Ecological Entomology

Ecological Entomology (2015), **40**, 150–158 DOI: 10.1111/een.12169

A meta-analysis of leaf-cutting ant nest effects on soil fertility and plant performance

ALEJANDRO G. FARJI-BRENER and VICTORIA WERENKRAUT Laboratorio Ecotono, CRUB-Universidad Nacional del Comahue-INIBIOMA-CONICET, Bariloche, Argentina

Abstract. 1. Leaf-cutting ants (LCAs) are considered as one of the most important agents of soil disturbances that affect vegetation patterns, but these assertions are based on isolated studies or anecdotal data. In this study, meta-analysis techniques were used to quantitatively analyse the generality of these effects and determine some of their sources of variation.

2. The results reveal the following: (i) LCA nest sites showed higher levels of soil fertility than control sites, but the key source of these nutrients is the refuse material rather than the nest soil itself; (ii) refuse material from external piles tended to be richer in nutrient content than refuse material from internal refuse chambers; (iii) nest sites from temperate habitats showed higher cation content than those located in tropical/subtropical habitats; and (iv) nest sites showed higher plant growth than adjacent non-nest sites (especially if plants have access to the refuse) but similar plant density and plant richness.

3. As LCAs improve nutrient availability in nest sites through the accumulation of refuse material, the location of the refuse will have a relevant role affecting vegetation. LCA species with external refuse dumps could benefit herbs, early vegetation stages and short-living plants, whereas those with internal refuse chambers could benefit long-living, large trees. However, the positive effect on individual plants does not extend to population and community levels. The foraging preferences of ants and the changes in microclimatic conditions around nests could act as selective ecological filters.

4. As refuse material from external piles and nest sites in temperate habitats tend to show higher fertility than refuse material from internal nest chambers and nest sites in tropical/subtropical habitats, LCA species with external refuse dumps in temperate regions could be of particular relevance for nutrient cycling and vegetation patterns.

Key words. *Acromyrmex*, ant nests, *Atta*, bioturbation, ecosystem engineers, soil disturbances.

Introduction

Some organisms are considered important components of ecosystems because they greatly affect the abundance and/or performance of other species through the modification and creation of habitats (‘ecosystem engineers’ *sensu* Jones *et al.*, 1994). Specifically, ecosystems engineers modify the availability of resources for other organisms by altering the physical environment. Numerous animals act as ecosystem engineers, altering the abundance, diversity and performance of plants through soil disturbances, including agoutis, wild pigs, armadillos, termites and ants. Of these, leaf-cutting ants (LCAs) are considered one of the most important ecosystem engineers because they act as soil and canopy disturbance agents due to their foraging activities, and because of the size and longevity of their nests (Wirth *et al.*, 2003; Rico-Gray & Oliveira, 2007; Farji-Brener & Tadey, 2009; Meyer *et al.*, 2011a,2011b, 2013; Leal *et al.*, 2014).

Leaf-cutting ants harvest a huge amount of plant material for growing a symbiotic fungus, which is the key protein source for the ant brood. To house the large fungus gardens and the

Correspondence: Alejandro G. Farji-Brener, Laboratorio Ecotono, CRUB-Universidad Nacional del Comahue-INIBIOMA-CONICET, Pasaje Gutiérrez 1125, (8400) Bariloche, Argentina. E-mail: alefarji@yahoo.com

© 2014 The Royal Entomological Society

enormous number of workers (up to eight million in the genus *Atta*), LCAs build long-lasting, colossal nest structures. For example, one typical adult *Atta* nest (e. g. 10–15 years old) can reach depths of 7 m, contain up to 8000 underground chambers and their mound may cover up to 150 m^2 (Hölldobler & Wilson, 2011 and references therein). During nest building and expansion, ants clear the nest area of understorey vegetation, cover the forest floor with large amounts of excavated soil, and accumulate a huge quantity of organic waste (i. e. the remaining plant material not useful for the symbiotic fungus; hereafter 'refuse material') either inside underground chambers or on the soil surface (Farji-Brener & Medina, 2000). All these ant activities disturb the soil around nests and affect soil nutrient availability, with concomitant effects on plants traits at individual, population and community levels.

It is generally accepted that LCAs enhance soil nutrient contents around the nest sites and that plants respond with increased growth, abundance and/or performance. For example, refuse material of LCA may have 80 times higher nutrient content than adjacent non-nest soils (Farji-Brener & Tadey, 2009 and references therein). Experiments and field measurements of natural isotopes demonstrated that plants can assimilate nitrogen from nest sites (Sternberg *et al.*, 2007; Farji-Brener & Ghermandi, 2008; Lescano *et al.*, 2012), increasing their biomas and fitness (Farji-Brener & Ghermandi, 2000, 2008; Moutinho *et al.*, 2003; Farji-Brener *et al.,* 2010; Sosa & Brazeiro, 2010; Saha *et al.*, 2012). However, several studies recently reported reduced soil nutrient availability in nest sites compared with non-nest sites (Bieber *et al.*, 2011; Madureira *et al.*, 2013; Meyer *et al.*, 2013). Accordingly, sometimes plants growing in nest sites did not have greater biomass or better performance than those located further away from nests (Schoereder & Howse, 1998; Moutinho *et al.*, 2003; Farji-Brener *et al.*, 2010). These contradictory observations question the commonly accepted view that LCAs always increase soil fertility and enhance plant performance in nest sites. Surprisingly, the few studies attempting to comprehensively summarise these topics use a qualitative approach or describe LCA effects as working hypotheses rather than reviewing the quantitative evidence (Fowler *et al.*, 1989; Wirth *et al.*, 2003; Rico-Gray & Oliveira, 2007; Farji-Brener & Tadey, 2009; Montoya-Lerma *et al.*, 2012; Leal *et al.*, 2014). Here, for the first time, we complemented these reviews with a meta-analysis of the literature on these topics. Specifically, we quantitatively determined whether LCAs affect soil fertility and plant performance, and the possible sources of variation of this effect.

The effect of LCAs on soil fertility and nearby plants could be affected by the place where ants deposit their refuse materials, ant genus, geographical location of nests, and the plant level of the organisation studied (i.e. individuals, populations or communities). First, refuse material from LCAs are a key source of organic matter and nutrients (Haines, 1978; Wirth *et al.*, 2003; Farji-Brener & Tadey, 2009). As nest soil samples used to measure nutrient content are often gathered at a superficial level (0–30 cm), only ant species with external refuse dumps might enhance the fertility of nest topsoil areas. Therefore, the location of refuse could play a critical role in explaining the variation found in soil fertility of nest sites. Secondly, ant genera with larger nests and greater worker density, foraging rate, capacity of soil turnover and organic waste production (e. g. *Atta*) may have greater effects on soil properties and nearby plants than species with smaller nests, lower foraging rate, reduced capacity of soil turnover and lower organic waste production (e. g. *Acromyrmex*). Thirdly, several abiotic and biotic characteristics change with latitude and may affect the ability of ants to improve soil nutrient content. For example, temperate habitats often show more extreme temperatures and fewer plant species than tropical/subtropical habitats. On the one hand, more extreme temperatures limit the foraging period of LCAs and decrease colony growth, reducing the ants' ability as soil modifiers. Additionally, a reduced availability of plant species often implies a reduced number of species harvested, which should decrease the nutrient content of the ant refuse (Tadey & Farji-Brener, 2007). From this perspective, the effect of ants on soil fertility could be greater in tropical than in temperate areas. On the other hand, nutrient content of leaves often increases with latitude (Oleksyn *et al.*, 2003; Reich & Oleksyn, 2004; Lovelock *et al.*, 2007); as the nutrient content of refuse material depends on the foliar nutrient content of the harvested plants (Moller *et al.*, 2011), ants from temperate areas should generate refuse with higher nutrient content than ants from tropical areas. In this scenario, ant nests in temperate habitats may enhance soil quality to a greater degree than nests in tropical regions. In sum, the geographical location of nests may greatly affect the strength and direction of the contribution of ant nests to soil fertility and, thus, to plant performance. Finally, enhanced soil fertility could differentially influence plants, depending on the level studied; nutrient patches could increase plant performance at individual and population levels, but decrease plant richness by favouring the dominance of certain species (Garrettson *et al.*, 1998; Farji-Brener, 2005). Overall, all of these factors could explain the conflicting results obtained by different studies.

Here we carried out a meta-analysis to better understand how LCAs affect soil fertility and plant performance. Specifically, we addressed whether the sampled substrate (refuse material or nest soils), location of refuse material (external or internal), LCA genus (*Atta* or *Acromyrmex*) and geographical nest location (tropical/subtropical or temperate habitats) affect the contribution of LCAs to soil fertility and the concomitant influence on plants.

Materials and methods

Data collection

We identified relevant studies by examining the reference section of recently published papers on the topic and by conducting keyword searches in Biological Abstracts, Current Contents, ISI Web of Science and Google Scholar databases using the words 'leaf-cutting ant nests' and/or 'soil fertility' and/or 'soil nutrients' and/or 'ant-nests effect on plants'. We also included our own unpublished records. We only included studies: (i) that compared soil fertility and/or plant traits and/or plant richness between LCA nest sites (treatment) and adjacent, non-nest sites (control); and (ii) that reported means, sample sizes and SEs or SDs for treatment and control to calculate effect sizes. In certain studies, numerical data of means and SDs/SEs were obtained from published figures using DATA THIEF software (http://www.datathief.org). Our final database included 34 independent studies conducted between 1975 and 2013 (Appendix S1). These 34 independent studies included 31 works from the published literature and three from our own unpublished records; 11 of those studies reported LCA effects on soil fertility, 10 on soil fertility and plant traits, and 13 only on plant traits. Therefore, the effects of LCAs on soil fertility were tested using 21 independent studies, and their effects on plant traits were tested using 23 independent studies. This number of studies is among the range of replicates used in other meta-analyses (see, among others, Koricheva, 2002; Morales & Traveset, 2009; Winfree *et al.*, 2009; Endara & Coley, 2011). Overall, our database included studies on 12 species (seven *Atta* and five *Acromyrmex*), which represent ~35% of LCA species, and a broad type of habitats such as tropical/subtropical savannas, dry and wet tropical/subtropical forests, temperate pastures, steppe and semi-desert scrublands. The diversity of our database reduced the chance of confounding effects (i.e. both *Atta* and *Acromyrmex* have species with internal and external refuse dumps distributed along tropical, subtropical and temperate habitats, but see the Results section).

Focal traits studied and moderator variables

We performed separate meta-analyses to assess the effect of LCA on three soil fertility traits and three plant performance traits. For soil fertility we evaluated separately the effect of LCA on: nutrients (C, N, P and K); cations (Al, Ca, Mg and Na); and pH. For plant performance we analysed separately plant growth (e.g. steam diameter, leaf and root biomass and/or plant height), plant density (e.g. plant cover and/or individuals/area), and plant species richness (plant species/area), to include effects at individual, population and community levels. Plant reproduction was not included because few studies measured this response variable.

For each data record, we classified whether nutrient/cation content or plant traits were measured on nest soils or on refuse material and, in the cases in which refuse material was the substrate analysed, whether this substrate was obtained from underground chambers or external piles (i.e. from species that deposit their organic waste in underground chambers or in external piles). We also classified each report according to ant genus (*Atta* or *Acromyrmex*) and geographical location (tropical/subtropical or temperate habitats). Therefore, our categorical moderator variables testing the effects of LCAs on soil fertility and plant traits were substrate (nest soils versus refuse), location of refuse material (external versus internal), ant genus (*Atta* versus *Acromyrmex*) and geographical zone (temperate versus tropical/subtropical habitats; hereafter 'latitude').

Calculation of effect sizes

First, we converted each pair of treatment and control observations from primary studies into a common measure of effect size, Hedges' *d*, and its associated variance 'var (*d*)' following Rosenberg *et al.* (2000). Hedges' *d* is an estimate of the standardised mean difference that is not biased by small sample sizes (Hedges & Olkin, 1985). Here, positive *d* values imply higher soil fertility/plant performance in nest sites compared with non-nest sites, whereas negative values entail the opposite trend (i.e. greater soil fertility and plant performance in control than in nest sites).

Primary studies often report estimates of more than one effect relating to a common focal trait (e.g. soil fertility is estimated via C, N, P and K nutrient content form the same soil sample). Therefore, the analysis of each nutrient as independent measures may involve potential pseudo-replication (Borenstein *et al.*, 2009; Mengersen *et al.*, 2013a). To avoid this problem, if a study contributed more than one effect to the same focal trait, we calculated a 'composite effect' for each focal trait (i.e. a separate 'composite effect' for soil nutrients, cations, pH, plant growth, density and/or species richness). The calculated 'composite effect' for each trait and study was the variance-weighted average of the available effect sizes for that trait, assuming a fixed-effects model (Mengersen *et al.*, 2013a). We calculated the variance of the mean of *m* effects (denoted X_i or X_j) following Mengersen *et al.* (2013a) as:

$$V\text{mean} = \left(\sum_{i=1}^{m} V_i + 2\sum_{i,j} V_{ij}\right)/m^2$$
$$= \left(\sum_{i=1}^{m} V_i + 2\sum_{i,j}\left(r_{ij}\sqrt{V_i}\sqrt{V_j}\right)\right)/m^2$$

where V_i represent the variance of X_i, V_{ij} represent the covariance between X_i and X_j, and r_{ij} represents the correlation between X_i and X_j (only one combination of a given pairing is required). As the correlation between the different traits measured is commonly unknown (e.g. it is not given in published results), we performed a sensitivity test by running two analyses, following Borenstein *et al.* (2009). In one analysis we assumed uncorrelated measures ($r = 0$) (i.e. that the estimates are fully independent of each other), and in a second analysis we assumed a perfect correlation between measures ($r = 1$). In the text, we present both *P*-values (lower and upper boundaries), but in figures we only show the upper boundary ($r = 1$) for simplicity and because both results (assuming no correlation and perfect correlation) were often consistent (i.e. showed similar *P*-values).

The final number of independent studies (IS) and effect size (ES) estimations used to calculate the 'composite effect' for each focal trait were: 19 IS and 66 ES for soil nutrient content; 13 IS and 29 ES for cation content; six IS and seven ES for pH; 13 IS and 26 ES for plant growth; 11 IS and 12 ES for plant density, and eight IS and 10 ES for plant richness.

Data analysis

We calculated the mean effect size for each focal trait running random effect models. We chose random-effect models *a priori*, because these models assume that studies differ by sampling error and random variation due to biological or environmental differences, and thus they are appropriate for ecological data

(Gurevitch & Hedges, 1999; Mengersen *et al.*, 2013b). As we chose random-effect models, we did not test for variation among study estimates (Q_t, total heterogeneity) because we were already assuming that it exists (see Mengersen *et al.*, 2013b). We calculated the bias-corrected 95% bootstrap confidence intervals (95% CI) based on 999 permutations (Adams *et al.*, 1997) to assess the significance of results. Effect size was considered significant at the 5% level when its 95% CI did not include zero (Rosenberg *et al.*, 2000).

For each focal trait, we tested whether moderator variables (e.g. substrate, ant genus, location of refuse and latitude) explained a significant amount of heterogeneity in the effect sizes by examining the *P*-values associated with Q_{between} (Q_b) statistics. This estimator describes the variation in effect sizes that can be attributed to differences between categories. We also calculated the bias-corrected 95% bootstrap confidence intervals (95% CI) for the different levels within categorical variables. All the analyses were performed in METAWIN 2.1 (Rosenberg *et al.*, 2000).

Publication bias

We tested for publication bias (i.e. the tendency of journals to favour publication of statistically significant results) in our effect size estimates using several methods. First, we inspected normal quantile plots which can be a useful diagnostic tool in meta-analyses to detect asymmetric distributions and/or the presence of gaps (Wang & Bushman, 1998). Secondly, we calculated Rosenthal's and Rosenberg's fail-safe numbers (i.e. number of studies that would have to be added to change the results of the meta-analysis from significant to non-significant; Rosenthal, 1979; Rosenberg, 2005). Thirdly, we generated funnel plots (Egger *et al.,* 1997) for visual inspection of the data, and tested for funnel plot asymmetry using the rank correlation test (Begg & Mazumdar, 1994). This test is used to examine whether the observed outcomes and the corresponding sampling variances are correlated. A high correlation would indicate that the funnel plot is asymmetric, which may be a result of publication bias. Finally, we applied the trim and fill method (Duval & Tweedie, 2000a,2000b) to estimate the number of studies missing from the meta-analysis due to the suppression of the most extreme results on the left and right sides of the funnel plot. The method augments the observed data so that the funnel plot is more symmetric and re-computes the summary estimate based on the complete data.

The normal quartile plots showed that effect sizes were normally distributed, suggesting there was no publication bias. The calculated fail-safe also showed robustness of our analyses against publication bias. Funnel plots and tests for plot asymmetry showed no tendencies for soil nutrients and plant growth, but a revealed asymmetry for soil cation content. The trim and fill method estimated no missing studies for cation content and plant growth on both sides and for nutrient content on the right side of the funnel plot, but estimated four missing studies for nutrient content on the right side of the funnel plot. Nonetheless, the estimated effect size was still statistically significant, suggesting that the apparent publication bias is insufficient to affect our results and interpretations. All publication bias analysis were performed using the METAFOR package in R (Viechtbauer, 2010), and detailed results are shown in Appendix S2.

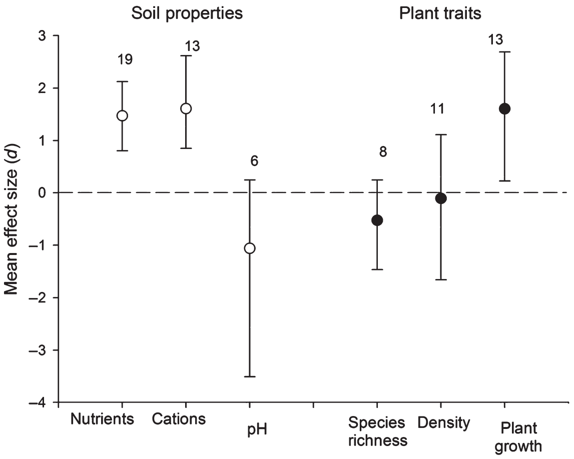

Fig. 1. Mean effect size (Hedge's *d*) and 95% bias-corrected bootstrap intervals of the effect of leaf-cutting ants on soil properties and plant traits. Numbers indicate sample sizes.

Results

Moderator variables were in general unrelated, reducing the probability of confounding effects. Specifically, ant genus was independent of latitude (tropical/subtropical versus temperate; Fisher's exact test, $P = 0.20$) and substrate type (nest soil versus refuse material; Fisher's exact test, $P = 0.71$). However, our data showed a dependence between ant genus and the location of refuse material sampled (Fisher's exact test, $P = 0.05$). This dependence was because all samples of *Acromyrmex* refuse in our database came from external piles, while samples from *Atta* refuse came from external piles and internal nest chambers.

General effects

Overall, LCAs had significant positive effects on soil fertility (Fig. 1). Specifically, nutrients and cation content were higher in ant-nest sites (which often include refuse material) than in non-nest sites (controls), but pH was unaffected by the existence of ant nests. On the other hand, nest sites showed higher plant growth than adjacent non-nest sites but similar plant density and plant richness (Fig. 1). Detailed mean effect sizes and confidence intervals are given in Table S1, Appendix S3.

Effect of moderator variables on soil properties: substrate, location of refuse, ant genera and latitude

Substrate. Refuse material had higher nutrient content than nest soils ($0.01 < P < 0.04$), but similar cation content ($0.64 < P < 0.75$).

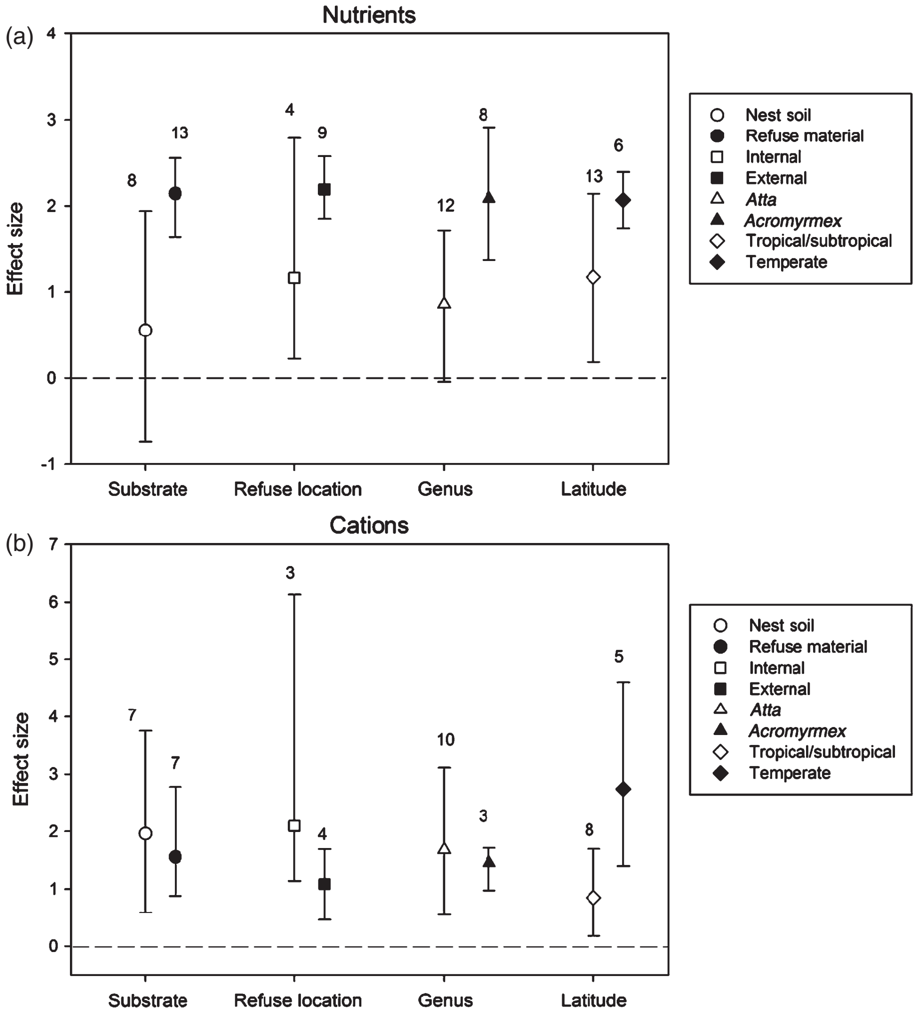

Fig. 2. Mean effect size (Hedge's *d*) and 95% bias-corrected bootstrap intervals of the effect of leaf-cutting ants on nutrients (a) and cation content (b) depending on type of substrate, location of the refuse dump, ant genus and latitude. Numbers indicate sample sizes for each category.

Location of refuse material. Refuse material from external piles tended to contain higher nutrient content than refuse material located in underground chambers ($0.06 < P < 0.19$), but similar cation content ($0.16 < P < 0.26$).

LCA genera. Acromyrmex nest soil sites tended to contain higher nutrient content than *Atta* nest sites ($0.05 < P < 0.06$), but similar cation content ($0.81 < P < 0.85$). However, the location of refuse may have caused this effect (see earlier).

Latitude. Temperate and tropical/subtropical nest sites did not differ in nutrient content ($0.16 < P < 0.28$), but temperate nest sites had higher cation content ($P = 0.01$). We were unable to explore the effect of categorical variables on soil pH due to the low number estimations ($n = 6$). In sum, nutrient content was higher in refuse material than in nest soils, tended to be higher in refuse material from external piles than in refuse material from internal chambers and was unaffected by latitude (Fig. 2a); and cation content was higher in nest sites from temperate than from tropical/subtropical regions, and was unaffected by substrate type, location of refuse material and ant genus (Fig. 2b). Details for number of studies and estimates used, mean effect sizes, confidence intervals, Q_b and associated *P*-values are given in Table S2, Appendix S3.

Effect of moderator variables on plant growth: substrate, ant genera and latitude

Plant growth tended to be higher on plants growing on refuse material than on nest soils ($0.06 < P < 0.07$). Confidence intervals of plant growth on refuse material did not include zero, while nest soils did. On the other hand, plant growth was unaffected by ant genus ($0.47 < P < 0.54$) and latitude ($0.37 < P < 0.44$; Fig. 3). We were unable to explore the effect of refuse location on plant growth due to highly unbalanced data. Details for number of studies and estimates used, mean

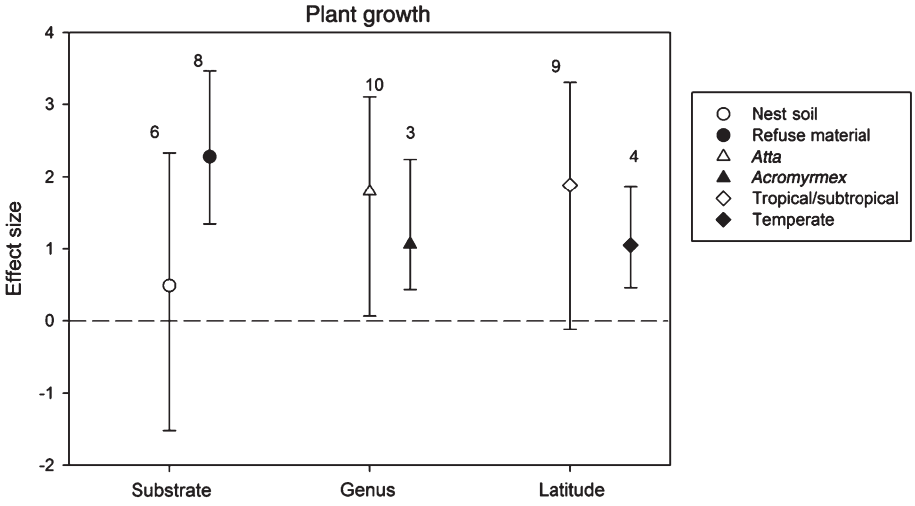

Fig. 3. Mean effect size (Hedge's *d*) and 95% bias-corrected bootstrap intervals of the effect of leaf-cutting ants on plant growth depending on type of substrate, ant genus and latitude. Numbers indicate sample sizes for each category.

effect sizes and confidence intervals, Q_b and associated P-values are given in Table S3, Appendix S3.

Discussion

Leaf-cutting ant nests have been commonly considered one of the most important agents of soil disturbances affecting vegetation patterns, but these assertions were based mostly on isolated studies, anecdotal data or qualitative reviews. Here, for the first time, we quantitatively confirmed the generality of these effects and determined some of their source of variation. Several patterns and trends emerged from this study. First, nutrient content was: (i) consistently higher in nest sites than in adjacent control, non-nest sites (however, the source of nutrients was the ants' refuse material, while the nest soil itself did not show higher nutrient concentrations); (ii) slightly higher in refuse material sampled from external piles than those sampled from internal underground chambers; (iii) slightly higher in *Acromyrmex* than *Atta* nests (but see later); and (iv) unaffected by latitude. Secondly, cation content was only affected by latitude; nest sites in temperate habitats had higher cation content than nest sites in tropical/subtropical habitats. Thirdly, plant growth was greater in nest sites than in adjacent non-nest sites, but again this effect appears to depend on substrate type: plants are often larger when established in refuse material than in ant nest soils. Plant growth was unaffected by both ant genus and latitude. Finally, both plant density and species richness were similar between nest sites and adjacent non-nest sites.

The first finding of this work is that the refuse material is the key source of nutrients by which LCAs improve soil fertility (Haines, 1978; Moutinho *et al.*, 2003; Farji-Brener & Ghermandi, 2004, 2008; Hudson *et al.*, 2009). This may bring together previous contrasting results where soil samples from LCA nests showed higher or lower nutrient content than adjacent non-nest soils. The location of the refuse material and its inclusion in soil samples may be the cause of the variation in the soil quality. Accordingly, those studies that showed higher nutrient content in nest sites sampled directly refuse materials or included them in their soil samples rather than sampling only ant nest soils (Farji-Brener & Ghermandi, 2000, 2008; Moutinho *et al.*, 2003; Verchot *et al.*, 2003; Hudson *et al.,* 2009). The studies finding similar or reduced nutrient content in nest soils than in adjacent non-nest soils gathered only nest top soils and did not include ant refuse materials in their samples (Bieber *et al.*, 2011; Madureira *et al.*, 2013; Meyer *et al.*, 2013). Two complementary reasons may explain why nest soils without refuse materials could have lower fertility (also discussed in Meyer *et al.*, 2013; Leal *et al.*, 2014). First, during nest construction and expansion, ants can move mineral soil with low nutrient concentration to the nest surface (Alvarado *et al.*, 1981). Secondly, ants heavily harvest almost all plants around the nest area, reducing the amount of leaf litter falling on nest topsoils (Farji-Brener & Illes, 2000; Hull-Sanders & Howard, 2003). Therefore, the strength and direction of the effects of LCA nests on top soil fertility are affected by the location of the refuse material.

The second finding is that refuse material situated in external piles tended to be richer in nutrient content than refuse material situated in internal chambers. Usually the soil biota responsible of soil mineralisation is more abundant and active in organic external piles than in isolated, dark underground waste chambers (Farji-Brener, 2010; Sousa-Souto *et al.*, 2012; Fernández *et al.*, 2014). Moreover, only external refuse dumps can receive litter fall that represents an extra input of nutrients and micro-biota. These two factors may contribute to explain why refuse material in external piles tend to have a higher nutrient content than those located in underground chambers. However, this result is not conclusive and needs to be considered as a working hypothesis rather than a verified pattern. One intriguing outcome was that *Acromyrmex* nests sites tended to have higher nutrient content than *Atta* nest sites. However, this tendency appears to be spurious because all refuse samples of *Acromyrmex* nests come from external piles while refuse from *Atta* nests comes from both locations, internal and external. As discussed earlier, it is probable that refuse material from external piles can contain higher nutrient pools than refuse material from internal nest chambers. Thus, the higher nutrient content in *Acromyrmex* nest sites may be an effect of the location of refuse rather than an effect of ant genus *per se*. Works that sample internal refuse dumps in *Acromyrmex* species are needed to balance the existing database and confirm this hypothesis.

The third finding is that ant nest sites from temperate zones were more cation-rich than ant nest sites located in tropical/subtropical zones. A probable explanation for this pattern is that cation content of leaves is often higher in temperate than in tropical/subtropical habitats (Oleksyn *et al.*, 2003; Reich & Oleksyn, 2004; Lovelock *et al.*, 2007). As the cation content of refuse material (the key fertility source of ant nest soils) depends on the foliar cation content of the harvested plants (Moller *et al.*, 2011), ants from temperate habitats should generate organic waste with a higher cation content than those from tropical areas. However, our analysis of potential publication bias regarding soil cation content suggested that our results on the effects of LCAs on this trait should be treated with caution.

Finally, our results confirm the general pattern that plants grow better in ant nest sites than in adjacent, non-nest sites. However, this positive effect on plant growth appears to be the consequence of the availability on refuse in nest sites, a result consistent with our evidence that refuse materials are the key source of nutrients. As discussed earlier, studies using natural isotopes demonstrated that plants near nest sites assimilate nitrogen from organic waste, enhancing their performance (Sternberg *et al.*, 2007; Farji-Brener & Ghermandi, 2008; Lescano *et al.*, 2012). Therefore, it is logical that plants established on refuse dumps grow better and faster than those on nest soils or adjacent non-nest soils.

The positive effect of LCA nests on individual plants apparently does not extend to population and community levels; plant density and species richness were similar between nest sites and adjacent non-nest sites. It is known that not all plant species respond equally to the excess of soil resources of refuse material (Farji-Brener *et al.*, 2010). Moreover, the enhanced nutrient availability created by LCAs (Farji-Brener & Tadey, 2009), the changes in microclimatic conditions generated by ant canopy harvesting (Corrêa *et al.*, 2010; Meyer *et al.*, 2011a,2011b), and the selective foraging capacity of leafcutters (Wirth *et al.*, 2003) may act like ecological filters for plant recruitment, thus affecting plant cover and diversity in nest areas by disfavouring particular species (Garrettson *et al.*, 1998; Farji-Brener, 2005; Silva *et al.*, 2012, Leal *et al.*, 2014).

Overall, the results of our meta analysis confirm certain patterns obtained for anecdotal and/or isolated studies and reveal some of their sources of variation, with unexplored potential consequences of LCAs on soil fertility and plant performance at regional level. As LCAs improve soil fertility through the accumulation of refuse materials, the type of plants affected and the ecological impact of these effects will ultimately depend on the location of this key nutrient source. On one hand, refuse material accumulated in external piles is temporarily unstable because of the effect of rain and wind (Hudson *et al.*, 2009), but it is easily accessible for small plants with superficial roots (Farji-Brener & Ghermandi, 2004, 2008; Farji-Brener *et al.*, 2010). Conversely, refuse material in internal nest chambers may be more temporarily stable but can only be accessed by the roots of large trees (Moutinho *et al.*, 2003; Saha *et al.*, 2012). In other words, refuse in external piles may benefit mainly herbs, early plant stages (seedlings and saplings) and short-living plants (annual or biannual), whereas refuse in internal nest chambers may benefit long-living, large trees. Accordingly, LCA nests with internal refuse chambers are often colonised by trees, promoting the formation of woody ‘islands’ in grass-dominated savannas and pastures (Jonkman, 1978; Farji-Brener & Silva, 1995; Sosa & Brazeiro, 2012), whereas LCA nests with external refuse dumps are often colonised by short-living plants (Farji-Brener & Ghermandi, 2004, 2008). Finally, if refuse material located in external piles is richer than that situated in internal nest chambers and if those nest sites are relatively more fertile in temperate than in tropical/subtropical habitats, LCA species with external refuse dumps that inhabit temperate regions should be of particular ecological relevance as an influence on nutrient cycling and vegetation patterns.

Acknowledgements

We thank S. Amador-Vargas and two anonymous reviewers for constructive comments that helped to improve the manuscript. This research was partially supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica (PICTO-UNRN 2010–0179 and PICT 2011–0701 to A.G.F.B. and V.W., respectively). No data was tortured during the progress of this study.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:
10.1111/een.12169

Appendix S1. Studies included in the meta-analyses.

Appendix S2. Details about publication bias analysis.

Appendix S3. Results of the overall meta-analyses for soil fertility and plant performance traits, including the effect of categorical moderator variables.

References

Adams, D.C., Gurevitch, J. & Rosenberg, M.S. (1997) Resampling tests for meta-analysis of ecological data. *Ecology*, **78**, 1277–1283.

Alvarado, A., Berish, C.W. & Peralta, F. (1981) Leaf-cutter ant (*Atta cephalotes*) influence on the morphology of andepts in Costa-Rica. *Soil Science Society of American Journal*, **45**, 790–794.

Begg, C.B. & Mazumdar, M. (1994) Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088–1101.

Bieber, A.G.D., Oliveira, M.A., Wirth, R., Tabarelli, M. & Leal, I.R. (2011) Do abandoned nests of leaf-cutting ants enhance plant recruitment in the Atlantic Forest? *Austral Ecology*, **36**, 220–232.

Borenstein, M., Hedges, L.V., Higgins, J.P.T. & Rothstein, H.R. (2009) *Introduction to Meta-Analysis*. John Wiley & Sons, New York, New York.

Corrêa, M.M., Silva, P.S.D., Wirth, R., Tabarelli, M. & Leal, I.R. (2010) How leaf-cutting ants impact forests: drastic nest effects on light environment and plant assemblages. *Oecologia*, **162**, 103–115.

Duval, S.J. & Tweedie, R.L. (2000a) Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics*, **56**, 455–463.

Duval, S.J. & Tweedie, R.L. (2000b) A nonparametric "trim and fill" method of accounting for publication bias in meta-analysis. *Journal of the American Statistical Association*, **95**, 89–98.

Egger, M., Smith, G.D., Schneider, M. & Minder, C. (1997) Bias in meta-analysis detected by a simple, graphical test. *British Medical Journal*, **315**, 629–634.

Endara, M.J. & Coley, P. (2011) The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology*, **25**, 389–398.

Farji-Brener, A.G. (2005) The effect of abandoned leaf-cutting ant nests on plant assemblage composition in a tropical rainforest of Costa Rica. *Ecoscience*, **12**, 554–560.

Farji-Brener, A.G. (2010) Leaf-cutting ant nests and soil biota abundance in a semi-arid steppe of northwestern Patagonia. *Sociobiology*, **56**, 549–557.

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. *Journal of Vegetation Science*, **11**, 453–460.

Farji-Brener, A.G. & Ghermandi, L. (2004) Seedling recruitment in the semi-arid Patagonian steppe: facilitative effects of refuse dumps of leaf-cutting ants. *Journal of Vegetation Science*, **15**, 823–830.

Farji-Brener, A.G. & Ghermandi, L. (2008) Leaf-cutting ant nests near roads increase fitness of exotic plant species in natural protected areas. *Proceedings of the Royal Society: Series B*, **275**, 1431–1440.

Farji-Brener, A.G. & Illes, A.E. (2000) Do leaf-cutting ant nests make "bottom-up" gaps in Neotropical rain forests? A critical review of the evidence. *Ecology Letters*, **3**, 219–227.

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.

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

Farji-Brener, A.G. & Tadey, M. (2009) Contributions of leaf-cutting ants to soil fertility: causes and consequences. *Soil Fertility* (ed. by D. P. Lucero and J. E. Boggs), Chapter 6, pp. 81–91. Nova Science Publishers, New York, New York.

Farji-Brener, A.G., Lescano, N. & Ghermandi, L. (2010) Ecological engineering by a native leaf-cutting ant increases the performance of exotic plant species. *Oecologia*, **163**, 163–169.

Fernández, A., Farji-Brener, A.G. & Satti, P. (2014) Moisture enhances the positive effect of leaf-cutting ant refuse dumps on soil biota activity. *Austral Ecology*, **39**, 198–203.

Fowler, H.G., Pagani, M.I., Silva, O.A., Forti, L.C. & Saes, N.B. (1989) A pest is a pest is a pest? The dilemma of neotropical leaf-cutting ants: keystone taxa of natural ecosystems. *Environmental Management*, **13**, 671–675.

Garrettson, M., Stetzel, J.F., Halpern, B.S., Hearn, D.J., Lucey, B.T. & Mckone, M.J. (1998) Diversity and abundance of understory plants on active and abandoned nests of leaf-cutting ants (*Atta cephalotes*) in a Costa Rica rain forest. *Journal of Tropical Ecology*, **14**, 17–26.

Gurevitch, J. & Hedges, L.V. (1999) Statistical issues in ecological meta-analyses. *Ecology*, **80**, 1142–1149.

Haines, B. (1978) Element and energy flows through colonies of the leaf-cutting ant, *Atta colombica*, in Panama. *Biotropica*, **10**, 270–277.

Hedges, L.V. & Olkin, I. (1985) *Statistical Methods for Meta-Analysis*. Academic Press, New York, New York.

Hölldobler, B. & Wilson, E.O. (2011) *The Leafcutter Ants: Civilization by Instinct*. W. W. Norton and Company, Inc., London, U.K.

Hudson, T.M., Turner, B., Herz, H. & Robinson, J.S. (2009) Temporal patterns of nutrient availability around nests of leaf-cutting ants (*Atta colombica*) in secondary moist tropical forest. *Soil Biology and Biochemistry*, **41**, 1088–1093.

Hull-Sanders, H.M. & Howard, J.J. (2003) Impact of *Atta colombica* colonies on understory vegetation and light availability in a neotropical forest. *Biotropica*, **35**, 441–445.

Jones, C.G., Lawton, J.H. & Shachak, M. (1994) Organisms as ecosystem engineers. *Oikos*, **69**, 373–386.

Jonkman, J. (1978) Nests of the leaf-cutting ant *Atta vollenweideri* as accelerators of succession in pastures. *Zeitschrift für Angewandte Entomologie*, **86**, 25–34.

Koricheva, J. (2002) Meta-analysis of sources of variation in fitness costs of plant anti-herbivore defenses. *Ecology*, **83**, 176–190.

Leal, I., Wirth, R. & Tabarelli, M. (2014) The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified neotropical forests. *Biotropica*, **46**, 516–528.

Lescano, N., Farji-Brener, A.G., Gianoli, E. & Carlo, T. (2012) Bottom-up effects may not reach the top: the influence of ant-aphid interactions on the spread of soil disturbances through trophic chains. *Proceedings of the Royal Society of London Series B*, **279**, 3779–3787.

Lovelock, C.E., Feller, I.C., Ball, M.C., Ellis, J. & Sorrell, B. (2007) Testing the growth rate vs. geochemical hypothesis for latitudinal variation in plant nutrients. *Ecology Letters*, **10**, 1154–1163.

Madureira, M.S., Schoereder, J., Teixeira, M.C. & Sobrinho, T. (2013) Why does *Atta robusta* (Formicidae) not change soil features around their nests as other leaf-cutting ants do? *Soil Biology and Biochemistry*, **57**, 916–918.

Mengersen, K., Jennions, M.D. & Schmid, C. (2013a) Statistical models for the meta-analysis of non-independent data. *Handbook of Meta-analysis in Ecology and Evolution* (ed. by J. Koricheva, J. Gurevitch and K. Mengersen). Princeton University Press, Princeton, New Jersey.

Mengersen, K., Schmid, C., Jennions, M.D. & Gurevitch, J. (2013b) Statistical models and approaches to inference. *Handbook of Meta-analysis in Ecology and Evolution* (ed. by J. Koricheva, J. Gurevitch and K. Mengersen). Princeton University Press, Princeton, New Jersey.

Meyer, S.T., Leal, I.R., Tabarelli, M. & Wirth, R. (2011a) Performance and fate of tree seedlings on and around nests of the leaf-cutting ant *Atta cephalotes*: ecological filters in a fragmented forest. *Austral Ecology*, **36**, 779–790.

Meyer, S.T., Leal, I.R., Tabarelli, M. & Wirth, R. (2011b) Ecosystem engineering by leaf- cutting ants: nests of *Atta cephalotes* drastically alter forest structure and microclimate. *Ecological Entomology*, **36**, 14–24.

Meyer, S.T., Neubauer, M., Sayer, E.J., Leal, I.R., Tabarelli, M. & Wirth, R. (2013) Leaf- cutting ants as ecosystem engineers: topsoil and litter perturbations around *Atta cephalotes* nests reduce nutrient availability. *Ecological Entomology*, **38**, 497–504.

Moller, I., Licht, H., Harholt, J., Willats, W. & Boomsma, J. (2011) The dynamics of plant cell-wall polysaccharide decomposition in leaf-cutting ant fungus gardens. *PLoS ONE*, **6**, 17506.

Montoya-Lerma, J., Giraldo-Echeverri, C., Armbrecht, I., Farji-Brener, A.G. & Zoraida, C. (2012) Leaf-cutting ants revisited: towards rational management and control. *International Journal of Pest Management*, **58**, 225–247.

Morales, C. & Traveset, A. (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters*, **12**, 716–728.

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.

Oleksyn, J., Reich, P.B., Zytkowiak, R., Kroleswki, P. & Tjoelker, M. (2003) Nutrient conservation increases with latitude of origin in European *Pinus sylvestris* populations. *Oecologia*, **136**, 220–235.

Reich, P. & Oleksyn, J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 11001–11006.

Rico-Gray, V. & Oliveira, P.S. (2007) *The Ecology and Evolution of Ant-Plant Interactions*. University of Chicago Press, Chicago, Illinois.

Rosenberg, M.S. (2005) The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis. *Evolution*, **59**, 464–468.

Rosenberg, M.S., Adams, D.C. & Gurevitch, J. (2000) *Metawin: Statistical Software for Meta-Analysis*. Sinauer Associates, Sunderland, Massachusetts.

Rosenthal, R. (1979) The 'file drawer problem' and tolerance for null results. *Psychological Bulletin*, **86**, 638–641.

Saha, A., Carvalho, K., Sternberg, L. & Moutinho, P. (2012) Effect of leaf-cutting ant nests on plant growth in an oligotrophic Amazon rain Forest. *Journal of Tropical Ecology*, **28**, 263–270.

Schoereder, J.H. & Howse, P.E. (1998) Do trees benefit from nutrient-rich patches created by leaf-cuttingants? *Studies on Neotropical Fauna & Environment*, **33**, 111–116.

Silva, P.S.D., Leal, I.R., Wirth, R., Melo, P.F.L. & Tabarelli, M. (2012) Leaf-cutting ants alter seedling assemblages across second-growth stands of Brazilian Atlantic forest. *Journal of Tropical Ecology*, **28**, 361–368.

Sosa, B. & Brazeiro, A. (2010) Positive ecosystem engineering effects of the ant *Atta vollenweideri* on the shrub *Grabowskia duplicate*. *Journal of Vegetation Science*, **21**, 597–605.

Sosa, B. & Brazeiro, A. (2012) Local and landscape-scale effects of an ant nest construction in an open dry forest of Uruguay. *Ecological Entomology*, **37**, 252–255.

Sousa-Souto, L., Jesus Santos, D., Ambrogi, B., Campos dos Santos, M., Braga-Bueno, M. & Pereira-Filho, R.E. (2012) Increased CO2 emission and organic matter decomposition by leaf-cutting ant nests in a coastal environment. *Soil Biology and Biochemistry*, **30**, 1–5.

Sternberg, L., Pinzon, M., Moreira, M., Moutinho, P., Rojas, E. & Herre, A. (2007) Plants use macronutrients accumulated in leaf-cutting ant nests. *Proceedings of the Royal Society of London Series B*, **274**, 314–321.

Tadey, M. & Farji-Brener, A.G. (2007) Indirect effects of exotic grazers: livestock decreases the nutrient content of refuse dumps of leaf-cutting ants through vegetation impoverishment. *Journal of Applied Ecology*, **44**, 1209–1218.

Verchot, L.V., Moutinho, P.R. & Davidson, E.A. (2003) Leaf-cutting and (*Atta sexdens*) and nutrient cycling: deep soil inorganic nitrogen stocks, mineralization and nitrification in Eastern Amazonia. *Soil Biology and Biochemistry*, **35**, 1219–1222.

Viechtbauer, W. (2010) Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, **36**, 1–48 [WWW document]. URL http://www.jstatsoft.org/v36/i03/ [accessed on 15 October 2014].

Wang, M.C. & Bushman, B.J. (1998) Using the normal quantile plot to explore meta-analytic data sets. *Psychological Methods*, **3**, 46–54.

Winfree, R., Aguilar, R., Vazquez, D., LeBuhn, G. & Aizen, M. (2009) Meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, **90**, 2068–2076.

Wirth, R., Herz, H., Rye, L., Beyschlag, W. & Hölldobler, B. (2003) *Herbivory of Leaf-Cutting Ants*. Springer-Verlag, Berlin, Germany.

Accepted 8 October 2014
First published online 12 December 2014