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Effects of leaf-cutting ant refuse on native plant performance under two levels of grazing intensity in the Monte Desert of Argentina

Noelia V. Cerda, Mariana Tadey, Alejandro G. Farji-Brener & María C. Navarro

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Cerda, N. V. (noelia_lra@hotmail.com) & **Navarro, M. C.** (mcecilianavarro@gmail.com): Universidad Nacional del Comahue, Escuela Superior de Salud y Ambiente, Licenciatura en Saneamiento y Protección Ambiental, 8300, Neuquén, Argentina
Tadey, M. (corresponding author, mtadey@conicet.gov.ar): Lab. Ecotono, CRUB, Universidad Nacional del Comahue, 8400, Bariloche, Argentina
Farji-Brener A. G. (alefarji@yahoo.com): Lab. Ecotono, Department of Ecology, CRUB - Universidad Nacional del Comahue, CONICET – INIBIOMA, 8400, Bariloche, Argentina

Abstract

Question: Low quantities of soil nutrients often restrict plant establishment and growth in arid lands and have been partially attributed to the scarcity of organic matter in these ecosystems. Refuse dumps from leaf-cutting ants are a natural source of organic matter; however, their effects on native plant performance have received limited attention to date. Do refuse dumps from leaf-cutting ants (*Acromyrmex lobicornis*) enhance the germination and growth of several native plant species and what is the potential influence of heavy grazing on this practice?

Location: Monte Desert, Neuquén Province, Argentina.

Methods: We collected fruits of five plant species and two types of substrate (ant refuse dump vs control soil) from two known paddocks with different livestock densities (high vs low). We sowed seeds, previously weighed, of each species in both substrates from both paddocks and monitored their development. We harvested emerged seedlings, documenting their age and measured their height, weight, number of leaves, root weight and root length.

Results: Seed weight was lower in the highly grazed paddock for all the plant species studied. However, seed weight did not affect germination rate. Refuse dumps from the less grazed paddock (i.e. with higher nutrient content) enhanced the vigour of seedlings from smaller seeds more often than those from the highly grazed paddock. Also, germination improved when both seeds and substrate were from the less grazed paddock. Vigour variables showed more complex results, but seedlings growing in refuse dumps tended to be more vigorous. Refuse dumps from the less grazed paddock (i.e. with higher nutrient content) enhanced the vigour of seedlings from the highly grazed paddock (smaller) more than the seeds from the less grazed paddock (larger).

Conclusions: Our results demonstrated that ant refuse dumps increased plant germination rate and improved performance of the most representative vegetation in the Monte Desert. Given that external refuse dumps from leaf-cutting ants are a renewable resource, very abundant and easy to collect, this substrate could be used as a free natural fertilizer in arid regions to restore and manage vegetation cover, especially in heavily grazed sites.

Introduction

Soil nutrients often restrict plant establishment and growth in arid lands (Mun & Whitford 1989; Satti et al. 2003). The low concentration of soil nutrients in desert soils has been partially attributed to the low quantity of organic matter, which results in low rates of mineralization (Fisher 1928). Therefore, the study of natural sources of organic matter and its effects on plant species performance may contribute to understanding the dynamics of vegetation maintenance in dry regions (Schlesinger et al. 1990).

Leaf-cutting ants are considered a major contributor of organic matter into arid ecosystems because they build big, long-lived nests that produce high amounts of organic

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waste. These ants collect huge quantities of fresh vegetation from a large area and carry them into their nest chambers where the plant material is degraded by a mutualistic fungus (Weber 1982). The waste material obtained from the fungal decomposition as well as dead ants and debris are removed from the fungus gardens to specific external or internal disposal areas (hereafter, refuse dumps). Refuse dumps are richer in organic carbon and nutrients than adjacent soils, generating nutritional hot-spots in the nest area (Farji-Brener & Illes 2000). This high availability of nutrients often increases plant abundance, diversity and productivity, and accelerates nutrient cycling in several ecosystems, including arid regions (Farji-Brener & Silva 1995; Farji-Brener & Ghermandi 2004, 2008; Haines 1978; Lugo et al. 1973; Moutinho et al. 2003). Hence, leaf-cutters may positively affect plant performance in deserts through their contribution to soil fertility.

Despite the recognized positive effects of refuse dumps on soil fertility, two factors remain almost unexplored experimentally: their effects on native plant performance and those factors that influence the quality of refuse dumps as natural fertilizer. There are few experimental studies of the effects of refuse dumps on plant performance, and results are conflicting. For example, recent studies showed that refuse dumps consistently increased the growth of exotic plant species, but native plant species showed a more heterogeneous growth response (Farji-Brener & Ghermandi 2004, 2008; Farji-Brener et al. 2010). Consequently, more experimentation is needed to determine to what extent and how refuse dumps may enhance native plant performance and establishment.

In addition, there is poor understanding of the factors that influence the nutrient content of refuse dumps and, therefore, the quality of this natural source of organic matter. This point is relevant for both basic and applied ecology. An understanding of which factors decrease the quality of refuse dumps may help to recognize variations in their effects on plant performance and improve their potential use as a natural fertilizer in restoration practices. Previous studies showed that the nutrient content of ant refuse dumps depends on the quality of the vegetation harvested by the ants, which in turn depends on the abundance of high nutrient quality plant species in the area (Bucher et al. 2004).

Heavy grazing appears to be one of the most relevant factors that indirectly reduces the quality of leaf-cutting ant refuse dumps. Grazing by exotic mammals often reduces the cover of palatable vegetation (Cingolani et al. 2005; Milchunas et al. 1988; Watkinson & Ormerod 2001), especially in arid lands, where vegetation development is water-limited. This consumption of highly preferred vegetation by livestock often shifts plant assemblages towards those with lower quality litter (Bestelmeyer 2006; Mazzarino et al. 1998; Rietkerk & van de Koppel 1997). The reduction in the availability of nutrient-rich vegetation also impacts on the diet composition of other organisms that use plants as food resources, such as leaf-cutting ants (Tadey & Farji-Brener 2007). This indirect effect of heavy grazing on the nutrient content of ant refuse dumps was recently documented in one of the larger arid regions of Argentina, the Monte Desert. In this region, heavy grazing strongly reduces plant richness and cover (Tadey 2006). This detrimental effect on plants affects the foraging activity of *Acromyrmex lobicornis* Emery, the only leaf-cutting ant species that inhabits the Monte Desert. As stocking rate increases, the number of plant species harvested by ants and the nutrient content of their external refuse dumps decreases (Tadey & Farji-Brener 2007). For example, an increment in the grazing intensity of only 0.06 individuals/ha has caused decreases in nutrient content of the ant refuse dumps of 50% nitrogen, 100% carbon and 200% phosphorus (Tadey & Farji-Brener 2007). These results suggest that livestock reduces plant richness and cover through grazing, affecting ant diet with the subsequent alteration of the nutrient content of their external refuse dumps. However, the consequences of this quality impoverishment of the refuse dumps as natural fertilizer on native plant performance remains untested.

In the present work, we experimentally determined whether the nutrient-rich organic waste of leaf-cutting ants enhances the germination and growth of several native plant species from the Monte Desert of Argentina, and analysed the influence of heavy grazing on this effect. We experimentally compared the effects of refuse dumps with known differences in quality on native plant species, collected from paddocks with low and high livestock abundance, having low (0.002 cattle ha^{-1}) and high (0.031 cattle ha^{-1}) grazing intensities, respectively.

Methods

Study site

The study area was situated in Neuquén province (39º17′S, 68º55′W), northwest Patagonia, Argentina. This is a temperate arid region with mean annual precipitation of 200 mm and mean annual temperature of 15°C (meteorological station of EL Chocón; see Tadey 2006 for more details). The vegetation of this area is a xerophytic scrubland dominated by *Larrea cuneifolia* (Cavanilles) and *L. divaricata* (Cavanilles) associated with other shrubs including *Atriplex lampa* (Gillies ex Moquin) D. Dietrich, *Bougainvillea spinosa* (Cavanilles) Heimerl, *Monttea aphylla* (Miers) Berthan et Hooker and *Prosopis alpataco* (Philippi) (Correa 1969–1998). Grasses represent <6% of plant cover, mostly *Stipa* sp.

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Acromyrmex lobicornis Emery is the only leaf-cutting ant species that inhabits Patagonia (Farji-Brener & Ruggiero 1994), and their nests are important components of this arid ecosystem (up to 180 nest ha^{-1}) (Tadey & Farji-Brener 2007). *A. lobicornis* nests reach depths of 1 m, and on the soil surface the ants construct a mound of twigs, soil and dry plant material above ground, which may reach 1 m in height and width. Inside the mounds, ants tend the fungus on which ant larvae feed. Refuse material is removed from the internal fungus garden and dumped on the soil surface. This refuse is regularly deposited in a flat pile on the soil surface near the mound, which makes it accessible to nearby plants and a renewable and convenient substrate to collect for restoration practices.

Methodology

During the spring of 2001, we selected two previously studied paddocks that differ in grazing intensity (high and low livestock abundance, see Tadey 2006) but with comparable climate, plant assemblage composition and edaphic characteristics (Del Valle 1998; León et al. 1998). Livestock composition was a mixture of cattle (*ca.* 40%), goats (*ca.* 50%) and horses (*ca.*10%). Livestock density was expressed as cattle ha^{-1} (1 horse = 1.25 cow, 1 goat = 0.17 cow and 1 sheep = 0.3 cow; see Vallentine 2001). Sampling was performed between October 2007 and March 2008. The paddock with the lower livestock density (hereafter, LOW) had 0.002 cattle ha^{-1} and the paddock with the higher livestock density (hereafter, HIGH) had 0.031 cattle ha^{-1}. Despite these stocking rates being relatively low compared to other systems, 0.03 cattle ha^{-1} has been shown to have a significant impact on vegetation in the study area (Tadey 2006). Paddocks were separated by approximately 40 km and have a grazing history of 20 ± 5 years (mean ± 1 SE). Paddocks did not differ in bare soil nutrient content (LOW: 0.24 ± 0.02% organic carbon (OC); 0.02 ± 0.0001% nitrogen (N); 4.6 ± 0.38 mgP kg^{-1} vs HIGH: 0.18 ± 0.04% OC; 0.02 ± 0.002% N; 3.1 ± 0.92 P mg kg^{-1}; mean percentage ± 1 SE are from Tadey (2006). Refuse dumps differed significantly in nutrient concentration between paddocks and soil (LOW: 16.2 ± 5.6% OC; 0.84 ± 0.4% N; 214,1 ± 127.9 mgP kg^{-1} vs HIGH: 9.6 ± 3.1% OC; 0.53 ± 0.17% N; 134.8 ± 117.9 mgP kg^{-1}, mean percentage ± 1SE see Tadey & Farji-Brener 2007 for more details). In each paddock, we sampled vegetation and ant nests within an area of *ca.* 6 ha located 300 m from the highway and more than 3 km from the house. Within a paddock, livestock grazed freely during the year and, given that paddocks lacked water points, grazing intensity was mainly uniform within each paddock.

Sampling of nests and adjacent soil

We randomly selected ten ant nests of *A. lobicornis* in each paddock and collected samples of their refuse dump material (hereafter RD) and adjacent non-nest soil. This material was used as a substrate for sowing seed of the selected plant species. Non-nest bare soil was collected, taking five sub-samples in different directions within a radius of 3 m from the ant nest located at the centre. Each soil sub-sample was collected at a depth from *ca.* 0 to 20 cm. Both substrates (RD and soil) were sterilized in a heat cabinet for 1 week at 70°C, to inactivate any seeds present. We collected and sterilized a total of 40 samples, 20 of RD and 20 of bare soil (ten nests per paddock).

Plant species selection and fruit sampling

We selected five plant species (*Monttea aphylla, Atriplex lampa, Gutierrezia solbrigii, Larrea divaricata* and *Chuquiraga erinacea*) to study the effect of RD on seed germination. The species were present in both paddocks and are representative of Monte vegetation (see Tadey 2006). At the end of the summer, we collected several matured fruits from ten plants per species from each paddock. We selected a sub-sample of 4000 seeds (800 seeds $species^{-1}$) to sow in each substrate (RD and soil) from the paddocks with LOW and HIGH livestock density. We prepared 200 pots per paddock, 100 with RD and 100 with soil (i.e. 400 pots, 200 with substrate from the LOW paddock and 200 with substrate from the HIGH paddock). We weighed the seeds of each plant species and sowed ten seeds per pot. We used plastic pots of 9-cm diameter and 15-cm depth for sowing. Sowing under near-natural conditions started in autumn and we observed seedling growth for a period of 1 year. Pots were watered at least once a week to maintain humidity and facilitate germination. At least 1 month after emergence, we collected the seedlings and measured vigour variables. We estimated germination rate as the number of seedlings emerged with respect to the number of seeds sowed per pot. We calculated seedling age (in days) from the time of harvesting, as not all seedlings appeared at the same time.

Statistical analysis

Livestock may indirectly affect seed traits through their effects on plants, and different plant species may respond differently to this pressure. Hence, we analysed the effect of seed origin (seeds from LOW and HIGH paddocks) and plant species (*Monttea aphylla, Chuquiraga erinacea, Atriplex lampa, Gutierrezia solbrigii* and *Larrea divaricata*) on seed weight using a two-way ANOVA. Both seed origin and plant species were considered as fixed factors as their selection was not random. We used plant species as a fixed factor to control for species effect.

To compare the germination level and seedling vigour in the different substrates (RD and soil) and how these may be affected by seed origin (LOW and HIGH paddocks), substrate origin (LOW and HIGH paddocks) and plant species (factors), we used two different general linear factorial ANOVA models (GLM; with four factors, one and two co-variables, and four interactions). The ANOVA performed on germination also considered the possible effects of seed weight as a co-variable, whereas the ANOVA used to analyse vigour variables considered both seed weight and seedling age (days since emergence), as co-variables, as not all the seedlings survived for the same time period. Germination was measured as the number of visible seedlings in the pot (each pot had ten seeds). Vigour variables measured were: number of leaves produced, height of seedling, length of main root, seedling weight (aerial part) and root weight. All data were log transformed ($\log_{10} = x + 1$) to meet ANOVA assumptions. As a consequence of low germination rates (see results), only some of all the possible ANOVA interactions were tested. We tested the following interactions in both ANOVAs: species × substrate; substrate × substrate origin; substrate × seed origin and substrate origin × seed origin.

Results

Seed weight

The mean (± SE) seed weight from the HIGH paddock was significantly lower than the LOW paddock (0.109 ± 0.008 g vs 0.13 ± 0.01 g, respectively, $F_{1,\ 389} = 162$, $P < 0.001$). The different species had different seed weights, as expected ($F_{4,\ 389} = 9011.51$, $P < 0.001$). Generally, all the species studied had heavier seeds in the LOW paddock (i.e. interaction between seed weight x origin; $F_{4,\ 389} = 45.6$, $P < 0.001$), except for *L. divaricata* and *G. solbrigii*, which had similar seed weights in both paddocks.

Co-variables

Seed weight and seedling age were weakly, positively associated ($R^2 = 0.06$, $F_{1,\ 91} = 6.18$, $P < 0.015$). Seed weight affected germination ($F_{1,8} = 6.64$; $P = 0.03$) but had no effect on the measured vigour variables (log leaves, log seedling height, log root length, log root weight and log seedling weight; all $P > 0.24$). Seedling age affected all of the vigour variables measured (all variables $P < 0.02$).

Germination

Only 93 seeds germinated from a total of 4000 seeds sown (*ca.* 2.5%). Germinated seeds were distributed among plant species as follows: 35 *C. erinacea*, 37 *A. lampa*, 13 *G. solbrigii*, 8 *L. divaricata* and no *M. aphylla*). Germination rate showed a complex interaction with the studied factors. Germination rate was similar between plant species ($F_{3,8} = 2.4$, $P = 0.15$), seeds from different origin (5.8 ± 1.1% vs 2.9 ± 0.5%; LOW vs HIGH paddock, respectively) and substrates from different origins (4.8 ± 1.2% vs 3.8 ± 0.6%; LOW vs HIGH paddock, respectively; all $P > 0.15$). Nonetheless, germination rate was significantly higher in RD than in non-nest soils ($F_{1,8} = 7.64$; $P = 0.025$; Fig. 1) for all plant species (substrate x species; $F_{3,8} = 1.57$; $P = 0.27$) and independent of seed origin (seed origin x substrate; $F_{1,8} = 0.03$; $P = 0.86$, Fig. 1). The highest germination rate was found in seeds from the LOW paddock growing in the substrate from the LOW paddock (i.e. seed origin x substrate origin; $F_{1,8} = 12.2$; $P = 0.008$; Fig. 2) and marginally significant in the RD from the LOW paddock (substrate x substrate origin; $F_{1,8} = 4.57$; $P = 0.06$; Fig. 1).

Vigour

Plant species also differed in their seedling vigour (all $P < 0.001$, except for root weight $F_{3,78} = 1,13$; $P = 0.34$). Seedling performance was not affected by substrate type (although all vigour variables had higher means in RD than in soil; sign test, $P = 0.06$; Table 1), or by substrate or seed origin in any of the studied species (all $P > 0.12$, except that seedling height was higher on the substrate from the HIGH paddock; $F_{1,78} = 7.03$, $P < 0.01$, see Table S1-2 in Supporting Information). Substrate and seed origin did not interact with substrate type (all $P > 0.10$; Table S2). However, vigour was enhanced when substrate origin interacted with seed origin. Seeds from the HIGH paddock showed more vigour when grown in substrate from the LOW paddock, whereas seeds from the LOW paddock showed similar growth for both substrate origins ($F_{\text{seedling weight}(1,78)} = 5.14$, $P = 0.03$; $F_{\text{root weight}(1,78)} = 14.03$, $P = 0.0003$, $F_{\text{root length }(1,78)} = 3.63$, $P = 0.06$; $F_{\text{seedling height }(1,78)} = 3.12$, $P = 0.08$, and $F_{\text{number of leaves }(1,78)} = 2.49$, $P = 0.12$; Fig. 3).

Discussion

Organic matter and nutrient availability often limit plant establishment and development in arid regions (Mazzarino et al. 1998). Here we demonstrate that the refuse dumps (RD) of leaf-cutting ants improved the germination rate and plant performance of several native plant species, and we describe the effect of heavy grazing on this process. These results have basic and applied implications. First, they experimentally demonstrate that leaf-cutting ants, through their positive effect

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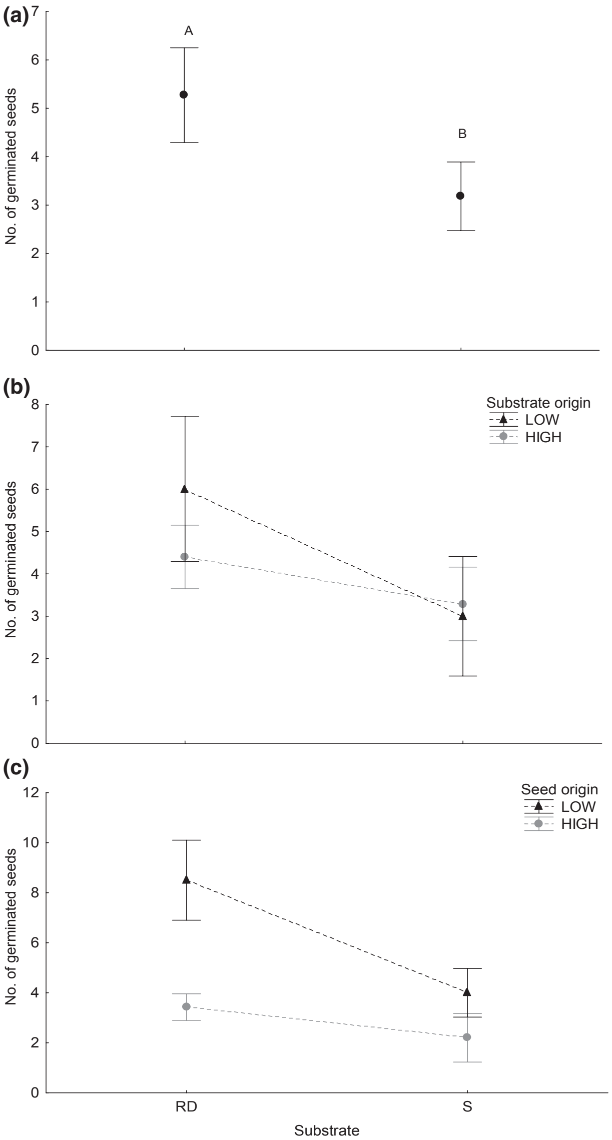

Fig. 1. (**a**) Mean number of germinated seeds (± SE) sown in each substrate (RD: refuse dump; S: soil). (**b**) Mean number of germinated seeds ± SE sown in each substrate per substrate origin (i.e. substrate from the less grazed paddock: LOW; and substrate from the highly grazed paddock: HIGH). (**c**) Mean number of germinated seeds (±SE) sown in each substrate per seed origin (i.e. seeds from the less grazed paddock: LOW; and seeds from the highly grazed paddock: HIGH). Different capital letters denote statistical difference (Tukey post-hoc test, $P < 0.05$).

on soil fertility, can indirectly benefit plant establishment and vegetation cover in arid communities. Second, given that external refuse dumps from leaf-cutting ants are a renewable resource that is easy to collect, our results suggest that this substrate is a potential candidate for use as a natural, free fertilizer in arid regions to restore and manage vegetation cover, especially in heavily grazed sites.

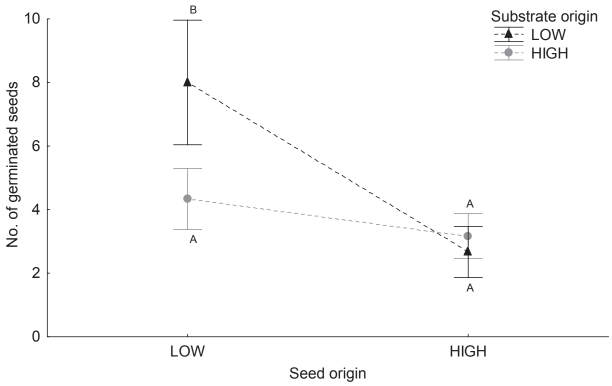

Fig. 2. Interaction between seed origin and substrate origin on germination rate. The mean number of germinated seeds (± SE) for each seed and substrate origin is shown (i.e. LOW: seeds/substrate from the less grazed paddock; HIGH: seeds/substrate from the highly grazed paddock); Capital letters denote statistical difference from a Tukey post-hoc test ($P < 0.05$).

Refuse dump material significantly enhanced the germination rate in all of the native plant species studied. The physical and chemical properties of RD appeared to improve seed germination. First, RD from leaf-cutting ants always had a higher nutrient concentration and organic matter content than adjacent soils (Farji-Brener & Ghermandi 2000, 2004). Seeds can utilize these high nutrient concentrations in the soil and increase their germination and seedling establishment, for instance through the breaking of seed dormancy by N (Hilhorst & Karssen 1989; Pérez-Fernández et al. 2000a; Rodríguez-Echeverría & Pérez-Fernández 2001). Second, RD retains more water than control soils (Farji-Brener & Ghermandi 2004, 2008) and water accessibility is a key factor that influences seed germination (Pérez-Fernández et al. 2000b, 2006). Because the presence of water and nutrients are both limiting factors in semi-arid regions (Mazzarino et al. 1998), RD provides an excellent substrate for plant germination.

Germination may also be affected by seed size. Bigger seeds may show higher germination rates due to a greater membrane integrity and increased energy availability in their endosperm (Zaidman et al. 2010). We found smaller seeds in the paddock with high livestock density. This suggests that the known negative effect of heavy grazing on vegetation may have flow-on effects for the next generation of plants. However, seed origin only affected germination rate in combination with substrate origin; seeds from the LOW paddock growing in substrate from the same paddock had the highest germination rate. This suggests that seed weight may influence germination rate in the context of high nutrient availability (i.e. in RD). However, we cannot conclude this from our current work, as we were unable to determine the three-way interaction between

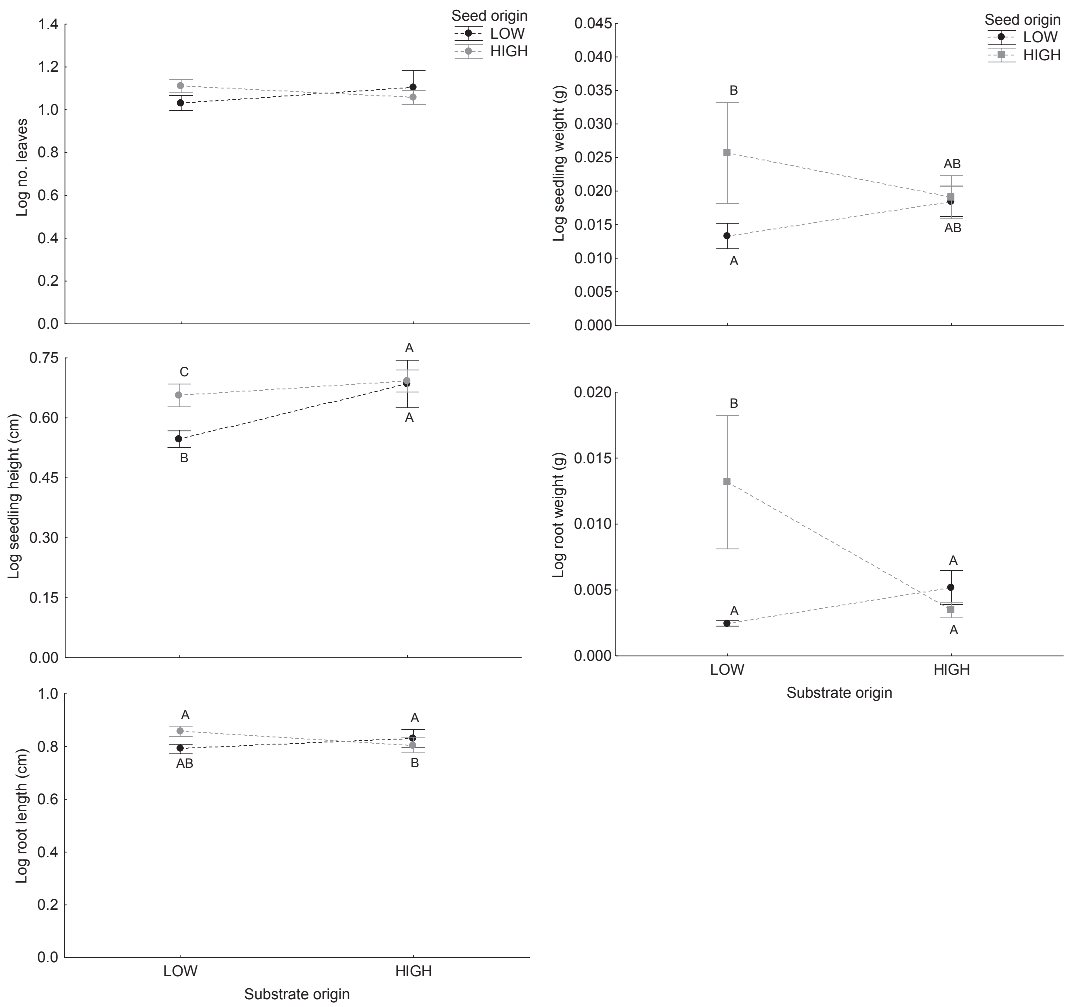

Fig. 3. Interaction between substrate origin and seed origin for five vigour variables measured on emerged seedlings. The figure shows the log-mean (±SE) of the variables for both substrate and seed origin (i.e. substrate/seeds from the less grazed paddock: LOW; and substrate/seeds from the highly grazed paddock: HIGH). Vigour variables are: number of leaves/seedling, log leaves; aerial height of seedling, log height; length of main root, log root length; aerial seedling weight, log seedling weight; and root weight, log root weight. Capital letters denote statistical difference (Tukey *post-hoc* test, $P < 0.05$).

seed origin, substrate origin and substrate due to low replication number (i.e. low number of emerged seedlings); although we do have indirect evidence supporting this suggestion. In accordance with this, we found that the highest germination rate occurred in refuse dumps that came from the less grazed paddock (i.e. RD with the higher nutrient content; interaction between substrate and substrate origin), while soil characteristics were similar in both paddocks (Tadey 2006). These two facts suggest that the RD substrate enhanced seed germination.

Surprisingly, vigour variables were not directly associated with the strong effect of RD detected on seed germination. The type of substrate did not significantly affect any of the five vigour variables examined. Nevertheless, seedlings growing in RD had higher mean values than those in soil for almost all of the variables measured (see Table 1). It is possible that we did not statistically detect the effect of RD on plant vigour because of low replication, as the percentage germination was only *ca.* 2.5% and was highly variable. The smaller seeds (i.e. those from the HIGH paddock) produced the most vigorous seedlings when grown in the substrate from the LOW paddock (i.e. with higher nutrient content), whereas larger seeds from the LOW paddock grew similarly regardless of substrate origin (from HIGH or LOW paddock), possibly because they have more nutrients in the endosperm (Zaidman et al. 2010). In other words, a high nutrient environment impacts more positively in smaller than in larger seeds. Smaller seeds may overcompensate when they grow in the nutrient-rich substrate, such as RD from the LOW paddock.

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Table 1. Log-mean (±SE) of five vigour variables measured on the seedlings growing in each substrate (RD: refuse dump, S: soil). Logleaves represents the logarithm of the number of leaves per seedling; log height is the height (cm) of the seedling; log root length, the length of the main root (cm); log seedling weight, aerial seedling weight (g) and log root weight, root weight (g). *N* is the number of replicates. Vigour variables often showed higher means in RD than in soil substrate (sign test, $N = 5$, $P = 0.06$).

Variable	Substrate		
	RD	Soil	RD>S
N	58	35	
Log leaves	1.11 ± 0.03	1.03 ± 0.03	+
Log height	0.64 ± 0.02	0.64 ± 0.02	=
Log root length	0.86 ± 0.01	0.81 ± 0.02	+
Log seedling weight	0.02 ± 0.003	0.01 ± 0.002	+
Log root weight	0.007 ± 0.002	0.003 ± 0.0002	+

Our results demonstrated that (1) RD from *A. lobicornis* nests improved germination rates and, potentially, establishment success of the most common native plants species of the Monte Desert of Argentina; and (2) heavy grazing reduces this potential positive effect. Particularly, this increment in plant performance is stronger if RD has higher nutrient content (e.g. if RD is sourced from areas with low grazing intensity) and when it is used to improve the germination and establishment of smaller seeds from seriously disturbed regions. Although more paddocks with contrasting stocking rates need to be sampled to confirm the effect of heavy grazing on the beneficial properties of ant refuse dumps on native plants, our data suggest that this may be particularly important in the Monte Desert region, where both plant germination and establishment are often restricted in terms of water and soil nutrient availability (Bertiller 1998). In this scenario, ant nests appear to be a more important source of organic matter and soil nutrients than other natural sources (e.g. plant litter). For example, in the study area RD is three- to17-fold richer in N and six- to 22-fold richer in OC than in the soil with plant litter found under vegetation, and between seven- and 50-fold richer than in bare soil (Carrera et al. 2005; Tadey & Farji-Brener 2007). Moreover, RD also retains water for longer periods than adjacent non-nest soils (Farji-Brener & Ghermandi 2004, 2008).

We confirmed that RD positively affected plants in near-natural conditions; however, this may also occur in natural situations. Other studies investigating *A. lobicornis* showed that plants that are naturally established in RD grow more and reproduce more successfully than those in adjacent non-nest soils (Farji-Brener & Ghermandi 2008). In our study area, we also observed that plants that naturally grow on RD produce more leaves and fruits (Tadey, M. unpub. data). Therefore, it is highly probable that the effect of RD we detected on plants growing in near-natural conditions may also occur in natural field conditions. Leaf-cutting ants are known for their defoliation capacity (Díaz et al. 1992) and are frequently catalogued as agroforestry pests (Weber 1982). However, we demonstrate that they also have the potential to aid in the germination and plant establishment of several native plant species. This nicely illustrates the twin ecological role of leaf-cutting ants in the regions that they inhabit.

In addition to the discussed ecological effects of leaf-cutter ants on native plants, our results have important applied implications. Leaf-cutting ants that have external refuse dumps are constantly depositing their organic waste onto the soil surface (Wirth et al. 2003), which creates a nutrient-rich substrate, providing a renewable resource that is easy to collect for restoration purposes. Ant nest density in the study area is high (61–180 nest ha^{-1}). In addition, a single leaf-cutting ant nest may produce up to 9.4 t of refuse material per year (Wirth et al. 2003). In summary, the high nutritional quality, positive effect on plants, great abundance and simplicity of collection make RD a potentially important natural fertilizer that could be used to improve germination and vegetation cover, especially in situations of elevated habitat degradation in arid lands.

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Supporting Information

Additional supporting information may be found in the online version of this article:

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Figure S1. Experimental design used for sowing.

Table S1. Mean ± SE for five vigour variables of seedlings growing in two different substrates with different seed origin, substrate origin and different plant species.

Table S2. Results from a general linear factorial ANOVA model performed to compare seedling vigour on different substrates with different seed origin, substrate origin and different plant species.

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