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1 **Stable isotope ratios in alpine rock ptarmigan and black grouse sampled along a precipitation**
2 **gradient**

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22 **Abstract**

23 Rock ptarmigan (*Lagopus muta*) and black grouse (*Tetrao tetrix*) are two closely related alpine bird
24 species that form relict populations in the European Alps. Besides manifold anthropogenic influences
25 in this region, global climate change is forecast to lead to significant changes in temperatures and
26 precipitation. We here analysed stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of feathers of both bird species
27 and their potential dietary plants across a longitudinal precipitation gradient in south-east
28 Switzerland.

29 Plant $\delta^{13}\text{C}$ was higher at higher altitudes and drier areas (coinciding with higher longitudes)
30 while plant $\delta^{15}\text{N}$ did not differ geographically. Black grouse $\delta^{13}\text{C}$ reflected the longitudinal pattern in
31 precipitation and plant $\delta^{13}\text{C}$, and there was no indication for a change in dietary composition with
32 precipitation (i.e. no significant changes in $\delta^{15}\text{N}$). In contrast, rock ptarmigan $\delta^{13}\text{C}$ were independent
33 of precipitation and plant $\delta^{13}\text{C}$ values and showed a significant increase in $\delta^{15}\text{N}$ towards drier areas,
34 suggesting a potential dietary shift.

35 In rock ptarmigan, we furthermore investigated intraspecific differences with age, between
36 males and females and among years, and did not find any biologically meaningful intraspecific
37 differences. Interspecifically, rock ptarmigan feathers had significantly higher $\delta^{13}\text{C}$ and lower $\delta^{15}\text{N}$
38 values than black grouse, reflecting a dietary segregation between both species. This may partly be
39 due to the higher altitudinal distribution of rock ptarmigan in combination with an altitudinal
40 gradient in plant $\delta^{13}\text{C}$. In addition, however, species also segregated in $\delta^{15}\text{N}$, most likely caused by a
41 higher proportion of invertebrate diet in black grouse.

42

43 **Keywords:** European Alps; global climate change; plant isotope; precipitation; stable isotope analysis

44

45 **Introduction**

46 As typical for mountainous areas, the European Alps show a high diversity of different climate zones
47 on a relatively small scale, mainly driven by the continuous temperature decrease with elevation
48 (Frey & Lössch 2004). In addition, the European Alps form a barrier against weather fronts, which
49 leads to higher precipitation at the edges and drier conditions in the central areas (Frey et al. 2004).
50 As temperature and moisture are important determinants of the ecological niche of plants and
51 animals (Begon, Townsend & Harper 2006), this diversity in Alpine climate habitats is also reflected
52 in the diversity of habitats and species. This richness in Alpine wildlife is, however, severely affected
53 by anthropogenic influences, such as the use of pastures and changes in grazing regimes
54 (Meusburger & Alewell 2008; Paschetta, La Morgia, Masante, Negro, Rolando et al. 2013; Patthey,
55 Signorell, Rotelli & Arlettaz 2012), installation of hydroelectric power plants (Fette, Weber, Peter &
56 Wehrli 2007; Truffer, Markard, Bratrach & Wehrli 2001), or recreational snow sport activities
57 (Braunisch, Patthey & Arlettaz 2010; Negro, Isaia, Palestrini, Schoenhofer & Rolando 2010). In
58 addition, the European Alps are one of the areas with the strongest observed warming trend
59 worldwide, regionally showing increases of 1–2°C of average annual air temperature during the 20th
60 century (Begert, Schlegel & Kirchhofer 2005; Parolo & Rossi 2008). Along with further temperature
61 increases, climate models for the European Alps predict changes in precipitation patterns, with
62 generally drier summers and wetter winters (including rain), and an increase of extreme weather
63 events including extreme rainfalls but also temporal droughts (Gobiet, Kotlarski, Beniston, Heinrich,
64 Rajczak et al. 2014; Zimmermann, Gebetsroither, Züger, Schmatz & Psomas 2013). These
65 climatological effects will lead to an elevation of the tree line, which is partly already visible
66 (Dullinger, Dirnböck & Grabherr 2004). In addition to land use changes we will likely observe a
67 reduction in size of high-alpine meadow habitats that goes along with an overall biodiversity loss
68 (Dirnböck, Dullinger & Grabherr 2003; Engler, Randin, Thuiller, Dullinger, Zimmermann et al. 2011).
69 Many of the now threatened high-altitude species are relict species that have survived in
70 mountainous areas since the last glacial period, boosting local biodiversity (Dirnböck, Essl & Rabitsch

71 2011; Ohlemüller, Anderson, Araújo, Butchart, Kudrna et al. 2008). Two prominent and closely
72 related species that form relict populations in the European Alps are rock ptarmigan (*Lagopus muta*)
73 and black grouse (*Lyrurus tetrix*). Rock ptarmigan are found in rocky areas above the tree line (from
74 about 1800 m and up to more than 3000 m) (Glutz von Blotzheim, Bauer & Bezzel 1973; Pernellet,
75 Korner-Nievergelt & Jenni 2015). In contrast, black grouse show a boreal distribution, with the main
76 alpine habitat being the upper forest edge, i.e. the area of the tree line (Glutz von Blotzheim et al.
77 1973). Populations of both species in the European Alps were considered as stable until the mid-
78 1990s (Peronace, Cecere, Gustin & Rondinini 2012; Schmid, Luder, Naef-Daenzer, Graf & Zbinden
79 1998). However, in the following decade rock ptarmigan declined by about 30% in both Switzerland
80 (Keller, Gerber, Schmid, Volet & Zbinden 2010) and Italy (Peronace et al. 2012). In the same period,
81 black grouse numbers declined by up to 20% in Italy (Peronace et al. 2012). Whether these declining
82 trends that also persist on a global scale (Storch 2007) are already caused by the effects of climate
83 change, or potentially other anthropogenic influences, is unknown. However, with ongoing climate
84 change effects, both rock ptarmigan and black grouse will have to track the shift of the tree line to
85 higher elevations, a trend that is already visible in rock ptarmigan (Pernellet et al. 2015). Especially
86 under warming scenarios exceeding 2°C, both species will furthermore suffer from a loss in suitable
87 habitat (Revermann, Schmid, Zbinden, Spaar & Schroder 2012; Zurell, Grimm, Rossmannith, Zbinden,
88 Zimmermann et al. 2012).

89 Both rock ptarmigan and black grouse feed predominantly on plants, especially on leaves and
90 buds of heather (Ericacea), including bilberry (*Vaccinium myrtillus*), mountain cranberry (*Vaccinium*
91 *vitis-idaea*) and black crowberry (*Empetrum nigrum*) as well as dwarf willows (*Salix herbacea*, *Salix*
92 *retusa*) and – especially young chicks – also on insects (Beeston, Baines & Richardson 2005;
93 Bertermann, Weber-Sparenberg, Pechura, Renard & Bergmann 1998; Glutz von Blotzheim et al.
94 1973; Lieser, Zakrzewski & Sittler 1997; Starling-Westerberg 2001). Habitat suitability models for the
95 presence of both species in the European Alps generally reflect the importance of patchy and
96 heterogeneous habitat structures for food and shelter (Favaron, Scherini, Preatoni, Tosi & Wauters

97 2006; Patthey et al. 2012; Zohmann & Wöss 2008). Schweiger et al. (2012) furthermore highlighted
98 the importance of dwarf shrubs for both species, and ant hills (reflecting an insect food source) for
99 black grouse. While current diet and habitat characteristics appear to be well known, it remains
100 open how the predicted changes in temperature, precipitation, reduced (and higher elevated)
101 suitable habitat and changed plant composition will affect diet and in the long term population
102 trajectories.

103 As a first step we here aim to infer how precipitation patterns may affect summer diet, by
104 comparing dietary changes along a distinct gradient in precipitation within the Swiss canton of
105 Grisons (Frei & Schär 1998; see Fig. 1). Stable isotopes, particularly the combination of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
106 isotope ratios provide a comprehensive picture of dietary relationships: $\delta^{15}\text{N}$ increases with each
107 trophic level and can therefore be used to assess the trophic position in the food web (reviewed in
108 Caut, Angulo & Courchamp 2009). In contrast, $\delta^{13}\text{C}$ varies with bed-rock and between C3 and C4-
109 plants (Fry 2006). Furthermore, $\delta^{13}\text{C}$ ratios in plants decrease with rainfall (Ferrio & Voltas 2005;
110 Stewart, Turnbull, Schmidt & Erskine 1995) and increase with elevation (Körner, Farquhar &
111 Roksandic 1988; Van de Water, Leavitt & Betancourt 2002). Feathers are a frequently analysed
112 tissue and reflect the diet during, or shortly prior to moult, as they remain metabolically inert after
113 their formation and even after death (Bearhop, Waldron, Votier & Furness 2002). Black grouse
114 moult their entire plumage, and rock ptarmigan their primary and secondary wing feathers during
115 the summer months, towards the end of the chick rearing period (Glutz von Blotzheim et al. 1973).
116 The feather samples analysed in this study therefore reflect the diet during summer.

117 By analysing precipitation patterns, plant and feather isotopes, we thus aimed to (1)
118 investigate potential differences in isotopic composition and consequently diet related to regional
119 precipitation patterns. Based on the literature, we expected plant $\delta^{13}\text{C}$ and consequently feather
120 $\delta^{13}\text{C}$ to increase along the precipitation gradient with longitude, but expected no changes in plant
121 and feather $\delta^{13}\text{C}$ with latitude. We furthermore expected generally higher $\delta^{13}\text{C}$ values in plant

122 samples at higher altitudes and consequently in feathers of rock ptarmigan compared to black
123 grouse.

124 Plant $\delta^{15}\text{N}$ values should not be affected by precipitation, and we therefore expected no
125 gradient in plant $\delta^{15}\text{N}$ with longitude or altitude. We had no specific expectations as to how
126 precipitation would affect dietary composition of rock ptarmigan and black grouse. However, if
127 precipitation would affect the food choice of either of the species, we expected to see a change in
128 $\delta^{15}\text{N}$ with longitude.

129 We further investigated (2) intra-specific dietary differences between adult and immature
130 birds and between adult males and females in rock ptarmigan. As juveniles have been described to
131 feed on a more insect-rich diet, we expected to find higher $\delta^{15}\text{N}$ values in feathers of immatures (i.e.
132 the first set of primaries and secondaries that is built when still guarded by the hens) compared to
133 adults (moulted in summer at the same time as chick rearing). Finally (3), we tested whether rock
134 ptarmigan and black grouse differ in their diet. Due to the differences in altitudinal distribution
135 during summer, we expected to find higher $\delta^{13}\text{C}$ values in rock ptarmigan than black grouse.
136 Assuming a similar diet in both species, we however expected similar $\delta^{15}\text{N}$ values in both species.

137

138

139 **Methods**

140 *Collection of feathers*

141 Feathers of black grouse and rock ptarmigan were obtained from several hunting districts within
142 Grisons (Fig. 1), during the hunting seasons between mid-October to end of November. Hunters
143 were obliged to deliver feather samples from shot black grouse (secondary wing feathers or body
144 coverts; in 2005–2007), and rock ptarmigan (secondary wing feathers; from 2008–2012) to the
145 Department of Wildlife and Fishery Service Grison. Feathers were stored in plastic bags along with
146 information about age and sex (identified from plumage characteristics). For black grouse, the
147 detailed coordinates of the shot locations were also registered, whereas for rock ptarmigan only the

148 municipality was noted. For subsequent analyses regarding locations, we therefore used the
149 principle town/village within the municipality for rock ptarmigan, but the detailed location for black
150 grouse.

151 Feathers from black grouse were solely from male birds, either adult or immature. Feathers
152 from rock ptarmigan were from both sexes and included both adults and immatures. In few cases (N
153 = 11 out of 190 feather samples in total), sex or age was not noted, and sample sizes among analyses
154 therefore vary slightly. In order to understand potential intra-specific variation in stable isotope
155 ratios across years, between sexes, and age (adults and immatures), we chose the municipality with
156 the highest number of shot animals (Pontresina; see location in Fig. 1) and analysed a total of 78
157 samples from rock ptarmigan, equally covering both sexes and age groups from the years 2008–
158 2012. To analyse the geographic effects and species differences, black grouse feathers from 2005 (N
159 = 53 samples) and rock ptarmigan feathers from 2009 (N = 59 samples) from across Grisons (N = 19
160 and 21 municipalities for black grouse and rock ptarmigan, respectively) were analysed (Fig. 1).

161

162 *Collection of plant samples*

163 Plants were sampled between 10th of July and 23rd of August 2013. Sampling locations were
164 distributed across Grisons matching the overall hunting locations of the birds (Fig. 1). Per location
165 (e.g. Vilan), we collected plant samples at one to two different plots, if possible at different
166 elevations (e.g. one at 1900 m, and one at 2300 m; see Table A1 for details about sampled plant
167 species per location). At each plot, we collected 3–5 samples per plant species, and ideally from at
168 least two species (depending on availability at the plot), generally one from the family Ericacea
169 (mostly bilberry, *Vaccinium myrtillus*) and the second either from Salicacea (*Salix reticulata*) or
170 Roseacea (*Geum montanum* or *Dryas octopetala*). Each sample consisted of one branch with several

171 leaves, and samples were collected from separate specimens that grew at least 3 m apart from each
172 other.

173 In order to estimate the isotopic variation among plant species also within the plant family
174 Ericaceae, we covered two locations (Ofenpass and Lukmanierpass; see Fig. 1) more closely and
175 analysed samples from 6 and 7 species, respectively.

176

177 *Stable isotope analyses*

178 Plant samples were dried in a drying oven at 50°C for at least 48 hours. We ground two leaves per
179 individual plant and mixed them thoroughly. Sub-samples (ca. 0.5 mg) were weighed into 0.3 x 0.5
180 mm tin capsules to the nearest 0.001 mg, using a micro-analytical balance.

181 For feather samples, we used the distal end of feathers. Only feathers free of visible
182 contamination (dirt or blood) were used. Feathers were rinsed with 75% ethanol and subsequently
183 with distilled water, and then dried in a drying oven at 50°C for at least 24 hours. Sub-samples of
184 0.8–0.9 mg were weighed into tin capsules.

185 Samples were combusted in a Pyrocube elemental analyser. The resulting CO₂ and N₂ were
186 separated by gas chromatography and admitted into the inlet of a Micromass (Manchester, UK)
187 Isoprime isotope ratio mass spectrometer (IRMS) for determination of ¹³C/¹²C and ¹⁵N/¹⁴N ratios.
188 Measurements are reported in δ-notation (δ¹³C and δ¹⁵N, respectively) relative to the Pee Dee
189 Belemnite (PDB) for carbon and atmospheric N₂ for nitrogen in parts per thousand deviations (‰)
190 using the formula

$$191 \delta (\text{‰}) = 1000 \times [R_{\text{sample}} / R_{\text{standard}} - 1]$$

192 Two sulfanilamides (iso-prime internal standards) and two Casein standards were used for
193 every seven unknowns in sequence. Internal laboratory standards indicated measurement errors
194 (SD) of $\pm 0.05\text{‰}$ for $\delta^{13}\text{C}$, 0.15‰ for $\delta^{15}\text{N}$.

195

196 *Weather data*

197 We obtained precipitation data from Grisons (108 weather stations; from the Swiss Federal Office of
198 Meteorology and Climatology, MeteoSwiss) as well as from 66 weather stations in adjacent areas
199 within Switzerland, Austria (from Zentralanstalt für Meteorologie und Geodynamik, ZAMG) and Italy
200 (from Agenzia Regionale per la Protezione dell' Ambiente della Lombardia, ARPA Lombardia). We
201 calculated the sum of precipitation of the months April through to August 2013, i.e. the growth
202 period of the year in which we collected plants for isotope analyses. We used these data to analyse
203 geographical differences in precipitation within Grisons (see Statistics, first step).

204

205 *Statistical analyses*

206 All statistics were performed in R (version 3.1.1; R Core Team 2014). We conducted linear models
207 (LM) and linear mixed effect models (LMM) using the packages lme4 (Bates, Maechler & Bolker
208 2011) and lmerTest (Kuznetsova, Brockhoff & Christensen 2014). We performed backwards-stepwise
209 model selection, subsequently removing non-significant variables from the models. P-values were
210 obtained from likelihood-ratio tests between models with and without the focal variable.

211

212 In the first step, we analysed potential differences in isotopic composition and consequently dietary
213 changes related to the regional precipitation pattern. We therefore (I) analysed the precipitation
214 pattern for the year 2013 in Grisons, running a LM with the sum of precipitation during the growth

215 period (April–August) as dependent variable. As explanatory variables, we included latitude and
216 longitude of the weather station as well as the two-way interaction term (latitude*longitude).

217 We then (II) took plant samples from all locations into account, and studied the effects of
218 latitude, longitude and elevation on plant isotopes. We ran LMMs separately for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ as
219 dependent variables. As elevation was significantly correlated with latitude and longitude (Pearson's
220 $R = -0.16$ and 0.43 , $P = 0.026$ and < 0.001 , respectively), we conducted models separately for
221 geographic location and elevation to avoid collinearity. The first set of models therefore contained
222 latitude, longitude and the interaction term (latitude*longitude) as explanatory covariates and plant
223 species as random effect. The second set of models contained only elevation as explanatory variable
224 and plant species as random effect.

225 For the two more intensively sampled locations of Lukmanierpass and Ofenpass, we also
226 tested whether isotope values differed among plant species. We therefore conducted LMMs with
227 plant species as fixed effect and the study site as random effect.

228 Finally (III), we investigated variation of rock ptarmigan (all data from 2009) and black grouse
229 (all data from 2005) isotope data with latitude and longitude. Again, we ran LMMs separately for
230 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. The starting global model contained species (as fixed factor) and both latitude and
231 longitude (each as covariates) and all possible interaction terms up to the three-way interaction
232 between species, latitude and longitude) as explanatory variables, as well as sex and age as
233 independent random variables.

234

235 In addition to these regional effects, we analysed in a second step potential intra-specific differences
236 in both rock ptarmigan and black grouse. For rock ptarmigan, we used a separate dataset which
237 contained data from the years 2008–2012 shot within the range of the municipality of Pontresina.
238 We used this dataset to investigate sex, age and year differences. LMs therefore contained sex, age

239 and year (each as fixed factor) and all possible two- and three-way interactions. For black grouse, we
240 exclusively used the data from 2005 from across Grisons.

241

242 Finally, in the third and last step, we analysed potential species differences between rock ptarmigan
243 and black grouse, using a LM with species as the only explanatory factor, applied to the dataset of
244 feather isotope data sampled across Grisons in the years 2005 (adult black grouse) and 2009 (adult
245 rock ptarmigan).

246

247 **Results**

248 *Geographical effects along a precipitation gradient*

249 Within Grisons, precipitation during the growth season 2013 (April to August) showed a significant
250 interaction between latitude and longitude (LMM: $F_1 = 12.12$, $P < 0.001$). Tested separately,
251 precipitation decreased with latitude (LMM: $F_1 = 9.90$, $P = 0.002$) and longitude (LMM: $F_1 = 90.04$, $P <$
252 0.001), thus the southern and western parts of the canton received more precipitation than the
253 northern and eastern parts, coinciding with the annual pattern of precipitation (Fig. 1).

254 Within the same geographical range, plant $\delta^{13}\text{C}$ increased significantly with elevation (LMM:
255 $F_1 = 28.65$, $P < 0.001$) (Fig. 2) and with longitude, i.e. from west to east (LMM: $F_1 = 23.98$, $P < 0.001$),
256 while there was no significant effect of latitude (LMM: $F_1 = 3.19$, $P = 0.090$) (Fig. 3). Plant $\delta^{15}\text{N}$ values
257 were not significantly affected by elevation (LMM: $F_1 = 0.07$, $P = 0.800$) (Fig. 2), latitude (LMM: $F_1 =$
258 2.09 , $P = 0.090$) or longitude (LMM: $F_1 = 0.38$, $P = 0.536$) (Fig. 4).

259 Focussing on the variation among plant species, we found a significant species effect on both
260 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (LMM: $F_{10} = 11.76$, $P < 0.001$ and $F_{10} = 2.36$, $P = 0.006$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively)
261 at the two intensively sampled locations of Lukmanierpass and Ofenpass (Table A1). Species
262 differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were also present within the family Ericaceae (LMM: $F_7 = 13.04$ and 2.07 ,

263 P < 0.001 and 0.028, respectively), and differences for $\delta^{13}\text{C}$ remained significant even within the
264 same genus: Mountain cranberry *V. vitis-idaea* and bilberry *V. myrtillus* sampled at the same study
265 plot (i.e. same geographic location and elevation) differed significantly in their $\delta^{13}\text{C}$ values (LMM: F_1
266 = 20.80, P = 0.002), but not in their $\delta^{15}\text{N}$ values (LMM: F_1 = 0.99, P = 0.348).

267 Analysing rock ptarmigan and black grouse data from across Grisons, we obtained a
268 significant three-way interaction term between species, latitude and longitude for both $\delta^{13}\text{C}$ (LMM:
269 F_1 = 3.77, P = 0.024) and $\delta^{15}\text{N}$ values (LMM: F_1 = 4.35, P = 0.018). We therefore continued with
270 separate analyses for both species.

271 In rock ptarmigan, feather $\delta^{13}\text{C}$ was independent of latitude (LMM: F_1 = 0.52, P = 0.444) and
272 longitude (LMM: F_1 = 0.122, P = 0.717; Fig. 3). Feather $\delta^{15}\text{N}$ was independent of latitude (LMM: F_1 =
273 0.08, P = 0.906) but increased with longitude (LMM: F_1 = 6.11, P = 0.013; Fig. 4).

274 In black grouse, the interaction term of latitude and longitude had a significant effect on
275 feather $\delta^{13}\text{C}$ (LMM: F_1 = 7.69, P = 0.005). Analysed in separate models, $\delta^{13}\text{C}$ increased with longitude
276 (LMM: F_1 = 6.99, P = 0.009) but was not affected by latitude (LMM: F_1 = 0.65, P = 0.412; Fig. 3).
277 Feather $\delta^{15}\text{N}$ was independent of latitude (LMM: F_1 = 1.33, P = 0.243) and longitude (LMM: F_1 = 0.12,
278 P = 0.729; Fig. 4).

279

280 *Intra-specific differences in rock ptarmigan and black grouse*

281 We tested intra-specific differences in rock ptarmigan with a 4-year dataset from the municipality of
282 Pontresina and found that feather $\delta^{13}\text{C}$ values were marginally lower in males than females (LM: F_1 =
283 4.44, P = 0.039; Fig. A1). Furthermore, the interaction between year and age (LM: F_1 = 2.59, P =
284 0.045; Fig. A2) was significant. Albeit significant, the overall differences were rather weak (Fig. A1 &
285 A2). Rock ptarmigan feather $\delta^{15}\text{N}$ values were not affected by either year, sex or age or any
286 interaction term (LM: all $F_1 \leq 1.69$, all P ≥ 0.164).

287 In black grouse sampled across Grisons, feather $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not differ between
288 adult and immature males (LM: $F_1 = 0.07$ and 0.01 , $P = 0.798$ and 0.986 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$,
289 respectively).

290

291

292 *Species differences between rock ptarmigan and black grouse*

293 Overall, adult rock ptarmigan had significantly higher $\delta^{13}\text{C}$ values (LM: $F_1 = 77.36$, $P < 0.001$) and
294 significantly lower $\delta^{15}\text{N}$ values (LM: $F_1 = 118.62$, $P < 0.001$) than adult black grouse, with isotopic
295 values of both species showing no overlap (Fig. 5).

296

297

298 **Discussion**

299 *Effect of latitude and longitude along the precipitation gradient*

300 Grisons shows a distinct precipitation gradient, with decreasing spring and summer rainfalls from
301 west to east. In agreement with our expectation, plant $\delta^{13}\text{C}$ increased with longitude, but not
302 latitude, and no effect of either latitude or longitude was found for plant $\delta^{15}\text{N}$. The same pattern was
303 found for black grouse feathers, suggesting that the dietary composition of black grouse did not
304 change along the precipitation gradient with longitude within the study area.

305 In contrast, in rock ptarmigan, we found a different effect: While $\delta^{13}\text{C}$ in feathers did not
306 differ with either latitude or longitude, $\delta^{15}\text{N}$ increased with longitude. The latter might suggest that
307 the proportion of insects in the rock ptarmigan diet increases from west to east, and therefore along
308 the precipitation gradient. Albeit significant, the effect itself was rather small and might on its own
309 be biologically hardly meaningful. In addition, however, the longitudinal effect of plant $\delta^{13}\text{C}$ was not
310 reflected in rock ptarmigan feather isotopes. Considering that rock ptarmigan diet appears to be
311 based even more on plants than that of black grouse (for which the effect was visible), this is
312 surprising. Potentially, these combined effects in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ therefore do suggest a change in

313 dietary composition and/or food sources of rock ptarmigan over the longitudinal precipitation
314 gradient.

315

316 *Intra-specific variation in isotopes in rock ptarmigan and black grouse*

317 Against our expectations, we found no age-dependent differences in dietary composition in either
318 rock ptarmigan or black grouse. Although invertebrate diet is expected to be common in immature
319 individuals, we found no differences in $\delta^{15}\text{N}$ (which would have indicated a more invertebrate-rich
320 diet in immatures). While young black grouse in Norway and northern England took substantially
321 more insect prey compared to adults (Starling-Westerberg 2001; Wegge & Kastdalen 2008), we are
322 not aware of any studies from the European Alps that compared the diet between adults and
323 juveniles. In rock ptarmigan, earlier studies from Greenland and the Alps suggested that dietary
324 segregation with age might be less strong compared to black grouse (Lieser et al. 1997 and literature
325 therein). Furthermore, potential differences in the diet might decrease with age of the juveniles, and
326 may not be present any more during formation of secondary wing feathers (which our isotope
327 analyses were based upon).

328

329 *Differences in the isotopic values and diet between rock ptarmigan and black grouse*

330 Black grouse had significantly lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ levels than rock ptarmigan, with no overlap
331 of isotopic values. As rock ptarmigan are distributed at higher altitudes than black grouse, and based
332 on our expectations (and findings) that plant $\delta^{13}\text{C}$ increases with elevation, we anticipated to find
333 the here-observed species-segregation in $\delta^{13}\text{C}$ independent of dietary preferences of both species.
334 Assuming a similar diet in both species, we however expected to find similar $\delta^{15}\text{N}$ values in both
335 species. Instead, black grouse feathers had on average 2.5 ‰ higher $\delta^{15}\text{N}$ values than rock ptarmigan
336 feathers. This difference cannot be ascribed to an effect of altitude, as plant isotopes did not differ in
337 $\delta^{15}\text{N}$ with elevation. Theoretically, the species differences in $\delta^{15}\text{N}$ could be explained by black grouse
338 feeding consistently on plant species with higher $\delta^{15}\text{N}$ values compared to rock ptarmigan. However,

339 variability in $\delta^{15}\text{N}$ was also large within plant species, even within the same study plots, i.e. within a
340 range of few metres (see standard deviations shown in Table A1). It therefore appears unlikely that
341 black grouse consistently selected for plant specimen with high $\delta^{15}\text{N}$, whereas rock ptarmigan did
342 the opposite. A more likely explanation for both the higher $\delta^{15}\text{N}$ values in black grouse and the larger
343 intra-specific variation in $\delta^{15}\text{N}$ in this species would be that black grouse feed on a higher proportion
344 of invertebrates compared to rock ptarmigan. Along the food chain, $\delta^{15}\text{N}$ increases with each trophic
345 step (DeNiro & Epstein 1981; Minagawa & Wada 1984). This trophic fractionation ranges between
346 approximately 2‰ and 5‰ (Post 2002). The difference in $\delta^{15}\text{N}$ between black grouse and rock
347 ptarmigan therefore represented a difference by about half to one trophic level, suggesting that
348 invertebrates make up a considerable amount of diet in black grouse. In contrast, diet in rock
349 ptarmigan can be expected to be based primarily on plants due to the rather small difference (on
350 average 2.22 ‰) in $\delta^{15}\text{N}$ between analysed plant material and rock ptarmigan feathers.

351 The literature so far ascribed a similar, mainly plant-based diet to adults of both of our study
352 species (Beeston et al. 2005; Bertermann et al. 1998; Glutz von Blotzheim et al. 1973; Lieser et al.
353 1997; Starling-Westerberg 2001). While we are unable to identify with certainty whether a
354 differential plant diet or the proportion of invertebrates cause the differences in $\delta^{15}\text{N}$, our data
355 clearly suggest a dietary segregation between black grouse and rock ptarmigan, which has not been
356 described previously.

357

358 *Potential caveats of this study*

359 We conducted stable isotope analyses only for a limited number of plant specimen and plant
360 species, not covering the entire food web of rock ptarmigan and black grouse. In particular, we did
361 not sample any invertebrates, which – with hindsight – could have helped with the interpretation of
362 our results. Variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ within and between plant species was large, also within
363 species and within study plots (see Table A1), and variation would likely be even larger under
364 inclusion of more plant specimen. Due to this strong variation and the high overlap of isotope values

365 among plant species, and further lack of knowledge about the fractionation factors between isotopic
366 ratios in the birds' food and their feathers, we decided against running stable isotope mixing models
367 as results would come with a large level of uncertainty. Consequently, we were, however, not able
368 to estimate specific dietary components in the diet of rock ptarmigan and black grouse.

369 Our sampling design was furthermore suboptimal in that plants, rock ptarmigan and black
370 grouse feathers had to be sampled in three different years. We therefore cannot rule out entirely
371 that long-term year-effects may have biased our results. However, we would like to emphasize that
372 1) there were no substantial and biologically meaningful year-differences in either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in
373 feathers from rock ptarmigan within the municipality of Pontresina; and 2) according to MeteoSwiss
374 the precipitation gradient with longitude in Grisons was present across the entire period for which
375 samples from either birds or plants were obtained. We therefore assume that despite the
376 suboptimal sampling design, our finding of dietary segregation between rock ptarmigan and black
377 grouse is robust.

378

379 *Conclusions / Implications of this study*

380 Alpine wildlife is severely affected by anthropogenic influences, from land use patterns to global
381 climate change that will affect temperature and precipitation patterns in the Alps. One aim of this
382 study was therefore to investigate the dietary changes along a precipitation gradient within the
383 central European Alps in both rock ptarmigan and black grouse. Overall, our data suggested a
384 potential effect of precipitation on the diet of rock ptarmigan and no effect on black grouse. The
385 more significant and slightly unexpected results of this study were, however, the dietary segregation
386 of rock ptarmigan and black grouse.

387

388

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396

397

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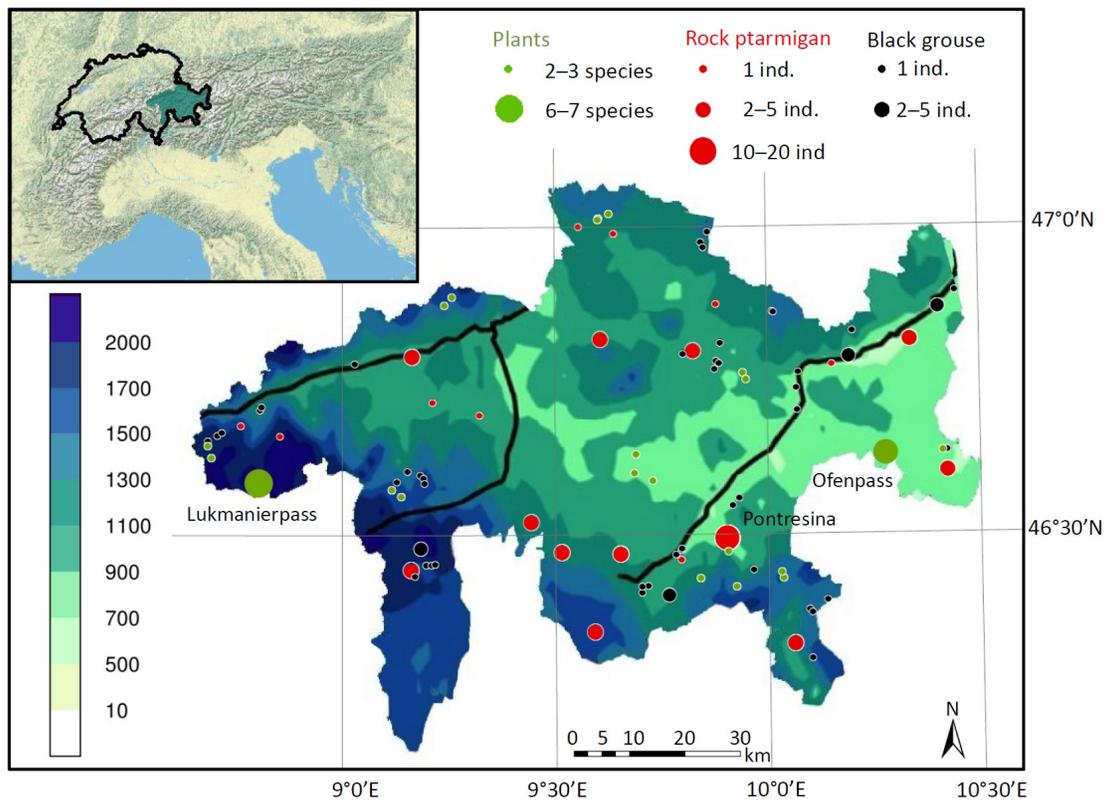
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517

518 **Figure Captions**

519

520 Fig. 1. Location of Switzerland (black frame) and Grisons (blue-green coloured) within the European
521 Alps (inserted plot in top-left corner). The main figure shows the sum of precipitation (in mm; see
522 legend on the left hand side) in the year 2013 in Grisons and sample locations of plants (green), rock
523 ptarmigan (red) and black grouse (black) feathers. For the two grouse species the size of the dots
524 reflects the number of sampled individuals in the year 2005 (black grouse) and 2009 (rock
525 ptarmigan, respectively). Lukmanierpass and Ofenpass (marked with large green dots) are the
526 locations where plants were sampled more intensively to compare interspecific isotopic variation.
527 Pontresina, the location where most rock ptarmigan were shot, is highlighted as samples from this
528 location were used for inter-annual and intra-specific comparisons of isotope ratios. A total of N =
529 137 feather samples of rock ptarmigan, N = 53 feather samples of black grouse and N = 197 plant
530 samples were analysed. Sum of precipitation as shown here is based on spatial interpolation of
531 weather station data (according to RhiresY v.1.0.; details in Frei et al. 1998). The background map
532 was modified based on a figure provided by MeteoSwiss.
533

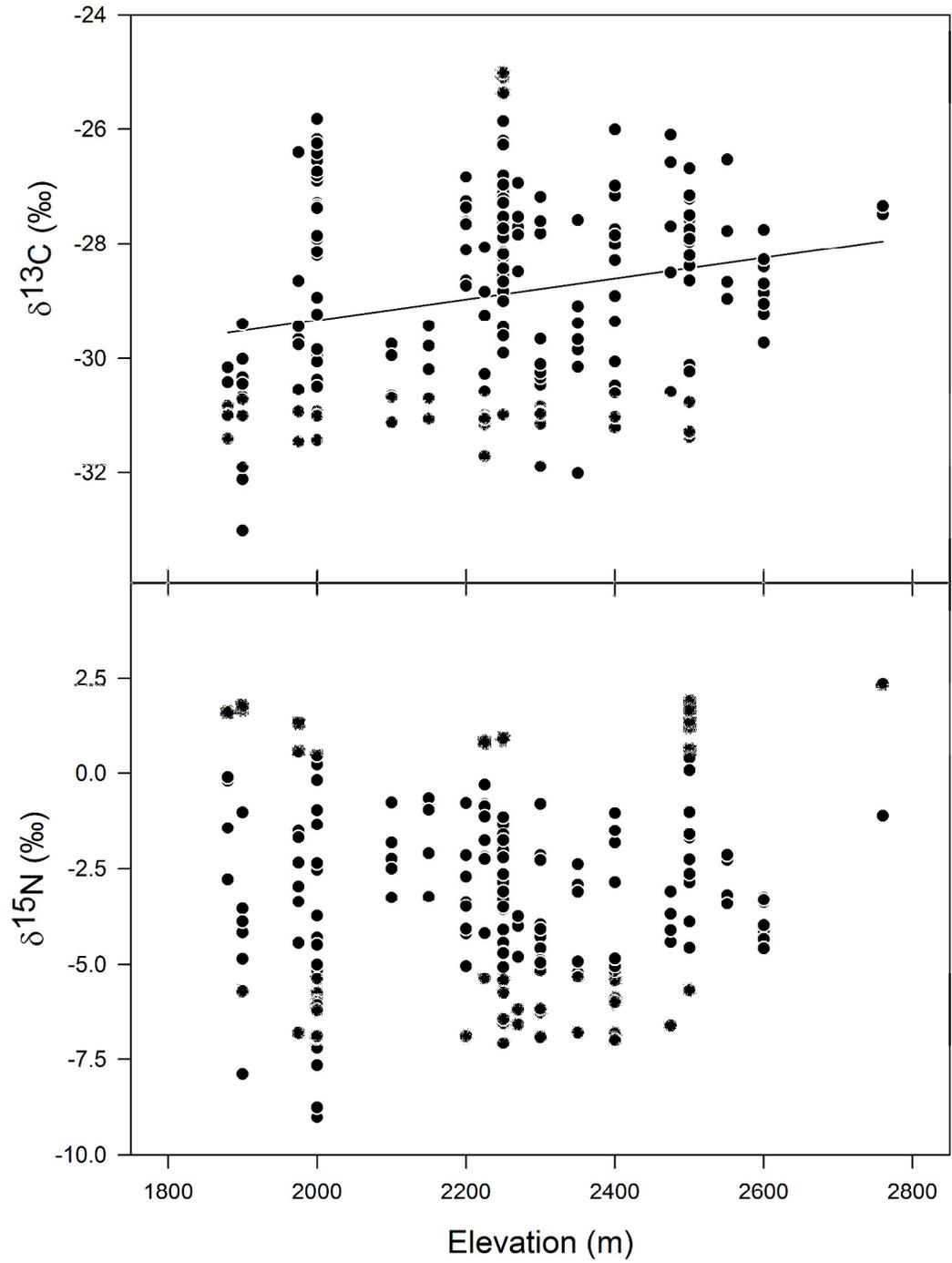


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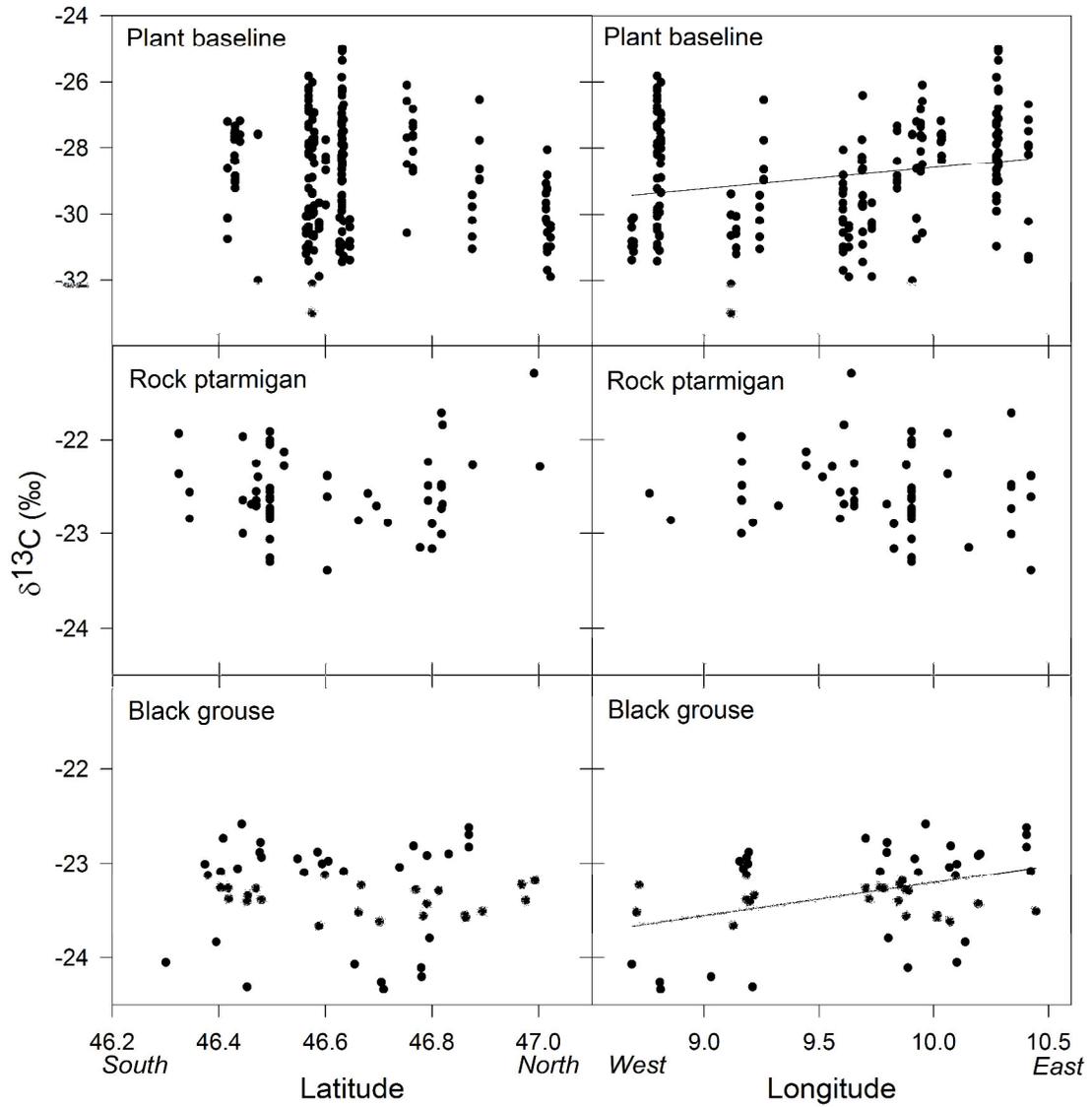
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537 Fig. 2. Effects of elevation on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of plants. Regression lines are shown for significant
538 effects only.
539



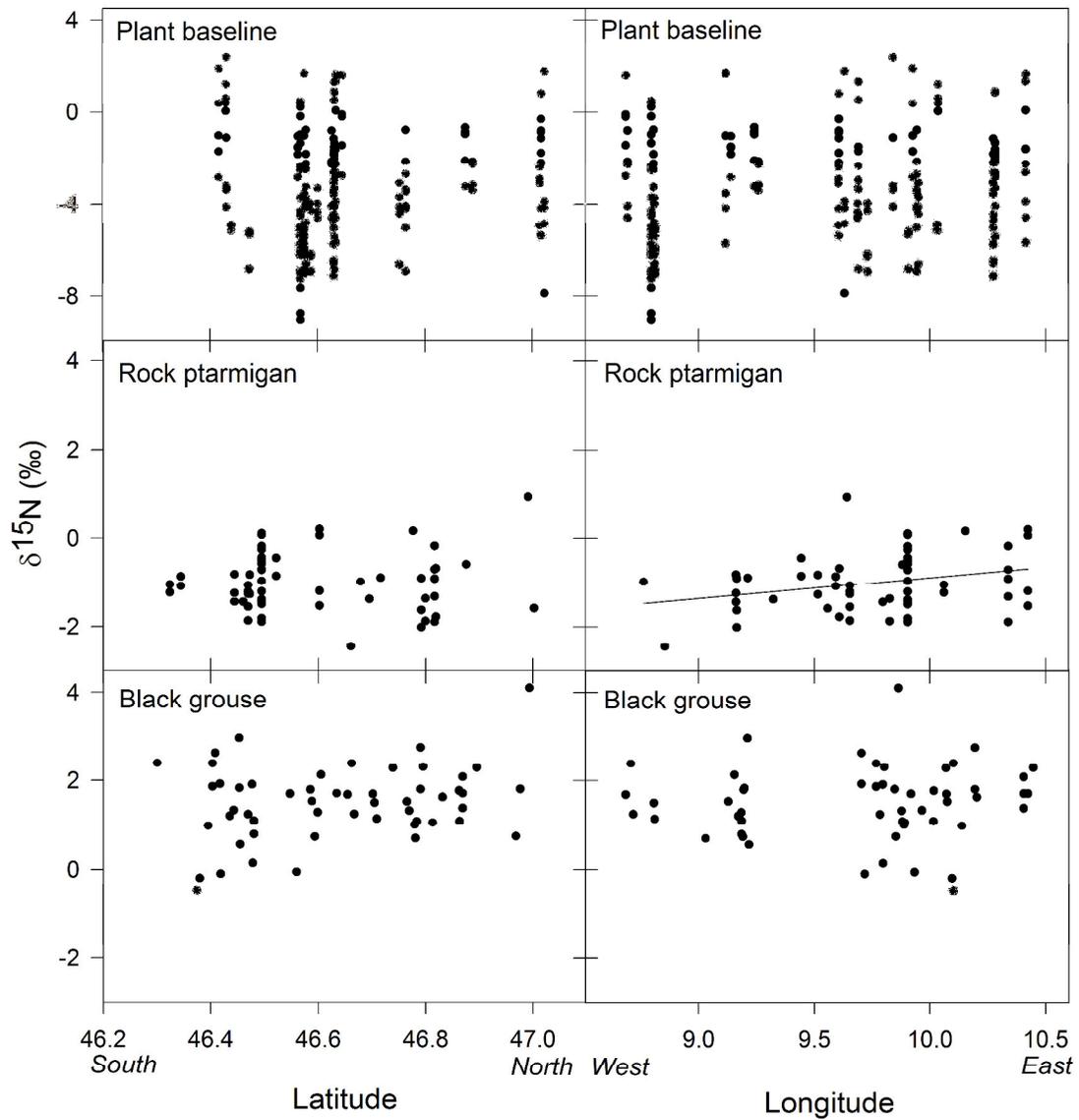
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542 Fig. 3. Effects of latitude and longitude on $\delta^{13}\text{C}$ of plants, rock ptarmigan and black grouse feathers.
543 Note that the scaling for $\delta^{13}\text{C}$ differs between the plants and the two grouse species. Regression
544 lines are shown for significant effects only.
545



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549 Fig. 4. Effects of latitude and longitude on $\delta^{15}\text{N}$ of plants, rock ptarmigan and black grouse feathers.
550 Note that the scaling for $\delta^{15}\text{N}$ differs between the plants and the two grouse species. Regression
551 lines are shown for significant effects only.
552



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