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ORIGINAL ARTICLE

Elucidating the trophic role of *Tapinoma ibericum* (Hymenoptera: Formicidae) as a potential predator of olive pests

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Abstract

Ants play a key role in improving the structure and function of local communities. They interact with plants, herbivores, predators and parasitoids and are able to change their trophic role in space and time. These features, however, make it difficult to establish the net trophic role of ants in agroecosystems. Here we aim to determine the isotopic enrichment and tissue incorporation rates in ants of the *Tapinoma nigerrimum* complex, which are found abundantly in olive orchards (i.e. *T. ibericum*), to determine their trophic role through stable isotopes analysis. We compared the isotopic signature of ants feeding on (1) natural diets, (2) experimental diets and (3) contrasting diets of ants inhabiting natural habitats and olive orchards with different management systems. Then, we contrasted our isotopic results on natural diets with the prey of ant foragers in olive orchards. Our results showed that (1) ants from olive orchards had an isotopic signature different from that of a hyper-predator; (2) the isotopic signature did not vary significantly amongst different management practices and (3) the diet of ants in nature varies greatly on preys according to resource availability, which may be reflected in isotopic signatures. Therefore, *T. ibericum* is a species that can potentially contribute to control olive pests without posing a risk to other natural enemies because commonly it is not a hyper-predator. The information presented here could be used by farmers and technicians to enhance local biological control planning and/or strategies in olive orchards.

KEYWORDS
ants, biological control, diets, olive orchards, stable isotopes analysis

1 | INTRODUCTION

Ants are key to improve the structure and functioning of local communities in many terrestrial ecosystems (Hölldobler & Wilson, 1990; Ottonetti et al., 2008). The beneficial effects of ants in agriculture are known since ancient times (304 A.D., Huang & Yang, 1987), as ants consume large numbers of pest insects, disturb pests during feeding and oviposition and increase soil quality and nutrients (Choate & Drummond, 2011). However, some ant species are involved in different mutualistic interactions with other arthropods that eventually affect plant health (e.g. the effects caused to citrus trees by *Pheidole pallidula*, *Lasius grandis* or *Linepithema humile* which

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decreased generalist predators of pests and promoted higher levels of pest parasitoids, Calabuig et al., 2015). Thus, making it difficult to determine if ant's effect balance in crops is positive or negative (Rosumek et al., 2009). Ants can also interact with plants, herbivores, predators and parasitoids (Vandermeer et al., 2002), they are able to change their trophic role in space and time (Mooney & Tillberg, 2005), and most of them are omnivorous, feeding on a wide range of resources (Ottonetti et al., 2008). Therefore, the net trophic role of ants in agroecosystems is difficult to establish (Vandermeer et al., 2002; Ottonetti et al., 2008; Feldhaar et al., 2008).

In olive orchards, different ant species can be found in the trees and the soil, in some cases at high abundance. One of the most abundant species in these orchards in the Iberian Peninsula had been identified as *Tapinoma nigerrimum* (Nylander, 1856) (Campos et al., 2011; Ruano et al., 2004; Santos et al., 2007), which belongs to a homonymous ant species complex. The *T. nigerrimum* complex is widely distributed in the circum-Mediterranean region, being very abundant in Western and Central Europe and Northern Africa (Seifert et al., 2017), including agroecosystems from these regions (Álvarez, 2021; Mansour et al., 2012). Along their distribution range within the Iberian Peninsula, the *T. nigerrimum* complex shows omnivorous behaviour, feeding on honeydew secretions and animal remains (Cerdá et al., 1989). Ants of *T. nigerrimum* complex behave as a dominant ant species in olive orchards too (Morris, Symondson, et al., 1998; Pereira et al., 2004; Redolfi et al., 2003). While sometimes they can act as herbivores, they can also feed on olive pests, therefore being a natural enemy (controller of pest) or sometimes feed on other predators therefore being a hyper-predator (Morris, Campos, et al., 1998; Pereira et al., 2004). This is of great importance because setting up biological control of pests—the use of natural organisms to maintain lower levels of pest populations, and ecological management within crops is a main goal for the European Union (IOBC, 2012).

A viable option to discern the trophic role of *T. nigerrimum* complex in Iberian olive orchards is through Stable Isotopes Analysis (SIA), using the stable isotopes (SI) nitrogen ^{15}N and carbon ^{13}C. In general, these two SI ratios suffer an enrichment in the tissues of the consumers with respect to their diet, due to the elimination of the lighter carbon ^{12}C isotope by breathing and nitrogen ^{14}N isotope by urine excretion (but see Spence & Rosenheim, 2005). Based on the premise 'you are what you eat', the ratio of nitrogen ^{15}N is used to estimate the trophic position of a species, and carbon ^{13}C is used to estimates the carbon source in its diet (Post, 2002). This technique has the potential to track energy or mass flow through ecological communities and help to discern complex trophic interactions, such as omnivory (Post, 2002). Studying the natural abundance of SI allowed us to evaluate trophic relationships (Morente & Ruano, 2022), infer animal diet types (Santi-Júnior et al., 2018) and assess species interactions (Caut et al., 2009). However, to do so, it is important to know the isotopic enrichment rate, which may change in different species (Quinby et al., 2020; Spence & Rosenheim, 2005), as well as the time needed for isotopes to be incorporated in the tissues of our species of interest (Franssen et al., 2017).

In this study, we aim to determine the isotopic enrichment and tissue incorporation rates in ants of the *Tapinoma nigerrimum* complex and infer their trophic role through SIA. Firstly, we investigated the enrichment and tissue incorporation rates of SIs ^{15}N and ^{13}C in laboratory conditions analysing and comparing the SI ratios from samples under natural and experimental diets. Secondly, we investigated whether there were differences between samples under natural diets from a natural habitat and olive orchards with different agricultural managements in different locations. Finally, we performed a direct in-field foraging sampling survey in nests of *T.* nigerrimum complex that allowed us to identify an inventory of prey species that foragers were carrying, which helped to understand the results of our SIA. We hypothesize that the ^{15}N levels and ^{13}C levels in artificial diets will be like those from ants found in field populations because ants might feed on similar proportions of a myriad of functional foods and that ^{15}N levels and ^{13}C levels will be affected by management and habitat.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

The study was conducted in 2010 and 2011 in the province of Granada, in Southern Spain. Climatic conditions in the region were for the year 2010, 15°C mean annual temperature, 27.55°C–11.1°C mean maximum and minimum temperatures from April to July, and 565.12 mm mean annual precipitation; and for the year 2011, 16°C mean annual temperature, 29.52°C–13.47°C mean maximum and minimum temperatures from April to July, and 368.82 mm mean annual precipitation (a slightly hotter and dryer year than the former) (https://www.ugr.es/~velilla/meteo-albayzin/). With respect to pest species incidence, the highest occurrence of *Prays oleae* adults/trap/day occurred between June 14 and 21 in both years, and the percentage of adults/trap/day was higher in 2011 (49%) than in 2010 (34.7%). The percentage of flowers with *P. oleae* larvae was higher in 2010 (8.9%) than in 2011 (1.7%) (RAIF, 2010, 2011).

Firstly, we collected 12 nests inhabiting olive orchards (locality: Arenales; April 2010), taking around 1000 ants to be reared in the laboratory. All nests were queen-right nests with a big proportion of brood. Secondly, we collected samples (approximately 100 ants per nest) of 103 nests in 6 locations (Arenales: same nests as above, Colomera, Dehesa del Generalife, Deifontes, Pinos Puente and Sierra Nevada all in the Granada Province, Spain) and at different times, coincident with the laboratory took of samples during the diet experiment (Table S1). The nests were selected from two different types of habitats: natural shrubby habitats at the Sierra Nevada National Park and olive orchards with different agricultural managements (organic, conventional and integrated) (Table 1) (Figure 1).

Ants were collected with an electrical entomological aspirator (Entomopraxis D702) In April, June and July, reported to be the months with the highest arthropod abundance in olive orchards (Ruano et al., 2004; Santos et al., 2007) and coincident with the

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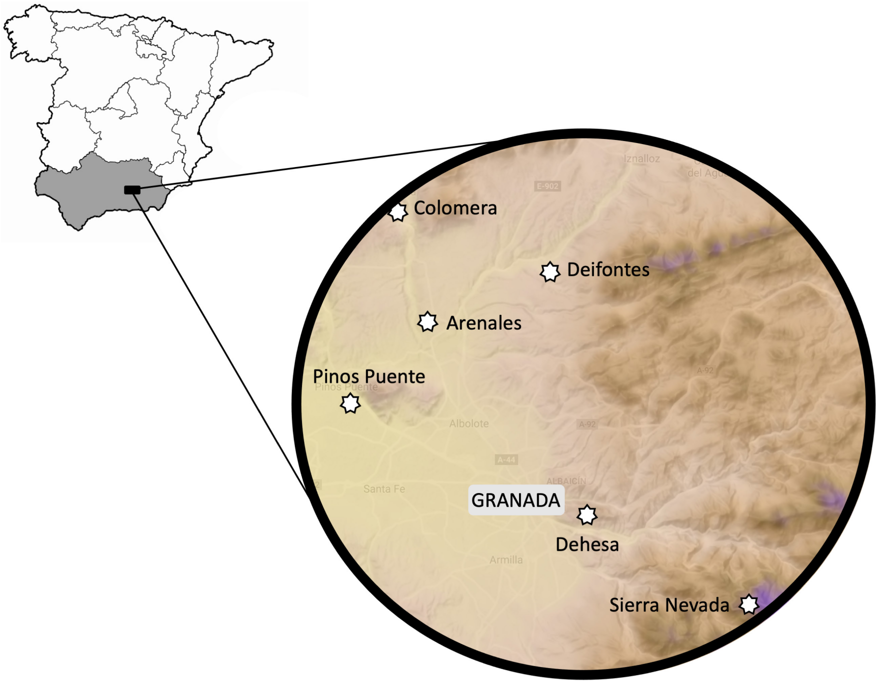
TABLE 1 Study areas and type of habitats in which *Tapinoma* nests were collected, in Andalusia, Spain.

Locality	Sampling points	Nests *n*	Type of habitat	Description of the habitat
Dehesa	2	38	Organic olive orchard	Shallow ploughed in April, natural vegetation patches and edges, rained fed
Deifontes	2	30	Organic olive orchard	Cover crop present, mowed April–July, drop irrigated
Arenales	2	18	Integrated olive orchard	Ploughed, no cover crop, sporadic flood irrigation
Pinos Puente	1	3	Integrated olive orchard	Not ploughed, herbicide application, cover crop stripes, rained fed
Colomera	2	8	Conventional olive orchard	Not ploughed, herbicide application, cover crop stripes, drop irrigation
Sierra Nevada	2	6	Native ecosystem	Shrubby natural habitat

FIGURE 1 Location of the study areas in southern Spain.



highest presence of *P. oleae*, one of the most important olive pests in the region. Ant nests were considered different when they were separated by at least 20 m, but their number varied per locality (Table 1). All nest samples collected in the field were stored alive in empty plastic vials (all individuals from the same nest in one vial) and transported to the Department of Zoology, University of Granada. The 103 samples of 100 individuals were frozen and maintained at −20°C.

We were aware that a previous study has determined that the *T. nigerrimum* complex actually includes four species (Seifert et al., 2017) that can be identified only by high-resolution methods of Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT) in which Nest Centroid Clustering plays a central role (Seifert et al., 2014). Thus, the ants classified as *T. nigerrimum* in Iberian olive orchards could belong to different species within the *T. nigerrimum* complex. To identify these ants at species level, specimens from olive orchards (all locations) and natural habitats were sent to Senckenberg Museum of Natural History (Görlitz, Germany) to be identified by NUMOBAT technique (for more detail on this technique, see Seifert et al., 2017).

2.2 | Experimental protocol and rearing

In our diet experiment, we reared ants, feeding them with one of the fourth next diets: a mixture of honey and yeast (also used as a basal diet until all the diets were available), adults/nymphs of the herbivore of cover crop plants *Aphis craccivora* (Hemiptera: Aphididae), larvae of the (1) olive pest species *P. oleae* (Lepidoptera: Praydidae) and (2) the carnivore insect *Chrysoperla carnea s.l.* (Neuroptera: Chrysopidae) which predates on *P. oleae* in olive orchards (Corrales & Campos, 2004). Diets were obtained in different manners. Especially, larvae of *C. carnea* were bought from BIOBEST (Sistemas Biólogicos S.L.). *Prays oleae* larvae and *A. craccivora* (aphids) were collected directly from olive orchards, the tree canopy and the cover crop of the olive farms, respectively. The phase of comparison among diets began when *P. oleae* larvae were available and abundant in the field (17 June), easy to collect and provide to the ant nests. All insects were maintained in the laboratory until ad libitum supply to their corresponding treatment nests.

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TABLE 2 Nests used in the diet experiment. It shows the different diet treatments used and the number of nests used per treatment per time. Note that two nests were missed throughout the experiment.

	Nests *n*			
Type of diet	**t1**	**t2**	**t3**	**t4**
Honey–yeast	12	2	2	2
A. craccivora	–	3	3	3
C. carnea	–	3	3	3
P. oleae	–	3	3	2

Each nest of the 12 nests transported to the laboratory (olive orchards, locality: Arenales, April; see above) was placed in an individual plastic container (see Figure S1) and maintained in a climatic chamber at 24°C (±2°C) mean temperature, 60% (±5%) relative humidity and light:dark period of 12:12 h. All nests were fed with the mixture of honey and yeast ad libitum over 2 months since the beginning of the experiment (t0) to standardize the baseline isotopic signature of all the nests and maintain them until *P. oleae* larvae were available in the field (June). Then, the nests were separated into four randomly distributed groups, and each group was fed ad libitum with one of the four assigned diets (honey and yeast, aphids, *C. carnea* or *P. oleae*). Around 20 workers were collected from every experimental nest at five different times: after field collection (t0, 23 April), and then 55 days (t1, 17 June), 73 days (t2, 5 July), 83 days (t3, 15 July) and 93 days (t4, 26 July) after the beginning of the experiment ($n = 100$ individuals per nest) (Table 2). Samples of workers were stored in empty plastic vials (all individuals from the same nest in one vial) and frozen and maintained at −20°C until the specimens were prepared for analysis (we used only workers because brood was not available in the time we feed the experimental diets).

2.3 | Stable isotopes analysis

The individuals of each sample were dried by lyophilization and pulverized manually in a mortar. Then, 0.4 mg of the resulting powder was encapsulated in tin tubes Eurovector 5 × 9 mm. The encapsulated samples were introduced in a gas chromatographer EUROVECTOR EURO EA 3000 which volatilizes the sample and the gases were passed throughout a column into a continuous flux mass spectrometer IRMS ISOPRIME Elemental Analyser. Analyses were conducted in the Laboratory of stable isotopes (LIE, Scientific Instrumentation Centre, University of Granada). The isotope composition of N and C was expressed using the δ notation relative to international standards (atmospheric N2 and caseine, respectively) that were reported per mil (‰) on the relative δ-scale and in reference to them. Standards were analysed every 10 samples to ensure the measurements were acceptable in terms of repeatability and to correct any possible deviations in measurements. Variability was accepted as valid under 0.2‰ values.

2.4 | Foraging sampling surveys

Forty nests from four different olive orchards (Deifontes) were monitored in spring–summer 2011. We recorded (1) data of trail activity (entering workers), that is the number of workers entering the nest with prey, therefore, a 5 min/trail/day survey. After testing that non-charged-with-prey ants regurgitated honeydew, ants entering the nest without prey were considered honeydew transporters. We also recorded (2) the abundance and nature of the prey carried by ant workers in a trail, therefore a 60 min/trail/day sample.

2.5 | Statistical analyses

The isotope composition of ^{15}N and ^{13}C was expressed using the δ notation relative to international standards. All statistical analyses were computed with the software R v4.0.5 (R Developmental Core Team, 2021). We analysed the trophic status of the *T. nigerrimum* complex by comparing, firstly, the overall signature of the experimental diets, as well as the natural diet signature, in all the Arenales nests ($n = 12$) by fitting two linear mixed models (LMMs) per SI (δ^{13}C or δ^{15}N), using SI signature as the dependent variable and diet as a fixed factor (i.e. samples of worker individuals that could carry out prey or honeydew within natural nests and experimental nests). To control for pseudo-replication and correct for non-balanced data, we included time as a random effect. LMMs fit was performed with the R package *nlme*. Secondly, we fitted another two LMMs per SI to compare the signature of the experimental diets across experimental times (as an ANCOVA), for which we used SI signature (δ^{13}C or δ^{15}N) as the dependent variable and diet, time and the interaction diet–time as fixed factors. To control for pseudo-replication, we included nest ID as a random effect. As the interaction diet–time was not significant (see Results), further differences between groups were inspected without it.

To determine whether the area and/or the agricultural management influence ant diet, we focused on the natural signature of ants, and thus, all isotopic samples from all nests collected in the field were used ($n = 103$, nests from the six localities) (Table 1). We fitted several linear models (LMs) using stable isotope signature (δ^{13}C or δ^{15}N) as the dependent variable and area or management as factors (separately). For each model, we tested for significant differences using the *F* test with the ANOVA function. Further differences between the groups in each model were tested using the Tukey (contrasts) post-hoc test with the *multcomp* package.

3 | RESULTS

Overall, a total of 103 samples of ant nests were collected from the field and from the 12 experimental nests, two were lost during the experiment (possibly due to queen death, hence, no broad production to maintain the colony). Unexpectedly, two species of *Tapinoma nigerrimum* complex were identified by NUMOBAT

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technique: *Tapinoma ibericum* Santschi, 1925, and *Tapinoma nigerrimum* (Nylander, 1856). However, *T. ibericum* was only found in the olive orchards samples, and conversely *T. nigerrimum* in the shrubby natural habitats of Sierra Nevada national park (Table 1).

3.1 | Differences in diet

LMM analysis showed significant differences between experimental diets for the δ^{15}N in *T. ibericum* ($F_{4,53}=6.461$, $p=0.001$). Ants that consumed strictly the predator *C. carnea* larvae had a significantly higher concentration of δ^{15}N (7.3‰ ± 0.4; Figure 2a) compared with the rest of the treatments (Tukey post-hoc test, $p<0.01$). The rest of treatments have a concentration of δ^{15}N not significantly different to the natural signature of *T. ibericum* (rhombus Figure 2a). LMM analysis also showed significative differences in the δ^{13}C signature ($F_{4,53}=6.33$, $p=0.001$). The signature of the natural diet had different concentration of δ^{13}C than the signature of ants fed with the herbivore *A. craccivora* (Tukey post-hoc test, $p=0.032$), the predator *C. carnea* ($p=0.001$), and the basal food mixture of honey and yeast ($p=0.001$) (Figure 2a). However, only the δ^{13}C signature of the natural diet was similar to the ants fed with the olive pest *P. oleae* (herbivore) (Figure 2a).

The detailed temporal analysis of isotopic experimental changes shows how after 39 days of eating their experimental diet (days between t1 and t4), ant isotopic signature changed for both isotopes. The temporal δ^{15}N enrichment ratio was of 2.1‰ between ants following the herbivore-based diets (5.2 ‰ ± 0.8) and the predator-based diets (7.3‰ ± 0.4) (see Table S2; Figure 3). Nevertheless, δ^{15}N concentration should not reach its maximum since the δ^{15}N concentration was higher in *C. carnea*-based diet (δ^{15}N = 7.85‰). Regarding δ^{13}C, its concentration decreased at a mean rate of −0.2 ‰ between ants following the herbivore-based diet (−24.6‰ ± 0.4) and the predator-based diet (−24.4‰ ± 0.6, see Table S2; Figure 4).

Figures 3 and 4 represent the SI signature pattern followed by ants consuming herbivores (predator ants) compared to that from ants consuming a carnivore (hyper-predator ants). The LMM analysis comparing the signatures of different diets across experimental times showed that for the δ^{15}N, only the effect of the diet was significant ($F_{3'21}=4.50$, $p=0.013$) but not the diet–time interaction ($F_{4'18}=0.79$, $p=0.545$). Only ants fed with *C. carnea* had different δ^{15}N compared with ants fed with *A. craccivora* (Tukey post-hoc test, $p=0.001$) and *P. oleae* ($p=0.010$). Concerning the δ^{13}C signature, neither the effect of time and diet nor their interaction were statistically significant.

Accordingly, throughout time, ants fed with the predator *C. carnea* larvae (i.e. the hyper-predator ants) increased their δ^{15}N signature

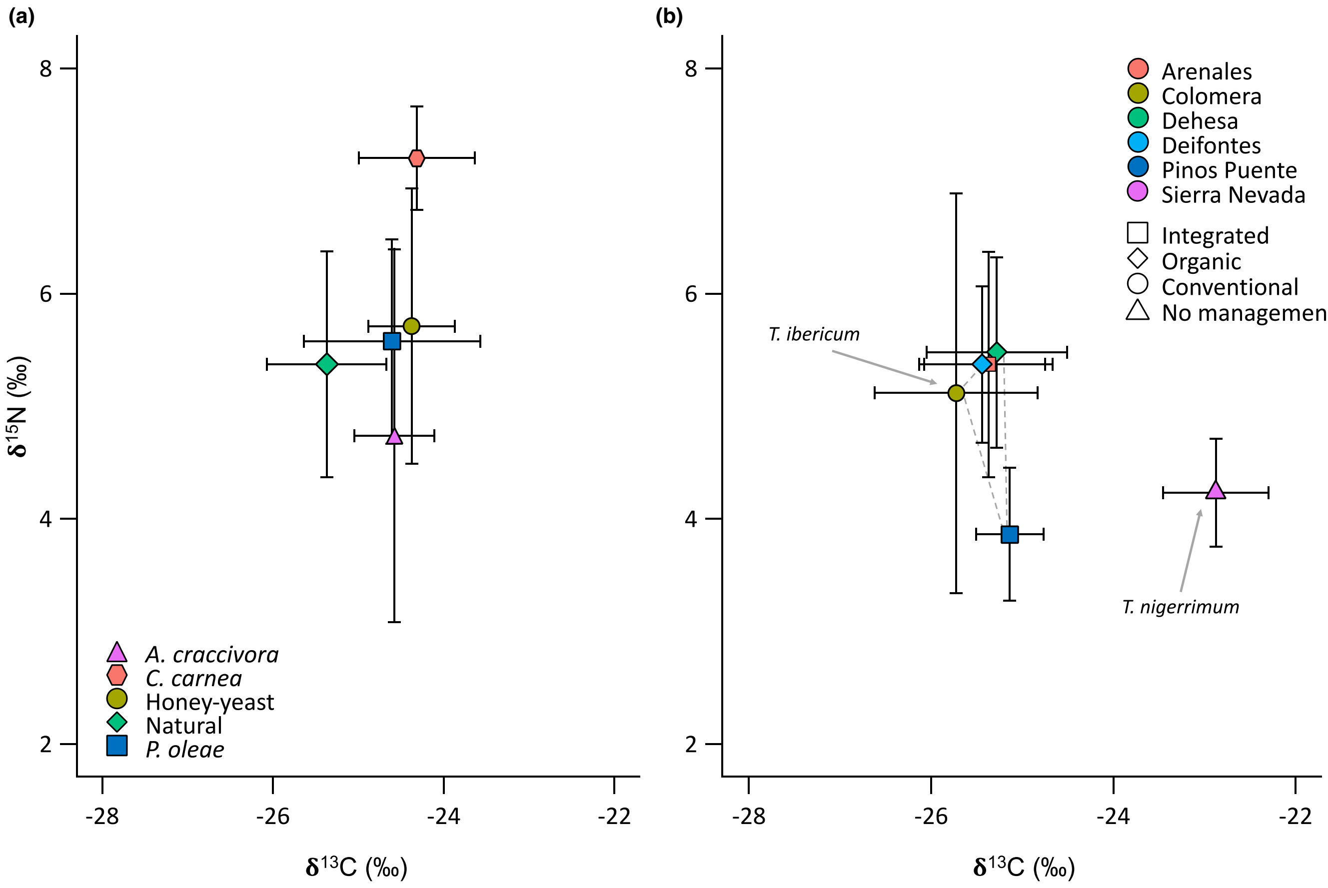

FIGURE 2 Overall isotopic signatures (mean δ^{13}C and δ^{15}N ± Standard deviation) of (a) experimental diets of *Tapinoma ibericum* and (b) natural diets of *T. ibericum* and *T. nigerrimum* from the different localities and types of orchards.

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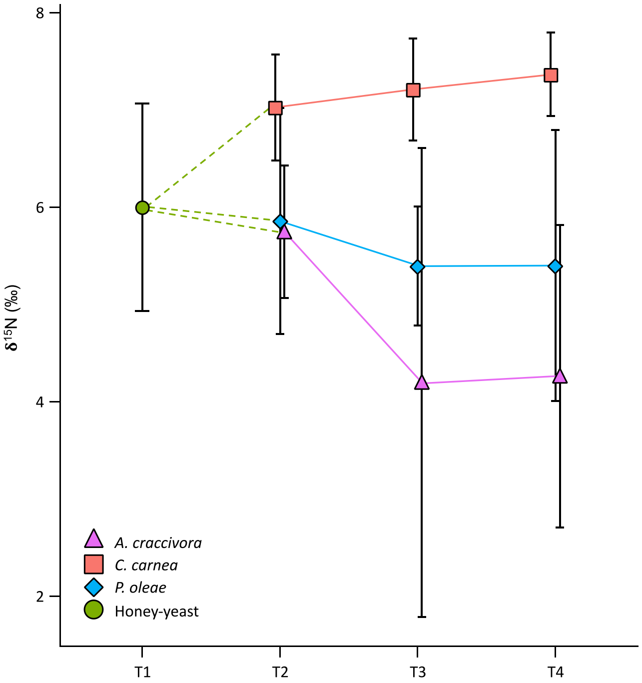

FIGURE 3 Isotopic signature and enrichment (mean $\delta^{15}N \pm$ Standard deviation) of experimental diets of *Tapinoma ibericum*, throughout experimental times.

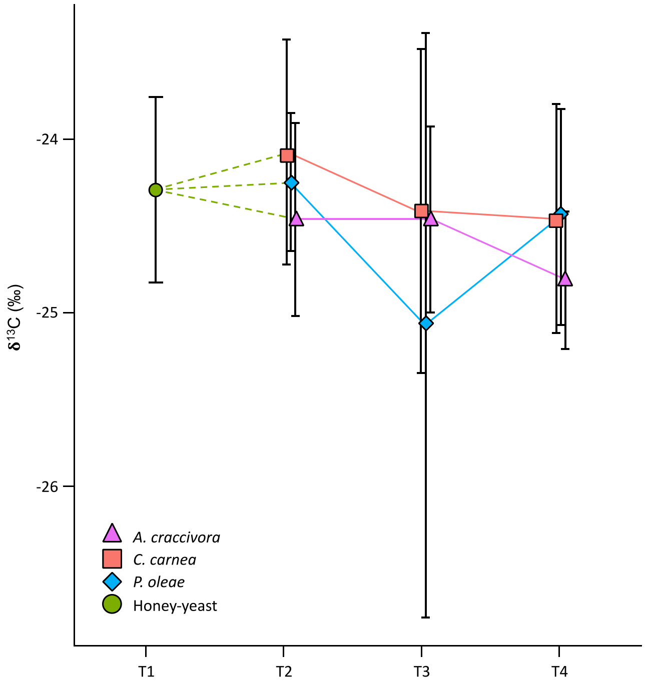

FIGURE 4 Isotopic signature and enrichment (mean $\delta^{13}C \pm$ Standard deviation) of experimental diets of *Tapinoma ibericum*, throughout experimental times.

in a continuous rate. Conversely, ants fed with herbivores decreased their $\delta^{15}N$ signature. The difference in the $\delta^{15}N$ signature between hyper predator ants feeding on *C. carnea* diet started to differentiate from that of predator ants feeding on herbivores (*A. craccivora* plus *P. oleae* diets) 1 week after switching from basal to experimental diets, at time point t2. At t3, *A. craccivora* and *P. oleae* diets also showed differences for $\delta^{15}N$, which remained in the time point t4. These results suggest that *T. ibericum* needs at least 20 days to integrate to its tissue the $\delta^{15}N$ of the diet that is consuming to be detectable when following any diet. On the other hand, although there are no significative differences among treatments through time for $\delta^{13}C$, there is one big decrease in *P. oleae* diet at t3. Ants at t2 started to feed on the new food after honey–yeast, so there is no difference regarding honey–yeast with those treatments. This suggest that there is some possible interference related to tissue assimilation regarding *P. oleae*, but also it is possible that some ants in that samples were not feeding on *P. oleae* nor the control diet at the time when the sample was taken, therefore that large departure between error bars and the lower mean.

3.2 | Differences between areas and managements

Study area had an effect on ant isotopic signature (natural signature). The $\delta^{15}N$ natural signature differed significantly between study areas ($F_{5,97} = 3.521$, $p = 0.005$), however, only Dehesa del Generalife had a significantly higher signature than Pinos Puente (Tukey post-hoc test, $p = 0.039$) and Sierra Nevada ($p = 0.025$). However, contrary to the results showed by study areas, the crop management did not show any significant differences in ant isotopic signatures (Figure 2b). Also, *T. nigerrimum*, found in shrubby habitats at Sierra Nevada, showed a significantly higher concentration of $\delta^{13}C$ compared with the rest of the ants collected in other areas ($F_{5,97} = 13.804$, $p = 0.001$; Tukey post-hoc test, $p < 0.001$). Based on these differences in isotopic signatures found between both species, this suggests that the trophic profile of *T. ibericum* ants inhabiting different olive orchards is similar independently of the crop management practices applied to the olive orchard, and that *T. nigerrimum* had a different type of diet than *T. ibericum* (Figure 2b), that is a different isotopic baseline might contribute to this difference.

3.3 | Foraging surveys

A total of 369 preys were recorded in all the 40 ant-trails (mean ± SD = 15.98 ± 10.06) (i.e. 60 min/trail/day surveys). Among the collected preys, we found 14 groups of arthropods, consisting in 34% were herbivores, 3.29% olive pests and 1.41% natural enemies. We also found miscellaneous remains (unidentifiable), larvae and other animal remains (Table 3). The most abundant group of preys were dead ants (23%), aphids (21.13%), miscellaneous remains (21.13%) and animal remains (19.72%). According to the data of ant activity in trails (i.e. 5 min/trail/day) the 4% of all ants recorded in this type of survey ($n = 5443$) carried a solid prey.

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TABLE 3 Abundance (relative abundance: RA) and variety of prey in ant-trails of *Tapinoma ibericum*, based on 60 min/trail/day surveys ($n = 40$ trails).

Order	Family/species	RA (%)
Araneae		0.63
Hemiptera	Aphididae	21.13
	Cicadomorpha	2.82
	Euphyllura olivina	3.13
	Heteroptera	1.10
Collembola		3.60
Coleoptera		0.78
Diptera		0.63
Embioptera		0.31
Hymenoptera	Formicidae	23.00
	Others	0.78
Lepidoptera		0.16
Neuroptera		0.47
Psocoptera		0.16
	Larvae (miscellaneous)	0.47
	Remains (animals)	19.72
	Remains (miscellaneous)	21.13

4 | DISCUSSION

SIA showed that *Tapinoma ibericum* had an (overall) isotopic signature compatible with a frequent consumer of herbivores, which included other *Tapinoma* ants and *Prays oleae*. Indeed, the isotopic signature of *T. nigerrimum* is very different from that of *T. ibericum* probably due to a different diet or an important baseline difference in both habitats. Both species are omnivores (Seifert et al., 2017) but based on our analysis, the former resembles more of a herbivore ant that can consume occasionally other herbivores (such as aphids). It is known that the ants of the *T. nigerrimum* complex act as predators (Cerdá et al., 1989; Morris, Campos, et al., 1998; Morris, Symondson, et al., 1998; Seifert et al., 2017), so this feature could be boosted by the type of habitats they inhabit and the fluctuant availability of different sources. However, *T. ibericum* appears to match better with a $\delta^{15}N$ profile of a herbivore predator and a natural enemy, also their $\delta^{13}C$ natural profile is compatible with the profile of ants submitted to an experimental diet based on *P. oleae* larvae. Moreover, *T. ibericum* did not match the profile of a hyper-predator (see Figures 3 and 4). With respect to the enrichment ratio during the diet experiment, $\delta^{13}C$ increased −0.2% and $\delta^{15}N$ 2.1% between herbivore and predator consumers in 39 days, but they did not surpass the signature of the different diets. Another useful information from our results for forthcoming SIA studies on ants is that changes in diet begin to be detectable after 20 days of feeding in a particular diet (see results).

Foraging surveys confirmed the varied diet of *T. ibericum* in olive orchards and its importance in the control of herbivores, including olive pests, although the year 2011 had a lower level of anthophagous *P. oleae* larvae available for *T. ibericum* (RAIF, 2011). An interesting aspect revealed by our study is the common consumption of corpses of other ants (even individuals of the same species) by *T. ibericum* in the field. This fact was impossible to control during the diet experiment and it could be responsible for a part of the variability found in SIA results for each diet. Interestingly, this variability was lower for ants fed with insects obtained from bio-factories where insects are reared with controlled diets (e.g. *C. carnea s.l.*) than for ants fed with natural insects collected from the field (e.g. *A. craccivora* and *P. oleae*).

Several studies pointed out that the olive moth *P. oleae* was consumed by ants (Álvarez et al., 2021), especially *T. nigerrimum* (Morris et al., 1999, 2002). This could be because *P. oleae* has its highest abundances between May and July (Villa et al., 2016). It is possible that before such a period of time *T. ibericum* might be feeding on the honeydew of herbaceous plants or aphids, and when the abundance of pest increases, they turn to feed on it (Morris et al., 1999, 2002). Hence, *T. ibericum* has got to invade the olive trees to feed on *P. oleae* (Álvarez, Morente, Oi, et al., 2019). In relation to this, Álvarez, Morente, Oi, et al. (2019) showed recently that ants living next to and within organic olive orchards tend to move from the natural adjacent vegetation to the olive trees mainly when the ground cover started to wither, which corresponds with the time that *P. oleae* lays the eggs on young olive fruits. Furthermore, the abundance and trophic interactions of *Tapinoma* ants within the canopy of olive trees can be boosted by mature ground covers and less pesticide use (Álvarez et al., 2021; Álvarez, Morente, Campos, & Ruano, 2019; Morente et al., 2018).

On the other hand, our results showed that *T. nigerrimum* tends to inhabit more natural ecosystems than *T. ibericum*, which supports the previous findings by Seifert et al. (2017). For example, our results suggest that in the region of the study *T. ibericum* is the species that inhabits olive orchards, which feeds on the same type of food no matter the type of agricultural management applied in the different olive orchards. This is of great importance because a predator that is not affected by management could be used to enhance local biological control planning and strategies. Nonetheless, our data do not show the actual isotopic signature in natural habitats of *T. ibericum*, an interesting issue to include in future studies.

Several studies have shown *T. nigerrimum* complex as the most abundant ant within olive orchards, sometimes representing more than 50% of the relative abundance among omnivores (Campos et al., 2011; Morris et al., 1999, 2002; Morris & Campos, 1999; Morris, Campos, et al., 1998; Morris, Symondson, et al., 1998; Pereira et al., 2004; Redolfi et al., 1999; Rodríguez et al., 2005; Santos et al., 2007), which makes it one of the strongest candidates for potential control *P. oleae*. Our analyses showed that in the field *T. ibericum* feed on *P. oleae* rather than *C. carnea*, and thus, this clarifies its role as a natural enemy. Nevertheless, the high variability showed by isotopic signatures is pointing to a varied omnivorous diet in nature. Though, it is important to point out that there could be antagonistic interactions with other predators of *P. oleae* such interactions can be appeased by less insecticide use (Morente et al., 2018) and modulated by ground covers (Álvarez et al., 2021;

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Álvarez, Morente, Campos, & Ruano, 2019; Álvarez, Morente, Oi, et al., 2019). Thus, as suggested by Mansour et al. (2017), even when there could be negative effects, ants should not be excluded from agroecosystems because the exclusion of a predator may alter the nature and intensity of predatory, competitive and mutualistic interactions among natural enemies (Pinol et al., 2012).

Overall, here we show a reliable framework of experimentation with SI on ants to disentangle trophic status and diets. Our results support the previous assumptions that the referred *T. nigerrimum* complex is beneficial for olive orchards in the south centre of the Iberian Peninsula. Of the two species identified here, *T. ibericum* is possibly the species that can potentially contribute to control *P. oleae* without being a hyper-predator in all types of olive orchards. However, our data only shows the trophic status of these ants in the time *P. oleae* is abundant as larvae in flowers and later as eggs in olive fruits, though, the same protocols followed here must be applied throughout all the year and through gradients of perturbation within habitats using adults and brood. Also, trophic interactions and trophic networks based on stable isotope analysis, and direct observations between ants and pests in olive orchards, should be investigated more thoroughly to clarify how *Tapinoma* ants may suppress olive pests.

AUTHOR CONTRIBUTIONS

Hugo Alejandro Álvarez: Formal analysis; validation; visualization; writing – original draft; writing – review and editing. **Antonio García-García:** Methodology. **Pedro Sandoval:** Methodology. **Rubén Martín-Blázquez:** Methodology; writing – review and editing. **Bernhard Seifert:** Methodology. **Alberto Tinaut:** Conceptualization; methodology; writing – review and editing. **Francisca Ruano:** Conceptualization; funding acquisition; methodology; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. There are no ethical concerns regarding the organisms used in this research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at Figshare, doi: 10.6084/m9.figshare.22100495.

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