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ASSESSING ANT DIVERSITY IN AGROECOSYSTEMS: THE CASE OF ITALIAN VINEYARDS OF THE ADIGE VALLEY

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Giannetti D., Schifani E., Castracani C., Ghizzoni M., Delaiti M., Penner F., Spotti F.A., Mori A., Ioriatti C., Grasso D.A. - Assessing ant diversity in agroecosystems: the case of Italian vineyards of the Adige Valley

Agroecosystems have gained a dominant position on worldwide land-usage, and therefore preserving their biodiversity is crucial for environmental sustainability. Ants are one of the most widespread groups of terrestrial arthropods, and, thanks to their significant diversification, they are considered as a good proxy group for biodiversity monitoring, also in agroecosystems. Vineyards are economically valuable cultures widespread worldwide, and hosting many ant species, that provide meaningful ecosystem services and disservices. Despite the important role that ants play in these agroecosystems, ant biodiversity in vineyards is still poorly studied, especially in Italy. In this context, we present a first detailed quantitative and qualitative assessment of the ant fauna of Italian vineyards from the Adige Valley based on pitfall traps data, and discuss the results in comparison with the few other similar assessments from Europe and other continents. We document an assemblage of 22 species (7-16 per orchard), mostly dominated by three disturbance-tolerant species (including an introduced species). Vineyards' ant faunas appear to be rather heterogeneous worldwide, mainly following local ecological and biogeographical constraints, and the role that most ant species play in these agroecosystems is presently unknown.

KEY WORDS: vines; biodiversity monitoring; myrmecofauna; Prealps.

INTRODUCTION

Since agriculture has become a dominant category of land usage worldwide, crop management practices have become a decisive factor to preserve the environment (TILMAN *et al.*, 2001; GREEN *et al*., 2005; TSCHARNTKE *et al.*, 2005; FIRBANK *et al.*, 2008). Overlooked for decades, insect and arthropod decline and its severe potential outcomes on ecosystems functioning recently attained much attention showing the need for a deeper commitment in the development of effective monitoring systems in contexts with different anthropic impacts (BURGIO & SOMMAGGIO, 2007; CAMPANARO *et al.*, 2011; BURGIO *et al.*, 2015; DIRZO *et al.*, 2014; HALLMANN *et al.*, 2017; LEATHER, 2017; PIZZOLOTTO *et al.*, 2018; HOMBURG *et al.*, 2019). While agricultural transformations may play a key in this process, diversity and distribution of the arthropodofauna in cultivated areas is still insufficiently documented.

Wine grapes (*Vitis vinifera* L.) are a widespread cultivated species of important economic value, whose cultivated surface is likely to increase in the future due to climate change (HANNAH *et al*., 2013; MORIONDO *et al.*, 2013). European vineyards alone cover 3.2 million ha representing 45% of the world's total areas under vines and 1.8% of the total utilized agricultural area. Over 20% of them is located in Italy (688,000 ha), representing about 5% of the total utilized agricultural surface (SAU) of the country (EUROSTAT, 2017; ISTAT, 2019). Under conventional management practices, establishment of viticulture is often associated with notable negative impacts on soil and local biodiversity, and thus may represent a serious conservation threat in certain contexts (ALTIERI & NICHOLLS, 2002; FAIRBANKS *et al.*, 2004; HILTY & MERENLENDER, 2004; COULOUMA *et al.*, 2006; HILTY *et al.*, 2006; HILDENBRANDT *et al.*, 2008; COLL *et al.*, 2011; LAWRENCE *et al.*, 2011; ROSADO *et al*., 2013). However, implementing correct agro-ecological practices can be an effective way to address some of these issues (VIERS *et al.*, 2012): for example, organic viticulture may allow richer communities of organisms to thrive, both within the vineyards themselves and in neighboring forested areas (e.g. GAIGHER & SAMWAYS, 2010; COLL *et al.*, 2012; KEHINDE & SAMWAYS, 2014; CAPRIO *et al*., 2015; MASONI *et al.*, 2017; DAANE *et al.*, 2018).

Due to their high diversity and strong ecological impacts, ants are considered an important group for biodiversity monitoring in both natural and anthropic impacted ecosystems, including agroecosystems (e.g. PECK *et al.*, 1998; DE BRUYN *et al.*, 1999; AGOSTI *et al.*, 2000; LACH *et al.*, 2010; GIBB *et al.*, 2017), where they provide impactful services and disservices. For example, they may control other arthropods, fungi or even weeds (e.g. RISCH & CARROL, 1982; BARAIBAR *et al.*, 2011; OFFENBERG & DAMGAARD, 2019) and favor foliar uptake of nitrogen (e.g. PINKALSKI *et al.*, 2018), but may also favor mutualistic pest species (e.g. PEKAS *et al.*, 2010; CALABUIG *et al.*, 2013; DAO *et al.*, 2014). As a result, ants can be employed as biocontrol agents in

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some cases (e.g. WAY & KHOO, 1992; PENG *et al.*, 2010; CHOATE & DRUMMOND, 2011; OFFENBERG, 2015; CASTRACANI *et al.*, 2017; SCHIFANI *et al.*, 2020), but they can be target of control strategies in other situations (e.g. TOLLERUP *et al.*, 2004; GREENBERG *et al.*, 2013). The balance between negative and positive effects of the ant presence in agroecosystems is variable, and it depends on many factors (e.g. STYRSKY & EUBANKS, 2006). In the last two decades, several studies began to investigate the role of ants in Italian agroecosystems and their possible use as bioindicators (e.g. CASTRACANI & MORI, 2006; OTTONETTI *et al.*, 2008; LA PERGOLA *et al.*, 2008; SANTINI *et al.*, 2011; MASONI *et al.*, 2017; CAMPOLO *et al.*, 2015; CASTRACANI *et al.*, 2015; SCHIFANI *et al.*, 2020). However, most contexts of the highly diversified Mediterranean agriculture remain currently unexplored in this sense.

Accounts of the ant fauna inhabiting vineyards are available through scattered checklists from very different geographic regions. For example, ant check-lists in Australian and South American vineyards were provided by CHONG *et al.* (2011) and ROSADO *et al.* (2012; 2013), while in Europe some assessments were provided by BELTRÀ *et al.* (2017) in Spain, GONÇALVES *et al.* (2017) in Portugal and MASONI *et al.* (2017) in central Italy (Tuscany region). However, European vineyards are found under several different climatic conditions. Italy offers a great variety of climatic conditions in this sense: on one hand, vineyards can be found under hot temperate and subtropical temperate climate in Sicily, while they are affected by a sub-continental climate in the Prealpine river valleys (FRATIANNI & ACQUAOTTA, 2017). While MASONI *et al.* (2017) offered a first assessment from an Italian area characterized by a sub coastal temperate climate, we decided to investigate vineyards' ants at the northernmost latitudes of Italian viticulture, considering that ants colonizing vineyards under a sub-continental climate have never been documented elsewhere in Europe. Therefore, we conducted a first qualitative and quantitative assessment of ant diversity in vineyards from the Prealpine Adige Valley in Italy in order to provide a baseline overview and compare the results to the accounts from other geographic regions.

MATERIALS AND METHODS

A total of 10 vineyards from the Adige Valley in northern Italy (region: Trentino-Alto Adige; cities: Rovereto and Trento), treated under conventional agriculture, were selected for this study (see Table 1).

The vines (Pinot grigio variety) were grown with a straight, single trunk and then trained onto a pergola system (Fig. I). The vineyards ground was permanently grass covered between the rows while chemical weed control was applied on a 50 cm strip under the vines. Grass was periodically mowed and mulched on place. Pest control was performed with repeated applications of fungicides and one or two insecticide treatments.

Our monitoring program was conducted from June to September 2016 (which is a good coverage of ants' activity season in the study area), focusing on two rows of each vineyard (each consisting of 16 vines). To obtain data on the arthropodofauna, we relied on pitfall traps (50-ml polypropylene Falcon vials) filled with 30 ml of propylene glycol. In each row, 12 traps were employed at a time: 4 placed on the vines' branches (B traps), 4 in the soil between two vines (S2 traps) and 4 at 1 m from the vines, between the rows (S1 traps) (Figs. II, III). Traps were replaced every 15 days, resulting in 7 sampling dates (from 07.06.2016 to 07.09.2016). Therefore, a total of 1680 traps were used (12 traps x 2 rows x 10 vineyards x 7 sampling dates).

Table 1- List of investigated vineyards.

Site name	Latitude and longitude	Altitude (m)
A: La Favorita	45.862860, 11.002325	175
B: De Bellat-Pulito	45.845952, 11.007980	155
C: Serravalle Campanella Alto	45.801827, 11.027747	210
D: Serravalle Campanella Basso	45.796905, 11.020143	135
E: Avio Depuratore	45.732027, 10.946634	130
F: Carnal Avio	45.739156, 10.941774	210
G: Avio Campei Alto	45.753609, 10.984719	175
H: Avio Campei Basso	45.752127, 10.984354	140
I: Marine	46.036315, 11.113790	215
J: Maso Grande Ravina	46.032534, 11.107437	260

Systematic identification was achieved using general dichotomous keys for arthopods and for soil microarthopods (CHINERY, 1986; AA VV, 2005). Specimens were recognized at different systematic levels depending on their taxon, but at least at order level. Ants were sorted and identified to species level and identification was achieved using the information provided by WAGNER *et al.* (2017) and SEIFERT (2018; 2020). Ants from the cryptic *Tetramorium caespitum* complex were initially not identified during 2016 as the taxonomy of this complex was still unclear (SCHLICK-STEINER *et al.*, 2006). After WAGNER *et al.* (2017) eventually provided taxonomic keys, only a part of the initial collection still remained in our possession. Since all available specimens were identified as *T. immigrans* (see Results), we refer as *T.* cf. *immigrans* to all the collected specimens from this group.

Species accumulation curves were computed using R 4.0.3 and the `specaccum()` function of the vegan package (OKSANEN *et al*., 2017; R CORE TEAM, 2021).

Fig. I - Vines grown with a single straight trunk and trained onto a pergola system in one of the investigated vineyards.

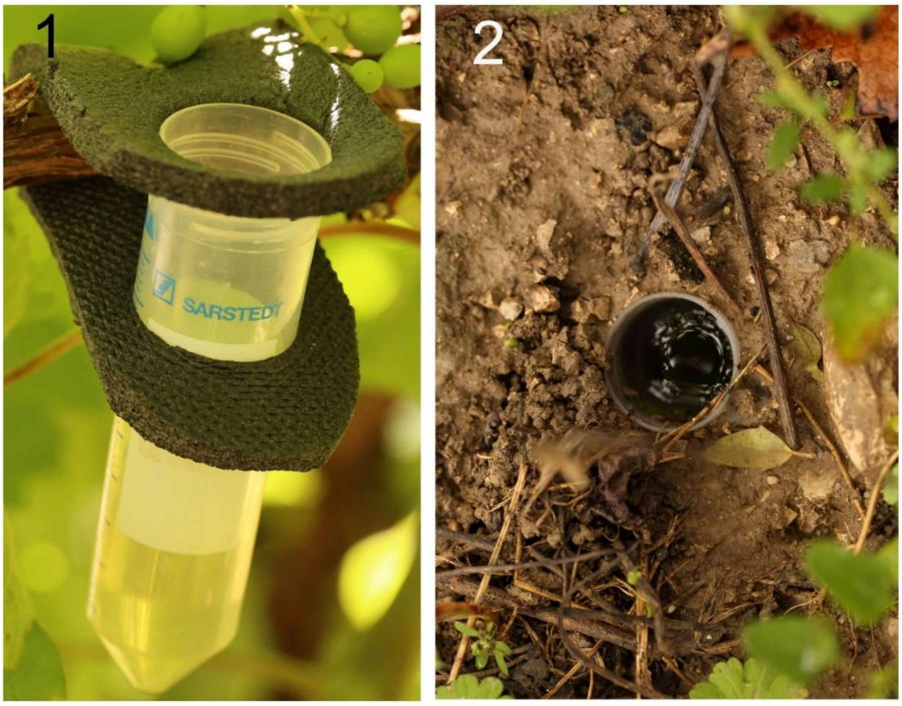

Fig. II - Pitfall traps placed on vines' branches (1) and in the soil (2).

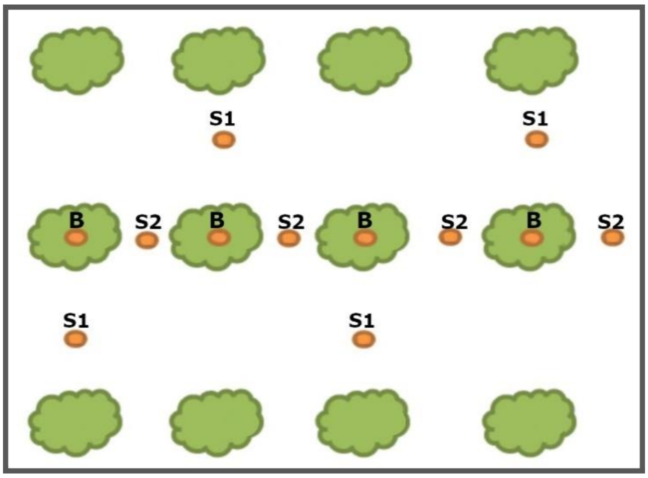

Fig. III - Traps placement in the vineyards' rows: B traps on the vines' branches, S1 traps between the rows and S2 traps between the vines.

Table 2 - Arthropod groups collected during the survey.

Class	Order	Tot. Ind. (n = 20,284)	% Ind.	Tot. Traps (n = 1,680)	% Traps
Arachnida	Acarina	135	0.7	85	5.5
	Araneae	1,210	6.5	526	31.3
	Opiliones	171	0.9	111	6.6
Crustacea	Isopoda	264	1.4	141	8.4
Hexapoda	Collembola	471	2.5	132	7.8
	Coleoptera, Adephaga	2,521	13.4	688	41.0
	Coleoptera, Polyphaga	1,137	6.1	395	23.5
	Coleoptera, larvae	401	2.1	243	14.5
	Dermaptera	168	0.9	115	6.8
	Diptera	1,036	5.5	473	28.1
	Hemiptera	274	1.5	202	12.0
	Hymenoptera, Formicidae	**10,501**	**56.0**	**1,228**	**73.1**
	Hymenoptera (other groups)	232	1.2	162	9.6
	Lepidoptera (adults)	21	0.1	18	1.1
	Lepidoptera (caterpillars)	85	0.4	65	3.9
	Neuroptera	9	0.0	9	0.5
Myriapoda	Diplopoda	25	0.1	19	1.3
	Chilopoda	74	0.4	67	4.0

RESULTS

A total of 20,284 specimens were retrieved from the traps and they were classified into 19 major groups representing 15 orders of Arachnida, Crustacea, Hexapoda and Myriapoda classes (Table 2).

Among these groups, ants were the most abundant, consisting in 56% of all of the collected specimens (10,501), and the most frequent, found in 73% of the traps, and these differences were averagely maintained through the entire sampling period (Table 2, Figs. IV, V).

Ants were represented by 22 species belonging to 16 genera and 3 subfamilies (Table 3).

The most abundant species, *F. cunicularia*, *L. niger* and *T.* cf. *immigrans* represented alone over 85% of the collected specimens, and among them *L. niger* was the most abundant during all the sampling dates (Figs VI, VII).

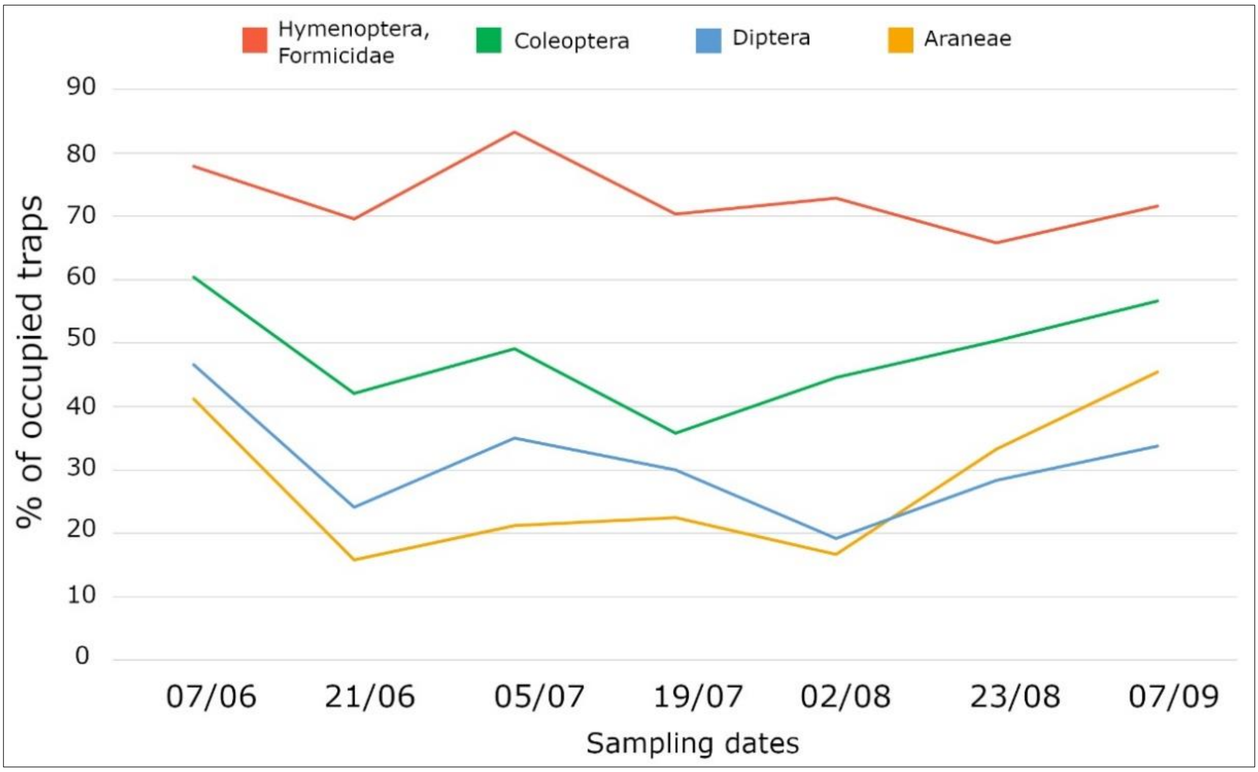

Fig. IV - Frequency of the four main arthropod groups among the traps retrieved in the seven sampling dates.

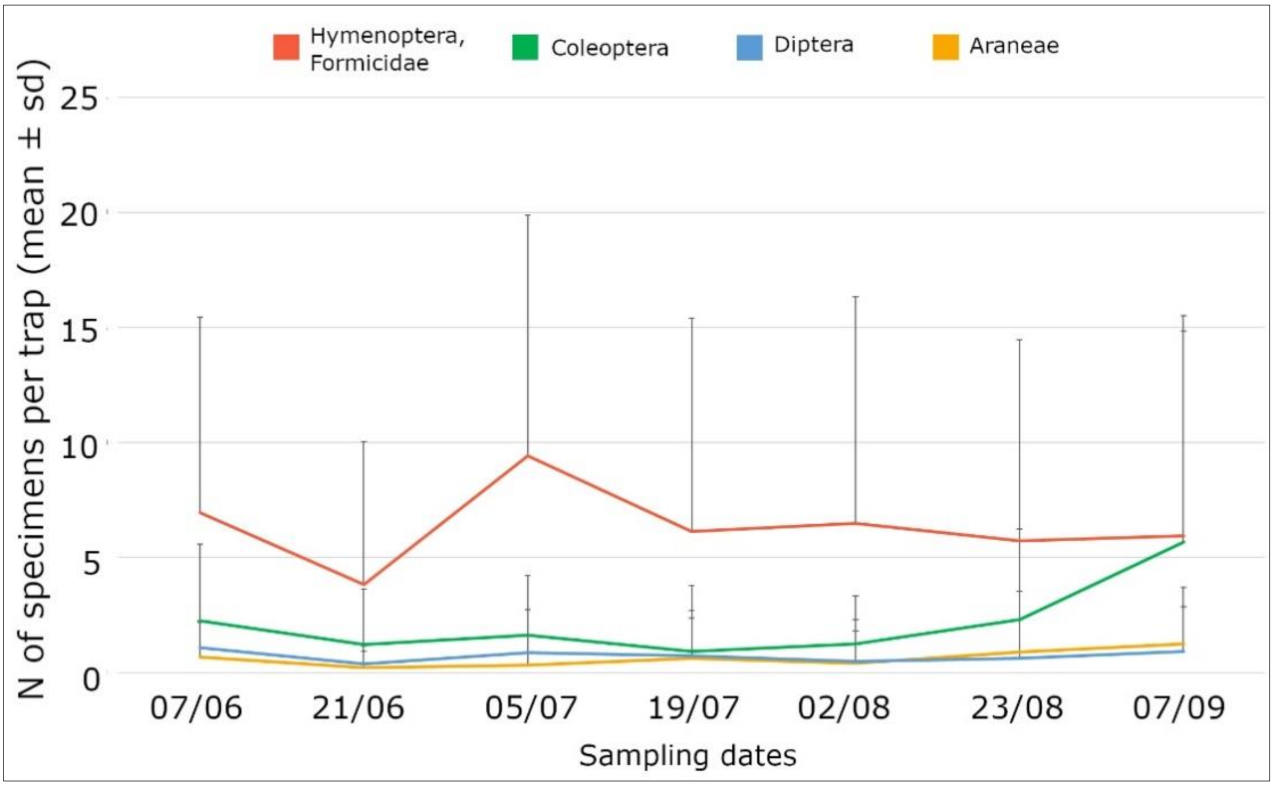

Fig. V - Arthropod specimens collected per trap during the seven sampling dates divided across the four main arthropod groups.

Table 3 - Ant species collected during the survey.

Subfamily	Species	Occupied sites (N=10)	Tot. Ind.	% Ind.	Branches traps (B)
Formicinae	*Camponotus aethiops* (Latreille, 1798)	1	4	0.0	
	Formica cinerea Mayr, 1853	4	19	0.2	X
	***Formica cunicularia* Latreille, 1798**	**10**	**1,169**	**11.1**	**X**
	Lasius emarginatus (Olivier, 1792)	2	4	0.0	X
	Lasius fuliginosus Latreille, 1798	3	4	0.0	
	Lasius myops Forel, 1894	3	7	0.1	X
	***Lasius niger* Linnaeus, 1758**	**10**	**6,170**	**58.7**	**X**
	Plagiolepis pygmaea (Latreille, 1798)	8	62	0.6	X
	Polyergus rufescens (Latreille, 1798)	3	64	0.6	
Myrmicinae	*Aphaenogaster subterranea* (Latreille, 1798)	5	9	0.1	
	Crematogaster scutellaris (Olivier, 1792)	4	4	0.0	X
	Messor ibericus Santschi, 1931	8	727	6.9	X
	Myrmica sabuleti Meinert, 1861	3	3	0.0	
	Myrmica specioides Bondroit, 1918	3	5	0.0	X
	Myrmecina graminicola (Latreille, 1802)	7	114	1.1	
	Pheidole pallidula (Nylander, 1849)	7	334	3.2	X
	Solenopsis fugax (Latreille, 1798)	9	132	1.2	X
	Strongylognathus testaceus (Schenck, 1852)	3	3	0.0	
	Temnothorax italicus (Consani, 1952)	5	9	0.0	
	Temnothorax unifasciatus (Latreille, 1798)	4	5	0.0	X
	***Tetramorium* cf. *immigrans* Santschi, 1927**	**10**	**1,643**	**15.6**	**X**
Ponerinae	*Hypoponera eduardi* (Forel, 1894)	3	5	0.0	

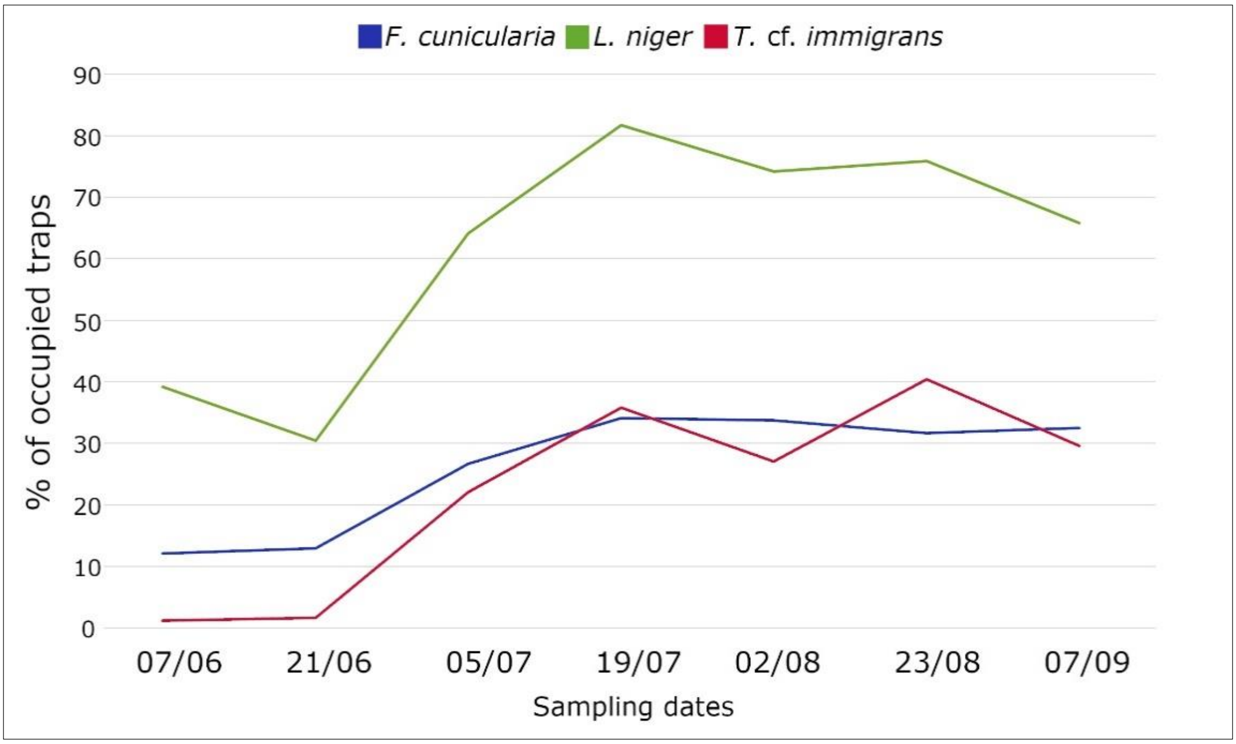

Fig. VI - Frequency on traps during the seven sampling dates of the three ant species detected in all orchards.

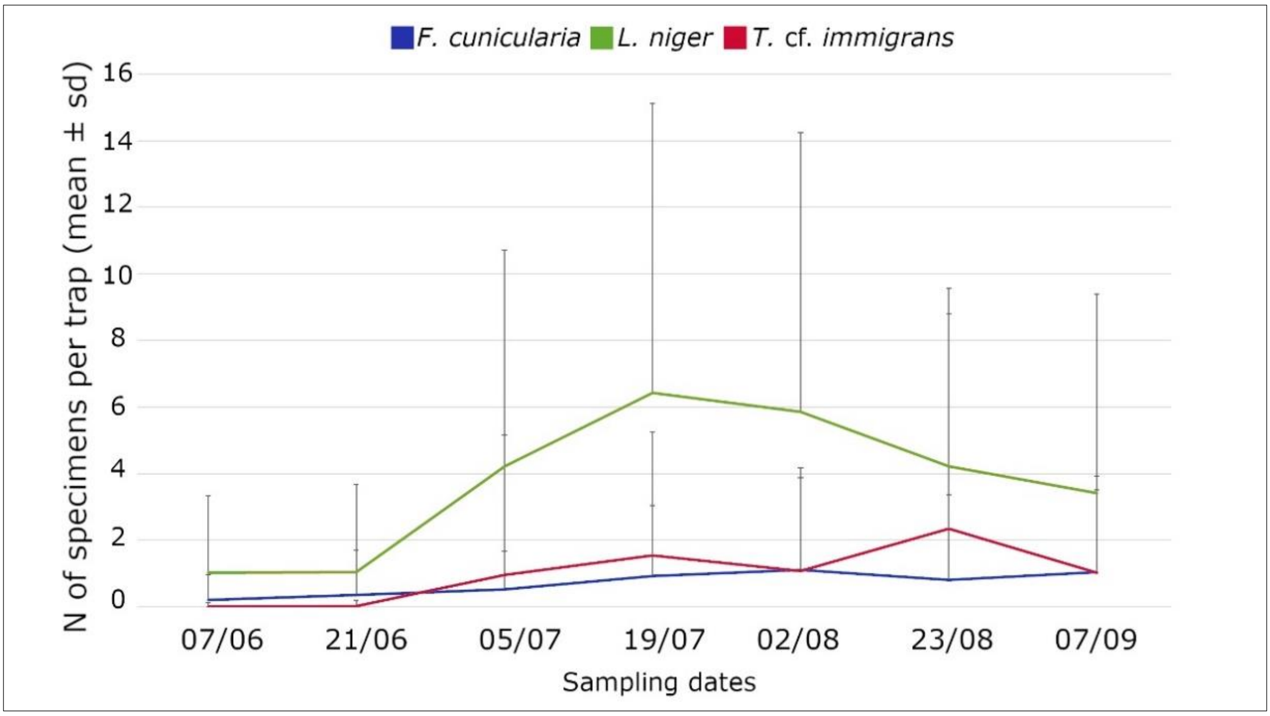

Fig. VII - Ant specimens collected per trap during the seven sampling dates for the three species detected in all vineyards.

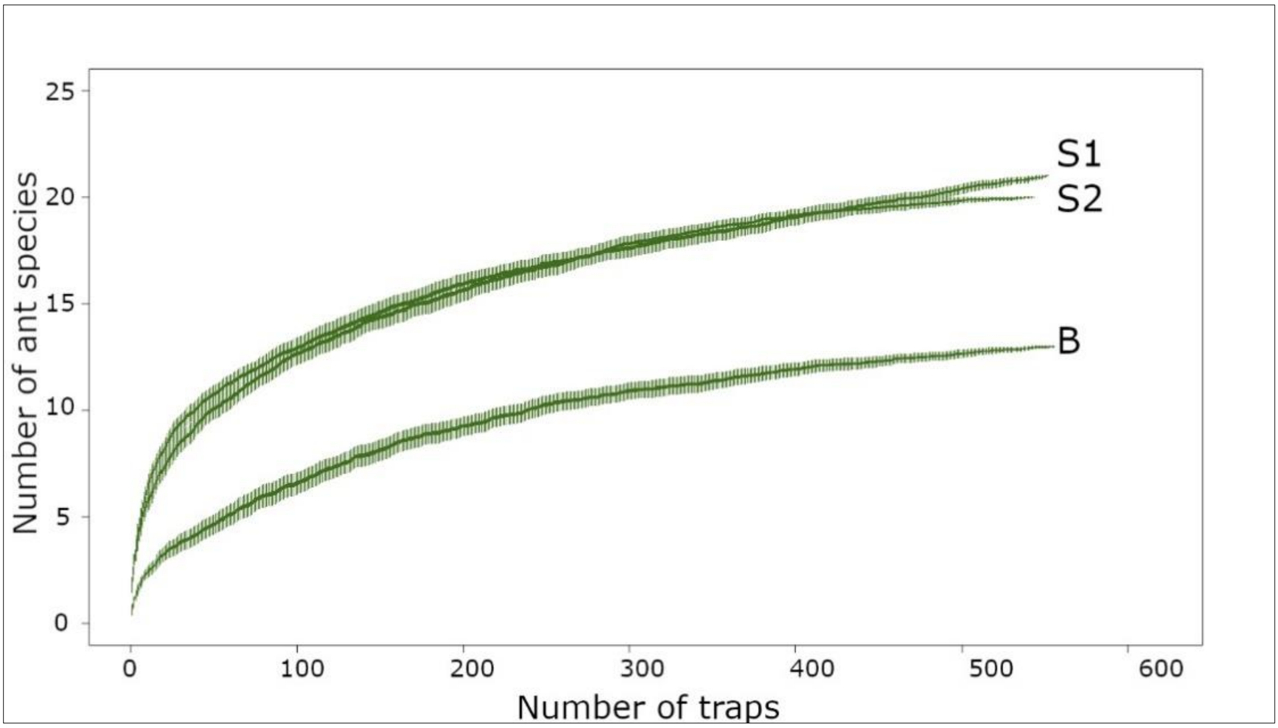

Fig. VIII - Species accumulation curves based on the number of ant species collected with traps placed in different positions. The vertical bars correspond to 30% of the standard error of the estimate.

The S1 and S2 traps yielded a comparable performance in terms of number of captured ant species (Fig. VIII), and together granted the detection of all of the species encountered during this study, while only a subset of 13 species was collected with B traps.

The number of species collected per vineyard varied from 7 to 16, while *F. cunicularia*, *L. niger* and *T.* cf. *immigrans* were found in every vineyard. Species accumulation curves showed that the sampling effort determined a clear plateau for most vineyards, with the exception of site I (Fig. IX; Table 4).

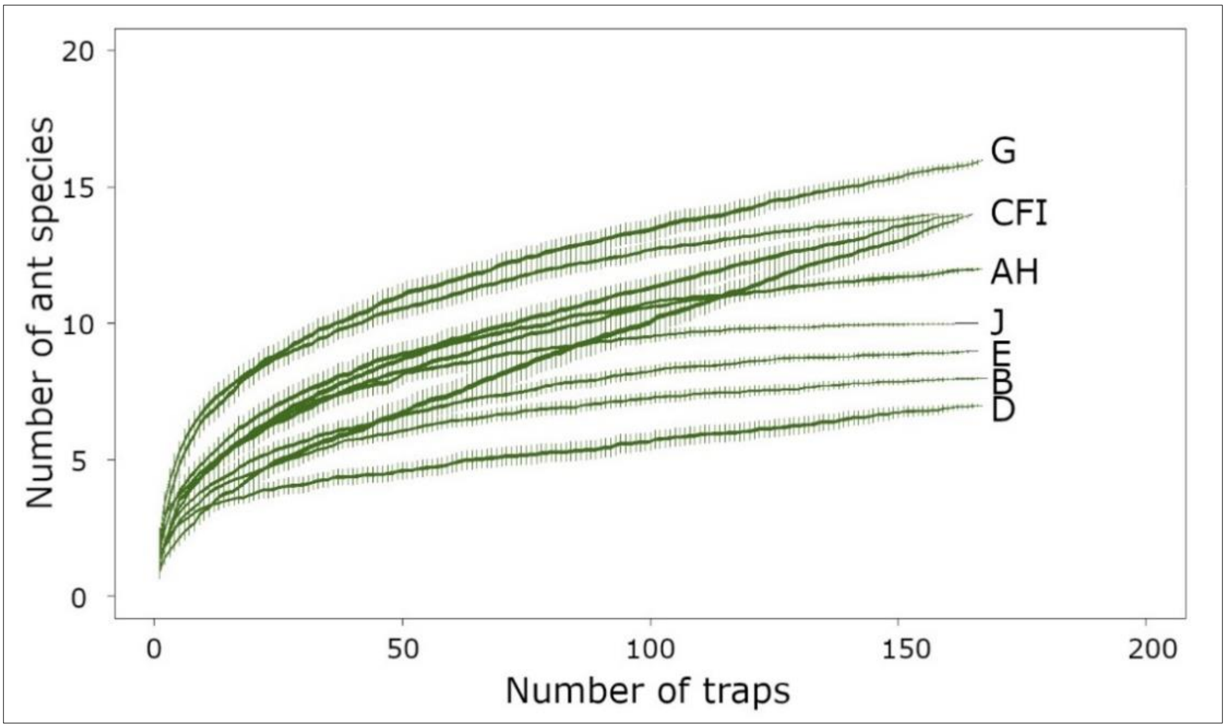

Fig. IX - Species accumulation curves based on the number of ant species collected at each site. The vertical bars correspond to 30% of the standard error of the estimate.

Table 4 - Diversity indexes of each site based on collected species and % of traps occupied by the three species present in each vineyard.

Site	**Species richness**	**Shannon index (*H*)**	**Equitability index (*E_H*)**	**% traps *F. cunicularia***	**% traps *L. niger***	**% traps *T.* cf. *immigrans***
A	12	1.60	.31	68	69	41
B	8	1.28	.25	3	64	26
C	14	1.87	.37	34	59	32
D	7	1.00	.19	23	77	16
E	9	1.35	.26	36	71	14
F	14	2.39	.47	23	41	33
G	16	2.46	.48	27	71	14
H	12	1.62	.32	17	55	26
I	14	1.14	.22	7	67	14
J	10	1.64	.32	28	51	11

DISCUSSION

As strongly documented from literature (e.g. AGOSTI *et al.*, 2000; LACH *et al.*, 2010), once again ants proved to be a convenient arthropod group for monitoring programs in agroecosystems, being consistently as the most abundant group in our survey. This study provides one of the few quantitative assessments conducted on the Italian ant fauna. We documented a moderately diverse fauna characterized by a high diversity of Formicinae and Myrmicinae (with a good diversity of *Lasius* genus, but also multiple species of *Formica*, *Myrmica* and *Temnothorax* genera) and by the notable absence of Dolichoderinae ants. We found an overwhelming prevalence of species characterized by very large distributions in Europe and beyond, only few Mediterranean taxa and no endemisms (JANICKI *et al.*, 2016; GUÉNARD *et al.*, 2017). This picture is not particularly different from that of recent surveys conducted on the Po Plain, but we detected no Eastern-Mediterranean species and even fewer Mediterranean or South European taxa (CASTRACANI *et al.*, 2020). Moreover, it is worth noting that three notoriously disturbance-tolerant species, *F. cunicularia*, *L. niger* and the *T.* cf. *immigrans* were the most abundant species, as it was observed elsewhere in Northern Italy (CASTRACANI *et al.*, 2020). While *T. immigrans* is probably an introduced species in Italy (CASTRACANI *et al.*, 2020), the numerical dominance of these three species likely reflects their ability to fill empty niches created by human activities (see ARNAN *et al.*, 2018; 2021). Only about half (54%) of the species we recorded was also detected on the vines themselves through the use of traps placed on their branches. Species like *C. aethiops* or *T. italicus*, which habitually visit plants (SEIFERT, 2018; GIANNETTI *et al.*, 2019), were probably only encountered on the soil just because of their low abundance. On the other hand, absence on the vines was expected for the social parasites such as the slave-maker *P. rufescens* or the inquiline *S. testaceus*, which are not active foragers. Other species such as *A. subterranea*, *H. eduardi* or *M. graminicola* were also not expected to climb into the vines because they usually forage on the soil surface or within the leaf litter (SEIFERT, 2018; GRASSO *et al.*, 2020). While *S. fugax* belongs to the same category (SEIFERT, 2018), its finding on vines was rather unexpected.

In comparison to the Australian and Brazilian vineyards' ant faunas, we detected much fewer ant species. CHONG *et al.* (2011) sampled 50 vineyards from different Australian regions, achieving a vast geographic coverage, and detected 147 species, estimating each vineyard to be inhabited by 30-40 species (but recording only 5-24 species in one of the sampled regions). At the same time, ROSADO *et al.* (2012) recorded 72 species in total in Brazil, from 21 to 50 per vineyard. Our numbers are far lower and the most represented genera are different from those detected in these surveys, but this is unsurprising considering the ecological and biogeographical patterns of ant diversity worldwide: ant diversity is notoriously higher in the tropics, where genera that are dominant in the temperate ecozone have a modest presence and vice-versa (e.g. see JANICKI *et al.*, 2016; GUÉNARD *et al.*, 2017).

On the other hand, possible comparisons with vineyards of the northern temperate ecosphere, which host more similar faunas, are not particularly numerous. For example, despite several papers dealt with peculiar ant species and their role in North American vineyards (e.g. KLOTZ *et al.*, 2003; TOLLERUP *et al.*, 2004; 2007; DAANE *et al.*, 2006; 2007; NONDILLO *et al.*, 2016; TOWNSEND *et al.*, 2016; WESTERMANN et al., 2016; COOPER et al., 2019), no data on vineyard overall ant diversity are available. In some other cases, an ant species check-list is provided, but it included only species that were observed foraging on the vines (BELTRÀ *et al.*, 2017). However, some interesting comparisons may be made with data published by GONÇALVES *et al.* (2017) from Portugal and MASONI *et al.* (2017) from Central Italy. The fauna from the Portuguese sites investigated by GONÇALVES *et al.* (2017) comprises 20 species in total, slightly less than ours, but the number of species per site is averagely much higher (15-20) than in our case, and so it is the number of species (9) common to all their 6 investigated vineyards. It is also a very different fauna in both ecological and biogeographic terms, consisting prevalently of species and genera associated with xero-Mediterranean climatic conditions and with a clear Western-Mediterranean characterization (e.g. *Cataglyphis* spp., West-Mediterranean *Camponotus* species such as *C. cruentatus* (Latreille, 1802) and *C. sylvaticus* (Olivier, 1792), the Iberian subendemic *Iberoformica* genus, *Aphaenogaster iberica* Emery, 1908 from the xerothermophilous *testaceopilosa* group, *Crematogaster auberti* Emery, 1869). On the other hand, MASONI *et al.* (2017) recorded a similar number of species (19) from 10 vineyards near Florence (Tuscany, Italy), and a slightly smaller number of species per vineyard than us (5-12). The ant assemblages documented by MASONI *et al.* (2017) present some relevant similarities such as a relatively high *Myrmica* diversity and the widespread presence of *F. cunicularia* and *M. ibericus*. Concerning the latter species, MASONI *et al.* (2017) refer to *M. structor* (Latreille, 1798), but Italy most likely only hosts its cryptic sister species *M. ibericus* (STEINER *et al.*, 2018; SCHIFANI *et al.*, 2021). However, there are also relevant differences, as the reduced diversity of *Lasius*, and, at the same time, the more widespread presence of thermophilous species such as *P. pallidula* and Mediterranean *Tapinoma* species from the Dolichoderinae subfamily.

The ecological role of the overwhelming majority of ant species that inhabit vineyards across the globe, including of those we detected in our survey, is still virtually unknown. Only three ant species, the worldwide spread invasive Argentine ant *Linepithema humile* (Mayr, 1868), the South American *L. micans* (Forel, 1908) and the North American *Formica perpilosa* Wheeler, W.M., 1913 have been the subject of several studies considering them as significant pests requiring control strategies in vineyards of California and Brazil (KLOTZ *et al.*, 2003; TOLLERUP *et al.*, 2004; 2007; DAANE *et al.*, 2006; 2007; SACCHETT *et al.*, 2009; NONDILLO *et al.*, 2016; WESTERMANN *et al.*, 2016; COOPER *et al.*, 2019). On the other hand, another invasive species, the red imported fire ant *Solenopsis invicta* Buren, 1972, was deemed a positive presence due to its significant predatory action on pest species in Texas' vineyards (TOWNSEND *et al.*, 2016).

In conclusion, ant communities in agroecosystems are diverse and often species-rich, and documenting their identities is crucial to assess the possible services and disservices that different species assemblages may yield. While vineyards are worldwide spread, their ant faunas are rather different from place to place according to local climatic and biogeographic factors, so that the few available data do not show clear patterns, which could have originated from strong homogenizing ecological constraints derived from viticulture *per se*. Further investigation will be required to understand how the fauna of the vineyards from the Adige Valley compares with that of other agroecosystems or natural habitats from the same region and how different management practices may influence it. It will also be important to assess what is the role that different ant species may play in these environments to improve management practices accordingly.

AUTHORS CONTRIBUTIONS

Conceptualization: CC, DG, DAG, CI; Data collection: MD, MG, DG, FP; Specimen processing and identification: CC, MG, ES, FAS; Data curation and analysis: CC, ES; Visualization: CC, DG, ES; Writing—original draft preparation: DG, ES; Writing—review and editing: CC, MD, DAG, CI, AM, FP, FAS; Supervision: DAG, CI, AM; Funding acquisition: DAG, CI, AM. All authors have read and agreed to the published version of the manuscript.

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