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Revised chronology and stable isotopic (Carbon and Nitrogen) characterization of Lahuradewa lake sediment (Ganga-plain, India): Insights into biogeochemistry leading to peat formation in the lake

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The Lahuradewa lake provided evidences for the earliest rice cultivation in central Ganga plains at ~8.3 ka BP using depth profiles of phytoliths and paddy field diatoms. Chronology of the lake profile was earlier constrained by six conventional radiometric ^{14}C dates. Organic matter was abundant throughout the Holocene and was expected to have recorded productivity changes controlled by monsoonal changes and/ or *in-situ* lake-biogeochemistry. To deduce these changes, here we measured total organic carbon (TOC) nitrogen (N) and sulphur (S) its concentration and their stable isotopes ($\delta^{13}C_{TOC}$, $\delta^{15}N$ and $\delta^{34}S$) in twenty eight sedimentary layers of the Lahuradewa lake. Besides, we also measured six additional ^{14}C dates by Accelerator Mass Spectrometry (AMS) to refine the chronology. All six AMS ^{14}C ages were found to be in excellent agreement with earlier reported radiometric ^{14}C dates. Using $\delta^{13}C_{TOC}$ and $\delta^{15}N$ variations, depositional history of the lake could be sub-divided into four phases (i) pre-agricultural or peat zone (~10,899 to 7,451 year BP) (ii) early-agricultural (~7,450 to 2,623 year BP) (iii) deteriorating monsoon (~2,633 to ~1,228 year BP) and (iv) the modern phase (~1,228 to present day). The pre-agricultural phase (black organic muddy sediment between 2.3 to 2.8 m; peat zone) was found to have strikingly higher TOC and N contents (~28% and ~2% respectively) with significantly enriched $\delta^{13}C_{TOC}$values (-17.5±0.5‰), lower $\delta^{15}N$ values (2.1±0.2‰) and $\delta^{34}S$ (9.6±0.9‰) is found only in this zone. We surmise that this pre-agricultural phase sequestered capacious amounts of atmospheric C (and N) by developing *Botrycoccus* algae, which produces natural hydrocarbons. Such algal growths in wetlands promote carbon sequestration (soaking greenhouse CO_2 gas) and act later as source(s) of renewable hydrocarbon biofuels.

Keywords: AMS Radiocarbon date, Stable C and N isotopes, Lake biogeochemistry, Paddy field diatoms, *Botrycoccus* algae, Phytolith taxa, Palaeo-agriculture

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INTRODUCTION

The Lahuradewa lake (26°46'N; 82°57'E) is situated adjacent to the Lahuradewa archaeological site of District Sant Kabir Nagar in Uttar Pradesh (central Ganga plain, India) (Thakur *et al.,* 2020). Excavations at the archaeological site were carried out between AD 2002-2006 by the experts comprising Directorate of Archaeology Uttar Pradesh, Lucknow University and Birbal Sahni Institute of Palaeobotany (now Palaeosciences) (BSIP henceforth) Lucknow (Tewari *et al.,* 2006). The lake should have been presumably feeding the inhabitants of Lahuradewa archaeological site by supplying potable waters as well as fertile arable land in its adjoining landscape. A ~2.8 metre deep vertical trench section (see field photographs from Chauhan *et al.,* 2009 and lithology as shown in Fig. 6 on eastern dried part of the Lahuradewa lake (~150 metres away from the Lahuradewa archaeological site) was dug and sampled at ~10 cm interval, yielding a total of twenty eight (28) samples. Initial chronology of this section was ascertained by six conventional radiometric ^{14}C dates obtained on sediment organic matter at BSIP Lucknow. All 28 samples were studied in detail for grain size distribution, pollen, phytolith and diatom assemblages (Chauhan *et al.,* 2009; Saxena *et al.,* 2006; 2013; Thakur *et al.,* 2020). Chief findings of these studies comprise multiple types of proxy evidences for presence of rice in its vicinity. Abundance of wild rice was found right from the beginning of the Holocene (~10 ka BP) followed by initiation of rice domestication (cultivation) beginning at ~8.35 ka BP. Inferences of rice cultivation were drawn from abundant phytoliths (of wild and cultivated variety) and paddy field diatoms. Although debateable, Lahuradewa lake studies present probably one of the oldest records of agriculture activity in the central Ganga Plain. Worldwide earliest evidence of domesticated rice (*Oryza sativa*) date back ~9000-8400 years BP from

China's Yangtze valley (Zheng *et al.,* 2016). At Lahuradewa, beginning of cultivated diatoms appeared at ~9,250 years ago (Thakur *et al.,* 2020). While archaeologists continue to debate oldest evidences of rice cultivation (Gross and Zhao, 2014), chronological methods applied and associated uncertainties significantly matter in such discussions. As chronology of the Lahuradewa lake was established using conventional beta decay counting based radiometric method that involve (a) higher sample amounts and (b) relatively larger uncertainties in the assigned ages, it becomes imperative to refine chronology using modern AMS method that involve better accuracy and precision. It is important to precisely date beginning of the agricultural landscape development in the Ganga plains, as farming activities have a symbiotic relationship with natural ecology and lead organizational human activities bringing climate-resilience in settler communities.

In addition to refining chronology, it is equally vital to investigate details of biologically available carbon (TOC) and nitrogen (N) stored in sediment organic matter as it may reveal insights into operative C and N cycling in atmosphere couple lacustrine environments. Sediment TOC and N may undergo variety of complex transformations which could be tracked using their stable isotopes ($\delta^{13}C_{TOC}$ and $\delta^{15}N$). Likewise, sedimentary sulfur (S) often found in anaerobic peat zones; $\delta^{34}S$ values could be utilized for gauging anoxic biogeochemistry in the water column/ sediment water interface/ pore waters of recent sedimentary layers. Modern agriculture plays a significant role in current human/ animal population to meet their food demands and has been projected for its compounding effects on climate (IPCC, 2019; Jia *et al.,* 2018). Vital clues about climate-ecology- agriculture interdependence could be gleaned from isotopic data aided palaeo-studies of prehistoric agriculture. Lahuradewa provides an ideal locale for investigating C and N isotopic variability in fresh water aquatic system owing to its capability of reconstructing climate-agriculture history of central Ganga plain going back in time all the way up to early Holocene. Despite issues with chronologies, archeobotanical analyses of the Lahuradewa lake have suggested Ganga plain as a plausibly independent centre of rice cultivation of the subspecies *indica* (*Oryza Sativa*) through a protracted and complex process since ~8,350 BP (Fuller *et al.,* 2007; Fuller, 2011; Murphy and Fuller, 2016).

With aforementioned issues in mind, we framed objectives of the present study as (i) to refine chronology of Lahuradewa lake sediments sequence using AMS ^{14}C (ii) to generate stable C and N isotopic data *i.e.* $\delta^{13}C$ of sediment organic material ($\delta^{13}C_{TOC}$) and $\delta^{15}N$ of sedimentary layers in order to assess inter-relationship between lake-biogeochemistry and already reported biotic data *e.g.* phytoliths, diatoms, and pollens counts. While, newly produced AMS ^{14}C dates reaffirm older chronological framework constrained by conventional radiometric dates (Saxena *et al.,* 2006), stable isotopic dataset reveals several hitherto aspects about organic matter deposition in the lake by providing palaeo-environmental conditions and processes especially in the pre-agricultural phase (10,899 to 7,451cal yr BP) and three distinct phase thereafter.

FRAMEWORK FOR UTILIZING STABLE C AND N ISOTOPIC DATA IN A FRESH-WATER LAKE ENVIRONMENT

Stable isotope ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) from lake sediments are excellent proxies to infer past changes in productivity, biogeochemical cycling of nutrients, water column temperatures etc. (Lücke *et al.,* 2003; Parplies *et al.,* 2008; Wu *et al.,* 2008). Applications have tremendously increased since anthropogenic amendments have started in catchment areas such as use of synthetic ammonium-nitrate fertilizers, irrigation, use of pesticides in agricultural practices. Modern lake systems face both organic and inorganic pollution like anthropogenic nitrogen loading of lakes and eutrophication (Elliott and Brush, 2006; Leavitt *et al.,* 2006). Nonetheless, for the period prior to AD 1950, we can assume lakes were mainly modulated by climate, ecology of the landscape, and its physical settings. Most of the in-land fresh water lakes are surrounded by terrestrial C_3 plants that are characterized by their $\delta^{13}C$ values varying between -24 to -34‰ with a mean -27‰ (Gunter Faure, 1998; Torres *et al.,* 2012). Organic matter at the surface of lake however, may have substantial contribution from *in-situ* produced organics, such as algae and lichens that normally have $\delta^{13}C$ values between -12 to -23‰ (Lange *et al.,* 1988; Huiskes *et al.,* 2006; Lee *et al.,* 2009). Aquatic plants of desert, salt marsh areas and tropical grasses have $\delta^{13}C$ values between -6 to -19‰ (Deines, 1980; Collins *et al.,* 2019). Where C isotopic composition of settling organic matter mainly mimic source information *i.e.* nature of primary productivity and carbonate chemistry of the lake, N isotopes of particulate phase mainly integrate influence of biogeochemical processes operative in the water column and sediments (Farmer *et al.,* 2021). Compared to C isotopes, N isotopes have rarely been used in freshwater lakes of Indian sub-continent to track biogeochemistry and N cycle dynamics (Singh *et al.,* 2020). N isotopic values would mainly be resultant of isotope fractionation effects of nitrification and denitrification processes (Lehmann *et al.,* 2002). No significant fractionation effect is seen on $\delta^{15}N$ values when NH_4^+ is produced, whereas microbial degradation of settling organic matter increases $\delta^{15}N$ of particulate phase (Altabet and McCarthy, 1985; Saino and Hattori, 1987; Owens and Law, 1989; Lehmann *et al.,* 2004; Hadas *et al.,* 2009).

In addition to $\delta^{13}C_{TOC}$ and $\delta^{15}N$ values, TOC/TN ratio or simply C/N ratios (henceforth) of particulate organic matter could be used for deducing source of organic matter and degree of biogeochemical cycling of C and N operative in the aquatic body. Higher C/N ratios are seen in land plants compared to those of lacustrine phytoplankton biomass (Meyers and Ishiwatari, 1995; Albuquerque and Mozeto, 1997). C/N ratios of soil organic matter produced mainly by phytoplanktons have low C/N ratios between 4 and 10. Contribution from vascular land plants, which are cellulose-rich and protein-poor, have enriched C/N ratios to 20 or greater. C/N ratios between 13–15 typically suggests a mixture of algal and vascular plant contributions, which could be an expected possibility for most of the lakes. Partial degradation of organic matter, however, can modify C/N ratios and would generally be identifiable by enriched these values (Ertel and Hedges, 1985; Meyers *et al.,* 1995; Frenette *et al.,* 1998; Meyers and Lallier-Verges, 1999).

MATERIALS AND METHODS

A 2.80 m deep core was dug on the eastern dried flank of the lake, about 150 m north of the Lahuradewa archaeological site (26°46'N; 82°57'E; Pokharia, 2011). All the samples were analysed for diatoms, pollens, phytolith assemblages and radiocarbon dating (Saxena *et al.,* 2006, 2013; Chauhan *et al.,* 2009; Thakur *et al.,* 2020). For AMS ^{14}C dating and δ^{13}C measurements, samples were powdered and decalcified in 10% HCl to remove the lithogenic carbonate fraction, were dried and grounded again into fine powder. Decalcified samples were used for determination of total organic contents (TOC) and δ^{13}C values following standard protocols (Agnihotri *et al.,* 2020). Bulk sediment samples (untreated) were used for TN, δ^{15}N, sedimentary S and its isotopes (TS and δ^{34}S) analyses. The isotopic data of δ^{13}C, δ^{15}N and δ^{34}S values are reported against international standards *viz.* V-PDB, atmospheric N_2 and V-CDT respectively, with an overall precision of ±0.2‰. Amino-n-caproic-acid (ACA) and IAEA-S2 were used as primary standards for calibration, while Sulfanilamide was repeatedly used as *in-house* control standard for reproducibility. Graphite targets were prepared using graphitization facility of the Radiocarbon dating and Isotope characterization Laboratory of BSIP. The ^{14}C contents were measured using AMS facility at Physical research laboratory (PRL-AURIS). All the samples were measured against the international standards (*e.g.* OX-II and IAEA-C3). Blank graphites were prepared using laboratory Anthracite powder ($C_{15}H_{11}O$).

RESULTS

The Lahuradewa Lake sediment core was composed of of dark mud with rootlets (0–90 cm), dark mud (90–200 cm), and black organic mud with more than 65% organic matter (peat) (200–280 cm). In this study, we have added six new AMS ^{14}C dates and contextualized them with previously reported six conventional radiometric ^{14}C dates (Saxena *et al.,* 2006). Both newly generated and already reported ^{14}C dates have been presented in Table-1 and Fig. 2A. Five AMS ^{14}C dates and six earlier reported radiometric dates were calibrated using *Oxcal 4.2* (IntCal20) (Bronk Ramsey, 2009; Reimer *et al.,* 2020). The modern ^{14}C date of sample LRD-2 was calibrated using *CALIBomb* (IntCal20) (Reimer and Reimer, 2021). Fig. 2B shows all calibrated ages with associated Bayesian uncertainties. For estimating Bayesian uncertainties, we have utilized clam version 2.4.0 in R studio. It is clear from Fig. 2A that all the AMS and LSC derived ages are in excellent agreement and allow us to infer that lake sediment sequence appears to have deposited with mainly with two distinct sediment rates *i.e.* younger part of the lake (up to ~150 cm) deposited with sedimentation rate of 0.06 cm yr^{-1}, while older portion was deposited with significantly lower sedimentation rate (0.014 cm yr^{-1}).

In addition to revisiting chronology of the lake sequence, we measured sediment TOC, TN and TS contents and their isotopic compositions (δ^{13}C, δ^{15}N and δ^{34}S). All the

Fig. 1. Map showing the study area, the Lahuradewa Lake. The site shows the water body along with adjoining landscape mainly utilized for agriculture at present.

Table 1. AMS and LSC radiocarbon dates of soil organic matter of lacustrine sediments in Lahuradewa (UP).

AMS measured Radiocarbon Dates

S. No.	AMS/ Graphitization Lab ID	Sample ID	Radiocarbon Age (years BP)	Radiocarbon Age 2σ (Cal years BP)	Radiocarbon Age Range 2σ (Cal years BP)	Calibrated Age Ranges 2σ (AD/BC)	(Median Age (AD/BC) ± Uncertainity (2σ)
1	AURIS-04238/ BSIP0608217	LRD-02	-24 ± 60	84±61 BP	1805-1927 AD	Modern	1866±61 AD
2	AURIS-04240/ BSIP0608214	LRD-06	1790 ± 67	1700±164 BP	1536-1864 BP	118-414 AD	266±148 AD
3	AURIS-04241/ BSIP0608215	LRD-13	2232 ± 60	2209±142 BP	2067-2351 BP	402-150 BC	276±126 BC
4	AURIS-04242/ BSIP0608216	LRD-15	2486 ± 78	2549±188 BP	2361-2737 BP	786-412 BC	599±187 BC
5	AURIS-04243/ BSIP0908213	LRD-25	6196 ± 112	7102±308 BP	6794-7410 BP	5376-4844 BC	5110±266 BC
6	AURIS-04244/ BSIP0908214	LRD-27	9638 ± 105	10966±274 BP	10692 -11240 BP	9290-8743 BC	9017±274 BC

LSC measured Radiocarbon Dates (Saxena *et al.*, 2006; Chauhan *et al.*, 2009)

S. No.	Lab ID	Sample ID	Radiocarbon Age (years BP)	Radiocarbon Age 2σ (Cal years BP)	Radiocarbon Age Range 2σ (Cal years BP)	Calibrated Age Ranges 2σ (AD/BC)	(Median Age (AD/BC) ± Uncertainity (2σ)
1	BS-2300	LRD-5	1040±100	926±191 BP	835-1017 BP	773-1215 AD	994±221 AD
2	BS-2302	LRD-8	1810±100	1706±283 BP	1423 -1989 BP	2-434 AD	218±216 AD
3	BS-2299	LRD-14	2180±90	2147±205 BP	1942-2352 BP	402 BC-8 AD	205±197 BC
4	BS-2981	LRD-24	7010±170	7851±329 BP	7522 -8180 BP	6230-5621 BC	5926±305 BC
5	BS-2215	LRD-26	8710±170	9838±383 BP	9455-10221 BP	8271-7586 BC	7929±343 BC
6	BS-2211	LRD-27	9210±170	10487±583 BP	10487-11070 BP	8868-7954 BC	8411±457 BC

aforementioned data are presented in Table 2. It is noteworthy that TS contents were below detection limits expect for bottom most peat section (Fig. 4C). Table-2 also comprises selected biotic proxy data from earlier publications such as phytolith counts (wild and cultivated variety) (Saxena *et al.,* 2006, 2013), pollen taxa (Chauhan *et al.,* 2009) and variety of diatoms (Thakur *et al.,* 2020). Depth profiles of measured geochemical and isotopic proxies were plotted against aforementioned biotic proxies in Figs. 3 and 4. This contextualization of geochemical data with biotic proxies was aimed to glean the role of lake-biogeochemistry vis-à-vis environmental/ agricultural changes occurring in the vicinity. Based on the variations in $\delta^{13}C_{TOC}$ and $\delta^{15}N$ values, it appears that depositional history of the Lahuradewa lake could be sub-divided into four time-windows:

Pre-agricultural phase (~10,899 to ~7,451 cal yr BP):

This phase represents bottom most part of the sedimentary sequence (~2.8–2.3 m; samples LRD 28 to 23 of Table-2; see Fig. 6) which is lithologically distinguishable for it was mainly comprised of black organic mud (peat layers) (Saxena *et al.,* 2006). These layers contained lowest phytolith counts (both wild and cultivated varieties) and paddy field diatoms (Fig. 3). Depth profiles of TOC, TN contents along with $\delta^{13}C_{TOC}$ and $\delta^{15}N$ values also showed distinctness of this zone (shown as grey colour bars; Figs. 3 and 4). Interestingly, this pre-agricultural phase is characterized by very high TOC and TN contents (going up to ~28.5% and ~2% respectively) compared to their respective averages for rest of the depth-profiles (with ~1.3% TOC and ~0.1% TN contents; Fig. 3C). Intriguingly, this higher TOC containing phase display significantly enriched $\delta^{13}C_{TOC}$ values (-17.8±0.5‰) compared to rest of the samples (~-23.0‰) (Figs. 3A, 4A). In contrast, N contents display much lower $\delta^{15}N$±0.2‰) compared to rest of the samples (averaging ~3.9‰) (Figs. 3B, 4B). It was also noteworthy and intriguing that this pre-agricultural phase showed presence of significant sedimentary S contents characterized by positive $\delta^{34}S$ isotopic values (9.6±0.9‰) (Fig. 4C). Lower $\delta^{15}N$ could be due to N fixation from atmosphere; that also explains enhanced TOC contents with enriched $\delta^{13}C$ values. Fresh water lake systems, in general, have atmospheric sources only for carbon capture through altered surface productivity (such as algal blooms) (Amundson *et al.,* 1998; Rantala *et al.,* 2016). Phytolith (especially cultivated variety) and diatom assemblage data (both paddy field and anthropogenic) reveal their minima (Fig. 3D-F). It appears that *Botrycoccus* algae likely conducted C and N sequestration in this phase which is evident in form of the pollen abundance pattern (Fig. 3G). This phase also shows consistently relatively higher C/N ratios (~15) (compared to the rest of the younger samples) (Fig. 4D). Sediment $\delta^{13}C$ values and C/N ratios are representing characteristics of *in-situ* biogenic deposition, which remained largely unaffected by post depositional changes as Fig. 5B does not reveal any systematic relationship between sediment $\delta^{13}C$ and C/N ratios.

Early-agricultural phase (~7,451 to ~2,623 cal yr BP):

This sedimentary sequence (~2.3–1.6 m; samples LRD 23 to 16 of Table-2) witnesses beginning and proliferation

Table 2. Stable isotopes ($\delta^{13}C_{TOC}$ and $\delta^{15}N$), and geochemical data TOC%, TN% and C/N ratio; and the relative abundances of Phytoliths, diatoms and Pollens recorded in Saxena *et al*. (2013), Chauhan *et al*. (2009) and Thakur *et al*. (2020).

S. No.	Name	Mean Depth (cm)	Cal. ^{14}C Age BP	TOC %	TN %	TS %	C/N ratio	C/S ratio	$\delta^{13}C$ (VPDB)	$\delta^{15}N$ (Air)	$\delta^{34}S$ (VCDT)	Anthropogenic diatoms	Paddy field diatoms	IpH index	Phytolith counts	wild phytolith taxa	cultivated phytolith taxa	*Botryococcus* (%)	Poaecae pollen (%)	P/C ratio
1	LRD-1	5	135	2.1	0.25	ND	8.2	ND	-27.0	2.7	ND	134	242	10.63	291	1	4	2	73.7	17.7
2	LRD-2	15	317	1.3	0.13	ND	9.9	ND	-26.9	3.6	ND	48	133	14.08	308	1	3	2	60.3	11.5
3	LRD-3	25	499	1.1	0.12	ND	9.3	ND	-26.0	1.9	ND	21	56	13.8	245	1	3	1	87	12.0
4	LRD-4	35	682	0.9	0.11	ND	8.5	ND	-24.5	3.7	ND	11	23	14.04	225	1	3	1	51.5	13.4
5	LRD-5	45	864	1.2	0.13	ND	9.6	ND	-24.9	3.5	ND	40	11	28.38	208	2	3	2	62.3	4.2
6	LRD-6	55	1046	0.9	0.10	ND	8.6	ND	-23.9	4.0	ND	66	105	12.61	231	2	3	3	57.2	14.3
7	LRD-7	65	1228	0.6	0.09	ND	6.7	ND	-23.6	4.4	ND	57	131	28.89	200	3	3	1	60.4	4.2
8	LRD-8	75	1410	0.8	0.08	ND	9.6	ND	-21.8	4.4	ND	57	58	37.41	179	2	2	1	61.3	2.9
9	LRD-9	85	1592	0.8	0.09	ND	8.8	ND	-21.6	3.6	ND	92	92	23.21	184	2	3	7	37.2	6.9
10	LRD-10	95	1775	0.4	0.06	ND	6.8	ND	-22.1	5.0	ND	7	26	14.73	234	3	4	7	40.4	12.0
11	LRD-11	105	1957	0.8	0.08	ND	9.6	ND	-21.9	4.1	ND	92	66	22.3	210	2	3	9	52	7.4
12	LRD-12	115	2139	1.0	0.08	ND	12.0	ND	-22.7	4.6	ND	114	77	15.58	191	3	2	1	38.5	12.9
13	LRD-13	125	2321	1.4	0.10	ND	14.0	ND	-22.8	4.0	ND	39	64	15.01	182	3	4	10	51.6	13.5
14	LRD-14	135	2503	1.0	0.09	ND	10.7	ND	-23.5	4.4	ND	17	177	21.83	164	5	2	17	36.6	7.4
15	LRD-15	145	2685	1.1	0.10	ND	11.0	ND	-23.7	4.2	ND	60	217	13.92	172	5	2	15	30.6	15.5
16	LRD-16	155	2623	1.2	0.09	ND	13.0	ND	-23.5	4.0	ND	192	162	17.52	192	6	3	8	34.6	8.6
17	LRD-17	165	3313	1.0	0.09	ND	11.5	ND	-22.8	5.2	ND	115	273	14.01	220	3	2	22	56.8	12.6
18	LRD-18	175	4003	1.1	0.10	ND	11.1	ND	-22.4	4.2	ND	140	165	38.01	144	2	1	19	48.6	2.8
19	LRD-19	185	4692	ND	ND	ND	ND	ND	ND	ND	ND	47	128	38.01	144	2	1	30	52.6	2.7
20	LRD-20	195	5382	1.4	0.10	ND	13.8	ND	-22.8	3.8	ND	8	135	30.95	178	3	2	18	35.3	5.5
21	LRD-21	205	6072	2.6	0.16	ND	16.4	ND	-21.7	3.1	ND	50	116	15.9	189	3	2	23	63.6	10.6
22	LRD-22	215	6761	4.5	0.29	ND	15.4	ND	-20.1	2.5	ND	55	268	16.41	168	4	2	19	65.3	12.7
23	LRD-23	225	7451	10.1	0.58	ND	17.4	ND	-17.2	2.4	ND	30	28	17.79	108	4	2	46	61.7	9.6
24	LRD-24	235	8141	8.4	0.56	ND	15.0	ND	-17.5	1.9	ND	15	25	19.59	81	5	3	54	71	6.5
25	LRD-25	245	8830	15.5	1.04	0.14	14.9	110.1	-16.7	1.9	9.2	0	21	29.05	71	3	0	66	69.6	7.5
26	LRD-26	255	9520	28.5	2.09	0.6	13.6	47.5	-18.2	2.1	9.0	0	27	19.35	52	2	0	4	71.3	9.1
27	LRD-27	265	10210	16.8	1.41	0.19	11.9	87.1	-17.8	2.0	9.2	0	38	17.97	31	0	0	41	62.4	ND
28	LRD-28	275	10899	10.2	0.63	0.12	16.2	88.4	-17.5	2.4	11.0	0	10	4.9	18	6	0	27	80	ND

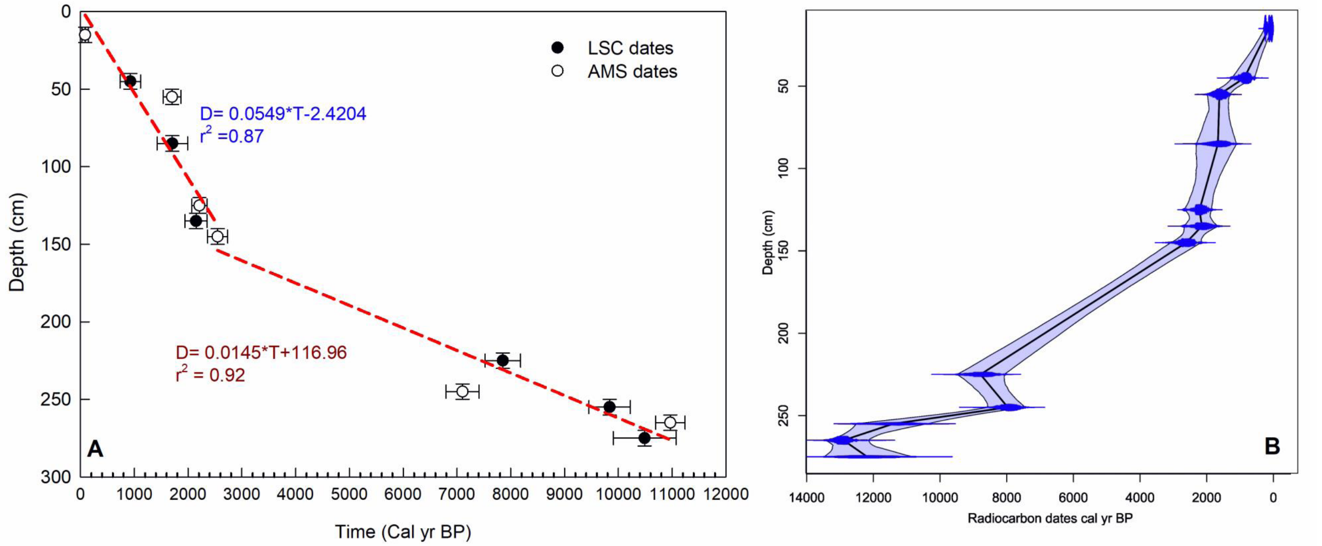

Fig. 2. (A) AMS and LSC radiocarbon dates of soil organic matter of lacustrine sediments in Lahuradeva (U.P.); (B) All calibrated ^{14}C ages along with associated uncertainties determined by B-chron model (using clam 2.4.0 version) with depth.

of agronomic activities as evidenced by arrival of phytoliths of cultivated rice, paddy field diatoms and anthropogenic diatoms (Fig. 3D-F). The $\delta^{13}C_{TOC}$ shows a steady depleting trend (from -20.1 to -22.8‰; Fig. 3A), while $\delta^{15}N$ values depict an increasing trend (2.5-5.2‰; Fig. 3B). Depth profiles of TOC, TN contents also display steady declining trends (from 4.5-1.0% and 0.29-0.09% respectively). This early-agricultural phase is shown by light green colour horizontal bars in Figs. 3 and 4. C/N ratios tend to be marginally lower (~13.6) compared to those for the pre-agricultural phase, but they are still higher compared to younger portion of the lake sequence (Fig. 4D). C/N ratios are susceptible to post-depositional changes in sedimentary environments (Liu *et al.,* 2020), however, in the studied lake profile, down core

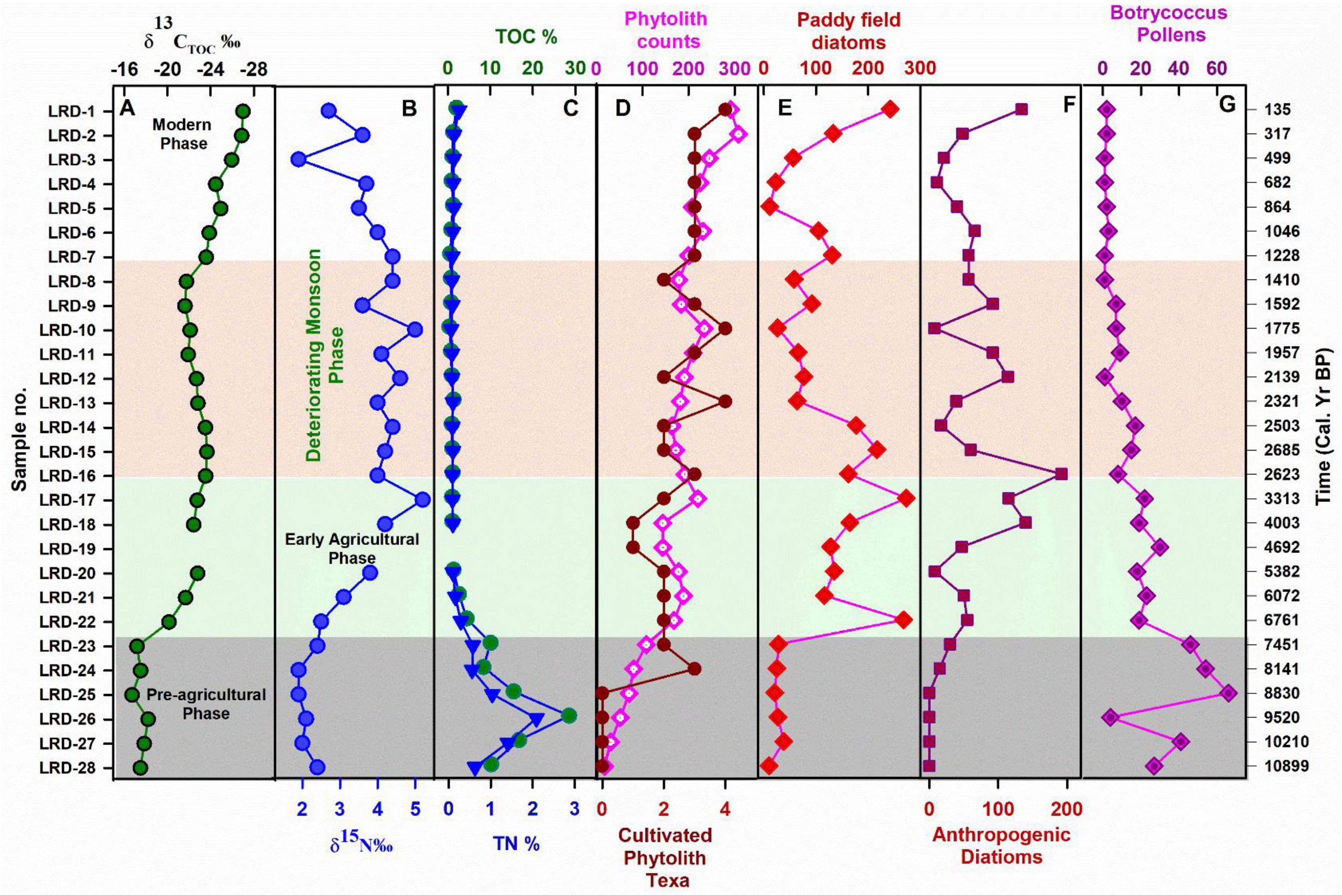

Fig.3. Depth profiles of stable isotopes ($\delta^{13}C_{TOC}$, $\delta^{15}N$ and $\delta^{34}S$), TOC and TN contents (panels A-C). Phytolith counts (Total and cultivated) are adopted from Saxena *et al.* (2006, 2013) (panel D). Paddy field and anthropogenic diatoms (panels E, F) were adopted from Thakur *et al.* (2020). Botrycoccus algae pollens counts shown in panel G were adopted from Chauhan et al. (2009).

variations of TOC and TN contents showing excellent correlation ($r^2 = 0.98$; Fig. 5A) hint against this possibility. TOC versus $\delta^{13}C_{TOC}$ and TN versus $\delta^{15}N$ cross plots while showing show two distinct sediment depositional phases, but there is no indicative trend of post-depositional (early diagenetic) changes (Fig. 5D). There is a conspicuous decline of *Botryococcus* algae as marked by its pollens (Fig. 3G). It is noteworthy that rice cultivation begun with dominant presence of *Botryococcus* algae and rice cultivation grew along proportion of algae appears to have declined.

Deteriorating Monsoon phase (~2,623 to ~1,228 cal yr BP):

This portion of sedimentary sequence (~1.6-0.7 m from samples LRD-16 to LRD-7) to have deposited under deteriorating monsoonal phase (shown by light pink colour horizontal bars; Figs. 3 and 4). Only a marginal declining trend in $\delta^{13}C_{TOC}$ values could be seen for this phase (~-21.1‰ from ~ -23.5‰; Figs. 3A, 4A), while $\delta^{15}N$ values remained steady at ~4.3‰ (Figs. 3B, 4B). Paddy field diatoms, anthropogenic diatoms and wild phytolith taxa show a conspicuous declining trend (Figs. 3E, 3F and 4E) most likely due to oscillating monsoonal changes in the lake vicinity. Only cultivated phytolith counts remained steady with some marginal variability (Fig. 3D). Waxing and waning of monsoonal conditions could be inferred from phytolith index (Iph%) (Prasad *et al.,* 2014) and Panicoidae/Chloridoidae (P/C) ratios (Saxena *et al.,* 2013). The higher values of Iph index denote aridity; whereas lower values of Iph index are observed during warm and wet conditions (Bremond *et al.,* 2005). Likewise, phytolith morphotypes belonging to subfamily Panicoidae indicate warmer and humid climate; whereas Chloridoidae sub-family indicate warmer and dryer climate (Saxena *et al.,* 2013; Sjöström, 2013). Hence, down core profiles of Iph and P/C ratios show inverse relationship in general. Significantly lower TOC and TN contents were recorded for this phase (~0.9% and 0.09% respectively; Fig. 3C). C/N ratios showed an overall decreasing trend from ~13 to 6.7 with an average of ~10.2 (Fig. 4D) which indicates increasing *in-situ* organic productivity in the lake (Tengberg *et al.,* 2003). During this monsoonal deteriorating phase, *Botryococcus* algae appear to reach its minima in this phase (Fig. 3G). The enriched $\delta^{13}C$ values during this phase hints water stressed C_3 vegetation (Godbold *et al.,* 2006). It is noteworthy that TOC content declined almost simultaneously along with *Botryococcus algae* (together $\delta^{13}C_{TOC}$ values) (see Figs. 3A,C,G). This association clearly hints *Botryococcus algae* had a conspicuous influence on lake's biogenic productivity. *Botryococcus* algae are generally prominent in natural stagnant shallow waters (Zippi, 1998); their decline

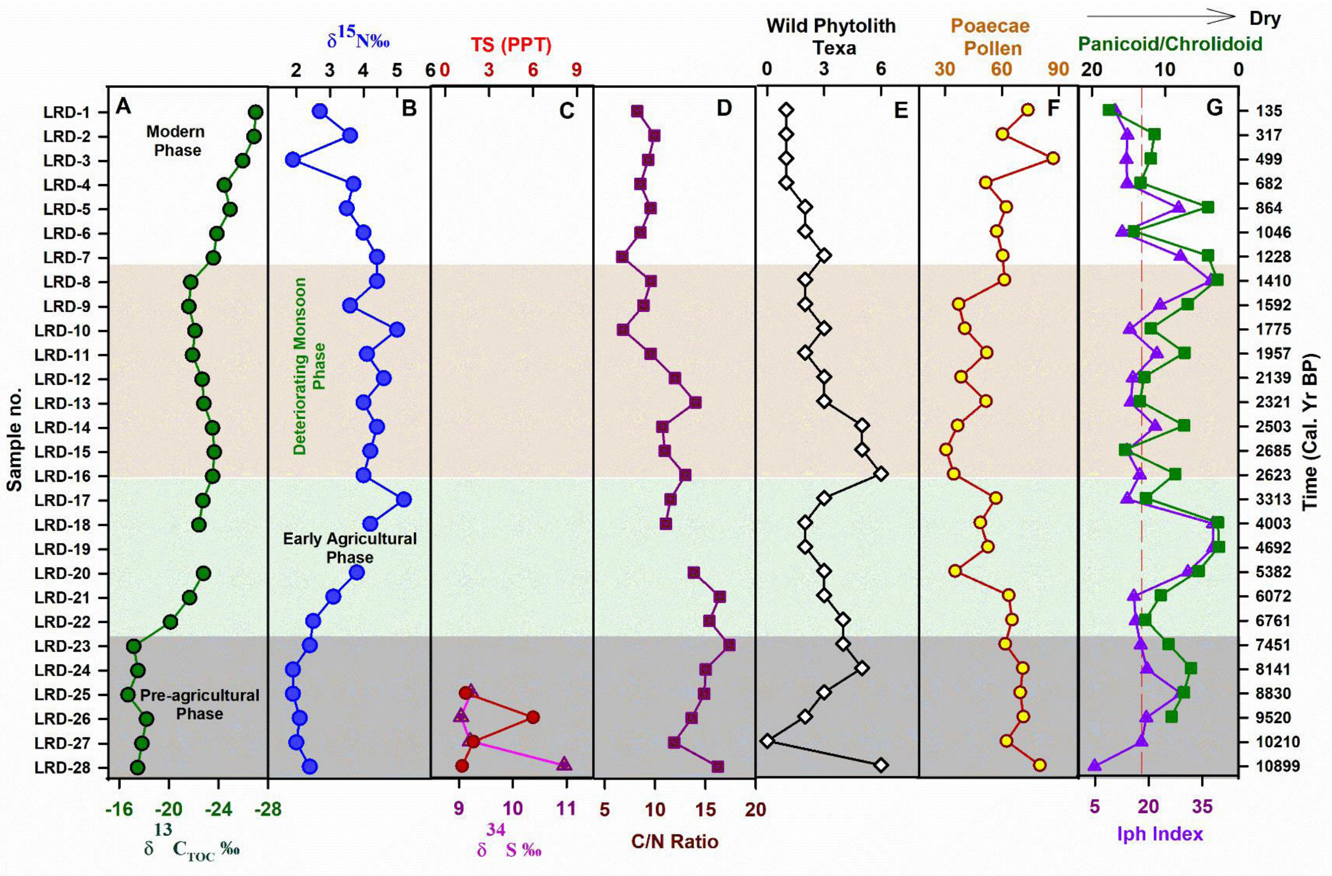

Fig. 4. Depth profiles of stable isotopes ($\delta^{13}C_{TOC}$, $\delta^{15}N$ and $\delta^{34}S$), TS % and C/N ratio (panels A-D). Phytolith counts (wild) are adopted from Saxena et al. (2006, 2013) (panel E). Poaecae (grass) pollens counts shown in panel F were adopted from Chauhan *et al.* (2009). Phytolith index (Iph) and P/C ratio shown in the panel G were adopted from Saxena *et al.* (2013).

could be due to enhanced human activities near the lake catchment (arrival of early agriculture).

Modern phase (Last millennium: ~1,228 cal yr BP to Present):

This phase represents top most portion of Lahuradewa lake sediment sequence (~0.7-0.1 m; from samples LRD-7 to LRD-1). In this phase, $\delta^{13}C_{TOC}$ values show a conspicuous declining trend (from -23.5 to -27.0‰) approaching typical $\delta^{13}C_{TOC}$ values of C_3 vegetation (Figs. 3A, 4A). This could be due to influence of intensification in Monsoon during the Mediaeval Warm Period. Similarly, $\delta^{15}N$ values also show a conspicuous decline from ~4.4‰ to ~2.0‰ (Figs. 3B, 4B). TOC and TN contents were typically ~1.2% and ~0.13% for this phase (Fig. 3C). Average C/N ratios were ~8.7 (Fig. 4D), normally obtained in modern day agricultural fields (Shi et al. 2017). For this modern phase, cultivated phytolith counts remain steady, while paddy field diatoms and anthropogenic diatoms show initially a decline followed by steady enhancing trends (Figs. 3D-F). *Botryococcus* pollens counts are at its minimum during this phase (Fig. 3G). This phase also registers a likely monsoonal fluctuation in its early portion (Fig. 4G). Declining $\delta^{15}N$ trend indicates here a plausible crop-diversification impact *i.e.* induction of leguminous crops (pulses) (typically grown during winter season) due to their ability to fix N from atmosphere (Kakraliya *et al.,* 2018). Introduction of leguminous crops may not have a discernible influence on $\delta^{13}C$ values (Agnihotri *et al.,* 2011). Chauhan et al. (2009) noted decline of forest groves likely due to reduced rainfall which resulted the lake as ephemeral. Despite this aridity, agriculture continued in the vicinity.

Fig. 5A-D shows cross- plots of TOC% versus TN%; $\delta^{13}C$ and C/N ratios; $\delta^{13}C_{TOC}$ versus $\delta^{15}N$; TOC versus $\delta^{13}C_{TOC}$ and TN versus $\delta^{15}N$ values of Lake sediments. From these, it is clear that sediment N content remained biogenic (associated with organic carbon) throughout the depositional history of the lake (Schubert and Calvert, 2001). Figs 5B and 5D ($\delta^{13}C_{TOC}$ versus C/N ratio cross plot; TOC versus $\delta^{13}C_{TOC}$ and TN versus $\delta^{15}N$ cross plots) reveal mainly two pools of samples (pre- and post- agricultural phase of the sedimentary deposit).

DISCUSSION

Revised chronology with AMS method

Accurate and precise radiocarbon dates have their

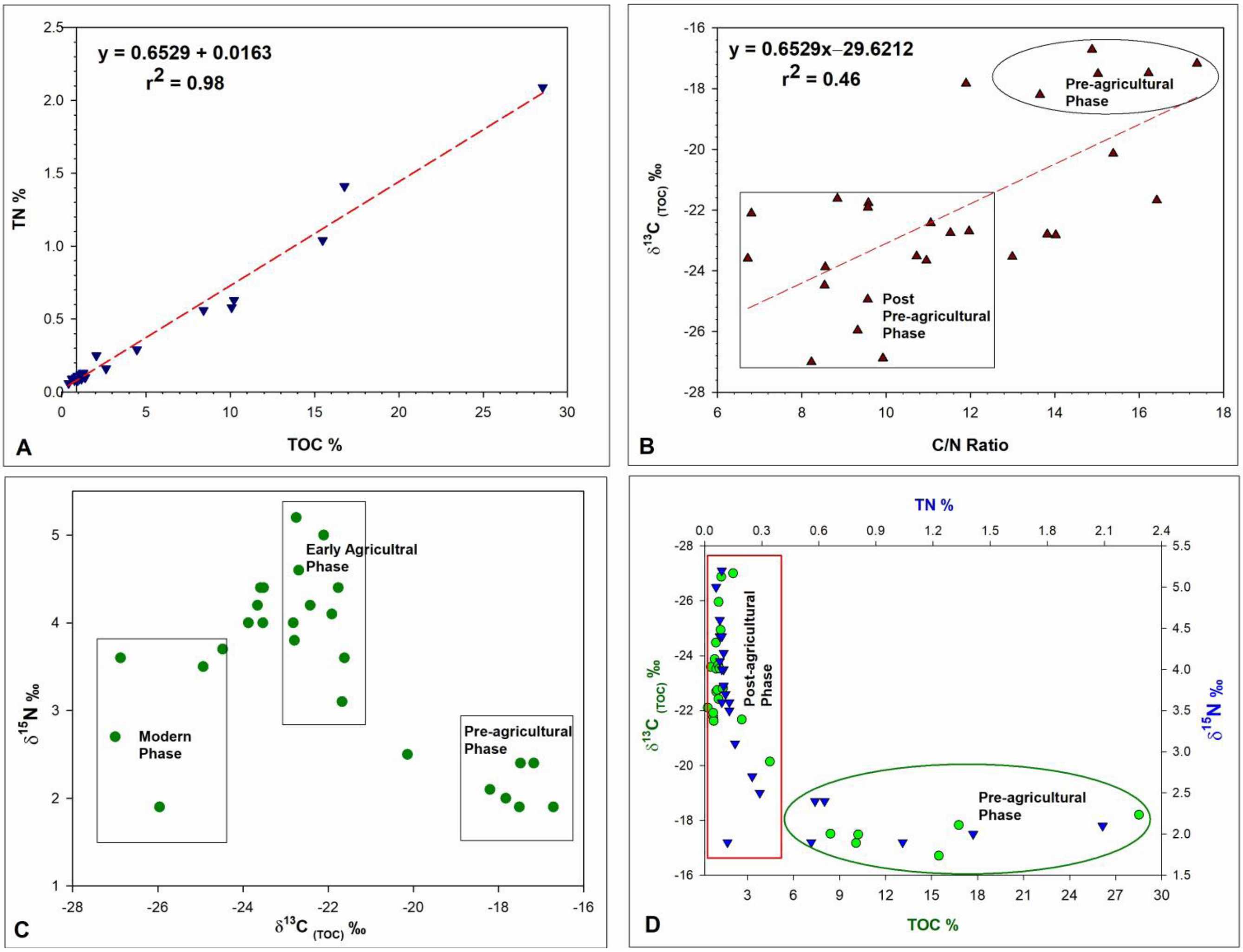

Fig. 5. (A) TN% versus TOC% plots of soil sediments of Lahuradewa Lake for whole sedimentary record from ~10,899 cal yr BP to Present. (B) δ^{13}C versus C/N ratio cross-plot showing two distinct pools of sedimentary phases. (C) $\delta^{13}C_{TOC}$ versus δ^{15}N cross-plot reveals three distinct sedimentary phases including a modern phase. (D) TOC versus $\delta^{13}C_{TOC}$ and TN versus δ^{15}N cross plots again show two distinct sedimentary phases.

immense importance in dating sedimentary sequences in order to glean temporal variability in climate/ environment and ecology in relation to biogeochemical changes in fresh aquatic or marine systems (Crosta and Koç, 2007; Hoffmann *et al.,* 2010; Hajdas *et al.,* 2021). Precision in ^{14}C dates become vital to decipher short term changes preserved in sedimentary records (Cronin, 1999; Ascough *et al.,* 2005). Changes spanning decadal timescales require minimum uncertainties on the assigned ^{14}C dates (Southon *et al.,* 2012; Zimmerman and Wahl, 2020).

In general, conventional radiometric method (β-decay counting based) involves labour intensive benzene preparation with larger amounts of sample size and yield assigned ages with relatively larger uncertainties (Norton and Woodruff, 2012; Agnihotri *et al.,* 2020). Introduction of AMS revolutionized radiocarbon dating as it requires significantly lower amounts of sample (in milligrams) and much reduced analysis-time (Bronk Ramsey, 2008; Krishnaswami and Lal, 2008). AMS produced ^{14}C dates have much lower uncertainties due to (i) lower sample size (requiring lesser amounts of chemicals for pre-cleaning) and (ii) direct counting of ^{14}C atoms rather than waiting for their beta decay (Hajdas *et al.,* 2021).

As chronology is very important aspect for constraining the early Holocene vegetation, initiation of early agriculture and its further advancements deciphered from the Lahuradewa lake sedimentary profile, we added six new AMS ^{14}C dates (Table-1). All the obtained AMS ^{14}C dates were found to be well in agreement with previously reported six conventional radiometric ^{14}C dates (Saxena *et al.,* 2006) (Fig. 2).This observation provides evidence for validity of conventional β-decay counting based radiometric method against modern AMS ^{14}C dates despite vast differences in their pre-requisite sample size and mode of detection. It must be however also mentioned here lithology of sediment is important which in case of Lahuradewa is clay-rich. Despite the excellent match, AMS chronologies are vital to minimize uncertainties associated with^{14}C dates and decipher short-term biogeochemical changes (in response to extreme climate changes) stored in palaeo-repositories (Andree *et al.,* 1986; Saarnisto, 1988; Grimm *et al.,* 2009).

δ^{13}C, δ^{15}N, δ^{34}S and C/N ratios and sequence development

δ^{13}C and C/N ratios of sedimentary layers depositing in fresh water aquatic realms (lakes) have ability to reveal relative changes in climate induced ecology and vegetation of the region by tracing source(s) of sediment organic matter (Meyers and Teranes, 2002). δ^{15}N values (in tandem with sediment N concentrations) help to deduce biogeochemical pathways of organic matter degradation during its settling and sedimentation (Lu *et al.,* 2010). Organic matter buried in the lake sediment could be derived primarily from the lake-catchment (allochthonous) and/or surface biogenic productivity developed within the lake system (autochthonous) (Meyers, 1994, 1997). A dominant allochthonous organic matter source is characterized by higher C/N ratios (>7.0) compared to those of *in-situ* organic productivity (Ptacnik *et al.,* 2010; They *et al.,* 2017). Enriched $\delta^{13}C_{TOC}$ and marginally higher C/N ratios characterize the bottommost section of the Lahuradewa Lake (Figs. 4A, D). It is note worthy that these sediment layers also contain conspicuously higher amounts of N contents with lower δ^{15}N values (~2‰; Fig. 3B), here possibility of N fixation from atmospheric source. The reported freshwater algae in Lahuradewa Lake sediments, *Botryococcus* generally have enriched δ^{13}C and lower δ^{15}N values with varying isotopic range from -10 to -23‰ having capability to fix atmospheric N (Alexander *et al.,* 2012; Mook *et al.,* 1974; Maberly and Spence, 1983; France, 1995; Burkhardt *et al.,* 1999; Marty and Planas, 2008).

The average δ^{13}C of organic matter in lake systems ~ −19.3‰, which indicates an *in-situ* (autochthonous) origin, more specifically enriched δ^{13}C (~ -17 to -16‰) generally found in biomass produced at the lake surfaces during summer months (March and September) (Gu *et al.,* 2006). Organic matter synthesis via aforementioned pathway is often limited by N which provids first order control on algal growth (Pinckney *et al.,* 2001). As a result, nitrogen fixation becomes feasible pathway for large algal biomass production (Williams and Carpenter, 1997). Hence, algal biomass produced in freshwater aquatic bodies is characterized by lower δ^{15}N values (ranging from–3 to +10‰, centring ~ 3‰) (France, 1995). Marginally higher C/N ratios (~14) likely indicate dominance of autochthonous origin of organic matter with minor proportion of allochthonous organic matter for bottom most peat section (pre-agricultural phase) of Lahuradewa lake (Fig. 4D). Interestingly, this bottom most section showed significant amounts of sedimentary S contents with average δ^{34}S ~9.6‰ (Fig. 4C). Degradation of *in-situ* produced organic matter (labile fraction) can lead microbial sulfate reduction pathway, and convert dissolved sulphates to elemental S or sulphides. Positive δ^{34}S values may have been manifested by bacterially mediated sulphate reduction (BSR) processes (Grossman and Desrocher, 2001; Choudhary *et al.,* 2009, 2013). More intrusive research is needed to understand complex S isotope biogeochemistry in fresh water aquatic bodies supporting high algal growth. δ^{13}C enrichment in the organic matter may also be manifested by preferential loss of ^{12}C during microbial degradation of organic matter in the peat section of Lahuradewa lake (Schelske and Hodell, 1995; Brenner *et al.,* 1999).

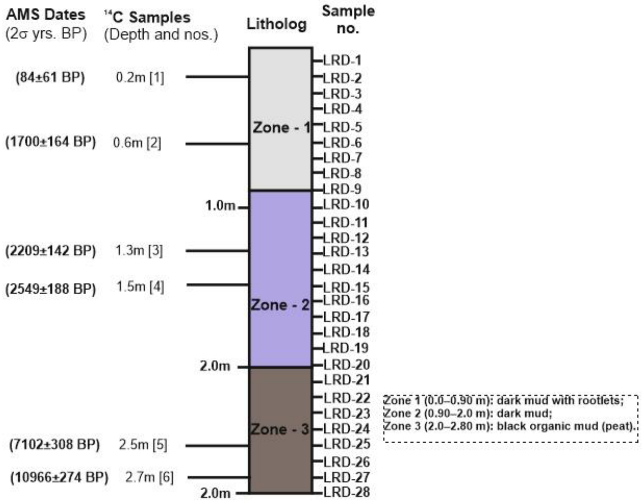

Fig. 6: Litholog of Lahuradewa lake sediment samples.

As aforementioned scenario (using enhanced TOC and TN contents and $\delta^{13}C_{TOC}$, δ^{15}N values) of pre-agricultural phase indicates towards the enormous sequestration of atmospheric C and N, likely mediated via algal community, Chauhan *et al.* (2009) provides a reliable confirmation for this contention, as it reported conspicuous prevalent domination of an algal community named as '*Botryococcus'* in the Lahuradewa lake. Pollen of this alga varied between ~26.5-80%. Plausible reason for enormous growth of this algal colony could be the undisturbed calm water conditions that prevailed after cool climate conditions during the 'Younger dryas' (Guy-Ohlson, 1992; Rasmussen *et al.,* 2006). Maximum bloom in the '*Botryococcus*' algae have been observed during or within the proximity to these cooling events and they appear to decline during the wetter periods (Kelts, 1988; Fig. 3G). This '*Botryococcus* algae' has capability to use the atmospheric C, N and dissolve HCO_3^- as carbon source, all of these sources manifest themselves as relatively enriched δ^{13}C values of biomass varying from -8.0 to -24.0 ‰ (median ~ -14.0‰); along with lower δ^{15}N values ranging between 1-4‰) (median ~2.5‰) (Tenaud *et al.,* 1989; Banerjee *et al.,* 2002; Alexander *et al.,* 2012). This study, hence, hitherto reveals high degree of C sequestration mediated by *Botryococcus* algae in central Ganga plains, leading to peat formations during early Holocene 10,999-7,451 cal yr BP. It must be noted that early Holocene period is known for wettest monsoonal conditions, driven by natural forcing factors especially highest solar insolation in tropical latitudes at ~9 ka BP (Gupta *et al.,* 2003). Intermittent dry and wet oscillations during pre-agricultural phase could be a plausible reason for blooms of *Botryococcus* algae which appears to have deposited at the surface of the lake. *Botryococcus* algae are known as precursor for hydrocarbons (Powell, 1986). In general, peat lands are known for sequestering large amounts of carbon; however, intermittent drought periods can result in carbon loss via respirations (Lund *et al.,* 2012). Both these processes can produce highly enriched δ^{13}C values of peat layers in sediments. Further, hydrocarbon release from sedimentary layers would favour additional enrichments of residual sedimentary organic matter (Gudin *et al.,* 1984; Urbanová *et al.,* 2013).

After the peat formation phase *i.e.* during the early-agricultural phase (7,451-2,623 cal yr BP) the $\delta^{13}C_{TOC}$ values appear to have declined, whereas $\delta^{15}N$ values enhanced. These values may be governed by nutrients and biogeochemical synthesis of organic matter (in fertile waters of the Lahuradewa lake) and supply of organic matter from cultivated croplands (paddy-fields) developed in the catchment (Rosenmeier *et al.,* 2004). It appears that the early agricultural phase was favoured in the vicinity 8,141-7,451 cal yr BP, as evidenced by earlier phytolith and diatoms studies in the same set of samples (Figs. 3D-F; Saxena *et al.,* 2013; Thakur *et al.,* 2020). More research is required to find answers to questions like 'was development of *Botryococcus* algae that promoted high amounts of carbon and bio-available nitrogen storage in the lake play key role for beginning of rice cultivation in the later (early agricultural) phase?' Direct evidences of agriculture (macro botanical remains based) in Lahuradewa archaeological site as well as nearby locales (Damdama) have indicated agriculture activities started in Ganga plains in the later 3rd millennium BC (~5,000 yr BP) (Pokharia, 2011).

We observed stability in both $\delta^{13}C_{TOC}$, $\delta^{15}N$ values together with rice phytolith counts between ~2,623 to 1,228 cal yr BP (LRD-16 to LRD-7); whereas paddy field origin diatoms and anthropogenic diatoms showed steady declining trends (Fig. 3). This phase may have been governed by a consistent decline in monsoonal precipitation. A declining phase of southwest monsoon in north India has been reported by Srivastava *et al.* (2017) by analysing a well dated peat sequence of Kedarnath Himalaya. They reported a weaker monsoon between ~1,400 to ~3,000 cal yr BP which is also corroborated by the other palaeomonsoonal studies (Gasse et al. 1991; Thompson et al. 2000; Fleitmann et al. 2003; Gupta et al. 2003). It was interesting to note that even though paddy field diatoms showed an overall decline in this phase most likely due to deteriorating monsoonal conditions in the region (Chauhan, 2003), cultivated phytolith taxa remained stable albeit a marginal waxing and waning. This possibly indicates human efforts to grow rice (without shifting crop type) despite prevailing aridity. It is reported from the Lahuradewa archaeological site that rice cultivation was traditionally practiced along with barley in the early agricultural phase (i.e. Neolithic ~4450 BP to early historic ~2150 BP), however, after ~2600 BP native settlers appear to continue with their agricultural activity with only rice (abandoning barley) till present day situation (Pokharia *et al.,* 2017).

During the Modern phase (~1,228 cal yr BP to present day; LRD-7 to LRD-1), the $\delta^{13}C_{TOC}$ values clearly appear to have declined further typifying modern day agricultural lands. $\delta^{15}N$ values also tend to decline (Fig. 3A, 4A) with steady C/N ratios ~8.7 (close to Redfield ratio of ~7). This phase is marked by conspicuous enhancing trends of diatoms (both paddy field and anthropogenic) with consistent phytolith counts of cultivated rice (Fig. 3D-F). These observations indicate that monsoonal conditions appear to have revived during this phase in central India. In modern day agricultural field, significant decline in $\delta^{15}N$ values could be anticipated due to application of synthetic inorganic ammonia based fertilizers (Bateman and Kelly, 2007). However, in present case of Lahuradewa lake, youngest declining trend $\delta^{15}N$ values might be resultant of arrival of leguminous crops that are capable of fixing atmospheric N_2 (Hardy *et al.,* 1971; Chiewattanakul, 2020).

CONCLUSIONS

Taken together, revising chronology using AMS method and measuring stable C and N isotopic values of sediment layers of the Lahuradewa lake provide several newer insights into biogeochemical changes occurring in a freshwater aquatic body of the central Ganga plain, where variety of proxy evidences for earliest rice cultivation have already been presented. Major conclusions of this study could be summarized as following-

All newly measured ^{14}C dates of sediment layers of Lahuradewa lake were found in excellent agreement with previously reported conventional radiometric ^{14}C dates (Saxena *et al.,* 2006). This finding reinforces chronology of earliest evidences of agriculture in central Ganga plain between ~8,830 to ~8,141cal years BP, which marginally pre-dates rice advent in the Yangtze River valley of southern China. This observation also validates utility of Liquid Scintillation Counter (LSC) derived conventional radiometric ^{14}C dates despite challenges arising from other suitable techniques like AMS.

All biogeochemical tracers *viz.* TOC, TN, C/N ratios along with $\delta^{13}C$ and $\delta^{15}N$ values indicate dominance of autochthonous organic matter (over allochthonous) throughout the Holocene.

Measured biochemical proxies reveal new insights into pre-agricultural phase (early Holocene) that sequestered capacious amounts of C and N from atmospheric source via production of *Botryococcus* algae. As this algae has been reported to be precursor of natural hydrocarbon gases and ability to sequester atmospheric CO_2, their intentional introduction in wetlands may greatly help climate change mitigation efforts (reducing carbon emissions) in concurrent climate change scenario.

Botryococcus alage possess a great potential for extraction of hydrocarbons that can be chemically converted into fuels (Moheimani *et al.,* 2013). In modern times, *Botryococcus* algae gets produced in blooms over the surface Lake Baikal (Siberia; 53.5587°N, 108.1650°E), where it releases sufficient amounts of oil on the surface of the lake which is collected with a special skimming apparatus and used as a source of fuel (Bellinger and Sigee, 2015).

It is noteworthy that $\delta^{13}C$ values alone, at times, could mislead climatic/ ecological interpretations and warrants dire need for other suitable biotic proxies to understand biogeochemistry of aquatic systems.

More intrusive research is needed towards understanding how lake environments respond to wetter phases of climate *i.e.* how (naturally) they switch to sequester atmospheric C and N. These insights are needed to attain carbon neutrality or minimizing carbon footprint of India by AD 2070 together with global emitters (Rogelj *et al.,* 2015).

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