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*Proc. R. Soc. B* 2012 **279**, 3779-3787 first published online 20 June 2012
doi: 10.1098/rspb.2012.1066

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# Bottom-up effects may not reach the top: the influence of ant–aphid interactions on the spread of soil disturbances through trophic chains

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Soil disturbances that increase nutrient availability may trigger bottom-up cascading effects along trophic chains. However, the strength and sign of these effects may depend on attributes of the interacting species. Here, we studied the effects of nutrient-rich refuse dumps of the leaf-cutting ant, *Acromyrmex lobicornis*, on the food chain composed of thistles, aphids, tending ants and aphid natural enemies. Using stable isotopes tracers, we show that the nitrogen accumulated in refuse dumps propagates upward through the studied food chain. Thistles growing on refuse dumps had greater biomass and higher aphid density than those growing in adjacent soil. These modifications did not affect the structure of the tending ant assemblage, but were associated with increased ant activity. In contrast to the expectations under the typical bottom-up cascade effect, the increase in aphid abundance did not positively impact on aphid natural enemies. This pattern may be explained by both an increased activity of tending ants, which defend aphids against their natural enemies, and the low capacity of aphid natural enemies to show numerical or functional responses to increased aphid density. Our results illustrate how biotic interactions and the response capacity of top predators could disrupt bottom-up cascades triggered by disturbances that increase resource availability.

**Keywords:** *Acromyrmex lobicornis*; biotic interactions; bottom-up cascades; mutualisms; soil nutrients

## 1. INTRODUCTION

Terrestrial disturbances have been widely recognized as forces that strongly influence the structure and dynamics of ecological communities [1]. Disturbances, via the reduction in plant biomass and the increase in resource availability, can directly impact on vegetation, modifying the relative abundance and richness of plant species [1–3], and also affect organisms from other trophic levels [4]. Disturbances that directly affect upper or basal levels of a food chain can trigger both top-down and bottom-up cascading effects, thus affecting organisms from distant trophic levels [5]. However, few studies have examined the pervasive effects of disturbances along trophic chains, which would help to gain a better understanding of the forces that structure species assemblages.

From a bottom-up perspective, a disturbance that changes quality, quantity or diversity of plants could also affect abundance or diversity of herbivores, and consequently affect populations of their natural enemies. Because most studies on bottom-up cascades report directional responses [5,6], it is expected that changes produced by a disturbance at the base of a food chain would spread with the same sign to higher trophic levels. For example, enhanced foliage sprouting after a flood caused an increase in the abundance of leaf beetles and their natural enemies [7]. However, the strength and the sign of disturbance effects might change through their propagation along a trophic chain. Disruptions of bottom-up cascades may be due to intrinsic characteristics of species or to the counteracting effects of biotic interactions, among other causes, acting as branch-off or resistances that dilute or interrupt the spread of disturbance effects. Bottom-up cascades may not reach upper trophic levels if consumers do not fully exploit the enhanced resource availability (e.g. by low consumer efficiency [8] or consumer satiation [9]). The occurrence of horizontal biotic interactions, such as competition and mutualism, could also alter the course and/or weaken the strength of trophic cascades [10,11]. Particularly, the mutualisms based on the exchange of (resource-dependent) food rewards and protection against natural enemies may affect the upward propagation of such positive cascades.

Ant–aphid interactions are a widespread mutualism [12,13] that constitutes a good model system to study how biotic interactions may affect the bottom-up cascade effects of disturbances. Aphids provide ants with

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2012.1066 or via http://rspb.royalsocietypublishing.org.

*Received* 9 May 2012
*Accepted* 30 May 2012

carbohydrate-rich honeydew, the waste product of its sugar-rich diet of plant sap; in return, ants defend aphids from predators and parasitoids [14]. Several factors could influence the extent of ant protection of aphids. First, aggressiveness and territoriality levels differ among ant species [15]. Second, the number of ant individuals that forage on a host plant, which is usually associated with colony density [16], may determine the efficiency of ant protection. Third, the type of threat to the 'aphid resource' may affect the ant defensive behaviour [17], with ants responding more aggressively/effectively to a greater threat to their resources. Finally, the quantity and/or quality of aphids (in terms of their honeydew) could influence the intensity of ant protection [18,19].

In northwest Patagonia, nests of the leaf-cutting ant *Acromyrmex lobicornis* are a common small-scale soil disturbance that generates spatial variation in nutrient availability [20]. Inside the nest, ants cultivate the fungus that larvae feed on; organic waste products from this activity, together with debris and dead ants, are accumulated in a pile on the soil surface near the nest (hereafter 'refuse dump'). Several studies have shown that refuse dumps of *A. lobicornis* contain two to eight times higher nutrient levels and better water retention capacity than adjacent soils [20]. In the study area, these refuse dumps are often colonized by two exotic thistle species, *Carduus thoermeri* and *Onopordum acanthium*, which are fast-growing species [21] that, in contrast with co-occurring native plants, are commonly infested by aphids. Thistles growing on refuse dumps are larger [21,22] and should be of enhanced quality for the development of aphid populations, which in turn may represent greater resource availability for both native tending ants [23] and aphid natural enemies. Thus, the small-scale disturbance generated by *A. lobicornis* could cascade up, but the actual outcome would depend on the responses of both tending ants and natural enemies to enhanced aphid populations, as explained above.

In this study, we documented the impact of a small-scale disturbance generated by *A. lobicornis* on the food chain composed of thistles, aphids, tending ants and aphid natural enemies. We first performed an isotopic tracer experiment to test whether the high nitrogen level present in refuse dumps propagates upward through the food chain. Then, in thistles growing on nutrient-rich refuse dumps and adjacent soils, we measured vegetative and reproductive biomass, and estimated aphid density, species richness and activity of tending ants, and both abundance and attack rates of aphid parasitoids and predators. Finally, we conducted field experiments to explore the mechanisms responsible for the observed patterns.

## 2. MATERIAL AND METHODS

### (a) *Study site*

We conducted this study in an area with herbaceous/shrub steppe vegetation, located at the eastern (driest) border of Nahuel Huapi National Park in north-western Patagonia, Argentina (41° S, 72° W). The mean annual temperature is 8°C and the average precipitation in a year is 600 mm. The sampling area is traversed by the Limay River and covers a belt of about 100 × 5000 m, parallel to the main road, where the nests of *A. lobicornis* are common. The dominant vegetation in the sampling area includes exotic species such as *Bromus tectorum*, *O. acanthium*, *C. thoermeri* and *Verbascum thapsus*, and native species typical of Patagonian steppes, such as *Pappostipa speciosa*, *Mulinum spinosum*, *Imperata condensata*, *Plagyobotris tinctoreus* and *Baccharis pingraea* [24].

### (b) *Thistles*

*Carduus thoermeri* (nodding or musk thistle) and *O. acanthium* (scotch thistle; Asteraceae: Cardueae) are among the most abundant exotic plants in the study area [20,22]. They invade a wide range of habitats and are considered serious weeds worldwide [21,25]. Both thistles are biennial, monocarpic herbs and have no vegetative reproduction. When a seed germinates, it forms a basal rosette that persists during the first year of life. Then, during the second year, each rosette gives rise to one or several stems, the plant produces numerous inflorescences, and then it dies.

### (c) *Aphids*

Two aphid species (Aphididae) belonging to the tribe Macrosiphini are common on adult thistles in the study area: *Brachycaudus cardui* (Linnaeus 1758) and *Uroleucon aeneum* (HilleRis Lambers 1939). Both show parthenogenetic reproduction and rapid population build-up. Mainly wingless asexual individuals form each colony, although there are some winged individuals, which are indicators of reduced host plant quality and/or aphid crowding [26].

### (d) *Tending ants*

In the study area, aphids on thistles are tended by four native ant species that inhabit the Patagonian region from Aluminé, Province of Neuquen (38° S, 71° W), to Esquel, Province of Chubut (42°5′ S, 71° W): *Dorymyrmex tener*, *Dorymyrmex wolffuegeli* (Dolichoderinae), *Brachymyrmex patagonica* (Formicinae) and *Solenopsis richteri* (Myrmicinae) [27]. *Dorymyrmex* spp. are generalist predators that live and forage in open areas or under rocks [27], and they have been observed feeding on protein or carbohydrate baits, showing an opportunistic behaviour. The nests of *B. patagonica* occur on a wide variety of habitats, especially under stones, in leaf litter or under piles of dead wood, and their diet consists of various insects, honeydew and other food sources [28]. *Solenopsis richteri* builds its nests in open areas and is an omnivorous species that feeds on honeydew, plant exudates, insects, spiders and seed oils [29]. Occasionally, some of these species coexist in the same host plant.

### (e) *Aphid parasitoids and predators*

Parasitoids are one of the most important groups of aphid-natural enemies and most of them belong to the subfamily Aphidiinae (Hymenoptera: Braconidae), which are solitary, aphid-specific endoparasitoids [30]. These parasitoids lay an egg inside the aphid; the emerging larva consumes the host tissues, and then spins a cocoon and pupates inside the dead aphid, whose exoskeleton hardens and turns brownish (hereafter, mummy). Mummies remain on the plant even after the emergence of adult parasitoids and have been successfully used to estimate parasitism rates [31].

Predator species from different insect families have been observed in the study area, including Coccinelidae (Coleoptera), Syrphidae (Diptera), Chrysopidae (Neuroptera) and small spiders, which is consistent with data for aphid predators in other habitats [32,33]. However, most of these individuals were observed only occasionally, except larvae and adults from the tribe Coccinellini (Coccinelidae), which were often observed on thistles with aphids. Contrasting with parasitoids, predators cause a direct and immediate reduction in aphid populations.

### (f) *Upward cascade of nitrogen*

To test whether the high nutrient level present in refuse dumps of *A. lobicornis* propagates upward through the food chain, we performed an isotopic tracer experiment. This consists of foliar-spraying branches and leaves of an individual of *Maytenus boaria* (Celastraceae), with a solution of $1\,\mathrm{g\,l^{-1}}$ urea (15N2, 98% atom). After that, we collected material highly enriched in $\delta^{15}$N and offered it to workers of six different nests of *A. lobicornis*. Then, at different times after the ants introduced the enriched material into the nests ($T1 = 10$ days, $T2 = 45$ days and $T3 = 300$ days), we collected samples of refuse dump material, thistles, aphids and tending ants associated with each nest to measure the isotopic ratio (or $\delta^{15}$N; see electronic supplementary material A for methodological details). To determine whether there was a temporal increase of $\delta^{15}$N values in the collected samples (waste material, thistles, aphids and ants), we used the average of $\delta^{15}$N for each substrate at each time, and analysed the data using the rank correlation coefficient Kendall's tau ($\tau$).

### (g) *Impact of nutrient-rich refuse dumps*

In order to evaluate the effects of small-scale disturbances generated by the leaf-cutting ant *A. lobicornis* on the food chain composed of thistles, aphids, tending ants and aphid natural enemies (parasitoids and predators), we carried out measurements of all of its components during the peak of aphid and ant activity (December–March) of 2008–2011. The measurements were taken on two types of substrates, which we consider as 'treatments': (i) soil adjacent to nests (control) and (ii) refuse dumps of *A. lobicornis* (treatment). Individuals of both thistle species used as focal plants (experimental units) were in their second and final year of life and were randomly selected within each treatment. Plants from soil adjacent to nests were randomly selected within a circular area (3–6 m radius) centred around the nest mound. Microsites of soil adjacent to nests are comparable in terms of slope steepness, degree of rockiness and physical and chemical properties (e.g. carbon, nitrogen and phosphorous; see [22]). Plants from refuse dumps were usually selected from different ant nests (in total, 67 refuse dumps). Occasionally, two plants (one of each thistle species) shared the same refuse dump (only 17% of 80 individual plants).

#### (i) *Thistles and aphids*

Because different plant parts may differ in quality to aphids [26], we divided each plant in three sections: leaves, stems and inflorescences. On each focal thistle (40 and 31 individuals of *O. acanthium* growing on refuse dumps and adjacent soils, respectively, and 35 and 30 individual of *C. thoermeri* growing on refuse dumps and adjacent soils, respectively), we measured plant height, and counted the number of leaves, stems and inflorescences. Then, in these focal plants and a few others used to increase the sample size (total: 41 and 32 individuals of *O. acanthium* growing on refuse dumps and adjacent soils, respectively, and 39 and 31 individuals of *C. thoermeri* growing on refuse dumps and adjacent soils, respectively), we estimated aphid density (aphids $\mathrm{cm^{-2}}$, nymphs $\mathrm{cm^{-2}}$ and winged aphids $\mathrm{cm^{-2}}$) using digital photographs. The estimation of aphid density was based on counts made in sub-groups of each population (hereafter 'food groups'). We considered as a 'food group' a spatially discrete set of individuals occupying a leaf, stem or an inflorescence. In each sample, we took 10 digital photographs of different food groups chosen at random in each focal plant (each photograph included a marked tape as reference). Then, we counted on each photo the total number of aphids, number of individuals in different nymphal instars and number of winged individuals per square centimetre for each photo. Finally, we averaged the values of each photo to get an estimate of aphid density per plant (total, nymphs and winged individuals).

For data analysis, we used multivariate two-way analysis of covariance (MANCOVA). Substrate (refuse dumps and adjacent soil) and thistle species (*O. acanthium* and *C. thoermeri*) were considered as fixed factors. The response variables for thistles were: number of leaves, stems and inflorescences; plant height was entered as covariate. The response variables for aphid density were total no. of individuals $\mathrm{cm^{-2}}$, proportion of nymphs and proportion of winged individuals. Response variables were transformed to meet assumptions of the analysis when necessary.

#### (ii) *Tending ants*

We assessed the structure of the native tending-ant assemblage on the same focal plants in which we measured aphids. On each focal thistle, we estimated ant richness and the activity of each ant species. The count of number of ants $\mathrm{min^{-1}}$ was performed five consecutive times, observing different plant parts, and the activity of each ant species was estimated as the sum of the number of ants counted in each minute (in a total of 5 min). We carried out the counts randomly between 8.00 h and 19.00 h, because preliminary samplings ruled out the existence of temporal segregation in the activity of ant species. Data were analysed using two-way ANCOVAs. We considered substrate (refuse dumps and adjacent soil) and thistle species (*O. acanthium* and *C. thoermeri*) as fixed factors, and plant height as covariate. Response variables, total ant activity (the sum of individuals of all species present in a plant) and activity of each species were transformed to meet analysis assumptions. Response variables that did not meet these assumptions (e.g. ant species richness) were analysed by non-parametric ANCOVA using ranks [34].

#### (iii) *Aphid natural enemies*

With regard to aphid parasitoids, we conducted measurements on the same focal plants in which we measured aphids and tending ants. We used the same digital photographs that were used to estimate aphid density, this time estimating mummy density (number of mummies $\mathrm{cm^{-2}}$) and calculating parasitism rates (mummy density/aphid density). We considered mummy density as an estimate of direct pressure of aphid parasitoids, while parasitism rates were a proxy for the probability of an aphid to be infected by a parasitoid. With regard to aphid predators, in 80 focal plants randomly selected from those used for parasitoid measures (26 and 15 individuals of *O. acanthium* growing on refuse dumps and adjacent soils, respectively, and 21 and 18 individuals of *C. thoermeri* growing on refuse dumps and adjacent soils, respectively), we estimated aphid predator abundance (number of predators per 5 min) and aphid predation pressure (aphid predator abundance/aphid density). We considered the abundance of predators as a measure of direct pressure on aphid population, while aphid predation pressure, weighted by aphid density, indicates the probability of an aphid to be consumed by a predator. To this end, we observed each plant for 5 min and counted the number of individuals belonging to different families referred to in the literature as aphid predators. Thistles on both substrates were observed during the same time to correct for possible differences in activity of organisms throughout the day. We occasionally observed individuals eating aphids, but these observations were scarce. Therefore, the actual aphid predation rate could not be estimated.

We used two-way ANOVAs for response variables related to aphid parasitoids (mummy density and parasitism rate), because these variables already included the effect of plant size, and ANCOVAs when the response variables corresponded to aphid predators (aphid predator abundance and aphid predation pressure), where plant height was used as covariate to control for plant size. Substrate (refuse dumps of *A. lobicornis* and adjacent soils) and thistle species (*O. acanthium* and *C. thoermeri*) were considered as fixed factors. All response variables were examined to meet ANOVA assumptions and transformed when necessary. To analyse the relationship between these variables and the activity of ants and aphid density, we performed Spearman non-parametric correlations (Rs).

To integrate the results obtained in this study and discriminate between direct and indirect effects, we conducted a path analysis considering the numbers of thistle leaves, aphid density, tending ant activity and attack pressure of aphid natural enemies (see electronic supplementary material B for methodological details).

### (h) *Field experiments*

We conducted two field experiments to study whether the abundance of aphids in individuals of *C. thoermeri* and *O. acanthium* was determined by resource quality (nutritional value of leaves) or quantity (number of leaves, stems and inflorescences). On the one hand, we modified the nitrogen content of thistles growing on adjacent soils by spraying them with 30 ml of an urea solution ($1\ \mathrm{g\,l^{-1}}$; $n = 15$). On the other hand, we manipulated the amount of plant biomass by pruning to 50 per cent all thistle components (leaves, stems and inflorescences) in plants growing on refuse dumps ($n = 13$). The response variable was the relative abundance of aphids, which was visually estimated according to the following categories, representing the percentage of plant covered by aphids: 0 (0%), 1 (less than 20%), 2 (20–40%), 3 (greater than 40–60%), 4 (greater than 60–80%) and 5 (greater than 80%). Aphid abundance was estimated in each focal plant before applying the treatment and a week later. At the same time, we estimated aphid abundance in untreated focal plants growing on both substrates (control). We have not performed an experiment to include the treatment of low nutrient quality and high biomass quantity. This could have been done by adding thistle stems, with leaves and inflorescences, to thistles growing on soil (trying to simulate the size of a thistle growing on refuse dumps of *A. lobicornis*). However, attempts to carry out this manipulation were unsuccessful because added parts dried quickly, resulting in a substrate unsuitable for aphid feeding. Another option would have been the 'removal' of N in thistles growing on refuse dumps, but we did not find an appropriate methodology to extract or dilute foliar nutrients. For data analysis, within each treatment we compared the response variable before and after treatment application using non-parametric Wilcoxon test for dependent samples. We also compared the abundance of aphids between focal plants growing on adjacent soils and between focal plants growing on refuse dumps before the application of their respective treatments. This was done using the Mann–Whitney non-parametric test for independent samples.

To check whether the increase in ant abundance could be explained by changes in aphid abundance, we randomly selected 11 individuals of *O. acanthium* (focal plants) growing on soil and colonized by aphids. We also estimated ant activity by counting the number of workers observed for 2 min. Then, we applied the 'aphid addition' treatment. This involved collecting a large number of aphids (over 100 individuals at different stages) from nearby thistles and placing them on different parts of each focal plant (whose previous relative abundance of aphids was less than 20%). One week later, we estimated again the ant activity in each focal plant. We compared the response variable before and after treatment application using the Wilcoxon test for dependent samples.

To test the effect of exclusion of the most common tending ant species (*B. patagonica* and *D. tener*) in a focal plant on (i) the others tending ants present in the study area and (ii) aphid natural enemies, we tried to perform two treatments of ant exclusion in the field (see electronic supplementary material C for methodological details).

## 3. RESULTS

### (a) *Upward cascade of nitrogen*

Nitrogen present in refuse dumps of *A. lobicornis* propagates upward through the food chain formed by thistles, aphids and tending ants. Ten days after the $^{15}$N-enriched leaves were introduced into the nest, the isotopic ratio ($\delta^{15}$N) from refuse dumps was 17 times greater than the reference value (see [22]). This ratio continued to increase; 45 days after, the $\delta^{15}$N in refuse dumps was 45 times greater than the expected natural ratio. Finally, values of $\delta^{15}$N in refuse dumps were close to reference values 300 days after the introduction of isotopic material into the nest (see electronic supplementary material D, figure D1).

The nitrogen detected in refuse dumps spread into the food chain; we detected an increase in $\delta^{15}$N with time in all of the studied components (thistles, aphids and tending ants; in all cases $\tau = 1$, $p = 0.04$, $n = 4$). Ten days after the treated *M. boaria* leaves entered into the nest, $\delta^{15}$N values of thistle, aphid and tending ant samples were similar to those of their natural proportions (obtained from [22,35]). However, after 45 days, we detected a significant increase in the isotope ratio of thistles ($\delta^{15}$N at T2 was four times higher than at T1 and reference value), aphids ($\delta^{15}$N at T2 tripled those at T1 and expected values for Homoptera) and tending ants ($\delta^{15}$N at T2 showed between 1.2- and 1.5-fold increase compared with T1 and values for ants that feed on honeydew). The increase in $\delta^{15}$N continued over the sampling time; we found $\delta^{15}$N values in thistle, aphid and tending ant samples taken at T3 even greater than those obtained at T2 (in all cases $\tau = 1$, $p = 0.04$, $n = 4$; see electronic supplementary material D, figure D1).

### (b) *Impact of nutrient-rich refuse dumps*

Individuals of both thistle species growing on refuse dumps of *A. lobicornis* had more leaves, stems and inflorescences that those growing on adjacent soils

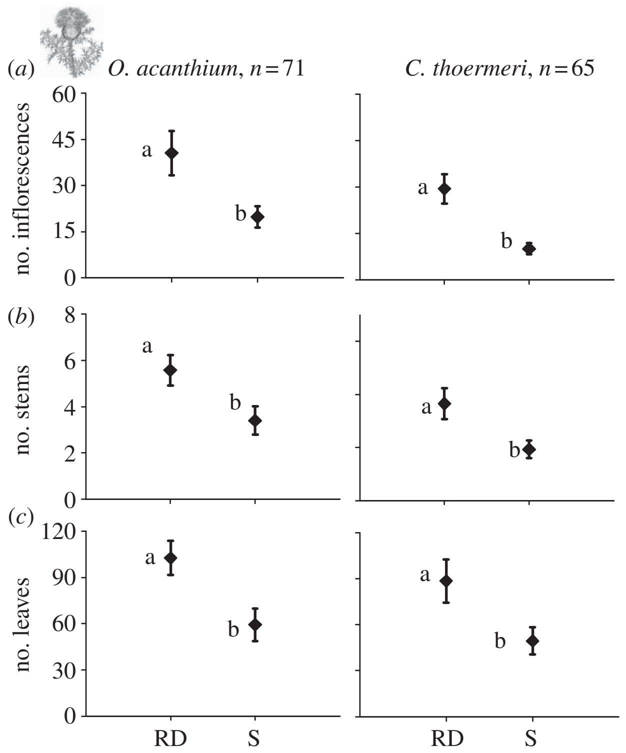

Figure 1. Mean number (± s.e.) of plant parts in *O. acanthium* and *C. thoermeri* thistles growing on refuse dumps of *A. lobicornis* (RD) and adjacent soils (S). (*a*) Number of inflorescences, (*b*) number of stems and (*c*) number of leaves. Different lower case letters indicate statistically significant differences between substrates (MANOVA, $p < 0.05$).

(Wilk's $\lambda = 0.92$, $F_{3,129} = 3.66$; $p < 0.05$; figure 1). The relative percentage of these plant parts differed between thistle species (Wilk's $\lambda = 0.88$, $F_{3,129} = 5.76$; $p < 0.001$). The number of leaves of both species was 80 per cent higher in plants growing on refuse dumps compared with plants growing on adjacent soils. However, plants of *O. acanthium* had 65 per cent more stems and 100 per cent more inflorescences when growing on refuse dumps, while plants of *C. thoermeri* had 90 per cent more stems and 200 per cent more inflorescences when they grew on refuse dumps.

Aphid density was affected by both the substrate on which thistles grew (Wilk's $\lambda = 0.58$, $F_{3,137} = 33.09$, $p < 0.0001$) and thistle species (Wilk's $\lambda = 0.88$, $F_{3,137} = 6.1$, $p < 0.001$). Plants of *O. acanthium* and *C. thoermeri* growing on refuse dumps had 145 and 230 per cent more aphids $\text{cm}^{-2}$ than plants growing on adjacent soils, respectively (figure 2*a*). The proportion of nymphs relative to the total number of aphids on a plant was 30 per cent higher in both thistle species when they grew on refuse dumps (figure 2*b*). There were few winged aphids, representing less than 5 per cent of total aphids in thistles growing on both substrates.

The mean number of tending ant species per plant was not affected by the substrate on which thistles developed ($F_{1,138} = 2.10$, $p = 0.15$) nor by thistle species ($F_{1,138} = 2.79$, $p = 0.10$; figure 3*a*). Most thistles with aphids were colonized by a single ant species: 64 per cent of 80 plants growing on refuse dumps and 66 per cent of 63 plants growing on adjacent soils. However, ant activity

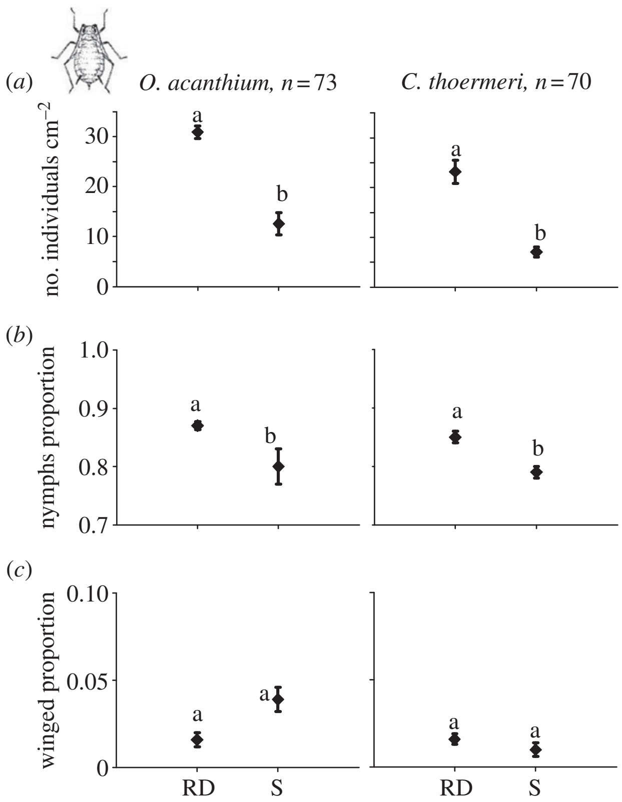

Figure 2. Mean (± s.e.) aphid abundance in *O. acanthium* and *C. thoermeri* thistles growing on refuse dumps of *A. lobicornis* (RD) and adjacent soils (S). (*a*) Total number of individuals $\text{cm}^{-2}$, (*b*) proportion of nymphs and (*c*) proportion of winged individuals. Different lower case letters indicate statistically significant differences between substrates (MANOVA, $p < 0.05$).

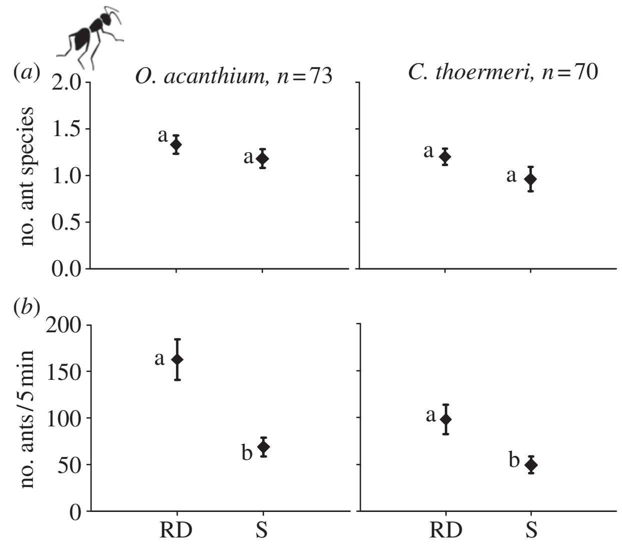

Figure 3. Species richness and abundance of tending ants in *O. acanthium* and *C. thoermeri* thistles growing on refuse dumps of *A. lobicornis* (RD) and adjacent soils (S). (*a*) Mean (± s.e.) number of ant species and (*b*) mean number (± s.e.) of tending ant individuals per 5 min. Different lower case letters indicate statistically significant differences (ANCOVA, $p < 0.05$).

was strongly affected by both substrate type ($F_{1,138} = 4.38$, $p < 0.05$) and thistle species ($F_{1,138} = 10.60$, $p < 0.01$, figure 3*b*). Individuals of *O. acanthium* and *C.*

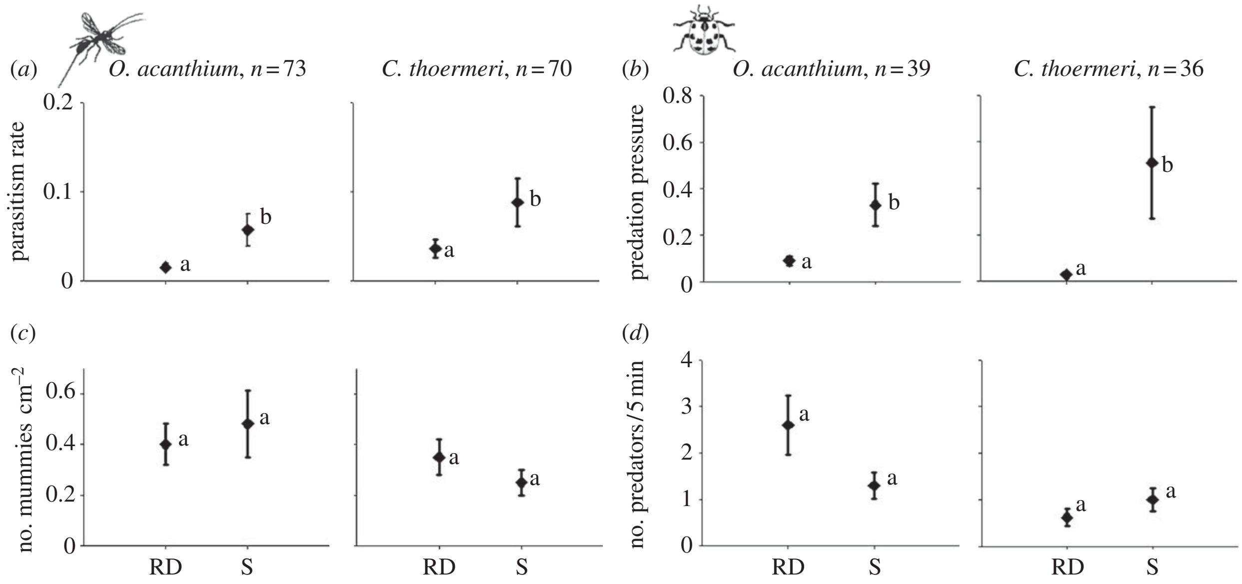

Figure 4. Attack pressure and abundance of aphid natural enemies. Mean (± s.e.) of (*a*) parasitism rate (mummy density/aphid density), (*b*) predation pressure (aphid predator abundance/aphid density), (*c*) density of mummies (number of mummies $\text{cm}^{-2}$) and (*d*) aphid predator abundance (number of predators per 5 min). Different lower case letters indicate statistically significant differences (ANOVA, $p < 0.05$ for parasitoid variables; and ANCOVA, $p < 0.05$ for predator variables).

*thoermeri* on refuse dumps had 136 and 98 per cent more ant activity, respectively, than those growing on adjacent soils. Particularly, the substrate on which thistles grew only influenced the activity of *B. patagonica* and *D. tener* ($F_{1,53} = 5.44$, $p < 0.05$ and $F_{1,48} = 8.33$, $p < 0.01$, respectively; see electronic supplementary material D, figure D2). However, when plant height was entered as covariate, only *B. patagonica* kept a differential activity between substrates ($F_{1,52} = 3.4$, $p = 0.07$).

The parasitism rate and the predation pressure on aphids varied with the thistles' substrate ($F_{1,137} = 9.9$, $p < 0.01$ and $F_{1,73} = 12.55$, $p < 0.001$, respectively) but were similar in the two thistle species ($F_{1,137} = 2.2$, $p = 0.14$ and $F_{1,73} = 0.22$, $p = 0.63$, respectively). Aphids on *O. acanthium* and *C. thoermeri* individuals growing on refuse dumps had 73 and 59 per cent lower parasitism rates, and 73 and 94 per cent lower predation pressures, respectively, than those on thistles growing on adjacent soils (figure 4*a*,*b*). Substrate type did not affect mummy density ($F_{1,137} = 0.18$, $p = 0.66$) or aphid predator abundance ($F_{1,73} = 0.005$, $p = 0.94$), but the latter was influenced by thistle species ($F_{1,137} = 9.9$, $p < 0.01$). Thistles on both substrates had low densities of mummies (approx. 0.4 mummies $\text{cm}^{-2}$ per plant; figure 4*c*) and aphid predator abundance was low too, but slightly higher in *O. acanthium* (average of 2.5 predators per 5 min per plant) than in *C. thoermeri* (on average, 1 predator per 5 min per plant; figure 4*d*). We found no significant association between aphid parasitism rate and ant activity ($Rs = -0.03$, $p = 0.67$, $n = 140$), whereas aphid predation pressure and ant activity were negatively associated ($Rs = -0.28$, $p < 0.05$, $n = 78$; see electronic supplementary material D, figure D3*b*,*d*). Finally, parasitism rate as well as predation pressure were negatively associated with aphid density ($Rs = -0.26$, $p < 0.001$, $n = 140$, and $Rs = -0.19$, $p = 0.08$, $n = 78$, respectively; see electronic supplementary material D, figure D3*a*,*c*). Interaction terms (i.e. substrate × thistle species) were not statistically different in all the analyses performed.

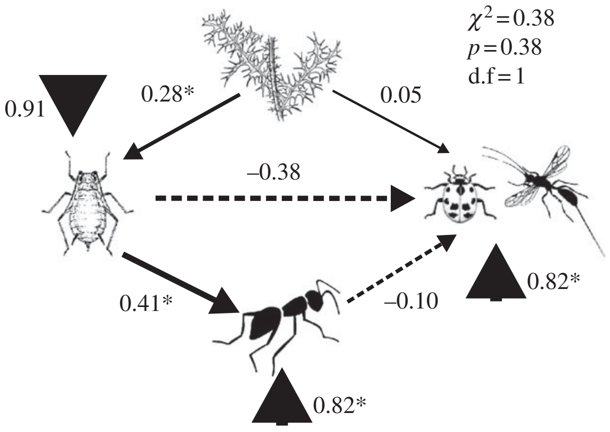

Figure 5. Path analysis of the relationship between number of thistle leaves, aphid density (no. of aphids $\text{cm}^{-2}$), ant abundance (no. of ants per 5 min) and attack pressure of aphid natural enemies (parasitism rate + predation pressure). Arrows represent direct causal effects. Coefficients are given for each path, and their value is also represented by arrow line thickness (the thicker the arrow, the higher coefficient value). Solid lines indicate positive effects; dashed lines indicate negative effects. Asterisk indicates significant path coefficients ($p < 0.05$). Unexplained variability is indicated with vertical arrows. The adequacy of models was evaluated based on model $\chi^2$, and associated *p*-values show direct causal effects.

Although not all coefficients were statistically significant, the path analysis summarizes well the relationships among thistles, aphids, tending ants and attack pressure of aphid natural enemies (figure 5). In general, the number of thistle leaves had a direct and positive effect on aphid density and, through this path, an indirect and positive effect on ant abundance. The attack pressure was influenced negatively and directly by aphid abundance and negatively and indirectly via tending ants.

### (c) *Field experiments*

A week after thistles growing on adjacent soils were fertilized with urea (increased quality to mimic the scenario on refuse dumps) the relative abundance of aphids increased by 40 per cent ($Z = 2.2$, $p < 0.05$, $n = 15$). Conversely, the relative abundance of aphids on unfertilized plants growing on control soil did not change after a week ($Z = 0.91$, $p = 0.36$, $n = 15$). When we experimentally reduced thistle biomass on refuse dumps (via pruning), aphid abundance showed a 30 per cent decrease ($Z = 2.37$, $p < 0.05$, $n = 13$). Thistles growing on refuse dumps without pruning did not show significant changes in aphid density throughout the experiment sampling time ($Z = 1.4$, $p = 0.16$, $n = 13$). Finally, one week after the experimental increment of aphid density (through 'aphid addition' treatment), ant activity increased by 150 per cent compared with values found before treatment application ($Z = 2.84$, $p < 0.01$, $n = 11$).

## 4. DISCUSSION

Refuse dumps from leaf-cutting ants are small-scale disturbances that enhance the availability of soil nutrients, and this direct effect on plants may propagate upward along a food chain, affecting herbivore populations and then higher trophic levels [5]. However, certain characteristics of each system may dilute or interrupt this bottom-up cascading effect. Our study, by using stable isotopes, clearly demonstrated that the nutrient increase generated by *A. lobicornis* propagates upward through the studied food chain. Earlier studies have shown that plants can benefit from the high nutrient content of refuse dumps from leaf-cutting ants [22,36]. The present study and a previous one [23] are the first evidence that the enhanced resource availability generated by leaf-cutting ants affects not only plants but also upper levels in a food chain. Here, we showed that growing on refuse dumps increases the biomass of *C. thoermeri* and *O. acanthium*, which leads to an increased abundance of aphids that feed on them. These modifications did not affect the structure of tending ant assemblage, but increased ant activity. In contrast with the expectations under the typical bottom-up cascade effects, the increase in aphid abundance did not positively impact on aphid natural enemies. Thistles on refuse dumps had similar density of mummies (parasitized aphids) and predator abundance to those growing on adjacent soils. Conversely, aphids on refuse dump thistles had lower parasitism rate (mummy density/aphid density) and reduced predation pressure (predator abundance/aphid density) than those infesting thistles on adjacent soil. This illustrates how changes in resource availability at the base of a food chain can trigger a bottom-up cascade and positively impact on other trophic levels, but also that the sign and strength of this effect may change along their spread through the trophic chain.

Our results and previous ones [20,22,23] show that growing on a better substrate increases the nutrient content and the abundance of the thistle species *C. thoermeri* and *O. acanthium*, which is consistent with results documented for a variety of plant species [37,38]. This enhanced plant quality (leaf nutrient content) and quantity (enhanced number of leaves, stems and inflorescences) in both thistle species occurs because soil nutrient content in refuse dumps of *A. lobicornis* is two to eight times higher than in natural soils [20], and plants on refuse dumps are able to use nutrients more efficiently [22,36]. These changes in plant quality and quantity should affect the abundance of insect herbivores that feed on thistles. Larger plants may sustain higher herbivore densities or be more attractive for female's oviposition [39], and enhanced plant quality improves herbivores' development rate, survival and/or fecundity, thus increasing their abundance [40,41]. Accordingly, we found that refuse dump thistles had higher aphid density than those established on adjacent soils. The results of our field manipulation of plant traits suggest that both enhanced plant quality and increased plant quantity explain the increased aphid population size. The latter probably occurs via an increase in reproductive rate, as suggested by the greater proportion of aphids in nymphal instars observed in both thistle species when growing on refuse dumps.

Enhanced aphid populations in thistles established on refuse dumps did not influence the species richness of tending ants, but increased their activity. Usually, only one tending ant species occurs in a thistle with aphids, regardless of the substrate. Ant territorial behaviour probably prevented increased aphid density from leading to the coexistence of several ant species on a single plant. Supporting this idea, several studies have shown that aggression, territoriality and competition are key factors structuring ant communities [17,42]. Moreover, Davidson [43] suggests that excess of carbohydrates, resulting from a diet rich in honeydew of Homopterans, is used by ants to defend their territory via offensive and defensive chemical weapons; this does not allow the coexistence of a richer ant assemblage. Enhanced aphid populations result in higher ant activity in refuse dump thistles. In agreement with this, other studies have shown that changes in host plant traits that affect aphid abundance influence their mutualistic ants [42,44]. However, the increase in tending ant activity depended on ant identity. Among the four ant species found in the study system, only *B. patagonica* and *D. tener* increased their activity in aphid-infested thistles growing on refuse dumps of *A. lobicornis*. Other factors besides aphid abundance may also affect ant foraging activities, such as the distance of ant colonies to aphid host plants [45], and the proximity and abundance of other plants hosting aphids [46], among others.

An increase in aphid abundance might enhance the abundance of aphid natural enemies, such as predators or parasitoids. However, the relative abundance of aphid natural enemies (measured as density of mummies and activity of predators) was similar between plants growing on refuse dumps (with high aphid density) and plants growing on adjacent soils (with low aphid density). These results are in contrast with the expectations under the classical food chain modelled by bottom-up forces, where increments in the abundance of basal trophic levels should translate into increments in higher trophic levels ([47] and references therein). Several mechanisms could explain why an increase in aphid density on thistles did not benefit predators or parasitoids. First, it has been shown that when resources are more abundant or more nutritious, ants may respond more aggressively to a threat [18,19]. In the study system, increments in aphid densities represent increased resource availability for tending ants, and thus ants would defend aphids more efficiently. Therefore, aphid natural enemies may not take advantage of an increased abundance of their prey. A decrease in predation pressure with increasing ant activity and the low parasitism rates at higher levels of ant activity support this idea. However, we did not find a strong negative relationship between ant activity and parasitism rate, as expected if ants effectively protect aphids against their natural enemies. This may be related to differences in protection effectiveness of different ant species. Second, if aphid natural enemies cannot respond to a significant increase in prey abundance, then enhanced growth of aphid populations may explain *per se* the lack of positive response of predators and parasitoids. Aphid natural enemies may not be able to exploit food resources beyond a given amount ('consumer satiation' [9]) or may have a low capacity to transform consumed energy into offspring ('low consumer efficiency' [8]), or other intrinsic factors, like the degree of specificity of the natural enemies, could limit their capacity to respond to increased resource availability. For example, the ladybird *Adalia bipunctata* (Coccinelidae) responds to increases in prey abundance by augmenting oviposition up to a maximum above which it is satiated, and egg production rate is constant and independent of prey abundance [48]. We found that parasitism rate and predation pressure are lower in thistles growing on refuse dumps, which had higher aphid density, than in thistles on adjacent soils; and that both parameters declined as aphid density increased. Third, low spatiotemporal predictability in aphid availability, owing to ephemeral aphid colonies and idiosyncratic phenology and life cycle of thistles, may hamper the detection of food resources by aphid natural enemies.

Trophic cascade theory proposes that changes generated by a disturbance at the base of a food chain controlled by bottom-up forces can spread upward and affect organisms in all trophic levels in the 'same sense' [5,6]. In this scenario, nutrient-rich refuse dumps of *A. lobicornis* should benefit all organisms belonging to higher trophic levels of the food chain. This was true for thistles growing on refuse dumps, aphids that colonized these thistles, and ants that tended these aphids, but not for aphid natural enemies. In this system, both branch-offs and resistances appear to alter bottom-up cascading effects. Particularly, the increased activity of tending ants, which could defend aphids against their natural enemies, and the low capacity of natural enemies to respond to a great increase in prey density, may affect both the strength and course of bottom-up disturbances. This illustrates how particularities of each system, such as mutualisms and the response capacity of top predators, may affect how disturbances that increase soil resources spread along trophic chains.

This research was partially funded by CONICET (PIP 1320) and ANPCyT (PICT 25314) to AGFB. The Biology Department of Penn State University provided travel support to T.A.C. and for stable isotope analyses. Comments from two anonymous reviewers greatly improved this manuscript.

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