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8 **Aridity drives coordinated trait shifts but not decreased trait variance across the**

9 **geographic range of eight Australian trees**

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60 All unpublished data is archived on Dryad (DOI: https://doi.org/10.6078/D1QQ5X) and relevant

61 data and code is posted on Github (https://github.com/leanderegg/EucTraits)

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68 **Summary:**

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70 • Large intraspecific functional trait variation strongly impacts many aspects of

71 communities and ecosystems, and is the medium upon which evolution works. Yet

72 intraspecific trait variation is inconsistent and hard to predict across traits, species, and

73 locations.

74 • We measured within-species variation in leaf mass per area (LMA), leaf dry matter

75 content (LDMC), branch wood density (WD), and allocation to stem area vs. leaf area in

76 branches (branch Huber value, HV) across the aridity range of seven Australian eucalypts

77 and a co-occuring *Acacia* species to explore how traits and their variances change with

78 aridity.

79 • Within-species, we found consistent increases in LMA, LDMC and WD, and HV with

80 increasing aridity, resulting in consistent trait coordination across leaves and branches.

81 However, this coordination only emerged across sites with large climate differences.

82 Unlike trait means, patterns of trait variance with aridity were mixed across populations

83 and species. Only LDMC showed constrained trait variation in more xeric species and

84 drier populations that could indicate limits to plasticity or heritable trait variation.

85 • Our results highlight that climate can drive consistent within-species trait patterns, but

86 that patterns might often be obscured by the complex nature of morphological traits,

87 sampling incomplete species ranges, or sampling confounded stress gradients.

88

89

90 **Introduction:**

91 Land plants exhibit astounding variation in both form and physiological function. The

92 identification of 'functional traits' as easily measured plant attributes that are proxies for plant

93 physiological function and performance has spurred the rise of the field of 'plant functional

94 ecology' and revealed some of the key causes and consequences of plant functional diversity

95 (Mooney *et al.*, 1978; Field, 1988; Reich *et al.*, 1997; Díaz *et al.*, 2016; Ma *et al.*, 2018). In

96 particular, across-species studies of plant traits have revealed global 'trait spectra' or 'trait

97 syndromes'—correlations between different plant traits indicative of coordination across various

98 aspects of plant physiology—that both illuminate trade-offs shaping plant evolution and provide

99 powerful tools for community and ecosystem ecological studies (Wright *et al.*, 2004; Reich,

100 2014; Ma *et al.*, 2018).

101 While functional ecology has largely been built on trait patterns among species, our

102 understanding of trait variation and trait coordination within individual species remains more

103 limited. Ecologists increasingly recognize that within-species trait variation can be a large

104 fraction of total trait variation (Albert *et al.*, 2010b; Siefert *et al.*, 2015), and that within-species

105 trait variation has large consequences for ecological and evolutionary processes (Laforest-

106 Lapointe *et al.*, 2014; Ahrens *et al.*, 2019a). Within-species variation in functional traits linked to

107 stress tolerance has been increasingly used to predict plant responses to global change

108 (Blackman *et al.*, 2017; Ahrens *et al.*, 2019a). Even as our appreciation of the importance of

109 intra-specific variation grows, a mounting body of perplexing results reveals the limits to our

110 understanding of within-species variation. For example, within-species trait responses to

111 environmental gradients have defied generalization by proving highly trait-specific and species-

112 specific (e.g. Schulze *et al.*, 1998; Albert *et al.*, 2010b,a; Vilà-Cabrera *et al.*, 2015; Rosas *et al.*,

113 2019), and sometimes even study specific (e.g. Martinez-Vilalta *et al.*, 2009; Laforest-Lapointe

114 *et al.*, 2014). Some possible explanations for these inconsistencies are: 1) that different taxa

115 employ different strategies of trait adjustment (Anderegg & HilleRisLambers, 2015) in which

116 case closely related species will show more consistent trait responses, 2) that geographic stress

117 gradients often confound multiple stressors, particularly drought and cold stress, and 3) that trait

118 adjustments over only a portion of a species range are often subtle and hard to detect without

119 sampling a species entire distribution (López *et al.*, 2016).

120 The between-species trait-trait coordination (consistent correlation among multiple traits)

121 that underpins theory about trait spectra does not necessarily hold within individual species

122 (Messier *et al.*, 2017; Anderegg *et al.*, 2018; Messier *et al.*, 2018). For instance, a recent analysis

123 of intra-specific trait coordination in saplings of temperate tree species found that essentially

124 none of the canonical trait relationships behind three classic theories of trait coordination held

125 among individuals within species (Messier *et al.*, 2018). In another example, strong between-

126 species trait-by-environment relationships and trait coordination didn't hold across populations

127 within those same species in northern Spain (Rosas *et al.*, 2019). Indeed, some important trait-

128 trait relationships can even reverse direction within- versus between-species (Anderegg *et al.*,

129 2018). This contrasting within- verse between-species trait coordination suggests that classical

130 explanations of trait correlations do not necessarily hold within-species, limiting their

131 applicability for predicting species' functional responses to climate change.

132   Additionally, patterns of trait variances within-species remain poorly understood. The

133 study of trait variance (rather than trait means) has a long history in community ecology (Kraft *et*

134 *al.*, 2014) and evolutionary studies, where heritable trait variance is the necessary precondition

135 for evolution. Yet trait variances have often been overlooked in the ecophysiological literature.

136 Ultimately, predicting plant responses to a shifting environment requires an improved

137 understanding of the amount of heritable trait variation, the capacity for trait plasticity, and the

138 trait-fitness links causing evolutionary selection within a species and/or performance differences

139 among species in a community (Richter *et al.*, 2011; Chevin *et al.*, 2012; Alberto *et al.*, 2013;

140 Franks *et al.*, 2014; Valladares *et al.*, 2014). As a first step towards this understanding,

141 observations of the size and spatial patterns of trait variances in different species and between

142 populations of the same species are critical (Molina-Montenegro & Naya, 2012; Lemke *et al.*,

143 2012; Siefert *et al.*, 2015). For instance, decreased amounts of trait variation in range-edge

144 populations of a species could indicate fundamental limits to trait plasticity or that the strength of

145 directional selection surpasses the rate of generation of genetic diversity (e.g. immigration,

146 mutation). Either would indicate limited potential for trait change in those populations in a

147 changing environment. Among species, environmental stress has been predicted to constrain

148 phenotypic plasticity (Valladares *et al.*, 2007) but also possibly increase the expression of

149 genetic variability in traits (Hoffmann & Merilä, 1999), and current evidence for either

150 increasing or decreasing within-species trait variation with drought stress is weak (Siefert *et al.*,

151 2015). Thus, understanding trait variances may be more critical to predicting potential plant

152 responses to climate change than trait means themselves.

153   While many of the plant physiological traits known to be most related to drought

154   resistance are time consuming to measure (e.g. xylem vulnerability to embolism, leaf turgor loss

155   point), a number of morphological "soft traits" have less direct but still clear links to drought

156   resistance and can be tractably assessed across hundreds of individuals and large geographic

157   areas. For instance, across species, increased leaf robustness quantified by Leaf Mass per Area

158   (LMA) and Leaf Dry Matter Content (LDMC) and stem robustness quantified by Wood Density

159   (WD) are often associated with xeric environments because their variation is partly driven by

160   anatomical adjustments that allow plants to maintain hydraulic function under increasingly

161   negative xylem pressures (Schulze *et al.*, 1998; Niinemets, 1999; Schulze *et al.*, 2006; Chave *et*

162   *al.*, 2009; Poorter *et al.*, 2009; John *et al.*, 2017; Li *et al.*, 2018b). The ratio of stem sapwood area

163   to leaf area or Huber value (HV) reflects the balance of hydraulic supply (sapwood area) relative

164   to hydraulic demand (leaf area), with high HV typically indicating increased hydraulic efficiency

165   and thus increased drought avoidance (Tyree & Ewers, 1991; Mencuccini & Grace, 1995; Li *et*

166   *al.*, 2019; Mencuccini *et al.*, 2019). Even though these morphological traits integrate numerous

167   drought- and non-drought-related anatomical traits, multiple tree species have been found to

168   adjust at least one of these traits depending on water availability (Martinez-Vilalta *et al.*, 2009;

169   Anderegg & HilleRisLambers, 2015; Rosas *et al.*, 2019). Moreover, in recent studies of

170   *Eucalyptus* species, changes in LMA, HV and WD were associated with more physiologically

171   direct mechanisms of drought tolerance such as changes in xylem anatomy and vulnerability to

172   embolism (Pfautsch *et al.*, 2012; Zolfaghar *et al.*, 2015; Pfautsch *et al.*, 2016; Li *et al.*, 2018b;

173   Pritzkow *et al.*, 2019), suggesting that they are relevant markers of drought resistance in

174   eucalypts. These traits are also central to our understanding of plant carbon allocation and carbon

175   stocks. For example, LMA is a key model parameter in many vegetation models for translating

176   carbon allocated to leaves into leaf area and WD is a critical component of carbon stock

177   estimation and prediction (Nabuurs *et al.*, 2008; Kovenock & Swann, 2018).

178   Within-species trait variation at landscape-scales is the result of some unknown

179   combination of genetic or 'ecotypic' variation among populations (G effects), plastic adjustments

180   to environmental gradients (E effects) and GxE interactions. Definitively disentangling these

181   components requires experiments such as provenance trials or common gardens (McLean *et al.*,

182   2014; Ahrens *et al.*, 2019b). However, because genetic variation among species is traditionally

183   larger than ecotypic variation within species, trait variation among related species in their native

184 habitat is often ascribed primarily to G effects. Thus, by sampling within-species and across

185 closely-related species, both genetic + plastic trait changes and primarily genetic trait changes

186 can be explored.

187   Here, we examine within-species variation in leaf and stem robustness and allocation

188 within closely related tree species across large gradients in water availability in the absence of

189 confounding freezing stress. We present a controlled test of predictions about intraspecific trait

190 variation across nested scales of organization, focusing on trait variation across aridity gradients

191 in Western Australia and Tasmania. Further, we minimize differences in species life history by

192 holding phylogenetic history relatively constant for seven core species ('eucalypts' from the

193 closely related *Eucalyptus* and *Corymbia* genera). We compare these patterns within eucalypts to

194 an unrelated species (*Acacia acuminata*) that co-occurs with the most xeric sampled eucalypt.

195 The specific questions we ask are:

196   1. Do leaf and stem tissues, and leaf vs stem allocation show consistent relationships with

197   water availability across the full aridity range of multiple closely related species? Or are

198   trait-environment relationship context dependent (e.g. present in xeric species but not in

199   mesic species)?

200   2. Do species consistently show coordination between leaf and stem robustness, and leaf to

201   stem allocation, and if so at what scale does this coordination emerge?

202   3. Is the total amount of within-species variation in leaf and stem traits more constrained in

203   dry sites (both within species across sites and across sister species with different aridity

204   niches), indicating potential limits to trait change in a drying climate?

205

206   Given their association with drought resistant phenotypes, we expected LMA, LDMC, WD

207 and HV to increase with aridity, resulting in coordinated trait changes across tissues. We

208 predicted consistent trait-environment relationships among closely related eucalypts, regardless

209 of whether they were mesic or xeric, but potentially less consistency between the eucalypts and

210 an unrelated *Acacia.* Alternatively, if trait adjustments are not consistent among eucalypts, we

211 predicted that xeric eucalypts and the xeric *Acacia* would show stronger trait patterns than mesic

212 eucalypts due to the increased biogeographic importance of drought stress in harsh environments

213 (MacArthur, 1972). In addition, assuming ongoing directional selection and a limit to both

214 plasticity and genetic variation near each species' dry range edge, we predicted that within-

215 species variation in these traits would decrease in higher aridity populations within a species. If

216 plasticity is constrained in harsh environments, we also predicted that xeric species would show

217 less within-species trait variation than mesic species.

218

219 **Methods:**

220 *Study site*

221 We collected trait data along two temperate aridity gradients (Figure S1), one in

222 southwest Western Australia (sampled November 2014) and one in Tasmania (sampled February

223 2016). Along each gradient, we identified three or four dominant eucalypt tree species (from the

224 *Eucalyptus* or *Corymbia* genera of the Myrtaceae family) that are easily identified in the field

225 and do not widely form cryptic hybrids or have notable subspecies within the sampled regions. In

226 Western Australia, we sampled *Eucalyptus marginata* Donn ex Sm., *Eucalyptus salmonophloia*

227 F.Muell., and *Corymbia calophylla* (Lindl.) K.D. Hill & L.A.S. Johnson. We also

228 opportunistically sampled the non-eucalypt *Acacia acuminata* Benth., which broadly co-occurs

229 with the most xeric sampled eucalypt, *E. salmonophloia.* In Tasmania we sampled *Eucalyptus*

230 *amygdalina* Labill.*, Eucalyptus obliqua* L'Hér.*, Eucalyptus ovata* Labill.*,* and *Eucalyptus*

231 *viminalis* subsp. *viminalis* Labill, all of which cover the majority of their global precipitation

232 range within Tasmania*.* All focal species are evergreen, dominant or co-dominant canopy trees

233 with the exception of *Acacia acuminata*, which is a small tree/tall shrub. All focal species are

234 common in multiple vegetation types and are both habitat generalists (i.e. not riparian-affiliated)

235 and soil type generalists with the exception of *E. marginata*, which is a habitat generalist but

236 principally occurs on ironstone-derived soils. Collectively, sampled sites spanned a mean annual

237 precipitation range of 328 to 1574mm/year (328 to 1189 mm in Western Australia, 584 to 1574

238 mm in Tasmania). Mean annual temperature spanned 8-20°C and elevation ranged from 24-620

239 m.a.s.l, with no site experiencing significant frost (mean coldest month minimum temperature

240 >0°C for all sites). Average site climate, soil, DBH (diameter at breast height as a proxy for tree

241 size) and stand basal area (measured for each tree with a variable radius forestry wedge prism,

242 Tasmania only) can be found in Table S1. Sampled tree size and (where measured in Tasmania)

243 stand Basal Area did not vary strongly with aridity for most species (Table S1). Climate data for

244 sampled plots, including mean annual precipitation (PPT), potential evapotranspiration (PET),

245 and moisture deficit (MD = PET – PPT), were extracted from the CHELSA 30 arc second

246   (~1km) gridded climate database of 1979-2013 climatologies (Karger *et al.*, 2017). Aridity Index

247   (P/PET) was also calculated but found to be almost perfectly collinear with MD(Figure S2). Soil

248   properties including soil depth and regolith depth, as well as % sand, silt and clay, total nitrogen

249   by mass, total phosphorus by mass, average water holding capacity, bulk density, and effective

250   cation exchange capacity (averaged over the top 60cm soil depth) were downloaded from the 3

251   arcsecond resolution (~90m) Soil and Landscape Grid of Australia (Grundy *et al.*, 2015), using

252   the *slga* R package (O'Brien 2019). Because soil properties were strongly collinear, we

253   performed a Principal Component Analysis (PCA) on the soil variables and used the first two

254   principal components (PCs) in analyses. The first PC explained 67% of soil variation and was

255   interpreted as 'soil fertility' because it loaded strongly (>0.3) with everything except depth of

256   regolith, depth of soil and water holding capacity. The second PC captured 12% of variation,

257   loaded strongly with water holding capacity and soil depth and was interpreted as 'soil depth'.

258

259   *Trait measurement*

260   We measured branch wood density (WD, g dry mass per cm^3 fresh volume), leaf mass

261   per area (LMA, g dry mass per cm^2 fresh leaf area) and leaf dry matter content (LDMC, g dry

262   mass per g fresh mass) as metrics of stem and leaf robustness, and terminal branch Huber value

263   (HV), the ratio of sapwood area to leaf area (mm^2 per cm^2), as a metric of investment in water

264   transport versus light capture. Trait measurements were collected in a nested hierarchical design

265   with four to five sites sampled per species to capture broad climate gradients, three plots per site

266   to capture topographic/edaphic variation, five trees per plot to capture within-population

267   variation, and three samples per tree to capture within-individual variation (Figure S1). For each

268   species, four to five forestry reserves, National Parks, State Forests, Nature Reserves, or

269   Conservation Areas were selected to cover as much of each species' precipitation range as

270   possible. Each species' precipitation range was determined using collection locations of

271   herbarium specimens, initially downloaded from Australia Virtual Herbarium

272   (www.avh.chah.org.au) for identifying sampling locations and later validated with expanded

273   occurrence records from the Atlas of Living Australia (see species climate distributions and

274   sampling coverage in Figure S3, ala.org.au). Edaphic variation within sites was captured by

275   locating three plots that were >500 m but <5 km apart and each containing more than five

276   individuals of the focal species within a 30 m radius. In each of the three plots, we sampled

277 within-population variation by collecting three sun exposed branches from the north side of each

278 of five mature, healthy individuals using pole clippers and pull ropes. Sampled individuals in a

279 plot were apparently healthy, canopy dominant trees at least 5m but less than 60m apart. Our

280 sample design resulted in 180-225 trait measurements per species.

281 From each branch, we collected a section ~8 mm in diameter for WD measurement, and a

282 terminal branch (first order branch collected at the point of branching) for leaf and HV

283 measurements. We selected terminal branches (typically ~1mm in diameter, see Table S2 for

284 details) with intact 'mature' leaves (i.e. fully expanded, not soft green new growth), though most

285 of the study species flush sporadically throughout the year (Davison & Tay, 1989; Heatwole *et*

286 *al.*, 1997) so it was not possible to perfectly control for leaf age. Sampling periods (Nov. in

287 Western Australia and Feb. in Tasmania) avoided large leaf flush events for all species with the

288 exception of *Corymbia calophylla* at two of its five sample sites. Samples were rehydrated in

289 moist ziplock bags in a cooler for at least 12 hours prior to trait measurement (Pérez-

290 Harguindeguy *et al.*, 2013). Bark was peeled from branch sections and WD quantified from

291 segments roughly 7 cm in length by dividing dry mass (following 72+ hrs drying at 70°C). WD

292 was weakly related to branch diameter for six species (Likelihood Ratio Test of a linear mixed

293 effects model with a fixed effect for diameter and site and random effects for plot and tree was

294 significant at alpha = 0.05 compared to an identical model without diameter), so diameter was

295 included as a covariate in models of WD for these species.

296 All leaves subtending the selected terminal branch were collected for measurement of

297 leaf area, LMA and LDMC. Total fresh one-sided leaf area (including petioles) of terminal

298 branch samples was measured with a flatbed scanner and ImageJ image processing software

299 (Schneider *et al.*, 2012). Leaves were then oven dried at 70°C to a constant weight (typically 48+

300 hrs) and their dry mass measured. Terminal twig basal diameter was measured just above the

301 swelling at the branch base after gently peeling back bark (except in *A. acuminata*, where bark

302 was difficult to distinguish from woody tissue). For each terminal branch HV, LMA, and LDMC

303 was calculated. Multivariate trait outliers were visually diagnosed by plotting all traits against

304 each other for each species and removed (n<10 per trait), as were LMA and LDMC values from

305 still expanding leaves (<10% of measurements).

306

307 *Statistics*

308

309   <u>Q1 – Trait-aridity relationships:</u> We tested for significant trait-environment relationships using

310   information-theory based model selection. For each species, we fit candidate linear mixed effects

311   models relating each trait to plot mean annual PPT, PET, MD, soil fertility (soil PC1), or soil

312   depth (soil PC2) with plot and tree random intercepts. We also included tree DBH (measured for

313   all species except for often multi-stemmed *Acacia acuminata* and for *E. salmonophloia* which

314   was missing measurements from two site) and the stand Basal Area around the focal tree

315   (measured for Tasmanian species with a wedge prism) as potential individual-level covariates

316   where measured. Because soil and climate variables were often collinear (Figure S2) and most

317   soil and climatic variation occurred among sites (n=4-5 per species) we only included models

318   with single soil/climate predictors and not multiple predictors or interactions to avoid overfitting.

319   We then compared the candidate models and a null model (with only plot and tree random effect)

320   using Akiake's Information Criterion corrected for small sample sizes (AICc) and selected the

321   model with the fewest parameters that was within 2 AICc of the model with the lowest AICc. We

322   quantified statistical significance of this model compared to the null model using Likelihood

323   Ratio Tests (LRT). Where a soil variable proved the best trait predictor, we also tested the

324   significance of the best climate model because soil and climate variables were often strongly

325   collinear (Figure S2). We visually checked the model fit and the validity of model assumptions

326   (e.g. normality of residuals, normality of random effects) using a variety of model criticism plots

327   (see code at https://github.com/leanderegg/EucTraits).

328

329   <u>Q2 – Trait coordination:</u> We assessed trait-trait covariation using multiple approaches. First, for

330   each species we tested for significant Pearson correlations between tree-level averaged traits for

331   all trait pairs and visualized the relationships with Standardized Major Axis (SMA) regressions.

332   Next, we assessed the distribution of trait-trait correlations for hierarchically nested data subsets

333   to assess at what level trait coordination emerges. For each trait pair for each species, this

334   involved calculating the Pearson correlations across the replicate branches within each tree,

335   across tree averages in each plot, across plot averages in each site, and across site averages, for

336   all eight sampled species. Lastly, we assessed the dominant mode of trait covariation across all

337   traits and species. We performed a Principal Component Analysis (PCA) on all branch-level trait

338   measurements with complete trait data (1400 branches), and assessed the trait loadings along the

339   first and second PC axes. We then calculated the PC score for all site-averaged trait values, and

340   assessed whether any PC related to site MD across species using a linear mixed effect model

341   including a fixed effect of MD and species random slopes and intercepts.

342

343   Q3 – Constrained variance at high aridity: We first examined whether more xeric species showed

344   less intraspecific trait variation than mesic species. For each species and trait, we quantified the

345   amount of trait variation at each nested scale using variance decomposition by fitting a linear

346   mixed effect model with a fixed intercept and random effects for site, plot, and tree. In this

347   formulation, the random effect variance parameters represent the between-site, between-plot in

348   site, and between-tree in plot variance (respectively), with the residual variance representing

349   samples within tree. We then characterized species' aridity niche based on occurrence records in

350   the Atlas of Living Australia, extracting the MD values for each occurrence from the CHELSA

351   gridded climatologies and calculating the range center (median MD) and dry range edge (90th

352   percentile MD) of each species' climatic distribution. We then used the species' range center or

353   dry range edge as an index of how mesic or xeric each species' range is. To test for among-

354   species patterns, we extracted the variance parameters for each eucalypt species (excluding

355   *Acacia acuminate*) and used linear models to relate species total trait variance (sum of all

356   variance components for a trait) to the species' dry range edge. We also tested whether

357   individual variance components decreased with increasing aridity by fitting linear models

358   relating species variance components to each species' driest range edge plus a fixed effect for

359   variance component (between-site, between-plot, between-tree, or within-tree) and a component-

360   by-MD interaction.

361   To test for decreasing trait variation with aridity within species (i.e. across populations),

362   we used AICc to determine whether the best trait-aridity mixed effect model (from Q1) for each

363   species and trait was improved by allowing the variance to change as either a power or

364   exponential function of the dominant climate variable, or to assume a different value for each

365   site. If AICc and LRTs suggested that a non-constant variance function improved the trait-

366   climate model, we classified whether the variance increased with aridity, decreased with aridity,

367   or showed variation between sites that was not aridity-related (i.e. the model with different

368   variances per site was the best model).

369   All analyses were performed in the R statistical environment (R Core Team, 2019),

370   version 3.6.0). Mixed effects models were fit using the *lme4* and *lmerTest* packages (Bates *et al.*,

371   2015; Kuznetsova *et al.*, 2017) for fixed variance models, or the *nlme* package for more

372   complicated variance structures (Pinheiro *et al.*, 2019). SMA regressions were fit using the

373   *lmodel2* package (Legendre, 2014). All data and analysis code is available in the Github

374   repository associated with this paper (https://github.com/leanderegg/EucTraits). Data are also

375   available in the Dryad data repository (DOI: https://doi.org/10.6078/D1QQ5X).

376

377   **Results**

378   *Do traits respond to aridity?*

379   For the majority of our examined species, most traits shifted in a way consistent with

380   greater drought resistance (increased WD, LMA, LDMC and HV) in higher aridity plots (Figure

381   1). All species showed significant trait-by-environment relationships for LMA and LDMC and

382   seven of the eight species showed significant trait-by-environment relationships for WD and HV

383   (Table S3). A measure of aridity (PPT, PET or MD) was the best predictor in 19 of 32 trait-by-

384   environment relationships, soil fertility in 9 of 32 and soil depth in 2 of 32. However, in all but

385   one of the trait-by-environment relationships where soil quality or depth was the best predictor,

386   precipitation was collinear to that soil variable and also a significant, if worse, predictor (Table

387   S3). Precipitation, potential evapotranspiration, moisture deficit and soil fertility were correlated

388   across plots for many, but not all species (Figure S2). Tree DBH was never a significant trait

389   predictor in final models, and stand Basal Area was only included as a covariate for one trait

390   (LMA) for two of the Tasmanian species (Table S3). Across the seven eucalypts, species mean

391   trait values also showed significant or marginally significant positive relationships with species

392   median MD, though for WD and LMA this was driven primarily by the driest species (Figure

393   S4).

394

395   *Are trait responses coordinated across tissues?*

396   Ubiquitous trait-by-environment relationships resulted in coherent trait coordination

397   across leaf and stem tissue, and coordination between leaf robustness and increased HV within

398   species (Figure 2). However, while consistent and often significant, these within-species trait

399   correlations were typically weak, with the mean within-species trait correlation being <0.5 for all

400 trait pairs except LMA and LDMC. Across tree-level trait averages, the majority of species

401 showed significant correlations between both WD and LMA (mean correlation of 0.33) and WD

402 and LDMC (mean correlation of 0.38; Figure 2a, 2b), though these were typically less strong

403 than the correlations between LMA and LDMC (mean correlation of 0.74; Figure 2c). Both leaf

404 traits were also positively correlated with HV, with mean correlations of 0.44 and 0.32 for LMA

405 and LDMC respectively. However, WD was only significantly correlated with HV in three

406 species. In the seven eucalypts, most species fell in roughly the same trait space, with more trait

407 variation within each species than across species (Figure 2). *Acacia acuminata* showed larger

408 HV, but similar trait correlations to the seven eucalypts (Figure 2d, 2e, 2f).

409   Within-species trait coordination only emerged when comparing traits across the most

410 disparate environments. The distribution of correlation coefficients at smaller spatial scales (e.g.

411 trait-trait correlations across individuals or branches within a plot, correlations across plots or

412 individuals within a site) typically had an interquartile range spanning zero for all trait pairs

413 except LMA-LDMC and HV-LMA (Figure 2g, Table S4). Only when comparing across site

414 mean trait values did the mean within-species correlation differ substantially from zero for most

415 trait pairs (Table S4). This decrease in correlation strength at smaller spatial scales was not

416 purely a result of smaller sampled trait variation, as there was often as much or more trait

417 variation within plots as across sites, and funnel plots did not show strong relationships between

418 correlation strength and sampled trait variance except for the relationship between HV and LMA

419 (Figure S5).

420   Even though trait coordination only emerged across large aridity gradients, the dominant

421 mode of trait variation in the entire dataset was a coordinated increase in tissue robustness and

422 HV, both within and among species, likely driven by decreasing water availability. In a PCA of

423 the entire branch-level dataset, the first principal component (PC1) explained 53% of the total

424 variance and was loaded reasonably equally with all four traits (Figure 3a). Additionally, for

425 each species the site-level average PC1 score was strongly related to site PPT and MD,(linear

426 mixed-effects models, p<0.0007 and 0.004, marginal R^2 = 0.51 and 0.53 respectively) though not

427 site PET. While the slopes differed between species (particularly for the driest eucalypt and the

428 *Acacia*) these patterns indicate that the coordinated increase in WD, LMA and LDMC, and HV

429 represented by PC1 was driven by water availability (Figure 3b). The second trait PC (PC2)

430   explained 26% of the variation, was largely unrelated to climate, and primarily differentiated the
431   seven eucalypts from *Acacia acuminata* (Figure S6).

432

433   *Is trait variation constrained at higher aridity?*

434   Evidence for increasingly constrained trait variation at higher levels of aridity was mixed,
435   both within and among species. Variance decomposition revealed huge variability in the total
436   amount and dominant scales of within-species trait variation (Figure 4). Variation between plots
437   in a site was almost always the smallest variance component. The relative contribution of within-
438   tree, within-plot and between-site variation differed drastically, however, depending on the trait
439   and species (Figure 4). The only exception was the consistently high amount of within-tree
440   variation in $\log_{10}$-transformed HV, which made up >40% of total trait variation in all species.
441   *Acacia acuminata* also tended to have much larger intra-specific trait variation than any of the
442   sampled eucalypts. Combining all trait data from all seven eucalypt species, within-species trait
443   variation represented between 31% (WD) and 72 % ($\log_{10}$(HV)) of total trait variation, and
444   between-site/climate-related within-species trait variation was over half the magnitude of inter-
445   specific variation in LDMC and $\log_{10}$(HV) (Figure S7).
446   Across species, there was limited evidence for decreased intraspecific trait variation in
447   more xeric species. In the seven eucalypts, total within-species trait variation was unrelated to
448   the aridity of a species' geographic distribution (the median MD of herbarium specimen
449   locations) for WD, LMA, and LDMC, but was marginally negatively related for LDMC (p=0.11;
450   Figure 5a-d). Most individual variance components were also unrelated to species aridity niche.
451   However, the amount of between-site variation was negatively related to species aridity niche for
452   HV (p=0.001) and marginally for LDMC (p=0.07; Figure 5e-h). Results were similar but slightly
453   more significant using the species' dry range edge (90th percentile MD) rather than niche center
454   (median MD). Results were also similar using trait coefficients of variation (CV=trait standard
455   deviation divided by trait mean) rather than trait variances, though the CV of HV was no longer
456   related to species mean MD but the CV of LMA decreased marginally with MD (p=0.067,
457   Figure S8).
458   Within-species, variance patterns moving from wet sites to dry sites also showed mixed
459   support for decreasing variance with increasing aridity. A few species did show constrained
460   within-tree and within-plot trait variation at drier sites in a few traits, consistent with an

461 increasingly strong environmental filter. However, most species for most traits showed no

462 change in trait variance across sites (Figure 6). LDMC showed the most consistent variance

463 constraint with aridity, with three of eight species showing lower trait variances at drier sites. HV

464 showed no aridity-related variance patterns in any species (Figure 6).

465

466 **Discussion**

467   Our extensive dataset of 1620 paired trait measurements demonstrated that increasing

468 aridity resulted in coordinated trait shifts. However, these shifts were only evident across large

469 aridity gradients. Moreover, despite ubiquitous aridity-related increases in trait means consistent

470 with high trait values being adaptive, we did not find decreasing trait variances with decreasing

471 water availability except in LDMC, providing little initial evidence for constrained plasticity or

472 limited genetic variation. Below, we discuss these results in greater detail.

473

474 *Mean trait shifts*

475   Shifts in leaf, stem, and allocation traits towards more drought resistant values at drier

476 sites were ubiquitous across the sampled species (Figure 1). Indeed, within-species trait shifts

477 due to some combination of local adaptation and plasticity were in the same direction and

478 sometimes even of the same magnitude as trait relationships across species (Figure S4),

479 particularly for the driest euclaypt (*E salmonophloia*) and for the trait HV. These shifts are

480 consistent with increases in tissue drought tolerance, and indeed all traits but LDMC have

481 previously been reported to show within-species patterns related to water availability either

482 geographically or experimentally in eucalypts (Li & Wang, 2003; Schulze *et al.*, 2006; Zolfaghar

483 *et al.*, 2014; McLean *et al.*, 2014). In *Eucalyptus obliqua,* geographic variation in LMA and HV

484 are associated with concurrent changes in physiological traits such as leaf turgor loss point,

485 xylem vessel wall thickness, and xylem vulnerability to embolism, primarily through plasticity

486 rather than genetic ecotypic variation (Pritzkow *et al.* 2019). However, these same traits often do

487 not show aridity-related variation within-species in other systems (Martinez-Vilalta *et al.*, 2009;

488 Fajardo & Piper, 2010; Richardson *et al.*, 2013; Laforest-Lapointe *et al.*, 2014; Vilà-Cabrera *et*

489 *al.*, 2015; Anderegg & HilleRisLambers, 2015; Rosas *et al.*, 2019). This may be in part due to

490 the nature of these morphological traits themselves. HV is directly relevant to the water balance

491 and hydraulic status of a plant (Whitehead & Jarvis, 1981; Trugman *et al.*, 2019), but traits like

492 wood density are only partially mechanistically linked to more drought-relevant physiological

493 traits such as xylem vulnerability to embolism (Lens *et al.*, 2010), and linkages have typically

494 only been shown among species rather than within species. However, complicated and

495 inconsistent trait-environment relationships are often found even for more labor intensive plant

496 hydraulic traits (Rosas *et al.*, 2019).

497   In the literature, it is more common to find changes in only a subset of traits than to find

498 the consistent trait coordination documented here. The lack of consistent trait-environment

499 relationships in the literature might be due to fundamentally different capacities of various clades

500 to adjust different tissue characteristics. For example, it is possible that eucalypts are a taxon

501 with anomalously large morphological plasticity or adaptability. Indeed, eucalypts have

502 demonstrated remarkably clean trait-environment patterns within and among species in

503 numerous studies (Schulze *et al.*, 1998; 2006; Pfautsch *et al.*, 2016; Li *et al.*, 2018a). However,

504 other methodological causes of the discrepancies in the literature warrant mentioning.

505   This study was unique in that it explicitly sampled as much of each focal species'

506 geographic aridity niche as possible, and because the aridity gradients in Australia are largely

507 unconfounded by freezing stress. Given that between-site, or climate-related trait variation is

508 often less than half of total within-species trait variation (Figure 4), sampling as broad of climate

509 space as possible may be critical to ensure that one can detect the climate signal from the

510 considerable noise. With the exception of *E. obliqua*, our collections covered the vast majority of

511 the precipitation space inhabited by all study species and the bulk of potential evapotranspiration

512 space (Figure S3).

513   Additionally, the confounding effect of cold stress may weaken trait-climate relationships

514 and obscure trait coordination. Morphological traits such as LMA are known to vary with

515 multiple environmental signals, including water availability, nutrient availability, and cold stress

516 (Poorter *et al.*, 2009). In our study, none of our sites experienced significant cold stress, though

517 soil quality and water availability co-varied (Table S1, Figure S2). While some patterns

518 documented here may be due to changes in nutrient rather than water availability (soil quality or

519 depth was the best trait predictor in ~1/3 of trait-environment relationships), these stresses tend

520 to have similar effects on morphology that may reinforce each other in our study. For example,

521 low nutrient availability and low water availability both tend to increase LMA in isolation

522 (Poorter *et al.*, 2009). However, in cold temperate study systems, cold stress and low water

523   availability tend to have the similar effect of increasing tissue robustness but are *negatively*

524   correlated on the landscape. We posit that studies focused on elevational gradients (Fajardo &

525   Piper, 2010; Anderegg & HilleRisLambers, 2015) and latitudinal gradients (Martinez-Vilalta *et*

526   *al.*, 2009) in the cold temperate zones are likely to see confounding effects of cold stress and

527   drought stress, particularly on leaf traits (González-Zurdo *et al.*, 2016; Niinemets, 2016). If stem

528   versus leaf allocation (i.e. HV) is less sensitive to cold stress than other morphological traits, this

529   could explain why HV shows more ubiquitous within-species patterns than other morphological

530   adjustments, as HV is responding to only a single stress gradient while leaf traits are responding

531   to the complex interactions between multiple stressors.

532

533   *Trait coordination*

534   We found that coordination across leaf, stem, and allocation traits related to aridity was

535   consistent across species and the dominant mode of trait variation in our study (Figure 2 & 3).

536   One implication of this coordination is that the effects of water stress are scaled to species

537   physiology, such that both mesic and xeric species must respond similarly to increasing water

538   stress at their dry range edge regardless of large differences in total water availability. Our seven

539   eucalypt species differed in the wetness of their range center by over 1100 mm of moisture

540   deficit (Figure 5). Yet all of them showed significant trait-by-aridity relationships and trait-trait

541   coordination.

542   The consistent trait coordination across leaf, stem, and allocation traits found here is also

543   reasonably unique in the literature. It is far more common for within-species trait coordination to

544   show variable and often unexpected patterns (Richardson *et al.*, 2013; Laforest-Lapointe *et al.*,

545   2014; Anderegg *et al.*, 2018; Messier *et al.*, 2018; Rosas *et al.*, 2019). However, while present in

546   our entire dataset (Figure 3), trait coordination only emerged at the largest of spatial and

547   ecological scales (Figure 2g). Indeed, even though variation between branches in a canopy and

548   between individuals within a plot constituted the majority of trait variation in the majority of

549   traits and species (25 of 32 species by trait combinations, Figure 4). Despite this, consistent trait

550   correlations only emerged across site-level trait averages in five of six trait pairs (Table S4).

551   These large-scale trait correlations suggests that leaf, stem, and allocation traits are at

552   best weakly mechanistically linked within species. Even when many axes of variation are held

553   constant by looking only within a species, the potential for compensating trait variation (e.g.

554 between roots and leaves) and the important but ultimately weak relationships of many

555 'functional traits' with either physiological rates or demographic outcomes should make weak

556 trait-trait relationships the norm and strong coordination the exception in land plants. Moreover,

557 given that functional traits may respond independently to different environmental stresses

558 (Anderegg *et al.*, 2018), it should be no surprise that consistent within-species trait coordination

559 has been so elusive in the literature.

560

561 *Patterns in trait variance*

562   In contrast to the ubiquitous patterns in trait means, we found less evidence for consistent

563 patterns in trait variances with aridity. Looking across the seven eucalypt species (i.e. focusing

564 primarily on the between species or G component), we found that HV and to a lesser extent

565 LDMC tended to be more constrained in xeric than mesic species but the same was not true of

566 LMA and WD. This pattern was more statistically significant for between-site variance than total

567 variance, suggesting that the component of trait variation controlled by climate was indeed

568 increasingly constrained at low water availability (Figure 5), perhaps indicating constrained

569 plasticity in harsh environments (Valladares *et al.*, 2007). However, this pattern only

570 sporadically scaled down to populations within species (where G, E and GxE effects are

571 possible), with almost half of species showing marked variance patterns across sites for LDMC,

572 rare variance constraints in HV, LMA and WD (Figure 6).. LDMC was somewhat unique among

573 the four traits, showing the most prevalent within-species variance patterns (Figure 6) and

574 decreasing climate-linked trait variances (Figure 5) and CVs (Figure S8) with aridity across

575 species. LDMC may therefore be a worthwhile trait to investigate alongside more detailed

576 physiological measurements in common garden work with *Eucalyptus* (Bourne *et al.*, 2017) and

577 other trees. For other traits, GxE effects (an adaptive increase in plasticity) may maintain

578 plasticity in dry-adapted populations, as found for LMA in a common garden study of

579 *Eucalyptus tricarpa* (L.A.S. Johnson) L.A.S. Johnson & K.D. Hill (McLean *et al.*, 2014).

580   If LMA, WD and HV are under selection in a warming world (which is likely given the

581 trait-by-aridity relationships within and between species), their variance patterns may be good

582 news for the adaptive and/or acclimatory potential of these species. The acclimatory potential for

583 HV may be particularly high, given the consistently high within-tree variation in this trait (Figure

584 4). Meanwhile, depending on the heritability of WD and LMA, which has often proven to be

585 high in angiosperms (Zobel & Jett, 1995; Poorter *et al.*, 2009) and specifically high for WD but

586 not LMA in *C. calophylla* (Ahrens *et al.*, 2019a), the reliably high within-plot variation (Figure

587 4) and lack of variance-by-aridity relationships (Figure 5 & 6) may indicate considerable

588 adaptive potential.

589   It should be noted, however, that a likely explanation for both the weak trait coordination

590 and the mixed variance patterns documented here is that selection is not happening on any of

591 these four traits directly, but rather on underlying anatomical traits that collectively determine

592 gross morphology. All four of the studied 'functional traits' integrate signals from many different

593 anatomical attributes that have a multitude of influences on actual physiological function

594 (Niinemets, 1999; Chave *et al.*, 2009; Poorter *et al.*, 2009; 2011). Thus, it is common for trait

595 variation in different environments to be driven by disparate anatomical changes that have

596 drastically different physiological consequences but result in identical trait values (e.g. Baird *et*

597 *al.*, 2017).

598   Within eucalypts, our results might indicate a constraint on the underlying anatomical

599 properties that drive variation in LDMC and HV, the two traits that did show decreased variance

600 in xeric species (Figure 5). However, a considerable amount of the total variation in both traits is

601 non-climatic (Figure 4, Figure S7), making it difficult to detect changes in trait variation at the

602 population level (Figure 6). This further highlights the importance of understanding the

603 underlying anatomical drivers of variation of these traits (Niinemets, 1999; Onoda *et al.*, 2017).

604 The trait-trait and trait-climate relationships documented here are unlikely to prove mechanistic

605 in the manner necessary for the parameterization of dynamic 'trait-based' vegetation models

606 without gaining a greater understanding of the root causes of this trait variation.

607

608 **Conclusion**

609   We found consistent and coordinated trait shifts towards drought resistance across the

610 aridity range of eight tree species. These findings are unique in the literature, in part because we

611 were able to explicitly sample complete aridity gradients that were not confounded by cold

612 stress. However, the compound nature of the gross morphological traits we measured resulted in

613 1) within-species trait coordination that only emerged across the most climatically disparate

614 individuals in a species and 2) fewer consistent patterns in the size of trait variances with aridity

615 than between trait means and aridity. Our findings imply considerable capacity for these species

to adapt and/or acclimate to increasing aridity with future climate change thanks to the substantial within-species variation in multiple traits that is significantly related to climate. Our work highlights outstanding questions about the anatomical mechanisms driving functional trait variation within species, as well as the need to disentangle conflicting effects of different environmental constraints (e.g. temperature versus nutrient versus water) on trait variation to develop a multi-scale understanding of plant functional ecology.

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849   Supporting information:

850   Table S1: Summary of site characteristics

851   Table S2: Huber Value branch characteristics

862

863   **Figure Legends**

864

865   **Figure 1:** Relationships between four leaf and stem traits and plot mean annual precipitation

866   (PPT) for eight tree species. Points show tree averages. Red crosses show *Acacia acuminata,* the

867   one non-eucalypt species. Trend lines show simple linear regressions of tree averages against

868   MD for each species. Species abreviations: *A. acu – Acacia acuminata, E. sal – Eucalyptus*

869   *salmonophloia, E. mar – E. marginata, C. cal – Corymbia calophylla, E. ova – E. ovata, E. vim –*

870   *E. viminalis, E. amy – E. amygdalina, E. obl – E. obliqua*

871

872   **Figure 2:** Correlations between leaf and stem traits across the aridity range of eight tree species

873   (a-f). Points show tree average trait values, and lines show Major Axis Regressions (solid lines

874   show significant correlations). Crosses show *A. acuminata*, the one non-eucalypt species. Trend

875   lines show SMA regressions per species (n.s. correlations are dashed lines). Numbers in the

876   upper left corners report the fraction of species showing significant trait-trait correlations with

877   the range of Pearson correlation coefficients in parentheses. Panel (g) shows the distribution of

878   correlation coefficients across all species for two example trait pairs, LMA vs WD (black) and

879   LMA vs LDMC (blue). Trait correlations typically had a mean near zero across branches or

880   across individuals within a site for all trait pairs except LMA vs LDMC. Species abreviations: *A.*

881   *acu – Acacia acuminata, E. sal – Eucalyptus salmonophloia, E. mar – E. marginata, C. cal –*

882 *Corymbia calophylla, E. ova – E. ovata, E. vim – E. viminalis, E. amy – E. amygdalina, E. obl –*

883 *E. obliqua*

884

885 **Figure 3:** PC loadings of a PCA including all branch measurements (a). Site average trait PC1

886 scores are strongly related to site moisture availability across eight tree species (b). PET =

887 potential evapotranspiration, PPT = precipitation. Trend lines show SMA regressions per

888 species. Species abreviations: *A. acu – Acacia acuminata, E. sal – Eucalyptus salmonophloia, E.*

889 *mar – E. marginata, C. cal – Corymbia calophylla, E. ova – E. ovata, E. vim – E. viminalis, E.*

890 *amy – E. amygdalina, E. obl – E. obliqua*

891

892 **Figure 4:** Variance decomposition of WD (a), LMA (b), LDMC (c), and $\log_{10}$-transformed HV

893 (d) measured across the aridity range of eight tree species. Colored bars show proportion of total

894 trait variance ("% trait Var") while gray bar shows absolute amount of variance per species ("Tot

895 trait Var"). The amount and dominant scale of trait variance differs considerably between species

896 for the same trait and between traits. However, variation between plots at a site was almost

897 universally the smallest variance component for all traits and species. Within-tree variation was

898 also always larger for $\log_{10}$-transformed HV than for all other traits. Species are ordered from

899 driest on the left to wettest on the right. Species abreviations: *A. acu – Acacia acuminata, E. sal*

900 *– Eucalyptus salmonophloia, E. mar – E. marginata, C. cal – Corymbia calophylla, E. ova – E.*

901 *ovata, E. vim – E. viminalis, E. amy – E. amygdalina, E. obl – E. obliqua*

902

903 **Figure 5:** The total amount of within-species trait variation (top row) and individual variance

904 components (bottom row, variance components multiplied by 100 for axis labels) of WD (a,e),

905 LMA (b,f), LDMC (c,g) and $\log_{10}$(HV) (d,h) of seven eucalypt species (excluding *A. acuminata*)

906 were rarely related to species aridity niche (here shown as the median moisture deficit of each

907 species' geographic distribution based on occurrence records in the Atlas of Living Australia).

908 Total trait variation in HV decreased marginally significantly in drier species, and climate-

909 related (between site) trait variation in LDMC and HV decreased significantly in drier species,

910 consistent with environmental filtering limiting constraining trait variation. Solid lines indicate

911 significant trends (alpha<0.05), dashed lines indicate near significant trends (0.15<alpha<0.05).

912

913   **Figure 6:** Summary of within-species variance patterns. Few of the seven eucalypt and one

914   *Acacia* species showed evidence of decreasing trait variation (red) at dryer sites, with even

915   LDMC (the trait in which this pattern is most prevalent) showing decreases in only 37.5% of

916   species and increases in 12.5% of species. "Non-aridity" signifies species that showed

917   significant site-to-site differences in trait variance that could not be explained by site aridity.
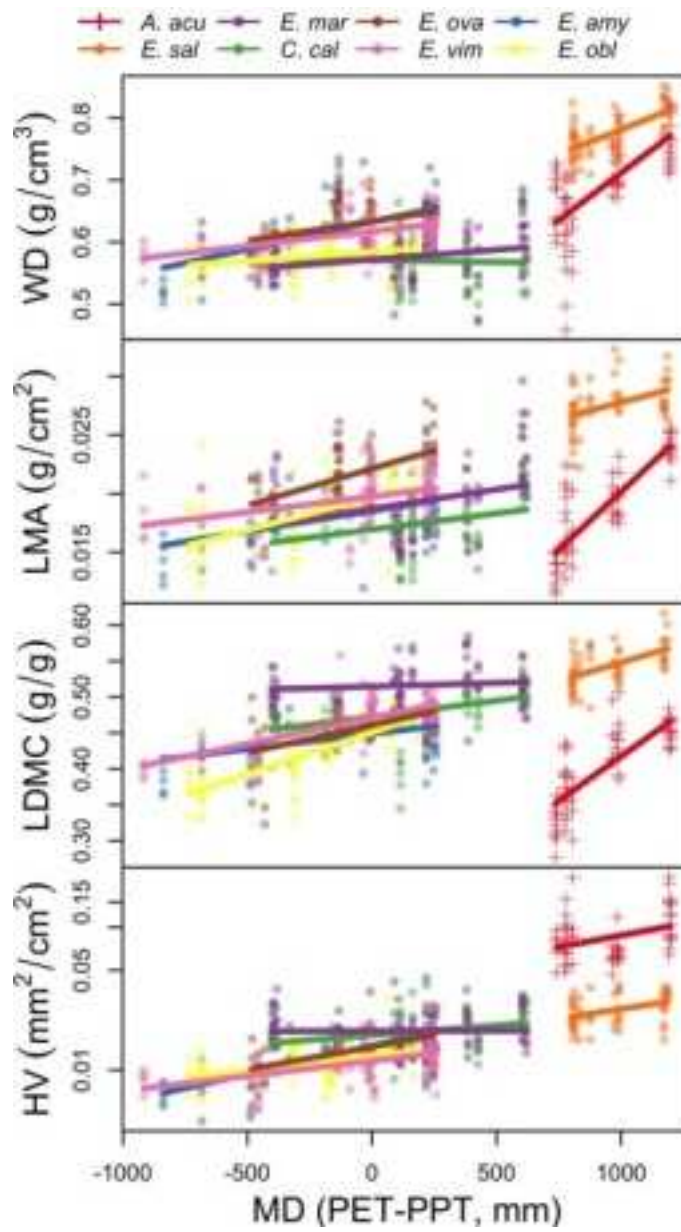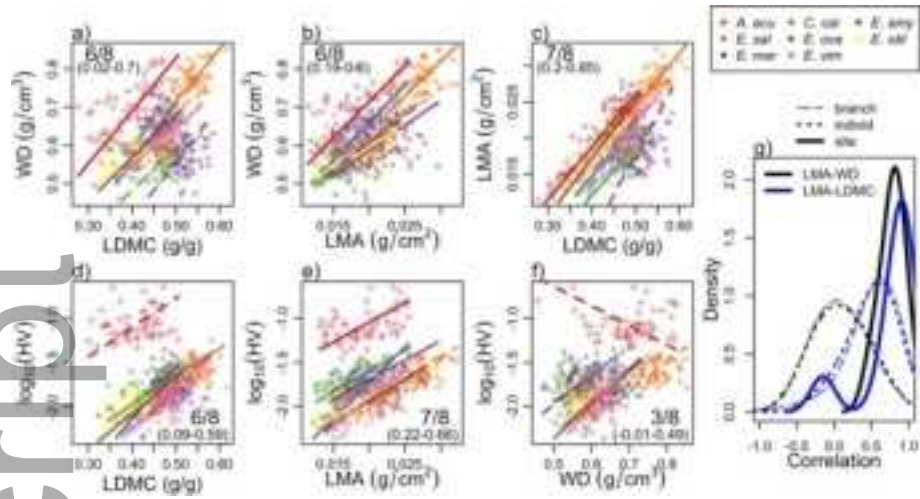
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a)

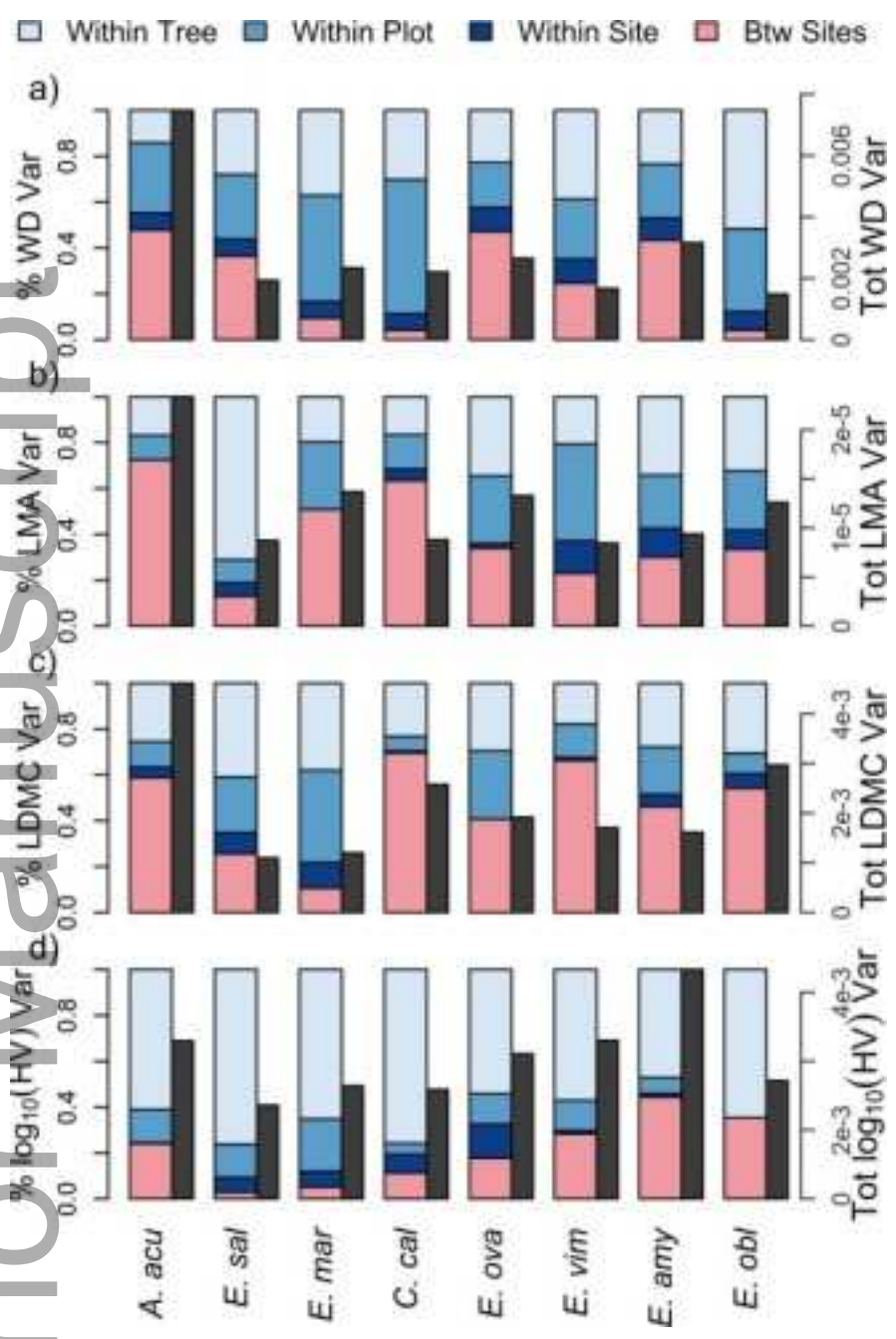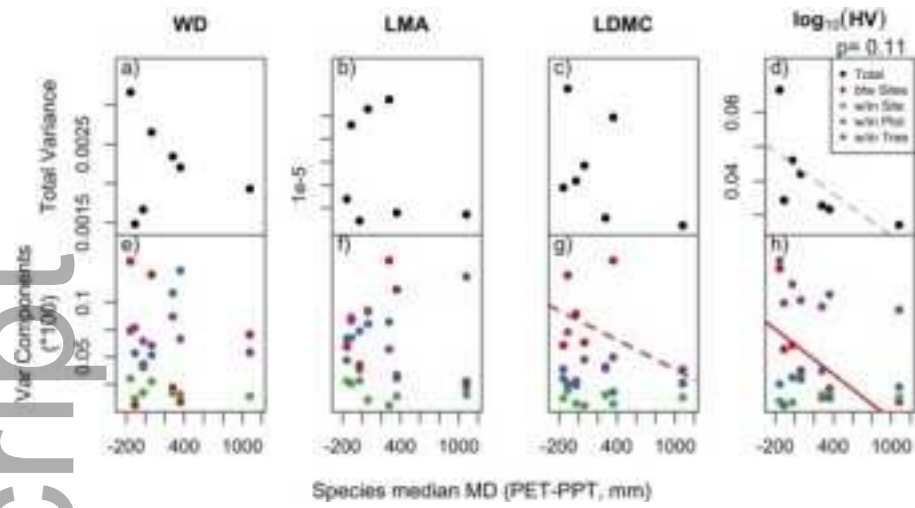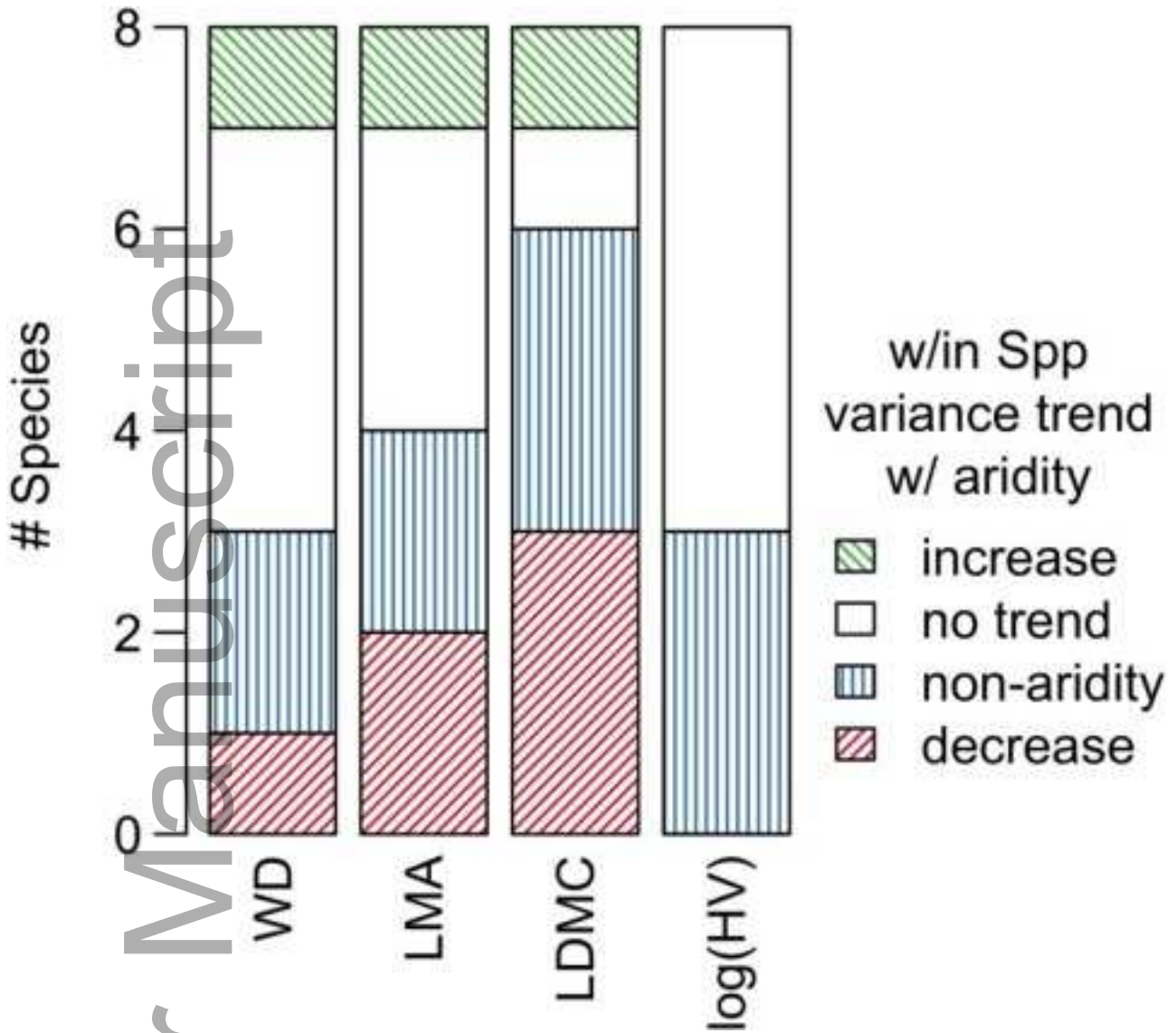|	PC1 (53%)	PC2 (26%)
WD	-0.53	0.29
LMA	-0.61	-0.22
LDMC	-0.48	-0.58
$log_{10}(HV)$	-0.34	0.73

b)
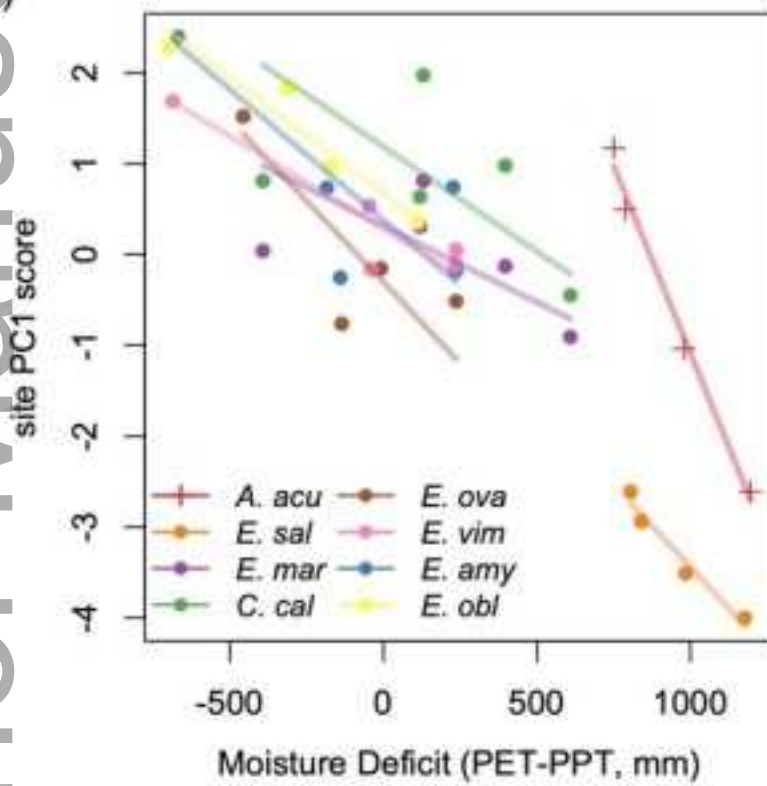
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