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Refuse pile turnover by harvester ants (Hymenoptera: Formicidae) increases seed density and seedling species richness in dry grasslands

Adeline BULOT, Erick PROVOST & Thierry DUTOIT

Abstract

Messor barbarus (LINNAEUS, 1767) ants are Mediterranean region seed predators. However, transported seeds can be found rejected in refuse piles around their nests, making this ant a seed dispersal agent. This raises the following questions: Do refuse piles affect seed distribution? Do their small-scale seed composition, richness, and density in autumn and winter differ from those of areas without refuse piles? Are there differences in the vegetation found the following spring in refuse piles that have survived the winter or on sites where they have been destroyed, compared with control areas without refuse piles? In a Mediterranean steppe, we measured autumn and winter seed banks in a greenhouse, both in refuse piles and in controls. The following spring, in situ seedlings from refuse piles, from refuse piles artificially destroyed (sieved) in winter, and from controls without refuse piles were recorded. Seedling species richness and density were significantly higher in autumn in refuse piles than in controls. Nevertheless, no increased seedling contribution from the transient seed bank was detected in the winter refuse piles. The following spring, natural persistent refuse piles showed no seedlings. However, seedling species richness and density were significantly higher in places where refuse piles had been sieved before in winter. The construction of refuse piles by *M. barbarus* locally concentrates seed density and seed species richness; for the first time, a positive impact on seedlings is observed in places where refuse piles were sieved before winter and the potential bias of this methodology must be now compared with actions of natural agents (surface runoff) that destroyed refuse piles in winter.

Key words: Seed dispersal, plant-insect interactions, harvester ant, *Messor barbarus*, Mediterranean grassland.

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Introduction

The mutualistic relationships that ants maintain with plants through seed dispersal have led to ants being recognized as keystone species (GILBERT 1980, GOVE & al. 2007). They are even considered to be the major invertebrate seed dispersers in terrestrial ecosystems (STILES 1980). Sensu lato, seed dispersal by ants is an active process defined by the term "myrmecochory" (SERNANDER 1906, HOWE & SMALLWOOD 1982). Some plants even develop a nutritious appendage on their seeds, which means they can be dispersed specifically by ants (SERNANDER 1906, BOND & SLINGSBY 1984, BEATTIE & HUGHES 2002, YOUNGSTEADT & al. 2009). Other plants may be spread by dyszoochory, where harvester ants forget or accidentally drop small amounts of non-elaiosome seeds during their foraging activity (HOBBS 1985, RISSING 1986, ARNAN & al. 2010).

Harvester ants can be found in a variety of regions including arid, semi-arid, and tropical areas and in various habitats (BROWN & al. 1979, MORTON & DAVIDSON 1988, JOHNSON 2001). They can have strong direct and indirect effects on regeneration and on population densities of plants (BROWN & HUMAN 1997, DEFALCO & al. 2009, ARNAN & al. 2012). Harvester ants play a particularly important role in the Mediterranean basin, where the genus *Messor* is the most abundant granivorous ant species (DÍAZ 1991, CERDÁ & RETANA 1994, AZCÁRATE & PECO 2007).

Because harvester ants are voracious seed predators, a large proportion of the seeds produced is destroyed by their consumption (ANDERSEN & ASHTON 1985, DETRAIN & PASTEELS 2000, SCHÖNING & al. 2004). Nevertheless, some authors maintain that if the number of available seeds

of a species is higher than the number of suitable sites for establishment, high seed predation does not necessarily imply limitations on the establishment of new individuals (ANDERSEN 1989, ERIKSSON & EHRLÉN 1992). Moreover, one indirect advantage could be the favoring of uneaten plant species through decreased competition with eaten species. In addition, rare events can compensate for damage caused by predation: Ants can lose, abandon, or reject a few seeds on their foraging trails (DETRAIN & TASSE 2000) or in the refuse piles around their nest (RISSING 1986, LEVEY & BYRNE 1993, VORSTER & al. 1994, DETRAIN & TASSE 2000, RETANA & al. 2004, AZCÁRATE & PECO 2007). The displaced seeds can then be dispersed over great distances in microsites favorable to the recruitment of seedlings (DEAN & YEATON 1992, VORSTER & al. 1994, ARNAN & al. 2010). Workers of the genus *Messor* are known to forage tens of meters from their nest (DETRAIN & al. 2000, RETANA & al. 2004, ARNAN & al. 2010, PLOWES & al. 2013). These seeds are also spared competition with the parent plant, seed predators and pathogens (BENTLEY 1977, HOWE & SMALLWOOD 1982). It is refuse piles that frequently contain a high abundance of viable seeds collected in a 30 m radius forage area (CERDAN 1989), deposited or rejected in error by worker ants (MAC MAHON & al. 2000) or after a final selection carried out inside the nest (MAJER & LAMONT 1985, DETRAIN & PASTEELS 2000).

Although dry grasslands are hot spots of biodiversity (WEIHER & KEDDY 1999, WILLEMS 2001, POSCHOLD & WALLISDEVRIES 2002, ALARD & POUDEVIGNE 2002, GIBSON 2009), their area, habitat quality and biodiversity have already been shown to have decreased drastically (DZWONKO & LOSTER 1998, LEE & al. 2001, ADRIAENS & al. 2006, DUTOIT & al. 2013), mainly due to 20th century changes in land use (grazing abandonment, cultivation, etc.) (SAUNDERS & al. 1991). This is particularly true of Mediterranean grasslands, strongly in need of conservation and ecological restoration (TRABA 2003, BONET 2004, BUISSON & DUTOIT 2006, JANIŠOVÁ 2011). However, implementing measures for ecological restoration requires a fuller understanding of an ecosystem's functioning and of its plants and animals, some of which can be used as "ecosystem engineers" to assist in the restoration of biodiversity after degradation (JONES 1997, FOLGARAIT 1998). Assessing seed dispersal by ants in particular may also help to understand the process of plant species assemblage in dry grasslands, with a view to preserving species.

Previous work has already revealed that ants, and especially the genus *Messor*, have an extensive impact on Mediterranean vegetation, including through the construction of refuse piles (LÓPEZ & al. 1993, DETRAIN & TASSE 2000, SÁNCHEZ & al. 2006, AZCÁRATE & PECO 2007). For example, although the density and species richness of the viable seed bank were found to be significantly higher in autumn in refuse piles of *Messor barbarus* ants than in soil samples without refuse piles, in the following spring, seedling species richness from seeds present in the surviving refuse piles was significantly lower than in surrounding areas (AZCÁRATE & PECO 2007). Thus, to date, no significant positive effect of ants on small-scale spatial vegetation redistribution in dry grasslands via refuse pile construction has been clearly demonstrated. Moreover, while soil fertility has been found to increase in refuse piles, especially through changes in texture, pH, organic material and nutrient content (P, N, K), refuse piles can also have negative effects on seed viability and emergence due to the extensive accumulation of soil and plant material in a small area (AZCÁRATE & PECO 2007). However, refuse piles have both temporal and spatial dynamics. In the French Mediterranean region, their size is at its maximum in autumn and most of them disappear naturally as a result of weathering over the winter (A. Bulot, unpubl.). This natural destruction of refuse piles in autumn and winter could play a role in increased seedling emergence following a phase of seed concentration in the former refuse piles. As a consequence, seedling regeneration needs to be assessed not only in refuse piles themselves but also on former refuse pile sites.

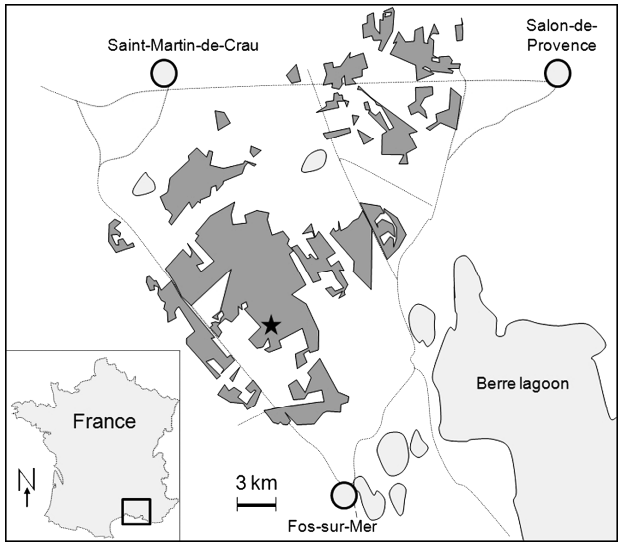

Fig. 1: Location of the plain of La Crau in south-eastern France and the study site (black star) in the National Nature Reserve outlined in dark grey.

The objectives of this paper will thus be to analyze the effects of *Messor barbarus* on small-scale seed distribution and seedling emergence in Mediterranean dry grassland. Our hypotheses are that: (1) Refuse piles have a significant impact on seed composition and significantly increase species richness and seed density in autumn and winter relative to the same quantities of soil without refuse piles, (2) where refuse piles have been artificially destroyed during winter, plant species richness and density significantly increase the following spring and (3) refuse piles maintained throughout winter significantly increase the number of seedlings, no doubt by creating environmental conditions favorable to the preservation of seeds (accumulation of organic dry matter, protection against predation and parasites, etc.).

Materials and methods

Study site

The plain of La Crau is a Mediterranean grassland located in south-eastern France (Bouches-du-Rhône), about 70 km west of Marseille (43° 30' 57.11" N, 4° 52' 21.72" E) (Fig. 1). This plain is considered as a "steppe" ecosystem and is typical of the rangelands of the Mediterranean basin (LE HOUÉROU 2001). Siliceous pebbles, washed down from the Alps, cover more than 50% of the soil surface, which has an extremely flat topography. An impermeable con-

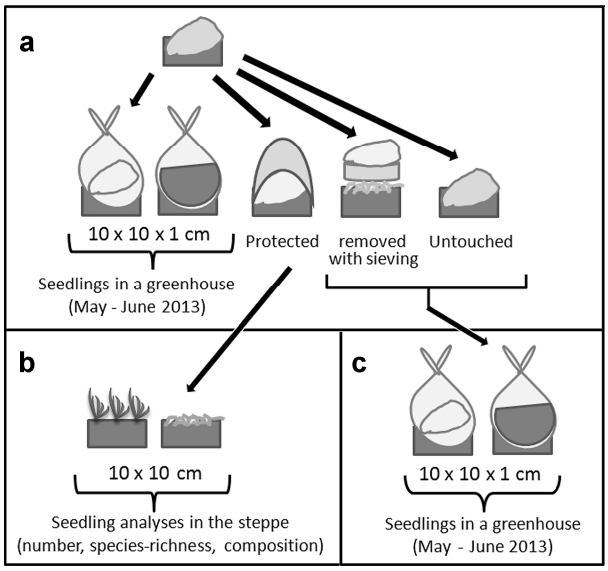

Fig. 2: Schema of the experimental design. (a) In autumn 2012 forty refuse piles of *Messor barbarus* georeferenced in the plain of La Crau: September to December 2012, ten 70 cm^3 samples from these refuse piles placed with ten 70 cm^3 soil samples without refuse piles for seed bank analyses, ten refuse piles removed with sieving, ten refuse piles protected with mosquito netting, and ten refuse piles were untouched. (b) April 2013 seedling analyses realized in the steppe where the refuse piles were sieved or untouched in autumn. (c) May to June, ten 70 cm^3 samples taken from the refuse piles protected in autumn, with ten 70 cm^3 soil samples without refuse piles for seed bank analyses.

glomerate of pebbles in a matrix of calcium carbonate 40 - 60 cm deep in the soil prevents plant root systems from reaching the water table (MOLLIEX & al. 2013). The Mediterranean climate is dry, with strong sunshine throughout the year (DEVAUX & al. 1983). Interacting effects of a shallow soil, a warm Mediterranean climate and the actions of living beings, such as centuries of itinerant sheep grazing (HENRY & al. 2010), have structured this species-rich plant community. The vegetation is composed of 50% annual species which germinate in autumn, overwinter as seedlings and grow in spring, flowering from March to May or in September (BOURRELLY & al. 1983). Plant cover ranges from 50% to 80% of the soil surface. On average, 30 to 40 plant species per square meter, with a maximum of 70 plant species per square meter, can be observed (RÖMERMANN & al. 2005). This xeric vegetation is composed mainly of stress-tolerant annual species, but perennial species such as *Brachypodium retusum* (PERS.) P. BEAUV. (Poaceae) and *Thymus vulgaris* L. (Lamiaceae), structure the plant community by their cover. The other most characteristic plant species are *Asphodelus ayardii* JAHAND. & MAIRE (Xanthorrhoeaceae), *Linum gallicum* L. (Linaceae) and *Stipa capillata* L. (Poaceae) (MOLINIER & TALLON 1950, DEVAUX & al. 1983, BUISSON & DUTOIT 2006).

Biological model

Messor barbarus (LINNAEUS, 1767) (Hymenoptera: Formicidae) is a granivorous ant species whose impact on seed dispersal and consumption has already been studied in the Mediterranean Basin (DETRAIN & TASSE 2000, AZCÁRATE & PECO 2007, BARAIBAR & al. 2011). *Messor barbarus* is the main granivorous ant species in the plain of La Crau (CERDAN 1989, CERDAN & al. 1990). This harvester species occurs naturally in the steppe ecosystem, as it does throughout the north-western area of the Mediterranean Basin (BERNARD 1968). In the plain of La Crau, its density is relatively high, with 60 to 80 nests per hectare (CERDAN 1989). *Messor barbarus* is a harvester ant which predates 5% to 20% of the total steppe seed production, and the seeds of 63% of the typical phanerogams of this steppe are collected and transported by this ant (CERDAN & al. 1990). The high polymorphism of the worker ants means they can transport a large variety of seeds: They have been found to carry from 0.2 mg to 60 mg (DETRAIN & al. 1996), with a maximum of almost 96 mg already measured in Spain (HENSEN 2002), over a distance of as much as 30 metres. As observed in Spain, seeds may thus be discarded, lost or ejected (DETRAIN & TASSE 2000, AZCÁRATE & PECO 2007), while conserving their capacity for germination (BONTE & al. 2003). Some of these uneaten seeds are found in refuse piles located near ant nest entrances (RISSING 1986, ANDERSEN 1988, LEVEY & BYRNE 1993, DETRAIN & TASSE 2000, BONTE & al. 2003, AZCÁRATE & PECO 2007, MARTÍNEZ-DURO & al. 2010). *Messor barbarus* was identified using the determination key in LEBAS (2014).

Experimental design

Autumn seed bank sampling: In late summer (September 2012), thus after peak seed production in Mediterranean dry grasslands (BOURRELLY & al. 1983) but before seed germination and the wet season, a total of forty refuse piles were sampled upon encounter at their maximum size on a site representative of the plain of La Crau, "Le Coussoul de Figuières" (≈ 8 ha, 43° 30' 52.17" N, 4° 52' 21.80" E). Of these forty refuse piles, we sampled the organic material and the first centimeter of the mineral soil measured with a centimeter ruler in ten refuse piles on 10 × 10 cm quadrats, i.e., much smaller than the minimum size of all the selected refuse piles. To ensure that both destroyed and intact refuse piles would be found the following spring, ten other refuse piles were randomly selected and sieved with a 2 mm mesh, to mimic natural winter destruction by removing fine organic and soil material; ten further refuse piles were covered with mosquito netting to protect them against natural destruction (surface runoff) during winter. We chose this approach because it was impossible to predict in autumn 2012 how many and which refuse piles would be destroyed during winter 2012 - 2013.

In the sieved refuse piles, seeds larger than 2 mm were collected in the sieve and replaced in the area formerly occupied by the refuse piles. Thus, only the dry matter accumulated in the refuse piles was removed. The last ten refuse piles were left undisturbed (Fig. 2a). To ensure that the refuse piles were near different nests, we selected nests spaced at least 10 m apart, as a nest can be four meters in diameter in the plain of La Crau (CERDAN 1989).

Simultaneously, the first soil centimeters from ten randomly distributed 10 × 10 cm areas with no refuse piles, ant nests or other signs of former ant activities were collected (control) (Fig. 2a). For each of the 20 soil samples (sites with and without refuse piles) collected during this

period, a volume of 70 cm^3, the minimum volume collected in one sample, was spread on a substrate composed of a 1 : 4 compost-vermiculite mix in germination boxes measuring 30 cm × 45 cm, without sieving. They were placed to germinate according to the concentrated seedling emergence method (TER HEERDT & al. 1996). All samples were randomly placed in a greenhouse for three months from September to December 2012 with no control of temperature and humidity. The samples were watered every day. They were then kept at 4°C for 15 days and subsequently returned to the greenhouse in January 2013, until no new germination was observed. In the greenhouse, germinations were identified (MULLER 1978), counted, and removed each week to measure the number of viable seeds, species richness and composition of each sample.

Spring seed bank sampling: In March 2013, the first soil centimeters were collected from 10 × 10 cm quadrats on the ten refuse piles protected by mosquito netting in autumn 2012. The same methodology was applied to ten quadrats (control) of 10 × 10 cm on randomly distributed steppe areas with no refuse piles, ant nets or other signs of ant activities. A volume of 70 cm^3 of each soil sample was spread and placed to germinate using the method described above (Fig. 2c). Identification of plant species was carried out using a determination key (TISON & al. 2009).

Spring seedling analysis: In April 2013, when most seedlings are identifiable in the field, they were identified and counted on ten 10 × 10 cm quadrats from each of these sampling areas described above: (1) ten natural refuse piles chosen at random and which had neither been destroyed nor protected by mosquito netting during winter, (2) ten areas where the refuse piles had been sieved in autumn 2012 and (3) ten areas (control) with no ant nests or refuse piles identified previously in 2012 (Fig. 2b). Vegetation and bare soil percent cover were visually estimated in each quadrat. Nevertheless, the opportunity to compare seedlings emergence in these non-expected naturally destroyed refuse piles in comparison with the autumn sieved refuse piles was not taken in this study and then, it is not possible to exclude a potential experimental artifact of the sieving method in comparison with the natural winter destruction.

Statistical analyses

In order to compare species composition among treatments, multivariate analysis was used based on a similarity matrix. Similarity analysis gives an overview of the relations among samples in terms of species abundance. Similarity among treatments was verified via multivariate similarity analysis (ANOSIM) (CLARKE & GREEN 1988), based on the Bray-Curtis index (BRAY & CURTIS 1957). ANOSIM analysis calculates the R statistic (degree of difference ($0 \leq R \leq 1$) between treatments, and the significance of R (p-value < 0.05). When the p-value is significant, the closer the R value is to 1, the more the treatments differ; and conversely, the closer the R value is to 0, the less the treatments differ. Then, the contribution of species to mean similarity was evaluated via SIMPER analysis (CLARKE 1993, CLARKE & WARWICK 2001). SIMPER analysis identifies the species contributing most strongly to patterns of similarity among treatments. Moreover, the same matrix of similarity was ordinated using Non-metric Multi-Dimensional Scaling (NMDS) based on the Bray-Curtis distance (BORCARD & al. 2011). The more similar two samples

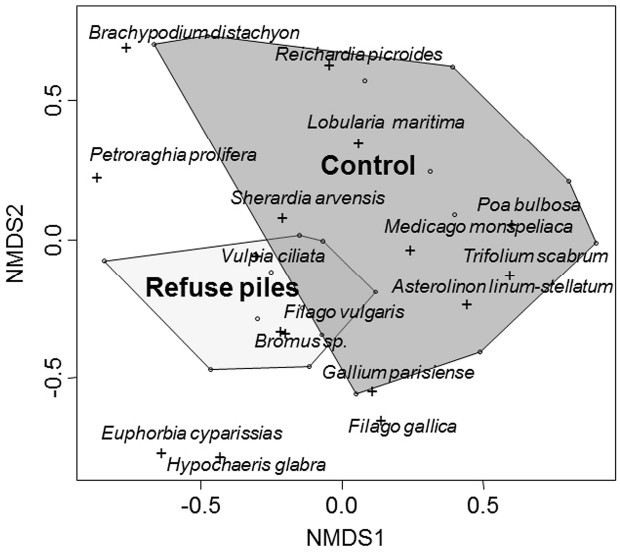

Fig. 3: Non-metric multidimensional scaling (NMDS) realized on the seedling analyses of ten 70 cm^3 samples from refuse piles and in ten 70 cm^3 soil samples without refuse piles (control) in autumn 2012. The ten samples of each treatment are grouped with polygons. To clarify, only the plant species most highly correlated to the two first axes are literally inscribed.

are in abundance, the closer they are to each other on the graph. A stress coefficient value of less than 0.1 means a good representation (FIELD & al. 1982). Data were square root transformed to reduce the effects of the most abundant species.

In order to compare species diversity among treatments, Shannon and Evenness indices of the distribution of species were calculated. The Shannon index is zero when the population is represented by a single species and becomes maximum when the population is evenly distributed among all species. The Evenness index, based on the Shannon index, varies between 0 and 1. Its value is maximum when the population distribution is homogeneous among all species and is minimal when one species dominates. The Evenness index indicates the level of diversity achieved by the theoretical maximum (PIELOU 1969).

In order to compare seedling plant species richness, seedling number, cover percentage of vegetation and bare soil and Shannon and Evenness indices among treatments, Student-t tests were used when the data followed a normal law according to Lilliefors tests. When conditions were not normal, non-parametric Wilcoxon tests were used. The alpha level used in distinguishing between significant and not significant was 0.05.

All statistical analyses were performed using the R software version 2. 15. 2. (THE R FOUNDATION FOR STATISTICAL COMPUTING 2012), with its packages for univariate analyses, and the additional packages ade4 (CHESSEL & al. 2004, DRAY & DUFOUR 2007) and vegan (OKSANEN & al. 2013) for multivariate analyses.

Results

Autumn 2012 seed banks: Multivariate analyses based on the abundance data of 46 species separates the samples taken in the refuse piles from the controls, but they remain similar, as illustrated by the NMDS ordination

Tab. 1: Characteristics of the viable seed banks in autumn 2012 and spring 2013; and characteristics of the seedlings identified in the field in spring 2013: samples taken in refuse piles and in the controls. Values are means ± standard errors; df corresponds to the degrees of freedom; the t-value (t), the W-value (W), and the p-value (p) resulting from t tests or Wilcoxon tests to test the effect of each treatment on each variable. Within a row, two boxes with a different letter are significantly different (p-value < 0.05).

	Autumn 2012 seedbanks			**Spring 2013 seedbanks**			**Spring 2013 field seedling analyses**		
	Student-t tests / Wilcoxon	**Refuse piles**	**control**	**Student-t tests / Wilcoxon**	**Refuse piles**	**control**	**Student-t tests / Wilcoxon**	**Sieved refuse piles**	**control**
Total number of seedlings	/	925	334	/	6	3	/	362	216
Mean number of seedlings	W = 90 p < 0.01	92.5 ± 6.22 a	33.3 ± 6.70 b	df = 17.66 t = -0.08 p = 0.94	1.0 ± 0.67 ns	1.05 ± 0.77 ns	df = 9.99 t = -1.97 p = 0.08	36.2 ± 0.75 ns	21.6 ± 3.19 ns
Species richness	df = 17.90 t = 2.88 p < 0.01	12.9 ± 0.35 a	7.5 ± 0.35 b	W = 30 p = 0.1	0.5 ± 0.26 ns	1.10 ± 0.44 ns	df = 13.99 t = -3.84 p < 0.01	13.4 ± 0.29 a	8.3 ± 0.53 b
Shannon Index	df = 12.17 t = 0.91 p = 0.38	0.82 ± 0.02 ns	0.74 ± 0.04 ns	/	/	/	df = 13.34 t = -3.64 p < 0.01	1.15 ± 0.01 a	0.87 ± 0.03 b
Evenness Index	df = 12.09 t = -1.35 p = 0.15	0.32 ± 0.01 ns	0.37 ± 0.02 ns	/	/	/	W = 27 p = 0.09	0.45 ± 0.004 ns	0.41 ± 0.01 ns
Vegetation cover (%)	/	/	/	/	/	/	df = 12.93 t = -2.66 p < 0.05	61.0 ± 4.25 a	34.0 ± 1.86 b
Bare soil (%)	/	/	/	/	/	/	df = 12.93 t = 3.48 p < 0.01	36.8 ± 3.98 b	70.5 ± 1.91 a

with a stress coefficient of 0.18. The NMDS ordination also shows that floristic composition and plant population distribution are more homogeneous in the treatment with refuse piles than in the controls (Fig. 3). The ANOSIM analysis confirms this result between these two treatments with an R statistic of 0.58 (p = 0.001). Thus, while there is a difference between the samples taken from the refuse piles and controls, this difference is small. Concerning the contribution of species to mean similarity, the SIMPER analysis shows the major contributors to be *Petrorhagia prolifera* (L.) P.W. BALL & HEYWOOD (Caryophyllaceae) (71.9%), *Medicago monspeliaca* (L.) TRAUTV. (Fabaceae) (69.3%), *Trifolium scabrum* L. (Fabaceae) (66.7%), *Filago gallica* L. (Poaceae) (64.1%), *Crassula tillaea* LEST.-GARL. (Crassulaceae) (61.5%), *Filago vulgaris* LAM. (Asteraceae) (58.3%), *Sherardia arvensis* L. (Rubiaceae) (54.4%), *Poa bulbosa* L. (Poaceae) (50.3%), *Gallium parisiense* L. (Rubiaceae) (46.0%), *Brachypodium distachyon* (L.) P. BEAUV. (Poaceae) (40.8%), *Lobularia maritima* (L.) DESV. (Brassicaceae) (34.0%), *Bromus* sp. (Poaceae) (26.9%**)** and *Vulpia ciliata* DUMORT. (Poaceae) (19.6%). The mean contribution of these 13 species is 51.1% (Appendix S1, as digital supplementary material to this article, at the journal's web pages).

In total, 1259 individuals germinated from the seed banks, representing a total of 49 species. A total of 925 seedlings representing 36 species were identified in the refuse piles, while in the control samples only a total of 334 seedlings representing 29 species were identified. 19 species are present only in the refuse piles and 13 only in the control samples (Appendix S1). Mean seedling number is roughly three times higher in the samples taken in refuse piles than in the controls, and mean seedling species richness is roughly twice as high in this treatment. Shannon and Evenness indices are very low and not significantly different (Tab. 1).

In the samples, the abundance of five species is significantly higher in the refuse piles than in the controls: *Bromus* sp. (Poaceae), *Filago vulgaris* LAM. (Asteraceae), and *Vulpia ciliata* DUMORT (Poaceae) with a p-value of less than 0.001; *Galium parisiense* L. (Rubiaceae) with a p-value of less than 0.01; and *Brachypodium distachyon* (L.) P. BEAUV. (Poaceae) with a p-value of less than 0.05 (Appendix S1).

Spring 2013 seed banks: Very few seeds germinated in the samples from the refuse piles protected by mosquito netting, making statistical analysis impossible. Nine individuals germinated from the seed banks, representing a total of four species. Six seedlings representing one species (*Lobularia maritima* (L.) DESV. (Brassicaceae) were only identified in the refuse piles protected during winter. In the controls, only three seedlings, representing three species (*Amaranthus albus* L. (Amaranthaceae), *Crepis vesicaria* L. (Asteraceae), *Diplotaxis tenuifolia* (L.) DC. (Brassicaeae), were identified (Tab. 1, Appendix S1).

Spring 2013 field seedlings: No germination was identified in the few remaining refuse piles, whether protected by mosquito netting or not. Only one of the ten untouched refuse piles was still intact after the winter. A wider investigation of the steppe in spring 2013 revealed no further untouched refuse piles intact. Most of the refuse piles were therefore destroyed during the winter. Nevertheless,

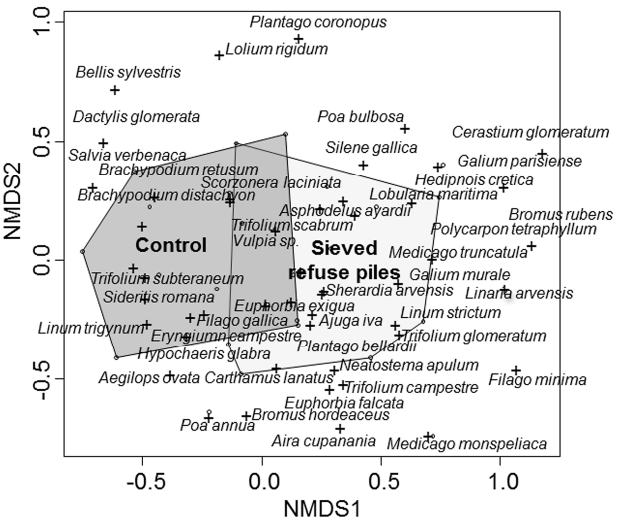

Fig. 4: Non-metric multidimensional scaling (NMDS) realized on the seedling analyses between the sieved refuse piles (light grey) and the control (dark grey) measured in the field in spring 2013.

the opportunity to compare seedlings emergence in these non-expected naturally destroyed refuse piles in comparison with the autumn sieved refuse piles was not taken in this study and then, it is not possible to exclude a potential experimental artifact of the sieving method.

Between the areas where the refuse piles were sieved and the control areas, multivariate analyses based on the abundance data of 50 species reveal high similarity (Appendix S1), as illustrated by the NMDS ordination with a stress coefficient of 0.23 (Fig. 4). The ANOSIM analysis confirms this result with an R statistic of 0.19 ($p = 0.014$). Concerning the contribution of species to mean similarity, the SIMPER analysis shows the strongest contributors to be *Filago gallica* L. (Poaceae) (78.2%), *Linum trigynum* L. (Linaceae) (71.0%), *Trifolium scabrum* L. (Fabaceae) (40.0%), *Gallium parisiense* L. (Rubiaceae) (69.0%), *Sideritis romana* L. (Lamiaceae) (66.9%), *Salvia verbenaca* L. (Lamiaceae) (64.9%), *Aegilops ovata* L. (Poaceae) (62.8%), *Bromus* sp. (Poaceae) (60.5%), *Brachypodium retusum* (PERS.) P. BEAUV. (Poaceae) (58.1%), *Trifolium subterraneum* L. (Fabaceae) (55.5%), *Sherardia arvensis* L. (Rubiaceae) (52.8%), *Ajuga iva* (L.) SCHREB. (Lamiaceae) (49.9%), *Hypochaeris glabra* L. (Asteraceae) (46.9%), *Evax pygmeae* (L.) BROT. (Poaceae) (43.6%), *Polycarpon tetraphyllum* (L.) L. (Caryophyllaceae) (36.1%), *Euphorbia exigua* L. (Euphorbiaceae) (32.1%), *Poa bulbosa* L. (Poaceae) (28.1%), *Vulpia ciliata* DUMORT. (Poaceae) (23.4%), *Lobularia maritima* (L.) DESV. (Brassicaceae) (18.6%), and *Brachypodium distachyon* (L.) P. BEAUV. (Poaceae) (13.5%). The mean contribution of these 20 species is 46.6% (Appendix S1).

Plant percent cover is significantly higher in the sieved refuse pile areas compared with the control areas (Student-t test, $df = 12$, $t = -2.66$, $p < 0.05$), while bare soil percent cover is significantly lower (Student-t test, $df = 13$, $t = 3.48$, $p < 0.01$) (Tab. 1).

In total, 578 individual seedlings were identified, representing 77 species. In the sieved refuse pile areas, 362 seedlings representing 45 species were identified, with 18 species identified only in this treatment. In the control areas, 216 seedlings representing 32 species were identified, with five species present only in these quadrats. Seedling numbers and species richness are roughly twice as high in the sieved refuse piles as in the controls, but the mean number of seedlings does not significantly differ between these two treatments (Tab. 1, Appendix S1).

The Shannon index is again very low but significantly different between the sieved refuse pile areas and the controls, being significantly higher in the former (p-value < 0.01). The Evenness index is also low but there is no significant difference between the refuse piles and the controls (p-value > 0.09) (Tab. 1).

The abundance of five species is significantly higher in the sieved refuse pile areas than in the control areas: *Poa bulbosa* L. (Poaceae) with a p-value of less than 0.01; and *Euphorbia exigua* L. (Euphorbiaceae), *Hedypnois cretica* (L.) DUM. COURS. (Asteraceae), *Lobularia maritima* (L.) DESV. (Brassicaceae) and *Polycarpon tetraphyllum* (L.) L. (Caryophyllaceae) with a p-value of less than 0.05 (Appendix S1).

Discussion

Despite being a voracious seed predator, *Messor barbarus* is often described as a good seed dispersal agent in the Mediterranean basin (CERDAN & al. 1990, DETRAIN & al. 1996, DETRAIN & TASSE 2000, SÁNCHEZ & al. 2006, AZCARÁTE & PECO 2007, PLOWES & al. 2013). Consequently, this ant could be a good partner in plant community restoration under ecological engineering (BULOT & al. 2014a), since it appears to play a major role in the distribution of annual plant populations in Mediterranean dry grasslands (WOLFF & DEBUSSCHE 1999). To confirm this seed dispersing role, we assessed how the ants' refuse piles affect seed viability and dispersal in a Mediterranean dry grassland, taking into account their temporal dynamics over one year.

Refuse piles are found here to have a significant impact on seed composition and significantly increase species richness and seed density in autumn compared to soil areas of the same size without refuse piles. 73.5% of the total seedlings counted were in the refuse piles, which also contained 14.3% more species than the areas without refuse piles. Our initial results thus confirm that the accumulation of seeds is the major effect of refuse piles, in agreement with previous studies both on *Messor barbarus* (see AZCÁRATE & PECO 2007) and on other granivorous ants (HOBBS 1985, RISSING 1986, LEVEY & BYRNE 1993, MACMAHON & al. 2000). This is to be expected, given that autumn follows the period of peak seed production in Mediterranean dry grasslands (BOURRELLY & al. 1983), and subsequent to the peak of *M. barbarus* activity there must be a maximum of seeds in the refuse piles (CERDAN 1989). These rejected seeds obviously remain viable in refuse piles during summer, since when placed in a greenhouse, many germinate (AZCÁRATE & PECO 2007). Besides protecting seeds against predators and pathogens (BENTLEY 1977, HOWE & SMALLWOOD 1982), refuse piles may offer a good microenvironment enhancing the survival and the germination rate of seeds (RISSING 1986, LEVEY & BYRNE 1993); AZCÁRATE & PECO (2007) showed that refuse piles can also increase soil fertility.

The fact that we found seedlings to be relatively well distributed among species, with proportions similar among refuse piles, may explain the absence of significant differ-

ences in species diversity. There was little difference in species composition between the refuse piles and the soil areas without refuse piles: thirteen most strongly contributing species accounted for 51.1% of mean similarity. However, species composition was more homogeneous in the refuse piles. A large quantity of Poaceae seeds was present in the refuse piles, for example *Vulpia ciliata* and *Bromus* sp., annuals producing many seeds. Seeds from these Poaceae species were certainly present in high density over the ant foraging area. As *Messor barbarus* is a generalist granivorous species which harvests large quantities of seeds present in great abundance (DETRAIN & al. 1996), we assume that the species composition reflects the activity of workers collecting, sorting and rejecting certain seeds (AZCARÁTE & PECO 2007). Thus, the significant differences in composition, species richness, and density we found between refuse piles and controls are likely due to the high concentrations in the refuse piles of seeds from the most abundant seed producers in the herbaceous plant community, which are here: *Vulpia ciliata*, *Lobularia maritima*, *Poa bulbosa*, and *Bromus* sp. Another explanation could be seed selection by *M. barbarus* workers during foraging. The refuse piles have not only more seeds but also larger seeds (or larger fruits) than the controls (AZCARÁTE & PECO 2005). Consequently, small-seeded species, even when highly abundant seed producers, may escape predation by ants.

Previous studies found very low seedling emergence on the persistent refuse piles (MARTÍNEZ-DURO & al. 2010), so it appeared interesting here to measure seedling emergence after their autumn and winter natural destruction as we try to do with an artificial destruction of the refuse piles with sieving. In spring 2013, very few seedlings emerged from either the protected refuse piles or the controls. In the protected refuse piles, only two species were present: *Lobularia maritima* and *Sanguisorba minor*, both belonging to the permanent seed bank (THOMPSON & al. 1997). Even though seed concentrations in refuse piles were high, no species with a transient seed bank were observed, despite environmental conditions favorable to seed conservation (accumulation of organic dry matter, protection against predation and parasites, etc.). However, the organic dry matter of refuse piles began to degrade even under the mosquito netting, too thin to protect the seeds. The seedling emergence of species with a transient seed bank in this ecosystem is therefore not enhanced by ants constructing these refuse piles.

We observed that the great majority of refuse piles did not survive the winter. The wet and windy conditions from autumn to spring documented for the plain of La Crau may therefore explain the marked deterioration of the refuse piles (DEVAUX & al. 1983). For this reason, seedling analysis was conducted only on areas where the refuse piles had been artificially destroyed by sieving, imitating their natural degradation as it was not possible to expect the natural destruction or persistence of each marked refuse piles before the winter. In these sieved refuse piles, plant species richness and seedling density were significantly higher in spring. 62.6% of the total seedlings were found in the sieved refuse piles, which contained 16.8% more species than the controls but these results were not compared to the total seedlings of naturally destroyed refuse piles which then cannot exclude an artifact from the sieving experimental methodology. The biomass proxy provided by plant cover and bare soil cover indicates a higher vegetation density in the sieved refuse piles. These results are in contradiction to other studies, where seedling emergence was extremely low and species richness decreased in persistent refuse piles the following spring (AZCARÁTE & PECO 2007, MARTÍNEZ-DURO & al. 2010). These authors sampled refuse piles which had survived the winter: They make the assumption that seed germination was limited by the thickness of the dry matter layer therein, so that seedling radicles and / or hypocotyls could not reach the soil and / or the light needed to grow (AZCARÁTE & PECO 2007, MARTÍNEZ-DURO & al. 2010). In support of this hypothesis, an inhibitory effect of litter on shoot emergence of small-seeded species was previously measured by SAYER (2005). We therefore conclude that when refuse piles are sieved, the dry matter layer becomes sufficiently thin to allow seedlings to emerge from the seeds deposited by ants in the refuse piles. Species composition was similar between the sieved refuse piles and the controls. Indeed, both the diversity and the similarity indices show that all species were present in relatively the same abundance and in the same proportions in the replicates of all samples (sieved refuse piles and controls).

Our findings shed light on the role of ants' refuse piles in spatial seed distribution at a local scale in Mediterranean dry grasslands. In autumn, worker ants harvest a great number of seeds, especially those of the greatest seed-producing species in the herbaceous plant community, which may then be discarded in refuse piles. Viable seeds are thus concentrated therein. They conserve their germination capacity, however, only during summer and early autumn, being protected against predators and pathogens (BENTLEY 1977, HOWE & SMALLWOOD 1982). Moreover, the refuse piles can increase soil fertility (AZCARÁTE & PECO 2007) and may represent favorable microsites allowing seeds to pass through dispersal and abiotic filters (LORTIE & al. 2004).

If the refuse piles have been artificially destroyed by sieving, the seeds can reach soil and light, germinate and grow, probably due to the absence or thinness of dry matter. As a consequence, future researches need to focus on the potential impact of this artificial method in comparison with the impacts of natural agents such as the surface runoff. In addition, the soil of naturally destroyed refuse piles provides a favorable microenvironment for the development of seeds, as previous studies showed (WHITFORD 1988, BEATTIE 1989, DEAN & YEATON 1993, MACMAHON & al. 2000, AZCARÁTE & PECO 2007). Previous studies also showed that the whole community may be subject to density-dependence regulation (GOLDBERG & BARTON 1992, BERTNESS & CALLAWAY 1994, GOLDBERG & al. 2001, WHITE & al. 2001, LORTIE & TURKINGTON 2002). Higher initial seed density can cause lower rates of seedling emergence (GOLDBERG & BARTON 1992, LORTIE & TURKINGTON 2002). However, in our case, seed accumulation in refuse piles does not seem to diminish the number of seedlings, which was significantly higher than in the controls.

Messor barbarus appears to be a seed dispersal agent in Mediterranean dry grasslands at a local scale. This harvester ant has a positive effect not only on seed distribution but also on seedling emergence when the ants' refuse piles were sieved in autumn. Thus, this ant could be a good

"ecosystem engineer" (JONES & al. 1997) to assist in restoration programs, provided that it promotes the desired species in a degraded area (BULOT & al. 2014b), even though the seed dispersal capacity of *M. barbarus* is limited by its 30 m radius foraging areas (CERDAN 1989). As the majority of refuse piles are naturally destroyed, artificial destruction of refuse piles by sieving does not appear to be necessary to improve vegetation restoration. Moreover, the impact should be intensified by the fact that the refuse piles have a relatively high percent cover (AZCARÁTE & PECO 2003) and that their distribution changes yearly.

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