

Cicadomorpha community (Hemiptera: Auchenorrhyncha) in different agroecosystems in the north of Portugal

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Abstract

1. The infraorder Cicadomorpha is a diverse group comprising several species considered important pests of economic crops and species that may act as vectors of plant pathogens. In Europe, the gram-negative bacterium *Xylella fastidiosa* is one of the most important and severe insect-borne plant pathogens associated with the infraorder Cicadomorpha.
2. Therefore, the knowledge of the abundance and diversity of native Cicadomorpha insect vectors related to the different agroecosystems is essential to design and implementing specific measures to control insect-borne plant pathogens.
3. In this work, in two consecutive years (2018 and 2019), five almond orchards, five vineyards, five olive orchards, and five scrublands distributed in the North of Portugal were sampled in three different periods (early summer, summer and autumn) to investigate species composition, richness, and diversity of the Cicadomorpha community. Also, in 2019, five lemon orchards were sampled.
4. A total of 6056 individuals were collected (2322 in 2018 and 3734 in 2019), belonging to 71 species of three families. Observing several considered pests or vectors of vicious pathogens within these species was possible. The confirmed vectors of *X. fastidiosa* (*Philaenus spumarius* (Linnaeus, 1758) and *Neophilaenus campestris* (Fallen, 1805)) were captured in all agroecosystems and, in general, with more abundance in autumn. The highest abundance, richness, and diversity of Cicadomorpha were observed in vineyards. However, these parameters (abundance, richness and diversity) significantly differed between the agroecosystem and sampling period.
5. Further research on how the composition of the vegetation cover shapes the Cicadomorpha community is essential to implement strategies to reduce the spread of insect-borne pathogens if they are introduced into agroecosystems.

KEYWORDS

Cicadellidae, insect-borne pathogens, *Philaenus spumarius*, *Xylella fastidiosa*

INTRODUCTION

Most microbial plant pathogens, including bacteria, fungi, and viruses, are transmitted and disseminated through the landscape by insect

vectors (Eigenbrode et al., 2018). Consequently, insect-borne plant pathogens are an increasing concern in worldwide agriculture since they are causal agents of devastating diseases that threaten the economy, diversity, and public health (Anderson et al., 2004;

Huang et al., 2020; Schneider et al., 2020; Tumber et al., 2014). Diseases derived from pathogens and phytophagous arthropods are estimated to be responsible for more than 20% of the world's crop yield losses, which could feed one billion people annually (Ye et al., 2021).

The infraorder Cicadomorpha is a highly diverse group with more than 30,000 species described worldwide (Dietrich, 2002). This infraorder comprises several species that are considered pests in important economic crops; that, through feeding and oviposition cause damage to plants (e.g., Alaserhat, 2021; Atakan, 2009; Backus, 1988; Decante & van Helden, 2006) while many of them act as vectors of plant pathogens (e.g. Chuche & Thiéry, 2014; Kyrkou et al., 2018; Nielson, 1968; Redak et al., 2004). For example, species of the infraorder Cicadomorpha are reported to transmit several phytoplasmas responsible for important economic diseases, such the 'Candidatus Phytoplasma vitis', 'Candidatus Phytoplasma phoenicium' and *Spiroplasma citri* (Abu Alloush et al., 2023; Bertaccini et al., 1995; EFSA PLH Plane, 2014). The phytoplasma 'Candidatus Phytoplasma vitis' is responsible for the Flavescence dorée (Bertaccini et al., 1995), one of the most important diseases in the European vineyards, and it is transmitted by the insect *Scaphoideus titanus* Ball (Chuche & Thiéry, 2014). 'Candidatus Phytoplasma phoenicium' is the causal agent of the almond witches-broom, a quarantine pest currently reported only in Iran and Lebanon (Abu Alloush et al., 2023). The phytoplasma *Spiroplasma citri* is transmitted by *Circulifer tenellus* (Baker, 1896) and is currently distributed in the United States, Northern Africa, the Mediterranean countries, and Southeast Asia, where it is responsible for the citrus disease known as "Stubborn disease" (EFSA PLH Plane, 2014). Furthermore, Cicadomorpha individuals can also transmit viruses and bacteria to monocots and dicot plants (Eigenbrode et al., 2018; Ye et al., 2021). However, in Europe, one of the most important and severe insect-borne plant pathogens associated with the infraorder Cicadomorpha is the bacterium *Xylella fastidiosa* Wells (Xanthomonadales: Xanthomonadaceae) (Huang et al., 2020).

Xylella fastidiosa is a xylem limit bacterium native to the Americas, responsible for several diseases in crops of economic importance, such as the Almond Leaf Scorch, Olive Quick Decline Syndrome, Pierce's disease in the vine and Citrus Variegated Chlorosis (Hopkins & Purcell, 2002; Saponari et al., 2013). Currently, there is no cure for this insect-borne pathogen, and it is expected that in the absence of proper control measures, the total economic loss in Europe can reach up to 1.9 billion Euros over the next 50 years (Schneider et al., 2020). Therefore, implementing sustainable measures to manage insect populations is perceived to be the main tool to limit the spread of this bacterium (Schneider et al., 2020). This bacterium is transmitted exclusively by xylem sap-feeding specialists (Almeida et al., 2005). They have sucking mouthparts (mandibular and maxillary stylets) that allow them to reach the xylem of plants, from which they ingest sap (EFSA, 2013). In Europe, more than 90 species are described as xylem sap-feeding specialists, and these insects belong to the infraorder Cicadomorpha superfamilies Cercopoidea and Cicadoidea and the family Cicadellidae (subfamily Cicadellinae) (Cornara et al., 2019; EFSA, 2013).

In Portugal, the knowledge of potential vectors of *X. fastidiosa* is still very incipient; however, there is an urgent requirement to know the diversity and abundance of potential vectors in the Portuguese agroecosystems since this pathogen was reported for the first time in 2019 in the north of the country, and several new outbreaks, have been reported (DGAV, 2022). In these outbreaks, the bacterium was confirmed in more than 80 host plants, including plants of economic importance, such as almonds, olives, vines, and citrus (DGAV, 2022; EFSA et al., 2022). These crops are characteristic of the landscape of the northern region of the country, where some have a long tradition and represent the main means of survival for farmers in the region. Therefore, establishing and spreading this bacterium throughout Portugal can have devastating effects on the cultural and agricultural sectors. The dissemination of this pathogen into a new area depends on host plant availability, climatic conditions, and the presence of its vectors (Almeida & Nunney, 2015).

Therefore, the objectives of the present study were to characterize the abundance and diversity of the Cicadomorpha community associated with almonds, olives, and citrus orchards, vineyards and natural areas surrounding the olive groves.

MATERIALS AND METHODS

Study area

Field surveys were conducted in five almond orchards, five vineyards, five olive orchards, five scrublands (surrounding the olive groves) in two consecutive years (2018 and 2019) and five lemon orchards (hereinafter referred to as citrus orchards), only in 2019, distributed in the North of Portugal (Figure 1). All agroecosystems have sustainable production systems. No herbicide, fungicide, or insecticide was applied during the sampling periods, and herbaceous vegetation cover was maintained in the inter-rows.

The herbaceous vegetation cover of the almond orchards was dominated by plant species such *Chrysanthemum segetum* L., *Anthemis cotula* L., *Calendula arvensis* L., *Coleostephus myconis* (L.) Rchb.f., *Lolium rigidum* subsp. *rigidum* Gaudin, and *Bromus madritensis* L.

The herbaceous vegetation of the vineyards was characterized by *Papaver* sp., *Avena barbata* Link in Schrad, *Cynodon dactylon* (L.) Pers., *Bromus tectorum* L., *Lathyrus angulatus* L., *Hypericum perforatum* L., *C. myconis*, and *Trifolium* sp.

The herbaceous vegetation of the olive orchards was dominated by plant species such *Sonchus tenerrimus* L., *C. myconis*, *Bromus diandrus* Roth, *C. dactylon*, *Medicago* sp., *Ornithopus compressus* L., and *Rumex bucephalophorus* L. The scrublands sampled were characterized by the herbaceous stratum dominated by plants belonging to Asteraceae and Poaceae families, the shrub stratum dominated by plant species such as *Cistus ladanifer* L., *Lavandula pedunculata* (Mill.) Cav., *Rubus ulmifolius* Schott, and *Crataegus monogyna* Jacq, and the tree stratum dominated by *Quercus rotundifolia* Lam., *Quercus pyrenaica* Willd., and *Arbutus unedo* L.

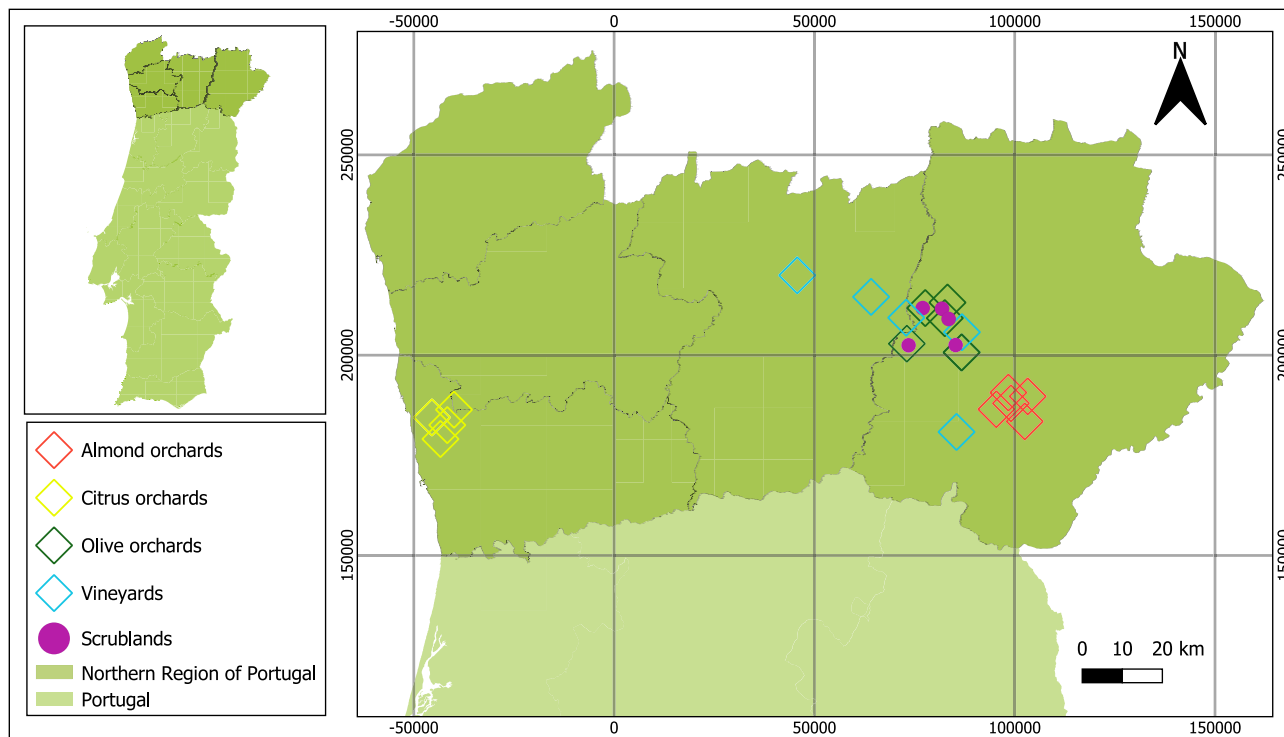


FIGURE 1 Location of the sampled agroecosystems. Map projected in ETRS89/PT-TM06.

And finally, the herbaceous stratum of the citrus orchards was dominated by plant species such *C. dactylon*, *B. diandrus*, *Echium plan-tagineum* L., *Crepis capillaris* (L.) Wallr., *Dactylis glomerata* L., *Hedera hibernica* (G. Kirchn.) Bean, and *R. ulmifolius*. More details of the study sites are provided in Table S1.

Sampling of insects

In each year and agroecosystem, adults of the Cicadomorpha infraorder were sampled in three different periods: early summer, summer, and autumn. The sampling of adults was performed in the natural ground vegetation and the canopy of the plants with an entomological sweep net (38 cm diameter). In each sampling date in all the sampling sites, randomly distributed over 1 ha, 10 samples of 10 consecutive sweeps were collected in the ground vegetation. Each sweep was performed by moving the entomological sweep 180 degrees. In the canopy of the plants of all sampling orchards, 10 samples of two sweeps in six randomly selected trees were analysed. Furthermore, 10 samples of 50 sweepings were collected in the canopy of the vineyards. Each sample was performed in a 40-m transect. The content of the sweepings was emptied into a plastic bag properly labelled and sealed. All samples were frozen at -20°C . The arthropods were separated under a stereoscopic microscope and preserved in ethanol 96% until further identification. For species identification, the male genitalia was dissected and placed in a heated solution of potassium hydroxide (KOH) at 10%. Subsequently, each genitalia was mounted in glass slides with glycerine and observed under a stereoscopic microscope.

The taxonomic classification was based on appropriate keys and illustrations (Biedermann & Niedringhaus, 2009; Dietrich, 2005; Dmitry, 2006; Le Quesne & Payne, 1981; Nielson, 1968), and confirmed by specialists. Voucher species were deposited in the collection of the Agriculture School of the Polytechnic Institute of Bragança.

Data analysis

The data for each year of the study, 2018 and 2019, were treated independently to avoid bias from the interannual variability. All statistical analyses and modelling were performed in R (R Core Team, 2020). The R package 'bipartite' was used to visualize the presence of the species in the different agroecosystems.

The total Cicadomorpha abundance (N), the species richness (S), and the Shannon index (H') per sampling period were calculated. The species richness was calculated as the number of species/morphospecies in each agroecosystem per sampling period, and the Shannon-Wiener index (H') was calculated according to the following formula: $H' = -\sum [P_i \log(P_i)]$, where $P_i = n_i/N$, n_i = the number of individuals of a species, N = a total number of individuals.

The effect of the agroecosystem and sampling period on the abundance and diversity of Cicadomorpha was investigated using general linear mixed models (GLMMs), followed by a post hoc multiple comparisons analysis ($\alpha = 0.05$). Agroecosystems (almond orchards, vineyards, olive orchards, scrublands, and citrus orchards), the sampling period (early summer, summer and autumn) and the interaction between the two terms were used as explanatory variables and the

abundance, richness and the Shannon index as response variables; the sampling plots were used as a random factor. Negative binomial distribution: quadratic parameterisation (nbinom2) was used to account for the overdispersion. The function `glmmTMB` from the “`glmmTMB`” package was used for fitting the models (Brooks et al., 2017). Models were validated using the `simulateResiduals` function from the “`DHARMA`” package (Hartig, 2023).

A non-metric multidimensional scaling (NMDS) was carried out using the Bray-Curtis's index (999 permutations) in order to assess the variability in the Cicadomorpha community along the Agroecosystems and sampling periods. In addition, a permutational multivariate analysis of variance (PERMANOVA) was performed, using the function `adonis` from the package “`vegan`” to corroborate the results of the NMDS.

A co-inertia analysis (“cross-table” multivariate analysis) was performed in order to determine the relationship between Cicadomorpha species/morphospecies and the agroecosystem and sampling period. This analysis was performed using the “`ade4`” package and the `table` value function to visualize the results.

Finally, species accumulation curves were drawn to assess the sampling effort in function of the number of sweeps performed. Species accumulation curves were computed using the `specaccum` function of the “`vegan`” package.

RESULTS

In total, 6056 individuals of the infraorder Cicadomorpha were collected, of which 2322 in 2018 and 3734 in 2019 (Table S2 and Table S3). Over the 2 years of study, 71 species/morphospecies were identified (54 in 2018 and 63 in 2019).

In almond orchards, 1691 individuals (701 in 2018 and 990 in 2019) belonging to 42 species (28 species in 2018 and 36 in 2019) were recovered. In 2018, *Fruticidia sanguinosa* (Rey, 1891) (204 individuals) was the most abundant species, followed by *Zygina schneideri* (Günthart, 1974) (143 individuals) and *Psammotettix* sp. (125 individuals). Whereas in 2019, the species: *Psammotettix* sp. (516 individuals), *Fruticidia bisignata* (Mulsant & Rey, 1855) (105 individuals), *F. sanguinosa* (87 individuals) were the most abundant. The species *F. bisignata*, *F. sanguinosa* and *Z. schneideri* were only present in the almond agrosystem (Figure 2).

In vineyards, 2908 individuals (1244 in 2018 and 1664 in 2019) belonging to 44 species (36 species in 2018 and 32 in 2019) were recovered. *Psammotettix* sp. (460 in 2018 and 321 in 2019), *Empoasca vitis* (Göthe, 1875) (311 in 2018 and 806 in 2019), and *Empoasca* sp. (Herrich-Schäffer, 1838) (149 in 2018 and 131 in 2019) were the most abundant species/morphospecies. Moreover, the species *E. vitis*, *Jacobiasca lybica* (Bergevin & Zanon 1922) and *S. titanus* (only in 2019) were only present in this agroecosystem (Figure 2).

In olive orchards, 568 individuals (208 in 2018 and 360 in 2019) belonging to 41 species (26 species in 2018 and 34 in 2019) were recovered. *Psammotettix* sp. was the most abundant morphospecies in both years of study (217 in 2018 and 204 in 2019). *Anoplotettix*

sp. (Flor, 1861) was the only captured just in the olive grove in both sampling years (Figure 2).

In the scrublands, 336 individuals (169 in 2018 and 167 in 2019) belonging to 31 species were recovered (20 species in 2018 and 27 in 2019). *Circulifer tenellus*, *Selenocephalus sacarroi* Rodrigues, 1968, and *Centrotus cornuta* Linnaeus, 1758 were the most abundant species; that were only present in this agroecosystem.

In the citrus orchard, 553 individuals belonging to 23 species were recovered. *Zyginidia scutellaris* (Herrich-Schäffer, 1838) (226 individuals) was the most abundant species, followed by *Psammotettix* sp. (92 individuals) and *P. spumarius* (55 individuals). Nevertheless, species such *Cicadella viridis* (Linnaeus, 1758) were only captured in the citrus agroecosystem (Figure 2).

Regarding the vectors and potential vectors of *X. fastidiosa*, these were present in different agroecosystems (Figure 2).

In 2018 and 2019, in the different sampling periods, the abundance and richness of Cicadomorpha significantly differed between agroecosystems (Table 1). In 2018, the agroecosystem vineyard showed the highest abundance and richness in the early summer and autumn (Figure 2). However, in summer, the almond orchard showed more abundance of Cicadomorpha than the olive orchard. Furthermore, the olive orchard showed a lower species richness in this sampling period (Figure 2). Concerning Shannon's index, the sampling period did not affect the Cicadomorpha diversity since only significant differences were observed between agrosystems. The olive orchard and the scrublands presented the lowest diversity ($p < 0.01$).

In 2019, the agroecosystem vineyard had a higher abundance of Cicadomorpha than the Citrus orchards and olive orchards in the early summer and autumn and a higher abundance than the scrublands in the summer. In addition, in general, citrus orchards, olive orchards and scrublands showed a statistically significantly lower richness and diversity (H') than the agroecosystem vineyard, except for scrublands in early summer (Table 1 and Figure 3).

NMDS suggested a change in the Cicadomorpha community between agroecosystems per sampling period in both years under study (Figure 4). Indeed, the PERMANOVA analysis showed significant differences between agroecosystems per sampling period ($df = 6$; $F = 2.15$; $p < 0.01$ for 2018, and $df = 8$; $F = 1.46$; $p < 0.01$ for 2019).

In order to further explore the Cicadomorpha species associated with each agroecosystem and sampling period and to evaluate the contribution of these aspects to the structure of the Cicadomorpha community, co-inertia analyses were performed (Figures 5 and 6). The results obtained in the co-inertia analysis corroborate with the previously observed in the NMDS and PERMANOVA analyses. In 2018, the Cicadomorpha community composition observed in the almond orchard, olive grove, and scrublands were distinctly different from that observed in the vineyard (Figure 5a). The species *Psammotettix* sp., *E. vitis*, and *J. lybica* were positively correlated with the agroecosystem vineyards, *F. sanguinosa*, *P. spumarius*, *N. camprestris*, and *N. lineatus* were positively correlated with the almond orchard. In addition, *C. comotus* was positively correlated with the scrublands and *Goniagnathus brevis* (Herrich-Schäffer, 1835) with the

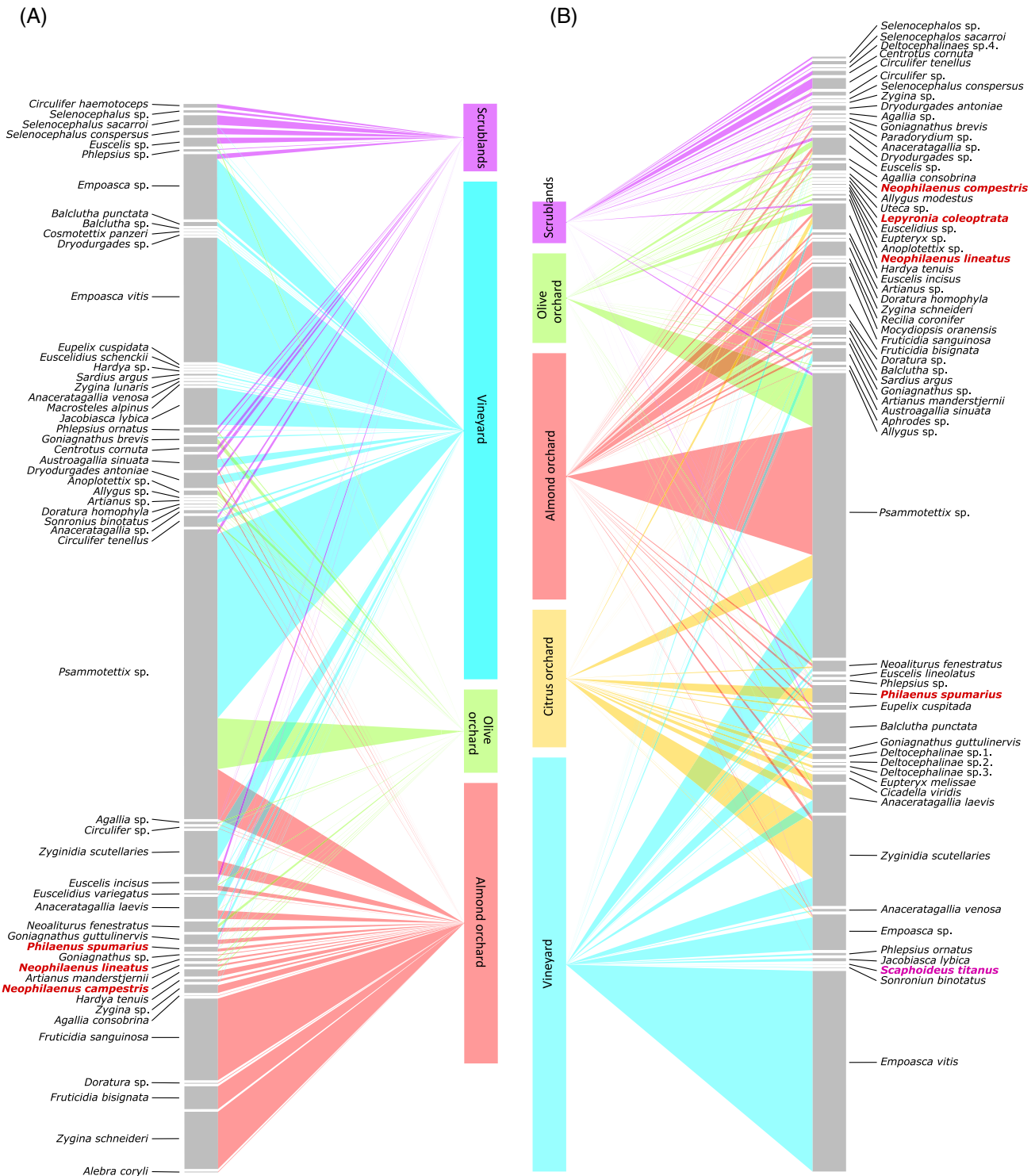


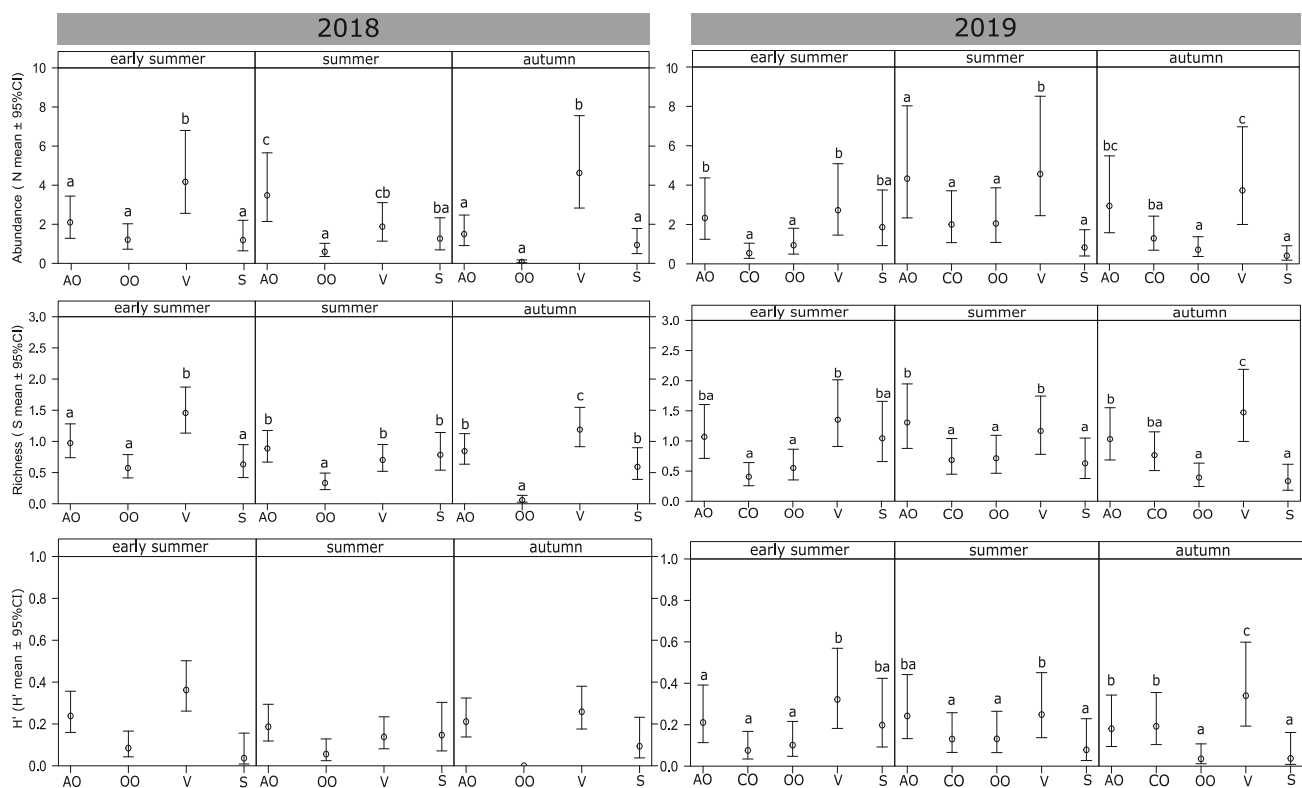
FIGURE 2 Cicadomorpha species/morphospecies–agroecosystem type interaction networks for (A) 2018 and (B) 2019. Red represents the potential vectors of *Xylella fastidiosa*, and pink is the vector of the Flavescence dorée phytoplasma. Information on the family and subfamily of each species is available at Tables S2 and S3.

olive orchard (Figure 5a). In 2019, vineyards and almond orchards showed a distinctly different community. In contrast, no differences were observed in olive groves and scrub communities. The species *J. lybica*, *E. vitis*, and *S. titanus* had a positive correlation with vineyards, the species *F. bisignata* and *Anaceratagalia* sp. had a

positive correlation with almond orchards, *C. cornuta* once again is positively correlated with scrublands. The potential vectors of *X. fastidiosa*, *N. campestris*, *N. lineatus*, and *L. coleoptera* were positively correlated with olive orchards, and *C. viridis* and *P. spumarius* were positively correlated with citrus orchards (Figure 5b).

TABLE 1 Results of the GMMs developed for the effect of the agroecosystem and sampling period as their interaction on the Cicadomorpha abundance, species richness and diversity (Shanon index (H')) in 2018 and 2019.

Explanatory variables	Response variable	2018			2019		
		df	χ^2	p	df	χ^2	p
Agroecosystems	Abundance	3	32.59	<0.01	4	14.16	<0.01
Period		2	20.03	<0.01	2	33.77	<0.01
Agroecosystems: period		6	66.07	<0.01	8	44.5	<0.01
Agroecosystems	Richness	3	40.38	<0.01	4	16.69	<0.01
Period		2	15.67	<0.01	2	1.23	0.53
Agroecosystems: period		6	37.37	<0.01	8	38.37	<0.01
Agroecosystems	H'	3	23.75	<0.01	4	8.96	0.06
Period		2	5.14	0.07	2	0.12	0.93
Agroecosystems: period		6	8.35	0.21	8	19.86	0.01

**FIGURE 3** Cicadomorpha abundance, species richness and diversity (Shanon index (H')) (mean \pm 95% CI) in the different agroecosystems (AO) almond orchard, (OO) olive orchard, (V) vineyard, (S) scrublands and (CO) citrus orchard per sampling period in 2018 and 2019.

The sampling period was also found to impact the structure of the Cicadomorpha community. In both sampling years, it was found that the community of Cicadomorpha present at the beginning of summer distinctly differs from that in summer and autumn (Figure 6). However, the Cicadomorpha community is not very different in the summer and autumn. In 2018, 30 species were positively correlated with the early summer; the most positively correlated species are *N. fenestratus*, *Artianus manderstjernii* (Kirschbaum, 1868) and species of the genus *Selenocephalus*.

Philaenus spumarius also showed a positive correlation with this sampling period (Figure 6a).

In 2019, 28 species showed a positive correlation with the early summer; species of the genus *Euscelis* and *Selenocephalus* were among the species that showed the highest positive correlation. Moreover, species such *N. campestris* and *P. spumarius* showed a positive correlation with autumn, while *C. viridis* presented a positive correlation with summer and autumn, whereas *N. lineatus* and *S. titanus* showed a positive correlation with summer (Figure 6b).

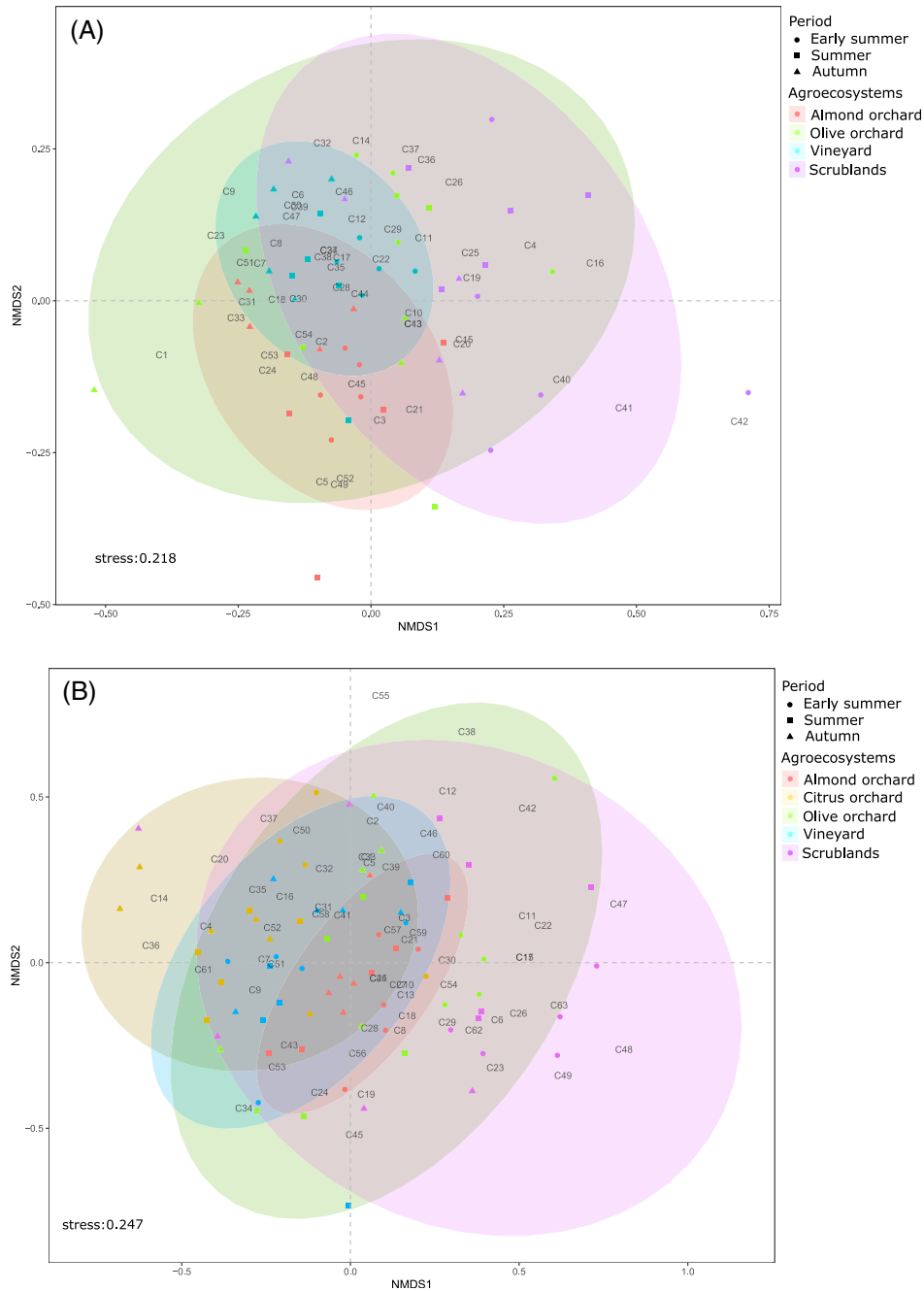


FIGURE 4 Non-metric multidimensional scaling (NMDS) analysis for Cicadomorpha abundance in the different agroecosystems and the sampling period in (A) 2018 and (B) 2019. The numbers within the panels correspond to the numbers of the species/morphospecies in Tables S2 and S3.

In general, the species accumulation curves tended toward stabilization (Figure S1), indicating that the sampling effort was adequate to detect most of the species of the Cicadomorpha community present in the sampled agroecosystems.

DISCUSSION

When insect-borne plant pathogens reach a new location, the spread throughout the landscape could be mediated by native insect vectors

(Redak et al., 2004). Therefore, knowing the abundance and diversity of native insect vectors associated with agroecosystems is essential to design and implementing specific measures to control insect-borne plant pathogens. Cicadomorpha species are well-known vectors of plant pathogens. In this study, the abundance and diversity of Cicadomorpha in several agroecosystems were studied in different sampling periods. In addition, the species and structure community associated with each agroecosystem were also studied.

Our results indicate that the agroecosystems of the north of Portugal can harbour abundant and diverse assemblages of

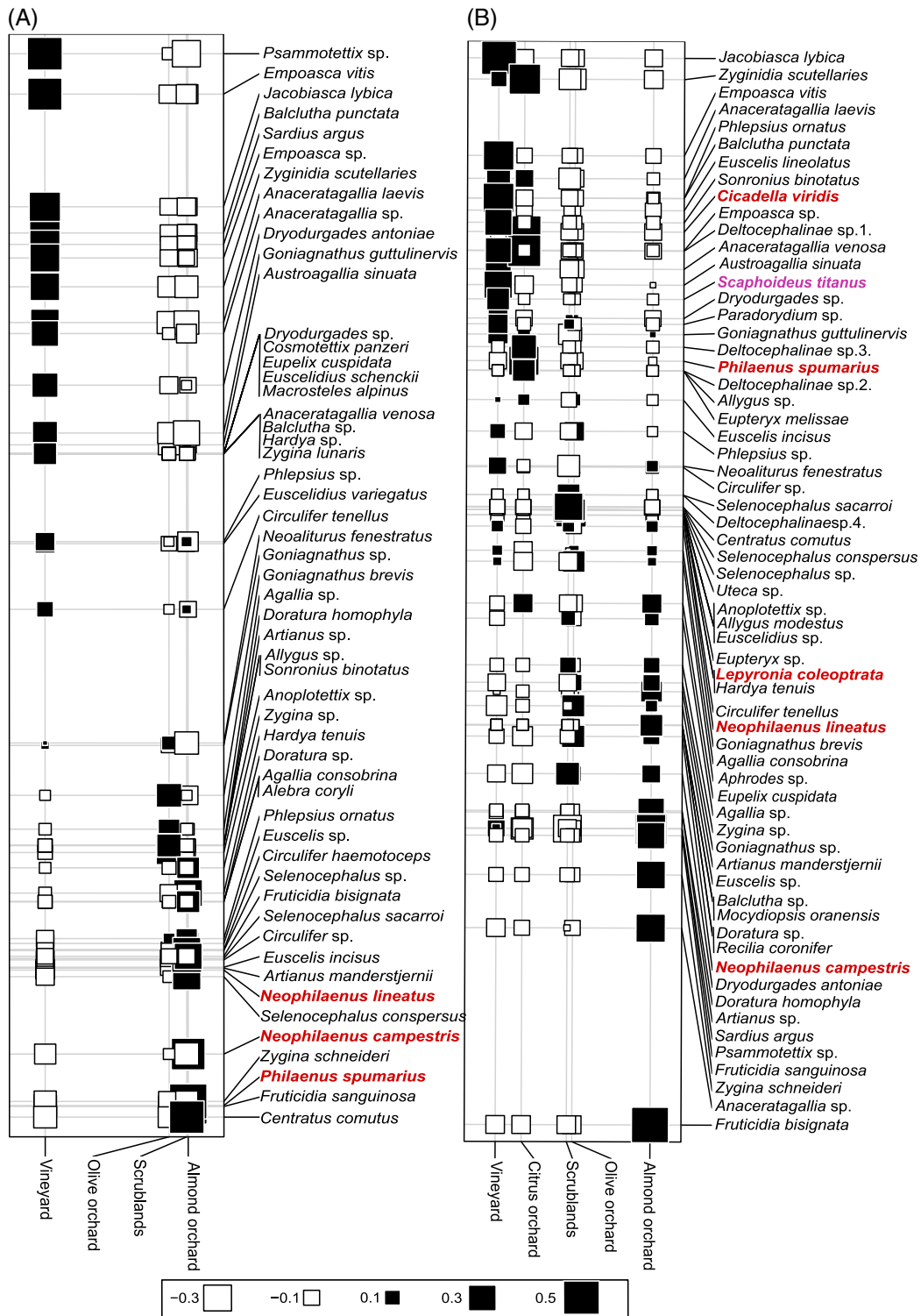


FIGURE 5 Co-inertia factorial map for the agroecosystems in (A) 2018 and (B) 2019. Black squares represent positive relationships, and white squares negative relationships. Square sizes are proportional to the magnitude of the correlation. Red represents the potential vectors of *Xylella fastidiosa*, and pink is the vector of the Flavescence dorée phytoplasma. Information on the family and subfamily of each species is available at Tables S2 and S3.

Cicadomorpha insects. The integration agroecosystem and period of samplings plays an essential role in shaping the structure and composition of the Cicadomorpha community. This might be associated with

their herbaceous host plants, which depend on the agroecosystems management and season (Aguyoh et al., 2004; Carpio et al., 2020; Villa et al., 2020). Also, Cicadomorpha species may have different

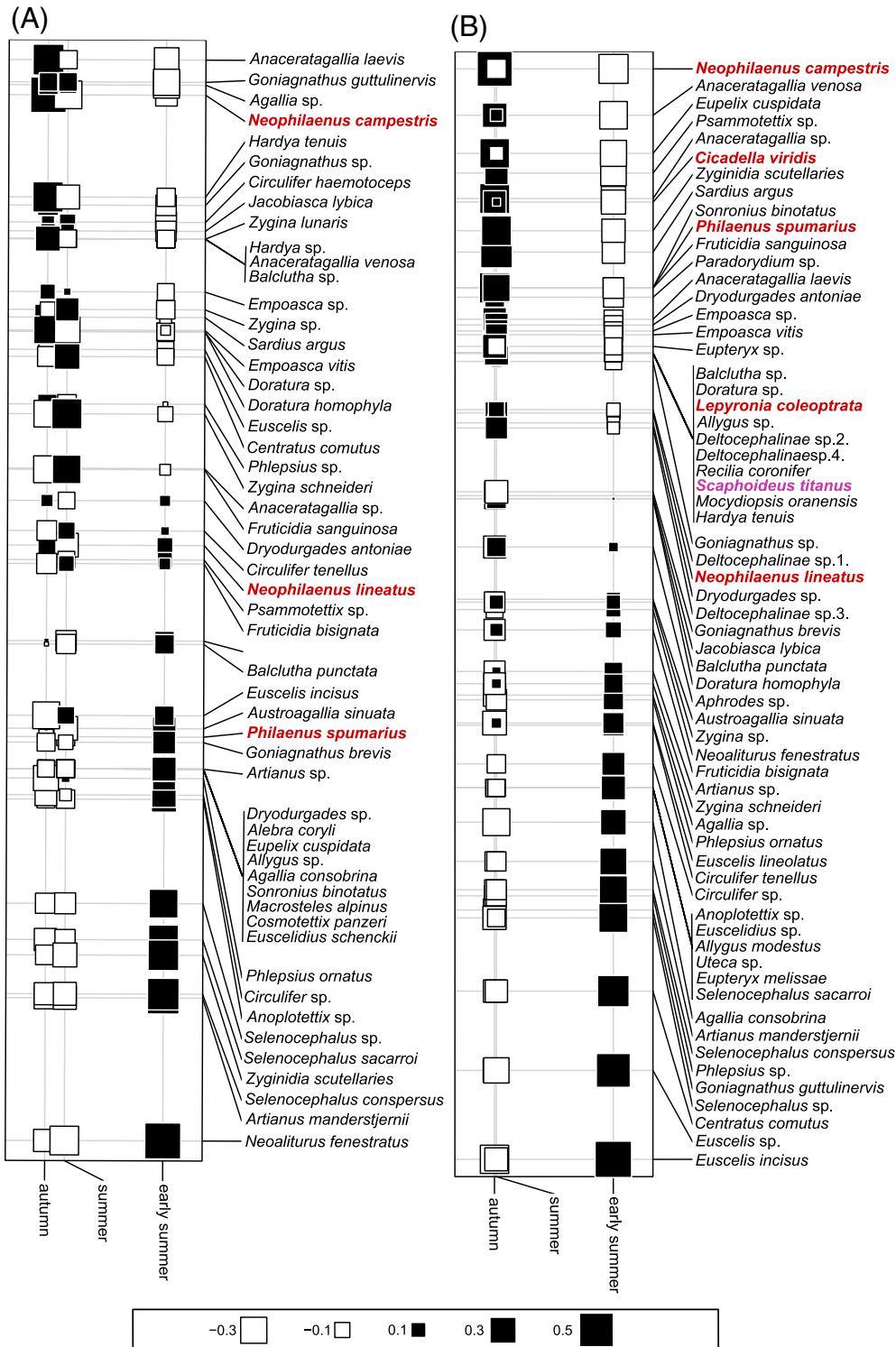


FIGURE 6 Co-inertia factorial map for the sampling period (A) 2018 and (B) 2019. Black squares represent positive relationships, and white squares negative relationships. Square sizes are proportional to the magnitude of the correlation. Red represents the potential vectors of *Xylella fastidiosa*, and pink is the vector of the Flavescence dorée phytoplasma. Information on the family and subfamily of each species is available at Tables S2 and S3.

biological cycles and phenology, which may differ with environmental conditions like relative humidity and temperature (Weaver & King, 1954; Whittaker, 1965). Some species only have one generation

per year; this is the case of the aphrophorids (e.g., Bodino et al., 2020; Morente et al., 2018), while others, like Cicadellidae, may develop more than one generation per year (e.g., Beok, 1972; Decante & van

Helden, 2006; Khfif et al., 2022). Furthermore, some species of Cicadomorpha exhibit a migratory behaviour, that is, they leave the main crop when the host plants of the herbaceous cover die or are cut, returning in autumn with the regrowth of the herbaceous cover after the first rains (Antonatos et al., 2021; Cruaud et al., 2018; Morente et al., 2018). These characteristics allow these insects to be present in the agroecosystems at different times throughout the year.

In vineyards, the agroecosystem with more abundance and diversity of Cicadomorpha individuals was dominated by the species *Psammotettix* sp., *Empoasca vitis* (Göthe, 1875), *Empoasca* sp. and *Jacobiasca lybica* (Bergevin & Zanon 1922). In fact, these species showed a positive correlation with this agroecosystem. Previous studies demonstrated that species of the genus *Psammotettix* tested positive for 'Candidatus Phytoplasma solani', the causal agent of Bois noir and Phytoplasmas of the aster yellows in vines (Quaglino et al., 2019). However, these herbivorous insects in Europe only cause great concern in the wheat, where species of *Psammotettix* can act as vectors for the persistent wheat dwarf virus (Lindblad & Sigvald, 2004).

Empoasca vitis and *J. lybica* are key pests in several European wine regions (Decante & van Helden, 2006; Fornasiero et al., 2016). These green leafhoppers feed on phloem tissues (Fornasiero et al., 2016), which reduces the possibility of acting as potential vectors of xylem-associated bacteria. However, when these species achieve heavy infestations in vineyards, they can result in severe yield losses (Decante & van Helden, 2006; Fornasiero et al., 2016).

Scaphoideus titanus is a univoltine species that feed specifically on vines. Native to the Nearctic Region, it is widely disseminated in Europe (Lessio & Alma, 2004); this species is known to be the vector of the phytoplasma agent of flavescence dorée, an economically important persistent grapevine disease (Chuche & Thiéry, 2014). However, in the present study, this species was captured in low abundance and only in 2019.

Furthermore, some of the genera recovered in the vineyard, such as *Austroagallia*, *Anaceratagallia*, *Euscelidius*, *Euscelis*, *Circulifer*, *Neoliturus*, and *Zyginidia* were previously reported as potential vectors of phytoplasmas of yellow grapevine disease (Batlle et al., 2000; Laviña et al., 2006; Minuz et al., 2013; Picciau et al., 2020; Quaglino et al., 2019; Riolo et al., 2007). Also, *P. spumarius* and *N. campestris*, efficient and competent vectors of *X. fastidiosa* (Cavaleri et al., 2019), were recovered in this agroecosystem, similar to those observed in other regions (e.g., Beal et al., 2021; Bodino et al., 2021; López-Mercadal et al., 2021). Nevertheless, its abundance was much lower than that reported in the literature. Indeed, Beal et al. (2021), Cornara et al. (2016), and Severin (1950) reported that *P. spumarius* could effectively transmit *X. fastidiosa* to vines.

Species of the subfamily Typhlocybinae and the genera *Psammotettix* were dominant in the almond orchard. This subfamily comprises serious agricultural pests due to the direct feeding injury they inflict on plants (Backus, 1988); when they reach high population levels, they feed on almond leaves, causing them to turn yellowish and curl up at the edges, leading to eventual drying and leaf fall (González-Zamora et al., 2021). *Asymmetrasca decedens* a highly polyphagous species capable of acting as a vector for 'Candidatus Phytoplasma

Phoenicium', a pathogenic plant bacterium responsible for almond witches-broom disease (Dakhil et al., 2011). Although this insect was already reported in Portugal (Coutinho et al., 2015), it was not captured in the sampled almond orchards.

In 2018 the highest abundance of *X. fastidiosa* vectors, namely: *N. campestris*, *N. lineatus*, and *P. spumarius* was observed in almond orchards. Still, in 2019, these insects were reduced, probably associated with the interannual variation.

To our best knowledge, except for *X. fastidiosa* (Saponari et al., 2013), the olive tree is not affected by other insect-borne pathogens. In this sense, the presence in this agroecosystem of species that have been reported to transmit pathogens, such as species of the genera *Neoliturus*, *Euscelis*, and *Anoplotettix* (Jakovljević et al., 2015; Laviña et al., 2006), is not alarming. However, in olive groves, four species of aphrophorids were captured, two confirmed vectors of *X. fastidiosa* (*P. spumarius* and *N. campestris*) (Cavaleri et al., 2019) and two whose transmission efficiency and competence are still unknown (*L. coleoptera* and *N. lineatus*). Although it is low in abundance compared to that observed in olive orchards from other regions (Antonatos et al., 2021; Bodino et al., 2020; Cornara et al., 2017; Morente et al., 2018; Tsagkarakis et al., 2018), their presence in this agroecosystem is alarming. *Xylella fastidiosa* does not need a latency period to be transmitted (Janse & Obradovic, 2010), and once acquired by the insect vector, it persists in the insect during its entire adult life (Almeida et al., 2005).

Our results suggest that olive orchards and scrublands have a similar Cicadomorpha community, probably because the sampled scrublands surround the olive groves. Scrublands may act as reservoirs of arthropods providing food and optional shelter from the main crop (Kubiak et al., 2022). Some studies carried out in olive groves in Spain, Corsica, and Greece demonstrated that in summer, *P. spumarius* and *N. campestris* migrate from olive groves to adjacent areas. This is probably related to the fact that on olive groves, the vegetation cover dies or is removed, and the insect migrates for a more favourable environment. As autumn approaches, after the first rains, they tend to return to the olive grove when there is regrowth of the herbaceous vegetation where these individuals lay the egg (Antonatos et al., 2021; Bodino et al., 2020; Cruaud et al., 2018; Morente et al., 2018; Tsagkarakis et al., 2018). Furthermore, Cornara et al. (2021) also described that natural and semi-natural areas surrounding cultivated orchards could act as *X. fastidiosa* vector reservoirs, sustaining their populations during the summer and possibly acting as both recipient and source areas for *X. fastidiosa*. This corroborates our data since *P. spumarius*, and *N. campestris* were recovered from the Scrublands in the early summer and summer, and the olive orchards were mainly present in the autumn. The same pattern is verified in the vineyards, almonds, and citrus orchards, where these two species were captured with greater abundance in autumn. This indicates that these vectors have a behavioural pattern in these agroecosystems similar to that observed in olive orchards.

The citrus tree is affected by several insect-borne pathogens (e.g., Coletta-Filho et al., 2020; EFSA PLH Plane, 2014) that can be transmitted by individuals of Cicadomorpha, such as *S. citri* and

X. fastidiosa. *Spiroplasma citri* is the causal agent of Citrus Stubborn Disease, the main vector of this pathogen, *C. tenellus* (EFSA PLH Plane, 2014); although this species was present in the other sampled agroecosystems, it was not captured in the sampled citrus orchards.

Xylella fastidiosa is responsible for Citrus Variegated Chlorosis, a very important disease in Brazil, where this bacterium is transmitted mainly by individuals of the Cicadellidae subfamily; however (Coletta-Filho et al., 2020), these vector species are absent in Europe (EFSA, 2013). *Cicadella viridis* is the most abundant Cicadellinae in Europe; although it cannot transmit *X. fastidiosa* to the olive tree (Bodino et al., 2022), there is still no evidence that it can transmit the bacteria in other agroecosystems. *Cicadella viridis* and *P. spumarius* were abundantly captured in the sampled citrus. It should be noted that in Portugal, *X. fastidiosa* has been reported in commercial citrus orchards (DGAV, 2022). Therefore, it is urgent to know the transmission capacity and efficiency of *C. viridis* and *P. spumarius* in citrus to implement proper control measures against these potential vectors.

CONCLUSIONS

Understanding and knowing the diversity and abundance of insects that may constitute important pests or play an important role in disseminating pathogens is the first step for implementing appropriate measures to combat the negative effects of these insects. In the present study, it was possible to verify that the agroecosystems under study could harbor great diversity and abundance of Cicadomorpha. Moreover, this diversity and abundance may vary depending on the agrosystem and time of the year. The confirmed vectors of *X. fastidiosa* (*P. spumarius*, and *N. campestris*) were recovered in all sampled agroecosystems in different abundances.

Further research on how the landscape, agricultural practices, and composition of the vegetation cover shape the Cicadomorpha community is essential to better understand what contributes to variations in the composition of the Cicadomorpha community, to implement strategies to reduce the spread of insect-borne pathogens if they are introduced into agroecosystems.

AUTHOR CONTRIBUTIONS

Isabel Rodrigues: Conceptualization; formal analysis; investigation; methodology; writing – original draft. **Paula Baptista:** Conceptualization; investigation; supervision; writing – review and editing. **José Alberto Pereira:** Conceptualization; funding acquisition; methodology; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Species accumulation curves based on the number of sweeps performed in the (a) almond orchards, (b) olive orchards, (c) Vineyards, (d) Scrublands and (e) Citrus orchards in the early summer, summer, and autumn in (1) 2018 and (2) 2019. The envelopes correspond to the 95% confidence interval.

Table S1. Agroecosystems' information: sampling dates (2018 and 2019) and metric characteristics.

Table S2. Total number (N) of Cicadomorpha adults collected in the canopy (c) and ground vegetation (gv) of the almond orchard, Citrus orchard, olive orchard, vineyard and scrublands in early summer (es), summer (s) and autumn (a) in 2018.

Table S3. Total number (N) of Cicadomorpha adults collected in the canopy (c) and ground vegetation (gv) of the almond orchard, Citrus orchard, olive orchard, vineyard and scrublands in early summer (es), summer (s) and autumn (a) in 2019.

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