Giacche Verdi Bronte

Soil arthropod biodiversity comparison between two agroecosystems in Bronte (Sicily)

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1. BACKGROUND

Most of the biodiversity of agroecosystems lies in the soil, which is the most diverse and complex ecosystem on the planet, containing nearly a quarter of the earth's diversity (Gunstone et al., 2021; Gonçalves et al., 2021). Soil arthropods represent a major part of soil fauna diversity. They play a crucial role in maintaining soil quality and health, providing ecosystem services essential for the sustainable functioning of natural and managed ecosystems, such as soil structure maintenance, decomposition of organic matter, humus formation, and regulation of pests and diseases (Caruso et al., 2006; Gonçalves and Pereira, 2012; Huber, 2009; Ruano et al., 2004). Specifically, arthropods have been used as indicators of a range of environmental features (Brown, 1997); for example, in the agroecosystems, spiders have been recommended as indicators for early detection of insecticide effects on predatory arthropods (Everts et al., 1989), acari of the suborder Oribatida can be used as bioindicators of disturbance or habitat status in agroecosystems (Behan-Pelletier, 1999), ant species have also been used as indicators for agroecosystems disturbance and condition (Peck et al., 1998). Taking into account the declines of biodiversity worldwide, especially arthropod communities, sampling and documenting their diversity in agricultural areas plays an important role in assessing the impact of different management techniques that can help in preserving populations or causing their declines (Sisterson et al., 2020). As the human population continues to grow, there is a need for research that focuses on agriculture without biodiversity loss side-effects.

The challenge is to identify insect conservation strategies that improve crop yields by balancing the mentioned ecosystem services provided by arthropods. Overuse of chemical control mechanisms like pesticides has been identified as the most impactful practice driving the loss of soil biodiversity in the last 10 years (FAO, 2020). This study aims to compare differences in the composition of soil surface arthropods in three locations in winter, using one distant olive grove and two adjacent agricultural fields (olive and almond). The olive fields are long-established groves, maintained without the use of any chemical substances, and have a significant amount of vegetation cover composed of grasses and wildflowers. Meanwhile, the young almond field, adjacent to one of the olive groves, is managed more intensively, with regular use of herbicides and barely any vegetation cover. With this study, we aim to evaluate differences in arthropod composition in the aforementioned locations with hypothetically greater diversity and evenness of arthropod orders in the less disturbed olive orchards.

2. MATERIALS AND METHODS

2.1 Study sites

The study area consisted of three agricultural lands: two olives fields (*Olea europaea* L.) and one almond field (*Prunus amygdalus* B.) (Fig.1). Olives A and almonds represent two adjacent fields with different management strategies belonging respectively to Giacche Verdi Bronte and a private landowner not affiliated to the association. Olives B represents another olive grove belonging to Giacche Verdi, about 600 m away from the two. Traps set in olives A covered an area of 0.25 hectares, almonds 0.34 hectares, and olives B 1.5 hectares. Both olive fields have had olive trees for over 20 years, are maintained without the use of pesticides and have considerably more vegetation cover than the almond field which is managed with regular use of Roundup. The area covered now by the almonds is also more disturbed as previous fruit trees were cut down in 2017, so the site does not function as a mature orchard yet and that could be a reason for a scarcity of vegetation cover.

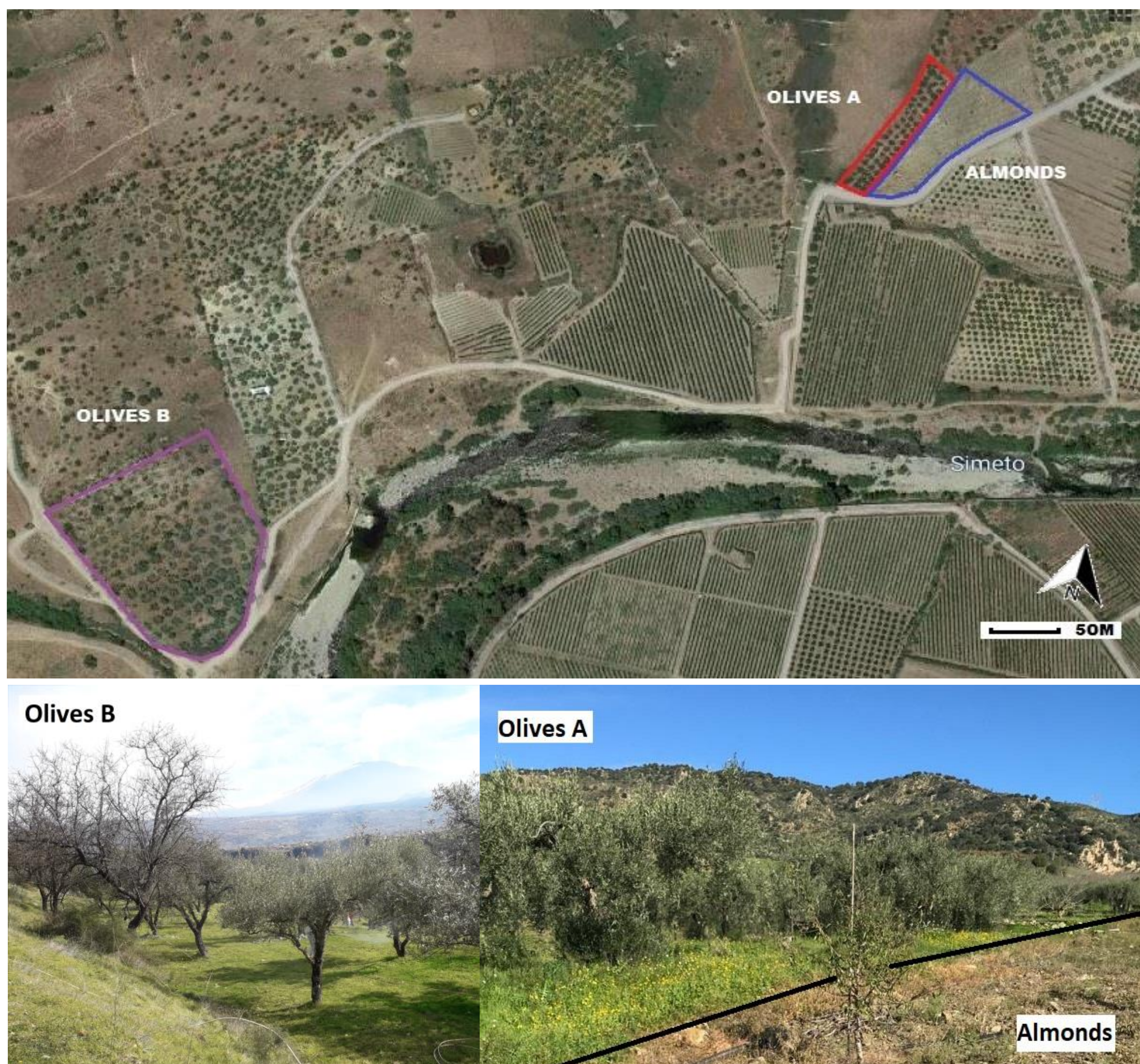


Figure 1. Three study areas in Bronte highlighted in purple, red, and blue.

2.2 Sampling method

We collected ground-dwelling arthropods using a pitfall trap method. The field visits were planned bimonthly for two consecutive days to set and collect the traps, leaving them over a 24-hour period from the end of November 2021 until the end of January 2022. A plastic cup filled with soapy water was placed in a dug-out hole with its top rim at ground level. We placed 15 traps randomly in each location using random numbers of steps (more detailed methods are described in Annex 4). The sampling of olives B started after the first two field visits because it was not planned as part of the study originally. This field was added as a comparison of a grove not influenced by a proximate use of pesticides.

We collected the cups' contents and later placed the detected arthropods in 80% alcohol per field for preservation and further identification. We analysed the samples with a 25x stereoscope, identifying them to order level based on the guide of Gibb and Oseto (2006), and separated them into different containers with alcohol. After collecting the data, we decided to identify Formicidae to species level (using the guides provided by antwiki.org and antweb.org such as Sanetra 1999; Seifert et al., 2012; Boer et al., 2013; Borowiec et al., 2015; Steiner et al., 2018) to analyse closer the ant community of the fields.

2.3 Data analysis

We focused only on adult stages for the analysis; larvae were excluded. All comparisons that include all three fields are based just on the last three sampling events when all fields were sampled at the same time. Simpson's Index of Diversity (1-D), Shannon Diversity Index (H), Pielou's evenness index (J), and Jaccard index of dissimilarity were used to assess arthropod order diversity, richness, and evenness within and between the sites. Simpson's Index of Diversity (max. value = 1) and Shannon Diversity Index both take into account richness and relative abundance, higher values represent more diversity. Pielou's evenness index ranges from 0 to 1, the closer to 1 the more even the dataset. For the ant composition, we used Shannon, Simpson, and additionally the Jaccard index of dissimilarity (which ranges from 0 to 1, the closer to 1 the less similar the data) to compare the sites using species presence/absence. Only presence-absence data was used because species were only identified after all collections were done, therefore their abundances could not be estimated for each sampling event to evaluate species dominance.

Principal Component Analysis (PCA) with Hellinger transformation of abundances was used to visually distinguish the representative orders between the fields. Mean relative abundances were calculated and plotted in stacked bar plots to compare the order composition of each field and determine the most abundant orders. The orders with a mean relative abundance of more than 5% were selected as the most representative orders shown in the plot comparisons. The coefficient of variation of each order was calculated to compare the variation in captures of the same orders selected for the mean relative abundance plot.

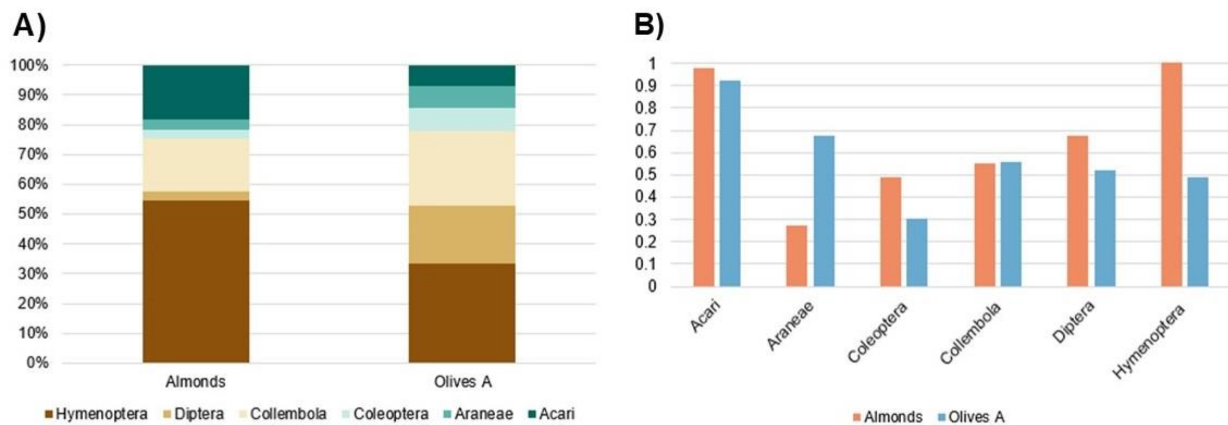
A mix of analyses of variances (ANOVA), Welch ANOVA and non-parametric Kruskal-Wallis tests were done to compare the variation of the data collected between each field and arthropod order using abundance as the response variable and field as the predictor variable. Square-root transformations were carried out when normality assumptions were violated. P-value adjustments were done to correct for the multiple comparisons using the Benjamini and Hochberg (BH) method (Jafari and Ansari-Pour, 2019). Tukey's post-hoc test was performed to further identify which fields were responsible for the significant differences. Significance was reported when $p<0.05$.

3. RESULTS

In total, 2715 individuals were collected and 14 orders were identified (Acari, Araneae, Chilopoda, Coleoptera, Collembola, Dermaptera, Diplopoda, Diptera, Hemiptera, Hymenoptera, Isopoda, Neuroptera, Opiliones, Thysanura, Thysanoptera) in all three localities altogether. More specifically, 603 individuals were collected in olives A and 1838 in almonds during five collection events, as well as 274 individuals in olives B after three collection events. At a taxonomic level, Hymenoptera was the numerically predominant order (45%) followed by Collembola (20%) and Acari (14%). The almond field had the highest order richness (14 orders), followed by olives A (12 orders) and olives B (9 orders).

3.1 Olives A and Almonds

Mean relative abundance of orders in almonds showed Hymenoptera accounted for more than 50% of collected arthropods, and more than 30% in olives A, making it the dominant order overall. Almonds were also more represented by Acari than olives A, while olives A had a large proportion of Diptera captured (Fig.2A). The coefficient of variation pointed out a difference between olives A and almonds in the orders Hymenoptera (58%), Araneae (40%), and Coleoptera (19%) (Fig. 2B). Based on the diversity indices, a higher order evenness and overall diversity were shown for olives A through the Pielou, Simpson, and Shannon indices (Fig. 2C).



C)

Diversity index	Olives A	Almonds
Simpson	0.804	0.655
Shannon	1.873	1.403
Pielou	0.754	0.532

Figure 2. A) Mean relative abundance comparisons of the six most representative orders between olives A and almonds. B) Coefficient of variation of the different orders captures compared between almonds and olives A. C) Comparison of diversity indices for olives A and almonds.

3.2. Principal Components Analysis (PCA)

The PCA of all three sites showed that they are represented by different arthropod order compositions, separating them into slightly distinct groups. Olives A was better represented by orders Isopoda, Dermaptera, Hemiptera, and marginally Diptera. Olives B seemed to have a higher representation of Opiliones, Coleoptera, Collembola, Araneae, and Diplopoda. Almonds had a greater affinity towards Hymenoptera, Acari, and Chilopoda (Fig.3).

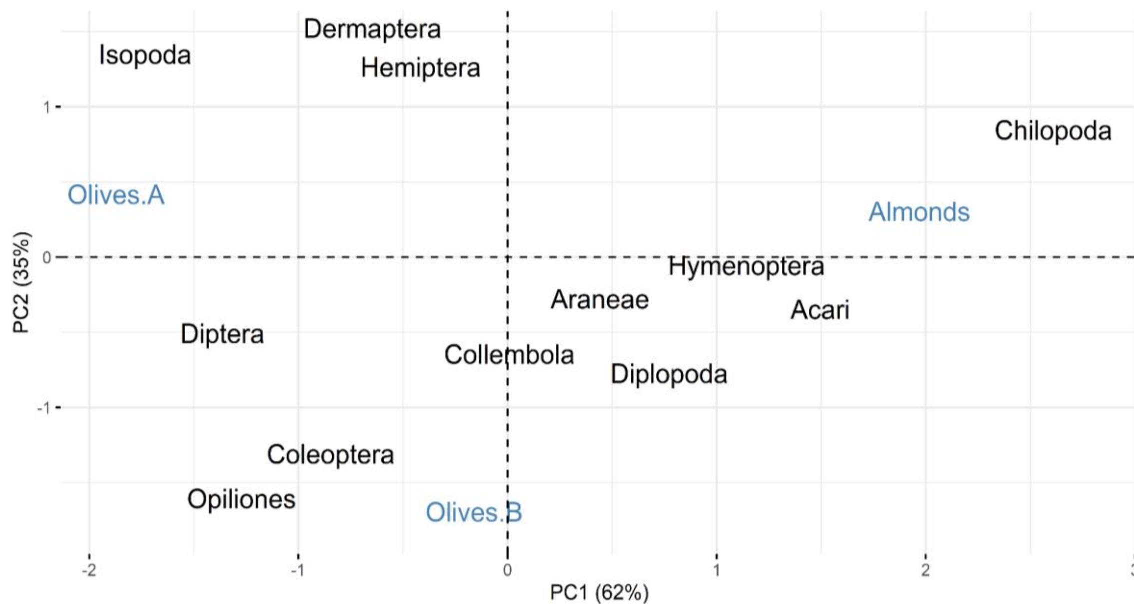
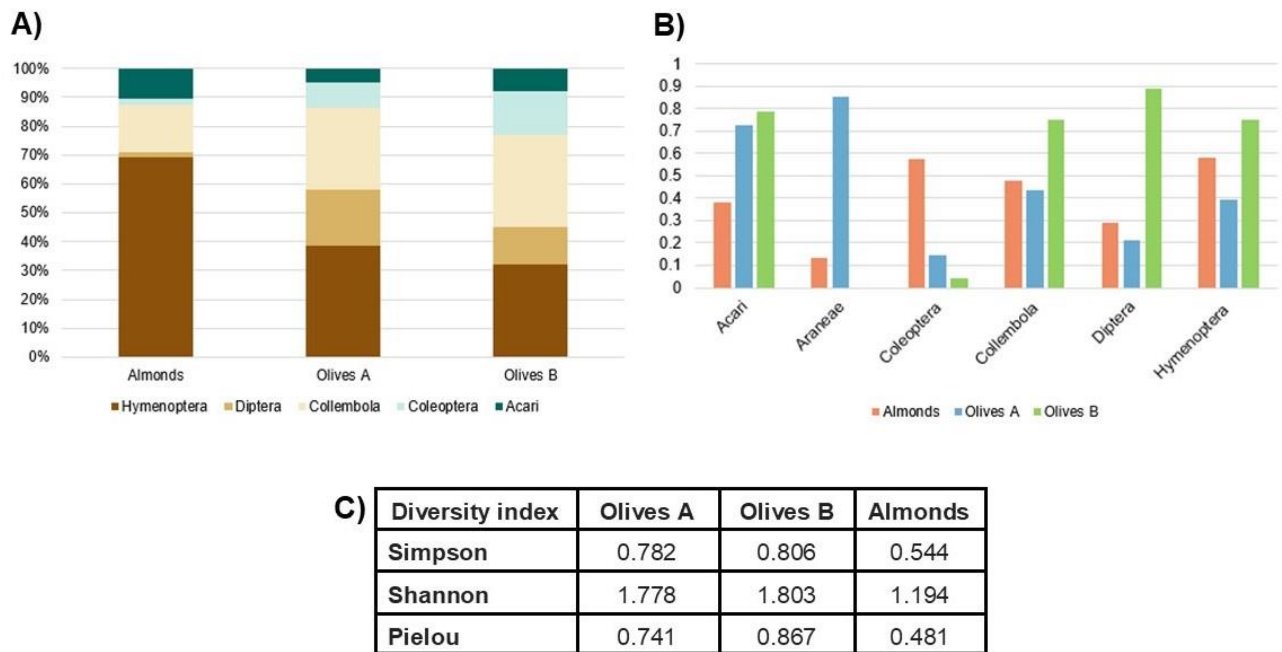


Figure 3. Principal Components Analysis biplot used to visualize the similarities between the sites based on the order composition.

3.3 Comparison of olives A, olives B and almonds

The abundance of Hymenoptera represented a large part (~70%) of the arthropods collected in almonds making their composition more homogenous. Olives A and olives B were similar in order proportions. Hymenoptera and Collembola accounted for more than half of the arthropod community in both olive fields (Fig. 4A). The coefficient of variation showed large variation differences between the three fields in all the orders. Olives B had the highest variation in all the orders except for Araneae and Coleoptera, which had almost no variation. Olives A had a high variation in Acari and Araneae, while almonds in Coleoptera and Hymenoptera (Fig. 4B). Diversity indices showed a higher order evenness and diversity in the olives B, followed by olives A, and least evenness in almonds respectfully (Fig. 4C).

We obtained high standard deviations, standard errors of the means, and coefficient of variance. High standard deviation and standard error show, that sample means are widely spread around the population mean (Annex 3).



Diversity index	Olives A	Olives B	Almonds
Simpson	0.782	0.806	0.544
Shannon	1.778	1.803	1.194
Pielou	0.741	0.867	0.481

Figure 4. A) Comparison of the mean relative abundance of the five most representative orders of each field. B) Coefficient of variation of the different orders captures compared between the three fields. C) Comparison of diversity indices for olives A, olives B, and almonds.

3.4 Formicidae

In total, 11 species, six genera, and three subfamilies of Formicidae were identified (Annex 6). The ant community shared similar diversity indices in all the fields (Table 1). Jaccard index for ants showed similar results between the three fields. Olives B and almonds showed the least different ant composition (Jaccard index = 0.3), followed by olives A and olives B (Jaccard index = 0.4), and lastly Olives A and almonds (Jaccard index = 0.5).

Table 1. Comparison of ant species diversity indices based on presence-absence data.

Diversity index	Olives A	Olives B	Almonds
Richness	9	7	8
Shannon (H)	2.197	1.946	2.079
Simpson (D)	0.889	0.857	0.875

3.5 Statistical tests

There was no sufficient evidence to find significant differences between almonds and olives A in any of the arthropod orders (p-value<0.5; Annex 1). Significant differences were found for Acari (F=7.11, df=2, adj. p-value=0.1044) and Hymenoptera (F=12.8, df=2, adj. p-value=0.04104) between the three fields (Annex 2). Tukey's test revealed that the significant differences were due to almonds having higher Acari (p-value=0.01, 0.01) and Hymenoptera (p-value=0.02, 0.01) abundances than olives A and olives B, respectively.

4. DISCUSSION

4.1 Composition of arthropod communities

Our study showed noticeable but mainly statistically insignificant differences between the two focus areas (i.e. almonds and olives A). The PCA showed that both of the olive sites shared more similarities with each other than with the almonds, which was supported by the diversity indices assessed. Specifically in the olive fields, we captured mainly Hymenoptera and Collembola. Other studies found diverse arthropod compositions, capturing mostly Hymenoptera (Santos et al., 2007), Collembola and Acari (Gonçalves and Pereira, 2012) Collembola, Acari, and Hymenoptera (Ruano et. al., 2004), Coleoptera and Hymenoptera (Gkisakis et al., 2016) or Coleoptera, Hymenoptera and Araneae (Lasinio and Zapparoli, 1993) in their pitfall traps. The most commonly captured orders seem to vary frequently, depending on multiple probable factors, such as the surroundings of the fields, season, and geographical location (Cotes et al., 2011; Gkisakis et al., 2016). On the other hand, our almond study site comprises very young trees and does not function as a mature orchard yet, therefore we could not compare the arthropod abundances to the scarce existing literature on functioning almond orchards.

Comparing our study sites based on the diversity indices, olive grove A was shown to have a higher overall diversity and evenness than almonds in the order composition, with no dominant order. It also showed that both olive groves were slightly more heterogeneous than almonds, starting with olives B having the highest evenness, followed by olives A (Fig.2 and Fig 4). This pattern might be present because of the surroundings of these fields (Cotes et al., 2011); olive grove A is in the vicinity of pesticide use, the disturbed, bare almond orchard on one side and a bare, plowed field on the other side. Meanwhile, olive grove B is not in the proximity of pesticide use, nor a plowed field. The low evenness of almonds is due to the higher captures of Acari and Hymenoptera individuals, making the composition more homogeneous in this disturbed area. We also found that some orders demonstrated relevant differences between fields in the mean relative abundance comparison. However, this was only significantly different when comparing the three last sampling events between all the fields and not between only almonds and olives A. A similar study in olive groves found that in differently managed fields, there were no significant differences in the soil-dwelling arthropod compositions, supporting our results (Gkisakis et al., 2016).

Since the almond field does not represent the habitat conditions of a mature orchard, we focused on the other aspects of this field to build our discussion instead, such as its low vegetation cover and the application of pesticides. Our results show indirectly that vegetation cover might positively affect the diversity of arthropods, with olives having higher diversity values than almonds. Likewise, previous studies have demonstrated that spontaneous vegetation cover provides the most diverse ecological niches and food resources for arthropod communities compared to bare soil (Gómez et al., 2018; Caprio et al., 2019; Gonçalves et al., 2020; Castro et al., 2021). Another explanation for the lower diversity in almonds could be due to the use of the herbicide Roundup, which has proven negative effects on arthropods, reducing their fitness and survival in the field (Cox, 1998; Saska et al., 2016). However, in our case, the highest abundance of organisms was obtained from the herbicide-treated site (i.e. the almond field, Fig 2A). This might mean that it has more generalist species that can still thrive in the glyphosate-treated areas (Svobodova et. al., 2018). Our study could be repeated after a couple of years to obtain more evidence after the almond trees grow and the possible residue and effect of the used pesticides will be gone.

More samples are also necessary to understand the reason for almonds having more Acari and Hymenoptera than the olive fields, which showed a weak significant difference between all three fields. For example, one of the reasons for a greater number of Acari individuals collected can be due to the fact that before one of the collection days, we were met with unexpected rainfall which caused 3 out of 15 cups in almonds to be filled with water and contain a higher than regular number of soil mites. The high proportion of Acari in almonds could also be explained by the short-generation time of these species, which allow them to recover quickly after events of disturbance (Prinzing et al., 2002).

Meanwhile, for the captures of order Hymenoptera, we believe the difference was due to the behaviour of the most commonly captured species. From this order >90% of the captures were of Formicidae, with a majority of them belonging to what we believe to be the species *Tapinoma nigerrimum* (Nylander 1856). This species has been commonly found in olive groves as the most abundant ant (Morris et al., 2002; Pereira et al., 2004; Santos et al., 2007; Gonçalves and Pereira, 2012). It might be possible that the great number of captures of this species in the almond field was due to its proximity to the olive field and that more disturbed landscapes (like the almost completely bare almond field) were found to capture higher abundances of Dolichoderinae ants, like *Tapinoma*, in pitfalls (Graham et al., 2008). *T. nigerrimum* is known to have large colonies with interconnected nests with thousands of workers that drive away other ant species from food sources because of its aggressive behaviour (Cerdá et al., 1989; Blight et al., 2010). Nevertheless, we did not find evidence of how the dominance of this species might have affected the captures of other species. The analysis done on the ant community showed that they are similar across the three fields. It was based just on presence-absence data; it would be more informative to account for species abundance data in the next sampling events, now knowing which species are mainly present.

4.2 Limits and recommendations

In our study, there were multiple limiting factors. One of them was the fact that collections took place throughout the winter months. Other studies have shown that capture rates of different orders change depending on the seasons (Gkisakis et al., 2016). The distribution of taxonomic groups and community structure of ground-dwelling arthropods is a dynamic time/season-related process linked to food availability and reproductive activity influencing the population sizes (Parker and Mac Nally, 2002; Lui et al. 2013, 2016). A continuous sample size across the seasons and an annual dataset would enhance our findings and represent more accurately the arthropod community structure.

Furthermore, the minimum preferred duration of the study would be 20 samples from each location (for our time-constrained period we only obtained five for almonds and olives A and three for olives B) to ensure optimal sampling and efficient comparison of ecological communities (Agosti et al., 2000). We obtained high SDs, SEs (Annex 3), and coefficients of variance (Fig. 2B; Fig. 4B), all of which show that sample means are widely spread around the population mean, and our results may not closely represent the real population. Therefore, more sampling is needed to have a more reliable dataset as tests showed that no significant differences can be drawn for almost any of the orders so far. The greater the sampling effort, the more individuals belonging to various taxonomic groups can be found (Agosti et al., 2000).

The weak significant difference in Acari and Hymenoptera abundances between all three fields has to be supported by further studies in the future with possible differences also for Araneae and Coleoptera based on the coefficient of variance between the fields (Fig. 4B). Moreover, the ANOVA performed for Acari between olives A and almonds had the highest F-value, which indicates that it has the greatest potential to have differences between the groups, even though the p-value was above 0.05. This also applies to Araneae, which had the highest F-value among the orders with a p-value>0.05 when comparing the three fields.

5. CONCLUSIONS

In conclusion, we found that both of the olive fields evaluated had higher arthropod biodiversity than the bare and pesticide-managed almond field. However, there were no significant differences between the abundances of the orders between the sites, except for the weak significance found for Acari and Hymenoptera that still requires more sampling to verify that they were not due to specific events that occurred during sampling. Our study would have to be continued over different seasons for a longer period of time to draw wider conclusions and strengthen the reliability of our initial findings pointing out a difference in the biodiversity of ground-dwelling arthropods between the less disturbed olive groves and the more disturbed almond orchard. This was just a preliminary study to create a basic picture of what can be found in these fields and the main differences between them. It would also be interesting to research more about the functions and importance of the arthropods found in these fields in the future.

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Annex 1. Statistical comparison of all the orders found in almonds and olives A.

Almonds vs. Olives A					
Order	Test	Test Statistic	df	Adj P-value	Transformation
Araneae	ANOVA	F = 2.466	1	0.4	
Opiliones	ANOVA	F = 1.923	1	0.4	
Acari	ANOVA	F = 7.166	1	0.4	SQRT
Collembola	ANOVA	F = 4.263	1	0.4	
Coleoptera	ANOVA	F = 0.318	1	0.6	
Thysanura	Kruskal-Wallis	Kruskal-Wallis chi-squared = 1	1	0.5	
Chilopoda	Kruskal-Wallis	Kruskal-Wallis chi-squared = 1	1	0.5	
Diplopoda	ANOVA	F = 0.328	1	0.6	
Hymenoptera	Welch ANOVA	F = 2.7964	1	0.4	
Dermaptera	Kruskal-Wallis	Kruskal-Wallis chi-squared = 0.81667	1	0.5	
Diptera	ANOVA	F = 3.582	1	0.4	
Hemiptera	ANOVA	F = 0.048	1	0.8	
Thysanoptera	Kruskal-Wallis	Kruskal-Wallis chi-squared = 1	1	0.5	
Isopoda	Kruskal-Wallis	Kruskal-Wallis chi-squared = 0.6	1	0.5	
Neuroptera	Kruskal-Wallis	Kruskal-Wallis chi-squared = 2.25	1	0.4	

Normality assumptions:

Shapiro-Wilk test on residuals (Abundance ~ Field)			
Order	Test Statistic	P-value	Transformation
Araneae	W = 0.95822	0.77	
Opiliones	W = 0.86599	0.09	
Acari	W = 0.93696	0.52	SQRT

Collembola	W = 0.98372	0.98	
Coleoptera	W = 0.96612	0.85	
Thysanura	W = 0.6247	0.00	SQRT
Chilopoda	W = 0.6247	0.00	SQRT
Diplopoda	W = 0.96111	0.80	SQRT
Hymenoptera	W = 0.91279	0.30	
Dermaptera	W = 0.76962	0.01	SQRT
Diptera	W = 0.94366	0.59	
Hemiptera	W = 0.90438	0.24	
Thysanoptera	W = 0.624	0.00	SQRT
Isopoda	W = 0.83023	0.03	SQRT
Neuroptera	W = 0.81415	0.02	SQRT

Equal variance assumptions:

Levene's test (Abundance ~ Field)				
Order	Test Statistic	df	P-value	Transformation
Araneae	F = 0.6919	1	0.43	
Opiliones	F = 1	1	0.35	
Acari	F = 1.8968	1	0.21	SQRT
Collembola	F = 2.2155	1	0.18	
Coleoptera	F = 0.8649	1	0.38	
Diplopoda	F = 3.1334	1	0.11	
Hymenoptera	F = 5.556	1	0.05	
Dermaptera	F = 1.2857	1	0.29	
Diptera	F = 0.6606	1	0.44	
Hemiptera	F = 0.2759	1	0.61	
Thysanura	F = 1	1	0.35	
Thysanoptera	F = 1	1	0.35	
Isopoda	F = 1	1	0.35	
Neuroptera	F = 2.6667	1	0.14	

Annex 2. Statistical comparison of all orders found on all three sites from the last three samples.

All three fields				
Order	Test	Test Statistic	df	Adj P-value
Araneae	ANOVA	F = 7.11	2	0.10
Opiliones	ANOVA	F = 0.875	2	0.51
Acari	ANOVA	F = 13.18	2	0.04*
Collembola	ANOVA	F = 2.636	2	0.36
Coleoptera	ANOVA	F = 1.108	2	0.47
Chilopoda	Kruskal-Wallis	Kruskal-Wallis chi-squared = 2	2	0.47
Diplopoda	ANOVA	F = 1.358	2	0.47
Hymenoptera	ANOVA	F = 12.8	2	0.04*
Dermaptera	ANOVA	F = 0.6	2	0.58
Diptera	ANOVA	F = 3.621	2	0.28
Hemiptera	ANOVA	F = 1.471	2	0.47
Isopoda	ANOVA	F = 1.5	2	0.47

*p-value < 0.05

Normality assumptions:

Shapiro-Wilk test on residuals (Abundance ~ Field)			
Order	Test Statistic	P-value	Transformation
Araneae	W = 0.9035	0.273	
Opiliones	W = 0.91353	0.341	
Acari	W = 0.89726	0.246	
Collembola	W = 0.93047	0.485	
Coleoptera	W = 0.89511	0.225	
Chilopoda	W = 0.72821	0.003	SQRT
Diplopoda	W = 0.84208	0.061	
Hymenoptera	W = 0.89502	0.225	SQRT
Dermaptera	W = 0.87463	0.148	
Diptera	W = 0.91086	0.322	
Hemiptera	W = 0.85786	0.091	
Isopoda	W = 0.89165	0.208	

Equal variance assumptions:

Levene's test (Abundance ~ Field)			
Order	Test Statistic	df	P-value
Araneae	F = 1.7547	1	0.25
Opiliones	F = 0.1667	1	0.85
Acari	F = 0.7269	1	0.52
Collembola	F = 0.4418	1	0.66
Coleoptera	F = 1.9808	1	0.22
Chilopoda	F = 1	1	0.42
Diplopoda	F = 2.5841	1	0.16
Hymenoptera	F = 3.2039	1	0.11
Dermaptera	F = 0.6	1	0.58
Diptera	F = 0.3813	1	0.70
Hemiptera	F = 0.6964	1	0.53
Isopoda	F = 1.625	1	0.27

Annex 3. Means, standard deviations and standard errors of the means for all collections and all sites.

	means olives A	SD olives A	SE olives A	means almond	SD almond	SE almond	means olives B	SD olives B	SE olives B
Araneae	8.20	5.54	2.48	12.80	3.49	1.56	5.00	0.00	0.00
Opiliones	1.60	1.52	0.68	0.60	0.55	0.24	1.67	1.15	0.67
Acari	7.80	7.19	3.22	65.00	63.49	28.40	7.33	5.77	3.33
Collembola	28.00	15.68	7.01	63.60	35.22	15.75	30.00	22.52	13.00
Coleoptera	8.60	2.61	1.17	10.00	4.90	2.19	14.33	0.58	0.33
Hymenoptera	37.40	18.23	8.15	194.80	209.68	93.77	17.00	5.20	3.00
Thysanura	0.00	0.00	0.00	0.20	0.45	0.20	0.00	0.00	0.00
Chilopoda	0.00	0.00	0.00	0.20	0.45	0.20	0.00	0.00	0.00
Diplopoda	2.60	1.52	0.68	6.40	8.20	3.67	3.67	0.58	0.33
Dermaptera	0.80	1.10	0.49	0.20	0.45	0.20	0.00	0.00	0.00
Diptera	21.40	11.19	5.01	10.40	7.02	3.14	12.33	10.97	6.33
Hemiptera	2.60	2.30	1.03	3.00	3.39	1.52	0.00	0.00	0.00
Tysanoptera	0.00	0.00	0.00	0.20	0.45	0.20	0.00	0.00	0.00
Isopoda	1.00	1.73	0.77	0.20	0.45	0.20	0.00	0.00	0.00
Neuroptera	0.40	0.55	0.24	0.00	0.00	0.00	0.00	0.00	0.00

Annex 4. Future reference study protocol.

Field materials:
White plastic cups
Plastic plates
1.5 litre bottle with soapy water
Container or bottles per field to collect the specimens from the pitfalls
Digging devices
Short sturdy sticks (x3 per trap)
Rocks or weights

Office materials:
White tray to separate specimens
Stereoscope
Soft forceps to pick up specimens from tray
Alcohol
Preparation:
Gather the number of cups and plates needed (15 for each field). Put 2-3 squirts of liquid hand soap in a bottle of water or passata jar and then fill it completely with water. Label the jars or containers that are going to be used to collect the samples for the next day with a field they were used in (i.e. Olives A, Almonds, Olives B). Pack all of this, a pair of gloves for each person, sticks, and the digging tools, in a basket or one of the white trays to take it to the field.

Sampling design:
We went in the morning around 9am to the field to place the traps and collected them the next day around the same time, preferably when there was no rain or strong winds. We used three fields in total, olives A, almonds and olives B. Olives A and almonds are next to each other and olives B is about 600 m away from both.

Random numbers were generated using a random number generator, like the one on Google, to place each of the traps in each field. The range of the steps differed per field because of their width. Olives A was the narrowest, so 1-25 steps following the olive tree row starting from the east side of the grove were used. In the almonds field, 1-45 steps were used, starting from the east side in the almond field and also following the rows of trees. If we reached the end of the field and there were still steps remaining, then we would turn around and count the remaining steps walking back to the starting point. Only adjacent parts of almonds and olives A were sampled to make a comparison of proximate parts, disregarding the upper part that did not contain many olive trees. We decided to sample the whole area of the olives B field in order to avoid the edge effect. We started from the first olive tree on the west side of the field, again following the row of olive trees, a trap was placed at random between 0 and 100 steps.

Once the steps were counted, we started digging a hole the same size and depth as the plastic cup. The cup was then placed in the hole and the borders were covered up to the top rim at ground level (no holes around). Three short sturdy sticks (we used the olive tree suckers that grow at the base of the tree to make the sticks) were inserted in three points in the ground, forming a triangular shape around the cup. The sticks should be around the same height so the plastic plate can be placed on top of them, forming a water and sun protecting roof over the cup. Then, a rock or weight (like a clump of clay) was put over the plate to prevent it from being blown away by the wind. The pitfall trap should look like figure A.

Figure A. Pitfall trap

The GPS location of each trap was taken using Google maps (for reference when collecting) and the cups were filled 1/4 with soapy water. This was done for each trap. The traps were left overnight for a total of 24-hours and collected.

Collection and preservation:

Trap collection was done by pouring down the whole content of the cups into a large container that can hold about the same volume as the soapy water poured into 15 cups. One or two large containers can be used to collect all the traps per field. The containers should be labeled with the field from which the traps were collected (i.e. Olives A, Olives B, Almonds). We used passata jars to carry the soapy water to the field the first day and then to collect the content of the traps the next day. All the parts of the pitfall traps were collected (i.e. plates, sticks, and cups) and taken back to the office for reuse in the next sampling event. The holes on the ground were covered again with soil and rocks after picking everything up.

Once back in the office, we would immediately need to transfer the arthropods from the soapy water to alcohol to prevent their exoskeleton from dissolving. To do so, we would pour some of the content from the passata jars into large white trays (shown in Figure B) and manually picked the arthropods with featherweight entomological forceps (the flexible ones) and placed them in one properly labeled container with 80% alcohol per field.

Figure B. Separation of specimens.

Identification:

After all the specimens were transferred to containers with alcohol, we started to identify them to order level under the stereoscope following the guide of Gibb and Oseto (2006) and previous entomological knowledge. Google searches were used to support our findings from the guide with real pictures. We made a separate container with 80% alcohol for each order and field (now stored in a cardboard box in the office). The total number of individuals collected per order, field, and date were all recorded in excel. We organized the data in columns by field and collection date and rows by orders (as shown in Figure C).

	O 23.12.21	O2 23.12.21	A 23.12.21	O 06.01.22	A 06.01.22	O2 06.01.22	O 20.01.22
Araneae (spiders)	5	5	17	2	13	5	13
Opiliones	0	1	0	1	1	1	2
Acari	11	4	61	4	27	4	3
Collembola (springtails)	26	17	40	26	71	17	52
Coleoptera (beetles)	12	14	12	10	16	14	9
Hymenoptera	26	14	123	61	336	14	53
Thysanura (silverfish)	0	0	0	0	0	0	0
Chilopoda	0	0	0	0	1	0	0
Diplopoda	3	4	1	4	8	4	1
Dermaptera	2	0	1	0	0	0	0
Diptera	29	6	9	23	6	6	19
Hemiptera	6	0	1	3	1	0	1
Tysanoptera	0	0	0	0	0	0	0
Isopoda (Armadillidiidae, Roly-poly)	4	0	0	0	1	0	1
Neuroptera	0	0	0	0	0	0	0
n (total amount of arthropods)	124	65	265	134	481	65	154

Figure C. Demonstration of how the gathered data is organised O-Olives A, O2- Olives B, A-Almonds

The identification of the ants was done first by looking at the Italian field guide of ants of antweb.org to see which ants looked similar to ours. Also, Schär et al., (2020) give a list of species that have been found and confirmed with DNA barcoding in Sicily, which is a good comparison reference for the possible species that were collected. Afterward, we did a more in-depth revision for species level by looking at specific identification keys per genus or sub-family. The keys can be found in antwiki.org by searching the species-genus or subfamily and “key”, antwiki will provide you with multiple nomenclature keys extracted from published scientific articles. Notes on the specimens that we captured and their identification can be found in the Google Drive mentioned in the supplementary information.

Data analysis:

Statistical analysis was undertaken using RStudio and Microsoft Excel to obtain diversity indices and test for data significance. We used the R codes generated by Gersey Vargas (Annex 5). For the diversity indices, the data was organized in columns by order and row by field, only the averages were used. For the statistical test, the raw data was organized in three columns: field, order and abundance (Figure D). For the PCA, the data was organized in columns by fields and rows by orders, only averages were used.

Field	Order	Abundance
Olives A	Araneae	5
Olives A	Araneae	2
Olives A	Araneae	13
Olives A	Opiliones	0
Olives A	Opiliones	1
Olives A	Opiliones	2
Olives A	Acari	11
Olives A	Acari	4
Olives A	Acari	3
Olives A	Collembo	26

Figure D. Demonstration on how to organise the data for R analysis.

All documents (excel with data and results, pictures of specimens, GPS positions of all the traps during each field visit, scientific papers cited) can be accessed through Google Drive following this link:
https://drive.google.com/drive/folders/1fq01UlgN1wvxpBgw2RQh2SAEsai66YF1?usp=sharing

Annex references:

Schär, S., Menchetti, M., Schifani, E., Hinojosa, J.C., Platania, L., Dapporto, L. and Vila, R., 2020. Integrative biodiversity inventory of ants from a Sicilian archipelago reveals high diversity on young volcanic islands (Hymenoptera: Formicidae). *Organisms Diversity & Evolution*, *20*(3), pp.405-416.

Annex 5. R codes

Diversity indices for the all orders Almonds vs Olives A:

```
Data <- read.csv("FieldvsOrder_Av_Data.csv")
orders <- Data[,2:16]
persabs<-decostand(orders, "pa") #pa makes the data into presence/absence data
richness<-specnumber(persabs)
H <- diversity(orders, index = "shannon")
Simpson <- diversity(orders, index = "simpson")
J <- H/log(specnumber(orders))
info_table<-cbind(Data$ï..Field,richness,H,Simpson,J)
write.csv(info_table, "diversity_info_last3.csv")
vegdist(persabs, method = "jaccard", binary = FALSE)
```

Diversity indices for the all orders of last three samplings of each field:

```
Data <- read.csv("FieldvsOrder_Data_last3.csv")
orders <- Data[,2:13]
persabs<-decostand(orders, "pa") #pa makes the data into presence/absence data
richness<-specnumber(persabs)
```

```
H <- diversity(orders, index = "shannon")
Simpson <- diversity(orders, index = "simpson")
J <- H/log(specnumber(orders))
info_table<-cbind(Data$ï..Field,richness,H,Simpson,J)
write.csv(info_table, "diversity_info_last3.csv")
vegdist(persabs, method = "jaccard", binary = FALSE)
```

Diversity indices for ants:

```
Ants <- read.csv("Ants_fields.csv")
species <- Ants[,2:12]
richness<-specnumber(species)
H <- diversity(species, index = "shannon")
Simpson <- diversity(species, index = "simpson")
J <- H/log(specnumber(species))
info_table <-cbind(Ants$Field,richness,H,Simpson,J)
write.csv(info_table, "diversity_info_ants.csv")
vegdist(species, method = "jaccard", binary = FALSE)
```

Statistical comparisons of variance Almonds vs Olives A:

```
Data3 <- read.csv("Raw_data_twocolumns_avso.csv")
Araneae <- Data3[Data3$Order == "Araneae",]
Opiliones <- Data3[Data3$Order == "Opiliones",]
Acari <- Data3[Data3$Order == "Acari",]
Collembola <- Data3[Data3$Order == "Collembola",]
Coleoptera <- Data3[Data3$Order == "Coleoptera",]
Thysanura <- Data3[Data3$Order == "Thysanura",]
Chilopoda <- Data3[Data3$Order == "Chilopoda",]
Diplopoda <- Data3[Data3$Order == "Diplopoda",]
Hymenoptera <- Data3[Data3$Order == "Hymenoptera",]
Dermaptera <- Data3[Data3$Order == "Dermaptera",]
Diptera <- Data3[Data3$Order == "Diptera",]
Hemiptera <- Data3[Data3$Order == "Hemiptera",]
Tysanoptera <- Data3[Data3$Order == "Tysanoptera",]
Isopoda <- Data3[Data3$Order == "Isopoda",]
Neuroptera <- Data3[Data3$Order == "Neuroptera",]

shapiro.test((aov(Araneae$Abundance ~ Araneae$Field))$residuals) #normal, p-value > 0.05
shapiro.test((aov(Opiliones$Abundance ~ Opiliones$Field))$residuals) #normal
shapiro.test((aov(sqrt(Acari$Abundance) ~ Acari$Field))$residuals) #normal with sqrt
transformation
shapiro.test((aov(Collembola$Abundance ~ Collembola$Field))$residuals) #normal
shapiro.test((aov(Coleoptera$Abundance ~ Coleoptera$Field))$residuals) #normal
shapiro.test((aov(sqrt(Thysanura$Abundance) ~ Thysanura$Field))$residuals) #not normal
shapiro.test((aov(sqrt(Chilopoda$Abundance) ~ Chilopoda$Field))$residuals) #not normal
shapiro.test((aov(sqrt(Diplopoda$Abundance) ~ Diplopoda$Field))$residuals) #normal with sqrt
transformation
shapiro.test((aov(Hymenoptera$Abundance ~ Hymenoptera$Field))$residuals) #normal
```

```
shapiro.test((aov(Hemiptera$Abundance ~ Hemiptera$Field))$residuals) #normal
shapiro.test((aov(Diptera$Abundance ~ Diptera$Field))$residuals) #normal
shapiro.test((aov(sqrt(Dermaptera$Abundance) ~ Dermaptera$Field))$residuals) #not normal
shapiro.test((aov(sqrt(Tysanoptera$Abundance) ~ Tysanoptera$Field))$residuals) #not normal
shapiro.test((aov(sqrt((Isopoda$Abundance)) ~ Isopoda$Field))$residuals) #not normal
shapiro.test((aov(sqrt(Neuroptera$Abundance) ~ Neuroptera$Field))$residuals) #not normal

leveneTest(Araneae$Abundance ~ Araneae$Field) #equal
leveneTest(Opiliones$Abundance ~ Opiliones$Field) #equal
leveneTest(sqrt(Acari$Abundance) ~ Acari$Field) #equal
leveneTest(Collembola$Abundance ~ Collembola$Field) #equal
leveneTest(Coleoptera$Abundance ~ Coleoptera$Field) #equal
leveneTest(sqrt(Diplopoda$Abundance) ~ Diplopoda$Field) #equal
leveneTest(Hemiptera$Abundance ~ Hemiptera$Field) #equal
leveneTest(Hymenoptera$Abundance ~ Hymenoptera$Field) #not equal
leveneTest(Diptera$Abundance ~ Diptera$Field) #equal
leveneTest(Thysanura$Abundance ~ Thysanura$Field) #equal
leveneTest(Dermaptera$Abundance ~ Dermaptera$Field) #equal
leveneTest(Tysanoptera$Abundance ~ Tysanoptera$Field) #equal
leveneTest(Isopoda$Abundance ~ Isopoda$Field) #equal
leveneTest(Neuroptera$Abundance ~ Neuroptera$Field) #equal

oneway.test(Hymenoptera$Abundance ~ Hymenoptera$Field, var.equal = FALSE) #not
significant
kruskal.test(Thysanura$Abundance ~ Thysanura$Field) #not significant
kruskal.test(Chilopoda$Abundance ~ Chilopoda$Field) #not significant
kruskal.test(Dermaptera$Abundance ~ Dermaptera$Field) #not significant
kruskal.test(Tysanoptera$Abundance ~ Tysanoptera$Field) #not significant
kruskal.test(Isopoda$Abundance ~ Isopoda$Field) #not significant
kruskal.test(Neuroptera$Abundance ~ Neuroptera$Field) #not significant
summary(aov(Araneae$Abundance ~ Araneae$Field)) #not significant
summary(aov(Opiliones$Abundance ~ Opiliones$Field)) #not significant
summary(aov(sqrt(Acari$Abundance) ~ Acari$Field)) #significant
summary(aov(Collembola$Abundance ~ Collembola$Field)) #not significant
summary(aov(Coleoptera$Abundance ~ Coleoptera$Field)) #not significant
summary(aov(sqrt(Diplopoda$Abundance) ~ Diplopoda$Field)) #not significant
summary(aov(Hemiptera$Abundance ~ Hemiptera$Field)) #not significant
summary(aov(Diptera$Abundance ~ Diptera$Field)) #not significant
```

Statistical comparisons of variance from the last three samplings of all the fields:

```
Data3 <- read.csv("Raw_data_twocolumns_all.csv")
Araneae <- Data3[Data3$Order == "Araneae",]
Opiliones <- Data3[Data3$Order == "Opiliones",]
Acari <- Data3[Data3$Order == "Acari",]
Collembola <- Data3[Data3$Order == "Collembola",]
Coleoptera <- Data3[Data3$Order == "Coleoptera",]
Thysanura <- Data3[Data3$Order == "Thysanura",]
Chilopoda <- Data3[Data3$Order == "Chilopoda",]
```

```
Diplopoda <- Data3[Data3$Order == "Diplopoda",]
Hymenoptera <- Data3[Data3$Order == "Hymenoptera",]
Dermaptera <- Data3[Data3$Order == "Dermaptera",]
Diptera <- Data3[Data3$Order == "Diptera",]
Hemiptera <- Data3[Data3$Order == "Hemiptera",]
Tysanoptera <- Data3[Data3$Order == "Tysanoptera",]
Isopoda <- Data3[Data3$Order == "Isopoda",]
Neuroptera <- Data3[Data3$Order == "Neuroptera",]

shapiro.test((aov(Araneae$Abundance ~ Araneae$Field))$residuals) #normal, p-value > 0.05
shapiro.test((aov(Opiliones$Abundance ~ Opiliones$Field))$residuals) #normal
shapiro.test((aov(Acari$Abundance ~ Acari$Field))$residuals) #normal
shapiro.test((aov(Collembola$Abundance ~ Collembola$Field))$residuals) #normal
shapiro.test((aov(Coleoptera$Abundance ~ Coleoptera$Field))$residuals) #normal
shapiro.test((aov(sqrt(Chilopoda$Abundance) ~ Chilopoda$Field))$residuals) #not normal with
SQRT transformation
shapiro.test((aov(Diplopoda$Abundance ~ Diplopoda$Field))$residuals) #normal
shapiro.test((aov(sqrt(Hymenoptera$Abundance) ~ Hymenoptera$Field))$residuals) #normal
with SQRT transformation
shapiro.test((aov(Hemiptera$Abundance ~ Hemiptera$Field))$residuals) #normal
shapiro.test((aov(Diptera$Abundance ~ Diptera$Field))$residuals) #normal
shapiro.test((aov(Dermaptera$Abundance ~ Dermaptera$Field))$residuals) #normal
shapiro.test((aov(Isopoda$Abundance ~ Isopoda$Field))$residuals) #normal
#removed Neuroptera, Thysanoptera and Thysanura because they only had 0s as values

leveneTest(Araneae$Abundance ~ Araneae$Field) #equal
leveneTest(Opiliones$Abundance ~ Opiliones$Field) #equal
leveneTest(Acari$Abundance ~ Acari$Field) #equal
leveneTest(Collembola$Abundance ~ Collembola$Field) #equal
leveneTest(Coleoptera$Abundance ~ Coleoptera$Field) #equal
leveneTest(Chilopoda$Abundance ~ Chilopoda$Field) #equal
leveneTest(Diplopoda$Abundance ~ Diplopoda$Field) #equal
leveneTest(Hemiptera$Abundance ~ Hemiptera$Field) #equal
leveneTest(Hymenoptera$Abundance ~ Hymenoptera$Field) #equal
leveneTest(Diptera$Abundance ~ Diptera$Field) #equal
leveneTest(Dermaptera$Abundance ~ Dermaptera$Field) #equal
leveneTest(Isopoda$Abundance ~ Isopoda$Field) #equal

kruskal.test(sqrt(Chilopoda$Abundance) ~ Chilopoda$Field) #not significant
summary(aov(sqrt(Hymenoptera$Abundance) ~ Hymenoptera$Field)) #significant
summary(aov(Dermaptera$Abundance ~ Dermaptera$Field)) #not significant
summary(aov(Isopoda$Abundance ~ Isopoda$Field)) #not significant
summary(aov(Araneae$Abundance ~ Araneae$Field)) #significant
summary(aov(Opiliones$Abundance ~ Opiliones$Field)) #not significant
summary(aov(Acari$Abundance ~ Acari$Field)) #significant
summary(aov(Collembola$Abundance ~ Collembola$Field)) #not significant
summary(aov(Coleoptera$Abundance ~ Coleoptera$Field)) #not significant
summary(aov(Diplopoda$Abundance ~ Diplopoda$Field)) #not significant
summary(aov(Hemiptera$Abundance ~ Hemiptera$Field)) #not significant
```

```
summary(aov(Diptera$Abundance ~ Diptera$Field)) #not significant
```

P-value adjustment:

```
#adjust p-values due to possible pairwise effect
pvalues_avso <- read.csv("Pvalues_avso.csv")
pvalues_all <- read.csv("Pvalues_all.csv")

#Benjamini and Hochberg (BH) is the most preferable approach is controlling false discovery rate as it not only reduces false positives, but also minimises false negatives.
p.adjust(c(pvalues_avso[,1]), method = "BH")
p.adjust(c(pvalues_all[,1]), method = "BH")
```

Post-hoc test:

```
acari <- aov(Acari$Abundance ~ Acari$Field)
hymen <- aov(sqrt(Hymenoptera$Abundance) ~ Hymenoptera$Field)
TukeyHSD(acari, conf.level=.95)
TukeyHSD(hymen, conf.level=.95)
```

PCA with averages from the last three samplings of all the fields:

```
install.packages("factoextra")
library(factoextra)
library(labdsv)
Data <- read.csv("OrdervsField_Data_last3.csv")
orders_h <- hellinger(Data[,2:4])
model <- prcomp(orders_h, center = TRUE, scale=TRUE)
dimnames(model$x)[[1]] <- Data$Order
summary(model)
plot(model)
fviz_pca_biplot(model, labelsize = 5, geom = "text", col.var = "steelblue", repel=TRUE, geom.var = "text", xlab="PC1 (62%)", ylab="PC2 (35%)")

png("C:/Users/gerse/Documents/Giacche Verdi/Biodiv assessment/R/PCA_all_last3.png", width=22, height=12, unit="cm", res=1000) #opens plotting into a new file of certain dimensions and resolution, don’t forget to add the name and file format at the end of the link
fviz_pca_biplot(model, labelsize = 5, geom = "text", col.var = "steelblue", repel=TRUE, geom.var = "text", xlab="PC1 (62%)", ylab="PC2 (35%)")
dev.off() #closes drawing (now you can open the file), this is to save the graphs as images
```

Annex 6. List of ant species found during the study and in which fields. 1 = present, 0 = absent.

Subfamily	Species	Almonds	Olives A	Olives B
Dolichoderinae	*Tapinoma* cf. *nigerrimum*	1	1	1
Formicinae	*Camponotus pilicornis siculus* Grandi, 1935	0	0	1
Formicinae	*Plagiolepis pygmaea* Latreille, 1798	0	1	1
Myrmicinae	*Aphaenogaster semipolita* Nylander, 1856	1	1	0
Myrmicinae	*Aphaenogaster subterraneosplendida* André, 1883	0	1	0
Myrmicinae	*Crematogaster laestrygon* Emery, 1869	1	1	1
Myrmicinae	*Crematogaster sordidula* Nylander, 1849	1	1	1
Myrmicinae	*Messor capitatus* Latreille, 1798	1	1	1
Myrmicinae	*Pheidole pallidula* Nylander, 1849	1	1	1
Myrmicinae	*Solenopsis latro* Forel, 1894	1	0	0
Myrmicinae	*Tetramorium* cf. *semilaeve*	1	1	0

Comment: *Tetramorium* cf. *semilaeve* was not fully determined because we believe it is most likely *Tetramorium semilaeve* André, 1883, but there is still some doubt if it is *Tetramorium punctatum* Santschi, 1927. Similarly, *Tapinoma* cf. *nigerrimum* can be either *Tapinoma nigerrimum* Nylander, 1856 or *Tapinoma erraticum* Latreille, 1798. Assistance from a Myrmecologist or somebody with more experience with Mediterranean or Sicilian ants would be beneficial to confidently determine which species they are.