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ORIGINAL PAPER

Ant invasion is associated with lower root density and different root distribution of a foundational savanna tree species

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Abstract Some invasive ants have worldwide distributions and impose substantial impacts on human society and native biodiversity. Yet we know little about how ants impact soil ecosystems in general, much less how soil ecosystems shift when invasive ants move in. We excavated the coarse roots of a monodominant savanna tree in invaded and uninvaded areas to test the hypothesis that the presence of invasive ants would be associated with changes in root distribution and biomass across the landscape. We found that in the presence of invasive ants, trees had a shifted distribution of lateral coarse roots, with proportionally less root biomass near the surface and far from tree stems. In addition, the density of lateral coarse-root biomass was ~20% lower for trees within invaded landscapes. Our results suggest that soil-nesting invasive ants can drive important changes in rooting strategy for a tree species that serves a foundational role in the biogeochemical cycles of vertisol savannas.

Keywords Ant acacia · Invasive soil macrofauna · *Pheidole megacephala* · Root functional traits · Soil ecosystem engineering · *Vachellia drepanolobium*

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Introduction

Soil macrofauna, such as earthworms, termites, and ants, act as soil engineers with understudied but potentially important influences on biogeochemical cycles (Cammeraat and Risch 2008; Jackson et al. 2017; Lavelle et al. 2020). Comparing soils on and off ant mounds, for example, has shown that ants can modify soil nutrients, aggregates, particle size, macropores, bulk density, and water infiltration (e.g., Nkem et al. 2000; Drager et al. 2016; Leite et al. 2018). By these modifications, ants can indirectly affect growth, nutrient content, and/or community composition of plant neighbors (e.g., Moutinho et al. 2003; Lafleur et al. 2005; DeFauw et al. 2008; Farji-Brener and Werenkraut 2017; De Almeida et al. 2020). Ants are hugely productive—estimated to comprise over a third of global insect

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biomass (Wilson and Hölldobler 2005; Schultheiss et al. 2022)—and can influence ecosystem processes as incipient or mature colonies, and even after colony death (reviewed by, e.g., Folgarait 1998; Del Toro et al. 2012). Ant effects on soil and its resident biota may thus apply to entire landscapes (Green et al. 1998), but such effects are rarely quantified (Lavelle et al. 2020; but see Zhong et al. 2021).

Invasive soil macrofauna create natural experiments to investigate unresolved impacts of these animals on ecosystems. For example, invasive earthworms (Lumbricidae) in North America increase soil nutrients, aggregation, and bioturbation (Frelich et al. 2006), and this direct interaction between earthworms and soil can increase greenhouse gas emissions (Lubbers et al. 2013). Earthworms also influence plant roots and aboveground growth (Springett and Gray 1997; Frelich et al. 2006), and Lubbers et al. (2013) noted that this influence has not been included in predictions for greenhouse gas emissions. Such indirect interactions, mediated by plants, may increase or even negate earthworm-driven soil gas emissions. Indeed, a key unresolved question is whether plant-animal interactions help drive the emergent effects of invasive soil macrofauna on terrestrial carbon and nitrogen cycling (Lubbers et al. 2013).

When invasive ants (Formicidae) establish a sufficiently large population, they can rapidly extirpate native ants, and the invasiveness of some ant species is enhanced by having large, distributed nests and foraging (Holway et al. 2002). The big-headed ant (*Pheidole megacephala*), for example, is an omnivorous ant of unknown origin (possibly native to Mauritius or another Afrotropical region; e.g., Fischer & Fisher 2013), which has invaded tropical and subtropical areas of all continents but Antarctica (Wetterer 2012). Nests of *P. megacephala* form dense nest networks that can cover tens of hectares (Hoffmann 1998) with low aggression between nests separated by as much as 49 km (Fournier et al. 2012). Since *ca*. 2000, *P. megacephala* has invaded “black cotton” vertisol savannas in Kenya (Riginos et al. 2015), which are dominated by *Acacia* [*Vachellia*] *drepanolobium,* an ant-defended foundational tree in these ecosystems (Riginos et al. 2009; Goheen and Palmer 2010). Within invaded black cotton savannas, *P. megacephala* extirpates these trees’ native symbiotic ants, resulting in high levels of damage by large herbivores. By facilitating herbivory, *P. megacephala* indirectly reduces tree carbon fixation (Milligan et al. 2021) and tree population growth (Hays et al. 2022).

In addition to its indirect effects, *Pheidole megacephala* also imposes direct negative effects on whole-tree-level carbon fixation and tree population growth of *A. drepanolobium*, but the mechanisms driving these direct effects are unclear. In a greenhouse study, nesting by *P. megacephala* around the roots reduced photosynthesis and stem carbohydrates of *A. drepanolobium* saplings even when ants were excluded from aboveground tissues (Milligan et al. 2022), but it is not known if these effects extend to the field or to mature trees. Although *P. megacephala* likely also affects tree belowground growth indirectly by facilitating aboveground herbivory—thereby limiting resources for root development (Wigley et al. 2019) and/or causing the tree to reallocate resources away from roots to support resprouting (Smith et al. 2018; Miranda et al. 2020)—an additional, direct negative effect could result from ants nesting in the soil near the plant. For example, omnivorous ants like *P. megacephala* could consume roots (Broekhuysen 1947; Shatters and Vander Meer 2000) or prune them to make room for their extensive chambers and tunnels (Broekhuysen 1947; Wetterer 2012).

Here we hypothesized that *A. drepanolobium* coarse root production and coarse rooting strategy differ in uninvaded and *P. megacephala*-invaded areas. We predicted that trees would have lower lateral coarse root density, altered lateral coarse root distribution, and shorter taproot length in *P. megacephala*-invaded savannas relative to uninvaded savannas. The potential consequences of such effects include alterations to landscape-scale carbon cycles in the vertisol-based savannas where *A. drepanolobium* is the dominant tree species. To determine if ant invasion is associated with differences in these key root traits in the field, we compared biomass, depth, and distribution of roots of randomly sampled trees within invaded and uninvaded savannas. We focused on coarse roots (≥ 2 mm diameter) because their architecture could be elucidated via excavation, and because they serve as vital proliferation structures to extend short-lived fine roots into nutrient patches for acquisition (summarized by Lambers et al. 2008).

Material and methods

Study system

We conducted the study at the Ol Pejeta Conservancy (OPC), located in Laikipia County, in Kenya's central plateau (0.0043° S, 36.9637° E; Fig. Sf1a). Approximately one third of the 360 km^2 conservancy property is classified as *A. drepanolobium* savanna or mixed bushland, underlain by clay vertisol soils (Adcock 2007). *Pheidole megacephala* has recently been expanding its distribution at OPC by ~50 m/year (Pietrek et al. 2021).

Root excavations

To compare the coarse root systems of *A. drepanolobium* trees in uninvaded and invaded field sites, we examined roots in invaded and uninvaded savannas using a comparative observational approach. Trees chosen for root excavation were sampled from three sites with active *P. megacephala* invasion fronts at OPC, separated from one another by > 3 km (for more details, see Palmer et al. 2021). Each site comprised an uninvaded 4-ha area located ≥ 1 km ahead of an invasion front, and an invaded 4-ha area located ≥ 1 km behind an invasion front and ≥ 1 km from habitat edges ($n = 10$ trees per site; $N = 30$). When they were established in 2016, invaded sites were equivalent distances from the nearest invasion front; based on the similar rate of expansion at each front (*ca*. 50 m yr^{-1}, Pietrek et al. 2021), we assumed that all invasions were of similar age (ca. 5 years in 2016), and those invasive populations persisted until and after this study. Although comparing uninvaded sites to sites that have been naturally invaded cannot demonstrate causality, this approach has produced accurate pictures of the community effects of invasive species when compared to before-after invasion approaches (Krushelnycky and Gillespie 2010). Prior to excavating roots, we confirmed that all study trees had similar heights (Mean ± SEM; 2.37 ± 0.07 m) and basal diameters (7.1 ± 0.9 cm) (Table S1). We also measured the crown length (longest side) and width (shortest side) to calculate tree crown area.

Trees in uninvaded and uninvaded areas were occupied either by the native ant species *Crematogaster mimosae* or by the invasive *P. megacephala*, respectively. *Crematogaster mimosae* is an aggressive, native, symbiotic ant that typically occupies ≥ 65% of mature *A. drepanolobium* at OPC. Invaded trees were located in areas where *Crematogaster* acacia-ants have been virtually extirpated by *P. megacephala* (Riginos et al. 2015). Trees were otherwise chosen at random. At OPC, the long vertical and lateral cracks in black cotton soil (DeCarlo and Caylor 2019) make it difficult to reliably identify individual *P. megacephala* nests. Nevertheless, trees in these "nest network" areas usually have *ca*. 10–20 *P. megacephala* workers patrolling the trunk during the day and *ca*. 50–100 workers can recruit to bait on tree trunks (P. Milligan pers. observations). Thus, we chose invaded trees without regard to their proximity to *P. megacephala* nests. We also did not quantify *P. megacephala* around tree roots at the start of our study, because we assumed that an instantaneous ant count at an arbitrary point in time would poorly correlate with tree rooting patterns that slowly accumulate over a ≥ 10-years-old invasion.

We measured tree coarse roots (≥ 2 mm diameter; henceforth, "lateral roots" or "taproots") in a 100-cm radius, 50-cm depth cylindrical volume of soil (henceforth, "cylinder") beneath each tree stem between December 2020 and May 2021 (Fig. S1b-d). To make our data comparable to those from other savanna trees, we precisely followed the methodology of a recent comparative study of coarse roots in South Africa (Zhou et al. 2020). We excavated all of the soil in the cylinder while leaving all of the roots intact (Figs. S1c and S1d). The taproot was identified as the single large root extending directly downward (Fig. S2) and was easily distinguished from lateral roots. Using a cylindrical coordinate system centered at the base of the tree, we quantified lateral root biomass every 20 cm of radial distance from the stem and every 10 cm of depth from the soil surface (Fig. S1b). The diameter of the taproot was measured every 10 cm, and 10-cm increments of the taproot were cut and weighed separately. Roots were dried at 50 °C to obtain dry biomass.

Statistical analyses

Analyses were done in R v.4.0.5 (R Core Team 2021). For all response variables summarized by tree individual ($N = 30$), we ran general linear mixed models in the package *glmmTMB* (Brooks et al. 2017), with invasion status as a fixed effect and site as a random

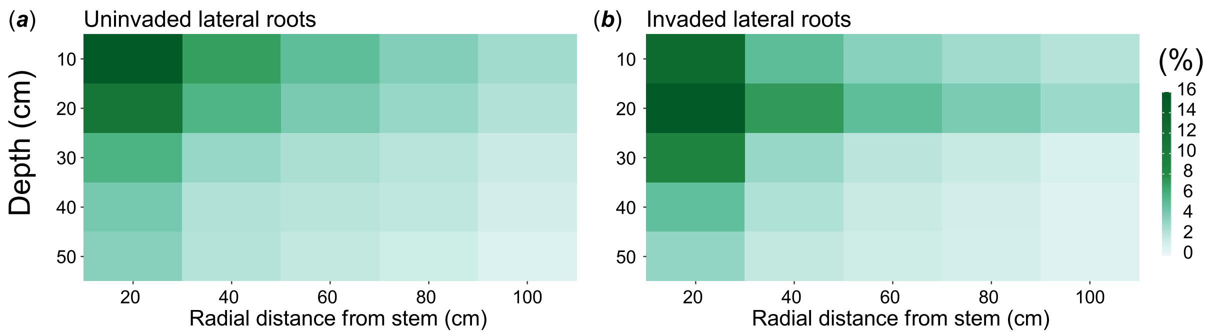

Fig. 1 Spatial distribution of lateral root biomass with depth and radial distance from the tree stem for trees in **a** areas that have not been invaded by *P. megacephala* and **b** areas where *P. megacephala* has been nesting in the soil for ~ 20 years

intercept. We used Welch's unequal variance t-tests to compare height, stem basal diameter, and canopy area between invaded and uninvaded trees. We visualized data to confirm that they approximated normal distributions.

To compare taproots between uninvaded and invaded areas, we compared potential rooting depth and the relative distribution of biomass in the 50-cm-deep cylinder. All taproot summary statistics correlated with a "deep:shallow ratio" (ratio of taproot diameters at 30 and 10 cm deep; Section 2 of Supplementary Information). This deep:shallow ratio has been recommended as a proxy for rooting depth (Zhou et al. 2020), where larger values indicate deeper taproots, so here we report the results on these ratios (see Section 2 of Supplementary Information for other taproot summary statistics).

To compare lateral roots between uninvaded and invaded areas, we assessed the distribution of biomass in the excavated cylinder and the root biomass density (g/cm^3) for each soil segment with a unique radial distance and depth in the cylindrical coordinate system (we refer to such segments as "quadrats," with 25 quadrats per tree). The relative distribution of biomass was estimated using W_b, a biomass-weighted rooting depth and a biomass-weighted radial distance of lateral roots from the stem (Zhou et al. 2020). Higher values of W_b indicate a greater proportion of lateral root biomass allocated to deeper soils or to farther away from the stem, respectively.

We characterized lateral root distribution in two ways. First, we calculated proportional lateral root biomass in each quadrat as $p = B/\text{TB} \times 100$, where B is root biomass for each quadrat and TB is total lateral root biomass in the entire excavated cylinder for each tree. These values were averaged per quadrat among all trees in uninvaded areas and in invaded areas to produce two 5×5 proportional biomass spatial matrices representing global means from uninvaded and invaded areas (Fig. 1). We compared these matrices statistically using a two-sample Syrjala test with 9999 permutations in the *ecespa* package (de la Cruz Rot 2008). The Syrjala test evaluates the null hypothesis that there is no difference between two spatial distributions using a bivariate generalization of the Cramér-von Mises nonparametric test (Syrjala 1996).

Second, to compare the density of tree lateral roots, we divided the grams of root by the quadrat volume for each of the 25 quadrats per tree. Root biomass densities were normalized by the mean of the root biomass densities in the respective quadrat for all 30 trees and log-transformed before analysis. In this analysis, we had 25 data points for each of the 30 trees ($N = 750$) (Section 3, Supplementary Information). We applied a general linear mixed model with a fixed effect of invasion, spatially correlated random effects representing our cylindrical coordinate system, and the additional random effects of tree identity and site in the *spaMM* package (Rousset and Ferdy 2014). The spatially correlated random effects accounted both for depth beneath the surface and the distance from the taproot of each quadrat.

Results

Trees in invaded sites had, on average, 37% smaller canopies than trees in uninvaded sites (Table S1; Welch's unequal variance t-test, $t = 3.71$, $\text{df} = 22.4$, $p = 0.0012$), but invaded and uninvaded trees did

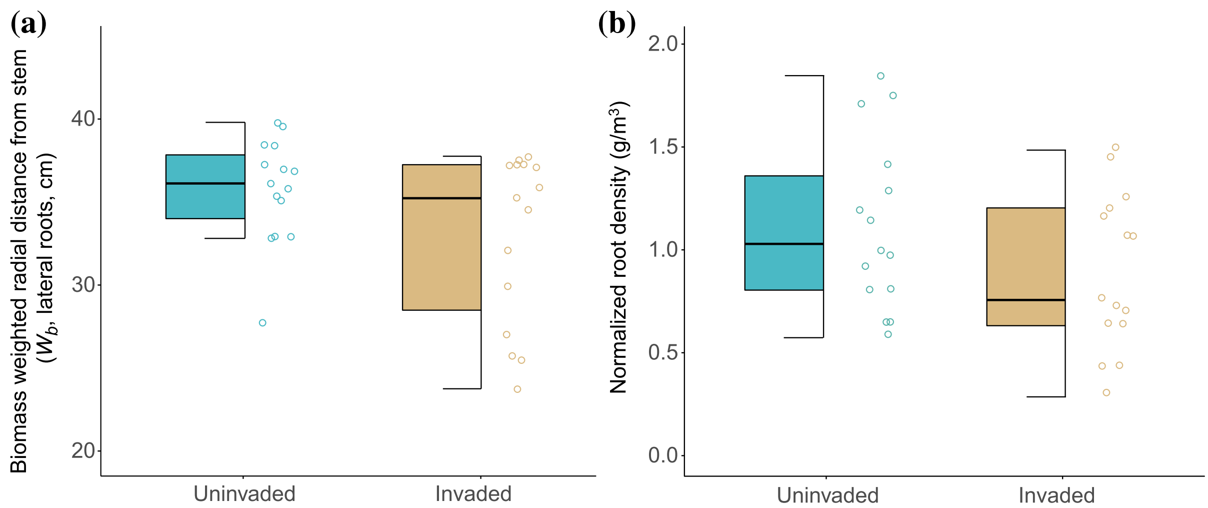

Fig. 2 Effects of ant invasion on **a** the biomass-weighted radial distance of lateral roots from the stem (W_b) and **b** the normalized lateral root density. For (**b**), lateral root densities were normalized, such that values > or < 1 indicate higher, or lower, than average lateral root density, respectively

not differ in other aboveground metrics (height: $t = 0.33$, df = 27.86, p = 0.75; trunk diameter: $t = 0.97$, df = 25.66, p = 0.34). We found no difference in the deep:shallow ratio of the taproot ($\chi^2 = 0.27$, df = 1, $p = 0.60$) between invaded and uninvaded areas (see also Section 2 of Supplementary Information).

Trees in *P. megacephala*-invaded areas had relatively more lateral root biomass close to the taproot and a lower density of lateral root biomass in the soil than trees in uninvaded areas. Lateral root biomass was distributed differently in the soil beneath trees in invaded areas (Fig. 1; Syrjala's Cramer-von Misses test $\varphi = 0.031$, $p < 0.04$). Post hoc comparisons of proportional lateral root biomass indicated that trees in uninvaded areas had relatively more biomass at a depth of 0–10 cm at distances of 20–40 and 40–60 cm away from the taproot and in the quadrat located 20–30 cm deep and 80–100 cm away from the taproot. Trees in invaded areas, by contrast, had relatively more biomass at a depth of 10–20 cm in the 20 cm nearest to the taproot. These differences contributed to relatively less biomass of lateral roots distributed at greater distances from the taproot in invaded areas than in uninvaded areas, as estimated by the W_b statistic for biomass-weighted radial distance (Fig. 2a; $\chi^2 = 3.29$, df = 1, $p < 0.07$). Finally, lateral roots were found at ~ 20% lower biomass densities (g/cm^3) in invaded soils than in uninvaded soils (Fig. 2b; $\chi^2 = 4.14$, df = 1, $p < 0.05$).

Discussion

Here we show that invasion by *P. megacephala*, a widespread ground-nesting ant, is associated with substantial differences in *A. drepanolobium* coarse lateral root biomass density and distribution. Based on *P. megacephala* literature in this and other systems, we speculate that these differences are attributable to nesting and other belowground activities of *P. megacephala*. Further studies manipulating both ant presence and herbivores are needed to determine if these belowground differences in root traits are directly driven by invasion, and if such direct underground consequences exacerbate or otherwise interact with the aboveground consequences of invasion-facilitated vertebrate herbivory (an indirect effect noted by Riginos et al. 2015; Milligan et al. 2021).

Our results have two major implications. First, *P. megacephala* may increase tree susceptibility to the savanna's periodic dry seasons and droughts. The lower biomass density and lower proportion of lateral roots near the surface, which are associated with the presence of *P. megacephala*, indicate that trees growing in invaded areas have a smaller framework for proliferating fine roots at shallow depths (Lambers et al. 2008). Moreover, tree fine roots are often damaged or sheared off by the tendency for vertisol soils to shrink and crack under dry conditions (Kidanu et al. 2005; Mekonnen et al. 2006), and thus we expect that a reduced coarse root framework will also limit the ability of *A. drepanolobium* to regrow its fine roots after periodic dry seasons. In addition, because water probably permeates only to shallow depths in vertisol soils (Zhou et al. 2020), reduced rooting near the soil surface may limit the tree's access to surface water. Second, because coarse roots represent major carbohydrate storage organs for these trees, less carbon may be stored in ant-invaded savannas. Our study tree is monodominant on vertisol savannas in East Africa (up to 98% of tree cover, Young et al. 1997), and our result emanates from the excavation of trees chosen randomly on invaded landscapes, which suggests that many trees are similarly affected. In fact, *P. megacephala* appears to cause *A. drepanolobium* population declines in both the presence and absence of herbivores (Hays et al. 2022), and the declines in herbivore-free areas seem likely to be driven partly by the changes in root allocation described here.

Invasive animals, including ants, are often speculated to alter terrestrial carbon via indirect interactions with plants. Invasive rats, for example, indirectly increased carbon storage and primary productivity on New Zealand islands by reducing seabird burrowing (Wardle et al. 2007). Likewise, increases in elephant herbivory caused by reduced ant defense in *P. megacephala* areas on OPC reduced tree carbon fixation by *ca*. 69% (Riginos et al. 2015; Milligan et al. 2021), which may limit carbon resources available for root growth. If invasion were associated with rooting differences solely through herbivore-mediated resource limitation, however, we would have expected to find lower root density throughout the root system. Instead, we observed lower root density for invaded trees mostly at shallow depths, suggesting that a direct invasion effect on rooting is concentrated in this zone. Many soil nesting ants tunnel to produce underground chambers, pruning roots along the way and altering soil bulk density and the distribution of soil resources (Moutinho et al. 2003; Drager et al. 2016; Leite et al. 2018). Invasive ants may likewise affect rooting by way of soil engineering (Holway et al. 2002; Ehrenfeld 2010), although to our knowledge this has not been shown. Interestingly, invasive *P. megacephala* ants may be particularly active nesters at shallow depths (nesting only in the top 13 cm of soil, Broekhuysen 1947), and they extirpate nearly all native ants in this region of Kenya (Riginos et al. 2015; Milligan et al. 2016). It thus seems possible that *P. megacephala* profoundly changes ant-soil and ant-root interactions at shallow depths throughout heavily invaded areas, thereby limiting the ability of *A. drepanolobium* roots to persist at these depths.

Surprisingly little is known about the natural history of *P. megacephala* despite its invasive reach (Holway et al. 2002; Wetterer 2012). Invasive *P. megacephala* in Cameroon forests are "opportunistic" nesters that only excavate large galleries around shallow grass and shrub roots (Fournier et al. 2012). At OPC, large *P. megacephala* colonies may also opportunistically excavate galleries and chambers at shallow depths around the stable structure provided by tree roots, as was previously described with wild forbs and greenhouse-reared tree saplings (Milligan et al. 2022). Careful study of *P. megacephala* nest architecture in these savannas would be useful but is logistically difficult (e.g., Moser 2006). Future studies should examine if invasive *P. megacephala* alters soil nutrients and bulk density near their mounds in the same manner as native *Pheidole* in other systems (Shukla et al. 2013; Wang et al. 2019), and if these changes can explain differences in tree rooting. Some other omnivorous invasive ants even feed directly on live plant roots (Broekhuysen 1947; Shatters and Vander Meer 2000), and *P. megacephala* has been anecdotally observed to cut and carry *ca.* 2 mm-thick roots from adult-tree root systems in shallow soil (P.D. Milligan, *personal observation*).

We also observed proportionally higher lateral root biomass close to the taproot (*i.e.*, lower radial-distance W_b) in invaded areas. Interestingly, 76% of all trees exhibited radial-distance W_b values within one standard deviation of the mean, suggesting that this statistic illustrates a strategy of lateral rooting for *A. drepanolobium* trees that is notably consistent, particularly for uninvaded trees, despite the many potential drivers of variation in tree belowground allocation. The difference correlated with invasion was thus driven by four trees in invaded areas whose lateral roots were clustered particularly close to the taproot, and by the lack of any trees in invaded areas with lateral roots particularly distant from the taproot. We speculate that variation in *P. megacephala* densities on the landscape resulted in excavating some trees where *P. megacephala* was at particularly high abundance, driving the lowest values of W_b, but that the pervasive influence of *P. megacephala* nests in invaded areas prevented any high values of W_b for invaded trees.

Evaluating the impacts of invasive species is challenging and can require multiple experimental approaches. Comparative observations do not demonstrate causality, but experimental approaches can sacrifice realism (Kumschick et al. 2015). Before-after-control-impact (BACI) studies, which measure responses before and after invasion, can require multiple years of data, and even then may underestimate the longer-term or lagging impacts of invasions (Krushelnycky and Gillespie 2010). For example, a BACI study here might not detect the effects of *P. megacephala* on plant root systems if such effects only manifest over longer (≥ 10 years) time scales. Nevertheless, as studies in different systems accumulate, we have made progress in understanding invasions and the impacts on native biodiversity (Kueffer et al. 2013). A similar multifaceted and integrative approach is needed to determine how invasive species affect ecosystems (Ehrenfeld 2010; Simberloff 2011), and this work needs to include extending a focus on below-ground impacts. Future investigations of soil engineering mechanisms underlying the changes in roots that we describe here will be critical to understanding the potential for invasive ants to affect primary productivity and carbon storage from within the soil.

Conclusions

Our results show that a soil-nesting invasive ant is associated with differences in coarse rooting of a savanna tree, with implications for other aspects of the tree's physiology and for the ecosystem processes that these foundational trees influence. Here we were not able to examine the possible knock-on effects of the differences in coarse rooting on tree fine rooting, but ongoing research in this system will use minirhizotrons to test if smaller coarse root networks in invaded trees limit the tree's capacity for fine roots as well. Little is known of plant rooting strategies in tropical savannas (Zhou et al. 2020), and root biomass and distribution are critically important to understanding ecosystem resilience to drought and savanna carbon cycles (Batjes and Sombroek 1997; Kristensen et al. 2021). Considering the outsized influence of the tree we studied here—*A. drepanolobium*—on nutrient cycles (Fox-Dobbs et al. 2010), understory productivity (Riginos et al. 2009), and even spatial characteristics like predator/prey visibility (Riginos and Grace 2008) and tree cover (Goheen and Palmer 2010) in its range, we encourage further studies of invasion-associated changes to the physiology and function of foundational trees, particularly of belowground differences that may be driven by invasive ant-soil and ant-root interactions. Such changes may cause large downstream effects on ecological processes and functions.

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Author contribution EP and TP conceived and designed the study with support from SM, BG, and AK. JL, JM, and PM conducted the experiments and measurements. PM and EP analyzed the data. EP, PM, and TP wrote the manuscript.

Data availability All of the data and analysis for this article are available at Dryad: https://doi.org/10.5061/dryad.5x69p8d7f.

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