

A Pugnacious ant (*Anoplolepis custodiens*) confounds ant assemblage responses to bush encroachment

Thinandavha Munyai¹, Stefan Foord², Rob Slotow¹, and Nomathamsanqa Mkhize¹

¹University of KwaZulu-Natal College of Agriculture Engineering and Science

²University of Venda

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Abstract

Habitat structure is a key determinant of variation in biodiversity. The effects of increased vertical and horizontal vegetation structure can result in marked shifts in animal communities. This is particularly true for ants in response to woody thickening, with predicted negative impacts on ant diversity. We used pitfall traps to study the response of epigeic ants in two co-occurring dominant habitats (closed and open) of an African savanna biome experiencing extensive woody thickening. Although species richness was higher in open habitats, evenness was significantly lower. Thickening explained significant amounts of variation in ant composition, but site-specific characteristics and seasonality were more important. These site-specific characteristics were largely linked to *Anoplolepis custodiens*, a species that were locally abundant in open habitats with clayey soils, where they often accounted for more than 90% of all ant activity. As *A. custodiens* also responds positively to disturbance, indiscriminate bush clearing could lead to knock on effects associated with the numerical and behavioural dominance of this species.

Introduction

The savanna biome dominates southern Africa, and is characterised by continuous grassy understory, a discontinuous woody overstory (Archer et al., 2017; Bond, 2019), and, therefore, considered a largely open ecosystem (Pausas & Bond, 2020). However, various anthropogenic activities (Osborne et al., 2018) pose a threat to this biome. One pervasive threat is that of woody plant encroachment into savannas and grasslands, linked to climate change in particular (Criado, Myers-Smith, Bjorkman, Lehmann, & Stevens, 2020). Over the past two centuries (Archer et al., 2017) encroachment rates have varied between -0.131 to 1.275% per year in South Africa (O'Connor, Puttick, & Hoffman, 2014), and 0.25% per year across the rest of Africa (Stevens, Lehmann, Murphy, & Durigan, 2017).

Broad-scale studies predict that woody plant encroachment threatens biodiversity (Parr, Gray, & Bond, 2012), reducing the quality and quantity of the habitat, and jeopardizing both ecological functioning and services. In communal grazing lands and protected areas, bush encroachment also reduce production forage and increase ectoparasites (Archer et al., 2017). Overall, biodiversity is strongly affected by woody plant encroachment, e.g. in Grampians National Park, southern Australia, species richness of herbaceous growth forms was significantly reduced (Price & Morgan, 2008) while bird communities in the Kingdom of Eswatini showed significant shifts in structure (Sirami & Monadjem, 2012). This has real implications for conservation managers that aim to maintain diversity.

Wingless arthropods and those with low dispersal ability will be the worst affected by local habitat change (Blaum, Seymour, Rossmanith, Schwager, & Jeltsch, 2009). However, gradients of shrub cover vary in their impact, with grasshopper richness peaking at intermediate shrub cover, but not affecting butterfly richness (Koch, Edwards, Blanckenhorn, Walter, & Hofer, 2015). Hering et al. (2019) and Blaum et al. (2009) reported similar mixed effects on beetles, with some families increasing in abundance and richness at

higher encroachment levels. Different metrics might also respond differently, e.g. shrub species in Australian woodlands were important drivers of arthropod community composition, but did not affect their abundance (Kwok & Eldridge, 2016). However, general consensus is that woody plant encroachment will have adverse effects on the ecological and ecosystem services (Eldridge et al., 2011) provided by arthropods, for example pollination (Kettenbach, Miller-Struttman, Moffett, & Galen, 2017; Lara-Romero, Garcia, Morente-Lopez, & Iriondo, 2016), and dung decomposition (Blaum et al., 2009).

Ants (Hymenoptera: Formicidae), are among the most abundant terrestrial arthropods (Hölldobler & Wilson, 1990), and perform a myriad of ecological functions (Wills & Landis, 2018) and ecosystem services (Del Toro, Ribbons, & Pelini, 2012). Their response to encroachment has rarely been investigated and varies geographically from increased abundance in the southern Kalahari (Blaum et al., 2009) and increased richness in the south western USA (Bestelmeyer, 2005). However, Parr et al. (2012) found no real differences between ant richness and activity in open and closed habitats in Hluhluwe-Imfolozi Park, but observed that more species were restricted to closed habitats and that these restricted taxa were largely predatory (80% in the forest compared to the 7% in open habitats). Their results mainly focus on these rare taxa. Although they analyzed compositional differences, the distance-based approach used leads to confounding location and dispersion effects and has been shown to not reflect community composition (Warton, Wright, & Wang, 2012). Here, we use generalized linear models and their multivariate extensions to model ant species abundance (Warton et al., 2012). Ant functional roles is largely the result of dominant taxa in ant assemblages. Here, we complement their study by focusing on the community as a whole, and ask (1) how seasonality, soil and structural habitat complexity interact to affect ant diversity in an African savanna biome experiencing extensive woody thickening, (2) and identify species associated with these differences.

Methods

Study area

The study was conducted in the Hluhluwe section of Hluhluwe-iMfolozi Park Park (900 km²), situated in Zululand, northern KwaZulu-Natal, South Africa (28.2198° S, 31.9519° E). The park receives rainfall ranging from 700 mm to 985 mm, usually in summer between October-March (Boundja & Midgley, 2010). Most of the park is covered with woodland savanna interspersed with shrub thicket. The majority of habitats vary from semi-deciduous forest on the northern side (Hluhluwe Game Reserve) to open savanna woodland (iMfolozi Game Reserve). The park is managed by Ezemvelo KwaZulu-Natal (KZN) Wildlife, and is a wildlife conservation area (Gray & Bond, 2013).

Site selection

Six-paired sampling sites were selected for this study: Gontshi (Gon), Isivivane (Isi), Magangeni (Mag), Mansinya (Man), Memorial gate (Mem), and Zincakeni (Zin). Replicates within each site were at least >300m apart while the actual sites were at least 2 km apart. Each site had four paired plots (one in the closed and open respectively), for a total of eight plots per site. The closed habitat plots were in the middle of this habitat, while the open replicates were 30 m away from the edge. In total there were 48 grids. Canopy cover was used to categorize the habitat type. As described in Parr et al. (2012), habitat-type with a *ca.* mean canopy cover of [?] 3% were treated as open savanna, and habitat-type with a *ca.* mean canopy cover [?] 55% were treated as closed habitat.

Ant sampling

Ground dwelling worker ants were sampled following a standardized pitfall trapping method as described in Munyai and Foord (2015). Sampling at each site was conducted in January 2017 (wet-hot season) and early October 2017 (dry-hot season). Pitfall traps (62mm) contained 50% solution of propylene glycol as a preservative that neither attracts nor repels ants (Munyai & Foord, 2012). There were ten pitfall traps in each plot. The pitfall traps were laid out in a 5 X 2 sampling grid and the pitfall traps were at least 10 m apart, and were left open for five days.

After collecting data, samples were washed in the laboratory, and the specimens counted, separated, and

stored in 70% ethanol. They were then identified to genus level using Fisher and Bolton (2016), and identified to species level where possible. Unidentified ants were given morphospecies number codes, i.e. genus name UKZN_01, 02, etc. (Munyai & Foord, 2015). Voucher specimens of each species are held in the School of Life Science, University of KwaZulu-Natal, and will later be accessioned into the Kwazulu-Natal Museum.

Environmental variables

Vertical and horizontal vegetation cover were quantified during each sampling period. The horizontal vegetation cover was quantified by visually estimating percentage area covered by bare ground, leaf litter, rocks, and vegetation within a 1 m² grid over each pitfall trap (Botes et al., 2006; Munyai & Foord, 2012). To quantify the vertical distribution of vegetation, number of hits (i.e., the number of contacts with vegetation) from the rod were recorded at 25 cm intervals using a 1.5 m rod. The 1.5 m rod was placed four times at 90deg angles from a pitfall trap, which was used as a central point. This method was used as a measure vertical vegetation complexity.

Soil samples were collected in October 2017 at each replicate, 10 subsoil samples were collected using a soil auger and then pooled for that replicate. A total of 48 soil samples were sent to the Research and Technology Development: Analytical service labs, in Cedara, Pietermaritzburg, for composition analysis (% organic carbon, % nitrogen and % clay), chemical content (phosphorus, potassium, calcium, manganese, zinc, magnesium and copper), and texture (% clay, % sand, % silt and % moisture).

Statistical Analysis

Collinearity between predictor variables (soil and habitat structure) was accounted for by doing a principle component analysis of the two sets of variables respectively. The first two axes of the PCA for soils explained 66.55 % of the variation, the first axis 51.98 %, and the second 14.57 %. PC1 was positively associated with more sandy soil and negatively associated with clay soils (Figure S1). The first two axes of the PCA for habitat structure explained 69.28 % of the variation, first axis, 41.92, and the second 27.36 %. The first axis was positively related to increased canopy cover, leaf litter, and more complex structure.

These principle components were used to model gradients in ant diversity in response to habitat structure and soil respectively. Two further categorical variables, habitat (open vs closed) and season (hot-wet and hot-dry) were also included. Species richness and effective number of species for Shannon diversity and Simpson's diversity (Jost, 2006) were modelled using Generalized Linear Mixed Models (GLMM), with site as the random factor and Poisson error distributions, a loglink function for richness and Gaussian error distributions, and an identity link function for Shannon and Simpson's effective number of species. The best model was identified using a Akaike Information Criterion, the lowest being the best. Models that were $< \Delta 2AIC$ were also included. The relative contribution of marginal (fixed, R^2_m) and conditional (fixed and random, R^2_c) in the variation explained were calculated for each model (Nakagawa & Schielzeth, 2013).

Compositional variation was modelled using a model-based multivariate approach where we fitted multivariate generalized linear models (GLMs) to ant species abundance data in the R package "mvabund" (Wang, Naumann, Wright, & Warton, 2012). Predictors included were similar to those included into the univariate GLMMs. This model-based approach deals with confounding mean-variance relationships typical of count data that are zero inflated (Warton, Thibaut, & Wang, 2017). Likelihood ratio statistics for each taxon were summed, that results in a community-level measure for each predictor. Correlation across species was accounted for by using the PIT-residual bootstrap method to derive p-values by resampling 999 rows of the data set (Warton et al., 2017). Predictors were included in the model individually to explore the marginal (variation explained by the predictor on its own) deviance explained.

Model fit for both univariate and multivariate models were evaluated by visually inspecting residual plots for deviations from normality, heteroscedasticity, systematics patterns, and autocorrelation. Ordination of ant assemblages was done using Bayesian ordination and regression (Hui, 2016). To aid visualization, samples for the two seasons were pooled.

Responses of ant assemblages to gradients in soil and habitat structure were modelled using Threshold

Indicator Taxa Analysis (TITAN) from the “TITAN2” package (Baker & King, 2010). This method uses standardized z -scores obtained from indicator species analysis (Indicator Value) to detect the species-specific change points, and the direction of response along a gradient (Baker & King, 2010; Costas, Pardo, Mendez-Fernandez, Martinez-Madrid, & Rodriguez, 2018). Increasing responses ($z+$) are distinguished from those decreasing ($z-$) at a specific change point (Baker & King, 2010). TITAN also estimates indicator reliability and the proportion of times that a taxon is given the same classification through bootstrapping, as well as uncertainty around the location of individual taxa and community change points (Baker & King, 2010).

Results

A total of 61 008 ant specimens were collected, comprising 74 species in 25 genera, and five subfamilies. Myrmicinae (47 species and eight genera) was the most diverse and abundant subfamily, followed by Ponerinae (13 species and eight genera), and Formicinae (15 species and six genera). The most speciose genera were *Tetramorium* (22 species), *Monomorium* and *Pheidole* each had six species, while *Camponotus*, *Lepisiota*, and *Pheidole* had five species each. Three quarters of all the ant individuals caught belonged to one species, *Anoplolepis custodiens*. This species, and those in the genus *Pheidole*, accounted for 90% of all the ants.

The best model for species richness included season and habitat (closed/open), with richness being significantly higher in the open habitat and during the wet season (Table 1). The second-best model included soil PC1, with very weak evidence for an increase in richness in more sandy soils (estimate = 0.014 ± 0.015). However, this trend was reversed when considering Hill no.1, with strong evidence that there was significantly less Shannon diversity in the open habitats. Similar to richness, Soil-pc1 was included in the second model, with weaker evidence for a positive response to sandy soil (estimate = 0.026 ± 0.05). The negative impact of open habitats became even more pronounced when Simpsons dominance was modelled. Soil-pc1 was also included in the second-best model. All three models explained around 22% of the variation observed, while at least half of this variation was linked to the random factor, i.e. site-specific characteristics (Table 1).

Site-specific characteristics not measured in this study explained the largest amount of variation in ant assemblage structure (Table 2, Figure 2). This impact of sites on assemblages was exemplified by the distinct ant assemblages comprised of *Odontomachus troglodytes*, *Tetramorium* UKZN_14 (*simillimum* gp.), and *Lepisiota* UKZN_02 (*capensis* gp.), associated with the sandy soils of Mansinya (Figure 2 & Figure S1).

Habitat followed seasons in explaining the most variation in species composition, particularly when considering conditional variation explained (Figure 2). None of the structural and soil variables explained significant amounts of variation beyond that explained by the previous three variables, although the first soil principle component (increasing sandy soil, see Figure S2), explained the third largest amount of marginal variation (Table 2). Similarly, no taxa were associated with any of the gradients in habitat structure, while eight indicator taxa were associated with the sand-clay gradient in the study site (Figure 3). *A. custodiens* notably preferred the clay soils across open and closed habitats. The activities of *Odontomachus troglodytes* increased in more sandy soil in both open and closed habitats, while *Anoplolepis custodiens* and *Tetramorium* UKZN_22 (*gabonense* gp.) had their highest activities in clay soil. *Myrmecaria natalensis*, *Bothroponera soror* and *Monomorium junodi* activities peaked in loamy soils.

Five species were distributed across all sites, and mostly sampled in both the closed and open habitat types. Three of these, *Pheidole* UKZN_01 (*megacephala* gp.), *Mesoponera caffraria* and *Monomorium* UKZN_03 were associated with open habitat types, while two species, *Pheidole crassinoda ruspolii* and *Tetramorium* UKZN_18 (*squaminode* gp.) were associated with closed habitat (Figure S3).

Discussion

Although site-specific characteristics and seasonality dominated ant assemblage structure, habitat type (open vs closed) had important impacts on ant richness and composition. Open habitat had significantly more species, but were less even than closed habitats; and, although ants responded to gradients in soil characteristics, responses to habitat structure changes were not gradual (Figure S4). This is evidenced by the relatively lar-

ger role of categorial classification (open versus closed) in explaining ant diversity. Responses to thickening are, therefore, predicted to be abrupt. This threshold response can partly be explained by the presence of the numerical dominant *A. custodiens* in open habitats of some sites that were mainly on clayey soils and *Odontomachus troglodytes* which is a specialised as well as aggressive predator in sandy soils.

Andersen (2019) reasoned that the amount of vegetation cover is an important driver of both flora and fauna communities. However in contrast to our study, Nooten, Schultheiss, Rowe, Facey, and Cook (2019) reported fewer ant species in open habitat. Notably their study aite was in highly altered urban gardens of Greater Sydney Region of south-east Australia. Pacheco and Vasconcelos (2012) also reported fewer species in the least structurally complex habitats of the Brazilian Cerrado. In contrast, Lassau and Hochuli (2004), Yusah and Foster (2016) and Ahuatzin et al. (2019) reported a negative response of ant species richness and diversity with habitat complexity. Similarly, Hethcoat et al. (2019) reported more open and warmer pasturelands supporting more ants than the compared primary or secondary forest.

Overall, habitat explained the third largest amount of conditional variation in the ant assemblages of this study. Similarly, across the western Soutpansberg mountains, vegetation types explained a significant amount of variation in ant assemblages (Munyai & Foord, 2012) . This is not surprising, given that habitat structure had been thought to determine the movements of ants as they are usually impeded by grass stems or woody twigs (Nooten et al., 2019). In the Amazon basin, Guilherme et al. (2019) reported the effects of habitat complexity on ant species foraging strategies and behaviour.

Not surprisingly, ant assemblages in the two habitats were significantly different. It is, however, expected that natural characteristics in a site, for example the amount of sunlight intensity, plant cover and overall variation in habitat structure must have a clear effect on the ant assemblage richness and composition (Campos, Soares, Martins, & Ribeiro, 2006). This is largely because of the effect of ground surface rugosity, which affects foraging activities, for example ant running speed (Greve, Blaha, Teuber, Rothmaier, & Feldhaar, 2019). In this study, closed habitat is largely structural more complex. In total, 24 % of the species were only recorded in the open habitat, compared to the 8 % restricted to closed habitat. This, once again, emphasises the important role of habitat openness, as detailed in Andersen (2019), and references there in, as a crucial driver of ant assemblage composition. However, this larger number of species in the open habitat could also be explained by the species-area effect. Currently, open Savannas dominate the Hluhluwe–iMfolozi Park is largely dominated by open savanna vegetation type (Charles-Dominique, Staver, Midgley, & Bond, 2015), covering >75% of the landscape. This in itself would explain the larger number of species observed in the open habitat.

One site, Mansinya, had a distinct ant assemblage and therefore highlighting on the importance of site-specific characteristics, particularly that related to soil characteristics. The Mansinya site is on sandy soil with specific species associated with this site (Figure S2). Similarly, sites on their own were the most influential predictors of ant assemblage structure, as suggested by both conditional and marginal effects (Table 1). Whether these differences are related to historical factors, spatial autocorrelation or variables that we did not measure is difficult to say and beyond the scope of this study. In general, Radnan, Gibb, and Eldridge (2018) noted that ants tend to be more responsive to small-scale alteration on the soil surface than overall changes in vegetation community composition. The influence of site-specific characteristics, namely, vegetational complexity, litter, and soil chemical properties were reported in Parui, Chatterjee, and Basu (2015) as the driver of variation in ant assemblage composition in the forested habitats of Eastern India.

The activities of the hyperabundant (72 % of the total abundance) *Anoplolepis custodiens* , increased in site with clay soils (Figure 3). In contrast, activities of *Odontomachus troglodytes* peaked in sandy soils. Three other species (*Myrmicaria natalensis* , *Bothroponera soror* and *Monomorium junodi*), were more active in loamy soils. Along an alluvial fan of the Tehuacán Valley of central Mexico, Rios-Casanova, Valiente-Banuet, and Rico-Gray (2006) reported high ant abundance in sandy soils. The latter study suggested that sandy soils and habitat complexity could have favoured the abundance of the dominant ants, which in turn could have also affected overall ant diversity patterns in their study. Bownes, Moore, and Villet (2014) also observed very large number of *Anoplolepis custodiens* in citrus orchards of the Eastern Cape, South Africa while *A.*

custodiens has been observed to nest in open and well insulated soils of the Karoo (Dean, 1992).

The high abundance, dominant behaviour, and impact of *A. custodiens* on ant diversity have been documented in several southern African studies (Mauda, Joseph, Seymour, Munyai, & Foord, 2018; Parr, 2008; Samways, 1983). Although this species is native to the region, it is well known to prey on a variety of other invertebrates (Keiser, Wright, & Pruitt, 2015), and also small livestock (Prins, Robertson, & Prins, 1990), it is also a major pest in agriculture landscapes (Addison & Samways, 2006; Addison & Samways, 2000). *A. custodiens* is also well known for tending honeydew producing pest insect species, and being nectar collectors, hence, regarded as one of the serious pests in the agricultural systems (Dean, 1992).

A. custodiens respond positively to disturbance (Addison & Samways, 2000; Mauda et al., 2018). Coupled with the formation of supercolonies in open shallow soils in particular (TC Munyai Personal observation), suggests that manual removal of trees and shrubs should not be done indiscriminately. The fact that this species can completely dominate open habitat (Addison & Samways, 2006), clearing of sites could facilitate invasion with negative impacts not only on ant diversity, but also ecosystem services. Particularly since *A. custodiens* is a polygynous (Samways, 1990), generalist predator of other invertebrates (Dean, 1992), and small vertebrates (Mauda et al., 2018; Prins et al., 1990), displacing other ant species (Mitchell, 2000), and control ant assemblage structure in the South African savanna (Parr, 2008).

A. custodiens is particularly active in clay soils, and clearing of woody species in the park need to be context specific. Exploring the response of *A. custodiens* to various woody species control measures, be it manual removal of trees and shrubs, using fire or browsing mammals as a management tool, should provide a better understanding of possible impacts. Generally though, we consider this a cautionary note around predicting local impacts based on broad-scale global patterns.

Conflict of interest

The authors declare on conflicts of interest regarding the publication of this paper. **Acknowledgments**

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Author contributions

TCM and SHF designed the study, analysed the data and wrote the paper. TCM and NNM collected the data, TCM identified the ants. TCM, NNM, RHS and SHF revised the article..

Conflict of Interest Statement

No conflict of interest among the authors.

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Table 1: Best models and the marginal and conditional variation explained based on Generalized Linear Mixed Effects Models (GLMM) of species richness, Shannon and Simpsons diversity. Shannon and Simpson indices are represented by there effective numebers

Model	Best model	AIC	Δ AIC (second best)	R^2_m	R^2_c
Species Richness	~Habitat* + Season*** Best model equation: $e^{0.15Open + 0.21Wet}$	549.84	1.3	0.11	0.23
Shannon (Effective number of species)	~Habitat** + Season Best model equation: $e^{-0.3Open - 0.15Wet}$	417.2	1.7	0.12	0.22
Simpsons (Effective number of species)	~Habitat** + Season Best model equation: $e^{-0.37pen - 0.19Wet}$	364.9	1.1	0.08	0.27

Table 2 : Conditional (Likelihood ratio and its significance) and marginal effects (Deviance) of predictors for ant assemblage in Hluhluwe

Predictor	Conditional effects Likelihood ratio (LR)	Marginal effects Deviance
Site	26.2**	705.1
Season	16.01**	1486.6
Habitat	15.38**	1503.8
Soil_pc1	9.99	1491.0
Str_pc1	10.07	1579.9



