



This is a repository copy of *A diet rich in C3 plants reveals the sensitivity of an alpine mammal to climate change*.

White Rose Research Online URL for this paper:  
<http://eprints.whiterose.ac.uk/139087/>

Version: Accepted Version

---

**Article:**

Bhattacharyya, S. [orcid.org/0000-0002-4335-0751](https://orcid.org/0000-0002-4335-0751), Dawson, D.A., Hipperson, H. [orcid.org/0000-0001-7872-105X](https://orcid.org/0000-0001-7872-105X) et al. (1 more author) (2018) A diet rich in C3 plants reveals the sensitivity of an alpine mammal to climate change. *Molecular Ecology*. ISSN 0962-1083

<https://doi.org/10.1111/mec.14842>

---

This is the peer reviewed version of the following article: Bhattacharyya S, Dawson DA, Hipperson H, Ishtiaq F. A diet rich in C3 plants reveals the sensitivity of an alpine mammal to climate change. *Mol Ecol*. 2018, which has been published in final form at <https://doi.org/10.1111/mec.14842>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

**Reuse**

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.



[eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk)  
<https://eprints.whiterose.ac.uk/>

**MOLECULAR ECOLOGY****A diet rich in C3 plants reveals the sensitivity of an alpine mammal to climate change**

Journal:	<i>Molecular Ecology</i>
Manuscript ID	MEC-18-0458.R1
Manuscript Type:	Special Issue
Date Submitted by the Author:	n/a
Complete List of Authors:	Bhattacharyya, Sabuj; Indian Institute of Science, Centre For Ecological Sciences Dawson, Deborah; University of Sheffield, Animal and Plant Sciences Hipperson, Helen; University of Sheffield, NERC Biomolecular Analysis Facility, Department of Animal and Plant Sciences Ishtiaq, Farah; Indian Institute of Science, Centre For Ecological Sciences
Keywords:	Alpine mammal, C3 Photosynthetic pathway, Climate Change, diet, metabarcoding, Himalaya

1 *Running head Title: Sensitivity of an alpine mammal to climate change*

2

3

4 **A diet rich in C<sub>3</sub> plants reveals the sensitivity of an alpine mammal to climate**  
5 **change**

6

7

8 Sabuj Bhattacharyya<sup>1,2</sup>, Deborah A. Dawson<sup>2</sup>, Helen Hipperson<sup>2</sup>, Farah Ishtiaq<sup>1</sup>

9

10

11 <sup>1</sup>*Centre for Ecological Sciences, Indian Institute of Science, Bangalore, 560012, India*

12 <sup>2</sup>*Department of Animal and Plant Sciences, Western Bank, Sheffield, S10 2TN, UK*

13

14

15 \*Corresponding author: Sabuj Bhattacharyya, bhattacharyyasabuj@gmail.com

16

17

18

19

For Review Only

20 **Abstract**

21 Plant-herbivore interactions provide critical insights into the mechanisms that govern the  
22 spatiotemporal distributions of organisms. These interactions are crucial to understanding  
23 the impacts of climate change, which are likely to have an effect on the population dynamics  
24 of alpine herbivores. The Royle's pika (*Ochotona roylei*, hereafter pika) is a lagomorph found  
25 in the western Himalaya, and is dependent on alpine plants that are at risk from climate  
26 change. As the main prey of many carnivores in the region, the pika plays a crucial role in  
27 trophic interactions. We examined topographical features, plant genera presence, and  
28 seasonal dynamics as drivers of the plant richness in the pika's diet across an elevational  
29 gradient (2600–4450 m). We identified 79 plant genera in the faecal pellets of pikas, of  
30 which 89% were forbs, > 60% were endemic to the Himalaya and 97.5% of the plant genera  
31 identified followed the C<sub>3</sub> photosynthetic pathway. We found that during the pre-monsoon  
32 season, the plant richness of the pika's diet decreased with increasing elevation. We  
33 demonstrate that a large area of talus supports greater plant diversity and, not surprisingly,  
34 results in higher species richness in the pika's diet. However, in talus habitat with deep  
35 crevices, pikas consumed fewer plant genera suggesting they may be foraging sub-optimally  
36 due to predation risk. The continued increase in global temperature is expected to have an  
37 effect on the distribution dynamics of C<sub>3</sub> plants and consequently influence pika diet and  
38 distribution, resulting in a significant negative cascading effect on the Himalayan ecosystem.

39

40 *Keywords: Alpine mammal, C<sub>3</sub> photosynthetic pathway, climate change, diet,*  
41 *metabarcoding, Himalaya, herbivore*

42

## 43 **Introduction**

44

45 Plant-herbivore interactions provide critical insights into diverse ecological processes  
46 shaping community dynamics across an array of fields linking co-evolution (Ehrlich *et al.*  
47 1964; Johnson *et al.* 2015), chemical ecology (Hay & Fenical 1988; Rasher *et al.* 2015),  
48 foraging and nutritional ecology (Raubenheimer *et al.* 2009; Wetzal *et al.* 2016). These  
49 interactions are key determinants of the mechanisms which govern a species' spatial  
50 distribution, abundance, community dynamics, primary productivity and function in the food  
51 web (Oerke 2006; Lurgi *et al.* 2012) and the impact of changing climate on landscape level  
52 patterns in a terrestrial ecosystem (Mulder *et al.* 1999). Determining the diet of a herbivore  
53 is fundamental to understanding trophic interactions and assessing dietary plasticity to  
54 climate change, and for developing effective monitoring, management and conservation  
55 strategies (Bernstein *et al.* 2007).

56 In alpine ecosystems, pikas (*Ochotona* spp.), small-bodied lagomorphs, are good examples of  
57 how a changing climate impacts plant-herbivore interactions. The American pika (*Ochotona*  
58 *princeps*) is a generalist herbivore whose diet relies heavily on alpine plants (Ray *et al.* 2012).  
59 However, climate induced changes in vegetation distribution and composition (Rodhouse *et*  
60 *al.* 2010; Wilkening *et al.* 2011; Jeffress *et al.* 2013; Erb *et al.* 2014) led to historic extinctions  
61 of local populations and recent range contraction (Beever *et al.* 2003, 2010; Stewart *et al.*  
62 2017). During the late Miocene, the reduction in availability of preferred C<sub>3</sub> diet plants was  
63 linked to the extinction of many pika genera in various parts of Africa, Eurasia and North  
64 America (Ge *et al.* 2012,2013).

65 The Royle's pika (*Ochotona roylei*) is a widely distributed alpine mammal found in rocky

66 areas at elevations ranging from 2,400–5,000 m in the Himalayan region. It is found on rock  
67 talus and is an obligate herbivore species with limited dispersal ability due to its small body  
68 size and fairly narrow ecological niches (Bhattacharyya *et al.* 2009, 2013, 2014a, 2014b,  
69 2015; Bhattacharyya & Smith 2018). The Royle's pika is a diurnal species and produces  
70 distinct piles of faecal droppings (Bhattacharyya *et al.* 2014a, 2014b). Similar to other  
71 lagomorphs in the Himalayan region, the Royle's pika does not hibernate and forage on  
72 alpine plants along the talus-vegetation interface throughout the year (Bhattacharyya *et al.*  
73 2009, 2013, 2014a, 2014b, 2015; Bhattacharyya & Smith 2018). Hence unlike other  
74 *Ochotona* species, the Royle's pika does not store hay for winter survival (Bhattacharyya &  
75 Smith 2018). Previous dietary analysis using visual observations indicated that forbs and  
76 grasses were preferred diet plants (Shrestha *et al.* 1999; Awan *et al.* 2004; Bhattacharyya *et*  
77 *al.* 2013), which have a significantly higher protein, lipid and moisture content (Ge *et al.*  
78 2012; Bhattacharyya *et al.* 2013). Pikas are able to consume plants with high toxic  
79 components including secondary metabolites (Bhattacharyya *et al.* 2013), which are usually  
80 avoided by large herbivorous mammals (Sorensen *et al.* 2005). In addition, the Royle's pika is  
81 the main prey for a range of carnivore species (*e.g.*, yellow-throated marten (*Martes*  
82 *flavigula*), Himalayan weasel (*Mustela sibirica*), snow leopard (*Panthera uncia*), red fox  
83 (*Vulpes vulpes*) in alpine and subalpine ecosystems in the Himalayan region (Robert 1977; Oli  
84 *et al.* 1994). Thus, pikas play a crucial role in plant-herbivore trophic interactions, and their  
85 extinction or range contraction is likely to have a significant negative cascading effect on the  
86 functioning of the whole ecosystem.

87 DNA metabarcoding using high-throughput sequencing enables the identification of dietary  
88 species using DNA extracted from faecal samples more accurately than visual observations

89 or conventional faecal analysis (Kartzinel *et al.* 2015). This technique has helped to identify  
90 herbivore gut contents, revealing cryptic functional diversity and niche partitioning (Kress *et al.*  
91 *et al.* 2015). Plastid genes such as *rbcL* (CBOL Plant Working Group 2009) and nuclear ribosomal  
92 internal transcribed spacer (ITS; Hollingsworth 2011) are commonly used for plant  
93 metabarcoding (Hollingsworth 2011). Using these techniques, we aimed to identify the diet  
94 plants using faecal pellets of Royle's pikas and examine the effects of talus characteristics,  
95 topography, and plant richness, abundance and seasonal dynamics (pre and post-monsoon)  
96 on diet, across five sites in the western Himalaya, India. Food availability (plant species'  
97 presence and abundance; Huntly *et al.* 1986; Dearing 1995, 1996; Wilkening *et al.* 2011;  
98 Bhattacharyya *et al.* 2013, Bhattacharyya *et al.* 2014a; Bhattacharyya and Ray 2015), habitat  
99 topography (elevation, slope, aspect; Walker *et al.* 1993; Deems *et al.* 2002; Wilkening *et al.*  
100 2011; Rodhouse *et al.* 2010; Gurung *et al.* 2017) and predation risk (rock cover, crevice  
101 depth, nearest talus distance; Calkin *et al.* 2012; Bhattacharyya *et al.* 2014 a, b; Castillo *et al.*  
102 2014; Bhattacharyya *et al.* 2015) significantly impact the foraging ecology of the Royle's pika  
103 and potentially influence access to nutritive plants, and thereby affect individual fitness  
104 (Bhattacharyya 2013). For talus dwelling *Ochotona* spp., talus size (area) and connectivity  
105 between talus are known to influence the habitat occupancy (Franken and Hik 2004). In  
106 Royle's pikas, high proportion of rock cover in talus habitat provides refuge from predation  
107 risk, which in turn increases their habitat occupancy and abundance (Bhattacharyya *et al.*  
108 2014a; Bhattacharyya *et al.* 2015). Topographical features determine the distribution and  
109 abundance of alpine plants (Bruun *et al.* 2009). The pre-historic distribution of small  
110 mammals (Ochotonidae), highly adapted to arctic or alpine environment was closely  
111 associated with preferred C<sub>3</sub> food plant diet which is high in protein and moisture content

112 (Ge *et al.* 2012). Whilst the interplay between plants and herbivores is a key determinant of  
113 community structure (see Wisz *et al.* 2013; Dolezal *et al.* 2016), climate induced expansion  
114 of C<sub>4</sub> plants is believed to have resulted in extinction and range contraction in pikas (Ge *et al.*  
115 2012). Furthermore, in highly seasonal montane habitats, dietary constraints in herbivores  
116 tend to be strongly linked to quality of forage available. In the Himalayan region, both plant  
117 quality and available biomass may act as constraints for pikas. Seasonal dynamics in diet  
118 selection can reflect dietary adaptations (plasticity) in a seasonal alpine habitat. Our Royle's  
119 pika diet analysis will provide insights into the plant genera selected during foraging and test  
120 the following hypotheses:

121 i) Seasonal difference in diet (pre-monsoon versus post-monsoon) will demonstrate dietary  
122 flexibility and the pikas will select plants with the highest nutrient gain possible from the  
123 feeding habitats available.

124 ii) Larger areas of talus habitat will result in more plant availability and will increase species  
125 richness of the diet.

126 iii) Talus characteristics such as rock cover, depth of crevices etc. would increase species  
127 richness in the diet by providing refuge from predators and allowing pikas to access larger  
128 foraging ground.

129 iv) Forbs and grasses constitute the largest proportion of their diet.

130 v) Pikas prefer diets rich in C<sub>3</sub> plants, which signify potential threat due to climate change as  
131 C<sub>3</sub> plants are dependent on high rainfall and low temperature.

132



### 133 **Materials and Methods**

#### 134 *Study area, collection of faecal pellets and habitat data*

135 The Royle's pika inhabits rock crevices in talus fields with a home range of approximately  
136 50m<sup>2</sup> (Kawamichi 1968; Bhattacharyya *et al.* 2015). During 2014–15, 104 rock talus habitats  
137 were surveyed and faecal pellets collected from five sites at elevations ranging between  
138 2,600 to 4,450 m above sea level (a.s.l): Chopta–Tungnath (TUN), Rudranath (RUD),  
139 Madmaheshwar (MAD: Kedarnath Wildlife Sanctuary), Har ki Doon (HAR: Govind Wildlife  
140 Sanctuary), Bedni-Roopkund (NAN: Nandadevi Biosphere Reserve; Figure 1) in Garhwal,  
141 Uttarakhand, India. The sampling was conducted in two seasons: post-monsoon (October to  
142 November 2014) in TUN, RUD, HAK and pre-monsoon (May to June 2015) in TUN, MAD and  
143 NAN.

144

145 Following Bhattacharyya *et al.* (2015), we selected 50m<sup>2</sup> survey plots in each talus habitat,  
146 and these were searched for fresh piles of faecal pellets (moist, dark brown/black). One site  
147 (HAK) had only old (> 2 months) faecal piles (light brown and dry). Faecal pellets (TUN pre-  
148 monsoon=42, post-monsoon=40; NAN=43; MAD=19; HAK=20; RUD=8) were collected in  
149 airtight 2 ml plastic tubes containing silica gel (Merek, India), and were labeled with date,  
150 sampling location and geographic coordinates (latitude and longitude). In addition, the total  
151 talus area (in m<sup>2</sup>) around each survey plot, distance from nearest talus (inter-talus distance,  
152 in m), depth of deepest crevices in the talus (< 0.5 m, 0.5–1 m, 1–1.5 m, > 1.5 m) and  
153 topographical features such as elevation, slope and aspect were recorded. For each 50m<sup>2</sup>  
154 plot, we visually estimated the proportion of rock cover, forbs (e.g., *Geum elatum*, *Potentilla*  
155 *atrosanguinea*), grasses (e.g., *Danthonia sp.*, *Poa sp.*), shrubs (e.g., *Rhododendron*

156 *campanulatum* and/or *Vivernum* sp.) and trees (e.g., *Abies pindrow*, *A. spectabilis*, *Quercus*  
157 *semecarpifolia*, *Rhododendron arboretum*; Van Hees & Mead 2000; Bhattacharyya *et al.*  
158 2015).

159

#### 160 *DNA metabarcoding of faecal pellets*

161 The Royle's pika's diet is rich in secondary metabolites (Bhattacharyya *et al.* 2013), which  
162 often inhibit downstream enzymatic reactions in Polymerase Chain Reaction (PCR; Weishing  
163 *et al.* 1995). Therefore, we homogenized 20–30 mg of the faecal samples and extracted DNA  
164 using QiaAmp DNA stool kit (Qiagen Inc., Germany) following minor modifications in the  
165 manufacturer's protocol (e.g. overnight incubation at 56°C with ASL stool lysis buffer; Qiagen  
166 Inc., Germany). As Royle's pika were the only lagomorph species present in the study area  
167 (Green 1985, Bhattacharyya 2013) with very distinct faecal pellets, the chances of  
168 misidentification of the faecal samples was ruled out.

169

170 We amplified the ITS2 region of plant nuclear DNA using primer pair UniPlantF (5'-  
171 TGTGAATTGCARRATYCMG-3') and UniPlantR (5'-CCCGHYTGAYYTGRGGTCDC-3';  
172 187–380 bp; Moorhouse-Gann *et al.* 2018) and the *rbcL* region of chloroplast DNA using  
173 primer pair *rbcLa*-F (5' ATGTCACCACAAACAGAGACTAAAGC-3' and *rbcLa*-R (5'-  
174 GTAAAATCAAGTCCACCRCG-3'; 553 bp; Levin *et al.* 2003, Kress *et al.* 2009, Yoccoz *et al.*  
175 2012). Primers had overhang adapter sequences added to the 5' end for the initial PCR  
176 amplification, following Campbell *et al.* (2015). This allows unique 6 bp dual index sequences  
177 to be added along with Illumina capture sequences to each sample in a subsequent PCR step  
178 prior to pooling samples for sequencing. Initial PCR amplifications were performed in 10 µL

179 reaction volumes including 5  $\mu\text{L}$  of Qiagen Multiplex PCR Master Mix (Qiagen, Manchester,  
180 UK), 1  $\mu\text{L}$  of each primer (1  $\mu\text{M}$ ), 2  $\mu\text{L}$  nuclease free water (ThermoFisher Scientific, Inc.) and  
181 1  $\mu\text{L}$  of DNA template. Reaction conditions were as follows: initial denaturation at 95°C for  
182 15 minutes; 44 cycles of 94°C for 90 seconds, 55°C for 30 seconds, 72°C for 60 seconds; final  
183 extension of 72°C for 10 minutes. We used DNA from Quaking grass (*Briza media*) as positive  
184 control and a negative control for each PCR run (Zarzoso *et al.* 2013). The PCR products  
185 amplified by each primer pair were separated on a 1% agarose gel stained with SYBR®Safe  
186 and amplicon size compared to a 100 bp ladder (Thermo Fisher Scientific, Paisley, UK) and  
187 amplification success assessed. In addition, each PCR product was quantified in BioAnalyzer  
188 (Agilent Technologies, Santa Clara, CA) to accurately estimate the amplicon size and DNA  
189 concentration. Only amplicons with clear visible band following electrophoresis were  
190 processed further. The ITS2 and *rbcl* PCR products were pooled in equal amounts and  
191 quantified using a Qubit (Thermo Fisher Scientific, Waltham, MA) to ensure approximately  
192 equal amounts of amplicon DNA was used in the second amplification step to attach the  
193 Illumina tags. This PCR amplification was performed in a 10  $\mu\text{L}$  reaction volume including 5  
194  $\mu\text{L}$  of Qiagen Multiplex PCR Master Mix (Qiagen, Manchester, UK), 0.5  $\mu\text{L}$  of forward and  
195 reverse Illumina Multiplex Identifier (MID) tagged or indexed primers (1  $\mu\text{M}$ ), 2  $\mu\text{L}$  DNase  
196 and nuclease free water (Thermo Fisher Scientific, Inc.) and 4  $\mu\text{L}$  of DNA template (pooled  
197 product from previous ITS2 and *rbcl* PCR). Reaction conditions were: initial denaturation at  
198 95°C for 15 minutes; 10 cycles of 98°C for 10 seconds, 65°C for 30 seconds, 72°C for 30  
199 seconds; final extension of 72°C for 5 minutes. The concentrations of these PCR products (2  
200  $\mu\text{L}$ ) were measured using a fluorimeter (Thermo Fisher Scientific, Paisley, UK) and pooled  
201 together in batches of 8 samples with similar concentrations. Each batch of pooled samples

202 was then purified following the Agencourt AMPure XP PCR Purification Kit protocol  
203 (Beckman Coulter Genomics, Aus.) to remove non-target DNA fragments and primer dimer,  
204 and eluted in 40  $\mu$ L of ultrapure water (Murray *et al.* 2015). Each cleaned pool was then  
205 analysed on a Tape Station (Agilent Technologies, US) to check for successful removal of  
206 primer dimer. Each pool was then serially diluted and quantified using quantitative PCR  
207 (Applied Biosystems, CA, USA) where a diluted custom synthetic oligonucleotide of known  
208 molarity was used as standard to determine the final volume of library to use for  
209 sequencing. The qPCR amplification was performed in a 25  $\mu$ L volume containing KAPPA  
210 library quantification kit (Kappa Biosystem, Inc.), 0.4  $\mu$ M of specific forward and reverse  
211 primer and 2  $\mu$ L of pooled amplicon library and using these reaction conditions: 95°C for 5  
212 minutes, followed by 35 cycles of 95°C for 30 seconds and 60°C for 45 seconds. Based on the  
213 Cycle Threshold (CT) values observed, each pool of 8 samples were combined in equimolar  
214 proportions (4 nM) to make a single library with unique Illumina tagged PCR product for all  
215 124 samples to run using the 500 cycle v2 (2 x 250bp paired-end reads) sequencing kit on  
216 the MiSeq Desktop Sequencer (Illumina, San Diego, CA).

217

#### 218 *Identification of plant diet from sequencing data*

219 The bioinformatic analyses were performed using Iceberg, the High Performance Computing  
220 Cluster at the University of Sheffield, UK. The paired-end reads were filtered for quality  
221 (minimum quality score 20 over a 4 bp sliding window) and any Illumina adapter sequences  
222 removed using Trimmomatic v 0.32 (Bolger *et al.* 2014), retaining only reads of at least 90 bp  
223 in length. For ITS2, filtered sequences were then aligned using FLASH (Magoč & Salzberg  
224 2011) and aligned sequences with matches to the ITS2 primer sequences only were

225 extracted and primer sequences removed using the “trim\_seqs” command in Mothur  
226 (Schloss *et al.* 2009). The ITS2 region was extracted from the whole amplicon sequence  
227 (which includes ~73 bp of 5.8S sequence, Moorhouse-Gann *et al.* 2018) using ITSx  
228 (Bengtsson *et al.* 2013). The “derep\_fulllength” and “uchime2\_denovo” commands were  
229 used in usearch software v 9.2.64 (Edgar 2010) to eliminate all sequences which had less  
230 than 10 copies per sample and any chimeric sequences. Each unique ITS2 sequence found in  
231 the dataset was then compared against the NCBI GenBank nucleotide database using the  
232 BLAST algorithm (Altschul *et al.* 1997) to assign a taxonomic unit for each plant sequenced  
233 identified. Only matches with at least a 97% identity to the reference sequence were  
234 retained for downstream analysis. The software MetaGenome Analyzer v 4 (MEGAN, Huson  
235 *et al.* 2016) was used against NCBI taxonomic framework for mapping and visualization of  
236 the BLAST results, keeping all default parameters for the LCA assignment algorithm except  
237 the bit score minimum support threshold, which was set at 1%. For *rbcl* the analysis pipeline  
238 differed slightly as we did not expect a region of overlap between the paired sequencing  
239 reads with these ~550 bp long amplicons. Instead, we used a custom script to reverse  
240 complement read 2 and combine with read 1 to form an *rbcl* sequence starting and ending  
241 with the primer sequences but having at least a 50 bp gap in the middle. Prior to performing  
242 the BLAST search we also clustered the dereplicated and non-chimeric at 97% sequence  
243 similarity using the “cluster\_fast” commands in usearch. Owing to limited reference plant  
244 database from the Himalayan region, we were able to identify plants only at genus level. The  
245 reliability of each molecular operational taxonomic unit (mOTU) corresponding to a specific  
246 plant genus was further evaluated against a published list of plants reported from the study  
247 area (Bhattacharya *et al.* 2007; Rai *et al.* 2012), and online plant [e.g. flora Himalaya

248 database (<http://www.leca.univ-savoie.fr/db/florhy/infos.html>), Dobremez *et al.* 2009] and  
249 biodiversity databases [e.g. The Global Biodiversity Information Facility  
250 (<https://www.gbif.org/>)], which provided information on elevation and geographic range of  
251 the plant genus. Plant genera not reported from the Himalayan region were not considered  
252 for further analysis.

253

#### 254 *Information of ecological and evolutionary linkages of plants in Royle's pika diet*

255 The adaptation ability, ecological requirements, physiology, and nutritional quality of plants  
256 often depend on their photosynthetic pathways (C<sub>3</sub>, C<sub>4</sub> or Crassulacean acid metabolism  
257 [CAM]; Ehleringer & Monson 1993). The photosynthetic pathway of plants belonging to the  
258 same genus is often considered to be the same (Osborne *et al.* 2014; Sage 2016). Therefore,  
259 we obtained information on photosynthetic pathways of pika food plants from published  
260 sources (Ge *et al.* 2012; Osborne *et al.* 2014; Bhattacharyya *et al.* 2016; Sage 2016), and  
261 information on evolutionary origin (e.g., endemic to Himalaya, Tropical, Holarctic) average  
262 upper and lower elevation distribution range of each diet plant genus was obtained from  
263 flora of Himalaya database (<http://www.leca.univ-savoie.fr/db/florhy/infos.html> ; Dobremez  
264 *et al.* 2009), Global Biodiversity Information Facility (<https://www.gbif.org/search>) and IUCN  
265 red list of threatened species online portal (<http://www.iucnredlist.org/>).

266

#### 267 *Statistical analysis*

##### 268 *Plant composition and seasonal dynamics in diet*

269 We compared the plant diet composition at family and genera level. We used Multiple  
270 Response Permutation Procedure (MRPP; Mielke *et al.* 1976; Zimmerman *et al.* 1985) to

271 understand seasonal variation in diet composition at plant genera level across multiple sites.  
272 We used contingency table analysis to test for heterogeneity in plant family in diet across  
273 sites, which was assessed by G-tests followed by partitioned analyses (Rohlf & Sokal 1995).  
274 In addition, we have used generalized linear models (GLMs) to understand the effect of  
275 elevation on plant diet richness (number of genera) across seasons and comparison of plant  
276 families across sites sampled in the same season. The significance of fixed effects was  
277 evaluated with Wald's  $\chi^2$  tests (Bolker *et al.* 2009). To understand if relative contribution of  
278 vegetation type (forbs, grass, shrub, tree) in pika diet is proportional to their availability in  
279 the environment, we conducted compositional analysis (Aebischer *et al.* 1993) using  
280 Adehabitat v 1.8.20 package in R (Calenge 2006). We restricted our analysis to the  
281 vegetation types which qualified within the criteria of a minimum of two data points greater  
282 than zero per vegetation category in the environment dataset (Calenge 2006). In addition,  
283 the contribution of each plant genus to overall pika diet with their corresponding  
284 evolutionary origin and distribution range was visually explored using boxplots.

285

#### 286 *Modelling effect of talus habitat on diet richness*

287 We investigated the effect of food availability (tree, shrub, grass and forbs cover), predation  
288 risk (talus area, distance between talus, depth of crevices) and talus topography (elevation,  
289 aspect, slope) on plant diet richness. We fitted 32 GLM models and used AIC-based multi-  
290 model inference to identify well-supported statistical models that describe the relationships  
291 between plant diet richness and biological parameters relevant to foraging ecology of  
292 Royle's pika (Table S1). Season was not incorporated as an explanatory variable in the model  
293 as not all sites were surveyed in both seasons. Model averaging was conducted using the

294 MuMin v 1.40.4 package in R (Barton & Barton 2018) on the model set generated from the  
295 global model, applying a threshold-corrected Akaike's information criterion ( $\Delta\text{AICc}$ ; Burnham  
296 and Anderson 2002) to select the best candidate model based on the lowest (AIC) values  
297 corrected for sample size bias, or AICc values ( $\Delta\text{AICc}$ ) > 2 units than the quality of other  
298 competing models. Alternatively, when two or more models had difference in  $\text{AICc} < 2$ , we  
299 used multimodel averaged estimates to check the validity of the top ranking model in each  
300 case, only including models with  $\Delta\text{AICc} < 2$  (Burnham & Anderson 2002). The relative  
301 importance (RI) of each parameter after model-averaging was calculated by summing  
302 Akaike's weight ( $w_i$ ) across all models in which the parameter was present. All data was  
303 checked for normality and corrected for over-dispersion if required. We tested for possible  
304 collinearity of the explanatory variables using Pearson's correlation analysis in the global  
305 model; the mean correlation was 0.14 and strongest was 0.64. All the correlated variables  
306 were used only in interactive models but not in additive models. All analyses were  
307 conducted in R v 3.3.3 (The R Foundation for Statistical Computing, [http://www.r-](http://www.r-project.org/)  
308 [project.org/](http://www.r-project.org/)).

309

## 310 **Results**

311 The Illumina run produced a total of 3,685,142 paired-end reads (2 x 250 bp), with an  
312 average of 29,719 reads per sample (range 172–113,816 reads, n=124).

### 313 *Plant composition and seasonal dynamics in pika diet*

314 We successfully obtained information from 110 fecal pellets (after controlling for data  
315 quality) representing 66 out of 104 sampled rocky talus plots. A total of 79 plant genera



316 (ITS2= 66, *rbcL* =13, 12 were common to both) were identified. We retrieved 62 genera and  
317 28 families of forbs, ten genera and one family of grass, three genera and three families of  
318 shrubs, three genera and three families of trees (Table S2).

319

320 Of the 32 plant families recorded in the pikas diet, Asteraceae, Poaceae, Primulaceae,  
321 Ranunculaceae, Rosaceae and Scrophulariaceae showed significant differences across sites  
322 in the mean proportion of occurrence within diet (Table 1). We found no significant  
323 difference in proportion of plant families in the pre-monsoon (GLM:  $F_{12}=111.42$ ,  $P < 0.25$ ) and  
324 post-monsoon season (GLM:  $F_6=49.74$ ,  $P < 0.08$ ). In the pre-monsoon season, we found  
325 significant differences between sites in the proportion of plant family per faecal sample  
326 (GLM:  $F_2=126.92$ ,  $P < 0.0001$ ; proportions listed in Table 1) with NAN having significantly low  
327 number of plant families ( $t=-4.18$ ,  $P < 0.001$ ), whereas TUN showed no difference ( $t=-1.5$ ,  $P <$   
328  $0.110$ ). In the post-monsoon season, the proportion of plants in the diet varied significantly  
329 across sites (GLM:  $F_2=37.61$ ,  $P < 0.011$ ) with RUD showing more plant families ( $t=2.84$ ,  
330  $P < 0.001$ ) than TUN ( $t=2.18$ ,  $P < 0.03$ ). However, high proportion of certain family (e.g.  
331 Asteraceae in RUD) in a site is possibly driven by low sample size.

332

333 Forbs constituted 89% of richness, which was significantly higher than both shrubs (7%) and  
334 grasses (3%) (GLM:  $F_2=48.91$ ,  $P < 0.03$ ). We found pre-monsoon composition of plant genera  
335 in diet varied significantly (delta obs. = 2.54, delta exp. = 2.56,  $A=0.006$ ,  $P < 0.05$ , Figure S1)  
336 across sites, whereas there was no variation in plant genera composition in the post-  
337 monsoon season. Across seasons within site, Tungnath (TUN) showed significant variation in

338 proportion of plant richness in diet (GLM:  $F_1=18.68$ ,  $P<0.0001$ ) with the pre-monsoon  
339 season having lower plant richness ( $t=-4.38$ ,  $P<0.0001$ ) than post-monsoon.  
340 Plant genus richness during pre-monsoon varied across elevation, where it increased with a  
341 decrease in elevation (Wald's  $\chi^2=9.51$ , d.f. = 1,  $P<0.001$ ); Figure 2). Comparing food  
342 availability in the environment with dietary results revealed that the Royle's pika prefers  
343 forbs (e.g. *Potentilla*) over shrubs (e.g. *Viburnum*) in talus habitat during the pre-monsoon  
344 season in TUN ( $\lambda=0.019$ ,  $P<0.01$ ) and NAN ( $\lambda=0.46$ ,  $P<0.01$  Figure S2), and  
345 during the post-monsoon season in TUN ( $\lambda=0.11$ ,  $P<0.01$ ) and HAK ( $\lambda=0.007$ ,  $P<$   
346  $0.05$ , Figure S2). No prominent preference for forbs or grasses was observed in MAD  
347 ( $\lambda=0.20$ ,  $P>0.05$ ). Overall, composition analysis of vegetation types in the  
348 environment and faecal samples across sites during pre- and post-monsoon indicated high  
349 preference towards forbs (pre-monsoon:  $\lambda=0.008$ ,  $P>0.01$ ; post-monsoon:  
350  $\lambda=0.10$ ,  $P>0.001$ ). Due low sample size in RUD, no site-wise vegetation comparison  
351 was conducted.

352

353 A high proportion (> 50%) of the plant genera (e.g., *Anaphalis*, *Berberis*) detected in both the  
354 pre-monsoon (TUN=52.02%, MAD=56.25%, NAN=56.67%) and post-monsoon (TUN=53.48%,  
355 RUD=64.28%, HAK=56.66%) seasons were endemic to the Himalayan region, followed by  
356 plants of Holarctic origin (e.g., *Deschampsia*, *Festuca*; pre-monsoon: TUN=22.97%,  
357 MAD=28.12%, NAN=25.0%, post-monsoon: TUN=26.35%, RUD=21.42%, HAK=26.66%) origin  
358 (Figure 3A & B; Table S2). The upper distribution limit of more than 90% of all pre and post-  
359 monsoon dietary plants was 3,500 m and above (Figure 4). A significant proportions of pre-  
360 monsoon (TUN=77.70%, MAD=75.32%, NAN=72.8%) and post-monsoon (TUN=74.49%,

361 RUD=80.00%, HAK=97.14%) dietary plants were found to have a lower altitudinal range of  
362 2,000 m to 1,000 m (Figure 5). C<sub>3</sub> plants constituted 97.5% of the pikas' diet with much  
363 smaller proportions being C<sub>4</sub> (1.25%) and CAM (1.25%; Table S2).

364

#### 365 *Effect of talus habitat on plant diet richness*

366 Model-averaged estimates derived from the 90% model set agreed with the best-  
367 approximating model with two variables: talus area cover and crevice depth, which were  
368 detected as significant predictors for diet richness; each having RI values of 1.0. Talus area  
369 has significant positive influence on plant diet richness. However, crevice depth showed a  
370 negative influence on plant richness (Table 2).

371

372

#### 373 **Discussion**

374 This is the first study of the diet for any Himalayan pika species that uses non-invasive  
375 sampling and metabarcoding. It allowed us to quantify genus richness and revealed cryptic  
376 aspects of functional diversity and useful insights into niche partitioning across an  
377 elevational gradient. Our study provides an excellent example of how DNA metabarcoding  
378 can be used in understanding diet and feeding preferences of an elusive herbivore species  
379 found in fragmented alpine terrain and is applicable for other herbivores dependent on C<sub>3</sub>  
380 and C<sub>4</sub> plants. We found DNA metabarcoding outperformed traditional methods by revealing  
381 the huge diversity of plant genera consumed by pikas and their reliance on species endemic  
382 to the Himalayas. Earlier studies based on traditional visual and microscopic observation

383 reported only 22 food plant genera for Royle's pika (Bhattacharyya *et al.* 2013), whereas our  
384 DNA metabarcoding results indicated more than 70 plant genera in the diet, which includes  
385 all of the 22 plant genera reported earlier. Forbs constituted the highest proportion of the  
386 diet. We demonstrate quantitative estimates of relative consumption of plant genera and  
387 capture fine-scale distribution across an elevational gradient, which has been difficult to  
388 detect using traditional methods of diet analysis. In addition, diet plant richness in the pre-  
389 monsoon season varied significantly across sites and decreased in higher elevation sites. We  
390 also demonstrate the effects of habitat characteristics, such as talus area and depth of  
391 crevices between rocks, on the genus richness in the Royle's pika's diet.

#### 392 *Plant composition and seasonal variation in Royle's pika diet*

393 Our study revealed that pikas exhibit dietary flexibility with high genus richness in their pre-  
394 monsoon diet at lower elevation, possibly due to a longer growing period in this habitat.  
395 Thick snow cover delays the beginning of the growing period of alpine plants in higher  
396 elevation areas (Inouye 2008). Therefore, plants experience comparatively longer growing  
397 periods at lower elevations. However, the lack of precipitation and low temperature during  
398 the post-monsoon period leads to the end of the growing period for alpine plants. This may  
399 explain why no variation in diet plant richness with elevation was observed during the post-  
400 monsoon season. Compared to other Asian pika species such as Northern pika (*Ochotona*  
401 *hyperborean*; Khlebnikova 1976; Revin & Boeskorov 1990), Royle's pikas show a preference  
402 for plants such as forbs (e.g., *Potentilla* spp., *Primula* spp. and *Anaphalis* spp.). The  
403 contribution of forbs in the pika's diet was found to be proportionately higher than its  
404 availability in the environment possibly due to the high nutrient value of forbs compared to

405 other vegetation types, such as grasses, shrubs and trees (Bhattacharyya *et al.* 2013).

406 Talus dwelling *Ochotona* species usually exhibit prominent hay building activity in the  
407 summer to cache food for survival during the winter (Hudson *et al.* 2008; Bhattacharyya &  
408 Ray 2015). This food caching helps in lowering the quantity of secondary metabolites during  
409 winter consumption (Dearing 1997). However, Royle's pikas do not collect or store plants as  
410 haypiles, but instead feed on dry leaves of shrub and tree species which have low plant  
411 secondary metabolites only during the resource-limited post-monsoon season. Himalaya  
412 experiences a relatively short snow covered period compared to other mountain regions,  
413 and food plants (mosses, lichens, dry leaves from trees and shrubs) are available during the  
414 post-monsoon and winter, which probably led to the weak hoarding behaviour observed in  
415 the Royle's pika. Its pre-monsoon diet is rich in nitrogen, moisture, low in secondary  
416 metabolites and probably more suitable for spring litter. Although, one of the caveats in this  
417 study was the inconsistent seasonal sampling across sites, which would have allowed us to  
418 explore seasonal differences within the sites more effectively.

#### 419 *Effect of habitat structure on plant diet richness*

420 In western Himalayan Royle's pika populations, plant diet richness increased with large talus  
421 area, whereas depth of crevices between rocks had a negative effect on plant diet richness.  
422 Plant richness in pika diet increased with talus area as larger areas have higher plant  
423 diversity (area-richness relationship). Large talus areas provide good cover and habitat both  
424 in terms of food availability and refuge from predators. Large talus provide pikas with many  
425 escape routes from predators, allowing pikas to forage on surrounding meadows and reduce  
426 the dependence on the availability of local vegetation. This may explain the higher plant diet

427 richness that was observed, as predation risk plays an important role in determining the  
428 food selection in Royle's pika (Bhattacharyya *et al.* 2013).

429 Predation risk has significant impacts on prey populations, either by direct predation-  
430 mediated mortality, or indirectly by altering their physiology and behaviour (Lima & Dill  
431 1990; Sinclair & Arcese 1995; Schmitz *et al.* 1997; Lima 1998). Large talus areas with high  
432 rock cover and vegetation help in forage selection by reducing other constraints such as  
433 energy demands (Stephens & Krebs 1986). Pikas balance predation risk associated with  
434 foraging activity against nutritional quality and availability of diet plants, a typical strategy  
435 found in central place foragers by exploiting nearby talus habitats (Huntly *et al.* 1986; Smith  
436 *et al.* 1990; Morrison *et al.* 2004; Bhattacharyya *et al.* 2013). Large rock talus fields with  
437 crevices possibly act as escape cover from predators and allow pikas to access extended  
438 foraging grounds and diverse array of food plants, and hence have a positive influence on  
439 diet richness. Furthermore, Royle's pikas are sensitive to high temperature, and stable  
440 microclimate talus habitat serves as a refuge from harsh climate as well as predators  
441 (Bhattacharyya *et al.* 2014b). Royle's pika utilises crevices in their talus habitat to build their  
442 nests (Bhattacharyya *et al.* 2015). The structure of rock talus and availability of small crevices  
443 (< 15 cm) appear to govern occupancy of Royle's pika habitat as it reduces predation risk;  
444 wide crevices probably make it easier for predators such as weasels and red foxes to catch  
445 pikas (Bhattacharyya *et al.* 2015). We found deep crevices had a negative effect on plant diet  
446 richness. The negative effect of deep crevices probably indicates foraging under fear  
447 response. The deep crevices often also have wider openings that increase predation risk  
448 from small size predators such as weasels and red foxes (Bhattacharyya *et al.* 2015). Hence,  
449 pikas inhabiting talus with deep crevices probably utilise the foraging ground less extensively

450 and have a narrow choice of plants. This could be a mechanism behind the avoidance of  
451 such taluses as restricted diet can further reduce individual fitness. These findings are in line  
452 with previous studies on the Royle's pika and other talus dwelling pikas, which tend to  
453 forage close to the talus habitat where predation risk is the least, and venture out to open  
454 meadows to forage only when talus patches are well connected (Holmes 1991; Roach *et al.*  
455 2001; Morrison *et al.* 2004; Bhattacharyya *et al.* 2013).

#### 456 *Ecological and Evolutionary linkages in the Royle's pika diet*

457 The majority (97.5%) of diet plants in this study were C<sub>3</sub> food plants, followed by C<sub>4</sub> (1.25%)  
458 and CAM (1.25%) plants. Previous research has shown that expansion of C<sub>4</sub> plants with low  
459 nutrient quality might have led to the distributional range contraction as well as extinction  
460 of herbivorous mammals dependent on C<sub>3</sub> plants during the late Miocene (MacFadden &  
461 Ceding 1994; Cerling *et al.* 1998; Ehleringer *et al.* 2002; Osborne & Beerling 2006; Osborne  
462 2008, Ge *et al.* 2012). The pre-historic distribution of pikas was closely associated with  
463 distribution of their preferred C<sub>3</sub> food plants, such as those with high protein and moisture  
464 content (Ge *et al.* 2012). Climate change has also been found to alter the distribution and  
465 abundance of alpine plants based on their temperature sensitivity (Scherrer & Körner 2011).  
466 Replacement of C<sub>3</sub> plants by C<sub>4</sub> plants across large landscapes during the late Miocene  
467 resulted in the extinction of a high number of pika species (Ge *et al.* 2012). Gottfried *et al.*  
468 (2012) suggested that the thermophylisation process could lead to the replacement of cold  
469 and moist environment plant species (e.g. C<sub>3</sub> plants) with an abundance of warm and dry  
470 environment plant species, (e.g. C<sub>4</sub>, CAM plants). Given that the Royle's pika diet consists of  
471 C<sub>3</sub> plant species (e.g., *Potentilla*, *Anaphalis*), the increase in temperature and rainfall patterns

472 are bound to influence the distribution range of plant species as well as pikas. In the past 25  
473 years (1982–2006), the Himalayan arc has experienced significant decreases in winter  
474 precipitation (17mm), which has resulted in an increase in mean annual (0.04–0.08°C per  
475 year), spring (0.02–0.08°C per year) and winter temperatures (0.03–0.04°C per year;  
476 Shrestha *et al.* 2012). This continued increase in temperature could have a significant effect  
477 on the distribution dynamics of C<sub>3</sub> species and potentially influence plant-herbivore  
478 interactions at a microhabitat level.

479 Alpine species adapted to cold climatic conditions are more vulnerable to global warming  
480 (Hughes 2000). Moritz *et al.* (2008) indicated significant changes in distributions and range  
481 contraction in mountain-dwelling small mammals. Walther *et al.* (2002) predicted upslope  
482 range shifts in animals to cope with changing (warming) environmental conditions. However,  
483 habitat fragmentation might not allow small mammals such as pikas to move their ranges  
484 fast enough to track shifts in suitable microclimates (Scholss *et al.* 2012; Ray *et al.* 2012).  
485 Given that the Royle's pika can cope with physiological stress (e.g., hypoxia) in high elevation  
486 environments, the upper limit of the distribution of diet plants (> 4,500 m) could still pose a  
487 threat to their survival and fitness. Isolated high altitude mountain habitats, also known as  
488 "Sky-islands", differ significantly in environmental conditions from intervening valleys  
489 (Shepard & Burbrink 2008). These intervening valleys were found to have significant impacts  
490 on the dispersal of cold-adapted mountain dwelling species, such as pikas, across sky-islands  
491 (Galbreath *et al.* 2010). Hence, the Royle's pika would potentially need to travel across the  
492 lowland valleys to find favourable habitat to cope with the changing environment. Apart  
493 from high sensitivity to warm environments (Bhattacharyya *et al.* 2014b) and predation risk  
494 (Bhattacharyya *et al.* 2015), absence of diet plants below 1,500 m elevation might hamper



495 such contemporary migration through lowland valleys. Furthermore, loss of distribution  
496 range and connectivity between habitat patches along with nutritional stress might also  
497 make small pika populations more vulnerable to both climatic changes as well as other  
498 threats, such as the susceptibility to infectious diseases (Hanski & Gilpin 1991; Harvell *et al.*  
499 2000; Epstein 2001; Biebach & Keller 2009).

500 India holds 28% of the flora which is endemic to the Himalayan region (Chitale *et al.* 2014).  
501 We found a high dependency of the Royle's pika on endemic plants, and 53–64% of dietary  
502 plant genera (e.g. *Geum*, *Fragaria*) are endemic to the Himalayan region. Increases in  
503 summer temperature and precipitation and the frequency of freeze-thaw cycles are  
504 predicted to have detrimental, multi-dimensional, and spatially variable impact on these  
505 endemic alpine plants (Dolezal *et al.* 2016). Recent research has suggested around 23.9% to  
506 41.34% reduction in the distribution of endemic plants in various biodiversity hotspots in  
507 Himalaya by 2080 (Chitale *et al.* 2014). Therefore, changes in the distribution and abundance  
508 of Himalayan endemic plants might have significant negative impacts on the overall  
509 nutritional ecology of the Royle's pika. Lack of a plant genetic reference library from the  
510 Himalayan region, especially from our study site, did not allow us to achieve species level  
511 identification of pika diet. Future research is needed to build a plant genetic resource  
512 reference library for insights to be gained at the plant species level.

### 513 **Conclusions**

514 This is the first study of the Royle's pika diet using DNA metabarcoding which provides an  
515 excellent example of genetic barcode use to understand feeding preferences in climate-  
516 sensitive herbivore. We were able to quantify the genus richness in the pika's diet in various

517 habitats and at different elevations. We revealed the high dependency of the pika on C<sub>3</sub>  
518 plants and plants endemic to the Himalayan region. We showed that seasonal differences in  
519 the diet are associated with elevation and that diet varies at different sites due to  
520 differences in topography and climate. A significant amount (> 89%) of the pika's diet  
521 consisted of forbs. The genus richness in the diet is strongly predicted by the size of the talus  
522 available to the pika for foraging, with large areas resulting in the highest levels of diversity  
523 observed in the diet. Crevice depth negatively influences plant diet richness due to increase  
524 predation risk. The continued increase in temperature could have a significant effect on the  
525 distribution of C<sub>3</sub> plants and reduce the amount of plant species available on which the pika  
526 can feed, which would be likely to impact both pika numbers and their distribution,  
527 especially in lower elevation habitats.

528

#### 529 **Acknowledgements**

530 This study was partially funded by the Department of Biotechnology Research Associateship  
531 Programme, the Pro-Natura Foundation, Japan and the Commonwealth Professional  
532 Fellowship Programme, UK to SB and Wellcome Trust/DBT India Alliance Fellowship (IA/I  
533 (S)/12/2/500629) to FI. We thank the National Biodiversity Authority, Government of India  
534 and the Department of Environment, Food and Rural Affairs (DEFRA) in the United Kingdom  
535 for providing permission to transport samples between India and the UK. We thank Mr  
536 Gabbar Singh Bisth for assistance during fieldwork. Dr. Gavin Horsburgh (University of  
537 Sheffield) kindly provided advice and assistance during the metabarcoding lab work. Prof.  
538 Terry Burke and the members of the Molecular Ecology Laboratory at the University of  
539 Sheffield provided helpful discussion. We thank Prof. Colin Osbourne (University of Sheffield)

540 for kindly providing information on plant photosynthetic pathways. We would like to thank  
541 three reviewers for constructive comments that improved the manuscript and Ms. Gayatri  
542 Anand for proofreading.

543

544

## 545 **References**

546

547 Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J.  
548 (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search  
549 programs. *Nucleic Acids Research*, 25(17), 3389–3402.

550

551 Aebischer, N. J., Robertson, P. A., & Kenward, R. E. (1993) Compositional analysis of habitat  
552 use from animal radio-tracking data. *Ecology*, 74(5), 1313–1325.

553

554 Awan, M. S., Minhas, R. A., Ahmed, K. B., & Dar, N. I. (2004) Distribution, food and habitat  
555 preferences of small mammals in Machiara National Park, district Muzaffarabad, Azad  
556 Kashmir, Pakistan. *Punjab University of Journal Zoology*, 19, 17–31.

557

558 Barton, K., & Barton, M. K. (2018) Package ‘MuMIn’. [http://cran.rproject.org/web/  
559 packages/MuMIn/index.html](http://cran.rproject.org/web/packages/MuMIn/index.html)

560

561 Beever, E. A., Ray, C., Mote, P. W., & Wilkening, J. L. (2010) Testing alternative models of  
562 climate mediated extirpations. *Ecological Applications*, 20(1), 164–178.

563

564 Beever, E.A., Brussard, P.F., & Berger, J. (2003) Patterns of apparent extirpation among  
565 isolated populations of pikas (*Ochotona princeps*) in the Great Basin. *Journal of Mammalogy*,  
566 84(1), 37–54.

567

568 Bernstein, L., Bosch, P., Canziani, O., Chen, Z., Christ, R., Davidson, O., Hare, W., Huq, S.,  
569 Karoly, D., Kattsov, V. & Kundzewicz, Z. (2007) IPCC, 2007: climate change 2007: synthesis  
570 report. Contribution of working groups I, II and III to the Fourth Assessment Report of the  
571 Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change,  
572 Geneva. < <http://www.ipcc.ch/ipccreports/ar4-syr.htm> >

573

574 Bhattacharya, T., Sathyakumar, S., & Rawat, G. S. (2007) Studies on animal habitat  
575 interactions in the buffer zone of Nanda Devi Biosphere Reserve. *Final Report. Dehradun,*  
576 *India: Wildlife Institute of India.*

577

- 578 Bhattacharyya, S. (2013) Habitat ecology of Royle's pika (*Ochotona roylei*) along an  
579 altitudinal gradient with special reference to foraging behaviour in western Himalaya. Ph.D.  
580 dissertation, Saurashtra University, Gujarat, India. pp. 120  
581
- 582 Bhattacharyya, S., Adhikari, B. S., & Rawat, G. S. (2013) Forage selection by Royle's pika  
583 (*Ochotona roylei*) in the western Himalaya, India. *Zoology*, 116(5), 300–306.  
584
- 585 Bhattacharyya, S., Adhikari, B. S., & Rawat, G. S. (2014a) Influence of snow, food, and rock  
586 cover on Royle's pika abundance in western Himalaya. *Arctic, Antarctic, and Alpine Research*,  
587 46(3), 558–567.  
588
- 589 Bhattacharyya, S., Adhikari, B. S., & Rawat, G. S. (2014b) Influence of microclimate on the  
590 activity of Royle's pika in the western Himalaya, India. *Zoological Studies*, 53(1), 73.  
591
- 592 Bhattacharyya, S., Dutta, S., Adhikari, B. S., & Rawat, G. S. (2015) Presence of a small  
593 mammalian prey species in open habitat is dependent on refuge availability. *Mammal*  
594 *Research*, 60(4), 293–300.  
595
- 596 Bhattacharyya, S., & Ray, C. (2015) Of plants and pikas: evidence for a climate-mediated  
597 decline in forage and cache quality. *Plant Ecology & Diversity*, 8(5-6), 781–794.  
598
- 599 Bhattacharyya, S. & Smith, A.T. (2018) *Ochotona roylei*. In: "Lagomorphs: Pikas, Rabbits, and  
600 Hares of the World of The World", Smith, A. T., Johnston, C. H., Alves, P. C., & Hackländer, K.  
601 (Eds.). John Hopkins University press. pp. 75–76.  
602
- 603 Biebach, I., & Keller, L. F. (2009) A strong genetic footprint of the re-introduction history of  
604 Alpine ibex (*Capra ibex ibex*). *Molecular Ecology*, 18(24), 5046–5058.  
605
- 606 Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., Wit, P.,  
607 Sánchez-García, M., Ebersberger, I., Sousa, F. & Amend, A. (2013) Improved software  
608 detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other  
609 eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and*  
610 *Evolution*, 4(10), 914-919.  
611
- 612 Bolger A, Lohse M, Usadel B (2014) Trimmomatic: A flexible read trimming tool for Illumina  
613 NGS data. *Bioinformatics*, btu170. URL <http://www.usadellab.org/cms/index.php>.
- 614 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., &  
615 White, J. S. S. (2009) Generalized linear mixed models: a practical guide for ecology and  
616 evolution. *Trends in Ecology & Evolution*, 24(3), 127–135.
- 617 Burnham, K. P., & Anderson, D. R. (2002) Model Selection and Multimodal Inference: a  
618 practical information-theoretic approach. New York: Springer-Verlag, pp. 488.  
619

- 620 Bruun, H. H., Moen, J., Virtanen, R., Grytnes, J. A., Oksanen, L., & Angerbjörn, A. (2006)  
621 Effects of altitude and topography on species richness of vascular plants, bryophytes and  
622 lichens in alpine communities. *Journal of Vegetation Science*, 17(1), 37–46.  
623
- 624 Calenge, C. (2006) The package “adehabitat” for the R software: a tool for the analysis of  
625 space and habitat use by animals. *Ecological Modelling*, 197(3-4), 516–519.  
626
- 627 Calkins, M. T., Beever, E. A., Boykin, K. G., Frey, J. K., & Andersen, M. C. (2012) Not-so-  
628 splendid isolation: modeling climate-mediated range collapse of a montane mammal  
629 *Ochotona princeps* across numerous eco regions. *Ecography*, 35(9), 780–791.  
630
- 631 Campbell, N. R., Harmon, S. A., & Narum, S. R. (2015) Genotyping in Thousands by  
632 sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon  
633 sequencing. *Molecular Ecology Resources*, 15(4), 855–867.  
634
- 635 Castillo, J. A., Epps, C. W., Davis, A. R., & Cushman, S. A. (2014) Landscape effects on gene  
636 flow for a climate-sensitive montane species, the American pika. *Molecular Ecology*, 23(4),  
637 843–856.  
638
- 639 Cerling, T. E., Ehleringer, J. R., & Harris, J. M. (1998) Carbon dioxide starvation, the  
640 development of C4 ecosystems, and mammalian evolution. *Philosophical Transactions of the*  
641 *Royal Society of London B: Biological Sciences*, 353(1365), 159–171.  
642
- 643 Chitale, V. S., Behera, M. D., & Roy, P. S. (2014) Future of endemic flora of biodiversity  
644 hotspots in India. *PLoS One*, 9(12), e115264.  
645
- 646 Dearing, M. D. (1995) *Factors governing diet selection in a herbivorous mammal, the North*  
647 *American pika (Ochotona princeps)*. PhD Thesis, Department of Biology, University of Utah,  
648 USA).  
649
- 650 Dearing, M. D. (1996) Disparate determinants of summer and winter diet selection of a  
651 generalist herbivore, *Ochotona princeps*. *Oecologia*, 108(3), 467–478.  
652
- 653 Dearing, M. D. (1997) The manipulation of plant toxins by a food-hoarding herbivore,  
654 *Ochotona princeps*. *Ecology*, 78(3), 774–781.  
655
- 656 Deems, J. S., Birkeland, K. W., & Hansen, K. J. (2002) Topographic influence on the spatial  
657 patterns of snow temperature gradients in a mountain snowpack. In: *Proceedings of the*  
658 *International Snow Science Workshop, Penticton, BC, Canada, 29 September-4 October 2002*.  
659

- 660 Dobremez, J.F., Shakya, P.R., Camaret, S., Vigny, F. & Eynard-Machet, R., 1967–2009 - Flora  
661 Himalaya Database, Laboratoire d'Ecologie Alpine, [http://www.leca.univ-](http://www.leca.univ-savoie.fr/db/florhy/)  
662 [savoie.fr/db/florhy/](http://www.leca.univ-savoie.fr/db/florhy/)  
663
- 664 Dolezal, J., Dvorsky, M., Kopecky, M., Liancourt, P., Hiiesalu, I., Macek, M., Altman, J.,  
665 Chlumska, Z., Rehakova, K., Capkova, K. & Borovec, J. (2016) Vegetation dynamics at the  
666 upper elevational limit of vascular plants in Himalaya. *Scientific Reports*, 6, 24881.  
667
- 668 Edgar. R. (2010) Search and clustering orders of magnitude faster than BLAST.  
669 *Bioinformatics*, 26(19), 2460–2461.  
670
- 671 Ehleringer, J. R., Cerling, T. E., & Dearing, M. D. (2002) Atmospheric CO<sub>2</sub> as a global change  
672 driver influencing plant-animal interactions. *Integrative and Comparative Biology*, 42(3),  
673 424–430.  
674
- 675 Ehleringer, J. R., & Monson, R. K. (1993) Evolutionary and ecological aspects of  
676 photosynthetic pathway variation. *Annual Review of Ecology and Systematics*, 24(1), 411–  
677 439.  
678
- 679 Ehrlich, P. R., & Raven, P. H. (1964) Butterflies and plants: a study in coevolution. *Evolution*,  
680 18(4), 586–608.  
681
- 682 Epstein, P.R. (2001) Climate change and emerging infectious diseases. *Microbes and*  
683 *Infection*, 3(9), 747–754.  
684
- 685 Erb, L. P., Ray, C., & Guralnick, R. (2011) On the generality of a climate-mediated shift in the  
686 distribution of the American pika (*Ochotona princeps*). *Ecology*, 92(9), 1730–1735.  
687
- 688 Franken, R. J., & Hik, D. S. (2004) Influence of habitat quality, patch size and connectivity on  
689 colonization and extinction dynamics of collared pikas *Ochotona collaris*. *Journal of Animal*  
690 *Ecology*, 73(5), 889–896.  
691
- 692 Galbreath, K. E., Hafner, D. J., Zamudio, K. R., & Agnew, K. (2010) Isolation and introgression  
693 in the Intermountain West: contrasting gene genealogies reveal the complex biogeographic  
694 history of the American pika (*Ochotona princeps*). *Journal of Biogeography*, 37(2), 344–362.  
695
- 696 Ge, D., Zhang, Z., Xia, L., Zhang, Q., Ma, Y., & Yang, Q. (2012) Did the expansion of C4 plants  
697 drive extinction and massive range contraction of micromammals? Inferences from food  
698 preference and historical biogeography of pikas. *Palaeogeography, Palaeoclimatology,*  
699 *Palaeoecology*, 326, 160–171.  
700
- 701 Ge, D., Wen, Z., Xia, L., Zhang, Z., Erbajeva, M., Huang, C., & Yang, Q. (2013) Evolutionary  
702 history of lagomorphs in response to global environmental change. *PLoS One*, 8(4), e59668.  
703

- 704 Gottfried, M., Pauli, H., Futschik, A., Akhalkatsi, M., Barančok, P., Alonso, J.L.B., Coldea, G.,  
705 Dick, J., Erschbamer, B., Kazakis, G. and Krajči, J. (2012). Continent-wide response of  
706 mountain vegetation to climate change. *Nature Climate Change*, 2(2), 111.  
707
- 708 Graham, M. H. (2003) Confronting multicollinearity in ecological multiple regression.  
709 *Ecology*, 84(11), 2809–2815.  
710
- 711 Green, M. J. B. (1985) Aspects of the ecology of the Himalayan musk deer. Doctoral  
712 dissertation. University of Cambridge, U.K.  
713
- 714 Group, C. P. W., Hollingsworth, P.M., Forrest, L.L., Spouge, J.L., Hajibabaei, M.,  
715 Ratnasingham, S., van der Bank, M., Chase, M.W., Cowan, R.S., Erickson, D.L.  
716 & Fazekas, A. J. (2009) A DNA barcode for land plants. *Proceedings of the National Academy  
717 of Sciences*, 106(31), 12794–12797.  
718
- 719 Gurung, D. R., Maharjan, S. B., Shrestha, A. B., Shrestha, M. S., Bajracharya, S. R., & Murthy,  
720 M. S. R. (2017). Climate and topographic controls on snow cover dynamics in the Hindu Kush  
721 Himalaya. *International Journal of Climatology*, 37(10), 3873–3882.  
722
- 723 Hanski, I., & Gilpin, M. (1991) Metapopulation dynamics: brief history and conceptual  
724 domain. *Biological Journal of the Linnean Society*, 42(1-2), 3–16.  
725
- 726 Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel,  
727 M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science*,  
728 296(5576), 2158–2162.  
729
- 730 Hay, M. E., & Fenical, W. (1988) Marine plant-herbivore interactions: the ecology of chemical  
731 defense. *Annual Review of Ecology and Systematics*, 19(1), 111–145.  
732
- 733 Hollingsworth, P. M. (2011) Refining the DNA barcode for land plants. *Proceedings of the  
734 National Academy of Sciences*, 108(49), 19451–19452.  
735
- 736 Holmes, W. G. (1991) Predator risk affects foraging behaviour of pikas: observational and  
737 experimental evidence. *Animal Behaviour*, 42(1), 111–119.  
738
- 739 Hudson, J. M., Morrison, S. F., & Hik, D. S. (2008) Effects of leaf size on forage selection by  
740 collared pikas, *Ochotona collaris*. *Arctic, Antarctic, and Alpine Research*, 40(3), 481–486.  
741
- 742 Hughes, L. (2000) Biological consequences of global warming: is the signal already apparent?  
743 *Trends in Ecology & Evolution*, 15(2), 56–61.  
744



- 745 Huntly, N. J., Smith, A. T., & Ivins, B. L. (1986) Foraging behavior of the pika (*Ochotona*  
746 *princeps*), with comparisons of grazing versus haying. *Journal of Mammalogy*, 67(1), 139–  
747 148.
- 748
- 749 Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.J. and  
750 Tappu, R. (2016) MEGAN community edition-interactive exploration and analysis of large-  
751 scale microbiome sequencing data. *PLoS Computational Biology*, 12(6), e1004957.
- 752
- 753 Inouye, D. W. (2008) Effects of climate change on phenology, frost damage, and floral  
754 abundance of montane wildflowers. *Ecology*, 89(2), 353–362.
- 755
- 756 Jeffress, M. R., Rodhouse, T. J., Ray, C., Wolff, S., & Epps, C. W. (2013) The idiosyncrasies of  
757 place: geographic variation in the climate–distribution relationships of the American pika.  
758 *Ecological Applications*, 23(4), 864–878.
- 759
- 760 Johnson, M. T., Campbell, S. A., & Barrett, S. C. (2015) Evolutionary interactions between  
761 plant reproduction and defense against herbivores. *Annual Review of Ecology, Evolution, and*  
762 *Systematics*, 46, 191–213.
- 763
- 764 Kawamichi, T. (1968) Winter Behaviour of the Himalayan Pika, *Ochotona roylei*. *Journal of*  
765 *faculty of Science Hokkaido, series VI Zoology*, 16(4), 582–594.
- 766
- 767 Kartzinel, T.R., Chen, P.A., Coverdale, T.C., Erickson, D.L., Kress, W.J., Kuzmina, M.L.,  
768 Rubenstein, D.I., Wang, W. and Pringle, R.M. (2015) DNA metabarcoding illuminates dietary  
769 niche partitioning by African large herbivores. *Proceedings of the National Academy of*  
770 *Sciences*, 112(26), 8019–8024.
- 771
- 772 Khlebnikova, I.P. (1976) Plant biomass used and stored by the northern pika in burned  
773 Siberian pine forests of the Western Sayan Mountains. *Ekologia* 2, 99–102 (in Russian).
- 774
- 775 Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., & Bermingham,  
776 E. (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot  
777 in Panama. *Proceedings of the National Academy of Sciences*, 106(44), 18621–18626.
- 778
- 779 Kress, W. J., García-Robledo, C., Uriarte, M., & Erickson, D. L. (2015) DNA barcodes for  
780 ecology, evolution, and conservation. *Trends in Ecology & Evolution*, 30(1), 25–35.
- 781
- 782 Körner, C. (2003) *Alpine plant life: functional plant ecology of high mountain ecosystems*.  
783 Springer Science & Business Media. pp. 298.
- 784
- 785 Levin, R. A., Wagner, W. L., Hoch, P. C., Nepokroeff, M., Pires, J. C., Zimmer, E. A., & Sytsma,  
786 K. J. (2003) Family-level relationships of Onagraceae based on chloroplast *rbcL* and *ndhF*  
787 data. *American Journal of Botany*, 90(1), 107–115.
- 788



- 789 Lima, S. L., & Dill, L. M. (1990) Behavioral decisions made under the risk of predation: a  
790 review and prospectus. *Canadian Journal of Zoology*, 68(4), 619–640.  
791
- 792 Lima, S.L. (1998) Nonlethal effects in the ecology of predator–prey interactions: what are the  
793 ecological effects of anti-predator decision-making? *Bioscience*, 48(1), 25–34.  
794
- 795 Lurgi, M., López, B. C., & Montoya, J. M. (2012) Climate change impacts on body size and  
796 food web structure on mountain ecosystems. *Philosophical Transactions of the Royal Society  
797 of London B: Biological Sciences*, 367(1605), 3050–3057.  
798
- 799 MacFadden, B. J., & Ceding, T. E. (1994) Fossil horses, carbon isotopes and global change.  
800 *Trends in Ecology & Evolution*, 9(12), 481–486.  
801
- 802 Magoč, T., & Salzberg, S. L. (2011) FLASH: fast length adjustment of short reads to improve  
803 genome assemblies. *Bioinformatics*, 27(21), 2957–2963.  
804
- 805 Mann, H. B., & Wald, A. (1942) On the choice of the number of class intervals in the  
806 application of the chi square test. *The Annals of Mathematical Statistics*, 13(3), 306–317.  
807
- 808 Mielke Jr. P. W., Berry, K. J., & Johnson, E. S. (1976) Multi-response permutation procedures  
809 for a priori classifications. *Communications in Statistics-Theory and Methods*, 5(14), 1409–  
810 1424.  
811
- 812 Moorhouse-Gann, R.J., Dunn, J.C., de Vere, N., Goder, M., Cole, N., Hipperson, H.,  
813 Symondson, W.O.C (2018). New universal ITS2 primers for high-resolution herbivory  
814 analyses using DNA metabarcoding in both tropical and temperate zones. *Scientific  
815 Reports*, 8(1), 8542.  
816
- 817 Moritz, C., Patton, J. L., Conroy, C. J., Parra, J. L., White, G. C., & Beissinger, S. R. (2008)  
818 Impact of a century of climate change on small-mammal communities in Yosemite National  
819 Park, USA. *Science*, 322(5899), 261–264.  
820
- 821 Morrison, S., Barton, L., Caputa, P., & Hik, D. S. (2004) Forage selection by collared pikas,  
822 *Ochotona collaris*, under varying degrees of predation risk. *Canadian Journal of Zoology*,  
823 82(4), 533–540.  
824
- 825 Mulder, C. P. H., Koricheva, J., Huss-Danell, K., Hogberg, P. & Joshi, J. (1999) Insects affect  
826 relationships between plant species richness and ecosystem processes. *Ecology Letters*, 2(4),  
827 237–246.  
828
- 829 Murray, D. C., Coghlan, M. L., & Bunce, M. (2015) From benchtop to desktop: important  
830 considerations when designing amplicon sequencing workflows. *PLoS One*, 10(4), e0124671.  
831
- 832 Naftz, D. L., Susong, D. D., Schuster, P. F., Cecil, L. D., Dettinger, M. D., Michel, R. L., &  
833 Kendall, C. (2002) Ice core evidence of rapid air temperature increases since 1960 in alpine

- 834 areas of the Wind River Range, Wyoming, United States. *Journal of Geophysical Research:*  
835 *Atmospheres*, 107(D13).
- 836
- 837 Oerke, E. C. (2006) Crop losses to pests. *The Journal of Agricultural Science*, 144(1), 31–43.
- 838
- 839 Oli, M. K., Taylor, I. R., & Rogers, M. E. (1994) Snow leopard *Panthera uncia* predation of  
840 livestock: an assessment of local perceptions in the Annapurna Conservation Area, Nepal.  
841 *Biological Conservation*, 68(1), 63–68.
- 842
- 843 Osborne, C. P., & Beerling, D. J. (2006) Nature's green revolution: the remarkable  
844 evolutionary rise of C<sub>4</sub> plants. *Philosophical Transactions of the Royal Society B: Biological*  
845 *Sciences*, 361(1465), 173–194.
- 846
- 847 Osborne, C. P. (2008) Atmosphere, ecology and evolution: what drove the Miocene  
848 expansion of C<sub>4</sub> grasslands? *Journal of Ecology*, 96(1), 35–45.
- 849
- 850 Osborne, C.P., Salomaa, A., Kluyver, T.A., Visser, V., Kellogg, E.A., Morrone, O., Vorontsova,  
851 M.S., Clayton, W.D. & Simpson, D.A. (2014) A global database of C<sub>4</sub> photosynthesis in  
852 grasses. *New Phytologist*, 204(3), 441–446. doi: 10.1111/nph.12942
- 853
- 854 Rai, I. D., Adhikari, B. S., & Rawat, G. S. (2012) Floral diversity along sub-alpine and alpine  
855 ecosystems in Tungnath area of Kedarnath wildlife sanctuary, Uttarakhand. *Indian Forester*,  
856 138 (10), 927.
- 857
- 858 Rasher, D. B., Stout, E. P., Engel, S., Shearer, T. L., Kubanek, J., & Hay, M. E. (2015) Marine  
859 and terrestrial herbivores display convergent chemical ecology despite 400 million years of  
860 independent evolution. *Proceedings of the National Academy of Sciences*, 112(39), 12110–  
861 12115.
- 862
- 863 Raubenheimer, D., Simpson, S. J., & Mayntz, D. (2009) Nutrition, ecology and nutritional  
864 ecology: toward an integrated framework. *Functional Ecology*, 23(1), 4–16.
- 865
- 866 Ray, C., Beever, E. & Loarie, S. (2012) Retreat of the American pika: up the mountain or into  
867 the void. Wildlife populations in a changing climate. University of Chicago Press, Chicago, IL.  
868 pp. 245–270
- 869
- 870 Revin, Y., & Boeskorov, G. G. (1990) Distribution and ecology of the northern pika (*Ochotona*  
871 *hyperborea* Pall.) in southern Yakutia. *Bulletin Moskovskogo Obshchestva Ispytatelej*  
872 *Prirody, Otdiel Biologii*, 95, 48–60.
- 873
- 874 Roach, W. J., Huntly, N., & Inouye, R. (2001) Talus fragmentation mitigates the effects of  
875 pikas, *Ochotona princeps*, on high alpine meadows. *Oikos*, 92(2), 315–324.
- 876
- 877 Roberts, T. J. (1977) *The Mammals of Pakistan*. London: Ernest Benn Ltd. pp. 561.

- 878 Rodhouse, T. J., Beever, E. A., Garrett, L. K., Irvine, K. M., Jeffress, M. R., Munts, M., & Ray, C.  
879 (2010) Distribution of American pikas in a low-elevation lava landscape: conservation  
880 implications from the range periphery. *Journal of Mammalogy*, 91(5), 1287–1299.  
881
- 882 Sage, R. F. (2016) Photosynthesis: Mining grasses for a better Rubisco. *Nature Plants*, 2(12),  
883 16192.  
884
- 885 Scherrer, D., & Körner, C. (2011) Topographically controlled thermal habitat differentiation  
886 buffers alpine plant diversity against climate warming. *Journal of Biogeography*, 38(2), 406–  
887 416.  
888
- 889 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski,  
890 R.A., Oakley, B.B., Parks, D.H., Robinson, C.J. and Sahl, J.W. (2009) Introducing mothur: open-  
891 source, platform-independent, community-supported software for describing and  
892 comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), 7537–  
893 7541.  
894
- 895 Schloss, C. A., Nuñez, T. A., & Lawler, J. J. (2012) Dispersal will limit ability of mammals to  
896 track climate change in the Western Hemisphere. *Proceedings of the National Academy of  
897 Sciences*, 109(22), 8606–8611.  
898
- 899 Schmitz, O. J., Beckerman, A. P., & O'Brien, K. M. (1997) Behaviorally mediated trophic  
900 cascades: effects of predation risk on food web interactions. *Ecology*, 78(5), 1388–1399.  
901
- 902 Shrestha, U. B., Gautam, S. & Bawa, K.S. (2012) Widespread climate change in the Himalayas  
903 and associated changes in local ecosystems. *PlosOne*, 7(5): e36741.  
904
- 905 Shrestha, K., Khanal, B., & Karki, J. B. (1999) Foraging and haying plants of Royle's Pika  
906 (*Ochotona roylei*: Lagomorpha) in far-west Nepal. *Journal of Natural History Museum*, 18, 3–  
907 13.  
908
- 909 Shepard, D. B., & Burbrink, F. T. (2008) Lineage diversification and historical demography of a  
910 sky island salamander, *Plethodon ouachitae*, from the Interior Highlands. *Molecular Ecology*,  
911 17(24), 5315–5335.  
912
- 913 Sinclair, A. R. E., & Arcese, P. (1995) Population consequences of predation sensitive  
914 foraging: The Serengeti wildebeest. *Ecology*, 76(3), 882–891.  
915
- 916 Smith, A.T., Formozov, N.A., Hoffmann, R.S., Changlin, Z. & Erbajeva, M.A. (1990) The pikas.  
917 In: Chapman J.A., Flux J.C., editors. Rabbits, Hares and Pikas: Status Survey and Conservation  
918 Action Plan, Gland, Switzerland: The World Conservation Union. pp. 14–60.  
919
- 920 Rohlf, F. J., & Sokal, R. R. (1995) Statistical Tables. W. H. Freeman and Company: San  
921 Francisco, California, pp. 199.  
922

- 923  
924 Sorensen, J. S., McLister, J. D., & Dearing, M. D. (2005) Plant secondary metabolites  
925 compromise the energy budgets of specialist and generalist mammalian herbivores. *Ecology*,  
926 *86*(1), 125–139.  
927
- 928 Stephens, D. W., & Krebs, J. R. (1986) Foraging theory. Princeton University Press. pp.239.  
929
- 930 Stewart, J. A., Wright, D. H., & Heckman, K. A. (2017) Apparent climate-mediated loss and  
931 fragmentation of core habitat of the American pika in the Northern Sierra Nevada,  
932 California, USA. *PloS One*, *12*(8), e0181834.  
933
- 934 Van Hees, W. W. S., & Mead, B. R. (2000) Ocular estimates of understory vegetation structure  
935 in a closed *Picea glauca/Betula papyrifera* forest. *Journal of Vegetation Science*, *11*(2), 195–  
936 200.  
937
- 938 Walker, D. A., Halfpenny, J. C., Walker, M. D., & Wessman, C. A. (1993) Long-term studies of  
939 snow-vegetation interactions. *BioScience*, *43*(5), 287–301.  
940
- 941 Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Fromentin, J.M.,  
942 Hoegh-Guldberg, O. and Bairlein, F. (2002) Ecological responses to recent climate change.  
943 *Nature*, *416*(6879), 389.  
944
- 945 Weising, K., Nybom, H., Wolff, K., & Meyer, W. (1995) DNA isolation and purification. In:  
946 DNA fingerprinting in plants and fungi, Boca Raton: Florida CRC Press. pp. 44–59
- 947 Wetzel, W. C., Kharouba, H. M., Robinson, M., Holyoak, M., & Karban, R. (2016) Variability in  
948 plant nutrients reduces insect herbivore performance. *Nature*, *539* (7629), 425.  
949
- 950 Wilkening, J. L., Ray, C., Beaver, E. A., & Brussard, P. F. (2011) Modeling contemporary range  
951 retraction in Great Basin pikas (*Ochotona princeps*) using data on microclimate and  
952 microhabitat. *Quaternary International*, *235*(1–2), 77–88.  
953
- 954 Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., & Dormann,  
955 C.F., Forchhammer, M.C., Grytnes, J.A., Guisan, A. & Heikkinen, R. K. (2013) The role of biotic  
956 interactions in shaping distributions and realised assemblages of species: implications for  
957 species distribution modelling. *Biological reviews*, *88*(1), 15–30.  
958
- 959 Yoccoz, N. G., Bråthen, K. A., Gielly, L., Haile, J., Edwards, M. E., Goslar, T., Von Stedingk, H.,  
960 Brysting, A.K., Coissac, E., Pompanon, F. & Sørnstebo, J. H. (2012) DNA from soil mirrors plant  
961 taxonomic and growth form diversity. *Molecular Ecology*, *21*(15), 3647–3655.  
962
- 963 Zimmerman, G. M., Goetz, H., & Mielke, P. W. (1985) Use of an improved statistical method  
964 for group comparisons to study effects of prairie fire. *Ecology*, *66*(2), 606–611.  
965

966 Zarzoso-Lacoste, D., Corse, E. and Vidal, E. (2013). Improving PCR detection of prey in  
967 molecular diet studies: importance of group-specific primer set selection and extraction  
968 protocol performances. *Molecular Ecology Resources*, 13(1), 117-127.

969

970

971

972

973 **Data Accessibility Statement**

974

975 GPS locations of all sample collection points, R code and data for generalized linear modeling,

976 DNA sequences generated during the study can be accessed in the Dryad database (

977 doi:10.5061/dryad.nt40m53).

978

979

980

981 **Authors' contribution**

982 SB collected the samples and performed the laboratory work; DAD and FI supervised the  
983 project; SB, HH and FI analysed the data; SB, DAD, HH and FI wrote the manuscript. All authors  
984 approved the final version of the manuscript.

985

986

987

988

989

990

991

992

993

994

995

996

997

998

999

1000 **Table 1:** Presence of plant families in the Royle's pika's diet across each sampled site (TUN:  
 1001 Tungnath; MAD: Madmaheswar; NAN: Bedni- Roopkund; RUD: Rudranath; HAK: Har ki  
 1002 Doon), with results from G-test (G statistics and p values), to estimate differences in the  
 1003 mean proportion of plant families within diet. Proportion of plant family is presented for  
 1004 each site; those highlighted in bold differ across sites at  $P < 0.05$ . \* Plant family not  
 1005 considered for comparison across sites as they were found only in one study site.  
 1006

Family	Total genus	Pre-monsoon			Post-monsoon			G	P
		TUN (n=27)	MAD (n=13)	NAN (n=32)	TUN (n=26)	RUD (n=4)	HAK (n=8)		
Apiaceae	5	3.70	0.00	6.25	11.54	25.00	0.00	1.74	0.41
Asteraceae	7	55.50	23.07	15.60	61.50	100.00	12.50	76.41	<b>&lt;0.0001</b>
Balsaminaceae*	1	0.00	7.69	0.00	0.00	0.00	0.00	*	
Berberidaceae*	1	0.00	0.00	6.25	0.00	25.00	0.00	*	
Betulaceae*	1	3.70	0.00	0.00	0.00	0.00	0.00	*	
Boraginaceae*	2	0.00	7.69	0.00	0.00	0.00	0.00	*	
Brassicaceae*	2	0.00	0.00	3.13	0.00	0.00	0.00	*	
Campanulaceae*	1	0.00	7.69	0.00	3.85	0.00	0.00	*	
Caprifoliaceae	2	11.11	0.00	3.13	7.69	25.00	0.00	2.69	0.26
Caryophyllaceae	4	14.81	15.38	15.63	18.52	15.38	0.00	4.54	0.47
Crassulaceae*	2	0.00	7.69	0.00	0.00	0.00	0.00	*	
Cyperaceae	1	11.11	23.08	9.38	14.81	50.00	0.00	6.9	0.22
Ericaceae	4	18.52	7.69	0.00	26.92	25.00	0.00	2.94	0.7
Fagaceae*	1	3.70	0.00	0.00	0.00	0.00	0.00	*	
Gentianaceae*	2	3.70	0.00	0.00	0.00	0.00	0.00	*	
Geraniaceae*	1	3.70	0.00	0.00	3.85	0.00	0.00	*	
Hypericaceae*	1	0.00	0.00	0.00	50.00	0.00	0.00	*	
Lamiaceae*	1	0.00	0.00	0.00	3.85	0.00	0.00	*	
Onagraceae	3	18.52	23.08	6.25	46.15	0.00	0.00	3.14	0.2
Papilionaceae*	2	11.11	0.00	0.00	0.00	0.00	0.00	*	
Poaceae	10	96.26	100.00	25.00	100.00	100.00	25.00	179.23	<b>&lt;0.0001</b>
Polygonaceae	4	0.00	30.77	6.25	3.85	0.00	0.00	2.32	0.31
Primulaceae	2	51.85	98.27	34.38	22.22	7.69	3.13	13.93	<b>&lt;0.001</b>
Ranunculaceae	4	59.26	92.31	9.38	30.77	25.00	12.50	41.89	<b>&lt;0.0001</b>
Rosaceae	6	96.30	92.31	56.25	92.31	100.00	25.00	210.07	<b>&lt;0.0001</b>
Rubiaceae	1	3.70	0.00	6.25	0.00	0.00	0.00	1.4	0.49
Salicaceae*	1	0.00	0.00	0.00	3.85	0.00	0.00	*	
Saxifragaceae	2	7.41	7.69	3.13	11.54	0.00	0.00	1.78	0.41
Scrophulariaceae	2	25.93	30.77	9.38	23.08	50.00	0.00	18.7	<b>&lt;0.002</b>
Orobanchaceae	1	18.52	15.38	3.13	3.85	0.00	0.00	4.2	0.11
Urticaceae*	1	0.00	0.00	3.13	3.85	0.00	0.00	*	
Violaceae*	1	0.00	0.00	3.13	3.00	0.00	0.00	*	

1007

1008

1009  
1010  
1011  
1012  
1013  
1014

**Table 2:** Model-averaged predictive models for effect of each parameter on plant diet richness with AIC < 2 and relative importance (RI) of each parameter.

Parameter	Estimate ( $\beta$ ) $\pm$ SE	Confidence Interval		Z value	P value	RI
		Lower (2.5%)	Upper (97.5%)			
Intercept	1.65 $\pm$ 0.04	1.56	1.73	37.87	<b>0.001</b>	
Talus area cover	0.17 $\pm$ 0.04	0.07	0.27	3.50	<b>0.001</b>	1.00
Depth of crevices	-0.13 $\pm$ 0.04	-0.22	-0.03	2.78	<b>0.01</b>	1.00
Talus area cover*						
Depth of crevices	-0.009 $\pm$ 0.03	-0.03	0.16	0.27	0.78	0.29

1015  
1016  
1017  
1018  
1019  
1020  
1021  
1022  
1023  
1024  
1025  
1026  
1027  
1028  
1029  
1030  
1031  
1032  
1033  
1034  
1035  
1036  
1037  
1038  
1039  
1040  
1041  
1042  
1043

1044

1045

**List of Figures**

1046

1047

1048

**Figure 1** Faecal pellet sampling sites in Garhwal, Uttarakhand, India

1049

1050

Code	site name	Altitude (above sea level a.s.l)	Long / Lat
1051	TUN Chopta-Tungnath	2,600–3,680 m,	30°28' to 31°32' N, 79°13' to 79°14' E
1052	RUD Rudranath	2,900–3,500 m,	30°28' to 31°31' N, 78°18' to 79°19' E
1053	MAD Madmaheshwar	3,000–3,300 m,	30°37'to 31°38' N, 78°12' to 79°13' E,
1054	HAR Har ki Doon	2,700–3,800 m,	31°06' to 31°09' N, 78°19' to 78°26' E,
1055	NAN Bedni-Roopkund	2,700–4,450 m,	30°11' to 30°15' N, 79°31' to 79°44' E.

1051

1052

1053

1054

1055

1056

1057

1058

**Figure 2** Plant diet richness across an elevation gradient in pre-monsoon (Premon) and post-

1059

monsoon season (Postmon) (HAK= Har ki doon; MAD= Madmaheshwar; TUN=Tungnath;

1060

RUD=Rudranath; NAN=Bedni-roopkund; full details of the study sites are provided in Figure

1061

1)

1062

1063

1064

**Figure 3** Evolutionary origin of plants per pellet sampled in pre-monsoon (A) and post-

1065

monsoon (B) diet of Royle's pika in the western Himalaya (CA: Centrasiatic; EH: Himalayan

1066

Endemic; HO: Holarctic; MA: South East Asiatic Malaysian; SJ: Sino Japanese or Eastern

1067

Asiatic; TR: Tropical; YU: South East Chinese; details of the study sites are provided in Figure

1068

1)

1069

1070

1071

**Figure 4** Upper distribution limit of plants detected per sample in pre-monsoon (A) and post-

1072

monsoon (B) diet of Royle's pika across elevation (Low=2,500–3,500 m; Medium=3,500–

1073

4,500 m; High=4,500–6,000 m; details of the study sites are provided in Figure 1)

1074

1075

1076

**Figure 5** Lower distribution limit of plants detected in pre-monsoon (A) and post-monsoon

1077

(B) diet of Royle's pika across elevation (Low=500–1,000 m; Medium=1,000–2,000 m; High

1078

=&gt; 2,000 m; details of the study sites are provided in Figure 1)

1079

1080

1081

1082

1083

1084

1085

1086

1087

1088



1089 **Figure 1** Faecal pellet sampling sites in Garhwal region of Uttarakhand, India

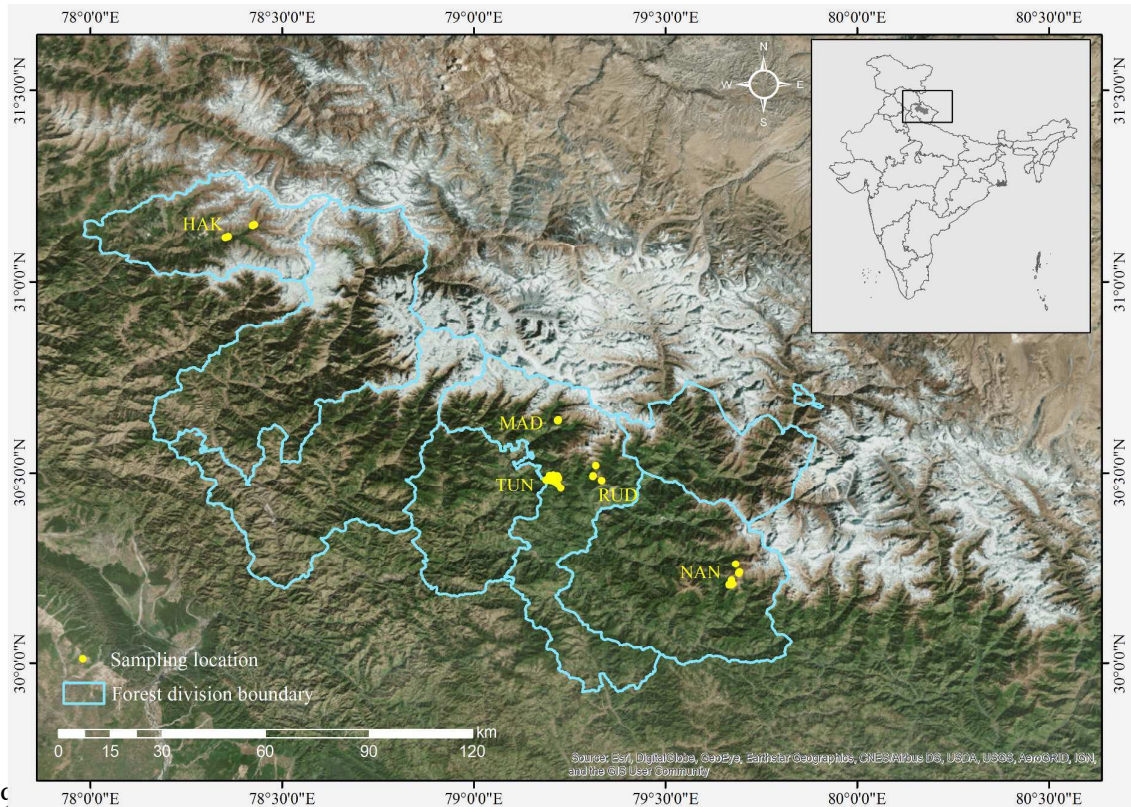
1090

1091

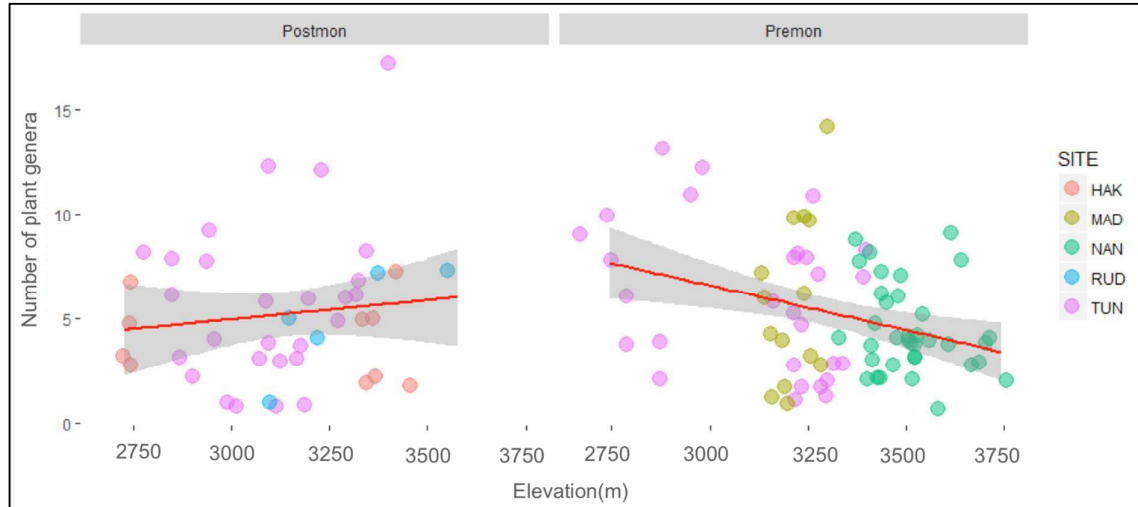
Code	site name	Altitude (above sea level a.s.l)	Long / Lat
1092	TUN Chopta-Tungnath	2,600–3,680 m,	30°28' to 31°32' N, 79°13' to 79°14' E
1093	RUD Rudranath	2,900–3,500 m,	30°28' to 31°31' N, 78°18' to 79°19' E
1094	MAD Madmaheshwar	3,000–3,300 m,	30°37' to 31°38' N, 78°12' to 79°13' E,
1095	HAR Har ki Doon	2,700–3,800 m,	31°06' to 31°09' N, 78°19' to 78°26' E,
1096	NAN Bedni-Roopkund	2,700–4,450 m,	30°11' to 30°15' N, 79°31' to 79°44' E.

1097

1098



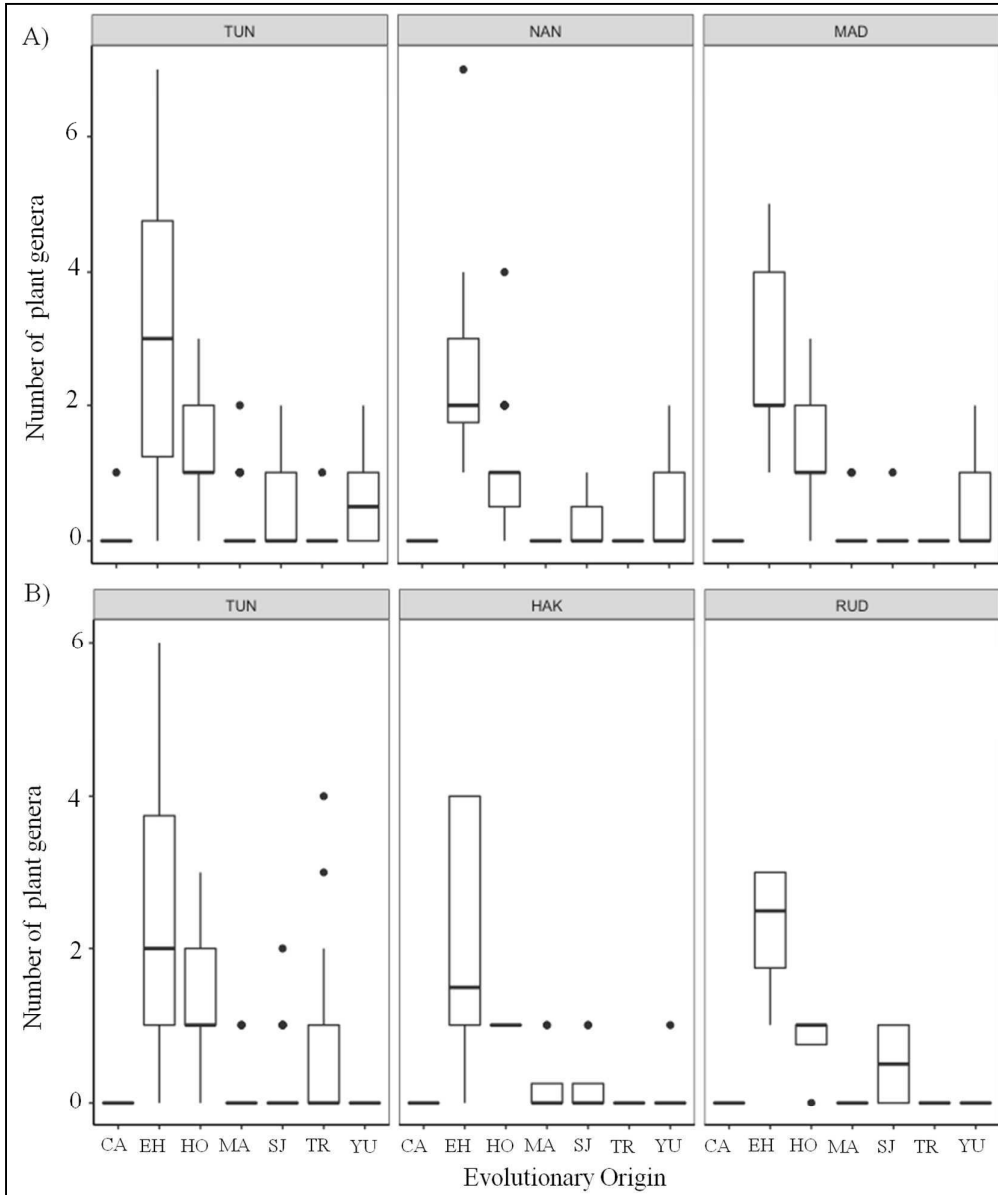
1110 **Figure 2** Plant diet richness across an elevation gradient in pre-monsoon (Premon) and post-  
1111 monsoon season (Postmon) (HAK= Har ki doon; MAD= Madmaheshwar; TUN=Tungnath;  
1112 RUD=Rudranath; NAN=Bedni-roopkund; full details of the study sites are provided in Figure  
1113 1)  
1114  
1115  
1116  
1117



1118  
1119  
1120  
1121  
1122  
1123  
1124  
1125  
1126  
1127  
1128  
1129  
1130  
1131  
1132  
1133  
1134  
1135

1136

1137 **Figure 3** Evolutionary origin of plants per pellet sampled in pre-monsoon (A) and post-  
 1138 monsoon (B) diet of Royle's pika in the western Himalaya (CA: Centrasian; EH: Himalayan  
 1139 Endemic; HO: Holarctic; MA: South East Asiatic Malaysian; SJ: Sino Japanese or Eastern Asiatic;  
 1140 TR: Tropical; YU: South East Chinese; details of the study sites are provided in Figure 1)  
 1141



1142

1143

1144

1145

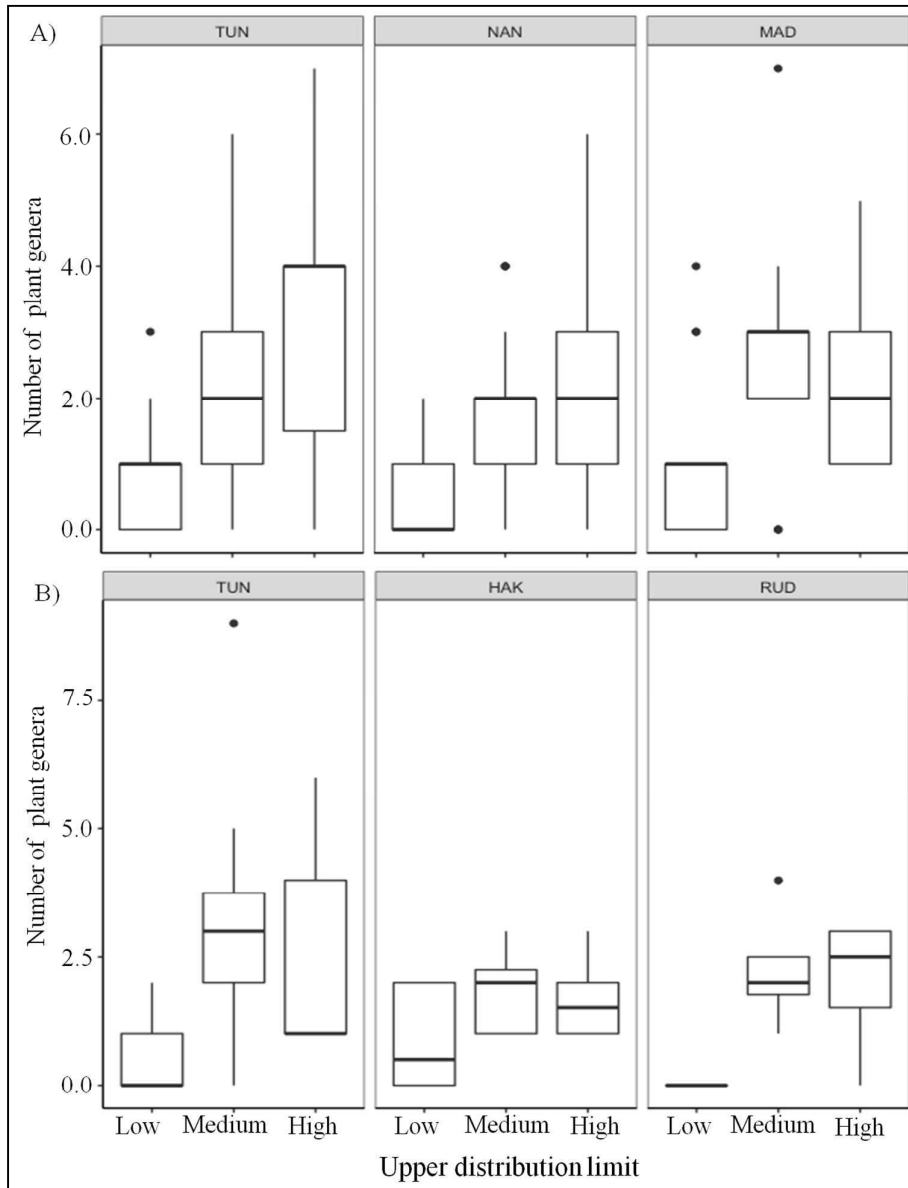
1146

1147

1148 **Figure 4** Upper distribution limit of plants detected per sample in pre-monsoon (A) and post  
 1149 monsoon (B) diet of Royle's pika across elevation (Low=2,500–3,500 m; Medium= 3,500–  
 1150 4,500 m; High =4,500–6,000 m; details of the study sites are provided in Figure 1)

1151

1152



1153

1154

1155

1156

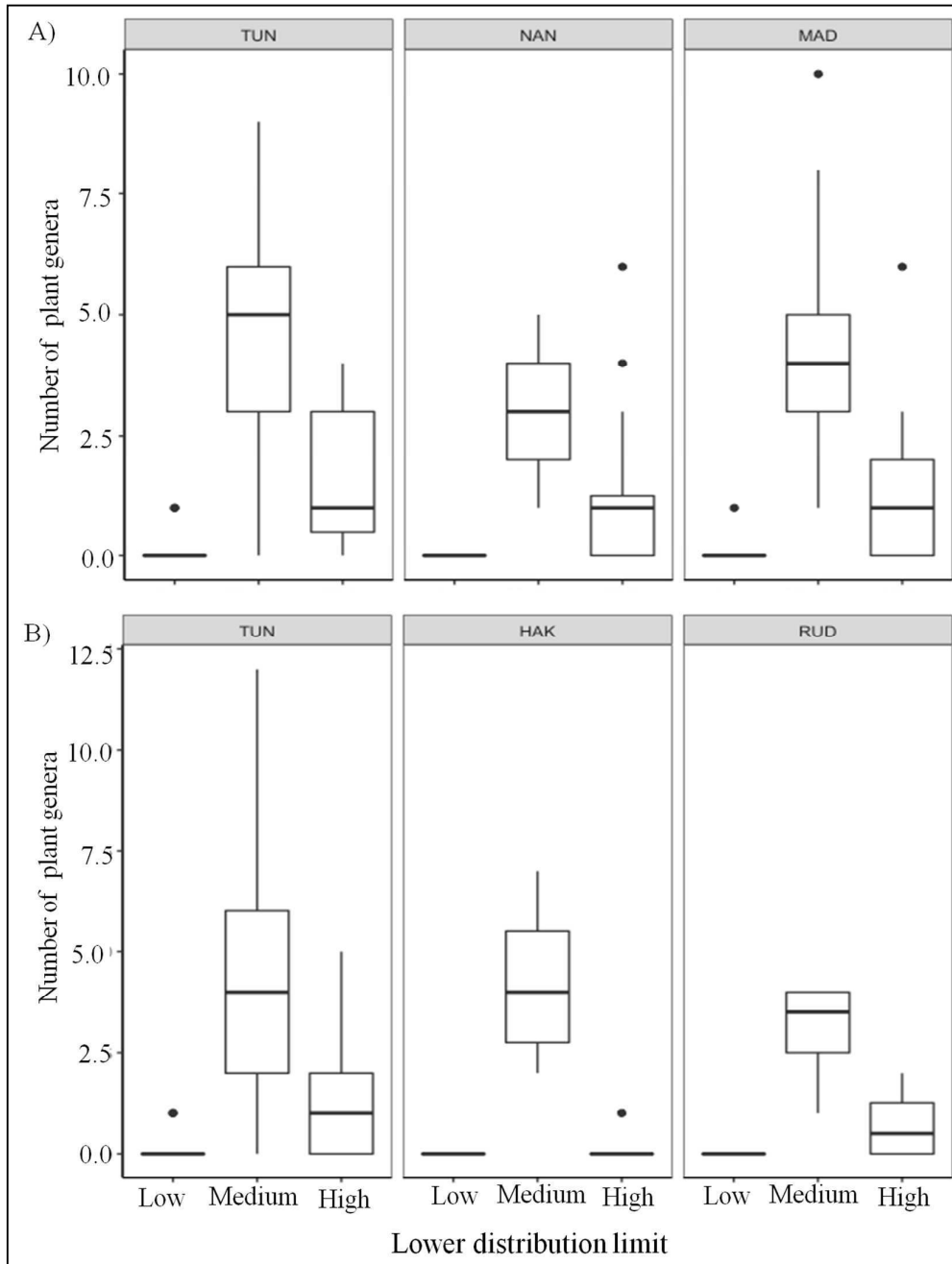
1157

1158

1159

1160

1161 **Figure 5** Lower distribution limit of plants detected in pre-monsoon (A) and post monsoon (B)  
 1162 diet of Royle's pika across elevation (Low= 500–1000 m; Medium=1,000–2,000 m; High =>  
 1163 2,000 m; details of the study sites are provided in Figure 1)  
 1164



1165  
 1166  
 1167  
 1168  
 1169