

Original Article

Trash to treasure: leaf-cutting ants repair nest-mound damage by recycling refuse dump materials

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Animals adjust their behaviors in response to changing environmental conditions because the costs and benefits of such behaviors change as conditions change. The reuse of materials from waste (i.e., recycling) rarely occurs in social insects because it may imply significant health risks and behavioral difficulties. However, the benefit of reusing may exceed its costs under certain circumstances. For the first time, we document that ants “recycle” refuse materials to repair nest-mound damage. We conducted a series of field measurements and experiments to test the hypotheses that fluctuations in this behavior in the leaf-cutting ant *Acromyrmex lobicornis* depend on 1) seasonal changes in the tendency to reject refuse (a proxy of changes in their pathogen levels), and/or 2) seasonal foraging restrictions. We 1) measured the rejection of foraging ants toward experimental refuse piles among seasons and 2) analyzed how mound condition, temperatures of fungus chamber and soil surface, and foraging activity explained this behavior using a classification tree, a powerful recursive partitioning method. Foraging ants showed similar rejection levels toward refuse piles in different seasons. Colonies repaired mound damage with refuse materials only during the hottest season and when they had low foraging rates, suggesting that ants recycle their refuse by a hierarchical set of decisions dependent on the risk of fungal damage and foraging restrictions. Repairing the mounds is essential during summer, when temperatures inside damaged mounds are lethal to their fungus cultures. However, these high temperatures also restrict the foraging activity, reducing the collection of building materials. Thus, colonies with lower foraging rates apparently use their refuse to repair mounds because this substrate requires less searching and carrying time. The use of refuse did not affect the colony growth rate. This illustrates how ants integrate information about food, hygienic and nest conditions through a novel and plastic behavior: recycling of their discarded materials. *Key words:* *Acromyrmex lobicornis*, ants, behavioral plasticity, hygienic behavior, nest-mounds, organic waste. [*Behav Ecol*]

INTRODUCTION

Animal behavior is molded by natural selection to minimize costs and maximize benefits (Krebs and Davies 1997). However, behaviors occur in the context of ever changing environments, and so costs and benefits also change. Behaviors that are inappropriate under one circumstance may become the best choice under another (Robinson et al. 2008). The capacity to adequately respond to changing conditions is known as behavioral plasticity (Chesson 1986). Although the study of behavioral plasticity is an important topic in evolution and ecology (Mercy and Burns 2010), our knowledge regarding how much flexibility exists, how it works, and when it matters is still limited (Gordon 2011). In this study, we describe a potentially harmful ant behavior that was never documented before: recycling of refuse materials to repair nest damage, and we explore why this behavior fluctuates temporarily.

The implementation of an adequate waste disposal strategy is vital at all levels of biological organizations (Hart et al. 2002b). Discarded materials usually are a source of pathogens and their accumulation may enhance the spread of diseases. This is especially true for social organisms that form dense

societies. As groups become larger, the manipulation and accumulation of waste and associated diseases pose an increasing threat to the group (Cremer et al. 2007). Leaf-cutting ants are an example of dense societies that discard a large quantity of organic material that represents a potential threat for the colony.

Leaf-cutting ants (*Atta* and *Acromyrmex*) use leaf fragments to cultivate a fungus in underground nest chambers. This symbiotic fungus is the main food source for the colony and has strict requirements for suitable growth: it depends nutritionally on the leaf fragments collected by the ants, it dies at high temperatures (Powell and Stradling 1986), and it is highly susceptible to parasitic fungi (Currie et al. 1999). Ant workers harvest large quantities of fresh vegetation (Wirth et al. 2003), build and maintain nest structures that keep the fungus gardens under favorable microclimatic conditions (Bollazzi et al. 2008), and remove infectious fungi and pathogenic waste to refuse dumps (Hart and Ratnieks 2001). Consequently, it might be expected that ant behaviors adjust to changes in food, weather, and hygienic conditions to maximize fungus growth and colony survival.

Leaf-cutting ants often exhibit adaptive flexible behaviors toward food and weather changes. For example, ants may concentrate their foraging on tree-fall gaps when the availability of palatable species in the surrounding matrix is low (Peñaloza and Farji-Brener 2003) and switch from

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diurnal to nocturnal foraging when soil temperatures are high (Rockwood 1975). Nevertheless, ants occasionally perform suboptimal behaviors under variable food and weather conditions without paying especially larger costs. Leaf-cutting ants sometimes gather novel plant resources potentially harmful for their fungus garden, but thereafter avoid their collection ("delayed rejection"; Herz et al. 2008). Ants sometimes forage under unsuitable weather conditions and are forced to drop the leaf fragments during heavy rain, but later they recover those fragments after the rain stops (Hart et al. 2002a). These behaviors imply the waste of a fraction of the foraging time, but they do not represent a high risk for colony survival. Conversely, suboptimal behaviors that potentially affect the survival of the fungus garden are potentially dangerous because the fungus is the only food source for the developing larvae (Powell and Stradling 1986).

The survival of the symbiotic fungus strongly depends on an adequate microclimate and on low levels of pathogens inside the nest. The fungus can suffer severe damage at humidity levels below 60% and above temperatures of 33 °C (Quinlan and Cherrett 1978; Powell and Stradling 1986). Accordingly, ants often relocate their fungus gardens to nest chambers where temperature and humidity are more suitable for their survival (Bollazzi and Roces 2002), and invest a considerable amount of energy in building, repairing, and maintaining nest structures that provide adequate microclimatic conditions (Kleineidam and Roces 2000; Bollazzi and Roces 2007, 2010). In addition, the fungus garden produces large quantities of waste such as degraded plant materials (hereafter, refuse), which is often contaminated with fungal competitors (i.e., *Escovopsis*) and other microorganisms potentially lethal to the fungus and to the ants themselves (Haeder et al. 2009 and references therein; Lacerda et al. 2010). Therefore, leaf-cutting ants allocate their waste in separate internal chambers or in external piles isolated from their fungus gardens, and also show hygienic behaviors that include a strict division of labor to reduce their contact with the garbage (Bot et al. 2001; Hart and Ratnieks 2001, 2002; Hart et al. 2002a, 2002b; Fernández-Marín et al. 2003; Ballari et al. 2007). Because the maintenance of an adequate microclimate and a relatively pathogen-free environment inside the fungus chamber are critical for colony survival, natural selection may favor the ability to detect changes in these conditions and to behave appropriately to reduce the risk of fungal mortality. Surprisingly, we observed that the leaf-cutting ants *Acromyrmex lobicornis* occasionally use their refuse instead of the usual building materials (e.g., dry twigs) to repair nest structures, a behavior that hypothetically implies a contamination threat for the fungus garden. Inspired by these observations, we conducted a series of field measurements and experiments to test the hypotheses that fluctuations in this behavior depend on 1) seasonal changes in pathogens levels in the refuse dump materials, and/or 2) seasonal foraging restrictions.

MATERIALS AND METHODS

Study area and species

The study area is located in Neuquén province in northwestern Patagonia (39°17'S, 68°55'W), Argentina. This is an arid temperate region with a mean annual precipitation ranging between 80 and 300 mm. The mean annual temperature is 15 °C, but the annual soil surface temperatures range between -10 °C (during winter) and 70 °C (during summer) (see Abraham et al. 2009). The vegetation is a xerophytic scrubland with considerable portions of bare ground. The leaf-cutting ant *A. lobicornis* Emery is widespread in temperate semi-deserts of South America reaching 44°S (Farji-Brener and Ruggiero 1994). This ant deposits refuse in external

dumps and builds mounds mostly made of dry twigs covering a single central fungus chamber, which they maintain at proper temperatures (Farji-Brener 2000; Bollazzi et al. 2008). Mounds are often damaged by mammals and sometimes partially collapse because of natural erosion. Mound damages have been associated with increased colony mortality in this ant species (Farji-Brener 2000), probably because they affect the microclimatic conditions inside the nest, and allow the entrance of pathogens to the fungus garden. Accordingly, ants regularly collect dry twigs and desiccated plant parts (hereafter, building materials) to repair damage and to expand the nest-mound (Farji-Brener 2000).

Methodology

All the measurements described below were performed in a total of 50 mature nests of *A. lobicornis*; 10 nests per season in summer, autumn, winter, and spring 2007, and summer 2008 (a total sampling period of 15 months). We analyzed whether foraging activity, ant diet composition, mound condition, and the temperatures of the internal fungus chamber and the soil surface explained the use of refuse materials for mound repairing, using a classification tree, a powerful recursive partitioning method (Breiman et al. 1984). Additionally, we measured 1) the occurrence of worker rejection toward experimental piles of refuse (a proxy for waste pathogen levels; see Farji-Brener and Sasal 2003; Ballari and Farji-Brener 2006; Lacerda et al. 2010; Waddington and Hedges 2010), and 2) the growth rate in a random sample of 30 nests with different status of mound repair (see below for methodological details).

Foraging activity, diet composition, and foraging rate

We monitored daily foraging activity of each nest every 2 h during 24 h. Nests were classified as active or inactive according to the presence of foragers. This procedure was repeated on 3 different days in each sampling season. To determine the foraging composition (food for the fungus vs. building materials), we counted all items transported by workers during a 5-min interval. This measure was performed on the 2 main foraging trails of each colony, every 2 h in 3 days during the period of foraging activity (nocturnal during the summer and diurnal during the rest of the seasons). Our sample effort was ~1800 min per nest (10 min × 12 sampling periods × 3 days × 5 seasons). The ant loads were classified as fresh plant material (for the fungus gardens) or building material (dry twigs for the mound). The relative importance of fresh plant and building materials during foraging was expressed as a proportion of each type of item over the total number of items. We used the number of laden workers (i.e., number of all fragments transported per 10 min per nest) and the period of daily activity (number of hours foraging per day) to extrapolate in each season the number of laden workers per trail per daily period of activity per nest. This measure was taken as the foraging rate of each colony (i.e., an estimation of the overall harvesting activity, which includes the collection of dry and green plant materials).

Nest-mounds, temperatures and the use of refuse dump materials to repair mound damage

During the visits to each nest we 1) measured 3 mound radius (long, wide, and height, in centimeter) and used the volume formula of the sphere to estimate the mound volume (MV) considering a mound as a hemisphere ($MV = [4/3 \times \pi \times r^3]/2$), 2) monitored whether the mound was intact or damaged, 3) estimated the relative area of mound damaged (in percentage) using digital photos and the software imageJ[®],

and 4) documented whether damaged mounds were repaired with building materials (i.e., dry twigs) or refuse materials. The use of refuse was easy to identify because of its texture and color (see Figure 1). Mound dimensions were used as a proxy for colony size (see Fowler et al. 1986), and the change in MV between 2007 and 2009 (in percentage) was used as an estimate of colony growth. We simultaneously measured the temperatures of the internal mound in the fungus garden (see Farji-Brener 2000; Bollazzi et al. 2008 for a detailed description of fungus gardens' location) and on the adjacent soil surface in 3 intact and 3 damaged nest-mounds (20–40% of damage) using HOBO® internal/external data loggers (Onset Computer Corporation, MA). Temperatures inside mounds were obtained using a thin cable for HOBO® external data loggers (\varnothing 3 mm). Cables were carefully inserted inside mounds in a non-destructive way. Preliminary sampling and a previous study (Farji-Brener 2000) showed that sensor insertion into the nest does not influence the accuracy of the information. Temperatures were measured every 15 min during 4 continuous days per season. Sampling days represented the typical weather conditions for the respective sampling seasons.

Field experiment: indirect evaluation of pathogens changes in refuse among seasons

Leaf-cutting ants are capable of detecting pathogens and of avoiding contact with contaminated substrates (Bot et al.

2001; Hart and Ratnieks 2001, 2002). It was possible that the levels of pathogens in external refuse piles might vary with weather changes (Ballari and Farji-Brener 2006), so we used the fluctuations in the rejection of refuse material by foraging ants as an indirect estimation of temporal changes in the amount of pathogens on this substrate. This measure has been employed in other studies to estimate the threat level of waste (Farji-Brener and Sasal 2003; Ballari and Farji-Brener 2006; Lacerda et al. 2010; Waddington and Huges 2010). In the 2 seasons with the most contrasting climates and ant foraging activity (autumn and summer), we blocked the ant traffic of 2 trails per nest placing experimental piles of refuse or soil (as a control for pile effect). Refuse samples were collected from 10 cm depth to the surface of the refuse dump of each nest to include all refuse microclimatic conditions that may affect their pathogenicity (Ballari and Farji-Brener 2006). Experimental piles were ~5 cm high and 15 cm wide. We counted the number of foragers that walked over the pile (i.e., a non-rejection behavior), returned through the same way before reaching the pile, and surrounded the pile (i.e., rejection behaviors) during a 10-min interval. In autumn and summer we performed a total of 28 trials (14 per treatment) using 5 randomly selected nests.

Statistical analyses

A classification tree was used to explore the fluctuations in the behavior of using refuse materials to repair mound damage.

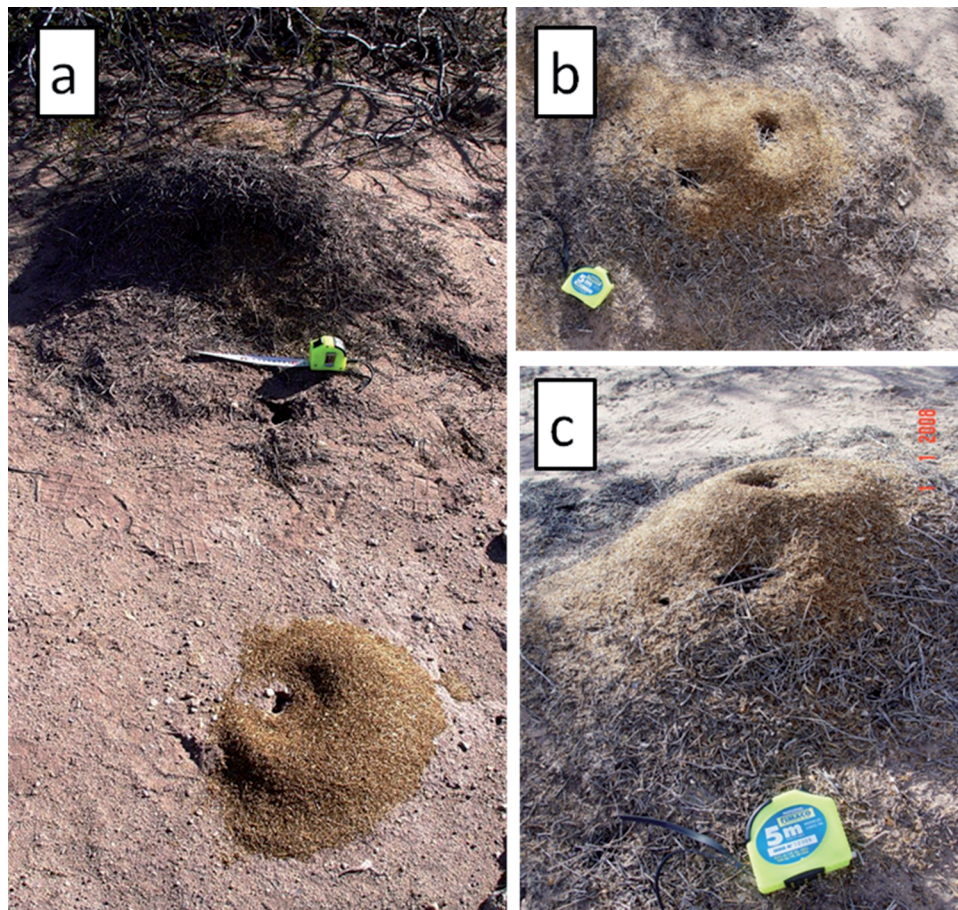


Figure 1

Photos illustrating nest-mounds of the leaf-cutting ant *Acromyrmex lobicornis* (a) repaired with usual building materials such as dry twigs (see also the usual location of refuse dumps on the soil surface near the nest-mound), and (b and c) repaired with refuse materials (see the upper section of the mound).

This is a binary recursive partitioning method that explains the variation of a single categorical response variable by 1 or more explanatory categorical or continuous variables; it is free of distributional assumptions and represents information intuitively (Breiman et al. 1984). The tree is constructed by repeatedly splitting the data, defined by a simple rule based on a single explanatory variable. At each split the data is partitioned into 2 mutually exclusive groups, each of which is as homogeneous as possible with respect to the response variable. The splitting procedure is then applied to each group separately. The objective is to partition the response into homogeneous groups, although keeping the tree reasonably small. Each group is typically characterized by the distribution of the categorical response. Trees are represented graphically, with a root node at the top, which represents the undivided data, and the branches and leaves beneath it (each leaf representing one of the final groups). Our categorical response variable was the use of refuse materials to repair mound damage or not. The explanatory variables were season, mound damage (in percentage), foraging rate, and the proportion of building material from the foraged items.

A blocked, repeated measures analysis of variance (ANOVA) was used to determine whether the rejection level of worker ants toward the experimental refuse piles depended on the season. Pile substrate (refuse or soil) was considered a main, fixed effect; nest a random effect, and season as the repeated measure factor. The response variable was the proportion of ants that returned and surrounded the experimental pile from the total number

of ants during a 10-min sampling period. A 1-way analysis of covariance (ANCOVA) was conducted to determine whether the status of the nest-mound influenced colony size. The difference (in percentage) between 2007 and 2009 MVs was the response variable; mound condition (repaired with building materials, $n = 12$; repaired with refuse dump, $n = 8$; or unrepaired, $n = 10$) was considered a main, fixed effect and the initial MV was the covariable. Prior to ANOVA analyses, the response variables were tested to meet the assumptions and arcsine square root transformations were used when necessary. Duncan post hoc comparisons of means were applied when the model was statistically significant ($P < 0.05$). All analyses were performed using the Software Statistica 7.0®

RESULTS

Foraging patterns of the leaf-cutting ants changed through the sampling period. No foraging activity occurred during winter, whereas the summer was the only season in which foraging was entirely nocturnal. Ants moved more slowly at night because of low soil temperatures (4 ± 0.30 s to walk along 10 cm of a trunk-trail at night vs. 1 ± 0.04 s at day, $n = 64$ ants, mean \pm SE), and summer showed the lowest overall foraging rate. Annual foraging rates (number of foraging ants per trail per day) varied between 3360 (summer) and 4608 (spring). The number of laden ants carrying building materials was strongly correlated with the number of laden ants carrying green or fresh plant material ($r = 0.88$, $P < 0.001$, $n = 180$

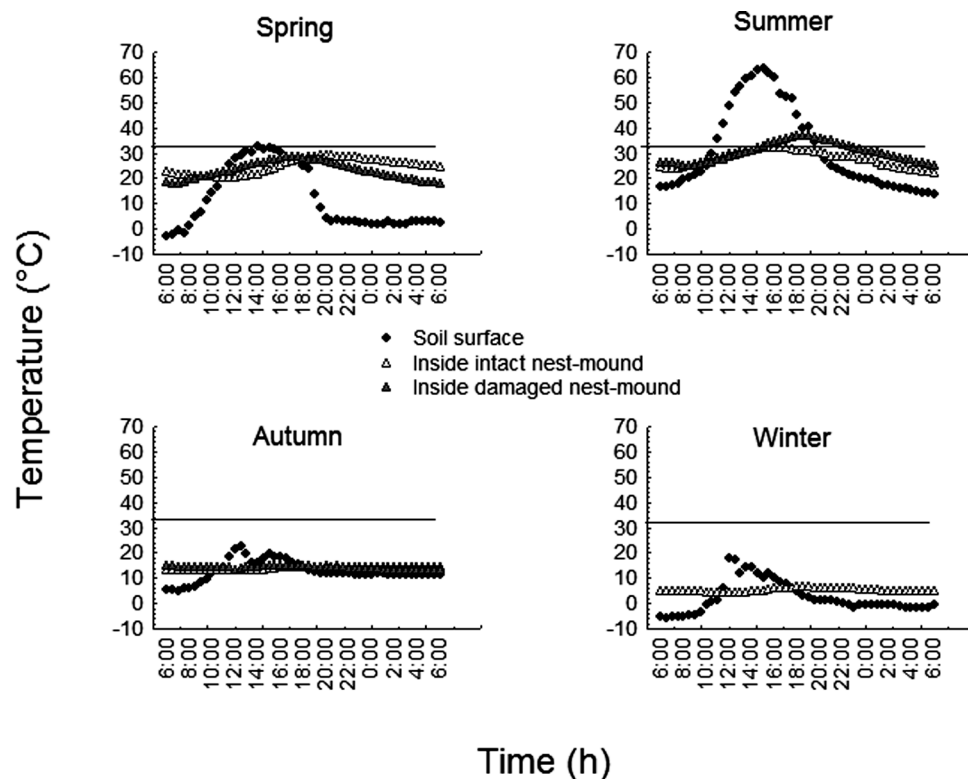


Figure 2

Seasonal and daily variation of temperatures on the soil surface (black circles) and inside damaged (grey triangles), and intact (white triangles) nest-mounds of the leaf-cutting ant *Acromyrmex lobicornis*. One daily cycle (24h) is shown. Values are means from 4 sampling days and 3 nests per category per season. The measures of dispersion around each value are not shown to improve figure clarity. The line represents the thermal limit for the symbiotic fungus that inhabits in the mound chamber; the fungus can suffer severe damage above temperatures of 33 °C. Note that damaged nest-mounds reached lethal daily temperatures only during summer. None of the sampled nests ($n = 10$ per season) showed damage during winter. Fungus chambers inside mounds repaired with building materials (e.g., dry twigs) showed similar temperatures as those repaired with reused material (data not shown).

sampling periods). Soil surface temperatures varied annually between $-6\text{ }^{\circ}\text{C}$ (winter) and $69\text{ }^{\circ}\text{C}$ (summer) whereas temperatures inside intact nest-mounds only varied between $5\text{ }^{\circ}\text{C}$ (winter) and $32\text{ }^{\circ}\text{C}$ (summer). Temperatures inside damaged mounds ranged between $13\text{ }^{\circ}\text{C}$ (autumn) and $42\text{ }^{\circ}\text{C}$ (summer) (Figure 2). Temperatures inside mounds in each season showed very little variation across nests in the same category (i.e., intact and damaged, in both cases with coefficients of variation of 0.04 ± 0.004 , mean \pm SE). No damaged mounds were found during the winter.

The use of refuse material for mound repairs depended on the season and the foraging rate of the ants. The classification tree concerning using refuse or not showed 2 splits, 3 terminal boxes (“leaves”) and a misclassification rate of zero (i.e., boxes containing no misclassified nests, Figure 3). The first split established that the use of refuse dump materials was only present in summer, when soil temperatures reached up to $70\text{ }^{\circ}\text{C}$, foraging was fully nocturnal, and damaged nest-mounds reached lethal temperatures for the symbiotic fungus every day (Figure 3). During the

summer, the second split established that only nests with the lowest foraging rate (i.e., with the lowest collection of building materials, see correlative results) used their refuse to repair mounds. The other explanatory variables were not selected by the model (Figure 3). On the other hand, foraging ants rejected more refuse than soil piles (0.93 ± 0.016 vs. 0.17 ± 0.016 , mean rejection rate \pm SE, respectively, $P < 0.001$), and showed a slightly higher rejection rate in summer than in autumn (0.56 ± 0.012 vs. 0.54 ± 0.012 , mean rejection rate \pm SE, respectively, $P < 0.04$). Nests also showed some variation in their rejection rates to soil and refuse piles ($0.09\text{--}0.34$ and $0.89\text{--}0.97$, respectively, $P < 0.01$). However, rejection rates of refuse piles were similar between seasons (substrate \times season $F_{1,22} = 0.001$, $P = 0.97$). In other words, refuse piles were equally highly rejected by foraging ants in summer and autumn (Table 1). All nests increased in size during the 3-year period. This increment was similar between nest-mounds repaired with building materials or refuse ($\sim 35\%$), but higher than non-repaired mounds ($\sim 10\%$) ($F_{2,26}$, $P < 0.001$, Table 2).

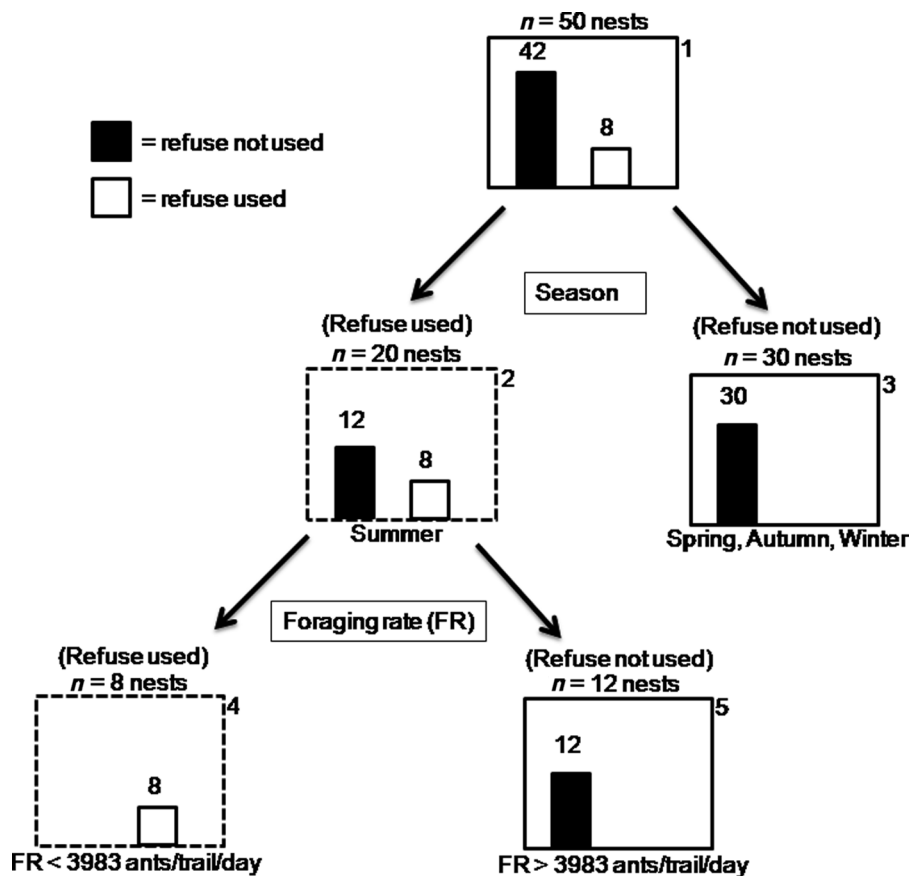


Figure 3 Classification tree depicting the use of refuse materials to repair nest-mound damage in the leaf-cutting ant *Acromyrmex lobicornis*. We sampled a total of 50 nests (10 nests per season along summer, autumn, winter, spring 2007, and summer 2008). Explanatory variables selected by the model were season and foraging rate. The model excluded mound damage and selected foraging rate instead of the percentage of building materials collected (these last 2 variables were strongly correlated, see text). Each of the 2 splits is labeled with the variable name. The categories or values that determined the split appear at the base of each box. Each box (i.e., leaf) is also labeled according whether refuse was predominantly used (dot line boxes) or not (solid line boxes). The length of the bars inside the box indicates the number of nests in each group (white bars = refuse used and black bars = refuse not used). The number outside the box (top right corner) indicates the box number. Initially, all 50 nests were assigned to the root node and tentatively classified as nests that did not use refuse materials to repair mound damage, because there were more nests in that category (42 vs. 8, see box 1). The split of the root node separates the nests sampled during summer (left, box 2) (which were tentatively classified as nests that used refuse), from those nests sampled in other seasons (right, box 3), which were classified as nests that did not use refuse. Similarly, the nests in box 2 were subsequently split depending on whether the foraging rate was < 3983 ants per trail per day (8 nests, box 4) or higher (12 nests, box 5). All nests in box 4 were classified as nests that used refuse, and all nests in box 5 were classified as nests that did not use refuse. Each of the 3 terminal boxes (boxes 3, 4, and 5) are pure, containing no misclassified nests.

Table 1
Rejection rates of the leaf-cutting ant *Acromyrmex lobicornis* toward experimental piles of refuse and soil (control) in 2 seasons, and the statistical results associated

Season	Soil pile	Refuse pile	
Autumn	0.16±0.02	0.92±0.02 (*)	
Summer	0.18±0.02	0.94±0.02 (*)	
Independent variables	Df	F	P
Substrate	1	1224.9	<0.001
Nest	4	4.1	0.01
Error	22		
Season (repeated measure)	1	4.7	0.04
Season x Substrate	1	0.001	0.97
Season x Nest	4	2.9	0.04
Error	22		

Rejection rates were calculated as the proportion of foragers that either returned through the same way or surrounded the experimental pile that we located blocking the main trail during a 10-min period (see MATERIALS AND METHODS). Values are mean ± SE. A blocked, repeated measures ANOVA were conducted to determine whether the rejection level of worker ants to their organic waste depended on the season. (*) = $P < 0.05$, Duncan post hoc test.

Table 2
Mound growth in nests of the leaf-cutting ant *Acromyrmex lobicornis* repaired with non-food materials, refuse, and non-repaired and the statistical results associated

Mound status			
Repaired with non-food material ($n = 12$)	Repaired with refuse ($n = 8$)	Non-repaired ($n = 10$)	
34.5±2.1 ^a	32.8±2.3 ^a	10.6±1.8 ^b	
Independent variables	Df	F	P
Initial MV	1	137.8	0.25
Mound status	2	1.4	<0.001
Error	26	2023.1	

Values are the difference between MV between the years 2007 and 2009 (mean ± SE, in percentage). One-way ANCOVA was conducted to determine whether mound growth depended on mound status. Initial MV was considered as a covariable.

^{a,b} Denote significant statistical differences (Duncan post hoc test).

DISCUSSION

Recycling of discarded materials might be an adaptive and flexible response to limit the waste accumulation generated by dense societies and to confront restrictions in the availability of resources. This behavior is rare in social insects presumably because the use of potentially pathogenic materials for new functions results in significant health risks and behavioral problems (Cremer et al. 2007). In this study, we documented that ants, under certain environmental conditions, can “recycle” their own refuse material to repair nest-mound damages.

Our results suggest that the use of refuse material for mound repair is determined by a hierarchical set of decisions dependent on the risk of fungal damage by heat and on foraging restrictions, rather than the other alternatives that we evaluated. Although we did not directly measure

the pathogenic level of refuse, it has been assumed that the rejection rate of forager ants to refuse material is a good estimation of its pathogenicity (Ballari and Farji-Brener 2006; Lacerda et al. 2010; Waddington and Huges 2010). Therefore, the similar rejection levels toward refuse materials between seasons partially discard the hypothesis that ants use refuse only in summer as a result of a seasonal decrease in its pathogenic level. However, seasonal changes in pathogenicity might still play a role. It has been shown that refuse from superficial layers are less rejected than those from inside the refuse dumps (Ballari and Farji-Brener 2006). This variable rejection level to waste according to their location is attributed to changes in pathogen activity; extreme aridity and high temperatures inhibit pathogen activity in external layers of refuse dumps although the conditions inside refuse dumps may favor the re-growth of the pathogens that have either survived or colonized from the upper layers of the mound of debris (Ballari and Farji-Brener 2006). Because our experimental refuse piles were a mixture of superficial and deep materials, the effect of a reduced pathogen activity in summer may be undetected by our experiment. A decrease in the availability of building materials to repair the mounds during the summer is another alternative explanation for this behavior. Although not formally tested, this hypothesis seems also improbable at first glance, because dry twigs are available year-round in the study area (personal observations). It may also be possible that refuse showed better physical properties than dry twigs to repair mounds. However, this does not explain the use of refuse only in the summer.

The high temperatures during summer could be responsible for the hierarchical set of decisions that determine the use of refuse to repair mounds. First, damaged mounds reached lethal temperatures for the symbiotic fungus only during summer, forcing ants to repair the mounds and bring down internal temperatures. The symbiotic fungus of *A. lobicornis* ants is probably more sensitive to higher than to lower temperatures normally encountered in its environment. Recent studies in species of northern temperate leaf-cutting ants demonstrated that cold-tolerance of the symbiotic fungus increased with winter harshness, indicating a selection for cold-tolerant fungi variants along a temperature cline at the northern distribution limit (Mueller et al. 2011). This selection for cold-tolerant cultivars may also occur in species at the southern limit of the leaf-cutting ants' distribution (Mueller et al. 2011). Therefore, mound damage during summer may jeopardize fungus survival in temperate populations of *A. lobicornis*. Second, extremely hot soil temperatures limit ant foraging activity (Whitford and Ettershank 1975; Mintzer 1979). High temperature increases oxygen consumption, water loss, and transport costs all reduce the foraging period of ants (Lighton and Feener 1989). High temperatures also increase the desiccation rate of leaf-fragments, reducing the quality of fresh plant material for fungal growth (Bowers and Porter 1981). Therefore, the high diurnal temperatures observed in the summer (almost 70 °C all moon) force leaf-cutting ants to forage at night. However, nocturnal foraging entails costs such as a decrease walking speed because of low temperatures, which ultimately reduces the overall foraging rate of the colony. The collection of building materials for mound repair may decrease during the summer either because ants show a low overall foraging rate or because under climatic restrictions ants give foraging priority to substrates on which to grow their fungus.

In sum, repairing the mounds is essential during summer because this is the only season when temperatures inside damaged mounds are potentially lethal to the fungus. However,

the high summer temperatures also restrict the ants' foraging activity, reducing the amount of building materials they can collect for mound repair. Faced with this dilemma, colonies with lower foraging rates use refuse material to repair damaged mounds because this substrate is structurally similar to the usual building materials (see Figure 1), but demands less searching and carrying time. The workers know the location of their refuse dump, and the distance between the refuse dump and the nest-mound is short (e.g., <1 m, see Figure 1). The potential hygienic cost of reusing refuse is ameliorated by the weather of arid environments, which often decreases the toxicity of refuse (Ballari and Farji-Brener 2006; Ribeiro and Navas 2007). The risk of colony contamination is also reduced because the manipulation of refuse is exclusively performed by specific workers (Ballari et al. 2007). In accord with this idea, we observed only waste-manage workers manipulating refuse material to repair mounds. The similar growth rates of nests repaired with building materials and refuse suggests that, at least in the summer, the potential sanitary costs of the use of refuse materials to repair mounds did not exceed their benefits as building resources. It is possible that the use of refuse to repair nests in other seasons implies some costs that were not measured in this work. When temperatures inside damaged mounds do not represent a lethal risk for the fungus culture and foraging is not limited, ants commonly used dry twigs instead of refuse. This intriguing, context-dependent trade off could be further explored in the future.

Nest structures can be considered as functionally versatile extensions of the animals' phenotypes (Hansell 2005). Building behavior and material selection for mound building are indicators of how ants cope with variations of their external environment (Aleksiev et al. 2007). It is known that ants can use leaf-fragments or vegetable debris to plug the circulation of dry air flows in nest-mounds (Bollazzi and Roces 2007, 2010a), collect leaves as building material for thatched nests (Bollazzi and Roces 2010b), use different soil particle sizes to improve the resilience of nest walls (Aleksiev et al. 2007), and deposit charcoal litter around nest entrances to modify soil temperature (Smith and Tschinkel 2007). Some termites include feces in their nest architecture (Emerson 1938); however, this is the first record of ants using the waste from their fungus gardens for building purposes and to regulate the internal environment of the nest. This behavior appears only in circumstances when the cost of collecting usual building materials for that purpose exceeds the potential cost of reusing degraded plant materials. These results reveal how ants integrate information about food, hygienic and nest conditions through a novel and plastic behavior: the recycling of discarded materials. Moreover, they emphasize how the relative costs and benefits of behaviors vary as circumstances change, supporting the importance of behavioral plasticity to deal with changing environmental conditions.

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