

Consistent responses to moisture stress within diverse mountain fynbos communities revealed by multi-year in situ physiological measurements

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1 **Title:** Consistent responses to moisture stress within diverse mountain fynbos communities
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12 **Authors' contributions:**

13 RPS, AGW and TED planned and designed the research. RPS conducted field work, analysed data,
14 and wrote the manuscript with input from AGW. DB assisted with data generation and data
15 analysis. All authors contributed to revising the manuscript.

16 **Key words:** drought, ecophysiology, plant water-use, sap flow, mountain fynbos

17

18 **Abstract**

- 19 ● Understanding climate change impacts on the Cape Floristic Region requires improved
20 knowledge of plant physiological responses to the environment. Prior studies examining
21 physiological responses of fynbos species have consisted of campaign-based
22 measurements, capturing snapshots in time of plant water relations and photosynthesis. We
23 examine conclusions drawn from these studies by tracking *in situ* physiological responses
24 of three species, representing three dominant growth forms (proteoid, ericoid, restioid),
25 over two years using miniature continuous sap flow technology, in combination with long-
26 term observations of xylem water potentials, gas exchange and assessments of xylem
27 vulnerability to embolism.
- 28 ● We observed considerable inter-specific variation in the timing and extent of dehydration-
29 induced declines in productivity. *Erica monsoniana* (a shallow-rooted ericoid) exhibited
30 steep within-season declines in sap flow and water potentials, and pronounced inter-annual
31 variability in total daily sap flux (J_s). *Protea repens* showed steady reductions in J_s across
32 both years, despite maintaining less negative water potentials. *Cannomois congesta* - a
33 shallow-rooted restioid - was least negatively impacted. Following rehydrating rain at the
34 end of summer we compared gas exchange recovery in the representative dry year with the
35 normal year, finding significant seasonal, but not species, effects. Loss of function in the
36 drier year was partially accounted for by loss of stem xylem transport capacity, but only in
37 *E. monsoniana* and *C. congesta*.
- 38 ● Hitherto unseen water-use patterns, including inter-annual variability of gas exchange that
39 were driven by contrasting water uptake properties, reveal that mountain fynbos species
40 use different mechanisms to cope with summer dry periods.

41 **Introduction**

42 Drought events in natural plant communities have the potential to alter plant community
43 composition and function by differentially impacting species mortality (Choat *et al.* 2018;
44 McDowell *et al.* 2020; Anderegg *et al.* 2015; Hammond *et al.* 2022). However, in addition to
45 extreme droughts that cause extensive plant die-off, there are many droughts that do not cause
46 mortality, but instead cause prolonged reductions in plant transpiration, carbon assimilation and
47 primary production, both during the dry period and after rainfall returns (McDowell *et al.* 2020;
48 Skelton *et al.* 2017; Garcia-Forner *et al.* 2016; Anderegg *et al.* 2015, West *et al.* 2008). While non-
49 lethal droughts may not result in visible plant mortality, they may still cause considerable loss of
50 function in plants, which may influence resilience to additional stressors and alter community
51 dynamics if species are differentially affected.

52 Estimating the extent of the loss of function caused by droughts in natural plant communities
53 requires robust mechanistic frameworks of plant response to the environment that are based on
54 quantitative physiology (Skelton *et al.* 2015; Martínez-Vilalta *et al.* 2014; Oren *et al.* 1999; Plaut
55 *et al.* 2012; Jackson *et al.* 2000; Sperry 2000). South Africa's Cape Floristic Region (CFR) is one
56 of the smallest global biodiversity hotspots, containing over 8500 species in an area of
57 approximately 90 000 km² (Allsopp *et al.* 2014). The combination of high species richness, high
58 environmental heterogeneity and strong interactions between climate and plant diversity (Cowling
59 *et al.* 2005; Procheş *et al.* 2005) makes the CFR an ideal location to examine plant functional
60 responses (West *et al.* 2012; Allsopp *et al.* 2014; Altwegg *et al.* 2014).

61 Previous work in mountain fynbos - a dominant vegetation type in the CFR - has revealed the key
62 role of growth form, rooting depth, and stomatal dynamics in determining plant exposure to
63 drought with implications for productivity. For example, deep rooted proteoids - the dominant
64 overstorey shrubs - have been found to exhibit little or no moisture stress throughout the year (Moll
65 and Sommerville 1985; van der Heyden and Lewis 1989; West *et al.* 2012) and have shown no
66 photosynthetic responses to irrigation (e.g., Herppich, *et al.* 1994; van der Heyden and Lewis
67 1990). Small- to medium-sized shallow rooted shrubs (ericoids) tend to display low seasonal
68 xylem water potentials (van der Heyden and Lewis 1989; Miller *et al.* 1983, 1984; West *et al.*
69 2012; Higgins *et al.* 1987), although they too have shown no photosynthetic response to irrigation

70 (van der Heyden and Lewis 1990). Water potentials in restioids - reed-like monocotyledons - tend
71 to remain relatively high (Miller *et al.* 1984; West *et al.* 2012), although seasonal declines have
72 been observed in some species (e.g. Moll and Sommerville 1985; van der Heyden and Lewis 1989)
73 and irrigation during summer has resulted in a 20-40 % increase in net photosynthetic rate in one
74 study species (van der Heyden and Lewis 1990). Furthermore, recent work has shown limited
75 plasticity in response to experimental changes in seasonal moisture availability in fynbos,
76 particularly in established shrubs (van Blerk *et al.* 2021a, b).

77 However, most prior studies examining physiological responses of mountain fynbos species have
78 consisted of campaign-based measurements capturing snapshots in time of plant water relations
79 and photosynthesis (West *et al.* 2012; Miller *et al.* 1983; Moll and Sommerville 1985; Herppich,
80 *et al.* 1994; von Willert *et al.* 1989; van der Heyden and Lewis 1989, 1990; Miller *et al.* 1984).
81 While highly informative, snapshots may be influenced by preceding conditions that may obscure
82 true patterns and drivers of plant function. Although the south-western corner of Southern Africa
83 is traditionally thought of as a winter-rainfall system, rain-bearing frontal systems occur
84 throughout the year. Thus, unlike other global regions with Mediterranean-type climates (e.g.,
85 California or the Mediterranean Basin), it is rare to go longer than two weeks without some
86 measurable precipitation (Agenbag *et al.* 2008; Cowling *et al.* 2005; Richardson and Kruger 1990;
87 Arnolds *et al.* 2015).

88 Additionally, clouds provide considerable moisture inputs during summer that could lessen plant
89 moisture stress between rainfall events (Marloth 1905; Nagel 1956). During the summer months,
90 rainfall events are often preceded by hot, dry, and windy conditions resulting from northerly
91 airflow from the interior of Southern Africa. These opposing systems result in strongly pulsed
92 atmospheric and soil moisture conditions that may vary over a matter of days, and mean that
93 snapshot campaign measurements may miss the full picture of dynamic plant physiology in this
94 region. To fully understand plant water use and foliar gas exchange in such a highly pulsed
95 environment and to decouple the effects of short-term inputs versus longer-term trends, it is
96 necessary to monitor these processes over multiple years.

97 The objective of this study was to quantify *in situ* physiological responses within mountain fynbos
98 of the CFR to identify patterns of plant response to dry conditions and enhance our understanding

99 of the potential impacts of drought on this system. Despite the known generalities of plant response
100 to drought found within these systems, several questions that remain unresolved. For instance, the
101 precise timing of physiological responses within a drought period (e.g., stomatal closure) may vary
102 between species, with unclear implications for productivity and coexistence (West *et al.* 2012,
103 Skelton *et al.* 2015). Further, physiological tolerance limits, such as xylem vulnerability to
104 embolism, may also vary between species, ensuring that dry periods can have varying impacts on
105 species (Paddock *et al.* 2013, Jacobsen *et al.* 2009).

106 In this study, we tracked *in situ* physiological changes in three sample species, representing the
107 three dominant growth forms in the fynbos, over two years. To do so, we used miniature external
108 (i.e., non-invasive) sap flow technology (Skelton *et al.* 2013) in combination with long-term
109 observations of water potential and leaf (or culm) gas exchange. In addition, we quantified key
110 functional traits of the three species, including xylem vulnerability to embolism. Our continuous
111 measurements allowed us to examine, in unprecedented detail, the diurnal and seasonal responses
112 of each species, enabling us to assess the accuracy and generality of the snapshot campaign
113 measurements previously captured in the region. Our results reveal that inter-specific, within-
114 season differences in xylem water relations drive variation in the timing and extent of dehydration-
115 induced declines in productivity. However, inter-specific variation in xylem vulnerability to
116 embolism ensures convergence in long-term water transport function under moderately dry
117 conditions.

118 **Materials and Methods**

119 *Study site and species*

120 The field site was situated at Jonaskop in the Riviersonderend Mountains (33° 56' 30.45" S and
121 19° 31' 34.18" E, 980 m elevation above sea level), located in the southwest corner of southern
122 Africa's CFR. The geological substratum is composed mostly of nutrient poor quartzitic sandstone
123 of the Table Mountain Group, which produces shallow, rocky, highly leached sandy soils. The site
124 receives 411 mm of rainfall on average annually, approximately 66% of which falls in winter
125 (April–Sept.) (Agenbag *et al.* 2008) (Table S1). Mean annual temperature is 13.6 °C, mean
126 minimum temperature of the coldest month is 3.6 °C and the mean maximum temperature of the
127 warmest month is 27.4 °C (Agenbag *et al.* 2008). Vegetation at the site is mountain fynbos

128 composed of a 2–3 m tall open canopy dominated by *Protea repens* (L.) L. (Proteaceae) and an
129 understory of ericoid shrubs and reed-like graminoids (Agenbag *et al.* 2008) (Plate 1). Three
130 species were selected for detailed physiological monitoring: *Erica monsoniana* L.f. (Ericaceae) is
131 a small- to medium-sized, small-leaved (ericoid) woody shrub; *P. repens* is a broad-leaved, woody
132 shrub; and *Cannomois congesta* Mast. (Restionaceae) is a reed-like rhizomatous perennial with
133 erect, lignified culms (Plate 1). In addition to being morphologically dissimilar, and therefore
134 representing high growth form diversity, these three species also represent the most dominant
135 growth forms in mountain fynbos.

136 *Micrometeorological monitoring station*

137 A micrometeorological station erected at the study site in 2011 (described previously in Skelton *et*
138 *al.* 2013) monitored environmental variables continuously throughout the study period. The
139 micrometeorological station was located on a level, slightly north-facing plateau and all measured
140 plant individuals were located within a zone of approximately 100 m radius from the station.
141 Briefly, the micrometeorological station consisted of a 3 m tall tripod (CM106, Campbell
142 Scientific, Logan, Utah, USA) and sensors, including a temperature and relative-humidity probe
143 (HMP45C, Campbell Scientific), a leaf-wetness sensor (237, Campbell Scientific), a tipping-
144 bucket rain gauge, and a soil water content profile probe (EnviroSMART, Campbell Scientific).
145 Meteorological variables were logged at half-hour intervals (CR1000, Campbell Scientific). Dew
146 events were defined as periods without rainfall when the resistance of the leaf wetness sensor fell
147 below 400 kOhms and the air temperature (T_{air} , °C) was within 0.5°C of the dew point temperature
148 ($T_{\text{dew_point}}$, °C), calculated as follows:

$$149 \quad T_{\text{dew_point}} = B * [\ln(\text{RH}/100) + A * T_{\text{air}} / (B + T_{\text{air}})] / (A - \ln(\text{RH}/100) - (A * T_{\text{air}})/(B + T_{\text{air}})),$$

150 Eqn 1

151 where RH is the relative humidity (%), $A = 17.625$ and $B = 243.04$ °C (Lawrence 2005).

152 To calibrate the soil moisture probe we assessed soil water content gravimetrically. Soil samples
153 collected approximately 1 m from the probe at depths of 20 and 40 cm using an auger were placed
154 in vials, which were then sealed and transported back to the laboratory. There the samples were
155 weighed, placed in a drying oven at 70 °C for 48 hours, and then weighed again. The relationship

156 between gravimetric soil moisture content (in grams of H₂O per gram of dry soil) and probe output
157 was strong, linear, and highly significant ($R^2 = 0.89$, $p < 0.0002$).

158 *Leaf gas exchange and xylem water potentials*

159 Predawn and mid-day xylem water potentials were measured on shoots or culms of at least five
160 individuals of each species at least once every month, from February 2012 until May 2014, using
161 a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA). Measurements were
162 made more frequently during periods of water stress, or when plants exhibited rapid changes in
163 xylem water potential (e.g., following rainfall). Midday measurements were made between 12:00
164 and 14:00 and predawn measurements were made approximately one hour before sunrise.

165 Leaf or culm carbon assimilation (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) were
166 measured using an Infra-Red Gas Analyser (Li-Cor 6400; Li-Cor BioSciences, Lincoln, NE,
167 USA). Measurements were taken concurrently with the midday water potentials. Light intensity in
168 the cuvette was set at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, humidity was maintained slightly below ($< 0.2 \text{ KPa}$)
169 ambient, and reference CO₂ concentration was held at $400 \text{ mmol m}^{-2} \text{s}^{-1}$. Leaf temperature was
170 maintained between 25 °C and 33 °C as calculated using energy balance equations to track ambient
171 conditions.

172 *Sap flow*

173 Sap flow was monitored for two summers (Oct. – Mar.) in at least five individuals of each species.
174 Sap flow was measured using miniature external (i.e., non-invasive) heat-ratio method (HRM)
175 gauges described in Clearwater *et al.* (2009) and Skelton *et al.* (2013). Sap flow gauges were re-
176 installed on each individual at the start of each new monitoring season (in Spring) to avoid
177 wounding or growth-mediated effects on the sap flow signal. Heat ratio values were quantified
178 every half hour for the duration of the season. Half hourly heat-ratio values were converted to heat
179 pulse velocity (v_h , cm s^{-1}), sap velocity (v_s , cm s^{-1}) and sap flux density (J_s , $\text{g cm}^{-2} \text{s}^{-1}$) using
180 equations from Skelton *et al.* (2013).

181 In addition, sap velocity was converted to sap flow-derived transpiration rate (E_{sf} , $\text{mmol m}^{-2} \text{s}^{-1}$)
182 using the relationship between sap velocity and transpiration (E , $\text{mmol m}^{-2} \text{s}^{-1}$) measured on
183 adjacent leaves or culms (see *Leaf gas exchange and xylem water potential* above). The

184 relationships between midday v_h and midday E measured on individual leaves were both strong
185 and highly significant in all individuals (Table S2).

186 *Sap flow derived stomatal conductance*

187 Sap flow derived stomatal conductance of sunlit leaves, G_{sf} ($\text{mmol m}^{-2} \text{s}^{-1}$), used as a proxy for
188 stomatal conductance of sunlit leaves (or culms), was calculated using the relationship between
189 sap flow-derived E_{sf} and vapour pressure deficit (VPD, kPa). G_{sf} can be determined from E_{sf} and
190 VPD in vegetation types with high aerodynamic conductance and low decoupling coefficient (for
191 a full discussion see Hogg & Hurdle, 1997), which applies to the open vegetation in this study:

$$192 \quad G_{sf} = (\alpha) E_{sf}/VPD; \quad \text{Eqn 2}$$

193 α , atmospheric pressure of water vapour equal to $\rho_w G_v T$, where ρ_w is the density of water (*c.* 1000
194 kg m^{-3}), G_v is the universal gas constant for water vapour ($= 0.462 \text{ m}^3 \text{ kPa kg}^{-1} \text{ K}^{-1}$) and T is air
195 temperature (Kelvin).

196 *Seasonal decline and recovery of plant function*

197 Mesic periods in October, prior to the onset of summer moisture limitation, provided a maximum
198 gas exchange reference period against which to quantitatively evaluate seasonal water use. Leaf or
199 culm stomatal conductance, assimilation rate and predawn water potential (MPa) of all individuals
200 confirmed that plant functionality during this early season window was at, or close to, a non-
201 stressed maximum of each growing season for all species. Within-season recovery was quantified
202 as the maximum % recovery of J_s or G_{sf} (relative to the Oct reference period) following a post-
203 summer return to pre-stressed predawn water potentials.

204 *Xylem vulnerability to embolism*

205 We collected large branches from individuals of *P. repens* and *E. monsoniana* and entire rhizomes
206 with >20 culms from individuals of *C. congesta* in December 2020. Samples were collected from
207 3+ healthy-looking individuals of each species. To avoid a potential artefact associated with cut,
208 open vessels in the woody species we ensured that the cut material was longer than the species'
209 maximum recorded vessel length. Vessels were short in *P. repens* (mean maximum vessel length
210 $= 21.1 \pm 2.1 \text{ cm}$, $n = 7$) and *C. congesta* ($15 \pm 0.0 \text{ cm}$, $n = 3$), but spanned the length of *E.*

211 *monsoniana* stems (> 50 cm, n = 3). To overcome this limitation, we cut stems of *E. monsoniana*
212 individuals below the root collar (maximum branch length ~2.5m). Upon excision, tension in the
213 xylem was relaxed by re-cutting the stems under water. Branches and rhizomes were placed in
214 large plastic bags with damp paper towels, which were then sealed to prevent further water loss
215 while being transported to the laboratory for processing.

216 We used the optical method described in Brodribb *et al.* (2016 a,b); Brodribb *et al.* (2017), and
217 Skelton *et al.* (2018) to generate xylem vulnerability curves for each species. Full details of the
218 method, including an overview of the technique, image processing, as well as scripts to guide
219 image capture and analysis, are also available at <http://www.opensourceov.org>. Briefly, as each
220 individual desiccated on a lab bench, we used flatbed scanners (Epson perfection V800 or V850
221 Scanner, Epson America) to generate a time series of images of an exposed section of the xylem
222 within small branches (diameter < 0.5 cm and always current year growth) or culms. Stems and
223 culms were scanned in reflective mode at 4800 dpi every five minutes over a period of a few days,
224 allowing us to detect embolism within the outer few layers of xylem in each sample. The small
225 sizes of the branches reduced the possibility that our method might have missed significant radial
226 variation in embolism within branches, although this possibility cannot be entirely excluded.

227 As branches or rhizomes dehydrated, we also quantified stem or culm xylem water potential. For
228 the two woody species we attached a stem psychrometer (ICT International, Armidale, Australia)
229 to each branch at more than 60 cm from the cut end of the main branch. Stem psychrometers were
230 sealed with high vacuum grease (Dow Corning Corp, Midland, MI, USA) to prevent moisture loss
231 and secured in place with Parafilm (Bemis NA, Neenah, WI, USA). Stem xylem water potential
232 was recorded every twenty minutes for the duration of the scanning process. We verified the
233 accuracy of the stem psychrometer readings for a subset of individuals by periodically measuring
234 leaf xylem water potential using a Scholander-type pressure chamber (PMS Instruments, Corvallis,
235 Oregon, USA). For *C. congesta* we quantified xylem water potential of culms attached to the same
236 rhizome as the scanned culms using a Scholander-type pressure chamber. While each excised culm
237 was being measured it was wrapped in moist paper towel and placed in a plastic bag to prevent
238 further water loss. Variation among neighbouring culms was slight (always < 0.1 MPa) indicating
239 that individuals were equilibrated.

240 Upon completion, image sequences were analysed to identify embolism events, seen as changes
241 in the reflection of the stem xylem. Image subtraction of subsequent images conducted in ImageJ
242 (National Institutes of Health, Bethesda, MD, USA) was used to reveal rapid changes in light
243 transmission or contrast produced by each embolism event. Slow movements of the stems or culms
244 caused by drying could easily be distinguished from embolism events and were filtered from the
245 analysis. Embolism events were thresholded, allowing automated counting of each event using the
246 analyse-stack function in ImageJ. From the thresholded stack of embolism events we could extract
247 a time-resolved count of embolism events (using the timestamp of each image). We then converted
248 the raw embolism counts to a percentage of total pixels embolized, producing a dataset of time-
249 resolved percent embolism. The time-resolved percent embolism data were combined with the
250 xylem water potential timeline to estimate the culm or stem xylem water potential associated with
251 each embolism event. Vulnerability to embolism was recorded as the relationship between percent
252 embolism and water potential (Ψ), and modelled using a sigmoid function:

$$253 \text{ Percent embolism} = 100 - 100 / (1 + e^{a(\Psi - b)}) \quad \text{Eqn 3}$$

254 where a corresponds to the sensitivity to decreasing water potential (proportional to the slope of
255 the equation) and b is the water potential associated with 50% embolism (P_{50} , MPa).

256 *Statistical analyses*

257 Relationships between total daily J_s and soil moisture or mean midday VPD were assessed using
258 mixed linear models using the “lmer” function in the “lme4” package in R v.3.0.2 (R Development
259 Core Team, 2016). The full model treated total daily J_s of each individual as a function of three
260 fixed factors (soil moisture or VPD, species and season) and a random factor
261 (species/season/individual).

262 We assessed inter-specific and seasonal (i.e., inter-annual) differences in plant physiological
263 variables (e.g., recovery of total daily J_s and G_{sf} , predawn and midday xylem water potential) and
264 traits (e.g., P_{50}) using ANOVA. This was done using the “aov” function in the “lme4” package in
265 R v.3.0.2. Minimum predawn and midday xylem water potential were expressed as functions of
266 species and season including a *species x season* interaction term. Recovery of total daily J_s or of
267 G_{sf} was also expressed as a function of species and season including a *species x season* interaction

268 term, with individuals treated as a random effect (intercept only). P_{50} was expressed as a function
269 of species only.

270 Post hoc analyses were conducted by comparing the full model to alternative models consisting of
271 fewer fixed factors and/or without interactions. This was achieved using ANOVA and Akaike's
272 Information Criterion (AIC) in R. In each case we selected the best fit model as the one with the
273 lowest AIC score that also differed statistically ($p < 0.05$) from the other models. For the ANOVA
274 tests, when significant main effects were established ($p < 0.05$) post-hoc pairwise comparisons
275 were made using Tukey's multiple comparisons of means tests.

276 **Results**

277 *Micrometeorological conditions*

278 We captured sap flow across two summers, the first being drier than the second (Figure 1). VPD
279 patterns at the study site tended to be similar across years (e.g., highest during mid to late summer
280 in both study years; Figure 1a), and soil moisture started high in both summers (e.g., approximately
281 10% at 70cm; Figure 1b). Soil moisture declined steadily throughout the first summer (2012/13;
282 Figure 1B), reaching the lowest levels observed in the study period in March 2013 (Figure 1b). By
283 comparison, soil moisture remained higher throughout 2013/14, despite a consistent drying period
284 between February and March 2014 (Figure 1b).

285 Soil moisture patterns tended to be driven by summer rainfall inputs: total summer rainfall in
286 2012/13 (90 mm) was less than half the amount received in 2013/14 (253 mm) (Figure 1c).
287 Although several small rain events (< 10 mm) were recorded during both summers, three large ($>$
288 20 mm) rain events were recorded in the summer of 2013/14 (Figure 1c). Overall, the total annual
289 rainfall in 2012 (293 mm) was also below ($< 70\%$) historical mean annual rainfall, while in 2013
290 the total amount (389 mm) was within 95% of the historical mean (Table S1). Dew and/or cloud
291 moisture was routinely recorded at the site, with most events lasting more than two hours (Figure
292 S1). Over the course of the study period, it was rare to go for longer than four days without
293 experiencing dew or cloud at the study site (Figure S1).

294 *Environmental drivers of within-season patterns of plant water use*

295 Total daily sap flux density (J_s) declined with the progression of summer in all three study species,
296 although there were notable inter-specific differences in within- and between-season responses
297 (Figure 1d-f). In the drier year, total daily J_s of *E. monsoniana* declined earliest and to the lowest
298 minimum (< 5% of maximum total daily J_s) of the three species (Figure 1d). *P. repens* and *C.*
299 *congesta* both maintained higher sap flow in the representative dry year compared to *E.*
300 *monsoniana* (Figure 1 e,f). Nevertheless, total daily J_s of *P. repens* declined steadily through the
301 measurement period in 2012/13, reaching as low as ~10% of maximum total daily J_s . Total daily
302 J_s of *C. congesta* was the least sensitive, remaining above 20% of maximum total daily J_s
303 throughout the measurement period in 2012/13. Total daily J_s remained high in the wetter year,
304 remaining above ~20% of maximum in all three species. However, steep declines in total daily J_s
305 were observed in *E. monsoniana* during dry spells, while more gradual declines were observed in
306 *P. repens* and *C. congesta* (Figure 1d-f).

307 Total daily J_s was positively linearly associated with soil moisture in all three species (Tables 1,
308 S3; Figure 2a-c), with the steepest response being observed in *E. monsoniana* (Table 1; Figure 2a).
309 There were significant seasonal effects on the relationship between total daily J_s and soil moisture
310 (Table S3), with the gradient of the response being steeper in all species in the representative dry
311 season compared to the normal year (Table 1; Figure 2a-c). A species by season interaction was
312 also observed (Table S3), where there were weak positive associations between total daily J_s and
313 soil moisture in the normal season in *C. congesta* and *P. repens* (Table 1; Figure 2b). Total daily
314 J_s was also positively linearly associated with VPD in all species (Table S3; Figure 2d-f). Similar
315 positive responses were recorded across seasons, although there was a significant species by
316 season interaction (Tables 1, S3; Figure 2d-f). We detected weaker positive associations between
317 total daily J_s and VPD in the representative dry season than in the normal year for *E. monsoniana*
318 and *P. repens* (Table 1; Figure 2 d,f).

319 Total daily J_s in *E. monsoniana*, but not the other two species, was responsive to summer rainfall
320 events. Figure 3 shows an example of summer rainfall that did not appear to reach the deeper soil
321 layers (Figure 3a) yet was associated with a sharp increase in J_s in *E. monsoniana* in the few days
322 following the event (Figure 3b). The small rain inputs did not elicit substantial responses in the
323 other two species (Figure 3c,d).

324 *Internal drivers of plant water use*

325 Seasonal patterns in sap flow-derived midday stomatal conductance (G_{sf}) were similar to the
326 overall patterns in total daily J_s in all three species (Figure 4). In the drier year, G_{sf} of *E.*
327 *monsoniana* declined earliest and to the lowest minimum of the three species (Figure 4a). G_{sf} of
328 *E. monsoniana* fell below 30% in late December 2012 and thereafter to a minimum of < 5% in
329 March 2013 (Figure 4a). In comparison *P. repens* and *C. congesta* both maintained higher G_{sf} for
330 longer in the representative dry year (Figure 4b,c). G_{sf} of *P. repens* declined to c. 30% in March
331 2013, approximately 84 days after *E. monsoniana* hit the same level. G_{sf} of *C. congesta* was the
332 least sensitive out of the three study species, falling to c. 30% of maximum G_{sf} in early April 2013,
333 about one hundred days after *E. monsoniana* had reached this value. G_{sf} remained high in the
334 normal year, declining to ~20% of maximum in *E. monsoniana*, but remaining above 30% in *C.*
335 *congesta* and *P. repens*.

336 Changes in mid-day stomatal conductance (g_s) measured at the leaf or culm level typically matched
337 those observed at the shoot level (G_{sf}) in all three sample species (Figure 4). Minor differences in
338 leaf/culm versus shoot values arose during periods of very low moisture availability or
339 immediately following rehydrating rainfall events, when leaf or culm g_s either declined lower (e.g.,
340 during drier periods) or rose higher (e.g., following rehydration) than G_{sf} (Figure 4).

341 *Recovery of stomatal conductance and water use following rehydration*

342 Following large rainfall events (> 15 mm) total daily J_s rose in all three species, but to different
343 extents (Table 2; Figure 5a). In the drier season, total daily J_s of *E. monsoniana* rose to c. 67% of
344 pre-stressed total daily J_s , similar to the recovery recorded for a similar event in the second, normal
345 season. Total daily J_s of *C. congesta* recovered to >75 % of pre-stressed total daily J_{sf} , less than the
346 95% recovery recorded in the second season. Recovery of total daily J_s of *P. repens* was the lowest
347 of the three species in both seasons, only reaching 57 % of pre-stressed total daily J_s in the drier
348 year and 62% in the normal season.

349 Recovery patterns of G_{sf} following large rainfall events differed from those for total daily J_s , in
350 that recovery of G_{sf} in all species was lower in the representative dry year in comparison to the
351 normal year (Table 2; Figure 5b). In the drier season, G_{sf} of *E. monsoniana* rose to c. 40% of pre-

352 stressed G_{sf} , half the recovery recorded for a similar event in the second, normal season. G_{sf} of *C.*
353 *congesta* recovered to 62% of pre-stressed G_{sf} , less than the 81% recovery recorded in the second
354 season. G_{sf} of *P. repens* was lower in the drier year, recovering to c. 47% of pre-stressed G_{sf} , less
355 than the 73% recovery recorded in the second, normal season.

356 *In situ changes in plant xylem water potential*

357 Minimum predawn and midday xylem water potentials differed between species and seasons
358 (Figure 6; Tables 3, S3). Predawn xylem water potentials remained high (i.e., less negative than
359 -1 MPa) and stable in *P. repens* and *C. congesta* but were more dynamic and declined lower ($<$
360 -1.5 MPa) in *E. monsoniana* (Figure 6a). Seasonal patterns of midday xylem water potentials were
361 similar to those observed for predawn xylem water potential (Figure 6b). Minimum midday values
362 were more negative in *E. monsoniana* (-4.0 ± 0.1 MPa in March 2013) than in *P. repens* ($-1.5 \pm$
363 0.03 MPa, $p < 0.000$) and *C. congesta* (-1.9 ± 0.26 MPa, $p < 0.000$), but did not differ between
364 the latter two species ($p = 0.94$) (Figure 6 and Table 3). Although minimum midday xylem water
365 potentials tended to be lower in the drier summer of 2012/13 ($p < 0.000$), there was a significant
366 species by season interaction (Table S3). Post-hoc tests revealed that only *E. monsoniana* was
367 significantly more dehydrated in 2012/13 than in 2013/14 (Table 3).

368 In all species, stomatal conductance declined rapidly with declining xylem water potential, with
369 stomata being mostly closed at water potentials above (i.e., less negative than) -2 MPa (Figure 7).

370 Predawn and midday xylem water potentials of all three species occasionally recovered to less
371 negative values throughout both summers (Figure 6). Partial recovery of xylem water potential
372 was associated with small moisture inputs (< 10 mm; see arrows in Figure 6). All three species
373 rehydrated to water potentials matching those of unstressed conditions following large (> 15 mm)
374 summer rainfall events (blue shaded areas in Figure 6).

375 *Predicted loss of function in the stem xylem transport system*

376 Stem or culm xylem capacity to withstand embolism differed between the three sample species
377 (Figure 7; $F = 16.36$, d.f. = 2, $p = 0.0023$). The water potential associated with 50 % embolism
378 varied from -2.3 ± 0.12 MPa in culms of *C. congesta* to -5.68 ± 0.78 MPa in stems of *E.*
379 *monsoniana* (Table 3). When combined with the seasonal minimum xylem water potential, the

380 vulnerability curves indicate that *C. congesta* and *E. monsoniana* individuals were likely to have
381 surpassed thresholds associated with embolism formation in culms/stems in 2012/13, but not in
382 2013/14 (Figure 7; Table 3). The predicted amount of embolism to have occurred in 2012/13 (i.e.,
383 from the combination of the mean stem/culm vulnerability curves and the minimum water
384 potentials from that season) was < 10% in both species (Figure 7; Table 3). When combined with
385 the seasonal minimum xylem water potential, the vulnerability curves indicate that *P. repens*
386 individuals were unlikely to have experienced embolism in the stem xylem in either 2012/13 or
387 2013/14 (Figure 7; Table 3).

388 **Discussion**

389 Our novel, multi-year sap flow dataset, collected on multiple growth forms simultaneously,
390 together with associated physiological measurements, provides unique insight into the ecology of
391 the CFR. Our study provides strong confirmatory evidence of consistent functional responses in
392 the fynbos across time and space, adding weight to the functional classifications inferred from
393 snapshot campaigns conducted on distinct species at different field sites. We also report on the
394 novel finding that despite co-occurring species with different growth forms experiencing quite
395 different responses to dehydration, these did not translate into substantial variation in recovery
396 following rehydration, primarily due to differences in xylem vulnerability to embolism and water
397 uptake characteristics. Below we discuss these results, with an emphasis on the inter-specific
398 differences in key functional traits that resulted in convergent functional outcomes. We conclude
399 with an evaluation of how this study informs our understanding of drought impacts in mountain
400 fynbos and other diverse ecosystems.

401 *Consistent functional responses observed across space and time*

402 Our highly temporally resolved dataset revealed functional responses to the environment in our
403 three species that were consistent with previous studies conducted on congenics at separate
404 locations and at separate times.

405 *Erica monsoniana* was most tightly coupled to shallow moisture dynamics through the summer,
406 showing rapid declines in sap flow and gas exchange as the surface soils dried. This was associated
407 with large declines in xylem pressure potentials over the summer, an observation consistent with

408 previous studies (Miller et al. 1983, 1984; West et al. 2012). The declines in water potential were
409 greater in the drier year, and may have resulted in xylem embolism in *E. monsoniana* (Figure 7),
410 highlighting the importance of summer moisture for this species. This is further supported by the
411 clear uptake of summer rainfall pulses by *E. monsoniana* (Figure 3) indicating the presence of
412 active roots in shallow soil layers during the summer. This conclusion is consistent with
413 observations that *Erica* species have shallow roots (e.g., *E. plukenetii* Higgins et al. 1987). Our
414 observations also suggest that prior observations showing a lack of response to irrigation in other
415 mountain fynbos species (e.g., van der Heyden and Lewis 1990) may be incomplete. For example,
416 van der Heyden and Lewis (1990) showed that *E. plukenetii* did not increase its photosynthetic
417 rates when irrigated concluding that it did not use the additional water as soon as it became
418 available in the soil and that it was not limited by low soil water availability during February. In
419 contrast, our results show that plant gas exchange capacity in *E. monsoniana* was sensitive to
420 summer rainfall, declining steeply during drying periods and responding rapidly to small summer
421 rainfall events (in the drier year). Such dynamic stomatal responses caused by rapidly changing
422 xylem water potentials suggest that not only does *E. monsoniana* have active roots in shallow soil
423 layers over the summer, but that it may also exhibit a peaking-type ABA response previously
424 documented in conifers (Brodribb et al. 2014).

425 In contrast, patterns of xylem water potential and sap flow decline (and recovery) in *P. repens*
426 were better explained by variation in soil moisture of deeper layers, consistent with previous
427 studies that have found proteoid species are deeper-rooted (e.g., *Leucadendron laureolum* in West
428 et al. 2012; *P. laurifolia* in Higgins et al. 1987). Unlike *E. monsoniana*, *P. repens* did not respond
429 to small summer rainfall events, suggesting that *P. repens* did not have active roots in shallow soil
430 layers over the summer. *P. repens* also maintained high xylem water potentials throughout both
431 summer periods, with only gradual declines to more negative xylem water potentials through drier
432 periods. Our sap flow and gas exchange data showed that this maintenance of high water potentials
433 was not solely a function of having access to deeper soil moisture but was in part due to stomatal
434 closure during the summer. This observation is consistent with the responses seen in the proteoid
435 *L. laureolum* (West et al, 2012), suggesting that maintenance of high water potentials is important
436 for the success of this functional type. A plausible reason is that they are weakly serotinous species
437 and reductions in xylem water potentials may trigger premature (i.e., not post-fire) seed release
438 with consequent reductions in reproductive fitness. However, having stomata closed during late

439 summer when temperatures are still high reduces the capacity for transpirational leaf cooling and
440 may induce thermal stress, particularly under windless days (Yates et al. 2008, Karpul and West
441 2016; Herppich et al. 1994). Thermal stress, if it occurs, is most likely to impact the broad-leafed
442 *P. repens* and not *C. congesta* or *E. monsoniana*. *C. congesta* has narrow, vertical culms, while *E.*
443 *monsoniana* has small, rolled leaves, which may promote efficient sensible heat loss, thereby
444 reducing the requirement for transpirational leaf cooling in summer.

445 Excavations done at the study site showed that the restioid, *C. congesta*, had dense, shallow (< 40
446 cm) adventitious root systems (Figure S2), consistent with *in situ* observations made on other
447 Restionaceae species (e.g., *Ischyrolepis* and *Elegia*, Higgins et al. 1987, *Hypodiscus* and
448 *Staberoha*, West et al. 2012). Furthermore, this species maintained high water potentials
449 throughout the summer periods and had the lowest inter-annual reduction in G_{sf} among the study
450 species. This too is consistent with previous observations of water potentials in mountain fynbos
451 Restionaceae subjected to drought (West et al 2012). Our explanation for the observation that *C.*
452 *congesta* remains hydrated even during periods with low rainfall is that these individuals can use
453 dew or cloud moisture inputs in addition to the soil moisture. Dew events lasting over two hours
454 were common at the study site (Figure S1) and we frequently observed droplets on the culms. The
455 vertical, reed-like culms direct moisture that condenses on the culm surface to the fibrous,
456 superficial roots. Previous research in other species of Restionaceae in mountain fynbos
457 communities (Marloth 1905, 1903; Nagel 1956) has shown that culms can trap considerable
458 amounts of moisture from the atmosphere, and this may result in a decoupling of surface soil
459 moisture during drought (West et al 2012). We suggest that this ability has importance for the
460 maintenance of plant function under periods without substantial rainfall. Maintenance of high
461 water potentials through the end of summer may be particularly important for nut-fruited species
462 like *C. congesta* that take 2 years to mature seeds in their canopy. Culms that desiccate below
463 ± 2.5 MPa tend to drop their seeds prematurely (pers. obs.). Establishing how long *C. congesta*
464 (and other Restionaceae species) can survive without dew/cloud moisture is a research priority for
465 understanding potential drought impacts in the CFR.

466 Overall, our three sample species displayed physiological responses to seasonal water limitation
467 that are consistent with the ericoid, proteoid and restioid functional type responses characterised
468 by West et al. (2012) under more severe experimental drought conditions. This is most noticeable

469 in the similar stomatal and xylem water potential responses observed for our species and for those
470 from the same growth forms but occurring in a different mountain fynbos community located >
471 100 km away from our study site. (Figure 7). Further, the reduced g_s seen in all three species during
472 two prolonged dry periods were consistent with prior observations of seasonal declines in g_s for
473 mountain fynbos species, including in congeneric species to our study species (e.g., *E. plukenetii*,
474 *P. laurifolia* and *C. acuminata* in Miller et al. 1983). This lower stomatal conductance was
475 associated with lower photosynthesis at the leaf level (Figure 7), indicating that dry summer spells
476 reduce the overall productivity of mountain fynbos communities.

477 *Convergence in recovery following rehydration despite differing minimum water potentials*

478 By comparing the within-season recovery of G_{sf} of a normal year with that of a representative dry
479 year, we were able to determine that gas exchange across all sample species was limited by up to
480 30% in the representative dry year. The species converged in their recovery of G_{sf} , despite the
481 substantial variation in minimum water potentials, due to the significant variation in xylem
482 vulnerability to embolism between our study species that matched the order of seasonal minimum
483 xylem water potentials (i.e., *E. monsoniana* \ll *P. repens* $<$ *C. congesta*). This resulted in all three
484 species maintaining similar, positive hydraulic safety margins from P_{50} . Co-variation of
485 physiological responses and functional traits suggests an evolved response to seasonal drying in
486 mountain fynbos communities that resulted in convergent recovery of differing functional types
487 during two “normal” years (i.e., not severe drought). This is unsurprising, given that these species
488 have evolved to exist in this climate. However, it is less certain whether this convergence would
489 continue should these communities be exposed to extreme drought induced by climate change as
490 discussed below.

491 *Mechanisms underlying low recovery of gas exchange*

492 Can low recovery of gas exchange in our study species be explained by drought-induced blockages
493 within the stem or culm hydraulic pathway, as has been found in other woody, evergreen plants
494 (Davis et al. 2013; Urli et al. 2013; Skelton et al. 2017; Sperry 2000; Tyree and Sperry 1988)?
495 Both shallow rooted species in this sample group of diverse growth forms (i.e., *E. monsoniana* and
496 *C. congesta*) exhibited small xylem hydraulic safety margins, and both were predicted to have
497 experienced permanent loss of function in the representative dry year. Such loss of hydraulic

498 function caused by xylem embolism is irrecoverable without new growth or positive root pressure.
499 However, the amount of embolism was predicted to have been low, and although this could explain
500 the slight loss of function in *C. congesta* culms in the drier year, it only partially explains the
501 observed declines in leaf function in *E. monsoniana*. Further, our results show that *P. repens*
502 maintained large stem hydraulic safety margins, indicating that of the three species it is the least
503 likely to incur hydraulic failure in stems, and that this is an unlikely cause of low recovery in this
504 species.

505 It remains to be fully determined what other physiological factors might be influencing low
506 recovery of gas exchange in *E. monsoniana* and *P. repens*. Blockages in other parts of the soil-
507 plant-atmosphere continuum could have caused sustained reductions in gas exchange capacity. For
508 example, lower hydraulic transport efficiency in other, unrelated species has been associated with
509 leaf embolism (Brodribb et al. 2016b) or lacunae formation in the leaf mesophyll tissue or in roots
510 (e.g., Scoffoni et al. 2014; Cuneo et al. 2016), including in other evergreen sclerophylls (Skelton
511 et al. 2017). Such blockages could have occurred in *P. repens* and *E. monsoniana* if leaf xylem
512 water potentials were lower than shoot xylem water potentials, or if the leaves or roots are more
513 vulnerable than stems. The latter – termed vulnerability segmentation - has previously been
514 observed in long-lived woody trees (e.g., Skelton et al. 2018), but is rarer in evergreen sclerophylls
515 (Smith-Martin et al. 2020).

516 Consistent seasonal progression of gas exchange across both years in *P. repens* suggests that
517 stomatal function could also be tied to genetically regulated growth phenology (e.g., Schley et al.
518 2022). Although *P. repens* can get water at depth during the summer (Higgins et al. 1987), nutrients
519 are quite limited for roots obtaining this water and it is slow growing during this time (Stock and
520 Lewis 1984). It is only in the spring when both water and nutrients (particularly nitrate) are
521 available when foliar function (and resultant growth) occurs. Such limited plasticity in foliar
522 function in *P. repens* is consistent with its limited growth responses to experimental changes in
523 seasonal moisture availability reported in a recent prior study (van Blerk et al. 2021a).

524 *How does our study inform our understanding of the potential impacts of future droughts on*
525 *mountain fynbos communities?*

526 Our study adds to the body of research indicating that 1) co-occurring fynbos species rely on subtly
527 different water sources, which may confer differential sensitivity to changing climate, and that 2)
528 simplifying the extra-ordinary species diversity of the region into key functional types such as
529 proteoid, ericoid and restioid provides consistent functional responses to change. The
530 disproportionate role that infrequent but substantial summer rainfall events play in maintaining gas
531 exchange capacity in *E. monsoniana* suggests that future reductions in rainfall amounts - or
532 changes in rainfall seasonality that are associated with less summer rainfall - are most likely to
533 negatively impact growth in this species. Further, *E. monsoniana*, but not *P. repens* and *C.*
534 *congesta*, had significantly smaller hydraulic safety margins in the representative dry year than in
535 the normal year, suggesting that it is the most likely to experience hydraulic dysfunction and loss
536 of function under extreme drought conditions. The three sample species displayed physiological
537 responses to seasonal resource limitation that are highly consistent with the ericoid, proteoid and
538 restioid functional type responses characterised by prior studies.

539 The predictions drawn from the physiological performances of our study species under moderate
540 drying conditions are consistent with previous experimental drought studies conducted in the CFR
541 in which plants were subject to more extreme drought. For example, our drought impact
542 predictions based on the *in situ* physiological responses match patterns of variation among species
543 in growth, flowering output and mortality following West et al.'s (2012) rainfall manipulation
544 experiment. After experimentally removing summer rainfall in their field manipulation study,
545 West et al. (2012) showed *Erica* species had reduced growth and flowering output, while restioid
546 and overstorey proteoid species displayed little treatment effect on growth, and flowering output.
547 This is well-aligned with our finding of the vital role that small, but regular summer rainfall events
548 play in the maintenance of function in *Erica*. *Erica* species suffered the most amount of branch
549 dieback and individual mortality, while restioids and proteoids suffered the least negative impact.
550 Another experimental drought study conducted on seedlings of several Proteaceae species
551 demonstrated that seedlings possess an ability to persist for prolonged periods without watering
552 (Arnolds *et al.* 2015), suggesting that West et al.'s observed responses apply across life history
553 stages in this functional type. Our study suggests that knowledge of the quantitative physiological
554 responses of a few species representing dominant functional types can be used to make meaningful
555 predictions about future drought impacts in hyper diverse plant communities such as the mountain
556 fynbos.

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Figures

Figure 1

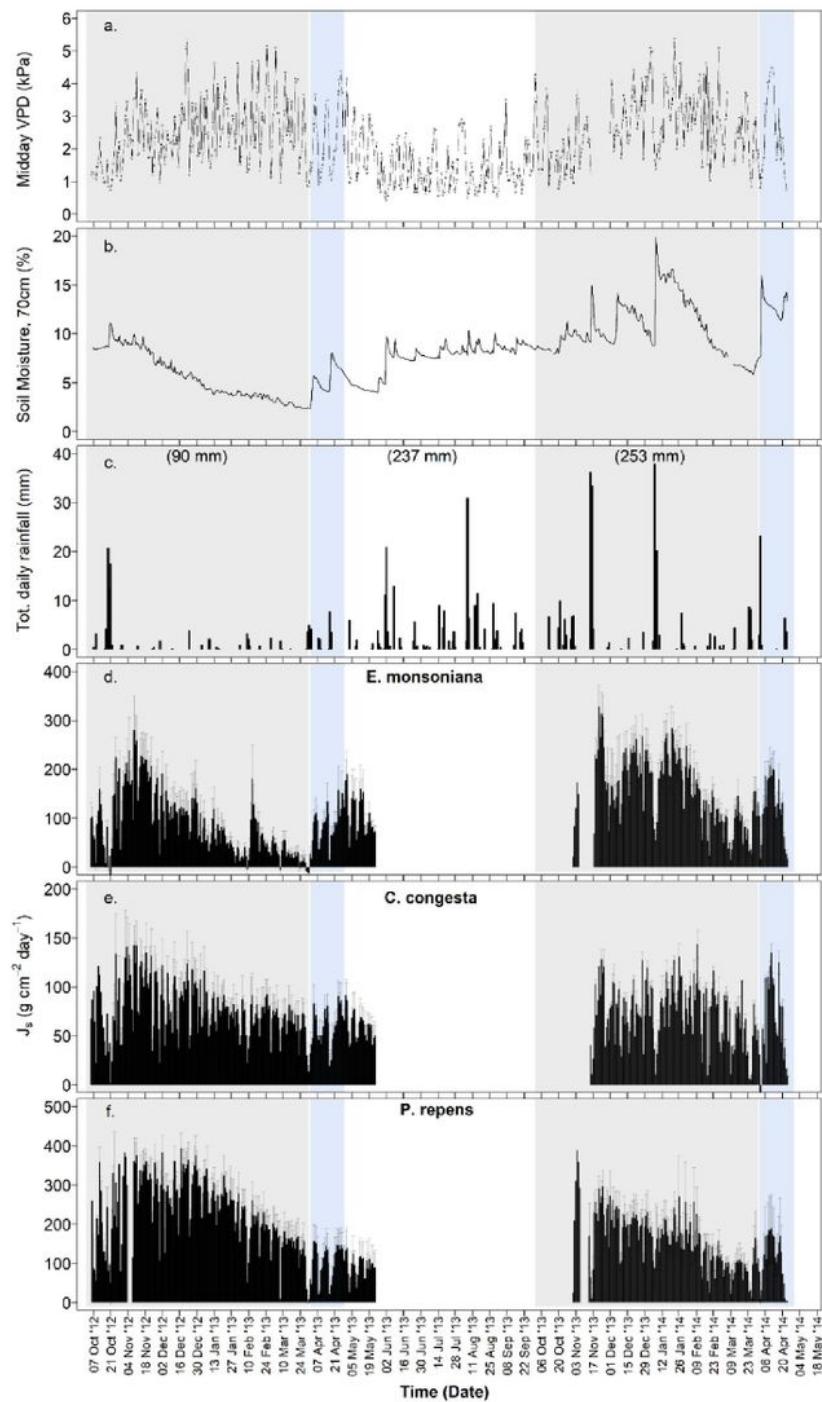


Figure 1

Timeline of environmental variables and total daily sap flux density (J_s) for each species recorded at the study site over the course of the study period. (a.) Mean daily midday vapour pressure deficit (VPD, kPa); (b.) soil moisture measured at a soil depth of 70cm (%); (c.) total daily rainfall (mm); and total daily sap

flux density (J_s , $g\ cm^{-2}\ day^{-1}$) for *Erica monsoniana* (d), *Cannomois congesta* (e) and *Protea repens* (f). Six-month total rainfall amounts (for summer 2012/13, winter 2013, and summer 2013/14) are indicated in parentheses. Grey background shading indicates summer periods, blue shading indicates periods when soil moisture recovered following rehydrating rainfall events (see Methods for further details).

Figure 2

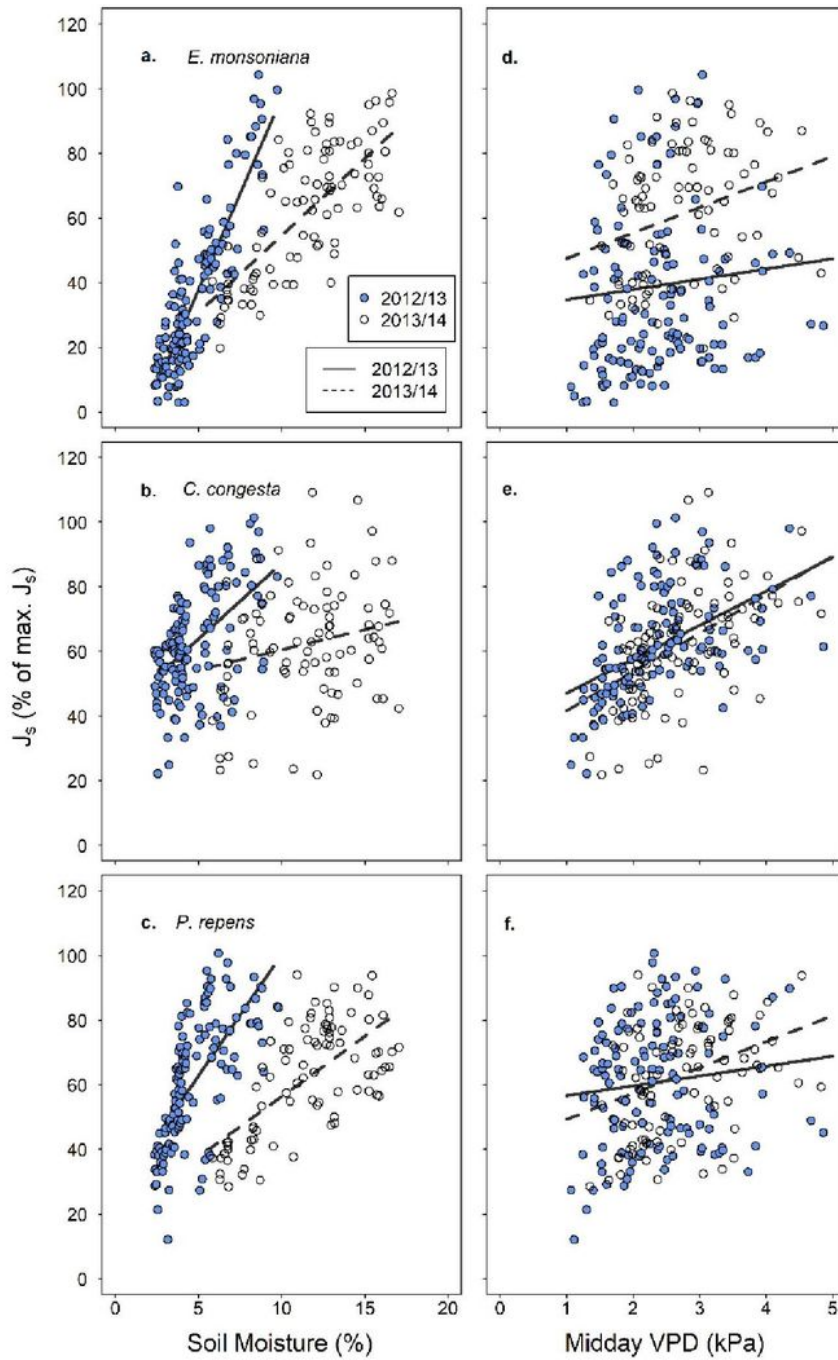


Figure 2

Relationship between total daily sap flux density (J_s) and soil moisture or midday vapour pressure deficit (VPD) for the three study species in the representative dry year (2012/13) and the normal year (2013/14). Relationships between variables were assessed using linear mixed effects models.

Figure 3

Timeline of environmental variables and total daily sap flux density (J_s) for each species recorded at the study site over mid-summer during the representative dry year (2013). (a.) Mean daily soil moisture measured at a soil depth of 70cm (%), total daily rainfall (mm), and duration of cloud/dew events; total daily sap flux density (J_s , $g\ cm^{-2}\ day^{-1}$) for *Erica monsoniana* (b), *Cannomois congesta* (c) and *Protea repens* (d).

Figure 4

Timeline of mean midday sap flow derived stomatal conductance (G_{sf} ; mean \pm s.e.) expressed as a percentage of pre-stressed maximum G_{sf} for *Erica monsoniana* (a), *Cannomois congesta* (b), and *Protea repens* (c). Grey shaded areas indicate periods in summer when plant xylem water potentials generally declined, blue shaded areas indicate periods when plant xylem water potentials recovered to pre-stressed values. Arrows indicate small summer rain events, vertical dashed lines indicate large rainfall events. For comparison, the leaf level stomatal conductance is also shown (red points; mean \pm s.e.).

Figure 5

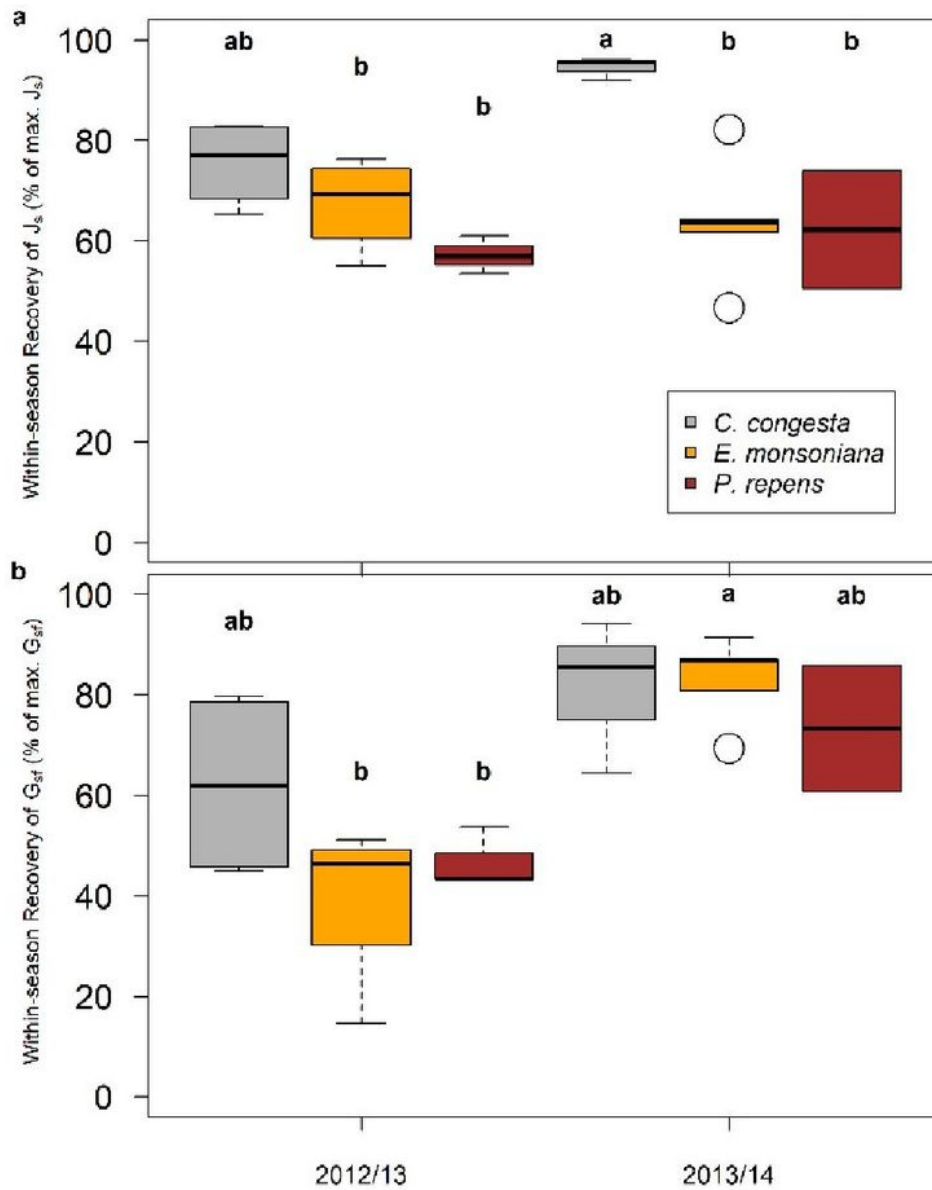


Figure 5

The recovery of total daily J_s (a) and G_{sf} (b) for each species in the representative dry year (2012/13) and the normal year (2013/14). Letters indicate statistical differences revealed by post-hoc tests.

Figure 6

Timeline of predawn and midday xylem water potential (mean \pm s.e., n = 5) for the three study species over the course of the two summer study periods. Grey and blue shading is the same as for Figure 1.

Figure 7

(a-c) Stomatal conductance and water potential envelopes for three Jonaskop study species (orange and red points) and Silver mine species from West et al. 2012 (grey points). Also shown are the xylem vulnerability curves for the three sample species: solid black lines are mean xylem vulnerability curves for each species, grey lines are individual curves, and filled white circles indicate the P50 values for each species. Minimum xylem water potentials for each species are also shown. Bars indicate the range between minimum predawn and minimum midday water potentials recorded in 2012/13 and 2013/14. (d-f) The relationship between stomatal conductance and carbon assimilation measured for all three species in the representative dry year (2012/13) and the normal year (2013/14). Grey lines are best fit models of the relationships.

Supplementary Files

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