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Patterns of floral resource use by two dominant ant species in a biodiversity hotspot

Natasha P. Mothapo · Theresa C. Wossler

Abstract Ecological dominance in ants is often fuelled by carbohydrate intake. Most studies have focused on the importance of invasive ant mutualistic associations with trophobionts whereas few studies have investigated the importance of floral nectar on invasion success. In this study, utilisation of temporarily available floral nectar by the invasive Argentine ant, *Linepithema humile*, was compared to that of the dominant native ant, *Anoplolepis custodiens*, within the Cape Floristic Region (CFR), a biodiversity hotspot. The effect of these two focal ant species on species composition and abundance of ground foraging ants as well as floral arthropod visitors in inflorescences of Proteacea species was assessed. Foraging activity, and trophic ecology inferred from the abundance of natural stable isotopes of Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$), and the ratio of Carbon to Nitrogen (C:N) were compared between the two ant species during three flowering periods. *Linepithema humile* significantly reduced the abundance and species diversity of both above-ground and floral arthropod species abundance and composition. *Linepithema humile* increased its foraging activity with increasing nectar availability, switching its diet to a more herbivorous one. *Anoplolepis custodiens* did not

respond as effectively to increasing floral nectar or negatively impact floral arthropod visitors. This study showed that the availability of floral nectar and ability of *L. humile* to more effectively utilise this temporarily available resource than native ants, can contribute significantly to the further spread and persistence of *L. humile* in natural environments in the CFR.

Keywords Diet switching · Cape Floristic Region · *Linepithema humile* · Floral nectar · *Anoplolepis custodiens* · Stable isotope analysis

Introduction

Resource availability is one of the most underappreciated factors contributing to invasion success of non-native ants in natural communities (Davis et al. 2000; Wilder et al. 2011a). Many invasive ant species rely heavily on carbohydrate based resources (Helms and Vinson 2002; Lach 2003; Ness and Bronstein 2004; Le Breton et al. 2007; Kay et al. 2010; Wilder et al. 2011b), obtaining honeydew through mutualisms formed with a variety of myrmecophilic arthropods or through nectar and extra floral nectaries from plants (Hölldobler and Wilson 1990; Holway et al. 2002; Davidson et al. 2003; Helms and Vinson 2008; Wilder et al. 2011a; Helms 2013). Carbohydrate resources provide a source of fuel for colony growth (Dussutour and Simpson 2008, 2012), improved survival (Lach et al. 2009) and have been shown to increase ant

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foraging activity and aggression (Grover et al. 2007; Helms and Vinson 2008; Savage et al. 2011; Wilder et al. 2011a, b; Shik and Silverman 2012), allowing invasive ants to dominate resources and compete with native ants (Davidson 1997; Grover et al. 2007; Tillberg et al. 2007). Carbohydrate resources are thus undoubtedly important to invasive ants in many systems because of the potential to influence establishment success (Ness and Bronstein 2004; Styrsky and Eubanks 2007; Rowles and Silverman 2009; Wilder et al. 2013). Indeed in two of the most notorious invasive ants, *Linepithema humile* and *Solenopsis invicta*, ecological dominance has been directly linked to carbohydrate availability and abundance (Tillberg et al. 2011; Savage et al. 2011; Wilder et al. 2013).

Carbohydrate supply may be a key limiting resource affecting survival and population growth of invasive ants as indicated by previous studies (Grover et al. 2007; Kay et al. 2010; Shik and Silverman 2012). Yet many of these studies are based on honeydew from trophobionts (Lach 2008; Gibb and Cunningham 2009; reviewed in Helms et al. 2010; Savage and Whitney 2011) and few focussed on floral nectar (Blüthgen and Fiedler 2004; Lach 2013). Floral nectar, although widely abundant, is a temporarily available resource and much work is needed to understand how ants respond or change their behaviour with respect to nectar availability and how this may impact invasion success for some ants (Lach 2013). Take for example how some invasive plant species show higher trait plasticity than similar native species, utilising fluctuating resources (e.g. such as soil nutrients) better and tolerating a wider range of environmental conditions (Dukes and Mooney 1999; Funk 2008). This consequently leads to these species having a greater advantage to establish and spread in the recipient community. Similarly, invasive ant species may also increase their chances of establishment by profitably responding to fluctuating resources and utilising those resources not consumed by resident species (Tilman 2004), or out competing resident species for those resources (Gibb and Cunningham 2009). The fluctuating resource availability theory posits that some communities are more invasible than others depending on the amount of unused resources with competition intensity between invading and native species inversely correlated with the amount of unused resources (Davis et al. 2000). An example of a fluctuating resource could be floral nectar (Lach 2013), which is

highly abundant and so can be partitioned among resident species (Davis et al. 2000; Tilman 2004). Yet, if the uptake of this resource by resident species is slower than the supply, then colonising species that respond quickly to this resource may improve their establishment or may compete for the resource (Dukes and Mooney 1999; Davis et al. 2000; McGlynn and Parra 2016). In their study on the foraging activity of an arboreal dominant ant, McGlynn and Parra (2016) showed access to floral nectar improved foraging success, which was positively correlated with increasing availability. Thus the abundant supply of floral nectar, although temporarily available, may allow invasive ants to fuel colony growth during flowering seasons, a period of high carbohydrate resource supply.

The Cape Floristic Region (CFR) is a globally renowned biodiversity hotspot characterised by high plant diversity and endemism (Cowling et al. 1996). The Fynbos biome is the largest of the CFR and is characterised by high diversity of nectar-producing Proteacea, and a high number of myrmerchorous plants (Cowling et al. 1996). Proteacea species of the Fynbos contain fairly large amounts of floral nectar and attract a wide range of nectarivorous birds, such as Cape sugarbirds, and insects which are important pollinators (Coetzee and Giliomee 1985; Visser et al. 1996; Rebelo 2001; Geerts and Pauw 2011). Lach (2013) compared the inflorescences of several Proteacea species and found that the invasive Argentine ant, *L. humile*, was better at exploiting floral nectar from Proteacea species since it dominated most of the sampled inflorescences compared to the native dominant, and keystone ant species, *A. custodiens,* and negatively affected floral visitors such as honeybees, lepidoptera and coleoptera responsible for pollination (Buys 2015; Lach 2007, 2008). Since *L. humile* is potentially more efficient at using Protea floral nectar than *A. custodiens* (Lach 2007, 2013), and poses a threat to pollinators (Lach 2008), we compared foraging patterns of these two ant species, assessed the effect of their presence on insect visitors to inflorescences of two Protea species, *Protea repens* and *Protea nitida*, and inferred the potential diet and trophic relationship of these two ant species throughout the flowering period using stable isotope analysis. Stable isotope analysis is a useful tool to reveal the trophic ecology and nutritional dynamics of ants (Feldhaar et al. 2010), particularly when studying how

invasive ants alter foodwebs (Tillberg et al. 2007; Menke et al. 2010). We hypothesise that as floral nectar availability increases, so too will the foraging activity of *L. humile* and that its trophic position will switch to reflect a carbohydrate rich diet during this time, indicating a flexible behavioural response with respect to their foraging ecology. If *L. humile* exploits this fluctuating resource more effectively than *A. custodiens*, it is likely that the availability of this resource will drive the successful spread of the Argentine ant and further increase the negative impacts it already has on the Fynbos biome. The aim of the study was to quantify the differences in foraging patterns and response to a fluctuating and highly abundant carbohydrate resource of two ecologically dominant ant species occurring within the Fynbos, namely the native ant *A. custodiens* and the invasive ant, *L. humile*.

Materials and methods

Ant species

Both species of ants form polydomous and polygynous colonies with a high number of aggressive workers (de Kock 1990; Holway et al. 2002), and have a high affinity for carbohydrate-rich resources (Lach 2013). *Linepithema humile* and *A. custodiens* distributions are mutually exclusive as demonstrated by pitfall trap and baiting data (Luruli 2007; Vorster 2011; Lach 2013). Therefore it is impossible to study these two species together in situ as they never occur in sympatry (Bond and Slingsby 1984; Witt and Giliomee 2004; Luruli 2007; Vorster 2011).

Study sites and plant selection

Study sites were selected in protected areas based on the abundance of nectar-producing Protea species, as well as the abundance of the native and invasive ant species. *Protea nitida* (M.) was selected as the study plant in the study area dominated by *L. humile* and *Protea repens* (L.) was selected as the study plant in the study area dominated by the native ant *A. custodiens*. The study plants were selected because they were the most common in the areas dominated by each of the ant species. Although both plants were present in both study areas, we focussed on sites with

largely monotypic stands of nectar producing proteacea. In this case, *P. nitida* was prevalent where *L. humile* was dominant, and *P. repens* was prevalent where *A. custodiens* occurred. *Protea nitida* and *P. repens* are pollinated by both insect and birds, and typically flower from March/April to August/September (Coetzee and Giliomee 1985; Cowling et al. 1996; Rebelo 2001). Both plants are characterised as Mesic Mountain Fynbos and Renosterveld (Cowling et al. 1996), and produce floral nectar, although the nectar properties differ in terms of volume, sugar concentrations are more similar (Geerts 2011; Geerts and Pauw 2011). Both these plant species have been shown to support a comparable species rich native ant community in natural areas free of Argentine ants (Donnelly 1983; Donnelly and Giliomee 1985; Luruli 2007). Both study areas had dense stands of the respective plant species which were of similar age (Cowling et al. 1996; Rebelo 2001). This study was conducted in two protected areas in the Boland Region of the Western Cape Province, South Africa, Helderberg Nature Reserve (HNR) (34°03′ S, 18°52′E) and Jonkershoek Nature Reserve (JNR) (34°58′ S, 18°56′E). The climate in both these reserves is similar to most of the Mediterranean type climate typical of the southern part of the Western Cape Region characterised by hot and dry summers (October–March) and, cold and wet winters (June–July) (de Kock 1990; Le Maitre et al. 1996; CapeNature 2010).

Sampling design

All sampling was conducted during three proteacea flowering periods, March/April–Early bloom, June/July–Peak bloom and September/October–Post bloom in 2011 and 2012. In 2012, the post bloom sampling was conducted in November for both areas due to the longer rainy season, as well as longer flowering season. In each reserve, 15 trees of *P. nitida* or *P. repens* were randomly selected, marked with red tape on a branch and the GPS coordinates recorded. The trees were at least 5–10 m apart and roughly the same height. Each tree was treated as a sampling unit and was sampled at each stage of the flowering period. The number of inflorescences that were open on each tree (floral density) was counted during the early, peak and post bloom sampling periods for both reserves. We also counted the number of nests within a 5 m radius around each tree during each of the flowering periods

Fig. 1 Argentine ants foraging on the inflorescence of *Protea nitida* during peak bloom. The flower exudes floral nectar which the ants feed on, both on the inside and outside of the flower. Photo credit, Eiriki Sunamura

to relate this to the abundance of ants foraging on the tree. The number of open inflorescences was used to infer the amount of nectar available, with an increase in the number of open flowers representing a concomitant increase in nectar availability (Geerts and Pauw 2011; Lach 2013). *Linepithema humile* foraged both inside and outside of the flowers of both species (Fig. 1, showing *L. humile* of *P. nitida*).

Ground activity and species composition of ants during the three flowering periods

Pitfall traps were used to assess the species composition and abundance of ground foraging ants in the two study areas, one dominated by Argentine ants while the other was free of Argentine ants, during each of the flowering periods. Pitfall traps, 50 ml plastic vials containing 25 ml of ethylene–glycol and water, were placed at 10 m intervals along four 100 m parallel transects separated by 25 m. Each trap was dug flush to the soil and left open for seven consecutive rain-free days. The traps were collected, brought back to the laboratory where the samples were washed, sorted and identified under a microscope (Zeiss, Stemi DV4) to species level where possible. The data were combined for the two sampling years and a Generalised Linear Model (GLZ) assuming a poisson distribution was used to determine whether there was a difference in the abundance of ground dwelling ant species across the three flowering periods. The estimation scale parameter used was the Pearson Chi Square to obtain more conservative variance estimates

and statistical significance. The analysis was conducted under the lme package in R (RStudio version 3.1.2).

Species composition and assemblage structure in *Protea nitida* and *Protea repens* inflorescences

Inflorescences of both *P. nitida* (n = 128) and *P. repens* (n = 142) were randomly collected from trees within the study area, bagged in labelled zip lock bags and brought back to the laboratory where they were frozen. The inflorescences were then dissected and all arthropod species (including coleopteran larvae) found were identified under a stereo microscope (Zeiss, Stemi DV4). Ants were identified to species level while beetles and other arthropod species were identified to family level, then stored in 70% ethanol for preservation. Multivariate analyses in PRIMER (Plymouth Routines in Multivariate Ecological Research, version 6: Plymouth Marine Laboratory, UK), were used to ascertain whether the presence of *L. humile* and *A. custodiens* had an effect on the arthropod species composition of *P. nitida* and *P. repens* inflorescences. Bray-Curtis Similarity Index analyses were used to assess the similarity matrix (Clarke and Gorley 2006). The abundance data were square-root transformed prior to analysis to balance the weight of contribution by common and rare species (Clarke and Gorley 2006). Analysis of Similarity (ANOSIM) was used to determine whether there was a statistically significant difference in the species composition of inflorescences of both plant species.

Foraging activity

Ant foraging activity (rates) was quantified by measuring ant traffic along a randomly selected branch on each of the 30 experimental *P. nitida* (n = 15) and *P. repens* (n = 15) trees, with a diameter greater than 10 cm for better visibility during each of the flowering periods. Only branches with high ant traffic (i.e. at least 15 ants per minute) were selected to standardise counts between the sampling units. A 4 cm line was drawn across the branch using white, fragrance free finger paint. Using a hand clicker, ants moving across the line in one direction were counted every two min during a 10 min period, and this was done at 0900 and 1100 hours in the morning; and at 1400 and 1700 hours in the afternoon for five days. We took

an average of two counts in the morning and for the afternoon and used these data for analysis. The Generalized Least Square Model (GLS) was used to determine the effects of floral density (number of open inflorescences) and nest density (number of nests within 5 m radius of the tree base) as continuous predictors, flowering period, sampling year and ant species as categorical predictors, on the variation in foraging activity. The dredge function was used to select the model that best explained foraging activity by the predictors. The best model, selected on the lowest Akaike Information Criterion, consisted of floral density, flowering period, sampling year and ant species. Posthoc analyses were conducted to identify the underlying differences. The analysis was conducted under the lme package in R (RStudio version 3.1.2).

Sampling for stable isotope analysis

In stable isotope analysis, the ratio of heavy to light isotopes (typically carbon and nitrogen) can be traced from primary producer to consumer with accumulation of nitrogen across trophic levels (Tillberg et al. 2006; Menke et al. 2010). The carbon to nitrogen ratio (C:N) represents the relative amounts of carbohydrate or protein assimilated by the organism (Smith and Suarez 2010), with higher C:N values indicating a high carbohydrate component to the diet whereas low C:N values indicate a high protein component to the diet (Fry 2006; Wilder et al. 2013). The sample ratios, heavy to light isotopes of C and N, are calculated using the following formula:

$$\delta X = \left[\left(R_{sample}/R_{standard} \right) - 1 \right] \times 1.000$$

The sample ratio is compared to the element specific standard, that is, the ratio of heavy to light isotopes of the sample to that of the standard. The standard for carbon is PeeDee Belemnite carbonate and atmospheric air for nitrogen (Post 2002; Tillberg 2004). Delta (δ) values are presented as per mil (‰) for both carbon and nitrogen isotopes.

Sample preparation

In each reserve, 15 nests each of *A. custodiens* and *L. humile* close to the experimental trees were located, disturbed and 10 individuals of *L. humile* and *A.*

custodiens from each nest were collected in an 8 ml glass vial (sample = 10 ants/vial, n = 15 samples per ant species), and killed in 95% ethanol. Storage times were less than one day to minimise the potential effect of the killing agent on $\delta^{13}C$ (Feldhaar et al. 2010). Leaf samples of each plant species were also collected for analysis, and ants were sampled from pitfall traps (a minimum of five individuals of each ant species per glass vial (n = three to five samples for each of the non-focal ant species). A number of non-ant arthropods were also collected randomly on the plants. All abdomens were removed from all arthropods collected to avoid recent feeding skewing the results and thus obscuring the effect on long-term resource assimilation information (Tillberg et al. 2006). All samples were dried in an oven at 60 °C for two days and then ground to a fine powder using a mortar and pestle, and stored in glass containers with desiccant until processing. Caterpillars and larvae were processed and ground whole. The samples were sent to the University of Cape Town Stable Isotope Unit where 1, 5 µg of each sample was weighed into small tin capsules and the $\delta^{13}C$ and $\delta^{15}N$ signatures of all samples were determined using a continuous flow Isotope Ratio Mass Spectrometer (IRMS; Delta Plus XP and DeltaV, both interfaced with elemental analysers and high temperature combustion devices), after sample combustion in on-line Carlo-Erba preparation. Beet sugar and Merck gelatine were used as standards, calibrated against International Atomic Energy reference materials (PeeDee-Belemnite and air for $\delta^{13}C$ and $\delta^{15}N$ respectively). The results are expressed in standard delta notation, $\delta X = \left(\left[R_{sample}/R_{standard} \right] - 1 \right) \times 1000$, where X is the element in question and R is the ratio of the heavy over the light isotope. Precision of replicate determinations for both carbon and nitrogen was ±0.05 ‰.

The two protea plant species did not differ in isotopic signatures (Pillai's Trace lambda = 0.12, $F_{(1, 28)} = 0.81$, $p > 0.05$), and were subsequently combined into one primary producer for the analysis. The isotopic signature, $\delta^{13}C$ and $\delta^{15}N$, of the plant, all ants, herbivores and predators under investigation were compared using a Two-factor Multivariate Analysis of Variance (MANOVA) to minimise chances of Type 1 error (Ottonetti et al. 2008). $\delta^{13}C$ and $\delta^{15}N$ values were both used as dependent variables. Pillai's Trace lambda was used as the test

statistic due to the violation of the assumption of equality of covariance (Box's M test was less than 0.05). Further, univariate F tests were conducted to compare $\delta^{13}C$ and $\delta^{15}N$ between species. The analysis was conducted using the Manova function under the lme stats package in R (RStudio version 3.1.2).

Trophic position and C:N ratio of *A. custodiens* and *L. humile*

The trophic position of *L. humile* and *A. custodiens* was estimated relative to that of known herbivores, predators and study plants within each study area, in order to overcome issues of spatial heterogeneity in resource acquisition between the two ant species (Post 2002; Feldhaar et al. 2010; Wilder et al. 2013). The trophic position of each ant species within the study area was calculated using the following formula from Lach et al. (2010) as adapted from Post (2002).

$$\rho_1 = \left[\delta^{15}N_{ant} - \delta^{15}N_{(2)} - \Delta N''\right] / \left\{\left[\delta^{15}N_{ant} - \delta^{15}N_{(2)} - \Delta N''\right] + \delta^{15}N_{(1)} + \Delta N' - \delta^{15}N_{ant}\right\}$$

$$\rho_2 = 1 - \rho_1$$

ρ_1 and ρ_2 represent the proportions of dietary inputs from two main sources, herbivores and predators; $\delta^{15}N_{(1)}$ and $\delta^{15}N_{(2)}$ represent the trophic position of herbivores and those of predators respectively. The $\delta^{15}N$ values were used to calculate the trophic position for each replicate for all ant species. We calculated site specific enrichment factors (ΔN), rather than use standardised fractionation factors (McCutchan et al. 2003; Fry 2006), by comparing mean fractionation/enrichment from plants to herbivores ($\Delta N'$), and from herbivores to predators (spiders) for each study area ($\Delta N''$). The trophic position (TP) of *L. humile* and *A. custodiens* was then calculated according to the formula below. The trophic position of plants was 1, and that of herbivores and predators was 2 and 3 respectively:

$$TP_{ant} = TP_{predator} + 1 - \left(TP_{predators} - TP_{plants}\right)\rho_1$$

The difference in trophic position (TP), and C:N ratio, of *L. humile* and *A. custodiens* over the flowering period was compared using the Generalised Linear Model (GLZ) function both assuming a quasi-poisson distribution under the lme package in R (RStudio version 3.1.2, R Core Team 2012). Statistical significance was accepted at $p < 0.05$ for all analyses.

Results

Ground activity and species composition of ants during the three flowering periods

The native ant, *A. custodiens*, co-existed with a higher diversity and abundance of native ant species than did the invasive ant, *L. humile* (Fig. 2a, b). Six native ant species were found with *A. custodiens* and only three native species with *L. humile*. The abundance of the ants in *P. repens* stands (Fig. 2a) varied significantly across the three flowering periods (Wald $\chi^2_{(12)} = 28.47$ $p < 0.01$). Pairwise comparison (LSD posthoc tests) showed that *A. custodiens* had highest abundances during the peak flowering period; *L. capensis* had the highest abundances during the early flowering period, while *Monomorium sp. 1* showed increased abundance in both peak and post bloom (Fig. 2a). The abundances of ant species in *P. nitida* stands also varied significantly across flowering period (Wald $\chi^2_{(3)} = 545.7$, $p \leq 0.0001$). Yet, only the abundance of *L. humile* and *Tetramorium quadrispinosum* varied significantly across the three flowering period; with *L. humile* having increased numbers during the peak bloom period while *T. quadrispinosum* shows increased numbers post bloom (Fig. 2b).

Species composition and assemblage structure in *P nitida* and *P. repens inflorescences*

Only 40% of *P. nitida* (52 of 128) and *P. repens* (57 of 142) inflorescences were occupied by ants (Table 1). *Protea nitida* inflorescences with ants were dominated by *L. humile*, while *P. repens* had *A. custodiens* followed by *Crematogaster sp. 1* and *Lepisiota capensis* (Table 1). Chrysomelidae beetles, Cucujoidea beetles and fruit flies (Diptera) were found in relatively large numbers in most of the inflorescences collected (Table 1, *P.repens* and *P. nitida*). *Linepithema humile* had the highest overall ant abundance in inflorescences, whereas a greater number of native ant species co-occurred on *P. repens*. *Linepithema humile* made up 95.5% of all Hymenopteran visitors to *P. nitida*, while *A. custodiens* made up 82.3% of
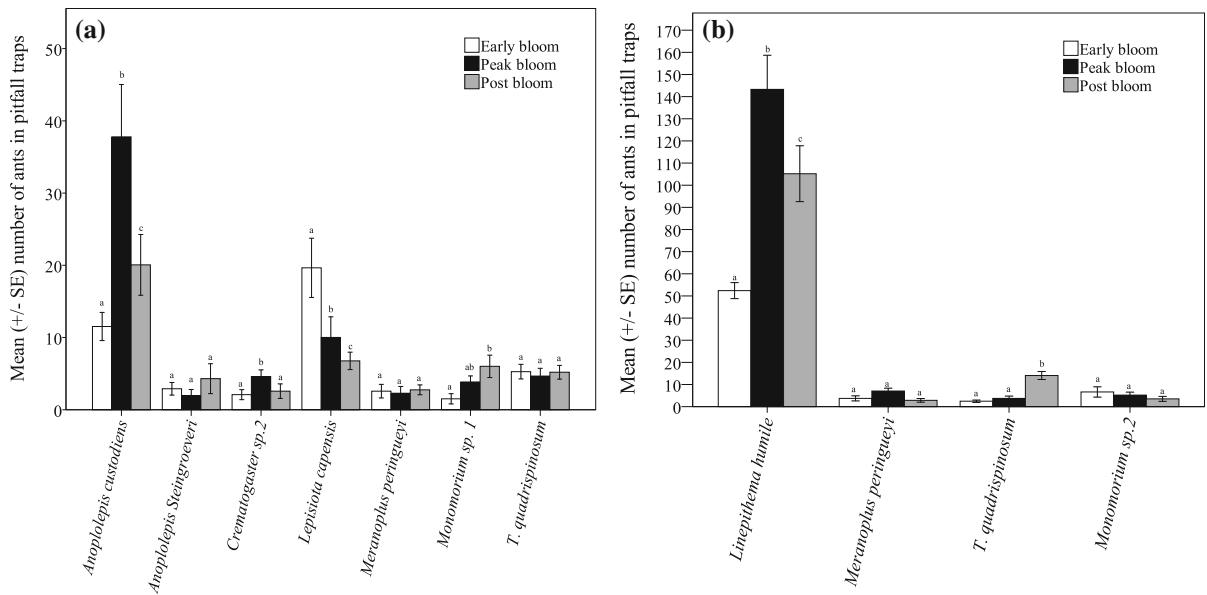
Fig. 2 Comparison of ant abundance in pitfall traps (**a** and **b**), across three flowering periods **a** in *P. repens* stands and **b** *P. nitida* stands. Significant differences indicated with *different letters above bars* (LSD posthoc comparisons) for those species that showed significant variation in abundance across the three flowering periods. *Linepithema humile* exclusively dominated the study site in terms of numerical abundance during all the sampling periods in *P. nitida* stands, while *Anoplolepis custodiens* and *Lepisiota capensis* were both numerically dominant in *P. repens* stands

Table 1 Abundance and composition of arthropods found in the inflorescences of *Protea nitida* and *Protea repens*

	P. nitida (128)			*P. repens* (142)		
	n	proportion (%)	Density/flower	n	proportion (%)	Density/flower
Anoplolepis. custodiens	0			1583	37.51	11.15
Linepithema humile	4564	18.68	35.66	–	–	–
Camponotus sp.1	5	0.02	0.04	–	–	–
Camponotus niveosetosus	121	0.50	0.95	34	0.81	0.24
Crematogaster sp.1	90	0.37	0.70	132	3.13	0.93
Lepisiota capensis	–	–	–	101	2.39	0.71
Lepisiota sp. 1	–	–	–	42	1.00	0.30
Apis mellifera capensis	1	–	0.01	32	0.76	0.23
Anthocoridae	1	0.004	0.01			
Chrysomelidae	19020	77.84	148.59	1367	32.39	9.63
Cucujoidea	153	0.63	1.20	157	3.72	1.11
Histeridae	–	–	–	129	3.06	0.91
Melolonthinae	8	0.03	0.06	5	0.12	0.04
Meloidae	13	0.05	0.10	3	0.07	0.02
Nitidulidae	15	0.06	0.12	–	–	–
Pentatomidae	12	0.05	0.09	–	–	–
Scarabaeidae	41	0.17	0.32	5	0.12	0.04
Curculionidae	2	0.01	0.02	–	–	–
Staphyinidae	25	0.10	0.20	290	6.87	2.04
Other arthropods						

Table 1 continued

	P. nitida (128)			P. repens (142)		
	n	proportion (%)	Density/flower	n	proportion (%)	Density/flower
Dermaptera (earwigs)	9	0.04	0.07	–	–	–
Thysanoptera(Thrips)	105	0.43	0.82	25	0.59	0.18
Diptera	244	1.00	1.91	309	7.32	2.18
Arachnida	5	0.02	0.04	6	0.14	0.04
Total	24434	100		4220	100	

Ants are identified to species level while beetles and other arthropods are identified to family level. The total number and the proportion (%) of each species of ants, beetles and other arthropods are given. Dashes denote when species were not found in inflorescences of a particular species

The very high arthropod densities found in P. nitida compared to P. repens is probably due to P. nitida's greater reliance on insect pollinators (Visser et al. 1992; Cowling et al. 1996; Rebelo 2001)

Hymenopteran visitors to *P. repens*. Ant species that were never found in inflorescences were assumed to be ground foragers only.

Protea repens flower visitors were less affected by the presence of *A. custodiens* with the arthropod assemblage showing 94% similarity to those inflorescences without *A. custodiens* (ANOSIM Global R = 0.06, $p < 0.05$). In contrast, the arthropod assemblage of *P. nitida* inflorescences was significantly altered by the presence of *L. humile*, with only 71% similarity between inflorescences with and without *L. humile* (ANOSIM Global R = 0.29, $p < 0.001$).

Foraging activity

Foraging activity was significantly affected by flowering period ($F_{(2)} = 298.58$, $p < 0.0001$, Fig. 3), floral density ($F_{(1)} = 23.35$ $p < 0.0001$), ant species ($F_{(1)} = 279.15$, $p < 0.0001$) and sampling year ($F_{(1)} = 64.01$, $p < 0.001$). Ant foraging increased significantly during the peak bloom ($\beta = 42.09 \pm 2.67$ t = 15.74 $p < 0.0001$) but was significantly reduced in the post bloom period ($\beta = -17.34 \pm 1.16$ t = -14.94 $p < 0.0001$) in comparison to the early bloom period. Foraging activity also differed between the two ant species, with *L. humile* showing increased foraging activity in comparison to the native ant *A. custodiens* ($\beta = 13.55 \pm 0.81$ t = 16.74 $p < 0.0001$). *Linepithema humile* foraged significantly more than *A. custodiens* throughout the flowering period (Fig. 3). Foraging activity was significantly higher in 2012 than in 2011 ($\beta = 4.34 \pm 0.54$ t = 8.01 $p < 0.0001$), while
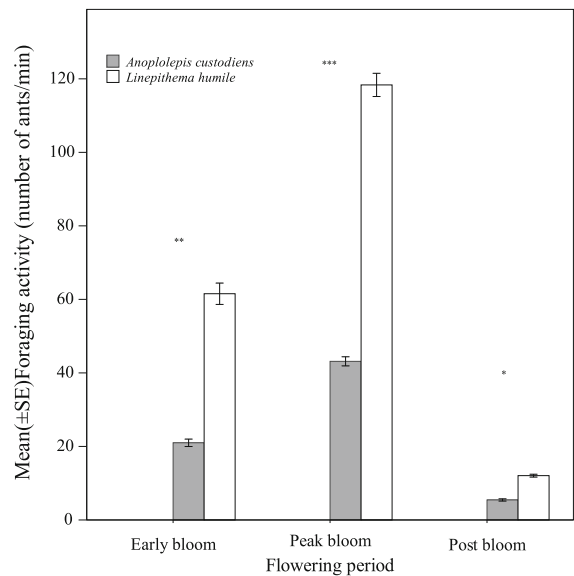

Fig. 3 Differences in the mean ($\pm$ SE) foraging activity of ants during the three flowering periods. Argentine ant activity on the plants was higher than that of *A. custodiens* across the flowering period. Significant pairwise differences shown as *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ based on Tukey HSD in GLS

foraging activity increased by a small but significant degree with increased floral density (number of open flowers) ($\beta = 0.35 \pm 0.02$ t = 4.84 $p < 0.0001$).

Stable-isotope analysis

The $\delta^{13}C$ values of most ant species and herbivorous arthropods were within the range of C3 plants (-24 to -34 ‰), suggesting that the basal source for carbon in this community is the plant (Fig. 4a–c). The isotopic

(a) Early bloom

(b) Peak bloom

(c) Post bloom

Mean(±) δ^{13}C
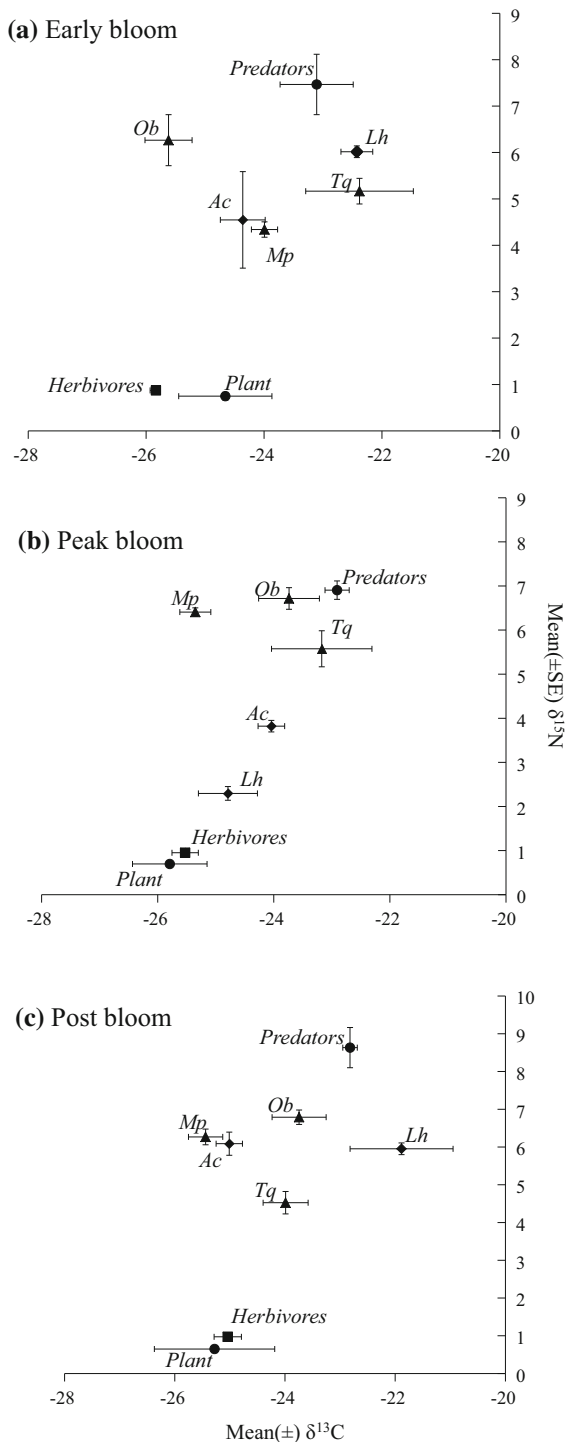
Mean(±SE) δ^{15}N

◀ **Fig. 4 a–c** Mean (±SE) δ^{13}C and δ^{15}N values of the plant as primary producer, herbivore as primary consumers, ant species, and predatory arthropod species as secondary and tertiary consumers, during the three flowering periods. Abbreviations: Ac (*Anoplolepis custodiens*), Lh (*Linepithema humile*), Mp (*Meranoplus peringueyi*), Tq (*Tetramorium quadrispinosum*), Ob (*Ocymyrmex barbiger*)

as well as the two metric variables, δ^{13}C (F$_{(7, 367)}$ = 19.96 $p < 0.0001$) and δ^{15}N (F$_{(7, 367)}$ = 357.53, $p < 0.0001$), varied significantly between the species sampled (Pillai's Trace lambda = 1.05 F$_{(14, 734)}$ = 57.63, $p < 0.0001$, Table 2). Flowering period also had an effect on the isotopic signatures of the species sampled (Pillai's Trace lambda = 0.11, F$_{(4, 734)}$ = 10.13, $p < 0.0001$) and δ^{15}N (F$_{(2, 367)}$ = 20.12, $p < 0.05$) but did not affect δ^{13}C (Table 2). Based on the effect size values, δ^{15}N explained a large percentage of the variation in isotopic signatures of the species under investigation.

The isotopic signatures of all species sampled were significantly different in the early, peak and post-bloom flowering periods (Table 2). Both δ^{13}C and δ^{15}N were significantly different between all species sampled at all three seasons (Table 2), with δ^{15}N explaining 87, 87.5 and 90% of the variation in isotopic signature at early bloom, peak bloom and post bloom respectively. Multiple pairwise comparisons (Tukey's HSD) showed that ants differed in isotopic signatures to that of plants, the herbivorous arthropods and predatory arthropods (spiders) at early bloom (Fig. 4a), while only *L. humile* was not significantly different from the herbivores in isotopic signature peak bloom (Fig. 4b). During post bloom, both *A. custodiens* and *L. humile* show a shift in their signatures and return to their early bloom positions, with *A. custodiens* showing a shift up to 6 ‰ δ^{15}N (Fig. 4c). It is likely that *A. custodiens* is utilising more protein based resources in the post bloom period.
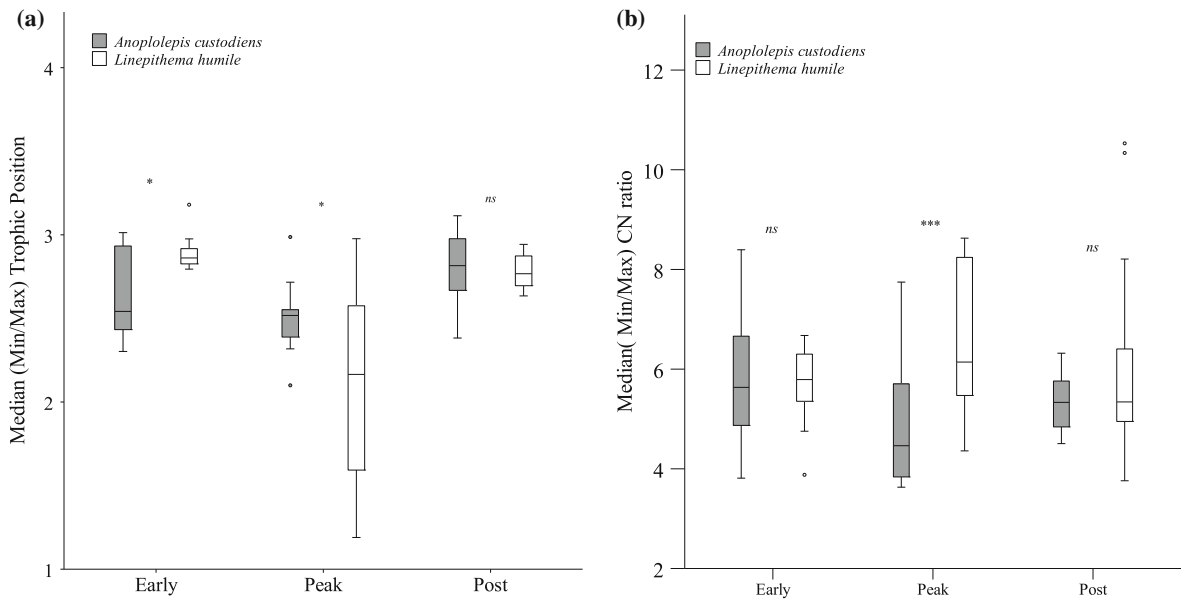
Trophic position and C:N ratio of *A. custodiens* and *L. humile*

The trophic position of *L. humile* and *A. custodiens* differed depending on flowering period (Wald $\chi^2_{(2)}$ = 17.06, $p < 0.05$). In the early bloom period, *L. humile* (2.89 ± 0.05) occupies a higher trophic

signatures varied between species and flowering period, the interaction effect of species and flowering period was significant (Pillai's Trace lambda = 0.48, F$_{(28, 734)}$ = 8.24, $p < 0.0001$). The isotopic signatures

Table 2 Manova results for comparison of stable isotope signatures (multivariate) and $\delta^{15}N$ and $\delta^{13}C$ (univariate) of the plant and arthropod species collected within the two study sites across three flowering periods

Test	Effect	Overall			Early			Peak			Post		
		Pillai's λ	$F_{(df,\,n)}$	P	Pillai's λ	$F_{(df,\,n)}$	P	Pillai's λ	$F_{(df,\,n)}$	P	Pillai's λ	$F_{(df,\,n)}$	P
Multivariate													
	Species	1.05	$57.63_{(14,734)}$	<0.0001	1.12	$22.31_{(14,246)}$	<0.0001	1.04	$19.44_{(14,250)}$	<0.0001	1.16	$23.55_{(14,238)}$	<0.0001
	FP	0.11	$10.13_{(4,734)}$	<0.0001									
	Species X FP	0.48	$8.24_{(28,734)}$	<0.0001									
Univariate													
$\delta^{15}N$													
	Species		$357.53_{(7367)}$	<0.0001		$117.12_{(7123)}$	<0.0001		$118.92_{(7125)}$	<0.0001		$37.62_{(7119)}$	<0.0001
	FP		$20.12_{(2367)}$	<0.05									
	Species X FP		$16.18_{(14,367)}$	<0.0001									
$\delta^{13}C$													
	Species		$19.96_{(7367)}$	<0.0001		$12.13_{(7123)}$	<0.0001		$7.26_{(7125)}$	<0.0001		$8.44_{(7119)}$	<0.0001
	FP		$1.31_{(2367)}$	>0.05									
	Species X FP		$3.47_{(14,367)}$	<0.0001									

Fig. 5 a, **b** Median (Min/Max) Trophic Position (*a*) and C:N ratio (*b*) for *A. custodiens* and *L. humile* over the three flowering periods. Significant pairwise differences in trophic position and C:N ratios compared using β and *t* test as part of the Generalised

Linear Model (GLZ): *ns* $p > 0.05$, '*$p < 0.05$, ***$p < 0.001$ based on pairwise Least Square Difference). *Circles* indicate outliers

position than *A. custodiens* (2.64 ± 0.03) (β = −0.32 ± 0.16, Z = 4.32, $p < 0.05$), whereas it is lower in trophic position than *A. custodiens* in the peak bloom period (*L. humile* (2.11 ± 0.08) *A. custodiens* (2.49 ± 0.05); β = 0.30 ± 0.08, Z = 4.04, $p < 0.05$). The ants did not differ in their trophic position during the post bloom period (*L. humile* (3.15 ± 0.05), *A. custodiens* (3.16 ± 0.04, Fig. 5a). Although both species are primary predators, the trophic position of *L. humile* shows more variation over the flowering period, with a lower trophic position at the peak flowering period matching the changes in the isotopic signature (Fig. 4a–c). This implies that *L. humile* is most likely responding to the increased floral nectar availability by foraging predominantly on this resource, reflected in the trophic position being closer to the herbivorous insects.

Linepithema humile and *A. custodiens* also differ in the C:N ratios (Wald $\chi^2_{(1)}$ = 5.97, $p < 0.05$), with *L. humile* having an higher C:N ratio than *A. custodiens* (Fig. 5b). This suggests that *L. humile* utilises more carbohydrate based resources than *A. custodiens*. The carbohydrate resource intake of *L. humile* was significantly higher in the peak (β = −0.28 ± 0.12, Z = 2.39, $p < 0.05$) but not in the early and post flowering periods (Fig. 5b).

Discussion

We found that the invasive Argentine ant, *L. humile*, utilised available floral nectar more actively and efficiently than the native dominant ant *A. custodiens*. The Argentine ant increased its foraging on floral nectar when it became available, switching its diet to rely on this floral nectar more so than *A. custodiens*. This evidence is supportive of the diet switching hypothesis (Tillberg et al. 2007) and reflects the trait flexibility of *L. humile* as an invasive species (Callaway and Aschehoug 2000; Wilder et al. 2013). A similar switch to a greater herbivorous diet on carbohydrate resources from honeydew producing arthropods has been shown for both *L. humile* (Tillberg et al. 2007; Menke et al. 2010) and the red imported fire ant, *S. invicta* (Helms 2013; Wilder et al. 2011a, b, 2013; Helms et al. 2010). To our knowledge, this study is the first to show this dietary response on plant-based carbohydrate resources, and further highlights the high dietary flexibility of this ant species and may contribute to its successful invasion in natural environments.

During periods of high nectar availability, peak bloom, $\delta^{13}C$ and particularly $\delta^{15}N$ of *L. humile* declined drastically and approaches the isotopic

signature of the herbivores and the plants, suggesting that the ant is potentially foraging exclusively on floral nectar. Yet, during periods of low nectar availability the isotopic signature of this ant was more similar to spiders and predatory ants. Even though the isotopic signature of *A. custodiens* did not fluctuate as drastically as that of *L. humile*, nonetheless the trophic position indicates that both species do exploit the high abundance of carbohydrates when available. The trophic position of both species lowers during the peak flowering period (Fig. 5a) but *L. humile* lowers significantly more, suggesting the native ant also has the ability to exploit this resource but does not respond to the change in availability as efficiently as *L. humile* does. The C:N ratio and trophic position of both ant species was the same at the post bloom period, but there was more variation in the relative amounts of carbohydrate assimilated by *L. humile*, suggesting that they continue to forage on carbohydrate resources, even though their trophic position is that of a predator. We did not quantify floral nectar availability from other Fynbos plant species, such as the Ericacea (Cowling et al. 1996), which could have been flowering at this time and may have provided an additional carbohydrate supply. A previous study by Lach (2007) found that a myrmecophilic treehopper species, *Beaufortiana sp.*, occurred on *P. nitida* at JNR, which she thought facilitated *L. humile*'s success in this area. However, we did not find this treehopper on any of the *P. nitida* sampled, nor did we observe any other honey-dew producing insects on either of the plant species during our sampling period or within a 30 month period of working in the area. It is likely that the myrmecophilic treehopper species did not successfully recolonize the area after the fire that occurred in 2009.

Trait plasticity has been shown in studies of invasive plants, where these plants were able to outperform native plant species in utilising fluctuating resources such as an influx of soil nutrients due to environmental perturbations within the environment (Funk 2008), as well as being better able to tolerate changes in environmental conditions (Dukes and Mooney 1999). Thus, this flexibility may benefit invasive species establishment success. Introduced species may benefit by responding more efficiently to changes in resource availability than resident species when competing for shared resources (Dukes and Mooney 1999; Davis et al. 2000; Mata et al. 2013), as

was the case with *L. humile* that showed a substantial shift in trophic position in response to carbohydrate availability during the peak bloom period (Tillberg et al. 2007; Wilder et al. 2013). Our foraging activity results also suggest that *L. humile* is flexible in their response to increased nectar availability, adjusting their foraging effort during peak bloom. Similarly, a greater number of workers of *Paraponera clavata*, an arboreal ant, respond actively to increased nectar availability fuelling carbohydrate intake and foraging success (McGlynn and Parra 2016). Thus, *L. humile* can potentially increase its spread within the Fynbos by being more effective at utilising these periodically available carbohydrate resources which have previously been shown to be essential for colony survival in this ant species (Savage et al. 2009; Wilder et al. 2011a, b, 2013; Shik and Silverman 2012).

The ability to switch diets is thought to be an important factor contributing to the success of *L. humile*, allowing it to utilise a wide range of resources as they become available in the recipient environment (Tillberg et al. 2007). Both short and long term studies show that *L. humile* can rapidly switch trophic position in response to available carbohydrate resources, particularly in response to honeydew (Tillberg et al. 2007; Menke et al. 2010; see also Wilder et al. 2013 in the case of *Solenopsis invicta*). Diet switching is considered an effective mechanism that allows these ants to maintain and grow their large supercolonies once they have successfully established (Tillberg et al. 2007). Similarly, *A. gracilipes*, the invasive yellow crazy ant, recruited large numbers of workers to artificial nectar sources and displayed high levels of aggression (Savage and Whitney 2011) suggestive of analogous responses to changing resource availability in invasive ant species (Kaplan and Eubanks 2005).

Little work has focussed on the importance of floral nectar to *L. humile* success in the Fynbos (Lach 2007, 2008, 2013). Lach (2013), using the occupancy of protea inflorescences by ants, contended that ants in the Fynbos were not effectively utilising the abundant floral resources, including *L. humile*. Similarly, we found ants in general only occupied a small proportion of inflorescences of both *P. nitida* (40.6%) and *P. repens* (40.1%), with *L. humile* having occupied 25% of *P. nitida* inflorescences whereas *A. custodiens* were found in only 19% of *P. repens* inflorescences. Furthermore, the studies by Lach (2007, 2008) suggested that *L. humile* was a threat to floral arthropods

that visit inflorescences of protea plants. In our study, *L. humile*, when present in flowers, was found in high abundances in the inflorescences of *P. nitida* and had a larger negative effect on the species composition through the displacement of floral visitors that are important in pollination, than did *A. custodiens*. These data suggest that native Fynbos ants are not utilising this available resource effectively, potentially providing a niche opportunity, with regards to resource availability, for *L. humile*. This floral resource is as yet not maximally exploited by *L. humile* but offers an available resource that can fuel colony growth (Tillberg et al. 2007; Dussutour and Simpson 2008, 2012) and contribute further to the invasion success of *L. humile;* as well as potentially explain the current distribution patterns of this ant in relatively undisturbed Fynbos areas (de Kock and Giliomee 1989; Lach 2007, 2008, 2013).

The numeric dominance of *L. humile* makes this ant an effective invader (Tsutsui et al. 2001; Helanterä et al. 2009). *Linepithema humile* dominated invaded sites and was found with only three indigenous ant species which were all found in much lower numbers (see also Donnelly and Giliomee 1985; Witt and Giliomee 2004; Luruli 2007). This pattern is similar to other introduced ranges where *L. humile* invasion leads to the decline of native ant species. In uninvaded sites within protected areas, a higher abundance and diversity of native ant species were found in areas containing both plant species used in this study (Donnelly 1983; Donnelly and Giliomee 1985; Luruli 2007). In this study A. *custodiens* did not compete with *L. humile* for the resource since they do not coexist (Luruli 2007), thus the exploitation of floral nectar by *A custodiens* was not hindered by competition from *L. humile*. Our result suggests that *L. humile* utilised more carbohydrate based resources than *A. custodiens* and is potentially exclusively foraging on floral nectar during the peak flowering period. These outcomes support our hypothesis that the carbohydrate rich floral resources available within the Fynbos are likely to fuel the spread of *L. humile* further within this region. Favourable abiotic conditions and the availability of floral resources from Proteacea, as well as the ants' effective foraging strategy may give *L. humile* the added potential to further spread into more pristine areas and speed up their rate of invasion. The lack of resource competition from native ants, and the negative impacts on native

arthropods associated with Proteacea by *L. humile* may indirectly threaten the future of this biodiversity hotspot.

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