

1 **Does nitrogen affect the interaction between a native hemiparasite and its native or**  
 2 **introduced leguminous hosts?**

3 **Robert M. Cirocco<sup>1</sup>, José M. Facelli<sup>1</sup> and Jennifer R. Watling<sup>1,2</sup>**

4 School of Biological Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

5 Manchester Metropolitan University, Manchester, UK

6 Author for correspondence:

7 *Robert M. Cirocco*

8 *Tel: +61 8313 5281*

9 *Email: [robert.cirocco@adelaide.edu.au](mailto:robert.cirocco@adelaide.edu.au)*

10 \*RMC, JMF and JRW conceived and designed the experiment. RMC performed the  
 11 experiment and analysed the data. RMC, JMF and JRW interpreted the analysis and wrote the  
 12 manuscript.

13 Brief heading: Native hemiparasite impacts overall growth of invasive but not native legumes  
 14 regardless of nitrogen.

Total word count (excluding summary, references and legends):	4,405	No. of figures:	5 (Fig. 5 in colour)
Summary:	200	No. of Tables:	3
Introduction:	1,028	No. of Supporting Information files:	1
Materials and Methods:	1,311		
Results:	643		
Discussion:	1,366		
Acknowledgements:	39		

15

16

## 17 **Summary**

- 18 • Associations between plants and N-fixing rhizobia intensify with decreasing nitrogen  
19 (N) supply and come at a carbon cost to the host. However, what additional impact  
20 parasitic plants have on their leguminous hosts' carbon budget in terms of effects on  
21 host physiology and growth is unknown.
- 22 • Under glasshouse conditions, *Ulex europaeus* and *Acacia paradoxa* either uninfected  
23 or infected with the hemiparasite *Cassytha pubescens* were supplied (HN) or not (LN)  
24 with extra N. Photosynthetic performance and growth of the association were  
25 measured.
- 26 • *Cassytha pubescens* significantly reduced maximum electron transport rates and total  
27 biomass of *U. europaeus* but not *A. paradoxa*, regardless of N. Infection significantly  
28 decreased root biomass of *A. paradoxa* only at LN, while the significant negative  
29 effect of infection on roots of *U. europaeus* was less severe at LN. Infection had a  
30 significant negative impact on host nodule biomass. *Ulex europaeus* supported  
31 significantly greater parasite biomass (also per unit host biomass) than *A. paradoxa*,  
32 regardless of N.
- 33 • We concluded that rhizobia do not influence the effect of a native parasite on overall  
34 growth of leguminous hosts. Our results suggest that *C. pubescens* will have a strong  
35 impact on *U. europaeus* but not *A. paradoxa*, regardless of N in the field.

36 **Key words:** Biomass, hemiparasite, legume, nitrogen, nodulation, photosynthesis, rhizobia,  
37 *Ulex europaeus*.

## 38 **Introduction**

39 Parasitic plants are globally important as they are found in a wide range of ecosystems and  
40 have profound effects on processes at the population, community and ecosystem levels (Press  
41 & Phoenix, 2005). They vary greatly in taxonomy, form and function, but all attach to either  
42 host stems or roots via haustoria (Press *et al.*, 1999). This structure joins the parasite to the  
43 host from which it extracts resources (Kuijt, 1969). Holoparasites access resources from the  
44 phloem and xylem of their hosts removing carbohydrate, water and nutrients but generally  
45 have very low photosynthetic ability (Stewart & Press, 1990). Conversely, hemiparasites  
46 typically access resources from the host xylem, and while being capable of photosynthesis  
47 they depend on their hosts for water, nutrients and other solutes (Press & Graves, 1995), as  
48 has been demonstrated for a range of host:parasite associations (e.g. Pate *et al.*, 1991; Pate,  
49 2001; Lu *et al.*, 2013, 2014).

50 Parasite effects on their hosts can range from negligible to host death and such outcomes can  
51 depend on a number of factors. One such factor is nutrient supply. For example, in some host  
52 species, high nitrogen (N) supply reduces the effect of the hemiparasite, *Striga hermonthica*,  
53 on host photosynthesis and growth, even to the point of eliminating it for *Sorghum bicolor*  
54 cultivar CSH1 (Cechin & Press, 1993; Cechin & Press, 1994), while in other cultivars or host  
55 species N does not influence the effect of this root hemiparasite (Gurney *et al.*, 1995;  
56 Aflakpui *et al.*, 1998; Sinebo & Drennan, 2001; Aflakpui *et al.*, 2002; Aflakpui *et al.*, 2005).  
57 These authors suggested that in their studies, insufficient amounts of N may have been added  
58 to influence the effects of *S. hermonthica* on its hosts. High N supply has also been found to  
59 dampen the effect of the stem holoparasites *Cuscuta campestris* and *Cuscuta reflexa* on  
60 growth of *Mikania micrantha* and *Ricinus communis*, respectively, but not for the *C. reflexa*-  
61 *Coleus blumei* association (Jeschke & Hilpert, 1997; Jeschke *et al.*, 1997; Shen *et al.*, 2013).  
62 At least for the *C. campestris*-*M. micrantha* association, the greater effect on host growth at  
63 low N supply was attributed to increased resource removal by the parasite in these conditions

64 (Shen *et al.*, 2013). It should also be kept in mind that the influence of nutrients such as  
65 nitrogen on the association is likely to be modified if other factors (e.g. water availability) are  
66 altered (Těšitel *et al.*, 2015).

67 The influence of N on host-parasite associations also becomes more complex when the host  
68 plants are N-fixers, such as legumes which form associations with rhizobia to obtain N at a  
69 cost of carbohydrate (Pennings & Callaway, 2002). When supplied with sufficient N, plants  
70 have low affinity for partnerships with rhizobia, while at low N, they have a greater  
71 engagement with these bacteria and this comes at a greater cost of carbohydrate (Lambers *et*  
72 *al.*, 2008). This may be compounded when legumes are also infected by a parasite, as  
73 carbohydrate may already be in short supply due to infection effects on host photosynthesis  
74 as well as direct removal of host carbon (C) by the parasite (Gurney *et al.*, 2002; Meinzer *et*  
75 *al.*, 2004; Shen *et al.*, 2007; Těšitel *et al.*, 2010). Thus, at low N supply, the combination of  
76 infection by a parasite and rhizobia, which may be the main N source for the host, may result  
77 in greater pressure on host carbon and ultimately growth.

78 One study investigating the effects of the stem holoparasite *Cuscuta reflexa* on the legume  
79 *Lupinus albus* found that nitrogen fixation, host growth and fruit setting were strongly  
80 suppressed by infection (Jeschke *et al.*, 1994). They attributed these decreases to carbon and  
81 nitrogen removal by the parasite from the host phloem, however, in this study plants were  
82 only supplied with nitrogen-free solution. Another study manipulated the nodulation status of  
83 *Dalbergia odorifera* infected with *Santalum album*, but did not include uninfected plants in  
84 the experiment (Lu *et al.*, 2013). Jiang *et al.* (2008) did include uninfected plants in their  
85 investigation into the effect of *Rhinanthus minor* on *Vicia faba* when colonised or not  
86 (provided with inorganic N) with rhizobia. However, while infection effects on host abscisic  
87 acid levels, nitrogen concentration and amino acid composition were quantified, there were  
88 no measures of host photosynthesis, growth or nodule biomass. There have also been a

89 number of studies investigating the influence of mycorrhizae (inoculated versus not  
90 inoculated) (Davies & Graves, 1998; Salonen *et al.*, 2001; Gworgwor & Weber, 2003; Stein  
91 *et al.*, 2009) on parasite effects on host growth and photosynthesis, but to our knowledge,  
92 there are none on the influence of rhizobia (high versus low colonisation) via manipulation of  
93 N supply which include measures of host growth or photosynthesis. This is a significant gap  
94 in knowledge considering that plants that form associations with N-fixing bacteria are  
95 common hosts of parasitic plants (Matthies, 1996). As below-ground process such as  
96 rhizobial interactions and root growth are very difficult to quantify in the field, glasshouse  
97 experimentation offers a practical and rigorous means to test the impact of combinations of  
98 parasite and rhizobial infection on hosts in isolation from numerous other factors found in  
99 nature.

100 Here we report results of an experiment investigating how N availability affected the  
101 association between the Australian native stem hemiparasite, *Cassytha pubescens* and two N-  
102 fixing hosts, a native (*Acacia paradoxa*) and an introduced weed (*Ulex europaeus*). We  
103 hypothesised that *C. pubescens* would have a greater effect on host performance at low N  
104 supply. This is because of carbohydrate limitations resulting from infection effects on host  
105 photosynthesis coupled with the additional C demand from rhizobia in these conditions.  
106 However, we also expected the impact of infection with *C. pubescens* would be greater in the  
107 introduced host, *U. europaeus*, than the native host, *A. paradoxa*. This is because *C.*  
108 *pubescens* has been found to negatively affect the performance of a number of introduced  
109 hosts, including *Cytisus scoparius* and *U. europaeus* much more than that of the native host  
110 *Leptospermum myrsinoides* (Prider *et al.*, 2009; Cirocco *et al.*, 2016a). Our study also  
111 provides the ability to compare responses of host species within the same family (under the  
112 same experimental conditions) to infection with a parasitic plant (Demey *et al.*, 2015).

## 113 **Materials and Methods**

114 *Study species*

115 *Cassytha pubescens* R. Br. (Lauraceae) is a perennial, stem hemiparasitic vine native to  
116 Australia (Kokubugata *et al.*, 2012) and abundant in the southern part of the continent. It has  
117 much reduced scale-like leaves on a coiling stem (0.5–1.5 mm in diameter) and attaches to  
118 host stems and leaves via multiple haustoria (McLuckie, 1924; Harden, 1990; Prider *et al.*,  
119 2009). *Acacia paradoxa* DC. (Fabaceae) is a perennial, evergreen, leguminous shrub native to  
120 southern Australia that grows on a range of soils and is often found in eucalypt-dominated  
121 woodlands (Cunningham *et al.*, 2011). *Acacia paradoxa* grows to *c.* 2.5–4 m in height and  
122 has dark green 0.8–3 cm long phyllodes (Harden, 1991).

123 *Ulex europaeus* L. (Fabaceae) is a perennial, evergreen, leguminous shrub *c.* 1.5–2 m in  
124 height that is native to Europe and Northern Africa (Clements *et al.*, 2001; Tarayre *et al.*,  
125 2007). It is a serious, introduced weed in more than 15 countries worldwide, including  
126 Australia (Lowe *et al.*, 2000; Clements *et al.*, 2001; Tarayre *et al.*, 2007). Its leaves, spines  
127 and stems are photosynthetic (Hill *et al.*, 1991; Clements *et al.*, 2001; Tarayre *et al.*, 2007).  
128 *Ulex europaeus* thrives in disturbed areas and grows well in nutrient poor sandy soils. Both  
129 *U. europaeus* and *A. paradoxa* are N-fixing and typically form associations with nitrogen-  
130 fixing bacteria from the genus *Bradyrhizobium* to obtain biologically reduced atmospheric N<sub>2</sub>  
131 in exchange for carbohydrate (Lawrie, 1983; Weir *et al.*, 2004; Thrall *et al.*, 2005). Images of  
132 all three experimental species are provided in the Supporting Information (Fig. S1).

133 *Experimental design*

134 *Acacia paradoxa* plants (*c.* 20 cm in height) were obtained from a commercial nursery and  
135 individually transplanted into 1.65 litre pots containing commercial soil (organic sandy loam,  
136 Supporting Information Table S1) in late April 2011. *Ulex europaeus* plants (*c.* 15 cm in  
137 height) were obtained from the field (Crafers, Mt. Lofty Ranges of South Australia:

138 35°27'41"S, 138°43'91"E), and were individually transplanted into 1.65 litre pots containing  
139 the same commercial soil in late January 2011. Throughout the experiment, plants were  
140 grown in the commercial soil mentioned. This soil was not inoculated with field soil in case  
141 of introducing any pathogens into the system. Further, although the commercial soil was not  
142 inoculated with any rhizobial strain this may be inconsequential as nodules were present on  
143 all experimental plants (total biomass of uninfected plants of both species in the high N (HN)  
144 treatment were similar with those in the treatment without additional N provided (LN), Fig.  
145 1a; and as expected, nodule biomass per unit root biomass was significantly higher at LN  
146 versus HN (independently affected by N, Tables 1 and 2)). All plants were provided with 400  
147 ml of liquid fertiliser (Nitrosol; Rural Research Ltd, Auckland, New Zealand; NPK 8:3:6)  
148 monthly (dilution factor and frequency in accordance with the manufacturer's directions).

149 Synchronous infection with *C. pubescens* of randomly selected individuals of both species  
150 was achieved in mid-June 2011 using the method described in Shen *et al.* (2010). Briefly,  
151 large *U. europaeus* plants already infected by *C. pubescens* were used as the source of  
152 infection, and the parasite was allowed to coil and attach to stems of experimental plants.  
153 Stems of *C. pubescens* attached to the newly parasitised plants were severed from the *U.*  
154 *europaeus* donor plant in early November 2011. The process of attachment took 4–5 months.  
155 Experimental plants were monitored for a further week to ensure that *C. pubescens* had  
156 successfully established on the hosts. All plants were then individually re-potted into 5 litre  
157 pots containing the same commercial soil in early December 2011.

158 Uninfected and infected plants of both species were randomly allocated into two N  
159 treatments. Plants in the high N treatment (HN) were provided with standard Hoagland's  
160 solution. Plants in the treatment without additional N (LN) were provided standard  
161 Hoagland's solution with KCl and CaCl<sub>2</sub> substituted for KNO<sub>3</sub> and Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O,  
162 respectively. All plants were randomly allocated into six blocks, each block containing all

163 combinations of treatments, and were re-randomised fortnightly to account for small light  
164 differences in the glasshouse. Plants were provided with 400 ml of standard (HN) or modified  
165 Hoagland's solution (LN) fortnightly. Nitrogen treatments ran from early February 2012 to  
166 mid-June 2012, lasting for 164 days. The experiment consisted of a full three-way factorial  
167 design with host species, infection and N at two levels each with six replicates for each  
168 combination of factors.

### 169 *Photosynthesis measurements*

170 Rapid light response curves for hosts and parasite were determined using a portable, pulse-  
171 modulated chlorophyll fluorometer (MINI-PAM, Walz, Effeltrich, Germany) fitted with a  
172 leaf-clip (2030-B, Walz, Effeltrich, Germany) (Supporting Information Fig. S2). Electron  
173 transport rate (ETR) was calculated as:

$$174 \text{ ETR} = \text{Yield} \times \text{PAR} \times 0.5 \times 0.84$$

175 Where Yield is PSII efficiency in the light, PAR is photosynthetically active radiation, 0.5  
176 signifies that two photons are required to transport a single electron and 0.84 is the  
177 absorptance factor for a standard leaf of an angiosperm (White & Critchley, 1999; Strong *et*  
178 *al.*, 2000). Actinic light levels were automatically increased in eight steps at 10 s intervals  
179 and included an initial measurement in darkness. Rates of electron transport were considered  
180 to be at their maximum ( $\text{ETR}_{\text{max}}$ ) at the same actinic light level within species where highest  
181 rates were consistently reached and most representative of replicates.  $\text{ETR}_{\text{max}}$  occurred at  
182 photon flux densities (PFD) of  $1904 \pm 23.31 \mu\text{mol m}^{-2} \text{s}^{-1}$  for *U. europaeus*,  $1308 \pm 20.41$   
183  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for *A. paradoxa*, and  $1439 \pm 12.85 \mu\text{mol m}^{-2} \text{s}^{-1}$  for *C. pubescens* on both hosts.  
184 *In situ* measurements of ETR were made between 11:00 and 13:00 on the youngest fully  
185 expanded spine or phyllode, depending on species, on a sunny day in mid-May 2012, 103



186 days after N treatments were imposed (DAT); and on *C. pubescens* 15 cm from the growing  
187 tip on a sunny day in mid-May 2012 (107 DAT).

188 Measurements of photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ) were obtained using a  
189 portable Ciras-2 gas-exchange system fitted with a PLC (5) conifer cuvette (PP Systems,  
190 Amesburg, MA). This cuvette enabled gas exchange measurements on the different  
191 photosynthetic organs (stems with spines or phyllodes) of *U. europaeus* and *A. paradoxa*.  
192 Measurements were made between 10:30 and 13:00 in early June 2012 (when days were  
193 sunny between 117-129 DAT), at mean PFD= $1278 \pm 4 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $n=32$  (Results are  
194 presented in Supporting Information Fig. S3 and Table S4).

#### 195 *Biomass and N concentration*

196 A destructive harvest was conducted at the end of the experiment in mid-June 2012, 164  
197 DAT. Nodules, roots, stems and spines (very few if any leaves present) of *U. europaeus*;  
198 nodules, roots, stems and phyllodes of *A. paradoxa*, and stems of *C. pubescens* were  
199 collected and oven dried at 70°C for three days. Nitrogen concentration of *U. europaeus*  
200 spines, *A. paradoxa* phyllodes and *C. pubescens* stems was determined by complete  
201 combustion gas chromatography at Waite Analytical Services (University of Adelaide), on  
202 final harvest oven-dried material.

#### 203 *Statistical analyses*

204 The variances of the data were homogeneous and the effects of infection with *C. pubescens*,  
205 N supply and host species were assessed using a three-way ANOVA. Where a three-way  
206 interaction was not detected, two-way interactions were considered e.g. Infection x Host  
207 species (uninfected plants at HN and LN pooled versus infected plants at HN and LN pooled  
208 for *A. paradoxa* compared with those of *U. europaeus*). A two-way ANOVA was

209 implemented to detect the effect of N and host species on parasite parameters. Where  
210 interactions were not significant, independent effects were then considered e.g. infection  
211 effect with *C. pubescens* (uninfected plants from both host species at HN and LN pooled  
212 versus infected plants from both host species at HN and LN pooled). Where effects were  
213 significant, a Tukey-Kramer HSD was used for pairwise comparisons of means. All data  
214 were analysed with the software JMP Ver. 4.0.3 (SAS institute Inc., 2000) and  $\alpha=0.05$ .

## 215 **Results**

### 216 *Growth, nodulation and N concentration*

217 Nitrogen did not have any interactive or independent effect on total or shoot biomass of either  
218 *U. europaeus* or *A. paradoxa* (Table 1, Fig. 1a, c). There was however, a species x infection  
219 interaction for total and shoot biomass (Table 1). Total and shoot biomass of infected *U.*  
220 *europaeus* was c. 60% less than that of uninfected plants (Fig. 1b, d). Infection had no effect  
221 on total or shoot biomass of *A. paradoxa* (Fig. 1b, d). In contrast to total and shoot biomass,  
222 there was a three-way interaction for root biomass (Table 1, Fig. 1e). Root biomass of  
223 infected *U. europaeus* in HN and LN was 56% and 36% lower compared with that of the  
224 respective uninfected plants (Fig. 1e). Root biomass of infected *A. paradoxa* in the LN  
225 treatment was 39% less relative to that of respective uninfected plants (Fig. 1e), but infection  
226 had no effect on root biomass of *A. paradoxa* in the HN treatment (Fig. 1e).

227 There were no treatment interactions for host leaf area, shoot:root ratio, nodule biomass or  
228 nodule biomass per g root biomass (Table 1). There was however, an independent effect of  
229 infection on leaf area (Table 1). Phyllode/spine area of infected plants on the whole was 42%  
230 less than that of uninfected plants (Table 2). There was also an independent effect of infection  
231 on nodule biomass (Table 1). Nodule biomass on roots of infected plants was 41% lower  
232 compared with that of uninfected plants (Table 2). There was an independent effect of species

233 on all four parameters. Spine area of *U. europaeus* was 70% lower relative to phyllode area  
234 of *A. paradoxa* (Table 2). Shoot:root ratio of *U. europaeus* was 48% lower than that of *A.*  
235 *paradoxa* (Table 2). Nodule biomass of *U. europaeus* was 43% lower compared with that of  
236 *A. paradoxa* (Table 2). Nodule biomass per g root biomass of *U. europaeus* was 58% lower  
237 relative to that of *A. paradoxa* (Table 2). This parameter was also independently affected by  
238 N treatment (Table 1). Nodule biomass per g root biomass of plants in LN ( $0.127 \pm 0.017$ )  
239 was 20% higher than that of plants in HN treatment ( $0.102 \pm 0.014$ ). Parasite biomass, both  
240 total and on a per g host biomass basis, was independently affected by species but not by N  
241 treatment (Table 3, Fig. 2a, b). Total parasite biomass on *A. paradoxa* was 63% less than it  
242 was on *U. europaeus* (Fig. 2a), and was nearly an order of magnitude lower per g of host on  
243 *A. paradoxa* than on *U. europaeus* (Fig. 2b).

244 There was no three-way interaction for host foliar N concentration (Table 1, Fig. 3a). There  
245 was however, an N x infection interaction for this parameter (Table 1). Host foliar N  
246 concentration of infected plants was not significantly different from that of uninfected plants  
247 in either HN or LN (Fig. 3b). However, foliar N of infected plants in HN was significantly  
248 higher compared with that of infected plants in LN treatment (Fig. 3b). There was also an  
249 independent species effect on N concentration of spines or phyllodes (Table 1). ‘Foliar’ N  
250 concentration of *U. europaeus* was 32% lower than that of *A. paradoxa* (Fig. 3c). There was  
251 no N x species interaction or independent effects on N concentration of *C. pubescens* stems  
252 (Table 3, Fig. 3d).

### 253 *Photosynthetic performance*

254 As with host total and shoot biomass, N had no interactive or independent effect on  $ETR_{\max}$   
255 of either *U. europaeus* or *A. paradoxa* (Table 1, Fig. 4a). There was however, a species x  
256 infection interaction for  $ETR_{\max}$  (Table 1). Infection decreased  $ETR_{\max}$  of *U. europaeus* by

257 46% while having no effect on that of *A. paradoxa*, regardless of N treatment (Fig. 4b). There  
258 was no interactive effect of N x species or any independent effects of these factors on ETR<sub>max</sub>  
259 of *C. pubescens* (Table 3, Fig. 4c).

## 260 **Discussion**

261 A simplified model of the C and N dynamics of the ‘host + parasite + rhizobia’ system is  
262 presented as a framework from which treatment effects can be interpreted (Fig. 5). The  
263 parasite as a partial and complete sink for C and N, respectively, may affect host C budget i.e.  
264 ‘whole plant photosynthesis’ (unit rate x ‘leaf area’) and thus, C supply to host roots +  
265 nodulation. The latter in turn dictates the host’s ability to acquire N which can also affect  
266 whole-plant C gain through its impact on foliar N concentration and rate of photosynthesis.

267 Our hypothesis that *C. pubescens* would have a greater effect on host performance under LN  
268 was supported by the root biomass data, although for the native not introduced host as  
269 expected. *Acacia paradoxa* root growth was affected by infection at only LN. This might be  
270 due to the 44% reduction in phyllode area resulting from infection in these conditions. This  
271 would result in lower whole-plant C gain, of which was evidently allocated to maintaining  
272 similar nodulation (at the expense of roots) and thus, an increased rate of nodulation i.e. g  
273 nod g root dwt<sup>-1</sup> relative to that of respective uninfected plants at LN. Consequently, infected  
274 *A. paradoxa* in LN was still able to obtain sufficient N to maintain foliar N (and  
275 photosynthetic performance) at the same level as found in other treatments and sustain  
276 similar overall growth compared with respective uninfected plants. This was likely enabled  
277 by small parasite demand for C and N from this host inferred from the much smaller *C.*  
278 *pubescens* supported by *A. paradoxa* relative to *U. europaeus* (Figs 2, 5).

279 In contrast to *A. paradoxa*, although *C. pubescens* negatively impacted root growth of *U.*  
280 *europaeus*, it was less severe at LN. The infection effect on root growth of *U. europaeus* may

281 be due to the parasite's significant impact on spine area and  $ETR_{max}$  of this host which would  
282 negatively affect its C budget (Fig. 5). But in contrast to *A. paradoxa* at LN, of that less  
283 available C it seems that *U. europaeus* allocated more toward root growth (Fig. 1), but with a  
284 56% decrease in nodule biomass and hence, lower rate of nodulation ( $g\ nod\ g\ root\ dwt^{-1}$ )  
285 relative to respective uninfected plants (Table 2). Thus, at LN, infected *U. europaeus* despite  
286 having decreased N-fixing capacity per unit root appears to have acquired adequate N supply  
287 to maintain similar N concentration relative to other treatment combinations by increased root  
288 biomass. This was also probably made possible by its total biomass being significantly lower  
289 than uninfected plants. That is, although parasite demand for C and N was presumably  
290 relatively large on this host (inferred from vigorous parasite growth), a much smaller infected  
291 *U. europaeus* would require less nitrogen than a much larger uninfected plant to maintain a  
292 relatively similar N concentration.

293 Within host species, LN plants were able to maintain similar foliar N concentrations as HN  
294 plants likely because they had significantly higher nodule biomass per gram root biomass,  
295 and thus, sufficient access to N from rhizobia in these conditions. Therefore, it makes sense  
296 that N treatment had no influence on  $ETR_{max}$ /total biomass of either host species and in turn  
297 no interactive effect with infection on these parameters. By contrast, Shen *et al.* (2013) found  
298 that the impact of *Cuscuta campestris* on total biomass of *Mikania micrantha* was more  
299 severe at low N. Parasites can affect host growth due to effects on host photosynthesis and/or  
300 resource removal. As Shen *et al.* (2013) found no significant N x infection interaction on host  
301 photosynthesis; they attributed the greater effect on host growth at low N to increased  
302 resource removal by *Cuscuta campestris* in these conditions. This difference between  
303 findings may be in part related to *Cuscuta campestris* and *C. pubescens* being holo and  
304 hemiparasites and or being associated with non-leguminous and leguminous hosts in these  
305 studies, respectively.

306 *Cassytha pubescens* significantly decreased nodule biomass of both species, regardless of N.  
307 By contrast, Tennakoon *et al.* (1997) found that nodule biomass on roots of *Acacia littorea*  
308 was unaffected by the root hemiparasite *Olox phyllanthi*. This difference may be due to  
309 infection having a significant effect on ETR<sub>max</sub> of *U. europaeus* and foliar area of both hosts  
310 in our study, whereas *O. phyllanthi* had no effect on either host photosynthesis or leaf area of  
311 its host (Tennakoon *et al.*, 1997). As a result, infected plants in our study may have had less  
312 C for rhizobia, which would explain why infection negatively impacted nodulation.

313 Another important finding of our study was that total biomass of *U. europaeus* but not that of  
314 *A. paradoxa*, was affected by *C. pubescens*, regardless of N. This is similar to other studies  
315 that have reported greater negative effects of native parasites on growth of introduced rather  
316 than native hosts (Prider *et al.*, 2009; Li *et al.*, 2012; Cirocco *et al.*, 2016a, b). Our results  
317 may be explained by the infection effect on photosynthetic performance of *U. europaeus*, but  
318 not that of *A. paradoxa* (Fig. 4b). It may also in part be due to more effective resource  
319 removal by the parasite from *U. europaeus* compared with *A. paradoxa*, resulting from a  
320 more effective haustorial connection to the introduced host (see Gurney *et al.*, 2003;  
321 Cameron *et al.*, 2006; Gurney *et al.*, 2006; Cameron & Seel, 2007; Rümer *et al.*, 2007). This  
322 is plausible considering that an earlier study with *C. pubescens* demonstrated that the  
323 radioactive phosphorous isotope <sup>32</sup>P was transferred more effectively across haustoria formed  
324 on the introduced host *Cytisus scoparius* (broom) than those on the native host *Acacia*  
325 *myrtifolia* (Tsang, 2010).

326 This idea is further supported by the fact that in our study, photosynthesis of the parasite was  
327 similar on both hosts, but the parasite grew significantly larger both in absolute and per unit  
328 host biomass terms on *U. europaeus* than *A. paradoxa* (Figs 4c, 2 a, b). Again, our finding  
329 builds on consistent reports that native parasites with indeterminate growth such as *C.*  
330 *pubescens*, grow much more vigorously on introduced versus native hosts (Prider *et al.*, 2009;

331 Yu *et al.*, 2011; Li *et al.*, 2012; Cirocco *et al.*, 2016a). Nitrogen was not found to influence  
332 parasite biomass in absolute terms nor on a per g host biomass basis. By contrast, Shen *et al.*  
333 (2013) found that biomass of *Cuscuta campestris* infecting *M. micrantha* was significantly  
334 greater at high than low N supply. Similarly, the root hemiparasite *Santalum album* grew  
335 significantly larger on the nodulated versus non-nodulated host *Dalbergia odorifera* (Lu *et al.*  
336 *et al.*, 2013, but see Jiang *et al.*, 2008). It appears that in these studies, hosts grew larger in  
337 response to high N/nodulation and so too did the parasites (Lu *et al.*, 2013; Shen *et al.*, 2013).  
338 Here, infected plants did not grow larger in HN relative to LN. This may be due to hosts  
339 being able to access sufficient nitrogen under LN, albeit by different mechanisms (increased  
340 root growth in the case of *U. europaeus*, and increased nodulation for *A. paradoxa*). This may  
341 explain why *C. pubescens* did not grow more in HN on either host.

342 Nitrogen had no influence on the effect of *C. pubescens* on photosynthetic performance of  
343 hosts, as was similarly found for the *Cuscuta campestris*-*M. micrantha* association (Shen *et al.*  
344 *et al.*, 2013). The negative effect of *C. pubescens* on ETR<sub>max</sub> of *U. europaeus* does not seem  
345 related to nitrogen stress as infected plants did not have a significantly lower foliar N  
346 concentration than uninfected plants. Although not significant, decreases in  $g_s$  of *U.*  
347 *europaeus* as a result of infection (Supporting Information Fig. S3c) may explain the impact  
348 of *C. pubescens* on photosynthetic performance of this host. Negative effects of *C. pubescens*  
349 on photosynthesis of the introduced *Cytisus scoparius* and native *Leptospermum myrsinoides*  
350 have been ascribed to decreases in stomatal conductance resulting from infection (Prider *et al.*  
351 *et al.*, 2009; Shen *et al.*, 2010). Importantly, our study revealed that *A. paradoxa* is the first  
352 native host studied whose photosynthesis was not affected by the native hemiparasite *C.*  
353 *pubescens*. In sum, the differential impact of *Cassiope pubescens* on photosynthetic  
354 performance and overall growth of these two legumes (irrespective of N), highlights the fact

355 that there can be variation within a functional group in terms of host responses/tolerance to  
356 infection.

### 357 **Acknowledgements**

358 Special thanks to Dr. Jane N. Prider, Hong T. Tsang, Elizabeth C. Maciunas, A/Prof. Robert J.  
359 Reid, Angela Cirocco and Michele Cirocco for all their assistance. Part funding for this  
360 experiment was provided by the Native Vegetation Council (56109204).

### 361 **Author Contribution**

362 \*RMC, JMF and JRW conceived and designed the experiment. RMC performed the  
363 experiment and analysed the data. RMC, JMF and JRW interpreted the analysis and wrote the  
364 manuscript.

### 365 **References**

- 366 **Aflakpui GKS, Gregory PJ, Froud-Williams RJ. 1998.** Uptake and partitioning of nitrogen  
367 by maize infected with *Striga hermonthica*. *Annals of Botany* **81**: 287–294.
- 368 **Aflakpui GKS, Gregory PJ, Froud-Williams RJ. 2002.** Growth and biomass partitioning  
369 of maize during vegetative growth in response to *Striga hermonthica* infection and  
370 nitrogen supply. *Experimental Agriculture* **38**: 265–276.
- 371 **Aflakpui GKS, Gregory PJ, Froud-Williams RJ. 2005.** Carbon (<sup>13</sup>C) and nitrogen (<sup>15</sup>N)  
372 translocation in a maize-*Striga hermonthica* association. *Experimental Agriculture* **41**:  
373 321–333.
- 374 **Cameron DD, Coats AM, Seel WE. 2006.** Differential resistance among host and non-host  
375 species underlies the variable success of the hemi-parasitic plant *Rhinanthus minor*.  
376 *Annals of Botany* **98**: 1289–1299.



377 **Cameron DD, Seel WE. 2007.** Functional anatomy of haustoria formed by *Rhinanthus*  
378 *minor*: linking evidence from histology and isotope tracing. *New Phytologist* **174**:  
379 412–419.

380 **Cechin I, Press MC. 1993.** Nitrogen relations of the sorghum-*Striga hermonthica* host-  
381 parasite association: growth and photosynthesis. *Plant, Cell & Environment* **16**: 237–  
382 247.

383 **Cechin I, Press MC. 1994.** Influence of nitrogen on growth and photosynthesis of a C<sub>3</sub>  
384 cereal, *Oryza sativa*, infected with the root hemiparasite *Striga hermonthica*. *Journal*  
385 *of Experimental Botany* **45**: 925–930.

386 **Ciocco RM, Facelli JM, Watling JR. 2016a.** Does light influence the relationship between  
387 a native stem hemiparasite and a native or introduced host? *Annals of Botany* **117**:  
388 521–531.

389 **Ciocco RM, Facelli JM, Watling JR. 2016b.** High water availability increases the negative  
390 impact of a native hemiparasite on its non-native host. *Journal of Experimental*  
391 *Botany* **67**: 1567–1575.

392 **Clements DR, Peterson DJ, Prasad R. 2001.** The biology of Canadian weeds. 112. *Ulex*  
393 *europaeus* L. *Canadian Journal of Plant Science* **81**: 325–337.

394 **Cunningham GM, Mulham W, Milthorpe PL, Leigh JH. 2011.** *Plants of western New*  
395 *South Wales*. Melbourne, AUS: Inkata Press.

396 **Davies DM, Graves JD. 1998.** Interactions between arbuscular mycorrhizal fungi and the  
397 hemiparasitic angiosperm *Rhinanthus minor* during co-infection of a host. *New*  
398 *Phytologist* **139**: 555–563.

399 **Demey A, De Frenne P, Baeten L, Verstraeten G, Hermy M, Boeckx P, Verheyen K.**  
400 **2015.** The effects of hemiparasitic plant removal on community structure and seedling  
401 establishment in semi-natural grasslands. *Journal of Vegetation Science* **26**: 409–420.

402 **Gurney AL, Press MC, Ransom JK. 1995.** The parasitic angiosperm *Striga hermonthica*  
403 can reduce photosynthesis of its sorghum and maize hosts in the field. *Journal of*  
404 *Experimental Botany* **46**: 1817–1823.

405 **Gurney AL, Press MC, Scholes JD. 2002.** Can wild relatives of sorghum provide new  
406 sources of resistance or tolerance against *Striga* species? *Weed Research* **42**: 317–324.

407 **Gurney AL, Grimanelli D, Kanampiu F, Hoisington D, Scholes JD, Press MC. 2003.**  
408 Novel sources of resistance to *Striga hermonthica* in *Tripsacum dactyloides*, a wild  
409 relative of maize. *New Phytologist* **160**: 557–568.

410 **Gurney AL, Slate J, Press MC, Scholes JD. 2006.** A novel form of resistance in rice to the  
411 angiosperm parasite *Striga hermonthica*. *New Phytologist* **169**: 199–208.

412 **Gworgwor NA, Weber HC. 2003.** Arbuscular mycorrhizal fungi-parasite-host interaction  
413 for the control of *Striga hermonthica* (Del.) Benth. in sorghum [*Sorghum bicolor* (L.)  
414 Moench]. *Mycorrhiza* **13**: 277–281.

415 **Harden G. 1990.** *Flora of New South Wales, Vol. 1*. Kensington, AUS: New South Wales  
416 University Press.

417 **Harden GJ. 1991.** *Flora of New South Wales, Vol. 2*. Kensington, AUS: New South Wales  
418 University Press.

419 **Hill RL, Gourlay AH, Martin L. 1991.** Seasonal and geographic variation in the predation  
420 of gorse seed, *Ulex europaeus* L., by the seed weevil *Apion ulicis* Forst. *New Zealand*  
421 *Journal of Zoology* **18**: 37–43.

422 **Jeschke WD, Bäuml P, R  th N, Czygan F-C, Proksch P. 1994.** Modelling of the flows  
423 and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and  
424 its host *Lupinus albus* L. II. Flows between host and parasite and within the  
425 parasitized host. *Journal of Experimental Botany* **45**: 801–812.

426 **Jeschke WD, Hilpert A. 1997.** Sink-stimulated photosynthesis and sink-dependent increase  
427 in nitrate uptake: nitrogen and carbon relations of the parasitic association *Cuscuta*  
428 *reflexa*–*Ricinus communis*. *Plant, Cell & Environment* **20**: 47–56.

429 **Jeschke WD, Baig A, Hilpert A. 1997.** Sink-stimulated photosynthesis, increased  
430 transpiration and increased demand-dependent stimulation of nitrate uptake: nitrogen  
431 and carbon relations in the parasitic association *Cuscuta reflexa*-*Coleus blumei*.  
432 *Journal of Experimental Botany* **48**: 915–925.

433 **Jiang F, Jeschke WD, Hartung W, Cameron DD. 2008.** Does legume nitrogen fixation  
434 underpin host quality for the hemiparasitic plant *Rhinanthus minor*? *Journal of*  
435 *Experimental Botany* **59**: 917–925.

436 **Kokubugata G, Nakamura K, Forster PI, Wilson GW, Holland AE, Hirayama Y,**  
437 **Yokota M. 2012.** *Cassytha pubescens* and *C. glabella* (Lauraceae) are not disjunctly  
438 distributed between Australia and the Ryukyu Archipelago of Japan—evidence from  
439 morphological and molecular data. *Australian Systematic Botany* **25**: 364–373.

440 **Kuijt J. 1969.** *The biology of parasitic flowering plants*. California, USA: University of  
441 California Press.

442 **Lambers H, Chapin III FS, Pons TL. 2008.** *Plant physiological ecology, 2<sup>nd</sup> edn*. New  
443 York, USA: Springer.

444 **Lawrie AC. 1983.** Relationships among rhizobia from native Australian legumes. *Applied*  
445 *and Environmental Microbiology* **45**: 1822–1828.

446 **Li J, Jin Z, Song W. 2012.** Do native parasitic plants cause more damage to exotic invasive  
447 hosts than native non-invasive hosts? An implication for biocontrol. *PloS One* **7**:  
448 e34577.

- 449 **Lowe S, Browne M, Boudjelas S, De Poorter M. 2000.** *100 of the world's worst invasive*  
450 *alien species: a selection from the global invasive species database.* Auckland, New  
451 Zealand: Invasive Species Specialist Group.
- 452 **Lu JK, Kang LH, Sprent JI, Xu DP, He XH. 2013.** Two-way transfer of nitrogen between  
453 *Dalbergia odorifera* and its hemiparasite *Santalum album* is enhanced when the host  
454 is effectively nodulated and fixing nitrogen. *Tree Physiology* **33**: 464–474.
- 455 **Lu JK, Xu DP, Kang LH, He XH. 2014.** Host-species-dependent physiological  
456 characteristics of hemiparasite *Santalum album* in association with N<sub>2</sub>-fixing and non-  
457 N<sub>2</sub>-fixing hosts native to southern China. *Tree Physiology* **34**: 1006–1017.
- 458 **Matthies D. 1996.** Interactions between the root hemiparasite *Melampyrum arvense* and  
459 mixtures of host plants: heterotrophic benefit and parasite-mediated competition.  
460 *Oikos* **75**: 118–124.
- 461 **McLuckie J. 1924.** Studies in Parasitism. I. A contribution to the physiology of the genus  
462 *Cassytha*, Part 1. *Proceedings of the Linnaean Society of New South Wales* **49**: 333–  
463 369.
- 464 **Meinzer FC, Woodruff DR, Shaw DC. 2004.** Integrated responses of hydraulic architecture,  
465 water and carbon relations of western hemlock to dwarf mistletoe infection. *Plant,*  
466 *Cell & Environment* **27**: 937–946.
- 467 **Pate JS, True KC, Rasins E. 1991.** Xylem transport and storage of amino acids by S.W.  
468 Australian mistletoes and their hosts. *Journal of Experimental Botany* **42**: 441–451.
- 469 **Pate JS. 2001.** Haustoria in action: case studies of nitrogen acquisition by woody xylem-  
470 tapping hemiparasites from their hosts. *Protoplasma* **215**: 204–217.
- 471 **Pennings SC, Callaway RM. 2002.** Parasitic plants: parallels and contrasts with herbivores.  
472 *Oecologia* **131**: 479–489.
- 473 **Press MC, Graves JD. 1995.** *Parasitic plants.* London, UK: Chapman & Hall.

474 **Press MC, Scholes JD, Watling JR. 1999.** Parasitic plants: physiological and ecological  
475 interactions with their hosts. In: Press MC, Scholes JD, Barker MG, eds.  
476 *Physiological Plant Ecology*. Oxford, UK: Blackwell Science Ltd., 175–197.

477 **Press MC, Phoenix GK. 2005.** Impacts of parasitic plants on natural communities. *New*  
478 *Phytologist* **166**: 737–751.

479 **Prider JN, Watling JR, Facelli JM. 2009.** Impacts of a native parasitic plant on an  
480 introduced and a native host species: implications for the control of an invasive weed.  
481 *Annals of Botany* **103**: 107–115.

482 **Rümer S, Cameron DD, Wacker R, Hartung W, Jiang F. 2007.** An anatomical study of  
483 the haustoria of *Rhinanthus minor* attached to roots of different hosts. *Flora-*  
484 *Morphology, Distribution, Functional Ecology of Plants* **202**: 194–200.

485 **Salonen V, Vestberg M, Vauhkonen M. 2001.** The effect of host mycorrhizal status on host  
486 plant–parasitic plant interactions. *Mycorrhiza* **11**: 95–100.

487 **Shen H, Hong L, Ye W, Cao H, Wang Z. 2007.** The influence of the holoparasitic plant  
488 *Cuscuta campestris* on the growth and photosynthesis of its host *Mikania micrantha*.  
489 *Journal of Experimental Botany* **58**: 2929–2937.

490 **Shen H, Prider JN, Facelli JM, Watling JR. 2010.** The influence of the hemiparasitic  
491 angiosperm *Cassytha pubescens* on photosynthesis of its host *Cytisus scoparius*.  
492 *Functional Plant Biology* **37**: 14–21.

493 **Shen H, Xu S-J, Hong L, Wang Z-M, Ye W-H. 2013.** Growth but not photosynthesis  
494 response of a host plant to infection by a holoparasitic plant depends on nitrogen  
495 supply. *PloS One* **8**: e75555.

496 **Sinebo W, Drennan DSH. 2001.** Vegetative growth of sorghum and *Striga hermonthica* in  
497 response to nitrogen and the degree of host root infection. *European Journal of Plant*  
498 *Pathology* **107**: 849–860.

499 **Stein C, Rißmann C, Hempel S, Renker C, Buscot F, Prati D, Auge H. 2009.** Interactive  
500 effects of mycorrhizae and a root hemiparasite on plant community productivity and  
501 diversity. *Oecologia* **159**: 191–205.

502 **Stewart GR, Press MC. 1990.** The physiology and biochemistry of parasitic angiosperms.  
503 *Annual Review of Plant Physiology and Plant Molecular Biology* **41**: 127–151.

504 **Strong G, Bannister P, Burritt D. 2000.** Are mistletoes shade plants? CO<sub>2</sub> assimilation and  
505 chlorophyll fluorescence of temperate mistletoes and their hosts. *Annals of Botany* **85**:  
506 511–519.

507 **Tarayre M, Bowman G, Schermann-Legionnet A, Barat M, Atlan A. 2007.** Flowering  
508 phenology of *Ulex europaeus*: ecological consequences of variation within and among  
509 populations. *Evolutionary Ecology* **21**: 395–409.

510 **Tennakoon KU, Pate JS, Fineran BA. 1997.** Growth and partitioning of C and fixed N in  
511 the shrub legume *Acacia littorea* in the presence or absence of the root hemiparasite  
512 *Olax phyllanthi*. *Journal of Experimental Botany* **48**: 1047–1060.

513 **Těšitel J, Plavcová L, Cameron DD. 2010.** Heterotrophic carbon gain by the root  
514 hemiparasites, *Rhinanthus minor* and *Euphrasia rostkoviana* (Orobanchaceae). *Planta*  
515 **231**: 1137–1144.

516 **Těšitel J, Těšitelová T, Fisher JP, Lepš J, Cameron DD. 2015.** Integrating ecology and  
517 physiology of root-hemiparasitic interaction: interactive effects of abiotic resources  
518 shape the interplay between parasitism and autotrophy. *New Phytologist* **205**: 350–  
519 360.

520 **Thrall PH, Millsom DA, Jeavons AC, Waayers M, Harvey GR, Bagnall DJ, Brockwell**  
521 **J. 2005.** Seed inoculation with effective root-nodule bacteria enhances revegetation  
522 success. *Journal of Applied Ecology* **42**: 740–751.

- 523 **Tsang HTS. 2010.** *Cassutha pubescens: germination biology and interactions with native*  
524 *and introduced hosts*. Masters Thesis, The University of Adelaide, Adelaide, SA,  
525 Australia.
- 526 **Weir BS, Turner SJ, Silvester WB, Park D-C, Young JM. 2004.** Unexpectedly diverse  
527 *Mesorhizobium* strains and *Rhizobium leguminosarum* nodulate native legume genera  
528 of New Zealand, while introduced legume weeds are nodulated by *Bradyrhizobium*  
529 species. *Applied and Environmental Microbiology* **70**: 5980–5987.
- 530 **White AJ, Critchley C. 1999.** Rapid light curves: a new fluorescence method to assess the  
531 state of the photosynthetic apparatus. *Photosynthesis Research* **59**: 63–72.
- 532 **Yu H, Liu J, He W-M, Miao S-L, Dong M. 2011.** *Cuscuta australis* restrains three exotic  
533 invasive plants and benefits native species. *Biological Invasions* **13**: 747–756.

534

### 535 **Supporting Information**

536 Additional supporting information may be found in the online version of this article.

537 **Fig. S1** Photos of uninfected, infected hosts and the parasite from the experiment.

538 **Fig. S2** Rapid light response curves of hosts and parasite.

539 **Fig. S3** Host gas exchange.

540 **Table S1** Analysis of the commercial soil.

541 **Table S2** Three-way ANOVA results for host growth measures, nodulation, nitrogen and  
542 maximum electron transport rates.

543 **Table S3** Two-way ANOVA results for parasite biomass, nitrogen and photosynthesis.

544 **Table S4** Three-way ANOVA results for host gas exchange.

545 **Table 1** *P*-values from three-way ANOVA for the effects of host species (Sp), infection with  
546 *Cassitha pubescens* (I) and nitrogen supply (N) on total, shoot and root biomass, foliar area  
547 (FA), shoot:root ratio (S:R), nodule biomass (Nod), nodule biomass g<sup>-1</sup> root biomass (Nod g<sup>-1</sup>  
548 root), foliar nitrogen concentration [N] and maximum electron transport rates (ETR<sub>max</sub>) of  
549 *Ulex europaeus* and *Acacia paradoxa*

	<b>Total</b>	<b>Shoot</b>	<b>Root</b>	<b>FA</b>	<b>S:R</b>	<b>Nod</b>	<b>Nod g<sup>-1</sup> root</b>	<b>[N]</b>	<b>ETR<sub>max</sub></b>
Sp	<b>0.016</b>	<b>0.0008</b>	<b>0.0005</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0005</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.944
I	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.003</b>	0.111	<b>0.001</b>	0.439	0.636	<b>0.0005</b>
Sp x I	<b>0.016</b>	<b>0.033</b>	<b>0.004</b>	0.176	0.230	0.590	0.769	0.227	<b>0.003</b>
N	0.420	0.340	0.863	0.528	0.668	0.175	<b>0.040</b>	0.890	0.954
Sp x N	0.310	0.408	0.125	0.522	0.770	0.236	0.409	0.382	0.219
I x N	0.693	0.660	0.959	0.895	0.245	0.773	0.691	<b>0.017</b>	0.546
Sp x I x N	0.226	0.356	<b>0.035</b>	0.508	0.261	0.291	0.084	0.540	0.080
Block	<b>0.034</b>	<b>0.032</b>	0.275	0.156	0.207	0.612	0.986	0.281	0.744

550 Significant effects are in bold; *F* and sum of square values are presented in Supporting  
551 Information Table S2.

552

553

554

555

556

557

558

559

560

561



562 **Table 2** Foliar area (FA: cm<sup>2</sup>), shoot:root ratio (S:R), nodule biomass (Nod: g dwt) and  
 563 nodule biomass g<sup>-1</sup> root biomass (Nod g<sup>-1</sup> root) of *Ulex europaeus* and *Acacia paradoxa*  
 564 either uninfected (minus) or infected (plus) with *Cassityha pubescens* and supplied (HN) or  
 565 not supplied (LN) with nitrogen

<b>Treatment</b>	<b>FA</b>	<b>S:R</b>	<b>Nod</b>	<b>Nod g<sup>-1</sup> root</b>
- HN <i>U. europaeus</i>	1175 ± 66	3.00 ± 0.07	2.68 ± 0.78	0.054 ± 0.013
- LN <i>U. europaeus</i>	1196 ± 90	2.96 ± 0.25	4.43 ± 0.40	0.094 ± 0.007
+ HN <i>U. europaeus</i>	462 ± 91	2.13 ± 0.16	1.08 ± 0.20	0.054 ± 0.011
+ LN <i>U. europaeus</i>	618 ± 96	1.92 ± 0.11	1.94 ± 0.38	0.069 ± 0.016
- HN <i>A. paradoxa</i>	3529 ± 639	5.19 ± 0.72	5.39 ± 0.93	0.177 ± 0.025
- LN <i>A. paradoxa</i>	3391 ± 739	4.45 ± 0.32	4.83 ± 0.49	0.150 ± 0.014
+ HN <i>A. paradoxa</i>	2521 ± 425	4.77 ± 0.56	3.51 ± 0.62	0.123 ± 0.014
+ LN <i>A. paradoxa</i>	1892 ± 513	5.02 ± 0.77	4.01 ± 0.96	0.211 ± 0.054
<b>Infection effect</b>				
uninfected	2323 ± 345a	3.90 ± 0.29	4.33 ± 0.39a	0.119 ± 0.013
infected	1346 ± 252b	3.38 ± 0.39	2.56 ± 0.38b	0.109 ± 0.018
<b>Species effect</b>				
<i>U. europaeus</i>	863 ± 85a	2.50 ± 0.13a	2.53 ± 0.36a	0.068 ± 0.007a
<i>A. paradoxa</i>	2883 ± 314b	4.85 ± 0.28b	4.46 ± 0.39b	0.163 ± 0.015b

566 No species x infection x nitrogen interaction for all parameters  $n=4-5$ ; significant  
 567 independent infection effect for FA and Nod; significant independent species effect for all  
 568 parameters  $n=19-20$ . Different letters denote significant differences (vertically) and data are  
 569 means ± 1SE.

570

571

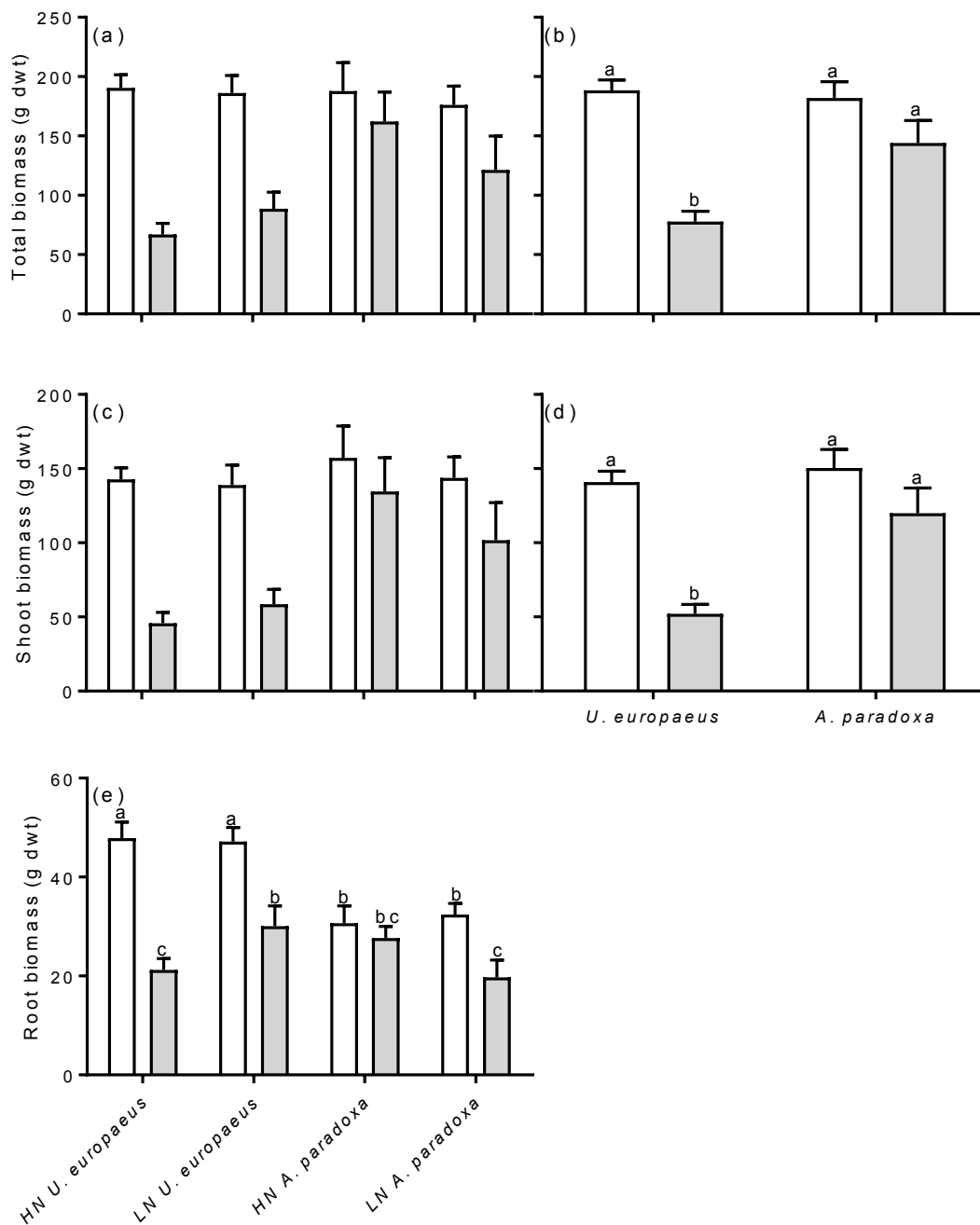
572

573 **Table 3** *P*-values from two-way ANOVA for effects of host species (Sp) and nitrogen  
 574 treatments (N) on parasite biomass, parasite biomass g<sup>-1</sup> host biomass, stem nitrogen  
 575 concentration [N] and maximum electron transport rates (ETR<sub>max</sub>) of *Cassitha pubescens*  
 576 infecting either *Ulex europaeus* or *Acacia paradoxa*

	<b>Parasite biomass</b>	<b>Parasite biomass g<sup>-1</sup> host</b>	<b>[N]</b>	<b>ETR<sub>max</sub></b>
Sp	<b>&lt;0.0001</b>	<b>0.0008</b>	0.395	0.069
N	0.628	0.599	0.566	0.844
Sp x N	0.733	0.746	0.860	0.078
Block	0.646	0.553	0.457	0.121

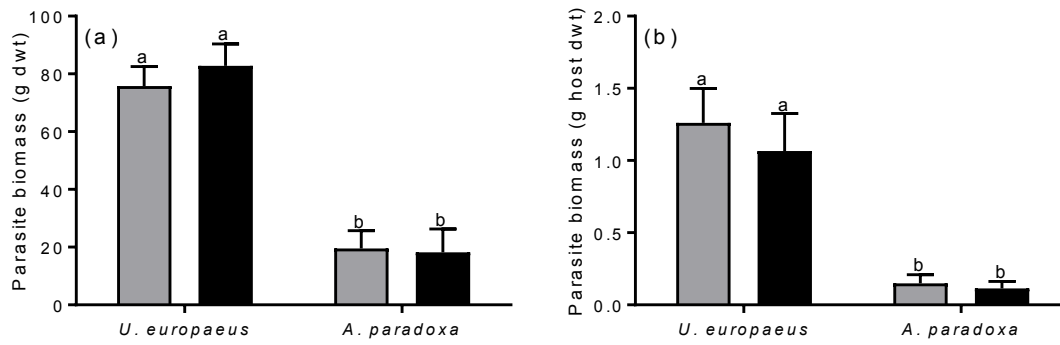
577 Significant effects are in bold; *F* and sum of square values are presented in Supporting  
 578 Information Table S3.

579  
 580  
 581  
 582  
 583  
 584  
 585  
 586  
 587  
 588  
 589  
 590



591

592 **Fig. 1** (a) Total, (c) shoot and (e) root biomass of *Ulex europaeus* or *Acacia paradoxa* either  
 593 uninfected (open bars) or infected (grey bars) with *Cassutha pubescens* and supplied (HN) or  
 594 not supplied (LN) with nitrogen. Species x infection effect on (b) total and (d) shoot biomass.  
 595 Different letters denote significant differences, data are means  $\pm$  1SE,  $n=4-5$  (a, c, e) and  
 596  $n=19-20$  (b, d).



597

598 **Fig. 2** (a) Parasite biomass and (b) parasite biomass per g host biomass of *Cassyth*  
 599 *pubescens* when infecting *Ulex europaeus* or *Acacia paradoxa* supplied (dark grey bars) or  
 600 not supplied (black bars) with nitrogen. Different letters denote significant differences  
 601 between species, data are means  $\pm$  1SE,  $n=5$  (a) and (b) (except *A. paradoxa* in no additional  
 602 N treatment,  $n=3$ ).

603

604

605

606

607

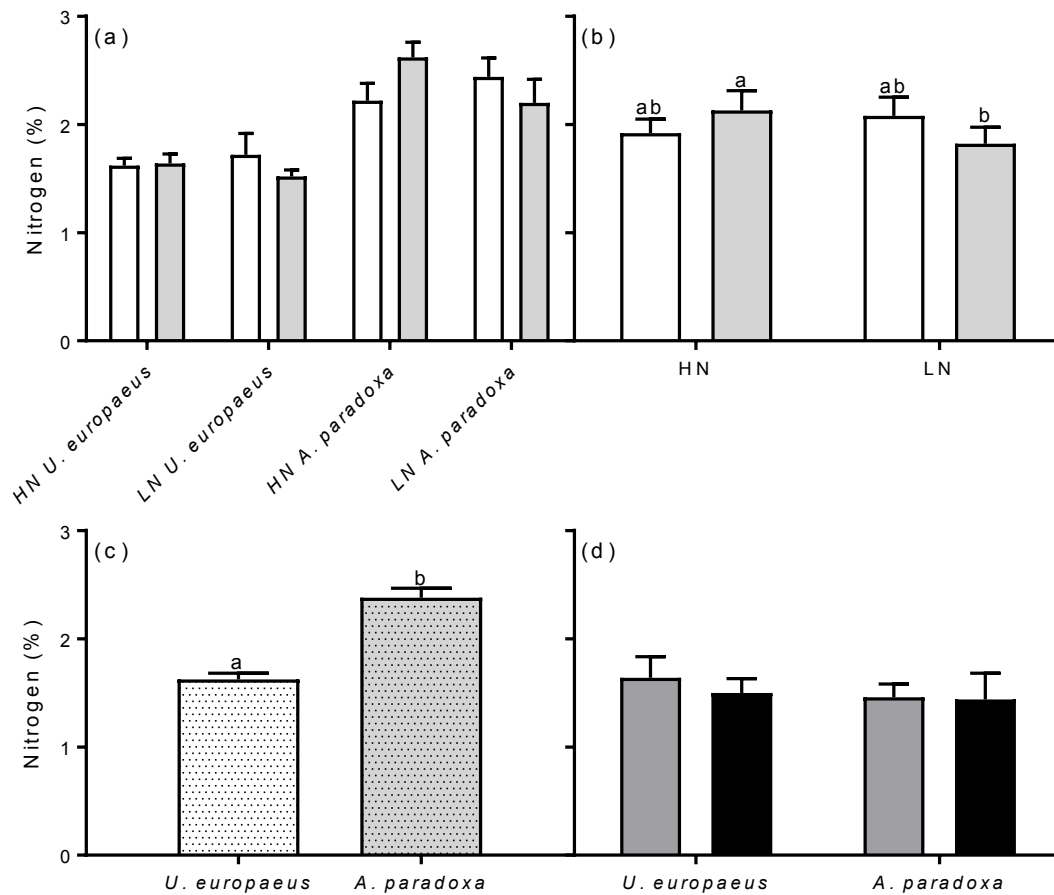
608

609

610

611

612

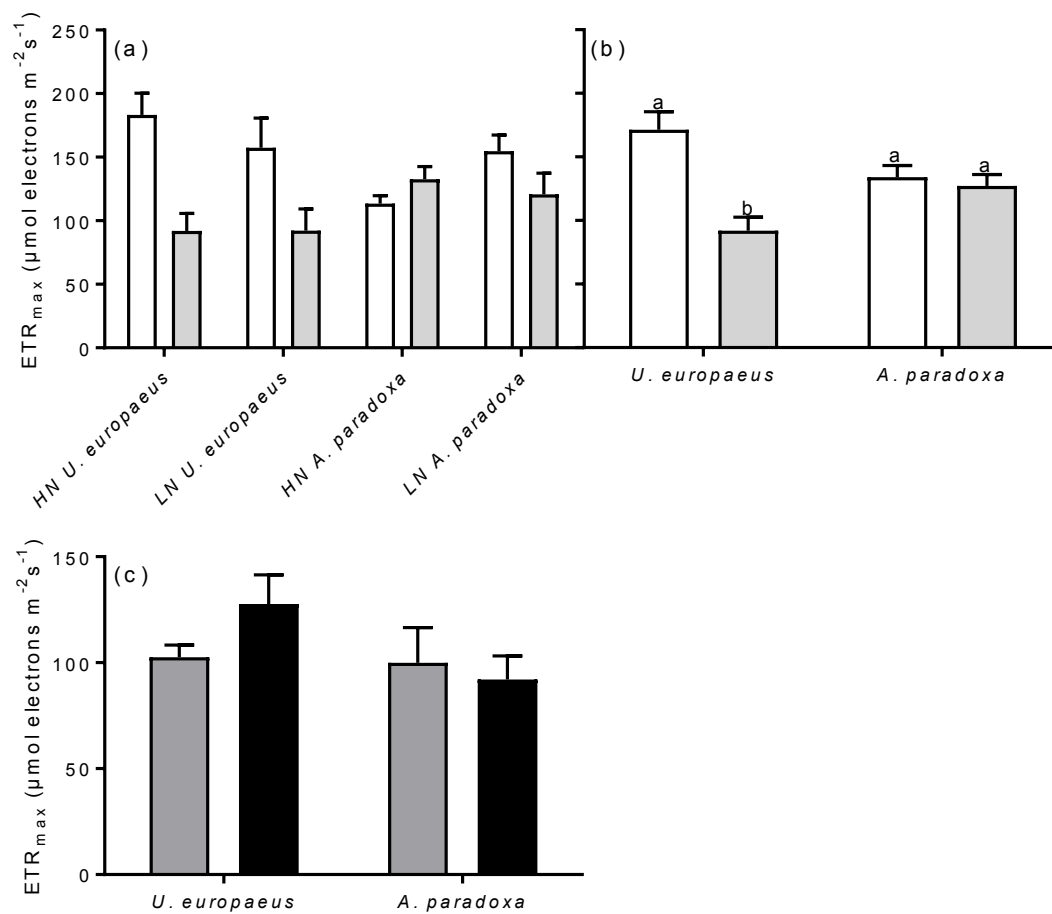


613

614 **Fig. 3** (a) Foliar nitrogen concentration of *Ulex europaeus* or *Acacia paradoxa* either  
 615 uninfected (open bars) or infected (grey bars) with *Cassythia pubescens* and supplied (HN) or  
 616 not supplied (LN) with nitrogen. (b) Nitrogen x infection effect for host foliar nitrogen  
 617 concentration. (c) Species effect for foliar nitrogen concentration of *U. europaeus* (dotted  
 618 open bar) and *A. paradoxa* (dotted grey bar). (d) Stem nitrogen concentration of *C. pubescens*  
 619 when infecting either host species supplied (dark grey bars) or not supplied (black bars) with  
 620 nitrogen. Different letters denote significant differences, data are means  $\pm$  1SE,  $n=4-5$  (a, d),  
 621  $n=9-10$  (b) and  $n=19-20$  (c).

622

623



624

625 **Fig. 4** (a) Maximum electron transport rates (ETR<sub>max</sub>) of *Ulex europaeus* and *Acacia*  
 626 *paradoxa* either uninfected (open bars) or infected (grey bars) with *Cassyltha pubescens*, and  
 627 supplied (HN) or not supplied with nitrogen (LN). (b) Species x infection interaction for host  
 628 ETR<sub>max</sub>. (c) ETR<sub>max</sub> of *C. pubescens* when infecting either host species supplied (dark grey  
 629 bars) or not supplied (black bars) with nitrogen. Different letters denote significant  
 630 differences, data are means  $\pm$  1SE,  $n=5-6$  (a),  $n=11-12$  (b) and  $n=4-6$  (c).

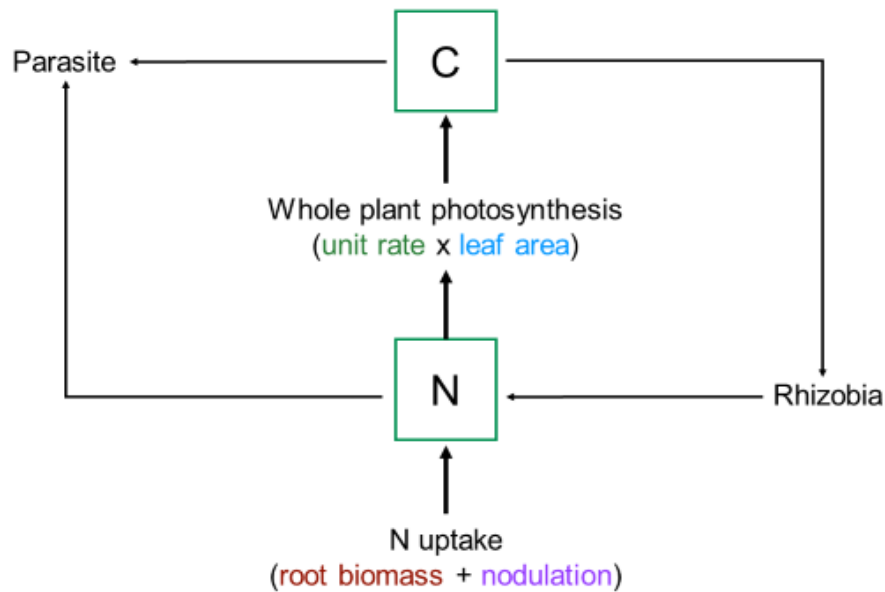
631

632

633

634

635



636

637 **Fig. 5** Simple model of carbon (C) and nitrogen (N) dynamics for a host+parasite+rhizobia  
 638 system. Host C and N pools are represented by the green boxes. C acquisition by the host is  
 639 determined by the unit rate of photosynthesis and the whole plant leaf area. Host N uptake is  
 640 determined by root biomass and the degree of nodulation. The parasite is a sink for both C  
 641 and N, while rhizobia are sinks for C, but contribute to host N uptake (as shown by the  
 642 arrows). The parameters, unit rate, leaf area, root biomass and nodulation are shown in  
 643 different colours to indicate that each can influence the host pools of either C (unit rate and  
 644 leaf area) or N (root biomass and nodulation) independently.