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► To cite this version:

T. de Almeida, Francois Mesleard, M. Santonja, Raphaël Gros, T. Dutoit, et al.. Above- and below-ground effects of an ecosystem engineer ant in Mediterranean dry grasslands. Proceedings of the Royal Society B: Biological Sciences, 2020, 287 (1935), pp.20201840. 10.1098/rspb.2020.1840 . hal-02946514

HAL Id: hal-02946514

<https://amu.hal.science/hal-02946514>

Submitted on 22 Oct 2020

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Above- and below-ground effects of an ecosystem engineer ant in Mediterranean dry grasslands

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Within a local assemblage, ecosystem engineers can have major impacts on population dynamics, community composition and ecosystem functions by transforming or creating new habitats. They act as an ecological filter altering community composition through a set of environmental variables. The impact of ants on their environment has been widely studied, but their multi-component effects (both trophic and non-trophic) have been rarely addressed. We investigated the roles of *Messor barbarus*, one of the commonest harvester ant species in south-western European Mediterranean grasslands. We analysed soil physico-chemical parameters, above-ground vegetation (e.g. species richness, plant community, micro-local heterogeneity, plant biomass) and above- and below-ground fauna (macrofauna, Collembola, Acari and nematodes). A clear and strong local impact of *M. barbarus* on soil, vegetation and fauna compartments emerges. The environmental filter is altered by modifications to soil physico-chemical properties, and the biotic filter by changes to plant communities and altered above- and below-ground fauna abundance, occurrence and community structure. The engineering activity of *M. barbarus* affects not only these separate ecosystem components but also the trophic and non-trophic relationships between them. By altering ecological filters at a local scale, *M. barbarus* creates habitat heterogeneity that may in turn increase ecological niches in these highly diverse ecosystems.

1. Introduction

One of ecology's major goals is to understand how organisms' interactions with each other and their environment impact species distribution and abundance. Some species, known as ecosystem engineers, are able to modify their community assembly. These engineers have major impacts on population dynamics, community composition and ecosystem functions [1,2] through environmental changes caused either by their physical presence or by their actions or interactions, transforming or creating new habitats [3]. The ecosystem engineer concept has, however, been the subject of debate, with some scholars arguing that all organisms engineer their environments to some degree [4]. It is therefore important to determine the nature and the magnitude of ecosystem engineers' impacts on local communities and their habitat.

Two distinct but linked hypotheses underpin studies on ecosystem engineers: (1) they affect a set of environmental conditions and (2) by modifying these conditions they act as an ecological filter altering community composition [5] by promoting a set of the other organisms' biological traits [6]. The ecological filter theory provides a conceptual framework for understanding the processes that determine which and how many species live in a particular locality. This theory explains local community composition and structure by a set of discrete and sequential mechanisms that constrain a larger species

pool of potential residents to the subset that occurs within a community, through three filters [7]. First, only a subset of the regional pool is available to colonize particular sites, depending both on the landscape matrix and on each species's dispersal ability and population size [8]. Second, only some species have the physiological capacity to withstand the prevailing environmental conditions. Finally, only some species are able to withstand certain biotic interactions that may depend, for example, on the availability of food resources [9] or the presence of natural enemies [10].

The ecological filter theory, which has been tested in a wide range of marine and terrestrial ecosystems (e.g. [7,11]), is particularly relevant to disentangle mechanistic and stochastic processes in the organization of local communities [12]. In terrestrial ecosystems, ants have been identified as ecosystem engineers that are able to act on all three filters. They affect plant communities by altering their dispersal capacities and modifying microenvironmental conditions [13]. By their structural engineering activities during nest construction and maintenance, ants may influence soil physico-chemical properties. Moreover, modifying the habitat structure may alter non-trophic relationships by creating habitat heterogeneity (e.g. [14]). Ants are also a key component of local trophic relations [15]. Their accumulation of organic material (e.g. seeds, plant tissues and insect carcasses accumulated on their refuse piles) increases the density of microbial and faunal decomposers [16] such as collembolans, with a cascading effect on their predators [17]. In addition, as predators, ants may directly or indirectly impact the abundance and diversity of other arthropods within an ecosystem [18].

Trophic and engineering functions are rarely considered in combination [19], and very few planned observations of engineer interactions to date even consider both functions [20]. Yet the engineering concept can contribute to theories of species coexistence. If engineers influence a set of environmental conditions, and if species selection and adaptation follow, then engineering should markedly enhance opportunities for niche differentiation, diversification and coexistence at the same or at multiple trophic levels [21]. While the impact of ants on their environment has been widely studied, only a few studies have addressed the multi-component effects (both trophic and non-trophic) of an ant species on its ecosystem [20].

In south-western European grasslands, the native granivorous ant *Messor barbarus* is known to redistribute seeds and to change soil physico-chemical properties [22] (see electronic supplementary material, Method S1). We recently demonstrated its capacity to improve the physical and chemical properties of a degraded soil in the Plain of la Crau, as well as its positive impact on plant communities and soil seed banks [23]. However, a global study that includes fauna and trophic and non-trophic relationships is necessary to obtain a comprehensive understanding of their role as engineers. This study assessed whether *M. barbarus* acts as an ecosystem engineer driving community assembly through trophic and non-trophic interactions. We conducted this study in xerophytic meadows of the Camargue delta regional Nature Reserve (south of France). We hypothesized that *M. barbarus* (1) modifies soil texture by redistributing soil particles, and increases soil nutrients through the accumulation of plant materials, (2) which in turn affects the plant community (e.g. increase in plant biomass and height) and

physiological status, and (3) increases above- and below-ground fauna abundance and occurrence and changes their community structure; and finally, (4) by its direct and indirect impacts on all these components, it changes trophic and non-trophic relationships.

2. Material and methods

(a) Study area

The study was carried out at the Domaine de la Tour du Valat in the Rhône delta, southern France (43°29' N, 4°40' E). This reserve is composed of almost 2700 ha of xero- and halophytic mixed meadows with patches of saline steppes and temporary marshes managed by traditional extensive cattle grazing [24]. Xerophytic meadows are a priority habitat (code 6220, European Union Habitats Directive, 1992). They are characterized by a high proportion of annual plant species including those characteristic of the Mediterranean region, such as *Brachypodium distachyon* (L.) P. Beauv., *Euphorbia exigua* L., *Plantago lagopus* L., *Trifolium scabrum* L., *Trifolium suffocatum* L., *Filago pygmaea* L. and *Catapodium rigidum* (L.) C. E. Hubb. [25].

The climate is Mediterranean with cold winters and warm dry summers; precipitation (average: 600 mm year⁻¹) occurs mainly during autumn and, to a lesser extent, spring [24].

(b) Experimental design

We randomly selected thirty of the largest *M. barbarus* active nests scattered over 10 randomly selected xerophytic meadows over an area of 262 ha extensively grazed by bulls (0.1 livestock units). The selected nests were class 4 on the Blanco-Moreno *et al.* [26] five-level scale (no class 5 were observed). This scale is based on a combination of factors: surface area occupied by the colony and number of entrances (class 4: nests covering 2–4 m² with 3–4 entrances). To ensure that we had applied this classification scheme accurately we measured the relevant parameters for each candidate nest. On each meadow, we selected the same number of ant-free patches as ant patches of 4 m² with no sign of ant activity (i.e. refuse piles, nests or tracks). They were located at a minimum distance of 5 m from a nest to avoid any border effect of colony activities [27].

Messor barbarus nest location is unpredictable, known to be strongly influenced by proximity to long-established colonies [26] rather than by soil properties (e.g. organic carbon (C), soil strength or aggregate distribution) [28], and distribution can be either regular or random [26]. Moreover, after their nuptial flight in autumn, mated queens land randomly. For the purposes of this study, therefore, differences in ecological variables between ant patches and ant-free patches are considered to result from the engineering activity of *M. barbarus*.

(c) Soil physico-chemical variables

We collected soil samples on the nest green belt (dense vegetation belt located on the refuse pile from the previous year) and randomly in the 4 m² ant-free patches by throwing the soil sample container. When it landed on bare soil area, which represented in average 18% of the patch, we threw it again. We sampled the top few centimetres of soil (about 200 g) [23] both in ant and ant-free patches in June 2018. To quantify both nitrate (N-NO₃) and ammonium (N-NH₄), about 50 g of soil was frozen and stored until analysis. The rest of the soil sample was air-dried and sieved (2 mm). Then, using standard international methods (Pipet method; NF X 316107) [29], we assessed particle-size distribution without prior decarbonization (clay (less than 0.002 mm), fine silt (0.002–0.02 mm), coarse silt (0.02–0.05 mm), fine sand (0.05–0.2 mm), coarse sand (0.2–2 mm))

and chemical properties (organic C, total nitrogen (total N), available phosphorus (available P) [30], calcium oxide (CaO), magnesium oxide (MgO), potassium oxide (K₂O), sodium oxide (Na₂O), pH_{KCl}, cation exchange capacity (CEC), C:N ratio, total organic matter (TOM)).

(d) Plant community

We sampled plant communities in 2 m × 2 m (4 m²) quadrats placed on the 30 selected nests (ant patches) and 30 ant-free patches in May 2018. The abundance of each plant species in each quadrat was defined according to a modified Braun-Blanquet scale [31] as follows: 0.2 = represented by a single individual in the 4 m² quadrat, 1 = covering less than 5%, 2 = covering between 5% and 25%, 3 = covering between 26% and 50%, 4 = covering between 51% and 75% and finally 5 = covering > 75%. In each quadrat, we also estimated both the percentage of bare soil based on canopy cover, and the vegetation height.

To assess the heterogeneity of the micro-local plant composition generated by ant activities around the nest (e.g. soil disturbed and deposited at the top of ant hills, refuse piles scattered around or ant 'trails') we placed three quadrats of 10 cm × 10 cm (0.01 m²) in each 4 m² quadrat. In the ant patches, they were placed as follows: one in the 'green belt', one in the 'bioturbated soil' (top of ant nest where soil is heavily disturbed) and one in an area showing neither of the two previous traces of ant activity. In ant-free patches, the 0.01 m² quadrats were placed randomly. In each 0.01 m² quadrat, we counted all plants in May 2018, when most seedlings can be identified [32]. Then for each of the 60 patches, a micro-local vegetation heterogeneity index based on the average of three Bray–Curtis index distances—a dissimilarity index varying between 0 and 1:0 for similar communities and 1 for distinct communities—was calculated between the three 0.01 m² quadrats.

We measured aboveground dry biomass by cutting the vegetation to ground level, then placing a 50 cm × 50 cm (0.25 m²) quadrat per 4 m² quadrat in the 'green belt' of ant patches and randomly in ant-free patches. The measurements were taken in May 2019 during the peak in plant productivity. Then, each sample was oven-dried at 40°C up to constant weight.

Plant community physiological status was assessed in May 2019 with a hand-held multi-parameter optical sensor Multiplex Research (Force-A, Orsay, France). This fluorometer uses fluorescence technology with multiple excitation (UV, blue, green and red) and detection wavelengths (yellow, red and far-red) to measure constitutive and induced leaf epidermal anthocyanins, chlorophylls and nitrogen balance indices (see electronic supplementary material, Method S2) [33,34]. Anthocyanins and flavonols are plant secondary compounds that can be affected by stress and thus reveal a physiological dysfunction. Without N limitation, a plant promotes its primary metabolism and synthesizes proteins (nitrogenous molecules) containing chlorophyll, and few flavonols (carbon compounds). The nitrogen balance index which corresponds to the chlorophyll: flavonols ratio is a useful indicator of N deficiency. We took 10 measurements on the 'green belt' in ant patches and at randomly selected points in ant-free patches.

(e) Above- and below-ground fauna

Fauna was sampled in spring 2018. Macrofauna was hand sorted from soil monoliths (25 × 25 × 25 cm) that were placed to maximize the cover of the green belt in the ant patches. In the ant-free patches, soil monoliths were placed randomly. Invertebrates were stored in 70% ethyl alcohol and then identified to order level and counted. Rare taxa—fewer than five individuals—were not considered.

Mesofauna was collected using two core-samples (5 cm diameter) from the upper 7 cm of soil from the 'green belt' of

ant patches and randomly from ant-free patches by throwing the sample container. When it landed on bare soil area, we threw it again. Collembola and Acari were extracted over a period of seven days using a modified high-gradient canister method [35] and stored in 70% ethyl alcohol. Collembola taxa were assigned to life-history groups (epedaphic, hemidaphic and euedaphic) according to Gisin [36]. Acari were divided into three suborders reflecting their trophic level: Oribatida, Gamasida and Actinedida.

Microfauna was sampled using the same protocol as for mesofauna. Nematodes were extracted over 2 days from moist soil using the Baermann funnel method [37] and a first count of live specimens performed in the subsequent days. They were then fixed in 4% formalin solution, mounted on glass slides under a microscope, and the first 200 individuals encountered were divided into trophic groups following Yeates *et al.* [38]. Nematodes that could not be assigned to a trophic group with certainty were classified in the group with the most similar morphological feeding structure.

(f) Soil respiration

In each patch, *in situ* soil C effluxes (g CO₂ m⁻¹ h⁻¹) (release of CO₂ from soils and plants due to production of CO₂ by leaves, roots, soil organisms and chemical oxidation of C compounds) were recorded in May 2019 with a portable infrared gas analyser (IRGA, EGM-4) connected to a closed soil respiration chamber (SRC-1) (PP Systems, Massachusetts, USA) before removal of aboveground vegetation. To prevent leakage of CO₂ into the air when placing the chamber on the grass, a PVC tube (10 cm diameter and 11 cm in height) was sunk 1 cm deep in the soil prior to measurement (see electronic supplementary material, Method S3).

(g) Statistical analyses

To test the effect of ants (fixed-effect predictor variable) on soil physico-chemical properties, soil respiration, plant community parameters and fauna abundance and occurrence, linear mixed-effect models (LMMs) or generalized linear mixed models (GLMMs) were selected according to the distribution of model residuals. Chlorophyll index, anthocyanin index, nitrogen balance index and plant species richness were fitted with a Gaussian distribution. All soil physico-chemical parameters, other plant community parameters and soil respiration analyses were fitted with a gamma distribution (R package 'lme4' [39]). Both mesofauna and microfauna abundance were fitted with a negative binomial distribution (R package 'MASS' [40]), while macrofauna abundance was fitted with a zero-inflated generalized linear mixed model (R package 'glmmTMB' with family 'nbinom2' [41]) and macrofauna occurrence with a binomial distribution (in g.m⁻² and ind.m⁻² respectively for plant biomass and fauna abundance). To assess differences in total abundance among the three trophic levels (decomposers, primary consumers (PC) and secondary consumers (SC)) and the ratio between them in ant and ant free-patches, GLMMs were selected, respectively, with a negative binomial distribution and a Gaussian distribution. The identity number of the xerophytic meadows was used as a random factor in all models.

Effect sizes were estimated using Cohen's *d* index with 95% confidence intervals for each variable normally distributed [42] (R package 'effsize', [43]). The formula was adapted for non-normal data [44]. The magnitude of the effects was assessed on the following scale: $|d| < 0.2$ 'negligible', $|d| < 0.5$ 'small', $|d| < 0.8$ 'medium', $|d| > 0.8$ 'large'. A positive Cohen's *d* indicates a higher response variable value in ant patches than in ant-free patches.

Changes in plant community composition were visualized via non-metric multidimensional scaling (NMDS) based on the

Table 1. Ant effects on plant community variables. Values are means \pm s.e.; $n = 30$. z - or t -values and associated p -values were obtained from LMM or GLMM with gamma distribution. *Italic values indicate significant differences between ant patches and ant-free patches.*

	z- or t-value	p-value	ant patches	ant-free patches
vegetation heterogeneity	−2.30	<i>0.02</i>	0.75 \pm 0.02	0.66 \pm 0.03
plant biomass (g/0.25 m ²)	−9.12	<i><0.001</i>	154.2 \pm 12.3	63.4 \pm 5.3
vegetation height (cm)	−2.83	<i>0.005</i>	9.2 \pm 0.7	7.1 \pm 0.5
species richness	−1.53	0.13	38.5 \pm 1.0	36.6 \pm 1.0
nitrogen balance index	−1.49	0.14	0.19 \pm 0.01	0.18 \pm 0.01
anthocyanin index	2.28	<i>0.03</i>	0.07 \pm 0.01	0.09 \pm 0.01
chlorophyll index	−2.80	<i>0.01</i>	1.52 \pm 0.04	1.38 \pm 0.03
bare soil percentage (%)	−0.03	0.98	18.5 \pm 2.6	17.8 \pm 1.9

Bray–Curtis dissimilarity index to ordinate the characteristics of plant communities (composition and abundance). Differences in plant community composition were tested by permutational multivariate analysis (PERMANOVA) using the Adonis function (R package ‘vegan’ [45]).

Path analyses (PA) were performed to evaluate the impact of ant presence on soil physico-chemical and vegetation parameters and trophic webs in both above- and below-ground compartments (R package ‘lavaan’ [46]). Path analysis is specific structural equation modelling (SEM) used to represent causal networks between several measured variables and to test model data consistency [47]. Because no statistically valid model linking the above- and below-ground compartments was obtained, we developed two conceptual models biologically relevant to above- and below-ground compartments (see electronic supplementary material, Method S4). For the above-ground compartment, we assessed the causal relationships between N–NH₄, clay content, vegetation heterogeneity and height, bare soil percentage, plant biomass and above-ground invertebrate abundances (epedaphic collembolans, Hemiptera, Araneae, plant feeders and Coleoptera predators) in both ant and ant-free patches. For the soil compartment, we assessed the causal relationships between vegetation height, plant bio-mass, clay content, TOM and soil invertebrate abundances (microfauna PC and SC, mesofauna PC and SC, earthworms, macrofauna detritivore and Geophilomorpha) in both ant and ant-free patches. The full models were simplified by stepwise exclusion of non-significant variables until a minimum adequate model was reached. The adequacy of each model was determined by non-significant differences between the predicted and observed covariance matrices (χ^2 -squared test, $p > 0.05$), low root mean squared error of approximation index (RMSEA < 0.1) and high comparative fit index (CFI > 0.90) [47].

All statistical analyses were performed using R software v. 3.6.1 (R Development Core Team, 2011).

3. Results

(a) Soil physico-chemical parameters

Clay, fine silt and coarse silt content were, respectively, 25%, 30% and 64% higher in ant-free patches than in ant patches, with a large effect from ants on coarse silt. Coarse sand was 17% higher in ant patches (see electronic supplementary material, figure S1 and table S1).

Available P and N–NH₄ content were, respectively, 51% and 39% higher in ant patches than in ant-free patches, with a large effect. By contrast, C:N ratio and CaO, K₂O, MgO, Na₂O and total N content were higher in ant-free

patches than in ant patches. No other significant differences were found (see electronic supplementary material, figure S1 and table S1).

(b) Plant community

At the 0.01 m² scale, vegetation heterogeneity was 14% higher (medium effect) in ant patches (table 1 and figure 1). At the 0.25 m² scale, plant biomass was 2.5 times higher (large effect) in ant patches (table 1 and figure 1). At the 4 m² scale, vegetation height was 30% higher in ant patches. We found no significant difference in plant community composition ($R^2 = 0.02$, pseudo- $F = 1.01$; $p = 0.43$) and plant species richness (table 1) between ant and ant-free patches.

The chlorophyll index was 10% higher in ant patches, while the anthocyanin index was 29% higher in ant-free patches (table 1).

(c) Above- and below-ground fauna

(i) Macrofauna

Abundances of Araneae and coleopteran predators were respectively three and four times higher (large effects) in ant patches than in ant-free patches (figure 1 and table 2). Abundances of total Coleoptera, Hemiptera and Isopoda were also higher in ant patches (table 2), but this effect was less marked (figure 1).

Occurrences of Araneae, coleopteran predators, Isopoda, Geophilomorpha, coleopteran detritivores and Oligochaeta were significantly higher in ant patches (table 2).

(ii) Mesofauna

Total abundances of Acari and Collembola were three times higher (large effect) in the ant patches (table 3 and figure 1), where abundances of all Acari and Collembola groups were also significantly higher (table 3), although less so for euedaphic collembolans than for the other mesofauna groups (figure 1).

(iii) Microfauna

Total abundance of nematodes was twice as high (large effect) in the ant patches (table 3 and figure 1), where abundances of all trophic groups were also significantly higher (table 3), although less so for omnivorous-predatory nematodes than for the other groups (figure 1).

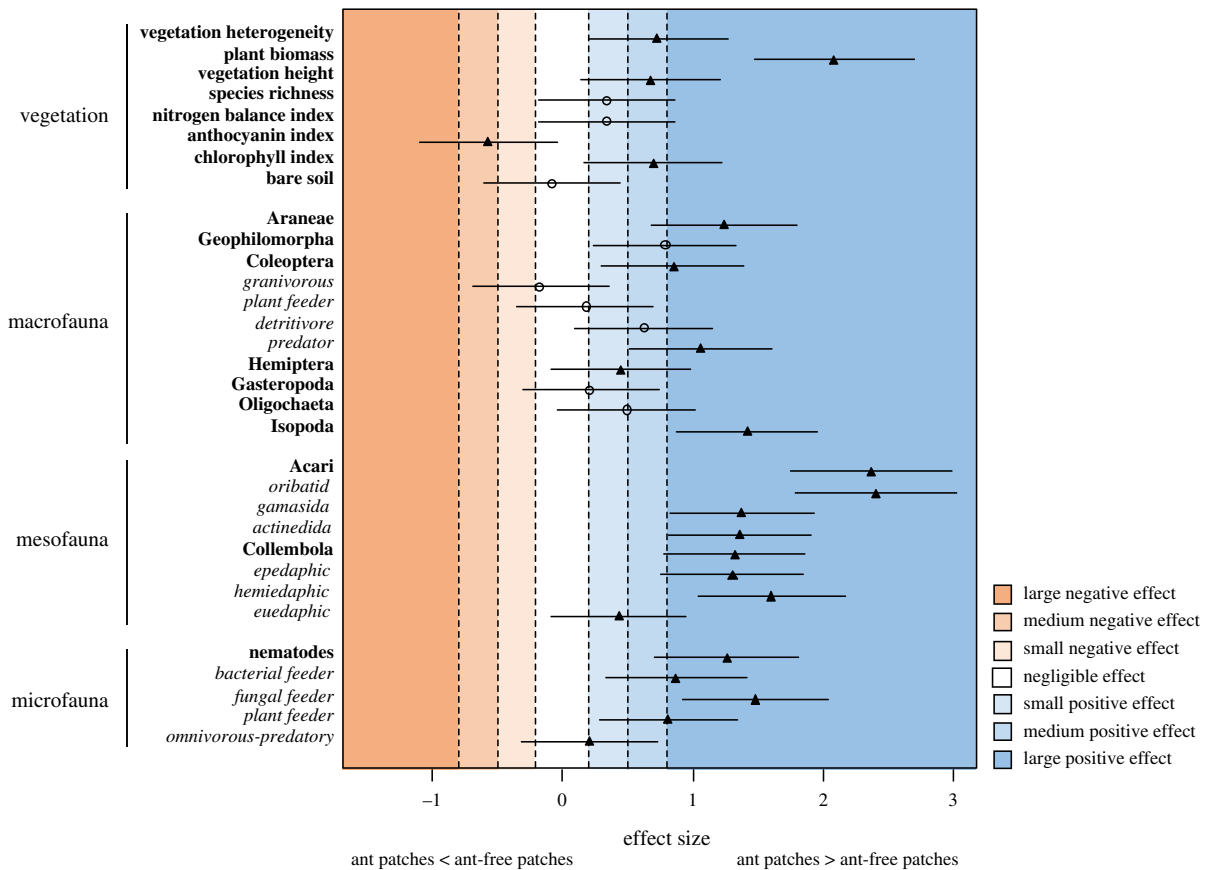


Figure 1. Effect size (Cohen's d) and 95% confidence intervals for the effect of ants on vegetation parameters and above- and below-ground fauna abundances (macrofauna, mesofauna and microfauna). Soil fauna subgroups are in italics. Black triangles represent the mean effect size for variables with a significant difference between ant and ant-free patches. Dots represent the mean effect size for variables with no significant difference between ant and ant-free patches. Effect size > 0 indicates higher values in ant patches than in ant-free patches; effect size less than 0 indicates lower values in ant patches than in ant-free patches. (Online version in colour.)

Table 2. Macrofauna abundance (ind m^{-2}) and occurrence analyses for ant and ant-free patches, spring 2018. Values are means \pm s.e.; $n = 30$. z -value and p -value were obtained from zero-inflated GLMM for abundance and from GLMM with binomial distribution for occurrence. Italic values indicate significant differences between ant patches and ant-free patches.

	abundance				occurrence	
	z -value	p -value	ant patches	ant-free patches	z -value	p -value
Araneae	-5.53	<0.001	80.6 \pm 11.3	25.6 \pm 5.2	-2.53	0.01
Geophilomorpha	-0.65	0.51	11.6 \pm 2.4	3.7 \pm 1.5	-2.70	0.007
Coleoptera	-4.00	<0.001	79.4 \pm 13.6	34.1 \pm 7.5	-1.30	0.19
<i>Granivorous</i>	0.95	0.34	7.7 \pm 5.3	13.3 \pm 6.1	0.48	0.63
<i>Plant feeder</i>	-0.97	0.33	10.5 \pm 3.7	6.4 \pm 2.1	-0.65	0.52
<i>Detritivore</i>	-1.46	0.15	11.0 \pm 3.1	2.6 \pm 1.3	-2.10	0.04
<i>Predator</i>	-4.72	<0.001	50.2 \pm 10.5	11.7 \pm 3.2	-2.28	0.02
Hemiptera	-2.60	0.01	51.9 \pm 10.8	27.2 \pm 5.5	-1.10	0.28
Gasteropoda	-0.50	0.62	9.4 \pm 2.8	7.5 \pm 2.8	-0.92	0.36
Oligochaeta	-1.02	0.31	21.5 \pm 5.6	16.5 \pm 4.2	-2.04	0.04
Isopoda	-3.83	<0.001	195.3 \pm 70.5	2.1 \pm 1.3	-3.74	<0.001

(d) Soil respiration

Soil respiration was twice as high in ant patches (2.39 ± 0.27) as in ant-free patches (1.23 ± 0.13) ($t = -4.64$, $p < 0.001$), with a large effect of ants (Cohen's $d = 0.96 \pm 0.55$).

(e) Trophic levels

Total abundance of decomposers (D) was three times higher in ant patches than in ant-free patches ($t = -5.70$, $p < 0.001$), while total abundances of PC and SC were twice as high in

Table 3. Mesofauna and microfauna abundance (ind m⁻²) analyses for ant and ant-free patches. Values are means \pm s.e.; $n = 30$. z-values and p -values were obtained from GLMM with binomial negative distribution. Italic values indicate significant differences.

	z-value	p-value	ant patches	ant-free patches
Acari	−8.98	<0.001	29 146.5 \pm 2 564.1	10 030.0 \pm 932.8
<i>Oribatid</i>	−7.24	<0.001	13 982.5 \pm 1 578.1	2 320.5 \pm 361.5
<i>Gamasida</i>	−5.34	<0.001	9 911.0 \pm 1 023.0	4 624.0 \pm 548.0
<i>Actinedida</i>	−4.51	<0.001	5 253.0 \pm 375.2	3 085.5 \pm 423.9
Collembola	−4.83	<0.001	47 804.0 \pm 7 660.2	16 583.5 \pm 4 718.9
<i>epedaphic</i>	−4.33	<0.001	3 578.5 \pm 576.6	1 275.0 \pm 239.9
<i>hemiedaphic</i>	−4.79	<0.001	32 147.0 \pm 4945.0	7 310.0 \pm 2 187.8
<i>euedaphic</i>	−2.00	0.05	12 078.5 \pm 3 696.6	7 998.5 \pm 2 659.7
Nematodes	−5.43	<0.001	372 274.5 \pm 40 792.8	186 660.0 \pm 15 830.0
<i>bacterial feeder</i>	−4.64	<0.001	249 283.0 \pm 34 142.3	127 300.8 \pm 10 543.8
<i>fungal feeder</i>	−4.48	<0.001	103 295.0 \pm 10 316.0	49 839.6 \pm 7 848.5
<i>plant feeder</i>	−2.57	0.01	18 840.3 \pm 3 223.3	9 205.3 \pm 1 884.4
<i>omnivorous-predatory</i>	−81.42	<0.001	855.9 \pm 294.3	314.2 \pm 92.7

ant patches (respectively, $t = -5.70$ and $t = -6.10$, $p < 0.001$). The D : PC and D : SC ratios were respectively 2 times and 1.5 times higher in ant patches than in ant-free patches (respectively, $t = -3.43$ and $t = -86.7$, $p < 0.001$), while the SC : PC ratio was not affected ($t = -0.54$, $p = 0.59$).

(f) Path analysis

In the above-ground compartment, we found more significant relationships in ant patches (figure 2a,b). Vegetation heterogeneity and height were important drivers of the ant patches' above-ground food web. Vegetation heterogeneity had a positive direct effect on predatory Coleoptera and Araneae abundances and, conversely, a negative effect on epedaphic collembolans. Vegetation height had a positive direct effect on epedaphic collembolans and plant feeder Coleoptera and negative effect on predatory Coleoptera. Predatory Coleoptera were positively affected by Hemiptera and plant feeder Coleoptera and negatively by Araneae, while Aranea was only dependent on Hemiptera. By contrast, on ant-free patches, predatory Coleoptera were negatively affected by plant biomass and positively by Araneae. Vegetation heterogeneity and vegetation height had less influence on the above-ground food web in ant-free patches. On both PAs, we observed that clay content had a strong positive effect on N-NH₄ content which in turn positively impacted the plant biomass.

For the soil compartment, ant patches revealed strong shifts in relationships across the food web (figure 2c,d). Both PA suggested that clay content could be an important driver of the food web, positively affecting TOM content and negatively affecting microfauna PC. Microfauna SC were strongly negatively affected by clay content in ant patches but positively affected in ant-free patches.

4. Discussion

This study reveals the strong local influence that *M. barbarus* has on both the environmental filter, by modifying soil

physico-chemical properties, and the biotic filter, by changing plant communities and altering above- and below-ground fauna abundance, occurrence and community structure. Its engineering activity affects not only these ecosystem components, but also the trophic and non-trophic relationships between them. These new results add to our previous findings on the positive effect of *M. barbarus* on soil and vegetation restoration in a degraded dry grassland [23], and highlight its central role in their ecosystem.

(a) Impacts on the environmental filter

The engineering activity of ants and their impacts on the environmental filter are directly related to specific functional traits called effect traits, such as colony size, nest location or size of workers' mandibles. For example, the capacity of ants to restructure soil depends on their physical capacity to transport material [48]. The large proportion of coarse sand particles ranging from 0.2 to 2 mm we recorded in *M. barbarus* nests may therefore be explained by workers' average jaw opening, which ranges from 0.80 to 2.80 mm [49].

Differences in soil nutrient concentration between ant and ant-free patches may arise from ant foraging as well as nest construction and maintenance [48]. The higher concentration of available P in ant patches can be explained by food collected in the nests and the subsequent increase in organic matter mineralization [23,48,50]. Their lower concentration of total N and other cations could be due to higher mineralization rates of organic matter [51], assimilation of N by plants and microbes [52] and/or replacement of the upper horizon with subsoil usually poor in organic matter [48,53]. Ant bioturbation may also lead to reduced Na₂O content [53]. Because sodium (Na⁺) can be fixed by clays [54], the measured decrease in clay content could lead to greater leaching of Na₂O in ant patches.

(b) Impacts on the biotic filter

Modifications to soil physico-chemical properties may indirectly affect the biotic filter by creating micro-

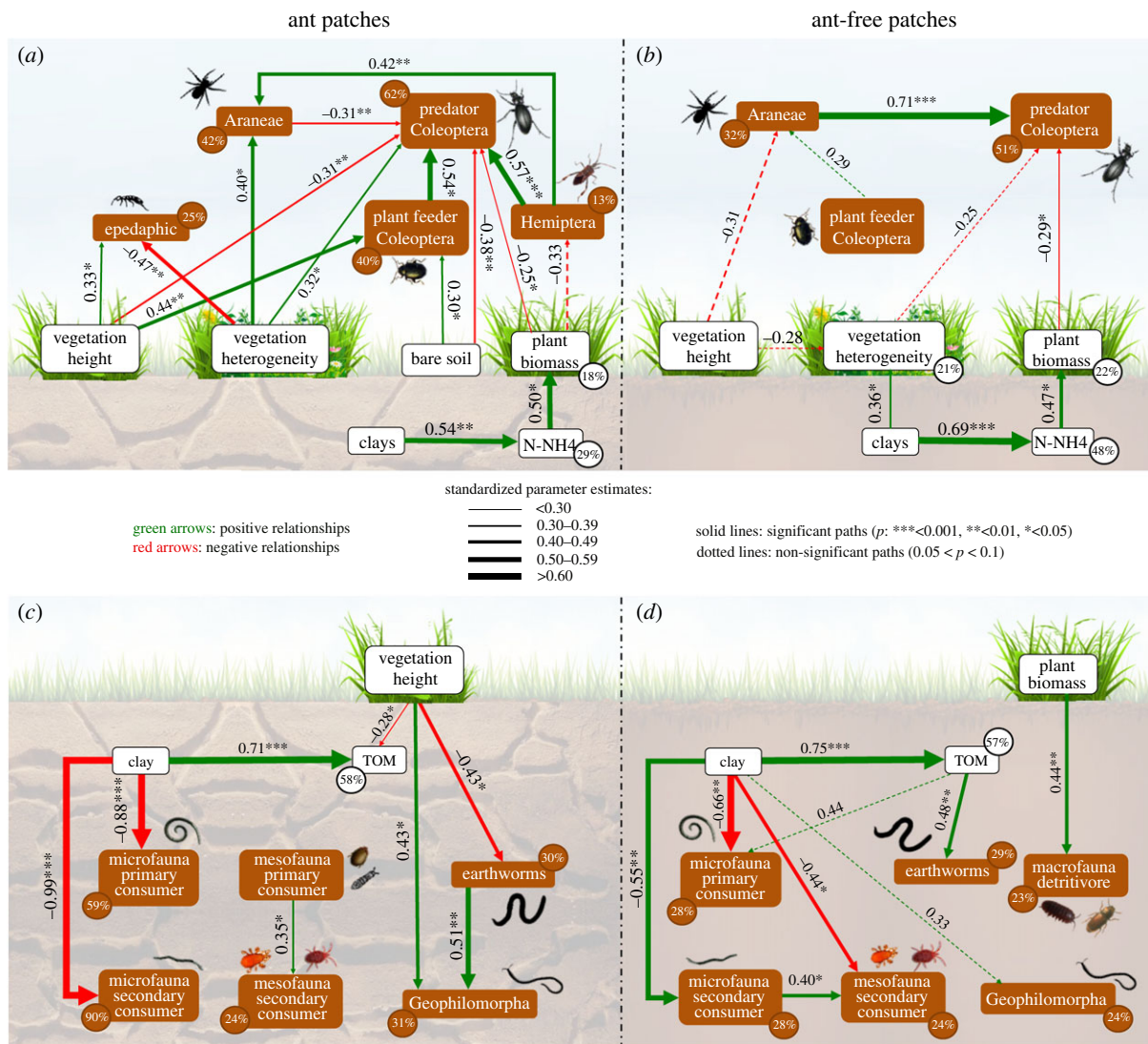


Figure 2. Path analysis models. Causal influences of vegetation heterogeneity and height, bare soil percentage, plant biomass, clay and N-NH₄ content on above-ground invertebrate abundance (epedaphic collembolans, Hemiptera, Araneae, plant feeder and predator Coleoptera) in ant patches (d.f. = 18, $\chi^2 = 18.83$, $p = 0.40$, RMSEA = 0.04, CFI = 0.99) (a) and ant-free patches (d.f. = 18, $\chi^2 = 18.02$, $p = 0.46$, RMSEA = 0, CFI = 1) (b). Causal influences of vegetation height, plant biomass, TOM and clay content on fauna abundance (microfauna PC and SC, mesofauna PC and SC, earthworms, macrofauna detritivores and Geophilomorpha) in ant patches (d.f. = 18, $\chi^2 = 17.78$, $p = 0.47$, RMSEA = 0, CFI = 1) (c) and ant-free patches (d.f. = 18, $\chi^2 = 23.05$, $p = 0.19$, RMSEA < 0.1 , CFI > 0.9) (d). Numbers next to arrows show standardized parameter estimates (see electronic supplementary material, table S2 and table S3). Percentages of explained variance are shown with dependent variables. (Online version in colour.)

environments favourable to plant growth. In general, burrowing animals such as ants create patches of disturbed soil that influence vegetation growth and contribute to spatial heterogeneity in plant communities [55]. Since we found no changes in plant community composition, the higher plant biomass [23], height and chlorophyll index in *M. barbarus* nest green belts may be explained by the higher proportions of N-NH₄ and available P, known to be responsible for higher plant productivity [56]. Moreover, the decrease in plant anthocyanin index (i.e. leaf anthocyanin content), probably due in part to the extremely reduced Na₂O content measured, reflects decreased environmental stress in these xerophytic meadows. It offers better conditions for less salt-tolerant species which could outcompete the more tolerant ones.

The high soil respiration we recorded in ant nests, in line with previous studies [57,58], is probably mainly due to ant respiration [57,58]. However, other possible contributing factors include increases in plant biomass, in root respiration

[59], in organic matter decomposition [50] and in organism abundances. In ant nest green belts, the abundances of organisms comprising each trophic level increased. Engineered patches with high rates of organic material accumulation serve as microhabitats for soil fauna. Above- and below-ground differences in fauna abundance were strong for the main decomposers, such as Isopoda, oribatid Acari or collembolans, which responded positively to the presence of *M. barbarus*. Similar positive effects from a *Messor* species have been documented for some of these organisms [16,60]. Interestingly, earthworms, also considered as ecosystem engineers, appear to have been unaffected by the presence of ants. The interaction between these two ecosystem engineers might have major local impacts and deserves to be thoroughly investigated.

Throughout the food web, increased abundances were observed in the ant nest green belts and no taxon was less abundant or frequent than in ant-free patches. Decomposer abundances increased more than those of PC and SC,

changing the ratio between the different trophic levels. The higher abundances of decomposers (e.g. Collembola) and PC (e.g. Hemiptera) may indirectly affect their predators [17]. In our study, the commonest predators were spiders and ground beetles, generalists feeding on taxa such as Hemiptera, Collembola and other Coleoptera [17,61]. Although most spider species are averse to ant predation, some have adapted ant-specific capture techniques and favour ants over other prey [62]. Such spiders might therefore benefit considerably from the presence of *M. barbarus*; for example, the obligate myrmecophagous spider *Zodarion elegans*, observed mainly in ant patches, which may feed on *Messor* species [63].

(c) Consequences on trophic and non-trophic relationships

Messor barbarus profoundly impacts the above- and below-ground compartments, modifying trophic and non-trophic relationships between organisms and between organisms and their environment. By increasing environmental heterogeneity, ants may affect food web organization by changing resource distribution patterns [6] or habitat structure. The consequences of these changes for above- and below-ground compartments differ. Here, the number of relationships increased in the above-ground compartment. Above-ground, vegetation heterogeneity and height were the main drivers of relationship complexity in *M. barbarus* patches. The increased heterogeneity and biomass of producers could be responsible for the increased abundance of some PC (e.g. plant feeder Coleoptera) and indirectly of their predators (e.g. Araneae and predator Coleoptera). However, these changes may also be directly related to an increase in potential habitats for some primary or secondary consumer organisms (e.g. plant feeder Coleoptera and Araneae).

Below-ground, clay content was the main driver of interaction complexity. However, the sign and the strength of the relationship between clay content and microfauna SC differed between ant and ant-free patches. Clays are known to affect soil moisture [64], an increase in clays is associated with an overall increase in soil moisture. In ant patches, the strong negative impact of clay content might suggest that ants provide drier microclimate habitats, filtering the assemblage of microfauna SC towards specialist species. A taxonomic and functional study should be carried out to confirm this hypothesis.

(d) The necessity of multi-component studies

Since its introduction, the concept of ecosystem engineer has generated considerable interest, but it is also a source of debate within the scientific community. The main concern is that all organisms affect their environments to some degree, which has an impact on other species [4]. This requires treating the effects of an organism on its environment and on other organisms as a coherent sequence of interactions and not as a collection of disconnected case studies [2,65]. A multi-component approach must therefore be favoured when studying the concept of ecosystem engineers. Such studies conducted on both terrestrial (e.g. kangaroo rats [66], earthworms [67]) and aquatic organisms (e.g. carp [68], crayfish [69]) have proven their value in studying the ecosystem engineer concept. Our multi-component study adds evidence, to the still limited literature, that some species can strongly affect their entire environment.

4. Conclusion

In Mediterranean dry grasslands, the effects of *M. barbarus* observed on above- and below-ground compartments illustrate the significance of the habitat alteration impact both on plant community structure and invertebrates and on their relationships. By altering both environmental and biotic filters at a local scale, *M. barbarus* creates habitat heterogeneity that may in turn increase ecological niches in these highly diverse ecosystems. Their impacts at a larger scale should be investigated for a clearer picture of this ecosystem engineer's ecological role.

Data accessibility. Data are available as part of the electronic supplementary material.

Authors' contributions. T.D.A., O.B., F.M. and T.D. conceived the ideas and designed the experiment; T.D.A., O.B. and R.G. collected the data; T.A., O.B. and M.S. analysed the data. T.D.A., M.S. and R.G. wrote the first draft. All authors contributed critically to the drafts and manuscript revisions and gave final approval for publication.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by Région Sud-PACA (PhD grant) and the Tour Du Valat Research Institute.

Acknowledgements. We thank two anonymous reviewers for their helpful comments that significantly improved this manuscript. We thank Emilie Mendes, Hugo Fontes, Loïc Willm and Thibaud Torel for field and laboratory assistance. Thanks to Marjorie Sweetko for editorial reviews.

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