



LETTERS

Mutualism disruption by an invasive ant reduces carbon fixation for a foundational East African ant-plant

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Abstract

Invasive ants shape assemblages and interactions of native species, but their effect on fundamental ecological processes is poorly understood. In East Africa, *Pheidole megacephala* ants have invaded monodominant stands of the ant-tree *Acacia drepanolobium*, extirpating native ant defenders and rendering trees vulnerable to canopy damage by vertebrate herbivores. We used experiments and observations to quantify direct and interactive effects of invasive ants and large herbivores on *A. drepanolobium* photosynthesis over a 2-year period. Trees that had been invaded for ≥ 5 years exhibited 69% lower whole-tree photosynthesis during key growing seasons, resulting from interaction between invasive ants and vertebrate herbivores that caused leaf- and canopy-level photosynthesis declines. We also surveyed trees shortly before and after invasion, finding that recent invasion induced only minor changes in leaf physiology. Our results from individual trees likely scale up, highlighting the potential of invasive species to alter ecosystem-level carbon fixation and other biogeochemical cycles.

Keywords

biological invasion, mutualism, ant-plant, photosynthesis, invasive ant.

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INTRODUCTION

Invasive insects can profoundly affect ecological communities, threatening biodiversity (Pimentel *et al.*, 2001; Englund 2008; Hill *et al.*, 2013), disrupting important ecological processes (forest canopy structure, biogeochemical cycles, Gandhi & Herms 2010; suppressing native foundational species, McGeoch *et al.*, 2015) and imposing large economic costs (Bradshaw *et al.*, 2016; Paini *et al.*, 2016). Invasive ants comprise > 240 species, and can affect the behaviour, functional role and abundance of their native counterparts (Holway *et al.*, 2002; Bertelsmeier *et al.*, 2017). In so doing, ants can restructure pollination networks (Vanbergen *et al.*, 2018), interrupt seed dispersal (e.g. Horvitz & Schemske 1986; Rodriguez-Cabal *et al.*, 2012) and pollination (e.g. Fuster *et al.*, 2020) and spread diseases in pollinator communities (Vanbergen *et al.*, 2018).

Although their community-level impacts are well documented and diverse, the consequences of ant invasions for biogeochemical cycles are poorly understood. In particular, effects of invasive ants might be expected to reverberate throughout ecosystems via shifts in carbon dynamics for several reasons. First, native ants, which are often displaced by invasive ants (Ness & Bronstein 2004; Milligan *et al.*, 2016), can increase the spatial variability of soil carbon (e.g. wood ants in subalpine forests, Risch *et al.*, 2005; Finér *et al.*, 2013) as a by-product of foraging and ground-nesting. Second,

invasive ants can feed on extrafloral nectar of host plants (Ness & Bronstein 2004; Lach *et al.*, 2009) and collect honeydew from heterospecific insect partners (Beardsley *et al.*, 1982; Zhou *et al.*, 2017; Demian, 2019; Anastasio 2020): both activities remove carbon from their host plant's active carbon pool that would otherwise support above-ground growth and development (Pringle 2016). Ant interactions with nectaries or with phloem-feeding insects can affect the carbon source–sink ratio of host plants (Albani *et al.*, 2010; Del-Claro *et al.*, 2016; Prior & Palmer 2018) which can affect leaf carbon exchange rates (Goldschmidt & Huber 1992; Nebauer *et al.*, 2011). Third, invasive ants can deter or facilitate herbivory on host plants with consequences for plant growth and overall canopy size (e.g. Savage *et al.*, 2009; Lach & Hoffmann 2011; Kulikowski II 2020), which may combine with changes in leaf carbon exchange rates to affect whole-plant carbon fixation. Finally, invasive ants could influence ecosystem carbon cycling by displacing the ant defenders of ant-plants, some of which are dominant primary producers in communities (e.g. devil's gardens, Frederickson *et al.*, 2005; *Acacia drepanolobium* savannas, Goheen & Palmer 2010), with potentially large effects on local carbon cycles.

We investigated how invasion by *Pheidole megacephala* Fabricius (the 'big-headed ant') affects carbon cycling in a widespread and monodominant foundation species, the whistling thorn tree (*Acacia drepanolobium*). *Pheidole megacephala* has invaded tropical and subtropical ecosystems around the

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world (Wetterer 2012), extirpating native ant mutualists (Ness & Bronstein 2004; Riginos *et al.*, 2015), forming facultative partnerships with phloem-feeding insects (e.g. Beardsley *et al.*, 1982; Gaigher *et al.*, 2013), and suppressing the abundance, distribution and diversity of many native insects (Ness & Bronstein 2004; Hoffmann & Parr 2008; Riginos *et al.*, 2015; Milligan *et al.*, 2016). In savannas underlain by clay-rich vertisols (i.e. 'black-cotton' savannas) in Laikipia, Kenya, *A. drepanolobium* comprises > 95% of woody cover (Young *et al.*, 1996) and forms obligate mutualisms with four native ant species (*Crematogaster mimosae* Santschi, *Crematogaster nigriceps* Emery, *Crematogaster sjostedti* Mayr and *Tetraponera penzigi* Mayr). Host plants produce extrafloral nectar and hollow spine domatia (e.g. Hocking 1970; Huntzinger *et al.*, 2004) which are consumed and occupied by single colonies consisting of thousands of defensive ants (Palmer 2004). The most common mutualist, *C. mimosae*, consumes nectar and honeydew (Prior & Palmer 2018) and reduces herbivory by large mammals (Stanton & Palmer 2011) including elephants (Goheen & Palmer 2010). In invaded habitats, *C. mimosae* mutualists are completely extirpated by *P. megacephala*, which does not deter herbivores (Riginos *et al.*, 2015).

Because native ant mutualists impose high continuous metabolic costs on their host plants but provide protection for their tree against destructive herbivory by elephants and other large herbivores, we expected that the replacement of defensive *C. mimosae* with non-defensive *P. megacephala* would cause distinct short- and longer-term consequences for *A. drepanolobium* trees. In the short-term, the removal of nectar- and honeydew-consuming *C. mimosae* by *P. megacephala* may free up carbohydrates, which would support leaf growth, photosynthetic upregulation, management of water (e.g. Inoue *et al.*, 2017; Zhang *et al.*, 2019) and other metabolic processes of the host tree (Wiley & Helliker 2012; Glanz-Idan & Wolf 2020). We also would not expect *P. megacephala* workers to act as a direct resource sink for the tree, because they fail to induce nectar production on host plants, resulting in a 55–98% decline in active nectaries on invaded trees (Riginos *et al.*, 2015; Palmer *et al.*, 2020): thus, host trees should experience energetic savings immediately after invasion. However, the loss of native ant mutualists increases the risk of rare but heavy damage by elephants and other large herbivores for *A. drepanolobium* trees (Goheen & Palmer 2010), such that over the longer-term, invaded communities experience more instances of mild to catastrophic herbivory (Riginos *et al.*, 2015). King & Caylor (2010) demonstrated that the prevention of herbivory by native ants influences photosynthetic rate of the host tree, but direct ant–plant interactions and the role of this invasive ant were not investigated in their study. These prior studies informed our predictions that not only (1) invasive *P. megacephala* affects leaf-level photosynthetic rates by removing an energetic sink for the host tree immediately after invasion, but also (2) *P. megacephala* reduces canopy-level photosynthesis over longer time scales by rendering trees vulnerable to canopy damage.

We conducted field experiments and observations over a 2-yr period to investigate how *P. megacephala* invasion affects carbon fixation in *A. drepanolobium*. Because the effects of invasion frequently lag behind the initial arrival of the invader

(Simberloff 2011), we evaluated both how *P. megacephala* influences host plant carbon fixation after a recent invasion (<1 year ago) and in 'longer-term' invasive sites (invaded *ca.* 5 years ago). We investigated these short- and longer-term impacts of invasion in wet and dry seasons during which host plant rates of photosynthesis can substantially differ (King & Caylor 2010). We addressed three research questions regarding *A. drepanolobium*: (1) Does leaf photosynthetic rate of *A. drepanolobium* change shortly after the extirpation of costly ant mutualists by *P. megacephala*? (2) Does leaf photosynthetic rate of *A. drepanolobium* further change in longer-term invasion sites, and how is that rate influenced by ant–plant and vertebrate herbivore–plant interactions? (3) How do vertebrate herbivores and invasive ants contribute to changes in canopy-level photosynthesis for longer-term invaded trees?

METHODS

Study site

Fieldwork was conducted from September 2016 to September 2018 at Ol Pejeta Conservancy ('OPC'; 0°0'52.62"N, 36°51'58.64"E, 1800 m above sea level). This 360-km² conservancy receives *ca.* 250–300 mm of rainfall in wet seasons (March to May; October to December, and intervening periods are typically dry and hot with monthly rainfall of *ca.* 30–50 mm (Ol Pejeta Conservancy Dept. of Wildlife Conservation, unpublished data). The OPC elephant population (*ca.* 130–300 depending on forage availability, OPC records) disproportionately imposes heavy damage on *A. drepanolobium* in areas where *P. megacephala* has invaded (Riginos *et al.*, 2015; Palmer *et al.*, 2020). Ground-dwelling *P. megacephala* ants have expanded from human habitation areas on OPC into black-cotton savannas for the past *ca.* two decades (Riginos *et al.*, 2015), where they occupy trees and soil. During this study, *P. megacephala* extended each monitored invasion front by *ca.* 50 m/yr (invading *ca.* 40–50 trees per 50 × 50 m area; Pietrek *et al.*, 2021).

Survey regime

We measured leaf gas exchange (photosynthesis and transpiration) in concurrent before–after–control–impact (BACI) and factorial experiments (Fig. S3) during rainy and dry seasons in 2017 and 2018. Both experimental designs are described below, followed by specific details of our plant physiology surveys. For each surveyed tree, we measured leaf water potential at mid-day and before dawn to (1) confirm assumptions that all sites had similar soil water status within each *ca.* 2-week survey period, (2) to confirm that our designations of 'wet' and 'dry' seasons were appropriate relative to studies of other East African acacias (Gebrehiwot *et al.*, 2005; Gebrekirstos *et al.*, 2006), and 3) to calculate leaf water potential range, which can be compared with leaf gas exchange rates to indicate changes in water management by the plant.

Before–after–control–impact experiment

To assess short-term impacts of *P. megacephala* invasion, we measured gas exchange rates and leaf water potential on the

same trees before and after invasion, and compared those to concurrent measurements on uninvaded trees that were protected by native *C. mimosae*. We surveyed trees in plots near the invasion front ('Transition' sites) before and after invasion, and also surveyed non-manipulated trees < 1 km from each Transition site ('Control' sites) that remained unaffected by *P. megacephala* range expansion over the course of the study. All sites were accessible to large herbivores. In the July 2017 dry season and November 2017 wet season, we surveyed 20–24 adult trees (1.5–2 meters tall) at each Transition (pre-invasion) and Control site. *Pheidole megacephala* workers expanded into Transition sites in December 2017, and we repeated surveys at each site in the May 2018 wet season and September 2018 dry season. Five trees were destroyed (evidently by elephants) between December 2017 and May 2018 and were excluded from analyses.

Factorial experiment comparing longer-term (>5 years) impact of invasion

In the factorial experiment, we tested direct and indirect effects of invasive *P. megacephala*, native *C. mimosae* and vertebrate herbivores on leaf and canopy gas exchange, and also compared gas exchange between longer-term invaded (*ca.* 5 years) and uninvaded tree stands. We measured leaf water potential and gas exchange rates in two dry (July 2017 and September 2018) and two wet seasons (November 2017 and May 2018). Treatment factors were large herbivores (present vs. excluded) and ants (present vs. excluded), resulting in four treatments (Fig. S3). We conducted our experiment in three sites where acacias had been invaded for *ca.* 5 years ('Invasion'; estimated from surveys of *P. megacephala* abundance from 2013 to 2015 and rates of expansion of nearby invasion fronts) and in 3 neighbouring (<2 km away) uninvaded sites with comparable tree density ('Uninvaded' sites). We constructed an electric fence enclosure at each site to exclude large herbivores (>20 kg) from a 50 × 50 m plot (0.25-ha) containing *ca.* 40 adult trees (1.5–2 meters tall). We marked 40 adult trees (1.5–2 m tall) in a plot of similar area and tree density *ca.* 200 m from each fenced plot to serve as the herbivore-present treatment. Each site comprised two plots, with a total of *ca.* 80 marked trees at each site. We fogged canopies of 20 trees in each plot with 0.6% alpha-cypermethrin (2–3 days in full sunlight, World Health Organization, 2013) to remove ants at the start of the experiment in 2016. To prevent the reestablishment of ant colonies, we applied sticky barriers (Tanglefoot® Insect Barrier, Contech Enterprises, Victoria, BC, Canada) to the trunks of those same trees (e.g. see Stanton & Palmer 2011). To maintain ant exclusion treatments, we reapplied sticky barriers as needed, and injected insecticide into domatia if they were colonised by foundress ant queens (recognisable by domatia holes sealed with carton material).

Tree physiological measurements

In the BACI and factorial experiments, we conducted all plant physiology measurements on fully-expanded leaves growing from non-lignified shoots in the unshaded sections of the

upper canopy. Leaf-level light-saturated photosynthetic and transpiration rates [henceforth 'leaf-level photosynthesis' ($A_{\max\text{-leaf}}$) and 'leaf-level transpiration' (E_{leaf})] were measured using a LI-6400XT Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NB) during sunny or partly cloudy days from 07:30 to 11:30. In longer-term Invaded and Uninvaded sites, we counted all mature leaves and measured leaf area of five randomly selected mature leaves for a random subset of 7–17 acacias per treatment in our factorial experiment in 2018 ($N = 102$ in wet season, $N = 61$ in dry season; means \pm SEM in Table S1) to estimate total leaf area. We then multiplied total leaf area by leaf-level photosynthesis and transpiration to estimate idealised light-saturated whole-canopy photosynthesis and transpiration capacities [henceforth 'canopy-level photosynthesis' ($A_{\max\text{-canopy}}$) and 'canopy-level transpiration' (E_{canopy})].

A_{\max} and E are calculated from gas exchange rates measured in the ideal environment within a controlled cuvette, and likely are higher than net photosynthesis and transpiration of a tree in naturally variable conditions (McGarvey *et al.*, 2004). We therefore compare these approximations at the leaf ($_{\text{leaf}}$) and canopy level ($_{\text{canopy}}$) to estimate relative differences in gas exchange for trees in our field experiments, but they do not estimate the absolute effect of invasion on carbon fixation.

We measured pre-dawn (ψ_{PD}) and mid-day leaf water potential (ψ_{MD}) on the same day as the gas exchange measurements for each study site using a Model 610 Plant Pressure Chamber (PMS Instruments, Corvallis, OR). Treatment means (\pm SEM) of ψ_{PD} and ψ_{MD} are in Tables S2 and S3. Wet season ψ_{PD} ranged from *ca.* -1.0 to -1.5 MPa and dry season ψ_{PD} ranged from *ca.* -1.9 to -2.1 MPa; studies of related tree species in the region recorded ψ_{PD} of *ca.* -2.0 MPa in dry conditions (Gebrekirstos *et al.*, 2006).

For each tree, we calculated diurnal leaf water potential range ($\Delta\psi$) as the difference between pre-dawn and mid-day leaf water potentials ($\Delta\psi = \psi_{\text{PD}} - \psi_{\text{MD}}$). $\Delta\psi$ demonstrates the range of viable water conditions that a leaf will experience (Gebrehiwot *et al.*, 2005; Gebrekirstos *et al.*, 2006): that range (negative with an upper limit of zero) is fundamentally created by stomatal water loss (Henry *et al.*, 2019) and made more negative by loss of vascular hydraulic conductivity (Lambers *et al.*, 2008; Scoffoni *et al.*, 2017). Plants will often remain within a species-specific $\Delta\psi$ (e.g. Gebrekirstos *et al.*, 2006), while photosynthesis and transpiration can vary without affecting $\Delta\psi$ as a result of osmotic or stomatal adjustments (Inoue *et al.*, 2017; Martínez-Vilalta & García-Fornier 2017; Hochberg *et al.*, 2018; Zhang *et al.*, 2019). Further details on our methods for measuring tree physiology parameters can be found in Note S1.

Statistical analysis

We used generalised linear mixed models (GLMMs) to analyse data in the BACI and factorial experiments. For the BACI experiment, we constructed individual GLMMs for each season (wet/dry) for $A_{\max\text{-leaf}}$, E_{leaf} and $\Delta\psi$. In the BACI GLMMs, sampling year (2017, 2018) and site type (Transition and Control) and their interaction term were fixed effects. We

included site as a random effect in all GLMMs. The BACI analysis produces three terms: effects of (1) site and (2) year, which indicate significant underlying differences between Transition and Control sites and interannual differences for all trees between survey years (respectively), and (3) an interaction term, which indicates if changes that occurred for leaf physiological traits between the 2017 and 2018 surveys were different for Transition trees (which were invaded at the end of 2017) and for Control trees. Only the interaction terms are discussed in the results section, while Site and Year (i.e. underlying differences) are reported in the supplement (Note S3). For the factorial experiment, we constructed separate GLMMs for each season (wet/dry) for $A_{\text{max-canopy}}$, E_{canopy} , $A_{\text{max-leaf}}$, E_{leaf} and $\Delta\psi$. In the factorial experiment GLMMs, invasion status (longer-term Invaded or Uninvaded) was a fixed effect, the exclusion of herbivores and ant occupants were fixed effects nested within invasion status, and we pooled data for the two dry seasons and for the two wet seasons.

Analyses were conducted using JMP Pro 15.1.0 (SAS Institute, Cary, North Carolina, USA). Further details on GLMMs are in Note S2.

RESULTS

Short-term effects of ant occupants and herbivores on *Acacia drepanolobium* physiology

In our BACI experiment during the wet season, on Control trees we found large declines between 2017 and 2018 in leaf-level photosynthesis (24%) and transpiration (20%), which significantly differed from minor declines for photosynthesis (4%) and transpiration (7%) between wet seasons for Transition trees (site \times year terms, photosynthesis: Fig. 1A; transpiration: Fig. 1C). Although leaf water potential range became more negative by 57% for Control trees and less negative by 3% for Transition trees from the 2017 to 2018 wet season, the site \times year term in our GLMM was only marginally significant (Fig. 1E).

In our BACI experiment during the dry season, we observed a decline between 2017 and 2018 dry seasons in leaf-level photosynthesis that was significantly larger for Transition trees (54%) than for Control trees (25%) (site \times year interaction term, Fig. 1B). Changes in leaf-level transpiration between dry seasons significantly differed for Transition trees (1% decline) and Control trees (35% increase) site \times year interaction term, Fig. 1D). Despite these different trends in photosynthesis and transpiration, we found no significant variation in leaf water potential range due to any GLMM factors during the dry season (site \times year interaction term, Fig. 1F).

Longer-term effects of ant occupants and herbivores on *Acacia drepanolobium* physiology

During wet seasons, we found significant differences in leaf- and canopy-level photosynthesis and transpiration as a function of invasion status, vertebrate herbivore exclusion from Invaded trees and *P. megacephala* exclusion (full ANOVA results in Table S5). Trees in stands which had been invaded for ca. 5 years had 16% lower photosynthesis (Fig. 2A) and 17%

lower transpiration (Fig. S1A) at the leaf level than trees in uninvaded stands. Scaled up to the canopy level, Invaded trees had 68% lower photosynthesis (Fig. 2A) and 54% lower transpiration (Fig. S1A) than Uninvaded trees. The exclusion of vertebrate herbivores from Invaded trees was associated with a 19% increase in photosynthesis (Fig. 3A) and a non-significant 13% increase in transpiration (Fig. S2A) at the leaf level, and was also associated with substantially higher canopy-level photosynthesis (365% higher; Fig. 3C) and transpiration (305% higher; Fig. 4A). Exclusion of *P. megacephala* ants did not significantly affect leaf-level photosynthesis (Fig. 3A) or transpiration (Fig. S2A), but their exclusion resulted in higher canopy-level photosynthesis (66% higher, Fig. 3C) and transpiration (52% higher; Fig. 4A). Despite these large differences between treatments in leaf and canopy transpiration, we only observed a significant but minor increase in leaf water potential range due to *P. megacephala* exclusion (14% less negative; Fig. 4C), and no significant differences due to invasion status or vertebrate herbivore exclusion from Invaded trees. Exclusion of *C. mimosae* and vertebrate herbivores from Uninvaded trees did not significantly impact leaf- or canopy-level photosynthesis or transpiration or leaf water potential range (Note S4).

During dry seasons, at the leaf level, we observed small increases in gas exchange and water potential range resulting from invasion status, herbivore exclusion from Invaded trees and *P. megacephala* exclusion, but no significant differences at the canopy level (Table S5). Invaded trees had significantly higher photosynthesis (13%; Fig. 2B) and transpiration (16%; Fig. S1B) at the leaf level, but we did not find significant differences in scaled-up canopy-level estimates of photosynthesis (Fig. 2B) or transpiration (Fig. S1B). Invaded trees also had slightly less negative leaf water potential range (7% less negative; Fig. S1D) during the dry season. The exclusion of *P. megacephala* from Invaded trees resulted in a minor but significant decline (17%) in dry season leaf-level transpiration (Fig. S2B), but *P. megacephala* exclusion did not significantly affect leaf- or canopy-level photosynthesis (Fig. 3B and D), canopy-level transpiration (Fig. 4B), or leaf water potential range (Fig. 4D). Similarly, exclusion of vertebrate herbivores was associated with a minor but significant decline (13%) in leaf-level transpiration, but no significant change in leaf- or canopy-level photosynthesis (Fig. 3B and D), canopy-level transpiration (Fig. 4B), or leaf water potential range (Fig. 4D). In contrast to trees from longer-term Invaded sites, Uninvaded trees did not exhibit different gas exchange or leaf water potential traits due to ant (*C. mimosae*) exclusion, herbivore exclusion or their interaction (Note S4).

DISCUSSION

Longer-term (ca. 5 year) invasion by *P. megacephala* reduced carbon fixation in *A. drepanolobium* by 69% during the growing (wet) season, consistent with other studies demonstrating that invasive species can alter fundamental ecosystem processes (a review of ecosystem functions affected by invasions: Hooper *et al.*, 2005; invasive insects reducing hardwood forest productivity: Kurz *et al.*, 2008; Albani *et al.*, 2010; Clark *et al.*, 2010; disruption of pollination by invasive insects:

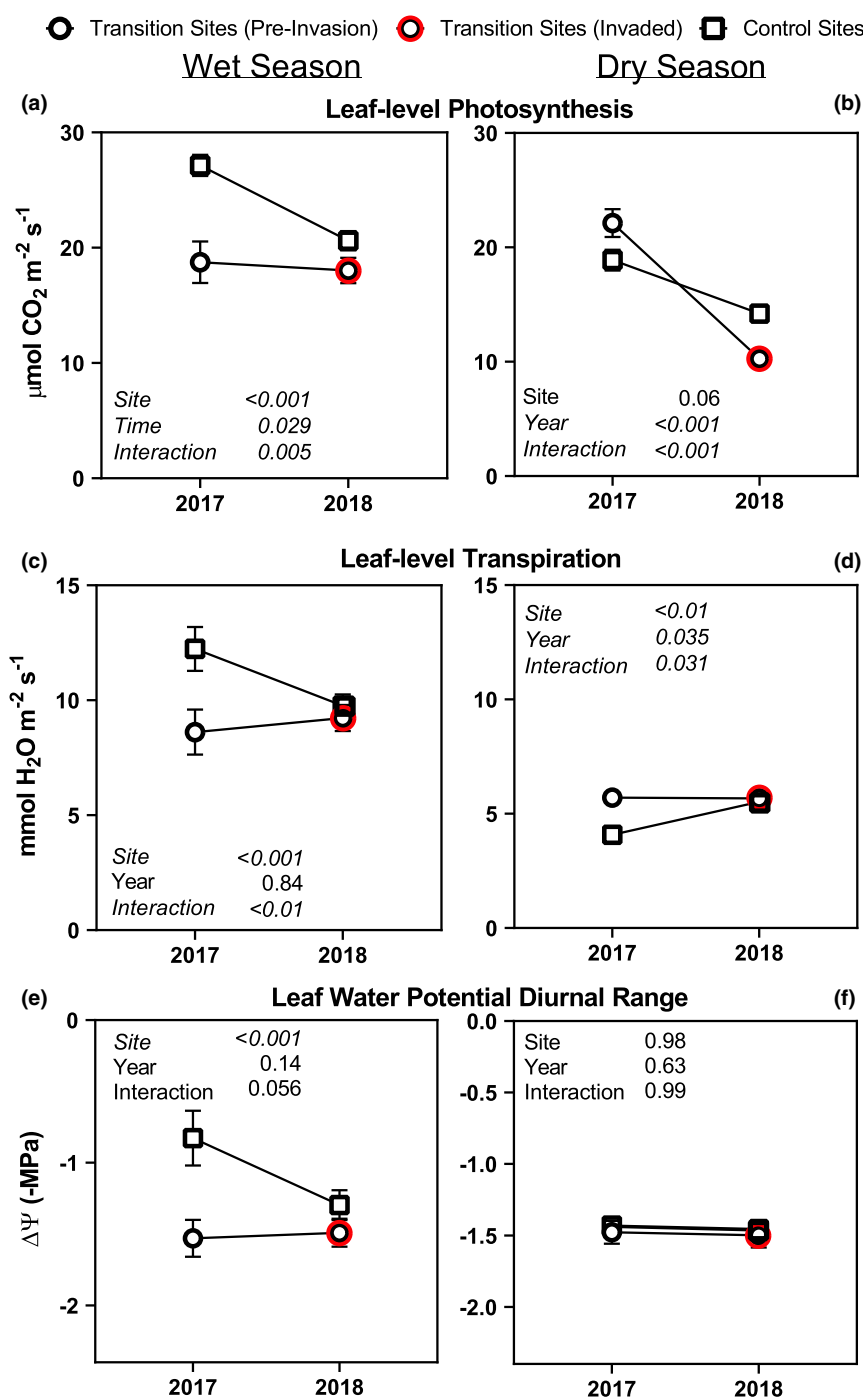


Figure 1 Leaf-level photosynthesis, leaf-level transpiration and diurnal leaf water potential range of *Acacia drepanolobium* trees in Transition sites (that were invaded by *P. megacephala* in December 2017 after initial wet and dry season surveys) and in paired Control sites (that were protected by *C. mimosae* throughout the study). Means of invaded Transition sites are surrounded by red circles. Effect tests are reported in each panel.

Morales *et al.*, 2017). The combination of shorter-term small or neutral effects of recent invasion on leaf-level photosynthesis with longer-term large reductions in canopy photosynthesis for trees that had been invaded for 5 years highlight that some ecologically relevant effects of invasive species can lag behind their initial appearance in a habitat (Crooks 2005; Strayer *et al.*, 2006; Simberloff 2011). Finally, the large

positive effect of vertebrate herbivore exclusion on canopy photosynthesis in invaded habitats demonstrates how invasive insects can indirectly interact with native fauna, including vertebrates, to substantially change carbon fixation for foundational plants.

Our results from Transition sites suggest that *P. megacephala* triggers minimal (if any) benefits for newly invaded

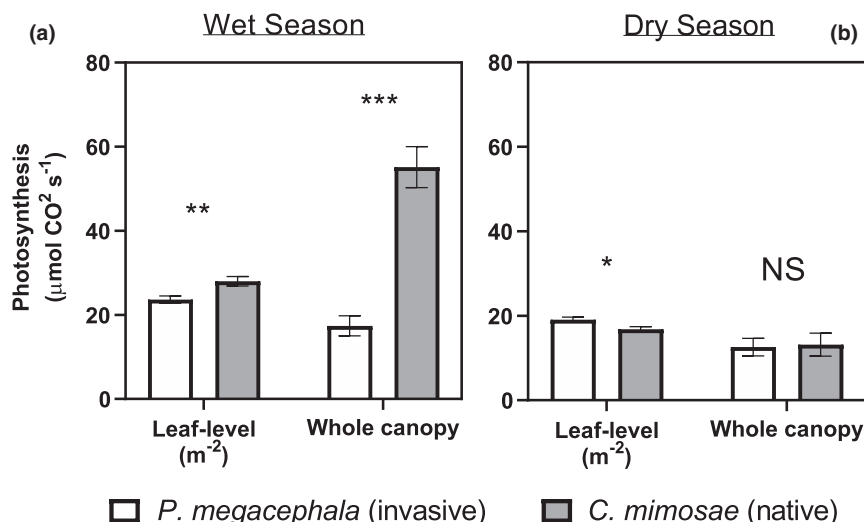


Figure 2 Differences in leaf- ($A_{\max-\text{leaf}}$; i.e. per-unit-leaf-area) and canopy-level ($A_{\max-\text{canopy}}$; i.e. canopy photosynthetic capacity) photosynthesis (means \pm SEM) of *P. megacephala*- vs. *C. mimosae*-occupied *Acacia drepanolobium* adults in wet and dry seasons at longer-term Invaded and Uninvaded sites. Results of pairwise comparisons are indicated as significant (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$) or not significant (NS).

trees, thus indicating that the loss of native ant mutualists has little direct effect on leaf-level photosynthesis in the short term. Wet season leaf-level photosynthesis and transpiration were nearly identical immediately before and after invasion for Transition trees, while the significant decline for both variables over the same period for Control trees may simply be driven by a decline in water availability for Control trees between the 2017 and 2018 wet seasons. Recently invaded trees experienced declines in dry-season leaf-level photosynthesis that were almost twice as large as declines for Control trees, perhaps because mixed feeders that forage on woody species during the dry season (Illius & O'Connor 1999) began to target their large and unprotected leafy canopies. The recent extirpation of *C. mimosae* by *P. megacephala* does not appear to cause increases in leaf-level photosynthesis, addressing our first research question about how the recent removal of native ant mutualists affects leaf photosynthetic rate. *Crematogaster mimosae* imposes a low net cost for the host tree compared to other ant species like *C. nigriceps* and *C. sjostedti* (Stanton & Palmer 2011), which may explain why their extirpation has no immediate effect on leaf physiology. Instead, our BACI experiment suggests that recently invaded trees become targets of herbivory during dry seasons, which may rapidly reduce leaf-level photosynthesis.

The longer-term effects of *P. megacephala* invasion on wet season carbon fixation is likely to affect how surviving invaded *A. drepanolobium* produce biomass and interact with other savanna species. In savannas that have been invaded for > 5 years, a 2-m tall tree has a canopy photosynthetic capacity of only ca. 31% of the carbon fixation per hour as a comparable uninvaded tree during the wet season, the primary growing period for many African acacias (Gourlay 1995). While ca. 20% of longer-term invaded trees at OPC experience complete destruction by large herbivores, ca. 60% experience mild to moderate levels of canopy damage (Riginos *et al.*, 2015): our findings apply to this large majority of trees

that survive for years after invasion. Reduced canopy-level photosynthesis can limit the availability of dissolved sucrose in above-ground tissue for *A. drepanolobium* (P. Milligan, unpublished manuscript), which is an important plant resource for growth (e.g. Wiley & Helliker 2012) and response to biotic and abiotic stress (e.g. Hummel *et al.*, 2010; Engelsdorf *et al.*, 2013). Low carbon availability can reduce population growth (McDowell 2011; Wiley & Helliker 2012), and likely contributes to *A. drepanolobium* population declines in longer-term invaded areas predicted by empirically derived demographic models of OPC trees (B. Hays, unpublished manuscript). Carbon limitation can prevent trees from producing above-ground biomass (Allen *et al.*, 2010; McDowell 2011), which would reduce the influence of *A. drepanolobium* on non-woody plant growth in its understory (Riginos *et al.*, 2009) and on the visibility of predators and prey in black cotton savannas dominated by *A. drepanolobium* (Riginos 2015). Also, carbon limited trees likely have fewer carbohydrates to support endosymbionts, which could affect nitrogen-fixing bacteria in *A. drepanolobium* roots that input N into black cotton soil (Fox-Dobbs *et al.*, 2010).

The interactive effect of invasion and vertebrate herbivory is likely the primary driver of leaf- and canopy-level photosynthesis and transpiration decline for longer-term invaded trees. The displacement of aggressive native *C. mimosae* by *P. megacephala* results in more intense browsing of *A. drepanolobium* by elephants (Riginos *et al.*, 2015), which suppresses canopy size for *A. drepanolobium* and for other savanna trees (Pellew 1983; Biggs & Jacobs 2002; Goheen & Palmer 2010), and thus reduces canopy-level photosynthesis. At the leaf-level, *P. megacephala* may function similarly to the native *C. sjostedti*, which provides negligible defence (Martins 2010) and is associated with low leaf-level photosynthesis when large herbivores are present (King & Caylor 2010). directly result from damage by herbivores (e.g. Delaney *et al.*, 2008) or indirectly result from the tree's production of antiherbivory

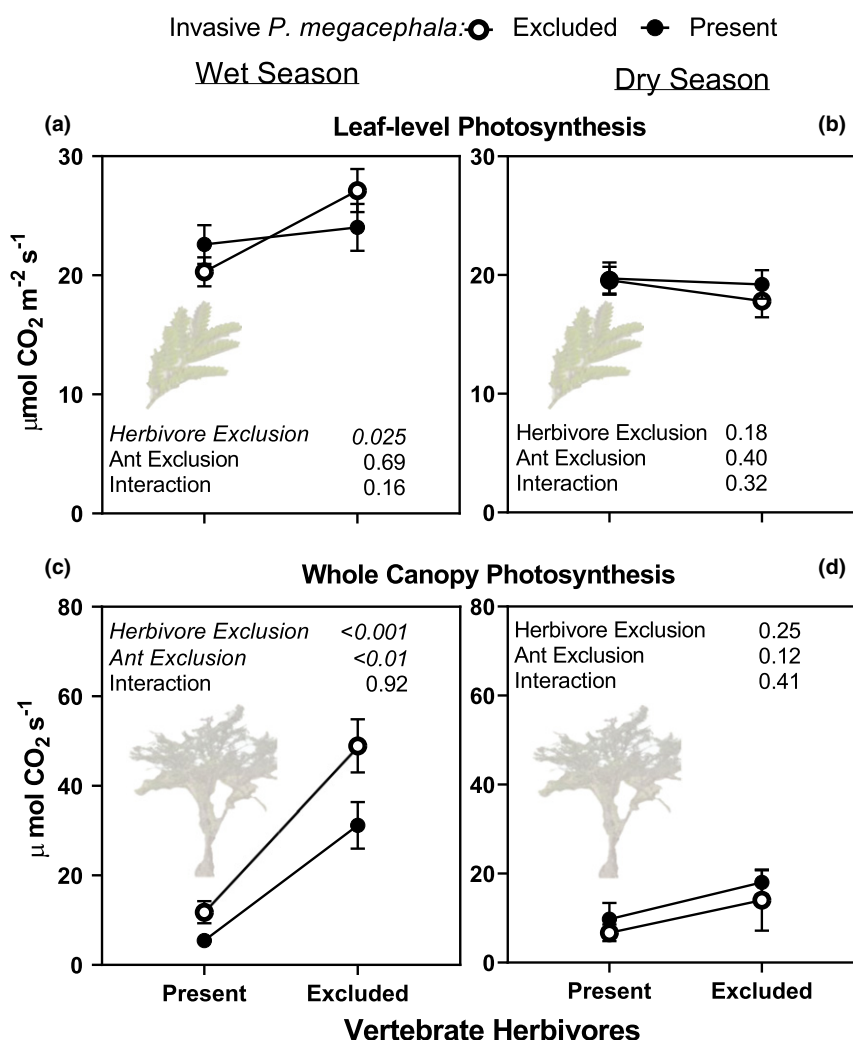


Figure 3 Differences in photosynthesis (means \pm SEM) of *P. megacephala*-occupied *Acacia drepanolobium* adults in a 2×2 full-factorial experiment (presence/exclusion of ants, large herbivores) conducted at 3 longer-term Invaded sites in wet and dry seasons. Photosynthetic indices are estimated at the leaf- ($A_{\text{max-leaf}}$) and canopy-level ($A_{\text{max-canopy}}$). Results of effect tests are reported in panels.

compounds in leaves as a response to damage (in this system: Ward & Young 2002; across plants: Ishida *et al.*, 2008; Sumbele *et al.*, 2012; Keenan & Niinemets 2016, see Note S5). While we did not observe reductions in leaf- or canopy-level photosynthesis on *C. mimosae*-removal trees in uninvaded areas, those trees may have benefitted from 'associational defence' (Barbosa *et al.*, 2009) with neighbouring trees that were occupied by defensive native ants (see Note S5).

Compared to vertebrate herbivores, invasive *P. megacephala* had smaller but significant direct negative effects on *A. drepanolobium* carbon fixation. This may be driven by *P. megacephala*'s habit of nesting around tree roots (P. Milligan, pers. observation), which can have large negative impacts on carbon fixation and leafy growth for *A. drepanolobium* saplings (Milligan *et al.* unpublished manuscript). *Pheidole megacephala* may also affect tree physiology through facultative interactions with other insect pests in the canopy, which were present in both ant-present and ant-excluded tree canopies > 8 months after canopies were initially fogged with

insecticide (P. Milligan, pers. observation). While we did not explicitly examine these relationships here, *P. megacephala* tends lycaenid caterpillars (pers. observation T. Palmer; a widespread association in Australia, Eastwood & Fraser 1999) and tolerates phloem-feeding cerambycid larvae (pers. observation P. Milligan) in the canopy. Infestations of phloem-feeding insects can affect the photosynthetic rates and water use efficiency of many plant species (Cockfield *et al.*, 1987; Meyer & Whitlow 1992; Haavik *et al.*, 2008; Golan *et al.*, 2015), which may further limit tree above- and below-ground productivity (Wiley & Helliker 2012).

While our finding of strong interactive effects of an invasive ant and vertebrate herbivores on carbon fixation of a dominant plant is novel, there are some parallels to be found with other systems. Herbivores are often more selective for high quality forage, such as the highly nutritious leaves produced by *A. drepanolobium* (Rubanza *et al.*, 2007), in other savanna systems (e.g. Roques *et al.*, 2001; Kos *et al.*, 2012; Abraham *et al.*, 2019). Thus, plants like *A. drepanolobium* with highly

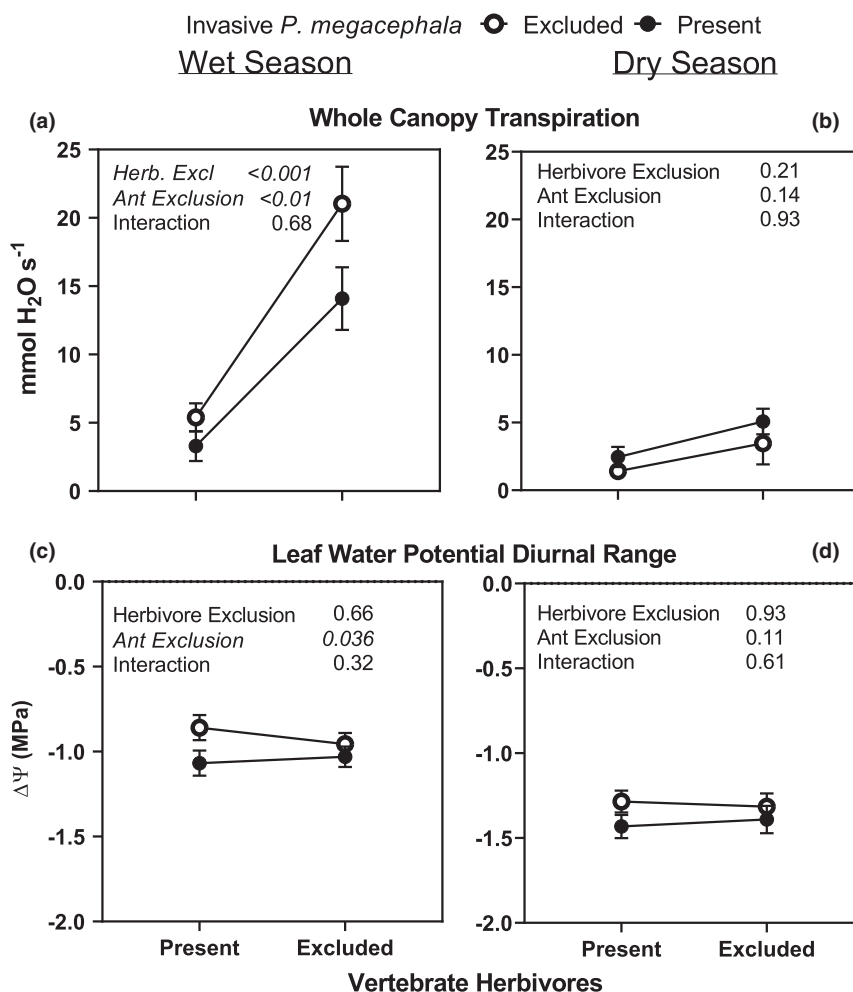


Figure 4 Differences in canopy-level transpiration (E_{canopy}) and leaf water potential diurnal range ($\Delta\psi$) (means \pm SEM) for *Acacia drepanolobium* adults in a 2×2 full-factorial experiment (presence/exclusion of ants, large herbivores) conducted at 3 longer-term Invaded sites in wet and dry seasons. Significant results of effect tests are reported in panels.

nutritious leaves may experience large changes in herbivore pressure if their deterrents against herbivory are disrupted by invasive insects. Chronic herbivory often imposes cumulative above-ground growth costs for plants in other systems (e.g. Kozlov & Zvereva 2017; Wilson *et al.*, 2018), and those increased metabolic costs can negatively affect leaf- and canopy-level photosynthesis (Wiley & Helliker 2012). McDowell (2011) reviewed how invasive insects contribute to carbon limitation and tree mortality during periods of environmental stress, but it is unknown whether interactive effects between invasive insects and native herbivores may be a more general phenomenon in terrestrial ecosystems.

The longer-term loss of photosynthetic capacity for ant-plants in invaded habitat reduces *A. drepanolobium*'s carbohydrate pool, which may affect other ecosystem processes to which this foundational tree contributes. For example, *A. drepanolobium* imports nitrogen into nitrogen-limited black cotton vertisols via nitrogen-fixing root endosymbionts (Fox-Dobbs *et al.*, 2010). If host plants reduce resource allocation to roots in invaded habitats, this could in turn reduce both nitrogen-fixing symbiont activity and soil

respiration (seen in a large-scale girdling experiment in boreal forests, Höglberg *et al.*, 2001). Potentially compounding this effect, elephants may reduce tree cover within invaded savannas over the longer term, further reducing nitrogen inputs into the system, as has been shown in other areas of East Africa where removal of acacia species reduces both the total content and mineralisation of C and N in soils (Glaser *et al.*, 2001). Finally, *A. drepanolobium* has density-dependent effects on the productivity of understory plants (Riginos *et al.*, 2009), and thus the resource availability and productivity of invaded trees may also be linked to understory productivity. By increasing the mortality (Riginos *et al.*, 2015) and decreasing the carbon fixation of this monodominant tree species, *P. megacephala* invasion may fundamentally alter carbon cycling and connected ecosystem processes in these savannas.

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AUTHORSHIP

PM, CR, JG and TP designed the study; PM, TM and TP devised the plant survey methods; PM conducted statistical analyses that were advised by TM, GJ and TP; PM and SC conducted fieldwork; PM wrote the manuscript, and all authors contributed substantially to revisions.

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DATA AVAILABILITY STATEMENT

All data from this project are available via Figshare : <https://doi.org/10.6084/m9.figshare.14049875.v1>.

REFERENCES

- Abraham, J.O., Hempson, G.P. & Staver, A.C. (2019). Drought-response strategies of savanna herbivores. *Ecol. Evol.*, 9, 7047–7056.
- Albani, M., Moorcroft, P.R., Ellison, A.M., Orwig, D.A. & Foster, D.R. (2010). Predicting the impact of hemlock woolly adelgid on carbon dynamics of eastern United States forests. *Can. J. For. Res.*, 40, 119–133.
- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M. *et al.* (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manage.*, 259, 660–684.
- Anastasio, O.E. (2020). Impacts of invasive ant-hemipteran interaction, edge effects and habitat complexities on the spatial distribution of ants. Clark University.
- Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A. & Szendrei, Z. (2009). Associational resistance and associational susceptibility: having right or wrong neighbors. *Annu. Rev. Ecol. Evol. Syst.*, 40, 1–20.
- Beardsley, J.W., Su, T.H., McEwen, F. & Gerling, D. (1982). Field investigations on the interrelationships of the big-headed ant, the gray pineapple mealybug, and pineapple mealybug wilt disease in Hawaii. *Proc. Hawaiian Entomol. Soc.* 24, 51–67.
- Bertelsmeier, C., Ollier, S., Liebhold, A. & Keller, L. (2017). Recent human history governs global ant invasion dynamics. *Nature Ecology & Evolution*, 1, 0184.
- Biggs, R. & Jacobs, O. (2002). The impact of the African elephant on marula trees in the Kruger National Park. *South African J. Wildlife Res.-24-Month Delayed Open Access*, 32, 13–22.
- Bradshaw, C.J., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A. *et al.* (2016). Massive yet grossly underestimated global costs of invasive insects. *Nat. Commun.*, 7, 1–8.
- Clark, K.L., Skowronski, N. & Hom, J. (2010). Invasive insects impact forest carbon dynamics. *Glob. Change Biol.*, 16, 88–101.
- Cockfield, S.D., Potter, D.A. & Houtz, R.L. (1987). Chlorosis and reduced photosynthetic CO₂ assimilation of *Euonymus fortunei* infested with euonymus scale (Homoptera: Diaspididae). *Environ. Entomol.*, 16, 1314–1318.
- Crooks, J.A. (2005). Lag times and exotic species: The ecology and management of biological invasions in slow-motion. *Ecoscience*, 12, 316–329.
- Delaney, K.J., Haile, F.J., Peterson, R.K.D. & Higley, L.G. (2008). Impairment of leaf photosynthesis after insect herbivory or mechanical injury on common milkweed, *asclepias syriaca*. *Environ. Entomol.*, 37, 1332–1343.
- Del-Claro, K., Rico-Gray, V., Torezan-Silingardi, H.M., Alves-Silva, E., Fagundes, R., Lange, D. *et al.* (2016). Loss and gains in ant–plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. *Insectes Soc.*, 63, 207–221.
- Demian, N. (2019). Ant-plant-hemipteran interactions between non-native species in Laikipia, Kenya. In: *Ecology and evolutionary biology* (ed Tarnita, C.E.). Princeton, NJ: Princeton University, Princeton University Senior Theses, pp. 1924–2020. <http://arks.princeton.edu/ark:/88435/dsp014m90dz338>
- Eastwood, R. & Fraser, A.M. (1999). Associations between lycaenid butterflies and ants in Australia. *Aust. J. Ecol.*, 24, 503–537.
- Engelsdorf, T., Horst, R.J., Pröls, R., Pröschel, M., Dietz, F., Hückelhoven, R. *et al.* (2013). Reduced carbohydrate availability enhances the susceptibility of *Arabidopsis* toward *Colletotrichum higginsianum*. *Plant Physiol.*, 162, 225–238.
- Englund, R.A. (2008). Invasive species threats to native aquatic insect biodiversity and conservation measures in Hawai'i and French Polynesia. *J. Insect Conserv.*, 12, 415. <https://doi.org/10.1007/s10841-008-9168-z>
- Finér, L., Jurgensen, M.F., Domisch, T., Kilpeläinen, J., Neuvonen, S., Punttila, P. *et al.* (2013). The role of wood ants (*Formica rufa* group) in carbon and nutrient dynamics of a boreal Norway spruce forest ecosystem. *Ecosystems*, 16, 196–208.
- Fox-Dobbs, K., Doak, D.F., Brody, A.K. & Palmer, T.M. (2010). Termites create spatial structure and govern ecosystem function by affecting N₂ fixation in an East African savanna. *Ecology*, 91, 1296–1307.
- Fredrickson, M.E., Greene, M.J. & Gordon, D.M. (2005). 'Devil's gardens' bedeviled by ants. *Nature*, 437, 495–496.
- Fuster, F., Kaiser-Bunbury, C.N. & Traveset, A. (2020). Pollination effectiveness of specialist and opportunistic nectar feeders influenced by invasive alien ants in the Seychelles. *Am. J. Bot.*
- Gaigher, R., Samways, M.J. & Van Noort, S. (2013). Saving a tropical ecosystem from a destructive ant-scale (*Pheidole megacephala*, *Pulvinaria urticae*) mutualism with support from a diverse natural enemy assemblage. *Biol. Invasions*, 15, 2115–2125.
- Gandhi, K.J. & Herms, D.A. (2010). Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biol. Invasions*, 12, 389–405.
- Gebrehiwot, K., Muys, B., Haile, M. & Mitloehner, R. (2005). The use of plant water relations to characterize tree species and sites in the drylands of northern Ethiopia. *J. Arid Environ.*, 60, 581–592.
- Gebrekiros, A., Teketay, D., Fetene, M. & Mitlöhner, R. (2006). Adaptation of five co-occurring tree and shrub species to water stress and its implication in restoration of degraded lands. *For. Ecol. Manage.*, 229, 259–267.
- Glanz-Idan, N. & Wolf, S. (2020). Upregulation of photosynthesis in mineral nutrition-deficient tomato plants by reduced source-to-sink ratio. *Plant Signal Behav.*, 15, 1712543.
- Glaser, B., Lehmann, J., Führböter, M., Solomon, D. & Zech, W. (2001). Carbon and nitrogen mineralization in cultivated and natural savanna soils of Northern Tanzania. *Biol. Fertil. Soils*, 33, 301–309.
- Goheen, J.R. & Palmer, T.M. (2010). Defensive plant-ants stabilize megaherbivore-driven landscape change in an African savanna. *Curr. Biol.*, 20, 1768–1772.

- Golan, K., Rubinowska, K., Kmiec, K., Kot, I., Górska-Drabik, E., Łagowska, B. *et al.* (2015). Impact of scale insect infestation on the content of photosynthetic pigments and chlorophyll fluorescence in two host plant species. *Arthropod-Plant Interactions*, 9, 55–65.
- Goldschmidt, E.E. & Huber, S.C. (1992). Regulation of photosynthesis by end-product accumulation in leaves of plants storing starch, sucrose, and hexose sugars. *Plant Physiol.*, 99, 1443–1448.
- Gourlay, I.D. (1995). Growth ring characteristics of some African Acacia species. *J. Trop. Ecol.*, 11(1), 121–140.
- Haaavik, L., Stephen, F., Fierke, M., Salisbury, V., Leavitt, S. & Billings, S. (2008). Dendrochronological parameters of northern red oak (*Quercus rubra* L. (Fagaceae)) infested with red oak borer (*Enaphalodes rufulus* (Haldeman)(Coleoptera: Cerambycidae)). *For. Ecol. Manage.*, 255, 1501–1509.
- Henry, C., John, G.P., Pan, R., Bartlett, M.K., Fletcher, L.R., Scoffoni, C. *et al.* (2019). A stomatal safety-efficiency trade-off constrains responses to leaf dehydration. *Nat. Commun.*, 10, 1–9.
- Hill, J.K., Rosengaus, R.B., Gilbert, F.S. & Hart, A.G. (2013). Invasive ants are fire ants drivers of biodiversity loss. *Ecol. Entomol.*, 38, 539.
- Hochberg, U., Rockwell, F.E., Holbrook, N.M. & Cochard, H. (2018). Iso/anisohydry: a plant–environment interaction rather than a simple hydraulic trait. *Trends Plant Sci.*, 23, 112–120.
- Hocking, B. (1970). Insect associations with the swollen thorn acacias. *Trans. R. Entomol. Soc. London*, 122(7), 211–255.
- Hoffmann, B.D. & Parr, C.L. (2008). An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biol. Invasions*, 10, 1171–1181.
- Högborg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högborg, M.N. *et al.* (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, 411, 789–792.
- Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D. & Case, T.J. (2002). The causes and consequences of ant invasions. *Annu. Rev. Ecol. Syst.*, 33, 181–233.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al.* (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecol. Monogr.*, 75, 3–35.
- Horvitz, C.C. & Schemske, D.W. (1986). Seed dispersal of a neotropical myrmecochore: variation in removal rates and dispersal distance. *Biotropica*, 18(4), 319–323.
- Hummel, I., Pantin, F., Sulpice, R., Piques, M., Rolland, G., Dauzat, M. *et al.* (2010). Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiol.*, 154, 357–372.
- Huntzinger, M., Karban, R., Young, T.P. & Palmer, T.M. (2004). Relaxation of induced indirect defenses of acacias following exclusion of mammalian herbivores. *Ecology*, 85, 609–614.
- Illius, A.W. & O'Connor, T.G. (1999). On the relevance of nonequilibrium concepts to arid and semiarid grazing systems. *Ecol. Appl.*, 9, 798–813.
- Inoue, Y., Ichie, T., Kenzo, T., Yoneyama, A., Kumagai, T. & Nakashizuka, T. (2017). Effects of rainfall exclusion on leaf gas exchange traits and osmotic adjustment in mature canopy trees of *Dryobalanops aromatica* (Dipterocarpaceae) in a Malaysian tropical rain forest. *Tree Physiol.*, 37, 1301–1311.
- Ishida, A., Nakano, T., Yazaki, K., Matsuki, S., Koike, N., Lauenstein, D.L. *et al.* (2008). Coordination between leaf and stem traits related to leaf carbon gain and hydraulics across 32 drought-tolerant angiosperms. *Oecologia*, 156, 193.
- Keenan, T.F. & Niinemets, Ü. (2016). Global leaf trait estimates biased due to plasticity in the shade. *Nature Plants*, 3, 16201.
- King, E.G. & Caylor, K.K. (2010). Herbivores and mutualistic ants interact to modify tree photosynthesis. *The New Phytologist*, 187, 17–21.
- Kos, M., Hoetmer, A.J., Pretorius, Y., de Boer, W.F., de Knecht, H., Grant, C. *et al.* (2012). Seasonal diet changes in elephant and impala in mopane woodland. *Eur. J. Wildl. Res.*, 58, 279–287.
- Kozlov, M.V. & Zvereva, E.L. (2017). Background insect herbivory: impacts, patterns and methodology. In: *Progress in botany Vol. 79* (eds Cánovas, F.M., Lüttge, U., Matyssek, R.). Cham: Springer, pp. 313–355.
- Kulikowski, A.J. II (2020). Ant-scale mutualism increases scale infestation, decreases folivory, and disrupts biological control in restored tropical forests. *Biotropica*, 52, 709–716.
- Kurz, W.A., Dymond, C.C., Stinson, G., Rampley, G.J., Neilson, E.T., Carroll, A.L. *et al.* (2008). Mountain pine beetle and forest carbon feedback to climate change. *Nature*, 452, 987–990.
- Lach, L., Hobbs, R.J. & Majer, J.D. (2009). Herbivory-induced extrafloral nectar increases native and invasive ant worker survival. *Popul. Ecol.*, 51, 237–243.
- Lach, L. & Hoffmann, B.D. (2011). Are invasive ants better plant-defense mutualists? A comparison of foliage patrolling and herbivory in sites with invasive yellow crazy ants and native weaver ants. *Oikos*, 120, 9–16.
- Lambers, H., Chapin, F.S. III & Pons, T.L. (2008). *Plant physiological ecology*. New York, NY: Springer Science & Business Media.
- Martínez-Vilalta, J. & García-Fornier, N. (2017). Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. *Plant, Cell Environ.*, 40, 962–976.
- Martins, D.J. (2010). Not all ants are equal: obligate acacia ants provide different levels of protection against mega-herbivores. *Afr. J. Ecol.*, 48, 1115–1122.
- McDowell, N.G. (2011). Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiol.*, 155, 1051–1059.
- McGarvey, R.C., Martin, T.A. & White, T.L. (2004). Integrating within-crown variation in net photosynthesis in loblolly and slash pine families. *Tree Physiol.*, 24, 1209–1220.
- McGeoch, M.A., Lythe, M.J., Henriksen, M.V. & McGrannachan, C.M. (2015). Environmental impact classification for alien insects: a review of mechanisms and their biodiversity outcomes. *Current Opinion in Insect Science*, 12, 46–53.
- Meyer, G.A. & Whitlow, T.H. (1992). Effects of leaf and sap feeding insects on photosynthetic rates of goldenrod. *Oecologia*, 92, 480–489.
- Milligan, P.D., Prior, K.M. & Palmer, T.M. (2016). An invasive ant reduces diversity but does not disrupt a key ecosystem function in an African savanna. *Ecosphere*, 7, e01502.
- Morales, C.L., Sáez, A., Garibaldi, L.A. & Aizen, M.A. (2017). Disruption of pollination services by invasive pollinator species. In: *Impact of biological invasions on ecosystem services* (eds Vilà, M., Hulme, P.). Cham, Germany: Springer, pp. 203–220.
- Nebauer, S.G., Renau-Morata, B., Guardiola, J.L. & Molina, R.-V. (2011). Photosynthesis down-regulation precedes carbohydrate accumulation under sink limitation in Citrus. *Tree Physiol.*, 31, 169–177.
- Ness, J. & Bronstein, J.L. (2004). The effects of invasive ants on prospective ant mutualists. *Biol. Invasions*, 6, 445–461.
- Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P. & Thomas, M.B. (2016). Global threat to agriculture from invasive species. *Proc. Natl Acad. Sci.*, 113, 7575–7579.
- Palmer, T.M. (2004). Wars of attrition: colony size determines competitive outcomes in a guild of African acacia ants. *Anim. Behav.*, 68, 993–1004.
- Palmer, T.M., Riginos, C., Milligan, P.D., Hays, B.R., Pietrek, A.G. & Jepkuri, N. *et al.* (2020). Frenemy at the gate: niche differences determine winners and losers in an invaded ant-plant mutualism. *Ecology*, 102, e03230.
- Pellew, R.A.P. (1983). The impacts of elephant, giraffe and fire upon the Acacia tortilis woodlands of the Serengeti. *Afr. J. Ecol.*, 21, 41–74.
- Pietrek, A.G., Goheen, J.R., Riginos, C., Jepkuri, N. & Palmer, T.M. (2021). Density dependence and the spread of invasive big-headed ants (*Pheidole megacephala*) in an East African savanna. *Oecologia*. <https://doi.org/10.1007/s00442-021-04859-1>

- Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., *et al.* (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agr. Ecosyst. Environ.*, 84, 1–20.
- Pringle, E.G. (2016). Integrating plant carbon dynamics with mutualism ecology. *New Phytol.*, 210, 71–75.
- Prior, K.M. & Palmer, T.M. (2018). Economy of scale: third partner strengthens a keystone ant-plant mutualism. *Ecology*, 99, 335–346.
- Riginos, C. (2015). Climate and the landscape of fear in an African savanna. *J. Anim. Ecol.*, 84, 124–133.
- Riginos, C., Grace, J.B., Augustine, D.J. & Young, T.P. (2009). Local versus landscape-scale effects of savanna trees on grasses. *J. Ecol.*, 97, 1337–1345.
- Riginos, C., Karande, M.A., Rubenstein, D.I. & Palmer, T.M. (2015). Disruption of a protective ant-plant mutualism by an invasive ant increases elephant damage to savanna trees. *Ecology*, 96, 654–661.
- Risch, A.C., Jurgensen, M.F., Schütz, M. & Page-Dumroese, D.S. (2005). The contribution of red wood ants to soil C and N pools and CO₂ emissions in subalpine forests. *Ecology*, 86, 419–430.
- Rodriguez-Cabal, M.A., Stuble, K.L., Guénard, B., Dunn, R.R. & Sanders, N.J. (2012). Disruption of ant-seed dispersal mutualisms by the invasive Asian needle ant (*Pachycondyla chinensis*). *Biol. Invasions*, 14, 557–565.
- Roques, K.G., O'Connor, T.G. & Watkinson, A.R. (2001). Dynamics of shrub encroachment in an African savanna: relative influences of fire, herbivory, rainfall and density dependence. *J. Appl. Ecol.*, 38, 268–280.
- Rubanza, C.D., Shem, M.N., Bakengesa, S.S., Ichinohe, T. & Fujihara, T. (2007). The content of protein, fibre and minerals of leaves of selected Acacia species indigenous to north-western Tanzania. *Archives Animal Nutri.*, 61, 151–156.
- Savage, A.M., Rudgers, J.A. & Whitney, K.D. (2009). Elevated dominance of extrafloral nectary-bearing plants is associated with increased abundances of an invasive ant and reduced native ant richness. *Divers. Distrib.*, 15, 751–761.
- Scoffoni, C., Sack, L. & Ort, D. (2017). The causes and consequences of leaf hydraulic decline with dehydration. *J. Exp. Bot.*, 68, 4479–4496.
- Simberloff, D. (2011). How common are invasion-induced ecosystem impacts? *Biol. Invasions*, 13, 1255–1268.
- Stanton, M.L. & Palmer, T.M. (2011). The high cost of mutualism: effects of four species of East African ant symbionts on their myrmecophyte host tree. *Ecology*, 92, 1073–1082.
- Strayer, D.L., Eviner, V.T., Jeschke, J.M. & Pace, M.L. (2006). Understanding the long-term effects of species invasions. *Trends Ecol. Evol.*, 21, 645–651.
- Sumbele, S., Fotelli, M.N., Nikolopoulos, D., Tooulakou, G., Liakoura, V., Liakopoulos, G. *et al.* (2012). Photosynthetic capacity is negatively correlated with the concentration of leaf phenolic compounds across a range of different species. *AoB Plants*, pls025. <https://doi.org/10.1093/aobpla/pls025>
- Vanbergen, A.J., Espíndola, A. & Aizen, M.A. (2018). Risks to pollinators and pollination from invasive alien species. *Nature Ecol. Evol.*, 2, 16–25.
- Ward, D. & Young, T.P. (2002). Effects of large mammalian herbivores and ant symbionts on condensed tannins of *Acacia drepanolobium* in Kenya. *J. Chem. Ecol.*, 28, 921–937.
- Wetterer, J.K. (2012). Worldwide spread of the African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). *Myrmecological News*, 17, 51–62.
- WHO (2013). *WHO specifications for public health pesticides: alphacypermethrin*. World Health Organization Geneva, Switzerland.
- Wiley, E. & Helliker, B. (2012). A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytol.*, 195, 285–289.
- Wilson, C.M., Schaeffer, R.N., Hickin, M.L., Rigsby, C.M., Sommi, A.F., Thornber, C.S. *et al.* (2018). Chronic impacts of invasive herbivores on a foundational forest species: a whole-tree perspective. *Ecology*, 99, 1783–1791.
- Young, T.P., Stubblefield, C.H. & Isbell, L.A. (1996). Ants on swollen-thorn acacias: species coexistence in a simple system. *Oecologia*, 109, 98–107.
- Zhang, Z., Huang, M., Zhao, X. & Wu, L. (2019). Adjustments of leaf traits and whole plant leaf area for balancing water supply and demand in *Robinia pseudoacacia* under different precipitation conditions on the Loess Plateau. *Agric. For. Meteorol.*, 279, 107733.
- Zhou, A.M., Liang, G.W., Zeng, L., Lu, Y.Y. & Xu, Y.J. (2017). *Solenopsis invicta* suppress native ant by excluding mutual exploitation from the invasive mealybug, *Phenacoccus solenopsis*. *Pakistan. J. Zool.*, 49, 133–141.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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