

1 **Title:** Coordination of photosynthetic traits across soil and climate gradients

2 **Running title:** Trait coordination across resource gradients

3 **Target journal:** *Global Change Biology*

4 Westerband, A.C.^{1*}, Wright, I.J.^{2,1}, Maire, V.³, Paillassa, J.³, Prentice, I.C.^{1,4,5}

5 Atkin, O.K.⁶, Bloomfield, K.J.⁴, Cernusak, L.A.⁷, Dong, N.^{1,4}, Gleason, S.M.⁸, Guilherme

6 Pereira, C.⁹, Lambers, H.¹⁰, Leishman, M.R.¹, Malhi, Y.¹¹, Nolan, R.H.²

7

8 **1** School of Natural Sciences, Faculty of Science and Engineering, Macquarie University,

9 North Ryde, NSW 2109, Australia

10 **2** Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797,

11 Penrith, NSW 2751, Australia

12 **3** Département des Sciences de l'environnement, Université du Québec à Trois-Rivières, CP

13 500, Trois-Rivières, Québec, G9A 5H7, Canada

14 **4** Georgina Mace Centre for the Living Planet, Imperial College London, Silwood Park

15 Campus, Buckhurst Road, Ascot SL5 7PY, UK

16 **5** Department of Earth System Science, Tsinghua University, Beijing 100084, China

17 **6** Australian Research Council Centre of Excellence in Plant Energy Biology, Research

18 School of Biology, The Australian National University, Canberra, ACT, 2601,

19 Australia

20 **7** College of Science and Engineering, James Cook University, Cairns, Qld 4878, Australia

21 **8** USDA-ARS Water Management and Systems Research Unit, Fort Collins, CO, USA

22 **9** Department of Civil and Environmental Engineering, Massachusetts Institute of

23 Technology, Cambridge, MA 02139, USA

24 **10** School of Biological Sciences, University of Western Australia, Perth, WA 6009,

25 Australia

26 **11** Environmental Change Institute, School of Geography and the Environment, University of

27 Oxford, Oxford, United Kingdom

28

29

30 *Corresponding author details:

31 Email: andreawesterband@gmail.com

32 Phone: +61448941204

33

34

35 ORCID iDs:
36 Andrea C Westerband: 0000-0003-4065-9689
37 Ian J. Wright: 0000-0001-8338-9143
38 Vincent Maire: 0000-0002-3245-2568
39 Jennifer Paillassa: 0000-0002-0683-4693
40 Iain Colin Prentice: 0000-0002-1296-6764
41 Owen K. Atkin: 0000-0003-1041-5202
42 Keith J. Bloomfield: 0000-0002-6492-4507
43 Lucas A. Cernusak: 0000-0002-7575-5526
44 Ning Dong: 0000-0003-0793-8854
45 Sean M. Gleason: 0000-0002-5607-4741
46 Caio Guilherme Pereira: 0000-0003-3288-8553
47 Hans Lambers: 0000-0002-4118-2272
48 Michelle Leishman: 0000-0003-4830-5797
49 Yadvinder Malhi: 0000-0002-3503-4783
50 Rachael H. Nolan: 0000-0001-9277-5142
51

52 **ABSTRACT**

- 53 • “Least-cost theory” posits that C₃ plants should balance rates of photosynthetic water
54 loss and carboxylation in relation to the relative acquisition and maintenance costs of
55 resources required for these activities. Here we investigated the dependency of
56 photosynthetic traits on climate and soil properties using a new Australia-wide trait
57 dataset spanning 528 species from 67 sites.
- 58 • We tested the hypotheses that plants on relatively cold or dry sites, or on relatively
59 more fertile sites, would typically operate at greater CO₂ drawdown (lower ratio of
60 leaf internal to ambient CO₂, C_i:C_a) during light-saturated photosynthesis, and at
61 higher leaf N per area (N_{area}) and higher carboxylation capacity (V_{cmax 25}) for a given
62 rate of stomatal conductance to water, g_{sw}. These results would be indicative of plants
63 having relatively higher water costs than nutrient costs.
- 64 • In general, our hypotheses were supported. Soil total phosphorus (P) concentration
65 and (more weakly) soil pH exerted positive effects on the N_{area}-g_{sw} and V_{cmax 25}-g_{sw}
66 slopes, and negative effects on C_i:C_a. The P effect strengthened when the effect of
67 climate was removed via partial regression. We observed similar trends with
68 increasing soil cation exchange capacity and clay content, which affect soil nutrient
69 availability, and found that soil properties explained similar amounts of variation in
70 the focal traits as climate did. Although climate typically explained more trait
71 variation than soil did, together they explained up to 52% of variation in the slope
72 relationships and soil properties explained up to 30% of the variation in individual
73 traits.
- 74 • Soils influenced photosynthetic traits as well as their coordination. In particular, the
75 influence of soil P likely reflects the Australia’s geologically ancient low-relief
76 landscapes with highly leached soils. Least-cost theory provides a valuable
77 framework for understanding trade-offs between resource costs and use in plants,
78 including limiting soil nutrients.

79 **KEYWORDS** Australia; least-cost theory of photosynthesis; nutrient-use efficiency;
80 optimality theory; plant functional traits; soil nutrients; soil phosphorus; trait coordination;
81 water-use efficiency

82

83 INTRODUCTION

84 Photosynthesis is a fundamental process in the global carbon cycle, governing flows of
85 energy (Friend et al., 2009; Smith & Dukes, 2013). Broad-scale influences of site climate on
86 photosynthesis and associated traits have been widely reported. For example, leaf nitrogen
87 concentration on an area basis (N_{area}) and area-based rates of light-saturated photosynthesis,
88 A_{sat} , are typically higher on relatively arid sites (Wright et al., 2005). Central to determining
89 rates of photosynthesis is the internal concentration of CO_2 within leaves (C_i), as the ratio of
90 intercellular to atmospheric CO_2 concentration ($C_i:C_a$) represents the balance between CO_2
91 demand (from the photosynthetic carboxylating enzyme, Rubisco) and supply (via stomata)
92 during photosynthesis. Typically, $C_i:C_a$ corresponding with A_{sat} shows clear patterning with a
93 variety of climate variables, being generally lower at arid, high-altitude and cold sites
94 (Cornwell et al., 2018; Dong et al., 2020; Prentice et al., 2014; Prentice et al., 2011).
95 Carboxylation capacity (V_{cmax}) considered at ambient temperatures tends to be higher at
96 warmer sites (Dong et al., 2022) and, at least within-species, is generally higher in summer
97 than in winter (Bloomfield *et al.*, 2018). Conversely, V_{cmax} normalized to a standard
98 temperature (commonly 25°C) tends to be lower in summer than in winter (Bloomfield et al.,
99 2018; Hikosaka et al., 2007; Lin et al., 2013) and declines with increasing growth
100 temperature (Dong et al., 2017; Scafaro et al., 2017; Togashi et al., 2018). Finally, stomatal
101 conductance to water, g_{sw} , shows little patterning with site climate, at least at a global scale:
102 individually or together, site temperature and precipitation explain $< 1\%$ variation in g_{sw} in
103 the global trait dataset of Wright et al. (2004b). Within C_3 woody angiosperms measured
104 across major terrestrial biomes, there is no relationship between g_{sw} and either mean annual
105 temperature (MAT), photosynthetically active radiation (PAR) or atmospheric vapor pressure
106 deficit (VPD) (Murray et al., 2019; Murray et al., 2020).

107 Broad-scale influences of soil properties on photosynthetic traits are less well
108 documented but this area of research is growing. Maire et al. (2015) found that A_{sat} increased
109 with increasing soil pH and decreased weakly with increasing soil organic C concentration
110 but had no relationship with soil N or available P concentration. N_{area} is higher on sites with
111 high soil pH (Dong et al., 2020; Maire et al., 2015) and negatively correlated with soil
112 organic C and soil total N concentrations, albeit weakly (Maire et al., 2015). Ordoñez et al.
113 (2009) reported higher mass-based nitrogen concentrations at sites with faster N-
114 mineralization rates (argued to be a more relevant index of plant available N than soil total N
115 concentration) but found no relationship between N_{area} and N-mineralization rate due to a
116 concomitant increase in leaf area per unit mass (specific leaf area, SLA), where N_{area} is N_{mass}

117 divided by SLA. Dong et al. (2020) reported lower $C_i:C_a$ on high-pH soils, as did Cornwell et
118 al. (2018) and Paillassa et al. (2020). Paillassa et al. (2020) explored the role of soil textural
119 properties and reported higher V_{cmax} coupled with higher g_{sw} on sites with high soil silt
120 content, lower $C_i:C_a$ on deeper soils, and higher $C_i:C_a$ in areas of high soil silt content, the last
121 of which was also reported by Cornwell et al. (2018). g_{sw} is higher on soils with low plant-
122 available P concentration (Maire et al., 2015), although studies on soil P effects are scarce.

123 Soil pH, often described as a “master soil variable”, has emerged as an important
124 explanatory variable in several studies of plant trait variation. Globally, soil pH tends to be
125 higher at more arid than at mesic sites (Slessarev et al., 2016), although in Australia acid soils
126 also occur at arid sites, likely owing to its low-relief landscape and the predominance of
127 highly leached, ancient soils (Kooyman et al., 2017). Previous studies have worked to
128 decouple the effects of pH and aridity. Presumably, the effect of pH on photosynthetic traits
129 relates to its influence on soil nutrient availability: broadly speaking, nutrient availabilities
130 are highest at mid-range pH values and lowest on extremely alkaline or acid soils. This can
131 occur via changes in solubility and oxidation states (Lambers & Oliveira, 2019), enzymatic
132 activity (Sinsabaugh & Follstad Shah, 2012; Sinsabaugh et al., 2008) and shifts in the activity
133 and diversity of soil micro-organisms involved in nutrient cycling (Fierer & Jackson, 2006;
134 Lauber et al., 2008). Hence, N and P availability are generally highest at intermediate levels
135 of soil pH, driving shifts in key plant functions, including photosynthesis.

136 “Least-cost” theory (Wright *et al.*, 2003) is a framework for understanding the
137 coordination of water and nutrient use during photosynthesis, and how it varies with site
138 climate and soil properties. Under this theory, photosynthesis is conceptualized as a
139 production process with two key inputs, N and water, which are associated with V_{cmax} and the
140 transpiration pathway, respectively. Based on standard microeconomics theory for a two-
141 factor production process, the optimal balance of these inputs – indicating the lowest total
142 cost for a given level of production – is set by the ratio of the *unit-costs* of the two resources.
143 A key concept of the theory is *substitutability*: in principle, plants can economise on water
144 use by “spending” more on leaf N (i.e., all else being equal, higher N_{area} at a given g_{sw} results
145 in higher V_{cmax} and hence lower $C_i:C_a$), or economise on N use by operating at a higher g_{sw} or
146 transpiration rate. Thus, the approach integrates the single-resource concepts of
147 photosynthetic nitrogen-use efficiency and water-use efficiency (Field et al., 1983; Lambers
148 & Oliveira, 2019; Smith et al., 1997).

149 Briefly summarizing, the following are key assumptions from least-cost theory
150 (Wright et al., 2003; Prentice et al., 2014; Wang et al., 2017): (1) the unit-cost for

151 carboxylation or N_{area} is set by the combined costs of acquiring soil nutrients needed for
152 photosynthetic enzymes and the respiratory costs of building and maintaining enzyme
153 function (e.g., protein turnover); (2) soil nutrients are more expensive to acquire when at
154 lower availability (e.g., from higher root construction costs; more carbon traded for nutrients
155 with mycorrhizas; higher costs associated with producing root exudates, such as carboxylates
156 or phosphatases); (3) the unit-cost for transpiration is set by the cost of acquiring soil water
157 and the respiratory costs of maintaining functional sapwood; (4) available soil water and
158 VPD affect plant water costs but also plant water demands, as transpiration is the product of
159 g_{sw} and VPD; (5) temperature affects Rubisco kinetics, which influences carboxylation costs
160 (as described above), and also the viscosity of water which influences water costs; and
161 finally, (6) elevation affects the saturated vapor pressure of water and hence VPD
162 (influencing water costs), and also gas partial pressures (Körner et al., 1991), ultimately
163 influencing the use of CO_2 versus O_2 by Rubisco and therefore carboxylation. Taken
164 together, the optimum balance between resource investments in transpiration and carbon
165 assimilation should thus depend both on soil properties and climate.

166 Assuming that site properties are the first-order controls on resource unit costs, typical
167 $V_{\text{cmax } 25}\text{-}g_{\text{sw}}$ and $N_{\text{area}}\text{-}g_{\text{sw}}$ ratios –and also $C_i:C_a$ – should vary predictably across
168 environmental gradients (and, conversely, there should be convergence in these traits among
169 co-occurring species). With successive iterations of least-cost theory, the predictions have
170 shifted from qualitative to quantitative (at least in regards to climate), with support
171 accumulating at regional and global scales. Wright et al. (2003) and Prentice et al. (2014)
172 observed, as predicted, that species from more arid or cooler sites in eastern Australia
173 typically operate with higher N_{area} and $V_{\text{cmax } 25}$ at a given g_{sw} , and at lower $C_i:C_a$. Wang et al.
174 (2017) generated quantitative predictions for the independent effects of site temperature,
175 aridity (VPD) and elevation on $C_i:C_a$ which were confirmed using a global dataset derived
176 from leaf $\delta^{13}\text{C}$ values (Cornwell et al., 2018). Dong et al. (2017) and Smith et al. (2019) have
177 successfully used least-cost theory combined with “photosynthetic coordination” theory
178 (Chen et al., 1993; Maire et al., 2012; Von Caemmerer & Farquhar, 1981) to predict climate-
179 driven patterns in $V_{\text{cmax } 25}$.

180 Here, we further investigate the effects of soil properties, primarily pH and total
181 phosphorus (hereafter, P) concentration but also additional proxies for fertility, in driving
182 photosynthetic coordination at a continental scale. In a global study (Paillassa et al., 2020),
183 we reported that plants on neutral to moderately alkaline soils (pH up to 8) had higher $V_{\text{cmax } 25}\text{-}g_{\text{sw}}$,
184 higher $N_{\text{area}}\text{-}g_{\text{sw}}$ and lower $C_i:C_a$ than plants on relatively acidic soils (pH as low as 4),

185 and that plants on deeper soils and soils with greater silt content had lower $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$, lower
186 $N_{\text{area-g}_{\text{sw}}}$ and higher $C_i:C_a$ than plants on shallow soils with little silt. These results were
187 interpreted as most likely reflecting lower unit-costs for acquiring water on silt-rich and deep
188 soils, and lower unit-costs for acquiring N on higher pH soils. Few studies have investigated
189 the role of soil pH in driving trait coordination, despite its importance for regulating nutrient
190 availability. In that previous study, climate and soil data were derived from global gridded
191 datasets. In the present study, we instead use a combination of measured and gridded soil data
192 and, importantly, we purposefully shift the focus to the soil P supply of a key limiting
193 nutrient for photosynthesis (Domingues et al., 2010; Peng et al., 2021; Reich et al., 2009).

194 Phosphorus plays a key role in leaf function in relation to P-rich bioenergetic
195 molecules (ATP, NADP etc), Calvin-Benson cycle intermediates (e.g., ribulose-1,5-
196 biphosphate), membrane lipids, and nucleic acids. On deeply weathered and infertile soils,
197 including those in Australia but also the tropics, P is a key limiting nutrient for plant
198 productivity, and geographic variation in soil P delineates native vegetation communities
199 (Beadle, 1954, 1966; Kooyman et al., 2017; Laliberté et al., 2014; Vitousek, 1984). Here we
200 address the aforementioned knowledge gaps, combining published and unpublished datasets
201 with *de novo* photosynthetic measurements, building a comprehensive photosynthetic trait
202 database for Australian native plants (536 species from 67 sites, Fig. S1).

203 Our aims were to understand the manner in which soils—and to a lesser extent
204 climate—have driven the coordination of photosynthetic traits, and to characterize trait-
205 environment relationships, focusing on soil pH, soil total P concentration, mean annual
206 precipitation (MAP), and mean annual temperature (MAT) for the Australian flora. Better
207 regional and global understanding of photosynthetic trait-environment relationships has the
208 potential to improving existing global vegetation models by expanding on the environmental
209 dependencies of traits. We focused on the effects of soil fertility via soil total P concentration
210 and soil pH, both of which presumably influence the unit-costs of N and carboxylation more
211 so than water costs, and we tested a number of key predictions (Fig. 1a). First, assuming—all
212 else equal—that the unit-costs of soil nutrients are lower on higher-P soils or higher pH soils,
213 we predicted that plants would increase their investment in N_{area} or V_{cmax} relative to g_{sw} in
214 these situations, and operate at lower $C_i:C_a$. We note that while extremely high pH soils
215 reduce soil nutrient availability (Lambers & Oliveira, 2019), Australian soils are
216 predominantly acidic compared with other arid regions of the world (Slessarev et al., 2016).
217 Second, we predicted the same trait-shifts (higher $V_{\text{cmax-g}_{\text{sw}}}$, higher $N_{\text{area-g}_{\text{sw}}}$, lower $C_i:C_a$) in
218 arid compared with wetter sites and on relatively colder compared with warmer sites. These

219 predictions arise from the assumption that the unit cost of water is greater at low rainfall and
220 high VPD, and that temperature affects the unit costs of both carboxylation and
221 photosynthetic water use (Prentice et al., 2014), as described above. The results from this
222 study will be of global significance, as they will clarify whether trait coordination patterns
223 observed at a global scale are consistent at a continental scale, in the context of locally
224 relevant soil properties.

225

226 **MATERIALS AND METHODS**

227 **Study system**

228 Australia is highly suited for this line of inquiry as there is wide environmental variation in
229 both soils and climate. The central portion of the continent (*ca.* 70% by land area) is arid to
230 semi-arid, while coastal regions vary from Mediterranean in the south to southwest, cool-
231 temperate in the south, temperate to tropical in the east, and wet-dry tropics in the far north.
232 Although Australian soils are on the whole ancient and nutrient-poor (He et al., 2021;
233 Kooyman et al., 2017; Viscarra Rossel & Bui, 2016), higher-nutrient soils punctuate the
234 landscape (de Caritat et al., 2011; Viscarra Rossel & Bui, 2016) and the Great Dividing
235 Range, which runs 3500 km north to south, approximately parallel to the east coast of
236 Australia, divides the mesic coastal regions from the arid interior. Furthermore, although
237 much of Australia has acidic soil, calcareous soils with high pH are also present across wide
238 areas (de Caritat et al., 2011), for example in southern Australia, resulting from repeated
239 marine incursions beginning in the Miocene era (Northcote & Wright, 1982; Taylor, 1994).
240 In this study, the majority of the sites were on acidic soils with low soil nutrient availability
241 (Table S1), which is representative of Australia but also relevant to other, similarly-leached
242 regions of the world.

243

244 **Field data collection**

245 Leaf trait data were collected on woody and non-woody plant species at three sites between
246 December 2018 and March 2019: Kidman Springs Research Station (tropical savanna,
247 sampled during the wet season; Northern Territory), Royal National Park (subtropical
248 rainforest; New South Wales), and Mount Keira (subtropical rainforest; New South Wales).
249 Latitude, longitude and climate data for these sites can be found in Table S1. These sites were
250 chosen to increase the number of samples within sites of moderately high total soil P

251 concentrations and moderately high soil pH (Fig. S2), compared with site coverage in our
252 compilation of literature data, described below. Ten soil samples (to 30 cm depth) were
253 collected at each site and air-dried prior to laboratory analyses (CSPB laboratory in Bibra
254 Lake WA, Australia) of soil pH in CaCl₂ solution (Rayment & Lyons, 2011) and total soil P
255 concentration via colorimetry, following Kjeldahl digestion (Rayment and Lyons Method
256 9A3b) .

257 We sampled seven to 28 species per site, randomly selecting three to eight individuals
258 per species and focusing on dominant woody and non-woody species (excluding C₄ plants).
259 Photosynthetic traits were measured using a Li-6800 gas exchange system (Li-Cor
260 Biosciences, Lincoln, NE, USA). Survey-style gas exchange measurements were made
261 between 0800 and 1400 hours on one leaf per plant. Young but fully expanded, undamaged
262 leaves were sampled from the most sun-exposed portion of each canopy. We measured light-
263 saturated (photosynthetic photon flux density of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) photosynthesis per unit
264 area (A_{area} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at an atmospheric CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$,
265 allowing leaves to remain in the chamber for several minutes. Leaf temperatures were
266 initially set to 25°C, although in many cases the temperature had to be increased above this to
267 prevent condensation in the cuvette. Mean leaf temperature was 29°C with 95% of
268 measurements made between 25°C and 35°C; relative humidity varied between 40 and 80%.
269 We also recorded stomatal conductance to water vapor (g_{sw} , $\text{mmol m}^{-2} \text{ s}^{-1}$) associated with
270 light-saturated photosynthesis, and the ratio of internal to ambient CO₂ concentration ($C_i:C_a$,
271 unitless). We note that gas exchange rates are sensitive to plant water status and can exhibit
272 pronounced temporal (e.g., diurnal, seasonal) variation. By measuring photosynthesis and
273 stomatal conductance in light-saturated leaves at a controlled temperature and humidity, we
274 reduced the amount of variation in the data by selecting favorable conditions for
275 photosynthesis inside the cuvette.

276 We also collected five or more outer canopy leaves per plant, sampling from multiple
277 branches up to 10 m above the ground, using an extendable pole pruner. Leaves were scanned
278 to estimate leaf area, dried at 60°C for a minimum of 72 hours and weighed to calculate leaf
279 mass per area (LMA, g m^{-2}). Samples were analysed for leaf N concentration (% mass basis)
280 by the Stable Isotope Core Laboratory at Washington State University, USA using an
281 elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA). Leaf N per area (N_{area} ; g
282 m^{-2}) was calculated as $N_{\text{area}} = N_{\text{mass}} \times \text{SLA}^{-1}$.

283

284 **Data compilation**

285 *Trait data*

286 We compiled field-measured photosynthetic trait data from published and unpublished
287 studies that employed similar standard methods to those described above, i.e. light-saturated
288 photosynthesis measured on young but fully-expanded, undamaged “sun” leaves at ambient
289 atmospheric CO₂ concentration, and relative humidity between 40 and 80%. See Table S2 for
290 a full list of source publications, noting that some of the trait data included herein are not
291 published. Further details regarding our field methods can be found below. For inclusion, a
292 dataset had to contain field-measured A_{sat}, g_{sw}, and C_i; where available we also extracted data
293 for leaf temperature (T_{leaf}), LMA, and N_{area}. We estimated carboxylation capacity at a
294 standardized temperature of 25°C (V_{cmax 25}) following the one-point method (De Kauwe et
295 al., 2016), which utilises T_{leaf}, A_{sat}, and C_i. We consulted the original publication or contacted
296 the data owners to determine the appropriate leaf temperature for studies where T_{leaf} was not
297 reported. If V_{cmax 25} from a CO₂-response (A-C_i) curve was provided, we used these data
298 rather than estimating V_{cmax 25} via the “one-point method” (De Kauwe et al., 2016); 179
299 measurements, or 6% of the original dataset. To ensure consistency in approach to estimating
300 V_{cmax}, R_{day} (CO₂ evolution from mitochondria in the light) was estimated as 1.5% of V_{cmax},
301 following De Kauwe et al. (2016), rather than from reported estimates of field-measured leaf
302 “dark” respiration (R_d, which were relatively scarce among the compiled datasets).

303 We visually inspected the data to find obvious errors (e.g., trait values < 0; C_i:C_a > 1)
304 and outliers, conservatively excluding from the analyses any observations with V_{cmax 25} > 500
305 μmol CO₂ m⁻² s⁻¹, and g_{sw} > 3000 mmol m⁻² s⁻¹. This resulted in the exclusion of nine
306 observations (seven for V_{cmax 25}, two for g_{sw}). These cut-offs were based on previously
307 published studies (Smith et al., 2019; Wright et al., 2004b).

308 In combination with the *de novo* field measurements described above, we amassed a
309 trait dataset for 3765 individuals of 528 species (85 families), sampled from 67 study sites
310 (Fig. S1 and Table S1). 152 species occurred at more than one site. On average, 11 species
311 were sampled per site, although this varied widely (Table S1). Species-mean trait values were
312 calculated at each site, although subspecies were kept separate, when reported. Taxonomy
313 followed The Plant List (accessed via <http://www.plantminer.com/>). 13 individuals could not
314 be identified beyond the genus level but were still included, and 20 species had names that
315 are taxonomically unresolved in The Plant List. The final dataset included a variety of growth
316 forms (mostly trees and shrubs but 28 herbaceous species, or 4.5% of the dataset), primarily
317 evergreen species, no winter-deciduous species, some drought deciduous species such as
318 *Toona ciliata* and *Melia azedarach*, and a mixture of N₂-fixing species (mostly Fabaceae but

319 also Casuarinaceae and Zamiaceae) and non-N₂-fixing species (84% of the dataset). There
320 were ten gymnosperm species, distributed among five families (Araucariaceae, Cupressaceae,
321 Zamiaceae, Podocarpaceae, Pinaceae). The original data compilation included five C₄ species
322 from the genus *Atriplex* and *Triodia* (Amaranthaceae and Poaceae, respectively) but these
323 were excluded from calculations of V_{cmax} as the one-point method is based on the Farquhar et
324 al. (1980) model of C₃ photosynthesis.

325

326 *Climate and soil data*

327 Long-term averages (1982 - 2002) of climate data (Table S3) were obtained for each site
328 from the ANUClimate model (Hutchinson et al., 2009) and TERN Ecosystem Modelling and
329 Scaling Infrastructure (eMAST) data products (Hutchinson et al., 2009; Xu et al., 2015), both
330 of which provide Australia-wide coverage at 0.01° spatial resolution, 1970-2012)
331 (<https://www.tern.org.au/>). We include a total of 21 soil and climate properties. Across the 68
332 study sites, MAT varied from 9.25 to 27.6°C, and MAP from 260 to 4390 mm (Fig. S2).

333 We had field-measured data for soil total P concentrations from 34 sites and for soil
334 pH (CaCl₂) from 28 sites. Otherwise we extracted modelled estimates of soil total P
335 concentration and pH (CaCl₂) from the TERN Soil and Landscape Grid of Australia (Grundy
336 et al., 2015; Viscarra Rossel et al., 2014) (<https://data.csiro.au/>), which offers Australia-wide
337 gridded data at a resolution of 3 arc sec (ca. 90 × 90 m pixels). We also extracted additional
338 soil properties known to influence soil fertility, including soil texture, soil organic matter
339 concentration and cation exchange capacity (Table S3). In the combined dataset, soil total P
340 concentration varied from 28.8 to 3790 ppm (mg kg⁻¹), and pH from 3 to 9. There were two
341 sites with exceptionally high (measured) soil P concentrations (Dorrigo National Park, NSW
342 and Curtain Fig National Park, QLD); without these sites, maximum soil P concentration was
343 1786 ppm.

344

345 **Statistical analyses**

346 We report results from all models with $P < 0.1$, noting those with $0.05 < P < 0.10$ as
347 “marginally significant”. All statistical analyses were carried out in R version 3.5.3 (R
348 Development Core Team, 2017).

349

350 *Testing predictions from least-cost theory*

351 V_{cmax 25-gsw} and N_{area-gsw} relationships at each site were summarized as standardized major
352 axis (SMA) slopes fitted with no intercept term (i.e., “forced” through the origin), using

353 untransformed data. These slopes, therefore, represent the average ratios of $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ and
354 $N_{\text{area-g}_{\text{sw}}}$ at each site (Wright et al., 2003). We conducted a slope heterogeneity test to assess
355 site differences, using ‘SMATR’ for R (Warton et al., 2006). Sites with low replication (<3
356 species per site) were left out from this analysis (two sites for $V_{\text{cmax } 25}$ and three sites for
357 N_{area}), resulting in 58 $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ slopes and 39 $N_{\text{area-g}_{\text{sw}}}$ slopes.

358 Next, bivariate and multiple linear regression analyses were used to quantify the
359 influence of soil and climate properties on $C_i:C_a$ as well as the $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ and $N_{\text{area-g}_{\text{sw}}}$
360 slopes. We tested how these traits varied in response to a total of 21 abiotic variables using
361 bivariate regressions but were unable to include the full suite of predictors in the multiple
362 regression due to multicollinearity and a lack of statistical power. For example, while VPD is
363 often considered an important variable driving photosynthetic trait coordination (Paillassa et
364 al. 2020), in this study it was highly correlated with both MAP and MAT. Similarly, soil pH
365 was correlated with soil N concentration, and soil P and N concentrations were correlated
366 with one another (Fig. S3). Therefore, we reduced the set of predictors in the multiple
367 regression to focus on soil pH and soil P concentration, which were not correlated (Fig. S3),
368 and also MAP and MAT, which were only weakly (positively) correlated (Fig. S2). We
369 selected soil P rather than soil N because soil P is a more strongly limiting soil nutrient for
370 plants within Australia (Beadle 1954, 1966). Soil pH and soil P were independently
371 correlated with MAT and MAP in opposing directions: soil P concentration was negatively
372 (albeit weakly) correlated with MAT and positively correlated with MAP, whereas soil pH
373 was positively correlated with MAT and negatively correlated with MAP. In other words,
374 relatively colder sites and sites with higher mean annual precipitation had a lower soil pH and
375 higher soil total P concentration, on average (Fig. S2). With the exception of $C_i:C_a$ in the
376 multiple regression, the dependent and independent variables were \log_{10} -transformed prior to
377 analyses to meet assumptions of normality.

378 From multiple regression analyses, we report the beta values for each predictor, i.e.
379 the regression weights for standardized variables, representing the change in the response
380 variable (in standard deviations) associated with a change of one standard deviation in a
381 given predictor, other predictors being held constant (Courville & Thompson, 2001;
382 Pedhazur, 1997). These partial effects were visualised with “added variable” (partial
383 regression) plots, created using the avPlots function in the ‘car’ package. Beta weight values
384 (hereafter, β) were calculated using the regr function in the ‘yhat’ package.

385 We also ran the above analyses after excluding species that were presumed to fix N_2
386 (Fabaceae, Casuarinaceae, Zamiaceae) as, on average, these species had notably higher leaf

387 N_{mass} and N_{area} than non-fixing species ($P < 0.001$, Fig. S4). However, the results (Fig. S5)
388 changed little compared with those from main analyses, the key difference being that soil P
389 exerted a stronger, positive effect the $N_{\text{area}}-g_{\text{sw}}$ relationship. Our overall conclusions were not
390 affected therefore these results are not discussed further.

391

392 *Quantifying climate and soil effects on photosynthetic traits*

393 Climate and soil effects on individual photosynthetic traits were quantified via ordinary least
394 squares (OLS) linear regressions, implemented using the `lm` function in base R. We
395 investigated relationships between the four focal plant traits ($C_i:C_a$, g_{sw} , $V_{\text{cmax } 25}$, N_{area}) and all
396 21 soil and climate variables. For this analysis, and for the subsequent partial regression
397 analysis, we included additional traits known to covary with N_{area} , including P_{area} , LMA, and
398 A_{area} . We also included photosynthetic phosphorus and nitrogen use efficiency, PPUE and
399 PNUE, respectively. In preliminary analyses, we tested quadratic fits between the focal traits
400 and soil pH, finding that the quadratic models for $C_i:C_a$, N_{area} , and $V_{\text{cmax } 25}$ had lower AIC ($>$
401 2) than the linear models but added very little explanatory power: R^2 values of quadratic
402 models ranged from 0.04 to 0.17, with a relative increase in $R^2 \leq 0.02$ for all traits. There was
403 no improvement in the model fit for g_{sw} . Because our study sites were dominated by acidic
404 soils (pH < 7) we had no *a priori* reason to expect non-linear relationships between soil pH
405 and nutrient availability, as typically occurs when comparing strongly acidic to strongly
406 alkaline soils (Maire et al. 2015). Therefore, we did not expect non-linear relationships
407 between soil pH and the focal traits and retained linear fits for all relationships. Leaf traits
408 and abiotic variables were \log_{10} -transformed prior to the statistical analyses to satisfy
409 assumptions of normality and homoscedasticity of the residuals.

410 We also evaluated trait-environment relationships using partial regression analyses on
411 models that included either the four key predictors above (soil P, soil pH, MAP, MAT) or
412 seven predictors (soil P, soil pH, soil N, MAP, MAT, VPD, and radiation), which were
413 selected because they are known to influence photosynthetic traits. Correlations between
414 abiotic variables were visualised using the `corrmat` function in the ‘`corrmat`’ package.

415

416 **RESULTS**

417 *Trait variation*

418 In the species-mean dataset, $V_{\text{cmax } 25}$ varied ca. 27-fold (from 5.8 to 156 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $n =$
419 636), g_{sw} varied ca. 150-fold (from 11.1 to 1670 $\text{mmol m}^{-2} \text{s}^{-1}$; $n = 664$), N_{area} ca. 19-fold
420 (0.55 to 10.6 g m^{-2} ; $n = 430$), and $C_i:C_a$ varied ca. four-fold (from 0.22 to 0.96; $n = 665$). The

421 notably wider range in g_{sw} was due to one exceptionally high value for *Eucalyptus miniata*
422 from Eamus and Prichard (1998). Excluding this g_{sw} would have resulted in a 90-fold
423 variation; however, we had no basis on which to exclude this value. If variation in traits was
424 compared in terms of the ratio of 97.5th to 2.5th percentiles, rather than maximum/minimum,
425 variation in g_{sw} was comparable to that in other traits (approx. 22-fold). By comparison, the
426 ratio of 97.5th to 2.5th percentiles for g_{sw} was ~ 23 in the global photosynthetic trait dataset of
427 Maire et al. (2015).

428

429 *Bivariate tests of least-cost theory*

430 $N_{area-g_{sw}}$ and $V_{cmax\ 25-g_{sw}}$ slopes varied widely across sites (slope heterogeneity $P < 0.001$,
431 Fig. 1b,c) where steeper slopes indicate that species are operating with higher $V_{cmax\ 25}$ or N_{area}
432 at a given rate of stomatal conductance to water vapor (Fig. 1a). Contrary to our expectation,
433 variations in these slopes were not associated with soil P concentration or soil pH in the
434 bivariate regressions (Fig. 2), and typically, the soil variables explained less than 5% of the
435 variation in the slopes. Similarly, we found no association between the slope relationships
436 and soil nitrogen (Soil N), bulk density of whole earth (BDW), soil organic carbon (SOC)
437 and the soil textural properties. The only variable that significantly influenced the slope
438 relationships was ECE, which exerted a positive effect on the slopes (Table S4), suggesting
439 that higher ECE increased nutrient availability and reduced nutrient costs relative to water
440 costs.

441 $C_i:C_a$ varied with both soil pH and soil P concentration in the expected manner, being
442 lower on average at sites with high soil P concentrations (Fig. 2i) or high pH (Fig. 2j). $C_i:C_a$
443 also decreased with increasing ECE, increasing SOC, and increasing clay content (and
444 increased with increasing silt and sand content) (Table S4), supporting our predictions (Fig.
445 1a).

446 With regards to climate, the $N_{area-g_{sw}}$ and $V_{cmax\ 25-g_{sw}}$ slopes were generally steeper at
447 drier sites (Fig. 2c,g) and at colder sites (Fig. 2d,h), as predicted. For example, $N_{area-g_{sw}}$
448 slopes were *ca.* six-fold steeper at 300 mm MAP than at 3000 mm MAP (0.04 vs 0.007,
449 respectively), and $V_{cmax\ 25-g_{sw}}$ slopes were *ca.* three-fold steeper (0.52 vs 0.19, respectively).
450 From the bivariate regressions, MAP explained 32% and 23% of the variation in $N_{area-g_{sw}}$ and
451 $V_{cmax\ 25-g_{sw}}$ slopes, respectively (Table S4, Fig. 2c,d). MAT explained 28% and 12% of the
452 variation in $N_{area-g_{sw}}$ and $V_{cmax\ 25-g_{sw}}$ slopes, respectively (Fig. 2d,h).

453 Also as predicted, species at drier sites and at relatively colder sites operated at lower
454 $C_i:C_a$ (Fig. 2k,l). Using a standard moisture index, the ratio of MAP to potential
455 evapotranspiration (Thornthwaite, 1948), gave similar results to using MAP alone (Table S4).

456 In general, climate variables explained a significantly greater percentage of the
457 variation in the $N_{\text{area-g}_{\text{sw}}}$ and $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ slopes ($0.005 \leq R^2 \leq 0.64$) than did the soil variables
458 ($0.004 \leq R^2 \leq 0.24$). Similarly, a greater amount of variation in $C_i:C_a$ was explained by
459 climate (max $R^2 = 0.12$) than by soil (max $R^2 = 0.08$).

460

461 *Multiple regression tests of least-cost theory*

462 Multiple regression analyses revealed some distinct patterns from the bivariate regressions
463 (Fig. 3). Together, the four environmental variables explained 52% of variation in $N_{\text{area-g}_{\text{sw}}}$
464 slopes, 36% of variation in $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ slopes, and 14% of variation in $C_i:C_a$. Comparing
465 standardized regression coefficients (β values in Fig. 3), MAP affected photosynthetic trait
466 coordination more strongly than the three other environmental variables. The effect sizes for
467 soil P concentration were of similar or slightly stronger magnitude to those for MAT, and
468 notably weaker than the precipitation effects.

469 After controlling for variation in other predictors via partial regression, the effect of
470 soil P concentration on the $N_{\text{area-g}_{\text{sw}}}$ and $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ relationship slopes became stronger than
471 what we observed in the OLS regression (i.e., $0.05 < P < 0.10$; Fig. 3a,e). The soil P
472 concentration effect on $C_i:C_a$ (Fig. 3i) was again negative, even when controlling for variation
473 in MAT, MAP and soil pH. These effects of soil P concentration were all in the predicted
474 direction (Fig. 1a). After controlling for variation in other predictors, soil pH still showed no
475 association with $N_{\text{area-g}_{\text{sw}}}$ and $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ relationship slopes (Fig. 3b,f) or $C_i:C_a$ (Fig. 3j).

476 For both sets of slopes, models including all four predictors indicated that the MAP
477 effect was strongly negative (in terms of β), and was stronger than that of MAT, soil P
478 concentration and soil pH (Fig. 3). That is, at a given MAT, soil P concentration or soil pH,
479 species at drier sites typically operated with higher $V_{\text{cmax } 25}$ or N_{area} at a given g_{sw} (Fig. 3c,g),
480 and also typically had lower $C_i:C_a$ (Fig. 3k) –all trends consistent with the expectation that
481 savings on photosynthetic water use can be achieved via increased investment in the N-rich
482 carboxylating enzyme, Rubisco. The MAT effects on the $N_{\text{area-g}_{\text{sw}}}$ and $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ slopes in
483 the bivariate regressions were no longer apparent once other environmental variables were
484 controlled (Fig. 3d,h). By contrast, a positive MAT effect on $C_i:C_a$ was observed when
485 controlling for other variables, as was the case in the bivariate analysis (Fig. 3l).

486

487 *Trait-environment relationships*

488 We quantified relationships between environmental variables and plant photosynthetic traits
489 including g_{sw} , $V_{cmax\ 25}$ and N_{area} , but also additional traits known to co-vary with the focal
490 variables (for the full suite of relationships, see Table S4). Species on low-P and on low-N
491 soils tended to have higher g_{sw} , N_{area} , and $V_{cmax\ 25}$ (Fig. 4), whereas species on high pH soils
492 (which in this dataset are expected to have higher soil nutrient availability) had higher V_{cmax}
493 $_{25}$ and higher N_{area} but exhibited no difference in g_{sw} (Fig. 4b). The higher $V_{cmax\ 25}$ and N_{area}
494 on low-P soils were likely driven by higher LMA on low-P soils (Table S4), as N_{area} was
495 positively correlated with both LMA $_{25}$ ($r = 0.75$, $P < 0.001$) and $V_{cmax\ 25}$ ($r = 0.37$, $P <$
496 0.001). Soil P concentration explained the highest percentage of the variation in $V_{cmax\ 25}$ ($R^2 =$
497 0.16) whereas soil N concentration explained the highest percentage of the variation in N_{area}
498 ($R^2 = 0.24$). On average, for a 10-fold decrease in soil P concentration, $V_{cmax\ 25}$ increased 1.5-
499 fold and g_{sw} two-fold. N_{area} showed a significant association with soil P concentration but
500 with little explanatory power ($R^2 = 0.02$; Fig. 4g).

501 Species at low-MAP sites (especially at $MAP < 1000$ mm) tended to have higher N_{area}
502 ($R^2 = 0.38$; Fig. 5g), which was by far the strongest correlation in this part of our analysis.
503 The higher N_{area} at low rainfall corresponded (as expected) to higher $V_{cmax\ 25}$ ($R^2 = 0.11$; Fig.
504 5d). By contrast, g_{sw} showed no relationship with MAP (Fig. 5a). The N_{area} -MAP scaling
505 slope of -0.39 indicates that for a 10-fold decrease in MAP, N_{area} increased nearly 2.5-fold, on
506 average. On average, there was a 1.5-fold increase in $V_{cmax\ 25}$ over this same interval in MAP
507 (log-log slope = -0.27). Species at warmer sites typically had higher g_{sw} but lower N_{area} (Fig.
508 5b,h), consistent with the predicted and observed MAT effect on N_{area} - g_{sw} slopes (Fig. 1, Fig.
509 3). That said, there was pronounced scatter in these relationships ($0.05 \leq R^2 \leq 0.07$). $V_{cmax\ 25}$
510 showed a marginally significant relationship with MAT (Fig. 5f) but with $< 1\%$ explanatory
511 power.

512 Of the remaining soil variables (Table S4), BDW ($0.10 \leq R^2 \leq 0.21$), SOC ($0.01 \leq R^2$
513 ≤ 0.23), and sand content ($0.01 \leq R^2 \leq 0.19$) explained the most variation in the focal traits.
514 Of the remaining climate variables, the results were idiosyncratic but VPD explained a
515 significant proportion of the trait variation ($0.06 \leq R^2 \leq 0.15$), as expected.

516 The partial regression analysis on the trait-environment relationships (Table S5) were
517 largely similar to the OLS regression with a few exceptions. First, the effect of soil P on N_{area}
518 was positive (rather than negative) when we accounted for the other abiotic variables.

519 Second, there was a significant negative effect of soil pH on g_{sw} and on $C_i:C_a$, where
520 previously these relationships were not statistically significant.

521

522 **DISCUSSION**

523 Despite the critical role of photosynthesis in driving the carbon cycle of terrestrial
524 ecosystems, we understand relatively little about how soil fertility influences the coordination
525 of photosynthetic traits, and the relative effects of soil versus climate. Here, we report the
526 effects of a globally-relevant, limiting soil nutrient, phosphorus (P), on photosynthetic trait
527 coordination. Previously, for four sites in Australia, we reported trait-shifts in relation to site
528 temperature and aridity that were consistent with predictions from least-cost theory (Prentice
529 et al., 2014; Wright et al., 2003). In a global study (Paillassa et al., 2020), we then examined
530 the interactive effects of soil and climate, focusing on pH and soil texture as indices of
531 fertility. Here we expand on the Australian study, including hundreds more species from
532 many more sites (67), a much wider range of climate variables and, very importantly, we
533 extend the analyses to soil nutrients also.

534

535 **Climate effects**

536 Using the largest Australian photosynthetic trait dataset to date, we generally observed that
537 climate effects were stronger than soil effects. We report strong climate-driven trait shifts in
538 line with previous studies and in line with our predictions. Most notably, with lower MAP we
539 observed higher N_{area} and $V_{cmax\ 25}$ at a given g_{sw} , higher N_{area} and $V_{cmax\ 25}$ overall, and lower
540 $C_i:C_a$. Although g_{sw} was not influenced by site precipitation, higher N_{area} and $V_{cmax\ 25}$ drove
541 the steeper $N_{area}-g_{sw}$ and $V_{cmax\ 25}-g_{sw}$ slope relationships across the precipitation gradient.
542 Steeper $V_{cmax\ 25}-g_{sw}$ and $N_{area}-g_{sw}$ relationships at drier sites underlie the lower $C_i:C_a$ in these
543 places, the higher carboxylation capacity (at a given g_{sw}) drawing leaf-internal CO_2 down to
544 lower concentrations. The lack of patterning of g_{sw} with respect to MAP accords with
545 “global” results from Wright et al. (2004b) and Murray et al. (2019; 2020). The uncoupling of
546 g_{sw} from MAP is interesting, as VPD is typically higher on more arid sites and g_{sw} increased
547 with VPD (Table S4), indicating higher transpiration rates when stomata are open. Similar to
548 the present study, global (Paillassa et al., 2020; Wang et al., 2017) and regional studies
549 (Bloomfield et al., 2019; Cernusak et al., 2011a; Cochrane et al., 2016; Wright et al., 2001)
550 have reported lower $C_i:C_a$, higher $V_{cmax\ 25}$ and higher leaf nutrient concentrations (especially
551 per unit area) in drier habitats.

552 Least-cost theory predicts that MAT affects both water and carboxylation costs. In
553 this study MAT effects matched our predictions but were weaker than those of MAP. Both
554 the MAT and MAP effects were stronger than soil effects—at least in the bivariate
555 relationships (see *Soil effects*). These findings suggest that environmental variables that solely
556 affect the unit-costs of N (and carboxylation) exert weaker effects on photosynthetic trait
557 coordination than do environmental variables that influence the unit-costs of water (i.e.,
558 MAP) or influence the costs of both water and carboxylation (i.e., MAT). Interestingly, we
559 also observed weaker effects of MAT relative to MAP on the $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ and $N_{\text{area-g}_{\text{sw}}}$
560 relationships—but not $C_i:C_a$ —when considered in a multiple regression framework. The
561 weaker effect size of MAT in the multiple regression likely resulted from collinearity
562 between MAT and either soil pH ($r = 0.22$) or MAP ($r = 0.25$), the latter of which are often
563 confounded, and can have compounding effects on plant functional traits. For example,
564 globally, species growing on relatively dry sites tend to have small leaves when the mean
565 temperature of the warmest month (TWM) is high, whereas species on wetter sites typically
566 have larger leaves when TWM is high (Wright et al., 2017). From bivariate regressions, we
567 also found that seasonality in temperature strongly (positively) influenced the $N_{\text{area-g}_{\text{sw}}}$ and
568 $V_{\text{cmax } 25\text{-g}_{\text{sw}}}$ slope relationships (Table S4), perhaps suggesting that more seasonal
569 environments have higher water costs.

570

571 **Soil effects**

572 Plants have various strategies that enhance their ability to cope with drought and nutrient-
573 deficiency, two properties that characterize much of the Australian continent. Indeed, a
574 significant proportion of Australian plants possess scleromorphic long-lived leaves with low
575 mass-based nutrient concentrations (Beadle, 1966; Lambers et al., 2010; Lamont, 1982;
576 Specht, 1969; Wright et al., 2004a; Wright et al., 2002) and highly-proficient nutrient
577 resorption (Wright & Westoby, 2003). That is, most Australian plant species are generally
578 positioned towards the ‘slow’ end of the leaf economics spectrum (Wright et al., 2004b).

579 In this study we considered the effects of soil fertility via soil total P concentration
580 and soil pH, both of which presumably influence the unit-costs of N and carboxylation more
581 so than water costs. Soil P concentration is a long-term site property that is strongly
582 determined by parent material and is widely used as an indicator of soil P status in Australian
583 ecology (Beadle, 1954, 1966; Fonseca et al., 2000; Kooyman et al., 2017). In contrast to soil
584 total N concentration, which is quite stable across time, soil N and P availability can vary
585 seasonally and also with plant nutrient-acquisition strategies, often reflecting root

586 morphology, the tendency for carboxylate release and associations with mycorrhizal fungi
587 (Lambers & Oliveira, 2019; Richardson et al., 2005; Turner, 2008). In the bivariate analyses
588 (Fig. 2), $V_{\text{cmax } 25}$ and g_{sw} showed clear negative relationships with soil P concentration ($R^2 =$
589 0.13-0.16) and the soil P effect was far greater than the soil pH effect overall. Because all of
590 g_{sw} , N_{area} and $V_{\text{cmax } 25}$ increased as soil P decreased, it makes sense that their ratios (the $N_{\text{area}}-$
591 g_{sw} and $V_{\text{cmax } 25}-g_{\text{sw}}$ slopes) show little pattern over soil P gradients. The negative relationship
592 between N_{area} and soil P in the OLS regression resulted from LMA being typically higher on
593 low-P soils (Table S4) and in this study, there was a positive relationship between LMA and
594 N_{area} ($r = 0.75$, $P < 0.001$, results not shown) and between N_{area} and $V_{\text{cmax } 25}$ ($r = 0.37$, $P <$
595 0.001, results not shown). We note, however, that the relationship between N_{area} and soil P
596 became positive when we accounted for the effects of soil pH, MAP, and MAT (Table S5),
597 which likely reflects the strong, negative effect of MAP on LMA and therefore N_{area} . A
598 positive relationship between N_{area} and soil P was also observed in partial residual plots
599 generated by (Peng et al., 2021), which utilized a global dataset that included Australia.

600 The high $V_{\text{cmax } 25}$ at low soil P concentration is novel and unexpected, whereas the
601 negative relationship between soil P and $C_i:C_a$ matched predictions from least-cost theory
602 (Table S4 and S5). Least cost theory also predicts that all else equal, $C_i:C_a$ and $V_{\text{cmax } 25}$ should
603 be inversely related (Wright et al. 2003), which we observed in the present study ($r = -0.15$, P
604 $= <0.001$, results not shown). In contrast to the $V_{\text{cmax } 25}$ -soil P relationship, the g_{sw} effect was
605 in line with our expectations: Maire et al. (2015) reported a negative association between
606 plant-available soil P concentration and g_{sw} , arguing that nutrient deficiency promotes greater
607 root production, increasing plant-available water and increasing g_{sw} and $C_i:C_a$. The authors
608 also suggested that stimulation of transpiration (and g_{sw}) on nutrient-deficient sites may
609 increase mass flow of soil nutrients to roots, ultimately enhancing leaf N and ultimately,
610 $V_{\text{cmax } 25}$ [i.e. the mass-flow hypothesis (Cernusak et al., 2011b; Cramer et al., 2009; Edwards
611 et al., 1998)]. Because the mobility of P is low compared with that of N, mass flow is more
612 likely to increase N uptake than P uptake and may only increase P supply on P-impooverished,
613 sandy soils with low P buffering capacity (Cernusak et al., 2011b; Huang et al., 2017).

614 Considering the importance of P for leaf metabolism, environmental properties that
615 affect the per-unit cost of P acquisition from the soil arguably also affect the unit cost of
616 carboxylation, vis-à-vis least-cost theory. The chief way that soil P is more expensive to
617 acquire on low-P soils is in terms of higher belowground expenditure, e.g., greater fine root
618 production, greater expenditure supporting mycorrhizal symbionts, greater expenditure on
619 root exudates that enhance access to recalcitrant pools of soil P (e.g., phosphatases; organic

620 acids released by cluster roots), and greater expenditure on cluster roots (Raven et al., 2018).
621 The latter are especially common in the Australian flora, particularly in the Proteaceae which
622 exhibit very high photosynthetic phosphorus-use efficiency (PPUE) (Denton et al., 2007;
623 Guilherme Pereira et al., 2019), i.e. rapid photosynthetic rates at low leaf P concentrations
624 (Lambers et al., 2012; Yan et al., 2019). In this study, we observed higher PPUE and higher
625 PNUE for plants growing on low-P soils (Table S4) and higher $V_{\text{cmax } 25}$ on low-P soils. High
626 PPUE may be accomplished by shifting allocation away from phospholipids towards
627 galactolipids (the latter being a key component of chloroplast membranes) and sulfolipids
628 that do not contain P (Lambers et al., 2012; Yan et al., 2019) with the transition from young
629 to mature leaves. Interestingly, Australian Proteaceae growing on P-deficient soils have been
630 shown to have low Rubisco activity but high levels of photosynthesis at low leaf P compared
631 to *Arabidopsis* (Sulpice et al., 2014). The reduction in Rubisco activity likely resulted from a
632 lower abundance of ribosomes and therefore lower rRNA levels, which may constrain the
633 synthesis of proteins, including Rubisco. Thus, Australian plants appear to be well-adapted to
634 low-P soils, as they maintain high levels of photosynthesis, high rates of carboxylation, and
635 high photosynthetic nutrient use efficiency in these environments.

636 Soil pH alters the solubility of soil minerals and causes shifts in community
637 composition of soil bacteria (Lauber et al., 2008), which in turn affects nutrient availability.
638 We thus included soil pH alongside soil total P concentration, due to its potential effects on
639 the unit cost of carboxylation. Against expectation, variation in soil pH had negligible effects
640 on the $N_{\text{area-gsw}}$ and $V_{\text{cmax } 25\text{-gsw}}$ relationships but $C_i:C_a$ did decrease with increasing pH,
641 albeit weakly ($R^2 = 0.03$), as predicted. While the pH effects were weak to negligible, the
642 pattern in $C_i:C_a$ matched global studies that showed strong modulation of $N_{\text{area-gsw}}$ and V_{cmax}
643 25-gsw relationships, $C_i:C_a$ and $\Delta^{13}\text{C}$ via soil pH (Cornwell et al., 2018; Paillassa et al., 2020;
644 Wang et al., 2017). $C_i:C_a$ is tightly determined by the balance between $V_{\text{cmax } 25}$ and g_{sw} , such
645 that the $V_{\text{cmax } 25\text{-gsw}}$ relationship is a function of the optimal $C_i:C_a$, which is itself a function
646 of water and N costs (Prentice et al., 2014). Therefore, it is possible that $C_i:C_a$ better reflects
647 costs associated with soil and climate properties than do the slopes, as it more directly
648 integrates N and water costs. This is evidenced by the observation that $C_i:C_a$ was sensitive
649 (statistically significant) to all four of the chosen environmental variables whether analyzed
650 in bivariate regressions or multiple regression. Although N_{area} was strongly influenced by soil
651 pH ($R^2 = 0.15$), g_{sw} was not (Fig. 4) and $V_{\text{cmax } 25}$ was only weakly affected ($R^2 = 0.02$). The
652 positive effect of pH on leaf N concentration suggests moderately higher soil nutrient
653 availability in less acidic soils, despite the negligible effect of soil pH on the $V_{\text{cmax } 25\text{-gsw}}$ and

654 $N_{\text{area-g}_{\text{sw}}}$ slopes. Maire et al. (2015) also found no relationship between g_{sw} and soil pH in a
655 global study and in general, the soil pH effects in this study were considerably weaker than
656 those reported at global scale (cf. Paillassa et al. 2020).

657 While the weak pH effects in the present study were unexpected, this suggests that
658 soil pH may not be an especially useful index of nutrient-acquisition costs in low-fertility
659 landscapes. We note that the partial regression analyses resulted in a statistically significant
660 negative effect of soil pH on g_{sw} and $C_i:C_a$, the latter of which matched our predictions,
661 indicating strong collinearities among the predictors. Nevertheless, soil pH does not underlie
662 variation in photosynthetic traits within this system to the extent that soil P does. The finding
663 that climate has a greater role in photosynthetic trait coordination than soil pH contrasts with
664 the findings of our companion study (Paillassa et al., 2020), and may reflect the
665 predominance of low pH/low nutrient sites in our Australian dataset (only 4 of 67 sites with
666 $\text{pH} > 7$).

667

668 **Implications for global studies**

669 By improving our understanding of photosynthetic trait-environment relationships at the
670 regional and global scale, there is the potential to enhance the conceptual basis and
671 parameterization of global vegetation models. For example, dynamic global vegetation
672 models (DGVMs) rarely incorporate variation in ecophysiological traits within Plant
673 Functional Types (e.g., across species or populations) or include environmental dependencies
674 of traits (Grimm et al., 2017; Scheiter et al., 2013; Verheijen et al., 2013; Yang et al., 2015).
675 We have now demonstrated that soil properties, namely soil P concentration and soil pH
676 (Maire et al., 2015; Paillassa et al., 2020), influence the coordination of ecophysiological
677 traits at a continental scale. These findings support increasing calls for soil properties to be
678 included in vegetation models (Norby et al., 2017) and could be further developed using
679 least-cost theory.

680 Within the least-cost theory framework, the first-order costs are set by site properties,
681 whole-plant respiration rates, and tissue chemistry (e.g., leaf N or Rubisco concentration). In
682 the formulation of least-cost theory by Prentice et al. (2014), optimal $C_i:C_a$ is proportional to
683 the ratio of two dimensionless parameters, a and b , which reflect the maintenance respiration
684 costs of transpiration and carboxylation, respectively. Paillassa et al. (2020) re-expressed the
685 cost functions to incorporate the effects of soil N and water supply, surmising that
686 maintenance respiration costs at a given transpiration rate or carboxylation rate should
687 increase when soil water or nutrients are scarce. But, of course, these are necessary

688 simplifications that do not account for all relevant costs. Most importantly, water and nutrient
689 unit-costs are presumably also affected by species life history traits. For example, information
690 regarding inter- and intraspecific variation in the ability to acquire soil water or nutrients via
691 alternative allocation or acquisition strategies, including root activity and depth, nutrient-
692 acquisition strategies (e.g., cluster roots vs. N₂-fixation vs mycorrhizal symbioses; Lambers
693 *et al.*, 2008), and wood permeability (Wright *et al.*, 2003), would likely help in the
694 interpretation of within-site variation in photosynthetic traits. For example, mycorrhizal
695 species may have lower N costs than non-mycorrhizal species, which would result in higher
696 N_{area} for a given g_{sw} in the mycorrhizal species. Regions dominated by mycorrhizal species
697 are thus expected to have species with higher N_{area}-g_{sw} slopes than regions where such species
698 are absent, even when these occur at similar soil nutrient levels.

699 Additional considerations are needed before we can quantitatively integrate the effects
700 of concentrations of soil P (or other metrics of fertility) in DGVMs. For example, one can
701 make assumptions about the extent to which different nutrients are *substitutable* [e.g. whether
702 species can “spend” more N belowground by investing in phosphatase enzymes to obtain
703 more soil P (Olander & Vitousek, 2000; Schleuss *et al.*, 2020; Treseder & Vitousek, 2001)],
704 or simply *coordinated*, and specify nutrient exchange rates in a currency that can also be
705 applied to water costs. It is also worth considering how additional soil properties influence
706 soil nutrient costs, as soil texture, which influences both the availability of nutrients and
707 water, seems also important for understanding geographic variation in photosynthetic trait
708 coordination (Paillassa *et al.*, 2020). For example, soils with higher silt content can hold more
709 water than sandy soils, reducing water costs, such that plants typically have higher g_{sw}
710 coupled with higher V_{cmax 25} on silty soils (Paillassa *et al.*, 2020). In this study, we found that
711 plants growing on silt-rich soils had higher g_{sw} and higher N_{area} and V_{cmax 25}, but similar slope
712 relationships (Table S4), indicating a proportionate increase in these traits, which cancelled
713 out. We also found a positive relationship between soil effective cation exchange capacity
714 (ECE) and the slope relationships, indicating that nutrient costs were lower with increasing
715 ECE, which is positively associated with soil nutrient availability. Lastly, we acknowledge
716 the important role of soil N in other regions of the world, which significantly influenced
717 individual photosynthetic traits (with the exception of C_i:C_a) but not trait coordination in this
718 study system. The negative relationship between leaf N and soil total N, which was also
719 reported by Maire *et al.* (2015), may result from low plant-available N if the soil organic
720 matter has a high C:N ratio (Parton *et al.*, 1988). Further consideration of long- versus short-
721 term indices of soil resources is also warranted. Here we focused on evolutionary adaptations

722 to soil nutrient pools rather than on acclimation to soil nutrient availabilities that can vary
723 tremendously over relatively short timescales. Future studies could consider the relative
724 strengths of short- and long-term controls on photosynthetic trait coordination, as this would
725 be useful for quantifying within-species variation in trait relationships.

726

727 ***Conclusions***

728 Rainfall and temperature are expected to change considerably over the coming decades,
729 regionally and globally, altering the availabilities of soil nutrients. While much is known
730 regarding how climate drives variation in photosynthesis, few studies have investigated soil
731 effects, although this is changing. Among our findings, the coordination of photosynthetic
732 traits in response to soil P concentration is especially novel, as it suggests a unique
733 contribution of a limiting soil nutrient that is independent of climate and soil pH. The simple
734 theoretical framework known as least-cost theory can thus be applied to low-nutrient regions
735 globally, e.g., highly weathered soils and tropical regions, where P limits productivity. By
736 considering the dependencies of plant traits on both climate and soils, we will better
737 understand the proximate and long-term controls of photosynthesis.

738

739 **ACKNOWLEDGEMENTS**

740 ACW acknowledges support from Macquarie University and from Australian Research
741 Council funding to IJW and ICP (DP170103410). Sampling in New South Wales was
742 approved under the NSW Scientific Licence SL102164, which was granted to ACW.
743 Fieldwork carried out by ACW in 2020 and 2021 was funded by the Hermon Slade
744 Foundation, HSF20047. We acknowledge Spud Thomas of the Kidman Springs and Victoria
745 River Research Station in the Northern Territory, for allowing us to collect data on site in
746 2019. VM and JP were supported by grants NSERC-Discovery (2016-05716). Data collection
747 in Northern Queensland was supported by European Research Council advanced investigator
748 grants GEM-TRAITS (321131) and T-FORCES (291585) under the European Union's
749 Seventh Framework Programme (FP7/2007-2013). OKA was supported by the ARC
750 (DP130101252 and CE140100008). Data contributed by SMG were funded by the ARC
751 Australian Laureate Fellowship (FL100100080/2011-2015).

752

753 **AUTHOR CONTRIBUTIONS**

754 IJW and ACW planned and designed the study. ACW carried out the fieldwork in 2018 and
755 2019 and analysed the data. ACW wrote the first draft with significant input from IJW. All
756 authors read and contributed to subsequent versions.

757 **DATA AVAILABILITY**

758 The data that support the findings of this study are openly available in DRYAD at
759 <https://doi.org/10.5061/dryad.j9kd51cgr>.

760 **REFERENCES**

- 761 Beadle, N. (1954). Soil phosphate and the delimitation of plant communities in eastern
762 Australia. *Ecology*, 35(3), 370-375.
- 763 Beadle, N. (1966). Soil phosphate and its role in molding segments of the Australian flora
764 and vegetation, with special reference to xeromorphy and sclerophylly. *Ecology*,
765 47(6), 992-1007.
- 766 Bloomfield, K. J., Cernusak, L. A., Eamus, D., Ellsworth, D. S., Colin Prentice, I., Wright, I.
767 J., . . . Cleverly, J. (2018). A continental-scale assessment of variability in leaf traits:
768 Within species, across sites and between seasons. *Functional Ecology*, 32(6), 1492-
769 1506.
- 770 Bloomfield, K. J., Prentice, I. C., Cernusak, L. A., Eamus, D., Medlyn, B. E., Rumman, R., . .
771 . Cleverly, J. (2019). The validity of optimal leaf traits modelled on environmental
772 conditions. *New Phytologist*, 221(3), 1409-1423.
- 773 Cernusak, L. A., Hutley, L. B., Beringer, J., Holtum, J. A., & Turner, B. L. (2011a).
774 Photosynthetic physiology of eucalypts along a sub-continental rainfall gradient in
775 northern Australia. *Agricultural and Forest Meteorology*, 151(11), 1462-1470.
- 776 Cernusak, L. A., Winter, K., & Turner, B. L. (2011b). Transpiration modulates phosphorus
777 acquisition in tropical tree seedlings. *Tree Physiology*, 31(8), 878-885.
- 778 Chen, J. L., Reynolds, J. F., Harley, P. C., & Tenhunen, J. D. (1993). Coordination theory of
779 leaf nitrogen distribution in a canopy. *Oecologia*, 93(1), 63-69.
780 doi:10.1007/bf00321192
- 781 Cochrane, A., Hoyle, G. L., Yates, C. J., Neeman, T., & Nicotra, A. B. (2016). Variation in
782 plant functional traits across and within four species of Western Australian Banksia
783 (Proteaceae) along a natural climate gradient. *Austral Ecology*, 41(8), 886-896.
- 784 Cornwell, W. K., Wright, I. J., Turner, J., Maire, V., Barbour, M. M., Cernusak, L. A., . . .
785 Santiago, L. S. (2018). Climate and soils together regulate photosynthetic carbon
786 isotope discrimination within C3 plants worldwide. *Global Ecology and*
787 *Biogeography*, 27(9), 1056-1067. doi:10.1111/geb.12764
- 788 Courville, T., & Thompson, B. (2001). Use of structure coefficients in published multiple
789 regression articles: beta is not enough. *Educational and Psychological Measurement*,
790 61(2), 229-248. doi:10.1177/0013164401612006
- 791 Cramer, M. D., Hawkins, H. J., & Verboom, G. A. (2009). The importance of nutritional
792 regulation of plant water flux. *Oecologia*, 161(1), 15-24.

- 793 de Caritat, P., Cooper, M., & Wilford, J. (2011). The pH of Australian soils: field results from
794 a national survey. *Soil Research*, 49(2), 173-182.
- 795 De Kauwe, M. G., Lin, Y. S., Wright, I. J., Medlyn, B. E., Crous, K. Y., Ellsworth, D. S., . . .
796 Rogers, A. (2016). A test of the 'one-point method' for estimating maximum
797 carboxylation capacity from field-measured, light-saturated photosynthesis. *New*
798 *Phytologist*, 210(3), 1130-1144.
- 799 Denton, M. D., Veneklaas, E. J., Freimoser, F. M., & Lambers, H. (2007). Banksia species
800 (Proteaceae) from severely phosphorus-impooverished soils exhibit extreme efficiency
801 in the use and re-mobilization of phosphorus. *Plant, Cell & Environment*, 30(12),
802 1557-1565.
- 803 Domingues, T. F., Meir, P., Feldpausch, T. R., Saiz, G., Veenendaal, E. M., Schrod, F., . . .
804 Lloyd, J. (2010). Co-limitation of photosynthetic capacity by nitrogen and phosphorus
805 in West Africa woodlands. *Plant, Cell & Environment*, 33(6), 959-980.
- 806 Dong, N., Prentice, I. C., Evans, B. J., Caddy-Retalic, S., Lowe, A. J., & Wright, I. J. (2017).
807 Leaf nitrogen from first principles: field evidence for adaptive variation with climate.
808 *Biogeosciences*, 14(2), 481-495. doi:10.5194/bg-14-481-2017
- 809 Dong, N., Prentice, I. C., Wright, I. J., Evans, B. J., Togashi, H. F., Caddy-Retalic, S., . . .
810 Lowe, A. J. (2020). Components of leaf-trait variation along environmental gradients.
811 *New Phytologist*.
- 812 Dong, N., Wright, I. J., Chen, J. M., Luo, X., Wang, H., Keenan, T. F., . . . Prentice, I. C.
813 (2022). Rising CO₂ and warming reduce global canopy demand for nitrogen. *New*
814 *Phytologist*.
- 815 Eamus, D., & Prichard, H. (1998). A cost-benefit analysis of leaves of four Australian
816 savanna species. *Tree Physiology*, 18(8-9), 537-545. doi:10.1093/treephys/18.8-9.537
- 817 Edwards, D., Kerp, H., & Hass, H. (1998). Stomata in early land plants: an anatomical and
818 ecophysiological approach. *Journal of Experimental Botany*, 255-278.
- 819 Farquhar, G. D., von Caemmerer, S. v., & Berry, J. A. (1980). A biochemical model of
820 photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, 149(1), 78-90.
- 821 Field, C., Merino, J., & Mooney, H. A. (1983). Compromises between water-use efficiency
822 and nitrogen-use efficiency in 5 species of California evergreens. *Oecologia*, 60(3),
823 384-389. doi:10.1007/bf00376856
- 824 Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial
825 communities. *Proceedings of the National Academy of Sciences*, 103(3), 626-631.
- 826 Fonseca, C. R., Overton, J. M., Collins, B., & Westoby, M. (2000). Shifts in trait-
827 combinations along rainfall and phosphorus gradients. *Journal of Ecology*, 88(6),
828 964-977.
- 829 Friend, A. D., Geider, R. J., Behrenfeld, M. J., & Still, C. J. (2009). Photosynthesis in global-
830 scale models. In *Photosynthesis in silico* (pp. 465-497): Springer.
- 831 Grimm, V., Ayllón, D., & Railsback, S. F. (2017). Next-generation individual-based models
832 integrate biodiversity and ecosystems: yes we can, and yes we must. *Ecosystems*,
833 20(2), 229-236.
- 834 Grundy, M. J., Rossel, R. A. V., Searle, R. D., Wilson, P. L., Chen, C., & Gregory, L. J.
835 (2015). Soil and Landscape Grid of Australia. *Soil Research*, 53(8), 835-844.
836 doi:<https://doi.org/10.1071/SR15191>
- 837 Guilherme Pereira, C., Hayes, P. E., O'Sullivan, O. S., Weerasinghe, L. K., Clode, P. L.,
838 Atkin, O. K., & Lambers, H. (2019). Trait convergence in photosynthetic nutrient-use
839 efficiency along a 2-million year dune chronosequence in a global biodiversity
840 hotspot. *Journal of Ecology*, 107(4), 2006-2023.

- 841 He, X., Augusto, L., Goll, D. S., Ringeval, B., Wang, Y., Helfenstein, J., . . . Yang, Y.
842 (2021). Global patterns and drivers of soil total phosphorus concentration. *Earth*
843 *System Science Data*, 13(12), 5831-5846.
- 844 Hikosaka, K., Nabeshima, E., & Hiura, T. (2007). Seasonal changes in the temperature
845 response of photosynthesis in canopy leaves of *Quercus crispula* in a cool-temperate
846 forest. *Tree Physiology*, 27(7), 1035-1041.
- 847 Huang, G., Hayes, P. E., Ryan, M. H., Pang, J., & Lambers, H. (2017). Peppermint trees shift
848 their phosphorus-acquisition strategy along a strong gradient of plant-available
849 phosphorus by increasing their transpiration at very low phosphorus availability.
850 *Oecologia*, 185(3), 387-400.
- 851 Hutchinson, M. F., McKenney, D. W., Lawrence, K., Pedlar, J. H., Hopkinson, R. F.,
852 Milewska, E., & Papadopol, P. (2009). Development and testing of Canada-wide
853 interpolated spatial models of daily minimum-maximum temperature and
854 precipitation for 1961-2003. *Journal of Applied Meteorology and Climatology*, 48(4),
855 725-741. doi:10.1175/2008jamc1979.1
- 856 Kooyman, R. M., Laffan, S. W., & Westoby, M. (2017). The incidence of low phosphorus
857 soils in Australia. *Plant and soil*, 412(1-2), 143-150.
- 858 Körner, C., Farquhar, G., & Wong, S. (1991). Carbon isotope discrimination by plants
859 follows latitudinal and altitudinal trends. *Oecologia*, 88(1), 30-40.
- 860 Laliberté, E., Zemunik, G., & Turner, B. L. (2014). Environmental filtering explains variation
861 in plant diversity along resource gradients. *science*, 345(6204), 1602-1605.
- 862 Lambers, H., Brundrett, M. C., Raven, J. A., & Hopper, S. D. (2010). Plant mineral nutrition
863 in ancient landscapes: high plant species diversity on infertile soils is linked to
864 functional diversity for nutritional strategies. *Plant and soil*, 334, 11-31.
865 doi:10.1007/s11104-010-0444-9
- 866 Lambers, H., Cawthray, G. R., Giavalisco, P., Kuo, J., Laliberté, E., Pearse, S. J., . . . Turner,
867 B. L. (2012). Proteaceae from severely phosphorus-impooverished soils extensively
868 replace phospholipids with galactolipids and sulfolipids during leaf development to
869 achieve a high photosynthetic phosphorus-use-efficiency. *New Phytologist*, 196(4),
870 1098-1108.
- 871 Lambers, H., & Oliveira, R. (2019). *Plant Physiological Ecology* (Third Edition ed.):
872 Springer Nature Switzerland AG.
- 873 Lambers, H., Raven, J. A., Shaver, G. R., & Smith, S. E. (2008). Plant nutrient-acquisition
874 strategies change with soil age. *Trends in ecology & evolution*, 23(2), 95-103.
- 875 Lamont, B. (1982). Mechanisms for enhancing nutrient uptake in plants, with particular
876 reference to mediterranean South Africa and Western Australia. *The Botanical*
877 *Review*, 48(3), 597-689.
- 878 Lauber, C. L., Strickland, M. S., Bradford, M. A., & Fierer, N. (2008). The influence of soil
879 properties on the structure of bacterial and fungal communities across land-use types.
880 *Soil Biology and Biochemistry*, 40(9), 2407-2415.
- 881 Lin, Y.-S., Medlyn, B. E., De Kauwe, M. G., & Ellsworth, D. S. (2013). Biochemical
882 photosynthetic responses to temperature: how do interspecific differences compare
883 with seasonal shifts? *Tree Physiology*, 33(8), 793-806.
- 884 Maire, V., Martre, P., Kattge, J., Gastal, F., Esser, G., Fontaine, S., & Soussana, J. F. (2012).
885 The coordination of leaf photosynthesis links C and N fluxes in C3 plant species. *Plos*
886 *One*, 7(6), 15. doi:10.1371/journal.pone.0038345
- 887 Maire, V., Wright, I. J., Prentice, I. C., Batjes, N. H., Bhaskar, R., van Bodegom, P. M., . . .
888 Ordonez, A. (2015). Global effects of soil and climate on leaf photosynthetic traits
889 and rates. *Global Ecology and Biogeography*, 24(6), 706-717.

- 890 Murray, M., Soh, W. K., Yiotis, C., Batke, S., Parnell, A. C., Spicer, R. A., . . . McElwain, J.
891 C. (2019). Convergence in Maximum Stomatal Conductance of C-3 Woody
892 Angiosperms in Natural Ecosystems Across Bioclimatic Zones. *Frontiers in Plant*
893 *Science*, 10. doi:10.3389/fpls.2019.00558
- 894 Murray, M., Soh, W. K., Yiotis, C., Spicer, R. A., Lawson, T., & McElwain, J. C. (2020).
895 Consistent Relationship between Field-Measured Stomatal Conductance and
896 Theoretical Maximum Stomatal Conductance in C3 Woody Angiosperms in Four
897 Major Biomes. *International Journal of Plant Sciences*, 181(1), 142-154.
898 doi:10.1086/706260
- 899 Norby, R. J., Gu, L., Haworth, I. C., Jensen, A. M., Turner, B. L., Walker, A. P., . . . Winter,
900 K. (2017). Informing models through empirical relationships between foliar
901 phosphorus, nitrogen and photosynthesis across diverse woody species in tropical
902 forests of Panama. *New Phytologist*, 215(4), 1425-1437.
- 903 Northcote, H., & Wright, M. (1982). Soil landscapes of arid Australia. In *Evolution of the*
904 *Flora and Fauna of Arid Australia* (pp. 15-21). Adelaide, Australia: Peacock
905 Publications.
- 906 Olander, L. P., & Vitousek, P. M. (2000). Regulation of soil phosphatase and chitinase
907 activity by N and P availability. *Biogeochemistry*, 49(2), 175-191.
- 908 Ordoñez, J. C., Van Bodegom, P. M., Witte, J. P. M., Wright, I. J., Reich, P. B., & Aerts, R.
909 (2009). A global study of relationships between leaf traits, climate and soil measures
910 of nutrient fertility. *Global Ecology and Biogeography*, 18(2), 137-149.
- 911 Paillasa, J., Wright, I. J., Prentice, I. C., Pepin, S., Smith, N. G., Ethier, G., . . . Cornwell, W.
912 K. (2020). When and where soil is important to modify the carbon and water economy
913 of leaves. *New Phytologist*.
- 914 Parton, W. J., Stewart, J. W., & Cole, C. V. (1988). Dynamics of C, N, P and S in grassland
915 soils: a model. *Biogeochemistry*, 5(1), 109-131.
- 916 Pedhazur, E. J. (1997). *Multiple regression in behavioral research: Explanation and*
917 *prediction* (3rd ed.). Fort Worth, Texas (USA): Harcourt Brace College Publishers.
- 918 Peng, Y., Bloomfield, K. J., Cernusak, L. A., Domingues, T. F., & Colin Prentice, I. (2021).
919 Global climate and nutrient controls of photosynthetic capacity. *Communications*
920 *Biology*, 4(1), 462. doi:10.1038/s42003-021-01985-7
- 921 Prentice, I. C., Dong, N., Gleason, S. M., Maire, V., & Wright, I. J. (2014). Balancing the
922 costs of carbon gain and water transport: testing a new theoretical framework for plant
923 functional ecology. *Ecology letters*, 17(1), 82-91.
- 924 Prentice, I. C., Meng, T., Wang, H., Harrison, S. P., Ni, J., & Wang, G. (2011). Evidence of a
925 universal scaling relationship for leaf CO₂ drawdown along an aridity gradient. *New*
926 *Phytologist*, 190(1), 169-180.
- 927 R Development Core Team. (2017). R: A language and environment for statistical
928 computing. Vienna, Austria: R Foundation for Statistical Computing.
- 929 Raven, J. A., Lambers, H., Smith, S. E., & Westoby, M. (2018). Costs of acquiring
930 phosphorus by vascular land plants: patterns and implications for plant coexistence.
931 *New Phytologist*, 217(4), 1420-1427.
- 932 Rayment, G., & Lyons, D. (2011). *Soil chemical methods: Australasia*. Clayton, VIC,
933 Australia: CSIRO Publishing.
- 934 Reich, P. B., Oleksyn, J., & Wright, I. J. (2009). Leaf phosphorus influences the
935 photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. *Oecologia*,
936 160(2), 207-212. doi:10.1007/s00442-009-1291-3
- 937 Richardson, A., George, T., Hens, M., & Simpson, R. (2005). Utilization of soil organic
938 phosphorus by higher plants. In B. Turner, E. Frossard, & D. Baldwin (Eds.), *Organic*
939 *phosphorus in the environment* (pp. pp. 165–184): CABI Publishing: Wallingford.

- 940 Scafaro, A. P., Xiang, S., Long, B. M., Bahar, N. H., Weerasinghe, L. K., Creek, D., . . .
 941 Atkin, O. K. (2017). Strong thermal acclimation of photosynthesis in tropical and
 942 temperate wet-forest tree species: the importance of altered Rubisco content. *Global*
 943 *change biology*, 23(7), 2783-2800.
- 944 Scheiter, S., Langan, L., & Higgins, S. I. (2013). Next-generation dynamic global vegetation
 945 models: learning from community ecology. *New Phytologist*, 198(3), 957-969.
- 946 Schleuss, P. M., Widdig, M., Heintz-Buschart, A., Kirkman, K., & Spohn, M. (2020).
 947 Interactions of nitrogen and phosphorus cycling promote P acquisition and explain
 948 synergistic plant-growth responses. *Ecology*, 101(5), e03003.
- 949 Sinsabaugh, R. L., & Follstad Shah, J. J. (2012). Coenzymatic stoichiometry and ecological
 950 theory. *Annual Review of Ecology, Evolution, and Systematics*, 43, 313-343.
- 951 Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C.,
 952 . . . Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale.
 953 *Ecology letters*, 11(11), 1252-1264. doi:10.1111/j.1461-0248.2008.01245.x
- 954 Slessarev, E. W., Lin, Y., Bingham, N. L., Johnson, J. E., Dai, Y., Schimel, J. P., &
 955 Chadwick, O. A. (2016). Water balance creates a threshold in soil pH at the global
 956 scale. *Nature*, 540(7634), 567-569. doi:10.1038/nature20139
- 957 Smith, N. G., & Dukes, J. S. (2013). Plant respiration and photosynthesis in global-scale
 958 models: incorporating acclimation to temperature and CO₂. *Global change biology*,
 959 19(1), 45-63.
- 960 Smith, N. G., Keenan, T. F., Colin Prentice, I., Wang, H., Wright, I. J., Niinemets, Ü., . . .
 961 Zhou, S.-X. (2019). Global photosynthetic capacity is optimized to the environment.
 962 *Ecology letters*, 22(3), 506-517. doi:10.1111/ele.13210
- 963 Smith, S. D., Monson, R. K., & Anderson, J. E. (1997). *Physiological ecology of North*
 964 *American desert plants*. New York: Springer.
- 965 Specht, R. (1969). A comparison of the sclerophyllous vegetation characteristic of
 966 Mediterranean type climates in France, California, and Southern Australia. I.
 967 Structure, morphology, and succession. *Australian Journal of Botany*, 17(2), 277-292.
 968 doi:<https://doi.org/10.1071/BT9690277>
- 969 Sulpice, R., Ishihara, H., Schlereth, A., Cawthray, G. R., Encke, B., Giavalisco, P., . . .
 970 Krohn, N. (2014). Low levels of ribosomal RNA partly account for the very high
 971 photosynthetic phosphorus-use efficiency of P roteaceae species. *Plant, Cell &*
 972 *Environment*, 37(6), 1276-1298.
- 973 Taylor, G. (1994). Landscapes of Australia: their nature and evolution. *History of the*
 974 *Australian vegetation: Cretaceous to recent*, 60-79.
- 975 Thornthwaite, C. W. (1948). An approach toward a rational classification of climate.
 976 *Geographical review*, 38(1), 55-94.
- 977 Togashi, H. F., Prentice, I. C., Atkin, O. K., Macfarlane, C., Prober, S. M., Bloomfield, K. J.,
 978 & Evans, B. J. (2018). Thermal acclimation of leaf photosynthetic traits in an
 979 evergreen woodland, consistent with the coordination hypothesis. *Biogeosciences*,
 980 15(11), 3461-3474.
- 981 Treseder, K. K., & Vitousek, P. M. (2001). Effects of soil nutrient availability on investment
 982 in acquisition of N and P in Hawaiian rain forests. *Ecology*, 82(4), 946-954.
- 983 Turner, B. L. (2008). Resource partitioning for soil phosphorus: a hypothesis. *Journal of*
 984 *Ecology*, 96(4), 698-702. doi:10.1111/j.1365-2745.2008.01384.x
- 985 Verheijen, L., Brövkín, V., Aerts, R., Bonisch, G., Cornelissen, J. H., Kattge, J., . . . Van
 986 Bodegom, P. (2013). Impacts of trait variation through observed trait-climate
 987 relationships on performance of an Earth system model: a conceptual analysis.

- 988 Viscarra Rossel, R., Chen, C., Grundy, M., Searle, R., Clifford, D., Odgers, N., . . . Kidd, D.
989 (2014). Soil and Landscape Grid National Soil Attribute Maps Release 1. v. 3. CSIRO
990 Data Collection (Publication no. <https://doi.org/10.4225/08/546F540FE10AA>).
- 991 Viscarra Rossel, R. A., & Bui, E. N. (2016). A new detailed map of total phosphorus stocks
992 in Australian soil. *Science of the Total Environment*, 542, 1040-1049.
- 993 Vitousek, P. M. (1984). Litterfall, nutrient cycling, and nutrient limitation in tropical forests.
994 *Ecology*, 65(1), 285-298.
- 995 Von Caemmerer, S. v., & Farquhar, G. D. (1981). Some relationships between the
996 biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, 153(4), 376-
997 387.
- 998 Wang, H., Prentice, I. C., Keenan, T. F., Davis, T. W., Wright, I. J., Cornwell, W. K., . . .
999 Peng, C. (2017). Towards a universal model for carbon dioxide uptake by plants.
1000 *Nature Plants*, 3(9), 734-741. doi:10.1038/s41477-017-0006-8
- 1001 Warton, D. I., Wright, I. J., Falster, D. S., & Westoby, M. (2006). Bivariate line-fitting
1002 methods for allometry. *Biological Reviews*, 81(2), 259-291.
1003 doi:10.1017/s1464793106007007
- 1004 Wright, I. J., Dong, N., Maire, V., Prentice, I. C., Westoby, M., Díaz, S., . . . Law, E. A.
1005 (2017). Global climatic drivers of leaf size. *science*, 357(6354), 917-921.
- 1006 Wright, I. J., Groom, P. K., Lamont, B. B., Poot, P., Prior, L. D., Reich, P. B., . . . Westoby,
1007 M. (2004a). Leaf trait relationships in Australian plant species. *Functional Plant
1008 Biology*, 31(5), 551-558.
- 1009 Wright, I. J., Reich, P. B., Cornelissen, J. H. C., Falster, D. S., Groom, P. K., Hikosaka, K., . .
1010 . Westoby, M. (2005). Modulation of leaf economic traits and trait relationships by
1011 climate. *Global Ecology and Biogeography*, 14(5), 411-421. doi:doi:10.1111/j.1466-
1012 822x.2005.00172.x
- 1013 Wright, I. J., Reich, P. B., & Westoby, M. (2001). Strategy shifts in leaf physiology, structure
1014 and nutrient content between species of high-and low-rainfall and high-and low-
1015 nutrient habitats. *Functional Ecology*, 15(4), 423-434.
- 1016 Wright, I. J., Reich, P. B., & Westoby, M. (2003). Least-Cost Input Mixtures of Water and
1017 Nitrogen for Photosynthesis. *The American Naturalist*, 161(1), 98-111.
1018 doi:10.1086/344920
- 1019 Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., . . . Villar,
1020 R. (2004b). The worldwide leaf economics spectrum. *Nature*, 428, 821-827.
1021 doi:10.1038/nature02403
- 1022 Wright, I. J., & Westoby, M. (2003). Nutrient concentration, resorption and lifespan: leaf
1023 traits of Australian sclerophyll species. *Functional Ecology*, 17(1), 10-19.
- 1024 Wright, I. J., Westoby, M., & Reich, P. B. (2002). Convergence towards higher leaf mass per
1025 area in dry and nutrient-poor habitats has different consequences for leaf life span.
1026 *Journal of Ecology*, 90(3), 534-543.
- 1027 Xu, T., Han, W., Hutchinson, M., Pauwels, J., Whitley, R., & Evans, B. (2015). eMAST R-
1028 Package collection (Dataset). [https://portal.tern.org.au/emast-r-package-
1029 collection/21637](https://portal.tern.org.au/emast-r-package-collection/21637)
- 1030 Yan, L., Zhang, X., Han, Z., Pang, J., Lambers, H., & Finnegan, P. M. (2019). Responses of
1031 foliar phosphorus fractions to soil age are diverse along a 2 Myr dune
1032 chronosequence. *New Phytologist*, 223(3), 1621-1633.
- 1033 Yang, Y., Zhu, Q., Peng, C., Wang, H., & Chen, H. (2015). From plant functional types to
1034 plant functional traits: A new paradigm in modelling global vegetation dynamics.
1035 *Progress in Physical Geography*, 39(4), 514-535.

1036

1037 **Figure legends**

1038

1039 **Figure 1.** An approach based on least-cost theory for understanding the co-optimisation of
1040 photosynthetic traits in relation to site properties. (a) The theory predicts that the optimal
1041 ratio of water and nitrogen (N) use during light-saturated photosynthesis (A_{sat}) depends on
1042 their relative costs of acquisition and use. Nitrogen use is represented by leaf N content per
1043 unit area (N_{area}) and carboxylation capacity ($V_{\text{cmax } 25}$). Water use is represented by stomatal
1044 conductance (g_{sw}). Blue dots represent site conditions where water costs are relatively greater
1045 than N costs, or alternatively, where N costs are relatively lower than water costs. $C_i:C_a$ is the
1046 ratio of leaf-internal to ambient CO_2 concentration and mediates the relationship between N
1047 use (and carboxylation) and water use. In this study, we found significant site-level variation
1048 in (b) photosynthetic N use versus water use, and (c) carboxylation versus water use, which
1049 we quantified using a slope fitted to a set of co-occurring species at each site, where each
1050 point represents a species-site mean. Each line was “forced” through the origin. Blue and
1051 purple tones represent sites with higher water costs and simultaneously lower N costs, while
1052 orange and red tones represent lower water costs and simultaneously higher N costs. Circles
1053 are non- N_2 -fixing species and triangles are N_2 -fixing species.

1054

1055 **Figure 2.** Linear regression plots of soil and climate effects on the (a)-(d) slope relationship
1056 between leaf nitrogen concentration (N) on an area basis, N_{area} , and stomatal conductance,
1057 g_{sw} , and the (e)-(h) slope relationship between photosynthetic carboxylation, $V_{\text{cmax } 25}$, and g_{sw} .
1058 (i)-(l) Relationship between $C_i:C_a$ and environmental variables. (a), (e), (i) Soil total
1059 phosphorus (Soil P, mg kg^{-1}) concentration, (b), (f), (j) Soil pH, (c), (g), (k) Mean annual
1060 precipitation (MAP, mm), and (d), (h), (l) Mean annual temperature (MAT, $^{\circ}\text{C}$). Red lines
1061 represent trend lines with 95% confidence intervals in grey and are only shown for
1062 statistically significant (P -values < 0.05) relationships. Notice the logarithmic scale to the
1063 axes. See Figure 3 for partial regressions.

1064

1065 **Figure 3.** Partial regression plots from multiple linear regression of soil and climate effects
1066 on the (a)-(d) slope relationship between leaf nitrogen concentration (N) on an area basis,
1067 N_{area} , and stomatal conductance, g_{sw} , and the (e)-(h) slope relationship between
1068 photosynthetic carboxylation, $V_{\text{cmax } 25}$, and g_{sw} . (i)-(l) Relationship between $C_i:C_a$ and
1069 environmental variables. (a), (e), (i) Soil total phosphorus (Soil P, mg kg^{-1}) concentration, (b),
1070 (f), (j) Soil pH, (c), (g), (k) Mean annual precipitation (MAP, mm), and (d), (h), (l) Mean
1071 annual temperature (MAT, $^{\circ}\text{C}$). Points in grey represent partial regressions with standard
1072 errors in grey and dark red lines are shown only for statistically significant relationships,
1073 where solid lines have $P < 0.05$ and dashed lines are marginally significant ($0.05 < P < 0.10$).
1074 P -values above each panel indicate the statistical significance of each variable in the multiple
1075 regression. Higher β values indicate a stronger effect size, where β values are the regression
1076 weights for standardized variables and represent the change in the slope value (in standard
1077 deviations) associated with a change of one standard deviation in a predictor while holding
1078 constant the value(s) of the other predictor(s).

1079

1080

1081

1082

1083 **Figure 4.** Trait-soil relationships from bivariate linear regression analysis. Points represent
1084 species-site means. All axes (except soil pH) have been \log_{10} -scaled. Abbreviations follow
1085 those in Table S1 and Figure 3. Red lines represent trend lines with 95% confidence intervals
1086 in grey and are only shown for statistically significant (P -values < 0.05 , solid line). In panel
1087 (b), the slope coefficient was <0.005 .

1088
1089 **Figure 5.** Trait-climate relationships from bivariate linear regression analysis. Points
1090 represent species-site means. All axes (except soil pH) have been \log_{10} -scaled. Abbreviations
1091 follow those in Table S1 and Figure 3. Red lines represent trend lines with 95% confidence
1092 intervals in grey and are only shown for statistically significant ($P < 0.05$, solid line, $0.05 < P$
1093 < 0.10 , dashed line). In panel (a), the slope coefficient was <0.005 .
1094
1095