



Original Articles

Plant sedimentary DNA as a proxy for vegetation reconstruction in eastern and northern Asia

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ABSTRACT

Plant DNA from sediments (sedDNA) are increasingly used to reconstruct the past vegetation composition, which contrasts with the few investigations on the relationship between the plant sedDNA signal and modern vegetation. Here, we applied broad-scale terrestrial plant sedDNA metabarcoding on surface sediments from 201 lakes in eastern and northern Asia to discuss the applicability as well as the limitations of using plant sedDNA metabarcoding for palaeovegetation studies. In total, 381 terrestrial plant taxa were determined with the universal plant primers of *trnL g* and *h*. Overall, plant sedDNA approach is able to retrieve major vegetation signals. The composition of plant sedDNA reflect well the vegetation types and related climate characteristics, and it also signals which are the dominant taxa in the vegetation. Our results indicate that plant sedDNA metabarcoding could be a reliable proxy of vegetation composition at a sub-continental scale and along large environmental gradients. But certain drawbacks such as limited taxonomic resolution, biases in the relative abundance of taxa, and a generally high variability of samples from similar vegetation types need to be solved before it can be widely applied to reconstruct palaeofloras.

1. Introduction

Ongoing climate change entails plant vegetation turnover and plant diversity changes (Chen et al., 2011; Dawson et al., 2011; Pauli et al., 2012; Nolan et al., 2018; Shukla et al., 2019). Due to logistical challenges and short vegetation periods, temporally, spatially, and taxonomically high-resolution vegetation data are still rare for remote alpine and arctic regions (Körner, 2003; Colella et al., 2020) where ecological risks caused by climate change are particularly high (Pang et al., 2017; Niskanen et al., 2019; Niu et al., 2019; Colella et al., 2020). New techniques are needed to provide a more comprehensive knowledge of spatial and temporal vegetation patterns in these regions.

Over the last two decades, advances in sequencing technology have permitted the analysis of plant DNA from sediments (sedDNA) (Capo et al., 2021), and thus both the reconstruction of the palaeoflora and the monitoring of modern terrestrial plant compositions (Shokralla et al.,

2012; Pedersen et al., 2016; Deiner et al., 2017; Ruppert et al., 2019). Sedimentary ancient DNA signals are increasingly used to investigate past vegetation dynamics (Alsos et al., 2016; Epp et al., 2015; Zimmermann et al., 2017a; Parducci et al., 2019; Liu et al., 2021), species invasions (Sjögren et al., 2017; Ficetola et al., 2018), plant diversity changes (Boessenkool et al., 2014; Zimmermann et al., 2017b; Clarke et al., 2019), and human presence and impact (Giguët-Covex et al., 2014; Pansu et al., 2015; Pedersen et al., 2016; Bremond et al., 2017; Xie et al., 2017; Li et al., 2019). This growing number of plant sedDNA-based palaeoecological studies contrasts with the few investigations into the relationship between the plant sedDNA signal and modern vegetation (Niemeyer et al., 2017; Alsos et al., 2018; Giguët-Covex et al., 2019). Therefore, the evaluation of modern plant sedDNA as an analogue for palaeovegetation is required.

It is under debate if the taphonomy of plant sedDNA can be used to infer major vegetation characteristics including vegetation composition

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(Seddon et al., 2014; Birks and Birks, 2016). Previous comparative studies have shown that plant sedDNA records only partly reflect aerially transported pollen assemblages but have a high overlap with fluviially transported macrofossil records, indicating a rather local origin for the plant sedDNA (Jørgensen et al., 2012; Pedersen et al., 2013; Parducci et al., 2015; Niemeyer et al., 2017; Sjögren et al., 2017). Direct comparisons of modern plant sedDNA from surface sediments against modern vegetation from several selected regions show that plant sedDNA generally reflects the vegetation composition of the catchment (Niemeyer et al., 2017; Alsos et al., 2018; Giguet-Covex et al., 2019). For example, a study analysing composition of vascular plant sedDNA and catchment vegetation at 11 lakes in northern Norway suggests that 17–49% (mean 31%) of the identifiable taxa recorded were detected with plant sedDNA, in which about 73% matched taxa recorded in vegetation surveys within 2 m of the lakeshore (Alsos et al., 2018). In another study, plant sedDNA metabarcoding results of surface sediments from 31 lakes in northern Siberia (Taymyr peninsula) captured a higher taxonomic richness than vegetation field surveys in the surroundings of the lakes (Niemeyer et al., 2017). Most lakes investigated by previous studies are small and with limited inflow and outflow streams. It is uncertain whether the conclusions drawn from those limited number of sites and regions also apply to other vegetation types and at a broader spatial scale.

Plant sedDNA-based vegetation studies face several challenges including the marker specificity and the completeness of the reference library (Jørgensen et al., 2012; Parducci et al., 2015; Birks and Birks, 2016; Parducci et al., 2017; Giguet-Covex et al., 2019). Because environmental DNA is highly degraded (Nielsen et al., 2007), barcodes for plant sedDNA should be short enough to allow successful amplification of degraded DNA (Taberlet et al., 2007). In palaeoecology, the

chloroplast *trnL g* and *h* primers are most commonly used (Voldstad et al., 2020), which allows an arctic plant to be identified to species, genus, and family levels with about 50%, 90%, and 100% identity, respectively (Taberlet et al., 2007). The broad applicability of the *g* and *h* universal plant primers and the established plant *trnL* reference libraries have been demonstrated for Arctic regions, but not yet investigated for other regions such as central Siberia and the Tibetan Plateau (Jia, 2020).

Biome distributions both in Siberia and on the Tibetan Plateau are known for their past and present sensitivity to climate change (Herzschuh et al., 2010; Cao et al., 2019; Zhong et al., 2019; Chen et al., 2020; Myers-Smith et al., 2020). The two regions are characterised by broad zonal and elevational vegetation types, constrained by large-scale climatic settings with limited human impact (Chang, 1981; Krestov, 2003; Walker et al., 2005; Zhang, 2007; Huang et al., 2017) and such by a high probability of vegetation change in the course of ongoing warming (Nolan et al., 2018), which contrasts with the lack of appropriate methods to collect spatially and temporally high-resolution data.

Here, we investigate vegetation composition derived from plant sedDNA analyses from 201 lakes using metabarcoding and compare the signals with information on climate and regional vegetation. The study area comprises two main regions, the treeline areas in north-eastern Siberia from the Taymyr peninsula (97°E) to Chukotka (168°E) and the central and eastern Tibetan Plateau, with some additional sites from central Yakutia and from northern and north-western China (Fig. 1). Our analyses focus on a basic scientific question of how well does the taxonomic composition of plant sedDNA reflect the dominant taxa and general composition of the vegetation. Additionally, we discuss the applicability as well as the limitations of using plant sedDNA metabarcoding for palaeovegetation studies in those two regions.

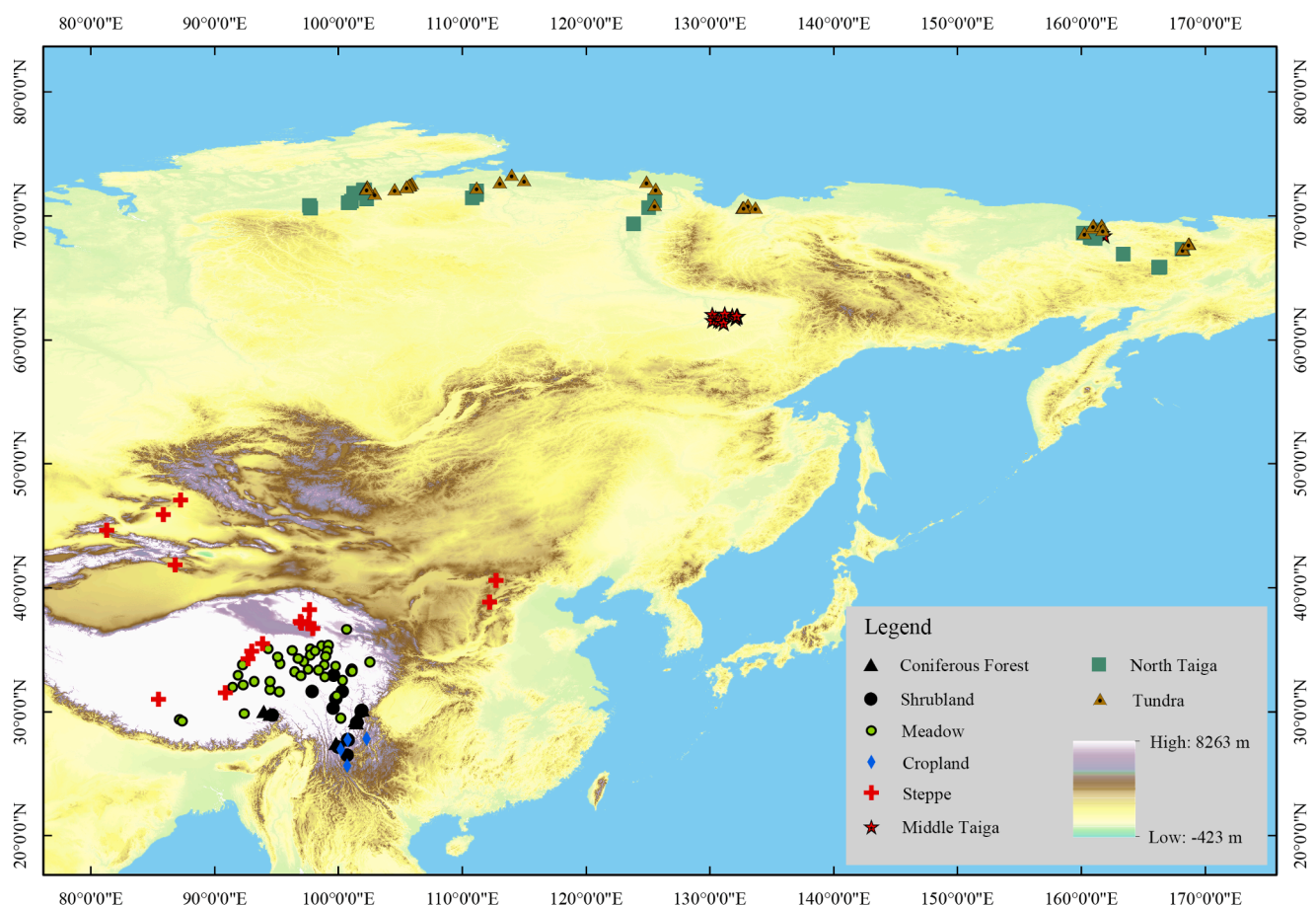


Fig. 1. Map of the study areas and sampling locations. The sampling sites are classified according to their surrounding vegetation types.

2. Materials and methods

2.1. Sample collection and environmental variables preparation

Sampling localities comprise lakes from Northern (which include expeditions to 07-SA, 09-Tik, 11-CH, 13-TY, 14-OM), Eastern (08-KO, 16-KP) and Central Siberia (05-Yak), Northern and Central China and Tibet (Fig. 1). Details about the sampled localities, including geographic coordinates, sampling time, physicochemical lake parameters, environmental parameters, and sampling methods were given in [Stoof-Leichsenring et al. \(2020\)](#). The samples we analysed were mapped in Fig. 1 and detailed information had been provided in Appendix A.

Information about the vegetation types (and dominant taxa) around the lakes was extracted from vegetation maps of Russia ([Stone and Schlesinger, 2004](#)) and China ([Zhang, 2007](#)). We assumed that plant sedDNA taxa originated mostly from the immediate surroundings of each lake ([Alsos et al., 2018](#); [Giguët-Covex et al., 2019](#)). Vegetation information was extracted by using the buffering function in R packages *raster* ([Hijmans, 2020](#)). The site-specific ring-buffer was measured between the sampling point to the furthest lake shoreline. For some sampling points with relatively small lake surface (radius < 50 m), this buffer was set to 100 m. Overall, eight vegetation types were retrieved, comprising three from Siberia and five from China (Appendix B). Most lakes were surrounded by only one vegetation type with a maximum of six dominant species. Only a few large lakes were surrounded by more vegetation types and dominant species, for example, two vegetation types – Meadow and Steppe – as well as 13 dominant species were recorded for Lake Qinghai.

In total, 57 lakes were surrounded by Tundra which was characterised by dwarf-shrubs (*Dryas punctata*, *D. octopetala*), erect shrubs (*Betula middendorffii*, *B. rotundifolia*, *Salix glauca*, *S. lanata*, *S. pulchra*), and grass (*Carex ensifolia* ssp. *arctisibirica*). Fifty-two lakes were in the North Taiga which was dominated by *Larix gmelinii* and *L. cajanderi*, while 24 lakes were in the Middle Taiga located in the larch forests of Central Yakutia and dominated by *L. gmelinii*. The direct vicinity of these latter lakes was thermo-karst lowlands (called *alaas*) with various herb taxa. Twelve lakes on the south-eastern Tibetan Plateau were surrounded by Coniferous Forest (Coni_Forest) with dominant species of *Abies forrestii*, *A. georgei*, *A. squamata*, *Picea brachytyla*, *P. likiangensis*, *Pinus yunnanensis*, *Juniperus indica*, *Rhododendron anthopogon*, *R. flavoflorum*, and *Kobresia*. Shrublands composed of dwarf-tree species such as *R. adenogynum*, *J. indica*, *Salix*, and *S. vaccinioides*, as well as herb species of *Kobresia* and *Polygonum macrophyllum* were found around 19 lakes on the Tibetan Plateau. In total, 46 lakes were surrounded by Meadow mainly comprising *Kobresia tibetica*, *K. setschwanensis*, *Carex*, *P. macrophyllum*, *Saussurea medusa*, *Stipa purpurea*, *Arenaria*, and *Potentilla fruticosa*. There were 23 lakes surrounded by Steppe which was dominated by *Achnatherum splendens*, *Agropyron cristatum*, *Artemisia desertorum*, *Stipa*, *Nitraria roborowskii*, *Krascheninnikovia compacta*, and *Ephedra przewalskii*. Eight lakes were surrounded by Cropland with diverse cultivated plants such as *Zea mays*, *Triticum*, and *Oryza*.

The climate parameters of each site were extracted from WorldClim 2 (www.worldclim.org), using average climate data for 1970–2000 at a spatial resolution of 30 s (ca. 1 km²). Several climate parameters were selected, including mean annual precipitation (P_{annual}), mean annual temperature (T_{annual}), mean temperature of July (T_{July}), and mean temperature of January (T_{Jan}). A variance inflation factor (VIF) was calculated where a VIF > 10 was assumed to indicate strong collinearity in the climate variables. We also performed the VIF test between climate parameters and vegetation types. After the VIF test, P_{annual}, T_{July}, T_{Jan} and vegetation types were kept for further numerical analyses. (Appendix C). The site-specific climate data were extracted using the R package *raster* ([Hijmans, 2020](#)) and the VIFs were calculated in the *usdm* package ([Naimi, 2017](#)).

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. \(2020\)](#). DNA extraction was carried out in the molecular genetic laboratories equipped for environmental and palaeogenetic DNA work at the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research. Extractions were carried out using the DNeasy PowerMax Soil Kit (Qiagen, Germany) and PowerMax Soil DNA Isolation kit (MoBio Laboratories, Inc., USA) following the manufacturer's instructions except for some modifications in the lysis step ([Zimmermann et al., 2017b](#)). PCRs were performed using the *trnL 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 1.25U Platinum® *Taq* High Fidelity DNA Polymerase (Invitrogen, USA), 1 × HiFi buffer, 2 mM MgSO₄, 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 were carried out in a TProfessional Basic thermocycler (Biometra, Germany) with initial denaturation at 94 °C for 5 min, 50 cycles at 94 °C for 30 s, 50 °C for 30 s and 68 °C for 30 s, and a final extension at 72 °C for 10 min. PCR products were purified with MinElute PCR Purification Kit (Qiagen, Germany), quantified with Qubit® 2.0 fluorometer (Invitrogen, USA) and pooled in equimolar concentrations for next-generation sequencing by external sequencing service at Fasteris SA, Switzerland. Samples were sequenced on four individual sequencing runs and were later merged into a single dataset for the final interpretation.

2.3. Filtering of sequence data and taxonomic assignments

A detailed description of the filtering and taxonomic assignment are given in [Stoof-Leichsenring et al. \(2020\)](#). The raw data of the four sequencing runs can be found in the Dryad repository (<https://doi.org/10.5061/dryad.k6djh9w4r>, [Stoof-Leichsenring et al. \(2020\)](#)). The bioinformatic assessment of the four single sequencing runs was conducted with the *OBITools* package ([Boyer et al., 2016](#)). We used *illumina-pairedend* to align forward and reverse reads, *ngsfilter* to assign sequence reads to samples, *obigrep* to remove sequences shorter than 10 bp in length, *obiuniq* to merge identical reads, and *obiclean* to remove probable PCR and sequencing errors. The program *ecotag* was used for taxonomic assignments based on the sequence reference database for Arctic and Boreal vascular plants ([Sønstebo et al., 2010](#); [Willerslev et al., 2014](#)), and a sequence reference database generated from the EMBL standard sequences (release 138). Only sequence types with 100% identity to reference sequences in either the Arctic and Boreal or EMBL databases were used for further analysis. Contaminated batches with obvious positive sequencing results in the blanks and no template controls (NTCs) were excluded. Primarily, the dataset included 623 plant DNA sequence types, including 69 non-plant sequences (including 4 planktic, 6 animals, 31 bacteria and 28 algae), 48 bryophytes, 61 aquatic plant and 445 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 plant sedDNA results with taxa data from the regional floras (<http://www.iplant.cn/foc/> and <https://www.gbif.org/>). This step allowed us to exclude 53 false assignments of terrestrial plant sedDNA sequences that were endemic to other regions or with no plausibility of growing in Siberia and China. Only sequences with a minimum read above 10 and samples with a total count higher than 1000 reads were kept (Appendix D). Sampling localities that produce read counts lower than 1000 were probably not suitable for terrestrial plant sedDNA preservation ([Parducci et al., 2017](#)). We therefore excluded such samples in this study but incorporate them into a discussion about the preservation of plant sedDNA in lake sediments along extensive environmental gradients (W. Jia, personal communication). Following these steps, 40 samples were excluded, and 201 samples were

used for further analysis.

2.4. Numerical analyses

Modern vegetation types, climate variables, and plant sedDNA results are included in numerical and statistical analyses. To minimise the bias of sampling size, the raw plant sedDNA data were resampled to 1000 reads without replacement (Birks and Line, 1992; Chao et al., 2014). This process was repeated 100 times. The percentage of each plant sedDNA taxon was calculated based on the mean of all resampling runs. Only taxa with a minimum percentage above 0.1% in at least one sample were included in the multivariate analyses. The percentage data was fourth-root transformed to stabilise variances and to optimise the ‘signal’ to ‘noise’ ratio in the dataset.

Non-metric multidimensional scaling (NMDS) was used to visualise the distances between the samples. Multi-response permutation procedure (MRPP) was applied to test the distance of plant sedDNA originating from different vegetation types, using the Bray-Curtis coefficient as a dissimilarity measure. Multivariate regression trees (MRT) modelling (De’Ath, 2002) was used to identify the composition of the plant sedDNA taxa and their corresponding vegetation types. The determination of tree size was guided by cross-validation and the smallest tree within one standard error of the best was selected (1-SE rule). Along with MRT, an indicator species analysis (Cáceres and Legendre, 2009) was done to assess the strength and statistical significance of the relationship between species occurrence/abundance and groups of sites, with the assumption that a *p*-value of < 0.05 indicates a significant relationship between sedDNA taxa and a vegetation type. Canonical correspondence analysis (CCA) was run (Kenkel and Orloci, 1986; Ter Braak, 1986) to investigate the relationship between the major patterns of plant sedDNA taxa composition and environmental variables. The *varpart* function in the R package *vegan* was applied to partition the variation of plant sedDNA composition with respect to the environmental variables (Oksanen et al., 2019). NMDS, MRPP, and CCA were all run in *vegan* (Oksanen et al., 2019), and the MRT model was produced

with *mvpart* (De’ath, 2014) and *MVPARTwrap* (Ouellette, 2013).

3. Results

3.1. General characteristics of the sedDNA dataset

The plant sedDNA results extracted from 201 samples comprised 7,766,578 reads from 381 terrestrial plant taxa with 100% identity (Appendix E). The number of reads in a single sample ranged from 1,008 to 284,177, with an average of 38,639. Of the taxa identified, 58.5% were to species level, 27.4% to genus level, and 14.4% to family or higher taxonomical levels. There were 64 families recognised from the plant sedDNA results, and 282 taxa appeared in at least three samples. A total of 270 taxa was over the threshold of 0.1% in one sample and included in the multivariate analyses. Taxa with the highest reads were Saliceae, Anthemideae, Asteraceae, *Betula*, and *Dryas*. Saliceae occurred mainly in samples from Siberia and Asteraceae mainly in samples from the Tibetan Plateau (Fig. 2).

3.2. Plant sedDNA composition and its relationship to vegetation and climate

Samples from a single vegetation type were mostly with a unique taxa composition (Fig. 2). Saliceae and *Dryas* dominated most spectra in samples from the Tundra which was also characterised by high percentages of *Betula*, *Rhododendron*, *Caltha*, and *Poinae*. Saliceae was abundant in samples from the North Taiga, while there were more tree and shrub taxa including *Larix* and *Alnus alnobetula* than in the Tundra. In samples from the Middle Taiga, the taxa composition was characterised by high percentages of herbaceous plants such as *Tephrosieris*, Asteraceae, and Anthemideae, with occasional tree and shrub taxa comprising *Larix*, *Pinus*, Saliceae, and *A. alnobetula*.

Samples from the Tibetan Plateau were mostly characterised by different taxa compositions than Siberia, although the samples from the Steppe shared similarities in their composition with those from the

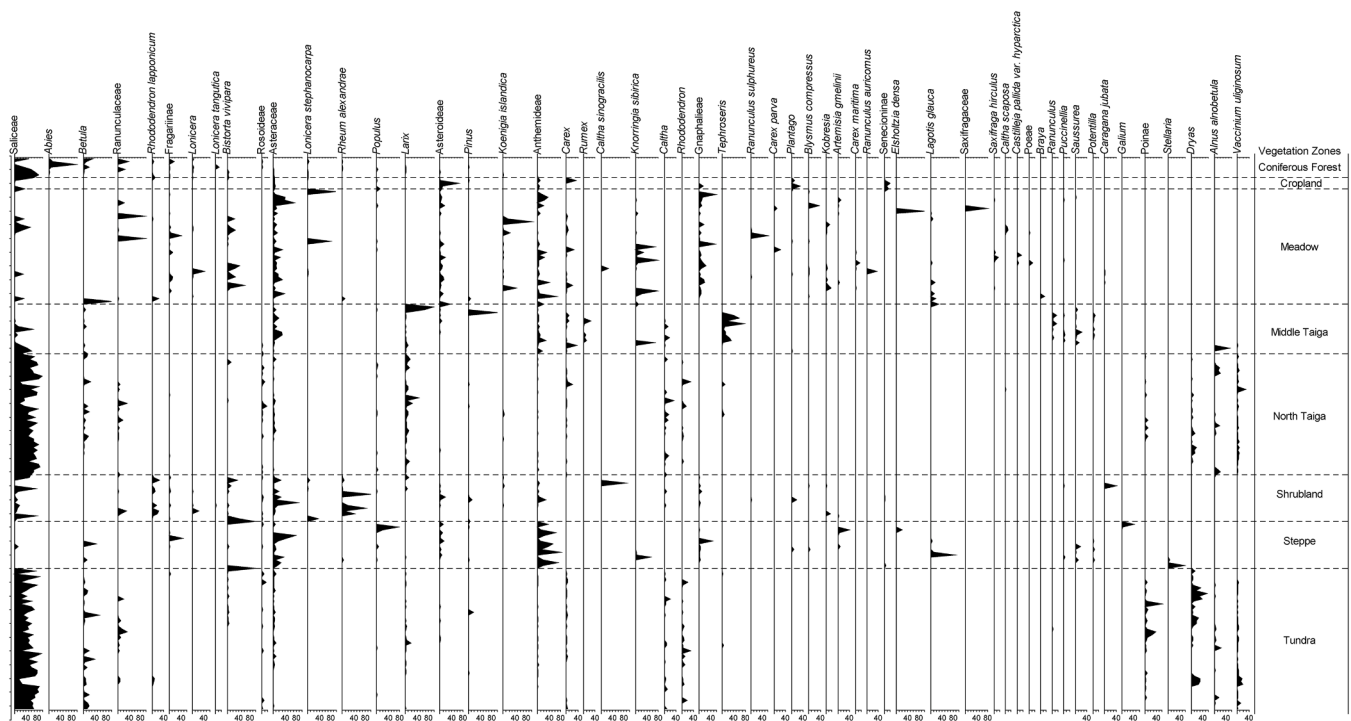


Fig. 2. Composition of plant sedDNA taxa for selected samples. Taxa with percentages above 1% in at least 5 samples are shown. Percentage is based on the total count of resampled data for each sample. Samples in Tundra, North Taiga and Middle Taiga are from Siberia, and samples in Steppe, Shrubland, Meadow, Cropland and Coniferous Forest are from Tibetan Plateau and China.

Middle Taiga including a high abundance of Anthemideae and Asteraceae. High percentages of Asteroideae, *Plantago*, Senecioninae, *Populus*, and *Carex* were recorded from samples from Cropland. In samples from Coniferous Forest, *Abies* and Saliceae were dominant, along with *Betula*, *Lonicera tangutica*, and Ranunculaceae. Percentages of *Rheum alexandrae*, Saliceae, Asteraceae, *Bistorta vivipara*, and *Rhododendron lapponicum* were high in samples from Shrubland. Taxa compositions in Meadow samples were highly variable, with high percentages of Asteraceae, Ganphalieae, *Knorringia sibirica*, *Bistorta vivipara*, *Kobresia*, *Potentilla anserina*, and *Koenigia islandica*.

NMDS ordination yielded a stress value of 0.185 indicating that taxa compositions of plant sedDNA could barely be distinguished by the distance matrix (Appendix F). It mainly separates samples from the Tibetan Plateau that were characterised by *Caltha sinogracilis*, *Carex maritima*, *Oxygraphis glacialis*, *Pedicularis cheilanthifolia*, and *Juncus* from samples from Siberia characterised by *Empetrum*, *Dryas*, *Ranunculus reptans*, and *Vaccinium uliginosum*. Samples from a single vegetation type were clustered in ordination space although overlaps exist. A slightly higher overlap occurred within samples from Siberia than samples from the Tibetan Plateau, which agreed with the dissimilarity measure results (Table 1). The compositional distances within a single vegetation type were high in samples from the Tibetan Plateau and in central Yakutia, suggesting strong compositional variations in samples from the same vegetation type. Conversely, the compositional differences between samples from northern Siberia were low, probably due to sharing of taxa of Saliceae, *Betula*, and *Alnus*, within the vegetation types of Tundra and North Taiga. MRPP results supported the ordination results (Appendix G) with the compositional variance of sedDNA samples from the same vegetation type generally being significantly smaller than the variance of samples originating from different vegetation types ($A = 0.1722$; $p < 0.001$).

The MRT results when including vegetation type as a constraining variable led to a tree with 6 leaves. Samples from the Tibetan Plateau and central Yakutia were separated from samples in arctic treeline regions, and each leaf represents a single vegetation type or a group of similar vegetation types (Fig. 3). Each leaf was characterised by significant indicator taxa ($p < 0.05$) as determined by indicator species analyses. Inferred indicator taxa were mainly consistent with literature-derived dominant plants. For example, *Kobresia* and *Knorringia sibirica*, the dominant taxa in Meadow areas on the Tibetan Plateau, were identified as indicator taxa for Meadow from the plant sedDNA. Likewise, *Dryas* was an indicator taxon for the Tundra, and *Abies* and *R. lapponicum* were indicative for Coniferous Forest and Shrubland, respectively. Accordingly, we conclude that plant sedDNA contains significant information about taxa composition of modern vegetation including indicator taxa.

Plant sedDNA taxa composition was significantly related to the modern vegetation and climate. The forward selection option within our CCA indicated that all the modern vegetation types and three climate parameters significantly influenced the compositional variation in the plant sedDNA taxa dataset, explaining 18% of the variation in total (Fig. 4) and 20% and 13% in Siberia and the Tibetan Plateau,

respectively (Appendix G). Vegetation type explained 5% of the variation when the effects of climate variables (7%) and their joint effects (8%) were removed in samples from Siberia. Similarly, vegetation type also explained 5% of the variation after removing the influence of the climate variables (3%) and their joint effects (5%) in samples from the Tibetan Plateau (Fig. 5). The relatively high contribution of vegetation type for both study regions supported the idea that plant sedDNA composition was mainly related to vegetation composition. Climate variables, however, could explain more unique variance than vegetation in samples from Siberia, with a high contribution from temperature, while precipitation contributed most to plant sedDNA compositional variations in samples from the Tibetan Plateau.

4. Discussion

Overall, we find that the vegetation composition revealed by plant sedDNA metabarcoding reflects well the vegetation types and related climate characteristics, and that dominant species in the vegetation tend to be indicator taxa in the sedDNA signals. As such, our results indicate that sedDNA can be a reliable proxy for vegetation composition on a sub-continental scale with broad environmental gradients. The results agree with restricted regional analyses of the relationship between plant sedDNA and modern vegetation (Alsos et al., 2016; Niemeyer et al., 2017; Alsos et al., 2018). However, our analyses also uncover certain limitations of the sedDNA approach to recording vegetation including a generally high variability in samples from similar vegetation types.

Our results show a clear relationship between compositional sedDNA signals and vegetation type. This is in line with previous studies where geographically isolated samples can produce a spatially distinct taxa composition via DNA metabarcoding (Yoccoz et al., 2012). Furthermore, characteristic vegetation gradients in the elevational and latitudinal treeline areas are reflected. *Abies*, *Rhododendron*, and Asteraceae are important vegetation components that depict an elevation pattern on the south-eastern Tibetan Plateau (Yu et al., 2001; Zhang, 2007; Liang et al., 2016), which is reflected by our sedDNA signals. The tundra-taiga transition in northern Siberia is mirrored by a decrease of *Dryas* and an increase of *Larix* in the sedDNA signals as has been described by Niemeyer et al. (2017).

Plant species that occur in one or a limited number of vegetation types can be regarded as diagnostic species and used for the identification of vegetation types (Chytrý et al., 2002; De Cáceres and Legendre, 2009). The indicator taxa identified by our analysis are generally dominant taxa in their principal vegetation type, for example *Artemisia* in Steppe (Liu et al., 2013), *Kobresia* in Meadow (Yu et al., 2001), *Abies* and *Rhododendron* in Coniferous Forest and Shrubland (Liang et al., 2016), *Larix* in Taiga, and *Dryas* in Tundra (Walker et al., 2005). Some taxa identified as indicator taxa are not named as dominant plant species in the atlases, but are known to occur in our study regions, such as *Artemisia gmelini* which occurs naturally in Steppe vegetation (Zhang, 2007) and *Populus* which has been widely planted in Chinese drylands (Pedersen et al., 2016).

The sedDNA compositions are constrained by both vegetation and

Table 1

Multi-response permutation procedure (MRPP) results of the significance of Bray-Curtis distances between different vegetation types.

	Coniferous Forest	Cropland	Meadow	Middle Taiga	North Taiga	Shrubland	Steppe	Tundra
Coniferous Forest	0.64							
Cropland	0.86 ***	0.66						
Meadow	0.85 ***	0.81 ***	0.77					
Middle Taiga	0.83 ***	0.83 ***	0.86 ***	0.69				
North Taiga	0.76 ***	0.87 ***	0.88 ***	0.73 ***	0.52			
Shrubland	0.75 **	0.82 ***	0.82 ***	0.85 ***	0.82 ***	0.77		
Steppe	0.89 ***	0.77 **	0.80 ***	0.83 ***	0.89 ***	0.84 ***	0.74	
Tundra	0.78 ***	0.87 ***	0.87 ***	0.76 ***	0.56 ***	0.83 ***	0.89 ***	0.55

Numbers on the diagonal represent the within group distance of every single vegetation type.

*** : significant at the 0.001 level; ** : significant at the 0.005 level.

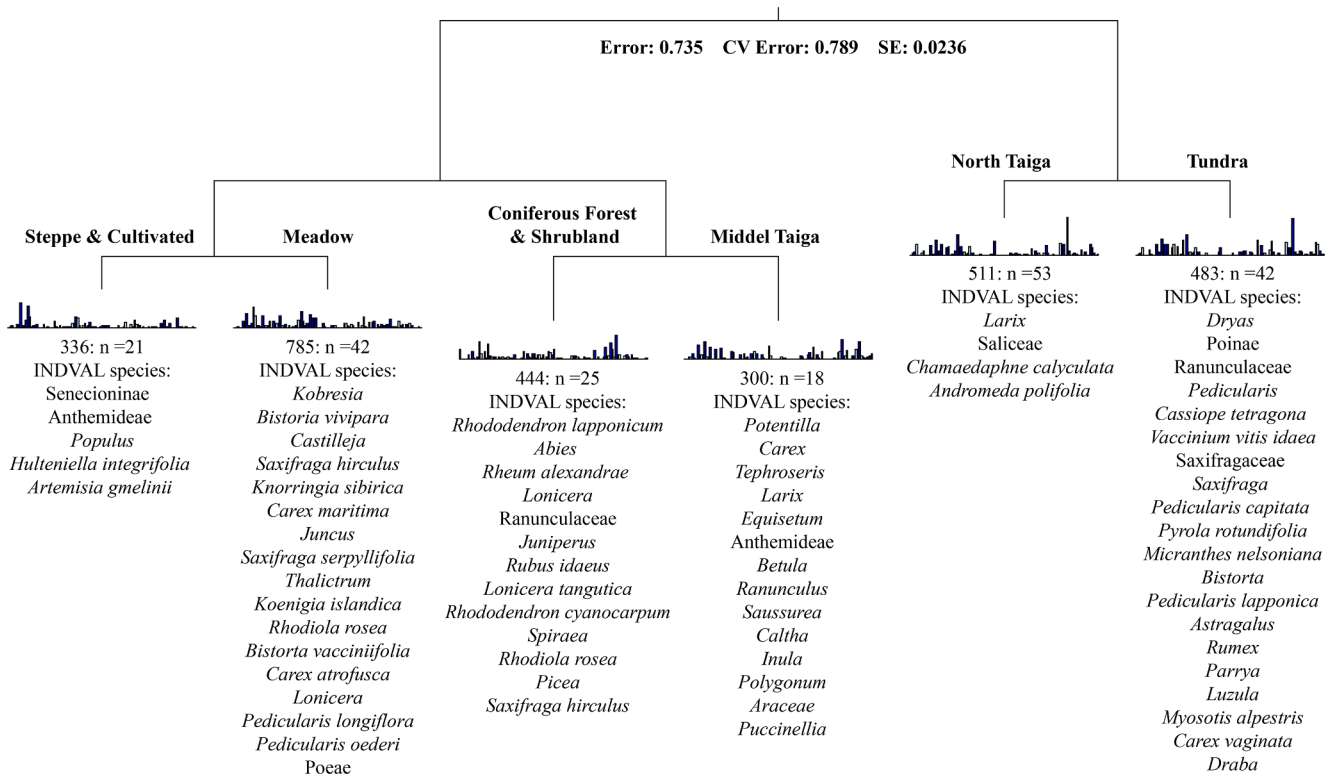


Fig. 3. Composition of each vegetation type determined by plant sedDNA with significant indicator taxa (INDVAL species) as determined by the multivariate regression trees (MRT) model ($p < 0.05$) listed below the corresponding leaves.

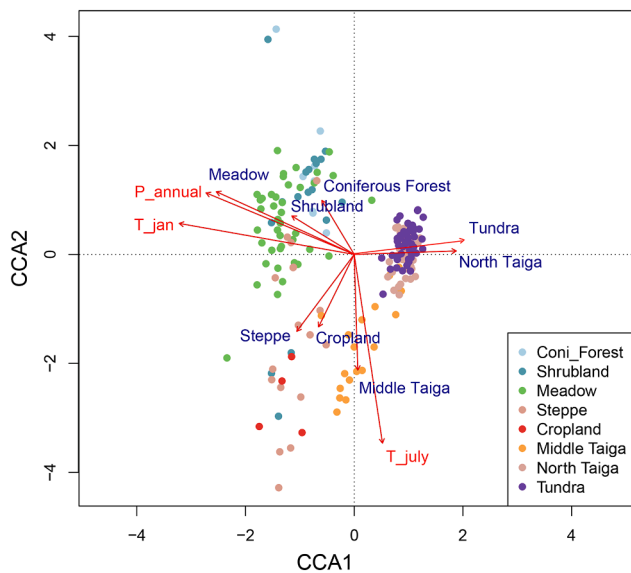


Fig. 4. Canonical correspondence analysis (CCA) of environmental parameters and plant sedDNA composition. P_{annual}: mean annual precipitation; T_{july}: mean July temperature; T_{jan}: mean January temperature.

climate conditions at broad scales, providing a basis for the potential application of sedDNA in palaeoclimate and palaeovegetation reconstructions. Plant sedDNA compositions are strongly constrained by temperature in Siberia while precipitation is the limiting variable on the Tibetan Plateau, consistent with results from previous bioclimatic analyses (Chang, 1981; Krestov, 2003; Paulsen and Körner, 2014).

Our metabarcoding results from central Yakutia hint at taphonomic limitations of sedDNA that affect its value as a proxy for vegetation

composition. Although central Yakutia is dominated by larch taiga, the sampling lakes are generally surrounded by *alaas*, which is mostly open steppe vegetation used for animal husbandry (Crate et al., 2017). The plant sedDNA composition will thus mainly reflect the *alaas* vegetation rather than the Taiga, as near-shore plants are expected to have a higher detection probability in sedDNA (Sjögren et al., 2017; Alsos et al., 2018). Similarly, aquatic plants tend to dominate the sedDNA if they are present (Stoof-Leichsenring et al., 2020). SedDNA from small lakes with limited inflow and outflow can record extremely local vegetation with high confidence. Conversely, large lakes with well-developed river system, such as Lake Qinghai, archive an integration from different vegetation sources, and the source of the eroded materials can strongly affect the DNA concentration resulting in a complex plant sedDNA signal (Evrard et al., 2019; Giguet-Covex et al., 2019).

Despite the consistency between plant sedDNA signal and vegetation types, certain drawbacks need further attention. Most of the dominant species can be recognised in our sedDNA signals, which matches our expectation that dominant species have high biomass and thus a chance to become deposited and preserved in lake sediments. However, the dominant species in the modern vegetation tend to be identified only to genus or family levels in the sedDNA results, for example *Abies georgei* is *Abies* in sedDNA, *Larix gmelinii* is *Larix*, and *Kobresia tibetica* is *Kobresia*. As the most commonly used marker for plant sedDNA metabarcoding, the *trnL* g and h primers balances the trade-off between short fragment length and taxonomic resolution (Taberlet et al., 2007; Taberlet et al., 2018). But, it fails to distinguish closely related species or intraspecific varieties (Shaw et al., 2005), as has been noted previously as well as in our result, with low taxonomic resolution for Poaceae, Cyperaceae, and Asteraceae. Low taxonomic resolution for families such as Amaranthaceae and Apiaceae are also apparent, probably due to the short sequence length (Fahner et al., 2016). Since those families are generally dominant plants on the Tibetan Plateau, the use of *trnL* g-h only will limit the taxonomic resolution for plant sedDNA based palaeovegetation study. We therefore suggest that the joint use of multi-primers including

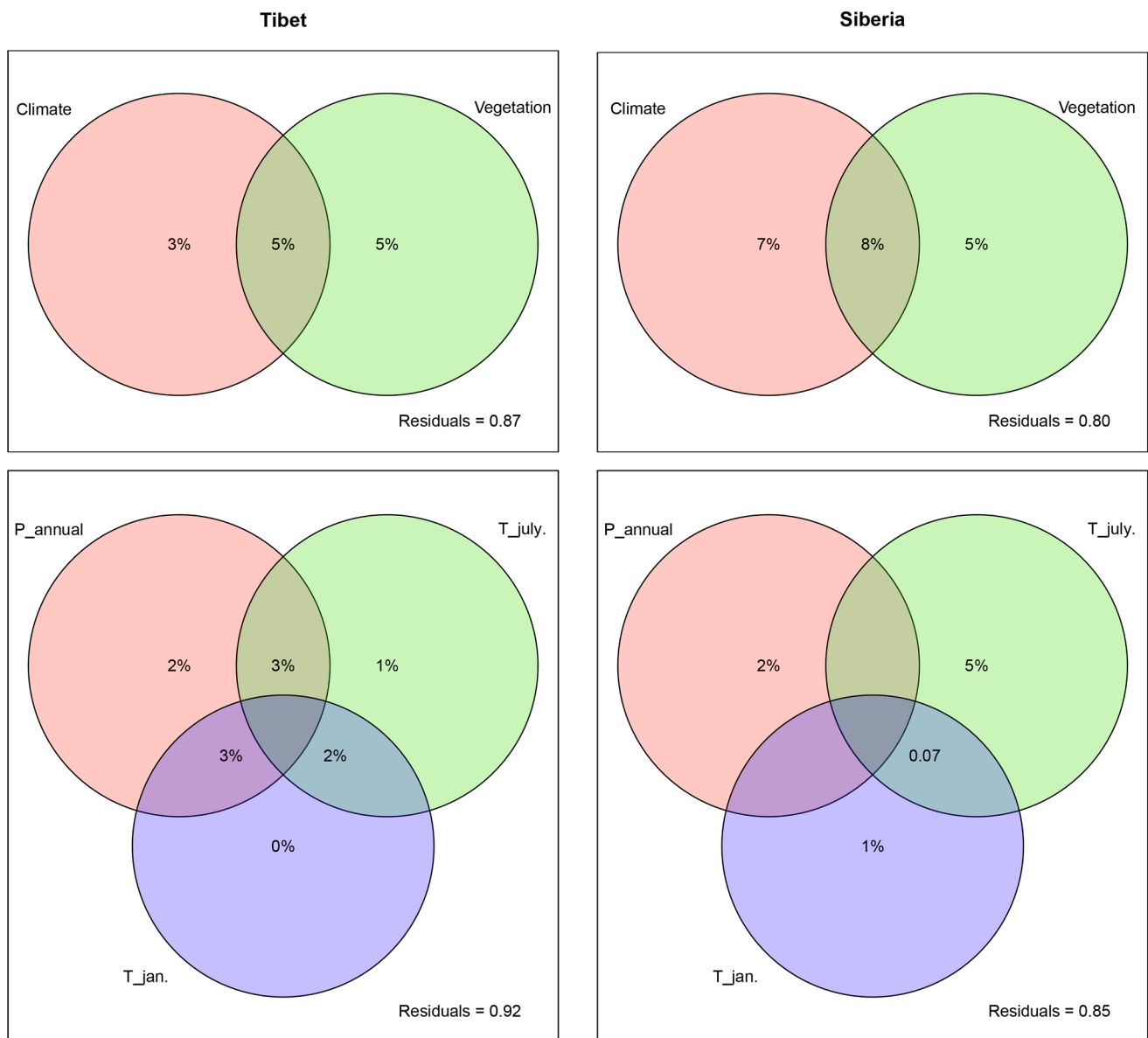


Fig. 5. Percent contributions of vegetation type and climate parameters to plant sedDNA taxonomic composition for the Tibetan Plateau (left) and Siberia (right). P_annual: mean annual precipitation; T_july: mean July temperature; T_jan: mean January temperature.

trnL g-h and ITS1 is necessary in future plant sedDNA researches studies to improve the taxonomic resolution (De Barba et al., 2014; Willerslev et al., 2014).

The completeness of the reference library affects the taxonomic resolution as well (Thomsen and Willerslev, 2015; McGee et al., 2019). The Arctic and Boreal *trnL* references used here were constructed from boreal and Arctic-European specimens (Sønstebo et al., 2010; Willerslev et al., 2014; Soininen et al., 2015). Using only this regional reference library would have resulted in many false-negative for samples outside northern Asia and using EMBL database likely lead some false positives in our results. The alpine flora of the Tibetan Plateau is a biodiversity hotspot (Wen et al., 2014), where 20% of the species are endemic (Liu et al., 2014). The absence of an open *trnL* reference library thus limits a more precise identification of samples from the Tibetan Plateau (Jia, 2020). Therefore, the establishment of plant DNA reference libraries for single regions in Asia would enhance the quality of sedDNA-based vegetation reconstruction.

Additionally, some species dominant in the modern vegetation around the lake were not traced by plant sedDNA spectra, for example

Bothriochloa ischaemum from Lake Daihai, probably because of taphonomic processes. Terrestrial plant sedDNA mainly derives from leaves, twigs, and seeds whose low dispersal and transport capacity strongly affect the plant sedDNA concentration and distribution (Alsos et al., 2018; Giguet-Covex et al., 2019). The lakes were only sampled once and at one point, which may not capture the full array of vascular plants growing in the catchment.

Our results do not provide evidence that taxa reads/percentages are a direct measure of a plant species' relative biomass or abundance, though this is not the focus of our investigation. Both the taphonomic process and protocol limitations can bias this quantitative relation (Taberlet et al., 2007; Sønstebo et al., 2010; Taberlet et al., 2018). SedDNA metabarcoding can potentially provide relative quantitative information, but needs *a priori* knowledge and additional experiments that might be too difficult to implement at large scales or in complex ecosystems (Taberlet et al., 2018). Instead, using sedDNA sequences as a direct analogue of vegetation type or to infer climate parameters might reduce the need for complete taxa assignment and still provide useful information. Relating time-sequenced plant sedDNA results from south-

eastern Tibet and Siberia to their closest analogue to modern vegetation based on plant sedDNA sequences and the vegetation types in our dataset demonstrates that sedDNA as a modern analogue is a promising tool for palaeovegetation reconstruction (S. Liu, personal communication). We acknowledge that more sophisticated analyses need to be performed to extract the vegetation signals from the huge amount of genetic data, but the approach relies less on the completeness of the taxonomic reference library and is less time-consuming than pollen analysis.

5. Data availability statement

The data that support the findings of this study are available in the [supplementary material](#) of this article. DNA sequence data can be retrieved at Dryad (<https://doi.org/10.5061/dryad.k6djh9w4r>).

CRedit authorship contribution statement

Kai Li: Investigation, Writing – original draft, Writing – review & editing. **Kathleen R. Stoof-Leichsenring:** Investigation, Methodology, Project administration, Writing – review & editing. **Sisi Liu:** Writing – review & editing. **Weihan Jia:** Investigation, Writing – review & editing. **Mengna Liao:** Writing – review & editing. **Xingqi Liu:** Funding acquisition, Investigation, Writing – review & editing. **Jian Ni:** Funding acquisition, Writing – review & editing. **Ulrike Herzschuh:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2021.108303>.

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