1 **Four years of experimental warming do not modify the interaction between subalpine**

2 **shrub species**

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23 **Abstract**

24 Climate warming can lead to changes in alpine plant species' interactions through the

25 amelioration of environmental conditions. Consistent with the stress-gradient hypothesis, many

26 studies have shown that release from environmental stress can lead to increased competition.

27 However, most of these studies were based on neighbour removal experiments, whereas the

28 response of natural communities has received less attention. We explored the effects of four

29 years of experimental warming with open-top chambers on *Vaccinium myrtillus* stands with

30 different neighbouring shrub species at the Pyrenean treeline. Our aim was to find possible

31 shifts in the interaction between *V. myrtillus* and its neighbours following warming that were

32 demonstrated through changes in *V. myrtillus* performance. We examined the effects of

33 warming on above-ground growth parameters, below-ground biomass and the C and N content

34 and isotope composition of *V. myrtillus* growing in pure stands, in stands mixed with *Vaccinium*

35 *uliginosum*, and in stands mixed with *Rhododendron ferrugineum*. We also analysed variations

36 in soil N pools and rhizospheric soil C/N ratios, and evaluated the effects of warming on the

37 neighbouring *V. uliginosum* in mixed plots. Our results showed that warming induced positive

38 changes in the above-ground growth of *V. myrtillus*, but not below-ground, while *V. uliginosum*

39 did not respond to warming. *Vaccinium myrtillus* performance did not differ between stand

40 types under increased temperatures, which indicates that warming did not induce any shifts in

41 the interaction between *V. myrtillus* and its neighbours. These findings contrast with many

42 studies in which species interactions changed when environmental conditions were modified,

43 and shows that the interaction between our study species may not be altered with warmer

44 temperatures at the Pyrenean treeline.

45

46

Introduction

47

48   In the recent decades, climate warming and land-use change (i.e. abandonment of

49   extensive grazing) have led to shrub encroachment processes in the treeline ecotone (Dullinger

50   et al. 2003, Rundqvist et al. 2011, Ropars and Boudreau 2012). The forecasted global air

51   temperature increase of 1.0–3.7 ºC by the end of the century (Collins et al. 2013) could

52   accelerate these processes. This could have a dramatic impact on Arctic and alpine tundra

53   ecosystems due to shifts in community composition and potential feedbacks to warming, such

54   as decreasing albedo or reducing radiative cooling at night or through the inputs of more

55   recalcitrant litter in the ecosystem (Hobbie 1996, Cornelissen et al. 2007, Myers-Smith et al.

56   2011, D'Odorico et al. 2013).

57   Many studies in Arctic and alpine ecosystems have shown the need to carry out species-

58   specific studies to understand vegetation changes with warming, since co-occurring species may

59   differ in their responses to increasing temperatures (Kudo and Suzuki 2003, Klanderud 2008,

60   Anadon-Rosell et al. 2014, Little et al. 2015, Yang et al. 2015). However, when studying

61   vegetation responses to temperature increase, it is also important to consider plant–plant

62   interactions, since they are one of the main drivers of community dynamics (Callaway and

63   Walker 1997). The stress-gradient hypothesis (SGH; Bertness and Callaway 1994) postulates

64   that competition is the major selective force in habitats with more benign environmental

65   conditions, whereas facilitation dominates in more severe environments. In fact, many studies in

66   cold regions across the globe have shown that plant interactions shift from facilitation to

67   competition as temperature increases, or in the opposite direction when temperature decreases

68   (Shevtsova et al. 1997, Choler et al. 2001, Klanderud 2005, Pugnaire et al. 2015, Wheeler et al.

69   2015, Olsen et al. 2016, amongst others). Nevertheless, most of these studies involved plant

70   removal experiments, and studies focusing on the effects of temperature changes on plant

71   interactions within natural communities are scarce (but see Dormann et al. 2004).

72   Shrubs are major components of tundra ecosystems. Amongst them, clonal dwarf shrub

73   species are of great importance in terms of Arctic and alpine vegetation cover, structure and

74   functionality. They present a complex network of subterranean rhizomes bearing fine roots, and

75   producing individual above-ground ramets. Thus, the below-ground system of clonal shrubs is

76   essential for their persistence and vegetative expansion, as well as an important source of soil

77   carbon (Cornelissen et al. 2014). Changes in the below-ground structure of dominant clonal

78   shrubs could translate into major changes in the community and ecosystem structure and

79   composition. Consequently, the study of below-ground responses to warming is an essential part

80   of the complex responses to temperature increase in Arctic and alpine areas. However, the

81   impacts of below-ground sampling and the difficulty encountered when attempting to identify

82   and separate roots from different species, together with the compromise of having studies

83   running for the longest term possible, explain why warming experiments including both above-

84   and below-ground plant measurements are less common (but see Hollister and Flaherty 2010

85   and Yang et al. 2015, amongst others).

86   Global warming can also have strong impacts on N mineralization, with effects on

87   nitrogen availability and, ultimately, plant growth (Bardgett and Wardle 2010). Several studies

88   in cold ecosystems have found an increase in the N pool size with warming (Chapin et al. 1995,

89   Hartley et al. 1999, Dijkstra et al. 2010, Dawes et al. 2011, Bai et al. 2013), which has been

90   related to an increase in the mineralization and decomposition processes at higher temperatures.

91   Since co-occurring species show different N preferences and N-acquisition strategies (Körner et

92   al. 2003, Pornon et al. 2007), shifts in the N pools can lead to changes in their niches that alter

93   their interactions.

94   *Vaccinium myrtillus* is a key species forming shrub patches that colonize subalpine and

95   alpine grasslands in the Pyrenees, where it grows close to the upper altitudinal limit of its

96   distribution (Bolòs et al. 2005), experiencing low temperatures and short growing seasons.

97   Warmer temperatures could favour the growth and expansion of this species in the treeline

98   ecotone, as has already been reported in a soil warming experiment in the Alps (Dawes et al.

99   2011, Anadon-Rosell et al. 2014) and in other warming experiments in the Arctic tundra

100   (Rinnan et al. 2009, Taulavuori et al. 2013). On the other hand, in line with the SGH, an

101   amelioration of the environment could induce changes in the interaction between this species

102   and its neighbours towards increased competition. Despite the numerous studies focusing on *V.*

103   *myrtillus* in tundra ecosystems, to our knowledge the effects that warming may have on the

104   interaction of this species with its neighbours have not been reported. Moreover, the previously

105   mentioned experiments on *V. myrtillus* have mainly focused on its above-ground responses to

106   warming, whereas below-ground responses have mostly been ignored.

107   At the treeline in the Central Pyrenees, *V. myrtillus* grows in pure patches (stands

108   hereafter) or in mixed stands together with *Vaccinium uliginosum* ssp. *microphyllum,* or

109   *Rhododendron ferrugineum*. The objective of this study was to investigate the above- (AG) and

110   below-ground (BG) responses of *V. myrtillus* to four years of passive warming and whether

111   warming induced changes in the interaction between *V. myrtillus* and its neighbouring shrub

112   species. For this purpose we assessed *V. myrtillus* phenology, AG and BG biomass, carbon and

113   nitrogen contents and isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$), soil inorganic N pools (ammonium

114   and nitrate) and nitrogen and carbon availability in the rhizosphere in different types of *V.*

115   *myrtillus* stands subjected to warming treatments. We hypothesized that warming would (i)

116   benefit *V. myrtillus* AG and BG growth, but that it would also (ii) increase the competition with

117   its neighbouring species, which would be manifested as a positive AG and BG growth response

118   of *V. myrtillus* to warming in pure stands but not in mixed stands, and also by a greater increase

119   in the soil inorganic N pool under warming in pure stands than in mixed stands.

120

121   **Materials and methods**

122   *Study area*

123   The study site was located at Eth Corrau des Machos, in the buffer zone of the Aigüestortes and

124   Estany de Sant Maurici National Park (Central Pyrenees, Catalonia, 31N 329, 472), on a N-

125   facing 10-15º steep slope at 2250 m a.s.l. The vegetation consisted of *Festuca eskia* Ramond ex

126   DC. and *Nardus stricta* L. grasslands mixed with patches of dwarf shrub heath dominated by

127   *Vaccinium myrtillus* L., *Vaccinium uliginosum* subsp. *microphyllum* (Lange) Tolm. and

128   *Rhododendron ferrugineum* L. The mean annual precipitation and mean annual temperature for

129   the study period (2010–2013) were 1223.1 mm and 2.7 °C, respectively. The mean monthly

130   precipitation and the mean temperature for the main months of the growing season

131   (June−August) were 99.1 mm and 10.2 ºC (obtained from a meteorological station at a nearby

132   location: La Bonaigua, 6.3 km away from the study site and at a similar altitude, run by the

133   Meteorological Service of Catalonia).

134

135   *Experimental design*

136   In July 2010 we established 30 plots of 1.1 m^2 combining a temperature and a coexistence

137   treatment. In 15 plots we placed an open-top chamber (OTC) similar to the model used in the

138   International Tundra Experiment (ITEX; Marion et al. 1997), which increased summer air

139   temperature by 1.1 ºC (measured by *ibuttons* placed at ground level); the other 15 plots served

140   as controls for temperature. Within each temperature treatment, five plots were assigned to pure

141   *Vaccinium myrtillus* stands (M stands), five to mixed stands of *V. myrtillus* and *V. uligiosum*

142   subsp. *microphyllum* (hereafter *V. uliginosum*; U stands) and five to mixed stands of *V. myrtillus*

143   and *Rhododendron ferrugineum* (R stands). The distance between two plots ranged from one to

144   a few metres (< 20 m), always ensuring that the studied patches were independent.

145

146   *Phenology and community composition*

147   In 2011 we labelled six *V. myrtillus* ramets per plot, which we monitored during the growing

148   seasons of 2011 and 2012 for a phenological survey. We recorded the following phenophases:

149   winter state, bud swelling, bud bursting, leaf expansion, shoot elongation, vegetative state, leaf

150   colour change, leaf shedding, leafless state and shoot winter colouring (brown-red coloration).

151   We visited the plots *ca.* once a month starting after snowmelt until late Autumn, when ramets

152   were leafless, and we recorded the presence of different phenophases in the six marked ramets.

153   We assigned an ordinal numeric code to all phenophases and calculated the average numeric

154   code per plot as the average score of the six ramets at each visit.

155   Plant community composition within the study plots was first recorded in 2011, by

156   estimating the cover of the main plant groups in each plot, i.e. shrubs and grasses. This was re-

157   assessed in September 2013 before the end of the experiment (Supplementary material

158   Appendix 1 Table A1).

159

160   *AG and BG biomass*

161   On the 3rd September 2013 we harvested five *V. myrtillus* ramets per plot (not corresponding

162   with those phenologically surveyed) plus five *V. uliginosum* ramets in U plots. We also dug out

163   their rhizomes (down to *ca.* 20 cm long) and the roots attached and collected six soil cores of 12

164   cm length x 4 cm diameter in each plot. Soil samples were kept in sealed plastic bags in a cool

165   box until freezing in the lab. Two of these cores were used for BG biomass measurements at the

166   plot scale; two were used for measurements of soil nitrate and ammonia content; and the other

167   two were used for rhizosphere carbon and nitrogen content and isotope composition analyses.

168   The two soil cores obtained for the same purpose from each plot were pooled together, therefore

169   we had one composite soil sample per plot for each type of measurement.

170   Once in the lab, we measured the ramet height of both *Vaccinium* spp. and counted the

171   scars left by the buds in each ramet to estimate their age. Then, we separated leaves, new shoots

172   (i.e. shoots grown in 2013), rhizomes and roots, and dried them at 60 ºC for 48 hours. Leaves

173   and new shoots were weighed for AG biomass measurements and subsequently used for

174   nitrogen and carbon content and isotope composition analyses. Rhizomes and roots were only

175   used for nitrogen and carbon content and isotope composition analyses, since BG biomass was

176   measured at the plot scale on material obtained from the soil cores. Since *Vaccinium myrtillus* is

177   a clonal plant with a long and complex rhizome network, we carried out BG biomass

178   measurements referring to a specific soil volume to make comparisons between warming

179   treatments and stand types possible. Soil cores for BG biomass measurements were sieved to

180   separate rhizomes, coarse roots (≥ 1 mm diameter) and fine roots (< 1 mm diameter). We dried

181   them in the oven at 60 ºC for 48 h and weighed them for BG biomass analyses.

182

183   *Carbon and nitrogen content and isotope composition*

184   For the analyses of C and N content and isotope composition of leaves, new shoots, rhizomes

185   and roots, we pooled together the material from all the harvested ramets of each plot for each

186   *Vaccinium* spp. Then we ground the material and weighed *ca.* 1 mg subsamples in small tin

187   capsules. The nitrogen and carbon contents of samples were determined using an elemental

188   analyzer (EA1108, Series 1; Carbo Erba Instrumentazione, Milan, Italy). The carbon and

189   nitrogen isotope composition of samples were determined using a Flash 1112 Elemental

190   Analyzer (Carbo Erba, Milan) coupled to an IRMS Delta C isotope ratio mass spectrometer

191   through a Conflo III Interface (Thermo-Finnigan, Germany). The results of carbon isotope

192   analyses are reported in per thousand (‰) on the relative δ-scale as $\delta^{13}C$, and refer to the

193   international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

194     $$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} \right) - 1 \qquad \text{(Eq. 1)}$$

195   where R is the $^{13}C/^{12}C$ ratio.

196   Carbon isotope discrimination ($\Delta^{13}C$) of shoot TOM (total organic matter) was

197   calculated from δ_a and δ_p (Farquhar et al. 1989) as:

198     $$\Delta^{13}C = \frac{\delta_a - \delta_p}{\delta_p + 1} \qquad \text{(Eq. 2)}$$

199   where a and p refer to air and plant, respectively.

200   Nitrogen results were also expressed in δ notation ($\delta^{15}N$) using international secondary

201   standards of known $^{15}N/^{14}N$ ratios (IAEA N_1 and IAEA N_2 ammonium sulphate and IAEA NO_3

202   potassium nitrate) relative to N_2 in air:

203     $$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} \right) - 1 \qquad\qquad (Eq.\ 3)$$

204   where R is the $^{15}N/^{14}N$ ratio.

205   All EA-IRMS analyses were performed at the CCiT of the University of Barcelona. The

206     $\delta^{13}C$ of CO_2 of the air and the $\delta^{15}N$ of the bulk soil were analysed in 2013 and were ca. -10.91‰

207   and ca. 7.33 ‰, respectively.

208

209   *Soil inorganic nitrogen pool*

210   Nitrate concentrations were measured following the UV method described by Kaneko et al.

211   (2010) by measuring the absorbance of KCl extracts from soils at 220 nm and 260 nm

212   wavelengths. Soil ammonia concentrations were measured by the conversion of ammonium into

213   the intense blue indophenol complex (IPC) using salicylate, following the methods used by

214   Kempers and Kox (1989).

215

216   *Rhizospheric soil analyses*

217   We carefully selected rhizomes and roots from the two soil cores collected for rhizosphere

218   analyses and separated the soil that was attached using a small paint brush. We ground the soil

219   and weighed *ca.* 3.5 mg subsamples in small tin capsules and analysed its carbon and nitrogen

220   content and isotope composition following the same procedure as for plant tissues.

221

222   *Statistical analyses*

223   We tested the effects of warming and stand type on *V. myrtillus* phenology, ramet height and

224   AG biomass using linear mixed effects models fitted with the restricted maximum likelihood

225   estimation method (REML). We included warming and stand type as fixed factors and plot as a

226   random factor. We used the same models for *V. uliginosum* variables, but in this case we only

227   used warming as a fixed factor. To test the effects of warming and stand type on the carbon and

228   nitrogen content and isotope composition of the different AG and BG tissues, BG biomass, soil

229   nitrate and ammonia contents and rhizosphere carbon and nitrogen content and isotope

230   composition we used simple linear model functions. We included ramet age as a covariate in

231   plant analyses when it was significant to account for possible age effects on our growth-related

232   response variables. This included the models for *V. myrtillus* height and AG biomass (except for

233   dry weight per shoot unit), plus the models for number of shoots and dry weight per shoot unit

234   for *V. uliginosum*. We tested for significance with analysis of variance tests and we graphically

235   evaluated the assumptions of normality and homoscedasticity of residuals (Zuur et al. 2009).

236   We double-checked with Shapiro and Bartlett tests when the visual evaluation of graphs was

237   difficult. We log-transformed data when necessary to satisfy these assumptions. Moreover,

238   when homoscedasticity of residuals was not met, we used the varIdent structure (Zuur et al.

239   2009) to account for the heterogeneity of variances among factor levels. In *V. uliginosum*

240   analyses, when both normality and homoscedasticity were not met, we used the non-parametric

241   Wilcoxon test. We considered effects significant at $P < 0.05$ and marginally significant at $0.05 >$

242   $P < 0.10$ to account for the relatively low replication. When we found significant differences

243   between stand types, we carried out Tukey HSD post hoc tests to determine those factor levels

244   that differed significantly. We performed all the analyses with R 3.1.2 (R Core Team 2015). For

245   linear mixed effects models we used the nlme package (Pinheiro et al. 2008); for graphical

246   evaluation of model assumptions we used the lattice package (Sarkar 2008); and for multiple

247   comparisons we used the multcomp package on linear mixed effects models (Hothorn et al.

248   2008) and the agricolae package on simple linear models (de Mendiburu 2010).

249

250   **Results**

251   *Phenology*

252   Warming advanced early-season vegetative phenology through an earlier onset of bud burst and

253   leaf expansion (see Supplementary material Appendix 1 Fig. A1). On the 21st May 2011 (day of

254   year, DOY, 141), *V. myrtillus* ramets in unwarmed plots were at the bud swelling phase,

255   whereas buds in ramets of warmed plots had already started bursting ($F_{1,24}$ = 3.92, P = 0.059). In

256   2012, *V. myrtillus* ramets in warmed plots were already expanding their leaves on the 14th June

257   (DOY 166), whereas ramets in unwarmed plots were still in the bud burst phenophase ($F_{1,24}$ =

258   6.59, P = 0.017). Monitoring later in the season for both years did not show any other

259   significant differences between warming treatments (see Supplementary material Appendix 1

260   Fig. A1 for visit dates). We only found significant differences between stand types (regardless

261   of the warming treatment) in September 2011, when ramets in pure plots were already shedding

262   the leaves whereas ramets in the other two stand types had just started changing their colour

263   prior to leaf shedding ($F_{2,24}$ = 9.31, P = 0.001). This advancement in senescence in pure plots

264   with respect to the other stand types was especially obvious in warmed plots (marginally

265   significant interaction for coexistence x warming, $F_{2,24}$ = 3.13, P = 0.062).

266

267   *Age and AG biomass of* Vaccinium *species*

268   Our age analyses confirmed that there were no differences in *V. myrtillus* ramet age between

269   warming treatments ($F_{1,24}$ = 0.16, P = 0.696) nor between stand types ($F_{2,24}$ = 1.89, P = 0.173).

270   Likewise *V. uliginosum* did not show differences in ramet age between warming treatments ($F_{1,6}$

271   = 0.01, P = 0.930).

272   After four years of warming, *V. myrtillus* ramets were 15% taller in warmed plots than

273   in unwarmed plots. There were no differences in ramet height between stand types or an

274   interaction between warming and stand type (Table 1). Similarly, there was no warming effect

275   on *V. uliginosum* height ($F_{1,6}$ = 0.07, P = 0.802).

276   *Vaccinium myrtillus* leaf biomass per ramet did not differ between warming treatments

277   (Table 1, Fig. 1a) but new shoot biomass was higher under warming than in control plots (Fig.

278   1b). The total above-ground biomass per ramet was also higher in warmed plots than in

279   unwarmed plots (Table 1, Fig. 1c). There were no differences between stand types or a stand

280   type x warming interaction for *V. myrtillus* AG biomass (Table 1). There were no differences

281   between warming treatments in terms of *V. uliginosum* leaf biomass ($F_{1,6}$ = 2.77, P = 0.147),

282   new shoot biomass ($F_{1,6}$ = 0.04, P = 0.849) or total AG biomass ($F_{1,6}$ = 0.39, P = 0.554,

283 Supplementary material Appendix 1 Fig. A2), although we found contrasting effects of

284 warming on the dry weight per shoot and the number of new shoots. Dry weight per shoot in *V.*

285 *uliginosum* was higher inside the OTCs than in control plots ($F_{1,6} = 6.42$, $P = 0.044$), whereas

286 the number of new shoots was higher in ramets from unwarmed plots ($F_{1,6} = 14.81$, $P = 0.009$).

287

288 Vaccinium myrtillus *BG biomass*

289 There were no effects of warming on *V. myrtillus* BG biomass (Table 2, Fig. 2). We only found

290 differences in rhizome and coarse root biomass between stand types. Plots with *R. ferrugineum*

291 showed lower rhizome biomass per soil volume than in the other two stand types (Table 2, Fig.

292 2a). Plots with *V. uliginosum* showed marginally significant greater coarse root biomass than

293 pure populations (Table 2, Fig. 2b). Fine root biomass did not differ between stand types (Table

294 2, Fig. 2c). We did not find any warming x stand type interaction for any of the BG

295 compartments analysed.

296

297 *Carbon and nitrogen content and isotope composition of AG and BG plant fractions*

298 Carbon concentration in *V. myrtillus* tissues was similar across warming treatments and stand

299 types for leaves, shoots and roots. Rhizomes, however, had greater carbon content under

300 warming than in control plots (Table 3, Fig. 3), which was not related to any rhizome biomass

301 increase under warming (see above). Carbon concentration values of *V. uliginosum* new shoots,

302 rhizomes and roots did not show any response to warming, but there was a marginally

303 significant positive effect of warming on leaf C concentration (Table 4, Fig. 5).

304 The δ^{13}C of *V. myrtillus* and *V. uliginosum* tissues did not differ between warming

305 treatments (Fig. 3, 5) but we found significant differences in the δ^{13}C of *V. myrtillus* tissues

306 between stand types. *Vaccinium myrtillus* δ^{13}C was lower in plots with *R. ferrugineum* than in

307 the other two situations of coexistence for leaves (only marginally significant), shoots and

308 rhizomes. There were no significant differences between stand types for the δ^{13}C composition of

309 roots (Table 3, Fig. 3), or any warming x stand type interaction.

310   There was no warming effect on the nitrogen content and δ^{15}N of any of the *V. myrtillus*

311   tissues, and only a very marginally significant interaction between warming and stand type in

312   the N content of *V. myrtillus* rhizomes, which was higher in control plots than in warmed plots

313   in U stands (Table 3, Fig. 4). However, we found significant differences between stand types.

314   Leaf N content was higher in R stands than in U stands, but this was not the case for any of the

315   other plant fractions. Leaf and shoot δ^{15}N values were higher in M stands than in the other two

316   stand types, which is consistent with a previous study carried out in the area (Anadon-Rosell et

317   al. in prep.). Finally, rhizome δ^{15}N values were also higher in M stands than in the other two

318   stand types, but only significantly higher than in R stands (Table 3, Fig. 4).

319   *Vaccinium uliginosum* shoots showed a significantly lower N content under warming

320   than in control plots, but this was associated with an increase in leaf N content under warming

321   (although the latter was not significant). δ^{15}N values did not differ significantly between

322   warming treatments (Table 4, Fig. 5).

323

324   *Soil inorganic N pools and rhizosphere C and N*

325   Soil nitrate content decreased by 36% in warmed plots compared with unwarmed plots ($F_{1,24}$ =

326   5.87, P = 0.023, Fig. 6a), but the ammonia content remained similar between warming

327   treatments (F = 0.45, P = 0.508, Fig. 6b). As a consequence, the nitrate/ammonia ratio

328   decreased by 27% under warming with respect to control conditions. There was no difference

329   between stand types or any interaction between warming and stand type for any of the two N

330   forms analysed.

331   The rhizosphere C/N ratio did not differ between warming treatments. However, it

332   differed between stand types, as it was higher in U stands than in the other two ($F_{2,24}$ = 7.99, P =

333   0.002, Supplementary material Appendix 1 Fig. A3). Both rhizosphere soil C and N content

334   were significantly higher in U stands than in R and M stands ($F_{2,24}$ = 5.81, P = 0.009 and $F_{2,24}$ =

335   3.64, P = 0.042, respectively), but the difference in the C content was greater than the

336   difference in N (data not shown). There was no significant warming x stand type interaction on

337   the rhizosphere C/N ratio ($F_{2,24}$ = 0.89, P = 0.422), but the high dispersion in the data could

338   have masked possible differences between warming treatments in U stands. Neither warming

339   nor stand type or their interaction had any effects on rhizospheric soil δ^{13}C and δ^{15}N values ($P >$

340   0.28).

341

342   **Discussion**

343   This study provides evidence that four years of passive warming did not lead to changes in the

344   interaction between *V. myrtillus* and its neighbours at our treeline study site. *Vaccinium*

345   *myrtillus* slightly benefitted from increased temperatures regardless of whether or not it was

346   growing with a neighbouring species, or the identity of this neighbour.

347   According to the stress-gradient hypothesis (Bertness and Callaway 1994), we expected

348   *V. myrtillus* to perform worse in warmed plots with neighbours than in pure stands due to

349   increased competition caused by the amelioration of the environmental conditions with

350   warming. However, our results indicate that, although the applied experimental warming of 1.1

351   ºC was sufficient to improve *V. myrtillus* growth conditions, it was not sufficient to change the

352   outcome of the interactions between *V. myrtillus* and its neighbouring shrubs. This contrasts

353   with previous studies in which the interaction between plant species shifted when temperatures

354   changed (both naturally and experimentally). Dormann et al. (2004) found that the interaction

355   between the rush *Luzula confusa* and the deciduous shrub *Salix polaris* changed with warming

356   in favour of *S. polaris* in Svalbard. In a removal experiment in Finse, Norway, Klanderud and

357   Totland (2005) found that the removal of the neighbour species negatively affected *Thalictrum*,

358   but not when the latter was inside an OTC, indicating that warming could affect the interaction

359   between these species. Callaway et al. (2002) also reported evidence of a shift from facilitation

360   at higher elevation sites to competition at lower elevation sites when removing neighbours of

361   target individuals at 11 different mountain sites across the world. In addition, a study in

362   seminatural grasslands across precipitation and temperature gradients in southern Norway found

363   increased competitive interactions with increasing temperature (Olsen et al. 2016). Most of

364   these studies consisted of removal experiments, which provide very important ecological and

365 functional information about the community and species studied (see review by Díaz et al.

366 2003). However, despite their numerous advantages and outcomes, removal experiments cannot

367 avoid the disturbance caused by the extraction of the desired species. In contrast, our approach

368 was based on naturally established populations and species, and the avoidance of any

369 disturbance caused by removing part of this natural assemblage allowed us to assess the natural

370 response of our study species to warming. According to our results, species interactions can be

371 less responsive to warming when studying them in their natural conditions and distribution.

372   *Vaccinium myrtillus*, a species that is responsive to temperature change (Rinnan et al.

373 2009, Taulavuori et al. 2013, Anadon-Rosell et al. 2014, Dawes et al. 2015), showed positive

374 growth responses to warming regardless of the neighbouring species. Its AG biomass was

375 increased under warming, which could be the result of a longer growing period caused by the

376 advancement of its early-vegetative phenology. A previous study on this same species in the

377 Swiss Alps showed that its increase in growth after six years of soil warming with heating

378 cables was not related to a longer growing period (Anadon-Rosell et al. 2014). The above-

379 ground phenology of ramets could be more affected by warming through OTCs than by soil

380 warming, since air temperature at canopy level may be higher in OTCs than in plots with

381 warmed soil. However, it could also be that other factors related to warming but not directly

382 linked to a longer growing season influenced *V. myrtillus* growth in our study, such as direct

383 warming effects on photosynthetic rates (Heskel et al. 2013, Fu et al. 2015) or higher N uptake

384 rates with increased temperatures, which would be supported by the lower soil nitrate values

385 found at our study plots under warming. Although there was no increase in the nitrogen content

386 of our study ramets with warming that would support the notion of increased N uptake rates, the

387 N increase could be diluted by the increased growth under warming. In fact, a study in the

388 Swedish Lapland by Hartley et al. (1999) found no response of *V. myrtillus* and *V. uliginosum*

389 leaf N concentrations to warming despite increased mineralization rates, which the authors

390 attributed to an increase in N in their study plants through increased biomass.

391   In our study, the BG biomass of *V. myrtillus* did not change with warming in any of the

392 stands analysed. Thus, the increase in *V. myrtillus* AG growth did not result in increased BG

393   growth. Moreover, BG interactions between our study species did not change with warming

394   either. Although OTCs mainly increase ground-level and air temperature, they have been found

395   to slightly increase soil temperature at 5 cm depth (Hollister et al. 2006) and even at 10 cm in

396   steppe ecosystems in Northern Mongolia (Sharkhuu et al. 2013). Hollister and Flaherty (2010)

397   found a BG biomass increase in *Salix rotundifolia* at a tundra site in Alaska after 3–4 years of

398   warming with OTCs, but Shaver et al. (1998) found no BG biomass increase after 6–9 years of

399   passive warming in another Alaskan wet sedge tundra, indicating contrasting BG responses to

400   warming depending on the study site and community composition. *Vaccinium myrtillus* can

401   expand its rhizomes several metres below-ground (Flower-Ellis, 1971); therefore our warming

402   treatment might not have reached a large enough area to capture the potential response of a

403   whole functional unit to warming, or a possible transfer of assimilates from AG parts might

404   have been diluted by the complex BG network of this species.

405   *Vaccinium uliginosum* has been shown to be less plastic in response to warming than *V.*

406   *myrtillus* (Richardson et al. 2002, Kudo and Suzuki 2003, Anadon-Rosell et al. 2014). This can

407   be attributed to the better adaptation of *V. myrtillus* to warmer temperatures, as shown by its

408   lower altitudinal range (Bolòs et al. 2005). Although the dry weight of new individual shoots of

409   *V. uliginosum* increased with warming, the number of shoots decreased, probably as a trade-off,

410   which led to an overall lack of AG biomass response to warming in this species. In fact, only

411   the leaf carbon content of *V. uliginosum* increased with warming, but the statistical significance

412   was marginal, and was not accompanied by any other changes in the performance of this shrub.

413   Our study provides evidence that although *V. myrtillus* is more responsive to warming than *V.*

414   *uliginosum*, when they coexist *V. myrtillus* does not benefit more from warming than when it

415   grows in pure stands.

416   The slightly lower N content in *V. myrtillus* rhizomes in warmed plots than in

417   unwarmed plots when coexisting with *V. uliginosum* indicates an increase in competition for N

418   with warming. In fact, competition for N in mixed stands of these two species under natural

419   conditions was found in a previous study at the same site (Anadon-Rosell et al. in prep.). The

420   higher rhizosphere C/N ratio in these mixed stands than in the other stand types further supports

421   the idea of the occurrence of natural competition for N without warming, which would be

422   increased with the higher temperatures inside the OTC. A study in the Swiss Alps found a

423   positive response of warming in *V. gaultherioides* (synonym of *V. uliginosum* subsp.

424   *microphyllum*) leaf N content but only a short-term positive response in *V. myrtillus* (Dawes et

425   al. 2011). On the other hand, contrasting effects were found in an experiment in the Swedish

426   Lapland, where *V. myrtillus* showed a positive response in terms of leaf N content to warming

427   whereas *V. uliginosum* responded negatively (Richardson et al. 2002). However, none of these

428   studies tested the effects of warming on these species' interactions and the ultimate effects they

429   would have on their performance. Our study demonstrates that although warming increased the

430   competition for N between *V. myrtillus* and *V. uliginosum*, this ultimately did not outbalance the

431   positive growth response of *V. myrtillus* to warming.

432   A meta-analysis of experimental warming effects on N pools in terrestrial ecosystems

433   based on 51 studies showed that warming increased N mineralization rates and N pools across

434   different ecosystem types (Bai et al. 2013). However, in our experiment soil nitrate decreased

435   with warming (regardless of the stand type). This could be explained by greater nitrate uptake

436   rates promoted by increased temperatures, since temperature has been proven to be a modulator

437   of plant N assimilation in previous studies (Laine et al. 1994, Volder et al. 2000). The lack of an

438   increase in the N concentration of plant tissues could be due to a dilution effect caused by the

439   greater biomass or to increased nitrate assimilation by other species (especially grasses, due to

440   their abundance), which were not assessed in this study. Another explanation for the reduced

441   nitrate concentrations in the OTCs could be earlier consumption of nitrate through an advanced

442   root phenology promoted by warming (Sullivan and Welker 2005, Nord and Lynch 2009). A

443   study by Rinnan et al. (2009) in a tundra heath dominated by *V. myrtillus*, *V. vitis-idaea* and

444   *Empetrum nigrum* in southwestern Finland found no increase in soil N content with warming

445   either, but there was a decrease in the soil NH_4^+ concentration inside the OTCs. The authors

446   argued that this reduction could reflect the increased efficiency of N uptake with warming. The

447   differing responses in the N form between that study and ours might reflect the preferential use

448   of a specific N form at different sites with different community composition, or a greater

449   availability of nitrate than ammonia at our study site.

450   In conclusion, four years of experimental warming had no effect on the interaction

451   between *V. myrtillus* and *V. uliginosum* or *R. ferrugineum*. *Vaccinium myrtillus* showed a

452   positive AG growth response to warming regardless of the neighbouring species, but no BG

453   responses were found. Although warming seemed to increase the competition for N between the

454   two *Vaccinium* species, their overall performance was not affected. This study shows that

455   species' interactions are not altered by warming at this treeline site and, thus, the performance of

456   these populations will probably not change due to mild warming in the near future.

457

467

468   **References**

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470   soil warming experiment at the alpine treeline. - Plos One doi:

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600

601

602 **Tables**

603 Table 1. Results of ANOVA for effects of warming and stand type on *Vaccinium myrtillus*

604 above-ground (AG) growth and biomass parameters at the ramet scale. Significant ($P > 0.05$)

605 and marginally significant ($0.01 > P > 0.05$) effects are in bold.

AG Variable	Factor/covariable	df	*F*	*P*
Height	Age	1, 118	26.47	**<0.001**
	Warming	1, 24	5.33	**0.030**
	Stand type	2, 24	1.89	0.173
	Stand type x warming	2, 24	0.16	0.857
Leaf biomass	Age	1, 118	12.00	**0.001**
	Warming	1, 24	2.38	0.136
	Stand type	2, 24	1.49	0.246
	Stand type x warming	2, 24	1.20	0.320
New shoots biomass	Age	1, 118	8.68	**0.004**
	Warming	1, 24	5.02	**0.035**
	Stand type	2, 24	1.38	0.271
	Stand type x warming	2, 24	0.37	0.693
Total AG biomass	Age	1, 118	32.41	**<0.001**
	Warming	1, 24	4.74	**0.040**
	Stand type	2, 24	1.77	0.193
	Stand type x warming	2, 24	0.16	0.857
No. of shoots	Age	1, 114	23.45	**<0.001**
	Warming	1, 23	0.26	0.613
	Stand type	2, 23	0.84	0.443
	Stand type x warming	2, 23	0.83	0.450
Dry weight/shoot	Age	-	-	-
	Warming	1, 23	3.85	**0.062**
	Stand type	2, 23	2.59	0.100
	Stand type x warming	2, 23	0.55	0.584

606

607

608   Table 2. Results of ANOVA for effects of warming and stand type on stand below-ground (BG)

609   biomass. Significant ($P > 0.05$) and marginally significant ($0.01 > P > 0.05$) effects are in bold.

BG Variable	Factor	df	F	P
Rhizome biomass	Warming	1, 24	2.98	0.100
	Stand type	2, 24	6.93	**0.004**
	Stand type x warming	2, 24	0.03	0.970
Coarse roots biomass	Warming	1, 19	0.91	0.352
	Stand type	2, 19	3.04	**0.071**
	Stand type x warming	2, 19	0.30	0.745
Fine roots biomass	Warming	1, 24	0.88	0.358
	Stand type	2, 24	0.41	0.667
	Stand type x warming	2, 24	0.57	0.575

610

611

612   Table 3 . Results of ANOVA for effects of warming (W) and stand type (ST) on the C and N

613   concentration and isotope composition (δ^{13}C, δ^{15}N) of *Vaccinium myrtillus* leaves, new shoots,

614   rhizomes and roots. *F*-values and *P*-values (in parentheses) are given. Significant ($P > 0.05$) and

615   marginally significant ($0.01 > P > 0.05$) effects are in bold. Between-groups degrees of freedom

616   were 1 for W, 2 for ST and 2 for ST x W. Within-groups degrees of freedom were 24, except for

617   root N concentration and rhizome C and N concentration (22) and root δ^{15}N (23).

Fraction	Variable	W	ST	W x ST
Leaves	C concentration	0.93 (0.344)	1.51 (0.242)	1.57 (0.228)
	N concentration	0.02 (0.884)	4.93 (**0.016**)	0.07 (0.931)
	δ^{13}C	0.90 (0.352)	2.72 (**0.086**)	0.85 (0.441)
	δ^{15}N	0.08 (0.780)	10.28 (**0.001**)	0.04 (0.960)
New shoots	C concentration	1.68 (0.207)	0.94 (0.404)	0.94 (0.404)
	N concentration	0.07 (0.793)	0.63 (0.540)	0.77 (0.472)
	δ^{13}C	0.07 (0.794)	8.16 (**0.002**)	1.85 (0.179)
	δ^{15}N	0.33 (0.571)	9.39 (**0.001**)	0.00 (1.000)
Rhizomes	C concentration	5.71 (**0.026**)	0.33 (0.723)	0.7 (0.509)
	N concentration	0.05 (0.829)	0.46 (0.637)	2.57 (**0.099**)
	δ^{13}C	0.42 (0.522)	8.78 (**0.001**)	0.03 (0.972)
	δ^{15}N	0.02 (0.884)	6.53 (**0.005**)	0.08 (0.921)
Roots	C concentration	0.21 (0.653)	0.43 (0.656)	0.56 (0.578)
	N concentration	1.69 (0.207)	0.62 (0.545)	1.19 (0.323)
	δ^{13}C	1.59 (0.218)	0.15 (0.860)	2.53 (0.101)
	δ^{15}N	0.21 (0.650)	2.04 (0.153)	0.19 (0.826)

618

619

620 Table 4. Results of ANOVA or Wilcoxon tests for the effects of warming on the C and N

621 concentration and isotope composition (δ^{13}C, δ^{15}N) of *Vaccinium uliginosum* leaves, new

622 shoots, rhizomes and roots. Significant ($P > 0.05$) and marginally significant ($0.01 > P > 0.05$)

623 effects are in bold.

Fraction	Parameter	df	*F/W*	*P*
Leaves	C concentration	1, 6	3.89	**0.096**
	N concentration	1, 6	2.57	0.160
	δ^{13}C	1, 6	0.01	0.934
	δ^{15}N	-	$W = 6$	0.686
New shoots	C concentration	-	$W = 6$	0.686
	N concentration	1, 6	13.91	**0.010**
	δ^{13}C	1, 6	0.03	0.871
	δ^{15}N	1, 6	0.35	0.575
Rhizomes	C concentration	1, 6	0.99	0.357
	N concentration	-	$W = 8$	1.000
	δ^{13}C	-	$W = 8$	1.000
	δ^{15}N	-	$W = 8$	1.000
Roots	C concentration	1, 6	0.00	0.997
	N concentration	1, 6	0.93	0.373
	δ^{13}C	1, 6	1.08	0.339
	δ^{15}N	1, 6	3.86	0.097

624

625

626 **Figure legends**

627 Figure 1. *Vaccinium myrtillus* AG biomass in different stand types (ST) and warming treatments

628 in September 2013 (W; $n = 5$, means + 1 SE are shown). Asterisks (**) show significant

629 differences at $0.05 > P > 0.01$. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R.*

630 *ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

631

632   Figure 2. BG biomass per soil volume at the plot scale in different stand types (ST) and

633   warming treatments in September 2013 (W; $n = 5$, means + 1 SE are shown). Asterisks show

634   significant differences (* marginally significant differences at $0.1 > P > 0.05$; ** significant

635   differences at $0.05 > P > 0.01$; *** significant differences at $P < 0.01$). M: *V. myrtillus* pure

636   stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V.*

637   *uliginosum* stands.

638

639   Figure 3. Carbon concentration and δ^{13}C of *V. myrtillus* tissues in different stand types (ST) and

640   warming treatments in September 2013 (W; $n = 5$, mean + 1 SE for concentrations and mean - 1

641   SE for isotope compositions are shown). Asterisks show significant differences (* marginally

642   significant differences at $0.1 > P > 0.05$; ** significant differences at $0.05 > P > 0.01$; ***

643   significant differences at $P < 0.01$). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R.*

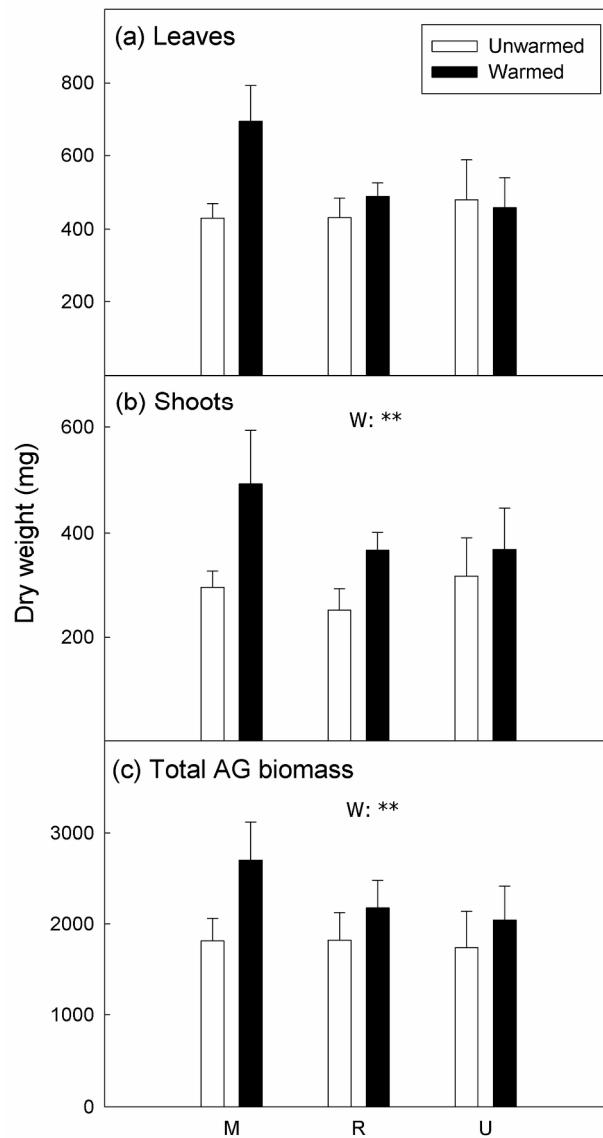644   *ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

645

646 Figure 4. Nitrogen concentration and $\delta^{15}N$ of *V. myrtillus* tissues in different stand types (ST)

647 and warming treatments in September 2013 (W; $n = 5$, mean + 1 SE for concentrations and

648 mean - 1 SE for isotope compositions are shown). For N concentration of rhizomes and roots

649 see the righthand Y-axis scale. Asterisks show significant differences (* marginally significant

650 differences $0.1 > P > 0.05$; *** significant differences at $P < 0.01$). M: *V. myrtillus* pure stands;

651 R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum*

652 stands.

653

654 Figure 5. Carbon and nitrogen concentrations and δ^{13}C and δ^{15}N of *V. uliginosum* tissues under

655 different warming treatments in September 2013 (W; *n* = 4, mean + 1 SE for concentrations and

656 mean - 1 SE for isotope compositions are shown). Asterisks (***) show significant differences

657 between warming treatments at *P* < 0.01.

658

659 Figure 6. Soil nitrate and ammonia content in our study plots in different stand types (ST) and

660 warming treatments in September 2013 (W; $n = 5$, means + 1 SE are shown). Asterisks (***)

661 show significant differences at $P < 0.01$. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with

662 *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

Figure 1. *Vaccinium myrtillus* AG biomass in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, means + 1 SE are shown). Asterisks (**) show significant differences at 0.05 > P > 0.01. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.
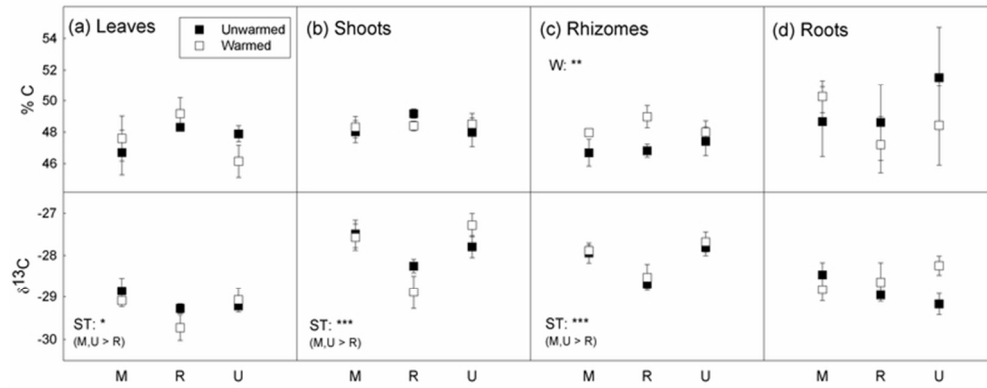150x283mm (300 x 300 DPI)
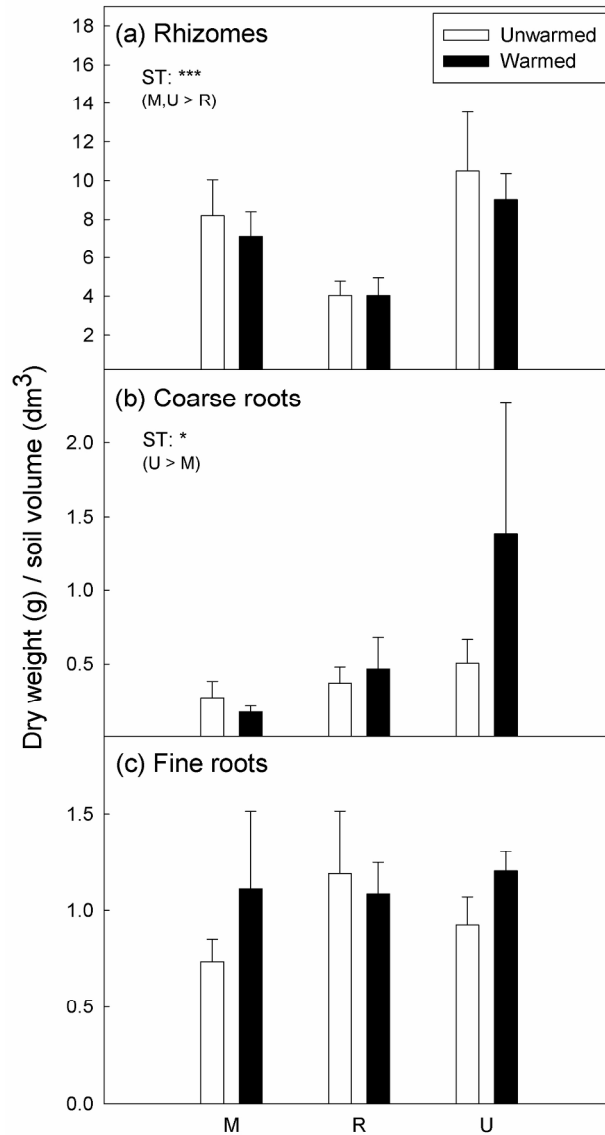
Figure 2. BG biomass per soil volume at the plot scale in different stand types (ST) and warming treatments in September 2013 (W; n = 5, means + 1 SE are shown). Asterisks show significant differences (* marginally significant differences at 0.1 > P > 0.05; ** significant differences at 0.05 > P > 0.01; *** significant differences at P < 0.01). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.
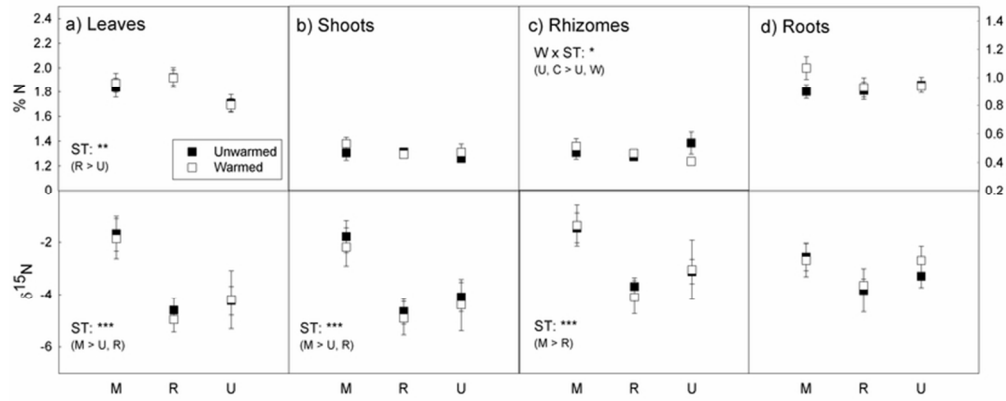
150x281mm (300 x 300 DPI)

Figure 3. Carbon concentration and $\delta^{13}C$ of *V. myrtillus* tissues in different stand types (ST) and warming treatments in September 2013 (W; *n* = 5, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). Asterisks show significant differences (* marginally significant differences at 0.1 > P > 0.05; ** significant differences at 0.05 > P > 0.01; *** significant differences at P < 0.01). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.
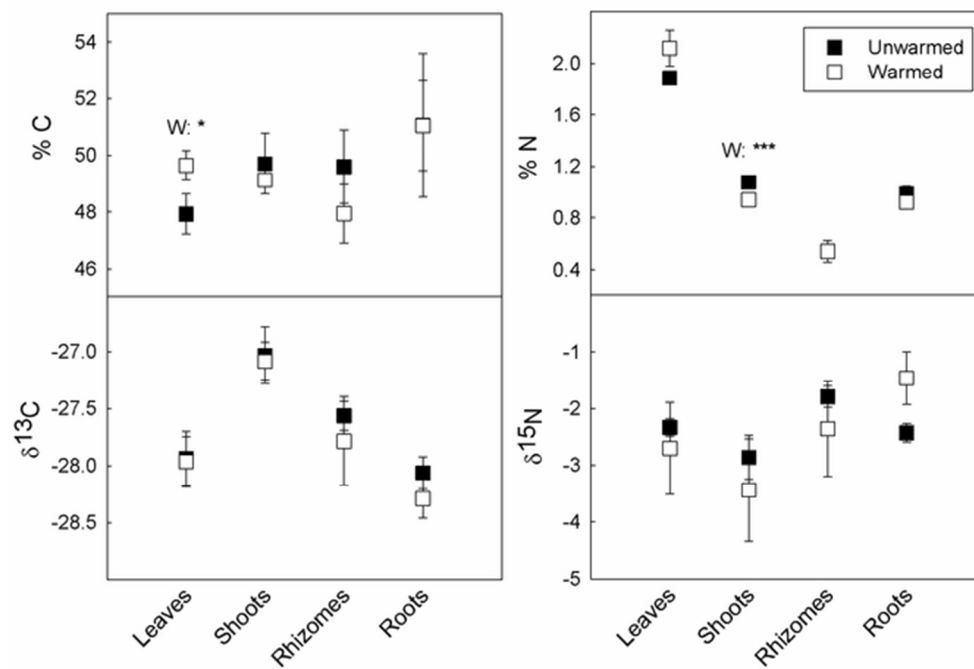67x27mm (300 x 300 DPI)

Figure 4. Nitrogen concentration and $\delta^{15}N$ of V. myrtillus tissues in different stand types (ST) and warming treatments in September 2013 (W; $n = 5$, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). For N concentration of rhizomes and roots see the righthand Y-axis scale. Asterisks show significant differences (* marginally significant differences $0.1 > P > 0.05$; *** significant differences at $P < 0.01$). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

69x29mm (300 x 300 DPI)

Figure 5. Carbon and nitrogen concentrations and $\delta^{13}C$ and $\delta^{15}N$ of *V. uliginosum* tissues under different warming treatments in September 2013 (W; *n* = 4, mean + 1 SE for concentrations and mean - 1 SE for isotope compositions are shown). Asterisks (***) show significant differences between warming treatments at P < 0.01.
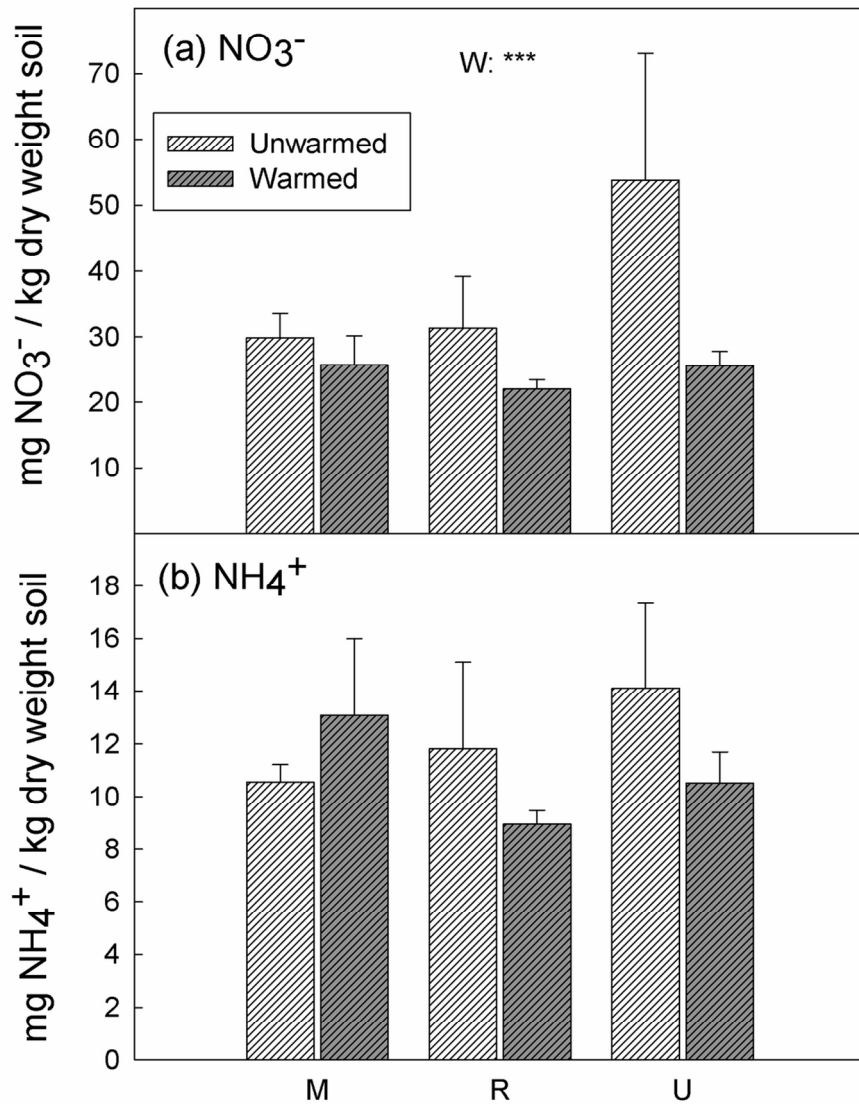57x41mm (300 x 300 DPI)

Figure 6. Soil nitrate and ammonia content in our study plots in different stand types (ST) and warming treatments in September 2013 (W; n = 5, means + 1 SE are shown). Asterisks (***) show significant differences at P < 0.01. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.
104x136mm (300 x 300 DPI)
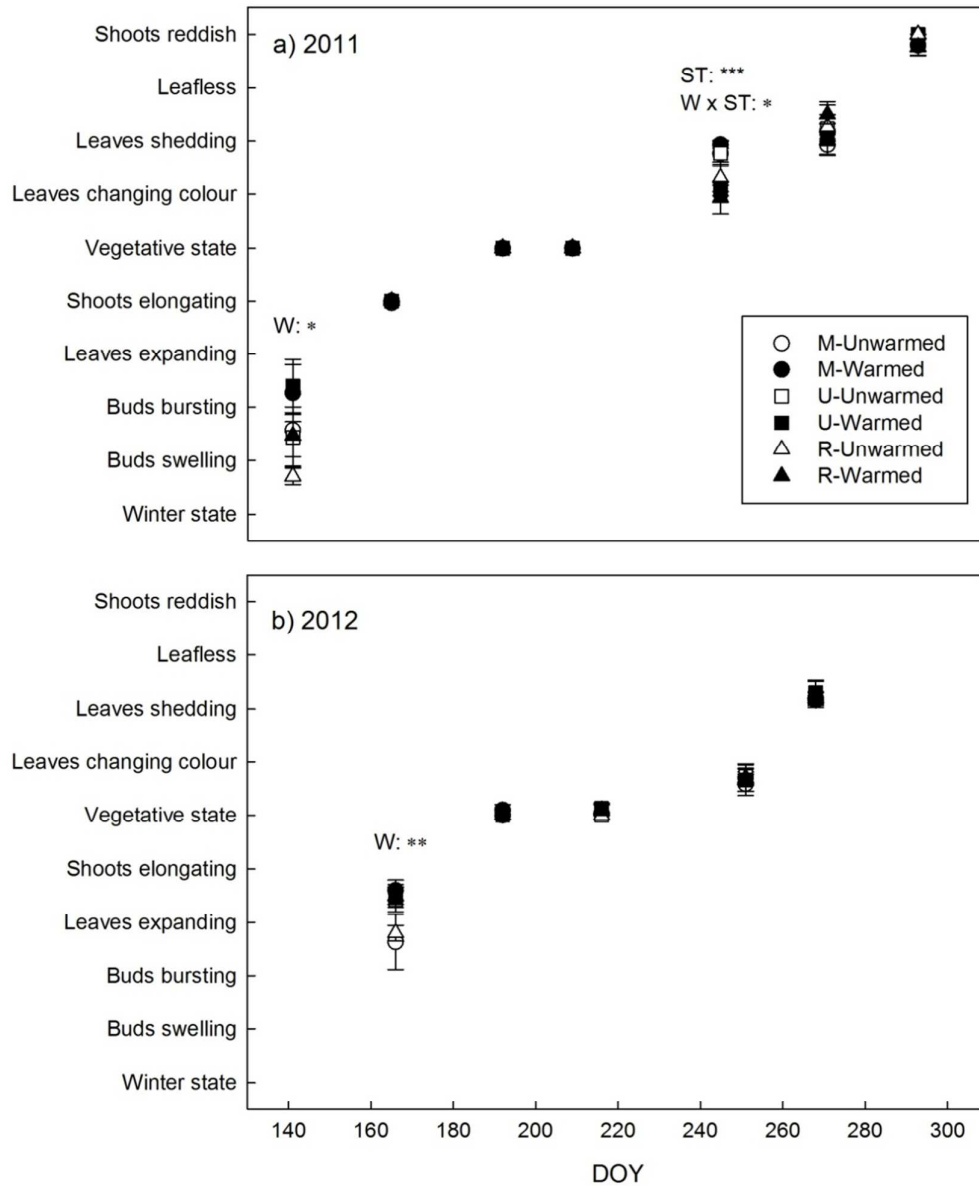
Supplementary Material Appendix 1

Figure A1. Vegetative phenology (mean phenophase calculated as the average numeric phenophase of six ramets per plot in each visit, day of year: DOY) of *Vaccinium myrtillus* at the study plots during the growing seasons of 2011 and 2012 under different stand types (ST) and warming treatments (W; $n = 5$, means +1SE are shown). Asterisks show differences between treatments (* marginally significant differences $0.1 > P > 0.05$; ** significant differences at $0.05 > P > 0.01$; *** significant differences at $P < 0.01$). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.
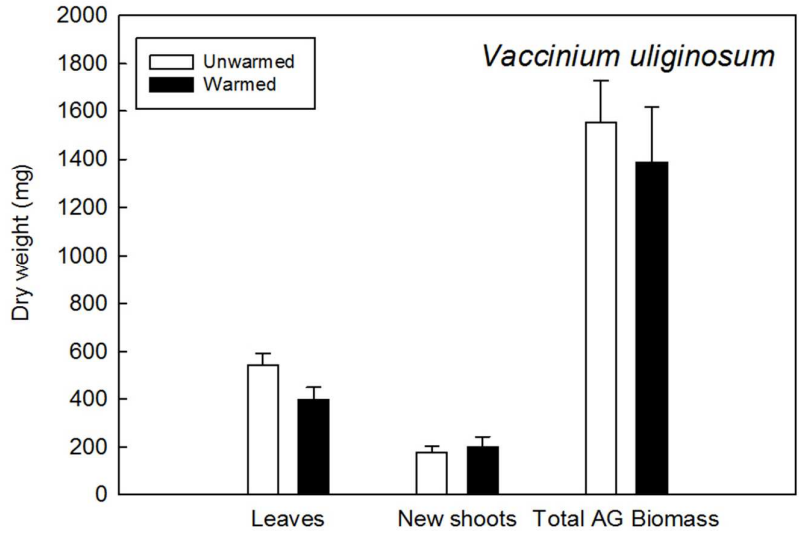
Figure A2. *Vaccinium uliginosum* above-ground (AG) biomass under different warming treatments in September 2013 ($n = 4$, means + 1 SE are shown). There were no significant differences between warming treatments.
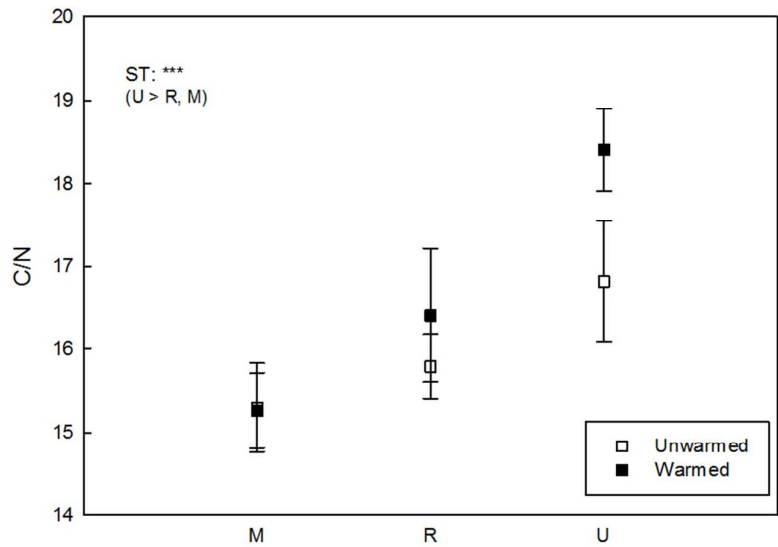

Figure A3. Rhizospheric soil C/N ratio for the different stand types (ST) and warming treatments in September 2013 (W; $n = 5$, means ± 1 SE are shown). Asterisks (***) show significant differences at $P < 0.01$. M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum* stands; U: *V. myrtillus* mixed with *V. uliginosum* stands.

Table A1. Percent cover of the three main shrubs and grasses for the different stand types and warming treatments (*n* = 5, means are shown) in 2011 (left) and 2013 (right). Forbs were only recorded in 2013.

Stand type	Warming treatment	*V. myrtillus*	*V. uliginosum*	*R. ferrugineum*	Grasses	Forbs
M	Control	70 69	0 0	0 0	28 20	- 8
(V. myrtillus)	Warmed	68 83	0 0	0 0	25 17	- 8
U	Control	52 43	48 44	0 2	14 23	- 7
(V. myrtillus + *V. uliginosum)*	Warmed	41 34	66 68	0 0	15 13	- 9
R	Control	43 44	0 1	73 82	20 17	- 6
(V. myrtillus + *R. ferrugineum)*	Warmed	40 49	0 0	77 75	12 11	- 6