Mid-late Holocene vegetation response to climatic drivers and biotic disturbances in the Banni grasslands of western India

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Abstract

Tropical grasslands and savannas are globally extensive, and are of significant environmental, economic, and ecological importance. These ecosystems are anticipated to be particularly sensitive to future changes in climate, and understanding how these systems have responded to climatic changes in the past can provide us with insights into their potential responses to future global change. In this study, the temporal dynamics of C₃-C₄ vegetation changes in response to changes in moisture availability, local fire events and changing levels of herbivory in a summer-rainfall region of Western India are reconstructed for the past ~4600 cal yr BP. Paleodata such as stable carbon isotope of bulk organic matter (δ¹³Corg), oxygen isotope from carbonate shells (δ¹⁸Oshell), macro-charcoal and herbivore dung fungal spores are reported from the retrieved cores of two wetland sites located in the Banni grasslands of Western India. Results show that vegetation in the Banni was composed mostly of C₃ vegetation from ~4600 to ~2500 cal yr BP, after which there was a decline in C₃ vegetation. From the late-Holocene to the present, there was a mix of both C₃ and C₄ vegetation, with C₄ grasses being more abundant in the ecosystem. These shifts were coincident with rainfall changes from more mesic conditions during ~4600 to ~2500 cal yr BP to more arid conditions towards the present as indicated by δ¹⁸Oshell isotope data. The period of increase in C₄ vegetation also coincides with a period of increased biotic disturbances in the ecosystem, particularly fire. Given the current scenarios of global warming, recurrent drought events and increased anthropogenic use of similar ecosystems, such studies can provide us insights into potential future trajectories of these ecosystems.
Keywords: tropical grassland; charcoal; dung fungal spore; isotope; monsoon; paleoecology.

1. Introduction

Tropical grasslands occupy ca. 20 % of the global land area (Ramankutty and Foley, 1999) and provide critical ecosystem services to nearly one fifth of the world’s human population (Scholes and Archer, 1997). Climate and land-use practices play a dominant role in maintaining the biodiversity and structural integrity of these ecosystems (Hansen et al., 2001; Sankaran et al., 2004, 2005; Bond, 2008; Bond and Parr, 2010), and it is thus not surprising that tropical grasslands are believed to be amongst the most sensitive biomes to future changes in climate and land-use (Sala et al., 2009; Morgan et al., 2011). However, the impacts of global environmental change on arid and semi-arid grasslands remain poorly understood compared to other ecosystems (Parr et al., 2014).

The composition and structure of grasslands and savannas are known to be controlled by multiple factors including resource availability (water and nutrients), disturbance regimes (fire and herbivory) and climatic variability (temperature and precipitation) (Scholes and Archer, 1997; Onkware, 2000; Sankaran et al., 2004, 2005; Bond, 2008; Lehmann et al., 2014). However, the relative importance of these drivers of vegetation change, and the way they interact to maintain vegetation phases or drive vegetation transitions, is still debated (Gillson and Ekblo, 2009). Even while a common set of factors determine the structure of these ecosystems across the globe, these factors interact differently across regions, such that site specific environmental and evolutionary histories drive regional variation in the relationship between woody vegetation, fire and climate (Lehmann et al., 2014). In this context, studies that span longer time scales of centuries or millennia are useful for developing site-specific understanding of factors driving historical changes in vegetation and ecosystem dynamics.
Holocene paleo-ecological studies of tropical semi-arid savannas of Africa, South America and Australia show that the mid- to late-Holocene period was marked by human settlements, settled agriculture, the introduction of pastoralism (e.g., Cochrane, 2010; Birks et al., 2014) and rainfall fluctuations (Dykoski et al., 2005; Birks et al., 2014). An investigation from a South African savanna region reveals that these ecosystems historically alternated between grassland phases maintained by lower levels of water availability, herbivory and less frequent fire events and woody-savanna vegetation phases maintained by increased water availability, herbivore browsing of tree communities, and more frequent/ intense fires (Gillson and Ekblom, 2009). Such studies, which use multiple paleoecological and paleoclimatic proxies like stable isotope ratios of carbon and oxygen (Lisiecki and Raymo, 2005; West et al., 2006), charcoal (Gavin et al., 2007; Colombaroli et al., 2014) and herbivore dung fungal spores (Ekblom and Gillson, 2010; Baker et al., 2012) establish clear links between past climate, disturbance regimes and vegetation changes. However, the timing and magnitude of climatic and disturbance factors are variable across tropical grasslands globally, and further, the effects of these factors on vegetation are also variable, depending on whether systems are mesic or arid (Veldman et al., 2015).

Quaternary paleoecology studies from the arid, semi-arid and sub-humid zones of Western India report shifts towards more arid climates and vegetation from the mid-Holocene (Singh et al., 1974; Prasad and Enzel, 2006; Prasad et al., 2014b). However, such climatic shifts are spatially and temporally variable across the region (Singh et al., 1974; Prasad et al., 1997; Enzel et al., 1999; Prasad and Enzel, 2006; Roy et al., 2009; Laskar et al., 2013; Prasad et al., 2014b). Further, archaeological studies suggest qualitative links between livelihood activities of human settlements and local environments through the Holocene (Possehl, 1999, 2002; Madella and Fuller, 2006). In this study, we examine mid-late Holocene changes in the climate, disturbance and vegetation dynamics of the Banni grassland, an edaphic as well as
climate-determined grassland in Western India. The Banni, one of the largest grassland ecosystems in Asia, has historically supported high levels of livestock and pastoralism, and continues to do so at the present time despite widespread degradation resulting from decades of erratic rainfall, invasive species spread and changes in edaphic and hydrological conditions. Here, we reconstruct the past C₃-C₄ vegetation dynamics in Banni using bulk organic carbon isotope records and past drivers of vegetation change using oxygen isotope analysis from carbonate shells, macro-charcoal analysis and herbivore dung fungal spore analysis from lacustrine sediments. Additionally, we used the lipid biomarker (n-alkane) distribution in the Banni core sediments to disentangle organic matter contributions to sediments. This study is first of its kind from the region, using multiple-proxies to develop a comprehensive understanding of environmental and paleo-grassland dynamics of the region from the mid- to late- Holocene. The questions that we try to address through this study are: 1) How did the balance of C₃-C₄ vegetation of the Banni grasslands change through the mid- to late- Holocene? 2) What was the role of past climatic fluctuations in mediating these vegetation changes? and 3) How were these vegetation changes related to past changes in disturbance regimes such as fire and herbivory?

2. Study Area-location, climate and vegetation

The Banni grassland (23°19’ to 23°52’ N latitude and 68°56’ to 70°32’ E longitude; Fig. 1), located south of the marshy salt flats of the Rann of Kachchh in the state of Gujarat in Western India, covers an area of nearly 3847 km² (Fig. 1). The region receives an average annual rainfall of ~317 mm from the southwest summer monsoon (Gujarat Institute of Desert Ecology (GUIDE), 1998). Temperatures in the Banni range between 49 °C in summer (May-June) and 10 °C in winter (January - February) (GUIDE, 1998).
**Fig 1.** (A) Locations and topography of sampling sites - Chachi & Luna and (B) Schematic representation of vegetation types around the wetlands (after GUIDE, 2011).

The Banni is a ‘grass and shrub savanna’ ecosystem in which grasses, shrubs, herbs and trees coexist in a mosaic of grassland and woodland patches (Patel and Joshi, 2011; Sankaran and Ratnam, 2013). The understory vegetation is composed of herbs, both salinity tolerant and intolerant (89 species comprising ~46 % of all the plant species in the site), grasses (37
species, ~19 % of all plant species) and shrubs (31 species, ~16 % of all plant species) (Patel and Joshi, 2011). There are 17 species of trees which account for ~9 % of all the plant species (Patel and Joshi, 2011) and the remaining 10 % is composed of other life forms such as climbers and sedges. The ecosystem supports nearly 17000 people and 57,000+ livestock (GUIDE, 2010) and comprises ~45 % of the permanent pasture and 10 % of the grazing land available in the state of Gujarat (Parikh and Reddy, 1997). The study area is located close to the zones of the early-mid Holocene Indus-valley civilization and is believed to have supported pastoralism for several centuries. In addition, the ecosystem is also shaped by its abiotic regime including recurring droughts, high annual seasonality and high soil salinity. The low elevation of the region and the lack of pronounced topographic gradients results in flooding and water logging across large sections of the Banni during the rainy season. Soils are generally fine textured with a high proportion of silt and clay (Singh and Kar, 2001), resulting in low permeability. The Banni is also characterized by the presence of several natural wetlands, which makes it a good model system for paleoecological studies.

3. Methods

3.1. Selection of study sites and sampling

Two sediment cores, one each from the Chachi wetland in the Eastern Banni (23°32' 46.788" N and 69° 51'9.936" E) and the Luna wetland from the Western Banni (23°42' 21.3834" N and 69°15'38.268" E) were collected for the study (Fig. 1). These are closed shallow wetlands fed by surface runoff during the monsoonal period. Water level in the wetland fluctuates in response to variability in precipitation with higher lake levels observed during the monsoon period and wetland drying occurring during drought conditions (Fig. 1). The topography of the study area is not highly variable (<15 m) with the catchment area of
the wetlands not more than 2 km in radius (Fig. 1A). Sediment cores were sampled from the deeper section of the lakes in summer when the lakes were completely dry.

The Chachi wetland is a shallow wetland with an area of ~17.5 km$^2$ and depth ranging from ~0.5 to 2 m. Sediment samples were collected from the walls of a trench at 5 cm intervals up to a depth of ca. 70 cm, and a PVC pipe of 6 cm diameter was used to collect sediments below (70 cm up to 142 cm depth), which were then subsampled at 4 cm intervals (Fig. 2A). Based on the lithology, the entire Chachi profile was divided into four sections: brown clay with organic matter (0 to ~74 cm), brown silty clay with silty patches between 74 cm and 95 cm, followed by dark brown silty clay up to 142 cm. The intermediate layers between 116 cm and 126 cm were composed of dark brown silty, sandy clay with black clayey patches. Mollusc shells were present throughout the core and the salinity (measured using Thermo Orion 5-Star multi-parameter meter) was 8.2 psu for the surface layers, which decreased to 1.3 psu in the deeper layers. The Chachi wetland is surrounded by Suaeda scrub and grassy vegetation with sparse cover of Prosopis juliflora and other herbaceous vegetation (Figs. 1B and 2A).

The Luna wetland is a shallow wetland with an area of ~0.02 km$^2$ and depth ranging from 0.5 m in the fringes to ~3 m towards the centre. Sediment samples up to 60 cm were collected from the walls of a trench followed by coring with a PVC pipe of 6 cm diameter up to 85 cm depth. The entire profile was sub-sampled at 6 cm intervals. Based on sediment lithology, the Luna core was divided into two major sections: (i) brown silty clay from the surface to 40 cm depth with gastropod shells in the surface layers (ii) brown clay with silt beneath. The salinity of the surface sediment was 2.5 psu which decreased to 0.2 psu in the deepest layer. The Luna lake is located in a grassland with other herbaceous taxa and sparse to moderate density of Prosopis juliflora (Figs. 1B and 2B).
The analyses of various proxies such as $\delta^{13}$C$_{org}$, macro-charcoal and herbivore dung fungal spore analysis in Chachi and Luna core sediments were undertaken on 30 and 15 samples respectively. However, $\delta^{18}$O$_{shell}$ was not analysed from Luna core, and $\delta^{18}$O$_{shell}$ values of Chachi core sediments were limited to 21 samples due to the absence of well-preserved carbonate shells (Cerithium sp) in the sub-samples.

3.2. Analytical methods

3.2.1. $\delta^{13}$C$_{org}$ isotopic analysis from organic sediments

$\delta^{13}$C$_{org}$ values have been widely used to estimate relative abundances of C$_3$ (mainly trees and shrubs) versus C$_4$ plant cover (grasses) in the past owing to differences in isotopic fractionation that arise from different carbon fixation pathways (Cerling et al., 1989; Leng and Marshall, 2004; Sanyal et al., 2004, Basu et al., 2015). For carbon isotope ratios and weight percent TOC analysis, powdered core sediment samples were pre-treated with 0.5 N HCl to remove inorganic (carbonate) carbon, following which samples were centrifuged in deionized water. The $\delta^{13}$C$_{org}$ and TOC (%) were measured in duplicate using an elemental analyzer (Flash EA 2000) coupled via a ConFlow IV interface on a MAT 253 (Thermo Fisher
Scientific) IRMS (Isotope Ratio Mass Spectrometer). Around 6 mg of sample material was weighed into tin capsules and introduced into the pre-filled conditioned reactor of an elemental analyzer (Flash EA 2000) through an auto-sampler. The sample filled tin capsule was flash-combusted at 1050 °C in an oxygenated environment, and the CO₂ produced during combustion introduced online into the MAT 253 Continuous Flow IRMS. The calibration and reproducibility of data was determined using Cellulose (IAEA-CH-3, −24.724±0.041 ‰) standard. The reproducibility for replicate analyses was ±0.1 ‰ for δ¹³Corg and all the δ¹³Corg data are reported relative to the VPDB (Vienna PeeDee Belemnite) scale.

3.2.2. Macro-charcoal analysis

Macro-charcoal, an important proxy for local fire events was extracted from all the sediment layers. The sediment samples were wet sieved in a 150 µm mesh sieve. The residue was then soaked in 5 % metaphosphate solution followed by soaking in 8 % hydrogen peroxide solution for 12 hrs to concentrate charcoal particles and bleach all non-charcoal organic matter in the sediment (Stevenson and Haberle, 2005; Schlachter and Horn, 2010; Colombaroli et al, 2014). Washed samples were then scanned under a stereomicroscope at a magnification of 15x to identify and count the charcoal particles. Charcoal accumulation rate (CHAR) was then estimated by multiplying the macroscopic charcoal concentrations (count/cm³) by sedimentation rate (cm/yr) giving number of particles cm⁻² yr⁻¹ (Zhang et al., 2015; Iglesias et al., 2016) for the samples. We have refrained from the use of CHAR values to statistically estimate the paleofire frequency of the region as our sampling resolution was relatively low (Blarquez et al., 2013; Yu et al., 2016). However, this proxy provides important information about paleofire activity in the area (Aleman et al., 2013).

3.2.3. Herbivore dung fungal spore analysis
Fossil spores of coprophilous fungi or herbivore dung fungi such as Sordaria-, Sporomiella-, Coniochaeta- type etc. (Fig. S1 in the Supplementary data) provide valuable information about past levels of herbivory in an ecosystem (Raper and Bush, 2009; Ekblom and Gillson, 2010; Baker et al., 2013). Fungal spore extraction was carried out following the standard protocols of Faegri and Iverson (1989) & Bennett and Willis (2001) involving a series of acid treatments (Hydrochloric acid (HCl) and Hydrofluoric acid (HF) and acetolysis (using a mixture of acetic anhydride and sulphuric acid (H₂SO₄) (Erdtman, 1943). After chemical extraction, slides were prepared and scanned under a compound light microscope (500x). Based on pollen rarefaction curves generated from both the cores (Fig. S2 in the Supplementary data), fungal spores were recorded until 600 pollen grains were counted from the Chachi core and until 150 pollen grains were counted from the Luna core (Birks and Line, 1992; Giesecke et al., 2012; Baker at al., 2013; Ekblom and Gillson 2010). Spore values are presented relative to the total pollen sum in each sediment layer (Ekblom and Gillson 2010). Fungal spores were identified based on published literature (van Geel et al., 2003; Graf et al., 2006; Ekblom and Gillson, 2010; Wood et al., 2011; Baker et al, 2013 and references therein).

3.2.4. Oxygen Isotope (δ¹⁸Oᵦₑᵦₑ) analysis from carbonate shells

The δ¹⁸O of endogenic carbonates in closed wetlands of the tropics are controlled by changes in precipitation and evaporation (Leng and Marshall, 2004). Oxygen isotopes were measured on the gastropod shell Cerithium sp. Powdered shell samples (ca. 0.2 to 0.5 mg) were reacted with 3 to 5 droplets of 99 % H₃PO₄ (MERCK) at 72 °C in the GasBench II (Thermo Fisher Scientific) equipped with a GC-PAL autosampler. Oxygen isotope ratios were measured from the evolved CO₂ in a MAT 253 IRMS. The CaCO₃ standard Z-Carrara (procured from Physical Research Laboratory, Ahmedabad, India) was calibrated via NBS-18 (δ¹⁸O = −23.2 ± 0.1 ‰) and provided an external precision of ± 0.1 ‰ (2σ) for δ¹⁸Oᵦₑᵦₑ values.
The oxygen isotope values of carbonate samples in this paper are reported in the conventional δ notation relative to the international standard VPDB.

3.2.5. n-alkane analysis

The distribution of n-alkanes in lacustrine sediments has been widely used as a proxy for identifying sources of organic matter (Meyers, 2003, Basu et al., 2017 and references therein). Approximately 7–8 g of powdered sediment samples was extracted for lipid biomarkers. Sediment samples were taken in a clean stainless steel cell and total lipid extracted (TLE) in an accelerated solvent extractor (Dionex, ASE350) using a dichloromethane/methanol (93:7) mixture at 100 °C and 1600 psi pressure for 15 min (2 cycles). The extracted TLE was concentrated in a Rotavapour (R-210, Buchi) by evaporating the dichloromethane and methanol mixture. The non-polar hydrocarbon fraction (n-alkane) was then separated from the TLE using silica gel column chromatography. The obtained n-alkane was concentrated through dry N$_2$ and made up to 0.5 ml (Ghosh et al., 2017).

The relative concentration of n-alkanes was measured using a Gas chromatography system (Agilent 7890A, GC system) equipped with a non-polar capillary column (HP5-MS, 30 m × 250 µm × 0.25 µm) and flame ionized detector (FID). Sample injection was carried out in 1:1 split mode with initial inlet temperature at 320 °C. The GC oven temperature started at 60 °C (held for 2 min) and was increased to 320 °C at 8 °C/min (held for 12 min). Individual n-alkanes were identified based on the characteristic retention time obtained from the Fluka n-alkane standard mixture (C$_{10}$–C$_{40}$; part no. 68281).

3.3. Quantification of organic sources using n-alkane indices

The calculation of the n-alkane indices was conducted based on carbon chain length variations specific to organic sources (Meyers, 2003). Long-chain n-alkanes (C$_{27}$–C$_{33}$) are well known as a source indicator of terrestrial organic matter, and short chain n-alkanes less
than C\textsubscript{21} are attributed to algae and photosynthetic bacteria (Cranwell, 1987; Meyers, 2003). Submerged and emergent aquatic plants are the main producers of mid-chain (C\textsubscript{21}, C\textsubscript{23}, and C\textsubscript{25}) n-alkanes (Ficken, 2000).

\(P_{\text{aq}}\), an n-alkane based index, was calculated to characterise organic matter sources (Ficken, 2000) for the Chachi core sediment samples. \(P_{\text{aq}}\) approximates the proportions of aquatic macrophytes (C\textsubscript{23} + C\textsubscript{25}) vs. emergent and terrestrial plant (C\textsubscript{23}+ C\textsubscript{25} + C\textsubscript{29} + C\textsubscript{31}) inputs to lacustrine sediments (Ficken, 2000). \(P_{\text{aq}}\) values >0.4 indicate a dominant fraction of sedimentary n-alkanes derived from submerged/floating plants (Ficken, 2000).

### 3.4. Chronology

Chronology of sediments were derived from \(^{14}\text{C}\) AMS radiocarbon dating of bulk organic matter from four sediment layers of the Chachi and three layers of Luna cores. Dating was carried out at the Radiochronology lab in the University of Laval, Canada. The calibration of radiocarbon dates was carried out using the OxCal 4.1 software (Bronk Ramsey, 2008; Bronk Ramsey and Lee, 2013) with the IntCal 13 calibration curve (Reimer, 2013). Age-depth models for the cores were developed by linear interpolation of the calibrated ages using the program CLAM 2.2 (Blaauw, 2010) in the R language environment (R Core Team, 2016) (Fig. 3).
3.5. Data analysis

Proxies used in this study for vegetation change ($\delta^{13}$C$_{org}$) and its drivers such as fire (CHAR) and herbivory (herbivore dung fungal spore) from both the sediment profiles were plotted as time series and the trend components of each of these time series were estimated by smoothing using a simple moving average of the order five using the ‘‘TTR’’ library (Ulrich, 2016) in the statistical package R (R Core Team, 2016).

We used a multiple linear regression with $\delta^{13}$C$_{org}$ value as response variable and CHAR, dung fungal spore abundance and $\delta^{18}$O$_{shell}$ as predictor variables to test for the effects of these biotic drivers on vegetation change. All three variables were used as predictors of $\delta^{13}$C$_{org}$ for the Chachi core, while only CHAR and herbivore dung fungal spore abundance were included for the Luna core given that $\delta^{18}$O$_{shell}$ values were not available for this core.

4. Results

4.1. Chronology of the sediments
AMS radiocarbon ages of organic carbon from four depths in Chachi and three depths in Luna profile are shown in Table 1. The “hard water effect” associated with the carbonate dominated lake catchment can result in anomalously old dates for bulk organic matter (Anoop et al., 2012; Mischke et al., 2013). However, the organic matter in the Banni core sediments were predominantly derived from terrestrial vascular plants (see section 5.1.2) that draw CO$_2$ from the atmosphere alone (in equilibrium with the ambient $^{14}$C activity). Hence the $^{14}$C dates from the Banni core sediments are free of any “hard water effect”. The $^{14}$C AMS dates from Chachi core are stratigraphically consistent, and linear interpolation shows that the core spans ca. 4600 cal yr BP.

In the Chachi lake profile, sediment accumulation rates are high until ca. 2500 cal yr BP (0.037 cm/yr) followed by gradual decrease towards the present (0.018 cm/yr) (Fig. 3A). In the Luna profile, sediment accumulation rates were relatively high across the profile (ranges between 0.04 and 0.096 cm/yr) with the deeper layer dating back to only 997 cal yr BP (Fig. 3B).

### Table 1. AMS radiocarbon dates from Chachi and Luna lake profiles

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Depth (cm)</th>
<th>Material</th>
<th>Laboratory code</th>
<th>$^{14}$C Age (yr BP)</th>
<th>Age cal yr BP (99.7%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chachi</td>
<td>25</td>
<td>Organic sediment</td>
<td>ULA-5049</td>
<td>1595±20</td>
<td>1472±40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>Organic sediment</td>
<td>ULA-5050</td>
<td>1895±20</td>
<td>1844±25</td>
</tr>
<tr>
<td></td>
<td>108</td>
<td>Organic sediment</td>
<td>ULA-5051</td>
<td>2770±20</td>
<td>2862±35</td>
</tr>
<tr>
<td></td>
<td>134</td>
<td>Organic sediment</td>
<td>ULA-3988</td>
<td>3910±20</td>
<td>4350±40</td>
</tr>
<tr>
<td>Luna</td>
<td>24</td>
<td>Organic sediment</td>
<td>ULA-5229</td>
<td>600±15</td>
<td>602±30</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>Organic sediment</td>
<td>ULA-4844</td>
<td>1015±15</td>
<td>938±10</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>Organic sediment</td>
<td>ULA-5230</td>
<td>1085±15</td>
<td>995±30</td>
</tr>
</tbody>
</table>
4.2. $\delta^{13}\text{C}_{\text{org}}$ and TOC analysis

The TOC values for the Chachi core ranges from 0.22 % to 1.3 %. The $\delta^{13}\text{C}_{\text{org}}$ values for the Chachi core show a mean of −23.1 ‰ (range: −32.3 ‰ to −20.3 ‰), with more negative values before ~2500 cal yr BP (Fig. 4A). The most negative $\delta^{13}\text{C}_{\text{org}}$ values occurred between 4000 and 2500 cal yr BP. The $\delta^{13}\text{C}_{\text{org}}$ values for the period from 2500 cal yr BP to the present ranged from −24.5 ‰ to −20.5 ‰ (Fig. 4A).

The TOC content of the Luna sediments fluctuates between 0.38 % and 0.99%. The $\delta^{13}\text{C}_{\text{org}}$ values for the Luna core show a mean of −18.5 ‰ (range: −20.9 ‰ to −17.0 ‰) (Fig. 4B). From ~1000 to ~650 cal yr BP, values ranged from −17.0 to −17.9 ‰, followed by a gradual decline from ~650 cal yr BP (Fig. 4B).

4.3. $\delta^{18}\text{O}_{\text{shell}}$ analysis

The $\delta^{18}\text{O}_{\text{shell}}$ values for the Chachi core range from −5.3 ‰ to +2.5 ‰ with an average value of −1.7 ‰. Lower isotope values were recorded before ~2700 cal yr BP (Fig. 4A). The highest $\delta^{18}\text{O}_{\text{shell}}$ values occurred during the time from ~2700 cal yr BP to ~1500 cal yr BP, followed by decreased values towards the present (Fig. 4A).

4.4. CHAR analysis

The analysis of CHAR (macro-charcoal particles cm$^{-2}$ yr$^{-1}$) from the Chachi core shows an increasing trend from ~3000 to ~1000 cal yr BP, followed by a decline (Fig. 4A). Likewise, CHAR for sediment in Luna shows a steep decline from ~1000 cal yr BP (Fig. 4B).

4.5. Herbivore dung fungal spore analysis

Common dung fungal spores recorded from the Chachi profile were Sporomiella - , Coneochaeta-, Sordaria- and Pleospora- types, and those from the Luna core included
Coneochaeta-, Cercophora-, Sordaria- and Arnium- types. Herbivore dung fungal spores were more abundant in sediments after ~1500 cal yr BP when compared to earlier time periods (Fig. 4A). However, from ~1000 cal yr BP, fluctuations in herbivore dung fungal spore abundances were more pronounced in the Chachi sediments (Fig. 4A) compared to the Luna sediments (Fig. 4B).

**Fig 4. Proxies in Chachi (A) and Luna (B) cores.** Carbon isotope ratios (δ^{13}C_{org}), Total organic carbon (TOC), Oxygen isotope ratios (δ^{18}O_{shell}), CHAR and herbivore dung fungal spore relative abundance are plotted against age. Dashed black lines represent five-point moving average filters.

### 4.6. n-alkane index

The calculated P_{aq} index based on chain length distributions ranged from 0.07 to 0.33 in the Chachi core sediments, with an average of 0.18 (Table S1 in the Supplementary data).

### 4.7. Role of CHAR, herbivore dung fungal spore and δ^{18}O_{shell} values on δ^{13}C_{org} values
Multiple regression analyses of the relationships between CHAR, herbivore dung fungal spore abundance, $\delta^{18}$O$_{sh}$ shell values and $\delta^{13}$C$_{org}$ values revealed that CHAR alone had a significant positive effect on $\delta^{13}$C$_{org}$ values ($p = 0.047$) in the Chachi core. Similarly, CHAR had a marginally significant effect on $\delta^{13}$C$_{org}$ values in the Luna core ($p = 0.09$). Thus, in both the Chachi and Luna cores, periods of greater relative abundance of C$_4$ vegetation (higher $\delta^{13}$C$_{org}$ values) were associated with higher fire frequencies.

5. Discussion

This multiproxy paleoecological study is amongst the first of its kind from the region, and sheds light on the drivers of long-term vegetation change in this tropical grassland ecosystem in Western India. The proxy for vegetation composition in Banni grassland, $\delta^{13}$C$_{org}$ shows more depleted values from ~4600 to ~2500 cal yr BP and more enriched values from ~2500 cal yr BP towards the present. These values are, in turn, associated with changes in proxies for other environmental factors including rainfall ($\delta^{18}$O$_{shell}$), fire (CHAR) and the levels of herbivory (herbivore dung fungal spore abundance).

5.1. Inferring paleo-rainfall and vegetation dynamics from stable isotope proxies

5.1.1. $\delta^{13}$C$_{org}$ values - proxy for C$_3$-C$_4$ vegetation

We contend that changes in terrestrial C$_3$ vs. C$_4$ plant abundances are likely to have been the major factor controlling $\delta^{13}$C$_{org}$ values in the Banni core sediments in our study. The $\delta^{13}$C isotopic composition of organic matter is predominantly used for source (C$_3$ versus C$_4$) apportionment in limnological systems to understand paleoclimate changes (e.g., Leng and Marshal, 2004). $\delta^{13}$C$_{org}$ values of C$_3$ plants typically lie between $-31.5$ ‰ to $-23$ ‰ (average $-28.57$ ‰), whereas C$_4$ plants show $\delta^{13}$C values of $-16$ ‰ to $-10$ ‰ (average $-13$ ‰) (Cerling et al., 1989; Cerling et al., 1997; Kohn, 2010, Basu et al., 2015). However, in lacustrine environments, $\delta^{13}$C$_{org}$ value may be influenced by (i) diagenetic changes in organic matter
(Macko and Estep, 1984) and (ii) eutrophication resulting in enriched $\delta^{13}C$ value of aquatic organic matter (Meyers, 1997; Leng and Marshall, 2004). Various workers have interpreted the down core increases and decreases of $^{13}C_{org}$ in terms of diagenetic alteration (Spiker and Hatcher, 1984; McArthur et al. 1992; Muzuka and Hillaire-Marcel, 1999). However, the fluctuating values of TOC observed throughout the Luna and Chachi cores indicates an absence of diagenetic alteration of organic matter. Similarly, we also exclude the role of eutrophication in the $\delta^{13}C_{org}$ variations of Chachi wetland as the low $P_{aq}$ values for the Chachi core sediments (Table S1 in the Supplementary data) indicate a predominance of land derived vegetational source in the sediment rather than aquatic productivity, suggesting that changes in $\delta^{13}C_{org}$ values in the Banni core sediments are likely to have been predominantly driven by changes in C$_3$ vs. C$_4$ plant abundance, with more C$_3$ signals from ~4000 to 2500 cal yr BP and more C$_4$ signals from ~2500 cal yr BP to the present.

5.1.2. $\delta^{18}O_{shell}$ values - proxy for rainfall

The oxygen-isotopic composition of carbonate shells is controlled by (i) the $\delta^{18}O$ values of the meteoric water, (ii) temperature at the time of carbonate precipitation, and (iii) balance of precipitation to evaporative loss (P/E ratio) (Talbot, 1990; Valero-Garcés, 1999; Leng et al., 2005; Dixit et al, 2014). The oxygen isotopic composition of carbonates in equilibrium shows a decrease of about 0.24‰ with a 1 °C increase in temperature (Craig, 1965). The observed ~8‰ (~5.3‰ to +2.5‰) variations in $\delta^{18}O_{shell}$ values in Chachi would therefore require a temperature change in the region >30 °C. Thus, we exclude temperature change as a potential factor controlling $\delta^{18}O_{shell}$ variations in the Chachi core as the estimated temperature change (>30 °C) is too large to be attributed to Holocene temperature changes for the region.

Besides temperature changes, the residence time of water in limnological systems is particularly important as evaporation processes could modify the isotopic composition of lake
water derived from precipitation (Leng and Marshall, 2004; Anoop et al., 2013). Variations in the balance of precipitation to evaporative loss (P/E ratio) have a significant effect on the isotope balance of lakes in arid regions, with enriched (high) values reflecting preferential evaporative loss of $^{18}$O (Talbot, 1990; Holmes, 2007). Variation in the total amount of monsoonal precipitation in the Banni region could have affected lake-water $\delta^{18}$O values by changing rainfall $\delta^{18}$O values and altering the hydrologic balance between evaporation and precipitation (P/E ratio) in the lake system. Previous investigations on the $\delta^{18}$O of the gastropod M. Tuberculata shells from the paleolake bed at Kotla Dahar in arid Northwest India have similarly interpreted changes in $\delta^{18}$O$_{shell}$ values in terms of variations in $\delta^{18}$O values of the lake water (P/E ratio) corresponding to changes in the Indian summer monsoon rainfall (Dixit et al, 2014). Depleted $\delta^{18}$O$_{shell}$ values in the Banni core sediments between ~4600 to 2700 cal yr BP are similarly likely to be associated with periods of high monsoonal rainfall, whereas with periods of reduced monsoonal rainfall from ~2700 cal yr BP towards the present resulting in enriched $\delta^{18}$O$_{shell}$ values.

5.2. Paleo-grassland dynamics and drivers of vegetation change

Data on $\delta^{18}$O$_{shell}$ values for Chachi suggest that this region experienced high rainfall from ~4600 to ~2700 cal yr BP, followed by an overall decline in rainfall till about ~1500 cal yr BP, while the period from ~1500 yr BP to present experienced a slight increase in precipitation. Overall, there appears to have been a gradual shift from more mesic to more arid conditions from ~4600 cal yr BP to the present.

Rainfall is a major regional driver of vegetation change, and $\delta^{13}$C$_{org}$ values across this same period show that vegetation composition in Banni grasslands remained a mixture of C$_3$ and C$_4$ vegetation throughout the mid-late Holocene, with a prominence of C$_3$ vegetation before ~2500 cal yr BP when the region received more rainfall. After ~2500 cal yr BP,
corresponding to a period of decreased rainfall, increased fire and herbivory, the vegetation shows a gradual shift to a C₄ dominated plant community. This trend reverses again with an increase in C₃ vegetation from ~1000 cal yr BP, alongside a slight increase in rainfall and a decrease in fire activity.

While these overall trends in C₃-C₄ vegetation change, rainfall, fire and herbivory show that there was an increased abundance of C₃ vegetation with more rainfall, and a dominance of C₄ vegetation with increases in aridity and fire, this is not a one to one correlation. This may either be because biotic responses have a time lag with respect to the environmental shifts (Vegas-Vilarrúbia et al., 2011) or because the multiple drivers of vegetation change and their complex interactions varied across this period.

The higher δ¹³C_{org} values indicative of dominance of C₄ vegetation after ~2500 cal yr BP with increases in aridity and fire (Fig. 4A) are however consistent with the known fact that drier conditions and frequent fires promote and maintain fire tolerant C₄ grasses in grassland and savanna ecosystems (Higgins et al., 2000; Bond et al., 2003; Parr et al., 2014). The transition to C₄ dominance ~2500 cal yr BP was also correlated with an increase in the levels of herbivory experienced in the system (Fig. 4A). Previous studies have established the presence of mature Harappan sites adjacent to Banni grassland during mid- to late- Holocene (Enzel et al., 1999; Prasad and Enzel, 2006; Prasad et al., 2014b; Raj et al., 2015). Literature on anthropogenic activities in Western India and adjacent regions suggest the geographical expansion of agricultural and pastoral communities from the hills of Baluchistan, North western Frontiers and Sindh during the mid- to late- Holocene further into the plains and newer sites as human population densities increased (Madella and Fuller, 2006), and late Holocene increases in aridity led to less arable hydro-climates in several regions (Madella and Fuller, 2006; Asouti and Fuller, 2008). This might have caused an increase in disturbance
factors such as fire and herbivory in the Banni grassland. The increases in CHAR and dung fungal spores (indicative of increased local fire and herbivory respectively) in the Chachi core from ~2500 cal yr BP are consistent with increased human activities in the landscape. It is therefore likely that both climatic and anthropogenic factors cumulatively influenced the vegetation composition over this period.

After ~1000 cal yr BP, there is an increase in the C$_3$ signal in both the Chachi and Luna cores, coincident with a marginal increase in rainfall and a decrease in fire activity. The decline in fire events could be indicative of changes in livelihood activities of the inhabitants in the landscape. Further, although the levels of herbivory have been fluctuating during this period, herbivore dung fungal spore abundance in sediments after ~1500 cal yr BP is typically much higher than before suggestive of increased livestock grazing in the landscape over the recent past. Increased C$_3$ abundance after ~1000 cal yr BP could also be a consequence of greater grazing pressure in the system, which has been shown to be associated with shrub encroachment in many rangeland systems (Roques et al., 2001; Eldridge, 2011).

5.3. **Regional comparisons of climate data through the Holocene**

Several studies on mid- to late-Holocene precipitation trends in the arid and semi-arid zones of Western India suggest a shift from mesic to arid conditions after ~3000 cal yr BP (Singh et al., 1974; Prasad et al., 1997; Roy et al., 2009; Prasad et al., 2014b; Banerji et al., 2015). Evidence from carbon isotopes of sedimentary leaf waxes from Godavari river catchment in central India also suggests that there was a consistent increase in aridity from ~4000 cal yr BP towards the present (Ponton et al., 2012). The high-resolution Holocene paleoclimate study from Lonar lake, central India provides evidence of prolonged drought between 2000 to 600 cal yr BP (Prasad et al., 2014a). A recent geochemical study from
Eastern India shows increased precipitation between 6800 and 3100 cal yr BP followed by a reduction in monsoonal strength towards the present (Ankit et al., 2017). Records from Western and NW Himalayas also show evidence of lower moisture availability during the mid- to late-Holocene from ~2700 cal yr BP to the present (Leipe et al., 2014; Mishra et al., 2015; Demske et al., 2016). According to our findings, the period between ~2700 to ~1000 cal yr BP was the most arid time period in the Banni with the climate ameliorating slightly after this towards the present (Fig 5).

Previous studies from other regions in the tropics also suggest increased monsoonal precipitation in the early and mid-Holocene in the northern tropics of Africa and Asia caused by high northern hemispheric summer insolation followed by a period of increased aridity caused by the southward shift in the summer positions of northern hemisphere Intertropical Convergence Zone (ITCZ) during the late Holocene (Gasse, 2000; Fleitmann et al., 2003; Gupta et al., 2003; Wanner et al., 2008). Although these regional trends through the mid- to late-Holocene in Africa were attributable to insolation, local factors overrode these patterns at smaller scales (Gasse, 2000; Birks et al., 2014). For example, West Africa experienced a gradual and continuous aridification since ~5000 yr BP, resulting in a southern shift of the savanna belt (Cochrane, 2010), which then intensified 3800-3300 yr BP, resulting in the development of savanna closely resembling current day vegetation (Lézine, 1989; Holmes et al., 1997; Salzmann, 2000; Salzmann et al., 2002). These interpretations are in line with molecular proxy records from East and West-central Africa (Schefuß et al., 2005; Berke et al., 2012). In contrast, South America experienced an opposite pattern (Birks et al., 2014), with the mid-Holocene during ~6000 to 3500 yr BP reported as a wetter period for the cerrado/savanna transitions in Carajas and Titicaca relative to the early Holocene (Behling and Hooghiemstra, 1999; Baker et al; 2001). Studies from Colombia also show an increase in precipitation from ~6000 yr BP to ~2500 yr BP along with a shift in vegetation from grassy
savanna to a more rainforest like vegetation, followed by a period of environmental stability with no pronounced change in precipitation until ~1000 yr BP. The period from ~1000 yr BP to the present is marked by strong and brief climatic events with a dry phase between ~1000 and 500 yr BP (Behling and Hooghiemstra, 2000; Marchant et al., 2001). Likewise, pollen evidence from tropical northern Australia suggests that effective precipitation (EP) was high during the mid-Holocene between ~5000 to ~4000 yr BP, followed by a decline in EP until ~3500 yr BP and then an increase towards the present (Shulmeister and Lees, 1995). Thus, regional and global patterns of climate across the Holocene are asynchronous across continental and regional scales (Fig 5). While the monsoon records from Asia and east and west central Africa are harmonic, rainfall records from the Southern hemisphere, especially Australia and South America, appear to differ from those of the Northern hemisphere.

**Fig 5. Regional and global comparison of Banni climate data with other paleoclimate records:** A. $\delta^{18}O_{\text{shell}}$ of Chachi core (Current study); B. palynology, phytolith and carbon isotope data from Wadhwana Lake in the semi-arid region of mainland Gujarat, Western India (Prasad et al., 2014b); C. presence of evaporite minerals in Lonar lake, Central India (Anoop et al., 2013; Prasad et al., 2014a); D. Al$_2$O$_3$ (wt%) from continental shelf sediments near Rushikulya river, Eastern India (Ankit et al. 2016); E. $\delta^{18}O_{\text{carb}}$ data from Tso Moriri Lake, NW Himalayas (Mishra et al., 2015); F. Carbon isotope data from biomarkers derived
from the Godavari catchment (Ponton et al., 2012); G. Palynological, isotope and molecular proxy records from tropical Africa (Salzmann et al., 2002; Schefuß et al., 2005; Berke et al., 2012); H. Palynological and $\delta^{13}C_{org}$ data from tropical South America (Behling and Hooghiemstra, 1999, 2000; Baker et al., 2001; Marchant et al., 2001) and I. Palynological data from tropical Northern Australia (Shulmeister and Lees, 1995).

6. Conclusions

This paleoecological study shows that vegetation composition in the Banni through the mid-late Holocene has shifted in response to regional rainfall availability. Specifically, a mesic ecosystem characterized by the coexistence of $C_4$ grasses and $C_3$ trees and shrubs between ~4600 to ~2500 cal yr BP appears to have shifted to a water-limited and disturbance (fire and herbivory) mediated rangeland dominated by $C_4$ grasses from ~2500 to about 1000 cal yr BP. From ~1000 cal year BP to the present, there appears to have been an increase in $C_3$ vegetation, which may have been mediated by an increase in precipitation and grazing, and a reduction in fire activity, in the landscape. Thus, this grassland ecosystem has been maintained by complex interactions among rainfall and disturbances such as fire and herbivory, especially since ~2500 cal yr BP.

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Appendix A. Supplementary data

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