

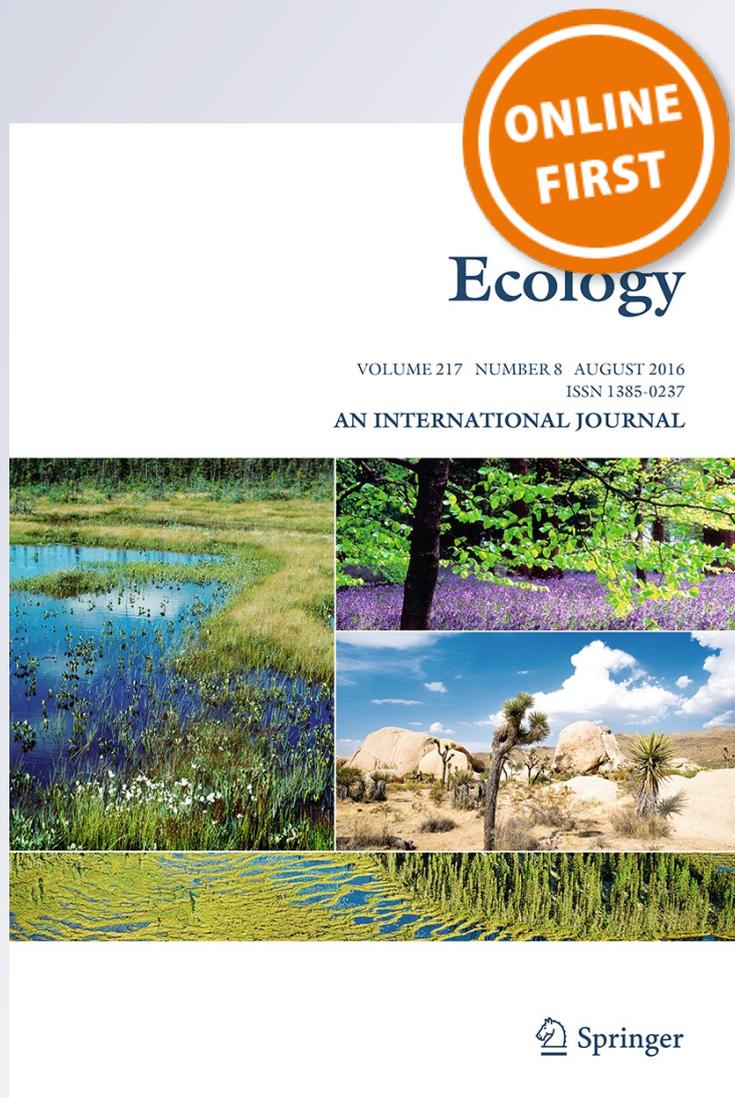
Vaccinium myrtillus stands show similar structure and functioning under different scenarios of coexistence at the Pyrenean treeline

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Abstract Plant–plant interactions are key drivers of treeline dynamics. At the Pyrenean treeline, the dwarf shrub *Vaccinium myrtillus* grows in pure stands or in mixed stands with *Vaccinium uliginosum* or *Rhododendron ferrugineum*. They form sparse shrub patches that colonize subalpine grasslands, having dramatic impacts on their structure and functioning. We investigated the role of the two co-occurring shrubs as possible modulators of the structure and performance of *V. myrtillus* in the Central Pyrenees. We analysed biomass, growth, functional parameters, age distribution, N and C concentrations and isotope compositions

($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of *V. myrtillus* ramets in pure stands, and stands mixed with *V. uliginosum* or *R. ferrugineum*. Volume, above-ground biomass and age of the ramets did not differ between stand types. We found lower $\delta^{13}\text{C}$ values, indicative of lower water-use efficiency (WUE), in leaves and shoots of *V. myrtillus* in stands with *R. ferrugineum* than in pure stands. The N content and $\delta^{15}\text{N}$ of *V. myrtillus* leaves and shoots in pure stands were higher than in mixed stands, pointing to a competition for N in mixed stands. Our results indicate that *V. myrtillus* competes for nutrients with its neighbours, but neither this competition nor the lower WUE affect its above-ground performance. Therefore, the interaction with co-occurring shrubs does not have a major effect on *V. myrtillus* structure and functioning at treeline and, consequently, should not be considered as a key driver on the dynamics of this species in the encroachment of subalpine grasslands.

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Keywords Biomass partition · Competition · Dwarf shrubs · Stable isotope composition · Treeline · Xylem growth rings

Abbreviations

IRMS Isotope ratio mass spectrometry
SPA Shoot photosynthetic area
TOM Total organic matter
 $\delta^{13}\text{C}$ Carbon isotope composition
 $\delta^{15}\text{N}$ Nitrogen isotope composition

Introduction

Plant–plant interactions represent one of the major selective forces driving population and community dynamics (Callaway and Walker 1997). At the treeline ecotone, shrubs have been identified as modulators of ecosystem dynamics (Batllori et al. 2009b; Grau et al. 2012). However, the role of plant–plant interactions in shrub communities has often been disregarded in favour of climate modulators (especially temperature; see the numerous warming experiments carried out at treeline areas, e.g. Michelsen et al. 1996; Xu et al. 2009; Kaarlejärvi et al. 2012; Anadon-Rosell et al. 2014). Shrubs can facilitate tree seedling survival and recruitment because they can exert a nurse effect when tree species are in their most vulnerable life stage (Gómez-Aparicio et al. 2008; Batllori et al. 2009b; Grau et al. 2012). Moreover, interactions between woody species can have a key role in the encroachment processes that have been reported during the last decades in alpine and Arctic tundra ecosystems (Eldridge et al. 2011; Myers-Smith et al. 2011).

In the last decades, the increase in temperature and the progressive abandonment of traditional land use practices (Tappeiner and Cernusca 1993; Gellrich et al. 2008; IPCC 2013) have caused a densification of woody plants at European alpine treelines (Stöcklin and Körner 1999; Camarero and Gutierrez 2004; Kullman 2005; Batllori and Gutiérrez 2008) and the re-colonization of abandoned pastures by shrubs (Didier 2001; Vicente-Serrano et al. 2004; Albert et al. 2008). This shrub encroachment, which is predicted to increase in response to the higher temperatures forecasted for this century (Walker et al. 2006; Meehl et al. 2007; IPCC 2013), plays an important role in the first stadia of tree re-colonization at treeline (Tasser and Tappeiner 2002; Targetti et al. 2010; Ninot et al. 2011). Therefore, studies focusing on shrub species interactions at treeline can provide essential understanding of the functioning of treeline ecosystem dynamics (Smith et al. 2003; Batllori et al. 2009b; Grau et al. 2012; Llambí et al. 2013).

In the subalpine belt of the Pyrenees, *Vaccinium myrtillus* has a substantial role in the transitional ecotone between *Pinus uncinata* forests and alpine grasslands (see Batllori et al. 2009a). In these areas, it grows close to the highest altitudinal limit of its distribution (Bolòs et al. 2005), where stressful

conditions related to low temperatures, wind abrasion, early-season frost and short growing seasons could lead to a prevalence of facilitative plant–plant interactions (Batllori et al. 2009b; Fajardo and McIntire 2011). Some studies in alpine and Arctic ecosystems have reported facilitative interactions between *Vaccinium myrtillus* and co-occurring species (Maillette 1988; Shevtsova et al. 1995), although allelopathic effects of *V. myrtillus* on some conifer species have also been reported (Mallik and Pellissier 2000; Talavera & Ninot, unpublished). At treeline in the Central Pyrenees, *Vaccinium myrtillus* forms sparse patches together with the similarly sized shrub *Vaccinium uliginosum* ssp. *microphyllum* and the taller shrub *Rhododendron ferrugineum*. These shrub communities have an important role on treeline dynamics due to their potential for colonizing subalpine grasslands. However, the role of *V. uliginosum* and *R. ferrugineum* as modulators of *V. myrtillus* population structure and performance and, ultimately, the effects that these interactions can have on treeline dynamics are poorly understood. Furthermore, studies on shrub interactions involving *Vaccinium* species have usually considered neighbour shrubs of similar dimensions (Maillette 1988; Shevtsova et al. 1995, 1997; Gerdol et al. 2000; Brancaloni and Gerdol 2006), whereas the effect of taller shrubs remains mostly unexplored (but see Pornon et al. 2007).

Carbon (C) and nitrogen (N) isotope compositions (i.e. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of plant tissues can be used to determine plant nutrient and/or water status. Natural abundance $\delta^{13}\text{C}$ values have been used to assess plant water-use efficiency (Farquhar et al. 1982), and $\delta^{15}\text{N}$ values may provide insight into the use of different sources of N (Ghashghaie et al. 2003; Tcherkez and Hodges 2008), the niche partitioning for N-acquisition (Pornon et al. 2007; Gundale et al. 2012) and differential isotopic fractionation of N in response to different mycorrhizal N uptake mechanisms (Emmer-ton et al. 2001). Moreover, C and N mass-based concentrations can be used as a measure of nutrient availability and dynamics (Aerts 1996; Killingbeck 1996; Palacio et al. 2007; Baptist et al. 2009; 2013; Muller et al. 2011; Vergutz et al. 2012). Previous studies have shown differences in the N-acquisition and N-use strategies between *V. myrtillus* and co-existing species such as *R. ferrugineum* or *Vaccinium vitis-idaea* (Pornon et al. 2007; Gundale et al. 2012).

Nevertheless, although studies on congeneric species have often shown that trait divergence and niche separation are strategies for coexistence (Maillette 1988; Vander Kloet and Hill 2000; Beltrán et al. 2012; Gundale et al. 2012), this has not been assessed for the co-occurring *V. myrtillus* and *V. uliginosum*.

We aimed to determine whether structure and functioning of *V. myrtillus* stands at the treeline ecotone are affected by the coexistence with *Vaccinium uliginosum* ssp. *microphyllum* (*V. uliginosum* hereafter) and *Rhododendron ferrugineum* and to find out the primary plant–plant interactions driving the performance of *V. myrtillus* in the area. To do so, we measured functional, growth and demographical parameters using growth measures, isotopic analyses and dendroecological techniques. We specifically aimed to answer the following questions: (i) does *V. myrtillus* compete for light with the taller shrub *R. ferrugineum* and, thus, presents greater spread (vertically and horizontally) when co-occurring with it?; (ii) does *R. ferrugineum* exert a facilitative effect on *V. myrtillus*, protecting it from environmental damage (e.g. wind damage and early-spring frost), translating into older, more vigorous *V. myrtillus* ramets in these mixed stands?; (iii) are growth and survival of *V. myrtillus* ramets from stands mixed with *V. uliginosum* comparable to those from monospecific stands due to the structural similarity between these two species?; and finally, (iv) does *V. myrtillus* compete for N with the other two shrubs despite the putative different N-acquisition strategy from *R. ferrugineum* and the suggested niche separation from *V. uliginosum*?

Materials and methods

Study area and species

The study site was located at Eth Corrau des Machos, in the buffer zone of the Aigüestortes and Estany de Sant Maurici National Park (Central Pyrenees, Catalonia, 31 N 329, 472). Sampled plots were located on a N-facing 10–25° steep slope at 2250 m a.s.l., slightly above the current treeline in the area, but with some trees scattered above. Climatic data from a nearby location (La Bonaigua, located 6.3 km away from the study site and at similar altitude) for the period 2007–2012 are a mean annual precipitation of 1154.9 mm and a mean annual temperature of 2.7 °C (Meteorological Service

of Catalonia, see detailed climatic data for the study period 2010–2012 in Fig. S1).

At this site, vegetation was composed of *Festuca eskia* Ramond ex DC. and *Nardus stricta* L. grasslands (Selino-Festucetum eskiae) mixed with patches of dwarf shrub heath dominated by *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. subsp. *microphyllum* (Lange) Tolm. and *Rhododendron ferrugineum* L. These communities are considered as serial stages in the succession to mountain pine (*Pinus uncinata* Ramond ex DC.) forests (Rhododendro-Pinetum uncinatae, i.e. grassland-shrubland-woodland-forest succession). These forests were formerly extensive in the area but are reduced at present due to anthropogenic deforestation.

Vaccinium myrtillus and *V. uliginosum* are both deciduous dwarf shrubs that reach ca. 15–35 cm height at our study site, whereas *R. ferrugineum* is a taller evergreen shrub that reaches 40–50 cm height.

Sampling design

In mid-September 2010, we evaluated the performance of *V. myrtillus* in three different stand types: pure stands (M stands), stands with *V. myrtillus* co-occurring with *V. uliginosum* (U stands) and stands with *V. myrtillus* co-occurring with *R. ferrugineum* (R stands). The percentage cover of each shrub species in the different stand types is shown in Table S1. For each stand type, we established five 20 cm × 20 cm plots, accounting for the small size but high density of the ramets. The distance between two plots ranged from one to a few metres (<20 m) but always ensuring that different plots belonged to different patches. We carried out this study on a ramet basis due to the clonal structure of *V. myrtillus*, which consists of a horizontal network of subterranean rhizomes from which aerial shoots arise (Ritchie 1956; Flower-Ellis 1971). We measured and clipped at ground level all *V. myrtillus* ramets grounded within each plot boundaries, and we subsequently stored them in sealed plastic bags kept in a portable cooler until we arrived at the laboratory.

Stand structure, above-ground growth and plant performance

To have an estimate of the ability of plants to compete for space at a stand scale, we calculated the volume of

all *V. myrtillus* ramets present in each plot. To do so, we measured their height and their canopy diameter (i.e. mean between the maximum and the minimum canopy diameters) and estimated the theoretical volume occupied for each ramet as a canopy cylinder ($V = \pi r^2 h$, Johnson et al. 1988).

To study above-ground biomass partition of individual ramets, we selected 12 ramets per plot when possible (except for one M plot, which only had 11 ramets, and two R plots, which only had 8 and 10 ramets), representative of the wide range of ramet sizes in the plots. We used the last three cohorts of shoots as the sampling unit for biomass allocation comparisons between stand types. Ramets younger than 3-year old were not used for biomass allocation measurements. The following fractions were detached and counted: leaves, new shoots (formed in 2010, Sh1), one-year-old shoots (formed in 2009, Sh2), two-year-old shoots (formed in 2008, Sh3) and main stem (>3-year old). We identified the different cohorts of shoots by the scars left by bud scales after shoot elongation. Before drying, we cut a 1.5 cm segment at the base of the stem of each ramet for counting xylem growth rings to determine ramet age (see below). We dried all the fractions at 70 °C for 72 h and weighed them to the nearest 0.001 g (with a Mettler Toledo PB303 Delta Range scale). We corrected the weight of the main stems by adding the weight of segments of similar dimensions to the part previously cut. For each ramet, we calculated the dry mass of Sh1, Sh2 and Sh3 relative to the two-year-old shoot unit, and we also calculated the total above-ground biomass. Moreover, for each ramet (except for ramets younger than 3 years), we measured the main stem diameter as the mean between the maximum and minimum diameters measured at the base of the stem.

We randomly selected one of the 12 ramets described above in three plots of each stand type and measured length and width of all shoots from the different cohorts to calculate shoot photosynthetic area (SPA). For this, we assumed shoot surface area as that of a tetrahedron, because young shoots of *V. myrtillus* show a tetrahedral shape. The greenness of the shoots gradually decreases as ramets age, but the three last shoot cohorts still show a clear green photosynthetic colour. We carried out linear regressions between photosynthetic area and shoot dry mass separately for each cohort and found positive significant correlations (Sh1: $R^2 = 0.96$, $P < 0.001$; Sh2:

$R^2 = 0.91$, $P < 0.001$; Sh3: $R^2 = 0.85$, $P < 0.001$, Fig. S2). Consequently, we used the obtained regression equations to estimate the photosynthetic area of each shoot cohort per two-year-old shoot unit for the rest of ramets.

Ramet age distribution

The structure and growth of *V. myrtillus* ramets depend on age (Flower-Ellis 1971). Thus, it is important to know and consider ramet age when performing comparative studies between *V. myrtillus* stands. To estimate the age of ramets and to identify differences in the above-ground age structure of different stands, we made cross sections of 20–25 μm thickness from the basal segments of *V. myrtillus* stems using a sledge microtome. Sections were stained with Safranin to emphasize the growth ring structure, subsequently rinsed with ethanol (75 %) for dehydration (Schweingruber and Poschlod 2005, modified) and mounted in DPX. We counted xylem growth rings under the microscope (Olympus CH2) to assess ramet age.

C and N concentration and isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

At the time of harvest, leaves were already senescent, and therefore we established another sampling period in August 2012. Then, we collected *V. myrtillus* leaf and new shoot samples in fifteen plots similar to those established in 2010, i.e. five plots for each stand type, and analysed their C and N concentrations and isotopic compositions. We sampled leaves and new shoots of five ramets per plot and pooled together the material of these ramets for each plot separately, both for leaves and new shoots. Thus, we had five replicates for each stand type and plant fraction. Samples were dried at 60 °C for 72 h, and subsamples were weighed in small tin capsules.

Samples were analysed to determine the C and N isotope compositions using a Flash 1112 Elemental Analyzer (Carbo Erba, Milan) coupled to an IRMS Delta C isotope ratio mass spectrometer through a ConFlo III Interface (Thermo-Finnigan, Germany). Results of C isotope analyses are reported in per thousand (‰) on the relative $\delta^{13}\text{C}$ and refer to the international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1, \quad (1)$$

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

C isotope discrimination ($\Delta^{13}\text{C}$) of shoot TOM (total organic matter) was calculated from δ_a and δ_p (Farquhar et al. 1989) as

$$\Delta^{13}\text{C} = \frac{\delta_a - \delta_p}{\delta_p + 1}, \quad (2)$$

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

N results are also expressed in δ notation ($\delta^{15}\text{N}$) using international secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios (IAEA N_1 and IAEA N_2 ammonium sulphate and IAEA NO_3 potassium nitrate) relative to N_2 in air:

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1, \quad (3)$$

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

N and C contents were determined using an elemental analyser (EA1108, Series 1; Carbo Erba Instrumentazione, Milan, Italy).

In August 2013, we collected air samples with a 50 ml syringe (SGE, Ringwood, Australia) and kept them in 10 ml vacutainers (BD vacutainer, Plymouth, UK) to analyse the $^{13}\text{CO}_2$ isotopic composition at the study site by gas–chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS) according to Nogués et al. (2004). The $\delta^{13}\text{C}$ of CO_2 of the air at the site in the Pyrenees was ca. -10.91 ‰. We also collected soil samples to determine soil N isotope composition at the study site and analysed them following the same technique as mentioned above for plant material. Soil $\delta^{15}\text{N}$ was ca. 7.33 ‰.

All the EA-IRMS and GC-C-IRMS analyses were performed at the CCiT of the University of Barcelona.

Statistical analyses

We used linear mixed effects models fitted with the restricted maximum likelihood estimation method (REML) to test the effects of the different scenarios of coexistence on *V. myrtillus* volume, canopy height and width, biomass partitioning, total above-ground biomass, SPA of each cohort of shoots, ramet age and stem diameter. We included stand type as a fixed factor and plot as a random factor. To account for the possible age effects on response variables, we

included ramet age as a covariate in the analyses of the effects of stand type on individual ramet traits when it was significant, that is for total above-ground biomass, stem diameter and SPA in Sh3. For the N and C concentrations and isotope compositions, we evaluated differences between stand type, plant fraction (leaves and shoots) and their interaction with Type III analysis of variance using the linear model function. We tested for Pearson's correlations between ramet age, stem diameter and total above-ground biomass.

For all statistical analyses, we visually evaluated the assumptions of normality and homoscedasticity of residuals (Zuur et al. 2009) and transformed response variables when necessary to reach these assumptions. When homoscedasticity of residuals was not met, we used the varIdent structure (Zuur et al. 2009) to account for the heterogeneity of variances among factor levels. Effects were considered significant at $P < 0.05$ and marginally significant at $0.05 < P < 0.10$ to account for the relatively low replication. When there were significant differences between stand types, we performed Tukey HSD post hoc tests to determine which factor level means differed significantly. All analyses were performed with R 2.15.2 (R Core Team 2012), using the nlme package (Pinheiro et al. 2008) for linear mixed effects models, the lattice package (Sarkar 2008) for graphical evaluation of the assumptions of the models, the multcomp package (Hothorn et al. 2008) for multiple comparisons on linear mixed effects models and the agricolae package (de Mendiburu 2010) for multiple comparisons on simple linear models.

Results

Stand structure, above-ground growth and performance

Ramet volume (calculated as an elliptical cylinder) did not differ between stand types (Tables 1, 2). Despite the average values for R stands were much higher than for the other two stand types, the great data dispersion in these R stands masked possible significant differences. The average ramet height and ramet canopy width did not differ between the three stand types either (Tables 1, 2), although the trend of higher values but higher dispersion in R stands was also evident.

Table 1 Mean (and standard deviation) for the parameters studied at plot scale (all the ramets in the plot)

Stand type	Ramet volume (cm ³)	Ramet height (cm)	Canopy width (cm)
M	223.04 (111.62)	14.46 (2.71)	3.24 (0.71)
R	1074.93 (1691.77)	15.57 (5.76)	5.89 (4.16)
U	137.07 (66.16)	12.21 (1.41)	3.02 (0.66)

M, *V. myrtillus* pure stands; R, *V. myrtillus* mixed with *R. ferrugineum* stands; U, *V. myrtillus* mixed with *V. uliginosum* stands

Table 2 Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) on *V. myrtillus* volume, height and canopy width studied in all ramets within each plot

Variable	df	F	P
Ramet volume	2, 12	2.07	0.17
Ramet height	2, 12	1.05	0.38
Ramet canopy width	2, 12	2.38	0.14

No significant differences were found

Similarly, total above-ground ramet biomass did not differ significantly between the three stand types. For ramets in M stands was 868.7 ± 195.9 mg (mean \pm SD), in R stands 761.9 ± 635.1 mg and in U stands 507.1 ± 123.6 mg (Table 3). However, we found significant differences in the biomass allocation patterns. *Vaccinium myrtillus* ramets growing with *R. ferrugineum* showed a lower biomass allocation to Sh3

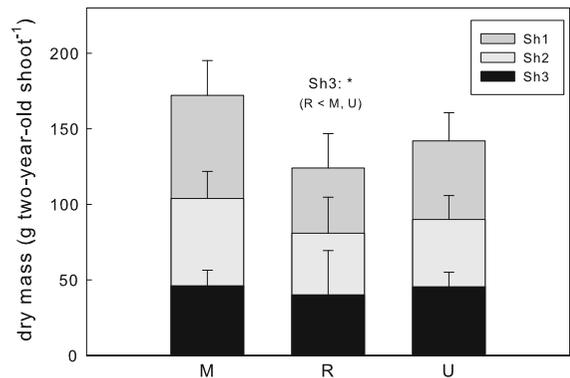


Fig. 1 *Vaccinium myrtillus* biomass allocation to the last three shoot cohorts in the three stand types. The asterisk shows significant differences between stand types ($0.01 < P < 0.05$), referred to Sh3 between R stands and M and U. Data are means + SD, $n = 5$. M, *V. myrtillus* pure stands; R, *V. myrtillus* mixed with *R. ferrugineum* stands; U, *V. myrtillus* mixed with *V. uliginosum* stands. Sh1 new shoots, Sh2 one-year-old shoots and Sh3 two-year-old shoots

Table 3 Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) on *V. myrtillus* biomass partitioning, total above-ground (AG) biomass, shoot photosynthetic area (SPA), age and stem diameter in selected ramets per plot

Variable	Factor/covariable	Df	F	P
Biomass partition				
Sh1	Stand type	2, 12	2.39	0.13
Sh2	Stand type	2, 12	1.86	0.20
Sh3	Stand type	2, 12	3.96	0.04
Total AG biomass	Age covariable	1, 152	100.08	<0.01
	Stand type	2, 12	1.92	0.19
SPA				
Sh1	Stand type	2, 12	2.31	0.14
Sh2	Stand type	2, 12	2.72	0.11
Sh3	Age covariable	1, 139	5.26	0.02
	Stand type	2, 12	4.53	0.03
Age	Stand type	2, 12	0.20	0.82
Stem diameter				
	Age covariable	1, 123	57.91	<0.01
	Stand type	2, 12	0.89	0.44

Significant differences ($P < 0.05$) in bold

Sh1 new shoots, Sh2 one-year-old shoots, Sh3 two-year-old shoots

than ramets in the other stand types (Table 3; Fig. 1). Although the biomass allocation to Sh1 and Sh2 tended to be lower too, differences between stand types were not significant for these two cohorts. Biomass allocation to Sh1 was 14 % higher than to Sh2, which was 9 % higher than Sh3 averaged across all stand types. Stem diameter did not differ between stand types either (Table 3).

SPA is related to the biomass allocation to each fraction. Therefore, it showed a similar pattern than biomass allocation: it was greater in Sh1, followed by Sh2 and Sh3 for the three stand types (Fig. 2). Moreover, SPA of Sh3 was higher in M and U stands than in R stands (Table 3; Fig. 2).

Ramet age distribution

Ramet age did not differ between stand types (Table 3; Fig. 3). The oldest ramet was 12-year old (U stand), but the majority of the ramets of each stand type were younger than 7-year old (70 % in M stands, 68 % in U stands and 61 % in R stands). Ramet age was positively correlated with stem diameter ($R^2 = 0.55$, $P < 0.001$) and total above-ground biomass ($R^2 = 0.53$, $P < 0.001$).

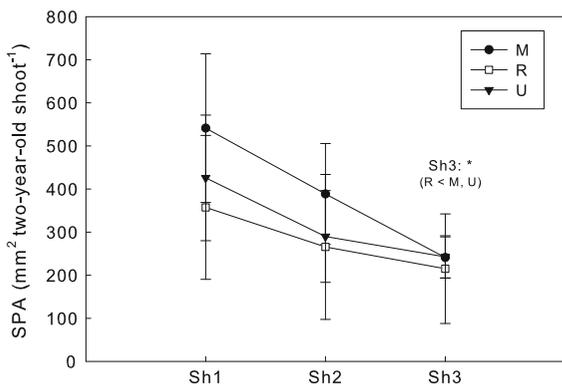


Fig. 2 Shoot photosynthetic area (SPA) of the last three shoot cohorts of *V. myrtillus* ramets in the three stand types. The asterisk shows significant differences between stand types ($0.01 < P < 0.05$), referred to *Sh3* between *R* stands and *M* and *U*. Data are means + SD, $n = 5$. *M*, *V. myrtillus* pure stands; *R*, *V. myrtillus* mixed with *R. ferrugineum* stands; *U*, *V. myrtillus* mixed with *V. uliginosum* stands. *Sh1* new shoots, *Sh2* one-year-old shoots and *Sh3* two-year-old shoots

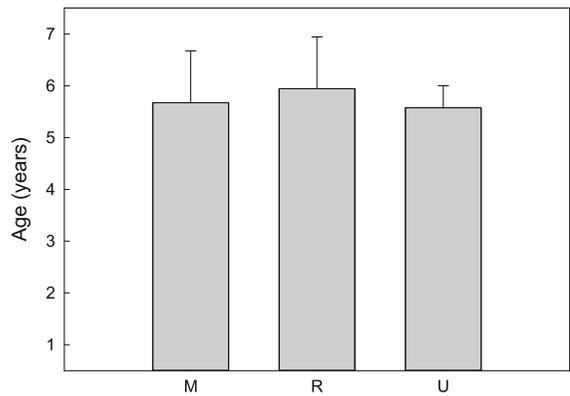


Fig. 3 Ramet age in the three stand types. No significant differences were found. Data are means + SD, $n = 5$. *M*, *V. myrtillus* pure stands; *R*, *V. myrtillus* mixed with *R. ferrugineum* stands; *U*, *V. myrtillus* mixed with *V. uliginosum* stands

C and N concentrations and isotope compositions ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of leaves and new shoots

We found significant differences in the C and N isotopic composition and N concentration of leaves and new shoots of *V. myrtillus* between stand types. $\delta^{13}\text{C}$ values of leaves and new shoots of *V. myrtillus* in pure stands and U stands were higher than those of ramets from R stands (marginally significant, Table 4; Fig. 4a; see Table S2 for ^{13}C discrimination values, $\Delta^{13}\text{C}$), and $\delta^{13}\text{C}$ values of new shoots were higher than values for leaves. The C content of leaves and new shoots did not differ between stand types, and it was higher in new shoots than in leaves (Table 4; Fig. 4b). The N content and $\delta^{15}\text{N}$ of leaves and new shoots of pure stands were higher than those of mixed stands (Table 4; Fig. 4c, d), and the N content was higher in leaves than in new shoots. The interaction between stand type and plant fraction did not have a significant effect on any of the parameters analysed.

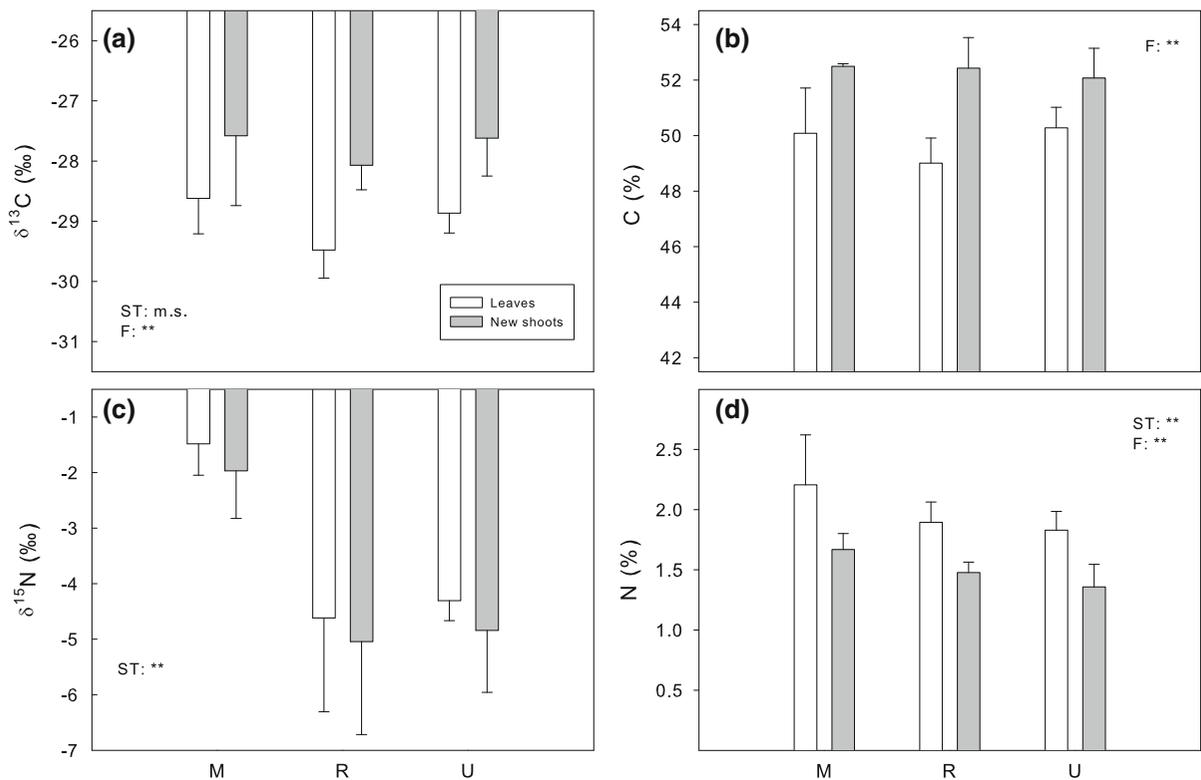
Discussion

Above-ground growth and performance

We did not find major differences in *V. myrtillus* volume, biomass or SPA between the different stand types, which indicates that *V. myrtillus* above-ground growth and performance is similar when it grows in pure and in mixed stands.

Table 4 Results of the analysis of variance tests of the effects of stand type (pure stands, stands mixed with *V. uliginosum* and stands mixed with *R. ferrugineum*) and plant fraction (leavesand new shoots) on *V. myrtillus* C and N isotope compositions ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and concentrations

Variable	Factor	Df	F	P
$\delta^{13}\text{C}$	Stand type	2, 24	2.93	(0.07)
	Fraction	1, 24	26.39	<0.01
	Stand type \times fraction	2, 24	0.20	0.82
$\delta^{15}\text{N}$	Stand type	2, 24	39.09	<0.01
	Fraction	1, 24	1.30	0.27
	Stand type \times fraction	2, 24	0.01	0.99
C concentration	Stand type	2, 24	0.86	0.44
	Fraction	1, 24	45.48	<0.01
	Stand type \times fraction	2, 24	1.58	0.23
N concentration	Stand type	2, 24	6.67	<0.01
	Fraction	1, 24	35.78	<0.01
	Stand type \times fraction	2, 24	0.19	0.83

Significant differences ($P < 0.05$) in bold and marginally significant differences ($0.10 > P < 0.05$) in bold in parentheses**Fig. 4** C and N isotopic compositions (a, c) and C and N concentrations (b, d) of leaves and new shoots of *V. myrtillus* in the three stand types. Data are means + (b, d) or – (a, c) SD. Asterisks (**) show significant differences between situations of coexistence ($P < 0.01$), and marginally significant differencesare indicated by m.s. M, *V. myrtillus* pure stands; R, *V. myrtillus* mixed with *R. ferrugineum* stands; U, *V. myrtillus* mixed with *V. uliginosum* stands; ST, stand type; F, plant fraction. For detailed explanation on the differences between factor level means, see the main text

Nevertheless, the higher values and greater spread of *V. myrtillus* volumes when growing with *R. ferrugineum* as compared to ramets growing with *V. uliginosum* or in pure stands suggest that *V. myrtillus* finds more heterogeneous conditions when growing with *R. ferrugineum*. *Vaccinium myrtillus* might face some competition for light when growing with the taller shrub *R. ferrugineum*, which is the reason why *V. myrtillus* would spread its shoots both laterally and upwards to occupy more space, a plasticity that would allow it to favourably compete to intercept more light (van Kleunen and Fisher 2001; Mourelle et al. 2001; Callaway et al. 2003; Lepik et al. 2005).

Although *V. myrtillus* ramets tended to show a reduced biomass allocation to the three last shoot cohorts in plots with *R. ferrugineum*, differences were only significant in two-year-old shoots, and they were not maintained in the next two shoot cohorts. These results could indicate that *V. myrtillus* experienced less favourable conditions in stands with *R. ferrugineum* in 2008 that caused a lower investment in shoot growth that year. Despite these differences, results for the younger shoot cohorts and total above-ground biomass suggest that the coexistence with the other shrub species does not have major effects on the above-ground growth of *V. myrtillus* in the study area.

The photosynthetic potential of *V. myrtillus* shoots is especially important during the cold season, when ramets are leafless, because the green shoots of this species remain partly active in autumn and spring, and can photosynthesize even under the snow cover (Körner 2003). SPA increased with shoot biomass, and therefore, shoots produced in 2008 also had a lower photosynthetic area in ramets growing with *R. ferrugineum*. However, apart from two-year-old shoots, *V. myrtillus* SPA was similar across stand types, indicating a similar productive potential irrespective of the coexistence with *V. uliginosum* and *R. ferrugineum*.

The lack of major significant differences in these functional and growth parameters, however, needs to be carefully interpreted. Although important differences between stand types were not occurring, some differences could be masked by the high dispersion in some of the studied parameters and the relatively low replication.

Ramet age distribution

The lack of differences between the three stand types in ramet age, together with the above-ground biomass

results, indicates that this species does not encounter a different environment for its ramets growth or turnover when coexisting with the other two shrubs than when growing in pure stands. We did not expect to find differences in the mean ramet age between *V. myrtillus* pure stands and stands with *V. uliginosum*, because the effects of the intraspecific interaction between *V. myrtillus* ramets would be functionally and structurally similar to the effects from the interspecific interaction between ramets of the two *Vaccinium* species (i.e. we did not expect *V. uliginosum* to exert any protection to *V. myrtillus*). However, our results indicate that we cannot assume that the taller *R. ferrugineum* exerts any facilitative effect on *V. myrtillus* ramets development at our study site either. Grau et al. (2013) found that *R. ferrugineum* reduced winter damage to tree seedlings at treeline sites in the Pyrenees. Also in the Pyrenees, we observed that *R. ferrugineum* had some kind of nurse effect on *V. myrtillus* in subalpine grasslands colonized by shrubs and in open shrublands, since the density of ramets growing next to *R. ferrugineum* was higher than far from it (unpublished data). Consequently, we expected to find older *V. myrtillus* ramets in plots with *R. ferrugineum*, but such a nurse effect was not detected at our study site.

All the stands analysed were formed by relatively young ramets, the oldest ones being only 12-year old. This indicates that we are dealing with patches where *V. myrtillus* has recently established or patches where a high above-ground turnover by environmental constrictions or disturbance maintains ramets at a young stage. The below-ground network of *V. myrtillus* rhizomes can spread and expand clonally and thus, be much older than the above-ground ramets (Flower-Ellis 1971). Therefore, only a long-term monitoring or a below-ground dendrochronological survey could corroborate whether we are dealing with a recent encroaching area where *V. myrtillus* could expand in the near future, or with older populations with a high above-ground turnover.

C and N concentration and isotope composition

Our results on C and N concentrations and isotope compositions showed differences between stand types, which evidence that competition occurred between *V. myrtillus* and its neighbours.

First, our results suggest that the WUE of *V. myrtillus* was reduced in the presence of *R. ferrugineum*. Several studies have reported lower values of $\delta^{13}\text{C}$ in leaves under shaded conditions than under full light (Gebauer and Schulze 1991; Berry et al. 1997; Le Roux et al. 2001; Duursma and Marshall 2006; Kranabetter et al. 2010). However, stomatal conductance can be reduced under shade (Gross et al. 1996; Forseth et al. 2001), leading to a lower discrimination against ^{13}C and higher $\delta^{13}\text{C}$ values in shaded plants. The observed lower $\delta^{13}\text{C}$ values in ramets coexisting with *R. ferrugineum* are probably explained by a greater difference in the assimilation rates between open and shady stands than the difference in stomatal conductance between these two situations (Carelli et al. 1999; Forseth et al. 2001). When growing with *R. ferrugineum*, *V. myrtillus* might have encountered more shade, which probably reduced its photosynthetic rate to a greater extent than the stomatal conductance (i.e. leading to lower WUE values). A study in the semiarid Colorado Plateau showed that shading by neighbour shrub species reduced the performance of the herbaceous perennial *Cryptantha flava* through a reduction of its photosynthetic rate and a non-proportional reduction of its transpiration and stomatal conductance, thus reducing its WUE (Forseth et al. 2001). In our study, we also observed a reduction of the WUE of *V. myrtillus* growing with *R. ferrugineum*, but its performance was not affected. As suggested by Forseth et al. (2001), temperature, vapour pressure deficit and transpiration rates may have also been lower under the shade of *R. ferrugineum*, which would explain why *V. myrtillus* performance was not altered. As expected, the C content was higher in new shoots than in leaves, since the former are permanent structures, whereas leaves are shed every year in this species.

The lower N content and the lower $\delta^{15}\text{N}$ values of leaves and new shoots in mixed stands than in pure stands suggest that *V. myrtillus* encountered a deficit in available N when growing with *R. ferrugineum* and *V. uliginosum* compared to pure stands (Olsrud et al. 2004; Craine et al. 2009). Wang and Schjoerring (2012) found a positive correlation between $\delta^{15}\text{N}$ and N concentration in leaves of ryegrass from intensively managed fields in Scotland, and Craine et al. (2009) also found evidence that foliar $\delta^{15}\text{N}$ increased with increasing N supply at a local and regional scale after reviewing data from 11,000 plants worldwide. These studies showed that foliar $\delta^{15}\text{N}$ values and foliar N

concentrations correlate and that they can both give information on the N availability.

The lower N content values in leaves and new shoots of *V. myrtillus* in mixed plots are probably explained by the competition for N with its neighbours, which might have limited the amount of N that *V. myrtillus* ramets could invest in their tissues. *Vaccinium myrtillus* and *R. ferrugineum* have shown different N-acquisition strategies in an experiment in a subalpine community in the French Pyrenees (Pornon et al. 2007), where *V. myrtillus* took up N early in the growing season, whereas *R. ferrugineum* showed a slower N uptake rate, which was maintained over the growing season. Moreover, a study on *V. myrtillus* and *V. vitis-idaea* in a boreal shrub community (Gundale et al. 2012) showed that the removal of one shrub species did not affect the isotopic signal of the other coexisting shrub, demonstrating that the niches of these species were not affected by the presence of their neighbours. Differences in the study species, community composition and soil N pools (both content and dynamics) between these studies and ours might explain the contrasting results. A N shortage can have dramatic impacts on plant performance, since the majority of the leaf N is allocated to the photosynthetic apparatus, and leaf N content and photosynthetic capacity are well correlated (Evans 1989). Thus, competition for N can have negative impacts on plant growth, as it has been reported in previous studies (Wilson and Tilman 1991; Li et al. 2015). However, despite the evidence of an existing competition for N in our study, *V. myrtillus* could clearly counteract the effects of this competition and grow as vigorously as in pure stands. These results suggest that competition did not lead to a sufficient N shortage that could negatively affect *V. myrtillus* growth. Further studies should be carried out to determine whether the lower $\delta^{15}\text{N}$ values in leaves and shoots of *V. myrtillus* from mixed populations were due to higher ^{15}N fractionation by mycorrhiza (Emmerton et al. 2001), to an increased proportion of N obtained by mycorrhiza (Hobbie et al. 2000), or to the use of different N sources (Michelsen et al. 1998) as a result of the competition with the other shrub species.

Concluding remarks

We did not find evidence that the performance of *V. myrtillus* at our study site was affected by the presence

of *R. ferrugineum* or *V. uliginosum*. Although *V. myrtilloides* seems to compete for N with both species, it can counterbalance these competition effects without compromising its growth. This is especially important to understand and predict landscape dynamics at treeline: the possible expansion of *V. myrtilloides* at this treeline site would not be tightly related to the presence of *V. uliginosum* and *R. ferrugineum*, but mostly driven by the own population dynamics, mediated by other biotic interactions and environmental factors.

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Supporting information

Table S1. Percentage cover (%) of the shrub species in each stand type. Data are means \pm 1 SE ($n = 5$). M: *V. myrtillus* pure stands; R: *V. myrtillus* mixed with *R. ferrugineum*; U: *V. myrtillus* mixed with *V. uliginosum*.

Stand type	<i>V. myrtillus</i>	<i>R. ferrugineum</i>	<i>V. uliginosum</i>
M	100 \pm 0	-	-
R	48 \pm 2.0	62 \pm 5.8	-
U	49 \pm 6.0	-	60 \pm 5.5

Table S2. $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ of leaves and new shoots TOM (total organic matter) for the three stand types studied. M: monospecific stands of *V. myrtillus*, R: stands of *V. myrtillus* mixed with *R. ferrugineum*, U: stands of *V. myrtillus* mixed with *V. uliginosum*. Values represent means \pm 1 SE ($n = 5$). The $\delta^{13}\text{C}$ of the CO_2 of the air was -10.91‰.

Stand type	Fraction	$\delta^{13}\text{C}$ (‰)	$\Delta^{13}\text{C}$
M	Leaf	-28.62 \pm 0.26	17.71 \pm 0.26
	Shoot	-27.58 \pm 0.52	16.67 \pm 0.51
R	Leaf	-29.48 \pm 0.21	18.57 \pm 0.21
	Shoot	-28.07 \pm 0.18	17.16 \pm 0.18
U	Leaf	-28.87 \pm 0.15	17.96 \pm 0.15
	Shoot	-27.62 \pm 0.28	16.71 \pm 0.28

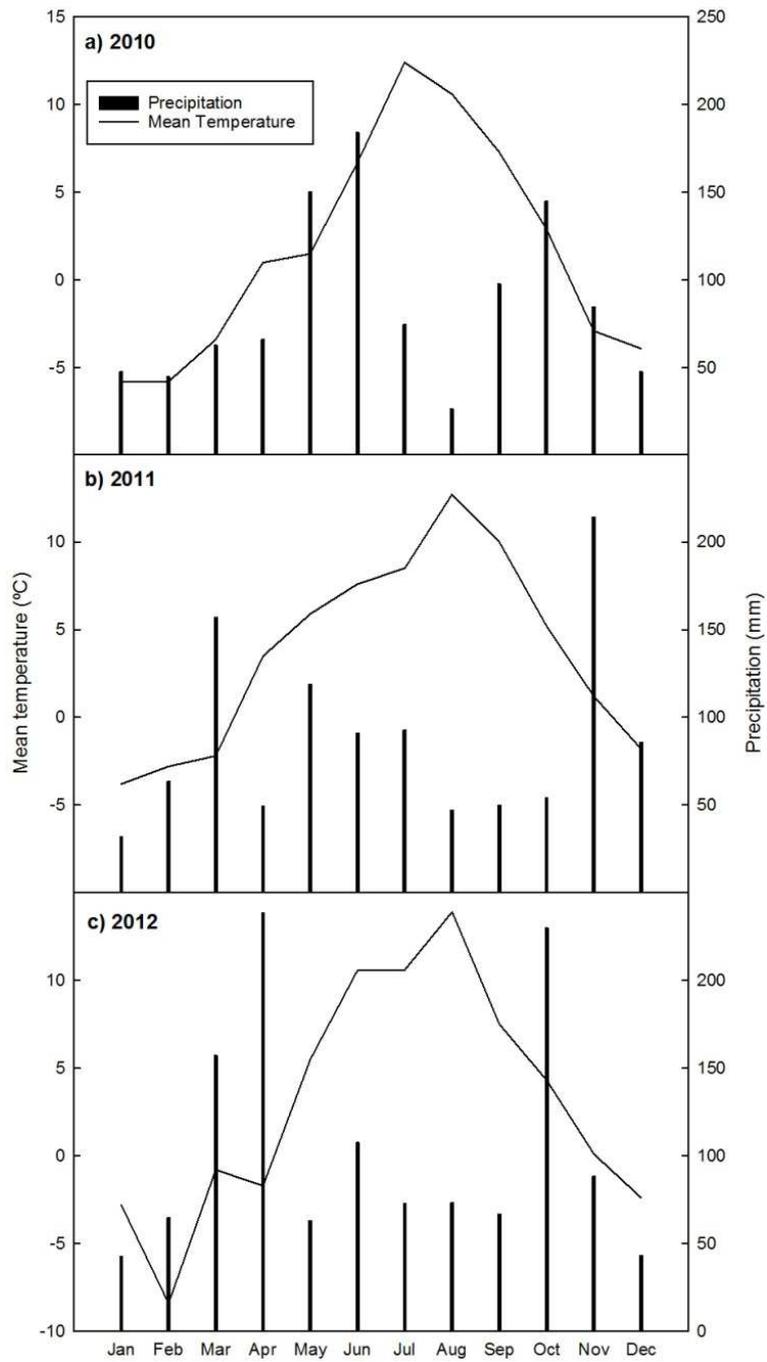


Figure S1. Monthly mean temperature (°C) and precipitation (mm) from 2010 to 2012 at La Bonaigua Station (Meteorological Service of Catalonia), located at 2266 m a.s.l., 6.3 km from the study site.

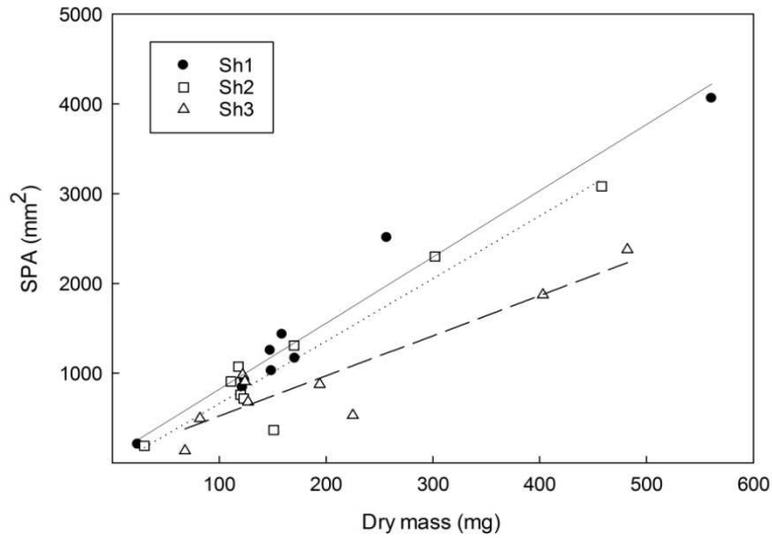


Figure S2. Correlations between shoot photosynthetic area (SPA, mm²) and shoot dry mass (mg) of new shoots (Sh1), one-year-old shoots (Sh2) and two-year-old shoots (Sh3) of *V. myrtillus*. We used the obtained regression equations to estimate SPA of the rest of the study ramets: $y = 7.38x + 79.66$ (Sh1), $y = 6.98x - 39.46$ (Sh2), $y = 4.48x + 73.26$ (Sh3).