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Phylogenetic diversity only weakly mitigates climate-change-driven biodiversity loss in insect communities

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Abstract

To help address the underrepresentation of arthropods and Asian biodiversity from climate-change assessments, we carried out year-long, weekly sampling campaigns with Malaise traps at

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different elevations and latitudes in Gaoligongshan National Park in southwestern China. From these 623 samples, we barcoded 10,524 beetles and compared scenarios of climate-change-induced biodiversity loss, by designating seasonal, elevational, and latitudinal subsets of beetles as communities that plausibly could go extinct *as a group*, which we call 'loss sets.' The availability of a published mitochondrial-genome-based phylogeny of the Coleoptera allowed us to compare the loss of species diversity with and without accounting for phylogenetic relatedness. We hypothesised that phylogenetic relatedness would mitigate extinction, since the extinction of any loss set would result in the disappearance of all its species but only part of its evolutionary history, which is still extant in the remaining loss sets. We found different patterns of community clustering by season and latitude, depending on whether phylogenetic information was incorporated. However, accounting for phylogeny only slightly mitigated the amount of biodiversity loss under climate change scenarios, against our expectations: there is no phylogenetic "escape clause" for biodiversity conservation. We achieve the same results whether phylogenetic information was derived from the mitogenome phylogeny or from a *de novo* barcode-gene tree. We encourage interested researchers to use this dataset to study lineage-specific community assembly patterns in conjunction with life-history traits and environmental covariates.

Key words: beetles, Coleoptera, OTU, phylogenetic diversity, Gaoligongshan, Hengduan Mountains, phylogenetic placement, barcodes

1. Introduction

Insect populations appear to be declining worldwide (Cardoso et al., 2020; Vaidyanathan, 2021; Abrego et al. 2021). Our particular interest here is to gauge the threat posed by climate change to arthropod communities. Their responses to changing climate are underrepresented in vulnerability assessments (Pacifici et al., 2015), and the requisite long-term time-series data of their community composition needed for such assessment are especially lacking in Asia (Sánchez-Bayo & Wyckhuys, 2019; Srivathsan et al. 2022). To start addressing this underrepresentation, we conducted year-long, weekly sampling campaigns with Malaise traps at different elevations and latitudes in Gaoligongshan (Chinese for Mt. Gaoligong, 高黎贡山) National Park in Yunnan province in southwestern China, part of the Hengduan Mountains biodiversity hotspot (Myers et al., 2000).

Gaoligongshan runs nearly 500 km north to south between 28° 30' N and 24° 40' N, paralleling the China-Myanmar border (Fig 1) and varying in elevation from 523 m above sea level to a snow cap at 5,128 m. Its vegetation types range from mixed coniferous-broadleaf forest in the north to subtropical forest in the south (Li & Li, 2020; Liu et al., 2021). The region's rugged topography has generated high species richness in plants (Li et al., 2000), vertebrates (Dumbacher et al., 2011; Yang et al., 2019), and arthropods (Liu et al., 2020; Yi et al. 2021). At 28,000 km^2 (7% of the area of Yunnan), Gaoligongshan contains at least 57.5% of birds, 61% of mammals, and 23% of ant species ever reported in Yunnan.

We barcoded beetles (Phylum: Arthropoda, Order: Coleoptera) from our Malaise trap samples to estimate the extent of biodiversity loss if seasonal, elevational, or latitudinal subsets of them are to be extirpated by a changing climate. We designate these subsets of beetles communities that plausibly could go extinct *as a group* as "loss sets". (1) *Latitudinal loss set*:

northern biota might be more at risk in response to global warming because these species tend to be replaced by poleward-moving tropical organisms ("tropicalisation", see Osland et al., 2021), while the southern biota might be more at risk if, for instance, heatwaves and forest fires become frequent (Neeraja et al., 2021; Ward et al., 2020). (2) *Elevational loss set*: mountainous species could migrate upslope in response to warming (Elsen & Tingley, 2015) while high-elevation endemics could go extinct (Wilson et al., 2007). (3) *Seasonal loss set*: either summer or winter specialists might be more at risk, depending on the effects of climate change on species physiologies and the phenologies of host plants (Ding & Gao, 2020; Schuldt et al., 2020; Abrego et al., 2021).

These latitudinal, elevational and seasonal loss sets map directly to the three predominant patterns predicted for vegetation responses to future climate change in the Hengduan Mountains: (1) a northern shift of plant distributions (Peng et al. 2022; He et al.2019a,b; Liang et al. 2018); (2) an upward shift in both treeline and alpine species (He et al. 2020; Tian et al. 2022; Liang et al. 2018); (3) and a 4-5°C increase of mean annual temperature by 2070, under the pessimistic high-emission scenario (RCP 8.5). To put this increase in context, the current temperature difference between mean summer and winter temperatures is around 13°C at Gaoligongshan (calculated from WorldClim CMIP5, 2.5 minutes resolution, Fick & Hijmans, 2017). Combining these effects, Liang et al. (2018) modelled the distribution shift of 151 representative plants in the Hengduan Mountains (under RCP 8.5) and predicted that by 2050, their distributions would on average shift 1 degree north and 400m upward. Since insect and plant distributions are correlated (reviewed in Zhang et al. 2016), we posit that it is reasonable for the same projected changes in climate to result in similar range shifts (and concomitant contractions and extinctions) in insects.

Our loss set approach complements existing approaches to species climate-vulnerability assessment such as correlation analysis (Araújo & Peterson, 2012), mechanistic modeling (Jenouvrier et al., 2009), and indicator scoring (Thomas et al., 2011) in that these methods are trying *to predict plausible loss sets*, whereas we are *measuring the amount of biodiversity contained in plausible loss sets*.

What makes this more than a species-counting exercise is that we take into account shared evolutionary history between species (Webb et al., 2002; Cavender-Bares et al., 2009; Davies, 2021). For instance, two loss sets might have no species in common but still share considerable evolutionary history if their members are closely related. The extinction of one loss set would result in the disappearance of all its species but only part of its evolutionary history, which is still extant in the remaining loss sets. We therefore hypothesise that while certain climate change scenarios (e.g. a 5°C rise in mean winter temperature causing the extinction of winter-adapted beetles, or replacement of northern species by those dispersed from the south) will drive biodiversity loss *by reducing species richness in one or more ecological communities*, shared phylogenetic history might mitigate that loss via the preservation of evolutionary history in other communities.

However, a test of the above hypothesis requires (1) a phylogeny that encompasses the focal taxon (here, Coleoptera) and (2) each sampled individual in each community to be genotyped and placed on the phylogeny (Ahrendsen et al., 2016; Jin et al., 2021; Kembel et al., 2011). For this reason, we individually DNA-barcoded the 10,524 beetles we collected, using a combination of Sanger sequencing and multiplexed individual barcoding (Creedy et al., 2020; Ratnasingham, 2019), and placed the barcodes on a published mitochondrial-genome-based phylogeny of the Coleoptera (Linard et al., 2018). Such "phylogenetic placement" (Barbera et al., 2019) on a

robust phylogeny has previously been shown to improve inference in community ecological studies (Jassen et al., 2018).

While the placement-based approach offers a robust phylogenetic position for each barcode, broad-taxon-coverage phylogenies that include the locus used for barcoding are not available for most taxa (which is why our study is limited to the beetles, despite them being a minority catch in Malaise traps, which more effectively sample Diptera, Lepidoptera, and Hymenoptera). We thus also asked whether we could achieve similar results with a gene-tree approach, i.e. a single-gene phylogeny constructed using only the DNA barcodes, following the routine use of single-gene phylogenies in microbiome community analysis (Lozupone & Knight, 2005). In other words, we asked whether phylogenetically informed ecological conclusions are dependent upon the choice of a robust reference phylogeny vs. an *ad hoc* barcode gene tree.

We compare these phylogeny-informed results to the non-phylogenetically-informed method of treating every species independently, which we call the Operational Taxonomic Unit (OTU) analysis because we clustered the 10,524 barcodes into a smaller number of self-similar sequence clusters (i.e. OTUs), which approximate species (Floyd et al., 2002; Blaxter et al., 2005).

Here we report that (1) communities clustered differently by season and latitude, dependent on whether phylogenetic information was accounted for, and that (2) compared with the non-phylogenetically informed OTU analysis, accounting for phylogeny only slightly mitigated the amount of biodiversity lost when loss sets were removed from the dataset, contrary to our expectation described above. In broader terms, phylogenetic diversity is highly correlated with species count and only weakly mitigates potential climate-driven biodiversity loss in Gaoligongshan beetle communities. We also report that (3) conveniently for the study of taxa

without a well-resolved phylogeny, we achieved the same results whether we conducted placement on a multi-locus reference phylogeny or used a single-gene phylogeny.

2. Materials and Methods

Our workflow is summarized in Fig 2.

2 1. Beetle sampling

We set up Malaise traps to sample flying arthropods in the north (Dulongjiang, 独龙江, 27.68˚N, 98.28˚E) and south (Baihualing, 百花岭, 25.31˚N, 98.80 ˚E) of Gaoligongshan. The two sites are 268 km apart (Fig 1D). The northern site has a higher diversity of seed plants (2816 vs. 1549 species in the south) and higher, more uniform precipitation: monthly average precipitation is 156.14 mm in the north (s.d. = 81.5) and 148.1 mm in the south (s.d. = 90.0). The northern site is also cooler than is the south: mean monthly temperature is 13.7 °C in the north (s.d. = 5.6) and 16.1 °C in the south (s.d. = 4.6). This information is visualized in Fig S1, plotted from 0.5 degrees meteorological data from the China Meteorological Data Service Center (http://data.cma.cn/site/index.html). Under a pessimistic climate-change scenario (RCP 8.5), by 2070, the mean annual temperatures of the northern and southern sites will increase by 4.1°C and 4.4°C, respectively, and the mean temperatures of the coldest quarter will increase by 4.4°C and 4.2°C, respectively (WorldClim CMIP5, 2.5 minutes resolution, Fick & Hijmans, 2017).

From June 2014 to May 2015, we set up six Malaise traps in a site in the south (four at 1400 m, two at 1800 m); from August 2015 to August 2016, we set up seven Malaise traps in a site in the north (five at 1400 m, two at 1800 m) (Fig 1EF). The extra trap at 1400 m in the north was inadvertently set up near a local *Amomum tsaoko* (Zingiberaceae) plantation, which we did not abandon. We used 95% ethanol as killing and preserving agent. Local farmers assisted by

changing sampling bottles weekly and storing bottles in -18 °C freezers that were given to them as compensation. We then periodically transferred stored bottles to a -80 °C freezer at Kunming Institute of Zoology (KIZ), China. At KIZ we replaced the collecting ethanol with 99.9% ethanol and hand-picked the beetles from the weekly sampling bottles into individual Thermo Scientific matrix 2D storage tubes, each with a unique 2D barcode.

2.2 DNA extraction and metabarcoding

We extracted DNA from each southern-site beetle following the glass fiber plate DNA extraction protocol of the Canadian Centre for DNA Barcoding (https://ccdb.ca/resources/), except for using a non-destructive protocol for tissue lysis (Tin et al., 2014). For the north-site beetles, we extracted DNA from a single leg of each larger individual (> 2 mm length) using the Tiangen kit (Tiangen Biotech, Beijing Co. Ltd., Beijing, China), and from whole bodies of the smaller individuals (≤ 2 mm) using the Qiagen kit (Qiagen, Hilden, Germany).

For all beetles, we first attempted to amplify the full DNA barcode (658 bp, COI-5P) using the LCO1490/ HCO2198 primer pair (Vrijenhoek, 1994). PCR was carried out in a 15 μL reactions consisting of 10.625 μL ddH2O, 1.5 μL 10X Buffer, 1.2 μL dNTP, 0.075 μL TaKaRa Taq, 0.3 μL of each primer (10 μM), 1 μL of DNA. PCR reactions were carried out under the following conditions: initial denaturation at 94 °C for 1 min, followed by 5 cycles of (94 °C for 1 min, 45 °C for 1 min, 72 °C for 1 min), 30 cycles of (94 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min) and a final extension of 72ºC for 5 min. For each extraction, we resolved 2 μl of PCR product by electrophoresis on a 2% agarose gel. Successful amplicons were Sanger-sequenced. For beetles that failed to generate a full-length amplicon with the above-mentioned protocol, we

repeated the PCR procedure with the mlCOIintF/ jgHCO2198 primer pair (313 bp, a subset of *COI*, Leray et al., 2013), with same reaction and PCR cycles.

If samples failed these two amplification attempts, they were amplified with the CFMRb primer pair (180 bp, a subset of *COI*, Jusino et al., 2019), which was then sequenced using a multiplexed individual barcoding protocol (Creedy et al., 2020). For every 96 samples that we planned to pool into a single library, we used 96 twin-tagged primer pairs to identify samples and eliminate tag-jump errors (Schnell et al., 2015; Yang et al., 2021). PCR was carried out in a 15 μL reactions same as the previous approach. For failed PCR runs, a second round of PCR was carried out in 15 μL reactions consisted of 5.9 μL ddH2O, 7.5 μL KAPA HiFi HotStart ReadyMix (2X), 0.3 μL of each primer (10 μM), and 1 μL of DNA. PCR products were pooled and gel-purified by using a Qiagen QIAquick PCR purification kit. In total, 3120 beetles were pooled into 35 libraries (96 beetles per library). The PCR products were sent to Novogene (Beijing, China) for library construction and 150 bp paired end sequencing on an Illumina NovaSeq 6000.

2.3 *Bioinformatics*

2.3.1 Read processing and OTU table

Read processing. Sanger-sequenced barcodes (i.e. those amplified from LCO1490/HCO2198 or mlCOIintF/jgHCO2198 primer pairs) were assembled in Geneious v10.2.6. For reads of multiplexed individual barcoded samples (i.e. those amplified with the CFMRb primer pair), we used AdapterRemoval v2.2.2 (Schubert et al., 2016) to trim Illumina adapters and Sickle v1.33 (Joshi & Fass, 2011) to remove reads with average Phred score less than 20. Reads were then demultiplexed using DTD (Double Tag Demultiplexer,

https://github.com/yoann-dufresne/DoubleTagDemultiplexer) and dereplicated on MBRAVE (http://mbrave.net, Ratnasingham, 2019). We translated dereplicated sequences to codons using gotranseq (https://github.com/feliixx/gotranseq) and chose the most abundant read without a stop codon as the sample barcode.

Community assignment. Our smallest unit of analysis was two Malaise-trap bottles from consecutive weeks pooled together (hereafter referred to as a "sample"). Each barcode was assigned a set of three environmental covariates: (1) elevation (1400 m or 1800 m); (2) latitude (north or south); and (3) season (spring, summer, autumn, winter). Samples from June, July, and August are summer; September, October, November are autumn; December, January, and February are winter; March, April, May are spring. To assign samples to month, we used the month in which the starting date of each biweekly sample fell. Samples were also assigned a week number.

OTU table construction. To quantify biodiversity loss using a count-based approach for barcoded samples, we used sumaclust 1.0.31 (Mercier et al., 2013) to group barcodes into 97%-similarity Operational Taxonomic Units (OTUs). If an OTU contained a barcode from a sample, it is deemed to be present in that community. We refer to the resulting sample × OTU matrix as an "OTU table". The cell values of the OTU table are the counts of barcodes (beetles) belonging to that row's sample and that column's OTU.

2.3.2 Phylogenetic placement

Maximum likelihood (ML) tree for phylogenetic placement. To quantify biodiversity loss using a phylogenetic-placement approach, we needed a robust reference tree. The most comprehensive beetle reference phylogeny to date has 10 partitions in its 19-loci alignment

(Linard et al., 2018), but multiple partitions (i.e. multiple sets of model parameters for the ML tree) are not supported in phylogenetic placement programs such as EPA-ng (Barbera et al., 2019). We thus needed to "downsize" the existing alignment of Linard et al. (2018) to a single partition and obtain a single set of parameters for its ML tree. We did this by trimming the alignment of Linard et al. (2018) down to a subset that contained only *COI*, *cytb*, *16s*, and *12s* regions (all three barcodes in our study are nested within *COI*, and all 4 loci are widely used in metabarcoding studies). The resulting alignment, along with the original tree from Linard et al. (2018), were used to re-optimize model parameters and to re-infer the best beetle ML tree ("*--evaluate*" command in raxml-ng-mpi v0.9.0, Kozlov et al., 2019). We used Ktreedist v1.0 (Soria-Carrasco et al., 2007) and TreeCmp v2.0-b76 (Goluch et al., 2020) to check if the new ML tree was significantly different from that of Linard et al. (2018). The new best ML tree (and its set of model parameters) were used for phylogenetic placement.

Phylogenetic placement. We placed every unique barcode onto the ML tree using EPA-ng v0.3.5 (Barbera et al., 2019). Each barcode (called "queries" in EPA-ng) could be placed on multiple edges of the tree with different likelihoods. We evaluated the certainty of each barcode's placement by looking at (1) the expected distance among all its placement locations ("*--edpl"* command in Gappa v0.5.0, Czech & Stamatakis, 2019) and (2) the likelihood weight ratios of all of its placements ("*--lwr"* command in Gappa). To extract a "placement tree", we represented each barcode as a pendant edge on its most likely placement position ("*--graft --fully resolve*" command in Gappa).

2.3.3 De novo tree construction

To investigate whether the result of biodiversity assessment is dependent on the availability of a robust reference phylogeny (previous section "*2.3.2 Phylogenetic placement*"), we also built a maximum-likelihood *de novo* gene tree from our (unique) barcode sequences only. We used ModelFinder (Kalyaanamoorthy et al., 2017) to estimate the best partition scheme, and searched for most likely tree in IQ-TREE v1.6.12 (Nguyen et al., 2015), with 1,000 iterations for ultrafast bootstrap approximation (Minh et al., 2013). We refer to this best gene tree as our *de novo* tree. This procedure mimics the single-gene tree approach widely used in microbiome community analysis (Lozupone & Knight, 2005). We compared the topology between *de novo* tree and our placement tree using Ktreedist v1.0 (Soria-Carrasco et al., 2007) and TreeCmp v2.0-b76 (Goluch et al., 2020).

2.4 *Statistical analyses*

Unless otherwise noted, statistical analyses were performed in R v3.6.3 (R Core Team 2020). We conducted two sets of analyses across all samples (Fig 2C): (1) we performed ordination analysis in the form of non-metric multidimensional scaling (NMDS, Kruskal, 1964) to visualize the compositional similarity across all samples; (2) we performed rarefaction analysis to compare how much biodiversity is lost when removing sets of ecological communities (see 2.4.2 below).

We carried out these analyses on the three outputs obtained above (Fig 2B): (1) the OTU table (see *"2.3.1 Read processing and OTU table"*), (2) the placement tree (see *"2.3.2 Phylogenetic placement"*), and (3) the *de novo* tree (see *"2.3.3 De novo tree construction"*).

2.4.1 Ordination with non-metric multidimensional scaling

With the OTU table, we calculated both Jaccard and Bray-Curtis dissimilarity among samples (Jaccard, 1912, Bray and Curtis, 1957, implemented as *"(distance = 'jaccard')*" or *"(distance = 'bray')*" option in "*--metaMDS*" command in vegan v2.5-6, Oksanen et al., 2019). In contrast, phylogenetically informed samples are represented as sets of tips on the placement tree and *de novo* trees. We generated dissimilarity matrices by measuring both weighted and un-weighted UniFrac distances between samples (Lozupone et al. 2010, implemented in "*--UniFrac*" command, *"weighted = FALSE"* or *"weighted = TRUE"* options in phyloseq v1.30.0, McMurdie & Holmes, 2013,). Since tree tips in our analysis are unique barcodes, unweighted UniFrac only accounted for unique barcode presence/ absence, while weighted UniFrac also accounted for the abundance of each unique barcode in each sample. We visualized these dissimilarity matrices with non-metric multidimensional scaling (NMDS, Kruskal, 1964, implemented with the "*--metaMDS*" command in vegan), with each point being a sample, scored for season (summer vs. winter), latitude (north vs. south), and elevation (1400 m vs 1800 m).

2.4.2 Comparing diversity loss scenarios

We define a loss set as the subset of all samples that share the same season, latitude, or elevation, these being subsets that could plausibly be lost *as a group* due to future climate warming. To avoid confusion, when we refer to a "summer loss set scenario" for example, we mean the whole dataset *minus* all summer samples. We have six loss set scenarios: (1-2) loss of all summer or of all winter samples; (3-4) loss of all northern or of all southern samples; and (5-6) loss of all 1400 m or of all 1800 m samples.

With the OTU table, we estimated the effect on total diversity of each loss set scenario by extrapolating abundance-based species richness with iNEXT.3D v0.0.1 (Chao et al. 2021, "*diversity=TD*" option in "*--iNEXT3D*" command). With the phylogenetically informed datasets, samples are tips on the placement and *de novo* trees, and we also used iNEXT.3D to extrapolate abundance-based Faith's phylogenetic diversity (Faith, 1992) ("*diversity=PD*" option in "*--iNEXT3D*" command).

3. Results

3.1 *Sampling and barcoding*

We hand-pulled 12,195 beetles (3,935 from the north and 8,251 from the south) out of 632 weekly Malaise trap bottles (a 93% bottle retrieval rate, while 44 bottles in the northern site were lost due to trap loss and transportation difficulties from December to March). See Table S1 for site and OTU information for each beetle.

Our Sanger-based and multiplexed individual barcoding attempt obtained 10,524 barcodes, 7,057 of which are unique. Of all the barcodes, 7,842 were full-length (658 bp) COI barcodes, 265 were mlCOIintF/jgHCO2198 barcodes of 313 bp in length, and 2,417 were CFMRb barcodes 180 bp in length. Of the 1,671 unbarcoded samples, 9 were discarded due to missing sample information, and 1,662 (910 south + 752 north) were discarded due to PCR failures or not being Coleoptera. Full-length barcodes, along with individual photographs and site information, are available on BOLD (see Data Accessibility Statement); the mlCOIintF/jgHCO2198 barcodes are included in Supplementary Material; and the CFMRb barcodes (and quality control summary) are available on MBRAVE (see Data Accessibility

Statement). The spatio-temporal and taxonomic distribution of all barcodes are visualized in Fig 3, Fig S2, Fig S3 and tallied in Table S2.

3.2 *OTU clustering*

A total of 10,258 out of 10,524 barcodes were clustered by 97% similarity into 2,822 OTUs. We omitted all 265 of our 313 bp mlCOIintF/jgHCO2198 barcodes because while they overlapped with full length barcodes, they did not overlap with CFRMb sequences (all three types of barcodes could not be clustered together to generate representative OTU sequences).

Table S1 contains the OTU assignment of each barcode, while Table S3 presents the OTU table. In summary, the north contains 1277 OTUs, and (with some overlap) the south contains 1597 OTUs. The summer contains 1796 OTUs, and the winter contains 205 OTUs. The 1400 m elevation contains 2110 OTUs, and the 1800 m elevation contains 1101 OTUs.

3.3 *Phylogenetic placement*

Our re-optimized, single-partition ML tree contained 4 loci, 3,091 alignment sites and 13,995 species tips (see Supplementary Material for the new ML tree and model parameters). The new ML tree had a similar genetic distance scale (K factor = 1.08 in `Ktreedist`; K factor of 1 indicates identical distance scale) to the original 10-partition tree. The Robinson-Foulds clustering value (RC = 0, measured in `TreeCmp`) indicated identical topology between the new and original trees.

All barcodes were placed onto the new ML tree (see Supplementary Material for our placement tree). The most likely placement position of 27.63% of the barcodes have higher than 0.96 likelihood weight ratio (LWR), indicating high certainty of placement (Fig S4A). A total of

43% of the barcodes have less than 0.44 LWR for their most likely placement, but 93.85% of those placements have an expected distance between placement locations <0.13 (Fig S4B). Since our ML tree has an average branch length of 0.087, a value of 0.13 means that multiple likely placements of the same sequence are on average within two branch lengths of each other.

3.4 *De novo tree*

See Supplementary Material for the *de novo* tree and its model parameters. Our *de novo* tree and placement tree are different in mean branch lengths (K factor = 0.56, see Soria-Carrasco et al. 2007 for interpretation of K factor) and topology (RC = 6895 for a total of 14112 edges, see Robinson and Foulds 1981 for interpretation of the RC value).

3.5 *Ordination*

We removed 11 samples that contained fewer than 20 OTUs for our ordination analysis. Non-metric multidimensional scaling (NMDS) of the OTU table dataset resulted in samples clustering by latitude (north vs. south) on the first axis and then by season (winter vs. summer) on the second axis (Fig 4A, Fig S5A). In contrast, when phylogenetic diversity was taken into consideration, communities clustered by *season* on the first axis and then by latitude on the second axis (Fig 4BC for unweighted UniFrac, Fig S5BC for weighted UniFrac). Compared with OTU-derived ordinations, phylogenetically informed ordinations showed considerably more overlap between northern and southern samples, indicating phylogenetic relatedness between the two latitudes. We found no signal of clustering by elevation (Fig S6A).

3.6 *Species diversity and loss set scenarios*

With the OTU table, the seasonal loss set scenarios result in little (summer) or no (winter) reduction of extrapolated species richness (Fig 5A) and diversity (Fig S7), whereas the latitude and elevation loss set scenarios do cause declines in extrapolated species-richness (Fig 5A). We then ask whether taking into account phylogenetic relatedness mitigates these declines. That is, upon the extinction of a loss set (e.g. North), is much of the phylogenetic history retained in the remaining part of the reserve (e.g. South)? Contrary to our expectations, the outcomes are nearly the same (Fig 5BC), with at most a small mitigation of diversity loss when phylogenetic relatedness is taken into account (Fig 5D).

4. Discussion

We generated a dataset of 10,524 barcoded, photographed beetles from year-long, weekly sampling using 623 Malaise trap bottles across both elevational and latitudinal gradients of Gaoligongshan (Figs. 3, 4). While further projects will build upon this dataset to explore coleopteran lineage-specific community assembly in conjunction with their functional traits and environmental covariates (see "*4.3 Future directions*" below), this work reports a broad-stroke visualization and analysis of community change over space and time. We asked how much species diversity would decline upon the removal of subsets of total beetle diversity (subsets grouped by season, latitude, or elevation) that could plausibly be lost together due to climate heating, and we asked whether the answer to this question would change depending on whether phylogenetic relatedness was taken into account (Fig. 5). Our main message is that there is no phylogenetic "escape clause" for biodiversity conservation. Below, we discuss our ordination

and rarefaction results, and we hypothesise why taking phylogeny into account does not materially mitigate species loss.

4.1 Count-based vs. phylogenetically informed matrices

The effect of incorporating phylogenetic information into community analysis is visible when comparing ordination results calculated from discrete OTUs vs. UniFrac distances (Fig 4A vs. 4BC). When analyzed as unrelated OTUs, the samples first cluster by latitude (north vs. south) and then by season (winter vs. summer) (Fig 4A). This is not surprising: out of 2,822 OTUs, only 52 were shared between the north and south samples; slightly more than that, 59 OTUs, were shared between winter and summer samples. In contrast, when phylogenetic information was accounted for, we observed the opposite pattern: samples first separated by season and then by latitude (Fig 4BC). Change in the predominant axis of variation from latitudinal to seasonal difference after incorporating phylogenetic information suggests that northern and southern beetles communities share more evolutionary history than do winter and southern beetles, which is hidden from the OTU dataset. Although yet to be examined in beetles, this pattern of lineage diversification along mountain valleys is a major theme of speciation in the Hengduan Mountains found in plants (Xing & Ree, 2017), vertebrates (Wan et al. 2021) and other insects (Wang & Pierce, 2022). Specifically, winter (and some summer) samples from the north and south overlapped (Fig 4BC, gray circles, gray dots, and green dots), reflecting shared evolutionary history amongst winter beetles across Gaoligongshan. We found no observable elevational clustering in our dataset (Fig S6A), likely in large part because the elevational difference is only 400m. Studies on plants, insects and birds in the same region show large

differences when communities are further apart in elevation (Sreekar et al., 2018; Liu et al. 2017; He et al. 2022).

However, although the ordinations revealed an effect of shared evolutionary history on sample similarities, we did not see this effect when undertaking the loss set scenarios (Fig 5). Our expectation was that with the OTU table, where species are treated as discrete entities, extrapolated species richness would decline in line with the number of species in a loss set, but with the *de novo* and placement trees, where phylogenetic relatedness is taken into account, extrapolated phylogenetic diversity would decline by relatively less upon removal of the same loss set. For example, removal of the species-rich summer loss set from the OTU dataset should cause a large decline in extrapolated species richness, while removal of the summer loss set from the *de novo*- and placement-tree datasets should cause less decline because some of the evolutionary history of the summer species would remain in other seasonal samples.

However, we did not observe convincing evidence for phylogenetic mitigation: for example, the spacings between the "all samples" and the "summer loss set" rarefaction curves are similar in size across Fig 5A-C. We observed, at most, small effect sizes in the 1400 m, South, and North loss set scenarios (Fig 5D).

Alternatively, one could argue that intersections of loss sets (e.g. "winter" + "south" + "1800 m") are more realistic biodiversity loss scenarios representing species co-existing in space and time. Any given community will be confronted by both rising mean habitat temperature and contracting high elevation habitat under future climate change. However, phylogenetic mitigation of such intersection loss sets is likely also to be minimal. For instance, the "winter-south-1800 m" set contains only 9 OTUs, which constitute 0.32% of all OTUs and 0.20% or 0.38% of phylogenetic diversity, on the *de novo* and placement trees, respectively.

The effective absence of phylogenetic mitigation recovers the theoretical expectation that PD is a generalized form of richness index (Chao et al. 2010). Studies have found high correlation between PD and species richness (Davies and Buckley 2011; Safi et al. 2011, Dias et al. 2020). In other words, in our dataset, we can use OTU or number of barcodes as a proxy number to rank loss sets by phylogenetic diversity (Table S2). This is also shown via inspection of Figure 3, in which the columns with the highest number of barcodes (colored bars) are in summer, 1400 m, and the south, which are the loss sets causing the largest declines in PD when removed (Fig 5C).

4.2 *Placement and de novo trees*

We carried out our analyses on beetles because this insect order had available a broad-coverage phylogeny, even though our trap bottles also contain many lepidopteran, hymenopteran and dipteran samples yet to be (meta)barcoded. With these taxa, future phylogenetic trees could be assembled *de novo* from barcodes, which is why we have tested whether lack of a multi-locus phylogeny prevents correct inference. Despite differences in tree topologies, we did not observe major differences between *de novo*-tree-derived and placement-tree-derived loss set scenarios (Fig 5). Both their PD values were highly correlated with OTU counts. These results suggest that barcode-gene trees can indeed be used to test for the effect of phylogenetic relatedness on community structure and assembly, although this conclusion requires further testing in different taxa. We also note that although both approaches generate similar ordination plots (Fig 4BC), there is a greater degree of clustering of the high-elevation, winter samples in the ordination based on the placement tree.

4.3 *Future directions*

While we report broad-stroke pattern of community diversity change across space and time in our dataset, we have not examined intraspecific and interspecific variations in response to climate change across taxa, such as thermal plasticity, microhabitat and dispersal ability (Forrest 2016). We invite interested researchers to utilize this dataset and take further investigations in two directions. Firstly, in this study we have not touched on the evolutionary assembly process at the sample (i.e. community) level, and their environmental covariates. At the sample level, metrics that reveal historical and climatic patterns of community assembly (while accounting for effect of species richness) include the standardized effect size of PD (Webb et al. 2008), as well as net relatedness index (NRI) and nearest taxon index (NTI) (Webb et al., 2002). We are interested in whether the community assembly process itself (e.g. a phylogenetically clustered vs. dispersed set of species in a community) has environmental or temporal drivers. Secondly, we encourage researchers with taxonomic expertise to explore our dataset by pairing ecological and phylogenetic covariates with trait information on, for instance, feeding habit (e.g. xylophagous vs. herbivorous vs. insectivorous) and microhabitat preference (e.g. leaf vs. bark dwelling)—while keeping in mind that Malaise traps are less likely than other methods to sample ground-dwelling beetles (Musthafa et al. 2022). It is possible that major lineages of beetles (e.g. Cerambycidae, Carabidae, Curculionidae) show convergent patterns of community assembly in response to environmental and temporal covariates; certain taxa might respond to climate change as a functional group (despite being analyzed in different spatiotemporal subsets in this study); it is also possible that functional subsets of the beetles (or, indeed, the other arthropod taxa captured by the Malaise traps) could exhibit the phylogenetic mitigation that we hypothesised would exist for the whole-beetle dataset that we have analysed here. We also note that climate

change could drive the divergence of phenologies between insects and their food plants (known as phenological mismatch or trophic asynchrony, Renner & Zohner, 2018), which is another potential driver of community-level diversity loss (Visser & Gienapp, 2019) that could be examined using our dataset in combination with local plant phenology dataset (e.g. Peng et al. 2022).

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Author Contributions

DY conceived the study. BL and AV generated the beetles reference phylogeny data. ZL, DY and ZW gathered field data, conducted experiments and analysis. ZL, DY and ZW prepared the manuscript, with input from BL and AV.

Data Accessibility

All barcodes can be downloaded from NCBI by their accession number (see Table S1).

Barcode information and OTU table are presented in the Supplementary Material.

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Figures

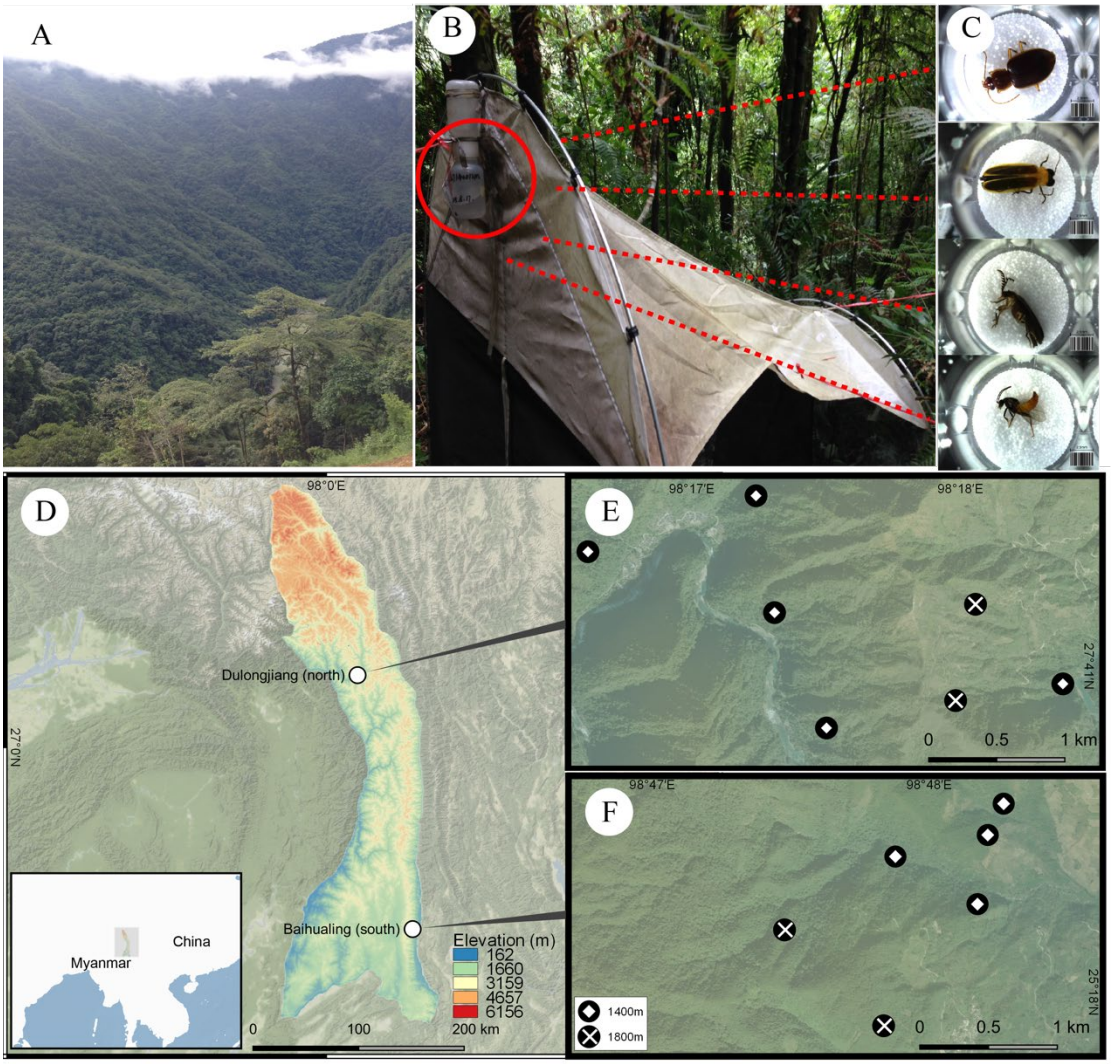

Fig. 1. Study area and sampling scheme. (A) Gaoligongshan (photo taken at northern site, around 1800 m, summer 2015. Photo credit: ZL). **(B)** One of 13 Malaise traps set up in our study, at northern site, 1800 m, in August 2015. The collection bottle (circled in red) was changed once per week. Photo credit: ZL. **(C)** Exemplars of beetles collected in Malaise trap bottles, from top to bottom: samples from family Carabidae, Lampyridae, Ptilodactylidae and Staphylinidae. **(D)** The range of Gaoligongshan, and our northern (Dulongjiang) and southern (Baihualing) site. **(E-F)** Topographical maps of northern and southern sites, with their Malaise traps set up at 1400 m and 1800 m.

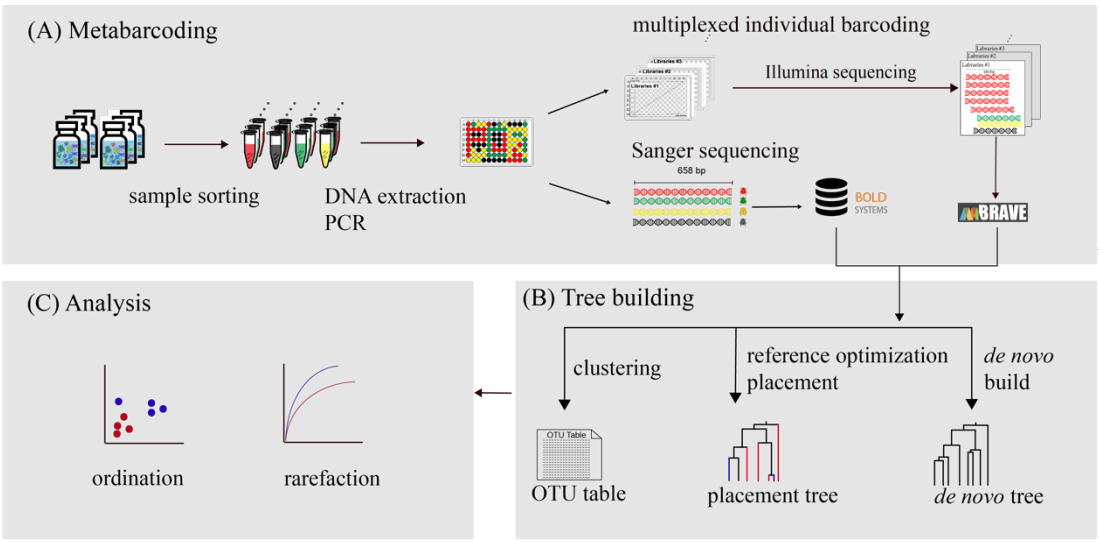

Fig 2. Study design. (A) Each beetle was barcoded with Sanger sequencing (658 bp or 313 bp) or high-throughput, multiplexed individual barcoding (180 bp). **(B)** Barcodes were used to construct an OTU table, a placement tree, and a maximum likelihood, *de novo* gene tree. **(C)** We conducted two sets of ecological analysis, using different barcode-derived inputs generated in the previous step.

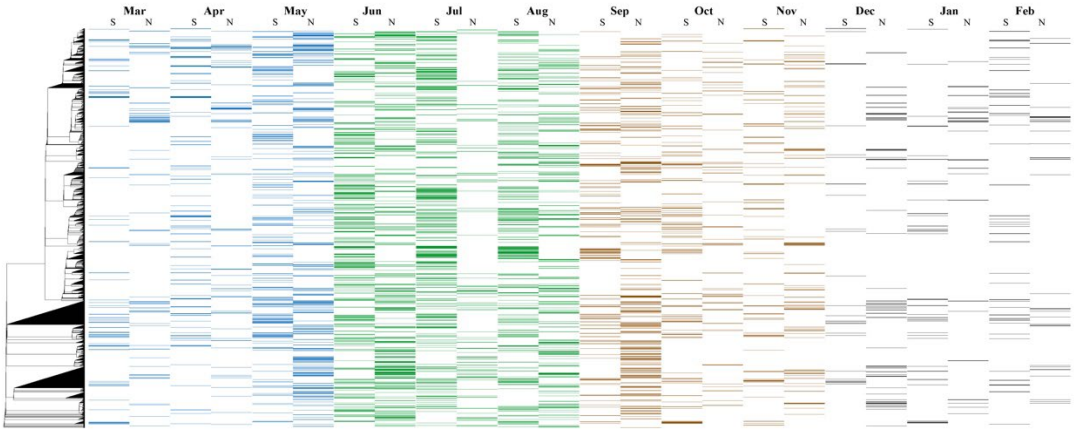

Fig 3. Unique barcodes generated from northern and southern sites at 1400 m, across the year. See Fig S2 for samples collected at 1800 m. The placement tree is positioned to the left, and the colored bars show the most likely placement position of each month's barcodes on the tree. Columns for "N" and "S" indicate northern and southern samples, while the different colors for barcodes indicate assigned seasons (blue for Spring, green for Summer, brown for Autumn, black for Winter).

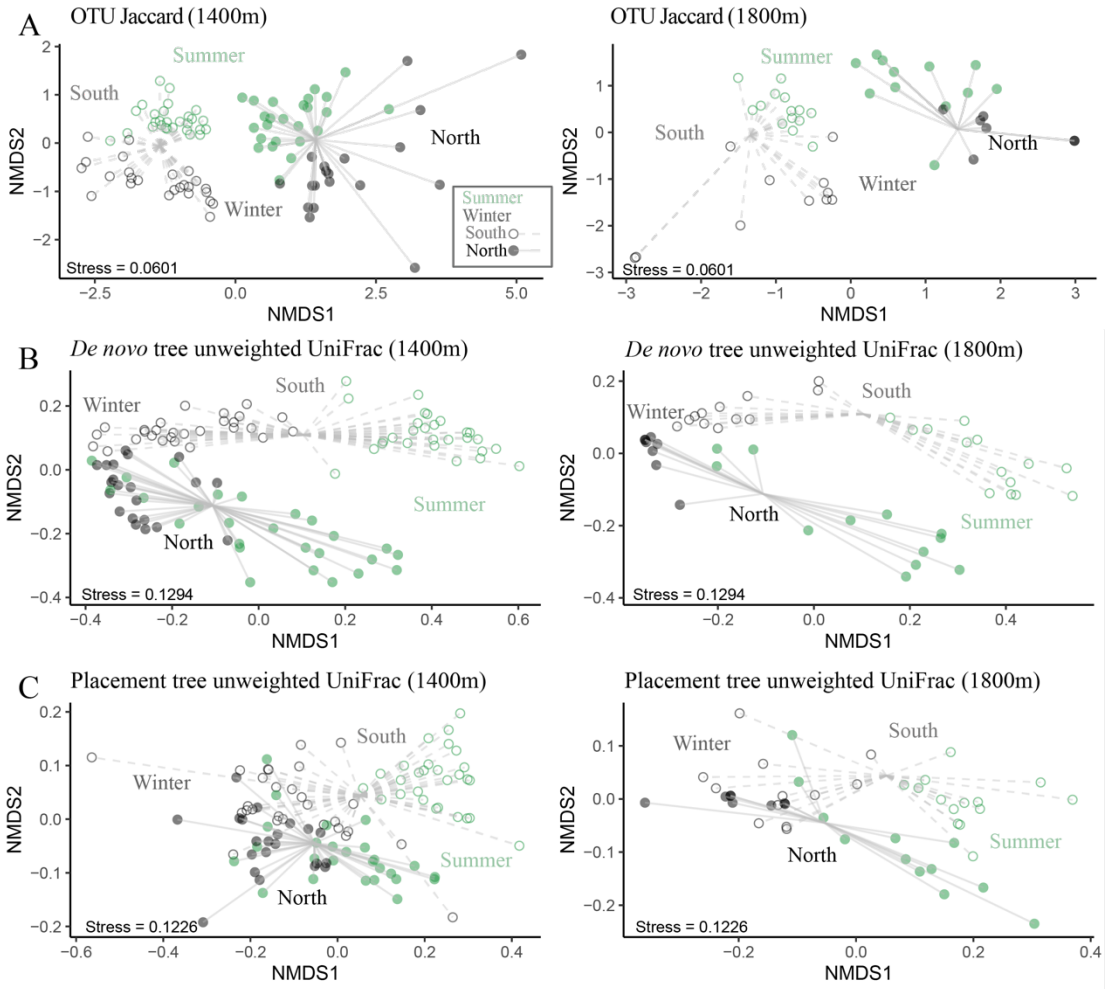

Fig. 4. Non-metric multidimensional scaling (NMDS) ordination for biweekly samples in winter and summer. See Fig S6BC for ordination including all four seasons. Ordinations highlighting elevational differences are shown in Fig S6A. **(A)** NMDS plot based on Jaccard distances between samples in the OTU table, shown separately for 1400 m (left) and 1800 m (right). For both elevations, samples first separate by latitude (north vs. south on NMDS axis 1), then by season (summer vs. winter on NMDS axis 2). (**B**) NMDS plot based on unweighted UniFrac distances on the *de novo* tree among samples, shown separately for those at 1400 m (left) and 1800 m (right). **(C)** NMDS plot based on unweighted UniFrac distances on the placement tree among samples, shown separately for those at 1400 m (left) and 1800 m (right). For both B and C, samples first separate by season and then by latitude. Moreover, the winter (and some summer) samples from the north and south overlap.

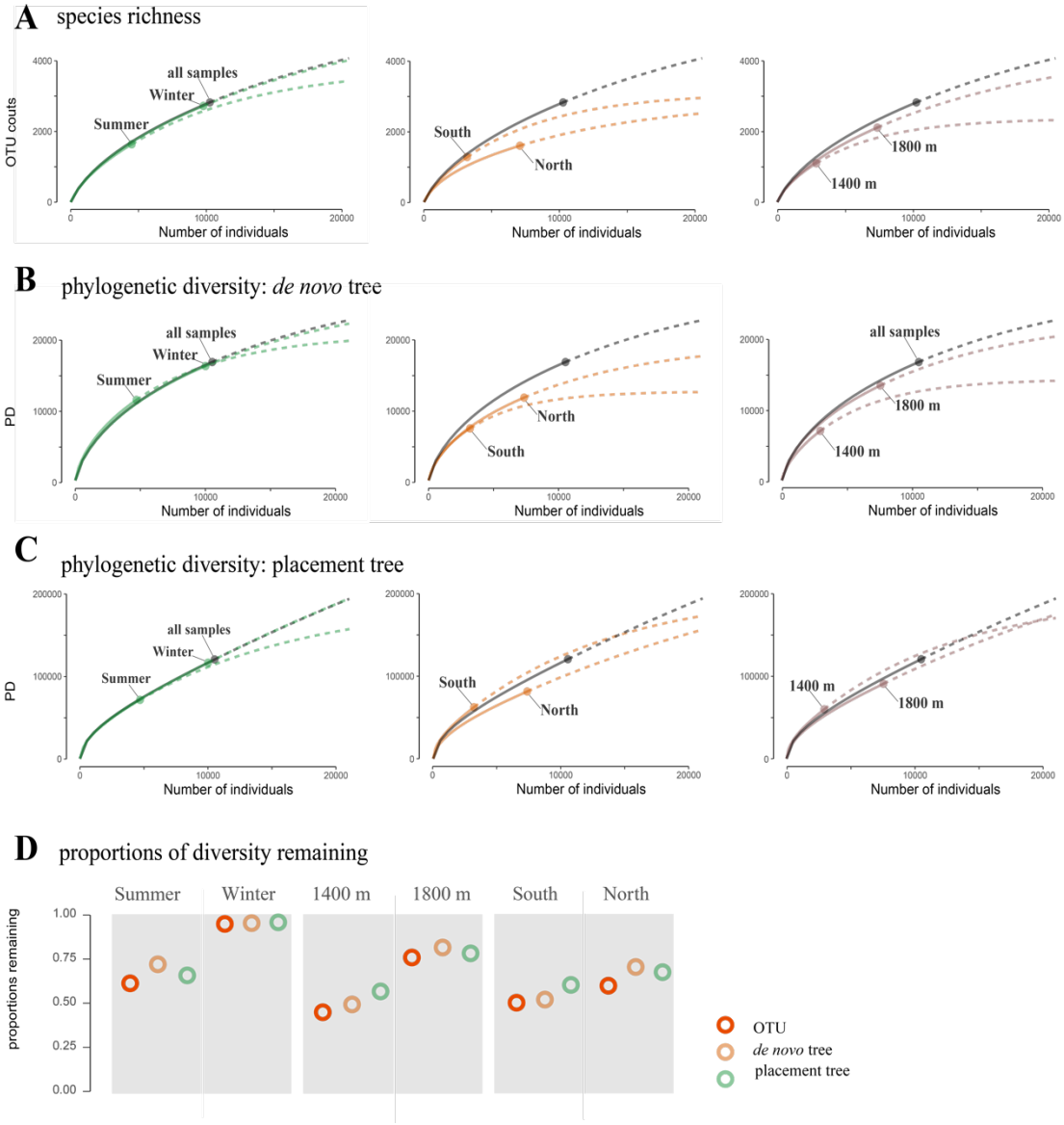

Fig. 5. Rarefaction results comparing loss set scenarios. (A-C) Rarefaction results comparing the species richness (A), *de novo* tree-based Faith's PD (B), and placement tree-based Faith's PD (C) among all samples and their seasonal (first column), latitudinal (second column), elevational (third column) loss sets. Solid lines are rarefactions and dashed lines are their extrapolations. The labels on the figure indicate sets that are subtracted from all samples. **(D)** Proportions of species richness or PD remaining after subtraction of each loss set. For instance, in the Summer box, all three ways of measuring diversity estimate a loss of about 40% when the summer samples are removed.