Secondary seed dispersal by ants in Neotropical cerrado savanna: species-specific effects on seeds and seedlings of *Siparuna guianensis* (Siparunaceae)

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Abstract. 1. Most woody plant species in tropical habitats are primarily vertebrate-dispersed, but interactions between ants and fallen seeds and fruits are frequent. This study assesses the species-specific services provided by ants to fallen arillate seeds of *Siparuna guianensis*, a primarily bird-dispersed tree in cerrado savanna. The questions of which species interact with fallen seeds, their relative contribution (versus vertebrates) to seed removal, and the potential effects on seedling establishment are investigated.

2. Seeds are removed in similar quantities in caged and control treatments, suggesting that ants are the main dispersers on the ground. Five ant species attended seeds. *Pheidole megacephala* ($\approx$0.4 cm) cooperatively transported seeds, whereas the smaller *Pheidole* sp. removed the seed aril on spot. Large (> 1.0 cm) *Odontomachus chelifer*, *Pachycondyla striata*, and *Ectatomma edentatum* individually carried seeds up to 4 m. Bits of aril are fed to larvae and intact seeds are discarded near the nest entrance.

3. Overall, greater numbers of seedlings were recorded near ant nests than in control plots without nests. This effect, however, was only detected near *P. megacephala* and *P. striata* nests, where soil penetrability was greater compared with controls. Soil nutrients did not differ between paired plots.

4. This study confirms the prevalence of ant–seed interactions in cerrado and shows that ant-derived benefits are species-specific. Ant services range from seed cleaning on the spot to seed displacement promoting non-random spatial seedling recruitment. Although seed dispersal distances by ants are likely to be shorter than those by birds, our study of *S. guianensis* shows that fine-scale ant-induced seed movements may ultimately enhance plant regeneration in cerrado.

Key words. Ant–seed interaction, diplochory, directed seed dispersal, fleshy diaspores, Formicidae, seedling establishment.

Introduction

Nearly 90% of the shrub and tree species in tropical environments have frugivorous vertebrates as their primary seed dispersers (Frankie *et al.*, 1974). Seed dispersal systems, however, can be quite complex and often involve dispersal services by multiple agents from different animal groups (Howe &

Smallwood, 1982). Particularly in tropical habitats, seed dispersal commonly involves sequential movements executed by distinct vectors, in a process known as diplochory or two-phase dispersal (Vander Wall & Longland, 2004). Fleshy fruits and seeds can reach the ground by falling spontaneously below the parent plant, dropped by frugivores during consumption in the tree's canopy or during dispersal, or embedded in frugivores' faeces (Byrne & Levey, 1993; Pizo & Oliveira, 1999). Once on the ground, fleshy diaspores (i.e. the unit of dispersal; van der Pijl, 1982) are commonly attended by a great variety of ant species that are attracted to their nutritious fruit pulp or

seed aril (Pizo & Oliveira, 2000; Passos & Oliveira, 2003). In tropical habitats, where fruitfall is heavy (Jordano, 2000) and ground-dwelling ants are remarkably abundant and diverse (e.g. Kaspari, 1993; Longino *et al.*, 2002), interactions between ants and fallen fleshy diaspores are prominent, and a range of ant-induced benefits to seeds and seedlings have been reported in forests and savannas (Rico-Gray & Oliveira, 2007, Oliveira *et al.*, 2017, and references therein).

The Neotropical cerrado savanna covers nearly 22% of Brazil and its physiognomy ranges from extensive grass-lands with scattered shrubs to forest woodlands with a scanty understorey of herbs and subshrubs (Oliveira-Filho & Ratter, 2002). Although most woody species in cerrado have fruits primarily dispersed by vertebrate frugivores (Gottsberger & Silberbauer-Gottsberger, 1983), interactions between ants and fleshy seeds/fruits are frequent on the ground and may involve around 70 ant species attending fallen diaspores of nearly 40 plant species (Christianini *et al.*, 2012).

Previous studies in cerrado have shown that ant activity at fallen fleshy diaspores can result in directed seed dispersal to nutrient-rich ant nests where seedling recruitment is enhanced (Leal & Oliveira, 1998; Christianini & Oliveira, 2009, 2010, 2013). As opposed to specialised ant–seed dispersal mutual-ism (i.e. myrmecochory; Manzaneda & Rey, 2009), consumption of fleshy, non-myrmecochorous diaspores (i.e. primarily vertebrate-dispersed) requires little specialisation by the ground ant community (but see Pizo & Oliveira, 2001; Christianini *et al.*, 2012). As such, because exploitation of fallen diaspores by ground-foraging ants is rather opportunistic (Pizo & Oliveira, 2000), ant-induced effects on seeds and seedlings can vary with the size of the diaspore relative to the size of the ant, with chem-ical cues of the fleshy portion, as well as with the ants' foraging behaviour and nest characteristics (Oliveira *et al.*, 2017).

Here, we assessed the species-specific dispersal services pro-vided by ants to fallen arillate seeds of *Siparuna guianensis* Aubl. (Siparunaceae), a primarily bird-dispersed tree in the cer-rado savanna of southeastern Brazil. Specifically, we addressed the following questions: (i) which ant species interact with fallen seeds of *S. guianensis*; (ii) what is the relative contribution of ants and vertebrates to seed removal on the cerrado floor; (iii) does the fleshy aril, rather than the seed, act as an ant attrac-tant; (iv) how far are seeds displaced by different ant dispersers; (v) overall, do ant–seed interactions affect the distribution of *S. guianensis* seedlings; and (vi) is there differential seedling estab-lishment among nests of different seed-dispersing ant species?

Materials and methods

Study site

Fieldwork was undertaken in a 470-ha cerrado reserve near Mogi-Guaçu, São Paulo state, southeastern Brazil (22°18′S, 47°11′W). Observations and experiments were carried out in the forest-like 'cerradão', whose physiognomy is composed of 50–90% of trees up to 10–12 m in height (Oliveira-Filho & Ratter, 2002). The climate of the region consists of a dry/cold season (winter) from April to September and a rainy/hot season (summer) from October to March. The annual temperature

ranges from 20.5 to 22.5 °C, and accumulated rainfall varies from 250–300 mm in the winter to 1100–1200 mm in the summer (data from 1961 to 1990 provided by the climatological station at the cerrado reserve).

The plant species

Siparuna guianensis (Siparunaceae) is a tree species (5–15 m tall) distributed from Central to South America, which com-monly occurs throughout the cerrado domain (Renner & Hausner, 2005; Valentini *et al.*, 2010). This monoecious species flowers from August to October and mature fruits occur from December to May (Valentini *et al.*, 2013; Magalhães, 2016). Fruits are globular capsules (*c.* 15 mm diameter) that dehisce to expose two to 12 white fruitlets (diameter 3–4 mm), which look like arillate seeds (Renner & Hausner, 1997), contrasting against the yellow fruiting receptacle (Fig. 1a). The chemi-cal composition of the seed aril, expressed as percentage of dry mass, consists of 28.4% carbohydrate, 13.2% lipid, 8.9% protein, and 12.3% ashes (methods follow AOAC, 2012). At least nine bird species and one marmoset species have been reported visiting fruiting trees of *S. guianensis* and consuming arillate seeds in Neotropical forests and cerrado, thus acting as primary seed dispersers (Simas *et al.*, 2001; Valentini *et al.*, 2010; Gonçalves *et al.*, 2015). Entire fruits and arillate seeds are occasionally dropped by frugivores beneath parent plants or fall spontaneously in great numbers on the cerrado floor (Gonçalves *et al.*, 2015; Magalhães, 2016).

Ant attendance to fallen seeds

Interactions between ants and fallen seeds of *S. guianensis* were recorded throughout the fruiting period, extending from December 2014 to March 2015. During this period we carried out four monthly systematic censuses of ants. In each sampling we distributed seeds at six ground stations (10 m from one another), along eight transects crossing the study area (10 m from one another). One seed of *S. guianensis* was placed per sampling station on white filter paper (4 × 4 cm) to facilitate visualisation on the leaf litter. Filter paper apparently had no effect on ant behaviour (e.g. Pizo & Oliveira, 2000; Passos & Oliveira, 2002). Diaspores were set at 08.00 and 20.00 hours and checked at 15-min intervals over 2 h, allowing us to record ant activity during the day and night. We registered the ant species attracted and their behaviour towards the arillate seeds of *S. guianensis*, including whether they removed the seeds and/or recruited nestmates to exploit them on the spot. Ants carrying seeds were followed until they entered their nests or disappeared in the leaf litter. The distance of seed displacement was then measured. Ant voucher specimens are deposited at the Museu de Zoologia da Universidade Estadual de Campinas (ZUEC, Campinas, Brazil; registration nos 3723 to 3727).

Seed removal by ants versus vertebrates

In order to evaluate the relative contribution of ants and vertebrates to seed removal on the cerrado floor, we performed
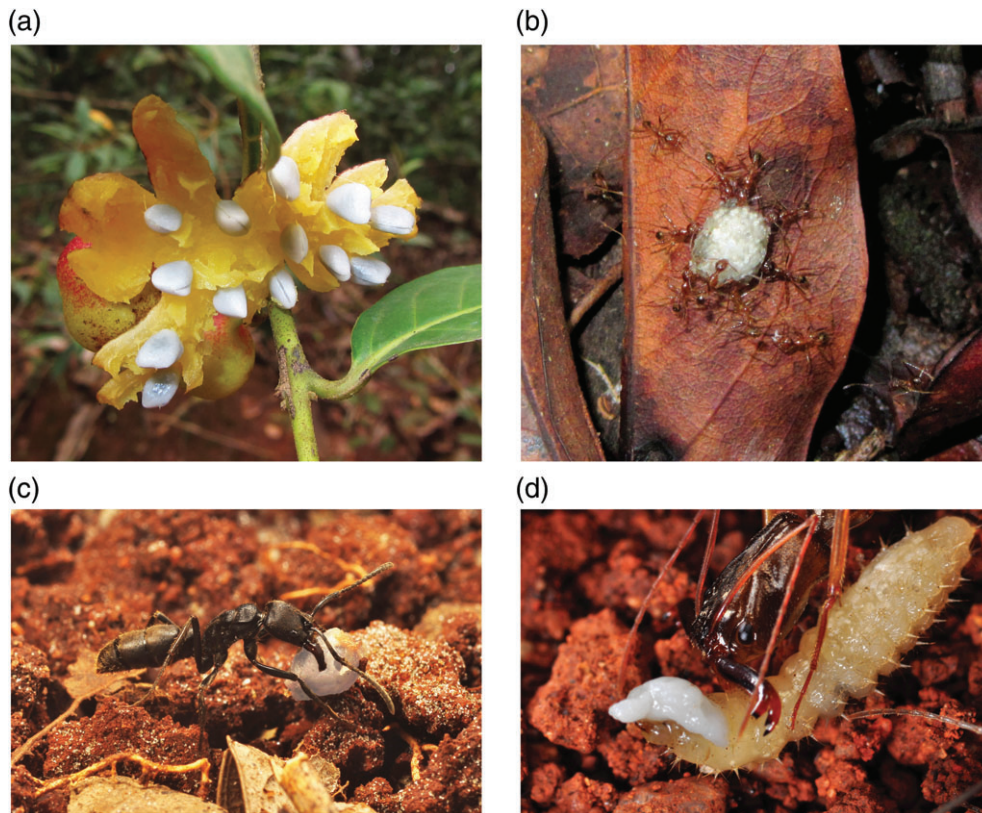
Fig. 1. Dispersal of *Siparuna guianensis* arillate seeds on the floor of a cerrado reserve in Mogi-Guaçu, southeastern Brazil. (a) Open mature fruit exposing seeds coated by a white aril that contrast against the yellow fruiting receptacle. (b) Recruited workers of *Pheidole megacephala* cooperatively displace a fallen seed. (c) *Pachycondyla striata* worker transports a seed to its nest. (c) *Odontomachus chelifer* worker feeds the nutritious aril to a larva inside the nest; cleaned intact seeds are eventually discarded near the nest entrance. Photo credits: (a, b) V.B. Magalhães; (c, d) H. Soares Jr. [Colour figure can be viewed at wileyonlinelibrary.com].

a paired experiment using exclosures during the fruiting period of *S. guianensis*. Vertebrates were excluded from seeds using wire cages (15 × 15 × 10 cm), fenced on top and the sides with mesh (1.5 cm) and staked to the ground (Pizo & Oliveira, 1998). Each experimental pair consisted of a set of 12 seeds on white filter paper (4 × 4 cm) placed directly on the floor under a wire cage (experimental treatment; vertebrates excluded) and an exposed set of seeds placed outside the cage, 15 cm away (open control; ants and vertebrates allowed). The density of seeds in each experimental category mimicked the natural pattern beneath fruiting trees, where several tens of seeds fall naturally or are dropped by vertebrate frugivores (Gonçalves *et al.*, 2015; Magalhães, 2016). Seeds were set out at 06.00 hours, and each experimental pair was placed at a random location beneath one fruiting tree of *S. guianensis* (n = 18 trees > 10 m apart from one another). Seeds used in the experiment were marked with a small dot of enamel paint (Testors, Rockford, Illinois) to differentiate them from naturally fallen seeds found in the same area (the paint had no detectable effect on ant behaviour; see Passos & Oliveira, 2002). After 12 h we recorded the number of seeds removed in each experimental category. A given seed was considered removed if it was not found within 30 cm of its original location.

Ant effects on seedling distribution

To investigate the spatial association between seedlings of *S. guianensis* and nests of seed-dispersing ants, in December 2014 we established paired experimental plots (0.5 × 0.5 m) on ant nests and on random control areas without nests (2.5 m away in a random direction from each nest; Passos & Oliveira, 2002). Twenty paired plots were established for each of the four ant species recognised as potential dispersers of *S. guianensis* seeds (based on samplings described earlier). The number of seedlings growing around and away from ant nests was recorded in March 2015, at the end of the fruiting season. We collected soil samples from paired experimental plots to analyse for chemical properties (total N, macronutrients, micronutrients, pH, organic matter) around and away from ant nests. Given that soil physical properties such as drainage and aeration may be altered around ant nests (Cammeraat & Risch, 2008), we also measured soil penetrability in nests and in adjacent control plots (n = 15 pairs). For each paired plot we released a sharpened wire stake (length 30 cm, diameter 1 cm, weight 190 g) from the inside top of a 1.5-m-high plastic PVC tube. The depth reached by the stake into the ground was the estimate of soil penetrability (Passos & Oliveira, 2004).

Table 1. Interaction between ants and seeds of *Siparuna guianensis* on the floor of a cerrado reserve in Mogi-Guaçu, southeastern Brazil.

Ant subfamily Species (worker length)	Ant behaviour towards seeds	No. of ant−seed interactions (total = 132)	Distance of seed displacement (m) (mean ± SE*)
Ectatomminae			
Ectatomma edentatum ($\approx$ 1.3 cm)	Remove seed individually to nest	19 (14%)	0.71 ± 0.69 (range = 0.20−2.70, n = 19)
Myrmicinae			
Pheidole megacephala ($\approx$ 0.4 cm)	Recruited ants remove seed to nest	30 (23%)	1.03 ± 0.81 (range = 0.10−2.90, n = 30)
Pheidole sp. ($\approx$ 0.2 cm)	Recruited ants feed on fleshy aril on spot	35 (27%)	0 (n = 35)
Ponerinae			
Odontomachus chelifer ($\approx$ 1.8 cm)	Remove seed individually to nest	24 (18%)	0.86 ± 0.73 (range = 0.10−4.00, n = 24)
Pachycondyla striata ($\approx$ 1.6 cm)	Remove seed individually to nest	24 (18%)	0.69 ± 0.46 (range = 0.15−1.60, n = 24)

*Displacement distances do not differ among ant species; one-way ANOVA, $F_{3,93}$ = 1.35, P = 0.26.
Monthly samplings were carried out during the fruiting season, from December 2014 to March 2015. Fresh arillate seeds were distributed on the ground along eight transects, totalling 96 seeds per month (2-h censuses, day and night).

Observations in captive ant colonies

We used captive colonies of seed-dispersing ant species to document food consumption by the ants and the fate of *S. guianensis* seeds inside the nest (Levey & Byrne, 1993; Passos & Oliveira, 2004). The following ant species were reared in captivity: *Odontomachus chelifer* (Latreille), *Pachycondyla striata* Fr. Smith, *Ectatomma edentatum* Roger, and *Pheidole megacephala* (Fabricius). One colony of each ant species was excavated in the study area and brought to the laboratory containing numerous workers (> 100) and plenty of brood (eggs, larvae, and pupae). Colonies were reared at 25 °C in artificial nests consisting of up to five glass test tubes (2 cm diameter × 15 cm) covered by translucid red shelters and containing water trapped behind a cotton plug (Hölldobler & Wilson, 1990). Each ant colony was connected to a foraging arena (30 × 20 cm) where the ants were fed every 2 days with dilute honey, beetle larvae, and artificial diet (Bhatkar & Whitcomb, 1970). During trials, the colonies were offered a few fresh arillate seeds of *S. guianensis*. After the ants discovered the seeds in the foraging arena and transported them into the nest tubes, consumption and treatment of the fleshy aril and seed were documented in intermittent observation sessions of 30–60 min.

Statistical analyses

Distance of seed dispersal by ants and seed removal rates by ants × vertebrates were analysed using one-way ANOVA. Differences in the number of seedlings and in soil properties in nests and in control plots were analysed with Wilcoxon paired signed-rank tests, first with all ant species together and then separately for each one of them. Homogeneity of variances and normality of residuals were checked graphically for all analyses and considered acceptable for the estimation of *P*-values. All analyses were performed using R version 3.2.3 (R Development Core Team, 2015).

Results

Five ant species were recorded interacting with seeds of *S. guianensis* on the cerrado floor, and all but one was able to transport the seeds to the nest (Table 1). Medium-sized *P. megacephala* workers (length $\approx$ 0.4 cm) consistently recruited nestmates to collectively transport the seed (Fig. 1b), whereas the smaller *Pheidole* sp. fed on the fleshy aril on the spot. The large poneromorphs (length > 1.0 cm) *O. chelifer*, *P. striata*, and *E. edentatum* individually carried the seeds up to 4 m inside their nests (Fig. 1c; Table 1).

Overall, the ants transported 74% (97 out of 132) of the seeds they found during the 2-h samplings. No significant difference was found in seed displacement distances among the four ant species observed removing seeds (Table 1; one-way ANOVA, $F_{3,93}$ = 1.35, P = 0.26). The caging experiment revealed that the quantity of seeds removed within 12 h was similar in the exclosure (mean ± SE = 11.16 ± 0.27) and control (11.05 ± 0.22) experimental categories (one-way ANOVA, $F_{1,34}$ = 0.10, P = 0.75), suggesting that ants are primarily responsible for seed removal on the cerrado floor. Because seeds were set at 06.00 hours, and rodents and ants can also be active at night (see Vieira *et al.*, 2010; Christianini *et al.*, 2007), it is possible that removal rates were slightly underestimated.

Observations with captive colonies confirmed that the fleshy aril rather than the seed acted as an ant attractant, and that attendance and transport of *S. guianensis* seeds by ants in the field characterise dispersal rather than granivory. Upon finding the arillate seeds on the foraging arena, workers of the four species typically carried the seeds into the nest and fed bits of the fleshy aril to larvae (Fig. 1d), sometimes also sharing the food with worker nestmates. Cleaned intact seeds were then discarded on refuse piles outside the nest tubes, with no evidence of seed predation by the ants.

By the end of the fruiting season, we found greater numbers of *S. guianensis* seedlings in the vicinity of ant nests than in control plots without nests (Fig. 2; Wilcoxon paired signed-rank test: V = 194.5, P < 0,001). Ant-induced seedling establishment, however, was species-specific and was only detected near the nests of *P. megacephala* (V = 18, P = 0.017) and *P. striata* (V = 21, P = 0.049) (Fig. 3). Higher seedling establishment in nests of *P. megacephala* and *P. striata* cannot be attributed to increased seed removal by these ants, as the four seed-dispersing species displaced seeds in similar proportions (Table 1; multinomial goodness-of-fit test: χ^2 = 2.505,
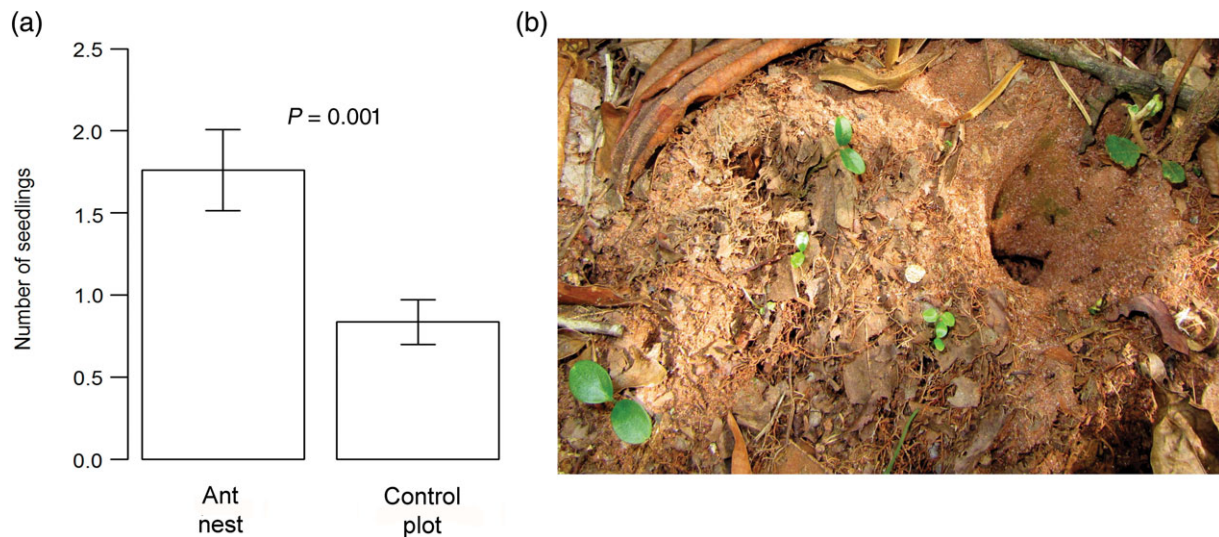
Fig. 2. Patterns of recruitment of *Siparuna guianensis* seedlings in a cerrado reserve in Mogi-Guaçu, southeastern Brazil. (a) Quantity of *S. guianensis* seedlings around ant nests and in control plots without nests. Wilcoxon paired signed-rank test: data are means ± SE; *n* = 80 paired plots (20 paired plots per species: *Pheidole megacephala*, *Pachycondyla striata*, *Odontomachus chelifer*, *Ectatomma edentatum*). (b) Seedlings of *S. guianensis* clumped near the entrance of a *P. megacephala* nest (photo by V.B. Magalhães). See also Fig. 3 for species-specific effects from different seed-dispersing ants. [Colour figure can be viewed at wileyonlinelibrary.com].

$P = 0.474$, d.f. = 3). For all ant species pooled together, soil penetrability was greater in nests (53.09 ± 1.46) than in control plots (45.09 ± 1.03) ($V = 585.5$, $P < 0,001$). If considered separately, soil penetrability was greater in nests of *P. megacephala* ($V = 42$, $P = 0.034$), *P. striata* (V = 26.5, $P = 0.003$), and *O. chelifer* ($V = 24$, $P = 0.001$) than in control plots without nests (Fig. 4). Chemical analyses of soil samples from ant nests and paired control plots did not differ for total *N*, macronutrients, micronutrients, pH, or organic matter (Table 2).

Discussion

Given that the quantity of *S. guianensis* seeds removed did not differ between the exclosure and control categories in the caging experiment, and no other invertebrate was seen attending the seeds, ground-dwelling ants can be confirmed as the main secondary seed dispersers of *S. guianensis* on the cerrado floor. Indeed, although the vast majority of plants in cerrado savanna are primarily dispersed by frugivorous vertebrates (Gottsberger & Silberbauer-Gottsberger, 1983), in recent decades several studies have revealed the prominence of the interactions between ants and fallen fleshy diaspores in this ecosystem, demonstrating ant-induced benefits to seeds and seedlings (Oliveira *et al.*, 2017, and references therein). Although the number of ant species seen interacting with fallen seeds of *S. guianensis* is smaller than that recorded for other cerrado plant species (Leal & Oliveira, 1998; Christianini *et al.*, 2007, 2012), our field observations confirmed that directed seed dispersal to nests is generally performed by a subset of more efficient, seed-carrying species of the ground-dwelling ant community (Passos & Oliveira, 2002, 2004; Christianini & Oliveira, 2010; Warren II & Giladi, 2014; Leal *et al.*, 2017). As reported for

other non-myrmecochorous diaspores, the behaviour towards fallen seeds of *S. guianensis* differed among ant species in accordance with their size relative to the diaspore. While small ants usually consume the fleshy portion on the spot, medium- to large-sized species are able to remove (individually or in groups of recruited workers) the diaspore to their nests, thus acting as effective secondary seed dispersers. Our data in the cerrado show that large *Odontomachus*, *Pachycondyla* and *Ectatomma* ants (size > 1.0 cm) transport *S. guianensis* seeds to their nests at considerable distances (for similar results, see Passos & Oliveira, 2002; Christianini & Oliveira, 2010). *Pheidole megacephala* (≈ 0.4 cm) recruits nestmates to displace seeds up to a distance of nearly 3 m away on cerrado soil (Table 1), corroborating other studies carried out in tropical forests (Levey & Byrne, 1993; Pizo, 2008; Bieber *et al.*, 2014) and cerrado (Christianini & Oliveira, 2010). This variation in foraging behaviour towards *S. guianensis* diaspores among ant species may result in different outcomes for seeds and seedlings (reviewed by Oliveira *et al.*, 2017; see below).

By displacing diaspores from beneath parent trees, ant dispersers can benefit the seeds by removing them from a predation-prone zone where seed and seedling mortality is high (Heithaus, 1981; Pizo, 1997). If the final seed fate is not an ant nest, seeds of *S. guianensis* can still benefit from ant-induced removal if they are dropped at variable distances from the parent plant, in more favourable microsites for seedling recruitment (see below). Therefore, ant-induced seed movements not only may rearrange the seed rain generated by primary seed dispersers (Roberts & Heithaus, 1986; Kaspari, 1993; Pizo & Oliveira, 1999), but could also potentially decrease seed predation and seedling competition under parent trees (Howe & Smallwood, 1982). Even if *S. guianensis* fallen seeds are not carried away by ants, continual removal of the fleshy seed aril
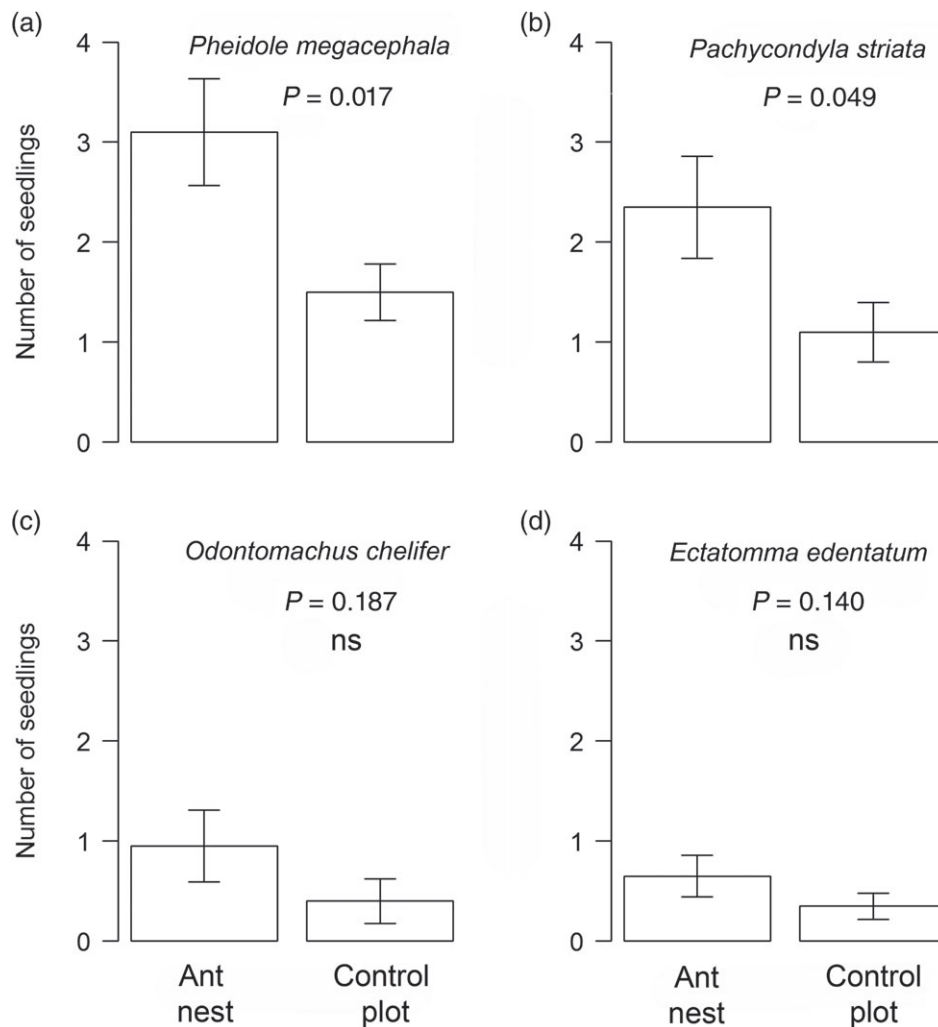
Fig. 3. Species-specific effects by seed-dispersing ants on recruitment of *Siparuna guianensis* seedlings in a cerrado reserve in Mogi-Guaçu, southeastern Brazil. (a, b) Increased numbers of seedlings are found in the vicinity of *Pheidole megacephala* (a) and *Pachycondyla striata* nests (b) compared with control plots without nests. (c, d) No significant effect on seedling recruitment is detected near *Odontomachus chelifer* (c) and *Ectatomma edentatum* nests (d). Wilcoxon paired signed-rank tests: data are means ± SE; *n* = 20 paired plots for each ant species.

('seed cleaning') on the spot by tiny ants such as *Pheidole* sp. 1 can be beneficial by reducing fungal attack to seeds on the humid leaf litter and facilitating germination (Oliveira *et al.*, 1995; Leal & Oliveira, 1998; Ohkawara & Akino, 2005).

Our observations using captive colonies confirmed that the four ant species seen in the field displacing diaspores of *S. guianensis* do not destroy the seeds inside their nests. Instead, the fleshy aril is fed to larval and adult nestmates before the intact seed is discarded around the nest entrance. Consumption of nutrient-rich aril by dispersing ants has also been recorded in other unspecialised ant–diaspore systems and has recently been shown to enhance larval growth (Pizo & Oliveira, 1998; Bottcher & Oliveira, 2014), as also recorded for ants consuming lipid-rich elaiosomes of true myrmecochores (Gammans *et al.*, 2005). In tropical forests, non-myrmecochorous diaspores comprise a wide range of sizes, and the chemical composition of the fleshy portion is also highly variable (Pizo & Oliveira,

2000; Galetti *et al.*, 2011). The lipid and protein contents of the seed aril or fruit pulp are known to be important determinants of ant attendance to fleshy diaspores on the ground of tropical forests, representing essential food sources for ant colonies (Horvitz & Beattie, 1980; Pizo & Oliveira, 2001; Passos & Oliveira, 2004). Lipids are major attractants of ants to fleshy diaspores and are regarded as important behavioural releasers inducing seed-carrying by ant dispersers (Beattie, 1985; Skidmore & Heithaus, 1988). Indeed, lipid- and protein-rich diaspores have been shown to be particularly attractive to large, primarily carnivorous poneromorph ants that use the nutritious fleshy portion to feed larval nestmates (Horvitz, 1981; Passos & Oliveira, 2002, 2004). In cerrado savanna, ground-dwelling ponerine ants have been shown to actively disperse fallen, lipid-rich arillate seeds of *Xylopia aromatica* (Annonaceae), a primarily bird-dispersed tree (Christianini & Oliveira, 2010; Oliveira *et al.*, 2017). Carbohydrate, lipids, and protein are the
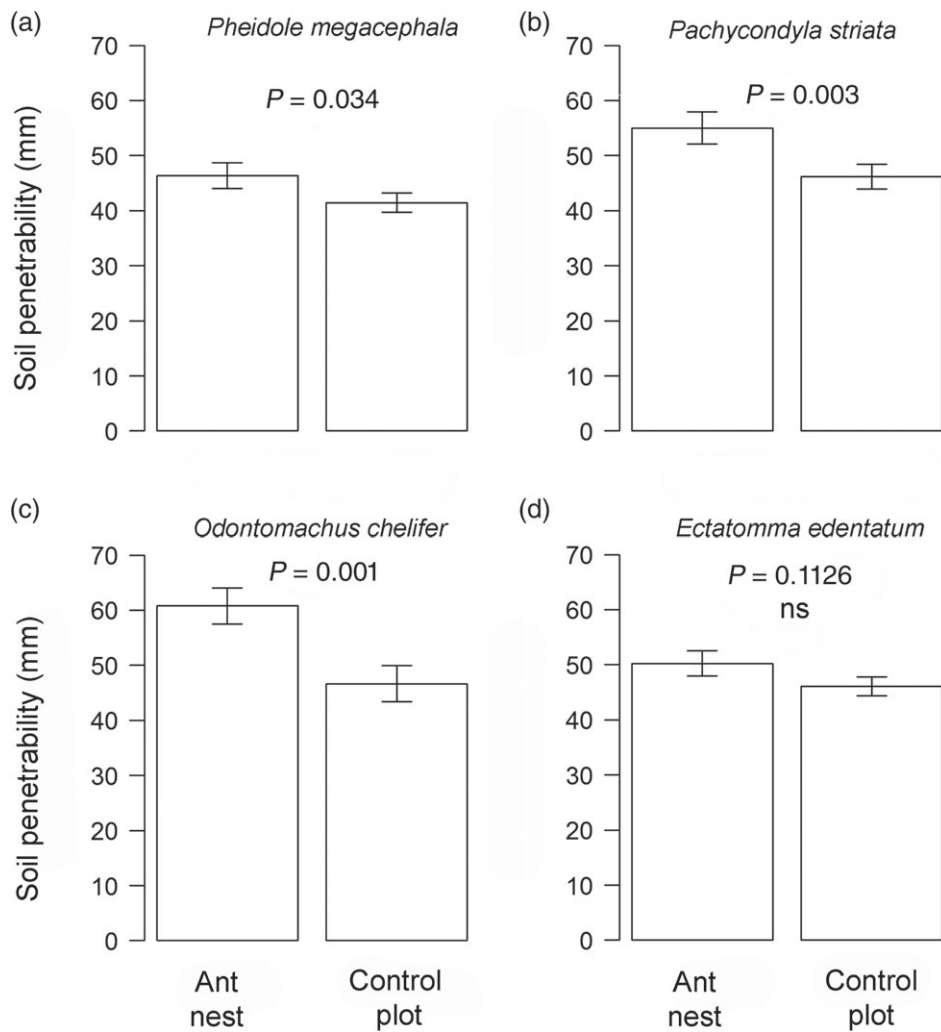
Fig. 4. Comparison of soil penetrability from ant nests of different seed-dispersing ants and random plots without nests, in a cerrado reserve in Mogi-Guaçu, southeastern Brazil. (a–c) Soil penetrability was significantly greater in nests of *Pheidole megacephala* (a), *Pachycondyla striata* (b) and *Odontomachus chelifer* (c), but not of *Ectatomma edentatum* (d). Wilcoxon paired signed-rank tests: data are means ± SE; *n* = 15 paired plots for each ant species.

major constituents in the seed aril of *S. guianensis* (28%, 13% and 9% of dry mass, respectively), suggesting their possible relevance for the diet of ground-dwelling ants in cerrado and their potential role as ant attractants to induce diaspore displacement (see also Pizo & Oliveira, 2000; Passos & Oliveira, 2003; Christianini *et al.*, 2007). Further experiments with captive colonies are needed to examine in greater detail if the consumption of *S. guianensis* arillate seeds can positively affect ant development (see Gammans *et al.*, 2005; Bottcher & Oliveira, 2014).

Our results using paired experimental plots revealed that overall ant activity at *S. guianensis* seeds ultimately affect seedling distribution, but also showed that this effect differs among ant species. *Pheidole megacephala* and *P. striata* acted as direct seed dispersers of *S. guianensis*, causing increased numbers of seedlings near their nests compared with control plots. Indeed, large ponerines are recognised as high-quality seed dispersers of fallen fleshy diaspores, enhancing seedling

establishment in tropical habitats (Oliveira *et al.*, 2017; Leal *et al.*, 2017, and references therein). Although *Pheidole* ants are commonly reported seed predators, their role in direct seed dispersal and seedling recruitment has also been documented in tropical forests (e.g. Levey & Byrne, 1993; Pizo, 2008; Gallegos *et al.*, 2014). Surprisingly, seed displacement by *O. chelifer* and *E. edentatum* ants had no significant effect on the distribution of *S. guianensis* seedlings, contrasting with other studies showing that seed-dispersing ants in these two genera can promote seedling recruitment in several plant species in different ecosystems, including cerrado (Horvitz, 1981; Passos & Oliveira, 2002, 2004; Christianini & Oliveira, 2010; Renard *et al.*, 2010; Leal *et al.*, 2017).

Seedlings are known to grow better around ant nests because these microsites are usually richer in nutrients than background soils (e.g. Andersen, 1988; Passos & Oliveira, 2002; Farji-Brener & Ghermandi, 2008; Farji-Brener & Werenkraut,

Table 2. Comparison of chemical soil variables from nests of seed-dispersing ants with those of random control plots, in a cerrado reserve in Mogi-Guaçu, southeastern Brazil.

Soil variable	*Pheidole megacephala*	Control	P	*Pachycondyla striata*	Control	P	*Odontomachus chelifer*	Control	P	*Ectatomma edentatum*	Control	P
Total nitrogen	1.19	1.15	0.477	1.34	1.22	0.136	1.25	1.20	0.615	1.2	1.3	0.248
Phosphorus	10.93	11.13	0.504	11.66	11.4	0.506	10.66	10.33	0.423	10.46	10.73	0.570
Potassium	0.96	0.94	0.700	1.11	0.99	0.069	1.06	1.09	0.666	1.04	1.08	0.394
Zinc	0.34	0.33	0.906	0.37	0.42	0.326	0.36	0.32	0.779	0.34	0.32	0.441
Iron	244.6	252.4	0.532	283.6	284.5	0.776	243.4	252.93	0.232	242.4	239.1	0.820
Manganese	2.06	2.24	0.733	2.09	2.02	0.363	2.09	2.21	0.932	1.74	1.77	0.900
Cooper	0.52	0.57	0.061	0.58	0.57	0.313	0.56	0.55	0.575	0.54	0.52	0.423
Calcium	8.13	3.26	0.132	4.13	2.86	0.272	2.60	3.46	0.480	3.66	3.2	0.593
Magnesium	1.26	1.13	0.463	1.26	1.26	1.000	1.06	1.33	0.108	1	0.93	0.592
Boron	0.33	0.32	0.706	0.45	0.42	0.451	0.47	0.49	0.550	0.39	0.38	0.670
Organic matter	30.53	32.06	0.362	30.06	35.06	0.570	34.86	34.06	0.924	33.46	32.93	0.690
H + Al	95.4	106.3	0.232	125.2	120.9	0.649	128.93	125.93	0.887	123.5	124.4	0.798
pH	3.52	3.54	0.798	3.48	3.46	0.600	3.56	3.56	0.844	3.45	3.46	0.888

Mean values of K, Ca, Mg, Na, Al, H + Al are given in mmol dm^{-3}; P, B, Cu, Fe, Mn, and Zn are given in mg dm^{-3}; organic matter is given in g dm^{-3}; and N is given in g kg^{-1}. *P*-values refer to Wilcoxon's paired-sample sign-rank tests; *n* = 15 paired samples for each ant species.

2017). Our chemical analyses, however, revealed that nutrients did not differ between paired soil samples of ant nests and random plots in forest-like 'cerradão', indicating that the nutrient-enriched microsite hypothesis may not always apply (see also Rice & Westoby, 1986; Bond & Stock, 1989). In fact, patterns of soil fertility may vary widely among adjacent cerrado physiognomies (Bueno *et al.*, 2018), and it is thus possible that ant nests in cerradão may not represent nutrient islands against background soils. On the other hand, physical tests showed that soils in the vicinity of *P. megacephala* and *P. striata* nests had greater penetrability than random soil samples. Given that seed-dispersing ants presented similar seed removal rates and seed displacement distances, improved performance of *S. guianensis* seedlings near nests of *P. megacephala* and *P. striata* might have resulted from decreased soil compactness at these microsites (see Passos & Oliveira, 2004; Giladi, 2006). Similar paired experiments on seedling performance in cerrado and sandy Atlantic forest indicate that the quality of ant nest microsites may differ with the plant and ant species involved, and that ant-derived beneficial effects may vary locally and across habitats (Passos & Oliveira, 2002, 2003; Christianini & Oliveira, 2013; Gallegos *et al.*, 2014; Bottcher *et al.*, 2016). Indeed, a number of abiotic and biotic factors could mediate seedling establishment in the vicinity of ant nests (see Horvitz & Schemske, 1986; Leal *et al.*, 2017; Leroy *et al.*, 2017). The observation of *P. megacephala* benefiting plant regeneration is surprising, given its invasive status and well-documented role at displacing native ants and disrupting ant–plant mutualisms worldwide (e.g. Blüthgen *et al.*, 2017; Lach, 2017; Ness & Holway, 2017, and references therein). Although aggressiveness by recruited *P. megacephala* might have prevented other native cerrado ants from exploiting fallen *S. guianensis* seeds, our results support Heil and González-Teuber's (2017) suggestion that some ant–plant interactions may suffer little from invasion by novel species and can even be re-established under the new ecological conditions.

In conclusion, the current study confirms the prevalence of interactions between ants and fallen fleshy diaspores in cerrado, and enhances the potential benefits from ants to seeds and seedlings of *S. guianensis*. Our results show that ant-derived benefits are species-specific, with ant services ranging from mere seed cleaning on the spot to seed displacement promoting non-random spatial recruitment of seedlings. Although seed dispersal distances by ground-dwelling ants are probably shorter than those by birds in cerrado savanna (see Christianini & Oliveira, 2009, 2010), our study with *S. guianensis* shows that fine-scale ant-induced seed movements could ultimately enhance plant regeneration at early developmental stages.

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