# Environmental controls on stable isotope ratios in New Zealand Podocarpaceae: implications for palaeoclimate reconstruction

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## Abstract

Stable isotope ratios of various proxies are widely used for palaeoclimate reconstruction, and it is often assumed that isotope ratios reflect vegetation abundance or type. However, very little research exists on the isotopic equilibration of extant biomes under variable environmental conditions. In this study, carbon and oxygen isotope ratios from leaves of various Podocarpaceae genera, endemic to New Zealand, are linked to environmental parameters from the Land Environments New Zealand model. The dominant influence on stable isotope ratios within the majority of Podocarpaceae studied here is vapour pressure deficit (VPD). A simple latitudinal trend does not exist, and neither temperature nor rainfall (decoupled from VPD) are major controls on the stable isotope ratios. The results suggest that modern spatial heterogeneity in VPD affects the stable isotope values of vegetation, and that historic VPD variability would change the stable isotope ratios of Podocarpaceae without necessitating a change in vegetation type, density, or productivity. This represents an alternative model for temporal isotope change within geochemical proxies, and reinforces the

23 need for increased stable isotopic research in modern plant ecosystems to better understand

24 modern, and eventually palaeoclimatic processes affecting the terrestrial biosphere.

25 **Keywords:**

26 Stable isotopes; New Zealand; Podocarps; leaves; environment; modern analogue;

27 palaeoclimate

28

## 1. Introduction

Stable isotope ratios of various climate proxy archives are used extensively in palaeoclimate reconstructions (Brookman and Ambrose, 2013; Brown et al., 2013; Bussell and Pillans, 1997; Denniston et al., 1999; Dorale et al., 2010; Frappier et al., 2002; Frappier et al., 2007; Loader et al., 2013; McCarroll and Loader, 2004; Royer et al., 2013), however not all the processes affecting these ratios are understood. Past research has ascribed isotopic ratio variability in some terrestrial climate proxies (e.g., lake sediments, speleothems) to either changes in vegetation type (Denniston et al., 1999; Dorale et al., 2010) or biomass density (Baldini et al., 2005; Cosford et al., 2009). Isotopic discrimination in plant biomass, especially leaves, may affect climate proxy records. Stable-isotope ratios of plant material (especially $\delta^{13}C$) will ultimately affect soil isotopic ratios. Thus any proxy whose formation is partially dependent on soil processes will subsequently respond to changes in the terrestrial biosphere. To maximise the accuracy of palaeoclimate proxy record interpretations our understanding on how modern isotopic ratios of vegetation, regionally, respond to environmental change is critical (Fig. 1).

Podocarpaceae are coniferous $C_3$ trees regarded as the Southern Hemisphere equivalent of Pinaceae (Brodribb, 2011). A review focussing on the distribution and characteristics of Podocarpaceae was presented by Turner (2012; see references therein), but to date, very little isotopic research has been produced on Podocarpaceae.

Diefendorf et al. (2010) proposed that to better interpret $\delta^{13}C$ in palaeo-records, modern populations of similar species must be investigated under different environmental conditions

54 such as temperature, water availability and $p\text{CO}_2$. More accurate datasets would require a

55 natural laboratory setting, and in this case New Zealand, which has a very varied climate,

56 offers an excellent opportunity to produce such a dataset for Podocarpaceae, as an example of

57 how isotopes may vary greatly within one family due to environmental conditions. This study

58 measures $\delta^{13}\text{C}$ of bulk leaf material and relates that directly to environmental influences.

59

60 The stable carbon isotope ratio of leaf material, hereafter referred to as $\delta^{13}\text{C}_{leaf}$, is controlled

61 by photosynthetic capacity (the rate of photosynthetic fixation of carbon) or stomatal

62 conductance (the rate of gas exchange between the atmosphere and interstitial leaf space via

63 the stomata). Differentiating between these two possibilities is achievable using the

64 relationship between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of modern leaves (Scheidegger et al., 2000). In

65 macrofossils, the $\delta^{18}\text{O}$ is a product of diagenesis and secondary alteration so it is not possible

66 to use that relationship to establish stomatal conductance or photosynthetic capacity as the

67 control for $\delta^{13}\text{C}$, except by comparing to a modern analogue. Once the relationship in modern

68 leaves is known, it can be extended to leaf macrofossils of the same family to model the

69 original $\delta^{18}\text{O}$ of that macrofossil. Therefore, the relationship between $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and

70 environmental factors is critical for modelling macrofossil $\delta^{18}\text{O}$. Sampling $\delta^{18}\text{O}$ of fresh

71 leaves allows interpretation of stomatal conductance and photosynthetic within each species

72 or genus. According to Scheidegger's conceptual model (Fig. 2), the relationship of $\delta^{18}\text{O}$ to

73 $\delta^{13}\text{C}$ indicates the dominance of stomatal conductance or photosynthetic capacity in $\delta^{13}\text{C}$

74 discrimination.

75

76 In this study, the isotopic variation of a number of species from the Podocarpaceae family

77 across New Zealand were investigated. Podocarp species native to New Zealand are

78 particularly useful because they are drought intolerant and their occurrence in the fossil

79  record have already been used to constrain climatic conditions: that climate is wet, mild and

80  lacking in extremes (Coomes and Bellingham, 2011). Additionally, macrofossils from these

81  species have been found in New Zealand and King George Island (Antarctica) (Fontes and

82  Dutra, 2010; Turney et al., 1999), thus Podocarpaceae have the potential to provide regional

83  palaeoclimatic information from the high-latitude Southern Hemisphere. Moreover, the study

84  provides an example of how isotope ratios can vary across a plant family, which should be

85  taken into account when studying other coniferous $C_3$ vegetation. We report that whole-leaf

86  carbon isotope ratios within the same genus (Podocarpaceae) vary according to local climatic

87  conditions, thus implying that they would change accordingly in the historic record due to a

88  climatic shift.

89

## 2. Methodology

### 2.1.  Field Area

92  Fieldwork was conducted in New Zealand, on both the North and South Islands. A large

93  proportion of the land area of New Zealand is set aside as National Parks, allowing a huge

94  area of native forest to flourish. With a wide variety of endemic and unique species, generally

95  unpolluted watercourses, and a large latitudinal range producing a variety of environmental

96  conditions, New Zealand is an ideal natural laboratory. Podocarpaceae do not grow in all

97  regions found in New Zealand, because they are: (1) intolerant to hard frost and therefore do

98  not grow at high altitude (with the exception of *P. alpinus* if conditions are correct); and (2)

99  subject to xylem cavitation under drought conditions and hence are not found in semi-arid

100 regions.  *Nothofagus* dominates colder climate forest biomes (Leathwick, 2001) and native

101 tussock grass dominates in semi-arid areas (Mark et al., 2003); furthermore, large-scale

102 deforestation since the human settlement of New Zealand has limited the geographical extent

103 of native forest, and not all Podocarpacae are found even where such native forests still exist.

104  Hence some areas of New Zealand have not been sampled and not all genera are sampled at

105  every site.

106

107  All samples for this study were collected within a time window of 5 weeks during July and

108  August of 2011. Sample types included leaves, leaflets, and water. After collection, leaf and

109  water samples were regrettably not kept in constant conditions; samples were parcelled and

110  sent by airmail to the UK in an uncontrolled environment and would have experienced

111  changes in temperature and pressure. For example, *Phyllocladus* samples changed colour

112  from green to brown during transit whilst all other specimens remained green.

113

114  Leaves or leaflets were taken from the family Podocarpaceae. The inclusion of the genus

115  *Phyllocladus* in this family is debated (Keng, 1978; Molloy and Markham, 1999; Wagstaff,

116  2004). Leaflets were sampled at approximately $1.5 - 2$ m above ground level and, where

117  possible, from the side of the tree likely to experience the most sunshine (for example at the

118  outside edge of a forested areas). Podocarpaceae are coniferous and do not drop their foliage

119  seasonally, but produce new growth at the tip. To ensure a consistent sampling strategy,

120  leaflets were taken from growth further down the branch, which grew approximately 2 - 3

121  years before. These stipulations were limited by the natural propensity of the trees to grow in

122  dense rainforest and to grow very tall (up to 60 m) with the foliage only at the top few metres.

123  This resulted in more sampling of juvenile trees which have morphologically different leaves

124  from the adult tree in a number of genera. Reference samples were obtained from Dunedin

125  Botanical Gardens where all genera in question were growing in the same conditions and at

126  the same altitude.

127

128     To sample water, 10 cm$^3$ plastic vials were filled from lakes, rivers and streams at ~30 cm

129     depth or base of water course, whichever was shallower. An air space of approximately 0.5

130     cm$^3$ was left at the top of each vial to allow for thermal expansion of the water whilst in

131     transit. Sampled waters include glacial melt, shallow and deep lakes, rivers, streams, and

132     water from hydrothermal areas.

133

134     **2.2.    Isotopic analysis**

135

136     All analyses were performed in the Stable Isotope Biogeochemistry Laboratory, part of the

137     Durham Geochemistry Centre at Durham University, UK. Whole-leaf $\delta^{13}C$ was determined

138     using a Thermo-Finnigan Delta V Advantage isotope ratio mass spectrometer (IRMS)

139     coupled with a Costech ECS 4010 Elemental Analyser. Samples of 0.3–0.5 mg were taken

140     from a leaf or leaflet from each location, placed within a 6mm x 4mm tin foil capsule and

141     sequentially dropped into a furnace where they were completely combusted. The resulting

142     gases were passed through a gas chromatography column for separation and then measured in

143     succession by the TCD detector. Isotopic measurements were initially taken from a transect

144     of a token sample for each species, in order to establish any significant variation in $\delta^{13}C$

145     within the leaf. Each species was sampled at the same point on the leaf and the same area of

146     growth to minimise any error from the natural variation in carbon within a leaf and tree. Data

147     accuracy is monitored through routine analyses of in-house standards, which are stringently

148     calibrated against international standards (e.g., USGS 40, USGS 24, IAEA 600, IAEA CH6,

149     UREA, SPAR): this provides a linear range in $\delta^{13}C$ between +2‰ and –47‰ VPDB.

150     Analytical uncertainty for $\delta^{13}C$ is typically ±0.1‰ for replicate analyses of the international

151     standards and typically <0.2‰ on replicate sample analysis.

152

153 Whole-leaf $\delta^{18}O$ was determined using a Thermo-Finnigan Delta V Advantage isotope ratio

154 mass spectrometer (IRMS) coupled with a Thermo-Finnigan High Temperature Conversion

155 Elemental Analyser (TC/EA). The method for preparing samples for the TC/EA was similar

156 to that for the EA; except the mass weighed out was between 0.5–0.6 mg and silver foil

157 capsules of 5mm x 3mm were used. The samples were introduced to the TC/EA where they

158 underwent pyrolysis to produce CO and $H_2$. These gases were separated using an isothermal

159 gas chromatography column and passed to the IRMS for measurement. Data accuracy was

160 monitored through routine analyses of international standards (e.g., IAEA 600, 601, 602): this

161 provided a linear range in $\delta^{18}O$ between +71.4‰ and –3.5‰ VSMOW. Analytical

162 uncertainty for $\delta^{18}O$ was typically ±0.1‰ for replicate analyses of the international standards

163 and typically <0.2‰ on replicate sample analysis.

164

165 Each water sample was prepared using a sterilized 0.45μm filter attached to a 5 cm$^3$ syringe.

166 Approximately 1 cm$^3$ of water was filtered into a glass vial and sealed using a septum cap.

167 Isotopic measurements of $\delta^{18}O$ and $\delta D$ were obtained by laser spectroscopy using a Los

168 Gatos Research DLT-100 liquid water isotope analyser (Berman et al. 2013). A suite of Los

169 Gatos Research water isotope standards was used and the analytical uncertainties for $\delta^{18}O$

170 and $\delta D$ were typically better than ±0.1‰ and ±0.3‰ respectively. Replicate analyses of the

171 samples were typically <0.15‰ for $\delta^{18}O$ and <0.3‰ for $\delta D$.

172

173 **2.3. Data Analysis**

174 Latitude and longitude of each sampling locale were entered into the Land Environments

175 New Zealand (LENZ) model, which then output modelled mean parameters used for this

176 study: mean annual temperature (MAT), mean minimum daily temperature of the coldest

177 month (TMIN), mean annual solar radiation (MAS) monthly water balance (R2PET), and

178    vapour pressure deficit (VPD). For accuracy and modelling details refer to Leathwick *et al.*

179    (2003a,b).

180

181    Bowen and Revenaugh (2003), and  Bowen and Wilkinson (2002) presented the Online

182    Isotopes in Precipitation Calculator (OIPC) based on data from the International Atomic

183    Energy Association and the World Meteorological Organization Global Network for Isotopes

184    in Precipitation. OIPC values have been calculated for all water sampling locations from this

185    study for comparison (see section 3.1).

186

187    Stable isotope data derived from leaves were organised by genus rather than species or family

188    for a number of reasons: *P. totara* and *P. hallii* display a high degree of hybridisation

189    between the two species (Leathwick, 2001; Leathwick and Whitehead, 2001), and *D.*

190    *cupressinum* and *D. dacrydioides* are the only examples of these genera native to New

191    Zealand. *P. ferruginea* is much more readily identifiable than *P. taxifolia* such that the former

192    is our representative for the genus *Prumnopitys*. Further mixture analysis highlighted the

193    significant difference between juvenile and mature *D. cupressinum* specimens; the mean and

194    standard deviations of $\delta^{13}$C from mature *D. cupressinum* versus juvenile specimens were well

195    predicted by the numerical model. Juvenile and mature *D. cupressinum* have significantly

196    different mean $\delta^{13}$C values, and due to the difference in morphologies between the juvenile

197    and mature stages, and the growing conditions favoured by Podocarpaceae in general, this

198    observation is discussed in detail in section 3.3.

199

200    The statistical analysis package PAST (Hammer et al., 2001) was used to compute normality

201    using the Shapiro-Wilk, Jarque-Bera and Chi$^2$ tests; the Shapiro-Wilk values were used in

202    conjunction with the latter two for confirmation. When datasets were normally distributed,

203 Pearson's regressions were used to link isotopes to environmental variables, and where

204 normality was rejected, Spearman's rank correlation coefficient was used. In each case *r*

205 values and *p* values were calculated with *p* values below 0.001 taken to be the limit for very

206 significant correlation, and 0.01 for significant.

207

208 Variability in regional climates due to landforms and weather patterns is very important for

209 the distribution of Podocarp forests (Leathwick, 2001; Leathwick and Whitehead, 2001;

210 Turner, 2012). Contour maps were created for the different environmental parameters used

211 and for the isotopic datasets generated in this study. By comparing these maps one can

212 qualitatively assess how the different environmental factors influence the correlation between

213 the maps. For example, the Tongariro National Park in the centre of the North Island and the

214 Southern Alps dividing the South Island are topographical highs, which strongly influence

215 weather patterns. The contour maps for the different environmental parameters and isotopic

216 datasets were generated using the contouring program, Surfer 10 (Golden Software).

217

218 ## 3.    Results & Discussion

219 ### 3.1.    Oxygen isotopes in New Zealand water samples

220 $\delta^{18}O_{water}$ measurements displayed significant positive correlation with latitude ($r^2 = 0.31$, $p =$

221 $2.5 \times 10^{-4}$) as well as significant negative correlation with elevation ($r^2 = 0.38$, $p = 3.8 \times 10^{-5}$).

222 Temperature is partially a function of latitude and elevation so regression analysis of $\delta^{18}O_{water}$

223 with mean annual temperature (mat) and the mean minimum temperature of the coldest

224 month (tmin) data from LENZ was conducted.   Strong and statistically very significant

225 positive correlations exist with both: $\delta^{18}O_{water}$ against MAT had an $r^2$ of 0.56 ($p = 9.26 \times 10^{-8}$)

226 and $\delta^{18}O_{water}$ against tmin results in 0.67 ($p = 6.03 \times 10^{-10}$). These values are consistent with

227 Rayleigh fractionation theory (Bowen and Wilkinson, 2002) even though these samples were

228    from streams, rivers, and lakes instead of direct precipitation and isoscapes reflect the

229    relationship between tmin and $\delta^{18}O_{water}$ (Fig. 3).

230

231    OIPC (Bowen and Wilkinson, 2002) uses latitudes, longitudes and elevation to model $\delta^{18}O$

232    precipitation, but this study sampled surface water so a degree of difference would be

233    expected between theoretical precipitation values and observed surface water values.

234    Although a strong correlation between OIPC and sampled $\delta^{18}O_{water}$ exists, it is not as strong

235    as might be initially expected ($r^2 = 0.49$). Sampled water displays more extreme values than

236    OIPC (Fig. 4) and the means and variances of these data are not statistically similar ($f =$

237    $2.9x10^{-9}$ and $t = 0.04$). OIPC calculates a monthly average $\delta^{18}O$ of precipitation which should

238    account for seasonality. Surface water end members are included in our data; glaciers and

239    estuaries were sampled. Sampled water also has a greater deviation from the global meteoric

240    water line than OIPC values mostly likely due to interaction of precipitation with

241    groundwater, aquifer units, and evaporation from rivers and lakes. The discrepancy in $\delta^{18}O$

242    range between them may stem from the effect of elevation where sampled values have a

243    much stronger and more statistically significant correlation with elevation than OIPC values.

244

245    **3.2.    Leaf Isotopes**

246    Water and leaves were not always sampled at the same locations so it is difficult to get an

247    overall estimate of how $\delta^{18}O_{water}$ affects $\delta^{18}O_{leaf}$, however for the sites at which both were

248    sampled, there is no significant correlation ($r^2 = 0.09$, $p = 0.4$, $n = 9$). No significant

249    correlation allows the application of Scheidegger's conceptual model (Scheidegger et al.,

250    2000) but it is still useful as a secondary source of inference supporting our conclusions.

251

252   Podocarpaceae leaf $\delta^{13}$C is positively correlated with monthly water balance ($r^2 = 0.18$, $p =$

253   $1.46\text{x}10^{-6}$, $n = 120$) and VPD ($r^2 = 0.13$, $p = 6.37\text{x}10^{-5}$, $n = 120$). Leathwick and Whitehead

254   (2001) demonstrated that the optimum VPD and root zone water deficit vary for different

255   species of Podocarpaceae in New Zealand, potentially explaining the observed weak (but

256   very significant) correlations; mixing of species and great diversity of environments may also

257   contribute. The statistical significance of the relationship between $\delta^{13}$C and $\delta^{18}$O within the

258   overall leaf data ($p = 0.07$) permits the use of the model produced by Scheidegger et al.

259   (2000), which indicates that stomatal conductance is a slightly stronger control than

260   photosynthetic capacity on $\delta^{13}$C of Podocarpaceae in New Zealand (Fig. 2). Analysis of each

261   genus separately corroborates this finding with stronger and more significant trends (section

262   3.3).

263

264   If leaf samples are considered  based on their locations on either the North or the South

265   Islands, it becomes apparent that $\delta^{13}$C and $\delta^{18}$O leaves for North Island have no significant

266   correlations to any environmental parameters considered here. Coomes and Bellingham

267   (2011) noted that Podocarpaceae thrive on post-catastrophic landscapes (e.g. volcanic)

268   because their superior ability to capture and retain nutrients from poor soils allows them to

269   outcompete angiosperms that would usually dominate on richer soils. On North Island,

270   Podocarpaceae are at greatest abundance on post-eruptive landscapes whereas on South

271   Island, landslides and floods allow them to regenerate their population while they temporarily

272   have the advantage over angiosperms. Geothermal activity is a major aspect of North Island

273   hydrology and has a significant impact of $\delta^{18}$O of source water (Clayton and Steiner, 1975)

274   which is likely to dampen correlations with environmental influences. $\delta^{13}$C data from South

275   Island Podocarpaceae are very significantly correlated with VPD ($r^2 = 0.27$, $p = 1.83 \text{ x}10^{-5}$, $n$

276   $= 63$) and water balance ($r^2 = 0.2$, $p = 2.78 \text{ x}10^{-4}$, $n = 63$). $\delta^{18}$O also has weak but significant

277  correlation with water balance ($r^2 = 0.15$, $p = 0.048$, $n = 51$). Water balance is inversely

278  correlated with VPD because they are both a function of precipitation and temperature. $\delta^{18}O$

279  and $\delta^{13}C$ are strongly correlated ($r^2 = 0.37$, $p = 0.007$, $n = 51$), which is consistent with

280  Scheidegger's model (Scheidegger et al., 2000) suggesting a primary control of stomatal

281  conductance over photosynthetic activity.

282

283  **3.3.    Segregated data by species or genera**

284  Initial $r$ and $p$ values of the entire data set displayed very little correlation to the

285  environmental parameters and mixing models indicated that at least two distinct normal

286  distributions existed within the data set). *Podocarpus* and *D. cupressinum* have significant

287  positive correlations with VPD and significant negative correlations with monthly water

288  balance in both $\delta^{18}O$ and $\delta^{13}C$ (Table 1), indicating a primary control of stomatal

289  conductance on $\delta^{13}C$, consistent with Scheidegger's model when $\delta^{18}O$ was regressed against

290  $\delta^{13}C$.

291

292  *D. dacrydioides* and *D. cupressinum* both have distinctive dimorphism between juvenile and

293  adult specimens. Mixture analysis for $\delta^{13}C$ *D. dacrydioides* indicated two normal

294  distributions within the data set although the correlation with VPD is significant throughout

295  the species. Acceptable sampling density of *D. cupressinum* provided an opportunity to look

296  for isotopic differences between juvenile and mature leaves. Juvenile leaf $\delta^{13}C$ and $\delta^{18}O$

297  display significant correlations with water balance and vapour pressure deficit (Fig. 5)

298  whereas mature leaf isotopes are not significantly correlated with the environmental

299  parameters considered (Table 2), although the two groups had statistically similar means. For

300  *D. cupressinum*, a juvenile form could therefore be a better palaeoclimate indicator than a

301  mature form because it appears to be much more sensitive to environmental stresses.

302

303 *P. ferruginea* $\delta^{13}C$ correlates with elevation rather than VPD and the $\delta^{18}O$ data indicate

304 influences from MAT, MAS, water balance and VPD, although the latter is the strongest and

305 most significant. $\delta^{18}O$ *P. ferruginea* display a correlation of 0.47 ($p = 0.007$, $n = 13$) with

306 $\delta^{13}C$ indicating again a consistent relationship between the two isotopes and a controlling

307 influence of stomatal conductance within this family.

308

309 Increased sunshine hours should cause an increased photosynthetic rate, which theoretically

310 would cause a more negative $\delta^{13}C$ ratio, but correlations with MAS are not evident in $\delta^{13}C$

311 for *Podocarpus*, *D. dacrydioides*, *D. cupressinum* and *P. ferruginea*. However, increased

312 sunshine hours would also cause an increased VPD; using the LENZ data, VPD and MAS

313 have a strong positive correlation ($r^2 = 0.4$, $p = 2.25 \times 10^{-14}$, $n = 118$). Increased VPD causes

314 stomata to close to minimise evaporation from the leaves hence $\delta^{13}C$ becomes less negative

315 due to decreased gas exchange resulting in Rubisco sequestering more $^{13}C$ than usual.

316 Because these two effects are opposed, the correlation with MAS would be highly dampened.

317 Coomes and Bellingham (2011) note that Podocarpaceae are shade tolerant and reach light

318 saturation at low thresholds compared with other C3 plants. *D. cupressinum* and *D.*

319 *dacrydioides* exhibit dimorphism with pendant adult leaves, reducing surface area of exposed

320 leaves exposed to direct sunlight and increasing the sunshine allowed into canopy areas; such

321 morphological adaptations would also decrease correlations with MAS.

322

323 *Phyllocladus* differs in response to most parameters compared to the other genera/species

324 studied here. $\delta^{13}C$ has a negative correlation with MAS but no other significant correlations

325 and $\delta^{18}O$ had no significant correlations with climatic parameters ($p > 0.1$). Unlike the other

326 plants, *Phyllocladus* $\delta^{18}O$ is negatively correlated to $\delta^{13}C$ although the correlation is not

327   significant ($r^2 = 0.21$, $p = 0.13$, $n = 12$). If we reject the significance of $\delta^{18}O$ to $\delta^{13}C$, it would

328   be reasonable to propose that neither photosynthetic capacity or stomatal conductance have a

329   significantly dominant influence on $\delta^{13}C$, however if significance is accepted, Scheidegger's

330   model would propose that photosynthetic capacity is the main controlling factor on $\delta^{13}C$

331   which would agree with the correlation with MAS (Fig. 6). Turney et al (1999) carried out a

332   similar study on *phyllocladus alpinus*, proposing VPD to be the main control on $\delta^{13}C$, but the

333   *Phyllocladus* results presented here are inconsistent with that hypothesis. 86% of Turney's

334   samples were from South Island whereas the majority of *Phyllocladus* samples from this

335   study were from North Island, and geological differences between the islands may affect the

336   results. *Phyllocladus* is disputed as a member of the Podocarpaceae family and is

337   morphologically different from the other genera studied here because it has flattened stems

338   called cladodes rather than leaves; it is therefore unlikely that *Phyllocladus* would have

339   identical stomatal characteristics to the other genera considered.

340

341   **3.4.     Potential for palaeoclimate reconstruction**

342

343   Presence of New Zealand Podocarpaceae in the fossil record constrains the palaeoclimatic

344   interpretation, because they currently thrive only in a relatively narrow climate band within

345   New Zealand. For Podocarpaceae forests dominated by these species to exist, the climate

346   must be wet and cool but without extreme cold, and periodic catastrophic events must occur

347   to allow regeneration of these conifers (Coomes and Bellingham, 2011). To further constrain

348   the vapour pressure deficit and water balance, $\delta^{13}C$ of macrofossils can be compared with the

349   modern day findings presented here. If *Phyllocladus* and a *Podocarp* fossil species occur in

350   the same sequence, information about both Mean Annual Sunshine hours and Vapour

351   Pressure Deficit of the palaeoclimate at time of deposition can be obtained.

352

353 The lack of strong latitudinal trends within these data and strong correlations with Vapour

354 Pressure Deficit suggest that topography-induced environmental conditions are more

355 important than latitudinally-induced conditions for affecting the stable isotope ratios of leaves

356 (Fig. 7). This reinforces the concept that palaeoclimate proxies are best interpreted

357 considering studies on stable isotopes in local vegetation.

358

359 ## 4. Conclusions

360

361 The diversity of altitudes, latitudes, and types of environments from which the samples were

362 obtained provides a robust test of the controls on stable isotope ratios of vegetation.

363 *Podocarpus*, *D. cupressinum*, *D. dacrydioides*, and *P. ferruginea* $\delta^{13}$C is primarily controlled

364 by stomatal conductance responding to Vapour Pressure Deficit variability. The relationship

365 between Mean Annual Sunshine hours and *Phyllocladus* $\delta^{13}$C demonstrates that

366 photosynthetic capacity is the dominant control. $\delta^{18}$O ratios support these conclusions

367 through statistical associations with Vapour Pressure Deficit and application of Scheidegger's

368 conceptual model. These results are relevant for palaeoclimate reconstructions using a range

369 of New Zealand proxies and macrofossils, and, when compared with $\delta^{13}$C and $\delta^{18}$O of other

370 species worldwide, to construct more detailed global isotopic gradients. Additionally, this

371 research suggests that local environmental conditions can directly affect climate proxy $\delta^{13}$C,

372 suggesting that interpretations based on $\delta^{13}$C proxies records should consider shifts in

373 vegetation type, density, and climate (Fig. 1), because they can operate simultaneously and

374 independently.

375

376 ## 5. Acknowledgements

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# 7. Captions

**Fig. 1**: *Simplified models for interpretation of $\delta^{13}C$ excursions. $\delta^{13}C$ becomes less negative in the direction of x-axes arrows. Model A: A shift from sparse to dense vegetation causes $\delta^{13}C$ to decrease (e.g., Baldini et al. 2005). Model B: A shift of vegetation type from forest to prairie will cause $\delta^{13}C$ to increase (e.g., Denniston et al., 1999). Model C: Model tested by this study where a change in climatic conditions such as vapour pressure deficit or sunshine hours can cause $\delta^{13}C$ shifts within a stable biosphere.*

**Fig. 2:** *For scenarios a-h, $\delta^{13}C$ is plotted against $\delta^{18}O$ and related to stomatal conductance($g_i$) and photosynthetic capacity ($A_{max}$) in a number of steps. Firstly the change in $\delta^{18}O$ is related to a change in vapour pressure deficit since $\delta^{18}O$ is controlled by stomatal conductance and stomatal conductance is primarily a function of relative humidity or in this study, vapour pressure deficit. Then $\delta^{13}C$ is linked to intecellular $CO_2$ concentration ($c_i$). In the 3$^{rd}$ step two possible causes for the change in ci are proposed in terms of changes in $A_{max}$ and $g_i$, and then the more likely one chosen by considering vapour pressure deficit. This results in an output model of relative dominance of $g_i$ or $A_{max}$. Adapted from Scheidegger et al. (2000).*

**Fig.3**: *Left: mean minimum temperature of the coldest month (LENZ) with sampling locations; green = leaves, blue = water, scale in °C. Right: $\delta^{18}O$ of sampled water with sampling locations..*

**Fig. 4**: *Data relative to Global Meteoric Water Line (green). OIPC $\delta^{18}O$ precipitation (blue) and $\delta^{18}O$ NZ water samples (red).*

**Fig. 5**: *Juvenile and mature D. cupressinum isoscapes. Colour scale is isotope ratio in ‰.*

**Fig. 6:** *Phyllocladus was sampled primarily from North Island and is correlated with Mean Annual Sunshine hours.*

**Fig. 7**: *Isoscapes a) Podocarpus leaf $\delta^{18}O$ b) Podocarpus leaf $\delta^{13}C$   c) Vapour Pressure Deficit d) Water Balance. Topographically controlled conditions provide a greater influence on leaf stable isotopes than latitudinal variations.*

**Table 1**: *Statistics for segregated data.*

**Table 2:** *Statistics for juvenile and mature D. cupressinum*

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Figure 1

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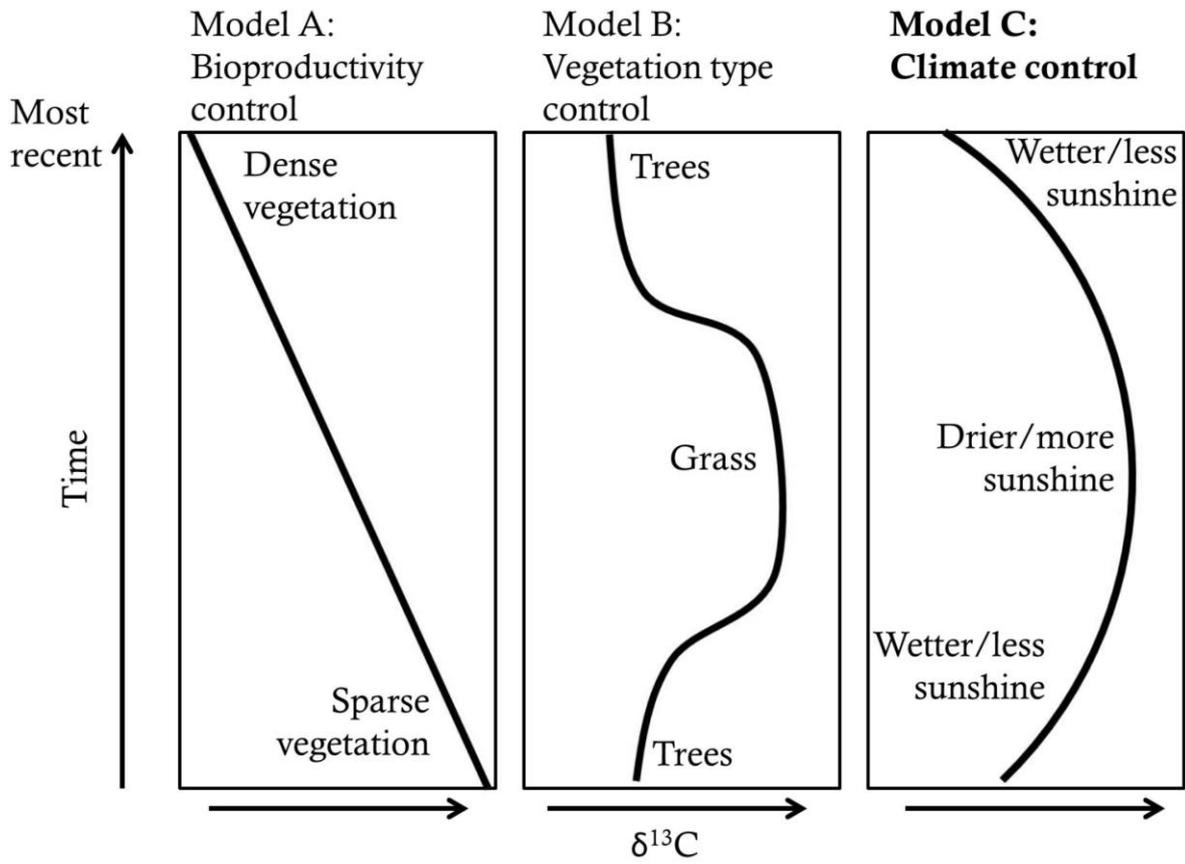
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Input:



| | a) | | b) | | c) | | d) | | e) | | f) | | g) | | h) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\delta^{18}O$ | ≈ | | ↑ | | ↑ | | ↑ | | ≈ | | ↓ | | ↓ | | ↓ | |
| VPD | ≈ | | ↑ | | ↑ | | ↑ | | ≈ | | ↓ | | ↓ | | ↓ | |
| $\delta^{13}C$ | ↑ | | ↑ | | ≈ | | ↓ | | ↓ | | ↓ | | ≈ | | ↑ | |
| $c_i$ | ↓ | | ↓ | | ≈ | | ↑ | | ↑ | | ↑ | | ≈ | | ↓ | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| $A_{max}$ | ↑ | ≈ | ↑ | ≈ | ↑ | ↓ | ≈ | ↓ | ≈ | ↓ | ≈ | ↓ | ↑ | ↓ | ↑ | ≈ |
| $g_i$ | ≈ | ↓ | ≈ | ↓ | ↑ | ↓ | ↑ | ≈ | ↑ | ≈ | ↑ | ≈ | ↑ | ↓ | ≈ | ↓ |
| Selection based on VPD change | x | | | x | | x | | x | | x | x | | x | | x | |

Output:
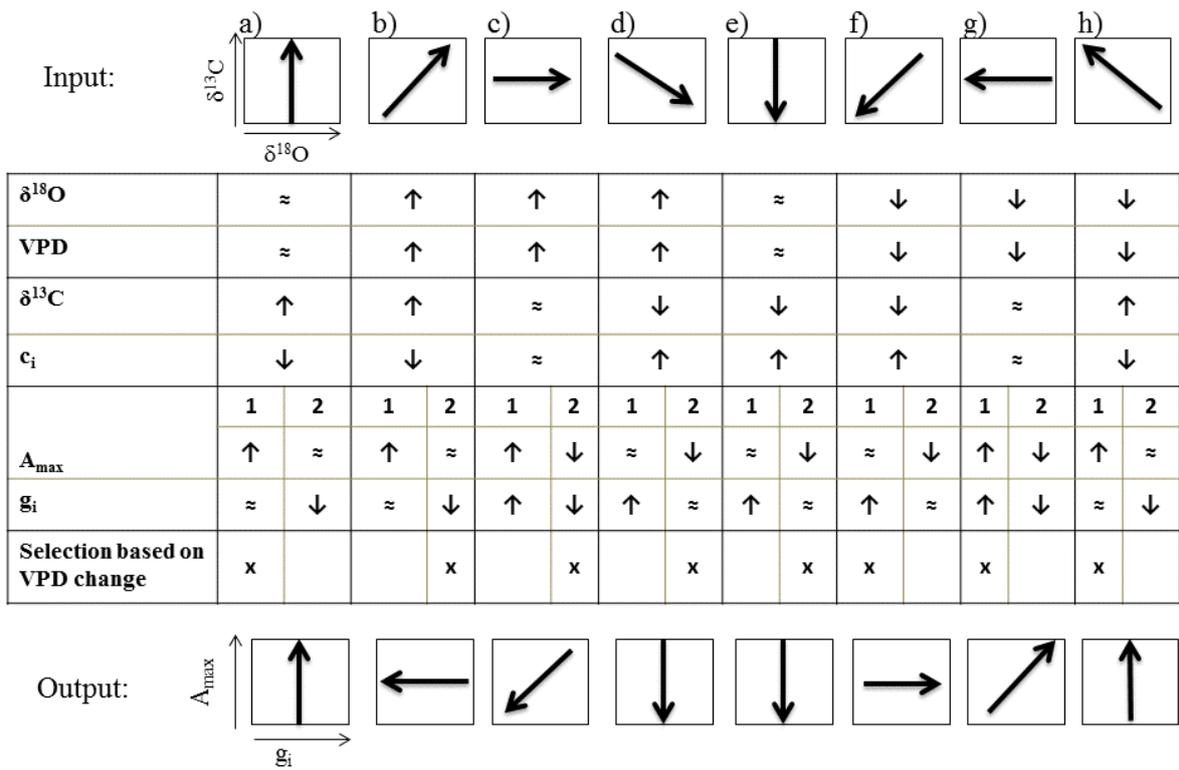


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515 Figure 2

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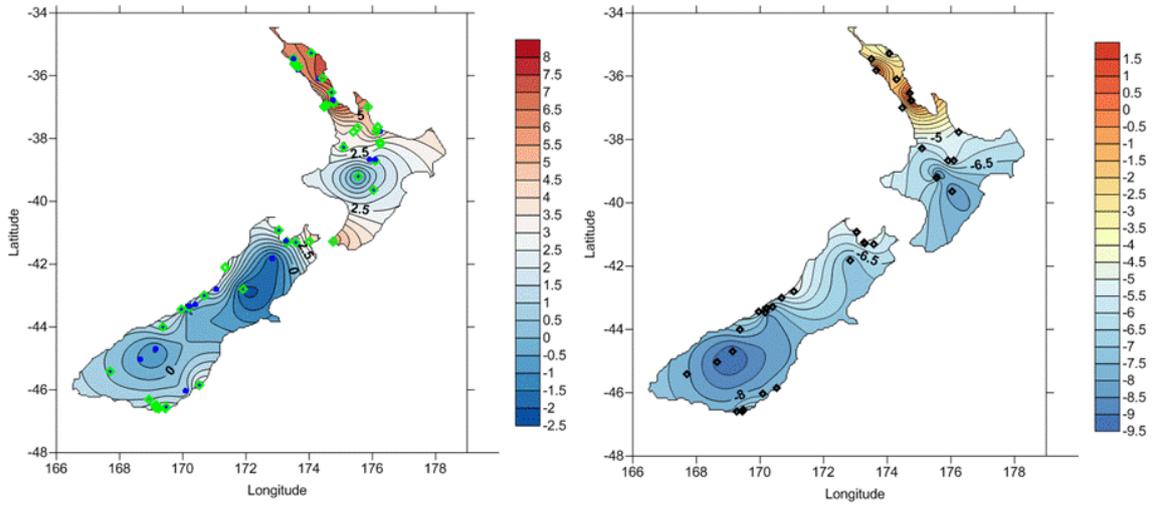


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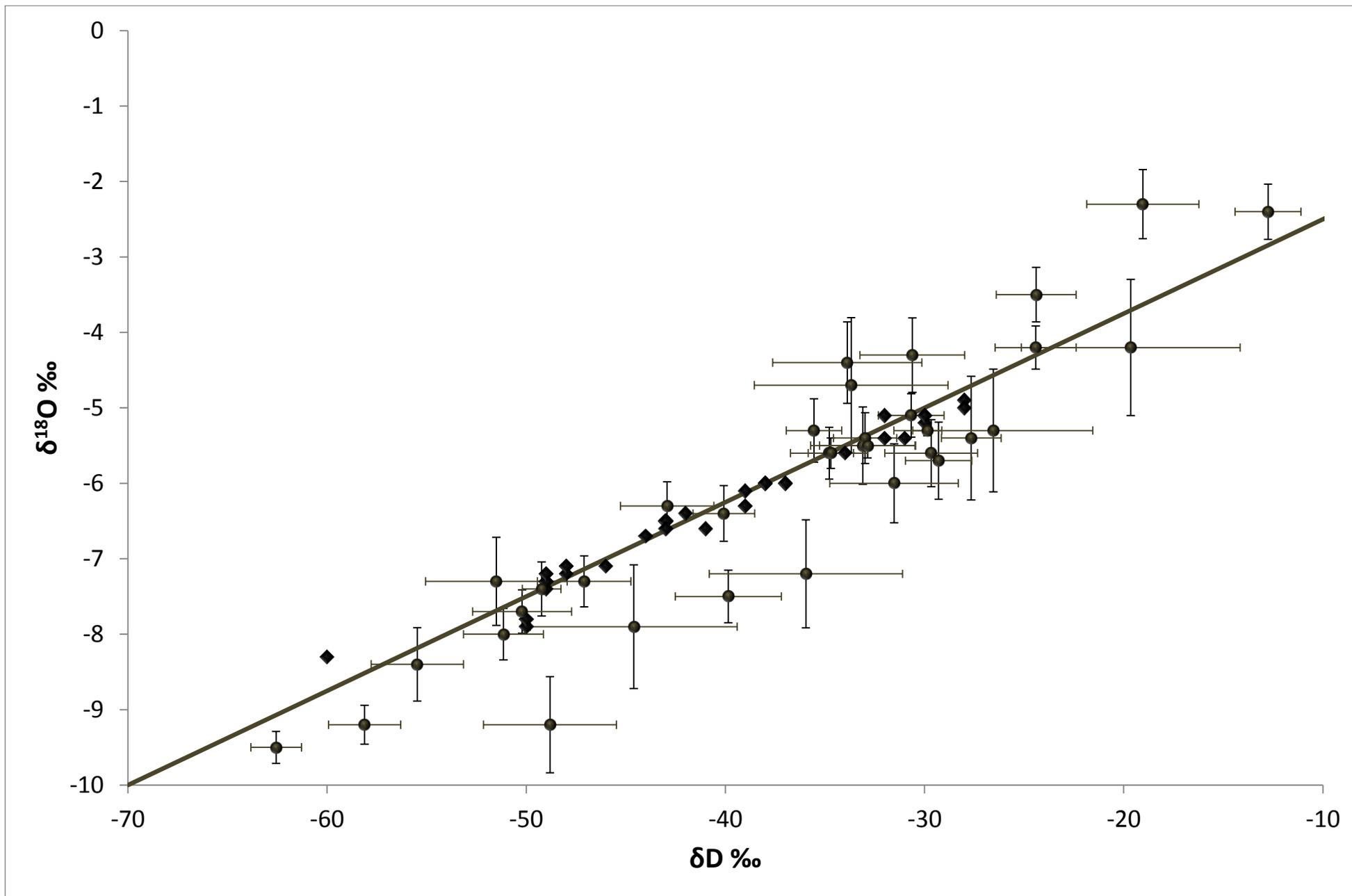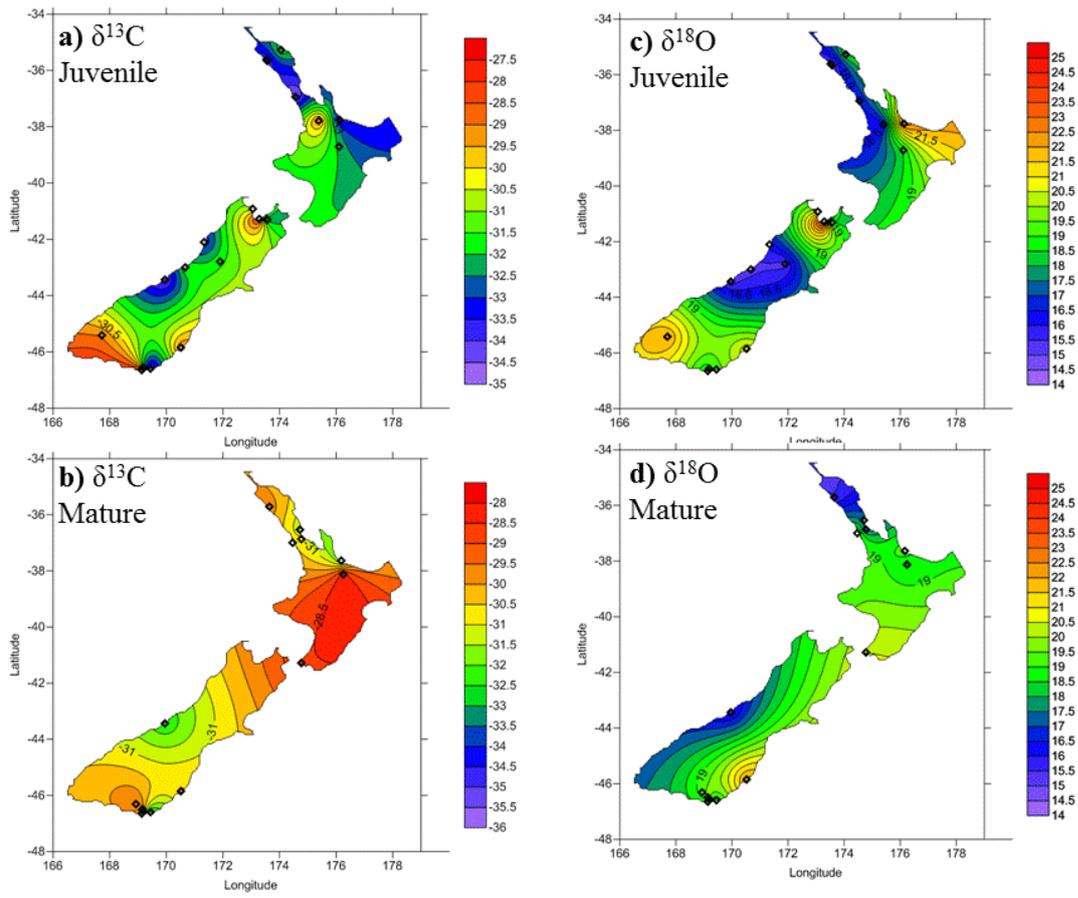
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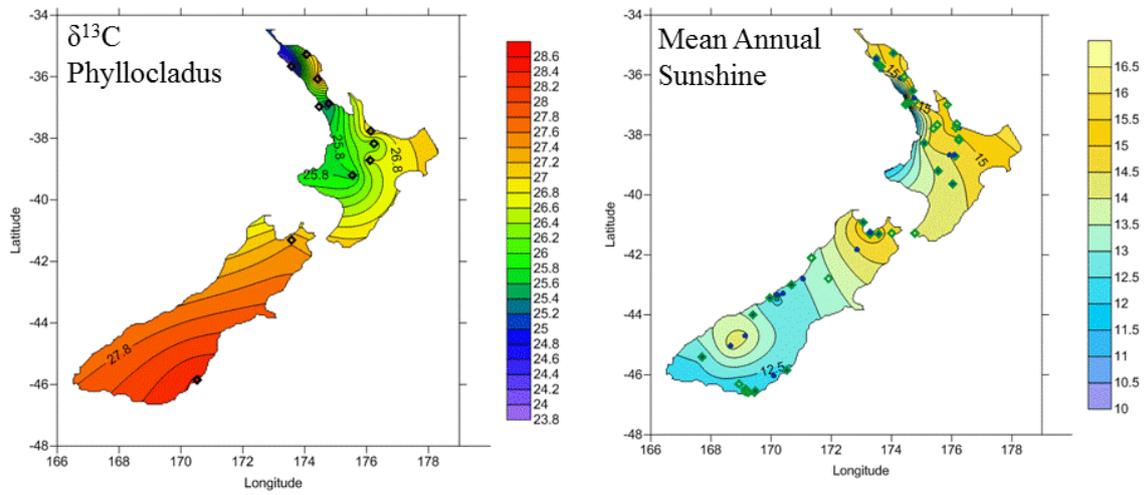


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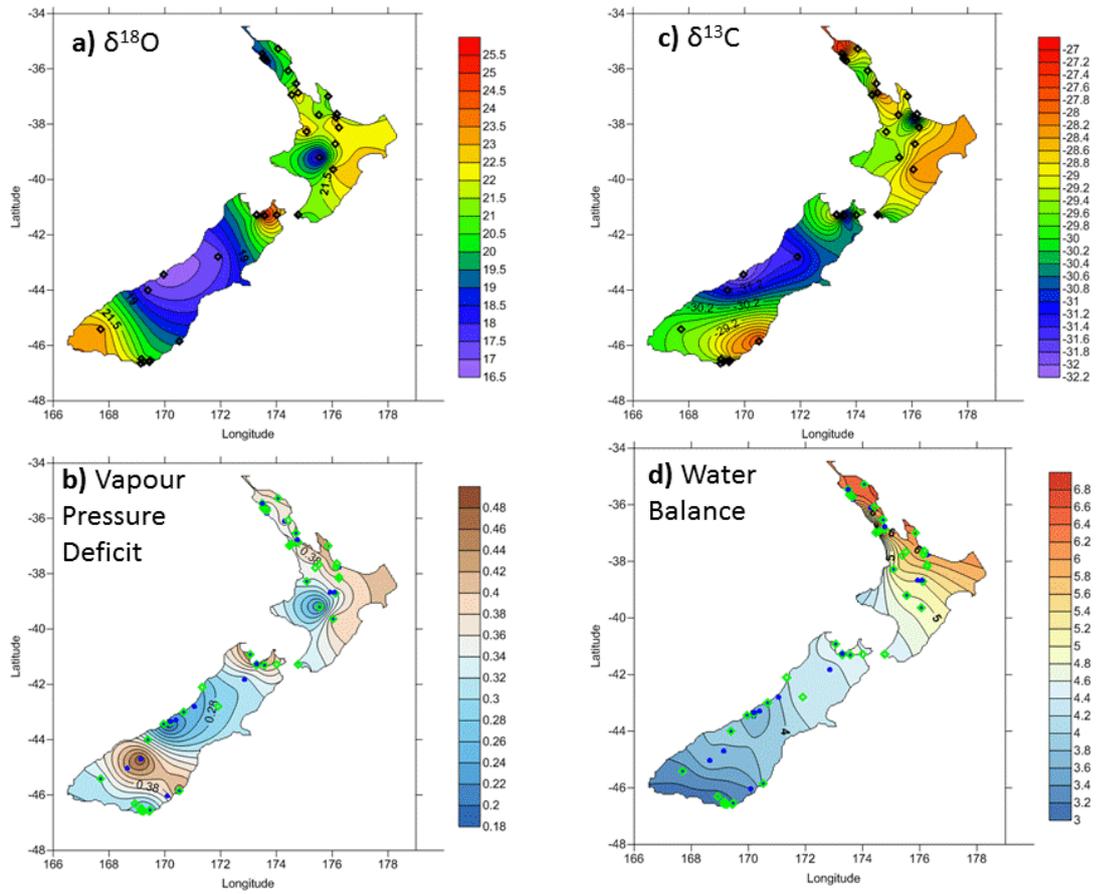
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| Data set | Isotope | Parameter | Direction of correlation | Strength of correlation | p value (significance) | n |
|----------|---------|-----------|--------------------------|-------------------------|------------------------|---|
| juvenile *d. cupressinum* | $\delta^{13}C$ | Water balance | negative | 0.16 | 0.092 | 19 |
| | | VPD | positive | 0.16 | 0.094 | |
| | $\delta^{18}O$ | Water balance | negative | 0.38 | $7.81 \times 10^{-3}$ | 17 |
| | | VPD | positive | 0.44 | $3.77 \times 10^{-3}$ | |
| mature *d. cupressinum* | $\delta^{13}C$ | no significant correlation | | | | 12 |
| | $\delta^{18}O$ | no significant correlation | | | | 10 |

**Table 2:** *Statistics for juvenile and mature d. cupressinum*

| Data set | Isotope | Parameter | Direction of correlation | Strength of correlation | $p$ value (significance) | $n$ |
|---|---|---|---|---|---|---|
| Podocarpus | $\delta^{13}C$ | Water balance | negative | 0.47 | $8.77 \times 10^{-4}$ | 38 |
| | | VPD | positive | 0.22 | 0.003 | |
| | $\delta^{18}O$ | Water balance | negative | 0.40 | $1.51 \times 10^{-4}$ | 31 |
| | | MAS | positive | 0.16 | 0.01 | |
| | | VPD | positive | 0.26 | $3.55 \times 10^{-3}$ | |
| d. cupressinum | $\delta^{13}C$ | Water balance | negative | 0.12 | 0.055 | 31 |
| | | VPD | positive | 0.10 | 0.079 | |
| | $\delta^{18}O$ | Water balance | negative | 0.33 | $1.68 \times 10^{-3}$ | 27 |
| | | VPD | positive | 0.37 | $7.01 \times 10^{-4}$ | |
| p.ferruginea | $\delta^{13}C$ | Elevation | negative | 0.26 | 0.074 | 14 |
| | $\delta^{18}O$ | MAT | positive | 0.28 | 0.06 | 13 |
| | | MAS | positive | 0.35 | 0.03 | |
| | | Water balance | negative | 0.32 | 0.04 | |
| | | VPD | positive | 0.70 | $1.73 \times 10^{-4}$ | |
| d. dacrydioides | $\delta^{13}C$ | Water balance | negative | 0.13 | 0.162 | 16 |
| | | VPD | positive | 0.25 | 0.048 | |
| Phyllocladus | $\delta^{13}C$ | MAS | negative | 0.20 | 0.143 | 12 |

*Table 1*: statistics for segregated data.