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Oecologia

Effective nut dispersal by magpies (Pica pica L.) in a Mediterranean agroecosystem

--Manuscript Draft--

Manuscript Number:	OECO-D-16-00918R1
Full Title:	Effective nut dispersal by magpies (Pica pica L.) in a Mediterranean agroecosystem
Article Type:	Plant-microbe-animal interactions – original research
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	José María Rey-Benayas
Response to Reviewers:	December 13, 2016 Dear Dr. Ballaré, Thank you very much for your email of November 20, 2016 concerning the review of the ms OECO-D-16-00918 “Effective nut dispersal by magpies (Pica pica L.) in a Mediterranean agroecosystem”. We thank as well the reviewers for their helpful comments, which have improved the clarity and precision of the manuscript. Virtually all the changes proposed by the reviewers have been incorporated into the attached revised version. We considered the comments carefully when preparing our revision, and provide responses to all of them on the pages below, with detailed explanations of the changes made and their locations in the text. We attach two versions of the revised manuscript, a pdf marking the changes (track changes) and a word documented without tracked changes. Please note that text lines in the responses below refer to the pdf, tracked version. Reviewer #1 We greatly appreciate the positive and constructive comments of Dr. Schupp and the annotated copy of the ms with corrections to improve the English writing. All changes and suggestions indicated by Dr. Schupp have been incorporated into the revised version. We provide a quick summary below: 1. Line 51. We have deleted “effective”. Dr. Schupp is right in his question, as there is no measure of effectiveness in this study. 2. Climatic data. Climatic data were obtained from a weather station placed in IFAPA, an agricultural research center with identical environmental conditions located in the same area (Vega de Granada), at 1.5 km from the study site. This information has been incorporated into the revised version of the ms for the period of data availability (lines 148-149). 3. Unclear sentence. Nut recovery tended to decrease with increasing distance to the feeder. This information has been clarified in the revised version of the ms (line 322). 4. The likely alternative will be rats… The video-cameras had night vision and recording was made both during day and night times. No rats were recorded removing nuts from the feeders.

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We cannot provide information on the relationship between seed mass and recovery rate. The nuts used to replace the radio-labeled nut were of similar weight. However, we did not mark each nut individually. In any case, we should bear in mind that the nuts dispersed by magpies (with a transmitter inside) were not those they might eventually recover later, as the "original" radio-tagged nut was replaced by one without transmitter. Thus, we do not think that this analysis should be done.
Finally, the same applies to the relationship between nut mass and germination or emergence probability; we did not label each individual nut that was used to replace the radio-tagged nut. In any case, the number of emerged seedlings (2) is too low to conduct this analysis.

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8. Lines 364-365 - you are not actually getting an accurate estimate of seed dispersal effectiveness… We appreciate and understand the concern of the reviewer. In this study we provide accurate estimates of the qualitative component of SDE (which is, in fact, a key issue to estimate SDE; e.g. Schupp et al. 2010). However, as noted by the reviewer, we cannot provide an accurate estimate of the quantitative component, even though we demonstrated that the magpies are active walnut dispersers. Consequently, we have made the necessary changes (most of them semantic) to use terminology with accuracy.

Reviewer #2.
We appreciate the comments of the reviewer and have improved details of the text that, together with the comments of the two other referees, make our specific hypothesis clearer. Nonetheless, contrary to what seems to be the impression of the reviewer, we consider this study to be a relevant contribution in the field of Ecology, particularly for seed dispersal and the interaction between scatter-hoarding birds and nut-producing trees. First, to our knowledge this is the first time that the precise fate of individual nuts dispersed by birds have been monitored until seedling emergence, and we provide key data for improved estimation of the qualitative component of seed-dispersal effectiveness. Second, we used a novel methodological approach to conduct our study. In this regard, we disagree that these are "now standard methods for the study of bird scatter-hoarding". To date, only a handful of studies have addressed nut dispersal by corvids using radio-tracking, including the one indicated by the reviewer. In any case, a key additional, linked new approach in our study, beyond the radio-tracking method per se, is the further monitoring of individually-tracked seed fates to determine seedling emergence, placing the study in the context of seed dispersal effectiveness. Third, this is the first precise report about the role of magpies as scatter-hoarding birds with a potential key role for tree regeneration. Although, as pointed out

by the reviewer, this could be expected from previous research, this is not a drawback of the study but rather a merit as we put together all previous evidence to generate a hypothesis and design an empirical study to test it. Altogether, we think we are providing novel results that constitute the first report, and will constitute a baseline, to expand our knowledge in a highly relevant plant-animal interaction for forest regeneration. We also explicitly formulated a general, relevant hypothesis and four objectives to corroborate it. Also note that there were more than just "a pair of magpies" dispersing nuts (although we cannot determine the exact number) and that the dispersal distance reached values within what is generally considered to be a long-distance dispersal. In summary, we are confident that our study is novel, and addresses a relevant issue in the field of Ecology.
We have included the study helpfully mentioned by the reviewer (line 107).

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We greatly appreciate the positive and constructive comments of the reviewer, including the observations on the novelty and relevance of the study. The reviewer raises as a single major concern the fact that we perform the study in a single location, which might limit statistical inference. Consequently, the recommendation is to treat the results on dispersal effectiveness with more caution, particularly in the Abstract and Discussion section. A similar concern is raised in his/her last comment. We agree with the reviewer that more caution is needed, and we have modified the text accordingly, although we also believe that our data are representative of magpie activity (several individuals were recorded simultaneously in the feeders). We explicitly point out in the Discussion section that our study is based on a single site, and that further studies and replication are needed to ascertain the role of magpies in seed-dispersal effectiveness. We have also modified some sentences of the Abstract according to the reviewer's indications. For example, in lines 18-19 we replace "…that the magpie is an effective scatter-hoarding disperser" by "…that magpies can be an effective scatter-hoarding disperser …". We hope these clarifications solve the major concern of the reviewer.

Other minor comments:
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Funding Information:	Ministerio de Economía y Competitividad (CGL2014-53308-P) — Dr. Salvador Rebollo Gobierno de Madrid (S2013/ MAE- 2719) — Dr. José María Rey-Benayas
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Sincerely yours,

Jorge Castro.

On behalf of all authors.

Effective nut dispersal by magpies (*Pica pica* L.) in a Mediterranean agroecosystem

Jorge Castro[1*], Mercedes Molina-Morales[2], Alexandro B. Leverkus[1], Loreto Martínez-Baroja[3], Lorenzo Pérez-Camacho[3], Pedro Villar-Salvador[3], Salvador Rebollo[3] and José M. Rey-Benayas[3]

[1] Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain.

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[3] Grupo de Investigación "Ecología y Restauración Forestal" FORECO. Departamento de Ciencias de la Vida, UD Ecología, Edificio de Ciencias, Universidad de Alcalá, 28805 Alcalá de Henares, Spain

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Author Contributions: JC, LPC, PVS, SR and JMRB conceived and designed the experiments. JC, MMM, AL and LMB performed the field work. JC, MMM and AL performed statistical analyses. JC wrote the first draft of the ms; all authors provided editorial advice.

Abstract

Scatter-hoarding animals such as corvids play a crucial role in the dispersal of nut-producing tree species. This interaction is well known for some corvids, but remains elusive for other species such as the magpie (*Pica pica*), an abundant corvid in agroecosystems and open landscapes of the Palearctic region. In addition, the establishment of the individual dispersed seeds, a prerequisite to determine seed-dispersal effectiveness, never before has been documented for the interaction between corvids and nut-producing trees. We analysed walnut dispersal by magpies in an agroecosystem in southern Spain. We used several complementary approaches, including video-recording of nut removal from feeders, measuring dispersal distance using radio-tracking (with radio transmitters placed inside nuts), and monitoring the fate of dispersed nuts to the time of seedling emergence. Magpies were shown to be highly active as nut dispersers. The dispersal distance averaged 39.6±4.5 m, with a range from 4.1 to 158.5 m. Some 90% of the removed walnuts were cached later, and most of these (98%) were buried in the soil or hidden under plant material. By the time of seedling emergence, ca. 33% of nuts still remained in the caching location. Finally, 12% of the cached nuts germinated, and 4% yielded an emerged seedling, which allowed the transition to the next regeneration stage. The results demonstrate for the first time that magpies can be an effective scatter-hoarding disperser of a nut-producing tree species, suggesting that this bird species may play a key role for the regeneration and expansion of broadleaf forests in Eurasia.

Key words: *Corvidae*, forest regeneration, *Juglans*, radio-tracking, scatter-hoarding, seed caching, seed dispersal effectiveness

INTRODUCTION

Seed dispersal of large-seeded species of paramount relevance in the context of temperate forests is largely ascribed to a plant-animal interaction in which a vertebrate vector is responsible for direct seed transport (Vander Wall 1990; Johnson et al. 1997; Pesendorfer et al. 2016). Several bird species from the *Corvidae* family are among the most active dispersers for these trees, acting as scatter-hoarding animals that cache seeds in a large number of locations across the landscape for later consumption, disperse a very large number of seeds, and usually cover distances exceeding hundreds or even thousands of meters (Bossema 1979; Darley-Hill and Johnson 1981; Lenda et al. 2012; Pesendorfer et al. 2016). A fraction of the seeds may remain un-recovered, providing the opportunity for seed germination and tree recruitment (Vander Wall 1990; Pesendorfer et al. 2016). In fact, the interaction between corvids and many tree species from the *Fagaceae* or *Juglandaceae* plant families is considered a key mutualistic relationship for the regeneration, colonization, and expansion of forests in the Northern Hemisphere, helping to explain the post-glacial migration and current distribution of temperate forests (Johnson and Webb III 1989; Johnson et al. 1997; Vander Wall 1990; Mosandl and Kleinert 1998; Pesendorfer et al. 2016).

The role of corvids in the transport of nuts has been noted since ancient times (e.g. Aristotle and Theophrastus; Thanos 1994), and for decades has been intensively studied in several species throughout the Holarctic region (e.g. Grinnell 1936; Richards 1958; Bossema 1979; Cristol 2005; Pesendorfer et al. 2016 [and references therein]). In the case of North America, at least seven species have been described as dispersers of nuts from *Fagaceae* or *Juglandaceae* species (Cristol 2005; Pesendorfer et al. 2016). However, the dispersal of large nuts such as acorns and walnuts by corvids in Eurasia is ascribed mostly to a single species, the European jay (*Garrulus glandarius* L.) (Bossema 1979; Pesendorfer et al. 2016), and to a much lesser extent to the rook (*Corvus frugilegus* L.) (Waite 1985; Källender 2007; Lenda et al. 2012). Knowledge of the role of other corvids in the regeneration of these tree species in the Palearctic is almost negligible. In particular, the black-billed magpie (*Pica pica* L., hereafter referred to as "magpie"), a common corvid in Eurasia, is considered to have little relevance for tree dispersal, as it is assumed to preferentially cache perishable food, while caching few nuts within short distances, and with a recovery time of only a few days (Henty 1975; Waite 1985; Birkhead 1991).

Several pieces of evidence, however, suggest that magpies might be noteworthy vectors in nut dispersal. It is well established that magpies cache food items (Henty 1975; Clarkson et al. 1986; Birkhead 1991), have the capacity to recall cache locations (Zinkivskay et al. 2008; Feenders and Smulders 2011) and have a well-developed hippocampus (Healy and Krebs 1992; Brodin and Lundberg 2003), a brain region linked to spatial memory and food-storing behaviour. Magpies have also been suggested to be the most likely dispersers of almond trees in agroforestry systems (Homet-Gutiérrez et al. 2015), and reports on acorn dispersal, although very scant, are available (Birkhead 1991). In short, several clues support the idea that magpies might have an influential role in nut dispersal for Eurasian tree species. However, to date, the magnitude of nut dispersal and recovery rate for this bird have never been documented.

Although many studies have addressed the dispersal of nut-producing trees by corvids (e.g. review by Pesendorfer et al. 2016), a gap in knowledge persists concerning the implications of this mutualistic interaction for forest regeneration. Studies reporting a link between the vector and the plant are based mostly on evidence arising from synchronic observations of dispersal and seedling-recruitment patterns (e.g. Mosandl and Kleinert 1998; Gómez 2003; Hougner et al. 2006; Castro et al. 2012; Lenda et al. 2012; Puerta-Piñero et al. 2012). This procedure has demonstrated beyond a doubt that the corvids are major vectors for nut dispersal. However, a fine-grained quantification of the effect of animal seed-dispersal vectors requires precise knowledge concerning the fate of the dispersed seed, an aspect seldom addressed in studies of seed dispersal (Schupp and Fuentes 1995; Schupp et al. 2010) and, as far as we know, never addressed for the interaction between corvids and nut-producing tree species. The use of radio-tracking with small transmitters embedded in the seed is a recent method to study nut dispersal (e.g. Pons and Pausas 2007; Tamura and Hayashi 2008; Morán-López et al. 2015). By replacing the transmitter-containing nut after dispersal by another non-manipulated nut able to germinate and continue with the recruitment processes, we might be able to monitor the magnitude of effective seed dispersal. Although this method could still underestimate the probability of recruitment in case the dispersed nuts are re-cached, it has the potential to provide a more accurate measure of the qualitative component of seed-dispersal effectiveness (*sensu* Schupp et al. 2010) and a more comprehensive picture of the role of corvids in the recruitment of nut-producing tree species.

In this study, we analyse the activity of magpies, a common corvid in open landscapes and agroforestry systems throughout Eurasia, in the dispersal of the common walnut (*Juglans regia* L.). Nut removal, dispersal distance, cache location, and seedling emergence were precisely monitored, providing the necessary framework to analyse the seed-dispersal effectiveness mediated by a vertebrate vector. Given the already known scatter-hoarding behaviour of magpies and their capacity to remember caching sites, together with observations made under field conditions supporting magpie nut dispersal (Birkhead 1991; Omat et al. 2015, author’s personal observations), we hypothesise that magpies are effective nut dispersers. Four specific questions were posed: 1) Do magpies disperse walnuts in the study area? 2) What are the characteristics of dispersal events in terms of habitat selection, caching type, and dispersal distance? 3) What is the recovery rate of cached nuts? And 4) what are the germination and emergence rates of unrecovered nuts? The response to these questions will allow us to determine an accurate value of the qualitative component of seed-dispersal effectiveness and the role of magpies as dispersers for a nut-producing tree.

MATERIALS AND METHODS

1. Study site and natural history of the system

The study was conducted in an agroforestry system located in the “Vega de Granada” (SE Spain, 37º 10' 03.43" N, 3º 36' 57.80" W), a flat and irrigated agricultural area of small-sized farms located at ca. 650 m a.s.l. The area is used mainly for crop production, mostly vegetables, maize, tree plantations, and pasture. The soil is deep and loamy, and the climate is Mediterranean-type, with hot, dry summers and mild winters. The mean annual rainfall is 394±71 L m^2 y^{-1} and the mean temperature 15.3±0.1 ºC (period 2006-2015, based upon climatic data from a meteorological station located at IFAPA Research Field Station, 1.5 km from the study site). Common walnut (*Juglans regia*; target plant species of this study) is traditionally grown in the farms of the area (presumably since Roman times; Buxó 1997), usually as scattered trees close to houses.

The study site was a 1.8-ha farm (hereafter referred to as “core site”) plus surrounding fields where nut dispersal could be registered with radio-tracking. The core site, which is used mostly for research purposes, is divided into three main areas (habitats, hereafter), namely 1) a broadleaf stand, 2) a pine stand, and 3) cropland (Fig. 1). The broadleaf habitat is a 7000-m^2 mixed tree plantation of poplar (*Populus* ×

euroamericana (Dode) Guinier, clone I-214) and hybrid walnut (*Juglans major* x *Juglans regia* MJ 209xRa) with an even number of individuals, all trees being evenly spaced at a planting density of 400 individuals ha^{-1}. Tree diameter at breast height in October 2015 was 27.6±0.3 cm for poplar and 9.6±0.2 cm for hybrid walnut. The pine habitat consisted of 2000 m^2 of Aleppo pine saplings (*Pinus halepensis* Mill.), evenly spaced at a density of 1200 individuals ha^{-1}. Saplings had a height of 1.95±0.04 m by October 2015, with lower branches touching the ground. The cropland habitat covers the rest of the core site area and is used for vegetable production (Fig. 1). It also contains some scattered fruit trees (3-6 m tall) such as plums, apples, pears, persimmons, fig trees and peaches, for a total of 34 individuals. The three habitat types were ploughed in late August 2015, one week before the start of this study.

The black-billed magpie is a corvid widely distributed across the Palearctic and is the most abundant corvid in southern Europe (Cramp and Perrins 1994; Martí and Del Moral 2003). It is particularly abundant in agroecosystems and open landscapes where other nut-dispersing corvids such as the Europen jay are usually absent (Martí and Del Moral 2003; Martínez 2011). The magpie is a common species in the Iberian Peninsula, but was absent in the study area until some years ago despite being common in nearby areas at distances of ca. 20 km. Regular bird sampling in the study area since 1985 (J.C.; unpublished data) showed that they appeared in low numbers (occasional individuals) in 2002 and started nesting in 2008. Their population has steadily increased since then, currently being a common breeding bird in the area. Coinciding with its arrival to the study site, the emergence of walnut seedlings in the fields became evident. In 2012 we made preliminary observations and confirmed that magpies were dispersing nuts picked directly from *J. regia* trees of the area. These observations were not methodical, but they led us to formulate the hypotheses and sampling design to conduct this study.

2. Sampling of the magpie-walnut interaction

We studied the interaction between magpie and walnut by using three complementary approaches: 1) monitoring the removal of non radio-tagged nuts offered in feeders, 2) monitoring the removal and dispersal distance of radio-tagged nuts offered in feeders, and 3) monitoring post-dispersal recovery rate and seedling recruitment for nuts that replaced the radio-tagged nuts. Nut dispersal was sampled in all cases within the period of natural nut ripening and dispersal in the study area. The coordinates of all dispersed nuts and feeders were marked with a GPS, which allowed dispersal distances to be calculated using Quantum GIS. For the core site, we also constructed an ortho-photo from 5-cm/pixel-resolution photos (Fig. 1).

The removal of non radio-tagged nuts placed in feeders was monitored from September 5 to October 26, 2015 (see Fig. S1 for feeder details). A total of 165 nuts were offered in bunches of 20 (occasionally 10 or 5; Table 1), and a video camera with a continuous recording system and day and night vision was placed at ca. 1.5 m from the nuts (Miniature Motion Activated DVR Resolution SSC-758HQ, coupled with Led Color Cameras SSC-56C36; Advance Security, Belleville, Illinois, USA). Also, we conducted non-systematic direct observations from a hide. A fraction of the nuts (120) were weighed before placing them in the feeders, and they were identified with a number on the shell using waterproof, permanent ink. Overall, this procedure was chosen as an initial method to test nut removal by magpies (i.e. before using radio transmitters) to reduce nut manipulation and potential distrust by magpies. It also allowed us to ascertain the disperser’s identity and activity.

The dispersal of radio-tagged nuts placed in feeders was monitored from October 25 to December 12, 2015. For this, a radio transmitter (PIP2 Tag Ag392; Biotrack, Wareham, Dorset, UK; weight: 2.2 g; mean life span: 3 months) was placed inside the nut, which allowed us to relocate dispersed nuts and to measure exact dispersal distances. Nut removal was also video-recorded with a movement-sensitive system (Moultrie M-990i; Moultrie Products, Alabama, USA) as well as with day and night vision. For each sample, the walnut shell was split open along the suture, a portion of the kernel of similar weight to the transmitter was excised, the transmitter with its antenna rolled up was placed inside the nut, and then the two halves of the nut were glued together with Loctite® (Supplementary Material 1, Fig. S2). Five transmitter-containing nuts were used, either in a single feeder or divided into groups of 2 and 3 nuts in the two feeders simultaneously. Eventually, we noted that magpies refused to pick some radio-tagged nuts from the feeders, which might have been due to desiccation or to any other cue that we could not identify. In those cases we changed the transmitter to another nut. Once removed from the feeders, the nuts with the radio-transmitter were located (usually within a few hours after dispersal) with the help of a radio-tracking receiver with a unidirectional Yagi antenna (Biotrack, Wareham, Dorset, UK) plus a hand-held metal detector (White's Auto-Scan Personal Search Detector, Tulsa, Oklahoma, USA) for exact location of the nut/transmitter, which is particularly necessary for buried nuts. The caching characteristics were categorized as: 1) Superficial, nuts left visible, on the soil surface; 2) Buried, nuts buried in bare soil; and 3) Under plant material, nuts hidden below leaf litter or below leaves of live vegetation, the latter including forbs, grasses, vegetables or the pine branches that were touching the ground in the pine habitat.

For the study of post-dispersal recovery rate and seedling recruitment, once a nut with a transmitter was located, the nut was placed back in the feeders, and a non-manipulated nut of similar weight was placed in the same location. The point where the nut was found was marked with a wooden stake (12 cm x 9 mm x 9 mm) 50 cm away from the nut, and a small metal rod was placed under the nut to facilitate later relocation with the metal detector. Approximately 6 months later (from 5 to 24 May 2016), coinciding with the period of seedling emergence in the study area, we sampled the status of all those nuts, considering as categories: absent (assigned as recovered), non-germinated, germinated, and emerged seedling. In the case of emerged seedlings we also noted seedling height.

4. Data analyses

We analysed differences in the weight among removed and non-removed nuts from the feeders with a one-way ANOVA. The effect of nut weight on dispersal distance was assessed with a linear mixed model in the lme R package (Pinheiro et al. 2016), using nut as a random effect because the same transmitter-containing nuts were placed in the feeders several times. Differences in caching types were analysed with contingency tests, and the effect of caching type on recovery rates with a glm with a binomial distribution. The effect of habitat type and distance from the feeder on nut dispersal was analysed with spatial statistics. This analysis was restricted to the core site given that in this area the habitats persisted through the study period, whereas outside the core site the crops changed from September to December, precluding definition of permanent habitats. For this, we fitted point process models with the spatstat R package (Baddeley & Turner 2005). The models considered a non-homogeneous Poisson process, with the density of dispersed nuts within the core site depending on two spatial covariates:

habitat type (broadleaf, pine or farmland) and a map of the distance of each 1 x 1 m pixel to the feeder. The performance of this model was assessed through likelihood ratio tests during model simplification. This procedure was performed for the nuts dispersed from Feeder 1, as the number of nuts from Feeder 2 was insufficient to perform this analysis. Analyses were performed with R version 3.1.1 in all cases (R Core Team 2014).

RESULTS

1. *Nut removal from feeders*

A total of 193 nuts were removed from feeders, including nuts with and without transmitters (68 and 125, respectively; Table 1). In 98% of the cases, the disperser was video-recorded or directly observed from the hide, and it was a magpie in all cases. Magpies in no case consumed nuts in the feeders, and the number of nuts removed was one in all dispersal events. The number of magpies observed simultaneously in the feeders ranged from 1 to 5. Removed nuts were heavier (9.44±0.17 g) than non-removed nuts (8.67±0.32 g; F=4.47, d.f.=1, 118, p=0.037). Overall, magpies showed a high activity, and were able to remove all or most of the nuts within a few hours (Table 1).

2. *Dispersal distance and caching characteristics*

Dispersal distance was measured for 66 radio-tagged nuts. Mean dispersal distance was 39.6±4.5 m, with a range of 4.1 to 158.5 m (Fig. 2a). Two nuts containing transmitters were not found despite thorough searching up to a distance of at least 300 m from the core site (not considered for analyses), and they were likely carried long-distances based on video-camera recordings of these nuts (see Supplementary Material 2 for a video of one of the cases). Nut weight did not affect dispersal distance (L.Ratio = 0.58, p = 0.45).

Of the dispersed nuts, 10.6% were not cached but rather consumed immediately after removal from the feeder (the nut was found open with the transmitter partially or entirely outside), whereas the remaining 89.4% (59 nuts) were cached. Of those, 55.9% were buried in the soil (at 1-3 cm depth in all cases), 42.4% cached under plant material, and only one (1.7%) was left on the ground surface (Chisq = 25.36, df = 2, p < 0.001). In all cases the nuts were cached individually. Two of the nuts cached under plant material were located on a roof, although still hidden below litter.

The point process models did not show a significant effect of habitat type on the density of dispersed nuts (Δ Dev = 0.94, df = 2, p = 0.63), but they showed a significant negative effect of distance from the feeder (Δ Dev = 114.62, df = 1, p < 0.001; Fig. 2b).

3. Nut recovery and seedling recruitment

By May 2016 we were able to determine the fate of 49 of the 59 cached radio-tracked nuts; the remaining 10 nuts were either lost (four sampling points within the core site could not be relocated) or dispersed outside the core site, where the ground was tilled before the time of sampling (thus provoking the loss of the sampling point). Of these 49 nuts, 67.3% were recovered, 20.4% did not germinate, 12.2% germinated (including emerged ones), and 4.1% produced an emerged seedling (Fig. 3).

Nut recovery was 73.5% in the cropland habitat, 57.1% in the broadleaf habitat, and 33.3% in the pine habitat, although there were no significant differences among habitat types (ChiSq = 3.79, d.f. = 2, p=0.15). Nut recovery tended to decrease with increasing distance to the feeder, with non-recovered nuts being at an average distance of 37.2±6.2 m from the feeder vs 27.5±4.3 m for the recovered nuts. Nonetheless, this trend was not significant (logistic regression, ChiSq = 1.61, d.f. = 1, p=0.20).

We could not unequivocally determine the animal that removed the cached nuts, but we often observed magpies recovering nuts in the study area, and found a large number of nuts consumed and opened in two valves as is characteristic in magpies (Homet-Guitérrez et al. 2015; author's personal observation). No other animal was directly observed removing the nuts dispersed by the magpies. Recovery activity spanned the entire study period, and we observed magpies consuming recovered nuts until early May 2016.

DISCUSSION

In this study, the magpie, an abundant corvid in Eurasian agroecosystems and open landscapes, proved to be an effective disperser of a large-seeded species, moving a large number of walnuts over dispersal distances that reached 158 m. Furthermore, a fraction of the nuts was not recovered after caching and resulted in effective early seedling recruitment. Magpies had previously been suggested as dispersers for nut-producing tree species such as oaks (Birkhead 1991) or almond trees (Homet-Gutiérrez et al. 2015), but this interaction had never been demonstrated or measured in the context of plant recruitment. Our study contributes to the understanding of the role of scatter-hoarding corvids in the regeneration of Eurasian forests, and provides for the first time precise information of the qualitative component of seed-dispersal effectiveness for the interaction between birds and nut-producing trees.

Nut dispersal

Most of the nuts that were offered in the feeders were quickly dispersed and cached, and only a small fraction (ca. 10%) was consumed just after removal. This behaviour is typical in scatter-hoarding animals, which display vigorous dispersal activity when the resource is abundant presumably in order to accumulate as much of it as possible during the short period of availability (Clarkson et al. 1986; Vander Wall 2001). After nut removal from feeders or trees, magpies also displayed a behaviour similar to that of other corvids (Bossema 1979; Cristol 2005). On arriving to a place to hide the nut, they wandered for a few seconds as if selecting the most preferable site, presumably checking for potential competitors that could steal the cached nut. In fact, in some cases they flew away with the nut and searched for another site. To cache the nut, they pushed it with the beak, sometimes hammering on it to bury it in the soil, and then they covered the site with soil or litter in such a way that the exact caching point became undetectable to the human eye (see Birkhead 1991 for a description of similar behaviour). In addition, the majority of the cached nuts were buried in the soil or hidden under plant material, both being microhabitats that may favour seed germination and seedling recruitment by reducing the risk of predation and desiccation (Bossema 1979; Vander Wall 1990, 2001; Gómez 2004). Furthermore, magpies preferred heavier nuts, therefore favouring a trait (large seed mass) that may enhances seedling establishment (Castro et al. 2006).

The observed dispersal distances lie within the lower range described for the rook, a corvid with a documented role in walnut dispersal (Lenda et al. 2012). Nonetheless, the body mass of the rook (around 500 g) is much larger than the mass of the magpie (around 200 g). In addition, two of the transmitters were lost, perhaps as a consequence of long-distance dispersal. In fact, we observed several events in which a magpie flying from a feeder with a nut was lost in the distance, likely far beyond the maximum dispersal distance recorded (Supplementary Material 2). In any case, 7.3% of the nuts were dispersed beyond 100 m, a distance and proportion great enough to support the contention that magpies can act as long-distance seed dispersers (Cain et al. 2000). Thus, magpies can play a relevant role in the expansion of nut-producing trees into new areas, a key step for the regeneration of the temperate forest (Pesendorfer et al. 2015).

The seed rain generated also supports the idea that magpies are effective dispersers in agroecosystems and agroforestry mosaics, since nuts were invariably cached alone, one by one, and were widely distributed throughout different habitats of the landscape. Some consequences of the spatial structuring of the seed rain for plant recruitment also seems plausible, as shown by the facts that caching density decreased with distance from the source (as it is ultimately expected in a cost-benefit trade-off; Clarkson et al. 1986), or that there were significant differences in caching characteristics. Nonetheless, our results are based in a single study site, which precludes generalization of patterns. Further studies including more study areas and larger sample size would be necessary to ascertain the relationship between habitat characteristics, dispersal distance, and its potential implication for effective long-distance dispersal. Radio-tracking, combined with the monitoring of the fate of seeds that replace the dispersed, radio-tagged seed, has proved to be an appropriate method to answer these questions.

Post-dispersal nut recovery and seed dispersal effectiveness

Radio-tracking also allowed us to obtain accurate estimates of nut recovery, which reached 67% ca. 8 months after dispersal. Magpies were the only animals observed recovering the nuts. Rodent pilfering of part of the nuts cannot be ruled out, but these animals appear to play a minor role in this system, as throughout the study period we found only three nuts with marks of chewing by rodents on the shell vs. a high number (not recorded) of nuts opened in two halves as magpies do. We cannot discount pilfering by other magpies, either, or the re-caching by the original magpie. In any case, the consequence for plant recruitment is that after nut dispersal by magpies, a large fraction (at least 32.6%) of the seeds remained on site until the following spring ready to start the next regeneration stage.

As a final result, 4% of the cached nuts rendered an emerged seedling, thus providing a net value of seed dispersal effectiveness up to the seedling stage that could generate recruitment. In addition, the number of emerged seedlings might have been higher if the final sampling had been conducted a few weeks later, as all the germinated (but not emerged) seedlings showed a healthy radicle protruding in the soil. In fact, since the arrival of magpies to the study area ca. 15 years ago, there is abundant walnut seedling emergence around the farms at distances of dozens to hundreds of meters from adult trees (authors' personal observation). Although this rarely translates into adult walnut recruitment due to yearly ploughing, it is very likely that walnut expansion would occur if ploughing were discontinued, as documented for example by Lenda et al. (2012) for walnut dispersed by rooks in abandoned farms in Poland.

In summary, this study demonstrates the relevance of magpies as scatter-hoarding dispersers of nut-producing trees, and for the first time provides an accurate estimate of seed-dispersal effectiveness for a bird-plant interaction that is crucial for the regeneration and expansion of temperate, large-seeded trees (Pesendorfer et al. 2016). Magpies are abundant in open landscapes such as agricultural land and successional shrublands, habitat types usually avoided by Eurasian jays for their nut-dispersal activity (Gómez 2003; Pons and Pausas 2007; Leverkus et al. 2016), and where jays are often rare or absent (Andrén 1990; Pons and Pausas 2008; Cramp and Perris 1994). This may increase the relevance of the magpie as a key species for the demography of nut-producing trees in anthropogenic landscapes where habitat fragmentation and reduced forest cover are common. In short, our results support the hypothesis that magpies act in the regeneration and expansion of the Eurasian temperate forest, thus increasing the number of corvid species with known key mutualistic roles for forest regeneration.

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	Date	Feeder	#Nuts offered	#Nuts dispersed
Non radio-tagged nuts	05/09/15	1	20	18
	06/09/15	1	20	19
	08/09/15	1	20	20
	09/09/15	1	20	15
	11/09/15	1	20	14
	13/09/15	1	20	6
	18/09/15	1	20	15
	21/10/15	1	10	9
	21/10/15	2	10	4
	26/10/15	1	5	5
			Σ=165	**Σ=125**
Radio-tagged nuts	25/10/15	1	5	4
			5	5
	26/10/15	1	5	1
	27/10/15	1	5	4
	28/10/15	1	5	5
	29/10/15	1	5	2
			5	2
	1/11/15	1	5	4
	2/11/15	1	5	3
	3/11/15	1	5	4
	5/11/15	1	5	2
	7/11/15	2	5	3
	9/11/15	2	5	3
	10/11/15	2	3	1
	10/11/15	1	2	2
	15/11/15	1	3	1
	17/11/15	1	5	5
	25/11/15	1	5	3
	01/12/15	1	5	5
	04/12/15	1	5	2
	10/12/15	1	5	3
	12/12/15	1	4	4
			Σ=102	**Σ=68**

Table 1. Summary of the number of nuts offered and removed from feeders during the study period.

Figure captions

Figure 1. Ortho-rectified aerial photograph of the study area, taken with a drone. The area surrounded by a dashed red line is the core site. The dashed yellow line delimitates the "broadleaf" habitat, the green dashed line the "Pine" habitat, and the rest of the area within the core site corresponds to the "Cropland" habitat. Yellow dots indicate the position of cached nuts dispersed from feeder 1, and green dots that of cached nuts dispersed from feeder 2. The orange triangles show the position of the feeders. One nut with a radio-transmitter was dispersed towards the east outside the area of the picture and is not shown here. The image was take with a GoPro 4 Black edition camera attached to a drone (Phantom 2 UAV) during a photogrammetric flight at 50 m height on 23rd January 2016. The photos were processed with the Agisoft PhotoScan 1.2.0 software, which was also used for the final 10-cm pixel-resolution image.

Figure 2. Distribution of radio-tagged nuts dispersed by magpies. A) Histogram showing the frequency of dispersal distances. B) Kernel-smoothed density of cached nuts encountered in the core site for radio-labeled nuts dispersed from Feeder 1 (marked as a red dot). The map shows the intensities of the point pattern generated by caching points within the plot. Density of caching points reduces with increasing distances from the feeder. The space occupied by gravel roads and buildings has been eliminated for the analysis.

Figure 3. Path diagram indicating the stages in the qualitative component of seed-dispersal effectiveness (SDE) for the magpie-walnut interaction. The numbers in the boxes indicate the number of nuts available for the next demographic transition (green boxes) and those that were lost for recruitment (red boxes). The yellow boxes indicate

the starting (total number of monitored nuts) and the ending points (number of emerged seedlings). Numbers in bracket show the transition probability for each stage from the number of dispersed seeds. The height of the seedlings was 12.0 and 14.8 cm, respectively. Data of the three habitat types have been pooled for simplicity. Not-recov. = Not recovered.

Supplementary Material 1

Figure S1. Characteristics of the feeders used in this study. The first feeder (Feeder 1 in Figure 1 of the manuscript) consisted of an almost flat roof of a chicken house placed at 2.20 m above the ground plus a wooden slat that prevented the nuts from rolling down (upper picture). The second feeder (Feeder 2 in Figure 1 of the manuscript) was a 30 × 40 cm wooden cage with a metal bottom, held 1.7 m from the ground by a metal post and located below the canopy of a walnut tree (bottom picture). We knew from previous observations that magpies foraged or perched regularly in both locations. The two feeders were placed 100 m from each other.

Figure S2. Details of radio-transmitter insertion into a walnut. The nut was split open with a knife, a portion of the kernel similar in weight to the transmitter was removed, the transmitter was inserted into the nut with the antenna rolled, and finally the two halves of the shell were glued together with superglue (Loctite®).

Supplementary Material 2. Video recording with a sensitive-movement camera in Feeder 1 on 27 October 2015, showing three magpies at once, and two of them retrieving a nut each (containing transmitters in this case). The time that appears in the video is local time, one hour ahead of solar time (thus, it was 8 am solar time). It can be observed that the second magpie that removed a nut flew westwards beyond a group of trees, being lost at a distance of ca. 130 m from the feeder. This transmitter was not found despite a thorough search at a distance up to 300 m, and might represent an event of long-distance dispersal.

Effective nut dispersal by magpies (*Pica pica* L.) in a Mediterranean agroecosystem

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Author Contributions: JC, LPC, PVS, SR and JMRB conceived and designed the experiments. JC, MMM, AL and LMB performed the field work. JC, MMM and AL performed statistical analyses. JC wrote the first draft of the ms; all authors provided editorial advice.

Abstract

Scatter-hoarding animals such as corvids play a crucial role in the dispersal of nut-producing tree species. This interaction is well known for some corvids, but remains elusive for other species such as the magpie (*Pica pica*), an abundant corvid in agroecosystems and open landscapes of the Palearctic region. In addition, the establishment of the individual dispersed seeds, a prerequisite to determine seed-dispersal effectiveness, never before has been documented for the interaction between corvids and nut-producing trees. We analysed walnut dispersal by magpies in an agroecosystem in southern Spain. We used several complementary approaches, including video-recording of nut removal from feeders, measuring dispersal distance using radio-tracking (with radio transmitters placed inside nuts), and monitoring the fate of dispersed nuts to the time of seedling emergence. Magpies were shown to be highly active as nut dispersers. The dispersal distance averaged 39.6±4.5 m, with a range from 4.1 to 158.5 m. Some 90% of the removed walnuts were cached later, and most of these (98%) were buried in the soil or hidden under plant material. By the time of seedling emergence, ca. 33% of nuts still remained in the caching location. Finally, 12% of the cached nuts germinated, and 4% yielded an emerged seedling, which allowed the transition to the next regeneration stage. The results demonstrate for the first time that magpies can be an effective scatter-hoarding disperser of a nut-producing tree species, suggesting that this bird species may play a key role for the regeneration and expansion of broadleaf forests in Eurasia.

Key words: *Corvidae*, forest regeneration, *Juglans*, radio-tracking, scatter-hoarding, seed caching, seed dispersal effectiveness

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INTRODUCTION

Seed dispersal of large-seeded species of paramount relevance in the context of temperate forests is largely ascribed to a plant-animal interaction in which a vertebrate vector is responsible for direct seed transport (Vander Wall 1990; Johnson et al. 1997; Pesendorfer et al. 2016). Several bird species from the *Corvidae* family are among the most active dispersers for these trees, acting as scatter-hoarding animals that cache seeds in a large number of locations across the landscape for later consumption, disperse a very large number of seeds, and usually cover distances exceeding hundreds or even thousands of meters (Bossema 1979; Darley-Hill and Johnson 1981; Lenda et al. 2012; Pesendorfer et al. 2016). A fraction of the seeds may remain un-recovered, providing the opportunity for seed germination and tree recruitment (Vander Wall 1990; Pesendorfer et al. 2016). In fact, the interaction between corvids and many tree species from the *Fagaceae* or *Juglandaceae* plant families is considered a key mutualistic relationship for the regeneration, colonization, and expansion of forests in the Northern Hemisphere, helping to explain the post-glacial migration and current distribution of temperate forests (Johnson and Webb III 1989; Johnson et al. 1997; Vander Wall 1990; Mosandl and Kleinert 1998; Pesendorfer et al. 2016).

The role of corvids in the transport of nuts has been noted since ancient times (e.g. Aristotle and Theophrastus; Thanos 1994), and for decades has been intensively studied in several species throughout the Holarctic region (e.g. Grinnell 1936; Richards 1958; Bossema 1979; Cristol 2005; Pesendorfer et al. 2016 [and references therein]). In the case of North America, at least seven species have been described as dispersers of nuts from *Fagaceae* or *Juglandaceae* species (Cristol 2005; Pesendorfer et al. 2016). However, the dispersal of large nuts such as acorns and walnuts by corvids in Eurasia is

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ascribed mostly to a single species, the European jay (*Garrulus glandarius* L.) (Bossema 1979; Pesendorfer et al. 2016), and to a much lesser extent to the rook (*Corvus frugilegus* L.) (Waite 1985; Källender 2007; Lenda et al. 2012). Knowledge of the role of other corvids in the regeneration of these tree species in the Palearctic is almost negligible. In particular, the black-billed magpie (*Pica pica* L., hereafter referred to as “magpie”), a common corvid in Eurasia, is considered to have little relevance for tree dispersal, as it is assumed to preferentially cache perishable food, while caching few nuts within short distances, and with a recovery time of only a few days (Henty 1975; Waite 1985; Birkhead 1991).

Several pieces of evidence, however, suggest that magpies might be noteworthy vectors in nut dispersal. It is well established that magpies cache food items (Henty 1975; Clarkson et al. 1986; Birkhead 1991), have the capacity to recall cache locations (Zinkivskay et al. 2008; Feenders and Smulders 2011) and have a well-developed hippocampus (Healy and Krebs 1992; Brodin and Lundberg 2003), a brain region linked to spatial memory and food-storing behaviour. Magpies have also been suggested to be the most likely dispersers of almond trees in agroforestry systems (Homet-Gutiérrez et al. 2015), and reports on acorn dispersal, although very scant, are available (Birkhead 1991). In short, several clues support the idea that magpies might have an influential role in nut dispersal for Eurasian tree species. However, to date, the magnitude of nut dispersal and recovery rate for this bird have never been documented.

Although many studies have addressed the dispersal of nut-producing trees by corvids (e.g. review by Pesendorfer et al. 2016), a gap in knowledge persists concerning the implications of this mutualistic interaction for forest regeneration. Studies reporting a link between the vector and the plant are based mostly on evidence arising from synchronic observations of dispersal and seedling-recruitment patterns (e.g. Mosandl

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and Kleinert 1998; Gómez 2003; Hougner et al. 2006; Castro et al. 2012; Lenda et al. 2012; Puerta-Piñero et al. 2012). This procedure has demonstrated beyond a doubt that the corvids are major vectors for nut dispersal. However, a fine-grained quantification of the effect of animal seed-dispersal vectors requires precise knowledge concerning the fate of the dispersed seed, an aspect seldom addressed in studies of seed dispersal (Schupp and Fuentes 1995; Schupp et al. 2010) and, as far as we know, never addressed for the interaction between corvids and nut-producing tree species. The use of radio-tracking with small transmitters embedded in the seed is a recent method to study nut dispersal (e.g. Pons and Pausas 2007; Tamura and Hayashi 2008; Morán-López et al. 2015). By replacing the transmitter-containing nut after dispersal by another non-manipulated nut able to germinate and continue with the recruitment processes, we might be able to monitor the magnitude of effective seed dispersal. Although this method could still underestimate the probability of recruitment in case the dispersed nuts are re-cached, it has the potential to provide a more accurate measure of the qualitative component of seed-dispersal effectiveness (*sensu* Schupp et al. 2010) and a more comprehensive picture of the role of corvids in the recruitment of nut-producing tree species.

In this study, we analyse the activity of magpies, a common corvid in open landscapes and agroforestry systems throughout Eurasia, in the dispersal of the common walnut (*Juglans regia* L.). Nut removal, dispersal distance, cache location, and seedling emergence were precisely monitored, providing the necessary framework to analyse the seed-dispersal effectiveness mediated by a vertebrate vector. Given the already known scatter-hoarding behaviour of magpies and their capacity to remember caching sites, together with observations made under field conditions supporting magpie nut dispersal (Birkhead 1991; Omat et al. 2015, author's personal observations), we hypothesise that

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magpies are effective nut dispersers. Four specific questions were posed: 1) Do magpies disperse walnuts in the study area? 2) What are the characteristics of dispersal events in terms of habitat selection, caching type, and dispersal distance? 3) What is the recovery rate of cached nuts? And 4) what are the germination and emergence rates of unrecovered nuts? The response to these questions will allow us to determine an accurate value of the qualitative component of seed-dispersal effectiveness and the role of magpies as dispersers for a nut-producing tree.

MATERIALS AND METHODS

1. Study site and natural history of the system

The study was conducted in an agroforestry system located in the “Vega de Granada” (SE Spain, 37º 10' 03.43" N, 3º 36' 57.80" W), a flat and irrigated agricultural area of small-sized farms located at ca. 650 m a.s.l. The area is used mainly for crop production, mostly vegetables, maize, tree plantations, and pasture. The soil is deep and loamy, and the climate is Mediterranean-type, with hot, dry summers and mild winters. The mean annual rainfall is 394±71 L m^2 y^{-1} and the mean temperature 15.3±0.1 ºC (period 2006-2015, based upon climatic data from a meteorological station located at IFAPA Research Field Station, 1.5 km from the study site). Common walnut (*Juglans regia*; target plant species of this study) is traditionally grown in the farms of the area (presumably since Roman times; Buxó 1997), usually as scattered trees close to houses.

The study site was a 1.8-ha farm (hereafter referred to as “core site”) plus surrounding fields where nut dispersal could be registered with radio-tracking. The core site, which is used mostly for research purposes, is divided into three main areas (habitats, hereafter), namely 1) a broadleaf stand, 2) a pine stand, and 3) cropland (Fig. 1). The broadleaf habitat is a 7000-m^2 mixed tree plantation of poplar (*Populus* ×

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euroamericana (Dode) Guinier, clone I-214) and hybrid walnut (*Juglans major* x *Juglans regia* MJ 209xRa) with an even number of individuals, all trees being evenly spaced at a planting density of 400 individuals ha^{-1}. Tree diameter at breast height in October 2015 was 27.6±0.3 cm for poplar and 9.6±0.2 cm for hybrid walnut. The pine habitat consisted of 2000 m^2 of Aleppo pine saplings (*Pinus halepensis* Mill.), evenly spaced at a density of 1200 individuals ha^{-1}. Saplings had a height of 1.95±0.04 m by October 2015, with lower branches touching the ground. The cropland habitat covers the rest of the core site area and is used for vegetable production (Fig. 1). It also contains some scattered fruit trees (3-6 m tall) such as plums, apples, pears, persimmons, fig trees and peaches, for a total of 34 individuals. The three habitat types were ploughed in late August 2015, one week before the start of this study.

The black-billed magpie is a corvid widely distributed across the Palearctic and is the most abundant corvid in southern Europe (Cramp and Perrins 1994; Martí and Del Moral 2003). It is particularly abundant in agroecosystems and open landscapes where other nut-dispersing corvids such as the Europen jay are usually absent (Martí and Del Moral 2003; Martínez 2011). The magpie is a common species in the Iberian Peninsula, but was absent in the study area until some years ago despite being common in nearby areas at distances of ca. 20 km. Regular bird sampling in the study area since 1985 (J.C.; unpublished data) showed that they appeared in low numbers (occasional individuals) in 2002 and started nesting in 2008. Their population has steadily increased since then, currently being a common breeding bird in the area. Coinciding with its arrival to the study site, the emergence of walnut seedlings in the fields became evident. In 2012 we made preliminary observations and confirmed that magpies were dispersing nuts picked directly from *J. regia* trees of the area. These observations were not

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methodical, but they led us to formulate the hypotheses and sampling design to conduct this study.

2. Sampling of the magpie-walnut interaction

We studied the interaction between magpie and walnut by using three complementary approaches: 1) monitoring the removal of non radio-tagged nuts offered in feeders, 2) monitoring the removal and dispersal distance of radio-tagged nuts offered in feeders, and 3) monitoring post-dispersal recovery rate and seedling recruitment for nuts that replaced the radio-tagged nuts. Nut dispersal was sampled in all cases within the period of natural nut ripening and dispersal in the study area. The coordinates of all dispersed nuts and feeders were marked with a GPS, which allowed dispersal distances to be calculated using Quantum GIS. For the core site, we also constructed an ortho-photo from 5-cm/pixel-resolution photos (Fig. 1).

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The removal of non radio-tagged nuts placed in feeders was monitored from September 5 to October 26, 2015 (see Fig. S1 for feeder details). A total of 165 nuts were offered in bunches of 20 (occasionally 10 or 5; Table 1), and a video camera with a continuous recording system and day and night vision was placed at ca. 1.5 m from the nuts (Miniature Motion Activated DVR Resolution SSC-758HQ, coupled with Led Color Cameras SSC-56C36; Advance Security, Belleville, Illinois, USA). Also, we conducted non-systematic direct observations from a hide. A fraction of the nuts (120) were weighed before placing them in the feeders, and they were identified with a number on the shell using waterproof, permanent ink. Overall, this procedure was chosen as an initial method to test nut removal by magpies (i.e. before using radio transmitters) to reduce nut manipulation and potential distrust by magpies. It also allowed us to ascertain the disperser’s identity and activity.

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The dispersal of radio-tagged nuts placed in feeders was monitored from October 25 to December 12, 2015. For this, a radio transmitter (PIP2 Tag Ag392; Biotrack, Wareham, Dorset, UK; weight: 2.2 g; mean life span: 3 months) was placed inside the nut, which allowed us to relocate dispersed nuts and to measure exact dispersal distances. Nut removal was also video-recorded with a movement-sensitive system (Moultrie M-990i; Moultrie Products, Alabama, USA) as well as with day and night vision. For each sample, the walnut shell was split open along the suture, a portion of the kernel of similar weight to the transmitter was excised, the transmitter with its antenna rolled up was placed inside the nut, and then the two halves of the nut were glued together with Loctite® (Supplementary Material 1, Fig. S2). Five transmitter-containing nuts were used, either in a single feeder or divided into groups of 2 and 3 nuts in the two feeders simultaneously. Eventually, we noted that magpies refused to pick some radio-tagged nuts from the feeders, which might have been due to desiccation or to any other cue that we could not identify. In those cases we changed the transmitter to another nut. Once removed from the feeders, the nuts with the radio-transmitter were located (usually within a few hours after dispersal) with the help of a radio-tracking receiver with a unidirectional Yagi antenna (Biotrack, Wareham, Dorset, UK) plus a hand-held metal detector (White's Auto-Scan Personal Search Detector, Tulsa, Oklahoma, USA) for exact location of the nut/transmitter, which is particularly necessary for buried nuts. The caching characteristics were categorized as: 1) Superficial, nuts left visible, on the soil surface; 2) Buried, nuts buried in bare soil; and 3) Under plant material, nuts hidden below leaf litter or below leaves of live vegetation, the latter including forbs, grasses, vegetables or the pine branches that were touching the ground in the pine habitat.

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For the study of post-dispersal recovery rate and seedling recruitment, once a nut with a transmitter was located, the nut was placed back in the feeders, and a non-manipulated nut of similar weight was placed in the same location. The point where the nut was found was marked with a wooden stake (12 cm x 9 mm x 9 mm) 50 cm away from the nut, and a small metal rod was placed under the nut to facilitate later relocation with the metal detector. Approximately 6 months later (from 5 to 24 May 2016), coinciding with the period of seedling emergence in the study area, we sampled the status of all those nuts, considering as categories: absent (assigned as recovered), non-germinated, germinated, and emerged seedling. In the case of emerged seedlings we also noted seedling height.

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4. Data analyses

We analysed differences in the weight among removed and non-removed nuts from the feeders with a one-way ANOVA. The effect of nut weight on dispersal distance was assessed with a linear mixed model in the lme R package (Pinheiro et al. 2016), using nut as a random effect because the same transmitter-containing nuts were placed in the feeders several times. Differences in caching types were analysed with contingency tests, and the effect of caching type on recovery rates with a glm with a binomial distribution. The effect of habitat type and distance from the feeder on nut dispersal was analysed with spatial statistics. This analysis was restricted to the core site given that in this area the habitats persisted through the study period, whereas outside the core site the crops changed from September to December, precluding definition of permanent habitats. For this, we fitted point process models with the spatstat R package (Baddeley & Turner 2005). The models considered a non-homogeneous Poisson process, with the density of dispersed nuts within the core site depending on two spatial covariates:

habitat type (broadleaf, pine or farmland) and a map of the distance of each 1 x 1 m pixel to the feeder. The performance of this model was assessed through likelihood ratio tests during model simplification. This procedure was performed for the nuts dispersed from Feeder 1, as the number of nuts from Feeder 2 was insufficient to perform this analysis. Analyses were performed with R version 3.1.1 in all cases (R Core Team 2014).

RESULTS

1. *Nut removal from feeders*

A total of 193 nuts were removed from feeders, including nuts with and without transmitters (68 and 125, respectively; Table 1). In 98% of the cases, the disperser was video-recorded or directly observed from the hide, and it was a magpie in all cases. Magpies in no case consumed nuts in the feeders, and the number of nuts removed was one in all dispersal events. The number of magpies observed simultaneously in the feeders ranged from 1 to 5. Removed nuts were heavier (9.44±0.17 g) than non-removed nuts (8.67±0.32 g; F=4.47, d.f.=1, 118, p=0.037). Overall, magpies showed a high activity, and were able to remove all or most of the nuts within a few hours (Table 1).

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2. *Dispersal distance and caching characteristics*

Dispersal distance was measured for 66 radio-tagged nuts. Mean dispersal distance was 39.6±4.5 m, with a range of 4.1 to 158.5 m (Fig. 2a). Two nuts containing transmitters were not found despite thorough searching up to a distance of at least 300 m from the core site (not considered for analyses), and they were likely carried long-distances based

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on video-camera recordings of these nuts (see Supplementary Material 2 for a video of one of the cases). Nut weight did not affect dispersal distance (L.Ratio = 0.58, p = 0.45).

Of the dispersed nuts, 10.6% were not cached but rather consumed immediately after removal from the feeder (the nut was found open with the transmitter partially or entirely outside), whereas the remaining 89.4% (59 nuts) were cached. Of those, 55.9% were buried in the soil (at 1-3 cm depth in all cases), 42.4% cached under plant material, and only one (1.7%) was left on the ground surface (Chisq = 25.36, df = 2, $p < 0.001$). In all cases the nuts were cached individually. Two of the nuts cached under plant material were located on a roof, although still hidden below litter.

The point process models did not show a significant effect of habitat type on the density of dispersed nuts (Δ Dev = 0.94, df = 2, p = 0.63), but they showed a significant negative effect of distance from the feeder (Δ Dev = 114.62, df = 1, $p < 0.001$; Fig. 2b).

3. Nut recovery and seedling recruitment

By May 2016 we were able to determine the fate of 49 of the 59 cached radio-tracked nuts; the remaining 10 nuts were either lost (four sampling points within the core site could not be relocated) or dispersed outside the core site, where the ground was tilled before the time of sampling (thus provoking the loss of the sampling point). Of these 49 nuts, 67.3% were recovered, 20.4% did not germinate, 12.2% germinated (including emerged ones), and 4.1% produced an emerged seedling (Fig. 3).

Nut recovery was 73.5% in the cropland habitat, 57.1% in the broadleaf habitat, and 33.3% in the pine habitat, although there were no significant differences among habitat types (ChiSq = 3.79, d.f. = 2, p=0.15). Nut recovery tended to decrease with increasing distance to the feeder, with non-recovered nuts being at an average distance

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of 37.2±6.2 m from the feeder vs 27.5±4.3 m for the recovered nuts. Nonetheless, this trend was not significant (logistic regression, ChiSq = 1.61, d.f. = 1, p=0.20).

We could not unequivocally determine the animal that removed the cached nuts, but we often observed magpies recovering nuts in the study area, and found a large number of nuts consumed and opened in two valves as is characteristic in magpies (Homet-Guitérrez et al. 2015; author's personal observation). No other animal was directly observed removing the nuts dispersed by the magpies. Recovery activity spanned the entire study period, and we observed magpies consuming recovered nuts until early May 2016.

DISCUSSION

In this study, the magpie, an abundant corvid in Eurasian agroecosystems and open landscapes, proved to be an effective disperser of a large-seeded species, moving a large number of walnuts over dispersal distances that reached 158 m. Furthermore, a fraction of the nuts was not recovered after caching and resulted in effective early seedling recruitment. Magpies had previously been suggested as dispersers for nut-producing tree species such as oaks (Birkhead 1991) or almond trees (Homet-Gutiérrez et al. 2015), but this interaction had never been demonstrated or measured in the context of plant recruitment. Our study contributes to the understanding of the role of scatter-hoarding corvids in the regeneration of Eurasian forests, and provides for the first time precise information of the qualitative component of seed-dispersal effectiveness for the interaction between birds and nut-producing trees.

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Eliminado: insights into both the qualitative and quantitative aspects of nut-dispersal effectiveness

Most of the nuts that were offered in the feeders were quickly dispersed and cached, and only a small fraction (ca. 10%) was consumed just after removal. This behaviour is typical in scatter-hoarding animals, which display vigorous dispersal activity when the resource is abundant presumably in order to accumulate as much of it as possible during the short period of availability (Clarkson et al. 1986; Vander Wall 2001). After nut removal from feeders or trees, magpies also displayed a behaviour similar to that of other corvids (Bossema 1979; Cristol 2005). On arriving to a place to hide the nut, they wandered for a few seconds as if selecting the most preferable site, presumably checking for potential competitors that could steal the cached nut. In fact, in some cases they flew away with the nut and searched for another site. To cache the nut, they pushed it with the beak, sometimes hammering on it to bury it in the soil, and then they covered the site with soil or litter in such a way that the exact caching point became undetectable to the human eye (see Birkhead 1991 for a description of similar behaviour). In addition, the majority of the cached nuts were buried in the soil or hidden under plant material, both being microhabitats that may favour seed germination and seedling recruitment by reducing the risk of predation and desiccation (Bossema 1979; Vander Wall 1990, 2001; Gómez 2004). Furthermore, magpies preferred heavier nuts, therefore favouring a trait (large seed mass) that may enhances seedling establishment (Castro et al. 2006).

The observed dispersal distances lie within the lower range described for the rook, a corvid with a documented role in walnut dispersal (Lenda et al. 2012). Nonetheless, the body mass of the rook (around 500 g) is much larger than the mass of the magpie (around 200 g). In addition, two of the transmitters were lost, perhaps as a consequence of long-distance dispersal. In fact, we observed several events in which a magpie flying from a feeder with a nut was lost in the distance, likely far beyond the

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maximum dispersal distance recorded (Supplementary Material 2). In any case, 7.3% of the nuts were dispersed beyond 100 m, a distance and proportion great enough to support the contention that magpies can act as long-distance seed dispersers (Cain et al. 2000). Thus, magpies can play a relevant role in the expansion of nut-producing trees into new areas, a key step for the regeneration of the temperate forest (Pesendorfer et al. 2015).

The seed rain generated also supports the idea that magpies are effective dispersers in agroecosystems and agroforestry mosaics, since nuts were invariably cached alone, one by one, and were widely distributed throughout different habitats of the landscape. Some consequences of the spatial structuring of the seed rain for plant recruitment also seems plausible, as shown by the facts that caching density decreased with distance from the source (as it is ultimately expected in a cost-benefit trade-off; Clarkson et al. 1986), or that there were significant differences in caching characteristics. Nonetheless, our results are based in a single study site, which precludes generalization of patterns. Further studies including more study areas and larger sample size would be necessary to ascertain the relationship between habitat characteristics, dispersal distance, and its potential implication for effective long-distance dispersal. Radio-tracking, combined with the monitoring of the fate of seeds that replace the dispersed, radio-tagged seed, has proved to be an appropriate method to answer these questions.

Post-dispersal nut recovery and seed dispersal effectiveness

Radio-tracking also allowed us to obtain accurate estimates of nut recovery, which reached 67% ca. 8 months after dispersal. Magpies were the only animals observed recovering the nuts. Rodent pilfering of part of the nuts cannot be ruled out, but these

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Eliminado: despite a reduction in caching density with distance from the source (as it is ultimately expected in a cost-benefit trade-off; Clarkson et al. 1986).

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Eliminado: nuts cached at larger distances from the feeder tended to have a lower recovery rate, which might be due to a lower pilfering rate with increasing distance (Clarkson et al 1986) or a reduction in memorization capacity and recovering success due to an exponential increase in foraging area. In any case, this trend was not significant, due possibly to low sample size.

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animals appear to play a minor role in this system, as throughout the study period we found only three nuts with marks of chewing by rodents on the shell vs. a high number (not recorded) of nuts opened in two halves as magpies do. We cannot discount pilfering by other magpies, either, or the re-caching by the original magpie. In any case, the consequence for plant recruitment is that after nut dispersal by magpies, a large fraction (at least 32.6%) of the seeds remained on site until the following spring ready to start the next regeneration stage.

As a final result, 4% of the cached nuts rendered an emerged seedling, thus providing a net value of seed dispersal effectiveness up to the seedling stage that could generate recruitment. In addition, the number of emerged seedlings might have been higher if the final sampling had been conducted a few weeks later, as all the germinated (but not emerged) seedlings showed a healthy radicle protruding in the soil. In fact, since the arrival of magpies to the study area ca. 15 years ago, there is abundant walnut seedling emergence around the farms at distances of dozens to hundreds of meters from adult trees (authors' personal observation). Although this rarely translates into adult walnut recruitment due to yearly ploughing, it is very likely that walnut expansion would occur if ploughing were discontinued, as documented for example by Lenda et al. (2012) for walnut dispersed by rooks in abandoned farms in Poland.

In summary, this study demonstrates the relevance of magpies as scatter-hoarding dispersers of nut-producing trees, and for the first time provides an accurate estimate of seed-dispersal effectiveness for a bird-plant interaction that is crucial for the regeneration and expansion of temperate, large-seeded trees (Pesendorfer et al. 2016). Magpies are abundant in open landscapes such as agricultural land and successional shrublands, habitat types usually avoided by Eurasian jays for their nut-dispersal activity (Gómez 2003; Pons and Pausas 2007; Leverkus et al. 2016), and where jays are

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often rare or absent (Andrén 1990; Pons and Pausas 2008; Cramp and Perris 1994). This may increase the relevance of the magpie as a key species for the demography of nut-producing trees in anthropogenic landscapes where habitat fragmentation and reduced forest cover are common. In short, our results support the hypothesis that magpies act in the regeneration and expansion of the Eurasian temperate forest, thus increasing the number of corvid species with known key mutualistic roles for forest regeneration.

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Acknowledgements

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	Date	Feeder	#Nuts offered	#Nuts dispersed
Non radio-tagged nuts	05/09/15	1	20	18
	06/09/15	1	20	19
	08/09/15	1	20	20
	09/09/15	1	20	15
	11/09/15	1	20	14
	13/09/15	1	20	6
	18/09/15	1	20	15
	21/10/15	1	10	9
	21/10/15	2	10	4
	26/10/15	1	5	5
			Σ=165	**Σ=125**
Radio-tagged nuts	25/10/15	1	5	4
			5	5
	26/10/15	1	5	1
	27/10/15	1	5	4
	28/10/15	1	5	5
	29/10/15	1	5	2
			5	2
	1/11/15	1	5	4
	2/11/15	1	5	3
	3/11/15	1	5	4
	5/11/15	1	5	2
	7/11/15	2	5	3
	9/11/15	2	5	3
	10/11/15	2	3	1
	10/11/15	1	2	2
	15/11/15	1	3	1
	17/11/15	1	5	5
	25/11/15	1	5	3
	01/12/15	1	5	5
	04/12/15	1	5	2
	10/12/15	1	5	3
	12/12/15	1	4	4
			Σ=102	**Σ=68**

Table 1. Summary of the number of nuts offered and removed from feeders during the study period.

Figure captions

Figure 1. Ortho-rectified aerial photograph of the study area, taken with a drone. The area surrounded by a dashed red line is the core site. The dashed yellow line delimitates the “broadleaf” habitat, the green dashed line the “Pine” habitat, and the rest of the area within the core site corresponds to the “Cropland” habitat. Yellow dots indicate the position of cached nuts dispersed from feeder 1, and green dots that of cached nuts dispersed from feeder 2. The orange triangles show the position of the feeders. One nut with a radio-transmitter was dispersed towards the east outside the area of the picture and is not shown here. The image was take with a GoPro 4 Black edition camera attached to a drone (Phantom 2 UAV) during a photogrammetric flight at 50 m height on 23rd January 2016. The photos were processed with the Agisoft PhotoScan 1.2.0 software, which was also used for the final 10-cm pixel-resolution image.

Figure 2. Distribution of radio-tagged nuts dispersed by magpies. A) Histogram showing the frequency of dispersal distances. B) Kernel-smoothed density of cached nuts encountered in the core site for radio-labeled nuts dispersed from Feeder 1 (marked as a red dot). The map shows the intensities of the point pattern generated by caching points within the plot. Density of caching points reduces with increasing distances from the feeder. The space occupied by gravel roads and buildings has been eliminated for the analysis.

Figure 3. Path diagram indicating the stages in the qualitative component of seed-dispersal effectiveness (SDE) for the magpie-walnut interaction. The numbers in the boxes indicate the number of nuts available for the next demographic transition (green boxes) and those that were lost for recruitment (red boxes). The yellow boxes indicate

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the starting (total number of monitored nuts) and the ending points (number of emerged seedlings). Numbers in bracket show the transition probability for each stage from the number of dispersed seeds. The height of the seedlings was 12.0 and 14.8 cm, respectively. Data of the three habitat types have been pooled for simplicity. Not-recov. = Not recovered.

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Supplementary Material 1

Figure S1. Characteristics of the feeders used in this study. The first feeder (Feeder 1 in Figure 1 of the manuscript) consisted of an almost flat roof of a chicken house placed at 2.20 m above the ground plus a wooden slat that prevented the nuts from rolling down (upper picture). The second feeder (Feeder 2 in Figure 1 of the manuscript) was a 30 × 40 cm wooden cage with a metal bottom, held 1.7 m from the ground by a metal post and located below the canopy of a walnut tree (bottom picture). We knew from previous observations that magpies foraged or perched regularly in both locations. The two feeders were placed 100 m from each other.

Figure S2. Details of radio-transmitter insertion into a walnut. The nut was split open with a knife, a portion of the kernel similar in weight to the transmitter was removed, the transmitter was inserted into the nut with the antenna rolled, and finally the two halves of the shell were glued together with superglue (Loctite®).

Supplementary Material 2. Video recording with a sensitive-movement camera in Feeder 1 on 27 October 2015, showing three magpies at once, and two of them retrieving a nut each (containing transmitters in this case). The time that appears in the video is local time, one hour ahead of solar time (thus, it was 8 am solar time). It can be observed that the second magpie that removed a nut flew westwards beyond a group of trees, being lost at a distance of ca. 130 m from the feeder. This transmitter was not found despite a thorough search at a distance up to 300 m, and might represent an event of long-distance dispersal.

Figure 1

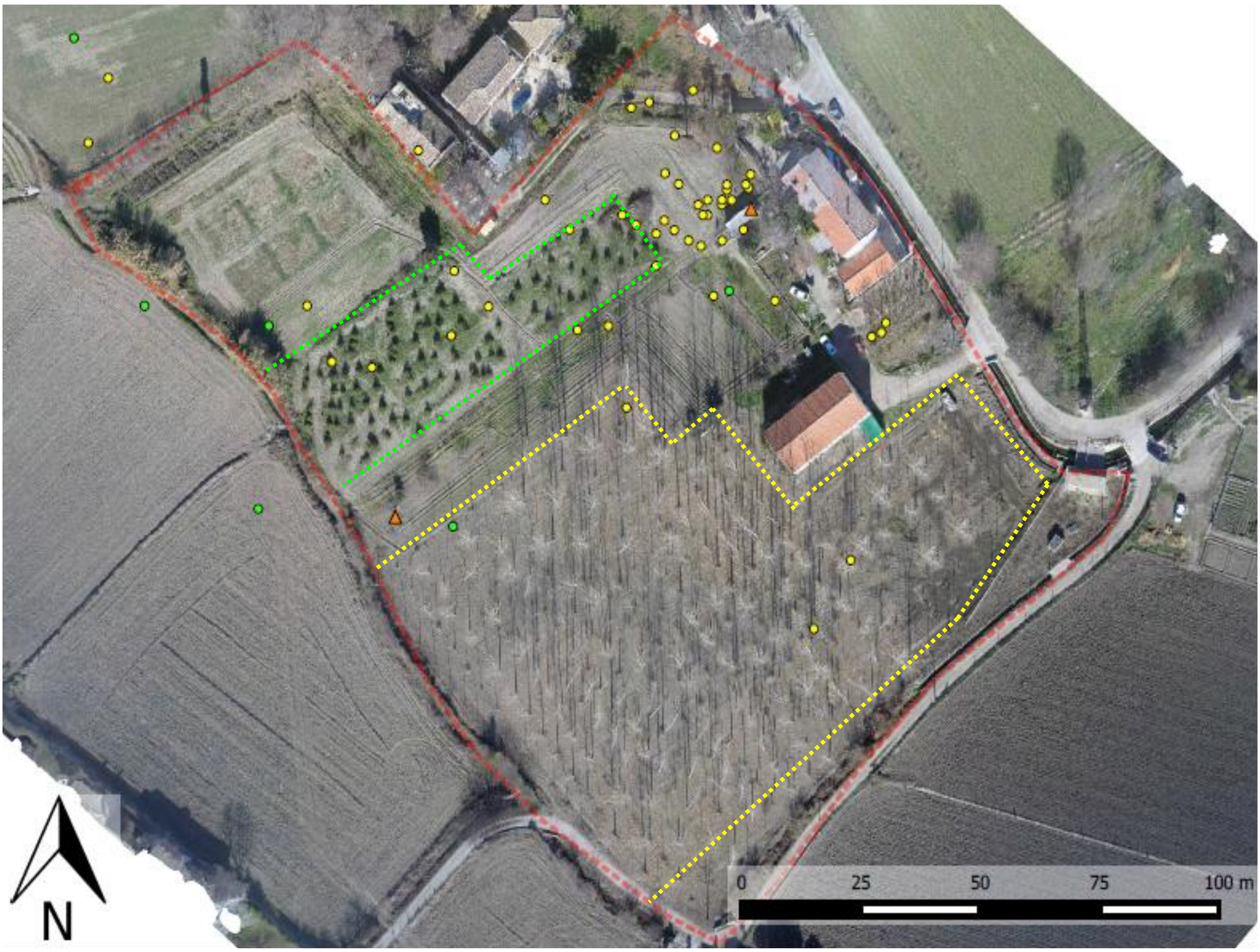


Figure 2

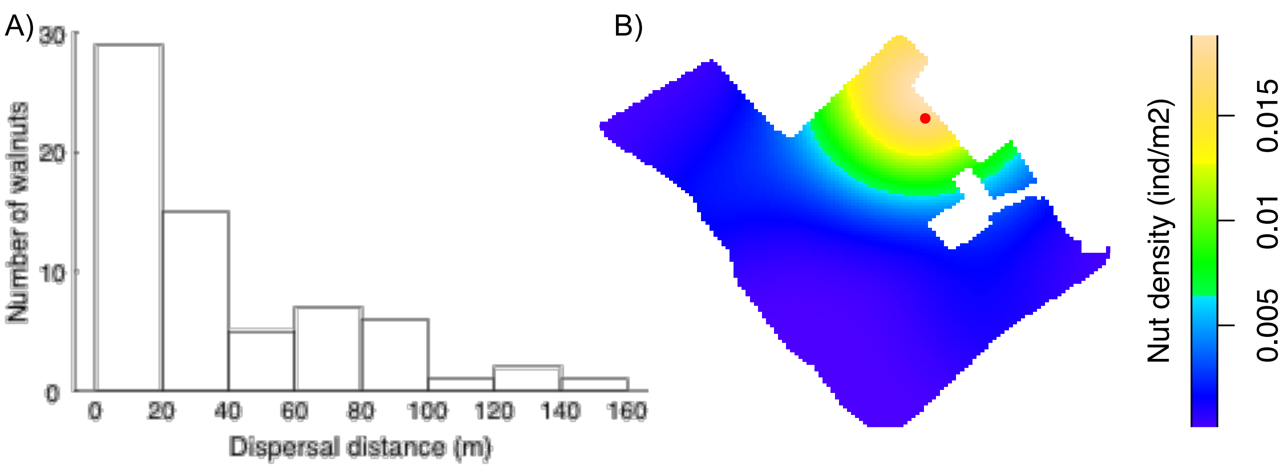
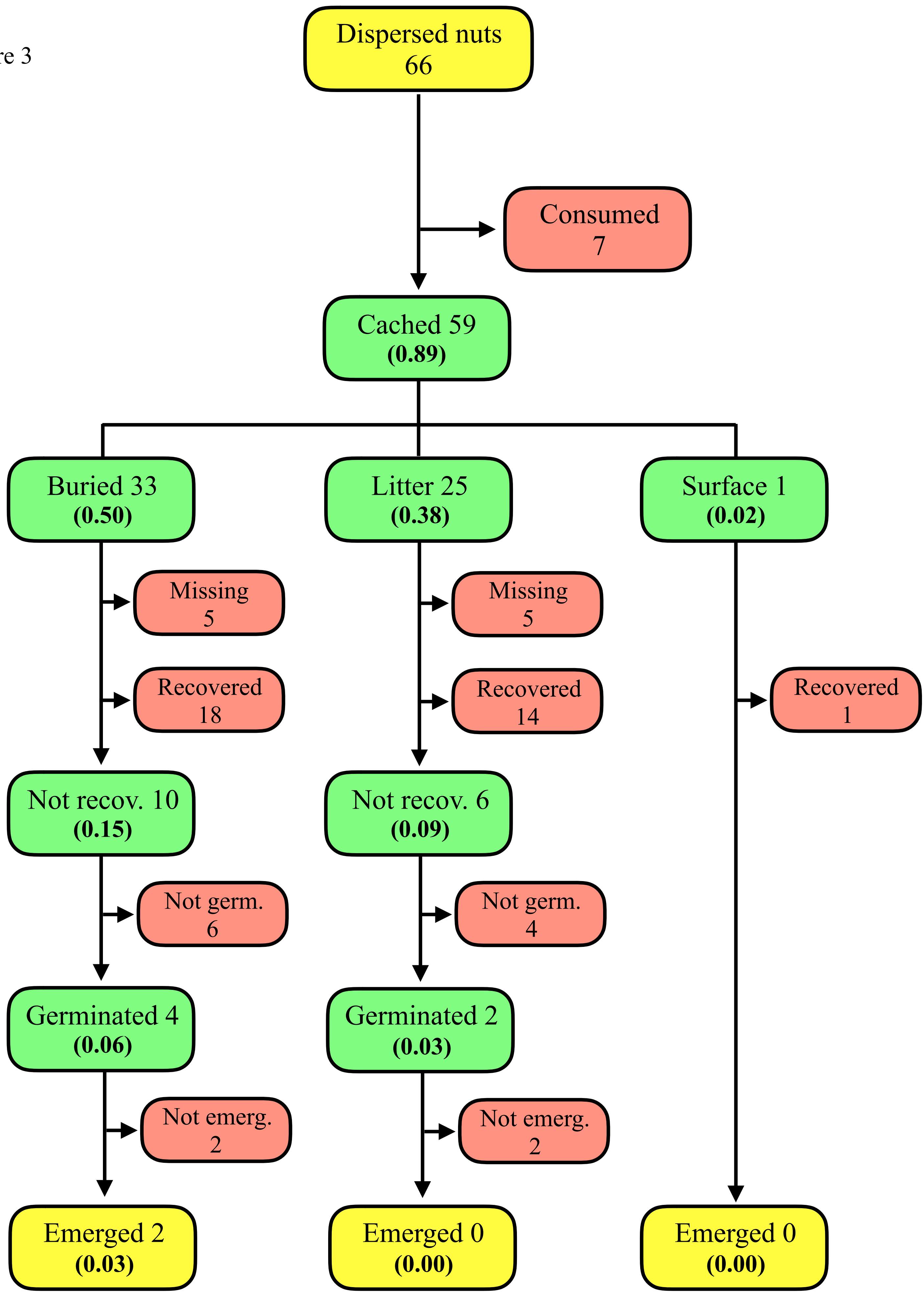

A)
Number of walnuts
30
20
10
0
0
20
40
60
80
100
120
140
160
Dispersal distance (m)
B)
Nut density (ind/m2)
0.015
0.01
0.005

Figure 3



Dispersed nuts
66
Consumed
7
Cached 59
(0.89)
Buried 33
(0.50)
Litter 25
(0.38)
Surface 1
(0.02)
Missing
5
Missing
5
Recovered
18
Recovered
14
Recovered
1
Not recov. 10
(0.15)
Not recov. 6
(0.09)
Not germ.
6
Not germ.
4
Germinated 4
(0.06)
Germinated 2
(0.03)
Not emerg.
2
Not emerg.
2
Emerged 2
(0.03)
Emerged 0
(0.00)
Emerged 0
(0.00)

Supplementary Material 1, Figure S1

Supplementary Material 1, Figure S2

Supplementary Material 2

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