Leaf-cutting ant nests near roads increase fitness of exotic plant species in natural protected areas

Alejandro G. Farji-Brener* and Luciana Ghermandi

Laboratorio Ecotono, INIBIOMA-CONICET and Centro Regional Universitario Bariloche,
Universidad Nacional del Comahue, 8400 Bariloche, Argentina

Understanding the mechanisms that promote the invasion of natural protected areas by exotic plants is a central concern for ecology. We demonstrated that nests of the leaf-cutting ant, *Acromyrmex lobicornis*, near roadsides promote the abundance, growth and reproduction of two exotic plant species, *Carduus nutans* and *Onopordum acanthium*, in a national park in northern Patagonia, Argentina and determine the mechanisms that produce these effects. Refuse dumps (RDs) from ant nests have a higher nutrient content than nearby non-nest soils (NNSs); foliar nutrient content and their ^{15}N isotopic signature strongly suggest that plants reach and use these nutrients. Both species of exotic plants in RDs were 50–600% more abundant; seedlings had 100–1000% more foliar area and root and leaf biomass; and adult plants produced 100–300% more seeds than nearby NNS plants. Plants can thus gain access to and benefit from the nutrient content of ant RD, supporting the hypotheses that enhanced resource availability promotes exotic plant performance that could increase the likelihood of biological invasions. The two exotics produce an estimated of 8 385 000 more seeds ha^{-1} in areas with ant nests compared with areas without; this exceptional increase in seed production represents a potential threat to nearby non-invaded communities. We propose several management strategies to mitigate this threat. Removal efforts of exotics should be focused on ant RDs, where plants are denser and represent a higher source of propagules.

Keywords: *Acromyrmex lobicornis*; ant debris; biological invasions; *Carduus nutans*; *Onopordum acanthium*; resource availability hypothesis

1. INTRODUCTION

Exotic plant species represent one of the most serious threats to native biodiversity and ecosystem function worldwide (Mack *et al.* 2000). An increase in the density of exotic plants can affect the properties of the habitats they invade in multiple ways, including the loss of native biota (Meiners *et al.* 2001) and changes in biological interactions (Traveset & Richardson 2006), disturbance regimes (Mack & D'Antonio 1998) and ecosystems processes (Sperry *et al.* 2006). Therefore, understanding the mechanisms that affect the establishment and spread of exotic plants is a central concern for ecology, for conceptual as well as practical reasons.

Many different mechanisms that promote invasions have been proposed, including disturbances, the creation of empty niches, microclimate changes, reduced biotic resistance, propagule pressure, enemy release, increased resource availability and phenotypic plasticity (Davis *et al.* 2000; Holway *et al.* 2002; Keane & Crawley 2002; Kennedy *et al.* 2002; Levine *et al.* 2004; Lockwood *et al.* 2005; Richards *et al.* 2006). Although considerable progress has been made in identifying a conceptual framework for the factors that drive plant invasions (Shea & Chesson 2002; Dietz & Edwards 2006), the mechanisms associated are still little understood and rarely confirmed by rigorous testing. For example, among the 150 papers of biological invasions reviewed by Levine *et al.* (2003), less than 5% evaluated the mechanisms that promote invasions. In natural protected areas, it is critical to understand the invasion mechanisms, since managing strategies will differ among different causative mechanisms (Gelbard & Harrison 2005). For example, if biotic resistance prevents plant invasion, it is critical to protect the vegetation that confers this resistance; if natural habitats are less invaded due to their isolation, it is vital to control the introduction of alien seeds; and if the spread of exotics is promoted by an increase in resource availability, it is crucial to identify and manage the agent that enhances those resources.

Areas around roads offer a good scenario for the study of both the theoretical and applied ecology of invasions (Safford & Harrison 2001; Gelbard & Harrison 2003). Since roadsides are spots of establishment and a source of exotic propagules, these areas allow us to identify the mechanisms related to the dispersal, establishment and persistence of invading populations. Nevertheless, relatively few studies have used roadsides to experimentally test mechanisms of biological invasions. In one of the best exceptions, Gelbard & Harrison (2005) examined whether propagule limitation or environmental variation was responsible for the invasion of the yellow starthistle (*Centaurea solstitialis*) in grassland near roads. Their results demonstrated that increased native grass cover was the key factor that inhibited invasion. Hence, the creation of soil disturbances appears to promote invasions in sites near roads. Other recent studies found that the success of exotic species was facilitated by nutrient addition (Rickey & Anderson 2004; Leishman & Thomson 2005). Overall,

*Author for correspondence (alefarji@crub.uncoma.edu.ar).

this new evidence together with previous experimental studies (Burke & Grime 1996; Davis & Pelsor 2001; Brooks 2003) suggests that improved soil nutrient patches, in concert with disturbances that reduce native plant cover, may help explain the invasions by exotic plants.

In Central and South America, one of the most important sources of soil nutrient patches are leaf-cutting ant nests (Farji-Brener & Illes 2000). Leaf-cutting ants collect huge quantities of fresh vegetation and carry it into their nest where it is used to feed their fungus gardens. The fungus develops on this plant substrate and parts of the fungal colonies are fed to the developing ant brood. The waste material from the fungal decomposition is removed from the fungus gardens to the specific external or internal disposal areas (hereafter, refuse dumps (RDs)). RDs are several times richer in organic carbon and other nutrients than adjacent soils, which generate nutritive hot spots around nests in several ecosystems (Haines 1975; Farji-Brener & Silva 1995; Farji-Brener & Ghermandi 2000; Moutinho *et al.* 2003; Wirth *et al.* 2003). Accordingly, plants are often more abundant and vigorous when growing near ant debris, suggesting that they use the nutrient content of RDs (Haines 1975; Farji-Brener & Silva 1995; Moutinho *et al.* 2003; Wirth *et al.* 2003; Farji-Brener & Ghermandi 2004). Nevertheless, the direct consumption of nutrients by plants from ant RDs has rarely been confirmed (Sternberg *et al.* 2006). In addition, leaf-cutting ants are more common in disturbed than in undisturbed habitats (Vasconcelos & Cherrett 1995; Farji-Brener 2001), such as roadsides (Vasconcelos *et al.* 2006). The occurrence of this important source of soil nutrients in areas with usually high abundance of exotics like roadsides is likely to promote biological invasions. But the possibility that leaf-cutting ants promote the spread of exotic plant species has rarely been documented (Coutinho 1982), and the underlying mechanism has never been established.

Invasive plants are one of the most important threats to the native biota in northern Patagonia (Aizen *et al.* 2002; Vázquez 2002). In this region, exotic plants are particularly abundant on roadsides, suggesting that roadsides are key areas for their dispersion (Rapoport 1991). In addition, the only leaf-cutting ant species that inhabits Patagonia, *Acromyrmex lobicornis*, is found more frequently near roads (Farji-Brener 2000). Previous studies showed that exotic plant species were especially abundant in ant nest sites (Farji-Brener & Margutti 1997). Here we investigated whether the presence of *A. lobicornis* nests near roadsides influences the abundance and fitness of the two most common exotic plant species, *Carduus nutans* (nodding thistle) and *Onopordum acanthium* (Scotch thistle) in a natural protected area of northern Patagonia, Argentina. We first tested whether these exotic plants are more abundant, grow faster or increase their fitness when growing on ant RDs, and then conducted experiments to explore the mechanisms responsible for the above-described patterns.

2. MATERIAL AND METHODS
(a) *Study site and species*
The study was conducted in the Nahuel Huapi National Park, located in Patagonia, Argentina (41° S, 72° W). This national park is one of the largest in Argentina (approx. 750 000 ha) and protects a series of natural habitats along a remarkable environmental gradient. The climate of this area is dominated by an abrupt west-to-east decrease in precipitation (varying from 3500 mm yr^{-1} in the western zone of the park to 500 mm in the eastern zone over the course of 150 km) due to the rain shadow effect of the Andes on the passage of moist, Pacific air masses. Vegetation types reflect this climatic pattern, forming three major habitats along the west-to-east gradient: forest, shrub and steppe. Trees of *Nothofagus* species, which grow in sites of 3500–1500 mm of mean annual precipitation dominate the forest in the west. Semi-arid shrub vegetation and forest of *Austrocedrus chilensis* and *Nothofagus antarctica* grow along the foothill zone, in sites of 1800–1400 mm of mean annual precipitation. In the eastern zone, the steppe is mainly composed of xerophytic shrubs and herbs that grow in sites of 800–300 mm of mean annual precipitation (Correa 1969–1998). This study was performed in the steppe located at the east (driest) border of the National Park along road 237, the main paved access to the national park. The mean annual temperature in this site is 8°C and the mean annual precipitation is approximately 600 mm. Soils are sandy loam Andisols developed from volcanic ashes and Aridisols. The dominant vegetation is a mix of native species typical of Patagonian steppes (e.g. *Stipa speciosa*, *Mulinum spinosum*, *Imperata condensata* and *Plagiobothrys tinctorius*) and exotics (e.g. *Bromus tectorum*, *O. acanthium*, *C. nutans* and *Verbascum thapsus*; Correa 1969–1998).

Carduus nutans (Asteraceae; musk or nodding thistle) and *O. acanthium* (Asteraceae; Scotch thistle) are both natives of Eurasia but have successfully colonized Australia, New Zealand and the Americas, where they are considered as serious weeds and cause economic and ecological damage (Shea & Kelly 1998; Qaderi *et al.* 2002). Both the species (hereafter CN and OA, respectively) are biennial, monocarpic herbs with the ability to colonize widely and disperse quickly (Austin *et al.* 1985; Beck 1999; Shea *et al.* 2005). An individual seedling of either species initially forms into a flat rosette in the first year and then, in the following year, it bolts and produces a single or several tall stems with several purple inflorescences. Plants die after flowering and there is no vegetative reproduction. Seeds are wind dispersed, but the papus detaches easily and seeds are rarely blown more than a few metres from the parent plant (Lee & Hamrick 1983; Shea & Kelly 1998). In the study site, CN and OA are common along roadsides, but they are especially abundant near *A. lobicornis* nests (figure 1).

Acromyrmex lobicornis Emery is one of the leaf-cutting ants with the widest latitudinal range, reaching from subtropical areas in southern Brazil and Bolivia (23° S) to northern Patagonia (44° S), where it is the only leaf-cutting ant species (Farji-Brener & Ruggiero 1994). *Acromyrmex lobicornis* occurs in a broad range of biomes, but in the study area it is more abundant in the driest region of the national park, reaching 43 nests ha^{-1} in roadside steppes (Farji-Brener 2000). *Acromyrmex lobicornis* nests reach depths of 1 m and externally consist of a single mound of twigs, soil and dry plant material of a maximum height and diameter of 1 m (figure 1). Organic debris from the fungus culture are removed from the internal fungus garden and dumped on the soil surface near the mound in a few large piles (Farji-Brener & Ghermandi 2000, 2004; figure 1).
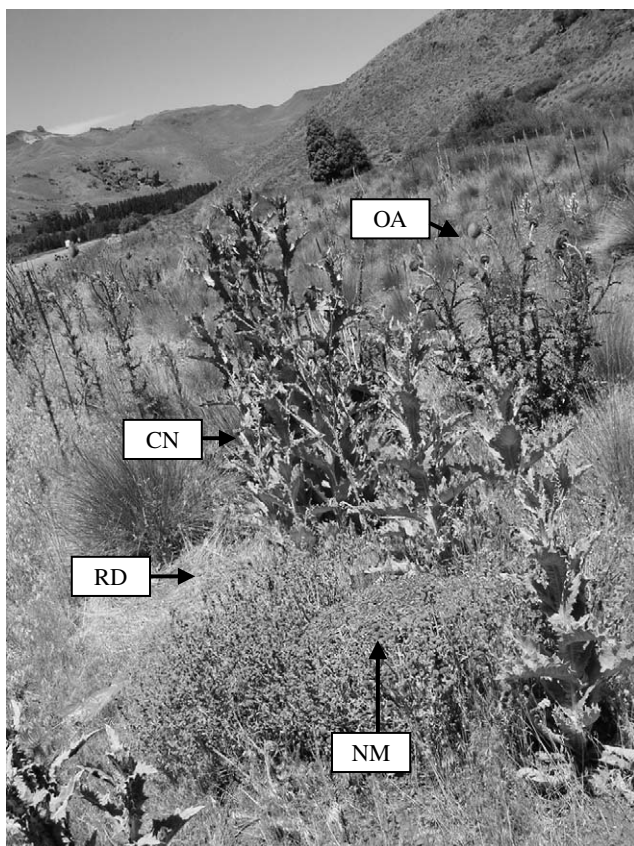
Figure 1. Plants of *C. nutans* (CN) and *O. acanthium* (OA) growing associated with external refuse dumps (RDs) from a nest of the leaf-cutting ant *A. lobicornis* near a road in northern Patagonia. NM, nest mound.

(b) *Methodology*

Field sampling was carried out along 50 km on both sides of the road 237, the main paved access to the national park, between 1997 and 2005. Our measurements involved approximately 40 000 seeds, 10 000 inflorescences, 300 plants and 50 ant nests. All plants, nests and soil samples were located within belts of approximately 20 m width to each side of the road. Additional field sampling and non-systematic observations were also carried out in sites distant from roads (see below). Mean distance ($\pm$s.d.) between nests was 10 ± 5 m. Non-nest and nest sites were environmentally comparable sites (e.g. similar slope steepness and degree of rockiness and disturbance). Plants from non-nest soils (NNSs) and NNS samples were randomly selected from each nest mound by selecting a random angle from 0 to 360° and random distance between 2 and 6 m using the algorithm proposed by Skalski (1987). Other methodological details and the statistical methods employed are explained in each case. All response variables were examined for normality and homogeneity of variance and transformed when necessary. Duncan *post hoc* comparison of means was employed when ANOVA results were statistically significant ($p < 0.05$). Bonferroni adjustments were made when necessary.

(c) *Plant abundance in nest and non-nest areas*

To evaluate the influence of nests on the abundance of CN and OA, we sampled the number of individuals of these species on 23 nests and on adjacent non-nest areas. Ant nests were randomly selected and a plot of 3×3 m was located around their mounds; RDs were always within the plots. The

plots of identical area but without nests (controls) were randomly chosen adjacent from each nest as explained above. In both plots, the number of plants of CN and OA were counted and compared with *t*-tests.

(d) *Chemical and physical characteristics of nest and NNSs*

To analyse the influence of ant nests on soil characteristics, we collected 10 soil samples from 10 active nest mounds (nest soils), 10 samples from external RDs and 10 soil samples from randomly selected sites 3–6 m away from the nest (NNSs). Each soil or refuse sample was a mix of four subsamples of refuse cores or soil cores of 10 cm diameter and 15 cm depth (approx. 300 ml). The samples ($n = 30$) were dried in the laboratory and sent to the Instituto Nacional de Tecnologia Agropecuaria for analyses. Extractable phosphorus (P), total nitrogen (N), organic matter (OM) content, carbon (C), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), pH and texture were measured in all samples following Bigham (1996). The percentage of silt, sand and clay was determined granulometrically. The soil properties of nest soils, RDs and NNS samples were analysed using a one-way MANOVA. Substrate was considered a fixed factor, response variables were the content of OM, C, N, P, K, Mg, Ca, Na, pH and percentage of silt, sand and clay.

(e) *Field measures of plant fitness*

We estimated plant fitness in the field from the number of inflorescences per plant and, in a randomly selected subset of individuals, from their seed production. We measured a total of 306 plants; 128 of CN (55 from RDs and 73 from NNSs) and 178 of OA (91 from RDs and 87 from NNSs). Plants in NNSs were randomly selected as explained above. Plants from RDs were usually selected from different ant nests. Occasionally, two to three plants established in the same nest were selected; in these cases, they were located in different refuse piles of the same nest. The total number of inflorescences per plant and plant height were measured in all individuals. For a subset of 200 plants (80 of CN, 34 from RDs and 46 from NNSs, and 120 of OA, 61 from RDs and 59 from NNSs), the diameter of each inflorescence (approx. 10 000) was measured to estimate the total number of seeds per plant. The number of seeds per inflorescence was estimated using a regression model with inflorescence diameter as the predictor of seed number. To do this, 60 inflorescences from 30 plants per species were randomly selected in the field (half growing in NNSs and the other half in RDs). For each plant, we measured the diameter of all inflorescences (in mm) and counted the total number of seeds per inflorescence; the percentage of viable seeds and seed weight was also measured. Aborted seeds were easily identified because the seed coats were empty and floated in water. Inflorescence diameter was thus converted into an estimate of seed production. We initially performed regressions for NNS and RD plants separately, but their slope was statistically indistinguishable ($p > 0.50$ for both the plant species). Therefore, plants growing in RDs and NNSs were grouped together. The final estimation formulae were for CN: $y = -820 + 31.3x$ and for OA: $y = -149 + 14.5x$, where y is the number of seeds and x is the diameter (in mm) of the inflorescence. These relationships were found to be very strong ($R^2 = 0.92$ for CN and 0.89 for OA, $F_{1,58} = 392$ and 364, respectively, all $p < 0.0001$). The number of seeds per plant was estimated as (the number of seeds/inflorescence) $\times$

(the total number of inflorescences/plant). For each plant species separately, the number of inflorescences and seeds was compared between RDs and NNS plants using ANCOVAs; substrate (NNSs and RDs) was considered a fixed factor and plant height was used as the covariate.

(f) Greenhouse measures of plant fitness and maternal effect

We estimated plant fitness in greenhouse experiments measuring several leaf and root parameters in seedlings planted in NNSs and RDs. We also determined whether the effect of the maternal growing substrate (NNSs and RDs) affected the performance of the next generation of seedlings. First, in the field, we collected 400 viable seeds from randomly selected inflorescences of 20 CN and 20 OA plants of 1–2 m height, half from individuals established in RDs and the other half from individuals established in NNSs. Second, we collected samples of RDs from 10 *A. lobicornis* nests and from NNSs as described previously. In the laboratory, all refuse and soil samples were sterilized in a drying chamber (80°C) for one week. Each RD and NNS sample from the same site was then divided in two subsamples and placed in a plot of 10×20 cm. Seeds from different: maternal origins were sown into the subsample plots; seeds from plants that grew in RDs and from plants that grew in NNSs. This experimental design was a 2×2 factorial experiment: factors were 'origin', with two treatment levels (seeds from plants established in NNSs or RDs), and crossed with 'substrate', the type of subplot (NNSs or RDs). For each plant species, we sowed a total of 200 seeds in the RD plots and 200 in the NNS plots ($n = 20$ plots, 10 seeds per plot). All the plots were watered every 2 days. The level of germination per plot was monitored until the end of the experiment (10 seedlings = 100%). After 12 weeks, four randomly selected individuals per plot were harvested to measure leaf and root characteristics (see below). All measurements were averaged per plot ($n = 10$ plots per treatment) and analysed using 2×2 factorial MANOVA. Both main factors (origin and substrate) were designated as fixed effects; the number of leaves, foliar area, and leaf and root dry biomass per plant were the response variables.

(g) Measures of nitrogen stable isotopes and nutrient contents in plant tissues

To verify whether plants can use the nutrients of RDs, we analysed the $\delta^{15}N$ composition of NNSs, RDs and plant tissues from CN individuals established on NNSs and RDs. Tracing a plant's nitrogen sources by the analysis of naturally occurring stable isotope ratios is a common practice in plant ecology (Thompson *et al.* 2005), but it has rarely been employed to confirm the use of nutrients from leaf-cutting ant debris by plants. Nitrogen exists in two stable, non-radioactive, isotopes (^{14}N and ^{15}N). The unit of isotopic ratio measurement, the delta value (δX), reflects the ratio of the heavier to the lighter isotope and is expressed as the deviation ppt from an arbitrary standard (in the case of N, atmospheric air). Thus, natural abundance of ^{15}N was calculated as $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of $^{15}N/^{14}N$ (Mariotti *et al.* 1982). Because the $\delta^{15}N$ of the plant tissues typically reflects the $\delta^{15}N$ of the nitrogen source, we employed this measure to verify the source of N in plants established in NNSs and RDs. In addition, foliar nutrients in CN and OA plants growing on NNSs and RDs were also measured. We sampled RDs

in nine ant nests and adjacent NNSs as described previously, and CN plants growing on these substrates (nine per substrate, 18 in total). Soil samples, refuse samples and plant tissues (leaves and stems) were dried, ground to a fine powder and sent for isotopic analyses to the University of Arkansas Stable Isotope Laboratory (UASIL). These samples, together with samples of OA plants from NNSs and RDs (nine per substrate), were also employed to analyse foliar content of N, P, C and K. The content of $\delta^{15}N$ was compared among sites using a one-way ANOVA. Foliar content of N, P, C and K was compared for each plant species separately with *t*-tests.

(h) Additional field experiments: the importance of propagule availability

Although *A. lobicornis* nests are more abundant near roadsides (Farji-Brener 2000; A. G. Farji-Brener 1997–2005, field observations), ant nests can also be found distant from roads. RDs of these nests are rarely colonized by exotic plants (A. G. Farji-Brener 2005, personal observation), but exotics are also less abundant far away from roads. Therefore, we used these nests to test whether this pattern was due to propagule limitation. In early September 2004 (beginning of the spring), we planted a total of 300 CN seeds in RDs from six ant nests (50 per nest); RDs from another six nests were employed as controls. All sampled nests were located in native plant communities approximately 250 m distant from roadsides; treated and control nests were interspersed within the study area. CN seeds were randomly gathered from the plants employed in the estimation of seeds (see above). During September 2005, we counted the number of CN seedlings in RDs from all the 12 nests. The number of CN seedlings in treated (i.e. planted) and control (i.e. unplanted) RDs was compared using a Mann–Whitney test.

3. RESULTS

(a) Plant abundance in nest and non-nest areas

Both CN and OA plants were more numerous in nest sites (i.e. in RDs) than in adjacent NNSs. While 7.7 ± 2.6 CN and 13.3 ± 3.3 OA were found in 9 m^2 around nests, only 3.9 ± 1.4 CN and 1.8 ± 0.6 OA (mean $\pm$ s.e.) were found in identical areas of adjacent NNSs ($n = 23$, $t = 2.17$ and 3.8, respectively, all $p < 0.04$).

(b) Chemical and physical characteristics of nest and NNSs

Samples of RDs, nest soils and NNSs showed different characteristics (Wilks' lambda = 0.013, $F = 7$, $p < 0.001$; appendix A in the electronic supplementary material). RDs were extremely nutrient-rich, showing much higher contents of OM, C, N, P, Ca, K, Mg and Na than nest and NNSs (table 1). Despite differences in bulk density (Farji-Brener & Ghermandi 2004), the relative content of silt, sand and clay of RDs was similar to nest and NNSs (table 1).

(c) Field measures of plant fitness

Plants of CN and OA, which naturally grew in RDs showed similar inflorescence diameters compared with those that grew in NNSs ($F_{1,77} = 0.87$, $F_{1,117} = 3.5$ for CN and OA, respectively, all $p > 0.07$), but higher mean number of inflorescences ($F_{1,125} = 19.8$, $F_{1,175} = 20.5$ for CN and OA, respectively, all $p < 0.0001$). This pattern

Table 1. Variation in chemical and physical parameters (mean±s.e.) between RDs, nest and NNSs. (For each row, different letters imply statistically different groups. The contents of clay, lime, sand, OM (organic matter), C (carbon) and N (nitrogen) are expressed in percentages; P (phosphorous) in ppm; Ca (calcium), Mg (magnesium), K (potassium) and Na (sodium) in meq/100 g (MANOVA table is shown in the electronic supplementary material, appendix A).)

	RD	nest soils	NNS
OM	13.8 ± 2.5^a	3.2 ± 0.3^b	2.5 ± 1.0^b
C	8.1 ± 1.4^a	1.9 ± 0.2^b	1.4 ± 0.2^b
N	0.76 ± 0.09^a	0.14 ± 0.02^b	0.13 ± 0.01^b
P	597.4 ± 61.4^a	80.3 ± 19.3^b	56.5 ± 4.5^b
Ca	20.6 ± 1.4^a	13.1 ± 1.1^b	13.1 ± 0.8^b
K	6.9 ± 1^a	1.0 ± 0.1^b	0.8 ± 0.1^b
Mg	6.7 ± 0.7^a	2.7 ± 0.3^b	2.6 ± 0.2^b
Na	0.14 ± 0.01^a	0.05 ± 0.05^b	0.09 ± 0.02^b
pH	6.9 ± 0.2	6.7 ± 0.06	6.8 ± 0.11
clay	9.7 ± 1.6	7.7 ± 0.7	7.7 ± 0.5
silt	22.5 ± 2.8	24.5 ± 1.5	26.9 ± 1
sand	67.7 ± 3.7	67.3 ± 2	65.3 ± 1.8

Table 2. Reproduction measures in field plants of *C. nutans* (CN) and *O. acanthium* (OA) that grew in RDs and NNSs. (*n*, number of plants sampled. Values are means±s.e. Values in parentheses indicated minimum and maximum. Inflorescence diameter was used as the predictor of the number of seeds used to estimate the seed production per plant. ANCOVA tables are shown in the electronic supplementary material, appendix B; $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.0001$.)

	CN		OA	
variable	RD	NNS	RD	NNS
n	55	73	91	87
height	130 ± 4.7 (70–200)	$116 \pm 3.6^*$ (47–180)	135 ± 4.3 (40–220)	$122 \pm 3.3^*$ (50–210)
inflorescences per plant	28 ± 3 (4–104)	$14 \pm 1^{***}$ (2–40)	66 ± 8 (2–414)	$22 \pm 3^{***}$ (2–111)
inflorescences per metre	21 ± 2	$12 \pm 1^{***}$	44 ± 5	$16 \pm 2^{***}$
n	30	30	30	30
aborted seeds (%)	20.4 ± 3.3	27.2 ± 4.7	44.2 ± 4.3	34.5 ± 4.3
seed weight (mg)	0.34 ± 0.1	0.36 ± 0.1	10.2 ± 0.3	$9.2 \pm 0.3^{**}$
n	34	46	61	59
inflorescence diameter (mm)	27.7 ± 1.2	26.1 ± 1.1	23.3 ± 0.5	23.8 ± 0.3
seeds/plant	$20\,209 \pm 1808$ (3844–85 255)	$9714 \pm 1554^{***}$ (2461–32 045)	$26\,281 \pm 3233$ (4129–1 333 396)	$9580 \pm 1259^{***}$ (1954–40 814)

was significant in ANCOVAs after controlling for plant height (appendix B in the electronic supplementary material). Plants of CN and OA in RDs showed one to three times more inflorescences than in NNS plants; the maximum numbers of inflorescences were also greater (table 2). Accordingly, seed traits followed a similar pattern. After controlling for plant height, the number of estimated seeds per plant was still approximately two to three times higher in plants that grew in RDs (table 2). The mean number of CN seeds in RD plants was approximately 20 000 versus 9700 in NNS plants ($F_{1,77} = 14.4$, $p < 0.001$) and 26 000 versus 9500 for OA plants ($F_{1,117} = 20.7$, $p < 0.0001$; table 2; appendix B in the electronic supplementary material). The maximum and minimum number of seeds per plant was extremely high in plants that grew in RDs (table 2). While the percentage of viable seeds was similar between plants from NNSs and RDs for both species ($t = 1.18$ and 1.56, and $p = 0.25$ and 0.13 for CN and OA, respectively), OA seed weight was slightly higher for plants that grew in RDs ($t = 1.52$ and 2.58, and $p = 0.14$ and 0.01 for CN and OA, respectively; table 2).

(d) Greenhouse measures of plant fitness and maternal effect

Although we verified the viability of OA seeds by standard methods, they never germinated in our plots. Therefore, we present the results from CN seedlings only. The level of germination was similar in all the plots (approx. 80%, $p = 0.65$), but four months after seedlings emerged they clearly differed among treatments (figure 2). Seedling traits largely depended on the substrate where they grew (Wilks' lambda = 0.22, $F_{6,27} = 16.1$, $p < 0.0001$), but not on their origin (Wilks' lambda = 0.72, $F_{6,27} = 1.7$, $p = 0.16$; appendix C in the electronic supplementary material). Seedlings that grew in RDs were larger than those that grew in NNSs, having 50% more leaves, up to 400% more foliar area, up to 700% more leaf biomass and up to 1000% more root biomass (figure 2). The interaction between substrate × origin was slightly significant (Wilks' lambda = 0.60, $F_{6,27} = 3.1$, $p = 0.02$; appendix C in the electronic supplementary material). Seedlings from NNS plants showed approximately 30% more foliar area when they grew in RDs than those from RD plants (figure 2).
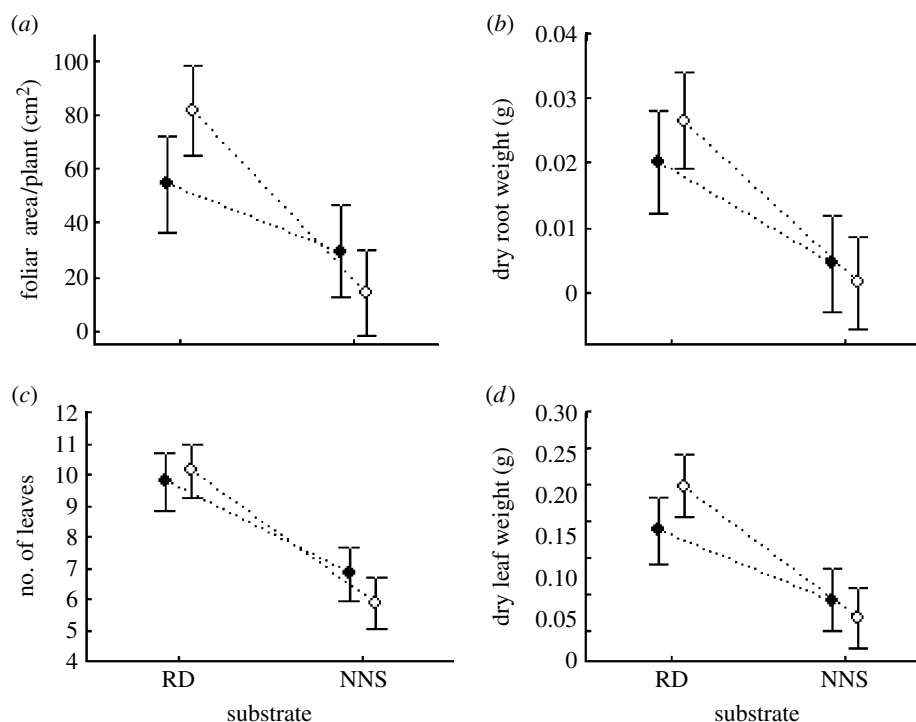
Figure 2. (*a*) Foliar area, (*b*) dry root weight, (*c*) number of leaves and (*d*) dry leaf weight in seedlings according to their origin (filled circles, seeds from plants growing in RDs and open circles, NNSs) and the substrate where they grew (RDs and NNS). MANOVA table is shown in the electronic supplementary material, appendix C.

(e) Measures of stable nitrogen isotopes and nutrient contents in plant tissues

Mean $\delta^{15}N$ value of NNSs was significantly higher than RDs, and higher in NNS plants than RD plants ($F_{3,32} = 6.4$, $p = 0.001$). Mean $\delta^{15}N$ values did not differ between plants that grew in NNSs and the soils nor between RD plants and RDs (figure 3). Additionally, the foliar content of nutrients was often higher in plants that grew in RDs than in NNSs. CN leaves from RD plants showed more C and N than NNS plants, but did not differ in K and P contents. Leaves of OA from RD plants showed more C, N and K than NNS plants; but did not differ in their P content (table 3).

(f) Additional field experiments: the importance of propagules availability

Seedlings of CN were more abundant in RDs where seeds were manually added than in RDs without seed addition. While treated RDs showed 18 ± 4 CN seedlings, nearby control RDs showed only 0.5 ± 0.3 CN seedlings (mean $\pm$ s.e., $Z = 2.9$, $p = 0.003$, U-test). The percentage of germination of seeds added in the field was approximately 40%.

4. DISCUSSION

There are three major findings in this study: (i) external RDs of *A. lobicornis* are an important source of soil nutrients, (ii) the two exotic plant species studied are more abundant and produce more seeds when they grow in RDs, and (iii) the mechanisms responsible for these effects appear to be related to the uptake of nutrients from RDs. These results have both theoretical and applied implications. First, they support the hypothesis that enhanced resource availability promotes exotic plant fitness that could increase the likelihood of biological invasions, for example, if the invasion is propagule-limited. Second, they
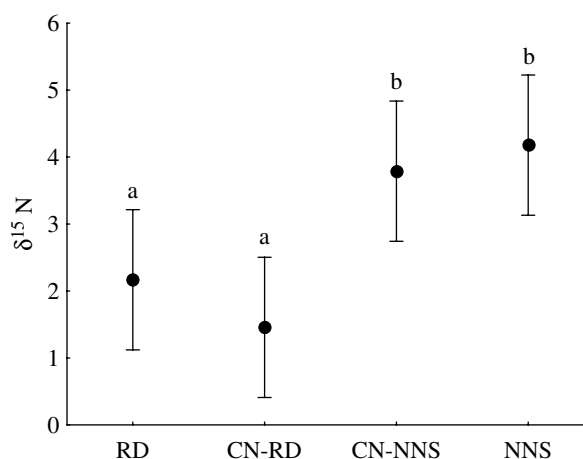
Figure 3. Mean ($\pm$s.d.) isotopic $\delta^{15}N$ value of NNSs, RDs and plant tissue from CN plants growing in NNSs (CN-NNSs) and in RDs (CN-RDs). Different letters imply statistically different groups (Duncan's *post hoc* comparison, $p < 0.05$).

can provide suggestions to mitigate the spread of these exotic plants into natural protected areas of northwest Patagonia.

RDs from *A. lobicornis* nests are soil nutrient hot spots; nutrient content in RDs was two to nine times higher than that in nearby NNSs. This level of nutrient improvement was similar to or higher than those reported for debris of other ant species, including leaf-cutters (Haines 1975; Farji-Brener & Silva 1995; Wagner *et al.* 1997; Moutinho *et al.* 2003; Wirth *et al.* 2003). This is particularly important in temperate habitats of South America, where available N and P are extremely low and thus plants are often N- and P-limited (Satti *et al.* 2003). Hence, RDs of *A. lobicornis* nests can potentially increase the reproduction of nearby plants if they can exploit this nutrient source.

Table 3. Nutrient foliar content of seedlings of *O. acanthium* (OA) and *C. nutans* (CN) that grew in RDs and NNSs. (C, carbon; N, nitrogen; K, potassium and P, phosphorous. All values are mean $\pm$ s.e. (in %). $^*p < 0.01$.)

	OA		CN	
	RD	NNS	RD	NNS
C	40.7 ± 0.4	$38.7 \pm 0.2^*$	40.7 ± 0.3	$37.8 \pm 0.6^*$
N	3.2 ± 0.2	$1.9 \pm 0.1^*$	2.6 ± 0.4	$1.9 \pm 0.5^*$
K	3.5 ± 0.2	4.1 ± 0.3	3.2 ± 0.3	$2.1 \pm 0.2^*$
P	0.25 ± 0.03	0.26 ± 0.01	0.15 ± 0.01	0.13 ± 0.01

Several studies have demonstrated a positive association between ant nests and plant fitness (Rissing 1986; Dean & Yeaton 1993; Wagner 1997). However, evidence that leaf-cutting ant nests positively affect plants is limited. Although leaf-cutting ants enhance available soil nutrients, stimulate root proliferation and often promote plant abundance and diversity (Haines 1975; Farji-Brener & Illes 2000; Farji-Brener & Medina 2000; Moutinho *et al.* 2003; Wirth *et al.* 2003; Farji-Brener & Ghermandi 2004; Sternberg *et al.* 2006), their effect on plant reproduction has never been confirmed and the responsible mechanisms unverified. This study provides the first experimental evidence that the high nutrient content of RDs is the cause of an increased plant reproduction.

Seedlings in RDs showed 4 and 10 times more foliar area and leaf and root biomass than in NNS seedlings (figure 2). Accordingly, adult plants that grew in RDs produced one to three times more seeds. This enhanced seed production was a result of a higher number of inflorescences. Three lines of evidence link this pattern with direct consumption of RD nutrients. First, leaf and root biomass were higher in RD seedlings relative to NNS seedlings despite the fact that they received equal amounts of water during the experiment. Second, some nutrients showed greater concentrations in foliar tissues of plants grown in RDs than those in NNSs. Third, the similar isotopic signature of ^{15}N in RD plants and RDs strongly suggests that plants assimilate nitrogen from this substrate (figure 3). RD plants may have a lower ^{15}N value than control plants because the greater nitrogen concentration in the RD caused plants to discriminate against ^{15}N during nitrogen uptake (Mariotti *et al.* 1982). Nonetheless, our results and those of a ^{15}N labelling experiment (Sternberg *et al.* 2006) strongly suggest direct uptake of nitrogen by the plant from ant RDs. Moreover, these plants can probably also take up other nutrients that occur at high concentrations in RDs. Some nutrients, such as P, K, Mg and Ca, may be as critical as N for plant growth, survival and reproduction (Larcher 1995). Other studies have also confirmed that plants can use nutrients from ant debris (Treseder *et al.* 1995; Sagers *et al.* 2000; Fischer *et al.* 2003). This phenomenon has been demonstrated in ant species that nest inside the plant and the accumulated debris is directly available to their host. This is the first case confirming that plants can both exploit and *benefit*, in terms of reproduction, from RDs of leaf-cutting ants. This advantage remains despite the risk of leaf-cutting ant herbivory. Leaves of plants of CN and OA outside RDs are sometimes harvested by the ants (Franzel & Farji-Brener 2000). But leaf-cutters tend to avoid contact with their own debris because it harbours micro-organisms harmful to the ant colony (Currie *et al.* 1999), and plants that grow in RDs are rarely attacked by leaf-cutting ants (Zeh *et al.* 1999).

Why *A. lobicornis* nests are more abundant near roadsides is not completely clear. Founding leaf-cutter queens prefer to nest in areas of bare soil (Vasconcelos 1990; Vasconcelos *et al.* 2006), and colonies established in the bare soil along roadsides may be more successful owing to the abundance of herbaceous plants and pioneer vegetation, which are key food resources (Farji-Brener 2001). On the other hand, some exotics may be better able than natives to exploit soil nutrient patches (Daehler 2003). Specifically, CN and OA are known for their fast growth and rapid gain of biomass when they grow in suitable micro-sites (e.g. enhanced nutrient patches, bare grounds, post-fire successions; Austin *et al.* 1985; Shea *et al.* 2005). Thus, on ant RDs, these species may grow quickly enough to suppress the establishment of other native seedlings, and produce large numbers of seeds.

Some of the effects demonstrated here may also occur in other species of the community and at larger spatial scales. Current experiments with several other native and exotic plant species show that exotics benefit more from RDs than natives (A. G. Farji-Brener 2007, unpublished data). Nests of leaf-cutting ants and exotic plants often occur in the same disturbed habitats such as road borders and secondary forests (Farji-Brener 2001; Vasconcelos *et al.* 2006). This co-occurrence occurs under a wide variety of environmental conditions, from temperate semi-deserts to tropical rainforests (Farji-Brener & Ruggiero 1994; Farji-Brener & Illes 2000). Other studies also confirm that ant nests in disturbed areas can promote the establishment of exotic species. In the Brazilian savannahs, the exotic grass *Melinis minutiflora* mainly colonizes open spaces provided by *Atta laevigata* nests (Coutinho 1982). In arid woodlands of Argentina, exotics are dominant in *Atta* nest areas (Bucher & Zuccardi 1967). Therefore, the use of nutrients from ant nests by invasive exotic plants may be more widespread than previously thought.

Our results indirectly support the resource availability hypotheses (RAH; Davis *et al.* 2000) as a mechanism of biological invasions. The RAH proposes that colonization is promoted by high resource availability; increases in invasion following experimental increases in resource availability provide direct support to this idea (Davis & Pelsor 2001; Daehler 2003). However, these studies often have manipulated soil nutrient content by adding commercial fertilizers (Burke & Grime 1996; Brooks 2003; Leishman & Thompson 2005). In natural ecosystems, nutrient patches are likely to be associated with decomposing OM. Thus, experiments using organically derived nutrient patches are more realistic than those involving additions of mineral salts (Tibbett 2000). Our study

reveals that natural events which increase soil nutrient content, such as ant dumps, can promote the fitness of exotics, demonstrating how the RAH may operate in nature.

Invasions comprise at least three main processes: colonization, establishment and spread into new areas (Mack *et al.* 2000). This work provides strong evidence supporting the first two processes and indirect evidence supporting the third one. We demonstrated that ant presence of RDs increase the ability of colonization and establishment (i.e. seed production and growth performance) by both exotic plant species. On average, nest sites showed 15 plants more than nearby sites, and each one produced approximately 13 000 seeds more than adjacent plants (table 2). The density of *A. lobicornis* in the study area was 43 nests ha^{-1} (Farji-Brener 2000), so a simple extrapolation suggests that CN and OA plants produce 8 385 000 more seeds ha^{-1} in areas with nests than in areas without nests. This enhanced seed production may promote the spread of exotic plants into areas distant from nest sites and road borders. First, copious seed production (i.e. 'seed swamping') provide a population level competitive advantage despite a theoretic *per capita* competitive inferiority (DiVittorio *et al.* 2007). Moreover, because a biological invasion is a probabilistic process, copious seed production allows exotics to colonize a greater proportion of available sites (Davis *et al.* 2000). Since we demonstrated that propagule availability may limit establishment of the exotic plants outside road verge areas, and the two exotics can grow successfully in non-ant nest sites; the large increase in seed production near roads in nest sites represents an increase in probability of establishment in communities that lack ant nests. Second, both leaf-cutting ants and exotic plants can disperse over relatively long distances during nuptial flights and wind or secondary dispersion via birds and mammals, respectively. Thus, exotics and leaf-cutting ants may colonize adjacent communities, especially sites previously disturbed by fire, humans and cattle (Vasconcelos & Cherrett 1995; Beck 1999), which are common in national parks. Third, in areas with leaf-cutters, neither CN nor OA is limited by ant foraging (Franzel & Farji-Brener 2000) and can successfully compete with natives outside RDs (A. G. Farji-Brener 2007, unpublished data); thus, this plant species has the potential to successfully invade native habitats. Accordingly, natural areas adjacent to road verges with high nest density had more exotic plants than natural areas adjacent to road verges with low nest density (A. G. Farji-Brener 1997–2005, field observations).

Understanding the causes of exotic plant invasions is critical in order to identify appropriate management strategies. Our results reveal that CN and OA increase their abundance and fitness by exploiting limiting nutrients from RDs. Management to prevent exotic plants should include the reduction of road verges and minimization of soil surface disturbances in habitats adjacent to roads. Reducing roadsides must decrease suitable nesting sites for ant queens and appropriate sites for the establishment of exotic seedlings. Prevention of soil disturbances in habitats distant to roads should minimize the expansion of these species into adjacent communities (Farji-Brener 2001; Gelbard & Harrison 2005; Vasconcelos *et al.* 2006). Therefore, natural communities adjacent to roads should be prioritized in conservation plans. Weed eradication would be most effective if efforts were focused on RDs, where plants are clumped and represent a higher source of exotic seeds. Planting native seedlings in RDs after exotics are removed might help prevent future invasions.

REFERENCES

Aizen, M., Vázquez, D. & Smith-Ramírez, C. 2002 Historia natural y conservación de mutualismos planta–animal del bosque templado de Sudamérica Austral. *Rev. Chil. Hist. Nat.* **75**, 79–97.

Austin, M., Groves, R., Fresco, L. & Kaye, P. 1985 Relative growth of six thistle species along a nutrient gradient with multispecies competition. *J. Ecol.* **73**, 667–684. (doi:10.2307/2260503)

Beck, K. G. 1999 Biennial thistles. In *Biology and management of noxious rangeland weeds* (eds R. Sheley & J. Petroff), pp. 145–161. Corvallis, OR: Oregon State University Press.

Bigham, J. M. 1996 *Methods of soil analysis*. Madison, WI: Soil Society of America, Inc.

Brooks, M. 2003 Effects of increased soil nitrogen on the dominance of alien plants in the Mojave Desert. *J. Appl. Ecol.* **40**, 344–353. (doi:10.1046/j.1365-2664.2003.00789.x)

Bucher, E. & Zuccardi, R. 1967 Significación de los hormigueros de *Atta vollenweideri* como alteradores del suelo en la provincial de Tucumán. *Acta Zool. Lilloana* **23**, 83–95.

Burke, M. & Grime, J. P. 1996 An experimental study of plant community invasibility. *Ecology* **77**, 776–790. (doi:10.2307/2265501)

Correa, M. 1969–1998. *Flora Patagónica*, vol. 7. Buenos Aires, Argentina: INTA.

Coutinho, L. 1982 Aspectos ecologicos de sauva no cerrado. Os murundus de terra, as características psamofiticas das especies de sua vegetaçao e sua invasao pelo Capim Gordura. *Rev. Brasilera Biol.* **42**, 147–153.

Currie, C., Scott, J., Summerbell, R. & Malloch, D. 1999 Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature* **398**, 701–704. (doi:10.1038/19519)

Daehler, C. 2003 Performance comparisons of co-occurring native and alien invasive plants: implications for conservation to agricultural weeds. *Biol. Conserv.* **84**, 167–180. (doi:10.1016/S0006-3207(97)00096-7)

Davis, M. & Pelsor, M. 2001 Experimental support for a resource-based mechanistic model of invasibility. *Ecol. Lett.* **4**, 421–428. (doi:10.1046/j.1461-0248.2001.00246.x)

Davis, M., Grime, J. & Thompson, K. 2000 Fluctuating resource in plant communities: a general theory of invasibility. *J. Ecol.* **88**, 528–534. (doi:10.1046/j.1365-2745.2000.00473.x)

Dean, W. & Yeaton, I. 1993 The influence of harvester ant *Messor capensis* nest-mounds on the productivity and distribution of some plant species in the southern Karoo, South Africa. *Vegetatio* **106**, 21–35. (doi:10.1007/BF00044856)

Dietz, H. & Edwards, P. 2006 Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology* **87**, 1359–1367. (doi:10.1890/0012-9658(2006)87[1359:RTCPCD]2.0.CO;2)

DiVittorio, C., Corbin, J. & D'Antonio, C. 2007 Spatial and temporal patterns of seed dispersal: an important determinant of grassland invasión. *Ecol. Appl.* **17**, 311–316. (doi:10.1890/06-0610)

Farji-Brener, A. G. 2000 Leaf-cutting ant nests in temperate environments: mounds, mound damages and mortality rates in *Acromyrmex lobicornis*. *Stud. Neotrop. Fauna Environ.* **35**, 131–138. (doi:10.1076/0165-0521(200008)35:2;1-9;FT131)

Farji-Brener, A. G. 2001 Why are leaf-cutting ants more common in early secondary forests than in old-growth tropical forests? An evaluation of the palatable forage hypothesis. *Oikos* **92**, 169–177. (doi:10.1034/j.1600-0706.2001.920120.x)

Farji-Brener, A. G. & Ghermandi, L. 2000 The influence of nests of leaf-cutting ants on plant species diversity in road verges of northern Patagonia. *J. Veg. Sci.* **11**, 453–460. (doi:10.2307/3236638)

Farji-Brener, A. G. & Ghermandi, L. 2004 Seedling recruitment in the semi-arid Patagonian steppe: facilitative effects of refuse dumps of leaf-cutting ants. *J. Veg. Sci.* **15**, 823–830. (doi:10.1658/1100-9233(2004)015[0823:SRIASP]2.0.CO;2)

Farji-Brener, A. G. & Illes, A. 2000 Do leaf-cutting ant nests make 'bottom up' gaps in neotropical rain forests? A critical review of the evidence. *Ecol. Lett.* **3**, 219–227. (doi:10.1046/j.1461-0248.2000.00134.x)

Farji-Brener, A. G. & Margutti, L. 1997 Patterns of plant species in relation to *Acromyrmex lobicornis* nest-mounds on roadside vegetation in northwest Patagonia. *Int. J. Ecol. Environ. Sci.* **23**, 37–47.

Farji-Brener, A. G. & Medina, C. 2000 The importance of where to dump the refuse: seed banks and fine roots in nests of the leaf-cutting ants *Atta cephalotes* and *Atta colombica*. *Biotropica* **32**, 120–126. (doi:10.1111/j.1744-7429.2000.tb00454.x)

Farji-Brener, A. G. & Ruggiero, A. 1994 Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical ranges sizes. *J. Biogeogr.* **21**, 535–543. (doi:10.2307/2845757)

Farji-Brener, A. G. & Silva, J. 1995 Leaf-cutting ants and forest groves in a tropical parkland savanna of Venezuela: facilitated succession? *J. Trop. Ecol.* **11**, 651–669.

Fischer, R., Wanek, W., Richter, A. & Mayer, V. 2003 Do ants feed plants? A ^{15}N labeling study of nitrogen fluxes from ants to plants in the mutualism of *Pheidole* and *Piper*. *J. Ecol.* **91**, 126–134. (doi:10.1046/j.1365-2745.2003.00747.x)

Franzel, C. & Farji-Brener, A. G. 2000 ¿Oportunistas o selectivas? Plasticidad en la dieta de la hormiga cortadora de hojas *Acromyrmex lobicornis* en el N–O de la Patagonia. *Ecología Austral* **10**, 159–168.

Gelbard, J. & Harrison, S. 2003 Roadless habitats as refuges for naïve grasslands: interactions with soil, aspect, and grazing. *Ecol. Appl.* **13**, 404–415. (doi:10.1890/1051-0761(2003)013[0404:RHARFN]2.0.CO;2)

Gelbard, J. & Harrison, S. 2005 Invasibility of roadless grasslands: an experimental study of yellow starthistle. *Ecol. Appl.* **15**, 1570–1580. (doi:10.1890/03-5362)

Haines, B. 1975 Impact of leaf-cutting ants on vegetation development at Barro Colorado Island. In *Tropical ecological systems, trends in terrestrial and aquatic research* (eds F. Golley & E. Medina), pp. 99–111. New York, NY: Springer.

Holway, D., Suarez, A. & Case, T. 2002 Role of abiotic factors in governing susceptibility to invasion: a test with Argentine ants. *Ecology* **83**, 1610–1619. (doi:10.2307/3071982)

Keane, R. & Crawley, M. 2002 Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.* **17**, 164–170. (doi:10.1016/S0169-5347(02)02499-0)

Kennedy, T., Naeem, S., Howe, M., Knops, J., Tilman, D. & Reich, P. 2002 Biodiversity as a barrier to ecological invasions. *Nature* **417**, 636–638. (doi:10.1038/nature00776)

Larcher, W. 1995 *Physiological plant ecology*, 3rd edn. New York, NY: Springer.

Lee, J. & Hamrick, J. 1983 Demography of two natural populations of musk thistle (*Carduus nutans*). *J. Ecol.* **71**, 923–936. (doi:10.2307/2259602)

Leishman, M. & Thomson, V. 2005 Experimental evidence for the effects of additional water, nutrients and physical disturbance in low fertility Hawkesbury Sandstone soils, Sydney. *J. Ecol.* **93**, 38–49. (doi:10.1111/j.1365-2745.2004.00938.x)

Levine, J., Vilá, M., D'Antonio, C., Duckles, J., Grigulis, K. & Lavorel, S. 2003 Mechanisms underlying the impacts of exotic plant invasions. *Proc. R. Soc. B* **270**, 775–781. (doi:10.1098/rspb.2003.2327)

Levine, J., Adler, P. & Yelenik, S. 2004 A meta analysis of biotic resistance to exotic plant invasions. *Ecol. Lett.* **7**, 975–989. (doi:10.1111/j.1461-0248.2004.00657.x)

Lockwood, J., Cassey, P. & Blackburn, T. 2005 The role of propagules pressure in explaining species invasions. *Trends Ecol. Evol.* **20**, 223–238. (doi:10.1016/j.tree.2005.02.004)

Mack, M. & D'Antonio, C. 1998 Impacts of biological invasions on disturbance regimes. *Trends Ecol. Evol.* **13**, 195–198. (doi:10.1016/S0169-5347(97)01286-X)

Mack, M., Simberloff, D., Londsale, M., Evans, H., Clout, M. & Bazzaz, F. 2000 Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol. Appl.* **10**, 689–710. (doi:10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2)

Mariotti, A., Mariotti, F., Champigny, M.-L., Amarger, N. & Moyse, A. 1982 Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of NO_3^- by Pearl Millet. *Plant Physiol.* **69**, 880–884.

Meiners, S., Pickett, S. & Cadenasso, M. 2001 Effects of plant invasions on the species richness of abandoned agricultural land. *Ecography* **25**, 215–223. (doi:10.1034/j.1600-0587.2002.250209.x)

Moutinho, P., Nepstad, D. & Davidson, E. 2003 Influence of leaf-cutting ant nests on secondary forest growth and soil properties in Amazonia. *Ecology* **84**, 1265–1276. (doi:10.1890/0012-9658(2003)084[1265:IOLANO]2.0.CO;2)

Qaderi, M., Cavers, P. & Bernards, M. 2002 Seed banks dynamics of *Onopordum acnathium*: emergence patterns and chemical attributes. *J. Ecol.* **90**, 672–683. (doi:10.1046/j.1365-2745.2002.00708.x)

Rapoport, E. 1991 Tropical versus temperate weeds: a glance into the present and future. In *Ecology of biological invasion in the tropics* (ed. P. Ramakrishnan), pp. 11–51. New Delhi, India: ISF.

Richards, C., Bossdorf, O., Muth, N., Gurevitch, J. & Pigliucci, M. 2006 Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* **9**, 981–993. (doi:10.1111/j.1461-0248.2006.00950.x)

Rickey, M. & Anderson, R. 2004 Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. *J. Appl. Ecol.* **41**, 888–896. (doi:10.1111/j.0021-8901.2004.00948.x)

Rissing, S. 1986 Indirect effects of granivory by harvester ants: plant species composition and reproductive increase near ant nests. *Oecologia* **68**, 231–234. (doi:10.1007/BF00384792)

Safford, H. & Harrison, S. 2001 Grazing and substrate interact to affect native versus exotic diversity in roadside grasslands. *Ecol. Appl.* **11**, 1112–1122. (doi:10.1890/1051-0761(2001)011[1112:GASITA]2.0.CO;2)

Sagers, C., Ginger, S. & Evans, R. 2000 Carbon and nitrogen isotopes trace nutrient exchange in an ant–plant mutualism. *Oecologia* **123**, 582–586. (doi:10.1007/PL00008863)

Satti, P., Mazzarino, M., Gobbi, M., Funes, F., Roselli, L. & Fernández, H. 2003 Soil N dynamics in relation to leaf-litter quality and soil fertility in northwestern Patagonian forests. *J. Ecol.* **91**, 173–181. (doi:10.1046/j.1365-2745.2003.00756.x)

Shea, K. & Chesson, P. 2002 Community ecology theory as a framework for biological invasions. *Trends Ecol. Evol.* **17**, 170–176. (doi:10.1016/S0169-5347(02)02495-3)

Shea, K. & Kelly, D. 1998 Estimating biocontrol agent impact with matrix models: *Carduus nutans* in New Zealand. *Ecol. Appl.* **8**, 824–832. (doi:10.1890/1051-0761(1998)008[0824:EBAIWM]2.0.CO;2)

Shea, K., Kelly, D., Sheppard, A. & Woodburn, T. 2005 Context-dependent biological control of an invasive thistle. *Ecology* **86**, 3174–3181. (doi:10.1890/05-0195)

Skalski, L. 1987 Selecting a random sample of points in circular field plots. *Ecology* **68**, 749. (doi:10.2307/1938483)

Sperry, L., Belnap, J. & Evans, R. 2006 *Bromus tectorum* invasion alters nitrogen dynamics in an undisturbed arid grasslands ecosystem. *Ecology* **87**, 603–615. (doi:10.1890/05-0836)

Sternberg, L., Pinzon, M., Moreira, M., Moutinho, P., Rojas, E. & Herre, A. 2006 Plants use macronutrients accumulated in leaf-cutting ant nests. *Proc. R. Soc. B* **274**, 315–321. (doi:10.1098/rspb.2006.3746)

Thompson, D., Bury, S., Hobson, K., Wassenaar, L. & Shannon, J. 2005 Stable isotopes in ecological studies. *Oecologia* **144**, 517–519. (doi:10.1007/s00442-005-0171-8)

Tibbett, M. 2000 Roots, foraging and the exploitation of soil nutrient patches: the role of mycorrhizal symbiosis. *Funct. Ecol.* **14**, 397–399. (doi:10.1046/j.1365-2435.2000.00417.x)

Traveset, A. & Richardson, D. 2006 Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol. Evol.* **21**, 208–216. (doi:10.1016/j.tree.2006.01.006)

Treseder, K., Davidson, D. & Ehleringer, J. 1995 Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature* **375**, 137–139. (doi:10.1038/375137a0)

Vasconcelos, H. L. 1990 Habitat selection by the queens of the leaf-cutting ant *Atta sexdens* in Brazil. *J. Trop. Ecol.* **6**, 249–252.

Vasconcelos, H. L. & Cherrett, J. 1995 Changes in leaf-cutting ant populations after the clearing of mature forest in Brazilian Amazonia. *Stud. Neotrop. Fauna Environ.* **30**, 107–113.

Vasconcelos, H. L., Vieira-Neto, E., Mundim, F. & Bruna, E. 2006 Roads alter the colonization dynamics of a keystone herbivore in neotropical savannas. *Biotropica* **38**, 661–665. (doi:10.1111/j.1744-7429.2006.00180.x)

Vázquez, D. 2002 Multiple effects of introduced mammalian herbivores in a temperate forest. *Biol. Invasions* **4**, 175–191. (doi:10.1023/A:1020522923905)

Wagner, D. 1997 The influence of ant nests on Acacia seed production, herbivory and soil nutrients. *J. Ecol.* **85**, 83–93. (doi:10.2307/2960629)

Wagner, D., Brown, M. & Gordon, D. 1997 Harvester ant nests, soil biota and soil chemistry. *Oecologia* **112**, 232–236. (doi:10.1007/s004420050305)

Wirth, R., Herz, H., Rye, I., Beyschlag, W. & Hölldobler, B. 2003 *Herbivory of leaf-cutting ants*. Berlin, Germany: Springer.

Zeh, J., Zeh, A. & Zeh, D. 1999 Dump material as an effective small-scale deterrent to herbivory by *Atta cephalotes*. *Biotropica* **31**, 368–371. (doi:10.1111/j.1744-7429.1999.tb00149.x)