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Context-dependent impacts of multitrophic invasions: Invasive ants potentially mediate predatory impacts on native ant biodiversity

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Abstract

Biological invasions are a primary driver of global change, yet the cumulative impacts of multiple invasive species across different trophic levels remain poorly understood. We investigated the impacts of two globally significant invasive ants, the red imported fire ant (*Solenopsis invicta*) and the yellow crazy ant (*Anoplolepis gracilipes*), and an invasive generalist predator, the greenhouse frog (*Eleutherodactylus planirostris*), on native ant assemblages in Hong Kong. Using field experiments and *in situ* diet analysis via stomach flushing, we tested how competition and predation can shape native ant assemblages. Our results confirmed that the invasive frog avoid both invasive ant species, with a complete avoidance of *A. gracilipes*. Importantly, this predator avoidance can lead to a shift in predation pressure onto native ants,

though the outcomes were context dependent. In *S. invicta*-invaded plots, competitive exclusion reduced native ant richness and altered community composition, which was reflected in the frog's diet as a trend towards fewer ants and more alternative prey. Conversely, in *A. gracilipes*-invaded plots where native ant diversity showed no significant reduction, frogs consumed a greater number of native ants, suggesting that predation pressure from frogs could be a greater threat. Our study demonstrates that the diet of an invasive generalist predator can be affected by the avoidance of invasive prey, and the combined effects of co-occurring invasive species can be modulated by the specific traits and interactions of the invaders. These findings highlight that multitrophic invasions can reshape native assemblages through complex indirect pathways, a critical consideration for predicting the magnitude of biodiversity loss in invaded landscapes.

Keywords: Co-invasion; Competition; Diet electivity; Functional traits; Predation

Introduction

Competition is among the longest-studied ecological interactions (Chase et al. 2002). Theoretical and empirical studies have shown how interspecific competition can structure biological communities (Schoener 1968; Cerdà et al. 2013; Fayle et al. 2015; Wong et al. 2025). Competition can be exacerbated in invaded communities, where initial conditions favour the invasive species' population growth in detriment to the native species (MacDougall and Turkington 2005). Therefore, invaded communities provide “natural experiments” to study community assembly patterns and processes *in situ* (Shea and Chesson 2002) and to examine and distinguish mechanisms that allow a population to establish and grow in a community. Other interaction types that structure communities, such as predation, however, are important modulators of the outcomes of competitive interactions (Chase et al. 2002; Chesson and Kuang 2008). Theoretical work supports a symmetric view of predation and competition as limiting factors to population growth in communities (Chesson and Kuang 2008). Furthermore, close interactions between invasive predator and invasive prey species can facilitate the persistence of both and drive species loss in invaded communities (Móréh et al. 2024; Móréh and Scheuring 2025). Empirical work on communities invaded by multiple predators is still limited. Yet, this scenario is becoming increasingly important as more ecologically distinct species are established outside their native ranges (Seebens et al. 2018).

The dynamics of co-invasions are complex and context-dependent, with outcomes ranging from additive to non-additive interactions (Jackson 2015, Ahmad et al. 2025). These outcomes are shaped by the overlap of invaders' niches and by interactions among invaders with resident species. When niches do not overlap, their combined effect is typically additive, representing the sum of their independent impacts. However, when niches overlap, interactions can become non-

56 additive. A global pattern identified through meta-analysis is a mean antagonistic (non-additive)
57 effect in animal co-invasions, in which the combined impact is less than the sum of the
58 individual impacts (Jackson 2015; Ahmad et al. 2025). This can occur through mechanisms such
59 as competitive or predation suppression between invaders, in which one species reduces the
60 impact of another, or tolerance among native species, in which the most sensitive species are
61 eliminated by the first invader, leaving the community less affected by subsequent invaders
62 (Jackson 2015, Ahmad et al. 2025).

63 In the continuum of co-invasion net effects, the dietary electivity – the choice of prey
64 relative to its environmental availability (Lechowicz 1980) – of an invasive generalist predator
65 can be a decisive factor shaping the dynamics of prey communities (Sih et al. 2010). A
66 preference for invasive prey can reduce the prey's impacts, as the predator acts as a biological
67 control agent; in contrast, preference for native prey can greatly exacerbate the impacts, since
68 these can become additive or synergistic (Ahmad et al. 2025). Ultimately, a diet of invasive prey
69 could lead to greater predator populations, which could generate apparent competition and other
70 effects across the ecosystem as a whole, exacerbating impacts on native species (Holt 1977;
71 Snyder and Evans 2006; Móréh and Scheuring 2025). This is exemplified by an invasive frog in
72 Hawaii, that heavily consumes ants (Olson and Beard 2012, Ferreira et al. 2015), which are all
73 introduced there, sustaining large frog populations that consume native prey. Thus, the net
74 impact of co-invaders is not a simple sum of individual effects but is shaped by invader
75 identities, their traits, and the resulting trophic interactions.

76 Ants are a common dietary source for many vertebrates and invertebrates (Redford 1987;
77 Caldwell 1996; Parmelee 1999; Cushing 2012; Cavalcanti et al. 2024). However, most vertebrate
78 diet studies do not provide complete ant identifications (to genus or species level), thus

hampering the study of the direct impacts of predators on this taxon (see Lee et al. 2017). The study of predation on invasive ants by native predators has been examined previously (Suarez et al. 2000; Glenn and Holway 2008; Robbins et al. 2013), especially for amphibians (Wanger et al. 2011; Konopik et al. 2014; Alvarez-Blanco et al. 2017; Alvarez-Blanco et al. 2021; Warren and Solomon 2022). In contrast, given the best of our knowledge, studies on the dynamics of invasive ant prey and invasive amphibian predators are scarce or non-existent.

Given the numerous possible outcomes in landscapes with co-invasions (Móréh et al. 2024; Ahmad et al. 2025), integrating ant assemblage collection with diet experimentation in natural communities enables us to assess the potential pressures of competition and predation on native ant assemblages. To this end, we developed an experiment using a model system of three widespread invaders in Hong Kong: the Caribbean generalist Greenhouse frog (*Eleutherodactylus planirostris*; Eleutherodactylidae), whose diet consists mainly of ants and other arthropods (Goin 1947; Stewart 1977; Olson and Beard 2012; Ferreira et al. 2015), and two invasive ants, Yellow Crazy Ant (*Anoplolepis gracilipes*, YCA) and Red Imported Fire Ant (*Solenopsis invicta*, RIFA), the latter being known for its aggressive dominance of resources and effects on native communities and the environment (Chan and Guénard, 2020; Wong et al. 2020; Hu et al. 2025). The dietary preference of this predator, in the presence or absence of invasive ants, remains unknown.

The above system enables testing for the potential effects of these three invasive species on the native ant assemblage, and the effect of the two invasive ants on the dietary patterns of the invasive frog, including the taxonomic and functional diversity of native ants in its diet. We describe and quantify the frog's diet under experimental conditions to determine whether the presence of invasive ants alters the frog’s diet and to identify how these multilevel interactions

could affect native ant assemblages. We hypothesise that the frog avoids (low electivity) both invasive ant species. Thus, consequently, we hypothesise that the frog's diet in invaded communities has a higher percentage of native ant species and non-ant taxa in terms of abundance and volume than in uninvaded communities. Furthermore, we predict that this effect on its diet is more pronounced in RIFA plots due to the joint effects of RIFA's stronger competitive exclusion of native ants and the frog's greater avoidance of this invasive ant.

Methodology

Invasive ant species

Anoplolepis gracilipes is an invasive species with significant ecological impacts (Lee and Yang 2022). Its influence on native assemblages and ecosystem functions remains to be tested, as evidence of environmental degradation and nuisance following its arrival and spread accumulates (see Table 2 in Lee & Yang, 2022). *Solenopsis invicta* is known for its significant impacts in North America (Lard et al. 2006). First detected in 2005 in Hong Kong and the Asian continent, its impacts are already known within agroecosystems, buildings, and public health (Chan and Guénard 2020), and it affects the functional diversity of native ant communities and their assembly mechanisms (Wong et al. 2020, 2021; Hu et al. 2025).

Ethical approval

We obtained approval from the Department of Health (DH) of the Hong Kong SAR government and the Ethics Committee of the University of Hong Kong to conduct the pilot (Online Resource 1; Figures S1 and S2) and main experiments described below. The approved reference codes are: (21-328) in DH/HT&A/8/2/3 Pt. 24 [BG] and (22-414) in DH/HT&A/8/2/3 Pt. 42 [FPR] for the

DH. The Ethics Committee protocol 22-141 was approved in April 2023. Details of procedures were duly reported to the authorities annually.

Study sites and environmental variables

Sites were chosen throughout Hong Kong based on two main requirements for the experiment: the presence of one of the two focal invasive ants (*A. gracilipes* and *S. invicta*) and, wherever possible, similar vegetation structure and/or environmental conditions. Two sites were chosen, featuring YCA, which were primarily located in secondary forests with patches of plantations of various exotic tree species. For RIFA, three sites were selected, primarily located in grasslands (both within and surrounding farms) and young secondary forests. None of the sites utilized were co-invaded by the two focal ants. Furthermore, we surveyed the presence of *E. planirostris* at the sites. This was an active visual survey conducted in transects (100 m walks in different areas of the sites) with the help of male call playback done in the early evening, their most active period, and random searches in the leaf litter and under logs and stones (Olson & Beard, 2012). The details of each site are listed in Online Resource 1 (Table S1).

We utilised the patchy distribution of the invasive ants to establish invaded and uninvaded plots at the same site (Figure 1). This facilitated data collection and enabled the randomisation of treatments in space. Each plot consisted of a 4 x 4-meter quadrat, and plots were typically spaced by more than 40 meters to ensure independence. However, due to site constraints, some plots were closer to each other, but never closer than 35 meters. Ant foragers of most species in the region will not go further than 5 m from their nest in search of food (Eguchi et al. 2004, Guénard et al., unpublished information), RIFA itself stays usually on a radius of up to 17 meters from its nest (Tschinkel 2011; Stringer et al. 2011), and YCA up to 32 meters (Hoffmann 2014). To

145 determine that a plot was uninvaded, we surveyed each potential area in a site before the
146 experiment with baits (sausage and honey) left for 30 minutes. The collected diet and the ant
147 sampling (pitfalls and leaf litter) after the experiment were used to confirm the classification of
148 both treatments. We considered a plot uninvaded only if RIFA or YCA were not found in all
149 three sampling sources.

150 The canopy cover of each plot was measured by taking a fisheye photograph of the canopy
151 with a camera held at eye level (~ 1.7 m above ground). Using the package 'hemispheR'
152 (Chianucci and Macek 2023), we extracted diffuse non-interceptance (DIFN) values, a measure
153 of canopy cover. Alongside the experiment, temperature and humidity near the soil surface were
154 measured every 10 minutes using an Elitech RC-51H data logger, as these factors are tightly
155 connected to insect activity (Deutsch et al. 2008).

156 ***Experiment setup***

157 Adult *E. planirostris* (Snout-Vent length > 14 mm) were collected on Lamma Island (around
158 22.224ºN, 114.111ºE) in a location where none of the invasive ants studied were found. Thirty
159 frogs were used in the experiment for each invasive ant species, represented by 15 plots with and
160 15 without their presence, totalling 60 frogs. Before the experiment, the frogs were kept in the
161 laboratory for no more than two days and one night in a moistened aquarium with leaf litter,
162 water *ad libitum*, and not being fed. All experiments were run from June to September,
163 especially after rainfall. After the experiment, the frogs were observed for 24 hours and returned
164 to their collection site. Additionally, to better understand and compare their diet with the
165 experimental one, 40 frogs were collected in the wild and had their diet retrieved. More details
166 on wild data collection are found in the Online Resource 1.

In the field, a metal cage frame (25 x 20 x 10 cm) covered with a bamboo-fibre mesh (mesh size 4 x 3 mm) was positioned in the centre of the plot (a 4 x 4 m quadrat) one hour before the frog introduction, secured in place flush to the ground with the help of tent pegs (Online Resource 1; Figure S3). The experimental cage was gently sprayed with water to reduce dehydration stress on dry days.

At approximately 18:00, one frog was introduced into the cage, along with a small water bowl. Being under field conditions, frogs had access to any arthropods that entered the cage. They were retrieved four hours later, and stomach flushing was conducted to collect the diet ingested. This time was kept to a minimum to limit potential stress on the animals, while still being sufficient for them to feed. Stomach flushing minimises mortality and health problems associated with previous techniques (e.g., dissection or anaesthesia), while ensuring accurate diet data. The procedure is described in detail by Solé *et al*. (2005). To adapt the method to the Greenhouse frog, we used a 20 mm-long, one mm-wide, soft plastic catheter attached to a 5 mL syringe. The procedure was repeated twice to ensure all items were retrieved. Finally, we measured their Snout-Vent Length (SVL) and mouth gap to the nearest 0.5 mm using a calliper. However, these two body measures were strongly correlated ($p = 0.0001$, $r = 0.872$); therefore, only SVL was used in the models.

After the cages were removed, the epigeic fauna was sampled using pitfall traps and a leaf litter sifting (Winkler method). The pitfalls consist of plastic cups with a 4.8 cm diameter, filled with 30 ml of ethylene glycol. Those were placed on the corners and centre of the plot, representing a total of five pitfall traps per plot and left operating for 48 hours. Leaf litter was collected from the four sides of the plot in quadrats measuring 1 m^2. The sifted leaf litter was dried in mini-Winkler bags for 96 hours. At two sites, Choi Yuen and Tsat Sing Kong, Winkler

extractors were not utilised due to the absence of leaf litter. Pitfall traps and Winkler extractors were chosen to properly assess the assemblages of the aboveground and leaf-litter arthropod communities (Lee & Guénard, 2019).

Laboratory procedures

The ants collected were point-mounted and identified to the species or morphospecies level using the Insect Biodiversity and Biogeography Laboratory (IBBL) reference collection and published taxonomic articles on local and regional fauna (e.g., Tang and Guénard 2023). We chose to focus on the impacts to the ant assemblage, which could be identified to the species level; furthermore, a significant percentage of this frog's diet in Hong Kong and elsewhere is composed of ants, indicating that this is a critical taxon to study the effects of multilevel invasions (Olson and Beard 2012; Ferreira et al. 2015; Fan et al. 2026; Ho et al. in prep.).

The same identification procedure was used for the other diet items. If they were dismembered, the heads were counted and matched with body parts, then measured. For all items, volume was estimated using their head or body width (whichever was wider) and body length. For more precision in measuring ants, we measured mounted specimens of the same species, when identified. The formula for volume estimation was $\nu = 4/3\ \pi\ \times l/2 \times (w/2)^2$, where l is the length and w is the width of the prey (Ferreira et al. 2015).

Ant trait measures were taken using a Leica M205C stereo microscope equipped with a Leica DFC450 digital camera and accompanying software (Leica Application Suite V4), to the nearest 0.01 mm. Beyond total length and head width, the width of the pronotum and the eye, and the length of the mandible, scape, leg, and mesosoma (Weber length) were measured. These

traits are linked to the performance of ants in their environment, including their metabolic rates, movement in leaf litter (physical constraints and mobility), and constraints of possible predators related to their size, thus connected with predation probability (Farjí-Brener et al. 2004, Silva and Brandão 2010, Schofield et al. 2016). Five individuals of each species were measured where possible; however, measurements from specimens collected in previous projects were used to complete the sample size for some species that did not reach five. In the case of the polymorphic RIFA, only minors were consumed by frogs, therefore, thirteen minors were measured.

Statistical analyses

All statistical analyses were conducted in R using packages developed for community ecology and mixed-model analyses. All models were fitted using the 'glmmTMB' function (Brooks et al. 2017), pairwise contrasts were generated with 'emmeans', and p-values were adjusted with Tukey's HSD.

To minimise the influence of the environment in our analyses, we separated the datasets for the diet analyses of RIFA and YCA sites. Furthermore, we assessed the environmental heterogeneity between sites and among plots at each site. The variables collected on-site during the experiment (average humidity, average temperature, and canopy cover) were evaluated with PERMANOVA (9,999 permutations). Different sites explained a significant amount of the variance (RIFA sites: $R^2 = 0.54$, $p = 0.0004$; YCA sites: $R^2 = 0.578$, $p = 0.0001$); however, within each site, no variation was found between invaded and uninvaded plots (all $p > 0.11$). To account for environmental variation, we reduced their dimensionality with PCA and included the first component, with an eigenvalue greater than one, in the models, following Wong et al. (2020).

Local and diet ant community

Ant assemblage richness and diversity per site, collected using pitfalls and Winklers, were assessed using Hill numbers based on presence-absence data from plots (incidence-frequency method), for each technique, using the iNEXT package in R (Hsieh et al. 2016). The ant assemblage richness of the plot and the diet were compared among invaded and uninvaded plots using generalised linear models with Poisson (or quasi-Poisson) distributions, depending on the response variable's dispersion. Furthermore, we compared differences in plot and diet ant community composition using PERMANOVA and PERMDISP. All analyses were performed with the invasive ant excluded from the data; thus, testing whether the invasive ant changes native assemblage richness and composition.

Ant impacts in the diet

We summarised the frogs' diet, both for the wild-collected and the experimental frogs, including the number of items, the number and percentage of ant items, the total volume, the total ant volume and its percentage in the diet. We assessed the impact of environmental variables (PCA1), frog body size (SVL), and invasive ant presence on the total number of items, total number of ants (without invasive ants), total volume, and ant volume percentage (without invasive ants), by applying GLMMs with Poisson (log link), beta (logit link), or gamma (log link) families, respectively.

The diet ant community-weighted mean (CWM) of total length and head width was calculated with ant incidence. For each invasive ant species and trait, we tested whether environmental PCA1 and the presence of invasive ants affected the trait's CWM using a GLMM with a Gaussian family and log link. Site and plot were the nested random factors (random

255 intercepts) in the models, which were eventually removed if model simplification improved the
256 fit considerably (AIC > 2).

257 Finally, the electivity of all the ant species found in the experimental diet, including the
258 two invasive ant species, was calculated using Kimmerer and Slaughter's electivity index, an
259 index that allows to estimate the uncertainty around its mean, important for count data and well-
260 aligned with our experimental design (Kimmerer and Slaughter 2021). For this analysis, we only
261 included the ant species that contained at least one individual in the frog's diet and plots that
262 contained enough repetitions of the environmental sampling (RIFA sites ≥ 3 pitfall traps and
263 YCA sites ≥ 7 pitfall traps and Winklers together).

264 Environmental abundance was based on ant incidence captured by pitfall and Winkler
265 samples in each plot. Total environmental incidence ('N', Kimmerer and Slaughter 2021) was
266 the total number of samples done in a plot (i.e., pitfalls and Winklers deployed and recovered),
267 and each species' environmental abundance was their incidence in each plot ('n_i'). Incidence was
268 used for the environmental part of the formula to avoid the problems with abundance data (i.e.,
269 ant abundance can be biased by nest proximity and species-specific sampling effects; Lee and
270 Guénard 2019) and due to distinct sampling effort among plots, due to rain and wild animals
271 interference.

272 Diet abundance was based on the total number of items consumed per frog ('M') and the
273 abundance of each species in the diet ('m_i'). Each pair of species found in the environment and
274 diet, or only in the environment, were counted as one observation; species not found in a plot
275 (environment) were discarded, regardless of being found in the diet of the frog on that plot. We
276 estimated the mean electivity index (X_i) and the credible interval around it for each observation.

A value below 0.5 can indicate weak to strong avoidance, and zero signifies total avoidance or interactions that did not occur. This uncertainty is captured by the Bayesian estimation of the binomial distribution of events occurring during the experiment. Credible intervals that do not cross 0.5 ensure us that an ant species was avoided or preferred in the frog's diet on a given plot.

We also grouped and compared average electivity estimates of native ants according to treatments, but only for those species with enough observations (two or more). The significance of the grouped electivity compared to a neutral preference was analysed with a log-odds ratio plot, in which observations crossing zero are non-significant; furthermore, we computed the posterior distribution of the difference between the two log odds ratios (Δ = log odds ratio invaded – log-odds ratio uninvaded). We considered a 95% credible interval of Δ excluding zero as evidence of difference in electivity between treatments.

Results

Throughout the experiment, nine frogs either did not eat or had contents that could not be identified, leaving 51 frogs (85%) for analysing the impact of multiple invasive species on native ant communities. Of these, 42 frogs (82.4%) had ants among their stomach contents. The summary of both diets is found in Table 1; more details about the wild and experimental diets are found in the Online Resource 1 (Tables S2 to S4).

Site- and plot-level ant community

A total of 149 ant species and morphospecies were collected across the five sites. Interpolation-extrapolation curves show that all sites harbour a high diversity of ants, with curves that did not converge in terms of richness (q = 0). In contrast, the curves converged when rare species were

given less weight (q = 2) (Online Resource 1: Figures S4 and S5). At the plot level, ant richness was significantly lower at RIFA-invaded plots (estimate = -0.36, p = 0.045) but not in YCA-invaded plots (estimate = 0.12, p = 0.283). Between invaded plots, YCA plots were significantly richer than RIFA plots (estimate = 1.079, p < 0.0001), and the pattern was the same for uninvaded plots, although the difference in richness was smaller (estimate = 0.602, p < 0.0001) (Online Resource 1: Figure S6). Regarding invaded and uninvaded plots in RIFA sites, significant differences in ant community composition were found (PERMANOVA: p = 0.022, R^2 = 0.055; PERMDISP: p = 0.77). No difference was found in YCA sites (PERMANOVA: p = 0.33, R^2 = 0.037; PERMDISP: p = 0.97).

Environmental variability and frog's SVL

Limited variation in environmental conditions was observed during the experiment, with temperatures ranging from 25.6 ºC to 29.2 ºC and humidity ranging from 88.5% to 100%. The environmental PCA1 did not significantly affect any of the variables tested in RIFA sites (quantity of items: p = 0.272; total volume: p = 0.168; quantity of ants: p = 0.56; ant volume percentage: p = 0.855), nor did the frog's SVL (quantity of items: p = 0.993; total volume: p = 0.896). For YCA sites, one variable was affected by PCA1, and that was quantity of ants (estimate = 0.353; p = 0.011), the remaining were not affected by it (quantity of items: p = 0.088; total volume: p = 0.114; ant volume percentage: p = 0.246). Frog's SVL also did not affect these variables in YCA sites (quantity of items: p = 0.697; total volume: p = 0.239). Therefore, PCA1 was retained in the models for ant quantity at YCA sites.

***Invasive ant species effect on the diet of* E. planirostris**

On average, the number of items and the total volume ingested were lower in the experimental frogs than in the wild frogs assessed (Table 1). A higher frequency and volume of ants were observed in the experimental diet (+20.2% in quantity and +15.3% in volume). In RIFA sites, no difference in the quantity of items ingested between frogs in invaded and uninvaded plots was observed (estimate = 0.077, p = 0.791), nor in the total volume ingested (estimate = -0.285, p = 0.49). In YCA sites, frogs in uninvaded plots ingested fewer items (estimate = -0.4145, p = 0.044) but of a similar total volume (estimate = -0.049, p = 0.94).

In total, 28 native ant species, plus *S. invicta,* were retrieved from frogs. In total, frogs consumed 15 species in RIFA sites, including *S. invicta*, while in YCA sites, the frogs consumed 17 species of ants, none of which were YCA. Among all ant species, four were found in the diet of frogs at both RIFA- and YCA-sites: *Crematogaster egidyi*, *Nylanderia* sp., *Pheidole pieli*, and *Ph. taipoana*. In RIFA sites, we observed no difference in the quantity of native ants ingested by frogs between invaded and uninvaded plots (estimate = 0.661, p = 0.171) (Figure 2a), nor in the percentage of native ants in the total volume (estimate = 0.429, p = 0.427) (Figure 2c). Ant richness was not different in the diet (estimate = 0.475, p = 0.187), nor was the community composition (PERMANOVA: $R^2 = 0.057$, p = 0.117). In YCA sites, frogs in uninvaded plots ingested fewer ants than in invaded plots (estimate = -0.825, p = 0.014) (Figure 2b); and no difference was observed between invaded and uninvaded plots for ant percentage of the total diet volume (estimate = -0.033, p = 0.952) (Figure 2d), in the richness of the ant community (estimate = -0.151, p = 0.6), and in its composition (PERMANOVA: $R^2 = 0.047$, p = 0.516).

In functional terms, ant CWM in the diet of frogs in invaded and uninvaded plots was not significantly different from each other in RIFA sites (Total length: Uninvaded: estimate = -0.11, p = 0.18; Head width: Uninvaded: estimate = 0.026, p = 0.717), neither in YCA sites (Total

342 length: Uninvaded: estimate = -0.136, p = 0.226; Head width: Uninvaded: estimate = -0.224, p =
343 0.169).

344 The mean electivity of both invasive species was low in the diets analysed, however, in
345 many plots the credible interval crossed the 0.5 line, indicating uncertainty in the electivity signal
346 (Table 2). YCA's mean electivity signal was of avoidance ($X_i < 0.5$), however, only three plots
347 had significant results, and these were the ones with a stronger presence of YCA (LFS8, YYW3,
348 and YYW5). Despite variability in values, most RIFA CI intervals were below 0.5, apart from
349 two plots, one with low diet item count (TSK14) and another with low incidence of RIFA (CY2).

350 Regarding the native ants and their electivity in invaded and uninvaded plots, it was not
351 possible to separate most species into avoided or preferred. In YCA sites, no significant shifts in
352 electivity happened between treatments for any species (Table S5), however, a few ants were
353 significantly avoided in both treatments (Figure 3). *Pheidole nodus* was preferred, though, in
354 invaded plots. In RIFA sites, none of the species had a significant shift in electivity between
355 treatments (Table S5), but *Nylanderia sharpii* and *Pheidole parva* were avoided in both
356 treatments (Figure 4).

357 **Discussion**

358 Multitrophic-level invasions are a complex phenomenon with multiple possible outcomes that
359 can reshape the composition and relative abundance of organisms across ecosystems (Jackson
360 2015; Ahmad et al. 2025). The intensity of these changes depends on the invaders' identities,
361 their interactions with each other, and with the species from the native community. Here, we
362 show that an invasive generalist predator generally avoids the invasive ant species studied and,
363 thus, have distinct diets depending on the local ant community, but this depends on the identity

of the invasive ant species. These potential combined effects, however, are species-specific and could still be unfolding while their distribution and overlap increase, providing chances for learning behavior. The combined impact of these three species, particularly for *E. planirostris* and *S. invicta*, is expected to increase as they spread through China (Khan et al. 2024) and as the frog predator learns to avoid them.

The diet of an invasive predator

The diet of the Greenhouse frog in Hong Kong is very similar to its diet in other regions of the world where it has been introduced (Goin 1947; Stewart 1977; Olson and Beard 2012; Ferreira et al. 2015; Fan et al. 2026). Although characterised as a generalist species, ants accounts for the bulk of the diet in all previously studied regions, including the native range (Goin 1947). The range of ant species found in the frog's diet, from *Carebara obtusidenta* (average Weber length = 0.32 mm) to *Diacamma rugosum* (average Weber length = 3.6 mm), shows that their ant prey is quite distinct in size and defence mechanisms. This wide range in ant prey size also indicates that both invasive species tested here, *S. invicta* (Weber length = 0.73 to 1.65 mm) and *A. gracilipes* (Weber length = 1.54 mm), could, in theory, be ingested by the invasive frog. Other common taxa in its diet, like mites, springtails, beetles, and isopods, were also important parts of the diet and are among the most commonly found arthropods in leaf litter and the top layer of soil. We also noted the importance of spiders in the frogs' diet, with this group being among the most abundant and active invertebrates in the leaf litter during summer (Kai and Corlett 2002). However, apart from ants, none of these taxa were consistently found in relatively high abundance or volume, consistent with the results of Ho and colleagues (submitted to BioInvasions Records).

Larger frogs consumed fewer but larger items. This is a typical pattern in animals (Cruz and Pires 2022; Meyer et al. 2025), aligning with predictions of Optimal Foraging Theory, and has been demonstrated previously for this species (Olson and Beard 2012). Furthermore, the observed average number of prey items in our study is similar to those previously reported in Hawaii and Florida (Goin 1947; Olson and Beard 2012; Ferreira et al. 2015). Although we did not measure their density in the sites, our observations indicate very high abundance of frogs when present. This means that the estimated invertebrate consumption rate of about 130 thousand invertebrates per hectare by Olson and Beard (2012) could apply to Hong Kong as well, with potential consequences for insect communities in these highly invaded areas. Currently, we do not know which species, if any, act as its predators in Hong Kong, nor whether this species competes with and displaces native frogs (Lee et al. 2016).

The impacts of an invasive predator and an invasive competitor

The impacts were different depending on the identity of the invasive ant species considered. Our first hypothesis on the electivity of the invasive ant species by the Greenhouse frog was confirmed, with both species avoided to varying degrees. Interestingly, RIFA was more consistently avoided than YCA, but YCA was not eaten at all. This could be explained due to the lower incidence of YCA in the environmental samples of most plots. Considering that YCA was present and active in their respective invaded treatment plots, especially in high incidence ones, it is highly likely that they interacted with the frog. Thus, we must consider that the YCA has anti-predator traits that safeguard it from active predators in the leaf litter, considering it has not evolved in sympatry with the Greenhouse frog. Most likely, these traits are its walking speed or chemical defence through formic acid spraying. However, as other larger ants were consumed, this suggests that speed is not the main limitation. Other chemically equipped ants avoid

predators by stinging (Warren and Solomon 2022) or by spraying chemical cocktails, which can be distasteful or lethal (Alvarez-Blanco et al. 2021). However, YCA was found in the diet of the same frog in Hawaii (Olson and Beard 2012); therefore, we hypothesise that the reduced availability of preferred ant species in the Hawaiian fauna may force the Greenhouse frog to choose this suboptimal prey.

The avoidance of both invasive ant species potentially shifted predation pressure onto native ants and other non-ant taxa. This aligned with our hypothesis that invaded communities would experience greater consumption of native ants and non-ant taxa. However, the outcome depended on the invasive ant present. In plots invaded by RIFA, frogs exhibited a non-significant trend toward consuming fewer, and a lower volume, of native ants, instead consuming more alternative prey, such as isopods, springtails, and arachnids, and some RIFA individuals. We hypothesize that this pattern likely results from competitive exclusion by RIFA, which reduces native ant abundance (Hu et al. 2025), thereby limiting their availability to frogs, a dynamic like that observed for horned lizards in California (Suarez et al. 2000), but another experiment should be designed to specifically test this. Our hypothesis is, however, reinforced by the fact that the competitive impact is evident at the site level, where RIFA-invaded plots had lower native ant richness and altered community composition compared to uninvaded plots. Consequently, the shift in the frog's preferred ant species between treatments is likely due to the changes in prey availability driven by RIFA. Interestingly, RIFA was still consumed (Table S4), suggesting it is not an unpalatable prey item, despite its painful sting. This further indicates that the frog's diet in RIFA plots reflects the reduced availability of native ants due to competition, rather than just an active avoidance of RIFA.

In YCA-invaded plots, a significantly higher frequency of ants was observed in the frog diet. The forested habitats where we conducted YCA experiments are rich in ant species, and no differences in ant community richness or composition were observed between invaded and uninvaded plots. Recent studies indicate that YCA has marginal impacts on leaf-litter ant species in the region (Hu et al. 2025), but may instead affect arboreal assemblages (Lee and Yang 2022). In our experiment, we observed similar ant species being preferred and avoided by frogs in both YCA-related treatments. We hypothesize that the native ants at YCA-invaded sites are more impacted by the predation pressure of the Greenhouse frog than by the competitive exclusion caused by the YCA.

The fact that most measured variable in the frog's diet did not change between the invaded and uninvaded plots, including the electivity of different native ant species, suggests that the invasive frog has a plastic diet that can adapt to different environments, contributing to its invasion success. When modelling invasion success and effects on native communities, Móréh and Scheuring (2025) showed that, theoretically, lower trophic levels are more likely to succeed in new food webs, and that close predation links with other invasive prey increase that success. Thus, the diet of the Greenhouse frog in Hong Kong, which includes invasive ant species, provides empirical data to support to these theoretical findings. Furthermore, to determine whether this interaction between both dampens their effects on native ant communities, additional experiments are needed. Previous meta-analyses, however, have shown that this is usually the case for animal co-invasions (Jackson 2015; Ahmad et al. 2025), and one reason is that the effect of the most impactful invader masks that of additional ones.

Finally, differences observed between the experimental and wild diets indicate that the experimental design was effective at capturing interactions between the invasive frog and its

prey, as 85% of frogs were retrieved with food in their stomach. Nonetheless, some modifications, such as larger cages and a longer field acclimation period, could be considered to ensure that caged frogs exhibit feeding patterns as close as possible to those of their wild counterparts. Overall, though, the diet diversity of caged frogs shows that this is a natural experiment that can be replicated, and that the results can be explored with a focus on different members of the prey community or for other invasive amphibian species. Nevertheless, limitations in our inference must be recognized as our design might confound invasive species impacts with other environmental impacts, like anthropogenic stressors (Kumschick et al. 2015), even though we controlled for it during site selection and statistical analysis. Thus, further experiments can be designed to disentangle these associations. Another limitation worth mentioning was our sampling survey, which failed to capture some of the species that were later found in the frog's stomach. This was especially important at RIFA sites, with eight mismatches (out of 264 combinations), that we attribute mainly to the impossibility of doing leaf litter sampling (Winkler) (Lee and Guénard, 2019). While YCA sites had four mismatches (out of 340), we consider this error rate to be within a reasonable boundary.

Future directions

This study addresses critical questions about the interactions between invasive animal species at different trophic levels and their potential impacts on native communities. Still, it also opens avenues for many other questions to come. Fortunately, the study system we used is highly amenable to experimentation and can be replicated almost anywhere. Frogs are usually generalist predators that commonly eat ants. They are also highly threatened, can be invasive, and can be easily sampled for diet. Ants, on the other hand, are among the most abundant insects in the world (Schultheiss et al. 2022), and we have a well-developed taxonomy for them and extensive

knowledge of foraging modes and defence strategies. Furthermore, the study of their functional traits is gaining traction, adding a new axis to understanding their interactions (Wong et al. 2019).

From here, we must invest in understanding how the new food webs formed by the addition of introduced species are structured and whether they create new stable states, which is dependent on the introduced species' traits. Functional traits and the trait-matching framework are promising for understanding and predicting these new interactions (Brousseau et al. 2018; Wong et al. 2019; Caron et al. 2022). Studies on ant invasions have used the functional trait framework and coexistence theory to better understand the impacts of single- or multiple-species invasions on native ant communities (Wong et al. 2020, 2021; Hu et al. 2025). To complement our study, it would be important to test the importance of learned feeding behaviour in the predator and the anti-predator repertoire of ant prey; furthermore, test the impacts of the invasive predator through exclusion experiments in areas already affected by it (Kumschick et al. 2015).

In conclusion, the impacts of multiple invasive species on native communities are not simply additive but are instead shaped by species-specific interactions across trophic levels. The invasive Greenhouse Frog avoids consuming two invasive ant species with which it co-occurs, particularly the RIFA. However, the ultimate impact on native ant communities depends on the identity of the invasive ant competitor. In areas invaded by the RIFA, native ants face greater threats from competitive exclusion. In contrast, in areas invaded by the YCA, where competitive impacts on native ant diversity are less pronounced, native ants could be experiencing higher predation pressure from the invasive frog. These findings underscore that the combined effects of co-invaders can reshape native communities through different pathways, where an invasive predator's avoidance of certain invasive prey could exacerbate its impact on native species.

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Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

FPR and BG contributed to the study conception and design. Material preparation, data collection and analysis were performed by FPR, MTH and BG. The first draft of the manuscript was written by FPR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Table 1 Summary of the diet observed during the wild data collection and experiments. The values presented consider only those stomachs that contained food. The percentage of ant abundance and volume is relative to the total. On invaded sites, the percentages of ant abundance and volume without invasive ants are shown in parentheses

	No. Frogs	No. Empty Stomachs	No. of items ± SD	Min. and max no. items	Ant abundance (%)	Total volume (mm^3)	Ant volume (%)
Wild Diet							
Aberdeen	10	1	6.11 ± 2.42	3 – 10	50.91	125.71	13.49
Chuk Ko Wan Tsui	10	0	10.1 ± 4.79	5 – 20	33.66	209.13	10.52
Lamma	10	1	15.33 ± 10.55	6 – 40	42.03	47.09	36.3
Lung Fu Shan	10	0	14 ± 10.15	2 – 32	67.14	226.97	30.35
Total	**40**	**2**	**11.42 ± 8.3**	**2 – 40**	**49.31**	**608.9**	**20.52**
Experimental Diet							
RIFA – Invaded	13	0	6.69 ± 4.03	1 – 14	52.87 (37.93)	101.29	29.6 (22.46)
RIFA – Uninvaded	17	3	7.21 ± 6.5	1 – 25	75.25	82.07	46.11
YCA – Invaded	16	3	7.39 ± 3.73	1 – 13	81.25 (81.25)	76.4	56.91 (56.91)
YCA – Uninvaded	14	3	5.91 ± 4.28	1 – 13	66.15	81.56	13.31
Total	**60**	**9**	**6.84 ± 4.71**	**1 – 25**	**69.63**	**341.31**	**35.79**

Table 2 Estimate of the mean electivity index (X_i) and 95% credible interval of (a) *A. gracilipes* (YCA) and (b) *S. invicta* (RIFA) in the diet of *E. planirostris*. Values smaller than 0.5 can indicate weak to strong avoidance and CI intervals that do not cross 0.5 are significant. The proportion in the environment ($Prop_e$) and proportion in the diet ($Prop_d$) are also given. Plots are arranged in increasing order of X_i

(a) Plot	***A. gracilipes* Mean X_i (CI 95% Range)**	**$Prop_e$**	**$Prop_d$**
LFS8	0.032 (0.001 – 0.137)	0.778 (7/9)	0 (0/12)
YYW3	0.044 (0.002 – 0.188)	0.667 (6/9)	0 (0/13)
YYW5	0.11 (0.003 – 0.438)	0.556 (5/9)	0 (0/6)
YYW6	0.167 (0.011 – 0.539)	0.333 (3/9)	0 (0/9)
LFS10	0.177 (0.011 – 0.623)	0.444 (4/9)	0 (0/5)
YYW4	0.248 (0.013 – 0.712)	0.222 (2/9)	0 (0/9)
YYW2	0.337 (0.026 – 0.839)	0.111 (1/9)	0 (0/8)
YYW15	0.338 (0.027 – 0.849)	0.111 (1/9)	0 (0/9)
YYW1	0.375 (0.025 – 0.891)	0.143 (1/7)	0 (0/5)
YYW9	0.444 (0.035 – 0.908)	0.111 (1/9)	0 (0/4)
(b) Plot	***S. invicta* Mean X_i (CI 95% Range)**	**$Prop_e$**	**$Prop_d$**
TSK4	0.018 (0.0002 – 0.096)	1 (5/5)	0 (0/10)
TSK3	0.041 (0.001 – 0.2)	1 (5/5)	0.125 (1/8)
TSK13	0.049 (0.001 – 0.257)	1 (5/5)	0 (0/3)
TSK2	0.073 (0.003 – 0.327)	1 (5/5)	0.286 (4/14)
CKWT2	0.081 (0.001 – 0.404)	1 (3/3)	0.166 (1/6)
TSK1	0.085 (0.003 – 0.355)	0.8 (4/5)	0.111 (1/9)
TSK14	0.22 (0.007 – 0.763)	1 (5/5)	0.667 (2/3)
CY2	0.281 (0.019 – 0.815)	0.25 (1/4)	0.077 (1/13)

Figure Captions

Fig. 1 Map of sites in Hong Kong where the experiment was performed. (a) In red, RIFA sites and in yellow, YCA sites. (b and c) Within YCA sites, the distribution of invaded (dark yellow) and uninvaded (light yellow) plots is shown. (d, e, and f) Within RIFA sites, the distribution of invaded (dark red) and uninvaded (red) plots is shown. The scale of different sites is not shown because they differ among each other. At sites (b), (e), and (f), the invasive frog was present

Fig. 2 Boxplot of the number of native ant items and percentage of native ant volume among the total diet volume found in the diet of frogs between invaded and uninvaded plots by RIFA (a and c) and YCA (b and d), respectively. Note the difference in scale between plots. Jittered points overlaying the boxplots represent the individual values observed

Fig. 3 The group-level posterior mean of the log-odds ratio of electivity (X_i) with 95% credible interval (CrI) for different native species observed in the diet of frogs, grouped by their observation in invaded and uninvaded RIFA plots. The dashed line at 0 represents the boundary value for significance of the frog's electivity for that species in that treatment; intervals that cross zero are inconclusive regarding the significance of the electivity signal

Fig. 4 The group-level posterior mean of the log-odds ratio of electivity (X_i) with 95% credible interval (CrI) for different native species observed in the diet of frogs, grouped by their observation in invaded and uninvaded YCA plots. The dashed line at 0 represents the boundary value for significance of the frog's electivity for that species in that treatment; intervals that cross zero are inconclusive regarding the significance of the electivity signal

Figure 1

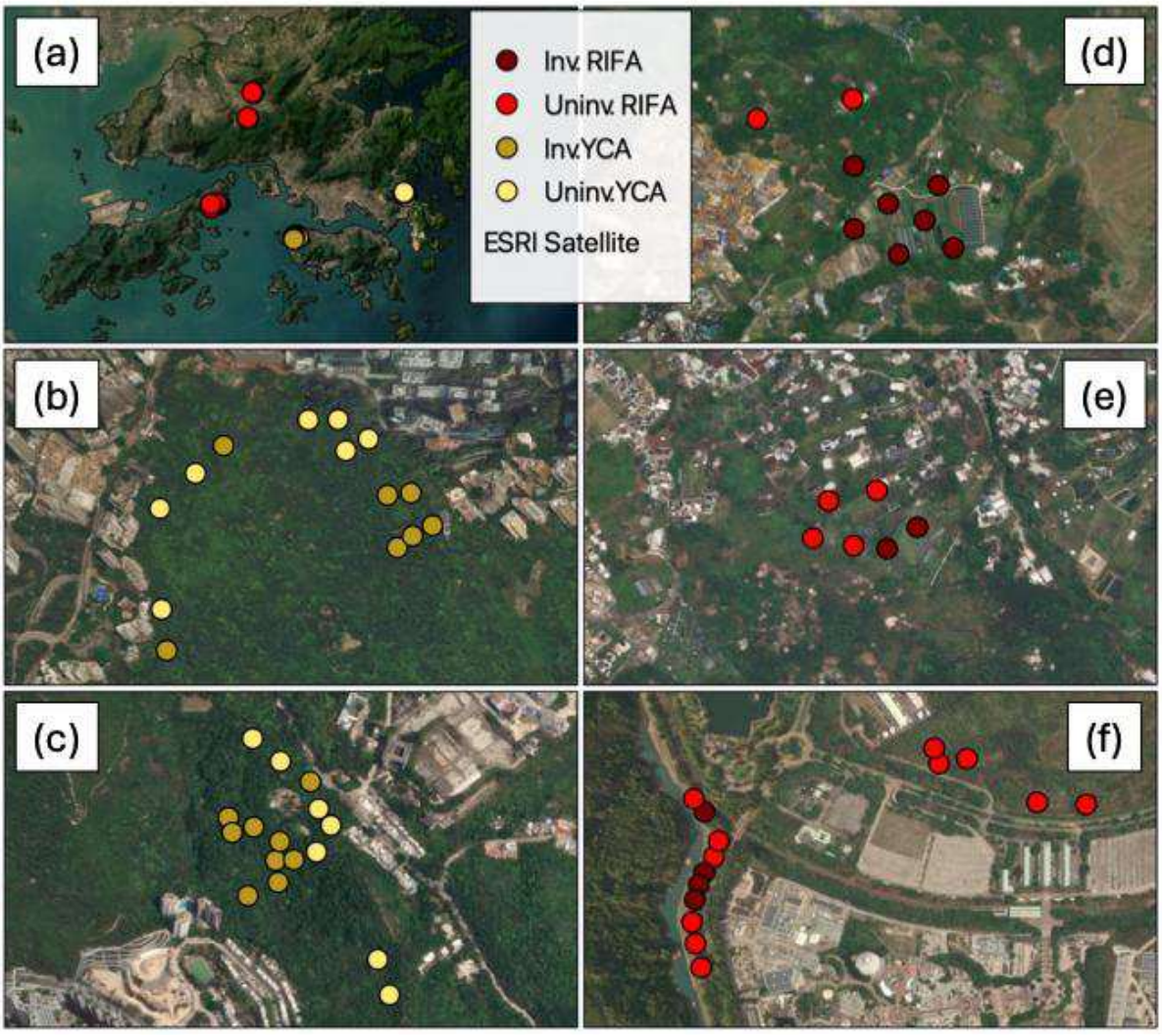


Figure 2

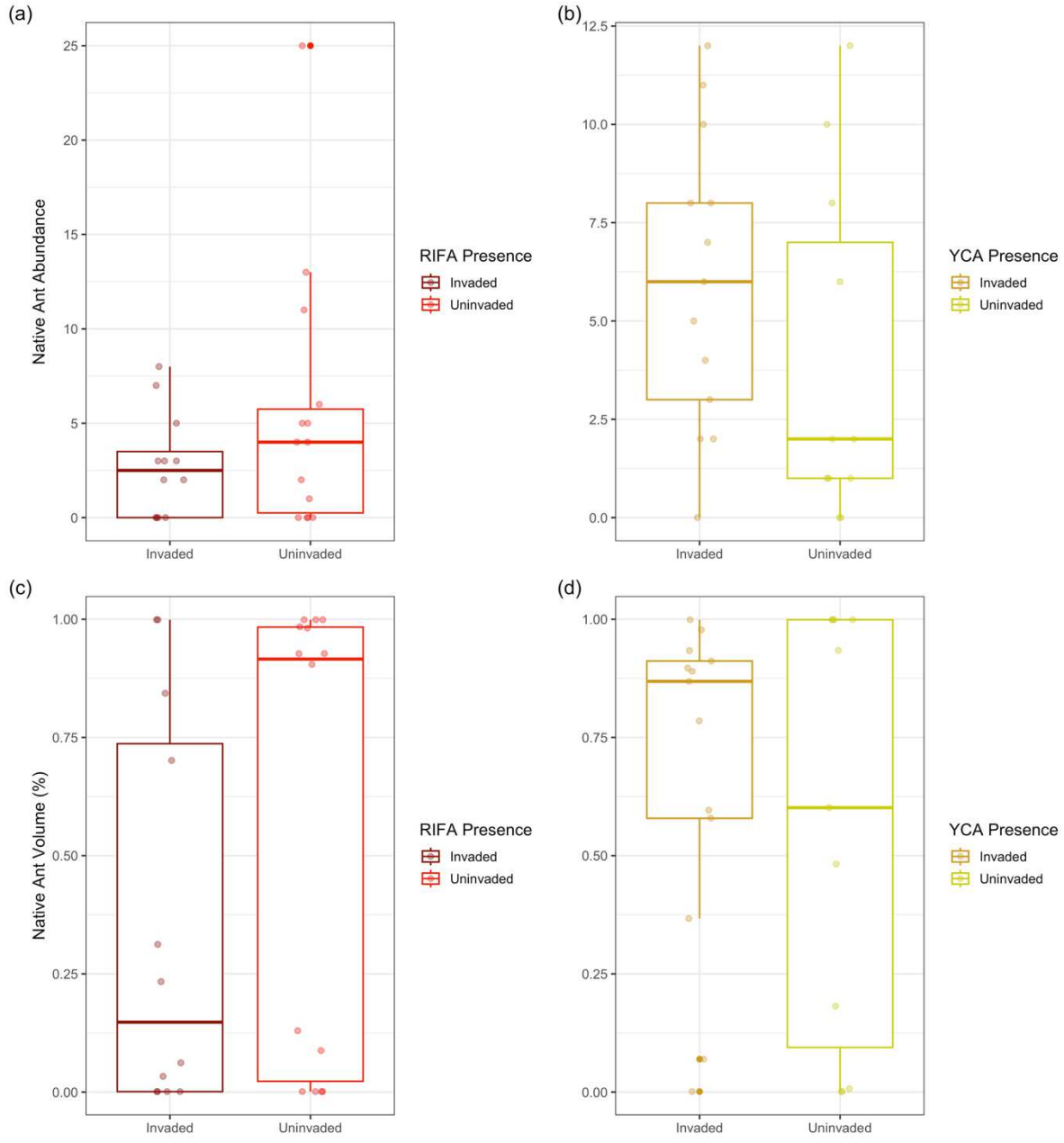
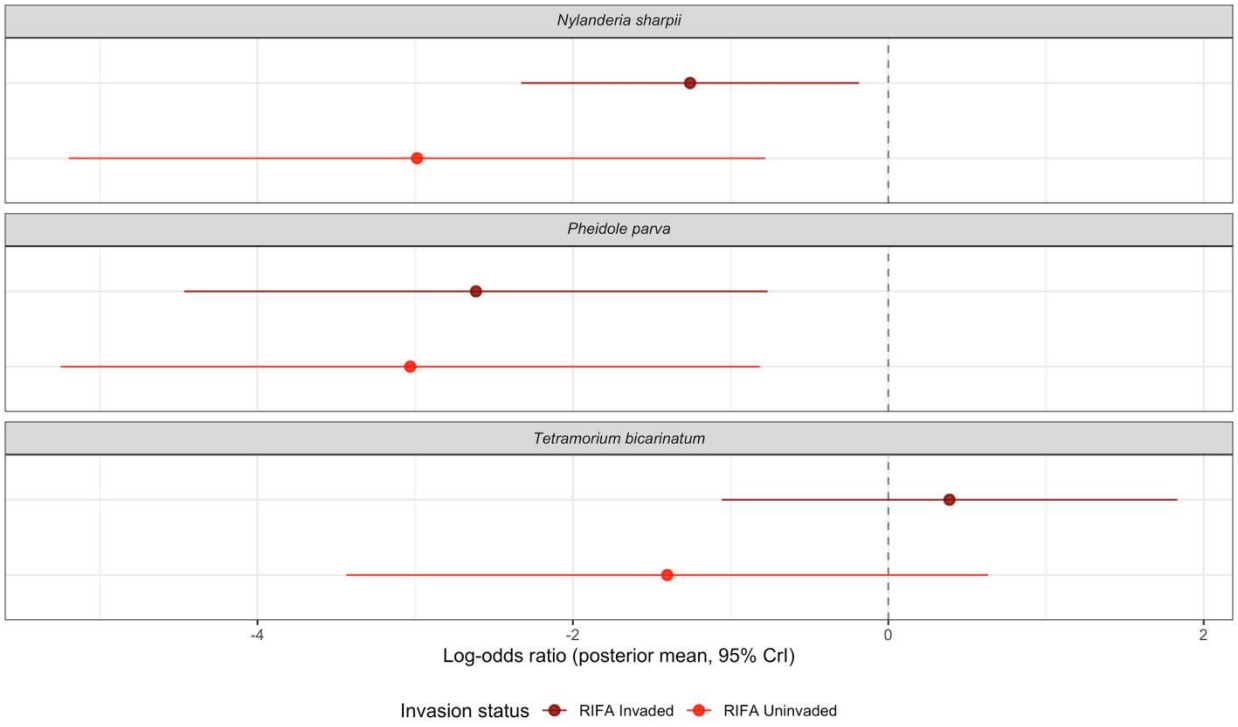

(a)
Native Ant Abundance
25
20
15
10
5
0
Invaded
Uninvaded
RIFA Presence
Invaded
Uninvaded
(b)
12.5
10.0
7.5
5.0
2.5
0.0
Invaded
Uninvaded
YCA Presence
Invaded
Uninvaded
(c)
Native Ant Volume (%)
1.00
0.75
0.50
0.25
0.00
Invaded
Uninvaded
RIFA Presence
Invaded
Uninvaded
(d)
1.00
0.75
0.50
0.25
0.00
Invaded
Uninvaded
YCA Presence
Invaded
Uninvaded

Figure 3


Brachyponera obscurans
Carebara affinis
Mayriella transfuga
Monomorium impexum
Monomorium psw CN01
Nylanderia IBBL 01
Nylanderia picta
Pheidole nodus
Pheidole pieli
Pheidole taipoana
Technomyrmex horni
-6
-4
-2
0
2
Log-odds ratio (posterior mean, 95% CrI)
Invasion status
YCA Invaded
YCA Uninvaded

Figure 4


Nylanderia sharpii
Pheidole parva
Tetramorium bicarinatum
-4
-2
0
2
Log-odds ratio (posterior mean, 95% CrI)
Invasion status
RIFA Invaded
RIFA Uninvaded

Supplementary Files

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- SuppInfo1.docx