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

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

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Summary

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

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

Introduction

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

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

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

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

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_2
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:

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

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

Uninfected and infected plants of both species were randomly allocated into two N treatments. Plants in the high N treatment (HN) were provided with standard Hoagland’s solution. Plants in the treatment without additional N (LN) were provided standard Hoagland’s solution with KCl and $CaCl_2$ substituted for KNO_3 and $Ca(NO_3)_2.4H_2O$, respectively. All plants were randomly allocated into six blocks, each block containing all

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

Photosynthesis measurements

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

$$ETR = Yield \times PAR \times 0.5 \times 0.84$$

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

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

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

Biomass and N concentration

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

Statistical analyses

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

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

Results

Growth, nodulation and N concentration

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

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

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

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

Photosynthetic performance

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

46% while having no effect on that of *A. paradoxa*, regardless of N treatment (Fig. 4b). There was no interactive effect of N x species or any independent effects of these factors on ETR_{max} of *C. pubescens* (Table 3, Fig. 4c).

Discussion

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

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

In contrast to *A. paradoxa*, although *C. pubescens* negatively impacted root growth of *U. 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 mainatin 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 *Olax phyllanthi*. This difference may be due to
309 infection having a significant effect on ETR_{max} 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 ^{32}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;

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

Nitrogen had no influence on the effect of *C. pubescens* on photosynthetic performance of hosts, as was similarly found for the *Cuscuta campestris-M. micrantha* association (Shen *et al.*, 2013). The negative effect of *C. pubescens* on ETR_{max} of *U. europaeus* does not seem related to nitrogen stress as infected plants did not have a significantly lower foliar N concentration than uninfected plants. Although not significant, decreases in g_s of *U. europaeus* as a result of infection (Supporting Information Fig. S3c) may explain the impact of *C. pubescens* on photosynthetic performance of this host. Negative effects of *C. pubescens* on photosynthesis of the introduced *Cytisus scoparius* and native *Leptospermum myrsinoides* have been ascribed to decreases in stomatal conductance resulting from infection (Prider *et al.*, 2009; Shen *et al.*, 2010). Importantly, our study revealed that *A. paradoxa* is the first native host studied whose photosynthesis was not affected by the native hemiparasite *C. pubescens*. In sum, the differential impact of *Cassytha pubescens* on photosynthetic 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.

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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 (^{13}C) and nitrogen (^{15}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_3
384 cereal, *Oryza sativa*, infected with the root hemiparasite *Striga hermonthica*. *Journal*
385 *of Experimental Botany* **45**: 925–930.
386 **Cirocco 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 **Cirocco 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Lambers H, Chapin III FS, Pons TL. 2008. *Plant physiological ecology, 2nd edn.* New York, USA: Springer.

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

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

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. *100 of the world's worst invasive alien species: a selection from the global invasive species database*. Auckland, New Zealand: Invasive Species Specialist Group.

Lu JK, Kang LH, Sprent JI, Xu DP, He XH. 2013. Two-way transfer of nitrogen between *Dalbergia odorifera* and its hemiparasite *Santalum album* is enhanced when the host is effectively nodulated and fixing nitrogen. *Tree Physiology* **33**: 464–474.

Lu JK, Xu DP, Kang LH, He XH. 2014. Host-species-dependent physiological characteristics of hemiparasite *Santalum album* in association with N_2-fixing and non-N_2-fixing hosts native to southern China. *Tree Physiology* **34**: 1006–1017.

Matthies D. 1996. Interactions between the root hemiparasite *Melampyrum arvense* and mixtures of host plants: heterotrophic benefit and parasite-mediated competition. *Oikos* **75**: 118–124.

McLuckie J. 1924. Studies in Parasitism. I. A contribution to the physiology of the genus *Cassytha*, Part 1. *Proceedings of the Linnaen Society of New South Wales* **49**: 333–369.

Meinzer FC, Woodruff DR, Shaw DC. 2004. Integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection. *Plant, Cell & Environment* **27**: 937–946.

Pate JS, True KC, Rasins E. 1991. Xylem transport and storage of amino acids by S.W. Australian mistletoes and their hosts. *Journal of Experimental Botany* **42**: 441–451.

Pate JS. 2001. Haustoria in action: case studies of nitrogen acquisition by woody xylem-tapping hemiparasites from their hosts. *Protoplasma* **215**: 204–217.

Pennings SC, Callaway RM. 2002. Parasitic plants: parallels and contrasts with herbivores. *Oecologia* **131**: 479–489.

Press MC, Graves JD. 1995. *Parasitic plants*. London, UK: Chapman & Hall.

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

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

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

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

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

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

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

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

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

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

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

Strong G, Bannister P, Burritt D. 2000. Are mistletoes shade plants? CO_2 assimilation and chlorophyll fluorescence of temperate mistletoes and their hosts. *Annals of Botany* **85**: 511–519.

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

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

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

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

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

Tsang HTS. 2010. *Cassytha pubescens*: *germination biology and interactions with native and introduced hosts*. Masters Thesis, The University of Adelaide, Adelaide, SA, Australia.

Weir BS, Turner SJ, Silvester WB, Park D-C, Young JM. 2004. Unexpectedly diverse *Mesorhizobium* strains and *Rhizobium leguminosarum* nodulate native legume genera of New Zealand, while introduced legume weeds are nodulated by *Bradyrhizobium* species. *Applied and Environmental Microbiology* **70**: 5980–5987.

White AJ, Critchley C. 1999. Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. *Photosynthesis Research* **59**: 63–72.

Yu H, Liu J, He W-M, Miao S-L, Dong M. 2011. *Cuscuta australis* restrains three exotic invasive plants and benefits native species. *Biological Invasions* **13**: 747–756.

Supporting Information

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

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

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

Fig. S3 Host gas exchange.

Table S1 Analysis of the commercial soil.

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

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

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

Table 1 *P*-values from three-way ANOVA for the effects of host species (Sp), infection with *Cassytha pubescens* (I) and nitrogen supply (N) on total, shoot and root biomass, foliar area (FA), shoot:root ratio (S:R), nodule biomass (Nod), nodule biomass g^{-1} root biomass (Nod g^{-1} root), foliar nitrogen concentration [N] and maximum electron transport rates (ETR_{max}) of *Ulex europaeus* and *Acacia paradoxa*

	Total	**Shoot**	**Root**	**FA**	**S:R**	**Nod**	**Nod g^{-1} root**	**[N]**	**ETR_{max}**
Sp	**0.016**	**0.0008**	**0.0005**	**<0.0001**	**<0.0001**	**0.0005**	**<0.0001**	**<0.0001**	0.944
I	**<0.0001**	**<0.0001**	**<0.0001**	**0.003**	0.111	**0.001**	0.439	0.636	**0.0005**
Sp x I	**0.016**	**0.033**	**0.004**	0.176	0.230	0.590	0.769	0.227	**0.003**
N	0.420	0.340	0.863	0.528	0.668	0.175	**0.040**	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	**0.017**	0.546
Sp x I x N	0.226	0.356	**0.035**	0.508	0.261	0.291	0.084	0.540	0.080
Block	**0.034**	**0.032**	0.275	0.156	0.207	0.612	0.986	0.281	0.744

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

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

Treatment	FA	S:R	Nod	Nod g^{-1} root
- HN *U. europaeus*	1175 ± 66	3.00 ± 0.07	2.68 ± 0.78	0.054 ± 0.013
- LN *U. europaeus*	1196 ± 90	2.96 ± 0.25	4.43 ± 0.40	0.094 ± 0.007
+ HN *U. europaeus*	462 ± 91	2.13 ± 0.16	1.08 ± 0.20	0.054 ± 0.011
+ LN *U. europaeus*	618 ± 96	1.92 ± 0.11	1.94 ± 0.38	0.069 ± 0.016
- HN *A. paradoxa*	3529 ± 639	5.19 ± 0.72	5.39 ± 0.93	0.177 ± 0.025
- LN *A. paradoxa*	3391 ± 739	4.45 ± 0.32	4.83 ± 0.49	0.150 ± 0.014
+ HN *A. paradoxa*	2521 ± 425	4.77 ± 0.56	3.51 ± 0.62	0.123 ± 0.014
+ LN *A. paradoxa*	1892 ± 513	5.02 ± 0.77	4.01 ± 0.96	0.211 ± 0.054
Infection effect				
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
Species effect				
U. europaeus	863 ± 85a	2.50 ± 0.13a	2.53 ± 0.36a	0.068 ± 0.007a
A. paradoxa	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.

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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^{-1} host biomass, stem nitrogen
575 concentration [N] and maximum electron transport rates (ETR_{max}) of *Cassytha pubescens*
576 infecting either *Ulex europaeus* or *Acacia paradoxa*

	Parasite biomass	**Parasite biomass g^{-1} host**	**[N]**	**ETR_{max}**
Sp	**<0.0001**	**0.0008**	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.

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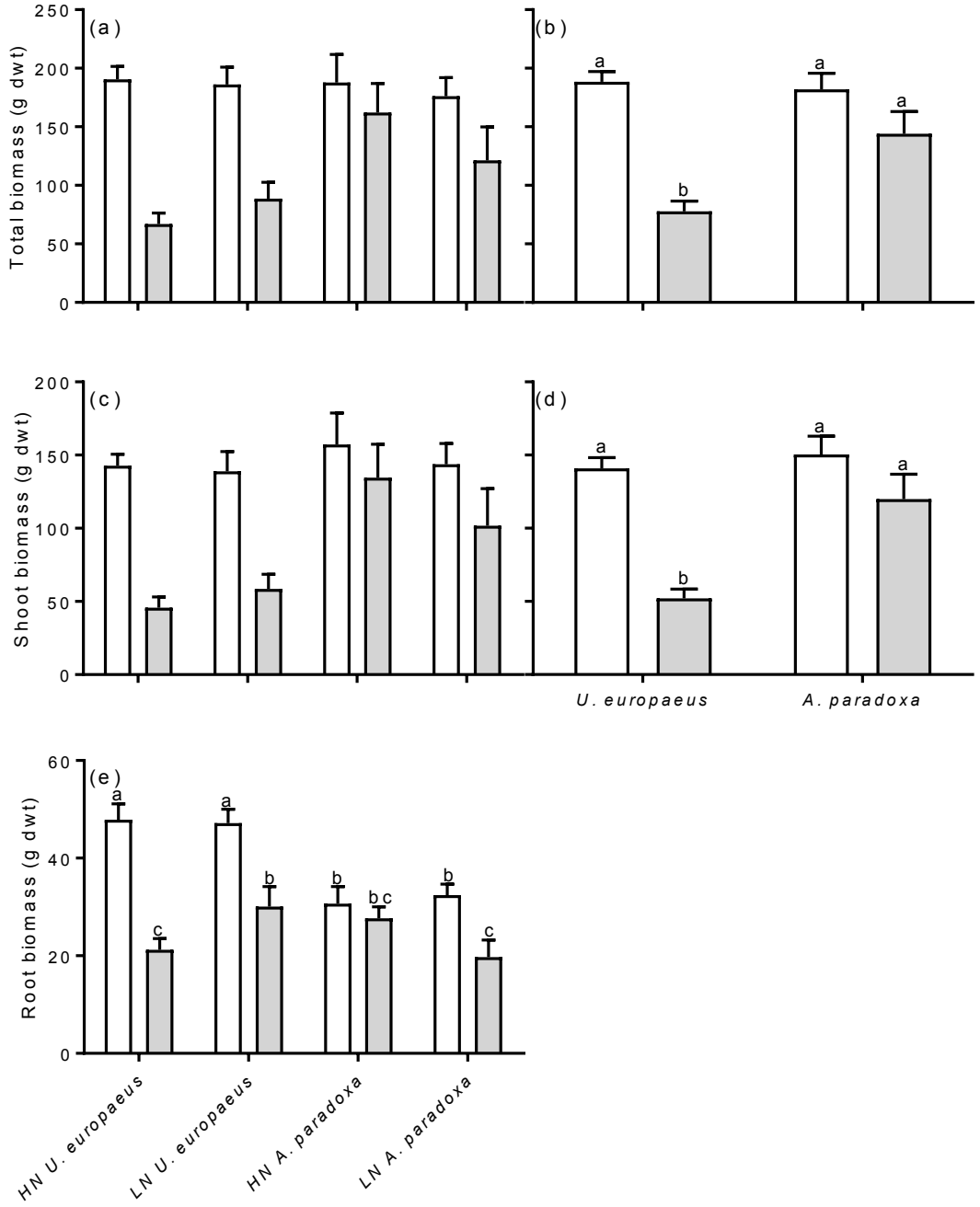

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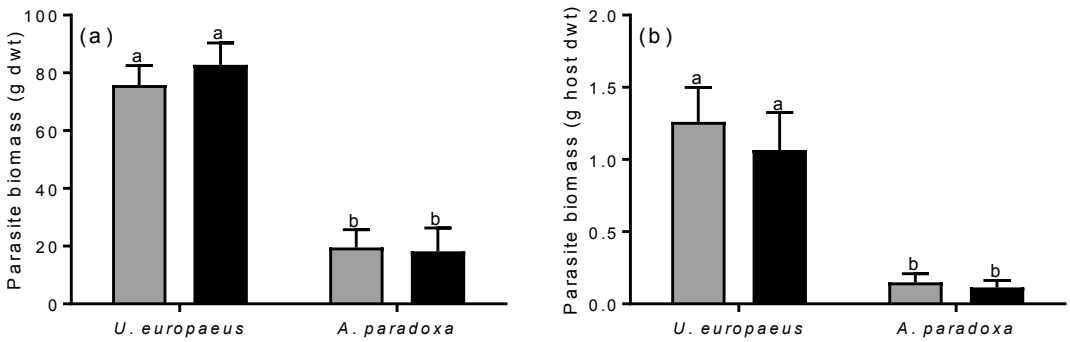
592 **Fig. 1** (a) Total, (c) shoot and (e) root biomass of *Ulex europaeus* or *Acacia paradoxa* either
593 uninfected (open bars) or infected (grey bars) with *Cassytha pubescens* and supplied (HN) or
594 not supplied (LN) with nitrogen. Species x infection effect on (b) total and (d) shoot biomass.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



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598 **Fig. 2** (a) Parasite biomass and (b) parasite biomass per g host biomass of *Cassytha*
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 ± 1SE, *n*=5 (a) and (b) (except *A. paradoxa* in no additional
602 N treatment, *n*=3).

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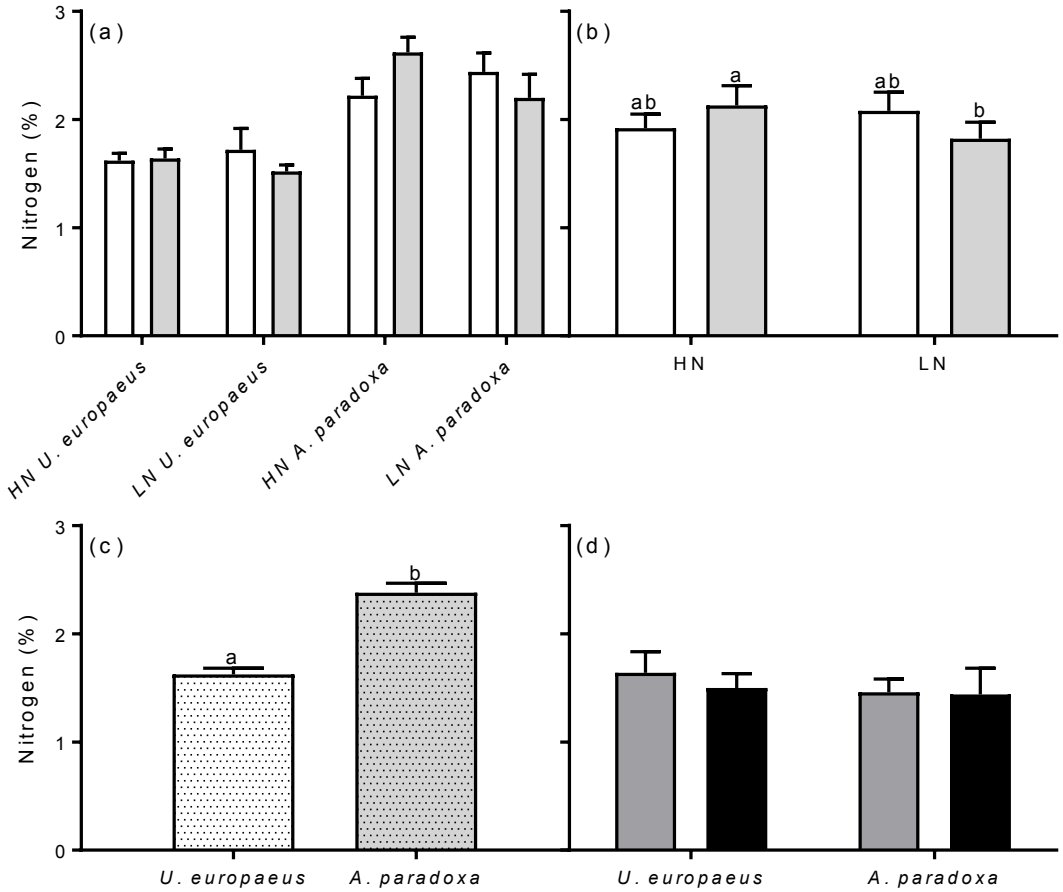


Fig. 3 (a) Foliar nitrogen concentration of *Ulex europaeus* or *Acacia paradoxa* either uninfected (open bars) or infected (grey bars) with *Cassytha pubescens* and supplied (HN) or not supplied (LN) with nitrogen. (b) Nitrogen x infection effect for host foliar nitrogen concentration. (c) Species effect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

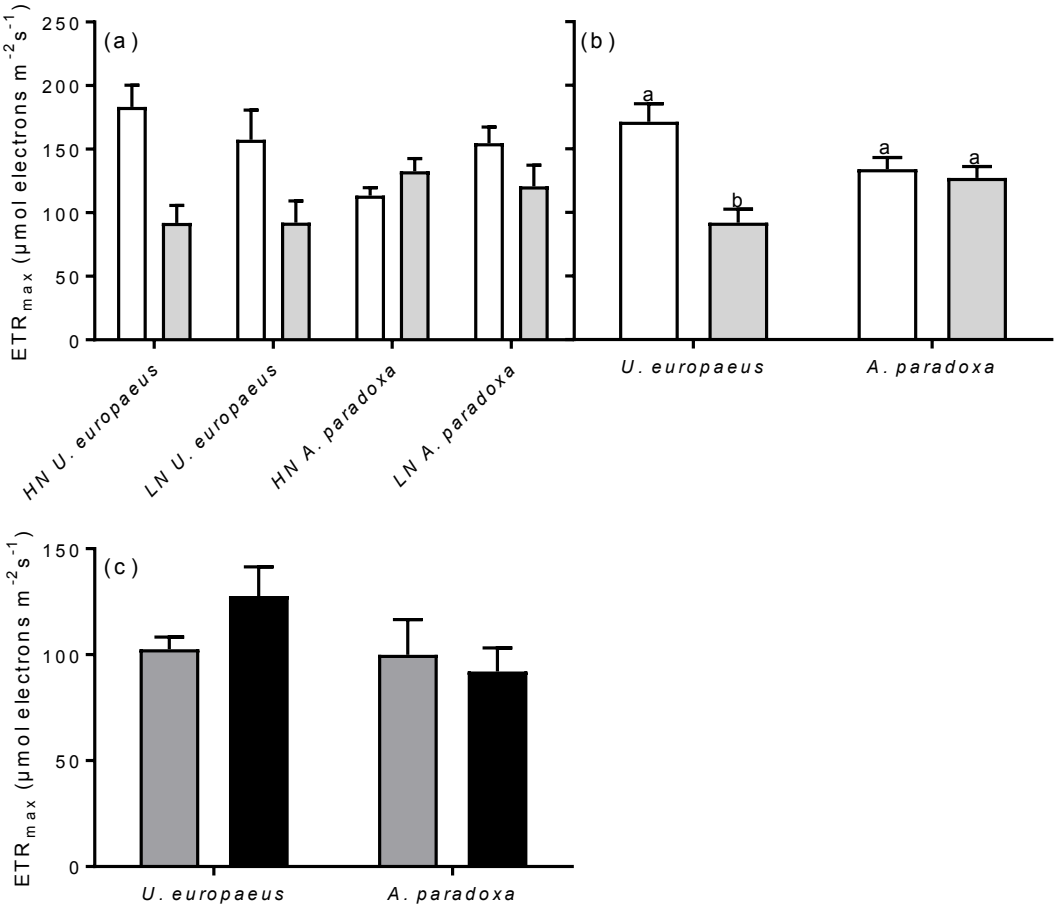
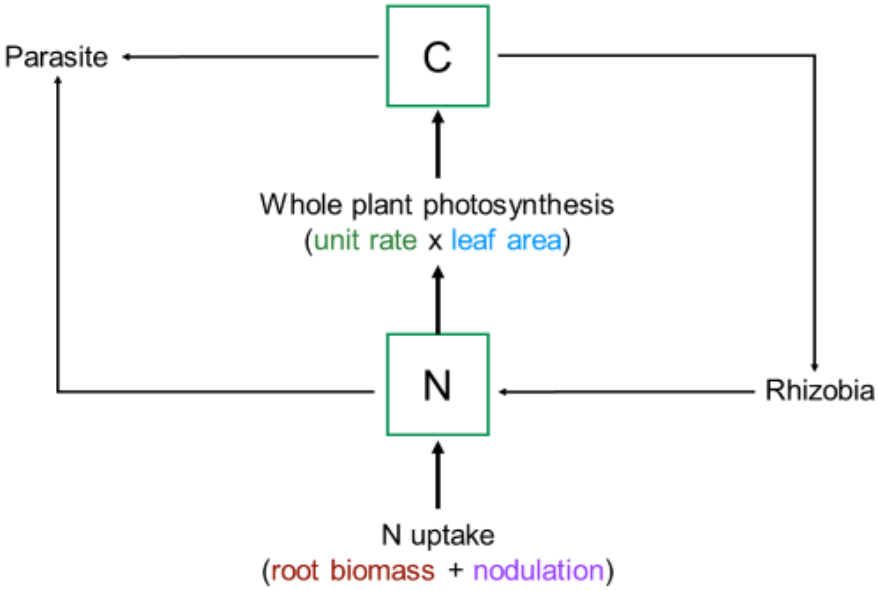


Fig. 4 (a) Maximum electron transport rates (ETR_{max}) of *Ulex europaeus* and *Acacia paradoxa* either uninfected (open bars) or infected (grey bars) with *Cassytha pubescens,* and supplied (HN) or not supplied with nitrogen (LN). (b) Species x infection interaction for host ETR_{max}. (c) ETR_{max} of *C. pubescens* when infecting either host species supplied (dark grey bars) or not supplied (black bars) with nitrogen. Different letters denote significant differences, data are means ± 1SE, n=5–6 (a), n=11–12 (b) and n=4–6 (c).



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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.