

Mutualism, herbivory, and invasive ants as seasonally dependent drivers of root surface area in a foundational savanna ant–plant

Patrick D. Milligan^{1,2} , Justin Rossiter³, Alina Zare^{3,4} , Todd M. Palmer^{2,5} , John Lemboi², Gabriella M. Mizell^{1,2,6} , John Mosiany², Corinna Riginos^{7,8} , Jacob R. Goheen^{2,9}  and Elizabeth G. Pringle^{1,2,6} 

¹Department of Biology, University of Nevada, Reno, Reno, NV 89557, USA; ²Mpala Research Centre, PO Box 555, Nanyuki, Laikipia, 10400, Kenya; ³Department of Electrical and Computer Engineering, University of Florida, Gainesville, FL 32603, USA; ⁴Artificial Intelligence and Informatics Research Institute, University of Florida, Gainesville, FL 32611, USA; ⁵Department of Biology, University of Florida, Gainesville, FL 32611, USA; ⁶Program in Ecology, Evolution and Conservation Biology, University of Nevada, Reno, Reno, NV 89557, USA; ⁷The Nature Conservancy, Lander, WY 82520, USA; ⁸Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA; ⁹Department of Natural Resource Ecology & Management, Iowa State University, Ames, IA 50011, USA

Author for correspondence:
Patrick D. Milligan
Email: patrickdmilligan@gmail.com

Received: 29 April 2025
Accepted: 25 August 2025

New Phytologist (2026) 249: 777–791
doi: 10.1111/nph.70553

Key words: ant–plant, biological invasion, cost–benefit tradeoff, herbivore extinction, mutualism, mutualism breakdown, root density, saturating benefits.

Summary

- Many plants are defended from herbivory by costly insect mutualists. Understanding positive associations between plants and mutualists requires a whole-plant perspective including roots.
- We hypothesized that root surface area increases with mutualist activity (to a saturation threshold) and recent rainfall but that this relationship shifts when herbivores are excluded. We also hypothesized that invasive ants limit root surface area and that mutualism breakdown driven by invaders blunts root responses to rainfall and herbivore exclusion.
- Using minirhizotrons (est. 2021), we surveyed root surface area of ant-acacias during a dry (2022) and then a wet (2023) season. Study plots either excluded or permitted vertebrate browsers, within a natural experiment comparing mutualist-defended ant-acacias to those invaded by a mutualism-disrupting ant.
- Root area increased with mutualist activity to a threshold, but this positive association was less apparent during rainy periods. Megabrowser exclusion increased overall root area but reduced the threshold for a positive association with mutualist activity and reduced the steepness of the root area–rainfall correlation. Ant-invaded acacias had smaller root areas that correlated less steeply with rainfall. Positive associations between insect defense and root area were thus contingent on rainfall, herbivory, and biotic invasion, drivers that are shifting under global change.

Introduction

Plant–insect mutualisms require investment of plant carbohydrates in insect partners, creating potential trade-offs with other carbon sinks and affecting plant physiology and performance. In exchange for carbohydrates, plants can gain services such as biotic defense. The net effect of biotic defense is typically assessed by measuring the change in herbivory or growth aboveground between defended and undefended individuals (Bronstein, 2001; Rosumek *et al.*, 2009). Yet, biomass belowground – that is root biomass – can represent approximately half of the biomass of a woody plant (Niklas, 2005), and roots capture critical water and nutrients (BassiriRad *et al.*, 2001). Any potential feedback that affects plant rooting is therefore important for long-term plant performance. Whereas aboveground herbivory has been linked to differences in tree root traits including surface area, carbohydrate storage, and responsiveness to fire and nutrient availability (see,

e.g. Wigley *et al.*, 2019; Heinze, 2020; Perkovich & Ward, 2021), the relationship between aboveground mutualists and root traits has not been characterized. Moreover, changes belowground may be difficult to infer from those aboveground because above- and belowground tissues can respond differently to the same stimuli (Bezemer & van Dam, 2005; Mundim *et al.*, 2017). For example, aboveground herbivory can stimulate regrowth aboveground but defenses (terpenoids) belowground, presumably for the distinct purposes of replacing biomass and safeguarding resources, respectively (Mundim *et al.*, 2017). Thus, determining how insect mutualists affect the fitness of long-lived plants such as trees requires a whole-plant perspective.

Climate change presents an urgent motive for understanding the relationship between tree physiology and mutualism. Yet, we lack even a basic understanding of how carbon allocation to insect mutualists fits into tree strategies (Hartmann & Trumbore, 2016; Pringle, 2016). Among other factors, variation in

rainfall changes tree physiological demands (Allen *et al.*, 2010), determines the size of labile carbon pools (Perkins & Keith Owens, 2003; Vogel *et al.*, 2008; Skarpaas *et al.*, 2016), and alters allocation priorities (e.g. Weber *et al.*, 2018; Huang *et al.*, 2019). Consequently, changes in rainfall could intensify or dampen allocation trade-offs by changing resource availability.

In addition to climate change, biodiversity loss can change the community context around defensive mutualisms with ramifications for how plants allocate finite resources to growth, reproduction, and defense. Herbivore pressure is often the stimulus that encourages host plants to offer incentives to insect defenders (Bronstein, 2001; Palmer *et al.*, 2008), but there are ongoing global declines in both invertebrate (Wagner *et al.*, 2021) and vertebrate herbivores (Ripple *et al.*, 2015). The loss of herbivore pressure cancels the benefits of biotic defense for the plant and may result in mutualism breakdown (Kiers *et al.*, 2010). Similarly, the introduction of exotic predators or competitors of native mutualists is expected to drive mutualism breakdown (Kiers *et al.*, 2010). As biodiversity loss and biological invasions change the fitness benefits and stability of tree–insect mutualisms, tree allocation to incentives for mutualists vs other aspects of growth, reproduction, and defense may also change.

The flexibility of belowground allocation is an understudied component of whether and how trees can reallocate their resources to buffer against climate change, biodiversity loss, and species invasions. There is considerable evidence that trees adjust aboveground physiological processes and traits in response to environmental variation. For example, host plants can modify carbon fixation, stomatal water loss, amino acids in phloem, and secondary metabolites in nectar in response to seasonal rainfall, insect mutualists, and/or herbivores (King & Caylor, 2010; Kremer *et al.*, 2018; Nepi *et al.*, 2018). In general, trees exhibit extensive root growth, especially at shallow soil depths (Lambers *et al.*, 2008) and in wetter conditions (Smucker & Aiken, 1992), as a strategy to enhance water absorption. Conversely, trees withstand dry periods by reducing or pausing new root growth and adjusting root anatomy (e.g. suberisation and vascular tissue reinforcement; Brunner *et al.*, 2015). In addition to their key roles in nutrient foraging (Giehl & von Wirén, 2014) and water stress mitigation (Aroca *et al.*, 2012), roots may act as a repository for resources that is largely safeguarded from fire, aboveground herbivores, and other destructive events such as windstorms (Peterson, 2019; Silvério *et al.*, 2019) and lightning strikes (Gora *et al.*, 2021). Roots thus play a central role in determining tree longevity (e.g. Andivia *et al.*, 2021; Chitra-Tarak *et al.*, 2021) and performance during adverse events (Trugman *et al.*, 2018), with implications for lifetime fitness.

The *Vachellia* (fmr. *Acacia*) *drepanolobium* ant–plant (henceforth, ‘acacia’) – an abundant tree on vertisol-underlain savannas across East Africa – is an ideal study species for testing hypotheses about rainfall-dependent trade-offs between mutualists and other resource sinks, including roots. The acacia’s ant mutualists exert a cost to the tree’s aboveground growth (Stanton & Palmer, 2011), and water is a limiting resource (Hesla *et al.*, 1985). Virtually, all mature acacias host colonies of defensive ants,

including *Crematogaster mimosae* (hereafter ‘native ant(s)’), the most widespread mutualist on these acacias in central Kenya (Young *et al.*, 1996). Native ants nest in the swollen spines (‘domatia’) of the acacias and consume extrafloral nectar secreted from glands along the rachis of acacia leaves. The ants aggressively repel large mammalian browsers such as African elephants (*Loxodonta africanus*) when they browse the host tree, and also attack invertebrate herbivores that would otherwise damage leaves and stems (Palmer & Brody, 2013). This defense allows acacias to form dense monocultures even in areas with intense herbivory (Young *et al.*, 1998). Rainy periods stimulate new foliar growth, on which acacias produce substantial nectar to support ant mutualists (Palmer & Brody, 2007). Given that rainfall stimulates ant–plant investment of labile carbon in domatia, nectar, and vegetative growth (see, e.g. Pringle *et al.*, 2013), we expected generally positive associations among rainfall, ant mutualist activity, and root surface area. Nevertheless, because defense of aboveground tissue by ants tends to saturate when ants (and other insects) increase in density beyond a certain threshold (i.e. the ‘return on investment’ diminishes) (Fonseca, 1993; Bronstein, 2001; Palmer & Brody, 2013), we might expect positive feedbacks for belowground biomass to saturate also.

Additionally, our study system is ideal for investigating whether megafaunal extirpations and biological invasions modulate any rainfall-dependent relationships between ant–mutualist activity and belowground biomass. Since at least the 1950s, the extirpation of large browsers in East Africa (Ripple *et al.*, 2015) and replacement with grazing livestock has reduced the value of defensive mutualisms for host trees (Palmer *et al.*, 2008). In the last 15–20 yr, native ants have also been extirpated from large tracts in central Kenya by invasive *Pheidole megacephala* ants (common name ‘big-headed ants’), which numerically overwhelm and extirpate the native ants. Invaded trees are vulnerable to elephants and other browsers and suffer chronic herbivory (Riginos *et al.*, 2015), which has been shown to deplete carbohydrate reserves in other tree species (Piper & Paula, 2020). Moreover, big-headed ants may also damage tree root systems directly (Milligan *et al.*, 2022, 2023b) by tunneling and nesting in the upper soil layers (Viles *et al.*, 2021).

We used minirhizotrons to determine how the root surface area of individual acacia trees is shaped by rainfall, native mutualist ant activity, browsing by megaherbivores, and the invasive big-headed ants. We surveyed the root surface area of ant–acacias during a 2022 dry season and a 2023 wet season; in both seasons, rainfall varied substantially among replicate sites, creating a fivefold gradient in quarterly rainfall. Acacias were located in study plots established in 2016 that either excluded or permitted megabrowsers (elephants, giraffes (*Giraffa camelopardalis*), and black rhinoceros (*Diceros bicornis*)). Study plots were split across a natural experiment comparing ant–acacias with intact mutualisms, on which ant activity levels varied naturally, to ant–acacias invaded by big-headed ants in c. 2010, which completely lacked ant defense. Our study combined these natural and manipulative experimental components to address the following questions.

How is ant mutualist activity associated with tree root surface area under variable rainfall and herbivory?

Although we hypothesized a positive association between ant mutualist activity and roots, we also hypothesized that increasing mutualist ant activity would eventually return diminishing benefits for belowground biomass in the same way it does for aboveground biomass (Palmer & Brody, 2013). We also hypothesized that, because larger ant colonies should be better able to repel herbivores and thereby preserve resources that may then support belowground biomass, the positive association between ant mutualist activity and root surface area would be strongest and steepest when megabrowsers were present. Because browsing encourages aggression and associated nectar consumption by native ants and thereby motivates root foraging for water and other resources, we further hypothesized that the presence of megabrowsers would steepen the correlation between recent rainfall and root surface area.

Does root surface area increase with ground-nesting ant abundance, and does this relationship persist after invasion by big-headed ants?

We hypothesized that we would find a positive association between native ground-nesting ant abundance and root surface area in uninvaded sites, driven by parallel responses to variation in primary productivity across our study arrays. Even though big-headed ants may also increase soil nutrient availability (Riginos *et al.*, unpublished data), nest construction by big-headed ants in shallow soils can directly reduce acacia rooting (Milligan *et al.*, 2023b), and we hypothesized that acacia root surface area would not increase with big-headed ant ground abundance, even within fenced plots where invasion has not facilitated vertebrate herbivory since 2016.

How do browsing and invasive ants interact to affect tree root surface area and response to rainfall?

To characterize the effects of invasion mediated by herbivores, we first characterized how herbivores affect acacia rooting in uninvaded savannas. Because browsing necessitates regrowth aboveground (Gadd *et al.*, 2001) and reduces labile carbohydrates (Piper & Paula, 2020) that could otherwise be used for growth belowground, we hypothesized that trees exposed to megabrowsers (i.e. from open plots) would exhibit root systems with lower surface area. And because browsed trees with limited resources may be less capable of maintaining root systems during dry conditions and/or may be more incentivized to rapidly grow roots during rainfall to capitalize on resource availability, we further hypothesized that the roots of trees in open plots would be more rainfall-responsive than the roots of trees from exclusion plots.

We predicted that these strategies would change as a consequence of ant invasion. Because megaherbivores browse more heavily on invaded trees than on trees protected by native ants, and this heightened browsing on invaded trees has the potential to deplete carbohydrate reserves in the long term, we predicted

that megabrowser exclusion in invaded acacia populations would have a smaller positive impact on tree rooting than in uninvaded acacia populations. Also, because of high levels of browsing and associated resource depletion in invaded areas, we further expected that chronic herbivory in these areas would reduce the responsiveness of tree rooting to rainfall, relative to uninvaded areas.

Materials and Methods

Study site

We conducted this experiment from May 2021 through June 2023 at the Ol Pejeta Conservancy (OPC; 0°0'52", 36°51'58"), a 360-km² conservancy in Laikipia County of central Kenya. The area is semiarid savanna and receives 80–100 mm of monthly rainfall in wet seasons and 30–50 mm of rainfall in dry seasons (c. 500–700 mm of annual rainfall). From 1960–2002, wet seasons typically occurred from March to May and October to December (Mpala Research Centre, public records), although this has become less predictable and consistent since the early 2010s. OPC contains a large elephant population (up to 300 individuals per OPC records 2016–2018), which browse, break, and kill acacias (*Vachellia* fmr. *Acacia drepanolobium* Harms ex Sjöstedt) in areas where big-headed ants (*Pheidole megacephala* Fabricius) have invaded and extirpated native ants (Riginos *et al.*, 2015; Palmer *et al.*, 2021). Big-headed ant invasion fronts at OPC have expanded from areas of human disturbance, and large tracts of the black cotton savanna have been invaded for over two decades (Riginos *et al.*, 2015).

We leveraged ongoing work at OPC, in which we have replicated paired megabrowser exclusion plots and open (unfenced) control plots at each of three uninvaded sites ('MU', 'NU', and 'SU') and three invaded sites ('MI', 'NI', and 'SI'; where 'M' and 'N' and 'S' denote an 'array' of paired uninvaded and invaded 'sites'; Supporting Information Fig. S1) from 2016 to 2023. *Crematogaster mimosae* Santschi occupy > 70% of mature acacias in the three uninvaded sites (P. Milligan, unpublished data), which are located c. 3–9 km away from one another (Fig. S1). In the three invaded sites, located 0.5–2 km away from the uninvaded sites, big-headed ants have disrupted the native ant protection mutualism since at least 2010 (Milligan *et al.*, 2021). Based on estimates that big-headed ants spread across black cotton savanna at OPC at a rate of c. 50 m yr⁻¹ (Pietrek *et al.*, 2021), we assume that these invaded sites have been invaded for over 10 years, whereas the uninvaded sites are likely to remain uninvaded for 10 more years. The electric fences exclude primarily megabrowsers (i.e. those approaching or exceeding a metric ton) from 0.25-ha square plots, while permitting smaller browsers (e.g. eland (*Taurotragus oryx*), impala (*Aepyceros melampus*), Grant's gazelle (*Nanger granti*)) to access plots. Boran cattle (*Bos (primigenius) indicus*) were regularly permitted to graze in the exclusion plots to maintain similar grass biomass inside and outside fences. Fenced plots each contained 40–50 mature (> 1 m tall) acacias. We designated a c. 1-ha unfenced plot 50 m from each fenced plot (Fig. S2), each containing c. 30 mature acacias

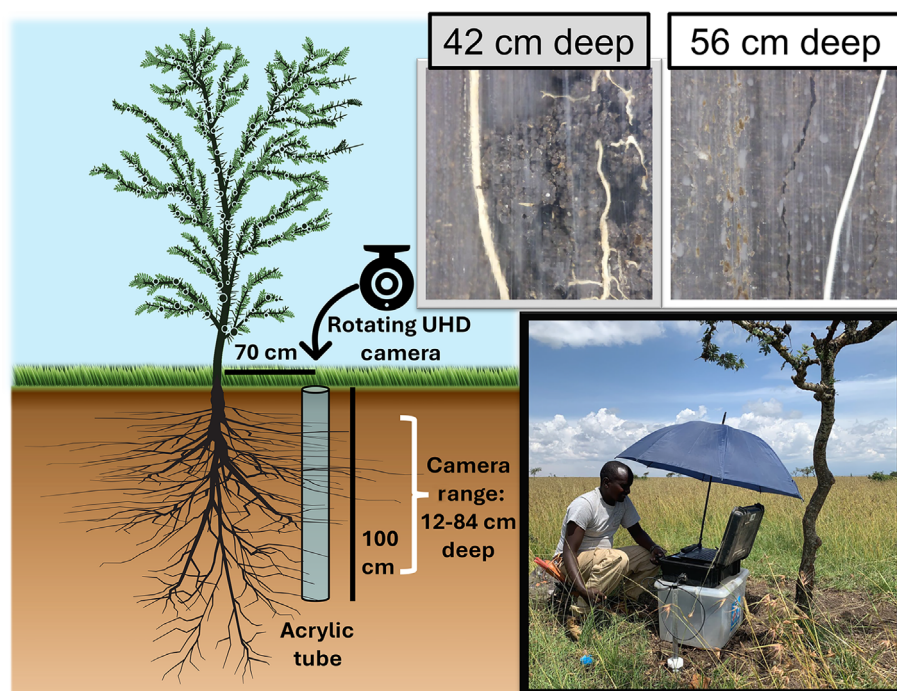


Fig. 1 Schematic of minirhizotron installation. (Left) Illustration of minirhizotron tube installment into the upper portion of an acacia (*Vachellia fmr. Acacia drepanolobium*) root system, with the tube installed 70 cm from the tree base at a 0° angle and nearly flush with the ground. (Upper right) example root images collected at 42 and 56 cm deep, reflecting typical higher root surface area at shallower survey levels. (Lower right) Author J. Mosiany is pictured collecting images of acacia roots from a minirhizotron tube in an uninvaded and unfenced site during a season of heavy rainfall. Some details are not to scale. Illustration elements by Ben Kolbus and arranged by P. Milligan.

in 2021. In sum, our experimental design consisted of megabrowser exclusion plots paired with open plots, on either side of an invasion front, for a total of 12 plots.

Minirhizotron installation and root surveys

To measure root surface area, we used minirhizotron root observation systems (Johnson *et al.*, 2001). Minirhizotrons can non-destructively quantify rooting strategies across seasons, illustrating how plants respond to herbivory, abiotic stress, and other adverse factors in water-limited systems such as black cotton savannas (e.g. Collins & Bras, 2007; Fensham & Fairfax, 2007). Within each of our 12 study plots, we selected four trees for minirhizotron installation ($n = 48$) in April 2021. Trees were selected that had similar heights (1.5–2.5 m tall), similar crown sizes ($c. 0.5 \text{ m}^3$), and no visible signs of recent browsing by megabrowsers or other mammals. All uninvaded trees were occupied by large native ant colonies (> 1000 workers) from at least April 2021 to July 2023. In invaded sites, big-headed ants nest in large numbers in the upper 15–20 cm of topsoil but forage in the acacia crowns (P. Milligan, pers. observations). All invaded acacia crowns had low abundances of big-headed ants (< 50 workers). To minimize the effects of intertree competition and to ensure that minirhizotrons would reflect the root systems of only one tree, most trees had no neighboring trees within a 5-m radius, and the small remainder of our trees had only a single tree neighbor that was at least three meters away. This selection criterion was necessary to link root image data with a single tree but also limits our inferences about how roots may respond to intraspecific competition when there are neighboring trees.

At 47 acacias, we installed a single clear acrylic minirhizotron observation tube in the root system, 0.7 m from the tree base, in

July 2021 (Fig. 1). One tube shattered in transit, and we randomly selected an uninvaded fenced tree at Site SU to exclude from our pool of 48 candidate trees. We chose not to install tubes at a 45° angle (which can improve quantification of root distribution per Bragg *et al.*, 1983) because hammering and then extracting the hollow drill bit at an angle appeared to widen the vertical and horizontal soil cracks (pers. observations during test installations, P. Milligan) that are naturally found across black cotton soil horizons (DeCarlo & Caylor, 2019). We were concerned that hollow gaps between the soil and tube surface could facilitate above-average root growth. By comparison, hammering and extracting the hollow drill bit at a 0° angle created more consistent soil-tube surface contact, and the installation angle could be easily standardized. After installation, we photographically surveyed the minirhizotron surface 12–84 cm deep to confirm tube-soil contact, check for scratches and large cracks and cavities, and create a library of reference images along the minirhizotron surface. Likely because of the disturbance of drilling and tube installation, this first survey photographed only soil and its images were not used for image processing or statistical analyses. We also measured the size of each focal tree at installation (will be described later). We allowed the tubes to settle for 10 months, then conducted one survey in May 2022 and a second survey in June 2023. Each minirhizotron was photographically surveyed once during each of these two surveys. One tube in an invaded unfenced plot was destroyed by wildlife between the 2022 and 2023 surveys, thus limiting our data from that tube to the May 2022 survey. During each survey, images were taken at 2-cm-depth increments (37 depth increments in total). At each depth, we took a single photograph facing toward the tree base, another away from the tree base, and one in each perpendicular direction, which altogether created a $c. 360^\circ$ view at each depth level (148

images constituting a single survey of each tree after installation, taken and in both May 2022 and June 2023). See Notes S1.1 for specifics on tube installation and maintenance, and see Notes S1.2 for camera details and photography protocol.

Surveys of aboveground covariates

We measured rainfall, tree size, ant activity, and ground-ant abundance during the experiment. We recorded weekly rainfall at each site throughout the experiment using a single rain gauge mounted on a wood post of each megabrowser exclusion fence. Rainfall was estimated cumulatively over the 3 months before each survey (see Notes S2, for rationale), and for brevity, we refer to this simply as ‘rainfall’ or ‘quarterly rainfall’ henceforth. Rainfall varied substantially among the six sites in the 3 months leading up to May 2022 (10–18 cm) and the June 2023 surveys (31–49 cm), and we thus treated rainfall as a continuous predictor in analytical models. Tree size in September 2021 was estimated in kg as a function of an allometric equation incorporating tree height and basal diameter developed at the neighboring Mpala Research Centre (E. Pringle and T. Palmer, unpublished data). We measured native ant activity during both photographic surveys (May 2022, June 2023) by selecting a random occupied domatium in the tree crown, designating a focal stipular spine, striking the domatium with a 21-g metal mechanical pencil three times in 3 s, and then counting the number of times a defending ant patrolled to the tip of that stipular spine from 0 to 30 s after the stimulus. Like in past studies in this system, we assume that this ant defense index is proportional to ant density on the tree, which is itself proportional to ant metabolic demand on its host (Fonseca, 1993; Palmer & Brody, 2013). Because our trees were size-matched with similar domatium density, we also assume that this ant defense metric is a reasonable index of relative metabolic demand from ants for trees with otherwise similar biomass and resource reserves. Finally, to determine the relationship between ground-nesting ants and root surface area, we also conducted monthly protein-baited transects to quantify the abundance of native ground-nesting ants and big-headed ants at each site from May to December 2022. We then calculated average monthly abundances of native ants and invasive ants for each site. See Notes S2, for further details.

Ranking study arrays by ground ant abundance

Big-headed ants often responded to protein baits in abundances over 10 times higher than those of native ants (see Notes S2, for ant abundance survey methods), in line with prior studies of big-headed ants (Wetterer, 2012; Milligan *et al.*, 2016). Big-headed ant workers composed 98.6% of all ground-nesting ants in invaded areas, whereas native ground-nesting ant abundance in uninvaded areas comprised workers from up to six morphospecies in the genera *Camponotus* and *Lepisiota*. Sites could be clearly ranked by site-level ground ant abundance (including all native and, where present, invasive ants) into a ‘low’ abundance array (paired sites ‘MI’ and ‘MU’), an ‘intermediate’ abundance array (paired sites ‘NI’ and ‘NU’), and a ‘high’ abundance array

(paired sites ‘SI’ and ‘SU’), such that the arrays themselves could be ranked from low to high ground ant abundance (‘M’ < ‘N’ < ‘S’) (see Table S1, for summary statistics of ant abundance from 2022 bait transects).

Convolutional neural network model training

We identified root and soil pixels in each survey photograph using a supervised computer vision model. Our computer vision model, a U-Net architecture convolutional neural network (CNN), was pretrained with PRMI (‘Plant Root Minirhizotron Imagery’) – a dataset of > 72 000 plant root images (Xu *et al.*, 2020) – and then further trained on 1522 images from our acacia dataset. The labeled dataset was randomly split into training (64% of labeled images), validation (16% of labeled images), and testing (20% of labeled images) groups. Images were preprocessed during training, with examples provided in Fig. S3. Across our 12 plots, the final model had an average precision of 0.909 ± 0.055 (SD) (rate of ‘true positives’), average recall of 0.528 ± 0.107 (SD) (rate of ‘true negatives’), and an overall F1 value (a harmonic mean of precision and recall) of 0.668. In other words, the CNN was highly conservative in what it would label as a ‘root’, and we are confident that pixels labeled as ‘roots’ were indeed roots. The CNN likely misidentifies fine root hairs and the outer edges of coarse roots as soil, meaning that our model likely underestimates root surface area across all trees.

From CNN pixel counts, we estimated root surface area at each depth level from 12 to 84 cm (the summed surface area from the four images at each depth; see details in Notes S3) and henceforth use root surface area (mm^2) as our response variable. Within the testing fold of 298 images, root surface area was estimated by both the expert user training the CNN (author JR) and the CNN itself through root annotation and segmentation. We fit Lin’s Correlation Coefficient (CCC) between these two estimates of root surface area using the DESCtools package in R. In line with our characterization of the model as conservative, a comparison of the line of best fit against a line of perfect concordance indicates that the CNN somewhat underestimates true root surface area (Fig. S4; Pearson’s correlation coefficient (precision) = 0.77; bias correction factor (accuracy) = 0.84).

Generalized additive models and sub-models

We used generalized additive models for location, scale, and shape (‘GAMLSS’) to estimate how rainfall, browsing, and big-headed ant invasion influenced acacia root surface area at each depth level. Importantly, generalized additive models allow for continuous variables to have nonlinear or ‘smoothed’ relationships with the response variable instead of following an assumption of linearity. This was ideal to accommodate the nonlinear trend in root surface area with depth (Fig. S5). We fit all root surface area models to a lognormal error distribution. In all models, we used the Rigby and Stasinopoulos fitting algorithm (Rigby & Stasinopoulos, 2005). We investigated model fit using diagnostic plots in the GAMLSS package to confirm normality of residuals and

homoscedasticity. What follows is the approach to statistical analyses for each of our three study questions.

(1) *How is ant mutualist activity associated with tree root surface area under variable rainfall and herbivory?* To characterize ant–plant rooting response to recent rainfall and the potentially saturating positive relationship between mutualist ant activity and root surface area, as well as how those two associations depend on browsing pressure by megaherbivores, we used data only from the three uninvaded sites within each array (for a total of 23 trees surveyed twice between May 2022 and June 2023) to construct a GAMLSS for root surface area at each depth level (37 levels from 12–84 cm deep) in R 4.3.1 (R Core Team, 2021), using the package GAMLSS (Rigby & Stasinopoulos, 2005). As fixed effects, this model included rainfall, native ant activity, fence treatment, and two interactions: rainfall $\times$ fence treatment and native ant activity $\times$ fence treatment. We included array ranked by ground ant abundance ('M' < 'N' < 'S'; limited to 'U' sites only) as an ordinal categorical predictor, and we included initial tree aboveground biomass (estimated allometrically in July 2021), survey depth, and native ant activity as nonlinear covariates using p-splines. We included tree ID as a random intercept. The model shown as an equation and a causal diagram, with justifications for expecting nonlinear relationships of root surface area with depth and host plant biomass, is found in Notes S4.1, and model predictions are also laid out in Table S2.

(2) *Does root surface area increase with ground-nesting ant abundance, and does this relationship persist after invasion by big-headed ants?* To test our hypotheses for Questions 2 and 3, we then constructed a GAMLSS of root surface area at each depth level using data from all six study sites (three uninvaded sites and their invaded counterparts within each array 'M', 'N', and 'S') (in equation and diagram form in Notes S4.2). Fixed effects included rainfall, fence treatment, invasion status, and all possible two- and three-way interactions among those three effects. We included array ranked by ground ant abundance ('M' < 'N' < 'S') as an ordinal categorical predictor that interacted with invasion status. This model omitted native ant activity because it is confounded with invasion status. All other predictors (tree aboveground biomass, survey depth, and tree-specific intercepts) were the same as the model described for Question 1.

For Question 2, we predicted that root surface area would be higher in arrays with higher ground-nesting ant abundance because both rooting and ant abundance should be positively affected by array-level productivity (Pringle *et al.*, 2019). However, for the invaded sites within the arrays, we predicted that root area would not increase with ant abundance because invasive ants in high abundance should suppress primary productivity (Coupe & Cahill, 2003) and because big-headed ant nest excavation may interfere with acacia root growth (Milligan *et al.*, 2023b). In other words, we expected to find a significant interaction term for invasion status and array. If this prediction were borne out, we would expect that the linear coefficient for 'array' would be positive, but that the coefficient of the interaction of array and invasion would be negative and significant (laid out with other model predictions in Table S3).

(3) *How does long-term big-headed ant invasion affect tree root surface area responses to megaherbivore exclusion and variance in rainfall?* Using the same model described above in Question 2, we first assessed whether acacias exposed to megabrowsers have lower root surface area in general, as they would if vertebrate herbivory reduced available resources for root growth. Because browsing may reduce acacias' capacity to maintain roots in dry seasons and/or incentivize acacias to grow roots rapidly when it rains, we also predicted that the association of root surface area with recent rainfall would be stronger and steeper for megabrowser-exposed trees.

However, because we hypothesized that years, if not decades, of elevated browsing caused by big-headed ant invasion (Riginos *et al.*, 2015) have reduced acacia resource reserves, we predicted that megabrowser exclusion from long-term invaded areas would be linked to only muted increases in root surface area. And because this hypothesized resource depletion for long-term invaded acacias may limit the capacity of acacias to capitalize on recent rainfall, we also predicted that the correlation between root surface area and rainfall would be less steep and less influenced by megabrowser exclusion for invaded trees than for uninvaded trees.

Unit conversion and partial effects plots

As a final data preparation step before model analyses and visualizations, we scaled our estimates of root surface area along the minirhizotron tube (*c.* 2034 cm²) to the surface area of the entire column of soil encircling the tree root system 70 cm from the tree base, 12–84 cm deep (*c.* 1.1 million cm²). Through uniform arithmetic, this converts units from the size of a minirhizotron (a very small perspective of a tree's root system) to a more biologically meaningful scale of a band of soil encircling an entire tree as if the minirhizotron completely encircled the tree 70 cm from its base. The conversion does not alter the relative distance between any data points; it simply upscales our index of root surface area by a constant.

To visualize how specific effects of interest influenced our model estimates for root surface area, we used each GAMLSS to predict tree-level root surface area while other model predictors were held constant at their median or reference values (Stasinopoulos *et al.*, 2024, pp. 107–108). This approach allows us to display partial effects from each model in units of upscaled tree-level root surface area (Figs 2–4) instead of as proportional impacts on the model outcome.

Results

Positive associations in the tree-ant mutualism under variable rainfall and herbivory

In sites with native ant mutualists, acacia root surface area increased with rainfall, and we found a positive correlation between root area and native ant activity in megabrowser-exposed trees – except when ant activity reached an upper saturating threshold in wet conditions (Fig. 2). For these acacias in open plots (our reference group in our analysis), our model estimated a $3.64\% \pm 0.43\%$ (SE) increase in root surface area per cm

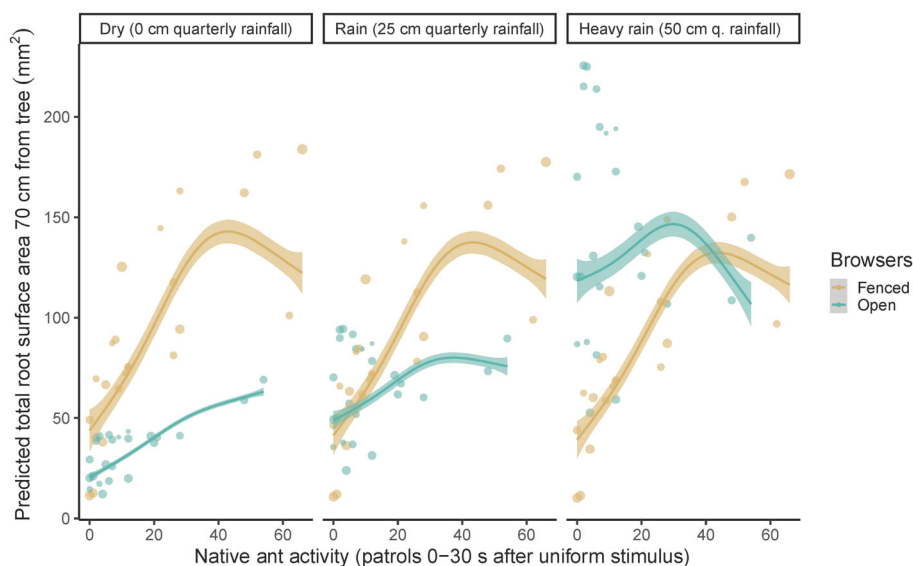


Fig. 2 Saturating positive relationship between mutualistic ant activity and root surface area. Variable nonlinear relationships of mutualist ant (*Crematogaster mimosae*) defensive activity with acacia (*Vachellia fmr. Acacia drepanolobium*) root surface area for host trees under different herbivore pressure (fenced vs open) and rainfall scenarios (dry, rainy, and heavy rain). The y-axis illustrates acacia total root surface area (mm^2) encircling the tree root system from 12 to 84 cm deep and 70 cm away from the tree trunk, as predicted by the first generalized additive models for location, scale, and shape (native ant only) with a pseudo- R^2 of 0.303. Points show root surface area predictions from the raw survey data and represent tree-level variance relative to the non-linear depth : root surface area relationship (Supporting Information Fig. S5), with adjustments for dry, rainy, and heavy rain conditions (L: 0 cm rainfall, C: 25 cm, and R: 50 cm, respectively). Curves show model predictions and 95% confidence intervals if all other continuous predictors (tree size, ground ant abundance) were 'excluded' (held constant at their median). Point size is proportional to tree initial biomass. Deviance outside those error envelopes is driven by excluded model predictors and unmeasured sources of variance.

rainfall ($\beta = 0.036$, $t = 8.34$, $P < 0.001$). We identified a positive association between root surface area and ant activity rate ($t = -3.95$, $P < 0.01$) for trees experiencing dry to light-rain conditions ($c.$ 0 and 25 cm rainfall; Fig. 2a,b). However, when rainfall was high ($c.$ 50 cm), root surface area had a positive association with ant activity at low and moderate ant activity rates ($c.$ 0–30 attacks per trial) that then saturated and became a negative association (Fig. 2c). This model predicted 30.3% of the variation in root surface area (pseudo- R^2).

When megabrowsers were excluded from trees with native ant mutualists, root surface area was still positively predicted by native ant activity up to a saturating threshold, but that relationship and threshold appeared to be consistent regardless of recent rainfall (Fig. 2). Unlike with trees in open plots, the positive association between root surface area and native ant activity for trees in exclusion plots always saturated at a threshold of $c.$ 40 ants attacking in the 30 s following a uniform domatium disturbance, which was consistent regardless of rainfall ($t = 4.84$, $P < 0.001$; Fig. 2). Root surface area within megabrowser exclusion plots did not depend on rainfall ($\beta = -0.114$, $-0.22\% \pm 0.73\%$ decline per cm rainfall), which differed from the association observed for trees in open plots ($t = -5.24$, $P < 0.001$).

Tree rooting has contrasting relationships with abundances of native vs invasive ants

Root surface area increased by $40.5\% \pm 7.85\%$ with each 'step up' in array rank by ground ant abundance in uninvaded sites

('MU' to 'NU', 'NU' to 'SU'; linear term for ordinal predictor; $\beta = 0.61$, $t = 8.18$, $P < 0.001$). By contrast, for acacias in invaded areas, root surface area remained relatively static with each 'step up' in array rank by ground ant abundance ('MI' to 'NI', 'NI' to 'SI'; $-14.19\% \pm 33.34\%$ change with each step), and this significantly differed from the ranked-array effect in uninvaded areas ($\beta = -0.51$, $t = -4.95$, $P < 0.001$). These array rank effects were not significantly changed by megabrowser exclusion in either invasion scenario (Fig. 3; fencing $\times$ array (linear): $\beta = 0.14$, $t = 0.95$, $P = 0.34$; fencing $\times$ invasion $\times$ array (linear): $\beta = 0.28$, $t = 1.36$, $P = 0.17$).

How does ongoing mutualism disruption change tree rooting responses to rainfall and herbivory?

Within our second model, which included the minirhizotron data from all six sites, root surface area of invaded acacias was substantially less responsive to rainfall than that of uninvaded acacias, and megabrowser exclusion had a positive effect on root surface area only among acacias protected by native ants. On its own, ant invasion was not linked to a difference in root surface area ($5.38\% \pm 20\%$ increase in root area among invaded trees, $\beta = 0.05$, $t = 0.28$, $P = 0.78$). Nevertheless, relative to uninvaded trees that exhibited $c.$ $3.41\% \pm 0.45\%$ more root surface area with each cm of rainfall (rainfall: $t = 7.51$, $P < 0.001$), invaded trees produced 37% less root area per each cm of rainfall ($2.16\% \pm 1.1\%$ increase per cm rainfall; invasion $\times$ rainfall: $t = -1.95$, $P = 0.05$). Furthermore, megabrowser exclusion

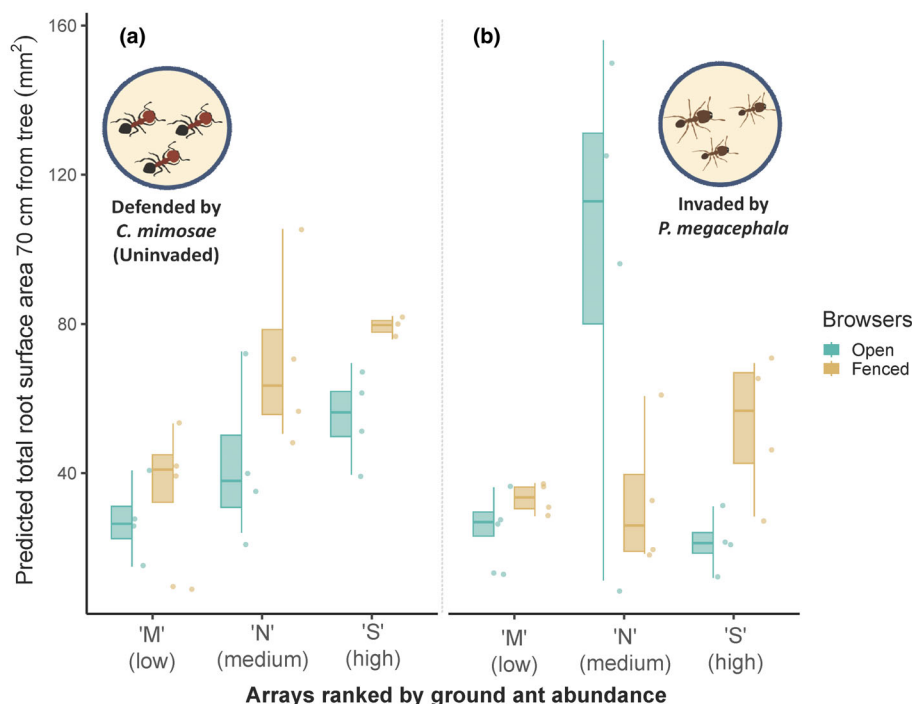


Fig. 3 Differential associations of acacia root surface area with native vs invasive ground-nesting ant abundance. Predicted total root surface area increased with array-level ground ant abundance, but only in uninvaded arrays. Panel a shows uninvaded (occupied by *Crematogaster mimosae*) acacias (*Vachellia fmr. Acacia drepanolobium*); panel b shows invaded (occupied by *Pheidole megacephala*) trees; boxes show median and quartiles and are separated by herbivore treatment (open vs fenced) and invasion status (uninvaded vs invaded); whiskers show the range of non-outlier points, and outliers are plotted as individual points outside of that whisker range. Arrays were ranked by ground ant abundance (low, medium, high). Each step up in ordinal abundance for uninvaded arrays was linked to a significant $40.5\% \pm 7.7\%$ increase in root area, whereas changes in ordinal abundance were not associated with a difference in root area for invaded arrays. Points and boxplots have been adjusted (using predictions from our overall generalized additive models for location, scale, and shape model) as if tree biomass and rainfall were held constant at their median.

from uninvaded acacias drove a doubling of root surface area ($114\% \pm 21\%$ increase in surface area when fenced; $\beta = 0.76$, $t = 3.9$, $P < 0.001$), whereas megabrowser exclusion from invaded trees did not ($-34.33\% \pm 57.72\%$ change in root surface area). Despite the large apparent difference in these effects of fencing in each invasion scenario, the interaction of ant invasion with megabrowser exclusion was only marginally significant ($\beta = -0.47$, $t = -1.75$, $P = 0.07$; Fig. 5).

We found no strong evidence for a three-way interaction among fencing, invasion, and rainfall ($\beta = 0.011$, $t = 1.3$, $P = 0.20$); rather, fenced, herbivore-excluded trees increased root surface area in response to rainfall only weakly (increasing only $1.15\% \pm 1.11\%$ per cm of rainfall, whereas rooting increased by $3.41\% \pm 0.45\%$ per cm of rainfall in our unfenced, uninvaded trees), and this effect was consistent in both uninvaded and invaded areas. This unified GAMLSS explained *ca.* 28.8% of the variance in root surface area (pseudo- $R^2 = 0.288$).

Discussion

Our field study demonstrates that interactions among savanna trees, insect mutualists, and megabrowsers shape root surface area and rooting responses to rainfall. We found evidence in support of our first hypothesis (Question 1) that ant mutualist activity has a positive association with host plant root surface area, but that this association is conditional on rainfall, megabrowser presence (and the associated benefit of mutualist activity), and the maintenance of ant activity (and associated metabolic cost of that activity) within a certain range. The positive association between ant activity and root area saturated when rainfall was particularly high, and/or exclusion of megabrowsers reduced the value of ant defense, and/or native ant defense was particularly high. We describe a saturation threshold between native ant activity (*c.* 40th

percentile) and root surface area that is approximately double the saturation threshold for the positive association between ant activity rates and the avoidance of vertebrate browser damage (*c.* 20th percentile) described previously in this system (Palmer & Brody, 2013).

We also found evidence in support of our hypothesis (Question 3) that invaded trees had weaker rooting responses to megabrowser exclusion and to rainfall than trees with intact biotic defenses. And although our study design was not focused on invasive ant abundance and behaviors in the soil, we also found evidence consistent with our hypothesis (Question 2) that the soil nesting habits of big-headed ants disrupt acacia rooting (Milligan *et al.*, 2022, 2023b). It is still difficult to generalize invasive ant impacts on plant performance and fitness because they often interact with novel host plants through complex or context-specific pathways (Lach, 2003): for example, *Anoplolepis gracilipes* and *Linepithema humile* steal host plant nectar yet provide no defense (Lach, 2005), and *L. humile* monopolizes but poorly disperses plant seeds (Devenish *et al.*, 2019). Our field experiment highlights the potential for common mechanisms, such as herbivory facilitation and soil/root disturbance, by which invasive ants can influence woody plant roots. Minirhizotrons provide long-term insight into how tree-ant mutualisms can impact below-ground tree physiology under different rainfall and herbivory regimes, as well as novel insights into how ant invasion impacts tree rooting.

Associations between tree investments in roots and biotic defense

Past studies of aboveground acacia traits and productivity and our present study of acacia rooting together suggest that ant defensive activity often facilitates but can compete with whole-

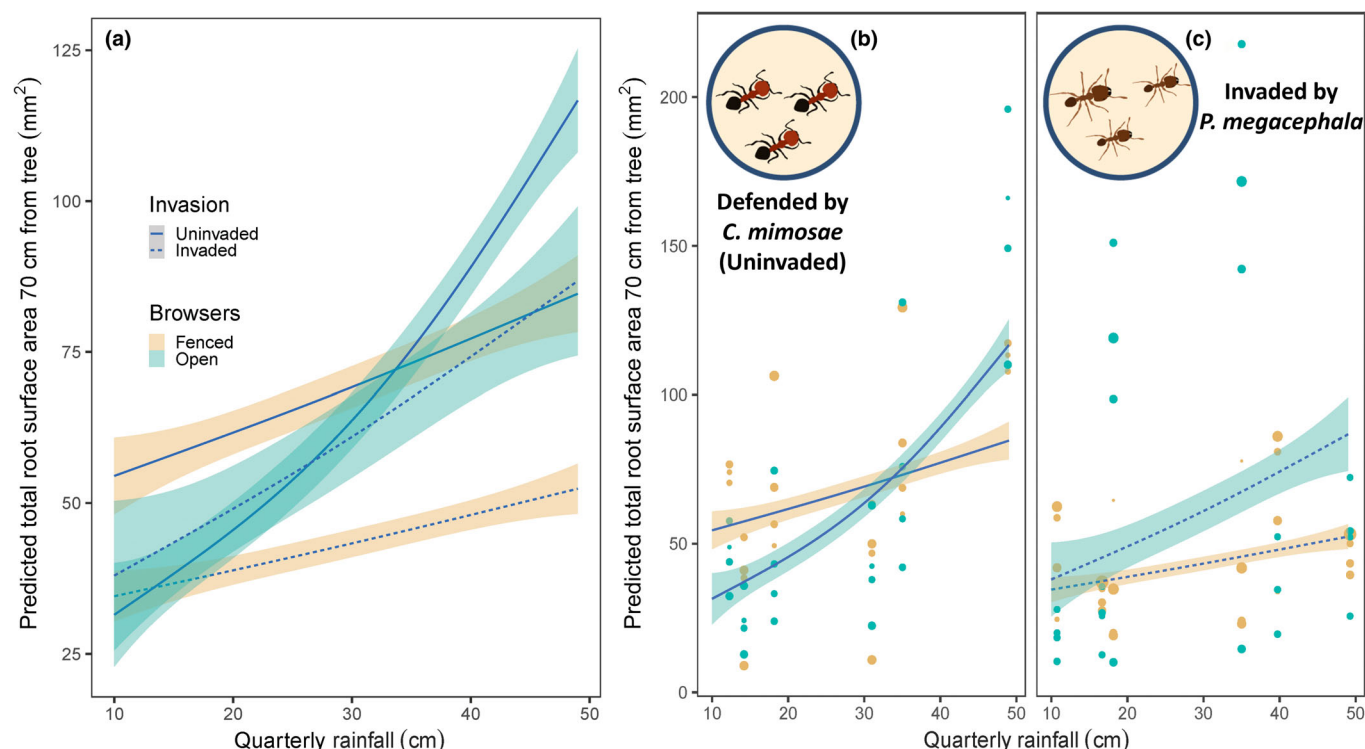


Fig. 4 Differential association of root surface area with rainfall for acacias in a factorial experiment crossing large browser presence/exclusion against intact/disrupted defensive ant mutualism. Acacia (*Vachellia* fmr. *Acacia drepanolobium*) root surface area increased with rainfall differentially based on tree exposure to vertebrate browsers (Open vs Fenced) and the status of the tree's biotic defense by mutualistic ants: model projections for root surface area–rainfall associations for all acacias are represented in (a) ($n = 93$; 47 acacias surveyed in 2022 and 46 in 2023); (b) displays data and projections only for trees with intact mutualisms with acacia-ants (*Crematogaster mimosae*) ($n = 46$; 23 acacias surveyed in both 2022 and 2023); and (c) displays data and projects only for trees in which the mutualism has been disrupted by big-headed ant (*Pheidole megacephala*) invasion ($n = 47$; 24 acacias surveyed in 2022 and 23 in 2023). Response to rainfall, which varied among sites within each survey, is expressed as predicted total root surface area (mm^2) along the outer surface of the 70-cm-radius volume of soil surveyed by the minirhizotron system from 12 to 84 cm deep. Lines show model predictions, with shaded regions showing the 95% confidence interval for those predictions; point size is proportional to tree initial biomass. Values were predicted by the generalized additive models for location, scale, and shape that tested the second and third set of hypotheses, with a pseudo- R^2 of 0.288; lines illustrate model estimates and SE for the partial effect of rainfall on root surface area within each experiment group. These lines and error envelopes become less representative of the net contrasts between treatment groups as rainfall increases (Fig. 5).

plant growth and performance in these ant-acacias. This may hold true across ant–plant protective partnerships.

Benefits of ant–plant mutualisms have been inferred in many other systems in which ant activity (and associated metabolic demand, when ants depend on host plants for food) correlates positively with host plant aboveground performance (e.g. herbivory, growth, and reproduction; Rosumek *et al.*, 2009; Trager *et al.*, 2010; Frederickson *et al.*, 2012). Ant–plants allocate substantial resources to domatia and/or food, but such defense often reduces herbivory on the host plant over the short (≈ 1 yr) (e.g. Heil *et al.*, 2001; Frederickson *et al.*, 2012) and long (> 5 yr) terms (Heil *et al.*, 2001; Gomes *et al.*, 2021), thereby maintaining photosynthetic surface area and reducing regrowth costs. Through these mechanisms, ant defense boosts acacia carbon fixation (Milligan *et al.*, 2021, 2023a), and that carbon influx can then support other resource sinks: even under high browsing pressure, these acacias can maintain large ant colonies and leafy crowns (Madden & Young, 1992) that fix twice as much carbon as undefended trees (Milligan *et al.*, 2023a). Here, we show that

ant mutualist activity can positively correlate with root surface area despite a lack of direct ant–root interaction, such that the positive influence of ants on host plants appears to extend belowground.

Focusing on roots allows us to examine an important, lesser-studied sink for fixed carbon and identify positive associations between trees and ants that may not be evident aboveground. Using ant activity metrics like we used here, Palmer & Brody (2013) found that the benefits of ant defense against elephants and galling midges saturate at a much lower threshold (10 ants per 30 s trial) aboveground than we found belowground (20–30 ants per 30 s trial). Acacias thus display a positive association between ant colony size and rooting even when ant abundance is higher than necessary to repel herbivores. Possible mechanisms for this result include that nutrient inputs from larger colonies (Wagner, 1997; Pinkalski *et al.*, 2015, 2018) could promote root growth, or perhaps insect/pathogen damage to the tree continues to decrease as ant density increases. Whatever the mechanism(s), our study establishes that positive associations in

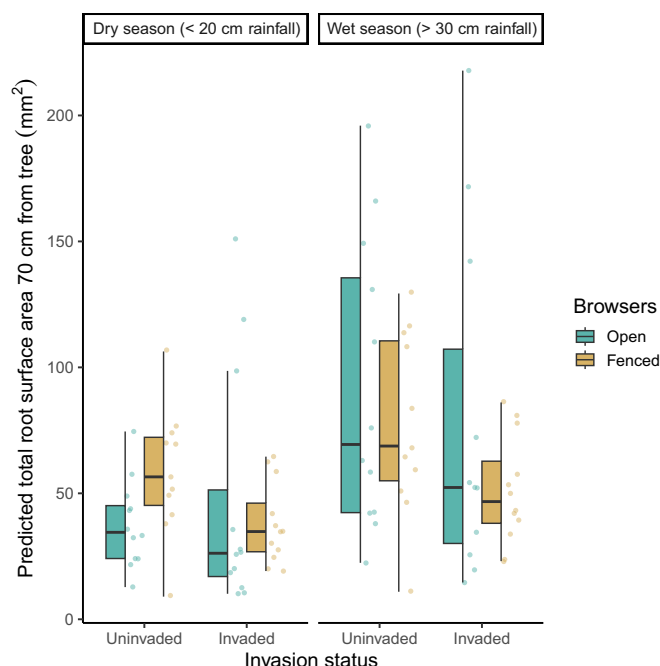


Fig. 5 Wet- and dry-season root surface area estimates for acacias in a factorial experiment crossing large browser presence/exclusion against intact/disrupted defensive ant mutualism. Acacia (*Vachellia fmr. Acacia drepanolobium*) root surface area increased with rainfall differentially based on tree exposure to vertebrate browsers (Open vs Fenced) and the status of the tree's biotic defense by mutualistic ants (intact mutualism with *Crematogaster mimosae* in Uninvaded plots, devoid of mutualists due to big-headed ants (*Pheidole megacephala*) in invaded plots). Boxes show medians and interquartile range, whiskers show the range of non-outliers, and outliers are plotted as individual points outside of whiskers. Whereas rainfall is treated as a continuous predictor in our models and in Fig. 4 because rainfall varied substantially among our six study sites in both survey months, this figure bins the data into dry (< 20 cm rainfall; 2022) and wet (> 30 cm rainfall; 2023) periods to visualize contrasts between experimental groups in the two minirhizotron surveys.

ant–plant partnerships can extend to belowground sinks that do not interact directly with ant mutualists. This is further evidence that biomass-guarding ant partners also increase the host tree resource pool (Milligan *et al.*, 2021), which may affect resource allocation strategies (Mizell *et al.*, 2023).

Importantly, however, ant activity rates and root surface area were decoupled under two conditions. First, ant activity was decoupled from root surface area when the activity rate (shown to be proportional to ant density, and therefore proportional to metabolic demand on trees of similar size; Fonseca, 1993; Palmer & Brody, 2013) was very high. Whereas ant colonies of low-to-moderate activity reduce elephant browsing and stem parasites with the same efficacy as more active/dense colonies (Palmer & Brody, 2013), larger colonies probably extract more carbohydrates (Stanton & Palmer, 2011). Nectar production is a substantial drain on the photosynthate budget of stressed ant–plants (Bronstein, 2001; Newman & Wagner, 2013). On trees with especially active ant colonies (> 40 ants responding to our standardized disturbance over 30 s), root surface area declined with

the ant activity rate, perhaps because host trees must reduce allocation to other sinks to support ant nectar consumption. It has been debated how often plants sanction their mutualist partners when costs outstrip benefits to the host (e.g. Weyl *et al.*, 2010; Kiers *et al.*, 2011): but, for example, host plants can reduce carbohydrates for suboptimal bacterial symbionts (Kiers *et al.*, 2003). However, in addition to consuming extrafloral nectar, native ants in our system can also tend sapsucking hemipterans (*Ceroplastes* sp. and *Hockiana* sp.) (Prior & Palmer, 2018), which exude carbohydrate-rich honeydew, and hemipteran abundance on branches positively correlates with native ant colony size (P. Milligan, unpublished data). Acacias may thus only partially control resource allocation to their ant colonies (Prior & Palmer, 2018). It is important to note, however, that even if large ant colonies limit short-term rooting, high-mortality wars of attrition with neighboring ant colonies (Palmer, 2004), which displace 8–10% of ant colonies per year (Palmer *et al.*, 2010), may periodically reduce colony size to an optimum that protects the tree and facilitates root growth, thus restarting the fidelity feedback loop (*sensu* Archetti *et al.*, 2011).

Second, positive associations between ant activity and root surface area weakened when rainfall was high (Fig. 2, right panel). Like with other tropical and temperate-zone trees (Green *et al.*, 2005; Cordeiro *et al.*, 2020; Dong *et al.*, 2022), recent rainfall positively correlated with root surface area. Under very high rainfall, however, higher root surface area likely yields diminishing benefits. Under wet conditions, ant colonies may continue to increase in density in conflict with the tree if ants control their carbohydrate budget via hemipteran tending. Alternatively, trees may produce more nectaries and nectar for ants if ants stimulate the production of vulnerable new acacia growth that follows heavy rains (Okello *et al.*, 2001; Pringle *et al.*, 2011). Combining minirhizotron surveys with plant water and carbon status surveys could determine whether acacias reduce root surface area investment because of carbon limitation or because of diminishing benefits of root investment for water acquisition. In addition, the development of computer vision models to distinguish coarse and fine roots from minirhizotron images, as well as the quantification of other root traits such as root specific length and diameter, could shed light on how aboveground interactions among host plants, ants, and herbivores influence root function.

The influence of herbivore regime on tree rooting strategies

In the absence of herbivores, root surface area increased strongly with ant activity from low to intermediate attack levels, indicating that a positive association between ant activity and root area persists but with a lower saturation threshold than in the presence of herbivores. Ants inside fences do not repel vertebrate herbivores, but they may still benefit their host tree by attacking other invertebrates, including branch-galling insects and stem-parasitizing beetles (Palmer & Brody, 2013). In addition, whereas ant attacks against elephants likely stimulate ant feeding because of the ants' energy expenditure, attacks against insects may have a comparatively smaller effect on ant carbohydrate consumption. Because acacia-ants consume their insect prey

(Palmer, 2003), they may seek out less EFN or honeydew following defense against insects: that is, the cost of defense may be 'paid' in part by the prey itself (but see, e.g., Ness *et al.*, 2009). Behavioral experiments are needed to determine whether acacia-ants change their nectar consumption (metabolic demand) following defense against different herbivore guilds, which could help explain our findings. Any defense that ants may provide against enemies other than vertebrate browsers could likewise reduce host plant compensation and maintenance costs (Stephens & Westoby, 2015; Kahuthia-Gathu *et al.*, 2018) and free up labile carbon for growth; however, this predation on insects in the tree crown may also stimulate ant nectar demand to maintain stoichiometric balance (Ness *et al.*, 2009; Arcila Hernández *et al.*, 2017).

Overall, vertebrate browsers caused a steeper correlation between rainfall and root surface area but decreased overall root surface area. These outcomes are in line with rooting responses to herbivory by other tropical trees, which often reduce rooting in dry periods (Green *et al.*, 2005; Cordeiro *et al.*, 2020) and expand roots to support compensatory regrowth in rainy seasons (Clarke *et al.*, 2013), but also can prioritize root maintenance over net root growth in response to herbivory (Mundim *et al.*, 2017). Tree rooting responses to aboveground herbivory can be contingent on the type and intensity of herbivory (Perkovich & Ward, 2021). Our study area hosts a relatively large population of elephants (1 individual for every 1.2 km²), and elephants are particularly destructive browsers of these trees. It is possible that ant-acacia rooting responses to aboveground herbivory are less evident in regions where herbivory is less of a threat than other factors such as fire (Wigley *et al.*, 2019). Considering that even stem damage by aboveground herbivorous insects has been linked to increases in root surface area for grasses and forbs (Heinze, 2020), future work should consider how different types of herbivory (damage type, intensity, and tissue) relate to ant-acacia rooting strategies. There are also other possible negative effects on rooting that are specific to megaherbivores: large mammals trample and compress cracks that form in dry soil (DeCarlo & Caylor, 2019), which may shear acacia roots. Untrampled roots inside megaherbivore exclusion fences may thus persist more consistently through time, requiring less rainy season expansion. Finally, rainfall's influence on tree rooting is potentially confounded with seasonal changes in browsing pressure. Because mixed feeders tend to graze rather than browse during rainy periods (Birkett & Stevens-Wood, 2005; Staver *et al.*, 2021), rainfall can decrease browsing pressure. Future work could profitably account for seasonal shifts in herbivore pressure that may underpin acacia rooting responses to rainfall.

Evidence consistent with direct and indirect negative effects of big-headed ants on root surface area and rooting responsiveness to rainfall and herbivory

There exist at least two, nonmutually exclusive explanations for the negative effects of big-headed ants on tree root area. The first is that soil bioturbation and nesting by big-headed ants may directly suppress acacia rooting (Milligan *et al.*, 2022, 2023b).

The positive association of root surface area with native ground ant abundance but not with big-headed ant ground-level abundance suggests that big-headed ants were counteracting otherwise positive associations between ant and root abundance that are driven by array-level productivity. Within the same model, we also found a marginally significant interaction between big-headed ant invasion and herbivore exclusion, suggesting an additional pathway: unlike uninvaded acacias, invaded acacias do not respond to a release from megaherbivore pressure either by increasing root surface area or with a change in rooting response to rainfall. These two mechanisms may result from two traits typical of invasive ants: alteration of soil disturbance regimes (Holway *et al.*, 2002); and extirpation of native ants (Holway *et al.*, 2002; Lach & Hooper-Bui, 2010; Siddiqui *et al.*, 2021), such as the defensive *Crematogaster* ants in this study.

Future studies investigating a direct interaction between big-headed ants and tree roots would benefit by tracking big-headed ant abundance at finer spatial resolution and longer temporal scales, while accounting for primary productivity and soil characteristics. Plant primary productivity frequently increases with ant abundance (noted in Amazonia by Pringle *et al.*, 2019), but when insect abundance surges during outbreaks as happens during invasions, it can suppress primary productivity (a temperate-zone meta-analysis by Coupe & Cahill, 2003). Big-headed ants in this study indeed reached abundances orders of magnitude higher than those of native ants. Big-headed ants disrupt soil and even destroy wires in other systems (Wetterer, 2012), and we have previously shown the coarse root density of invaded *V. drepanolobium* acacias is reduced in the 0- to 20-cm stratum of soil (Milligan *et al.*, 2023b) where big-headed ants typically nest (Broekhuysen, 1947). A second, not mutually exclusive, explanation for the lack of association between invasive ant abundance and root surface area is that big-headed ants, like other *Pheidole* ants, increase soil C, N, and P (Shukla *et al.*, 2013; C. Riginos unpublished data), thereby increasing root foraging efficiency. The survey depth of our minirhizotron system (12–84 cm deep) was too deep to characterize tree and grass root patterns resulting from nutrient input by invasive ants in the uppermost *c.* 10 cm of soil, where such modifications may be strongest, and the impacts of big-headed ants on vertisol soil chemistry warrant future work. Nevertheless, we believe that existing evidence supports our hypothesis that invasive ant soil presence disrupts tree rooting because it explains both why invaded trees have lower hydraulic conductivity (Milligan *et al.*, 2021) and why root surface area does not correlate positively with inferred site productivity in invaded areas. The consistent evidence for this mechanism in our work suggests that big-headed ants may directly disrupt tree rooting in other invaded ecosystems.

Tree rooting responded weakly to megabrowser exclusion in invaded areas, suggesting that trees with a long history of invasion accumulate a cost of chronic herbivory. Chronic damage may shift trees to an alternative, resource-limited rooting strategy that is less responsive to rainfall and herbivory. Vertebrate browsers remove substantial tree biomass in invaded areas (Riginos *et al.*, 2015), which may shrink the tree's labile carbon pool. Although invaded trees maintain *c.* 1.5% more starch in their

stems (P. Milligan and E. Pringle, unpublished data), that small increase in concentration does not offset their systemic biomass loss and reduction in the overall storage pool (Riginos *et al.*, 2015; Milligan *et al.*, 2021). An integrative ecophysiological approach, tracking carbohydrate allocation and outcomes across the whole tree, could determine whether carbohydrate limitation in invaded acacias limits root growth (Schutz *et al.*, 2011; Miranda *et al.*, 2020), and/or slows the rooting response to megabrowser exclusion (i.e. big-headed ants may reduce acacia 'resilience' as defined by Holling, 1973; Gunderson, 2000). Repeated and expanded minirhizotron surveys could also characterize time lags in the invaded-acacia rooting response to megabrowser exclusion. Acacia photosynthesis declines 5–10 yr after invasion, rather than immediately after invasion (Milligan *et al.*, 2021). Tree belowground adjustment may lag yet further behind aboveground adjustments (Du & Fang, 2014). This may explain why megabrowser exclusion from invaded tree stands increased leafiness and carbon fixation within 2–3 yr (Milligan *et al.*, 2021) but did not increase acacia rooting after 7+ years (Fig. 5).

Conclusions

To understand positive associations that reinforce plant–insect mutualisms, we need field experiments that connect mutualist cost to whole-plant host benefit, as well as examinations of how such connections respond to global change (McGlynn, 1999; Vidal *et al.*, 2021; Wong *et al.*, 2023). Here, we describe a positive linkage between defensive ant mutualist activity and host plant rooting that delivers host benefits across a much wider range of ant activity (here assumed to reflect ant density in the tree crown) than we would have inferred from evidence of aboveground herbivory reduction alone. Herbivore extinction has accelerated across the globe (Ripple *et al.*, 2015), and climate change is expected to lengthen droughts and shorten heavy rains in East Africa (Haile *et al.*, 2020; Wainwright *et al.*, 2021). Associations between biotic defense and host plant root surface area may be particularly altered by global change (Kiers *et al.*, 2010; Vidal *et al.*, 2021). Root surface area responded more strongly to rainfall when mutualists and/or herbivores were present, implying that mutualism breakdowns and herbivore declines will influence ant–plant rooting responses to ongoing changes in rainfall. Focusing on well-conserved traits of invasive ants – soil bioturbation, predation of native insects – can refine predictions for how this third agent of global change will impact plant performance. Plant–insect mutualisms and their breakdown have consequences for plants' abilities to forage, store resources, and support regrowth, with the potential to impact the persistence of tree populations.

Acknowledgements

The authors thank Sami Akiba and Jesper Christensen for assisting with data collection. The authors thank Samuel Mutisya and Benard Gituku for providing logistical support by Ol Pejeta Conservancy's dedicated and talented staff throughout the project. The authors thank Cosmas Nzomo and Nelly

Palmeris for providing additional logistical support from Mpala Research Centre during minirhizotron installation, and Oreste Casadio for providing extensive mechanic services for drilling equipment. This work was financially supported by NSF EAGER DEB-1935498 to EGP and NSF DBI-2010075 to PDM. Large language models were not used in any way to produce this article.

Competing interests

None declared.

Author contributions

CR, JRG and TMP conceived of the initial field experiment at OPC begun in 2016. EGP and TMP conceived of the minirhizotron project at OPC begun in 2021. PDM and JL designed and built the minirhizotron infrastructure, and PDM, JL, GMM and JM collected the data. JR and AZ trained the computer vision model and processed the minirhizotron images to estimate root surface area. PDM conducted the statistical analyses with input from EGP and GMM, and PDM wrote the initial manuscript. All authors contributed to revisions of manuscript drafts and approved the final manuscript. Three anonymous reviewers provided valuable feedback to this manuscript.

ORCID

Jacob R. Goheen  <https://orcid.org/0009-0008-0235-4490>
 Patrick D. Milligan  <https://orcid.org/0000-0003-1515-4638>
 Gabriella M. Mizell  <https://orcid.org/0000-0002-1922-5985>
 Todd M. Palmer  <https://orcid.org/0000-0001-5710-9750>
 Elizabeth G. Pringle  <https://orcid.org/0000-0002-4398-9272>
 Corinna Riginos  <https://orcid.org/0000-0001-9082-5206>
 Alina Zare  <https://orcid.org/0000-0002-4847-7604>

Data availability

The data and scripts used to do the analyses and produce figures in this manuscript can be accessed at doi: [10.6084/m9.figshare.28861334.v2](https://doi.org/10.6084/m9.figshare.28861334.v2). The U-Net used to differentiate roots from soil in minirhizotron images is catalogued at <https://github.com/GatorSense/PlantRootSeg> (Xu *et al.*, 2020) with instructions on how the model could be trained and applied for other image sets. Before training and the use of PlantRootSeg, the imagery from this study was normalized during pre-processing. The default parameters outlined in the PlantRootSeg repository were used in training for the model used in this study. An example set of model weights found using this data and process is archived on Zenodo (<https://zenodo.org/records/3462856>).

References

- Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH *et al.* 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate

- change risks for forests. *Adaptation of Forests and Forest Management to Changing Climate* 259: 660–684.
- Andivia E, Villar-Salvador P, Oliet JA, Puértolas J, Dumroese RK, Ivetic V, Molina-Venegas R, Arellano EC, Li G, Ovalle JF. 2021. Climate and species stress resistance modulate the higher survival of large seedlings in forest restorations worldwide. *Ecological Applications* 31: e02394.
- Archetti M, Scheuring I, Hoffman M, Frederickson ME, Pierce NE, Yu DW. 2011. Economic game theory for mutualism and cooperation. *Ecology Letters* 14: 1300–1312.
- Arcila Hernández LM, Sanders JG, Miller GA, Ravenscraft A, Frederickson ME. 2017. Ant–plant mutualism: a dietary by-product of a tropical ant's macronutrient requirements. *Ecology* 98: 3141–3151.
- Arco R, Porcel R, Ruiz-Lozano JM. 2012. Regulation of root water uptake under abiotic stress conditions. *Journal of Experimental Botany* 63: 43–57.
- BassiriRad H, Gutschick VP, Lussenhop J. 2001. Root system adjustments: regulation of plant nutrient uptake and growth responses to elevated CO₂. *Oecologia* 126: 305–320.
- Bezemer TM, van Dam NM. 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology & Evolution* 20: 617–624.
- Birkett A, Stevens-Wood B. 2005. Effect of low rainfall and browsing by large herbivores on an enclosed savannah habitat in Kenya. *African Journal of Ecology* 43: 123–130.
- Bragg PL, Govi G, Cannell RQ. 1983. A comparison of methods, including angled and vertical minirhizotrons, for studying root growth and distribution in a spring oat crop. *Plant and Soil* 73: 435–440.
- Broekhuysen GJ. 1947. The Brown House Ant (*Pheidole megacephala*, Fabr.) in South Africa. *Bulletin of the Department of Agriculture, South Africa* 266: 1–40.
- Bronstein JL. 2001. The costs of mutualism. *American Zoologist* 41: 825–839.
- Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C. 2015. How tree roots respond to drought. *Frontiers in Plant Science* 6: 547.
- Chitra-Tarak R, Xu C, Aguilar S, Anderson-Teixeira KJ, Chambers J, Detto M, Faybishenko B, Fisher RA, Knox RG, Koven CD *et al.* 2021. Hydraulically-vulnerable trees survive on deep-water access during droughts in a tropical forest. *New Phytologist* 231: 1798–1813.
- Clarke PJ, Lawes M, Midgley JJ, Lamont B, Ojeda F, Burrows G, Enright N, Knox K. 2013. Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist* 197: 19–35.
- Collins D, Bras R. 2007. Plant rooting strategies in water-limited ecosystems. *Water Resources Research* 43: W06407.
- Cordeiro AL, Norby RJ, Andersen KM, Valverde-Barrantes O, Fuchslueger L, Oblitas E, Hartley IP, Iversen CM, Gonçalves NB, Takeshi B. 2020. Fine-root dynamics vary with soil depth and precipitation in a low-nutrient tropical forest in the Central Amazonia. *Plant-Environment Interactions* 1: 3.
- Coupe MD, Cahill JF Jr. 2003. Effects of insects on primary production in temperate herbaceous communities: a meta-analysis. *Ecological Entomology* 28: 511–521.
- DeCarlo KF, Caylor KK. 2019. Biophysical effects on soil crack morphology in a faunally active dryland vertisol. *Geoderma* 334: 134–145.
- Devenish AJM, Gomez C, Bridle JR, Newton RJ, Sumner S. 2019. Invasive ants take and squander native seeds: implications for native plant communities. *Biological Invasions* 21: 451–466.
- Dong N, Zhou J, Yan G, Liu G, Xing Y, Wang Q. 2022. Effects of long-term nitrogen addition and precipitation reduction on the fine root dynamics and morphology in a temperate forest. *European Journal of Forest Research* 141: 363–378.
- Du E, Fang J. 2014. Linking belowground and aboveground phenology in two boreal forests in Northeast China. *Oecologia* 176: 883–892.
- Fensham R, Fairfax R. 2007. Drought-related tree death of savanna eucalypts: Species susceptibility, soil conditions and root architecture. *Journal of Vegetation Science* 18: 71–80.
- Fonseca CR. 1993. Nesting space limits colony size of the plant–ant *Pseudomyrmex concolor*. *Oikos* 67: 473–482.
- Frederickson ME, Ravenscraft A, Miller GA, Arcila Hernández LM, Booth G, Pierce NE. 2012. The direct and ecological costs of an ant–plant symbiosis. *The American Naturalist* 179: 768–778.
- Gadd ME, Young TP, Palmer TM. 2001. Effects of simulated shoot and leaf herbivory on vegetative growth and plant defense in *Acacia drepanolobium*. *Oikos* 92: 515–521.
- Giehl RFH, von Wirén N. 2014. Root nutrient foraging. *Plant Physiology* 166: 509–517.
- Gomes JMTI, Solar R, Blüthgen N, Vasconcelos LH, Silva EBL, Oliveira NK, Moreira ZM, Campos IR. 2021. Multiple effects of mutualistic ants improve the performance of a neotropical ant–plant: A long-term study with the Cecropia-Azteca system. *Basic and Applied Ecology* 57: 78–90.
- Gora EM, Bitzer PM, Burchfield JC, Gutierrez C, Yanoviak SP. 2021. The contributions of lightning to biomass turnover, gap formation and plant mortality in a tropical forest. *Ecology* 102: e03541.
- Green J, Dawson L, Proctor J, Duff E, Elston D. 2005. Fine root dynamics in a tropical rain forest is influenced by rainfall. *Plant and Soil* 276: 23–32.
- Gunderson LH. 2000. Ecological resilience – in theory and application. *Annual Review of Ecology, Evolution, and Systematics* 31: 425–439.
- Haile GG, Tang Q, Hosseini-Moghari S-M, Liu X, Gebremicael TG, Leng G, Kebede A, Xu X, Yun X. 2020. Projected impacts of climate change on drought patterns over east Africa. *Earth's Future* 8: e2020EF001502.
- Hartmann H, Trumbore S. 2016. Understanding the roles of nonstructural carbohydrates in forest trees – from what we can measure to what we want to know. *New Phytologist* 211: 386–403.
- Heil M, Fiala B, Maschwitz U, Linsenmair KE. 2001. On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants. *Oecologia* 126: 395–403.
- Heinze J. 2020. Herbivory by aboveground insects impacts plant root morphological traits. *Plant Ecology* 221: 725–732.
- Hesla BI, Tieszen HL, Boutton TW. 1985. Seasonal water relations of savanna shrubs and grasses in Kenya, East Africa. *Journal of Arid Environments* 8: 15–31.
- Holling CS. 1973. Resilience and stability of ecological systems. *Annual Review of Ecology, Evolution, and Systematics* 4: 1–23.
- Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ. 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33: 181–233.
- Huang J, Hammerbacher A, Weinhold A, Reichelt M, Gleixner G, Behrendt T, van Dam NM, Sala A, Gershenson J, Trumbore S *et al.* 2019. Eyes on the future – evidence for trade-offs between growth, storage and defense in Norway spruce. *New Phytologist* 222: 144–158.
- Johnson MG, Tingey DT, Phillips DL, Storm MJ. 2001. Advancing fine root research with minirhizotrons. *Environmental and Experimental Botany* 45: 263–289.
- Kahuthia-Gathu R, Kirubi Thungu D, Wangu L, Kimani R. 2018. Wood-boring beetles associated with *Acacia xanthophloea* in Nairobi and Machakos Counties, Kenya. *PLoS ONE* 13: e0188773.
- Kiers ET, Denison RF, Kawakita A, Herre EA. 2011. The biological reality of host sanctions and partner fidelity. *Proceedings of the National Academy of Sciences, USA* 108: E7.
- Kiers ET, Palmer TM, Ives AR, Bruno JF, Bronstein JL. 2010. Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters* 13: 1459–1474.
- Kiers ET, Rousseau RA, West SA, Denison RF. 2003. Host sanctions and the legume–rhizobium mutualism. *Nature* 425: 78–81.
- King EG, Caylor KK. 2010. Herbivores and mutualistic ants interact to modify tree photosynthesis. *New Phytologist* 187: 17–21.
- Kremer JMM, Nooten SS, Cook JM, Ryalls JMW, Barton CVM, Johnson SN. 2018. Elevated atmospheric carbon dioxide concentrations promote ant tending of aphids. *Journal of Animal Ecology* 87: 1475–1483.
- Lach L. 2003. Invasive ants: unwanted partners in ant–plant interactions? *Annals of the Missouri Botanical Garden* 90: 91–108.
- Lach L. 2005. Interference and exploitation competition of three nectar-thieving invasive ant species. *Insectes Sociaux* 52: 257–262.
- Lach L, Hooper-Bui LM. 2010. Consequences of ant invasions. In: *Ant ecology*. Oxford, UK: Oxford University Press, 286.
- Lambers H, Chapin FS, Pons TL. 2008. *Plant physiological ecology*. New York, NY, USA: Springer.
- Madden D, Young TP. 1992. Symbiotic ants as an alternative defense against giraffe herbivory in spinescent *Acacia drepanolobium*. *Oecologia* 91: 235–238.

- McGlynn TP. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. *Journal of Biogeography* 26: 535–548.
- Milligan PD, Martin TA, John GP, Riginos C, Goheen JR, Carpenter SM, Palmer TM. 2021. Mutualism disruption by an invasive ant reduces carbon fixation for a foundational East African ant–plant. *Ecology Letters* 24: 1052–1062.
- Milligan PD, Martin TA, Pringle EG, Prior KM, Palmer TM. 2023a. Symbiotic ant traits produce differential host-plant carbon and water dynamics in a multi-species mutualism. *Ecology* 104: e3880.
- Milligan PD, Martin TA, Pringle EG, Riginos C, Mizell GM, Palmer TM. 2022. A soil-nesting invasive ant disrupts carbon dynamics in saplings of a foundational ant–plant. *Journal of Ecology* 110: 359–373.
- Milligan PD, Palmer TM, Lemboi JS, Mosiany J, Mutisya S, Gituku B, Kibungei A, Pringle EG. 2023b. Ant invasion is associated with lower root density and different root distribution of a foundational savanna tree species. *Biological Invasions* 25: 1683–1691.
- Milligan PD, Prior KM, Palmer TM. 2016. An invasive ant reduces diversity but does not disrupt a key ecosystem function in an African savanna. *Ecosphere* 7: e01502.
- Miranda JC, Rodríguez-Calcerrada J, Pita P, Saurer M, Oleksyn J, Gil L. 2020. Carbohydrate dynamics in a resprouting species after severe aboveground perturbations. *European Journal of Forest Research* 139: 841–852.
- Mizell GM, Kim T, Sullivan BW, Lemboi JS, Mosiany J, Palmer TM, Pringle EG. 2023. Carbon allocation in an East African ant-acacia: field testing a ^{13}C -labeling method for evaluating biotic impacts on the carbon cycle. *Plant Ecology* 224: 953–963.
- Mpala Research Centre Mpala Research Centre weather and climate records.
- Mundim FM, Alborn HT, Vieira-Neto EHM, Bruna EM. 2017. A whole-plant perspective reveals unexpected impacts of above- and belowground herbivores on plant growth and defense. *Ecology* 98: 70–78.
- Nepi M, Grasso DA, Mancuso S. 2018. Nectar in plant–insect mutualistic relationships: from food reward to partner manipulation. *Frontiers in Plant Science* 9: 1063.
- Ness JH, Morris WF, Bronstein JL. 2009. For ant-protected plants, the best defense is a hungry offense. *Ecology* 90: 2823–2831.
- Newman JR, Wagner D. 2013. The influence of water availability and defoliation on extrafloral nectar secretion in quaking aspen (*Populus tremuloides*). *Botany* 91: 761–767.
- Niklas KJ. 2005. Modelling below- and above-ground biomass for non-woody and woody plants. *Annals of Botany* 95: 315–321.
- Okello BD, O'Connor TG, Young TP. 2001. Growth, biomass estimates, and charcoal production of *Acacia drepanolobium* in Laikipia, Kenya. *Forest Ecology and Management* 142: 143–153.
- Palmer TM. 2003. Spatial habitat heterogeneity influences competition and coexistence in an African acacia ant guild. *Ecology* 84: 2843–2855.
- Palmer TM. 2004. Wars of attrition: colony size determines competitive outcomes in a guild of African acacia ants. *Animal Behaviour* 68: 993–1004.
- Palmer TM, Brody AK. 2007. Mutualism as reciprocal exploitation: African plant–ants defend foliar but not reproductive structures. *Ecology* 88: 3004–3011.
- Palmer TM, Brody AK. 2013. Enough is enough: the effects of symbiotic ant abundance on herbivory, growth, and reproduction in an African acacia. *Ecology* 94: 683–691.
- Palmer TM, Doak DF, Stanton ML, Bronstein JL, Kiers ET, Young TP, Goheen JR, Pringle RM. 2010. Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proceedings of the National Academy of Sciences, USA* 107: 17234–17239.
- Palmer TM, Riginos C, Milligan PD, Hays BR, Pietrek AG, Maiyo NJ, Mutisya S, Gituku B, Musila S, Carpenter S *et al.* 2021. Frenemy at the gate: invasion by *Pheidole megacephala* facilitates a competitively subordinate plant ant in Kenya. *Ecology* 102: e03230.
- Palmer TM, Stanton ML, Young TP, Goheen JR, Pringle RM, Karban R. 2008. Breakdown of an ant–plant mutualism follows the loss of large herbivores from an African savanna. *Science* 319: 192–195.
- Perkins SR, Keith Owens M. 2003. Growth and biomass allocation of shrub and grass seedlings in response to predicted changes in precipitation seasonality. *Plant Ecology* 168: 107–120.
- Perkovich C, Ward D. 2021. Aboveground herbivory causes belowground changes in twelve oak *Quercus* species: a phylogenetic analysis of root biomass and non-structural carbohydrate storage. *Oikos* 130: 1797–1812.
- Peterson CJ. 2019. Twenty-five years of aboveground biomass and carbon accumulation following extreme wind damage in an old-growth forest. *Forests* 10: 289.
- Pietrek AG, Goheen JR, Riginos C, Maiyo NJ, Palmer TM. 2021. Density dependence and the spread of invasive big-headed ants (*Pheidole megacephala*) in an East African savanna. *Oecologia* 195: 667–676.
- Pinkalski C, Damgaard C, Jensen K-MV, Peng R, Offenberg J. 2015. Quantification of ant manure deposition in a tropical agroecosystem: implications for host plant nitrogen acquisition. *Ecosystems* 18: 1373–1382.
- Pinkalski C, Jensen K-MV, Damgaard C, Offenberg J. 2018. Foliar uptake of nitrogen from ant faecal droplets: an overlooked service to ant-plants. *Journal of Ecology* 106: 289–295.
- Piper FI, Paula S. 2020. The role of nonstructural carbohydrates storage in forest resilience under climate change. *Current Forestry Reports* 6: 1–13.
- Pringle EG. 2016. Integrating plant carbon dynamics with mutualism ecology. *New Phytologist* 210: 71–75.
- Pringle EG, Akçay E, Raab TK, Dirzo R, Gordon DM. 2013. Water stress strengthens mutualism among ants, trees, and scale insects. *PLoS Biology* 11: e1001705.
- Pringle EG, Dirzo R, Gordon DM. 2011. Indirect benefits of symbiotic coccids for an ant-defended myrmecophytic tree. *Ecology* 92: 37–46.
- Pringle EG, dos Santos TF, Gonçalves MS, Hawes JE, Peres CA, Baccaro FB. 2019. Arboreal ant abundance tracks primary productivity in an Amazonian whitewater river system. *Ecosphere* 10: e02902.
- Prior KM, Palmer TM. 2018. Economy of scale: third partner strengthens a keystone ant–plant mutualism. *Ecology* 99: 335–346.
- R Core Team. 2021. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rigby RA, Stasinopoulos DM. 2005. Generalized additive models for location, scale and shape (with discussion). *Applied Statistics* 54: 507–554.
- Riginos C, Karande MA, Rubenstein DI, Palmer TM. 2015. Disruption of a protective ant–plant mutualism by an invasive ant increases elephant damage to savanna trees. *Ecology* 96: 654–661.
- Ripple WJ, Newsome TM, Wolf C, Dirzo R, Everatt KT, Galetti M, Hayward MW, Kerley GIH, Levi T, Lindsey PA *et al.* 2015. Collapse of the world's largest herbivores. *Science Advances* 1: e1400103.
- Rosumek FB, Silveira FAO, de S. Neves F, de U. Barbosa NP, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160: 537–549.
- Schutz AEN, Bond WJ, Cramer MD. 2011. Defoliation depletes the carbohydrate reserves of resprouting *Acacia* saplings in an African savanna. *Plant Ecology* 212: 2047–2055.
- Shukla RK, Singh H, Rastogi N, Agarwal VM. 2013. Impact of abundant *Pheidole* ant species on soil nutrients in relation to the food biology of the species. *Applied Soil Ecology* 71: 15–23.
- Siddiqui JA, Bamsile BS, Khan MM, Islam W, Hafeez M, Bodlah I, Xu Y. 2021. Impact of invasive ant species on native fauna across similar habitats under global environmental changes. *Environmental Science and Pollution Research* 28: 54362–54382.
- Silvério DV, Brando PM, Bustamante MMC, Putz FE, Marra DM, Levick SR, Trumbore SE. 2019. Fire, fragmentation, and windstorms: a recipe for tropical forest degradation. *Journal of Ecology* 107: 656–667.
- Skarpaas O, Meineri E, Bargmann T, Pötsch C, Töpper J, Vandvik V. 2016. Biomass partitioning in grassland plants along independent gradients in temperature and precipitation. *Perspectives in Plant Ecology, Evolution and Systematics* 19: 1–11.
- Smucker AJM, Aiken RM. 1992. Dynamic root responses to water deficits. *Soil Science* 154: 281–289.

- Stanton ML, Palmer TM. 2011. The high cost of mutualism: effects of four species of East African ant symbionts on their myrmecophyte host tree. *Ecology* 92: 1073–1082.
- Stasinopoulos MD, Kneib T, Klein N, Mayr A, Heller GZ. 2024. *Generalized additive models for location, scale and shape: a distributional regression approach, with applications*. Cambridge, UK: Cambridge University Press.
- Staver AC, Abraham JO, Hempson GP, Karp AT, Faith JT. 2021. The past, present, and future of herbivore impacts on savanna vegetation. *Journal of Ecology* 109: 2804–2822.
- Stephens AEA, Westoby M. 2015. Effects of insect attack to stems on plant survival, growth, reproduction and photosynthesis. *Oikos* 124: 266–273.
- Trager MD, Bhotika S, Hostetler JA, Andrade GV, Rodriguez-Cabal MA, McKeon CS, Osenberg CW, Bolker BM. 2010. Benefits for plants in ant–plant protective mutualisms: a meta-analysis. *PLoS ONE* 5: e14308.
- Trugman AT, Detto M, Bartlett MK, Medvigy D, Anderegg WRL, Schwalm C, Schaffer B, Pacala SW. 2018. Tree carbon allocation explains forest drought-kill and recovery patterns. *Ecology Letters* 21: 1552–1560.
- Vidal MC, Anneberg TJ, Curé AE, Althoff DM, Segreaves KA. 2021. The variable effects of global change on insect mutualisms. *Current Opinion in Insect Science* 47: 46–52.
- Viles HA, Goudie AS, Goudie AM. 2021. Ants as geomorphological agents: a global assessment. *Earth-Science Reviews* 213: 103469.
- Vogel JG, Bond-Lamberty BP, Schuur EAG, Gower ST, Mack MC, O'Connell KEB, Valentine DW, Ruess RW. 2008. Carbon allocation in boreal black spruce forests across regions varying in soil temperature and precipitation. *Global Change Biology* 14: 1503–1516.
- Wagner D. 1997. The influence of ant nests on Acacia seed production, herbivory and soil nutrients. *Journal of Ecology* 85: 83–93.
- Wagner DL, Grames EM, Forister ML, Berenbaum MR, Stopak D. 2021. Insect decline in the Anthropocene: death by a thousand cuts. *Proceedings of the National Academy of Sciences, USA* 118: e2023989118.
- Wainwright CM, Finney DL, Kilavi M, Black E, Marsham JH. 2021. Extreme rainfall in East Africa, October 2019–January 2020 and context under future climate change. *Weather* 76: 26–31.
- Weber R, Schwendener A, Schmid S, Lambert S, Wiley E, Landhäusser SM, Hartmann H, Hoch G. 2018. Living on next to nothing: tree seedlings can survive weeks with very low carbohydrate concentrations. *New Phytologist* 218: 107–118.
- Wetterer JK. 2012. Worldwide spread of the African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). *Myrmecological News* 17: 51–62.
- Weyl EG, Frederickson ME, Yu DW, Pierce NE. 2010. Economic contract theory tests models of mutualism. *Proceedings of the National Academy of Sciences, USA* 107: 15712–15716.
- Wigley BJ, Staver AC, Zytowski R, Jagodzinski AM, Wigley-Coetsee C. 2019. Root trait variation in African savannas. *Plant and Soil* 441: 555–565.
- Wong MKL, Economo EP, Guénard B. 2023. The global spread and invasion capacities of alien ants. *Current Biology* 33: 566–571.
- Xu W, Yu G, Zare A, Zurweller B, Rowland DL, Reyes-Cabrera J, Fritschi FB, Matamala R, Juenger TE. 2020. Overcoming small minirhizotron datasets using transfer learning. *Computers and Electronics in Agriculture* 175: 105466.
- Young TP, Okello B, Kinyua D, Palmer TM. 1998. KLEE: a long-term multi-species herbivore exclusion experiment in Laikipia, Kenya. *African Journal of Range and Forage Science* 14: 94–102.
- Young TP, Stubblefield CH, Isbell LA. 1996. Ants on swollen-thorn acacias: species coexistence in a simple system. *Oecologia* 109: 98–107.

Supporting Information

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

Fig. S1 Overview map of study area.

Fig. S2 Plot-level maps of minirhizotrons.

Fig. S3 Example training images for convolutional neural network.

Fig. S4 Lin correlation coefficient check for computer vision analysis of root images.

Fig. S5 Non-linear trend of acacia root surface area with decreasing depth.

Notes S1 Detailed minirhizotron installation and maintenance methods.

Notes S2 Survey details of acacia aboveground covariates.

Notes S3 Unit conversion from root pixel counts to surface area.

Notes S4 Analytical models in equation and diagram forms.

Table S1 Site- and array-level abundances of ground-nesting ants.

Table S2 Notes S4 model predictions in table forms.

Table S3 Notes S4 model predictions in table forms.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

Disclaimer: The New Phytologist Foundation remains neutral with regard to jurisdictional claims in maps and in any institutional affiliations.