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Biological invasions are disrupting mutualisms worldwide. In a Kenyan savanna, the invasive ant *Pheidole megacephala* has disrupted a foundational mutualism between the tree *Acacia drepanolobium* and native ants that previously deterred herbivory by mega-browsers. I studied the consequences of this mutualism disruption on populations of *A. drepanolobium*.

First, I created a novel method of estimating tree biomass to quantify changes in tree canopies. I analyzed photographs of small trees to create a regression of destructively sampled tree biomass as a function of tree pixel area and diameter. This method outperformed a published allometric relation based on diameter alone ($R^2 = 0.86$, $R^2 = 0.68$) and also confirmed higher above ground biomass in the herbivore-exclusion plots than in unfenced plots ($p < 0.001$). As such, my new technique offers an accurate and cost-effective complement to existing methods for tree biomass estimation at small scales.

Second, I used demographic analyses to quantify the consequences of the mutualism disruption at a population scale. Ant invasion exacerbated population declines of *Acacia drepanolobium*. Ten-year predictions from matrix models showed large population expansions driven by higher reproduction and survival after the removal of browsers. Demographic effects of *P. megacephala* arose through decreased survival and reproduction of adult trees. Contrary to expectation, the extirpation of metabolically costly *Crematogaster* mutualists did not result in higher rates of population growth for trees protected from browsing ungulates. My results indicate that invasive ants impose a cost that exceeds that of native mutualists while providing no protection from mega-browsers.

Consequences of mutualism disruption for an African acacia: synergies between
mega-browsers and big-headed ant invasion.

By

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Chapter 1 - Using Photography to Estimate Above-Ground Biomass of Small Trees

Abstract

Quantifying tree biomass is an important research and management goal across many disciplines. For species that exhibit predictable relationships between structural metrics (e.g., diameter, height, crown breadth) and total weight, allometric calculations produce accurate estimates of above-ground biomass. However, such methods may be insufficient where inter-individual variation is large relative to individual biomass and is itself of interest (for example, variation due to herbivory). In an East African savanna bushland, we analyzed photographs of small (<5 m) trees from perpendicular angles and fixed distances to estimate above-ground biomass. Pixel area of trees in photos and diameter were more strongly related to measured, above-ground biomass of destructively sampled trees than biomass estimated using a published allometric relation based on diameter alone ($R^2 = 0.86$ versus $R^2 = 0.68$). When tested on trees in herbivore-exclusion plots versus unfenced (open) plots, our predictive equation based on photos confirmed higher above ground biomass in the exclusion plots than in unfenced (open) plots ($p < 0.001$), in contrast to no significant difference based on the allometric equation ($p = 0.43$). As such, our new technique based on photographs offers an accurate and cost-effective complement to existing methods for tree biomass estimation at small scales with potential application across a wide variety of settings.

Introduction

Allometric relationships enable the estimation of above-ground biomass of trees from structural measurements (e.g., diameter, height, crown breadth; Chave et al. 2005, Henry et al. 2011, Pastor et al. 1984, Young et al. 1964). This approach is most useful for individuals of large size which exhibit little variation in structure relative to overall biomass (e.g. rainforest trees). However, in populations with greater structural heterogeneity relative to total biomass, allometric relationships may be unreliable (Antonio et al. 2007, Dutcã et al. 2017). Such variation among individuals can arise from a number of factors, including structural modification of trees due to herbivory (Whitham & Mopper 1985), parasitism (Stanton et al. 1999), competition (Poorter et al. 2012), or abiotic conditions (Copenhaver & Tinker 2014). Therefore, in systems where structural heterogeneity is both large relative to individual biomass and itself of interest to researchers, methods that accurately quantify such variation are needed.

Recent advances in remote sensing technologies have made it possible to rapidly quantify such individual variation. LiDAR (Light Detection And Ranging) can generate highly accurate (<1 cm spacing) point clouds from which 3D models of trees can be constructed (Raumonen et al. 2015, Yau et al. 2012) and their biomass estimated (Gonzales et al. 2018, Popescu 2007). However, LiDAR is prohibitively expensive for many, with a standard sensor costing \$115,000 from the manufacturer (Rieglusa.com). Commissioning airborne LiDAR surveys may be cheaper but still costs tens of thousands of dollars. These techniques may be cost effective if large tracts of land need to be surveyed, however for smaller scale studies they are unsuitable. In an attempt to balance affordability, simplicity, and accuracy, we developed a technique to estimate above-ground biomass via photography and freely available image analysis software.

We sought to reliably assess above-ground biomass of *Acacia (Vachellia) drepanolobium*, a small (<5 m tall) savanna tree that forms monodominant stands across large tracts (100s-1000s of km²) in central Kenya (Young et al. 1997). As both a nitrogen fixer (Fox Dobbs et al. 2010) and a key component of several large mammals' diets (Birkett 2002, Kartzinel et al. 2015), *A. drepanolobium* is an important driver of ecosystem function. It is also a myrmecophyte (ant plant) which may host any of four intensely competing ant species offering varying degrees of protection against herbivores in exchange for food (extra-floral nectar) and shelter (modified stipular spines) (Palmer et al. 2008, Palmer et al. 2010). Because the various species of ant occupants differentially modify the architecture of *A. drepanolobium*, trees of the same trunk diameter can have drastically different canopy shapes (Stanton et al. 1999). In addition, elephants can dramatically alter tree canopy by ripping off large segments during feeding, removing anywhere from 10-100% of branches (Figure 1). As a result, variation amongst *A. drepanolobium* can be as large as the total biomass of individual trees. For example, two trees of equal diameter may differ in biomass by orders of magnitude when one tree has had its entire canopy removed via elephant herbivory. We developed our photographic technique to quantify this variation due to herbivory and ant occupant. Accordingly, we trained our method on trees with multiple species of ant occupant and validated the method in replicated unfenced and herbivore-exclusion plots.

Methods

Study Site

We worked at Mpala Research Centre (0° 17' 54.0" N 36° 52' 16.4" E) and Ol Pejeta Conservancy (0° 02' 01.7" N 36° 52' 59.9" E) in Laikipia County, Kenya. Here, as in many other parts of East Africa underlain by black cotton soils, *A. drepanolobium* forms the vast majority

(~98%) of tree cover (Goheen & Palmer 2010, Pringle et al. 2016, Young et al. 1997).

Throughout most of its range, *A. drepanolobium* exhibits variable canopy volume and a maximum height of 3-5 m (Okello et al. 2001); trees >3 m are rare at our study sites.

Tree Selection

We selected a sample of thirty *A. drepanolobium* trees at Mpala Research Centre, ranging from 0.5-2.5 m tall and with diameters from 3-10 cm. We measured height and diameter; we measured diameter at 30cm above the ground and marked the position with red paint. To account for variation in tree architecture, we selected trees that were occupied by the most common species of ant symbionts (Stanton et al. 1999). We selected 10 trees occupied by the less common *Crematogaster nigriceps*, which tend to exhibit smaller, more condensed architectures, and 20 trees occupied by the more common *C. mimosae*, which reach a greater height but have sparser canopies.

Photo Acquisition

Using a 4-megapixel Nikon Coolpix 4500 mounted on a 1 m tripod, we took two photos of each tree at perpendicular angles to account for anisotropy. For each photo, the camera was placed 4m from the tree and aligned either due north or east as measured by a high accuracy GPS compass (Garmin GPSMAP 64st). In cases where obstacles prevented camera placement due north or due east, both photo points were offset equally to maintain perpendicular orientations. We then used a bubble level to adjust the tripod until the camera was level relative to the ground. We also included a ruler at a fixed position for scale. The ruler was placed equidistant between the two photo points, 3.5 m from each point and 0.5 m from the tree. Once the camera and ruler were situated, a large, red-fabric sheet was erected behind the tree to maximize contrast (Figure 2).

The photograph was taken at minimum zoom (38 mm focal length in 35 mm camera equivalent) and at maximum resolution (2,272 x 1,704 pixels) in manual mode, so that aperture and shutter speed could be manipulated for maximum contrast between tree and sheet. We repeated this process for each tree for a total of 60 photos (2 photos per tree for 30 trees).

Destructive Sampling

After the trees had been photographed, they were cut down and all components above the diameter measurement were collected in large bags for drying (Okello et al. 2001). To ensure that photo pixels and their associated areas corresponded to actual canopy size, for each tree we measured the sum of the lengths of all tree branches >2 cm in diameter (hereafter “running branch length”). The tree components in bags were left out in the sun during the dry season and weighed every week until measurements stabilized; they were measured for another two weeks after this point to ensure constant dry weight had been reached. After two months, all trees had achieved a constant weight and final dry weight measurements were taken.

Photo Analysis

We attempted to isolate trees from background using automated methods for photo analysis in three different software packages: ImageJ, ArcGIS, and GIMP. In ImageJ, we used several auto-thresholding algorithms, which binarize an image into background and object pixels based on different mathematical approaches. In ArcGIS, we used both supervised and unsupervised maximum likelihood classifications. Comparing the resultant classifications visually, we found that analyzing photos manually in GIMP (GNU Image Manipulation Program), a freely available image editing software, was the most accurate means of isolating trees from background (Figure 3).

We used the following procedure in GIMP. First, photos were cropped to include only the portion of the tree above the red-painted diameter mark. Then, we used the “select by color” tool to select and delete all pixels with color values similar to a sample of pixels from the (red) background sheet. This process was iterated until only the tree pixels remained in the photo (hereafter ‘pixels’). The resolution of the original photo could be determined using the included ruler (cm²/pixel). The area of the tree was then calculated from this known scale and the total number of pixels remaining in the photo (hereafter ‘area’).

Data Analysis

Using individual tree dry weight as our response variable, we created two competing multiple linear regression models. The predictors of the two models were a series of covariates plus either photo pixels or area (since area was derived from photo pixels, they could not both be included in the same model, Equations 1 and 2).

$$\text{Biomass (kg)} \sim \text{Pixels} + \text{Diameter (cm)} + \text{Height (m)} + \text{Running Branch Length (cm)} + \text{Ant Species}$$

Eq. 1

$$\text{Biomass (kg)} \sim \text{Area (cm}^2\text{)} + \text{Diameter (cm)} + \text{Height (m)} + \text{Running Branch Length (cm)} + \text{Ant Species}$$

Eq. 2

The pixel values in perpendicular photos of the same tree were averaged to create the model variable; the same was done for area. The final candidate model was determined via backwards stepwise model selection by AIC using the stepAIC function from the MASS package in R (Venables and Ripley 2002). We evaluated the accuracy of the model by k-fold cross validation, splitting the 30 test trees into 5 groups and evaluating a model created from 80% of the data against the remaining 20%, repeated 1,000 times (Kuhn 2019). The predictions of the final

regression model were compared to an existing allometric equation for *A. drepanolobium* (Okello et al. 2001, Equation 3).

$$Biomass (kg) = e^{\ln(\text{diameter}) * 2.2949 + 4.7997} / 1000 \quad \text{Eq. 3}$$

We performed all statistical analyses using R statistical software (R core team 2018); regressions were carried out using the `lm()` function and relative importance of variables was assessed with the “relaimpo” package (Ulrike, 2006).

Model Validation

Finally, we used the regression to predict biomass for selected trees within twelve 0.5-ha herbivore-exclusion plots of a separate experiment started in 2017 at Ol Pejeta Conservancy. Half of the plots were fenced to keep out elephants and other large (>30 kg) ungulates, and half were left unfenced. Paired fenced and unfenced plots are separated by less than 50m to control for effects of precipitation and soil, and all plots were located in the same 37.5 km² area. A stratified random sample of tagged trees within these plots have been measured annually for a separate demographic study. We used a subset of these trees to validate our model: those that could be physically photographed (i.e. weren't obstructed by other closely growing trees) and were in the same 0.5-2.5 m height range as the trees used in model training. We photographed ten trees in each plot (for a total of 120 trees). On a windless day, it took ~1.5 hours to photograph 10 trees; therefore, to photograph all trees within a 0.5 ha plot (60-70) under ideal conditions would take ~12 hours. The plots had been fenced for two years by the time of photographing and showed significant differences in tree measurements (Table 1); we therefore expected differences in tree biomass between the unfenced and fenced areas. Finally, we applied Okello et al.'s regression to the same trees for comparison.

Results

Running branch length was positively correlated with tree area calculated from photographs ($r = 0.90$) and significantly related to model predicted biomass ($R^2 = 0.83$, $p < 0.001$), demonstrating that photo derived area accurately represents tree canopy area.

The final (best) regression model for tree biomass included only diameter and tree area in photos, with $R^2 = 0.86$ after cross validation (Table 2). Ant occupant was not a significant variable in the model, nor was there a significant difference in biomass based on ant species (2-sided t-test, $p = 0.43$). Area was a slightly better predictor of biomass ($R^2 = 0.86$ vs $R^2 = 0.85$) and was used instead of pixels, since they were highly collinear. Height was highly correlated with diameter ($r = 0.77$) and only accounted for a small amount of variation not accounted for by diameter ($R^2 = 0.0056$).

The allometric equation of Okello et al. explained less variation ($R^2 = 0.68$, $RMSE = 2.97$) than our regression ($R^2 = 0.86$, $RMSE = 1.36$, Figure 4). Squared residuals of the allometric predictions were significantly greater than our regression predictions (1-sided t-test, $p = 0.02$).

Finally, average individual tree biomass within herbivore-exclusion plots, as modeled by our photographic regression, was significantly greater (1-sided t-test, $p < 0.001$) in fenced plots (mean = $7.75 \text{ kg} \pm 0.50 \text{ SEM}$) than in open plots (mean = $4.52 \text{ kg} \pm 0.51 \text{ SEM}$). However, biomass modeled by the Okello et al. equation for the same subset of trees did not show a significant difference (1-sided t-test, $p = 0.43$) between fenced plots (mean = $9.44 \pm 0.70 \text{ SEM}$) and open plots ($9.20 \pm 1.33 \text{ SEM}$, Figure 5). Nor did the allometric equation show a significant difference in biomass when applied to all trees within plots (1-sided t-test, $p = 0.48$).

Discussion

Our photographic technique accurately predicted above-ground biomass of *A. drepanolobium* and was a substantial improvement over an existing allometric equation. Using this method, we were able to quantify the significant difference in above-ground biomass between unfenced and herbivore-exclosure plots, attributable to herbivore browsing. This contrast was apparent from a visual survey of the plots and was reflected in significant differences in tree height and diameter. However, the biomass estimates from the existing allometric equation of Okello et al (2001) did not accurately capture these differences, demonstrating the need for a complementary method to quantify changes in biomass due to herbivory. In addition, we did not find an effect of ant occupant, suggesting that differences in architecture induced by ants do not affect total biomass. Our photographic method provides an important extension to existing methods for quantifying changes in above-ground biomass.

In Laikipia and other regions of Kenya, *A. drepanolobium* is a key component of several large mammals' diets, including elephants (*Loxodonta africana*), reticulated giraffes (*Giraffa camelopardalis reticulata*), and black rhino (*Diceros bicornis*) (Birkett 2002, Kartzinel et al. 2015). Additionally, *A. drepanolobium* fixes nitrogen and partially drives nutrient dynamics and forage quality (Fox-Dobbs et al. 2010). Tracking changes in this acacia's biomass is therefore important for understanding both food availability for browsers and forage quality for all herbivores. This is particularly pertinent because *A. drepanolobium* in Laikipia County may experience wide scale changes in abundance and cover due to increasing disturbance from invasive species (Riginos et al. 2015), charcoal harvesting (Okello et al. 2001), and land use change (Muriithi 2016).

Across most savannas, tree biomass and cover are important drivers of ecosystem structure and function (Holdo et al. 2009). Trees provide food for browsers, fix nutrients in soil, serve as habitat for arthropods and nesting sites for birds, and modify mammal movement and habitat use. Therefore, accurately measuring tree biomass is not only a desirable goal in itself but will also enhance our understanding of savanna ecology and aid in the management of endangered species. Yet characterizing abundance, biomass, and size structure of trees has been a long-standing challenge in savanna ecosystems (Archer 1996, House et al. 2003), particularly for remote sensing approaches (Munyati et al. 2011). While there have been photographic techniques developed to measure vegetative cover or shrub biomass (Louhaichi et al. 2010, Louhaichi et al. 2017), these studies were conducted in arid regions in which low vegetation (forbs and shrubs) stood out starkly against a background of bare earth when viewed from above. In contrast, savannas are characterized by a matrix of grass that can be spectrally confused with the trees of interest (Cho et al. 2012). Likewise, a similar method (Ter-Mikaelian & Parker 2000) measured biomass on small, relatively isotropic seedlings that were not structurally altered by herbivory. But larger trees (1-3 m) present more 3-dimensional complexity and may suffer from significant asymmetry due to herbivory; consequently, they need to be photographed from multiple angles at ground level.

Our method is substantially less expensive than LiDAR, costing only a few hundred dollars for a camera, tripod, and backdrop. It is ideal for small scale projects in which it is inexpensive to employ 3-5 personnel to survey trees, although windy conditions can make holding the contrast backdrop physically taxing. However, our method is more laborious than LiDAR, and could not realistically be used to measure trees at scales of 10's or 100's of hectares. In cases where larger scales are of interest, our technique will provide indispensable ground truth measurements by

which to calibrate other forms of remote sensing, including LiDAR or aerial biomass estimates (Shepaschenko et al 2019). In sum, our method provides an accurate, cost-effective, and relatively efficient complement to existing methods for detecting changes in above-ground biomass of trees across space or through time.

A major obstacle is the extensive photo processing time required to classify photos of trees manually. If an accurate algorithmic classification scheme could be implemented, it would reduce the time investment considerably. Although our classification of photos was necessarily subjective, it was still considerably more accurate than any of the algorithmic approaches we attempted. Finally, those intending to use this technique should opt for the highest resolution (megapixel) camera available, as this will increase the accuracy of results.

Beyond savannas, accurately and efficiently estimating biomass of small trees should be useful for forest managers quantifying understory biomass or comparing total biomass of a single species at different life stages (Hubau et al. 2019). In particular, it will be useful for measuring change in biomass of individual trees over time, allowing for more precise calculation of growth rates under different environmental conditions. A similar photographic technique was used to quantify tree architecture and measure similarity of traits between individuals in a study of herbivore community assembly (Barbour et al. 2015). Any study in which researchers wish to quantify browsing more accurately than commonly used qualitative metrics will also benefit from this method. We hope that this technique will find broad use with anyone seeking to measure above-ground biomass of relatively small (<5m) trees.

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Figures and Tables



Figure 1. Two photographs of the same tree in 2017 (left) and 2019 (right) showing the extent of elephant damage on canopy.



Figure 2. Photograph setup in the field, with camera situated 4m from the target tree and oriented to 0° .

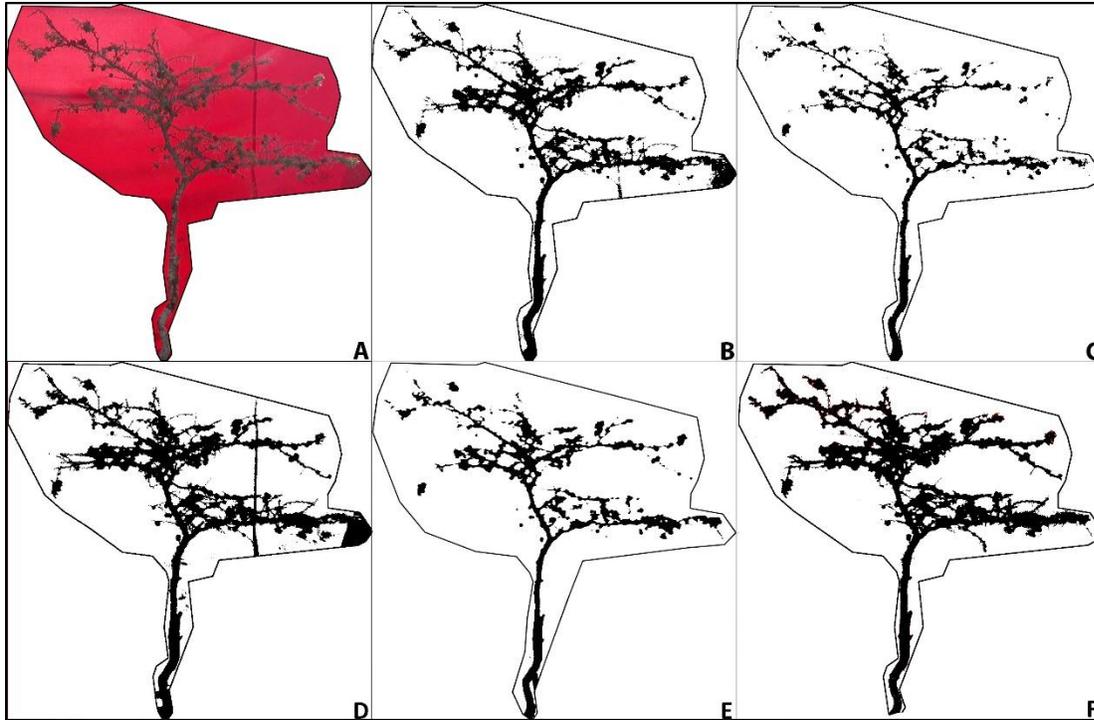


Figure 3. An individual *A. drepanolobium* photo: cropped (A), auto-thresholded using the three best algorithms in ImageJ (IsoData, Minimum, and Otsu, B-D), classified using a supervised maximum likelihood classification in ArcGIS (E), and manually classified in GIMP (F).

Table 1. Means and standard errors about means for tree measurements in the experimental plots used for model validation, with 380 trees in fenced plots and 385 trees in open plots.

	Fenced		Open		2-Sided T-Test
	Mean	SEM	Mean	SEM	
Height (m)	1.83	0.06	1.30	0.06	$p < 0.001$
Diameter (cm)	5.00	0.17	4.38	0.20	$p = 0.02$
Basal Area (cm ²)	28.57	1.77	27.42	2.25	$p = 0.69$
Pixel Area (cm ²)	8,714.97	630.40	3,342.64	472.08	$p < 0.001$

Table 2. Parameters for the final regression, with $R^2 = 0.86$.

	Parameter Estimate	Standard Error	Probability	Variation Explained
Intercept	-3.240	1.072	<0.01	
Area	0.0005259	0.00009901	<0.01	46%
Diameter	0.9941	0.2343	<0.01	40%

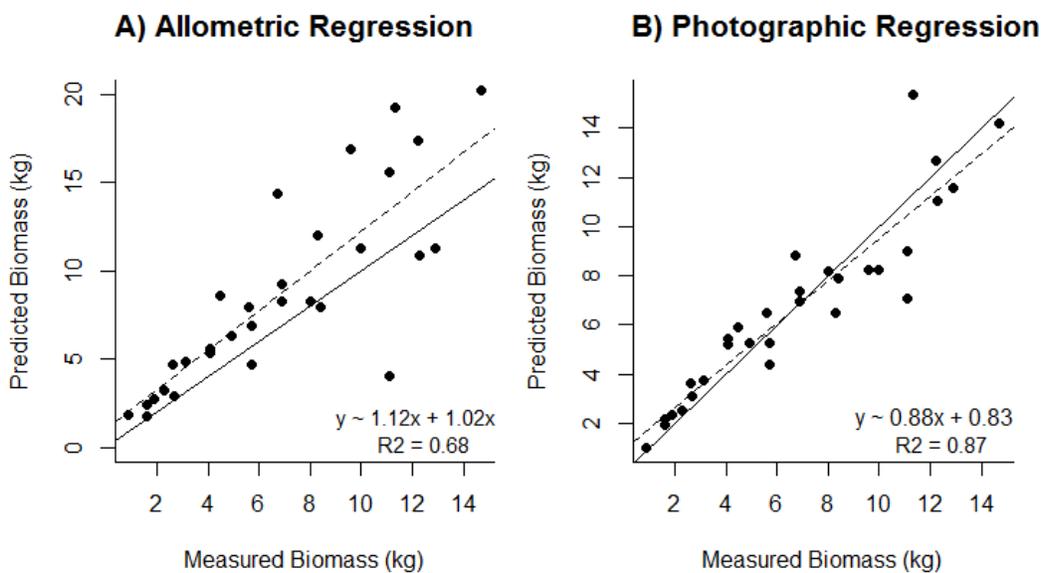


Figure 4. Plots showing measured tree biomass on the x-axis and model predicted biomass on the y-axis. The solid line represents a perfect 1-1 model and the dashed line represent simple linear regressions ($n = 30$ trees) between measured weights and weights predicted by the (A) Okello et al. allometric equation and (B) the photographic regression of the current study. Linear regression equations and R^2 values are included. The photographic regression ($R^2 = 0.86$) performed better than the allometric equation ($R^2 = 0.68$). Note that the linear regression of the allometric equation (A) falls wholly below the 1-1 line.

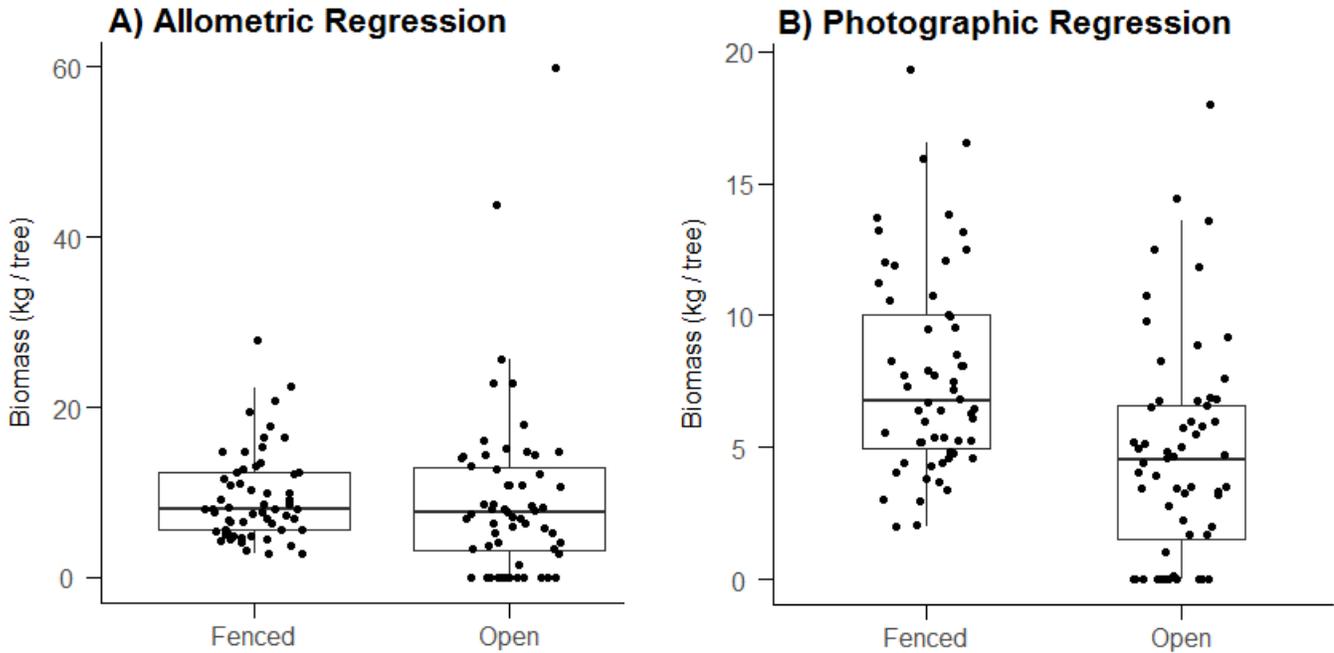


Figure 5. Boxplots showing the distribution of modeled biomasses for individual trees, pooled by fenced plots ($n = 58$ trees) and open plots ($n = 59$ trees). Predictions of the Okello allometric equation (A) do not show a significant difference between fenced and open plots (1 sided t-test, $p = 0.43$). Predictions of the photographic regression (B) do show significantly greater biomass in fenced plots (1-sided t-test, $p < 0.001$).

Chapter 2 –

Demographic consequences of mutualism disruption: synergies between mega-browsers and big-headed ant invasion drive declines of an African acacia

Abstract

Biological invasions are disrupting mutualisms worldwide, leading to reverberating consequences for ecosystems. In a Kenyan savanna, the invasive big-headed ant (*Pheidole megacephala*) has disrupted a foundational mutualism between the whistling-thorn tree (*Acacia drepanolobium*) and the native ants that previously deterred browsing by large mammalian herbivores. We quantified the demographic consequences of this mutualism disruption both in the presence of and following the exclusion of browsing ungulates. Ant invasion exacerbated population declines of the monodominant *Acacia drepanolobium*, although this reduction was small compared to the population increases caused by exclusion of browsing ungulates. Ten-year predictions from matrix models showed large population expansions driven by higher reproduction and survival after the removal of browsers. Demographic effects of big-headed ants arose through decreased survival and reproduction of adult trees. Contrary to expectation, the extirpation of metabolically costly *Crematogaster* mutualists did not result in higher rates of population growth for trees protected from browsing ungulates. Our results indicate that invasive ants impose a cost that exceeds that of native mutualists while providing no protection from mega-browsers. Across landscapes, we expect that invasion by big-headed ants will reduce *A. drepanolobium* populations, but that the magnitude of this effect will hinge on the local abundance and diversity of browsing ungulates.

Introduction

Invasive species are widespread and negatively impact ecosystems and biodiversity around the world (Mack et al. 2000, Early et al. 2016). Evidence of direct negative effects via competition or predation is abundant (Brown et al. 2002, Vila and Weiner 2004, Albins and Hixon 2008, Doherty et al. 2016), and even direct positive effects of facilitation have been observed (Rodriguez 2006, Schlaepfer et al. 2011, Padovani et al. 2020). However, because invasive species become part of the community, their effects on species interactions between native species are indirect and therefore more difficult to quantify (Tylianakis et al. 2008). In particular, studies quantifying changes to species interactions at the population level, where interactions may be buffered against environmental change (Chamberlain et al. 2014) are rare. Mutualisms, the species interactions most changeable by environmental context (Chamberlain et al. 2014), are useful for quantifying effects of invasive species.

Mutualisms are widespread and ecologically important species interactions, shaping both ecosystem structure and function through such processes as seed dispersal (Sekercioglu 2006, Nichols et al. 2008), nutrient cycling (Wilson et al. 2009, van der Heijden et al. 2015), and community succession (Bertness and Callaway 1994, Clay and Holah 1999). In cases involving foundational species like coral or kelp, such interactions create and maintain entire ecosystems (Stachowicz 2001, Bruno et al. 2003, Ellison et al. 2007). But, increasingly across the globe, species invasions are disrupting mutualisms (Christian et al. 2001, Traveset and Richardson 2014). Among the most common are cases in which invasive species disrupt pollination or seed dispersal mutualisms, either by consuming reproductive parts of plants or by displacing native mutualists via competition or predation (e.g., Vázquez and Simberloff 2004, Traveset and Richardson 2006, Aizen et al. 2014, LeVan et al. 2014, Rogers et al. 2017). In other instances,

invasive species alter soil properties through competition with mycorrhizae or by altering soil chemistry via allelopathy (Shah et al. 2009, Traveset and Richardson 2014). Yet, we have a poor understanding of demographic consequences following mutualism disruption (Howe 1989, Hoeksema and Bruna 2015).

Ant-plant protection interactions – in which ants protect plants from herbivores in exchange for some combination of food and shelter – are a common type of symbiosis that have helped build our understanding of the ecology and evolution of mutualisms (e.g., Bronstein 1998, Palmer et al. 2003, Heil and McKey 2003, Trager et al. 2010, Mayer et al. 2014). These mutualisms are increasingly subject to disruption by invasive species around the world (Kiers et al. 2010). Quantifying the demographic consequences of the disruption of such a mutualism by an invasive species will enhance our understanding of the effects of invasive species on native species interactions.

Across vast swathes of savanna in central Kenya (100's to 1,000's of km²), the foundational tree *Acacia (Vachellia) drepanolobium* forms nearly monodominant stands on poorly drained 'black cotton' soils, typically composing >95% of woody plant cover (Young et al. 1997). *Acacia drepanolobium* is a myrmecophyte, hosting ant symbionts that deter catastrophic (lethal) herbivory by elephants (Goheen and Palmer 2010, Palmer and Brody 2013). In exchange for shelter (swollen-thorn domatia) and food (extrafloral nectar), ants provide protection by aggressively swarming in response to browsing by large herbivorous mammals (Palmer et al. 2008, Palmer et al. 2010). While this defense is minimally effective against other species of browsing mammals (Palmer and Brody 2013), ant symbionts are a major deterrent of elephant (*Loxodonta africana*) browsing, and thereby stabilize tree cover across entire landscapes (Goheen and Palmer 2010). However, provisioning of extrafloral nectar and swollen-

thorn domatia comes at a metabolic cost to host plants: trees with ant symbionts grow more slowly and produce fewer fruits than those from which ant symbionts have been removed (Stanton and Palmer 2011, Palmer and Brody 2013). Thus, as with all obligate mutualisms, there exists a tradeoff between benefits received and costs paid by each participant, the balance of which is determined by environmental context (Boucher et al. 1982).

In recent years, the foundational ant-acacia mutualism has been disrupted by the advent of an invasive ant, *Pheidole megacephala*. Commonly known as the big-headed ant, this species is widespread throughout the tropics and subtropics (Wetterer 2012). By forming supercolonies, these invasive ants are able to dominate expansive areas (Fournier et al. 2012, Pietrek et al. *in review*), exterminating native invertebrates and diminishing biodiversity (Hoffman 1998, Wetterer 2007). In the Laikipia region of Kenya, *P. megacephala* initially established in areas of human activity and spread into the surrounding savanna, extirpating the native *Crematogaster* ants that most effectively defend host trees (Riginos et al. 2015). However, the ground nesting *P. megacephala* neither inhabit nor defend trees, leaving *A. drepanolobium* vulnerable to browsing; as a result, trees whose native symbionts have been expunged suffer increased catastrophic herbivory at the trunks of elephants (Riginos et al. 2015).

The Laikipia region is a ca. 10,000 km² woody savanna ecosystem in the central highlands of Kenya. Laikipia contains no formally protected areas; instead, it is a mosaic of private properties which vary in their tolerance for wildlife (Georgiadis et al. 2007). Conservancies house an abundant and diverse suite of large mammalian browsers (e.g. giraffes - *Giraffa camelopardalis reticulata* and black rhinoceros – *Diceros bicornis*; hereafter, and along with elephants, we refer to these collectively as ‘mega-browsers’). Wildlife intolerant properties, by contrast, fence out mega-browsers and are managed largely for livestock (primarily cattle -

Bos taurus indicus) production, which do not browse acacias (Odadi et al. 2007). Invasive *P. megacephala* occur on both conservancies and wildlife-intolerant properties and have the potential to spread across each (Riginos and Goheen, pers obs). Thus, quantifying the demographic mechanisms by which ongoing *P. megacephala* invasion affects acacia populations is key to understanding how the landscape may change in coming years.

We undertook a multi-year, large-scale demographic experiment to quantify the interactive effects of ant invasion and herbivory by mega-browsers on populations of *A. drepanolobium*. Such combinations of experimental manipulations and demographic methods are particularly useful for isolating the effects of multiple environmental factors on individual species (Maclean et al. 2011, Louthan et al. 2018). Through our experimental design, we simulated four scenarios based on the 2x2 combination of *P. megacephala* invasion and exposure to mega-browsers. For tree populations exposed to mega-browsers in uninvaded areas, we expected population growth rates (λ) not to differ statistically from 1.0 (stability) over the course of four years (Scenario 1, Figure 1). This scenario represents a pre-invasion reference population. Following exclusion of mega-browsers in uninvaded areas, we expected λ to exceed 1.0 since trees would be protected (although they still pay the metabolic cost of mutualism; Scenario 2, Figure 1). In *P. megacephala* invaded areas in which tree populations were exposed to mega-browsers, we expected populations to decrease ($\lambda < 1$) due to the combination of browsing and loss of protection by native *Crematogaster* ants (Scenario 3, Figure 1). Finally, in invaded areas where mega-browsers were excluded, we expected tree populations to exhibit the highest λ because they were freed from both the metabolic costs of the mutualism and herbivory by mega-browsers (Scenario 4, Figure 1).

Methods

Study System

Ol Pejeta Conservancy (hereafter OPC) is a 360 km² property in Laikipia County, Kenya, managed for wildlife conservation, tourism, and cattle ranching. The average elevation is 1,810 m and the average annual rainfall (2016-2020) is 809 ± 18 mm (mean ± standard error). The conservancy is situated almost entirely on heavy clay, black-cotton soils dominated by *A. drepanolobium* (Wahungu et al. 2009). The property has higher herbivore biomass than other properties in Laikipia and contains an array of cattle, grazers, and browsers. OPC is also the largest black rhino sanctuary in East Africa which, alongside giraffes, rely on *A. drepanolobium* as the primary part of their diet (Kartzinel et al. 2015).

The invasive *P. megacephala* likely arrived at OPC before 2005 (Riginos 2015). Initially, *P. megacephala* established around areas of concentrated human activity; they have since spread into the surrounding savanna. The spatial distribution of *P. megacephala* invasion on OPC used to guide our experimental design was mapped in a concurrent study which measured ant responses to baiting along transects (Pietrek et al. *in review*).

Experimental Design

In January of 2017, we established twelve 50 m² study plots across OPC in a 2x2 factorial design. For each of three blocks of replicated plots, a pair of plots was established on each side of an invasion front (the furthest extent of the spread of *P. megacephala*). Two plots were located between 0.5-2.5 km behind an invasion front (hereafter ‘invaded plots’ or ‘+ Invasion’) and two plots were located a similar distance beyond the same invasion front (hereafter ‘uninvaded plots’ or ‘- Invasion’). Since *P. megacephala* spread at a rate of ~100 m per year (Pietrek et al. 2020), this distance ensured both that uninvaded plots would not be invaded during

the course of the study and that invaded plots represented longer-term (~10 years') effects of invasion. To exclude mega-browsers, we constructed electrified fences around half of the invaded plots and half of the uninvaded plots. The fences consisted of two metal wires ~0.4 and 0.8 m above the ground with short lengths of wire (~0.5 m) projecting outwards from the top wire at regular intervals. These fences effectively excluded mega-browsers and other large herbivores while enabling smaller (≤ 10 kg) herbivores to access the plots. Since most grazing wildlife were also excluded, we periodically grazed cattle within fenced plots to maintain grass height at a level comparable to surrounding areas, thereby controlling for competitive suppression of tree growth by grasses (Riginos 2009). In sum, we had three replicates of four treatments based on the two-way combination of *P. megacephala* invasion and exposure to mega-browsers: +Invasion/+Mega-browsers, +Invasion/-Mega-browsers, -Invasion/+Mega-browsers, and -Invasion/-Mega-browsers.

Data Collection

Each January from 2017-2020, we collected demographic data on a structured sample of trees within study plots. We divided trees into five broad categories based on height (<0.5 m, 0.5-1.5 m, 1.5-2.5 m, 2.5-3.5 m, >3.5 m) and recorded data for 10 trees randomly selected within each category, within each plot. Our selection encompassed nearly all of the trees >2.5 m but only a sample of trees <0.5 m. *Acacia drepanolobium* readily coppices after severe damage by mega-browsers, such that previously large trees might be drastically reduced in height. Because we believed they might perform differently than trees of similar size, we also recorded data for 10 coppices in each plot, which were defined as trees <0.5 m tall that had clearly been taller (based on their diameter) prior to being browsed. It could sometimes be difficult to distinguish this

difference, such that some non-coppice trees <0.5 m may in fact have been coppices. We collected data on a maximum of 60 trees within each plot, although not all plots contained 10 trees >3.5 m (range = 1-10 trees >3.5 m per plot). Each year, we measured height, diameter at 30 cm above ground level (or as close as possible for trees <0.5 m and coppices), ant occupant, and mortality. We marked locations of diameter measurements with paint to ensure accuracy in the locations of measurements in subsequent years. We ensured mortality events represented true tree deaths, and not delayed conversion to coppices, by revisiting dead trees in subsequent years to check for any growth. Over the course of the study, there were a handful of times when elephants broke the fences and entered mega-browser exclosure plots; if study trees were damaged, we stopped recording data for these trees and instead followed a replacement tree of similar size in subsequent years (16 trees).

At OPC, *A. drepanolobium* reproduce between July - September. From May through September, we visited each tree to record whether they were reproductive (defined as any presence of flowers, flower buds, or fruits). Seeds of *A. drepanolobium* dangle from arils within fruits and are dispersed on windy days when mature (Goheen et al. 2007). So, for each tree found to be reproductive and also producing fruit, we returned on a weekly basis until fruits dehisced and then collected all fruits by hand. We wore gloves while collecting fruits to avoid scent contamination that might deter post dispersal seed predators and bias our estimates of germination (Goheen et al. 2004). Seeds were then separated from fruits and subsequently weighed and counted; any seeds with damage from bruchid beetles were discarded to avoid biasing germination (Goheen et al. 2004). To measure seed survival, we then scattered the seeds in 1 m² plots randomly placed beneath parent trees and monitored for germination. To avoid contamination of germination plots by seeds from nearby, non-study trees, we removed the fruits

from these trees and scattered them outside of the study plots. Any germinants within our 1 m² plots were marked with an individually numbered nail and subsequently monitored bi-weekly for up to 6 months, at which point individuals were assumed to have recruited to the sapling stage (Goheen et al. 2010).

Demographic Analyses

We followed a stage-based matrix projection approach (Caswell 2001, Morris and Doak 2002), with all analyses carried out in R statistical software (R Core Team). First, we selected a classifying variable by creating five vital rate regressions (growth, variance in growth, survival, probability of reproduction, and number of seeds produced given reproduction) using either height or diameter alone as our predictor variable. For this and all other analyses, data were pooled across treatment replicates. Variance in growth was calculated as the square of model residuals from the growth regression. Because it consistently produced higher R^2 values than diameter, we used height as the classifying variable in our regressions and matrices.

We created global models of the five vital rates as a function of tree height and treatment; we also included year as a random effect using the *lme4* package in R (Bates et al. 2015). Building regressions from observed data to predict vital rates across a range of tree heights (including heights not observed directly) reduces uncertainty in matrix elements and produces more accurate estimates of population growth (Gross et al. 2006). For model evaluation, we followed an information-theoretic approach and used Akaike's Information Criterion adjusted for small sample sizes (AICc; Burnham and Anderson 2002). We then used AICc selection in the *dredge* function to determine the top candidate model (Barton 2020). For survival, probability of reproduction, and seed production, there were multiple models with $\Delta\text{AICc} < 2.0$. In these instances, we ensured that no changes in the directionality of model parameters occurred and

then averaged all models within 2.0 AICc of the best model (model with $\Delta\text{AICc} = 0$). We did not create a regression for seed survival (i.e., germination and subsequent recruitment to saplings) both because of limited sample sizes and because we did not expect seed survival to differ based on tree height (although there may have been variation among years that we did not capture). Instead, we pooled seed survival data across years and used a single value per treatment (Appendix S1: Table S1).

We divided trees into 63 classes of 0.1 m height increments when constructing projection matrices, which achieves precision comparable to integral projection models (Shriver et al. 2012). Because inclusion of coppices as a distinct class did not significantly alter population growth rates, we did not differentiate between coppices and non-coppices (i.e., juvenile trees < 0.5 m) in matrix projections. The final regressions were used to predict vital rate values for each of these 63 height classes. To incorporate both model and parameter uncertainty into our estimates of λ , we used the `mvrnorm` function in the MASS package (Venables & Ripley 2002) to make repeated draws from parameter error distributions (Louthan et al. 2018). These were normal distributions with means equal to the regression parameter estimates and standard deviations equal to the variance-covariance matrix of regression parameters. In cases with a single top model, we performed a total of 1,000 draws for the parameters. In cases where there were multiple top models (i.e., multiple models with $\Delta\text{AICc} < 2$), we drew from the distributions of each of the top models in proportion to their model weights, with the sum of draws totaling to 1,000. The averages of these 1,000 estimates were used in the final model for predicting values of vital rates for each particular height class.

Predicted values from vital rate regressions were entered into a 64x64 population projection matrix (63 height classes, plus seeds). We created a single matrix for each treatment

and each transition year for a total of 12 projection matrices. Our data represent an intermediate breeding census (Morris and Doak, 2002) since reproduction data were collected at a different time of year from survival and growth data. Therefore, to calculate fertility matrix elements (the top row of the projection matrix), we multiplied adult reproduction by the probability of adult survival from census data collection to time of reproduction. We assumed that this probability of survival would be equal to overall adult survival for each height class. As such, the top cell of each column in the matrix (with the exception of seeds, which do not reproduce) was: Probability of Reproduction * Seed Production * Probability of Adult Survival. All cells below the first row for adult trees represent the probability of a tree transitioning from height class i to height class j , equal to $Growth_{j,i} * Survival_i$. Growth probabilities were calculated from a normal distribution with a mean equal to the average height at time $t + 1$ of a tree starting at height class i at time t (predicted from the growth regression). The standard deviation of the normal distribution was equal to the square root of model predicted growth variance for a tree of height class i . The first column, representing seed transition probabilities, contained only the value for seed survival, as seedlings only recruited to the smallest height class (0.10 – 0.19 m trees).

We used the popbio package (Stubben and Milligan 2007) to calculate deterministic growth rates (λ) for each year and treatment combination. Using the three transition years per treatment, we also calculated stochastic λ values for each treatment. Using the same stochastic approach, we also calculated predicted population sizes for each treatment 10 years in the future. We then calculated the expected reproductive value of trees by height. This is the average number of new trees (≥ 0.1 m tall) that a tree of a given height is expected to contribute to the population over its remaining lifespan.

Reproductive values and λ values are prospective analyses: both make predictions by assuming the observed (i.e., current) conditions represent future conditions. However, all environments – and savanna ecosystems in particular - exhibit variability over time. Therefore, and to complement these prospective analyses, we conducted a retrospective analysis (a Life Table Response Experiment [LTRE]) to quantify the relative contributions of observed vital rates at different tree heights in generating differences in (deterministic) λ values among treatments (Caswell 2001, Bruna and Oli 2005, Ali et al. 2018). We first examined the elasticities (the proportional change in λ with a small change in a vital rate) as a function of height to determine the potential of different vital rates to affect λ . We then carried out an LTRE analysis to calculate the contribution of vital rates to the differences in λ between our reference condition (- Invasion/+Mega-browsers) and each of the other three treatments. We first did this for each of the five vital rates pooled across all 64 height classes. This entailed starting with the projection matrix (averaged across years) for the reference condition and substituting values from the treatment being compared, one vital rate at a time. Growth rates (λ) were then determined for the altered projection matrix and compared to the original λ of the reference condition. We then examined the relative importance of height class pooled vital rates over time. For each year and treatment, we created a regression of differences in λ from whole vital rate substitutions as a function of which vital rate was changed and all possible interactions (Equation 1). For these regressions, growth and growth variance were paired as ‘growth’ and probability of reproduction, seed production, and seed survival were merged as ‘reproduction’.

$$\text{Difference in } \lambda \sim \text{Growth} * \text{Survival} * \text{Reproduction} \quad \text{Equation 1}$$

We then performed an ANOVA on each regression. We calculated the relative importance of each vital rate and interaction as the proportion of each vital rate’s sum of squares out of the total

sum of squares. Finally, we repeated the LTRE process for height class-specific vital rates, swapping out individual values within each matrix element, one vital rate at a time. The LTRE approach assumes additivity amongst the height class-specific vital rates: that is, that the sum of all height class-specific contributions should closely approximate the overall contribution of the vital rate, pooled across all height classes. However, because of interactions between vital rates within the projection matrix, changes to a single height class-specific vital rate may produce non-additive effects on λ , particularly when λ values are large. Therefore, we present the absolute values only for the overall contributions of vital rates, pooled across height classes. For height class-specific contributions of vital rates, instead of absolute values, we present contributions as a percentage of the summation of all height class specific contributions for a given vital rate.

Results

Consistently, our best-supported regression models for vital rates included an effect of mega-browsers; an effect of ant invasion was retained in models for survival, probability of reproduction, and seed production (Table 1, Figure 2). *P. megacephala* did not have an effect on regressions for growth or variance in growth, so no effect of *P. megacephala* was manifested through these vital rates in the matrix models. Interactions between exposure to mega-browsers and tree height featured in all vital rate regressions except survival, such that slopes of vital rate regressions differed with the presence of mega-browsers (Figure 2A-D). There was also an interaction between ant invasion and mega-browsers' presence for probability of reproduction, seed production, and survival (Figure 2C-2E).

Mega-browsers and *P. megacephala* reduced both deterministic and stochastic λ (Figure 3). Trees protected from mega-browsers exhibited stable or growing populations while trees

exposed to mega-browsers exhibited declining populations. *P. megacephala* invasion further reduced both deterministic and stochastic λ , but these effects were small relative to those of mega-browsers (Figure 3). Nevertheless, reductions in stochastic λ attributable to *P. megacephala* invasion resulted in pronounced differences in predicted population sizes and size structure 10 years in the future (Figure 4A). This is partially driven by the higher reproductive values of trees in uninvaded areas (Figure 4B). Larger trees (≥ 3.5 m) in areas protected from mega-browsers and not invaded by *P. megacephala* had 25-60% higher reproductive values than similarly protected trees in invaded areas (Figure 4B).

Our LTRE analysis with vital rates pooled among height classes showed that mega-browsers suppressed λ primarily by reducing growth and survival (Table 2). Similarly, *P. megacephala* invasion suppressed λ by reducing survival regardless of exposure to mega-browsers, but also reduced seed production where mega-browsers were excluded (Table 2). While there was some interannual variation, growth and survival were consistently the most important drivers of λ across all three transition years (Appendix S1: Figure S1).

When separated by height classes, LTRE contributions revealed the differential importance of trees of varying heights in driving λ . Such contributions are influenced both by vital rate regressions (Figure 2) and demographic elasticities (Appendix S1: Figure S2). For trees exposed to mega-browsers, λ was most elastic to changes in growth and survival of the smallest trees (< 0.5 m) and was relatively inelastic to reproduction (i.e., both probability of reproduction and seed production). Trees protected from mega-browsers exhibited low elasticity overall, but elasticity was variable across height classes for reproduction (Appendix S1: Figure S2).

Our LTRE analyses mirrored these patterns: trees < 1.5 m contributed the most to differences in λ between treatments and the reference condition (-Invasion/+Mega-browsers) for

growth, growth variance, and survival (Figure 5). This was partially due to the generally low survival rates in the reference condition and relatively large differences between survival rates in the reference condition and rates in the other treatments (Figure 2E). Additionally, variance in growth was near zero for the smallest trees in populations exposed to mega-browsers (Figure 1B). Since the slopes of the growth regressions (averaged across all three years) were less than one (Figure 1A), a variance near zero precludes the possibility of growth, resulting in low λ values. To further illustrate the impact of survival of small trees on λ , we set survival of the 10 smallest height classes (0.1-1.1 m) in populations exposed to mega-browsers as equal to those in populations protected from mega-browsers, causing population growth to stabilize ($\lambda \sim 1$; Appendix S1: Table S2). Manipulating other vital rates in the same manner did not produce such large increases in λ , reinforcing the importance of survival of small trees.

Discussion

Invasion by big-headed ants reduced population growth of *A. drepanolobium*. In contrast to our expectations (Figure 1), however, this reduction in population growth occurred regardless of the presence of mega-browsers. Unlike native *Crematogaster* ants, big-headed ants neither defend trees nor do they stimulate extrafloral nectar production (Riginos et al. 2015); thus, we predicted that population growth would increase for trees protected from mega-browsers following invasion by *P. megacephala*. Instead, we found that tree populations in invaded areas performed worse, even in the absence of mega-browsers; from this we infer that, in addition to removing native-ant defenses against browsing damage, *P. megacephala* are imposing a direct (metabolic) cost to individual trees. This is supported by recent evidence from greenhouse experiments showing that *P. megacephala* are attacking root systems of *A. drepanolobium*

(Milligan et al. *in review*). In sum, the costs and benefits of mutualisms are a product of the environmental contexts in which they evolved and the effects of their disruption will similarly be shaped by environmental context (Boucher 1982, Bronstein 1994, Palmer et al. 2008, Hoeksema runa 2015).

Although big headed ants suppressed population growth in all areas where they were present, their effect on trees manifested through different demographic pathways depending on the presence or absence of mega-browsers. Tree populations in invaded areas had lower survival than their uninvaded counterparts regardless of browsing pressure, however the difference in survival and its effect on λ were greater when trees were exposed to mega-browsers. Similarly, while trees protected from mega-browsers exhibited increased reproductive values regardless of invasion, trees in uninvaded areas had higher seed production and seedling survival. While invasion by *P. megacephala* is not strong enough to cause negative population growth alone, it compounds the destructive effects of browsing while limiting the beneficial effects of its removal.

In contrast to the demographic effects of *P. megacephala* invasion, those of mega-browsers were sufficiently strong to switch population growth from positive ($\lambda > 1$) to negative ($\lambda < 1$), in accordance with previous studies (Maclean et al. 2011). Suppression of λ by mega-browsers was largely driven by lowered survival and growth of the smallest (<0.5 m) trees: when survival rates for these were set equal to those of trees protected from mega-browsers, populations were nearly stabilized. Similar studies have found browsers limit population growth of acacia trees by suppressing growth and survival of saplings (Augustine and Mcnaughton 2004, Western and Maitumo 2004). Additionally, in previous studies on *A. drepanolobium*, browsing pressure forced the reallocation of energy from reproduction to defensive investment (spines),

further reducing λ by limiting reproduction (Goheen et al. 2007, Maclean et al. 2011). Mega-browsers thus exert a strong effect on all vital rates of *A. drepanolobium*, though their effect varies by tree size.

Reference populations (-Invasion/+Mega-browsers) indicate that, at least over the course of our four-year study, *A. drepanolobium* populations are declining. By contrast, populations in a nearby study system (~50km) are stable (Goheen and Palmer 2010); this discrepancy may be attributable to higher densities of mega-browsers on Ol Pejeta Conservancy. Tree populations in savanna ecosystems are often regarded as non-equilibrial, where variability in browsing, fire, and rainfall prevent canopy closure (Sankaran et al. 2004, Holdo et al. 2009, Ratnam et al. 2011, Daskin et al. 2016, Pellegrini et al. 2017). Mega-browsers, in concert with other factors (e.g., fire, drought, or invasive species), can limit or reduce tree abundance and cover, sometimes preventing regeneration (Western and Maitumo 2004, Baxter and Getz 2005, Riginos et al. 2015). In our study area, tree populations exposed to mega-browsers in *P. megacephala* invaded areas are declining more rapidly than trees in uninvaded areas. In a savanna ecosystem that relies on a balance of browsing and regeneration to regulate tree-grass dynamics (Goheen et al. 2010, Goheen et al. 2018), the exogenous influence of *P. megacephala* may compromise tree cover that was previously maintained by the native mutualism (Goheen and Palmer 2010). Additional years of data would have allowed us to quantify longer term population dynamics and better contextualize the relative importance of invasion. Four years of demographic data is not ideal for a species that can live for 100 years. However, our data collection spanned a range of environmental and phenological variation, including both drought and heavy rainfall years as well as highly reproductive and completely non-reproductive years. As such, we are confident that our models incorporate realistic environmental variation for our study system.

Across Laikipia, the native ant-acacia mutualism is essential for maintenance of the woody savanna ecosystem. *Acacia drepanolobium* is directly consumed by several globally endangered mega-browsers (e.g., black rhinoceroses and giraffes; Riginos and Grace 2008, Kartzinel et al. 2015). Additionally, *A. drepanolobium* is essential habitat for several species of birds and lizards (Pringle et al. 2015, Gregory et al. 2010, Carpenter et al. *in prep*), and influences large carnivore predation (Ng'weno et al. 2019, Kamaru et al. *in prep*). Reductions in tree cover by mega-browsers may further limit trees' ability to regenerate by suppressing reproduction of adult trees as well as growth and survival of saplings (Western and Maitumo 2004, Goheen et al. 2007). As elephant populations in Laikipia increase (Ogutu et al. 2016) and *P. megacephala* continue to spread (Pietrek et al. *in review*), *A. drepanolobium* populations are likely to decline in the future. Interventions to increase survival of small trees and allow them to grow to larger, less vulnerable, sizes might be used to bolster population growth rates and stabilize acacia populations. A recent study showed that *A. drepanolobium* saplings will grow rapidly in years of heavy rainfall when protected from browsing pressure (LaMalfa et al. *in review*). The combination of targeted fencing of small trees with high rainfall years or irrigation could offset increasing populations of mega-browsers.

Big headed ants have reduced populations of a monodominant tree both directly (by lowering survival and reproduction) and indirectly (by disrupting a foundational mutualism that otherwise enhances tree survival in the face of browsing pressure). Future research may document the cascading impacts of reductions in *A. drepanolobium* populations on community and ecosystem properties, including nutrient cycling, fire return intervals, and patterns of biodiversity. Other anthropological impacts, from climate change to hunting, alter the abundance, distribution, and diversity of species linked to mutualisms (e.g., Hoegh-Guldberg et

al. 2007, Terborg et al. 2008, de Fouw et al. 2016, Rosin and Poulsen 2016). Across the globe, mutualism disruption is accelerating the ongoing declines of biodiversity (Kiers et al. 2010, Aslan et al. 2013). Further research that elucidates the demographic mechanisms by which such mutualism breakdowns occur, and links those mechanisms to whole communities and ecosystems, will be vital to informing conservation and restoration strategies.

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Figures and Tables

Table 1. Best supported models for vital rate regressions after averaging all top candidate models ($\Delta AICc < 2.0$). A random effect of year (1|year) is included in each model.

Vital Rate	Distribution	Best-Supported Model
Growth	Normal	$\text{height}_{Y2} \sim \text{height}_{Y1} + \text{mega-browsers} + \text{mega-browsers}:\text{height}_{Y1} + (1 \text{year})$
Variance in Growth	Normal	$\text{variance} \sim \text{height}_{Y1} + \text{mega-browsers} + \text{mega-browsers}:\text{height}_{Y1} + (1 \text{year})$
Survival	Binomial	$\text{survival} \sim \text{height} + \text{mega-browsers} + \text{BHA invasion} + \text{mega-browsers} * \text{BHA invasion} + (1 \text{year})$
Probability of Reproduction	Binomial	$\text{prob. reproduction} \sim \text{height} + \text{mega-browsers} + \text{BHA invasion} + \text{height} * \text{mega-browsers} + \text{height} * \text{BHA invasion} + \text{mega-browsers} * \text{BHA invasion} + \text{height} * \text{mega-browsers} * \text{BHA invasion} + (1 \text{year})$
Seeds Produced by Reproductive Trees	Normal	$\text{seed production} \sim \text{height} + \text{mega-browsers} + \text{BHA invasion} + \text{height} * \text{mega-browsers} + \text{mega-browsers} * \text{BHA invasion} + (1 \text{year})$

Table 2. Summed LTRE contributions to differences in λ between treatments and the reference condition (-Invasion/+Mega-browsers) for each vital rate, averaged across all 3 transition years. Standard errors are included with each averaged contribution. Because the vital rate regressions for growth and variance in growth did not include a slope parameter for ant invasion, the contributions of these vital rates are identical in the treatments without mega-browsers and zero in the invaded treatment with mega-browsers.

	Growth	Growth Variance	Survival	Probability of Reproduction	Seed Production	Seed Survival
+Invasion/-Mega-browsers	0.041 ± 0.032	-0.0001 ± 0.0001	0.053 ± 0.007	0.0006 ± 0.0005	0.0004 ± 0.0004	-0.0003 ± 0.0003
+Invasion/+Mega-browsers	0	0	-0.021 ± 0.003	-0.0002 ± 0.0001	-0.0003 ± 0.0003	-0.0002 ± 0.0002
-Invasion/-Mega-browsers	0.041 ± 0.032	-0.0001 ± 0.0001	0.056 ± 0.008	-0.0001 ± 0.0001	0.0013 ± 0.0001	0.0003 ± 0.0003

Figure 1. Hypothesized population growth rates under four scenarios resulting from the pairwise combination of exposure to mega-browsers and BHA invasion. We expected that populations protected from mega-browsers (-Mega) would increase, while those exposed to mega-browsers (+Mega) would remain stable or decline. Because BHA kill native *Crematogaster* ants that defend host trees, and because trees must pay a metabolic cost to house *Crematogaster*, we expected BHA invasion (+Inv) to increase population growth for trees protected from mega-browsers, but to decrease population growth for trees exposed to mega-browsers.

Figure 2. Vital rate regressions averaged across years as a function of tree height. For growth and variance in growth, the slope of the regression is affected only by mega-browser presence and not by BHA invasion. Both mega-browsers and BHA reduced seed production and survival of adult trees. For probability of reproduction, there is an interaction between mega-browser presence, BHA invasion, and height. Small trees protected from mega-browsers have higher probabilities of reproduction in invaded areas than in uninvaded areas, but the reverse is true for large trees. For trees exposed to mega-browsers, the trend is opposite.

Figure 3. Deterministic and stochastic population growth rates (λ) for the four study treatments. Error bars around stochastic λ values represent 95% confidence intervals. Stochastic λ values are significantly different among all treatments. Both mega-browsers and BHA reduce λ ; mega-browsers depress λ more strongly than BHA.

Figure 4. A) Stochastic projections of population sizes and distributions for the four experimental treatments 10 years into the future (2030). Note the difference in y-axis scales for treatments

exposed to mega-browsers (right) and protected from mega-browsers (left). Initial distributions of population sizes for the projections were taken from trees in 2020. Initial seed numbers were taken from averages of seed production in 2017 and 2019, the two years of reproduction in our dataset. Total projected population sizes are: +Invasion/-Mega-browsers, 399 trees; +Invasion/+Mega-browsers, 54 trees; -Invasion/-Mega-browsers, 1,220 trees; -Invasion/+Mega-browsers, 87 trees.

B) Reproductive values calculated from population projection matrices, averaged across all 3 transition years. Values represent the average number of individuals recruited to the population by a tree of a given height class over their remaining lifespan. Thus, a tree that is 6.3m tall in a -Invasion/-Mega-browsers plot is expected to recruit 52 new individuals while a tree of equal height in a +Invasion/-Mega-browsers plot is expected to recruit only 32 new individuals.

Figure 5. Percentage of each height class of the total LTRE contribution to differences in λ between the reference condition (-Invasion/+Mega-browsers) and other treatments, per vital rate. Positive numbers represent height classes that contribute to a positive difference in λ relative to the reference condition; negative numbers represent height classes that contribute negatively relative to the reference condition. Contributions were calculated in 0.1 m height classes and then aggregated into larger classes for ease of display. There is no contribution to differences in λ between the reference condition and +Invasion/+Mega-browsers due to growth or growth variance because there was no effect of BHA invasion in those vital rate regressions.

Figure 1

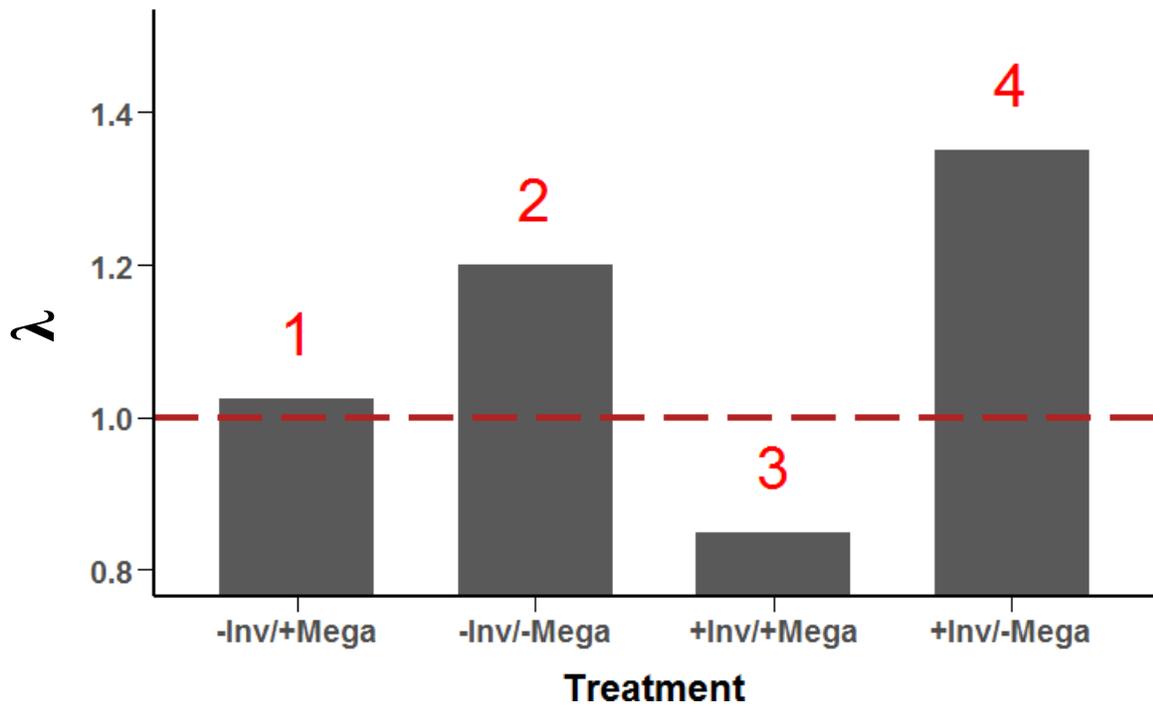


Figure 2

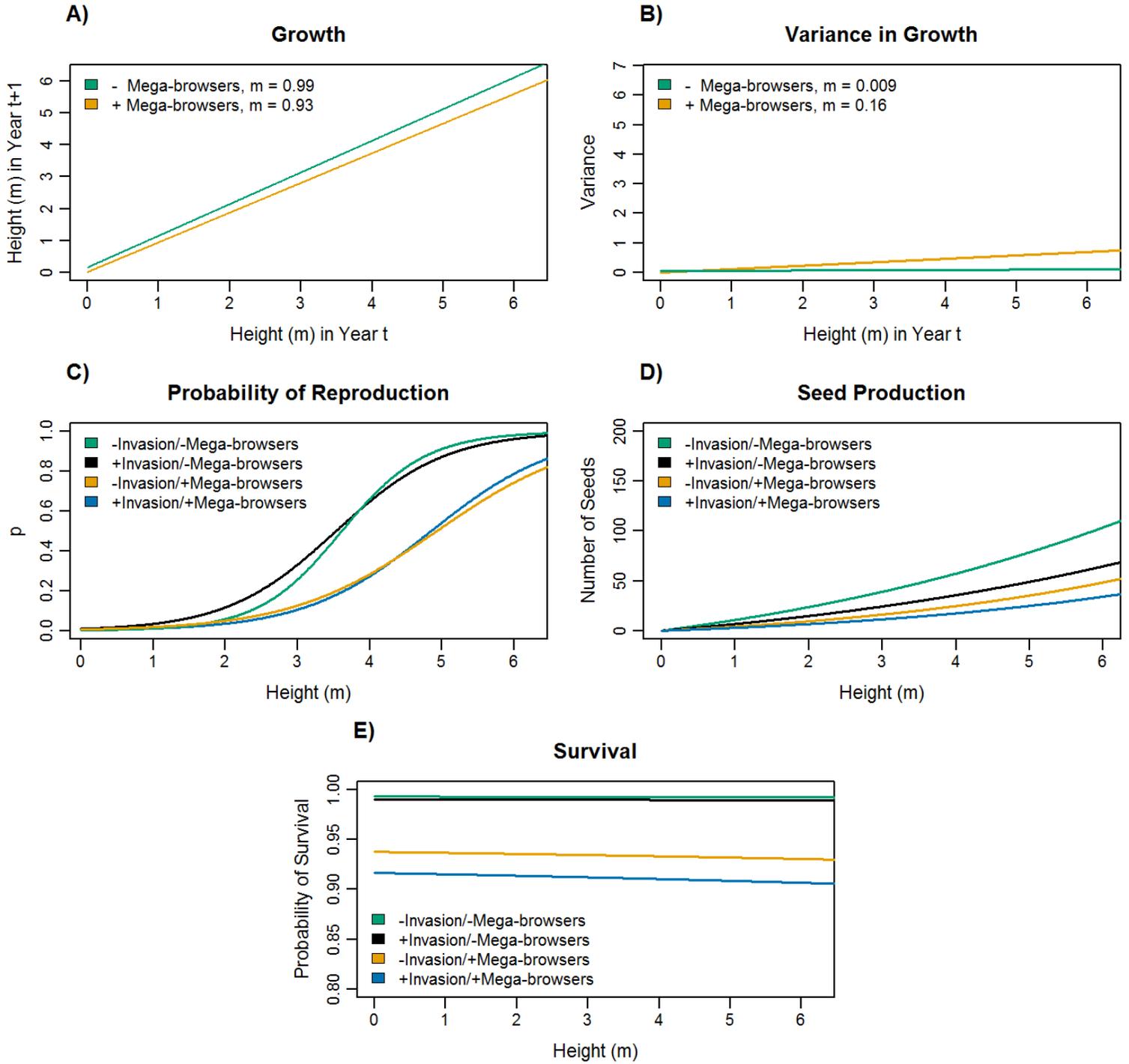


Figure 3

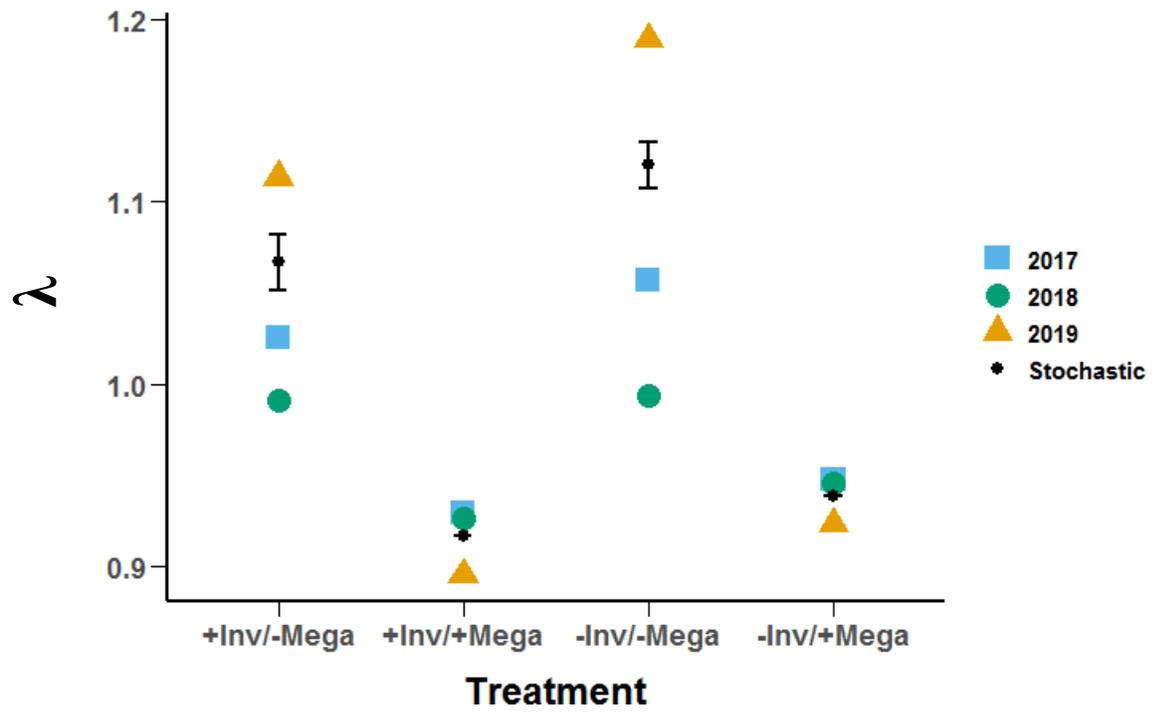


Figure 4

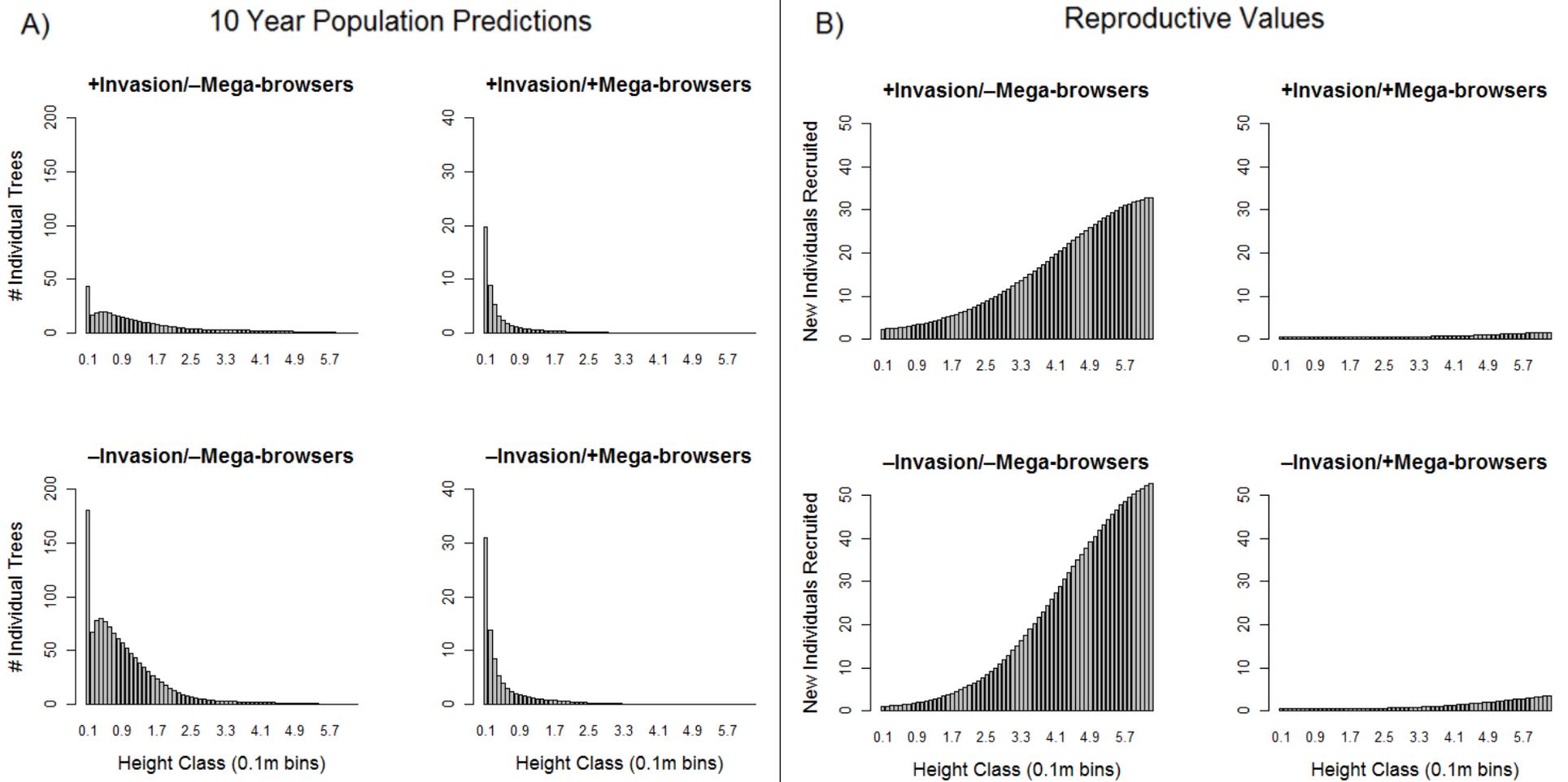
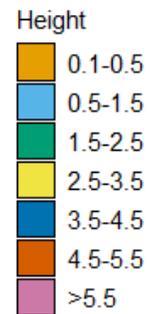
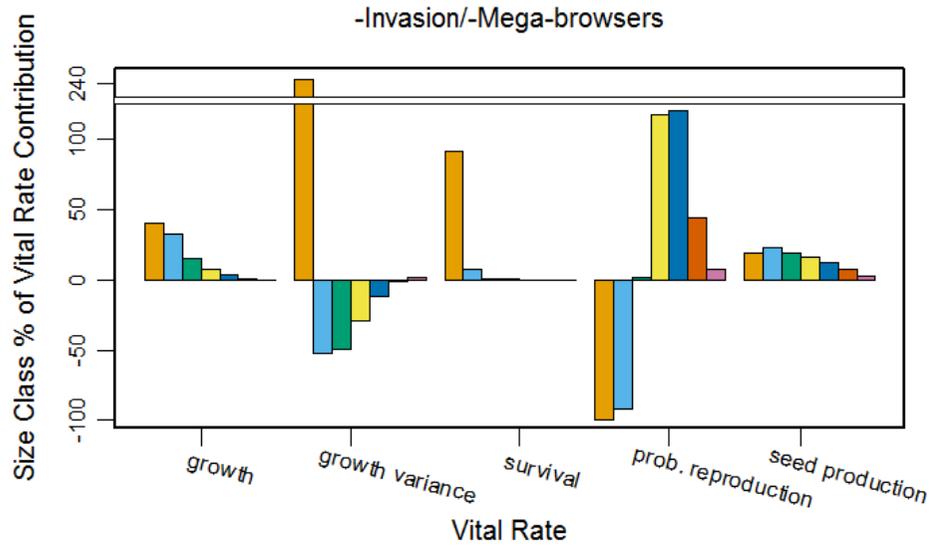
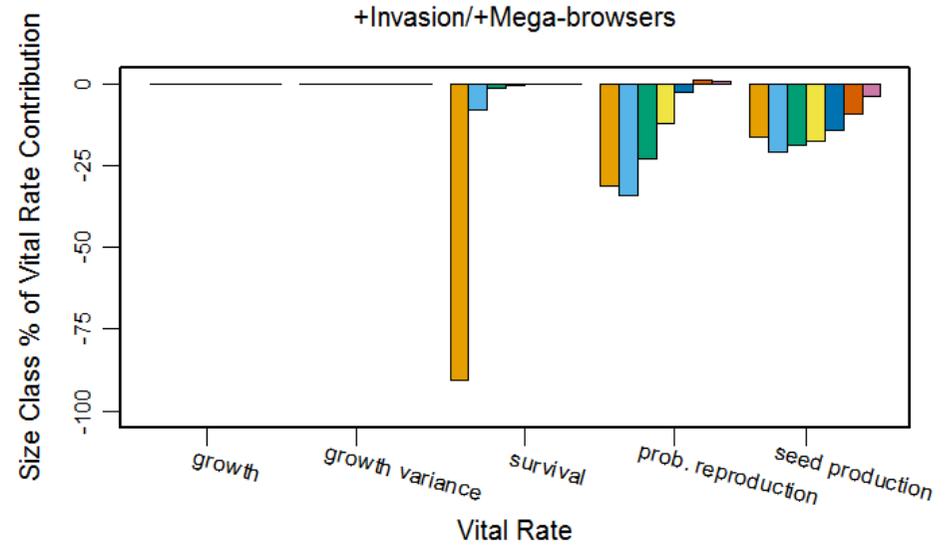
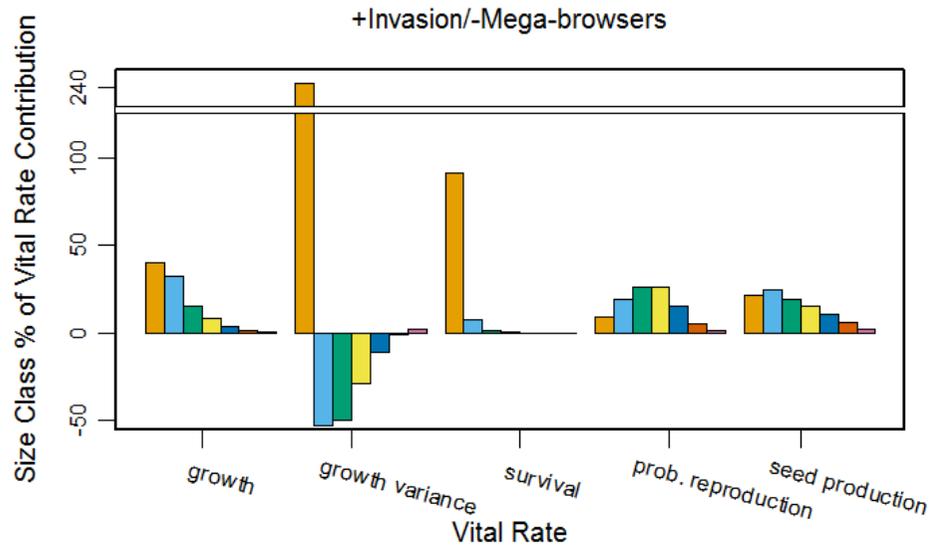


Figure 5



Chapter 2 – Appendix

Supporting Information. 2020. Demographic pathways of mutualism disruption: synergies between mega-herbivory and big-headed ant invasion on an ant-acacia symbiosis. Ecology

Appendix S1: Figures and Tables

Table S1. Seed survival rates (i.e., rates of germination and recruitment to the sapling stage) and standard errors for the four experimental treatments.

Treatment	Seed Survival	Standard Error
+Invasion/-Mega-browsers	0.012	0.002
+Invasion/+Mega-browsers	0.015	0.002
-Invasion/-Mega-browsers	0.034	0.004
-Invasion/+Mega-browsers	0.021	0.002

Table S2. Stochastic λ values and 95% confidence intervals after changing survival rates of the ten smallest size classes in treatments exposed to mega-browsers to be equal to survival rates of treatments protected from mega-browsers.

Treatment	λ	95% CI
+Invasion/+Mega-browsers	0.989	0.00005
-Invasion/+Mega-browsers	0.992	0.0002

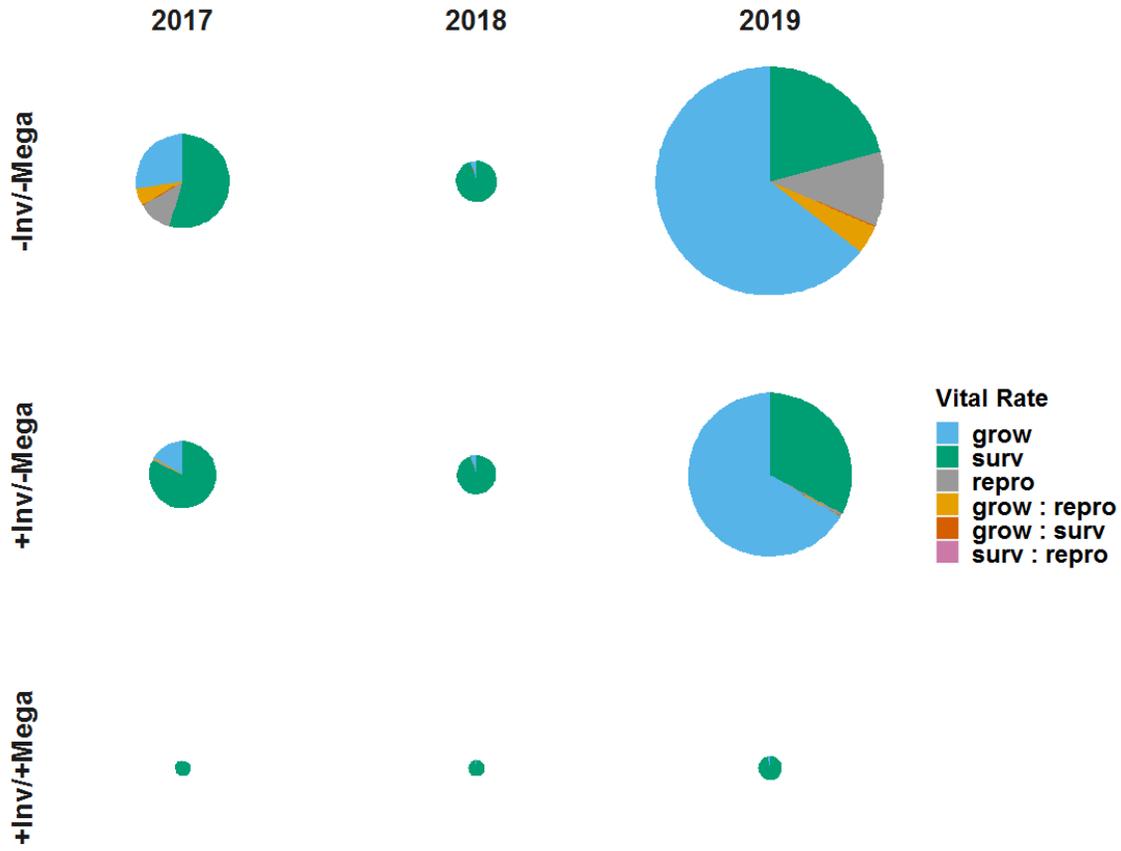


Figure S1. LTR contributions of each vital rate and interactions to differences in λ between the reference condition (-Invasion/+Mega-browsers) and each remaining treatment. Pie chart sizes are scaled by the total difference in λ between the treatment and reference condition. The two growth vital rates (growth and variance in growth) were combined, as were the three reproduction vital rates (probability of reproduction, seed production, and seed survival). The first row represents the effect of removing mega-browsers from the reference condition. The second row represents the effect of removing mega-browsers and adding invasive ants. The third row represents only adding invasive ants.

Survival makes the greatest contribution to differences in λ in years with little or no reproduction (2017 and 2018). In 2019, a year of high reproduction, growth was the largest contributor in treatments protected from mega-browsers, which had much higher levels of reproduction than the reference condition. For 2019, survival remains the largest contributor to λ in +Invaded/+Mega-browsers because growth for this treatment does not differ from the reference condition, and reproduction was comparable to the reference condition.

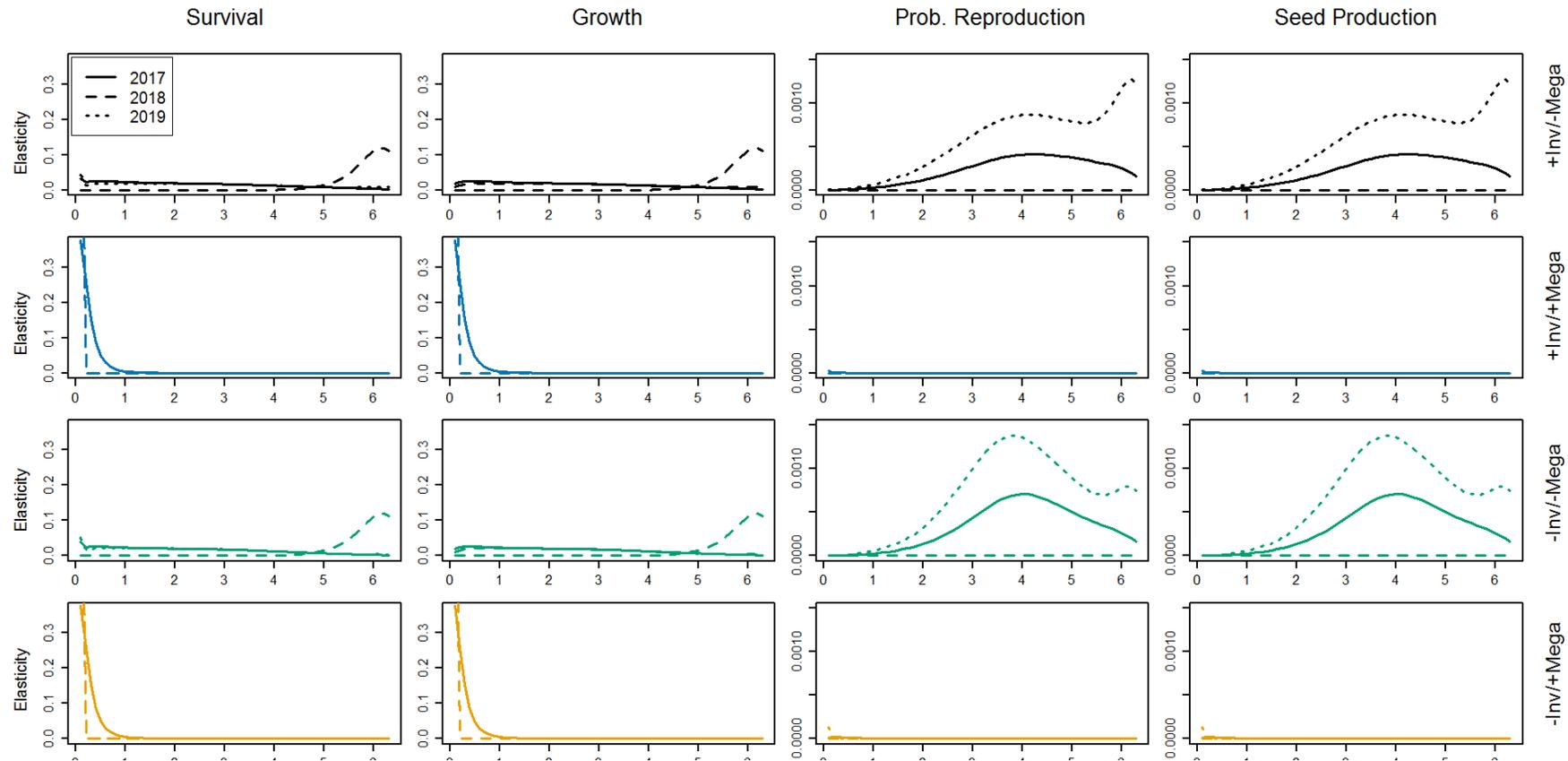


Figure S2. Elasticity of survival, growth, seed production, and probability of reproduction as a function of tree height. Note the differences in scale between survival/growth and seed production/probability of reproduction. The values for growth are the sums of elasticities for all possible transitions from a given size class to all others. Elasticities are calculated for each transition year, shown by different line types.

Across most years and all treatments, survival and growth of the smallest trees has the greatest proportional effect on λ , though this effect is substantially greater for trees exposed to mega-browsers. There is virtually no elasticity for the reproductive vital rates in plots exposed to mega-browsers. By contrast, the elasticity of reproduction rates is comparatively high in plots protected from mega-browsers, even if they are still much lower than survival and growth elasticities. There is some interannual variability in these treatments, with higher elasticity in a year of high reproduction (2019), less elasticity in a year of less reproduction (2017), and no elasticity in a year without reproduction (2018).

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