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Harvester ant nest distribution depends on soil disturbance regime

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A B S T R A C T

Nest densities of harvester ants (*Messor barbarus*) are high in rain-fed cereal fields in north-eastern Spain where the ants remove large quantities of seeds, contributing to reductions in weed populations. The distribution of harvester ant nests within a field can influence the effectiveness of ants as weed seed predators because areas with low ant nest density have lower weed seed removal rates. Tillage can disturb or even kill ant colonies and may be an important factor explaining the distribution of nests within fields. During the summers of 2011–2013, the number of nests in a 50 × 50 m area in 4 tilled and 3 no-till fields were counted. Tilled fields were disturbed twice a year, in November before cereal seeding and in July, after cereal harvest, whereas no-till fields had no soil disturbance. Ant nests were evenly spaced in no-till fields whereas nests were randomly distributed in tilled fields. Our results provide evidence that no-till in cereal fields promotes a more even distribution of *M. barbarus* nests, which should result in higher and more regular levels of weed seed predation across the field.

1. Introduction

Harvester ant, *Messor barbarus* L. are voracious weed seed predators and can significantly contribute to reductions in weed abundance in in rain-fed cereal fields in northeastern Spain (Baraibar et al., 2009, Westerman et al., 2012). Populations of *M. barbarus* are high and nest densities can range from 150 to up to 1100 nests ha^{-1} (Baraibar et al., 2011). The ant's foraging range can extend up to 30 m from the nest (Baraibar pers. obs), however their effectiveness as weed seed predators is highest near nest entrances where forager densities are highest. Torra et al. (2016) measured predation rates of weed seed patches of different sizes and found consistently high predation rates throughout the studied area except for those patches located in areas with low abundance of nests, or located further away from the nest entrances. Similarly, Comas et al. (2016) studied the relationship between ant nest distribution and weed species richness and cover in a no-till field in the same region and found that areas with high nest density, and especially with large nests, tended to have lower weed species richness and attributed these results to higher seed predation rates in those areas. Regularly spaced ant nests throughout the field should promote more uniform weed seed predation and improve weed control by *M. barbarus*. Therefore, understanding the causes of ant nest distribution may inform management practices to promote more regular nest spacing and enhance weed seed predation.

Soil disturbance, such as tillage, has the potential to decrease nest survival and influence spatial distribution of nests, especially if done in vulnerable early stages of a colony. In Northeastern Spain, tillage performed prior to winter cereal sowing in November occurs right after *M. barbarus* queen ants have mated and started establishing new colonies. These new colonies are very shallow and thus, very vulnerable to the effects of tillage. As the colony grows, it expands deeper into the soil and the queen is safely located on the deepest chambers. Tillage in the summer, after cereal harvest, is not as likely to kill the queen but it will kill workers and destroy the upper part of the nests, which will need to be rebuilt. This disturbance may slow the growth of colonies of all ages, and prevent colonies reaching their largest potential size. Therefore, tillage may result in lower nest densities and smaller average nest sizes in tilled fields compared to no-till fields (Baraibar et al., 2009). Although summer tillage may be considered a weed management strategy, it is not clear that its benefits outweigh those associated with decreased weed seed predation (Baraibar et al., 2017) and higher nest mortality.

The spatial distribution of nests may also be shaped by tillage or the absence of it. In no-till fields, where nests are not disturbed, intraspecific competition for nesting sites and resources may be the main force structuring *M. barbarus* communities, resulting in a regular ant nest distribution (Levings and Traniello, 1981, Wiernasz and Cole, 1995). Contrary, in tilled fields, repeated disturbance may limit nest

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establishment, survival and colony interaction, resulting in nests distribution being driven by the random effect of tillage, and not by intraspecific interactions among them.

In this study, we compared the distribution of *M. barbarus* ant nests in 4 tilled and 3 no-till cereal fields in northeastern Spain over three years. We predicted that: 1) tillage would result in decrease colony density due to increased mortality (especially of new and/or shallow colonies), 2) the size distribution of colonies would vary between no-till and tilled fields, as tillage may slow down colony growth, and 3) *M. barbarus* nest distribution would be regular in no-till fields and random in tilled fields. Because small ant nests make up for the larger proportion of *M. barbarus* nests in agricultural fields (Blanco-Moreno et al., 2014, Comas et al., 2016), the spatial distribution of nests between no-till and tilled fields may largely depend on the location of smaller nests. Therefore, we assumed that nest distribution could change across years within the same field but we hypothesized that patterns of ant nest distribution would be largely driven by tillage or the absence of it.

2. Materials and methods

2.1. Study area

During the summers of 2011–2013 the number of nests in 4 tilled and 3 no-till fields were counted in Catalonia, northeastern Spain. Tilled fields were located in Balaguer (41° 46′ 16″ N - 0° 45′ 12″ E), Bellmunt (41° 46′ 34″ N – 0° 58′ 35″ E), Vilanova de Bellpuig (41° 35′ 34″ N – 0° 58′ 45″ E) and Almenar (41° 46′ 36″ N – 0° 32′ 3″ E) and received a tillage operation after cereal harvest in July and another one before seeding the field in November, both with a chisel plow working vertically to a depth of around 20 cm. No-till fields were in Vilanova de Bellpuig (41° 35′ 17″ N – 0° 58′ 37″ E), Agramunt (41° 46′ 10″ N – 1° 4′ 42″ E) and Bellmunt (41° 46′ 10″ N – 1° 4′ 42″ E). Fields were visited in August, once the cereal had been harvested and tillage was completed. In each field, a 50 m by 50 m area was flagged and all *M. barbarus* ant nests were counted and located with a GPS with centimeter precision (Leica Geosystems AG, Heerbrugg, Switzerland). Entrances of counted nests were sprayed with paint to prevent double counting. Nest counting was done from sunrise until temperatures reached around 30 °C, when ant activity is reduced by heat. Only colonies with active workers were counted. Each nest was given a subjective measurement of size that ranged from 1 (smallest) to 5 (largest) based on the number of entrances, area occupied and size of the ant workers, following Baraibar et al. (2011).

2.2. Data analysis. Spatial statistics

2.2.1. Analysing the spatial point structure of ant nests

The distribution of harvester ant nests (point locations) was analysed using a spatial function derived from point process theory. The spatial structure of a point pattern can be described by various summary characteristics. We considered the Ripley's *K*-function and, in particular, an estimator of this function $\hat{K}(t)$ (see Diggle, 2013) to describe point patterns. We used this correlation function to characterise each replicated point pattern (ant nest distribution in each field) and to compare them in order to detect significant differences.

2.2.2. Estimating the K-function from the replicated point patterns

Once the ant nest point patterns were characterised by the *K*-function, we defined a global estimator of these functions for each experimental group, i.e. no-till and tilled. Diggle (2013) suggests to obtain a global estimator of $K_i(t)$ for a given distance *t*, for each of the experimental groups, *i* = 1, 2 via

$$\hat{K}_i(t) = \sum_{j=1}^{r_i} n_{ij}\hat{K}_{ij}(t)/\sum_{j=1}^{r_i} n_{ij} \tag{1}$$

where r_i is the number of point pattern replications for the *i*-th experimental group and n_{ij} and $\hat{K}_{ij}(t)$ are the number of points and an estimator of the *K*-function for *j*-th pattern within the *i*-th experimental groups, respectively. Once a global estimator for each experimental group was obtained, we evaluated the variability of these estimators via their variance. Although the value of the *K*-function is not affected by the intensity of the point process under analysis (in this case, the density of ant nests), and thus the weighted average (1) is an appropriate estimator where not necessarily all the replicated point patterns would have the same number of ant nests, the variance of this global estimator does depend on the point intensity (Ripley, 1988). As such, the variability associated to each $\hat{K}_i(t)$ is affected by the number of points of each replicated pattern. Diggle (2013) proposed a bootstrap procedure to obtain an approximation of the sample variance of $\hat{K}_i(t)$ This procedure is based on the difference between the global *K*-function and the *K*-function obtained for each replicate, i.e. the residual between the global and the replicate *K*-functions. This procedure provides a bootstrap approximation for the sample variance of $\hat{K}_i(t)$. For a detailed explanation of this procedure see Diggle (2013) pages 92–93. The procedure can provide a first and intuitive visual method to detect differences between the two experimental groups.

2.2.3. A Bootstrap approach to detect differences between experimental groups; a formal test

To test the hypothesis that $K_i(t) = K(t)$, we considered the test proposed by Diggle et al. (1991) and Diggle (2013), based on a statistic loosely analogous to the between-treatment sum of square in an analysis of variance defined as

$$D = \sum_{i=1}^{2} n_i \int_0^{t_0} w(t)\{\hat{K}_i(t)-\hat{K}_0(t)\}^2 dt \tag{2}$$

where $w(\cdot)$ and t_0 are a weighting function and a constant to be chosen, and $\hat{K}_0(t) = \sum_{i=1}^{2} n_i\hat{K}_i(t)/\sum_{i=1}^{2} n_i$ are a weighted average of the two $\hat{K}_i(t)$, as $K_i(t)$ is in fact the expectation of $\hat{K}_{ij}(t)$. Now as the null sampling distribution of *D* is intractable, for a design-based inference we can consider again a resampling method. This bootstrap method allowed us to obtain a set of $D_2, ..., D_s$ values under the null hypothesis and the rank of our empirical *D* value among this set provided the significance level for our test. For a detailed explanation of this procedure see Diggle (2013) pages 96–97.

2.3. Data analysis. Ant nest size

A generalized linear mixed regression model with a Poisson error structure was used to describe *M. barbarus* nest size between no-till and tilled fields in R (R Core Team 2017). Tillage system was used as a fixed effect and field and year were considered random effects. Nest size distribution between no-till and tilled fields was compared using Kolmogorov-Smirnov test with p < 0.05.

3. Results and discussion

Ant nest counts did not differ between no-till and tilled fields, although there was considerable variability within each group with 129 nests (standard deviation = 59.1) and 98 (sd = 53.4) nests in no-till and tilled fields, respectively, which corresponds to an ant nest density of 515 and 393 nests ha^{-1} (Table 1). These results did not support our hypothesis that nest densities would be higher in no-till fields but they are in the range of those expected in the area (Baraibar et al., 2009, 2011). Even if some research has reported higher nest densities in no-till fields (Baraibar et al., 2009), nest densities in no-till fields are not stable in time. Baraibar et al. (2011) reported that nest density was affected by the number of years a field has been in no-till, such that nest densities increase after no-till adoption until a field has been in no-till for 12 years and decrease thereafter (Baraibar et al., 2011). No-till fields

Table 1
Ant nests for each replicated point pattern for the two experimental groups.

	Number of nests in 50 × 50 m area	Ant nest density (nests/ha)
No-till fields (average)	128.66	514.7
Agramunt 2011	79	316
Agramunt 2013	72	288
Bellmunt 2011	237	948
Bellmunt 2013	127	508
V. Bellpuig 2012	124	496
V. Bellpuig 2013	133	532
Tilled fields (average)	98.2	393
Balaguer 2011	171	684
Balaguer 2012	114	456
Balaguer 2013	56	224
Bellmunt 2013	98	392
Almenar 2011	74	296
Almenar 2012	180	720
V.Bellpuig 2012	46	184
V. Bellpuig 2013	47	188

used in this experiment had been so for 25 years (in Agramunt) and 8 years (in Bellmunt and Vilanova de Bellpuig), and as a result, nest densities in Agramunt were much lower than in the other no-till fields and may explain the lack of significant difference between no-till and tilled fields.

Even though nest density in no-till and tilled fields was the same, size structure differences were found. Differences were due to variation in size distribution (D = 0.11795, p-value < 0.001, Fig. 1) rather than to average nest size, which was 2.3 and 2.0 respectively in no-till and tilled fields (z value = 1.57, p = 0.117). In no-till fields 70% of the colonies were small (sizes 1 or 2), while in tilled fields small nests represented 79% of all colonies (Fig. 1). More small colonies and fewer larger ones in tilled fields are possibly the result of slower growth rates
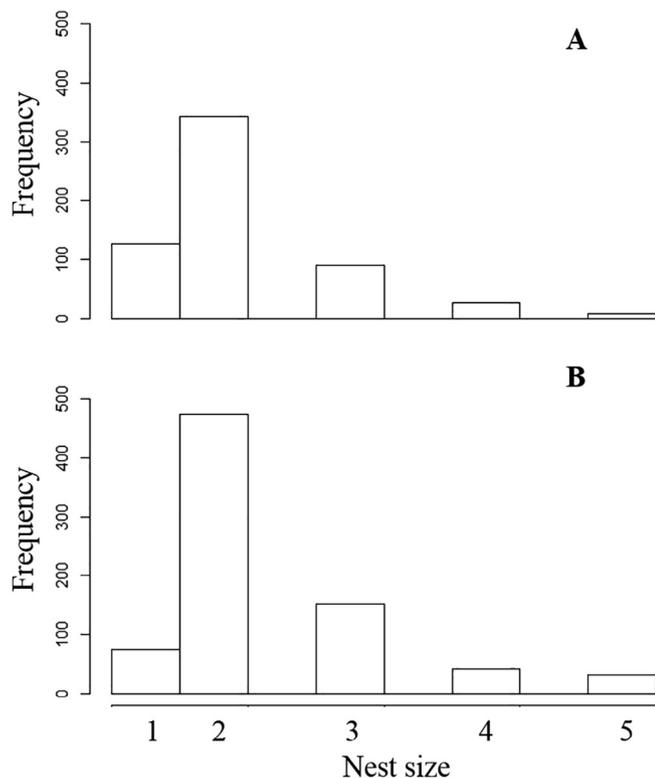

Fig. 1. *Messor barbarus* nest size distribution in tilled (A) and no-till (B) cereal fields in northeastern Spain. Sizes follow a subjective scale from 1 (smallest) to 5 (largest), based on the area occupied by the colony, the number of entrances, worker size and the number of active ants.

caused by recurrent tillage events.

Spatial distribution of nests also differed between no-till and tilled fields (bootstrap procedure with $t_0 = 12$ meters, $w(t) = t^{-2}$ and 1000 resamples resulted in a bootstrap p value of 0.015). Under no-till, ant nests tended to be more evenly distributed for inter-point distances $t < 5$, whilst in the tilled fields, ant nest were distributed at random, thus confirming our hypothesis. The resulting values of Ripley's function ($\hat{K}_{1j}(t) - \pi t^2$) for the no-till point patterns are shown in Fig. 2A. This shows that most of the resulting functions are similar and distributed around zero for any inter-point distance. Inspection of the pooled estimated functions with bootstrapped plus minus two pointwise standard error limits for the non-till fields (Fig. 2C) showed that zero lies outside both bootstrap standard errors limits (black dashed lines) for distances $t < 5$ meters, suggestive of small-scale regularity. Inspection of resulting values of Ripley's functions for the tilled fields (Fig. 2B) highlighted that these functions are far more variable than the no-till field functions, and that no apparent spatial structural difference appears from these 8 functions. The pooled estimated function and bootstrapped plus minus two pointwise standard error limits for tilled fields are shown in Fig. 3D and highlight that both bootstrap standard errors limits (black dashed lines) contain the zero value almost for all the analysed distances thereby suggesting that on average the spatial structures of these point patterns are random. Comparison of both experimental groups and corresponding bootstrapped plus minus two pointwise standard error limit shows that in both cases the pooled function (grey line) lies outside these limits and that that this is especially true for no-till fields (Fig. 2C), suggesting that ant nests spatial structure depends on the type of tillage.

These results are in agreement with Blanco-Moreno et al. (2014), who reported a regular distribution of ant nests in a no-till field in the same area in Spain. Ecological theory suggests that the spatial pattern of a population is the result of both dispersal and survival (reviewed in Wiernasz and Cole, 1995). In the case of *M. barbarus*, dispersal originates when queens fly out from their nest to mate. This event is highly localized in space and likely to result in a clumped distribution of ant nests, because newly mated queens do not fly or move very far. Competition with established colonies, first for space and later for resources, tends to shift the spatial pattern to a random distribution and finally a regular or over-dispersed distribution (Wiernasz and Cole, 1995). In the absence of disturbance, these biotic interactions and nest territoriality may be the main factors explaining nest distribution in no-till fields (Blanco-Moreno et al., 2014, Gordon and Kulig 1996). Regular ant nests distributions are the most common distribution in natural systems (Levings and Traniello, 1981). A no-till field may somehow resemble a natural setting in the sense that soil is not disturbed, although it is certainly different in many other ways, especially regarding food resources and management. In contrast, nest distribution in tilled fields is likely being influenced by tillage. Since fields are tilled twice every year, tillage may act as a recurring filter that randomly kills or severely disturbs colonies, and may be more important in shaping ant nest distribution than biological interactions. Nest distribution in a tilled field may never be able to reach a regular pattern because ant nests are continuously disturbed and colony mortality and turnover may be higher than in no-till fields. Populations of other seed feeding insect species such as carabid beetles or other ant species have been shown to be lower in tilled versus no-till fields (Robertson et al., 1994, Menalled et al., 2007, Trichard et al., 2013) but to our knowledge, this is the first study to report the effect of tillage on harvester ant nest distribution.

Differences in nest size structure between no-till and tilled fields may also help explain the spatial distributions observed. Large colonies exhibit more territoriality than smaller colonies and tend to have larger exclusion areas (areas from which other colonies are completely excluded) and interaction zones (areas with a low probability of finding another nest) (Blanco-Moreno et al., 2014). A lower proportion of large nests in tilled fields may have resulted in more space available for founding queens to establish small colonies and contributed to the
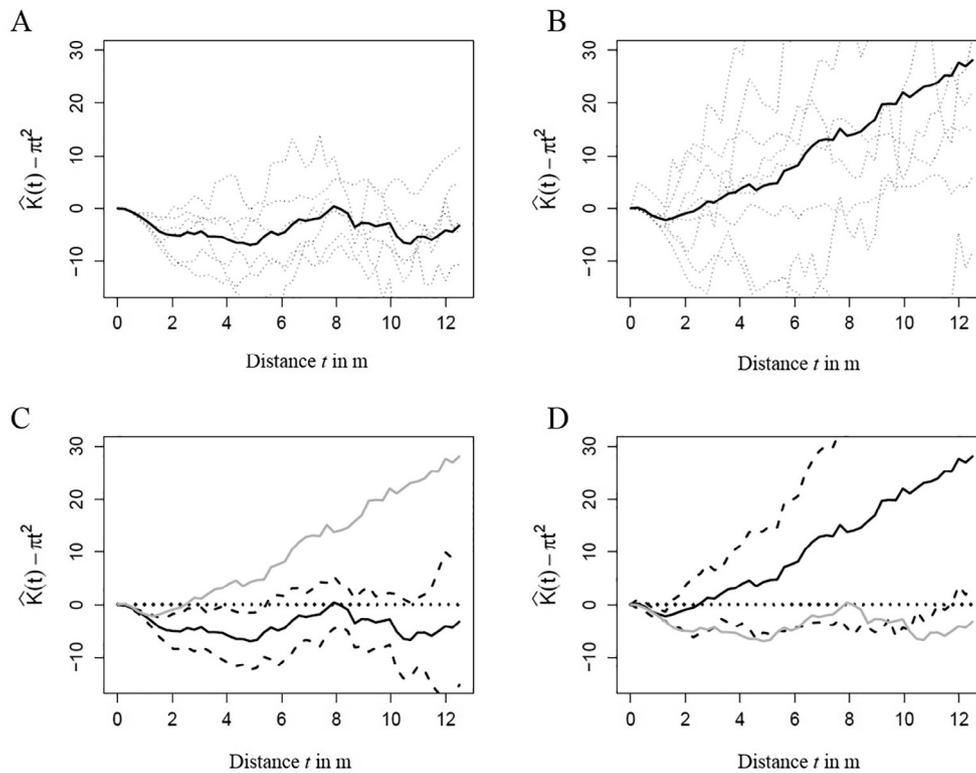
Fig. 2. Resulting estimator of the Ripley's K-function for j-th pattern within the i-th experimental groups ($\hat{K}_{ij}(t)-\pi t^2$) functions (dashed lines) and pooled estimator (black line) of the Ripley's K-function for each of the experimental groups $i = 1, 2$ ($\hat{K}_i(t)-\pi t^2$) for ant nests point pattern for no-till (A) and tilled fields (B). Functions in no-till fields (A) are similar and wandering around zero for any inter-point distance whereas they are far more variable in tilled fields (B). (C): pooled estimators $\hat{K}_1(t)-\pi t^2$ (black line) and $\hat{K}_2(t)-\pi t^2$ (grey line) for no-till fields, and (D): pooled estimators $\hat{K}_2(t)-\pi t^2$ (black line) and $\hat{K}_1(t)-\pi t^2$ (grey line) for tilled fields, both with bootstrapped plus and minus standard error limits (dashed lines). Graph C shows how the zero line (dotted) lies outside both bootstrap standard errors limits (dashed lines) for distances $t < 5$ meters, suggesting a regular distribution of ant nests.
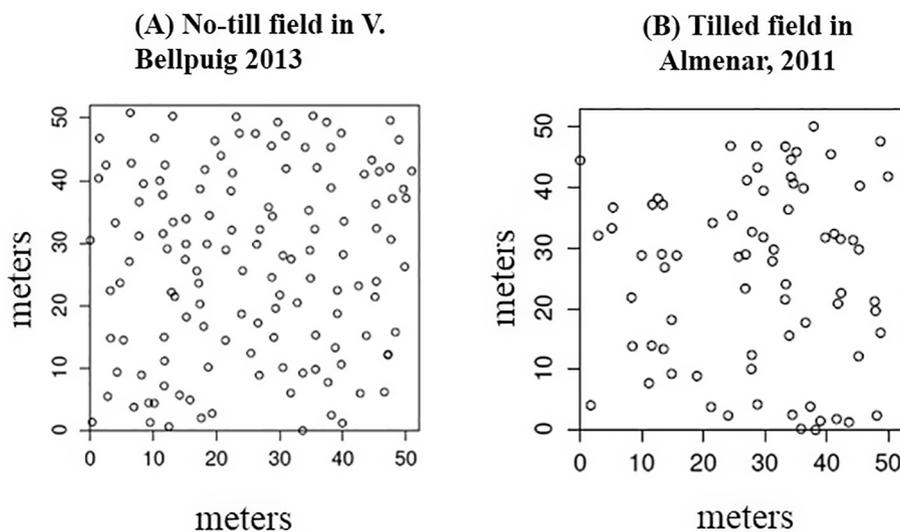
Fig. 3. *Messor barbarus* nest distribution patterns in a 50 × 50 m grid for a no-till (A) and a tilled field (B). Nests in no-till fields were regularly distributed while nests were randomly distributed in tilled fields.

random distribution observed. In this regard, Gordon and Kulig (1996) also found that smaller colonies of *P. barbatus* were more likely to be close to other small colonies than to large ones.

Our results provide evidence that even if harvester ant nest densities in no-till fields may not be necessarily higher than in tilled ones, no-till promotes larger nests and a more regular distribution. Larger nests can collect, store and ultimately consume larger amounts of weed seeds than smaller nests, thus decreasing weed seed input into the soil seed

bank. Further, if nests are regularly distributed, the likelihood that an area or a weed seed patch is not found and exploited by ants, is lower than if nests are randomly distributed (Comas et al., 2016, Torra et al., 2016). Decreasing tillage intensity is a feasible strategy in semi-arid systems of northeaster Spain and no-till adoption has steadily increased in the last years (Recasens et al., 2016). While reduced costs together with increased water savings and soil protection may be the main reasons for increased no-till adoption, increased weed seed predation

may be an added benefit that contributes to more sustainable weed management (Baraibar et al., 2009). Tillage, especially before cereal seeding, may still be a valuable strategy to control summer weeds and ensure a good soil to seed contact, but there are opportunities to decrease tillage frequency by avoiding the stubble tillage in July (Baraibar et al., 2017). Our results show that decreasing soil disturbance can promote a more regular nest distribution and can be a potentially powerful strategy to increase populations, and removal rates of a native and effective biological control agent such as *M. barbarus* harvester ants.

4. Declarations of interest

None.

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