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Yellow crazy ants (*Anoplolepis gracilipes* [Smith, F., 1857]: Hymenoptera: Formicidae) threaten community of ground-dwelling arthropods in dry evergreen forests of Thailand

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Abstract

Anoplolepis gracilipes is a widespread invasive species in tropical regions, posing a serious threat to native fauna. However, there is a lack of comprehensive field investigations into the negative impact of this species on ground-dwelling arthropods (GDAs). Herein, GDA orders, native ant species, and the abundance of native ant nests were compared between invaded (IVA) and uninvaded (UVA) areas in a dry evergreen forest in the Sakaerat Biosphere Reserve, Thailand. Pitfall traps was used to collect GDAs, including ants. Ant nests were surveyed using direct sampling and food baits. In total, 8,058 GDAs belonging to 13 orders were collected from both areas. Within the IVA, the abundance levels of centipedes, isopods, spiders, cockroaches, termites, beetles and ants were lower than those in the UVA. In addition, the frequency levels of occurrence of centipedes, isopods, cockroaches and termites were lower in the IVA than in the UVA. In total, 83 species of native ants were collected, and the more diverse ant population was in the UVA (66 species) compared to the IVA (49 species). The abundance and frequency of occurrence levels of seven ant species were significantly higher in the UVA than in the IVA. In total, 4,431 nests were found. Nest abundance of native ants in four nest categories and nest occurrence in two nest categories were significantly higher in the UVA than in the IVA. These results clearly indicated that invasion of *A. gracilipes* adversely affected the GDA community, especially native ant species and their nest abundance and occurrence.

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Introduction

Invasive ant species are a serious threat to the biodiversity of native species communities worldwide (Lach and Hooper-Bui, 2010). Many invasive ant species tend to be distributed in large areas with high numbers of nests and workers after successful invasion (Haines and Haines, 1978; Holway et al., 2002; Abbott et al., 2005). Throughout the invaded area affected by ant invasion, there are usually substantial declines in the biodiversity and abundance of various native species including birds, amphibians, reptiles, crabs and arthropods (Matsui et al., 2009; Lach and Hooper-Bui, 2010; Green et al., 2011). Invasive ant species can also negatively affect the biodiversity of native species through other mechanisms, such as creating increased competition for food and space (Lowe et al., 2000; Matsui et al., 2009; De Fisher and Bonter, 2013).

Yellow crazy ant (*Anoplolepis gracilipes*) is one of the world's worst invasive species (Lowe et al., 2000). This species can be found throughout tropical Asia and in the moist lowlands and tropical islands of the Indian and Pacific Oceans (Abbott et al., 2005; Wetterer, 2005; Mezger and Pfeiffer, 2011). *A. gracilipes* is highly competitive with native species for food and can affect native species through direct predation in invasion areas. It attacks native crabs (Vanderwoude et al., 2000; Sarnat, 2008), birds (O'Dowd et al., 2003; Gerlach, 2004; Sarnat, 2008) and arthropods (Hill et al., 2003; Mezger and Pfeiffer, 2011; Hoffmann et al., 2014; Kaiser-Bunbury et al., 2014; Stork et al., 2014;), thereby reducing the abundance and richness of these species. Studies have reported secondary effects on ecosystem processes caused by *A. gracilipes* (Vanderwoude et al., 2000; Sarnat, 2008; Lach and Hooper-Bui, 2010). For example, O'Dowd et al. (2003) reported that the number of red crabs on Christmas Island has been reduced substantially because of *A. gracilipes* predation. Consequently, the reduction in the number of red crabs altered the structure of the ground vegetation and the thickness of the litter layer because red crabs mainly consume fallen leaves and seedlings, thereby acting as a keystone species in the decomposition and regeneration processes for the ecosystem. Subsequently, this alteration caused secondary invasions by other invasive species, such as the giant African land snail, centipedes and snakes. Many studies have documented the negative effects of *A. gracilipes* on islands (Haines and Haines, 1978; Hill et al., 2003; Gerlach, 2004; Sarnat, 2008) and in savannahs (Hoffmann and Saul, 2010), as well as their impact on disturbing tropical forests (Mezger and Pfeiffer, 2011), oil-palm plantations (Kaiser-Bunbury et al., 2014) and cacao agroforests (Bos et al., 2008). However, few studies have been conducted in the tropical primary forests that represent biodiversity hotspots and are home to numerous endemic and endangered species (Myers et al., 2000). Such areas may even harbour species not yet identified (Erwin, 1982). Thus, if *A. gracilipes* causes serious harm to native animals in tropical primary forests, a domino effect could occur that has the potential to alter the biodiversity of these ecosystems.

Ground dwelling arthropods (GDAs) perform a number of ecological services for soil organisms in ecosystems (Folgarait, 1998; Stork et al., 2014). In addition, GDAs play an important role in soil food webs (Folgarait, 1998) and the decomposition of organic matter (Bignell and Eggleton, 2000; Yamada, 2004). Their nesting behaviour maintains soil fertility, which is crucial for soil nutrient dispersal (Folgarait, 1998) in soil and gasses emission from soils (Hasin et al., 2014; Ohashi et al., 2017). Biodiversity of GDAs and their abundance can also be ideal ecological indicators of habitat disturbance because of their short generation times (Kremen et al., 1993). The impact of *A. gracilipes* on GDAs has been the subject of various studies (Hill et al., 2003; Hoffmann and Saul, 2010; Hoffmann et al., 2014), but the results tended to differ between environments. Hoffmann and Saul (2010) reported non-significant differences in abundance and richness of native GDAs in savannahs, thickets and forests in Australia between invaded and uninvaded areas. Conversely, Hill et al. (2003) reported that an increase in arthropods species, such as scale insects, ant-crickets and cockroaches, in a coconut plantation on an African island following invasion by *A. gracilipes*.

The aim of the current study was to infer the impact of *A. gracilipes* on native organisms in a dry evergreen forest in Thailand. In particular, the main objectives were to compare areas that had been invaded by *A. gracilipes* with those in which this species was absent, with respect to: (1) the GDA community, (2) the ant community and (3) the abundance of ant nests and the occurrence of native ant species.

Materials and Methods

Study areas

This study was conducted in a dry evergreen forest (DEF) of the Sakaerat Biosphere Reserve (SERS; 14°30′N, 101°56′E), situated approximately 500 m above sea level in northeastern Thailand. The DEF covers 64% of the natural forest area in the SERS (Trisurat, 2009). The study area had a gentle slope of less than 10°. Two areas were selected with similar plant communities located 100–200 m apart; one had a prevalence of *A. gracilipes*' nests and workers, while the other contained no *A. gracilipes* from 2008 to 2011 (Hasin, 2008; unpublished data). Six 40×40 m^2 sample plots were established in the DEF. Three of the six sample plots were in previously invaded areas (IVA) where *A. gracilipes* was present, and their genetic structure characterized an invasive population (unpublished data), while the other three plots were in uninvaded areas (UVA) where *A. gracilipes* was absent. A series of different-sized subplots (5 m^2 and 10 m^2 plots) was set up within each sample plot to quantify the diversity and composition of the arthropod community (in the 10 m^2 plots) and ant nest abundance (in the 5 m^2 plots). In all study areas, the dominant trees were *Hopea ferrea* Laness (1886) and *H. odorata* Roxb (1811), which formed a closed canopy with heights in the range 23–40 m. Lower level vegetation consisted of *Hydnocarpus ilicifolius* King (1896), *Aglaia pirifera* Hance (1877), *Memecylon caeruleum* Jack (1820), *M. ovatum* Sm.(1812), *Ixora barbata* Roxb. ex Sm. (1811) and *Randia wittii* Craib (1911). The soil texture consisted of loam and clay loam, derived from sandstone (Lamotte et al., 1998). Soil porosity and available water capacity in the 0–50 cm depth layer were 50%–67% and 6–24 mm, respectively (Murata et al., 2009).

In the period 2000–2009, the mean annual precipitation, temperature and relative humidity at the SERS meteorological station were 978 mm, 26.3°C and 88.3%, respectively. The climate was characterized by a dry season from November to May (< 50 mm rainfall per month) and a wet season from June to October. During the study period for this research (1 November 2011–31 October 2012), the mean ± SD values for monthly precipitation, temperature and relative humidity were 19±17.8 mm, 24.5±2.03°C and 78.2±3.1%, respectively, during the dry season (November 2011–May 2012) and 166.8±63.6 mm, 27.4±1.5°C and 85.5±1.4%, respectively, in the wet season (June 2012–October 2012). Mean annual precipitation, temperature, and relative humidity during the entire period were 1237 mm, 25.8°C, and 81.7%, respectively.

Ground dwelling arthropod sampling

The diversity and abundance of GDAs were measured based on pitfall trapping. Each pitfall trap consisted of a plastic container (7 cm diameter, 10 cm depth) buried in the ground. The rim of each trap was level with the soil surface and the trap was half-filled with ethylene glycol to act as a short-term arthropod preservative. In each 40×40 m sample plot, 16 pitfall traps were established, with one trap positioned at the center of the 10 m^2 plots. Each trap was covered with a 5 cm^2 board with approximately 4 cm gaps above the ground to prevent rain interference. The traps were left in the field for 48 hr. Pitfall traps were set once for each season. The sampling was done separately during the dry season (November 2011–May 2012) and the wet season (June–October 2012). During the wet season, the GDA sampling was done 2 d after rain to minimize the direct negative affect of rain on the diversity and abundance of arthropods.

Ant nest sampling

Ant nests were sampled within each 5 m^2 plot using two methods: food bait and direct sampling. The food bait consisted of 10 cm × 10 cm pieces of white cotton containing 3 g of mixed food (canned tuna, peanut butter, honey dilution to 30% volume per volume with water). Three bait pieces was randomly placed approximately 2 m apart within each 5 m^2 plot. The bait was left for 45 min and then any ant trails of worker ants carrying food from each bait station were traced back to their nest. Direct sampling consisted of a visual search for ant nests on the ground by one collector for 5 min in each 5 m^2 plot. At each nest, ant activity was confirmed by carefully inspecting the inside of the nest and looking for any brood. Nest surveys using both baiting and direct sampling were conducted in the morning at 0800 hours to 0500 hours. A sample of 20 individuals from each nest was collected for species identification. Ant nest sites were classified into five types: litter layer (LL), rotten log (RL), underground nest (UG), tree trunk hole/crack (TT) and under substrate (US).

Identification of ground-dwelling arthropods

Arthropods in the pitfall traps were sorted to ordinal-level richness and ants were identified to species-level richness. Arthropod orders were identified using the systematic keys of Aoki (2015) and the taxonomic expertise of the authors. Ants were identified by referencing the insect collection at the Department of National Parks, Wildlife and Plant Conservation (DNP), Thailand, the systematic keys of Bolton (1994, 2003) and reliable digital resources (http://www.antweb.org and http://www.antbase.de). Species identification of some ant specimens was confirmed by ant taxonomist experts.

All ant individuals were identified to species/morpho-species. Arthropod groups were identified to the ordinal-level for most arthropods, sub-order for termites and cockroaches and family for ants. The number of individuals for each ant species and arthropod order was determined for analysis.

Data analyses

Community composition of GDAs and ants was evaluated using three variables: richness (R), abundance (A) and frequency of occurrence (F) with the ordinal-level for arthropods (F_{ar}) and at the species level for ants (F_{ant}) in each sample plot during both the dry and wet seasons using data from the pitfall traps (species/order and number of individuals therein). Richness was calculated as the number of arthropod orders or ant species, while abundance (A) was the number of individuals for each arthropod order or ant species. Frequency of occurrence (F, as a percentage) was used to quantify the percentage of traps in which the most frequent ant species or arthropod order in each sample plot was found (McCune and Grace, 2002; Hasin and Tasen, 2020). Frequency was calculated using Equation 1:

$$F_{(ar\ or\ ant)} = \frac{\text{Number of occupied pitfalls}}{\text{Total number of pitfall}} \times 100 \quad (1)$$

where $_{ar}$ represents orders of arthropods and $_{ant}$ represents species of ants.

A Student’s t test was used to determine the differences between invaded and uninvaded areas to an ordinal level or sub-ordinal level richness for arthropods, and in species richness for ants. The data from the two seasons were combined for these analyses. Because of the interaction between arthropod groups, habitats and seasonal variations in abundance and occurrence of GDA including ants, it was necessary to include sampling periods and arthropods group or species for data analyses. Thus, general linear models (GLMs) were used to explore the effects of variable factors on the arthropods and ants. In the case of arthropods, the A_{ar} or F_{ar} of the arthropod in order-level were used as dependent variables with study areas, with the other arthropod order and season as fixed factors. The GLMs were used for ants, in which A_{ant} and F_{ant} of ants were used as dependent variables with the species of ant, season and study areas used as fixed factors. In all these analyses, the data collection of *A. gracilipes* was excluded.

Nest abundance (A_{nest}) was the number of nests within a 5 m^2 plot. The frequency of nest occurrence for the ant species (F_{nest}) was calculated using Equation 2:

$$F_{nest} = \frac{\text{Number of occupied 5 m}^2\text{plots}}{\text{Total number of 5 m}^2\text{plots}} \times 100 \quad (2)$$

The GLMs were used to investigate the effects of variable factors on the abundance and frequency of occurrence of ant nests. The A_{nest} or F_{nest} of ants were used as a dependent variable with study areas, nest catalogues and seasons as fixed factors. The data collection for *A. gracilipes* nests was excluded. The normality and equality of variances for all data were tested using Shapiro-Wilk’s and Levene’s tests, respectively, before conducting all analysis of variance. Non-normally distributed variables were transformed using $\log_{10}$ (x+1) to improve normality before analysis. Bonferroni pairwise comparisons were used as post-hoc tests. Statistical analysis used the SPSS software (version 24; SPSS Inc.; Chicago, IL, USA).

Results

Difference between ground-dwelling arthropod communities

Across all study areas, 8,058 GDAs belonging to 13 orders were collected in pitfall traps (Table 1). The most abundant group was ants (70% of all individuals), followed by beetles (16%). GDA ordinal- richness did not differ between the UVA and IVA groups (t-test, $p > 0.01$, Fig. 1A).

The GLM revealed that GDA abundance (A_{ar}) differed significantly between study areas and seasons as well as among GDA orders (Table 2). Significant interaction between study areas and arthropod orders were found for A_{ar} (Table 2). The A_{ar} values for six of the nine arthropod groups were lower for the IVA than for the UVA (Fig. 2A; centipedes, $p = 0.01$; isopods and spiders, $p = 0.03$; cockroaches and ants, $p = 0.001$; termites, $p = 0.00002$).

The GLM revealed significant differences in the frequency of occurrence levels for GDAs (F_{ar}) between study areas and among GDA orders (Table 2). However, the season had no significant effect (Table 2). A significant interaction was detected between study areas and GDA orders (Table 2) for the frequency of occurrence for GDAs. The F_{ar} of five arthropod groups (centipedes, isopods, spiders, cockroaches, termites) was lower for the IVA than the UVA (Fig. 2B).

Differences in ant communities

In total, 5,062 individuals belonging to 83 species were collected using pitfall traps. The four most abundant species in the UVA were *Carebara affinis* (n = 481 individuals), accounting for 15.4% of the total abundance of all ant species combined (n = 3,122), *Pheidole hongkongensis* (n = 430; 14%), *Odontoponera denticulata* (n = 400; 13%) and *Carebara diversa* (n = 390; 12.5%). For the IVA, only one species, *A. gracilipes,* recorded a high number of individuals (1,063), accounting for 55% of the total abundance of all ant species combined.

Table 1 The list of ground-dwelling arthropods groups found in dry evergreen forest in Sakaerat Environmental Research Station

Group	Order/sub-Order (Families)	Abbreviation
Centipedes	Scutigeromorpha (Scutigeridae), Lithobiomorpha (Lithobiidae), Geophilomorpha (Geophilidae)	CH
Millipeds	Polydesmida (Paradoxosomatidae, Spirobolidae)	DI
Woodlouse	Isopoda (Oniscidae)	IS
Pseudoscorpion	Pseudoscorpionida (Cheliferidae)	PS
Earwigs	Dermaptera (Forficulidae)	DR
Spider	Araneae (Theridiidae, Salticidae, Agelenidae, Lycosidae, Araneidae)	AR
Harvestmen	Opiliones (Morphotaxons: 2 familly)	OP
Cockroaches	Blattodea (Blattellidae, Cryptocercidae, Blaberidae)	BL
Termites	Blattodea (Termitidae; *Macrotermes* spp., *Odontoterrmes* spp.)	TE
Bugs	Hemiptera (Cydnidae, Reduviidae)	HE
Beetle	Coleoptera (Bostrichidae, Carabidae, Curculionidae, Elarteridae, Nitituidae, Scarabaeidae, Staphylinidae, Tenebrionidae and morphotaxons: 4 familly)	CO
Cricket, Grasshopper	Orthoptera (Gryllidae, Tridactylidae, Acrididae, Tettigoniidae, Tetrigidae)	OR
Ant	Hynenoptera (Formicidae)	HY

Table 2 Statistical results for various factors on abundance (A_{ar}) and frequency of occurrence (F_{ar}) of ground-dwelling arthropods taxa, using ground dwelling arthropod (GDA) groups, season and study area as fixed factors. The dataset of *Anoplolepis gracilipes* was excluded from analyses.

Source of variation	Dependent Variable							
	A_{ar} (individuals/plot)				F_{ar} (%)			
	d.f.n.	d.f.d.	F	*p*-value	d.f.n.	d.f.d.	F	*p*-value
GDA	8	53	88.0	< 0.001	8	53	34.5	< 0.001
Season	1	53	5.1	0.03	1	53	1.7	0.19
Study area	1	53	45.8	< 0.001	1	53	22.1	< 0.001
GDA×season	8	53	0.2	0.98	8	53	0.3	0.95
Study area×season	1	53	1.2	0.27	1	53	3.0	0.09
GDA×study area	8	53	2.2	0.042	8	53	3.0	0.007
GDA×study area×season	8	53	0.4	0.90	8	53	1.1	0.39

d.f.n. = degrees of freedom in numerator; d.f.d. = degrees of freedom in denominator; F = F value based on the ratio of mean squares. The tests were considered significant at $p < 0.05$.

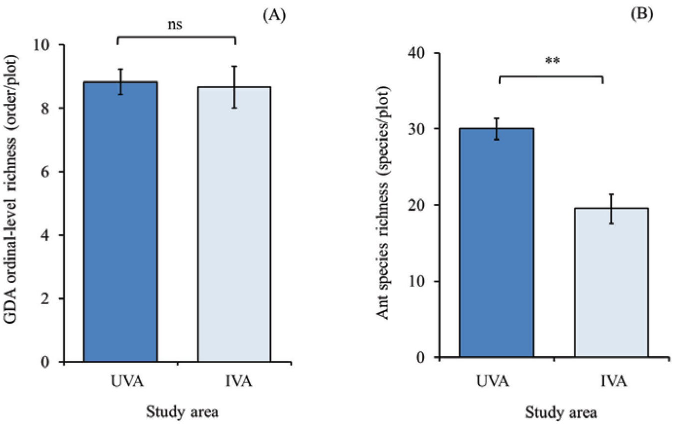

Fig. 1 Means (±SE; $n = 6$) of: (A) ground-dwelling arthropods (GDA) ordinal-level richness; (B) ant species richness, where UVA = uninvaded area and IVA = invaded area;significant difference is indicated with ** ($p < 0.01$) and ns indicate non-significant difference ($p > 0.05$).

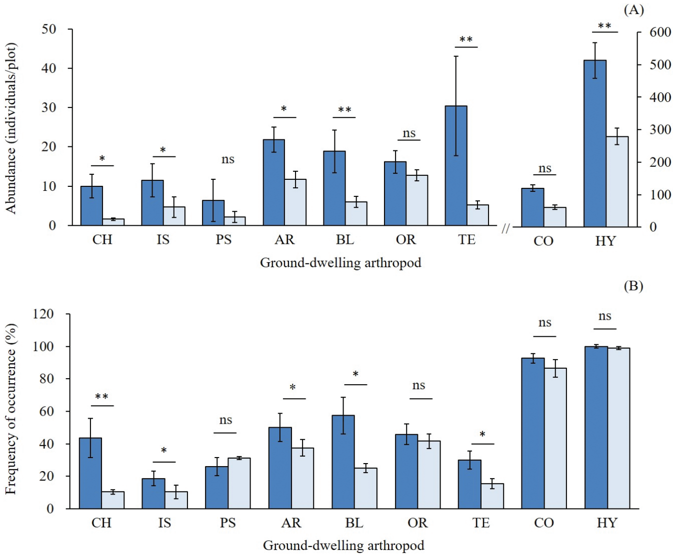

Fig. 2 Means (±SE) of ground-dwelling arthropods: (A) abundance; (B) frequency of occurrence, where blue column = uninvaded area and light blue column = invaded area; significant differences are indicated with * ($p < 0.05$) and ** ($p < 0.01$); ns = non-significant difference ($p > 0.05$); abbreviations are shown in Table 1.

Sixty-six species of ants were found in the UVA and 49 species in the IVA. Mean ant species richness was significantly higher in the UVA than the IVA (t-test; $p < 0.05$; Fig. 1B). Significant effects of study area, season and ant species (Table 3) were found for ant abundance (A_{ant}). A significant interaction between study areas and ant species was detected for the A_{ant}. The abundance (A_{ant}) of 11 ant species (*C. affinis*, *C. diversa, M. pharaonis, O. denticulata, Nylanderia* sp.2, *Pheidole parva*, *P. hongkongensis*, *P. plagiaria*, *Pseudolasius* sp.1, *Tetramorium lanuginosum, Tetramorium* sp.8) was lower for the IVA than the UVA (Fig. 3A). Conversely, the abundance of two ant species (*M. pharaonis* and *Nylanderia* sp.2) in the UVA was lower than in the IVA. The mean abundance ± SE of ants was also significantly higher during the wet season (455 ± 63) than during the dry season (211±15).

There were significant effects for study area, season and ant species (Table 3) for the frequency of occurrence for ants (F_{ant}). A significant interaction was detected between study area and ant species for F_{ant}. The F_{ant} values for 12 ant species (*C. affinis*, *C. diversa, E. astuta*, *M. pharaonis, O. denticulata, Pachycondyla* sp.3, *Nylanderia* sp.2, *P. parva*, *P. hongkongensis*, *Pheidole* sp.2, *Pseudolasius* sp.1, *T. lanuginosum*) were lower in the UVA than in the IVA (Fig. 3B). Conversely, the F_{ant} values of three ant species (*M. pharaonis, Nylanderia* sp.2*, Pheidole* sp.2) in the UVA were lower than in the IVA. F_{ant} in the dry season (65% ± 11.0) was lower than in the wet season (78% ± 10.0).

Effect on nest abundance and occurrence of native ant species

In total, 4,431 nests belonging to 66 ant species were found (Appendix Table 1). Most nests belonged to *O. denticulata* with 1,796 nests (41% of total nest abundance), followed by the nests of *A. gracilipes* (824 nests; 19%) and *Odontomachus rixosus* (219 nests; 10%). The GLM detected significant effects of nesting type, season and study area (Table 4) on nest abundance (A_{nest}). There was a significant interaction between study area and season for A_{nest}. The mean abundance of nests was 1.5 and 2.0 times higher in the UVA than in the IVA and in the dry season than in the wet season, respectively (Fig. 4A). A_{nest} in the UVA during the wet season was higher than during the dry season, whereas there was no significant difference in A_{nest} in the IVA between seasons. There was a significant interaction between study area and nesting type. The A_{nest} values in the IVA were significantly lower in four nesting types (LL, RL, UG US) than in the UVA (Fig. 5A).

Significant effects for study area and nesting types (Table 4) were detected for the frequency of nest occurrence (F_{nest}), whereas season had no significant effect on F_{nest} (Table 4). There was a significant interaction between study area and season. The mean F_{nest} was 1.7 times higher in the UVA than the IVA during the wet season (Fig. 4B). F_{nest} was also slightly higher in the UVA than the IVA during the dry season, but the difference was not significant. F_{nest} in the UVA during the wet season was 1.4 times higher than during the dry season, but there was no significant difference between the wet and dry seasons in the IVA. There was a significant interaction between study area and nesting type (Table 4). F_{nest} in the IVA was lower in two (RL and US) of the five nesting types than in the UVA (Fig. 5B).

Discussion

For the study areas, the abundance (A_{ar}) of seven GDA groups (centipedes, isopods, spiders, cockroaches, termites, beetles, ants) differed between UVA and IVA. In addition, differences in the frequency of occurrence (F_{ar}) were found for four GDA groups (centipedes, isopods, cockroaches, termites), suggesting that these differences between the two study areas were likely the result of invasion by *A. gracilipes*.

Table 3 Statistical results for various factors on abundance (A_{ant}) and frequency of occurrence (F_{ant}) of ants, using number of ant species, season and study area as fixed factors, where the dataset of *Anoplolepis gracilipes* was excluded from analyses.

Source of variation	Dependent Variable							
	A_{ant} (individuals/m^2)				F_{ant} (%)			
	d.f.n.	d.f.d.	F	p-value	d.f.n.	d.f.d.	F	p-value
Species	80	112	8.1	< 0.001	80	112	4.7	< 0.001
Season	1	112	8.1	0.005	1	112	13.5	< 0.001
Study area	1	112	20.1	< 0.001	1	112	0.6	0.05
Study area×season	1	112	2.1	0.16	1	112	0.6	0.46
Species×season	49	112	0.9	0.61	49	112	0.7	0.94
Species×study area	27	112	3.5	< 0.001	27	112	2.2	0.002
Species×season×study area	13	112	1.2	0.33	13	112	0.7	0.77

d.f.n. = degrees of freedom in numerator; d.f.d. = degrees of freedom in denominator; F = F value based on the ratio of mean squares. The tests were considered significant at $p < 0.05$.

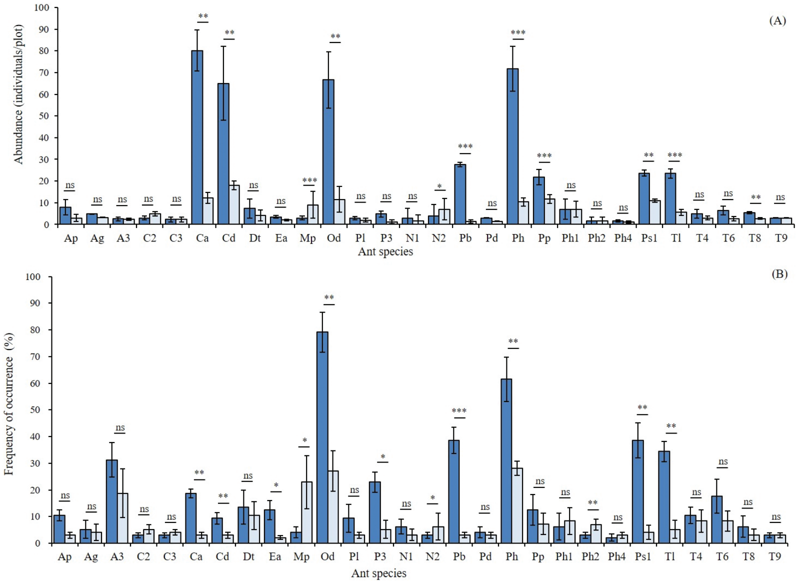

Fig. 3 Means (±SE) of each ant species: (A) abundance; (B) frequency of occurrence, where blue column = uninvaded area and light blue column = invaded area; significant differences are indicated with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$); ns = non-significant difference ($p > 0.05$), Ap = *Aphaenogaster* sp.1, Ag = *Anochetus graeffei*, A3 = *Anochetus* sp.3, C2 = *Crematogaster sp.*2, C3 = *Crematogaster* sp.3, Ca = *Carebara affinis*, Cd = *Carebara diversa*, Dt = *Dolichoderus thoracicus*, Ea = *Ectomomyrmex astutus*, Mp = *Monomorium pharaonis*, Od = Odontoponera denticulata, Pl = *Brachyponera luteipes*, P3 = *Pachycondyla* sp.3, N1 = *Nylanderia* sp.1, N2 = *Nylanderia* sp.2, Pb = *Pheidole parva*, Pd = *Pheidole dugosi*, Ph = *Pheidole hongkongensis*, Pp = *Pheidole plagiaria*, Ph1 = *Pheidole* sp.1, Ph2 = *Pheidole* sp.2, Ph4 = *Pheidole* sp.4, Ps1 = *Pseudolasius* sp. 1, Tl = *Tetramorium lanuginosum*, T4 = *Tetramorium* sp.4, T6 = *Tetramorium* sp.6, T8 = *Tetramorium* sp.8 and T9 = *Tetramorium* sp.9

Table 4 Statistical results for various factors on nest abundance (A_{nest}) and occurrence of native ant species (F_{nest}), using nest sites, seasons and study area as fixed factors, where dataset of *Anoplolepis gracilipes* was excluded from analyses.

Source of variation	Dependent variable							
	A_{nest} (nests/plot)				F_{nest} (%)			
	d.f.n.	d.f.d.	F	*p*-value	d.f.n.	d.f.d.	F	*p*-value
Nest site	4	40	102.2	< 0.001	4	40	64.2	< 0.001
Season	1	40	4.4	0.04	1	40	1.4	0.25
Study area	1	40	27.5	< 0.001	1	40	15.9	< 0.001
Nest site×season	4	40	1.9	0.13	4	40	0.9	0.49
Study area×season	1	40	4.5	0.04	1	40	5.3	0.03
Nest site×study area	4	40	6.2	0.001	4	40	3.9	0.01
Nest site×study area×season	4	40	0.9	0.49	4	40	1.5	0.35

d.f.n. = degrees of freedom in numerator; d.f.d. = degrees of freedom in denominator; F = F value based on the ratio of mean squares. The tests were considered significant at $p < 0.05$.

Appendix Table 1 Number of ant species and nests found in each nest location, with number of *Anoplolepis gracilipes* nests in parentheses

Nest location	Total number of ant species				Total number of ant nests			
	Dry season		Wet season		Dry season		Wet season	
	UVA	IVA	UVA	IVA	UVA	IVA	UVA	IVA
Litter layer	9	7	18	3	89	162(64)	145	44(4)
Rotten log	4	7	25	6	40	71(43)	160	104(74)
Soil	38	39	31	36	784	471(0)	978	572(0)
Termite mound	0	1	3	1	0	186(186)	13	223(223)
Tree trunk hole/crack	4	9	13	14	8	84(57)	29	114(70)
Under substrate	6	1	6	3	20	41(41)	28	51(49)
Total	43	46	48	45	941	1015	1340	1118

UVA = uninvaded area; IVA = invaded area

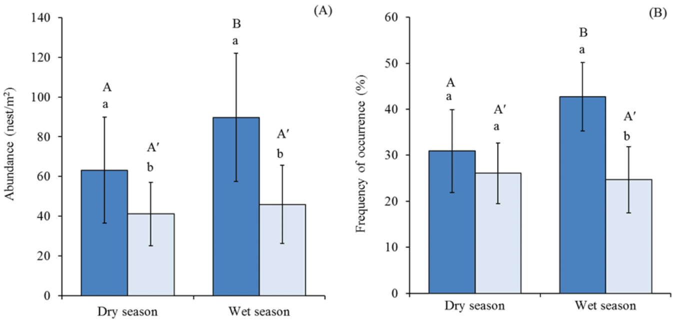

Fig. 4 Means (±SE) of ant nests for each season with dataset of *Anoplolepis gracilipes* excluded: (A) abundance; (B) frequency of occurrence, where blue column = uninvaded area (UVA) and light blue column = invaded area (IVA), different lowercase letters above columns indicate significant ($p < 0.05$) differences between UVA and IVA areas and different uppercase capital letters (A and B for UVA, A′ and B′ for IVA) indicate significant ($p < 0.05$) differences between seasons within each study area.

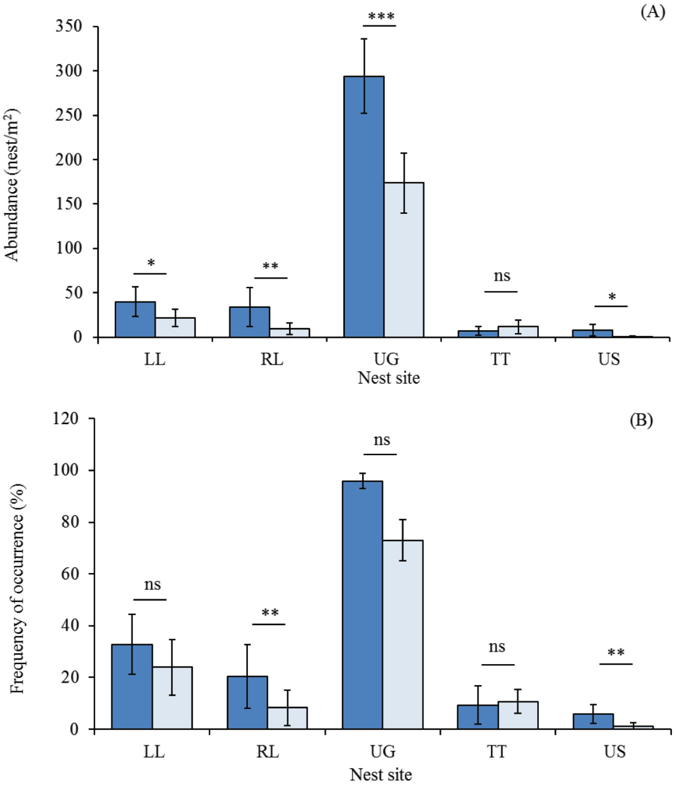

Fig. 5 Means (±SE) of ant nests for each nesting type with dataset of *Anoplolepis gracilipes* excluded and dataset of ant nests in termite mounds excluded: (B) abundance; (A) frequency of occurrence, where blue column = uninvaded area and light blue column = invaded area; significant differences are indicated with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$); ns = non-significant difference ($p > 0.05$); LL= litter layer; RL= rotten log; UG= underground nest; TT = tree trunk hole/crack; US = under substrate

There were significantly higher A_{ant} and F_{ant} values in the UVA than the IVA for *O. denticulata*, *E. astuta*, *Pachycondyla* sp.3, *Pheidole parva*, *P. hongkongensis*, *P. plagiaria*, *C. affinis*, *C. diversa, Pseudolasius* sp.1, *T. lanuginosum* and *Tetramorium* sp.8. These findings suggested that the abundance and occurrence of native ants and GDAs might be reduced because of the occurrence and abundance of *A. gracilipes*, which contrasted with the results of other studies that showed no significant differences between ant groups, wherein the abundance of native GDAs, including scale insects, ant-crickets and cockroaches, increased in *A. gracilipes* invasion areas (Hill et al., 2003; Hoffmann and Saul, 2010).

There are two possible ways in which *A. gracilipes* can negatively impact the richness, abundance and frequency of occurrence for GDAs and native ants: 1) predation (Lowe et al., 2000; Hill et al., 2003; Hoffmann et al., 2014; Kaiser-Bunbury et al., 2014; Stork et al., 2014) and 2) the displacement of other arthropod nests (Cooling et al., 2015). In the invaded areas in the current study, *A. gracilipes* workers frequently carried dead and living bodies of GDAs, including native ants, back to their nests (data not shown), which was concordant with the reports of Lowe et al. (2000), Hill et al. (2003), Hoffmann et al. (2014), Kaiser-Bunbury et al. (2014). and Stork et al. (2014) These other studies showed that *A. gracilipes* was a serious predator of native GDAs, including ants. Cooling et al. (2015) reported that *L. humile* decreased the abundance and occurrence of native millipedes by competing for spaces under rocks and dead wood, which were also suitable millipede nesting spaces. The current study identified that centipedes, isopods, spiders, cockroaches, termites and beetles were absent or less abundant in the IVA, especially in the litter layer, rotten logs and spaces under substrates (rocks or dead wood), where GDAs may prefer to shelter, nest or forage, as was reported also by Traniello and Leuthold (2000) and Basset et al. (2015). In the current study, there were high A_{nest} and F_{nest} values of *A. gracilipes* for abandoned mounds of termites (*Macrotermes* spp.) in the IVA (Appendix Table 1). These results supported suggestions that the nests of *A. gracilipes* can drive rapid change in the nest abundance and occurrence by replacing nests of land bird (Matsui et al., 2009).

Given the lower values of A_{ar} and F_{ar} for GDAs found in the IVA, it is possible that there might be a negative impact of *A. gracilipes* on ecosystem processes in future scenarios, because some of the GDA species that were displaced by *A. gracilipes* play important roles in ecosystem processes, particularly mound-building termites as decomposers (Yamada, 2004) in the current study area. Furthermore, the nesting behaviors of ants and termites maintain the carbon and nutrient cycles in tropical forest soil (Yamada et al., 2006; Hasin et al., 2014; Ohashi et al., 2017). Therefore, it is possible that the rate of decomposition and the cycling of carbon and other nutrients in the soil have been altered in the IVA. This point could be clarified by future research in terms of examining the effects of *A. gracilipes* on the rate of decomposition and the cycling of nutrients in the forest soils before and after the invasion of *A. gracilipes*.

The results in the current study revealed that the presence of *A. gracilipes* may increase the introduction regarding the A_{ant} values of two ant species (*M. pharaonis* and *Nylanderia sp.2*) and the F_{ant} values of three ant species (*M. pharaonis*, *Nylanderia* sp.2, *Pheidole* sp.2). Notably, one of these (*M. pharaonis*) is an invasive ant species in Asia and is a significant pest in households and agricultural areas (Gotzek et al., 2012; Kumar et al., 2015; Centre for Agriculture and Bioscience International, 2016). Finally, the nest abundance of native ants (A_{nest}) in four nest categories (LL, RL, UG, and US) and of nest occurrence (F_{nest}) in two nesting types (RL and US) in the UVA were higher than in the IVA, indicating the possible negative influence of *A. gracilipes* on the A_{nest} and F_{nest} values of native ants. To date, no direct effects have been reported on the A_{nest} and F_{nest} values of native ants by invasive ant species. Thus, the current findings revealed that the negative effects on native ant species may occur due to invasion by *A. gracilipes*. The possibility of negative effects in the IVA can be explained by the lower values of A_{ar} and F_{ar} for native ants found in the IVA compared to the UVA. In addition, nest establishment of native ants was particularly poor in LL, RL, UG and US, where *A. gracilipes* nests saturated the IVA.

In conclusion, the current results revealed that *A. gracilipe*s may have a secondary impact on forest areas in at least two ways: 1) by altering terrestrial ecosystem processes such as decomposition and nutrient cycling in the soil and 2) by facilitating secondary invasion by other invasive ant species. Climate change may cause invasion by *A. gracilipes* in high latitude areas and tropical primary forests (Wetterer, 2005; Bertelsmeier et al., 2015; Jung et al., 2017). Thus, the community composition and biodiversity of these ecosystems will face high risk because they are biodiversity hotspots and specific habitats for many endemic and endangered species (Myers et al., 2000). Therefore, it is necessary to monitor the impact of this invasive species in these areas. Further studies will also be required to prevent biodiversity loss from ant invasion.

Conflict of Interest

The authors declare that there is no conflict of interest.

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