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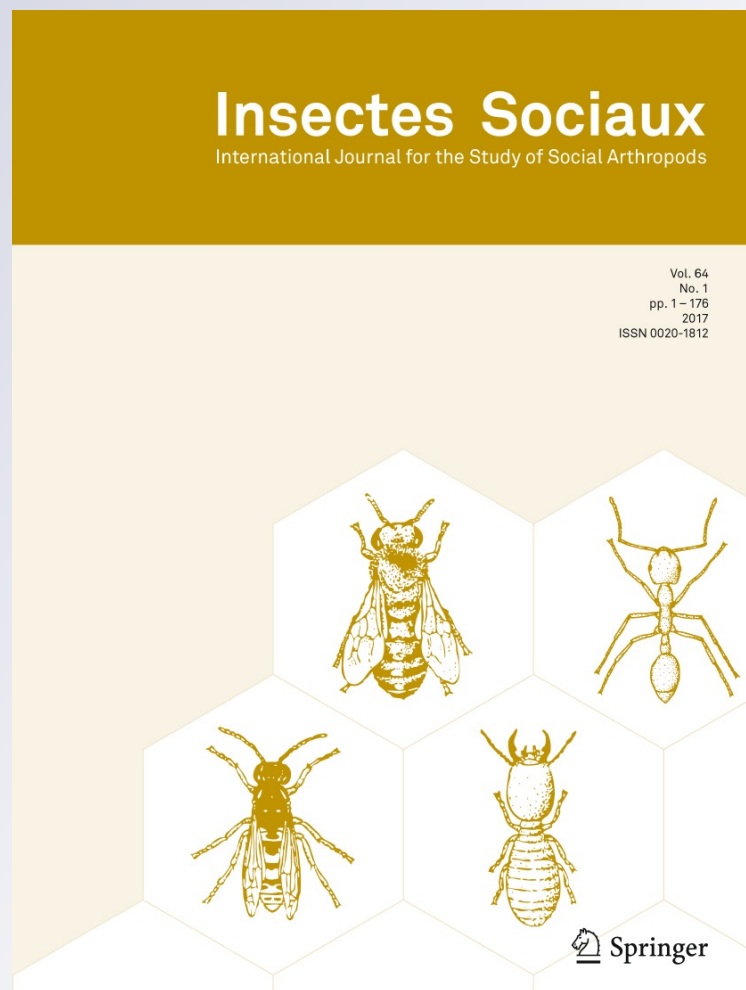
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# Consequences of leaf-cutting ants on plant fitness: integrating negative effects of herbivory and positive effects from soil improvement

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**Abstract** A better understanding of plant–herbivore relationships should integrate negative and positive effects of consumers on plant fitness. We studied the effect of a major insect herbivore (leaf-cutting ants, LCA) on plant fitness in several species of Monte Desert, assessing both the direct negative effect of ant defoliation and the indirect positive role of ants as soil improvers. To estimate the negative and positive effects on plants, we sampled 7000 plant fragments carried by the ants and analyzed the nutrient contents of refuse dumps in 15 ant nests. We estimated plant fitness measuring ~10,000 flowers and ~3600 fruits of 122 individuals from 7 of the most common plant species within the ant foraging area. First, we compared the fitness between plants growing on the nutrient-rich refuse dumps and co-specifics on adjacent non-nest soils. Second, using hierarchical confirmatory path analysis model, we analyzed how herbivory and nutrient content of refuse dumps affected plant fitness. We found that the fitness of plants growing on refuse dumps (a) was similar than those growing on adjacent non-nest soils, and (b) was unaffected by ant harvesting or by increments in nutrient content in a consistent way. In this dry habitat, the effect of LCA on plant fitness was complex and species-dependent. To explain this pattern, we discuss

the role of plant compensation to foliar damage, the limited plant response to soil nutrients due to water stress and the potential high abundance plant consumers on ant refuse dumps. Our results illustrate the complexity of herbivore–plant interactions emphasizing the need of measuring direct and indirect effects of herbivory in field conditions to validate greenhouse experiments.

**Keywords** *Acromyrmex lobicornis* · Ant foraging · Indirect effects · Monte desert · Nest effects · Patagonia · Plant fitness · Structural equation models

## Introduction

Animals interact with plants in multiple ways, challenging the traditional and simple classification of species as mutualistic or antagonistic. The same animal species may benefit or damage its plant partner species through different mechanisms and/or according to the ecological context. For example, *Manduca sexta* is a sphingid moth that pollinates *Datura stramonium*, but whose larvae feed on their leaf tissues (Adler and Bronstein 2004). Many animal species can act as seed dispersers or seed predators of the same plant species under different ecological circumstances (Retana et al. 2004). This complexity is relatively well known in herbivore–plant interactions, where plants may benefit from herbivory by increasing plant fitness or inducing anti-herbivore defenses against more detrimental herbivores just after defoliation (Agrawal 2000; Heil 2014). Also, consumers may benefit plants by the deposition of nutrient-rich waste products at the bottom of attacked plants (Owen 1980). For instance, aphids deposit sugar honeydew beneath their host plants, providing an energy source for free-living nitrogen-fixing bacteria (Stadler et al. 1998). Although this indirect and positive effect

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of consumers on plants is widespread in nature, it remains poorly understood (Jaremo et al. 1999). The question of whether fertilization compensates for foliar loss has been assessed experimentally by measuring plant responses under nutrient addition and foliar removal treatments (Hawkes and Sullivan 2001; Pirk and Farji-Brener 2013). An estimation of these effects under field conditions is still needed to assess the validity of the experimental results.

Leaf-cutting ants (hereafter, LCA) are ideal organisms to evaluate the overall effect of herbivores on plants under natural conditions. These ants annually consume a larger number of plant species harvesting high quantities of vegetal material (Wirth et al. 2003; Herz et al. 2007; Costa et al. 2008, and references therein). High defoliation levels of LCA often negatively affect harvested plants, increasing plant mortality, and decreasing plant fitness (Terborgh et al. 2001; Montoya-Lerma et al. 2012). However, at the same time, LCA generate large amounts of nutrient-rich organic waste, which may indirectly benefit plants. These ants collect huge quantities of vegetation from a large area that are transported to their underground nest chambers and decomposed by a mutualistic fungus. The waste material from the fungal decomposition (hereafter, refuse) is accumulated in external piles or internal chambers (Farji-Brener and Medina 2000). This refuse is several times richer in organic carbon and nutrients than non-nest soils, enhancing the growth and fitness of plants around the nest (reviewed in Farji-Brener and Werenkraut 2015). Therefore, ant harvesting may directly damage a focal plant but the same plant may indirectly benefit from the nutrient-rich refuse dumps. Although these effects are simultaneous in nature, they have been mainly studied separately (Rico-Gray and Oliveira 2007; Montoya-Lerma et al. 2012 and references therein). Hence, the net effect of LCA on plants in nature remains controversial (Farji-Brener and Werenkraut 2015), as well as, which factors will determine whether this net effect is positive, negative or neutral under field conditions.

The balance between negative and positive consequences of LCA on plants will depend on several factors (Leal et al. 2014). Plant identity may influence the negative impact of LCA on plants because not all plant species (nor even individuals) are equally harvested. LCA exhibit a strong preference for certain species and for individual plants within a species (Howard 1990). Particularly, LCA prefer water-stressed plants, leaves with high nutrient, and energetic content, and avoid plants and leaves with higher levels of chemical and physical defenses (Vasconcelos and Fowler 1990; Meyer et al. 2006; Hölldobler and Wilson 2011; Ribeiro Neto et al. 2012). Additionally, plant species may intrinsically differ in their capacity to tolerate herbivory (Paige and Whitham 1987). Therefore, the interplay between plant identity and defoliation level will determine the consequences of foliar damage on plant fitness. On the

other hand, plant identity, location, and the level of nutrient content of refuse dumps may influence the positive effects of LCA through soil improvement. Refuse dumps from different nests may vary in the concentration of nutrients (Tadey and Farji-Brener 2007) and the response of plants toward increased nutrient levels is often species-specific (Farji-Brener et al. 2010). Finally, only plants established near refuse dumps can reach to this nutrient-rich source but all plants around the LCA nest are potentially affected by defoliation (Vasconcelos 2002; Meyer et al. 2011). In sum, plant identity and proximity to refuse dumps are both key factors affecting the balance between positive and negative effects of LCA on vegetation.

The LCA *Acromyrmex lobicornis* Emery, that inhabits Monte Desert, Argentina, is a suitable species to study the balance between negative and positive effects of LCA on plant fitness under natural field conditions. These ants deposit their organic waste on the soil surface, facilitating refuse material collection for nutrient analysis and the measurements of plant distances to this nutrient-rich source. Monte Desert physiognomy consists of approximately 30 % of bare soil with sparse xeric shrub vegetation, making easy to sample the ant foraging activity and to identify harvested plants. A previous study in the same area showed that the nutrient content of refuse dumps and the degree of foliar damage may vary widely among colonies and plant species, respectively (Tadey and Farji-Brener 2007). These conditions allow analyzing of how ant harvesting and soil improvement through refuse dumps affect plant fitness. Here, we determine the overall effect of *A. lobicornis* on the fitness of the seven most common plant species in the Patagonian Monte Desert. We assessed both the potential positive effect of soil enrichment and the negative effect of ant defoliation by (a) comparing the fitness of foraged plants located near and distant from the refuse dumps, and (b) evaluating the relative importance of *A. lobicornis* as herbivores and soil improvers on the fitness of seven plant species using hierarchical confirmatory path analysis model and D-sep test (Shipley 2009). If the role of *A. lobicornis* as soil improver is more relevant than its role as primary consumer, we expect that: (a) plants growing near refuse dumps showed higher fitness than those growing in adjacent, non-nest soils and, (b) the effect of ants on plant fitness through refuse dumps will be positive and stronger than the negative effect via defoliation in the path analysis.

## Materials and methods

### Study area and species

The study area is located in NW Patagonia, in Neuquén province (39°17'S, 68°55'W), Argentina. This is a

temperate arid region with a mean annual precipitation between 80 and 300 mm. The mean annual temperature is 15 °C with a minimum temperature of −13 °C in winter and a maximum of 43 °C in summer (AIC, meteorological station of EL Chocón). The vegetation of this region is characterized by a xerophytic shrubland dominated by *Larrea cuneifolia* (Cavanilles) and *L. divaricata* (Cavanilles) associated with other xerophytic or aphyllous shrubs such as *Monttea aphylla* (Miers) Berthan et Hooker, *Bougainvillea spinosa* (Cavanilles) Heimerl, *Chuquiraga erinacea* (Don), *Prosopis alata* (Philippi), and *Atriplex lampa* (Gillies ex Moquin) D. Dietrich, among others (Correa 1969). Grasses, mostly represented by species in the genus *Stipa*, are scarce in this region (<6 % of plant cover) (Tadey 2006).

*Acromyrmex lobicornis* Emery is the only leaf-cutting ant species that inhabits arid regions of Patagonia (Farji-Brener and Ruggiero 1994), and is considered a key component of herbivore community of this region despite their moderate abundance (Tadey and Farji-Brener 2007). *A. lobicornis* nests reach depths of 1 m; on the soil surface, the ants construct a mound made of twigs, soil, and dried plant material, which may reach a height of 0.5 m and width of 1 m. Inside this mound, ants tend a fungus on which ant larvae feed. Refuse material is removed from the internal fungus garden and dumped onto the soil surface. This refuse material is deposited on a pile near the mound (~50 cm), which makes it accessible for nearby plants (Farji-Brener and Ghermandi 2004, 2008, Appendix S1, Figure S1a).

## Sampling

To determine the effect of ant harvest and soil improvement of *A. lobicornis* on plant fitness we analyzed, during 2005–2006: (a) diet composition of *A. lobicornis*, (b) nutrient content of refuse dumps and adjacent non-nest soils and, (c) plant fitness of the seven more common species within ant foraging area (Appendix S1, Table S1 and Figure S2). The distance between colonies ranged from 15 to 30 m and the total extent of sampled area was ~5 ha. Diet composition of *A. lobicornis* was considered as a reliable proxy of the degree of damage generated by LCA on the studied plant species. In this dry habitat, it is difficult to quantify ant herbivory directly on plants. Xeric plants of this region present tiny leaves and LCA collect entire leaves or flowers without leaving evident cutting marks as they do in plants from more humid habitats (Wirth et al. 2003, see also Appendix S1, Figure S1). Although diet composition is an indirect estimation of LCA foliar damage and may be less accurate than direct measures, it allowed us to include a large number of plant species and individuals to better understand the overall effects of LCA on plant assemblage.

## Composition of the leaf-cutting ant diet

To determine *A. lobicornis* diet, we counted and identified all items carried by ant workers to the nest during a 10-min interval in 1–3 foraging trails per colony ( $n = 15$  nests, see also Appendix S1, Figure S1b). This sampling was performed every 2 h in 3 days during the period of maximum foraging activity (nocturnal in the summer, diurnal for the rest of the seasons). This methodology was repeated in each of the four seasons. Our sampling effort was ~1440 min per nest (10 min × 12 sampling periods × 3 days × 4 seasons) and the identification of ~7000 leaf fragments carried by ants. This procedure is widely employed to determine diet composition and foraging preferences of LCA (Farji-Brener 2001; and references therein). Foraged items were identified at the best possible taxonomical level (85 % to species level), because ant workers collect entire leaves or parts of flowers. We also confirmed the identity of the plant species by following unloaded ant foragers from the nest entrance to their target plants (Appendix S1, Figure S1c-d). The colony foraging rate was estimated as the total number of carried fragments per nest weighted by sampling effort. This measure was used as a proxy of colony size, because bigger colonies often showed higher foraging rate (Wirth et al. 2003; Herz et al. 2007; Costa et al. 2008). The harvesting level of *A. lobicornis* for each plant species studied per nest was estimated directly by their number of fragments carried weighted by sampling effort.

## Nutrient content of refuse dumps and non-nest soils

To determine the nutrient content of refuse dumps and non-nest soils, we sampled refuse dumps of the 15 colonies and adjacent non-nest soils and analyzed the content of organic Carbon (C), total Nitrogen (N), extractable Phosphorus (P), Potassium (K), and Magnesium (Mg). Samples from each substrate (refuse dumps and non-nest soil) consisted of four subsamples collected between 0 and 20 cm of depth (~300 g each one). Refuse piles ranged between 0.5–1 m of diameter and up to 30 cm height. Non-nest soils were sampled 1–3 m away from the nests on bare soil. Refuse and soil samples were air-dried and analyzed in the laboratory. The following analyses were conducted (Sparks et al. 1996): exchangeable + soluble cations (Mg, K, Ca) extracted with 1 M NH<sub>4</sub>OAc at pH 7 and determined by atomic absorption; extractable P in 0.5 M NaHCO<sub>3</sub> at pH 8.4 (soil solution 1:20) and determined by the ascorbic acid-molybdate method. Soil subsamples that passed a 0.5 mm sieve were analyzed for total C and N with a CN analyzer (Flash EA 1112 Thermo Electron Corp.).



## Measurements of plant fitness

We estimated plant fitness of the seven most abundant species in the foraging area of *A. lobicornis* (*Atriplex lampa* (AL), *Bougainvillea spinosa* (BS), *Grindelia chilensis* (GCH), *Gutierrezia solbrigii* (GS), *Larrea divaricata* (LD), *Lycium chilense* (LCH), and *Monttea aphylla* (MA)) (Correa 1969; Tadey 2006, 2015) (Appendix S1, Table S1 and Figure S2). As discussed previously, to estimate the negative (i.e., via defoliation) and positive (i.e., via soil enrichment) effects of LCA on focal plants, we compared plant fitness in plants naturally established on ant refuse dumps and co-specifics on adjacent non-nest soils. We assumed that both plant categories had similar chances to be attacked by the ants because they were all within the ant foraging area, but only plants established near refuse dumps may benefit from the increased nutrient content of this substrate. Accordingly, the number of plants sampled for species was restricted by their presence near refuse dumps at each ant colony. We estimated the fitness of AL in 10 ant nests (20 plants, 10 on refuse dumps, and 10 on adjacent non-nest soils), BS in 8 nests (16 plants), GCH in 7 nests (14 plants), GS in 15 nests (30 plants), LD in 11 nests (22 plants), LCH in 5 nests (10 plants), and MA in 5 nests (10 plants). Consequently, we estimated the fitness of 122 plants from 7 species. Plants on non-nest soils were selected inside the colony foraging range, between 3 and 5 m from the nest. Along the sampling season, we followed ant foraging trails to confirm that ants harvested all sampled plants. We considered refuse dump plants, those individuals directly growing on this substrate (e.g., Appendix S1, Fig. S1a) or near it (up to 1.5 m); plants at that distance can reach this nutrient-rich source through their roots as it is generally accepted that canopy projection depicts root system projection (Mazzarino et al. 1998; Sternberg et al. 2007). On each selected plant we measured: (a) plant height, (b) number of flowers or inflorescences per branch length (hereafter, flower density), and (c) the number of fruits/flower (fruit set). In small species (<60 cm), we counted the total number of flowers and fruits per plant and measured the total length of all branches. In larger plants species, flower density was estimated counting the number of flowers in three randomly selected branches. Length of these branches was measured and plant fitness was estimated as flowers  $\text{cm}^{-1}$  of branch. Fruit set was estimated counting the number of fruits in the same sampled branches. Flower and fruit production was estimated every 2 weeks during the spring (September–December) and summer (November–March).

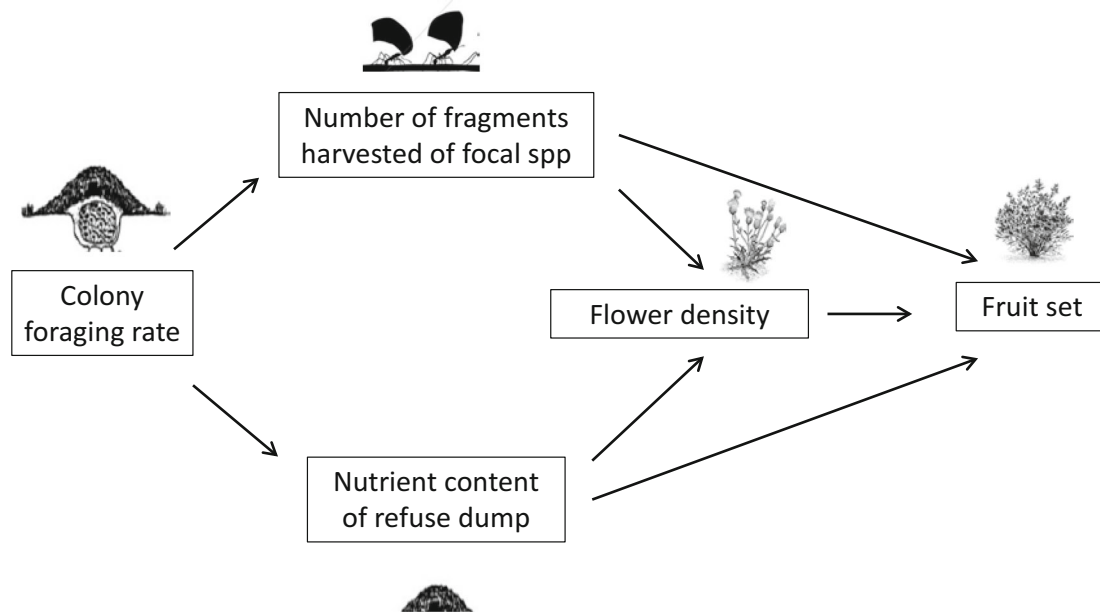
## Statistical analyses

We compared the fitness of plants located in refuse dumps and co-specifics in adjacent non-nest soils using a three-way analysis of co-variance (ANCOVA) with type III sum of

squares (SS) for unbalanced design. Substrate (refuse dumps vs. non-nest soils) was considered as fixed factor, plant species and site (colony foraging area) were random factors, and plant height was used as co-variable. Flower density and fruit set were the response variables. LSD method was used in post hoc comparisons.

We used hierarchical confirmatory path analysis models to evaluate the role of ants as consumers and soil improvers on the fitness of the focal plant species growing on refuse dumps. This method allows estimating causal and non-causal components from total variation through modeling direct and indirect relationships between variables (Shipley 2009). We constructed a general model where the net effect of LCA on a focal plant species could be partitioned into different effects (Fig. 1). Only plants growing on refuse dumps were analyzed with path analysis because plants growing far away from refuse cannot reach and benefit from this nutrient-rich source. Our hypothetical model proposed that the overall colony foraging rate (i.e., a proxy of its size) may affect the amount of harvested fragments of a target plant species. This may directly affect flower density and fruit set and/or through consumption or reducing photosynthetic plant ability. Also, the foraging rate on a focal plant species may indirectly affect fruit set through their effect on flower density (e.g., reducing pollinator attraction). On the other hand, we propose that the colony foraging rate affects the nutrient content of refuse dumps (e.g., larger nests contribute more than smaller nests to soil improvement). The nutrient content of refuse dumps may directly affect flower density and fruit set; or indirectly affect fruit set by affecting flower density, as previously mentioned. We performed a principal component analysis (PCA) to order the 30 soil samples (15 refuse dumps and 15 control soils) according to their content of C, N, K, P, Ca, and Mg to summarize nutrient content in one variable of substrate fertility. The first axis of the principal component analysis explained 82 % of the variance (Appendix S1, Table S2a). This axis was positively correlated with almost all the elements analyzed ( $0.93 < r < 0.97$  all  $P < 0.001$ , Appendix S1, Table S2b) and was interpreted as a gradient of soil fertility (Appendix S1, Fig. S3). Therefore, in the SEM, we used the axis scores on the first axis of PCA as an estimation of fertility of refuse dump samples.

Because of the hierarchical nature of our data (i.e., individuals within plant species), we analyzed our path analysis using a generalized multilevel path model using the “d-separation” test (Shipley 2009). All variables were log transformed to meet normality assumptions. The pathways were evaluated by their estimated slope values and the associated probabilities in the hierarchical regressions. We generated all possible independence claims (i.e., directional separations or d-sep’s) based on the path diagram depicted in Fig. 1. D-sep consists of a pair of variables that,



**Fig. 1** Hypothetical path model of causal relationship among variables involved in direct and indirect effects of LCA on plant fitness (flower density and fruit set). We proposed that the colony foraging rate (i.e., their size) may affect the amount of harvested fragments of a target species. This may directly affect flower density and fruit set either through consumption and/or reducing photosynthetic ability. Also, the foraging rate on a focal plant species may indirectly affect

fruit set through their effect on flower density (e.g., reducing pollinator attraction). On the other hand, we propose that the colony foraging rate will affect the nutrient content of refuse dumps (e.g., larger nests contribute more than smaller nests to soil improvement). This nutrient content may directly affect flower density and fruit set, or indirectly affect fruit set by affecting flower density

according to a given path model, are expected to be statistically independent after accounting for the effect of other variables (Shipley 2009, see also Appendix S1, Fig. S4). The regressions between variables accounted for random effects of the plant species. When appropriate, a “weights” function was used to correct residual variance heterogeneity between species ( $\text{varIdent}$  (form =  $\sim 1/\text{species}$ ) (Zuur et al. 2009). All statistical analyses of the path model were conducted using  $\text{lme}$  function of the library  $\text{nlme}$  and  $\text{lmer}$  function of the library  $\text{lme4}$  in R program (R Core Team 2013). Model fit was calculated comparing  $C$  ( $C = -2\ln_{i=1}^k \Sigma(p_i)$ ) to a  $\chi^2$  distribution with  $2k$  degrees of freedom (where  $k$  is the number of independent claims). This equation combines all the null probabilities ( $p_i$ ) for each independence claim. The model is rejected if  $C$  is below the chosen significance level.

We also conducted a separate set of structural equation model (SEM) procedures (one per plant species) for heuristic reasons, as a way of illustrating the idiosyncratic responses of each species to LCA effects included in our conceptual model (Appendix S1, Fig S5). Since our sample size for each plant species was limited by the number of individuals found near refuse dumps, we only analyzed those plant species with enough replication (AL, LD, BS, and GS). These results are not conclusive since small sample sizes may produce biased estimations and model testing. Nevertheless, path coefficients may be calculated as

standardized regression coefficients with conventional least square regression procedures allowing summarizing the relationships among a set of variables as in our hypothetical general model in a coherent way (see Vazquez and Simberloff 2004). The significance of the path coefficients were tested by the  $t$  tests for the parameter estimates in the regression analysis (Appendix S1, Fig. S5).

## Results

### Composition of leaf-cutting ant diets

The number and amount of species harvested varied among ant colonies. *A. lobicornis* colonies harvested a minimum of 5 and a maximum of 15 plant species through the sampling year, and the plant species studied represented up to the 70 % of their diet (50–70 %, min–max). Overall, *A. lobicornis* heavily harvested plants of GS ( $80 \pm 12$ ) and LD ( $52 \pm 13$ ), moderately plants of MA ( $17 \pm 6$ ), GCH ( $15 \pm 8$ ), and BS ( $10 \pm 4$ ), and scarcely LCH ( $4 \pm 2$ ) and AL ( $3 \pm 1$ ), (mean fragments/10 min  $\pm 1$  SE).

### Chemical analyses of refuse dumps and non-nest soils

The nutrient content of refuse dumps varied among nests but it was always higher than adjacent, non-nest soils

(Appendix S1, Table S3). Refuse dumps showed  $6.6 \% \pm 0.7$  of C,  $0.3 \% \pm 0.05$  of N,  $97 \text{ mg/kg} \pm 14$  of P,  $0.4 \text{ g/kg} \pm 0.04$  of Mg, and  $1.7 \text{ g/kg} \pm 0.15$  of K (mean  $\pm$  SE). Adjacent non-nest soils showed  $0.4 \% \pm 0.03$  of C,  $0.02 \% \pm 0.002$  of N,  $3.5 \text{ mg/kg} \pm 0.3$  of P,  $0.09 \text{ g/kg} \pm 0.002$  of Mg, and  $0.2 \text{ g/kg} \pm 0.02$  of K. Overall, refuse dumps were 17, 15, 28, 5, and 8 times higher in C, N, P, Mg, and K content than adjacent non-nest soils, respectively (all  $P < 0.05$ , Appendix S1, Table S3). Accordingly, the scores of refuse dump samples in the PCA differed greatly from those of non-nest soil samples (Appendix S1, Fig. S3).

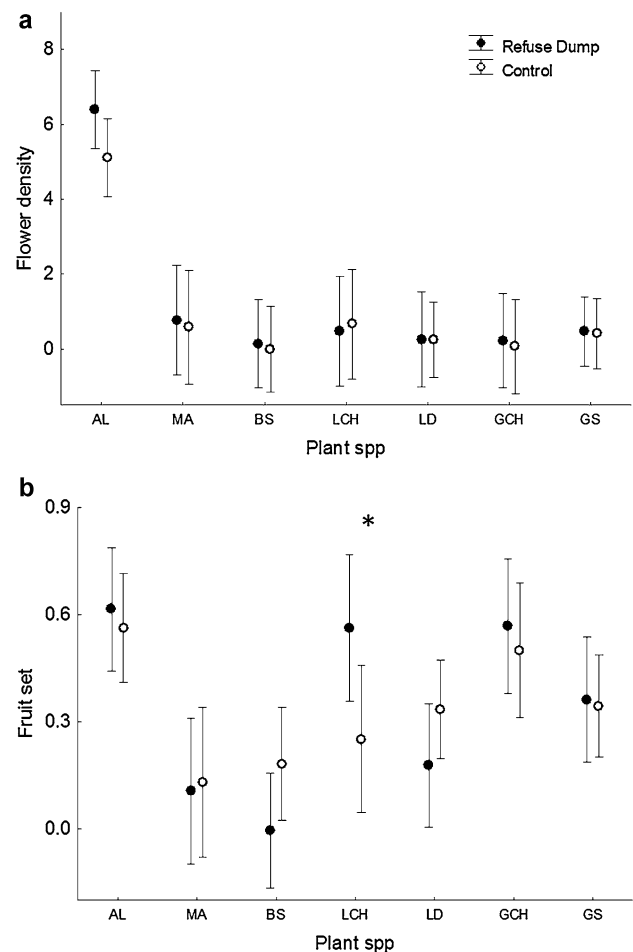
### Plant fitness

Plant fitness was estimated by sampling  $\sim 10,000$  flowers and  $\sim 3600$  fruits from 122 individuals in 7 plant species within the ant foraging area. Plant fitness varied greatly among species but it was similar between plants growing on RD and co-specifics on adjacent non-nest soils (Fig. 2). Flower density and fruit set were similar among different nest areas and plant size (Fig. 2 and Appendix S1, Table S4). Only fruit set tended to respond differently to the substrates depending on the plant species; LCH showed greater fruit set in RD than in adjacent non-nest soil ( $P = 0.02$ ); whereas BS showed the opposite trend ( $P = 0.12$ ) (Fig. 2b).

### Path analysis

The hypothetic hierarchical model proposed fitted well the data ( $P = 0.62$ , Fig. 3). As expected, the overall foraging rate of a colony positively affected the number of fragments harvested of a focal plant species (slope = 0.82,  $P = 0.001$ ) and the nutrient content of refuse dumps (slope = 0.11,  $P = 0.08$ ). However, none of these effects strongly influenced plant fitness. The number of fragments harvested by *A. lobicornis* directly and positively affected flower density, but this effect was weak (slope = 0.04,  $P = 0.01$ ). Flower density also marginally and positively affected fruit set in a direct way (0.04,  $P = 0.02$ ). The direct effect of the number of fragments harvested by *A. lobicornis* on fruit set was statistically non-significant. On the other hand, changes in the nutrient content of refuse dumps did not affect flower density but slightly fruit set (Fig. 3, Appendix S1, Table S5 and Fig. S4).

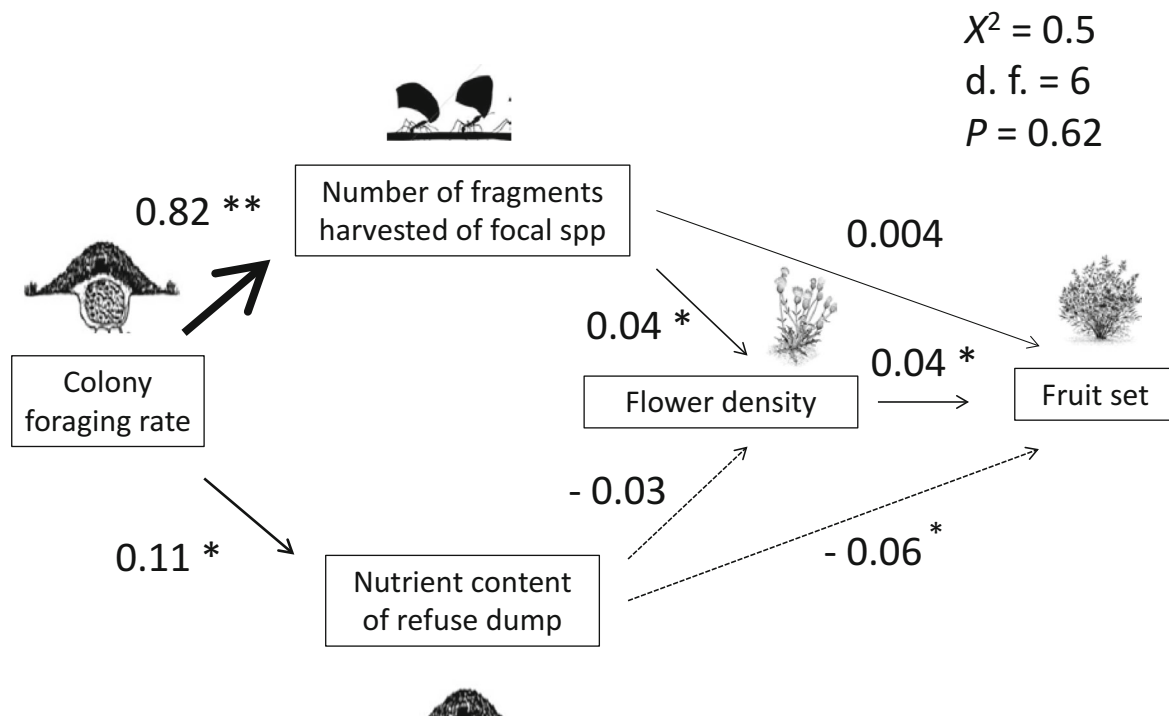
In the path analyses performed per each plant species separately, the number of fragments harvested of a focal plant species positively depended on the colony foraging rate in all the plant species analyzed (Appendix S1, Fig. S5). However, the strength and sign of the other proposed causal relationships depended on plant species identity (see also Appendix S1, Table S5 and Fig. S5). Plant fitness of two



**Fig. 2** Comparison of flower density (flower per cm of branch) and fruit set (fruits/flowers) between plants naturally growing on refuse dumps of *A. lobicornis* and adjacent non-nest soils for seven plant species in Monte Desert. (\*)  $P < 0.05$ . AL = *Atriplex lampa*, BS = *Bougainvillea spinosa*, GCH = *Grindelia chilensis*, GS = *Gutierrezia solbrigii*, LCH = *Lycium chilense*, LD = *Larrea divaricata*, MA = *Monttea aphylla*. Error bars denote the 95% confidence intervals

species (LD and GS) was apparently not affected by ant foraging or their fertilizer activity; none of the paths connecting both the number of fragments harvested and the nutrient content of refuse dumps with flower density and fruit set were statistically significant (Appendix S1, Fig. S5). Accordingly, the explained variance of plant fitness was extremely low for these two plant species ( $R^2 = 0.02$ – $0.15$ ). On the other hand, *A. lobicornis* affected plant fitness of two other species studied but in different ways. First, AL showed a decrease in flower density with increments in the nutrient content of refuse dumps ( $-0.71$ ,  $P < 0.05$ ). However, changes in flower density did not affect fruit set. The proposed model moderately explained changes in flower density and fruit set ( $R^2 = 0.53$  and  $0.25$  respectively). Second, BS plants showed an increment in flower density with the increase in number of fragments





**Fig. 3** Results of the path model tested. Coefficients (slopes and  $P$ ) for each path are besides the corresponding arrow. The strength and sign of coefficients were represented by the thickness of the arrow; solid

lines indicate positive effects and dotted lines, negative effects. Significance of path coefficients ( $0.01 < P < 0.05$  and  $P < 0.01$ ) are indicated with \* and \*\*, respectively

harvested ( $0.60$ ,  $P < 0.05$ ). Increments in nutrient content of refuse dumps did not affect flower density. Thus, changes in flower density were moderately explained by the model ( $R^2 = 0.38$ ). Paths related with fruit set in this plant species were impossible to estimate because  $\sim 90\%$  of sampled flowers did not produce fruits (Appendix S1, Fig. S5).

## Discussion

The ultimate outcome of plant–herbivore interactions is best understood when addressing both the direct negative effect and indirect positive effect of consumers. First, studies only assessing direct effects represent an incomplete picture of the mechanisms that model biotic interactions (Wootton 1994). Second, direct and indirect effects with opposite signs may result in null net effect and, therefore, be undetectable (Dyer and Leoturneau 1999). Here, we studied the overall effect of LCA on plant fitness, including their direct role as defoliators and their indirect role as soil improvers, with a great sampling effort. We found that almost all plants growing on nutrient-rich refuse dumps of *A. lobicornis*: (a) showed similar fitness than those growing on adjacent non-nest soils, and (b) were mostly not affected by ant harvesting neither by the presence of refuse dumps. Surprisingly, *A. lobicornis* affected the fitness of few plant species but in opposite direction to our expectation: flower

density increased with harvest in BS and decreased with nutrient increments in AL. Our findings suggest that in this desert habitat, the effect of LCA on plant fitness depended on plant identity and habitat context, illustrating the complexity of plant–herbivore interactions in nature.

## Harvest activity of LCA and plant fitness

We proposed that *A. lobicornis* negatively affects plant fitness in the studied plant species through defoliation. LCA often decrease plant reproduction by directly collecting flowers or harvesting photosynthetic tissues (Vasconcelos and Cherrett 1997; Wirth et al. 2003; Costa et al. 2008; Falcão et al. 2011; Montoya-Lerma et al. 2012; Mundim et al. 2012). However, we found that increments in the number of fragments harvested did not affect plant fitness in almost all the species studied. For some plant species (e.g., AL), this pattern could be explained by a very low defoliation level. However, flower density and fruit set were also unaffected in the two more harvested species (GS and LD). It also may be possible that plant species from arid lands are adapted to higher levels of defoliation because their long-time interaction with mammalian herbivores (Milchunas et al. 1988). A tolerance to high levels of herbivory or the fact that in arid lands defoliation by ants is much lower than those produced by livestock (Guillade and Folgarait 2015) may explain why defoliation levels of LCA had no effect on

plant fitness. Moreover, the only statistical significant relationship between the ant harvest activity and flower density was positive (BS), suggesting that this species can over-compensate insect herbivory (Trumble et al. 1993).

### Soil improving activity of LCA and plant fitness

Both our comparative (i.e., ANCOVA) and correlative (i.e., path analyses) approaches suggest that for almost all the studied species, the enhanced soil nutrient content generated by *A. lobicornis* did not increase plant fitness in field conditions. Plants growing on refuse dumps had similar fitness than those growing on adjacent control soils, and increments in nutrient content of refuse dumps did not cause larger flower density or fruit set. These results contrast with greenhouse experiments and field measurements which often show an enhanced fitness in plants growing on refuse dumps (Cerda et al. 2012; Farji-Brener and Werenkraut 2015). Several reasons may explain these conflicting results. First, water availability is more limiting than soil nutrients for plants in this arid region. Arid lands are water-limited and micro-sites with enhanced nutrients may not increase plant fitness under condition of water stress (Labraga and Villalba 2009; Fernandez et al. 2014). Accordingly, the studies describing positive effects of refuse dumps on plant fitness were performed in greenhouse conditions without water restriction (Farji-Brener and Ghermandi 2004, 2008; Farji-Brener et al. 2010) or in habitats where water is not a limiting factor (Moutinho et al. 2003; Saha et al. 2012). Second, native species that normally grow in poor soils may not respond consistently to increased nutrient contents. For example, in a greenhouse experiment with plant species from arid lands of northern Patagonia, only 40 % of native plants showed better performance in refuse dumps compared with 85 % of exotic plants (Farji-Brener et al. 2010). Finally, plants growing on refuse dumps may be more defoliated than those growing in the surroundings because of their proximity to the nest and the rapid loss of the known deterrent effect of refuse in dry habitats (Farji-Brener and Sasal 2003; Ballari and Farji-Brener 2006). The higher foliar damage in plants growing on refuse dumps may counteract their positive effect of enhancing substrate nutrients. However, because we estimated ant diet by counting leaf fragments and not directly by quantifying defoliation on standing plants, we cannot confirm this idea. Overall, water stress, the potentially limited response of native plant species to an enhanced soil nutrients and the higher probability of damage may explain why growing in refuse dumps did not increase plant fitness of the sampled species under field conditions.

Surprisingly, increased nutrient content of refuse dumps reduced flower density in AL. Several hypotheses may explain this result. First, high levels of refuse dump

nutrients may attract and/or contain plant natural enemies. For example, two external refuse dumps of *Atta mexicana* sampled in arid deserts from México contained 1331 insects from 49 species, including herbivores, detritivores, and flower/seed/fruit predators (Rojas 1989). Second, the high levels of nutrients in refuse dumps may trigger an overmuch flower production. Competitions among reproductive units and subsequent abortion are likely to increase with the number of reproductive units initiated (Stephenson 1981). Finally, other indirect, non-studied interactions may negatively affect plant fitness, like the proliferation in refuse dumps of micro-biota potentially detrimental for plants (Scott et al. 2010).

### Concluding remarks

Leaf-cutting ants are recognized for their defoliation capacity, as well as, for their ability to improve the nutrient content of soils (Farji-Brener and Illes 2000; Costa et al. 2008; Montoya-Lerma et al. 2012; Leal et al. 2014; Farji-Brener and Werenkraut 2015). However, only few studies integrate both roles and quantitatively analyze the net effect of LCA on plant fitness in field conditions. With a large sampling effort, we found that in this arid system the overall effect of LCA on plant fitness depended on plant identity, ranging from neutral to slightly positive or negative. Contrary to our expectations, there was not an overall detrimental effect on plant fitness from ant harvest activity or net beneficial effects from soil fertilization. Field studies in humid habitats often found a net positive effect of LCA on plant productivity near nests via enhanced soil fertility (Lugo et al. 1973; Haines 1978; Moutinho et al. 2003; Sosa and Brazeiro 2010). Since moisture disproportionally enhances the positive effects of refuse dumps on plants (Farji-Brener and Ghermandi 2004; Fernández et al. 2014), the strength and sign of LCA net effects may depend on the balance among precipitation, soil fertility, and herbivory pressure, which may vary along environmental gradients (Young et al. 2013; Leal et al. 2014; Farji-Brener and Werenkraut 2015). These contrasting results among humid and dry environments suggest that the net impact of LCA on plants (i.e., detrimental via foraging or beneficial via soil improvement) depends on habitat context (Leal et al. 2014). Our findings illustrate the complexity of herbivore–plant interactions, and emphasize the necessity of measuring direct and indirect effects of herbivory in field conditions to validate greenhouse experiments.

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