environmental changes. Fluorescent analyses determined OM from both cores to have similar amino acid-like signatures; however, more comprehensive molecular characterisation showed only 12 % overlap in molecular formulae, with Byrd OM being more chemically labile. Microbial diversity in both cores was low, and together with predicted metabolic capabilities, differed significantly between communities. Variation in OM composition and microbial diversity reflects changes in environmental sources and deposition patterns onto the Antarctic Ice Sheet during distinct climate periods, with OM composition potentially shaping microbial communities post-deposition. Combining detailed microbial and OM composition analyses created a unique window into the past, providing a way to characterise carbon composition and potential metabolic processes as a function of environmental change.

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Introduction

In reconstructing palaeoclimatic conditions, entrapped gases, dissolved chemicals, and dust have served as millennial-scale climate proxies in ice cores (Mahowald *et al.*, 1999; Petit *et al.*, 1999). More recently, the integration of biology into palaeoclimate research is gaining recognition, with distinct microbial assemblages and fluorescent organic matter (OM) signatures in ice indicative of depositional events related to climate (Miteva *et al.*, 2009, 2015; D'Andrilli *et al.*, 2017). We hypothesise that OM composition is conserved across climate periods, providing a chronological record in ice, similar to trapped gases and inorganic compounds.

Here we present OM molecular composition and microbial community structure within two sections from the Byrd Station (Byrd) and West Antarctic Ice Sheet (WAIS) Divide deviation #3 (WD_3) ice core sections (Fig. S-1) from the Last Glacial Maximum (LGM) and last deglaciation (LD) periods. Collecting ice cores for biological analyses is challenging due to significant contamination risks from drilling, retrieval, and processing. Rightfully, focus has been placed on developing rigorous decontamination protocols (Christner *et al.*, 2005; Miteva *et al.*, 2014), essential for producing credible results. Therefore, stringent next generation sequencing protocols and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) were applied in concert with bulk characterisation metrics (see Supplementary Information) to link deep ice core microbial assemblages and OM molecular composition to different climate periods.

Results

The ice core sections, Byrd and WD_3, correspond to approximately 20.5 ka BP (before present 1950) (Pedro *et al.*, 2012) and 14.5 ka BP (WAIS Divide Project Members, 2013), respectively. Decontamination removed hydrocarbon-based drilling fluid constituents, with diesel range organic concentrations below detection in both samples (Table S-1). The organic carbon (OC) concentration in the Byrd sample was 445 µM, ~38 times higher than the younger WD_3 (11.8 µM; Table S-2). Concentrations of Cl^-, NO_3^-, and SO_4^{2-} were approximately two times higher in Byrd compared to anion concentrations in WD_3 (Table S-2). Phosphate (PO_4^{3-}) concentrations were 2.77 µM for Byrd and below detection limit in WD_3. Both Byrd and WD_3 OM

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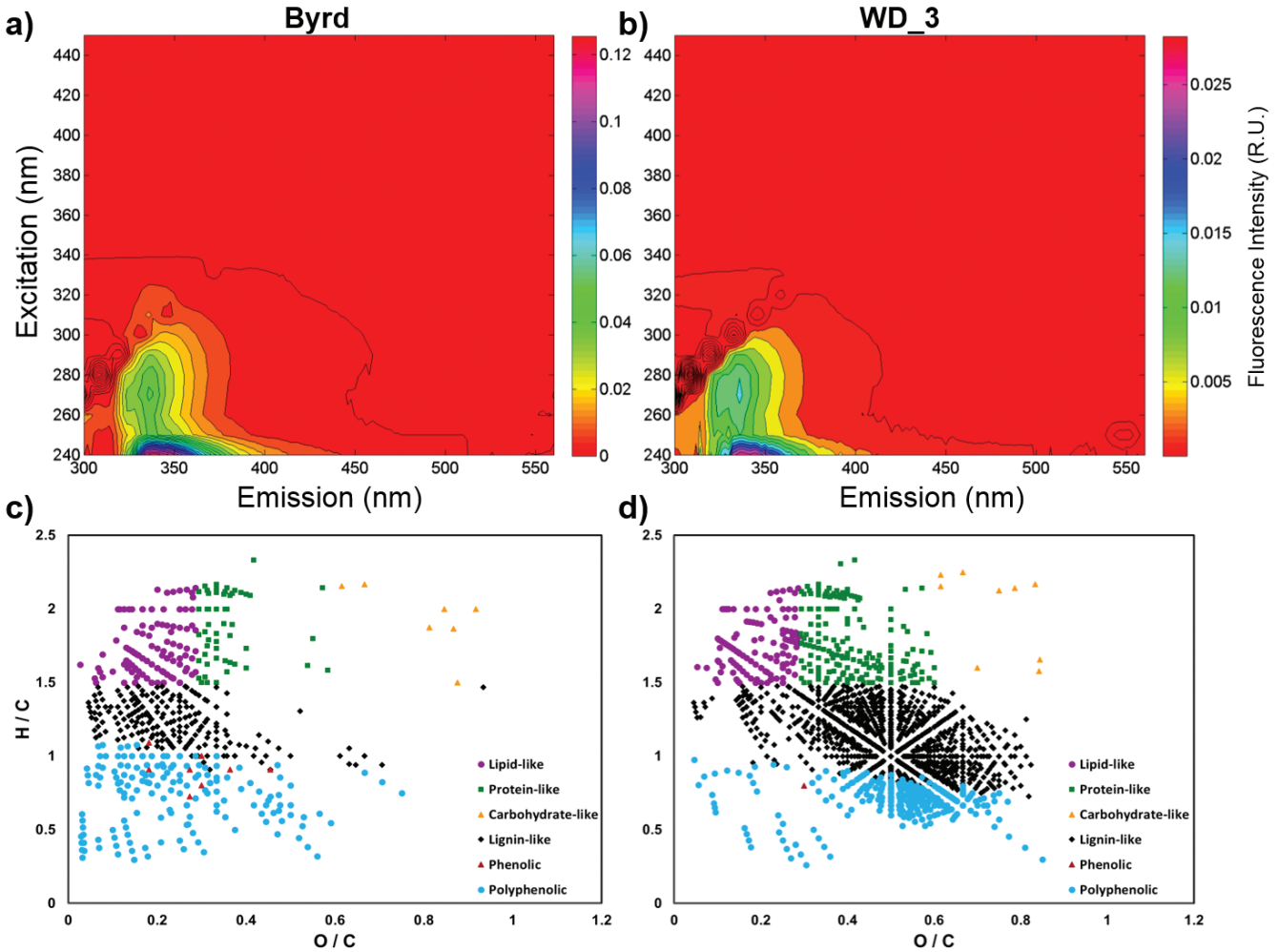

Figure 1 Organic matter (OM) characterisation of Byrd and WAIS Divide deviation #3 (WD_3) Antarctic ice cores by **(a-b)** fluorescence spectroscopy and **(c-d)** Fourier transform ion cyclotron resonance mass spectrometry van Krevelen diagrams.

exhibited fluorescence in nearly identical regions of the excitation emission matrices; however, intensities differed by an order of magnitude (EEMs; Fig. 1a–b). Fluorescing OM was situated at low excitation and emission wavelengths (Ex: 240–300 nm and Em: 300–400 nm) with maxima in regions characteristic of tryptophan-like chemical species (Coble *et al.*, 1990).

OM composition was comprised of $C_cH_hO_o$ (63.1 % and 74.4 %) and $C_cH_hO_oS_1$ (22.6 % and 19.3 %) chemical species for Byrd and WD_3, respectively. Other molecular combinations containing N and/or S comprised <15 % for both samples (Table S-3). Only 12.2 % of the molecular formulae identified in both ice cores were compositional matches (Fig. S-2). Byrd OM showed a wide range of hydrogen saturation (H/C: 0.3–2.4) at lower oxygenation, indicative of reduced OM species (Fig. 1c). Conversely, more oxygenated molecular species were observed for WD_3 OM, characteristic of lignin-like, protein-like, and polyphenolic compounds (Fig. 1d). Overall, Byrd OM exhibited more labile molecular formulae (D'Andrilli *et al.*, 2015) compared to WD_3 OM (33.1 % *versus* 21.3 %).

Microscopic analysis identified bacterial cells in both cores, with filamentous cells only found in Byrd (Fig. S-3). Bacterial cell concentrations ranged $1.92 \times 10^5 \pm 1.23 \times 10^4$ cells mL^{-1} for Byrd and $1.28 \times 10^4 \pm 2.48 \times 10^3$ cells mL^{-1} for WD_3. Bacterial cell abundances in the outer ice core layers removed during decontamination were two orders of magnitude greater.

Examination of 16S RNA gene amplicons, post quality control and blank subtraction, produced 220,899 and 5,991 high quality bacterial sequences, clustering into 66 and 22 operational taxonomic units (OTUs) for Byrd and WD_3. Overall, microbial assemblages differed significantly (Libshuff; $P < 0.001$) between cores, with only two shared OTUs. Taxonomic analysis at the class level (Fig. 2) showed that the Byrd microbial assemblage was dominated by *Flavobacteria* (74 %) and *Gammaproteobacteria* (16 %), while *Gammaproteobacteria* (67 %) and *Bacilli* (25 %) prevailed in WD_3. The dominant microbial phylotypes from the two cores were phylogenetically similar to taxa found in modern icy environments. OTUs 1 and 5, accounting for 65 % and 7 % of the Byrd assemblage, had ≥97 % 16S rRNA gene sequence identity to Antarctic strains *Flavobacterium micromati* and *Rhodoferax antarcticus* (Madigan *et al.*, 2000; Van Trappen *et al.*, 2004). OTUs 8 and 12 encompassed 32 % and 4 % of the WD_3 library, with 96 % and 97 % sequence identity to *Pseudomonas proteolytica* from Antarctic ponds (Reddy *et al.*, 2004). An unclassified *Actinobacter* from Arctic sea ice (99 % sequence match, Brinkmeyer *et al.*, 2003), made up 9 % and 32 % of the Byrd and WD_3 microbial assemblages. Cumulatively, these abundant, yet low numbers of OTUs described 81 % and 67 % of the microbial assemblages within Byrd and WD_3. Chao diversity was 43.5 (26.2–117.8; 95 % CI) and 156.3 (105.4–273.1; 95 % CI) for WD_3 and Byrd; ~two times greater than the observed number of OTUs. The Inverse Simpson estimated low biodiversity across both samples (Byrd: 2.2–2.2; WD_3: 3.9–4.0; 95 % CI) when compared to other deep ice core communities (Miteva *et al.*, 2015).

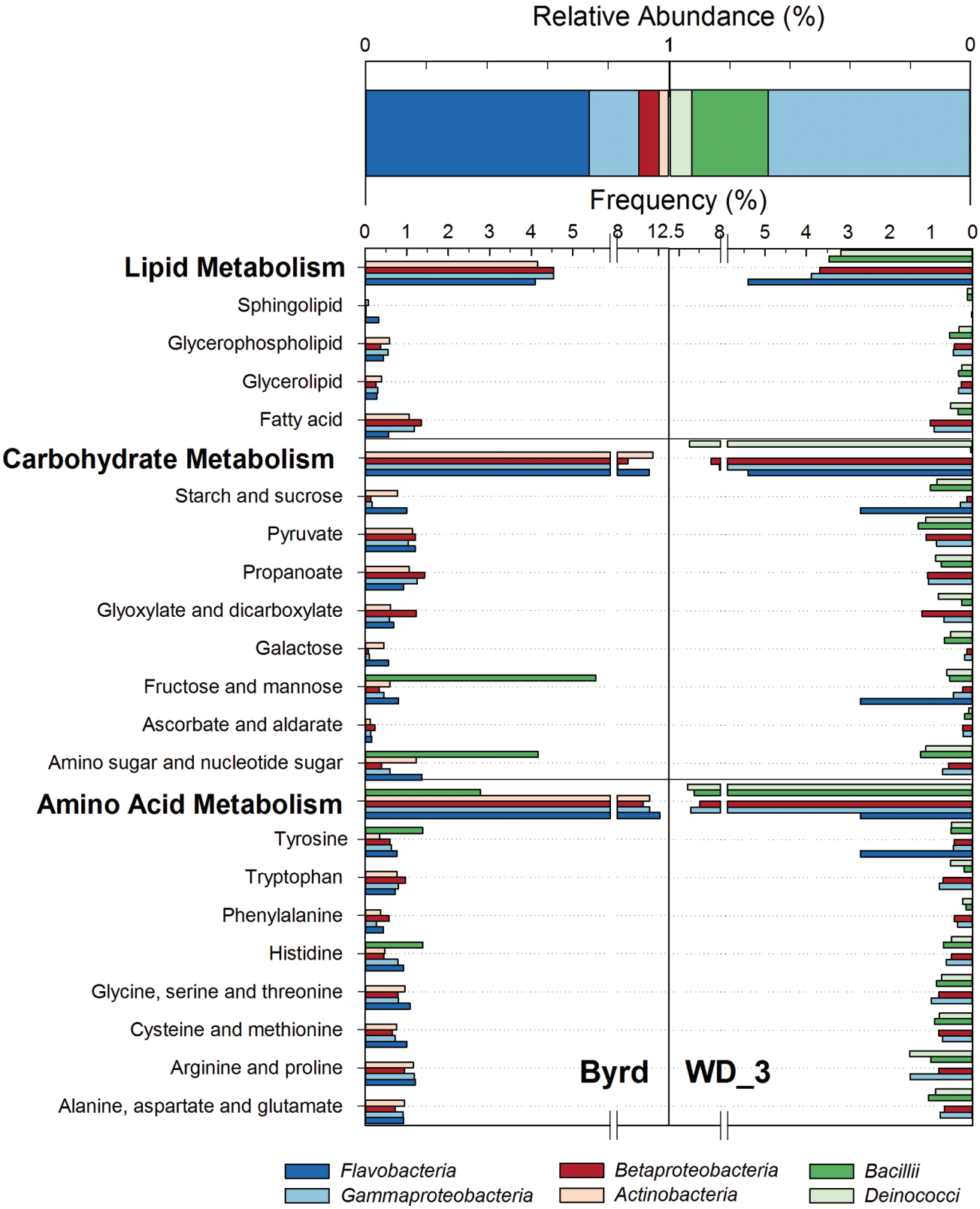

Figure 2 **Top**: Relative abundance of 16S rRNA gene sequences representing the distribution of microbial assemblages within the Byrd and WAIS Divide deviation #3 (WD_3) ice core sections. **Bottom**: Comparisons of predicted genes involved in lipid, carbohydrate, and amino acid pathways for the dominant bacterial classes.

Using 16S marker gene datasets, 57 % (Byrd) and 51 % (WD_3) of *in silico* predicted genes were attributed to metabolism. Of the 64 generally assigned functional classes, 58 of the predicted functions differed significantly between the two microbial assemblages ($P < 0.012$). Of relevance were carbohydrate (Byrd: 10.1 %, WD_3: 10.0 %), lipid (Byrd: 3.7%, WD_3: 3.5 %), and amino acid (Byrd: 9.8 %, WD_3: 9.7 %; Fig. 2) metabolism.

Discussion

Studies proposing OM as climate proxies have come from aquatic sedimentary records (*e.g.*, Meyers, 1994; Pailler and Bard, 2002; Cartapanis *et al.*, 2016). Fundamental differences exist, however, between sedimentation processes and aeolian deposition in these environments. Varying amounts of dust in ice attest to both hydro- and lithospheric changes on the continents and long distance transport (Palais and Legrand, 1985; Mahowald *et al.*, 1999; Petit *et al.*, 1999; Sigl *et al.*, 2016). Ion loads in Byrd and WD_3 corroborate these reports, with OM quantity and quality assessments, alongside traditional climate indicators, proving suitable as palaeoecological markers.

Although the limited data impose constraints on broader generalisations, the concentration, fluorescence intensity, and molecular composition of OM stored in the examined cores varied on glacial-interglacial timescales (Fig. 3). Fluorescent OM chemical species and intensities were consistent with a larger dataset of LGM and LD comparisons (D'Andrilli *et al.*, 2017). Byrd OM consisted of reduced chemical species of less degraded carbon character, indicative of greater bioavailability. Correlations between less degraded OM fractions (*i.e.* amino sugars, amino acids, and non-humic-like chemical species) and aridity have previously been reported (Scott *et al.*, 1998), agreeing with our interpretation of the OM molecular characteristics (higher chemical lability) found in the drier climate of the LGM. Conversely, OM in WD_3 was representative of more lignin-like species (Fig. 3). Although other compounds (carboxyl-rich alicyclic molecules and material derived from linear terpenoids) can occupy the lignin-like region on the van Krevelen diagram, the presence of more lignin-like species may reflect vegetation shifts between glacial-interglacial periods (Lamy *et al.*, 1999).

While we argue that transport and deposition patterns of biotic and abiotic material onto the ice sheet is the result of climate induced changes at the source, there is evidence for post-depositional OM processing in near surface environments (Antony *et al.*, 2017). Further, the chemical composition of OM is believed to drive microbial community structure and underlying metabolic strategies (McCarren *et al.*, 2010; Landa *et al.*, 2016). *Flavobacteria*, for instance, are largely involved in the degradation of amino acid-like OM (Kirchman 2002). Predicted lipid, carbohydrate, and amino acid pathways identified in *Flavobacteria* and *Actinobacteria* suggest genetic evidence for the utilisation of more bioavailable OM and its degradation products; traits which were significantly less ($P < 0.022$) prominent in *Bacillii* and *Deinococci* (Fig. 2). Hence, variations in OM composition between Byrd and WD_3 (Fig. 1c–d and Fig. S-2) could favour microbial communities based on their metabolic capabilities; a concept of community evolution supported by recent findings on microorganisms in Antarctic snow (Antony *et al.*, 2016). Although metabolic functions can be inferred from 16S RNA gene datasets (Bowman and Ducklow, 2015), obvious limitations exist compared to more detailed 'omics' studies; thus, our interpretations should be viewed as highly conservative, first approximations.

Overall, phylogenetic diversity in the Byrd and WD_3 cores were low compared to microbial communities found in Arctic ice during similar time periods (Miteva *et al.*, 2004, 2009, 2015), potentially from higher deposition rates on the

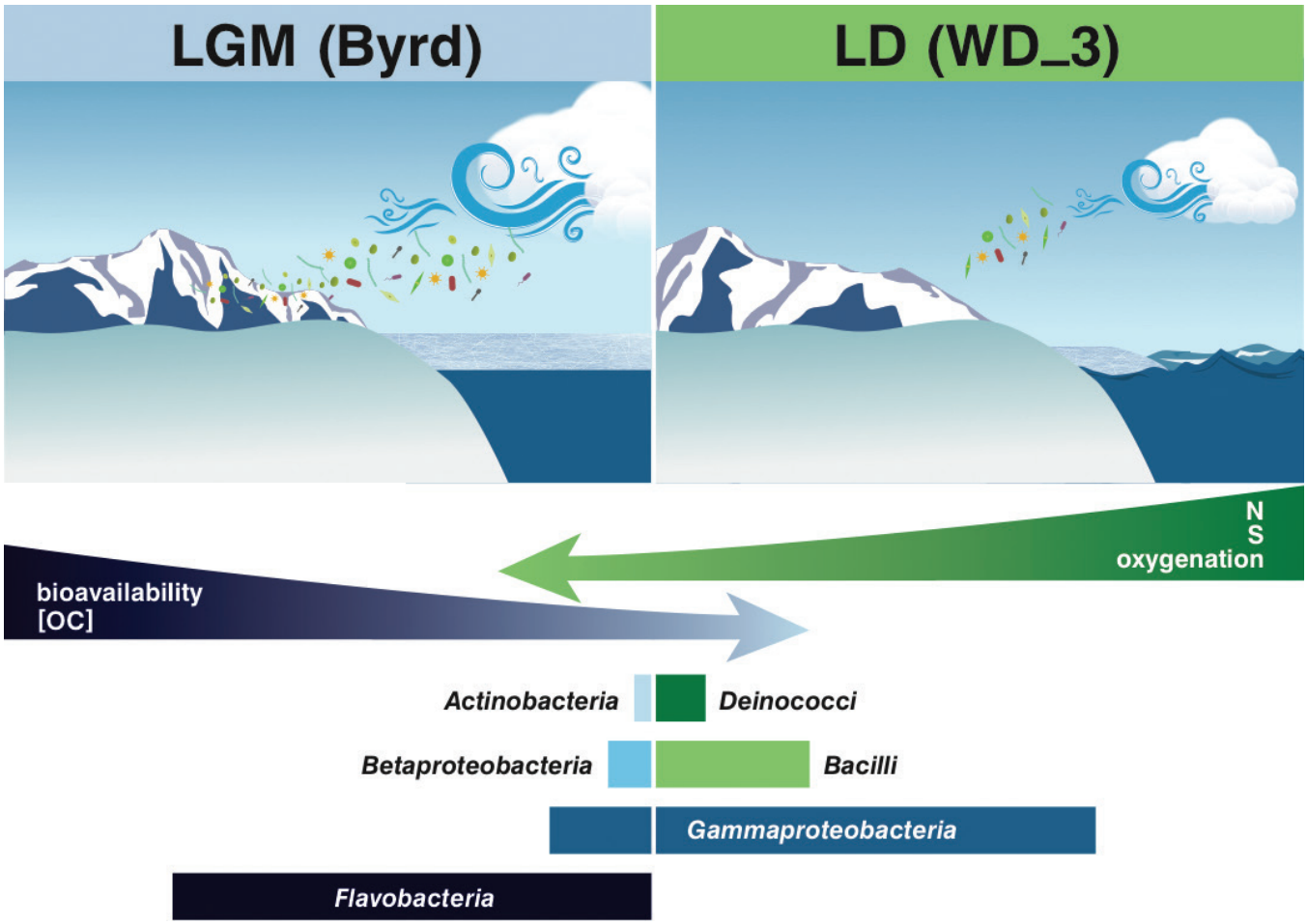

Figure 3 Schematic depicting Antarctic palaeoecological markers of organic carbon (OC) and microbial community structure from the Last Glacial Maximum (LGM) and last deglaciation (LD).

Greenland Ice Sheet (GIS) (Mahowald *et al.*, 1999). Winds from nearby land masses intersect the GIS (Biscaye *et al.*, 1997), while distant locations (*i.e.* Patagonia) have been suggested as the source for palaeo-deposits in Antarctica (*e.g.*, Sugden *et al.*, 2009). Assuming a positive correlation between dust and attached microorganisms in ice (Abyzov *et al.*, 1998; Miteva *et al.*, 2009), effects of climatic and environmental changes on their distribution would be expected. From their study on the vertical profile of microorganisms in ice, Zhang *et al.* (2006) concluded that less dust and microbial species were deposited during warmer periods. The three-fold lower numbers of OTUs and lower dust concentrations in the WD_3 core (Sigl *et al.*, 2016) follow this trend. It is important to note that although both cores were retrieved from West Antarctica (separated by ~161 km), aerosols over ice sheets may differ between locations (Rothlisberger *et al.*, 2000).

Conclusion

The onset of a warmer climate during the LD changed the southern polar front activity and ocean-atmospheric patterns circumventing Antarctica, inevitably affecting aerosol composition, long distance transport, and inland penetration of impurity-burdened air masses (Broecker and Denton, 1989; Morse *et al.*, 1998). Distinct chemical and biological signatures were detected in the Byrd and WD_3 ice cores, unique to each climate period. Major ion concentrations were consistent with values reported for Antarctic ice from different climate periods (Palais and Legrand, 1985; Rothlisberger *et al.*, 2000; Wolff *et al.*, 2010; Sigl *et al.*, 2016). Dissimilarities in cell and phylogenetic abundances, and OC concentrations collectively support our view of different deposition patterns onto the Antarctic Ice Sheet during distinct climate periods. Changes in the OM composition between the LGM and LD may further reflect changes in environmental sources (Lamy *et al.*, 1999). Differences in predicted metabolic capabilities are proposed to result from variations in LGM and LD OM composition. Therefore, similar to other palaeo-ecological materials, OM composition and microbial assemblages in ice may preserve past environmental conditions, and merit future investigation as palaeoclimate indicators.

Acknowledgements

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Additional Information

Supplementary Information accompanies this letter at www.geochemicalperspectivesletters.org/article1732

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