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Application of plant DNA metabarcoding of lake sediments for monitoring vegetation compositions on the Tibetan Plateau

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Abstract Benefiting from the rapid development of environmental DNA (eDNA) technologies, sedimentary DNA (sedDNA) emerges as a promising tool for monitoring plant compositions in remote regions. The Tibetan Plateau (TP), renowned for its harsh environment and numerous ponds and lakes, presents a potentially demanding region for the application of sedDNA on vegetation investigations. Here, we used the *g* and *h* universal primers for the P6 loop region of the chloroplast *trn*L (UAA) intron to amplify plant DNA in surface sediments from 59 ponds and small lakes on the southwestern TP. The applicability and limitations of using plant DNA metabarcoding for modern vegetation monitoring and palaeo-vegetation reconstructions have been assessed by comparing sedDNA, pollen, and vegetation survey data. Our results showed that plant DNA metabarcoding recorded 186 terrestrial taxa, of which 30.1% can be identified at the species level. The plant sedDNA approach can effectively disclose the dominant plant taxa (including Asteraceae, Cyperaceae and Poaceae) and significant vegetation assemblages in the vicinity of the investigated sites. The number of taxa and taxonomic resolution of plant sedDNA exceeded that of pollen analysis (75 taxa detected, 5.3% can be identified at species level). Unlike pollen that retains a broad spectrum of regional plant signals (including *Pinus* and *Artemisia*), plant sedDNA mirrors very local plants, underscoring its utility in local vegetation monitoring and reconstructions. To conclude, plant DNA metabarcoding of (small) lake sediments warrant increased attention in the future for local vegetation monitoring and reconstructions on the TP.

Keywords Sedimentary DNA (sedDNA), Metabarcoding, Pollen, Vegetation composition, Tibetan Plateau

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1. Introduction

Tibetan Plateau (TP), the roof of the world and the Asian Water Tower (Sun et al., 2012), is characterized by broad zonal and elevational vegetation types (Chang, 1981). With a remarkable richness of over 13,000 vascular plants species (Integrated Scientific Expedition to Tibetan Plateau, 1985–1987; Editorial Committee of the Flora Qinghaiica, 1996–1999), the plateau harbors a unique set of terrestrial ecosystems. Serving as a vital ecological barrier to surrounding areas, the plateau is exceptionally important for maintaining ecological security in China (Zheng and Yao, 2004). However, the wide-spread alpine grassland ecosystems on the plateau are highly ecologically fragile and climate-sensitive

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(Xiong et al., 2021). Investigation and monitoring of spatial and temporal variations in ecosystems and environments at different scales on the TP have gained high priority under the ongoing global climate change and increased anthropogenic disturbances (Wang et al., 2022).

Implementing vegetation field works on the TP always represents a challenging task. During the first Tibetan Plateau scientific expedition and research (1950s–1980s), a tremendous amount of large-scale vegetation surveys had been carried out by numerous botanists (Zhong, 1954; Zhang and Jiang, 1973; Li et al., 1981; Zheng and Chen, 1981; Wang and Li, 1982; David, 1983; Li et al., 1987; Wang, 1988), contributing foundational insights into plateau botany and plant distributions. The recent launch of the second Tibetan Plateau scientific expedition and research marks a good initiation to enhance our understanding of the vegetation characteristics on the TP and its interaction with the environment from a multidisciplinary perspective (Yao et al., 2022). Nevertheless, traditional vegetation survey on the TP is time-consuming and costly due to logistical challenges in the remote alpine regions. In some cases, challenges such as the presence of closely related species and variations in phenological periods often impede efficient and standardized field surveys. Furthermore, morphological identification highly relies on the prior knowledge of taxonomic expertise. All those limitations inherent in traditional vegetation monitoring on the TP have generated the pressing need for high-efficiency and alternative approaches.

Environmental DNA (eDNA) provides a non-invasive way to obtain information on species compositions from environmental samples. Over the past decade, plant DNA metabarcoding of lake sediments (Capo et al., 2021) has been increasingly used for vegetation reconstruction (Jørgensen et al., 2012; Giguet-Covex et al., 2014; Willerslev et al., 2014; Alsos et al., 2016; Parducci et al., 2018; Clarke et al., 2020; Huang et al., 2021; Liu et al., 2021a) and modern terrestrial plant monitoring (Pedersen et al., 2016; Deiner et al., 2017; Ruppert et al., 2019). The major advantages of plant sedimentary DNA (sedDNA) are that more rare taxa could be identified and it is well representative of investigated sites (Niemeyer et al., 2017; Alsos et al., 2018; Liu et al., 2021a). However, uncertainties persist regarding the taphonomy of plant DNA extracted from lake sediments and its correlation with modern vegetation, particularly when compared with Quaternary pollen analysis (Birks and Birks, 2016; Giguet-Covex et al., 2019; Jia et al., 2022a; Jia et al., 2022b). Studies on plant sedDNA from surface soils have demonstrated the DNA signals can mirror soil-grown plants and positively correlate with above-ground biomass (Yoccoz et al., 2012). However, soil-derived plant DNA tends to provide a highly localized vegetation signal (< 1 m) (Edwards et al., 2018) and may exhibit mixed temporal origins (Ariza et al., 2022). In contrast, a direct comparison of the taxonomic overlap between plant sedDNA and modern vegetation indicates that plant sedDNA generally reflects the vegetation composition within the catchment (Niemeyer et al., 2017; Alsos et al., 2018; Giguet-Covex et al., 2019). Plant DNA metabarcoding of surface sediments from 31 small lakes in northern Siberia (Taymyr peninsula) captured a higher taxonomic richness than those of vegetation field surveys around the lakes (Niemeyer et al., 2017). Similarly, another study comparing the composition of vascular plant sedDNA with catchment vegetation at 11 small lakes in northern Norway suggested that 17%–49% (mean 31%) of the identifiable taxa recorded were detected with plant sedDNA, in which approximately 73% matched taxa recorded in vegetation surveys within 2 m of the lakeshore (Alsos et al., 2018). The composition of plant sedDNA can effectively reflect vegetation types, related climate characteristics, and the dominant taxa in the vegetation. However, the signal of plant sedDNA is more complicated for large lakes with well-developed runoffs (Li et al., 2021). High erosion rates, along with a well-developed hydrographic network, had been proposed as factors positively affecting the representation of catchment flora (Evrard et al., 2019; Giguet-Covex et al., 2019; Jia et al., 2022a). Therefore, the plant sedDNA from small lakes with limited inflow and outflow streams can authentically detect vegetation within the proper catchment area (Sjögren et al., 2017; Alsos et al., 2018) and can be a suitable method for monitoring vegetation compositions at the local scale.

The TP has a vast lake area exceeding 50,000 km^2 (Zhang et al., 2019), in which small-size lakes and ponds (less than 1 ha, sometimes with strong seasonality) accounting for 5.93% of the total areas, with the number more than 40,000 (Yang et al., 2022). Small lakes (including ponds hereafter) are abundant ecosystems along broad ecological gradients (De Meester et al., 2005). Despite their ecological significance, the application of plant DNA metabarcoding as an emerging proxy in ecological studies on the TP remains limited, and the relationships between sedDNA from small lakes and the surrounding modern vegetation on the TP are not yet fully understood. Additionally, while both pollen and sedDNA have been suggested as robust proxies for Quaternary vegetation change, comparative studies of these two proxies on the TP are lacking. Hence, comparative assessments of the pollen analysis and sedDNA signals to ascertain their applibility for monitoring modern vegetation compositions on the TP are still needed.

Here, we conducted a comprehensive study by collecting surface sediments from 59 small lakes on the southwestern TP. By analyzing plant sedDNA signals, pollen compositions and vegetation survey data, we aim to elucidate the following key questions: (1) whether plant sedDNA accurately reflects the vegetation composition around the lake; (2) what are the differences between sedDNA and pollen in monitoring local and regional vegetation signals; (3) what are the current

applicability and limitations of plant DNA metabarcoding for modern vegetation monitoring and ancient vegetation studies on the TP.

2. Materials and methods

2.1 Sediment sampling

Field works were conducted during July and August on 2019 and 2020 (Figure 1), respectively. A total of 59 sampling sites were included, comprising 26 isolated ponds, 18 small-sized lakes, and 15 fluvial ponds. Surface sediments were collected from the uppermost 0.5 cm of water-substrate interface each site using sterile knives and bags. All samples were kept cool during field trip and subsequently frozen at −18°C in the laboratory until DNA extractions.

During the field work, we recorded information on the surrounding vegetation and dominant plant species (Appendix Table S1, https://link.springer.com). Vegetation information around the lake would be recorded when the lake radius was less than 50 m, as in the case of isolated and fluvial ponds. For the small-sized lake, the vegetation information was gathered within the circle with a radius of 10 m from the center of the sample site (Appendix Figure S1, https://link.springer.com). Since it was with great difficult to distinguish every species during the field work, we opted to record higher taxonomic units to minimize the identification errors (e.g., *Carex* sp. recorded as *Carex*). Most of the sampling sites were located in the alpine meadow and steppe regions. In the alpine meadow regions, the plant compositions around the lake/pond typically exhibited high frequencies of *Kobresia*, *Carex*, *Poa*, *Leontopodium*, *Arenaria*, *Ranunculus* and Poaceae taxa. Samples from alpine steppe regions displayed high frequencies of *Artemisia*, Asteraceae, Amaranthaceae, *Carex praeclara*, *Stipa* and *Potentilla*. Additionally, halophytes plants such as *Knorringia sibiricum* could be found in some saline-lake shores.

2.2 Molecular genetic laboratory work

A detailed description of DNA extraction, PCR amplification, purification, pooling, and preparation for next-generation sequencing were given in Stoof-Leichsenring et al. (2020). All DNA works were carried out in environmental DNA laboratory at Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research in Potsdam. Each DNA extraction batch included nine sediment samples (5–10 g/sample) and one extraction control, which was treated with a partially modified protocol of DNeasy® PowerMax® Soil Kit (Qiagen, Germany) (Epp et al., 2019). PCRs were performed using the *trn*L *g* and *h* universal plant primers amplifying the short and variable P6 loop region of the chloroplast *trnL* (UAA) intron (Taberlet et al., 2007). The PCR reactions contained 1.25 U Platinum® Taq High Fidelity DNA Polymerase (Invitrogen, USA), 1×HiFi buffer, 2 mM $MgSO_4$(1 M=mol L^{-1}), 0.25 mM mixed dNTPs, 0.8 mg Bovine Serum Albumin (VWR, Germany), 0.2 mM of each primer and 3 μL DNA in a final volume of 25 μL. PCRs settings included initial denaturation at 94°C for 5 min, 40 cycles at 94°C for 30 s, 50°C for 30 s and 68°C for 30 s, and

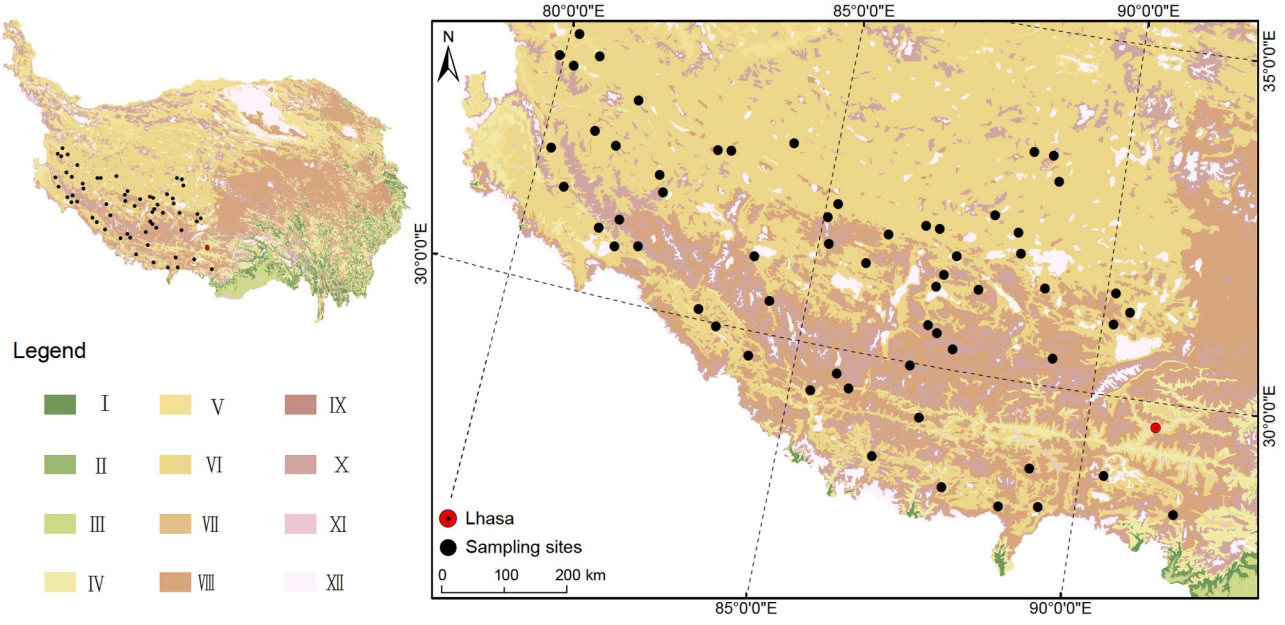

Figure 1 Location of the sampled lakes indicated by modern vegetation map of Tibetan Plateau. The vegetation data were from Vegetation Atlas of China (1:1,000,000; Editorial Committee of Vegetation Map of China, 2007). Ⅰ, needleleaf forest; Ⅱ, needleleaf and broadleaf mixed forest; Ⅲ, broadleaf forest; Ⅳ, scrub; Ⅴ, desert; Ⅵ, steppe; Ⅶ, grass-forb community; Ⅷ, meadow; Ⅸ, marsh; Ⅹ, alpine vegetation; ⅩⅠ, cultivated vegetation; ⅩⅡ, land without vegetation.

a final extension at 72°C for 10 min. A negative template control (NTC) was included for each PCR batch which included nine DNA extractions and one extraction control. Four PCR replicates were conducted. PCR products were purified with DNeasy® MinElute® PCR Purification Kit (Qiagen, Germany), quantified with Qubit® 4.0 fluorometer (Invitrogen, USA) and pooled in equimolar concentrations for next-generation sequencing by external sequencing service at Fasteris SA, Switzerland.

2.3 Sequencing data filtering and taxonomic assignment

The sequencing data were processed using the OBITools 3.0.1 (Boyer et al., 2016). The main filtering procedures were: (1) align forward and reverse reads using '*illuminapairedend*', (2) assign sequence to the corresponding sample using '*ngsfilter*', (3) remove sequences shorter than 10 bp and with <10 counts using '*obigrep*', (4) merge and count the identical sequences using '*obiuniq*', (5) remove punctual PCR errors using '*obiclean*', and (6) taxonomic assignment with '*ecotag*' based on the EMBL nucleotide sequence database (release 143). The database was created by '*ecoPCR*' function, an 'in silico' PCR (Ficetola et al., 2010) with the *g*/*h* primers on the EMBL nucleotide database (release 143), allowing five mismatches between the primers and the targeted reference sequences. After that, the ecoPCR output was filtered by taxonomic levels with '*obigrep*' and was dereplicated for redundant sequences with '*obiuniq*'. Finally, the ecoPCR was converted into an ecoPCR database with '*obiconvert*' which made it usable for '*ecotag*' function.

Only sequence types with 100% identity to reference sequences in the EMBL databases were used for further analysis. No sequences were detected in the extraction blank and NTCs indicated the absence of significant contamination during the laboratory procedures. Finally, the dataset comprised 238 plant DNA sequence types, including 4 non-plant sequences (algae), 3 bryophytes, 16 aquatic plant and 215 terrestrial plant sequences. Sequences assigned to hydrophytes and bryophytes were excluded, allowing us to concentrate on terrestrial vascular plants. Filtering criteria were applied by comparing the terrestrial sedDNA results with taxa data from the regional floras (http://www.iplant.cn/foc/). This step enabled the exclusion of falsely assigned 29 terrestrial plant sequences. To further reduce noise, only sequences with a minimum count above 5, sequences appeared at least in three PCR replicates, and samples with a total count higher than 1000 reads were kept. Sampling localities that produce read counts lower than 1000 were probably not suitable for terrestrial plant sedDNA preservation (Parducci et al., 2017). Following these steps, 2 samples were excluded, and 57 samples were used for further analysis (Table S1).

2.4 Pollen analysis

For each lake surface sediment, pollen samples were prepared using the dense-media separation, including HCl (10%), KOH (10%, 90°C for 10 min), $ZnBr_2$ (solution of density ~2.20), and acetic anhydride with H_2SO_4 (9:1, 80°C) for 3 min. To estimate the pollen concentration, a *Lycopodium* spore tablet (n=20,848) was added to each pollen sample. At least 400 pollen grains were identified with Microscope ZEISS scope A1 at 430 and/or 600 x. The pollen percentages of trees, shrubs and herbs were calculated based on the total terrestrial pollen.

2.5 Statistical analyses

Following data filtering, we compared taxonomic assemblages from sedDNA with the taxa recorded in the vegetation surveys and pollen data. To facilitate this comparison, taxa identified in the vegetation surveys and plant sedDNA data were homogenized to the taxonomic resolution of the pollen. And the comparison was done at the lowest resultant taxonomic level with only presence/absence data (taxa richness).

Modern vegetation information, pollen, and plant sedDNA results were utilized for further numerical and statistical analyses. We first used the iNEXT package (Hsieh et al., 2016) to estimate the taxonomic richness in pollen and plant sedDNA and to test whether the pollen and sequence counts adequately captured the diversity in each sample. Prior to calculation, the plant sedDNA data were transformed and merged into the higher taxonomic units when the raw taxon name has multiple sequence types. For example, taxa such as Asteroideae, Anthemideae and Gnaphalieae were all consolidated into Asteraceae. To minimize the bias of sampling size, the raw plant sedDNA data were resampled to minimum sample size (n=4000) without replacement with 100 repeats (Birks and Line, 1992; Chao et al., 2014). The percentage of each plant sedDNA taxon was calculated based on the mean of all resampling runs. To allow visual comparison of the different records and abundance changes, percentage bar plots of vegetation, pollen counts, and plant sedDNA composition were produced using the TILIA software (Grimm, 1992).

Taxa occurring in at least 0.5% of five samples were selected for the principal component analysis (PCA). The relative proportions of pollen and plant sedDNA data were double square-root transformed before PCA calculations. Procrustes analysis was then employed to assess the differences between pollen and plant sedDNA based on the site and species scores of the first two PCA axes of the two datasets using 'procrustes()' and 'protest()'. When looking for species associations, Legendre (2005) suggested transforming the species abundance to control for differences in total abundance among places, producing more monotonic

correlations between species. Before the concordance analysis among species, the presence of floristic groups was verified through grouping analysis with the species association method (Legendre, 2005). Partitioning of the species were based on the first two PCA axes for pollen and sedDNA using 'kml()'. Spearman's correlations between the species were analyzed, and a concordance global test was applied among the species of each group. The number of groups was determined based on the concordance of occurrence among species, with a posteriori analysis conducted to identify which individual species were concordant with one or some of the other species of the group.

3. Results

3.1 Plant sedDNA detected local plant compositions

The plant sedDNA results extracted from 57 samples comprised 13,643,475 reads from 186 terrestrial plant sequences with 100% identity. Individual sample reads ranged from 4,049 to 1,134,450, averaging 239,359 reads per sample. Of the sequences identified, 30.1% were assigned to species level, 33.9% to genus level, and 36.0% to family or higher taxonomical levels. There were 38 families recognized from the plant sedDNA results, of these, 99% were retrieved from three or more independent samples. Among these families, Asteraceae exhibited the highest diversity with 33 unique sequences, followed by Poaceae (15), Rosaceae (13), Fabaceae (12), Boraginaceae (12) and Ranunculaceae (10) (Figure S2). Rarefied richness per sample varied between 1 and 59 taxa relative to a sequence reads of 4,000 (Figure 2). Almost all the lake samples show saturation in the rarefaction curves (Figure S3). The sample site with the highest terrestrial plant richness was located in the southern part of the study area. In addition, other sample sites in the central and eastern parts of the study area also had high richness, while the western part of the study area showed relatively low richness (generally < 10) (Figure 3).

The plant sedDNA signals mainly comprise herbaceous taxa such as Asteraceae, *Carex*, Cyperaceae, Polygonaceae, and Rosaceae. Asteraceae and *Carex* were particularly prevalent, constituting mean percentage of 20.8% and 19.5%, respectively. *Knorringia sibirica* (mean 13.1%), *Potentilla anserina* (mean 6.7%) and Poaceae (mean 1.6%) were consistently present across most samples. Amongst, high percentages of *Knorringia sibirica* (50%−100%) could be found in salt marsh sites. Shrub taxa consisting of *Dasiphora fruticosa*, Caprifoliaceae and *Caragana jubata* were rarely distributed, while other arboreal taxa such as *Pinus* and Salicaceae were identified within individual samples. A total

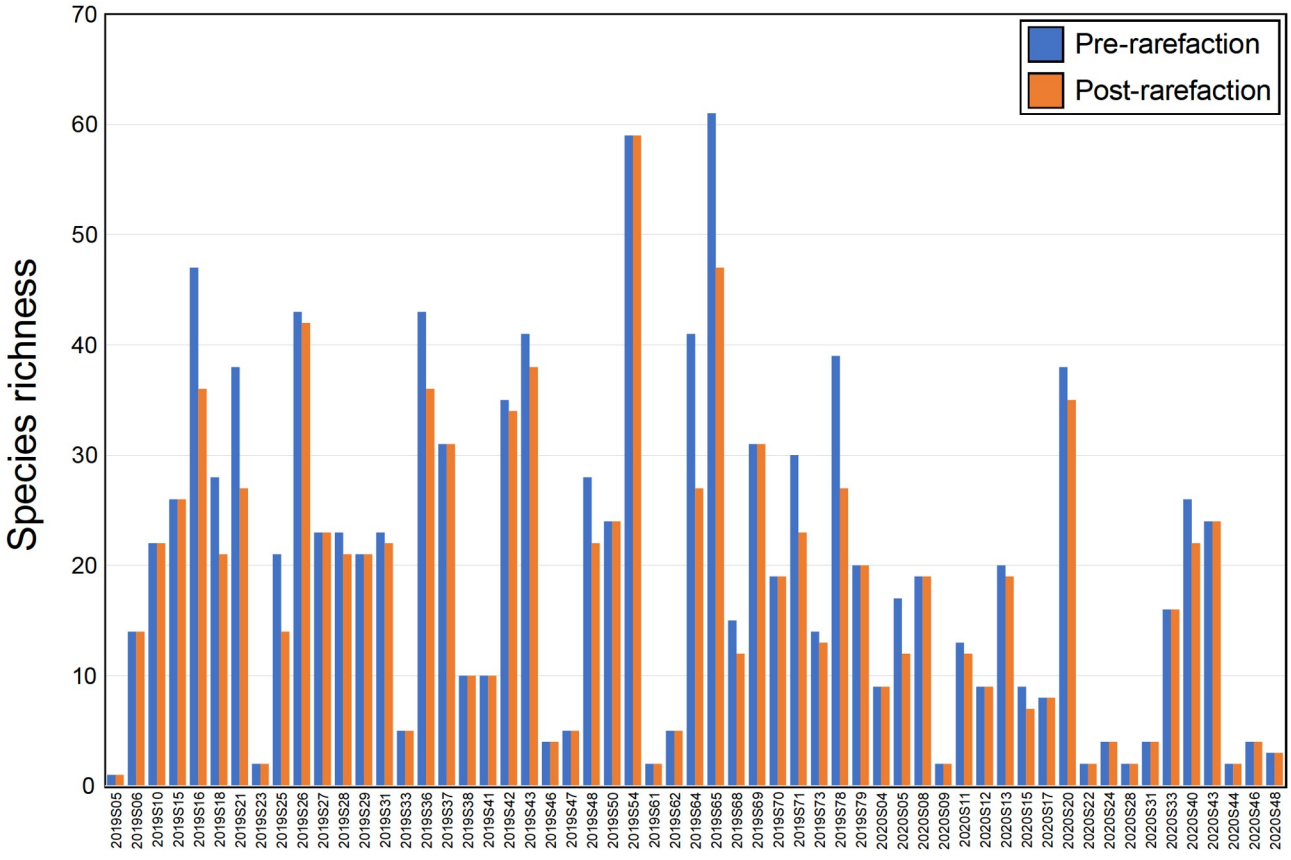

Figure 2 The pre-rarefied and rarefied richness of terrestrial plants based on the sedDNA at each site are indicated by the bar plot.

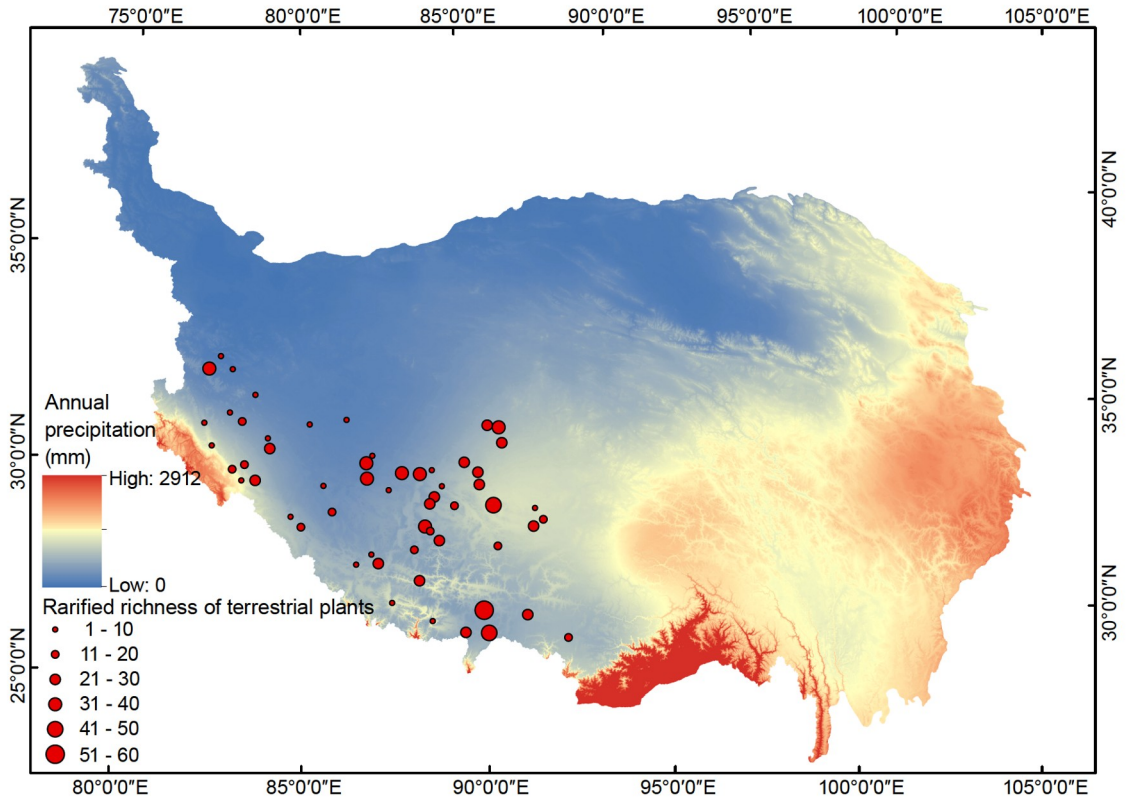

Figure 3 The rarefied richness of terrestrial plants based on the sedDNA. The background map of annual precipitation was extracted from the 1-km monthly precipitation datasets for China (1901–2021) (Peng, 2020).

of 22 taxa exceeded the threshold of 0.5% in at least five samples and were thus included in the multivariate analyses (Figure 4).

PCA ordination of plant sedDNA data, with the first two axes explaining 31.4% of the total variance, reflected the characteristics of the different vegetation compositions (Figure 5). Plants consisting of *Carex*, *Potentilla*, and *Thalictrum* were clustered in the first quadrant, representing of the main components of the meadow. Most xerophyte herb like Poaceae, Asteraceae, *Artemisia* and other widespread alpine steppe forbs appeared in the fourth quadrant. Meanwhile, *Polygonum sibiricum* and *Blysmus compressus* and Ranunculaceae were distributed on the left side of the plot. Considering that the Ranunculaceae thrives in freshwater environments whiles *Polygonum sibiricum* grows in saline-alkali shoreland regions, the second and third quadrants represented freshwater and saline habitats, respectively.

3.2 Pollen analysis revealed regional plant signals

In total, 75 terrestrial vascular plant taxa belonging to 42 families were identified by pollen. Only 5.3% of pollen taxa could be identified to species level, 33.3% to family level, and 61.4% to genus level. Among the 42 families detected, Rosaceae stood out with 8 unique pollen types, followed by Ranunculaceae with 6, Pinaceae and Betulaceae, each exhibiting 5 unique pollen types each (Figure S4). Rarefied pollen taxa richness varied between 10 and 33. Rarefaction curves of all samples did not show an asymptotic response within a count sum of 500 (Figure S3).

Herbaceous taxa, mainly comprising Cyperaceae, *Artemisia*, Poaceae and Amaranthaceae, dominated the pollen assemblage. The pollen of Cyperaceae prevailed in most samples, with mean percentage of 49.2%. Pollen of *Artemisia* (mean 7.1%), Poaceae (mean 5.5%) and Amaranthaceae (mean 3.4%) were consistently present across all samples. Arboreal pollen primarily consisted of *Pinus*, recognized in all samples with a mean percentage of 15.5%. High percentages of Cupressaceae (30%−85%) could be found in four samples from river valley sites. Other arboreal taxa such as *Abies*, *Picea*, *Betula* and *Quercus* were identified within individual samples. A total of 27 taxa surpassed the threshold of 0.5% in at least five samples and were thus included in the multivariate analyses (Figure 6). These taxa collectively represent more than 90% of the total pollen grains.

The first two PCA axes explained 26.3% of the total variance in pollen assemblages (Figure 7). In the first quadrant, tree and shrub taxa such as *Tsuga*, *Hippophae*, Rosaceae, *Betula* and *Quercus*, alongside herbaceous taxa comprising

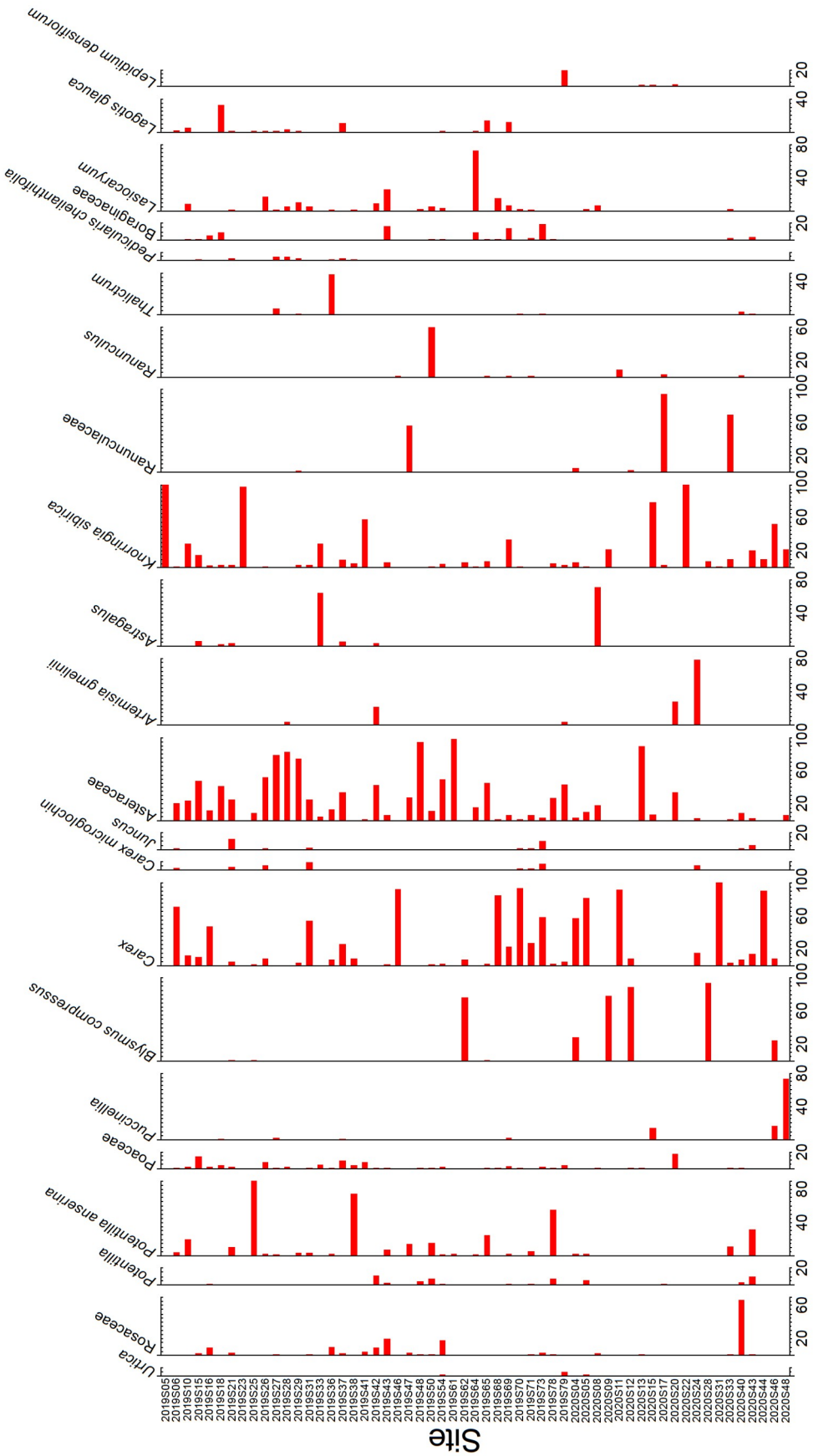

Figure 4 Percentages of selected taxa/sequences of sedDNA from 57 lakes located in the southwestern Tibetan Plateau used in our statistical analyses.

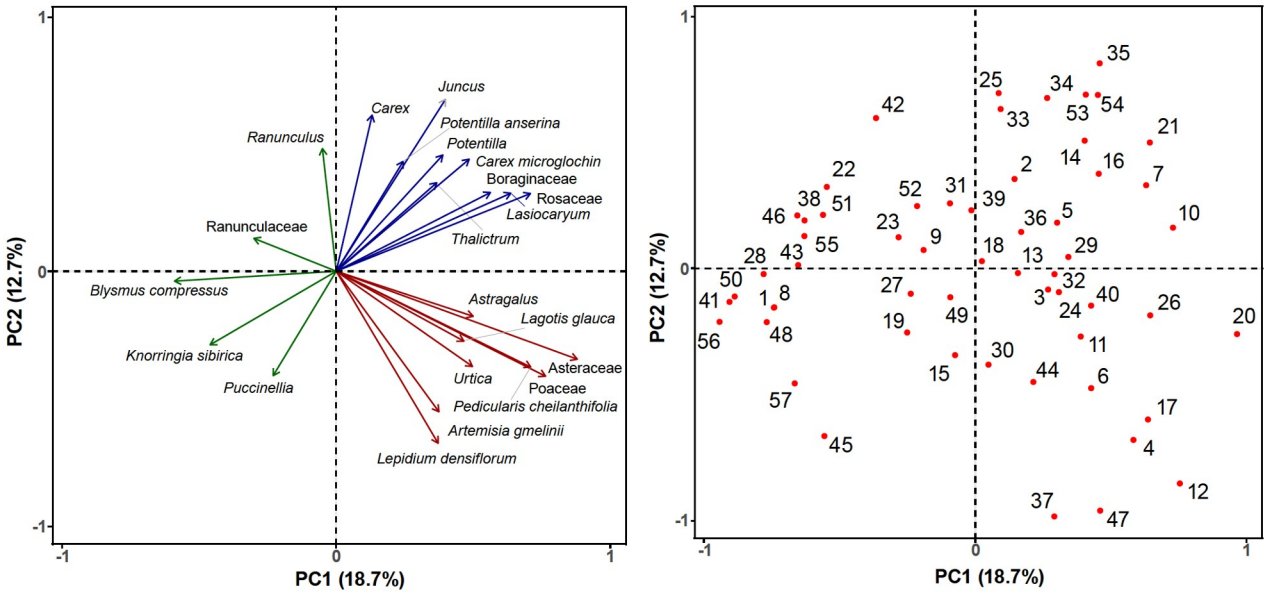

Figure 5 Biplots of the two principal component analyses of the plant sedDNA data indicating that different vegetation types can be distinguished by plant sedDNA.

Aster, Gentiana and Brassicaceae, were clustered together, probably indicating alpine shrubland vegetation. Cyperaceae and Ranunculaceae were positioned in the second quadrant, representing alpine meadow vegetation. Cupressaceae pollen, located in the third quadrant, corresponded well with their elevated percentages in specific sites. In the fourth quadrant, pollen types with high disseminate abilities grouped together, including arboreal pollen of *Picea*, *Abies* and *Pinus*, as well as xerophyte herb pollen like Amaranthaceae, *Artemisia*, *Chrysanthemum* and Poaceae. Thus, the fourth quadrant likely corresponded to areas with sparse vegetation on TP.

3.3 Plant sedDNA and pollen comparison and their relationships to modern vegetation

Comparison between plant sedDNA, pollen and vegetation showed that all the three methods recorded a rather similar assemble of Poaceae, Cyperaceae, and Asteraceae (Figure 8). However, subtle distinctions of each approach could be found as well. Weak correlation between the pollen and sedDNA of other herbaceous plants exist, while most tree and shrub signals could only be found in pollen data. The sedDNA showed a fair relationship to the vegetation records (kappa=0.37, $p<0.01$), while pollen showed a weaker relationship (kappa=0.19, $p<0.01$).

Results of the Procrustes rotation analyses and associated PROTEST (Figure 9) indicated significant correspondence between the scores of the sites for pollen and sedDNA ($p<0.05$). In contrast, we found no significant fit between the pollen and sedDNA species scores ($p=0.261$).

In general, the Spearman correlation between the species of sedDNA was commonly low with $r\leqslant|0.60|$ and pollen data. Concordance analysis indicated that one or some of the species were concordant with one or some of the other species in each sedDNA (Kendall's $W=0.13957$, $p<0.0001$ after 9,999 permutations) and pollen (Kendall's $W=0.12088$, $p<0.0001$ after 9,999 permutations) dataset. Three and six taxa groups were determined by Kendall's concordance test (W) on plant sedDNA and pollen, respectively. And all the groups rejected the null hypothesis (H_0) (Tables S2 and S3). The groups of sedDNA did not have species with the paired average of Spearman's negative correlation (Table S2).

4. Discussion

Our work backs up the good fits between the results obtained by the three distinct proxies—vegetation survey, pollen and sedDNA analyses—utilized for recording vegetation composition in the vicinity of 57 small-size lakes (ponds) located along the southwestern TP. Along with the forthcoming applications of sedDNA metabarcoding in biodiversity monitoring and palaeo-vegetation reconstruction on the TP (Jia et al., 2022a), our work assumes significance and timeliness within this field and holds promise for advancing related research endeavors. Notably, our adoption of a conservative filtering approach for plant sedDNA data, involving 100% identification, multiple positive confirmation and strictly native species, bolsters the reliability of our results and offers potential for further refinement.

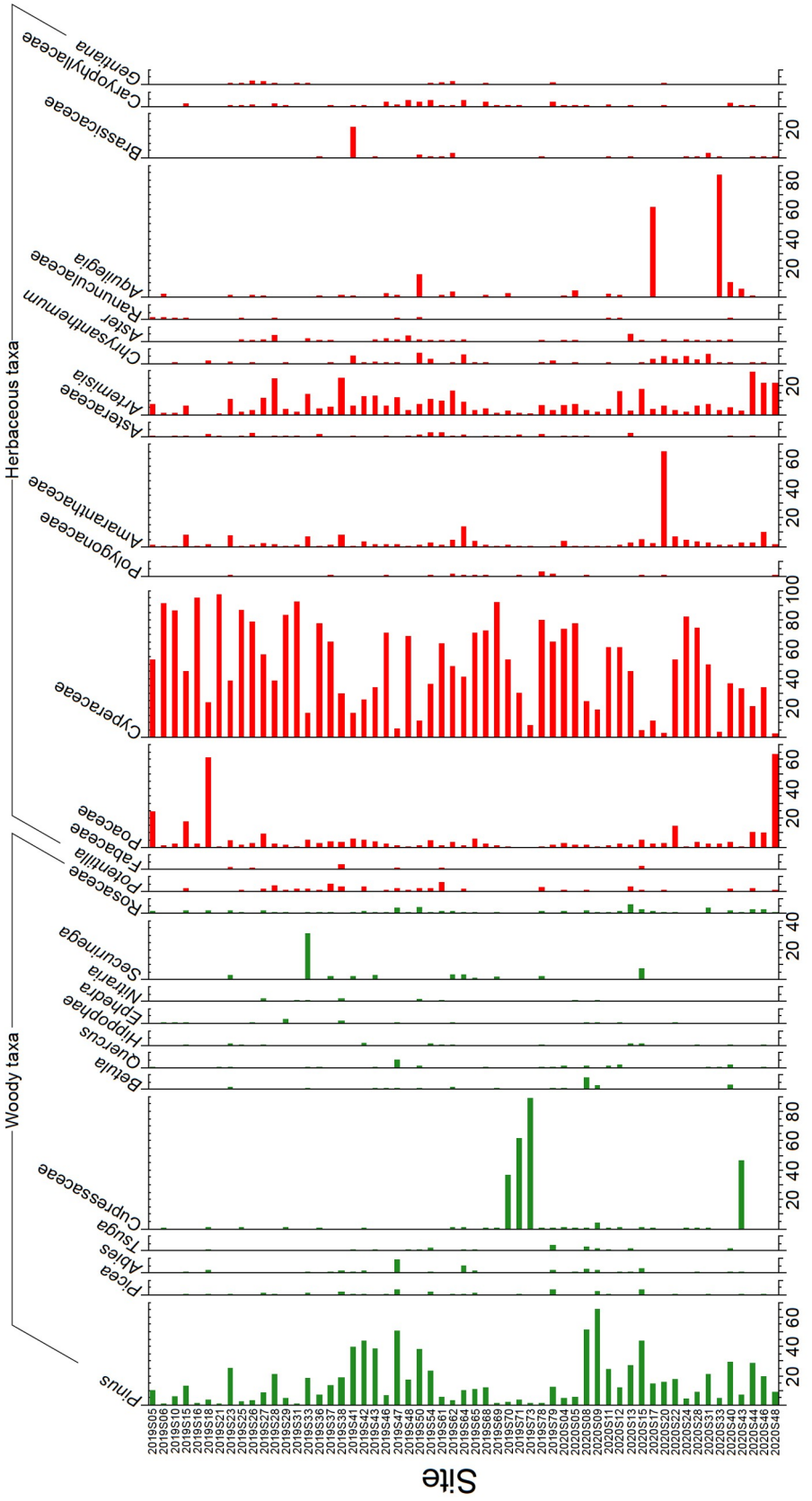

Figure 6 Percentages of selected taxa/sequences of pollen from 57 lakes located in the southwestern Tibetan Plateau used in our statistical analyses.

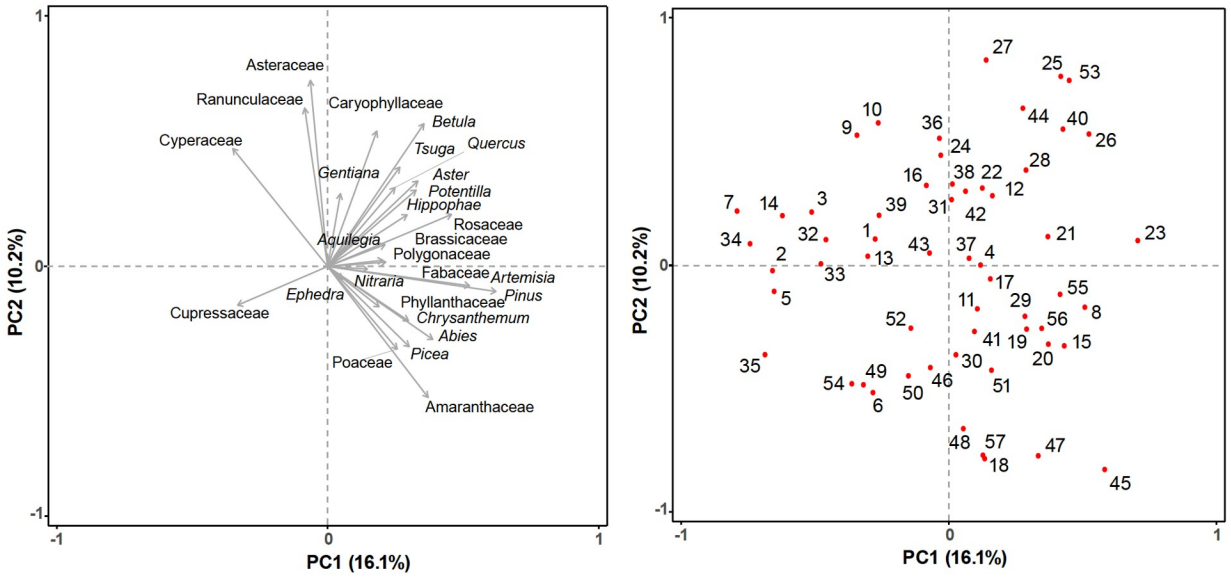

Figure 7 Biplots of the two principal component analyses of the pollen data indicating that pollen can distinguish the vegetation types different from plant sedDNA.

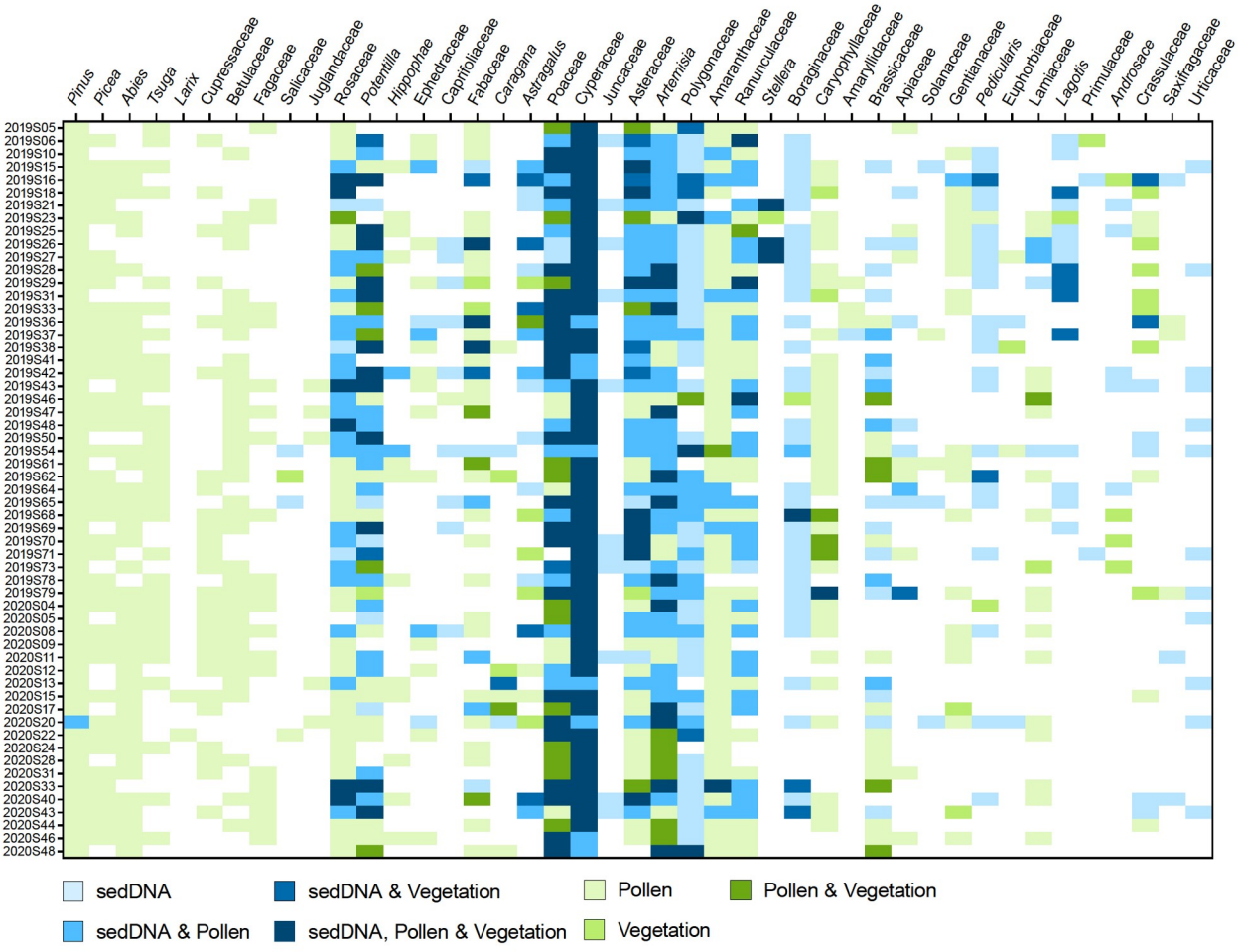

Figure 8 Comparison between plant sedDNA, pollen and vegetation, the agreement shown by Cohen's kappa between plant sedDNA and pollen was 0.20 (p<0.01), while Cohen's kappa between plant sedDNA and vegetation was 0.37 (p<0.01), and Cohen's kappa between pollen and vegetation was 0.19 (p<0.01).

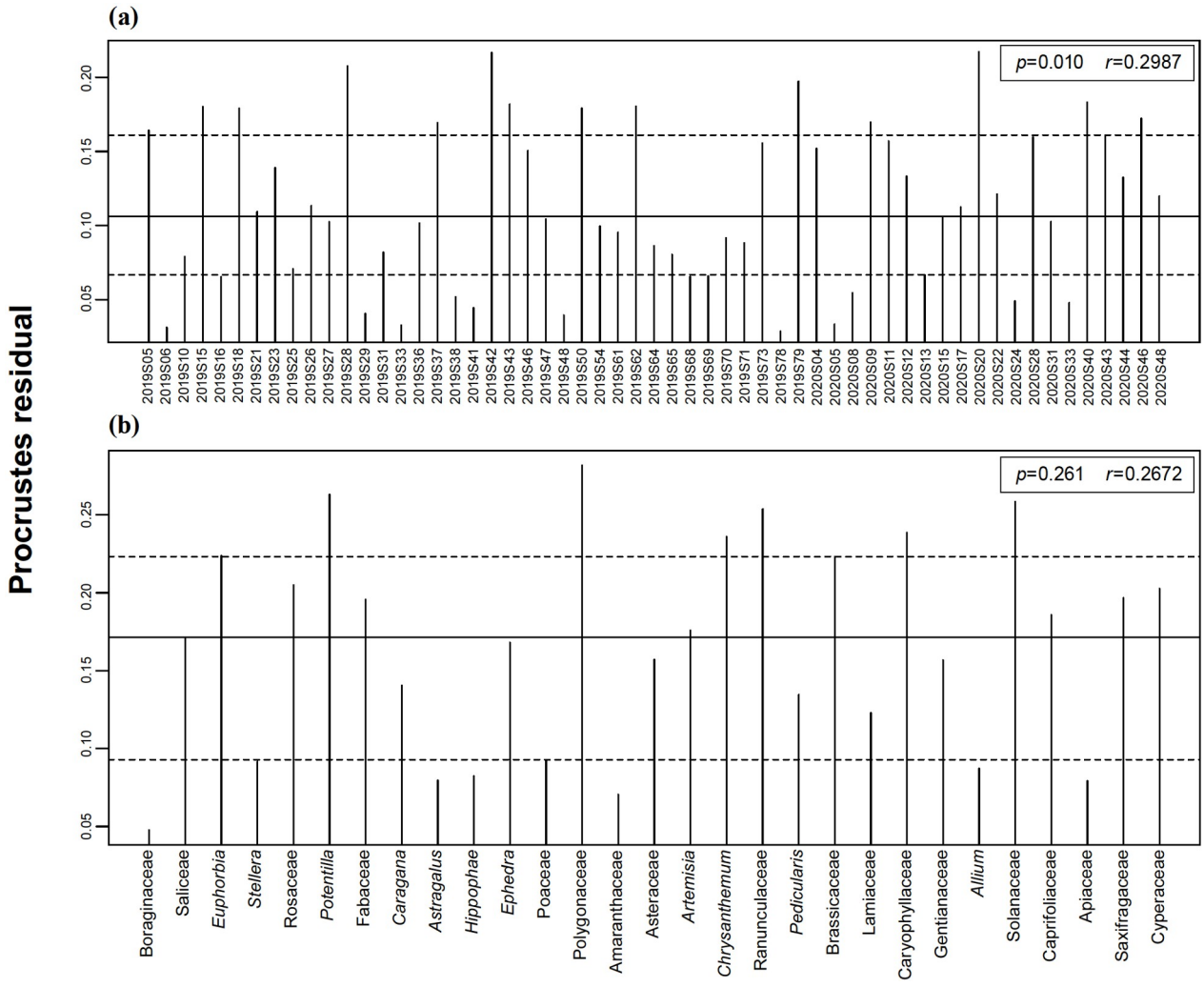

Figure 9 PROCRUSTES residuals plot of comparison (a) between PCA site scores of plant sedDNA and pollen and (b) between respective PCA taxa scores. Dashed and solid lines are the first, second and third quartiles. The *p*-value indicates the likelihood of the relationship occurring by chance and the *r*-value reflects the correlation between the two ordination results by superimposition.

4.1 Application of sedDNA metabarcoding for plant monitoring

Our results confirmed the considerable potential of plant sedDNA in monitoring modern vegetation. In line with modern vegetation composition, plant sedDNA extracted from small lakes can effectively capture the herbaceous plant signals around the sampling sites. Most of the abundant plant taxa can be recognized in the sedDNA signals, which was as expected that dominant species have higher biomass and thus a greater chance to be deposited and preserved in lake sediments (Li et al., 2021). The plant sedDNA approach clearly distinguished different vegetation types and habitat characteristics based on sedDNA assemblages, i.e., significantly correlated taxa within alpine meadow, steppe and wetland (Figure 5 & Table S2). This imply that the plant sedDNA could be use in monitoring plant compositions in areas characterized by a mosaic distribution of alpine meadow and steppe vegetation.

Unexpectedly, certain species dominant in the modern vegetation around the lake were not authenticated by plant sedDNA spectra, especially for *Kobresia*. Our result failed in recognizing Cyperaceae signals (8 Carex in 9 unique sequences) and yielded low taxonomic resolutions in Poaceae, Asteraceae, Ranunculaceae, Rosaceae and Amaranthaceae. The limitation may stem from the *trn*L *g* and *h* primers that cannot distinguish closely related species or intraspecific varieties (Shaw et al., 2005), or the long sequence length of Cyperaceae that cannot be preferentially amplified by PCR (Alsos et al., 2018). It is noteworthy that despite the low taxonomic resolution in plant families mentioned above, the taxonomic richness within each family remained plausible. The sedDNA metabarcoding therefore provides more unique sequences and taxonomic information than that of vegetation survey records, potentially enabling the detection of more rare plant taxa not recorded in the vegetation surveys.

However, the incompleteness of modern reference database may impede the application of sedDNA in detecting rare species in plant monitoring. Therefore, it is necessary to joint use multi-primers, such as specific primers for Cypercaceae, Poaceae, and Asteraceae (De Barba et al., 2014; Willerslev et al., 2014) and to establish the regional plant DNA reference database (Jørgensen et al., 2012; Parducci et al., 2015; Pedersen et al., 2015; Alsos et al., 2022) for the TP to improve the taxonomic resolution (Jia et al., 2022a).

Small lakes could be a proper choice for modern vegetation assessment via plant DNA metabarcoding. Sediments are suitable substrates for eDNA-based plant assessments in terrestrial environments, as most extracellular (released from the degradation of plant tissues and the lysis of plant cells) and intracellular (from plant fragments and living cells) plant DNA are gathered or ultimately deposited in these substrates (Parducci et al., 2017; Rodriguez-Ezpeleta et al., 2021). Hitherto different environments, including soil, sediments, glacier and air that had been used for eDNA and sedimentary ancient DNA studies, plant DNA in lake sediments own the special strengths in proper source area, coherence to contemporary plant signals and easy to access. In addition, we find higher consistency between sedDNA and modern vegetation than that investigated from soil with very local plant signals (Edwards et al., 2018) and large lakes which has large source area with complicated vegetation signals (Giguet-Covex et al., 2019). Combining its cost-effectiveness, high consistency with local plants, low dependance on morphological identification, and outstanding performance in detecting more taxa, the plant DNA metabarcoding from small lakes thus provides good potential for modern vegetation monitoring on the TP.

4.2 Comparison of sedDNA and pollen in capturing plant signals

Both pollen and sedDNA have been suggested as robust proxies for Quaternary vegetation reconstruction (Birks and Berglund, 2018; Edwards, 2020), yet their respective capacities for detecting plant communities and mirroring vegetation characteristics vary (Birks and Birks, 2016). In our results, the plant DNA metabarcoding yielded more than twice as many taxa as recorded by pollen and classified more taxa at lower taxonomic levels. Obviously, the plant sedDNA approach is outweighed the pollen analysis in both higher taxonomic resolution and the detection of rare taxa. This is consistent with recent studies that plant sedDNA can record more plant taxa than pollen (Niemeyer et al., 2017; Liu et al., 2020; ter Schure et al., 2021; Garcés-Pastor et al., 2022), probably benefits from the improvement of reference database. In comparison to sedDNA, our pollen data exhibits greater spatial homogeneity with a steadily high proportion of Cyperaceae, *Artemisia*, and woody taxa. By contrast, their relative abundance in the sedDNA records varies, confirming that the main origin of sedDNA is not pollen (Jørgensen et al., 2012; Pedersen et al., 2013; Parducci et al., 2015; Parducci et al., 2018). Unlike plant sedDNA that provides rather local vegetation composition, pollen analysis represents the diversity of regional vegetation (Prentice et al., 1996; Sugita, 2007a; Sugita, 2007b; Birks and Berglund, 2018), but it cannot support the diversity measure of local plant communities in our study region.

Based on our results, both plant sedDNA and pollen can reveal different plant communities and/or vegetation types. Plant sedDNA is able to distinguish wetland habitat, alpine meadow and steppe communities clearly (Table S2). The low Spearman's correlation among the dominant species of each sedDNA group can be attributed to the fact that species represent some degree of physiological and morphological adaptation along broad ecological gradients (Legendre, 2005; Rocha et al., 2022), and to that the sedDNA capture exactly plant community level signals (Giguet-Covex et al., 2014; Parducci et al., 2018; Clarke et al., 2020). The six pollen groups provided more vegetal information than sedDNA (Table S3). Group 1 and Group 2 overwhelmingly consisted of the anemophilous taxa, relating to regional vegetation signals. *Pinus* and *Artemisia*, with high production and transmit ability (Nadezda et al., 2006; Klemm et al., 2013), had been identified in nearly all the modern samples (Yu et al., 2001; Shen, 2003; Lu et al., 2011). Similar pollen assemblages like Group 2 could be found in Yajiang Valley, where the drought-tolerant species such as Amaranthaceae occored in the bottom valley, while coniferous trees are distributed on the slopes of the upper reaches of the valley (Zhang, 1978). Group 3 and 4 distinctly indicated the presence of alpine shrubs. High altitude heat deficiency restricts tree growth, resulting in shrubby canopy of tree taxa as *Quercus* and *Betula* (Carrion and Dupre, 1996). Cupressaceae, mostly occurring in the Hengduan Mountains of the eastern Tibetan Plateau (Zhang et al., 2018), but also scattered along the dry valleys of Yarlung Tsangpo River (Debreczy et al., 2011), leads to its strong over-representation in specific sites (Figure 6). Group 5 comprised both shrubs and forbs growing in dryer conditions that we infer the ecotone. Group 6 constantly occurred in alpine meadows.

The main reason contributing to the disparity between sedDNA and pollen results is their source areas. The higher consistence between plant sedDNA and modern vegetation indicated that most of the DNA signals originate from very local sites. This is quite similar with previous works from Niemeyer et al. (2017) and Alsos et al. (2018). However, it's worth noting that the source area of lake sedDNA should be larger than modern soil DNA, which gives a highly local vegetation signal (Edwards et al., 2018). For samples from riverain ponds, plant signals from upper reaches may also contribute to our final results (Giguet-Covex et al., 2019).

Different from sedDNA mirroring local plant community composition, pollen assemblage recorded plant composition across a much larger region. The high Spearman correlations of Group 1 and 2 with other species suggested they are quite disseminated and evenly distributed in our study region. As common element of the far-distance wind-transported component of pollen spectra due to the characteristics of anemophilous and high pollen production, taxa such as *Pinus* and *Artemisia* may obscure the pollen-based plant richness signals (Meltsov et al., 2011) as well as the vegetation compositions (de Klerk et al., 2009). These differences are clearly reflected by the Procrustes analyses, which showed no significant correlation between species scores of pollen data and sedDNA data (Figure 9).

The inconsistency between plant sedDNA and pollen in our result was quite different from that elucidated by from Niemeyer et al. (2017). This might be attributed to the different geographical setting and vegetation composition. As the most important geological landscape, the geographical setting of the TP harbors numerous unique habitats with diverse micro-climate and endemic plant communities (Xu et al., 2019). This inconsistency underscores the necessity for further more modern plant sedDNA studies on the TP. Pollen analysis, based on the use of extensive modern and fossil datasets along with well-developed methods and models, remains the primary choice for regional vegetation and climate reconstructions on the TP. Nonetheless, plant sedDNA performs well in recording local taxonomic richness and vegetation composition at the community level, thus serving as a supplementary approach to traditional pollen analysis.

4.3 Prospects and pitfalls of the application of plant sedDNA on the TP

The application of sedDNA metabarcoding from small lakes in monitoring the spatial and temporal dynamic patterns of vegetation would be expected to provide a comprehensive assessment of plant diversity and conservation management. However, several drawbacks in our work, including the sample size within each lake, the limitations of PCR amplification, and the lack of a suitable reference library, must be considered. If targeting on the better resolution for plateau vascular plants in plant monitoring, employing multiple primer combinations is advisable. The flora of the TP comprises one of the most important alpine biodiversity hotspots in the world, with more than 20% of the total species being endemic (Liu et al., 2014). The absence of complete reference library does matter when applying plant DNA metabarcoding into monitoring vegetation on the TP. We should also notice that an increasing number of reference barcode sequences are becoming available in public databases, such as The Germplasm Bank of Wild Species buildup by the Institute of Kunming Botany, Chinese Academy of Sciences (Li et al., 2010), which could provide more potential metabarcodes for future studies on the TP. Another important issue is the reliance of metabarcoding on PCR amplification. There are some potential biases in the current PCR-based analyses, such as humic substances in soils and sediments that assumed to be a major inhibitor of amplification, primer mismatch and polymerase selection, thus fails in providing the absolute abundance of particular groups (Jia et al., 2022b). Following the suggestion from Tkacz et al. (2018), incorporating the synthetic spikes directly into the environmental samples might be a solution to get absolute abundance of amplicon families.

The sedDNA provides botanists with an additional and powerful tool for investigating the long-term variations of plant composition and plant diversity on the TP. Liu et al. (2021a) used ancient sedDNA records to assess the changes in whole biological groups since the last deglaciation on the southeastern Tibetan Plateau, and further analyzed plant ancient sedDNA assemblages from lake sediment core with modern plant sedDNA assemblages using the modern analogue technique (Liu et al., 2021b). It is found plant ancient sedDNA-based quantitative reconstruction of vegetation types was more reliable than the pollen-based results. To date, most studies suggest that the source of pollen is more regional (Prentice et al., 1996; Sugita, 2007a; Sugita, 2007b; Birks and Berglund, 2018) and insufficient to reveal local vegetation changes, as well as the disturbances of human activities to natural vegetation, while plant sedDNA signals have a higher local or extra-local component (Niemeyer et al., 2017; Alsos et al., 2018). However, systematic investigations into the relationships between plant sedDNA and vegetation on the TP are rare. The differences between plant sedDNA and pollen analysis discussed above enhance our confidence on plant sedDNA and will catalyze its application in vegetation reconstructions on the TP. Unlike the regional representation of pollen, sedDNA signal represents information on local vegetation compositions. This is important in investigating the history of local vegetation change and its responses to abrupt climate change as well as to human disturbances. However, the small lakes would be inappropriate candidates when aiming to study the multi-millennia vegetation changes due to their fragile life in face of dramatic climate change. Thus, the medium or large lakes become the better option in adopting plant DNA metabarcoding to reconstruct long-term vegetation dynamics. When doing so, researchers also need to pay attention to the modern processes (including source, transfer, deposit and preserve processes) of plant DNA.

5. Conclusions

We investigated the relationship between plant sedDNA

signals, pollen assemblages and modern vegetation on the southwestern TP. Our results highlighted the potential of plant DNA metabarcoding technique in elucidating plant assemblages and diversity around the lake. Plant sedDNA from small-size lakes (or ponds) with limited inflows and outflows could precisely reflect the dominant herbaceous plants and local vegetation composition around the sampling sites. Plant sedDNA captured higher richness at a better taxonomic resolution than pollen. Different from pollen that contains many regional plant signals, plant sedDNA showed very local plants at the community level, thus resulting in significant differences in species assemblages identified by two proxies, which further indicates that the previous conclusions obtained at high latitudes might not be applicable to the southwestern TP. More systematic lake surveys are still needed to fully understand the mechanisms of plant sedDNA modern process, as well as how the physicochemical conditions of lakes affect plant sedDNA preservation.

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