Diversity of diatom and carbon isotope characterization of soil organic matter in extreme climate, Sikkim Himalaya, India

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Analysis of spatial variation of diatom assemblages and characterization of carbon isotopic composition of surface soil/sediment samples along three high-altitude transects (Chopta, Lashar and Gurudongmar valleys) in North Sikkim, Higher Himalaya have been done to delineate the ecological information (modern analogue). We have studied the variation in the distribution of diatom species and stable carbon composition in three different valleys having variable climatic conditions ranging from sub-humid to semi-arid. The results reveal that the biotic components respond appreciably towards varying environmental conditions. The spatial distribution of diatoms in surface sediments seems to be controlled by physical parameters such as temperature, water and nutrient availability. The overall $\delta^{13}C$ values vary from -25‰ to -29‰, suggesting a C_3-dominated vegetation in the region. Higher $\delta^{13}C$ values are observed in Gurudongmar samples that are characterized by less moisture and low temperature. The $\delta^{13}C$ values suggest that the source of organic matter in soil/sediment is contributed by open grasslands (C_3 grasses). The isotope values clearly demonstrate an increase in $\delta^{13}C$ values along with a progressive increase in elevation and decrease in precipitation. The present data will serve as an important archive for future correlations in palaeoclimatic studies.

Keywords: Carbon isotopes, diatom distribution, extreme climate, modern analogue, soil organic matter.

STABLE carbon isotope ($\delta^{13}C$) values and diatoms are among the most important proxies used for understanding the past climate dynamics[1–5]. However, like pollen, the understanding of modern isotopic composition and diatom distribution in the topsoil/sediments (modern analogue) from different climatic regimes of continental India and especially in the Higher Himalaya is lacking[6]. It is suggested that the climate exerts dominant control on spatial distribution of flora and fauna; hence the characterization of modern analogue is an essential step and

should be done in case of every proxy used for studies related to the past climate reconstruction[7–10]. The Higher Himalayan region with an elevation range of ~3000 to more than 8000 m amsl experiences a transitional climate between the semi-arid (dry) northern Trans Himalayan region and sub-humid southern Himalayan region[11,12]. The flora and fauna of such sensitive zones respond to slight changes in climate variability and this is also recorded in the sedimentary archives[13]. In order to better understand the effects of climate change on both abiotic and biotic elements of different ecosystems, it is important to first understand the present-day dynamics[14–16].

In view of the limited instrumental data records from the Himalayan region, the use of proxies is required to understand the present-day ecosystem dynamics and its response to past climatic variability. In high mountainous terrain, each proxy bears a limitation owing to the extreme climate and does not allow all modes of climate variability signatures to be captured. Therefore, different proxy records are studied simultaneously to infer the past climate. Biotic proxy indicators such as carbon isotope ($\delta^{13}C$) and diatoms are promising and direct indicators of climate as they respond to subtle changes in the environmental conditions owing to their high sensitivity to numerous environmental variables[13,17–20]. The carbon isotopic signatures of organic matter associated with soil/sediments (soil organic matter; SOM) have been widely used for understanding the vegetation types (C_3, C_4 and CAM) and are an important tool to reconstruct the present and past climatic conditions of any region[21]. Similarly, most of the diatom species have a narrow tolerance towards site-specific characteristics, thus making them good indicators for palaeoecological reconstructions[19,22–24]. Their occurrence is also affected by altitudinal gradients, including climate, geology, topography, anthropogenic activities such as agriculture and use of water resources[25].

The Higher Himalayan region (>4000 m amsl) is characterized by alpine vegetation dominated by grasses[26,27]. The grass family (Poaceae) is highly diversified in terms of photosynthesis, and both C_3 and C_4 species co-occur in
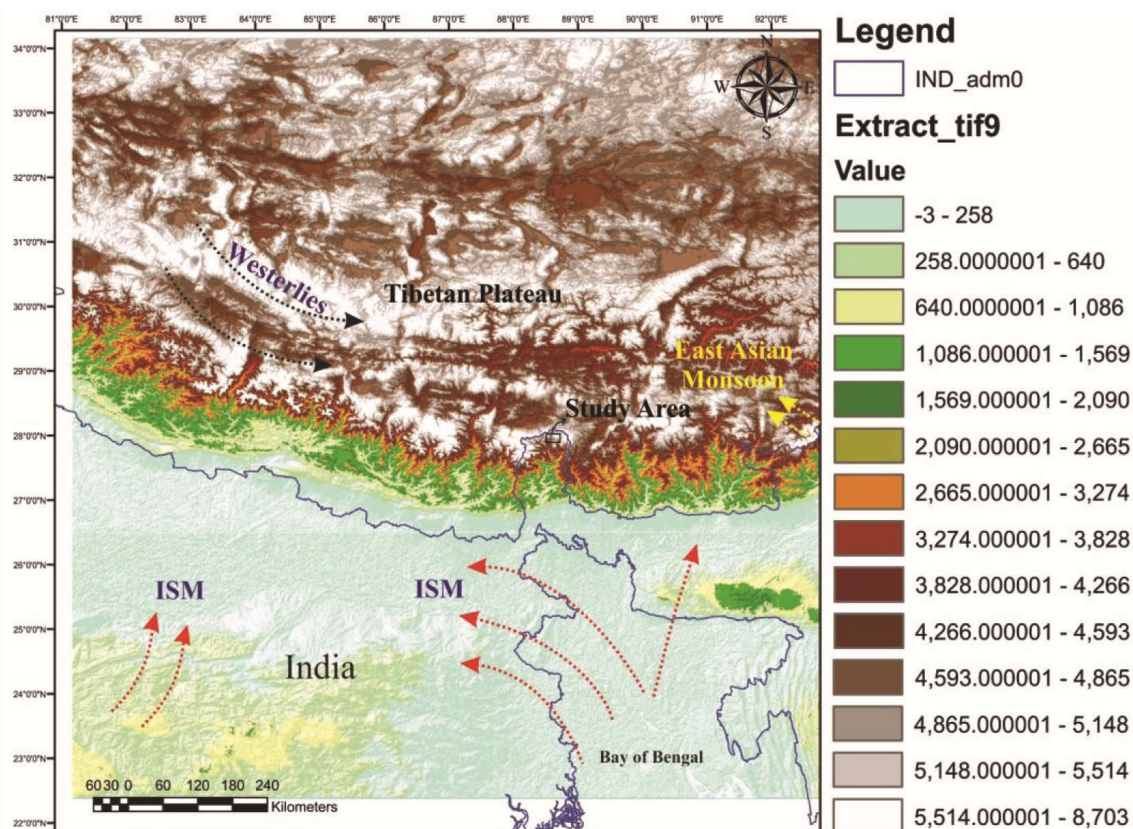
Figure 1. Shuttle radar topographic mission (SRTM) digital elevation model (DEM) showing the location of Sikkim (study area) and different weather systems, i.e. the Indian summer monsoon (ISM) and mid-latitude westerlies.

the tropical regions. In such cases, C_3 grasses show clear tendency to dominate in relatively more humid as well as cooler areas. These ecological separations of C_3 and C_4 grasses have been seen across the latitudinal and altitudinal gradients in the tropical region, and gradient of water availability and seasonality[21–28]. Hence, the isotopic study is being increasingly used to understand photosynthetic pathway of plants as well as palaeovegetational/climatic reconstruction.

In view of this, the present study delineates the diatom diversity in extreme climatic condition and characterizes the of $\delta^{13}C$ values of SOM in surface sediments from three different localities in the alpine zone of North Sikkim, Eastern Himalaya, India. The results will help to understand the relationship between modern conditions and the biotic analogues that will be useful in efficient interpretation of past $\delta^{13}C$ and diatom assemblages for palaeoecological and palaeoclimatic reconstructions.

Study area

The study of modern stable carbon isotope composition of SOM and diatom assemblages was undertaken in North Sikkim, Higher Himalaya, India (Figure 1). Sikkim has a steep altitudinal gradient (~8300 m elevation differ-

ence over 100 km distance) resulting in complex topography that significantly controls the local weather patterns and gives rise to distinctive microclimatic niches (Figure 2)[29,30]. This study has been undertaken in the Chopta, Lashar and upper Tista (up to Gurudongmar lake) valleys (27°03′41″N to 28°7′34″N lat and 88°3′40″E to 88°57′19″E long) (Figure 2). Climatologically, the Chopta and Lashar valleys are located in the transitional zone of southern sub-humid Himalaya with characteristics of low to moderate vegetation and the northern semi-arid zone that includes Gurudongmar (Figure 3). The Chopta and Lashar valleys, lying at the boundary of the tree line, are broad, U-shaped, pro-glacial valleys. Geomorphologically, these valleys are occupied by glacial and glaciofluvial deposits like prominent moraines and outwash sediments. The Gurudongmar (lake) area is located at an altitude of ~5150 m amsl in the upper catchment of Tista watershed. It is one of the largest moraine dammed glacial lakes in the Sikkim Himalaya covering an area of about 1.08 sq. km. The outlet of the lake is towards NNW direction and melt water of the lake is one of the sources of Chhombo River[31]. This area has scanty vegetation and experiences harsh semi-arid climate[32,33]. The main plant species found in extreme cold regions of Sikkim (like Gurudongmar) are *Elymus* L. sp., *Kobresia* Willd. sp., *Festuca* L. sp., *Poa* L.
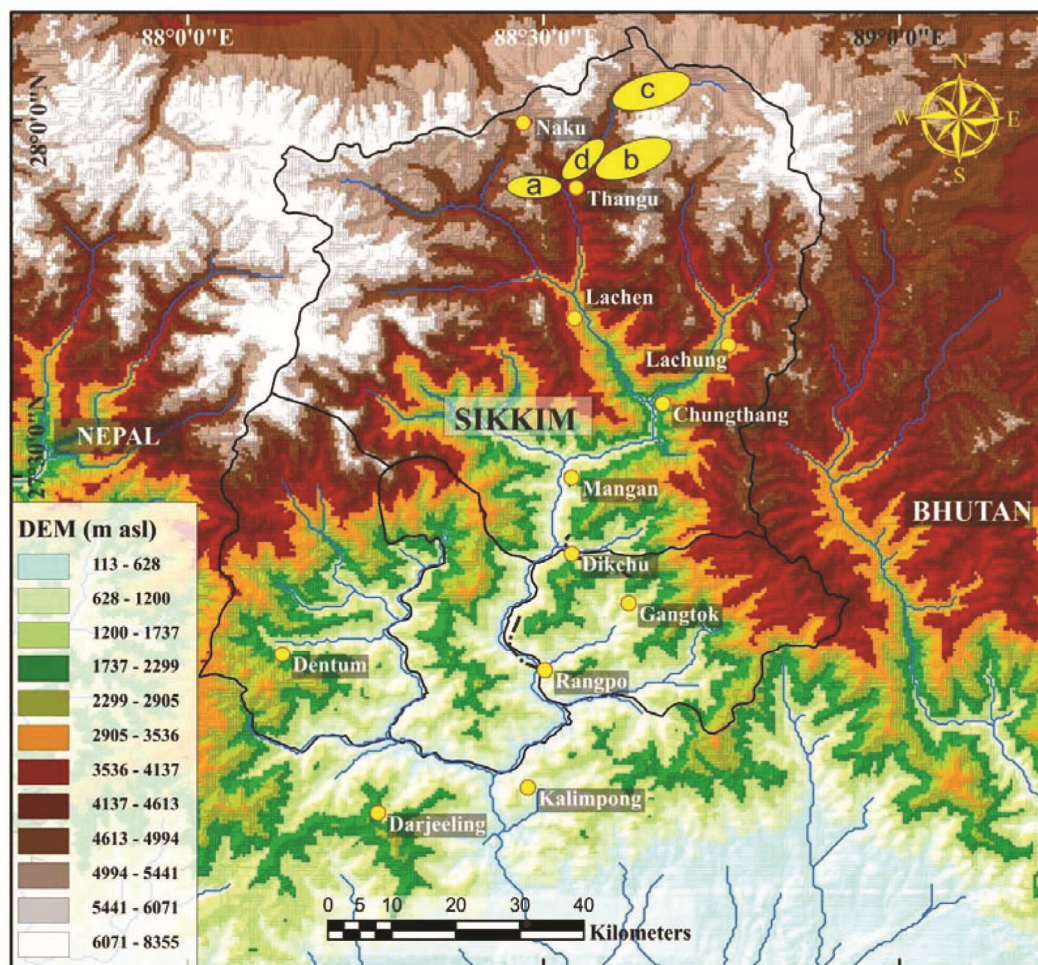
Figure 2. SRTM-DEM of Sikkim showing the state and district boundaries (black line), major drainages (blue lines), important towns/settlements (yellow dots) and sample locations sites (yellow ellipses) described in Figure 3.

sp., *Acronema* Falcon. ex Edgew., *Aconitum* L. spp., *Gentiana* L. sp., *Primula* L., *Saxifraga* L., *Picrorhiza* Royle *Nardostachys grandiflora* DC[34] (CCS-Sikkim-2005). Being in the trajectory of the Bay of Bengal branch of the Indian summer monsoon (ISM), the Sikkim Himalaya experiences a humid climate in the southern parts, whereas towards the north (higher altitude) the ISM precipitation progressively decrease due to orographic effect[1] and at the expanse of winter precipitation (snowfall) which is contributed by the southern branches of the mid-latitude westerlies[35–37].

Vegetation

Lashar and Chopta valleys situated at an elevation of ~4000 and 4500 m amsl respectively, mark the upper limit of the tree line and shelters alpine vegetation. Different high-altitude species like *Primula* L., *Juniperus* L., *Rhododendrons* L., *Meconopsis* Vig., *Potentilla* L. etc. are present in the study area[31]. *Aconitum hookeri* Stapf, *Agrostis* L. sp., *Astragalus* L. sp., *Oxytropis* DC. sp. etc.

are other predominant species growing in this area. The area extending beyond Thangu (above 4000 m amsl) is the Himalayan rainshadow zone of cold desert that merges with Tibetan plateau. Above ~4000 m amsl, the slopes are mildly undulating and the ground is mostly covered with scrubs like *Rhododendron setosum* D.Don, *Rhododendron nivale* Hook.f., *Rhododendron anthopogon* D.Don, *Juniperus indica* Bertol, *Juniperus recurva* Buch.-Ham. ex. D.Don, etc. The herb species prevalent in the area include *Bergenia ligulata* (Wall.) Engl., *Corydalis* DC. sp., *Gaultheria* L. sp., *Gentiana* L. sp., *Ephedra* L. sp., *Arenaria* L. sp., *Lactuca* L. sp., *Taraxacum* F.H.Wigg. sp., *Saussurea* DC. sp. and *Aster* L. sp.[31].

Methodology

Diatom sample preparation

A total of 35 modern surface samples were collected from the high-altitude transitional zones of fairly vegetated Chopta-CHS (24 samples; BSIP museum sample
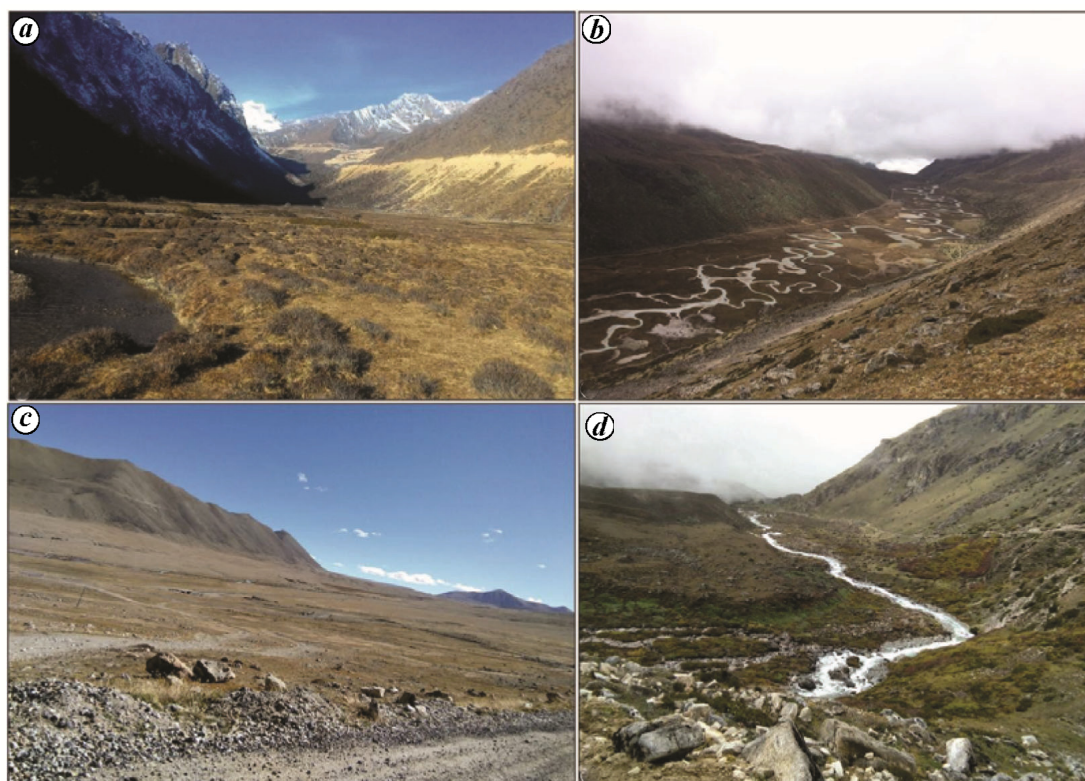
Figure 3. Field photographs. *a*, Facing the upstream direction showing the Chopta valley, North Sikkim, with alpine scrub vegetation demarcating the upper limit of the tree line. *b*, Facing the downstream direction of the Lashar valley, showing relatively lesser vegetation. *c*, Gurudongmar region with alpine barren condition. *d*, Transition zone showing vegetation change from alpine scrub to alpine barren for the transect towards Gurudongmar.

accession no. 9051), Lashar-TVM (five samples; BSIP museum sample accession no. 9221) and semi-arid Gurudongmar-SK (six samples; BSIP museum sample accession no. 9221) valleys in North Sikkim during October 2017 to delineate the diatom diversity changes in extreme climatic regions (glacial environments). In order to extract diatoms, ~2–3 g of dry sediment sample was treated with hydrochloric acid (HCl) to dissolve and remove the carbonates. Subsequently, the samples were treated with H_2O_2 to remove organic matter. The samples were decanted and washed with distilled water to remove any acid used in the process. Four slides of each sample were prepared on a hot plate using mounting material[38,39]. The identification, classification and counting of diatoms were carried out following standard procedures[40–42]. For better representation, TILIA and TILIA graph software were used for the preparation of range chart and CONISS was applied for cluster analysis[43] (Figure 4).

Isotopic sample preparation

The samples that were collected for diatom analysis were used for isotopic analysis as well. About 1 g of the sediment sample was taken after coning and quartering, and was finely powdered to clay size particles and poured into 50 ml centrifuge tubes. The samples were treated with 5% HCl solution (three times) for the removal of carbonates and washed with Milli-Q water using a centrifuge (~3000 r/min) to remove acid and soluble salts. The decarbonated samples were then dried in a hot-air oven with temperature fixed at 45°C. The oven-dried samples were again powdered with an agate mortar to loose clumps that might have formed during drying. All the acid-treated powdered samples were individually packed into tin capsules and introduced into the pre-filled and conditioned reactor of Elemental Analyser (Flash EA 2000 HT) through an autosampler. The CO_2 gas produced through the combustion was introduced into the Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS, MAT 253) coupled with Con–Flow IV interface for isotopic analysis. The reproducibility of samples was checked by repeat measurements. References gas was calibrated using International Atomic Energy Agency (IAEA) CH-3 and carbon isotopic data reported against Vienna Pee Dee Belemnite (VPDB). International standards (CH-3, and CH-6 as well as sulphanilamide) were run to check the accuracy for CO_2 measurements with an external precision of 0.1‰ (1σ). All samples were analysed in the Stable Isotope Laboratory, Birbal Sahni Institute of Palaeosciences (BSIP), Lucknow.

Table 1. Diatoms with abbreviations/codes used for statistical analysis

Diatom genus	Codes used in statistical analysis	Diatom species
Melosira	Mel	*M. varians*
Cyclotella	Cyc	*C. meneghiniana, C. striata*
Aulacoseria	Aul	*A. granulata*
Pinnularia	Pin	*P. borealis, P. gibba, P. virdis, P. biceps*
Eunotia	Eun	*E. camelus, E. diodon, E. arcus, E. monodon*
Amphora	Amp	*A. copulata*
Hantzschia	Han	*H. amphioxys*
Nitzschia	Nit	*N. clausii, N. palea*
Craticula	Cra	*C. cuspidata*
Gomphonema	Gom	*G. parvulum, G. gracile*
Neidium	Nei	*Neidium* sp.
Achnanthidium	Ach	*A. minutissimum*
Tabellaria	Tab	*T. flocullosa*
Navicula	Nav	*N. virdis, N. tenera, N. lancecolata*
Encyonema	Enc	*E. hustedtii*
Caloneis	Cal	*C. brevis*
Stauroneis	Sta	*S. circumborealis, S.* cf. *kriegeri*
Achnanthes	Ach2	*A. brevipes*
Frustulia	Fru	*F. saxonica*
Diatoma	Dia	*D. vulgaris*
Cymbellonitzschia	Cym	*Cymbellonitzschia* sp.
Cocconeis	Coc	*C. placentula*
Sellaphora	Sel	*S. pupula*
Fragillaria	Fra	*F. rinoi* sp.
Cymbella	Cym	*C. aspera*
Anomoeneis	Ano	*A. sphaerophora*
Surirella	Sur	*S. gemma*
Diploneis	Dip	*D. ovalis*
Hippodonta	Hip	*H. capitata*
Epithemia	Epi	*Epithemia* sp.
Bacillaria	Bac	*B. paradoxa*
Gyrosigma	Gyr	*G. acuminatum*
Synedra	Syn	*S. ulna*
Luticola	Lut	*Luticola* sp.

Numerical analyses

PCA, redundancy analysis (RDA) and HCA were carried out to study variations and environmental drivers for these variations among the diatom species, and how the species are clustered respectively. We used detrended correspondence analysis (DCA) to choose whether linear or nonlinear (unimodal) ordination technique needs to be employed. Environmental gradient lengths using the different diatom genera (Table 1) were calculated from DCA and the DCA axis length score was found to be 2.3 SD. This indicates that environmental gradient is small (less than 3 SD) and suggests employing the linear model of ordination analysis, i.e. PCA to understand the variability among 34 genera and the factors that account for the variability. As altitude and $\delta^{13}C$ (‰) values were available, both were used as explanatory variables. It is to be noted that although $\delta^{13}C$ is not an explanatory variable in its strict sense, we used it as an indicator of water availability which is a driving force. However, these two explanatory variables are not the only controlling parameters for the variation in diatom genera, but other environmental factors also play a role.

Further, RDA, a constrained ordination analysis was used to know the relationship between altitude and $\delta^{13}C$ to the biotic dataset. All the genera were used in the analyses with Hellinger transformation to reduce the impact of taxa with zero values[44,45]. All the analyses were done in R platform (R Core Team, 2014) using vegan package from CRAN project[46].

Results

Diatoms

A total of 34 genera with three planktic and 31 benthic forms have been recorded in this study (Table 1 and Figure 4). It was observed that samples lying close to the river and moist places showed excellent diatom response both in assemblage and frequency; however, samples from the semi-arid zone did not yield rich assemblage (<50 cells). The major genera encountered in terms of frequency and abundance were *Pinnularia, Eunotia, Amphora, Nitzschia, Hantzschia, Achnanthes*, etc. (Figure 5). TILIA 1.7 and cluster analysis (CONISS) were used for
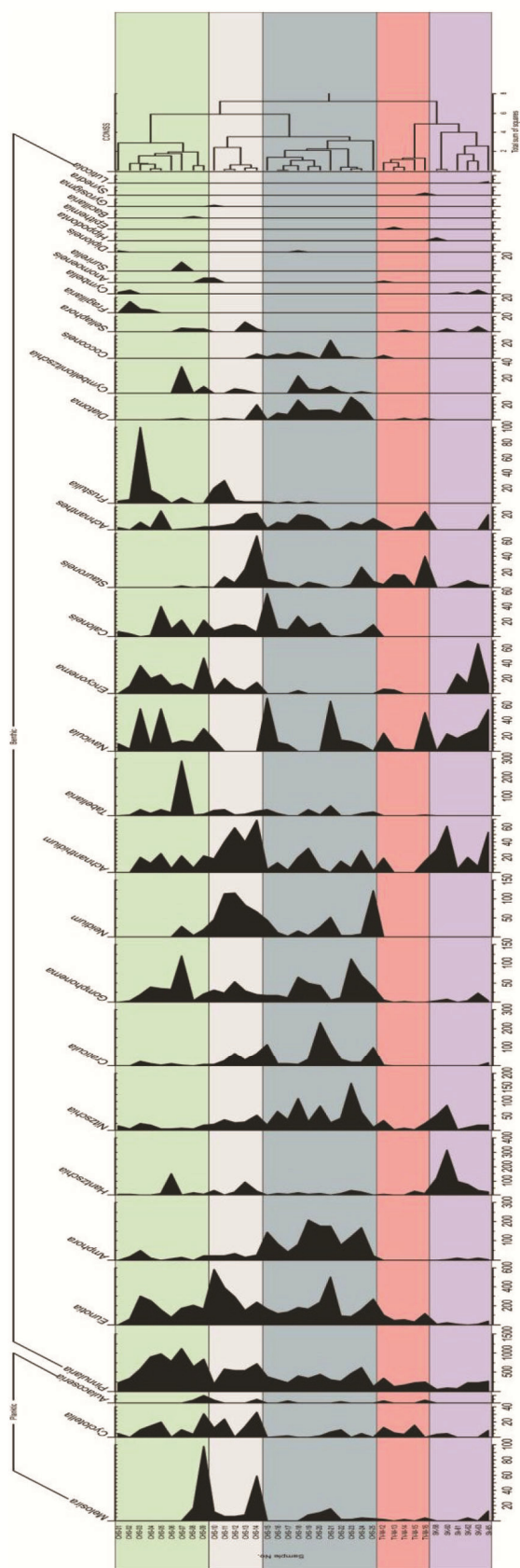
Figure 4. Frequency distribution of diatom genera in the surface samples from the Chopta, Lashar and Gurudongmar valleys with zonation based on cluster analysis.

differentiating and grouping the diatom assemblage of the studied locations (Figure 4). The Tilia plot shows dominance of pennate (benthic) forms over the centric (planktic), and the ratio of centric to pinnate is very low (<1). On the basis of cluster analysis, the assemblage has been broadly categorized into three major groups, i.e. Chopta (CHS), Lashar (TVM) and Gurudongmar (SK) and are described below.

Zone 1 – Gurudongmar (SK-65 to SK-58). This zone comprises samples collected in a transect along the Gurudongmar valley. Samples SK-58 to SK-60 were collected near the Gurudongmar lake and SK-61 to SK-63 were collected in the downstream area that represents the transitional zone, i.e. vegetated (grasses) to non-vegetated (~90% barren) belt. Sample SK-65 has been collected further downstream (~2 km upstream of Chopta) in a fairly vegetated (grasses) area. Among the benthic forms, *Pinnularia* is represented in the highest frequency (~272) in samples SK 62, 63 and 65. Similarly, *Hantzschia* shows extremely high values (~315) in sample SK-60 and moderately high in sample SK-58 (~120 counts) lying close to the Gurudongmar lake outlet. Other benthic forms like *Nitzschia* and *Achnanthidium* are recorded in low frequency ranging from 4 to 88 counts, and *Navicula*, *Encyonema*, *Eunotia*, *Gomphonema* and *Amphora* are represented in low frequencies (~4–54). The planktic forms such as *Melosira* and *Cyclotella* are sporadically represented.

Zone 2 – Lashar valley (TVM-12 to TVM-16). The samples collected from the Lashar valley yielded both benthic and planktic diatoms. *Pinnularia* shows the highest frequency with ~162–345 counts among all samples. *Eunotia* is recorded in fairly good frequency in samples TVM-16 (~120) and TVM-12 (~105), while it is scantily recorded in TVM-13 to TVM-15 (<60 counts). In TVM-16, the diversity of few benthic diatoms like *Navicula*, *Nitzschia*, *Achnanthes*, *Stauroneis* and *Hantzschia* increases moderately, ranging from 15 to 50 counts. Other benthic diatoms like *Encyonema*, *Craticula*, *Cocconeis*, *Tabellaria*, *Diatoma, Epithemia* and *Synedra* are recorded in very low frequencies (~2–4). The planktic forms such as *Cyclotella*, *Melosira* and *Aulacoseira* are represented sporadically in this zone.

Zone 3 – Chopta valley (CHS-1 to CHS-25). The samples collected from the Chopta valley (marshy outwash plain) have yielded a rich diatom assemblage. On the basis of diatom frequency, this zone has been further categorized into three subzones (3a–3c).

Zone 3a (CHS-15 to CHS-25). This includes both benthic and planktic forms. The benthic form *Pinnularia* shows the highest frequency (~623, CHS–24) followed by *Eunotia* (~90–503), *Amphora* (~23–210), *Craticula* (~9–229),
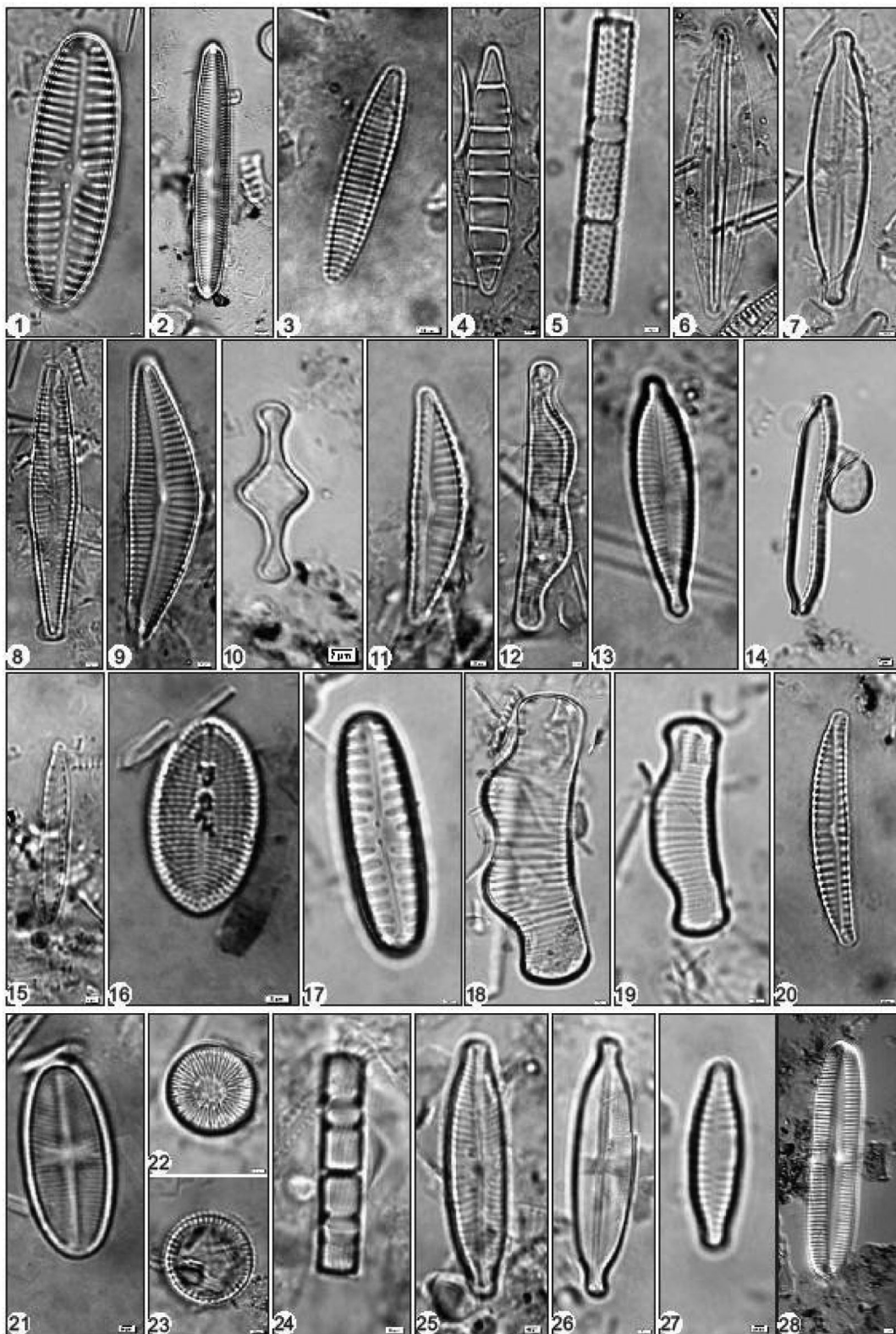
Figure 5. (1, 17) *Pinnularia borealis*; (2) *Pinnularia divergens*; (3) *Nitzschia amphibia*; (4) *Diatoma vulgaris*; (5) *Aulacoseira granulta*; (6) *Frustulia saxonica*; (7, 26) *Stauroneis circumborealis*; (8) *Gomphonema gracile*; (9) *Cymbella aspera*; (10) *Tabellaria flocculosa*; (11) *Encyonema hustedtii*; (12) *Eunotia diodon*; (13) *Gomphonema parvulum*; (14) *Hantzschia amphioxys*; (15) *Nitzschia clausii*; (16) *Cocconeis placentula*; (17) *Eunotia camelus*; (18) *Eunotia monodon*; (19) *Eunotia praerupta*; (20) *Amphora copulate*; (21) *Stauroneis* cf. *kriegeri*; (22) *Cyclotella striata*; (23) *Cyclotella meneghiniana*; (24) *Melosira varians*; (25) *Navicula lanceolata*; (26) *Fragilaria rinoi* sp.; and (28) *Pinnularia virdis*.

Nitzschia (~10–165) and *Gomphonema* (~5–113 counts). *Navicula, Achnanthidium, Tabellaria, Caloneis, Hantzschia, Diatoma, Stauroneis, Cocconeis* and *Cymbellonitzschia* are sporadically recorded (~4–69). However, *Encyonema* and *Diploneis* are encountered only in sample CHS-18 in low frequencies (~2–4). The planktic diatoms *Melosira, Cyclotella* and *Aulacoseira* are sporadic.

Zone 3b (CHS-10 to CHS-14). The benthic forms *Pinnularia* and *Eunotia* both show dissimilar behaviour and are recorded in low to high frequency (~147–727). *Neidium, Achnanthidium, Craticula, Nitzschia* and *Gomphonema* also show significant values (~157–429). However, *Hantzschia, Nitzschia, Amphora, Stauroneis, Tabellaria, Achnanthes, Frustulia, Caloneis, Encyonema, Diatoma, Sellaphora, Navicula* and *Cymbellonitzschia* show moderate to low counts. The planktic forms like *Cyclotella* and *Melosira* are occasionally present, but did not record more than 59 counts. In samples CHS-10 and CHS-15, the presence of *Aulacoseira is* recorded in extremely low counts (~4–5).

Zone 3c (CHS-1 to CHS-9). The samples CHS-1 to CHS-9 record the highest frequency of *Pinnularia* (~229–1119 counts) compared to all other zones, followed by *Eunotia* (~60–350). The other benthic diatoms, namely *Amphora, Hantzschia, Nitzshia, Craticula, Gomphonema, Neidium, Achnanthes, Tabellaria, Navicula, Encyonema, Caloneis, Frustulia* and *Achnanthidium* show variable frequencies ranging from ~2 to 100 in most of the samples. The present range of diatoms also varies indifferently in many samples and may reflect environmental biases. The planktic form *Melosira* is recorded only among two samples with the highest frequency in sample CHS-9 (~98) and lowest the frequency in CHS-8 (~17). Similarly, *Aulacoseira* is also recorded only in two samples, viz. CHS-9 (~10) and CHS-8 (~4), whereas *Cyclotella* is sporadically recorded with variable frequency throughout this zone.

$\delta^{13}C$

The $\delta^{13}C$ values of SOM in the Chopta valley range between –25.8‰ and –27.7‰ with an average of –26.6‰. In case of the Lashar valley, almost similar values ranging between –25.8‰ and –27.3‰ with an average of –26.5‰ have been recorded. On the other hand, the $\delta^{13}C$ values of SOM in the Gurudongmar valley are relatively higher and range from –24.9‰ to –26.8‰ with an average of –25.9‰.

Ordination analysis

PCA shows that first five components could only account for 52% (17.6%, 10.2%, 9.8%, 7.4% and 6.5%) of total variation in the genera data from different sample locations, as suggested by the scree plot (Figure 6, Table 1). RDA shows that a variation of 13.2% can only be constrained by $\delta^{13}C$ and altitude, and the remaining variation (86.8%) is left unconstrained. Both altitude (RDA 1–69.9%, $r^2 = 0.669$) and $\delta^{13}C$ (RDA 2–30.1%, $r^2 = 0.56$) could explain 13.2% variability together in the diatom species data (Figure 7). Both the parameters are found to be significant with *P*-value = 0.001. However, altitude was found to be significant than $\delta^{13}C$ that is governing the variability in distribution of diatoms at different altitudes.

HCA was carried out to see how these samples at three different localities are clustered based on dissimilarities between the diatom genera using the observed characteristics. Figure 6 *b* and Figure 6 *c* show the results as a dendrogram and phylogram of unrooted type respectively. In Figure 6 *d*, PCA and HCA results are shown together for comparison. We have observed that more than two clusters explain the data redundantly. All the analyses were carried out in R platform (R Core Team, 2014), and the CRAN packages used were vegan[46] and ape[47].

Discussion

Conventionally, modern analogues are used in palaeo-(vegetation/climate) reconstructions to understand modern pollen–vegetation relationship in surface sediments. However, this approach is lacking in other proxy climate reconstruction studies from the Indian subcontinent. We propose that for every proxy reconstruction, understanding the present-day dynamics of the proxy and extant environmental condition is a pre-requisite. Towards this, we have made an effort to understand the distribution of diatoms and $\delta^{13}C$ values along three climatologically different valleys (Chopta, Lashar and Gurudongmar) in North Sikkim, Higher Himalaya.

The diatom species reported in this study are dominated by pennate (benthic) forms, while the centric (planktic) forms are scanty. It has been observed that the samples collected near Gurudongmar show small diatom diversity. Diatom species like *Hantzschia, Pinnularia* and *Nitzschia* show dominance over centric forms, viz. *Cyclotella* and *Melosira*, indicating low water conditions in the region. The low turnout of *Eunotia* species also advocates for water scarcity. The total sum of diatoms dominated by benthic forms suggests nutrient availability owing to change in meltwater discharge during summer and winter seasons[25,37].

In the Lashar alpine valley, the diatom assemblage is dominated by *Pinnularia* followed by *Eunotia*, but with varying frequencies. Diatoms such as *Navicula, Nitzschia, Achnanthes, Stauroneis* and *Hantzschia* are recorded in low to moderate frequencies. The planktic forms such as *Cyclotella, Melosira* and *Aulacoseira* are sporadically
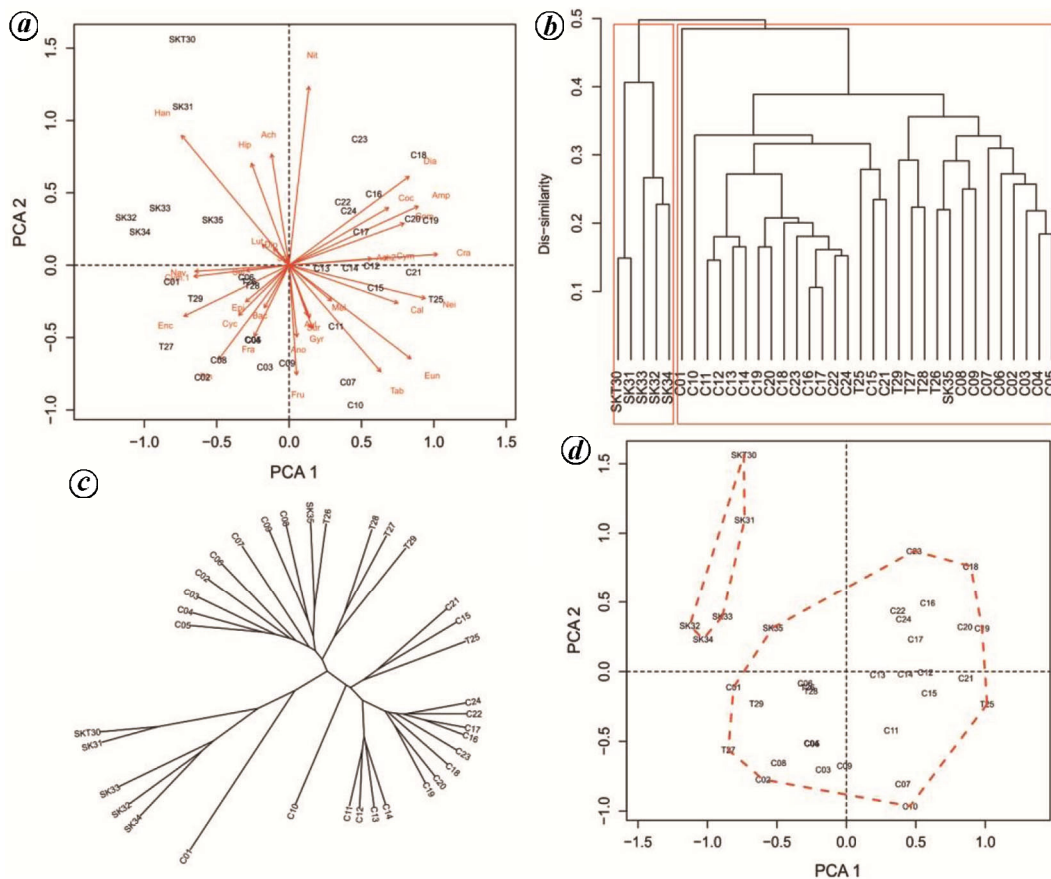
Figure 6. Statistical analysis. *a*, Biplot to visualize the principal component analysis (PCA) results. PCA was done based on the diatom genus of each sample. The diatom genera abbreviations/codes are given using the first three letters (Table 1). The red arrowed lines are the characteristics of the genera. *b*, Dendrogram of the sample locations from the three alpine valleys (C, Chopta; T, Lashar: SK, Gurudongmar). *c*, Phylogram of the sample locations from the three alpine valleys based on the dissimilarity indices between the species using hierarchical cluster analysis (HCA). *d*, Combination of the results obtained from PCA and HCA. *Abbreviations/codes for diatom genera used in statistical analysis are given in Table 1.

represented in the assemblage. The overall representation suggests that the region experiences a cold wet environment with very low human activity[48,49]. Also, the depositional environment shows pristine water conditions with low to moderate nutrient availability. It is also observed that the diatom variation is subjected to available water conditions that may have resulted due to landscape variations[48].

The Chopta Valley (marshy outwash plain) shows a significant heterogeneity in diatom assemblage. Samples CHS-15 to CHS-24 (collected in the downstream part of the valley) are dominated by the benthic diatoms. This may be attributed to the close proximity of the melt water stream and high nutrient availability (marshy landscape). The planktic forms are represented in low counts, suggesting extremely shallow water depth. The high counts of *Pinnularia*, *Eunotia*, *Amphora*, *Craticula*, *Nitzschia* and *Gomphonema* further advocate for pelagic conditions prevailing in the valley under marshy conditions. The availability and diversity of *Eunotia* suggest pristine

water conditions. Anthropogenic activity can be interpreted to some extent from *Navicula*, *Achnanthidium*, *Tabellaria*, *Caloneis*, *Hantzschia*, *Diatoma*, *Stauroneis*, *Cocconeis* and *Cymbellonitzschia*, and is in agreement with the field observations as this valley is being used as a pasture land by the locals[25]. In zone-3b comprising samples collected along the melt water stream (upstream part of the valley), a significant increase in *Cyclotella* and *Melosira* is observed, indicating enhanced melt water availability. The moderate rise in *Achnanthidium*, *Nitzschia*, *Gomphonema*, *Tabellaria*, *Achnanthes*, *Frustulia* and *Cymbellonitzschia* suggests increased human activity associated with grazing practices. The zone-3c samples were collected along the northern boundary (foothill) of the valley. This zone records the highest frequency of *Pinnularia* followed by *Eunotia* with varying frequencies of *Amphora*, *Hantzschia*, *Nitzshia*, *Craticula*, *Gomphonema*, *Neidium*, *Achnanthes*, *Tabellaria*, *Navicula*, *Encyonema*, *Caloneis*, *Frustulia* and *Achnanthidium*, suggesting a more drier depositional environment with
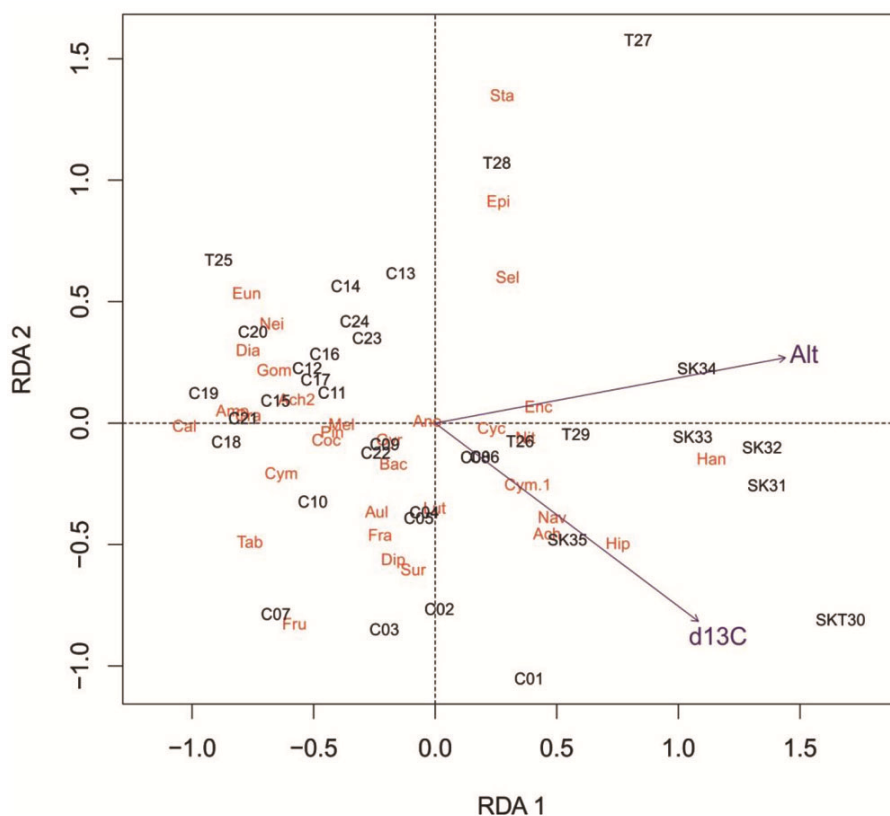
Figure 7. Redundancy analysis using diatoms as the response variable, and altitude and $\delta^{13}C$ as explanatory variables. Numerics are the sample number ($C - n$, where n varies from 1 to 24 for samples from Chopta valley, T-25 to 29 is for samples from Lashar valley and SK-30 to 35 for samples from Gurudongmar) and capital texts (first three letter of the genera) are the first three-letter code diatom genera.

fluctuating water levels. *Melosira* is recorded in higher frequency only in sample CHS-9, which may be attributed to moist environment. The results demonstrate the role of moisture availability in diatom diversity under microclimatic conditions. The diatom assemblage shows a significant contrast in different samples, suggesting a less diversified assemblage towards the hill slope as a result of sloping environments which may have allowed limited water availability for diatom growth[25,48].

The study area is located at an altitude of >4000 m amsl (alpine meadow) and is dominated by C_3 vegetation. The carbon isotopic composition of modern C_3 plants exhibits a wide range from –20‰ to –36‰ (ref. 21). Such a large range in $\delta^{13}C$ values can be explained by a physiological response to aridity (anomalously high $\delta^{13}C$) as well as a low light levels plus leaf litter recycling (anomalously lower $\delta^{13}C$). Considering the typical global average $\delta^{13}C$ values (about –26‰ to –27‰) of C_3 plants, the $\delta^{13}C$ values of SOM in these three Himalayan valleys suggest that sources of organic matter are from open grasslands at high altitudes consisting of C_3 grasses.

The spatial distribution of $\delta^{13}C$ values and the associated present-day environmental condition of the three valleys show a strong relationship between water availability and isotopic values. The isotopic values (SOM) of

Chopta and Lashar valleys that are located just above the present-day tree line and have a moderate annual precipitation (~1100 mm/year) are fairly low (–25.8‰ and –27.7‰, avg. –26.6‰)[13]. However, the Gurudongmar valley samples show relatively higher values ranging between –24.9‰ and –26.8‰ with an average of –25.9‰. As expected, the isotope values show low moisture availability and are in agreement with the semi-arid condition of Gurudongmar valley.

We have compared the average isotopic values (SOM) from the three valleys with the available regional as well as global carbon isotopic records. Modern isotopic analogues from the Indian subcontinent are lacking, except for a few from the Gangetic Plain[28,50,51] and the Himalaya[1]. The $\delta^{13}C$ values of modern C_3–C_4 plants from the different part of the Gangetic Plain have been analysed, and majority of $\delta^{13}C$ values of C_3 plants in different locations of the Ganga Plain vary from −28‰ to −32‰ (refs 28, 50 and 51). These lower values are in agreement with the $\delta^{13}C$ values of the tropical region. In comparison to the tropical region, the $\delta^{13}C$ values of SOM in the higher altitude of the Himalayan region are less negative. Higher $\delta^{13}C$ values found in the present study are characteristic of the temperate regions and vary between –25‰ and –29‰. The difference in $\delta^{13}C$ values between the

Gangetic Plain and Higher Himalaya can be explained by a sharp decline in the moisture availability (ISM) along a south to north transect attributed to the Himalayan topography. Apart from moisture availability, lower temperature in the Himalayan region leads to lower ratios of stomatal conductance to photosynthesis by decreasing the ci/ca ratio (ratio of partial pressure of CO_2 inside the leaf to that of the atmosphere), and is implicated for higher $\delta^{13}C$ values in these areas[52]. The present study demonstrated a sharp contrast in both diatom assemblages and $\delta^{13}C$ values at microclimatic level. Hence, we propose that before taking up palaeoclimatic/environmental studies in different climatic regimes of the Higher Himalaya, understanding the response of different proxies to present-day climatic conditions (modern analogues) is essential, and this practice should be followed.

Conclusion

The present study gives some important insights on the diversity of diatoms in different environmental settings under extreme/harsh climatic conditions along with the characterization of $\delta^{13}C$ values of SOM. The main conclusions drawn from this study are as follows:

- The diatom diversity shows a significant response to moisture availability within a single valley and hence suggests the role of microclimatic condition.
- Lower diatom diversity is a response to low water availability as observed in Gurudongmar samples and hill slope samples of Chopta valley.
- This $\delta^{13}C$ values suggest that the source of organic matter in soil/sediments is contributed by open grasslands (C_3 grasses).
- The higher $\delta^{13}C$ values recorded in Gurudongmar samples advocate for moisture deficiency and lower temperatures.
- The present data provide a base for future palaeovegetation and palaeoclimate reconstruction from the region.

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