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Consistent benefits of fungicide reduction on arthropod predators and predation rates in viticulture: a five-year experiment

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Abstract For reliable pest suppression, benefits of habitat management for natural enemies of agricultural pests need to be consistent over time. Unfortunately, most research projects allow only for one or two years of data collection. Here, we present a five-year study on effects of fungicide reduction and altered plant architecture on arthropod abundances and natural pest control in an experimental vineyard. The vineyard rows were divided into eight groups, half of which were trained in vertical shoot position ("trellis system") and the other half as semi-minimal pruned hedge ("minimal pruning"). Every row was divided in three sections receiving three different plant protection intensities, respectively, with fungicides certified for organic viticulture. In each year we sampled arthropods from the grapevine canopy by standardized leaf collection and beat-sheet sampling, and exposed baits of a major grapevine pest (*Lobesia botrana*) to assess natural pest control. Arthropods, in particular predators, benefited from reduced fungicide sprayings and in turn promoted natural pest control. In contrast, effects of minimal pruning were less strong, and restricted to the leaf mesofauna, earwigs and leafhoppers. Across the five study years with their variable weather conditions, we conclude that the advantages of reduced fungicide sprayings in fungus-resistant varieties are consistent over time.

Keywords Organic viticulture · Fungus-resistant grape varieties · Minimal pruning · Natural pest control · *Lobesia botrana* · Phytoseiidae · Eriophyidae

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Introduction

Arthropods are involved in important ecosystem services such as natural pest regulation. However, many species are sensitive to agricultural practices. For example, soil disturbances such as tillage, chemical inputs like pesticides and fertilizers, and mechanical harvesting strongly impair arthropod communities (Attwood et al. 2008; Sánchez-Bayo and Wyckhuys

2019). Nonetheless, habitat management can improve environmental conditions such as the availability of alternative food and shelter, particularly for beneficial arthropods (Landis et al. 2000). In contrast to annual crops, perennial crops such as orchards and vineyards are stable habitats with continuous vegetation cover. However, they can receive high levels of pesticide input (Bakker et al. 2022). In temperate regions with humid climate where pathogen pressure is high, vineyards typically receive 12–15 fungicide sprayings per year (Pertot et al. 2017; Reiff et al. 2023). Beneficial arthropods like parasitic wasps, ants, spiders, as well as predatory mites and beetles, are susceptible to fungicides (Thomson and Hoffmann 2006; Nash et al. 2010). Thus, frequent fungicide treatments may impede natural pest control in vineyards. A promising approach to fostering arthropod biodiversity and natural pest control is the cultivation of fungus-resistant grape varieties which allows to reduce fungicide applications by 60–100% (Pertot et al. 2017; Reiff et al. 2021a; Thiollet-Scholtus et al. 2021). However, the amount of wine produced from fungus-resistant varieties is still low, due to limited acceptance of new varieties by consumers (Wiedemann-Merdinoglu and Hoffmann 2010; Nesselhauf 2018; Borrello et al. 2021).

Another approach for a more sustainable viticulture is minimal pruning. While in traditional trellis systems 85–98% of the annual growth are pruned in winter (Sommer et al. 1995), minimally pruned vineyards are characterized by high amounts of wooded shoots that persist over the years. Thereby, canopies of minimally pruned vines sprout more quickly, develop a full leaf canopy sooner, and have higher volumes due to increased numbers of shoots and nodes compared to plants in traditional trellis systems (Sommer et al. 1995; Intrieri et al. 2011; Kraus et al. 2018). However, denser canopies can be less permeable to pesticide applications. Further, this shift in vine architecture results in altered microclimatic conditions (Pangga et al. 2013; Kraus et al. 2018). As a consequence, minimal pruning may amplify pathogen pressure of fungal diseases such as powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*). Arthropods may also be affected by microclimatic conditions. A denser and more complexly structured canopy may provide additional shelter for beneficial arthropods and promote top-down effects on herbivores (Langellotto and Denno 2004). For instance, some spiders and mites overwinter in tree bark and on branches (Bower and Snetsinger 1985; Duso and Vettorazzo 1999) and their densities may thus be enhanced by higher abundance of wooded branches in minimal pruned vineyards.

For reliable pest suppression, effects of habitat management on natural enemies need to be consistent over time. Unfortunately, most research projects allow only for one or two years of data collection (Estes et al. 2018). Some snapshots already highlight the benefits of reduced fungicide sprayings and minimal pruning on arthropod predators and natural pest suppression (Pennington et al. 2017, 2018, 2019). However, these studies lack evidence for long-term validity. To fill this gap, we investigate the single and combined effects of reduced fungicide sprayings in fungus-resistant grape varieties and altered grapevine architecture in minimally pruned vineyards on arthropods in five successive years. Since exposure to pesticides is highest in the grapevine canopy, we focussed on biodiversity sampling from the foliage, including both mesofauna and macrofauna. We hypothesize that arthropod abundances and natural pest control in the grapevine canopy is enhanced by both fungicide reduction and minimal pruning.

Materials and methods

Study site

The experiments took place in an experimental vineyard of the Julius Kühn-Institute in Siebeldingen, Germany (49° 13’ 8.22’’ N, 8° 2’ 25.8’’ E). Inter-row distance was 2 m and grapevine spacing was 1 m. The vineyard was planted with four different *Vitis vinifera* cultivars: ‘Reberger’ (red), ‘Villaris’ (white), ‘Felicia’ (white) and ‘Gf 84–58-988’ (red) which are resistant against powdery mildew and downy mildew. Cultivating fungus-resistant varieties allowed for reduced plant protection regimes while maintaining healthy plants. The varieties were used as replicates of the pruning and plant protection treatments and to represent a diversity of cultivars. Specific differences between these four varieties were beyond the scope of this study. Each variety was cultivated in six to ten rows, half of which were trained in vertical shoot position (VSP; “trellis system” hereafter) and half were trained in semi-minimal pruned hedge (SMPH;

"minimal pruning" hereafter). Each of these rows was again divided into three sections which received different plant protection regimes by using a plot sprayer to avoid spray drift to adjacent rows (Tunnel plot sprayer ABS 6/25-TU, Christian Schachtner Fahrzeug und Gerätebau, Ludwigsburg, Germany). Thus, each combination of plant protection intensity and pruning system was replicated four times in the different varieties, resulting in 24 treatment plots (see supplementary Figure S1 for a detailed plan). We chose a spraying regime with products certified for organic viticulture. The regime consisted of standard (10–13), reduced (4–7) or minimal (2–4) sprayings of Funguran progress® (350 g copper per kg [copper hydroxide]), Netzschwefel Stulln (796 g sulfur per kg) and VitiSan® (9949 g potassium bicarbonate per kg) per season. Under standard sprayings, fungicides were applied weekly between May and August following a standardized scheme. The number of sprayings and spraying intervals varied between years depending on phenological stages of the grapes and pathogen pressure, following recommendations of viticultural advisory authorities (supplementary Table S1). While conventional spraying regimes require a change of products between sprayings, the use of an organic spraying regime allowed us to spray the same products every time. This way, the impact of one spraying on the arthropod fauna was as standardized as possible. However, organic spraying products must be applied before disease incidence to allow adequate protection, which leads to relatively frequent sprayings in the studied region. No insecticides, acaricides or any other foliage spray (e.g., growth regulators, fertilizers) were applied in the vineyard, which allowed us to ascribe spraying effects solely to fungicide applications.

Leaf mesofauna

Mites were sampled during the vegetation period between May and October. Sampling frequency differed between study years, resulting in two sampling dates in 2015, seven sampling dates in 2016, five sampling dates in 2017, and four sampling dates in 2018 and 2019 (supplementary Table S2). At each sampling, we randomly selected 25 leaves from different vines in each of the 24 plots. Collected leaves were washed onto a filter paper following Hill and Schlamp (1984). All mites were counted and identified to family level using a stereomicroscope (Zeiss, Jena, Germany). As the focus of our study was mostly on functional aspects, we identified only a subsample of adult individuals to species level every year using the preparation method described by Krantz (1978) and the keys introduced by Schruft (1972), Karg (1994), Schliesske (1995), and Tixier et al. (2013). After the mites were washed off, the leaf area was determined using a leaf area meter (Li-COR, Modell 3100 area meter, Lincoln, NE, USA). Leaf area was used to calculate mite densities per square meter of leaf area, which was necessary due to differing leaf sizes of the four varieties being affected by the pruning method. We counted beneficial mites (Acari:Tydeidae and Phytoseiidae), the phytophagous mites *Colomerus vitis* Pagenstecher and *Calepitrimerus vitis* Nalepa (Acari:Eriophyidae), thrips (Thysanoptera) and immature leafhoppers (*Empoasca* sp., Hemiptera: Cicadellidae).

Macrofauna

Arthropods were sampled during the vegetation period between April and October. Sampling frequency differed between study years, resulting in three sampling dates in 2015, five sampling dates in 2016 and 2018, nine sampling dates in 2017, and four sampling dates in 2019 (supplementary Table S2). We took samples of the whole grapevine canopy using a beat-sheet with a diameter of 72 cm (beat-sheet by Dynort, bioform Dr. J. Schmidl E.K., Nürnberg, Germany). The sheet was placed under the vines while they were shaken vigorously. All arthropods falling on the sheet were collected and stored in 70% ethanol for further identification. We repeated the shaking on ten vines per plot in 2015–2017 and 20 vines per plot in 2018 and 2019, respectively. All arthropods were counted and identified at least to order level using a stereomicroscope (Zeiss, Jena, Germany) and the identification key by Schaefer (2017).

Predation rate assessment

To assess the natural pest control potential in the vineyard, we used eggs of the grape berry moth (*Lobesia botrana* Denis & Schiffermüller) (Lepidotpera:Totricidae) as a proxy, since it is of major concern in a global context (Benelli et al. 2023). Viticulture in the study area was more or less insecticide-free because the grape berry moth was controlled with mating disruption, and *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), a vector of the grapevine phytoplasma disease flavescence dorée, was not present. For rearing of *L. botrana* we followed Markheiser et al. (2018). Inside of the rearing containers, retainers were installed to allow for oviposition on exchangeable polyethylene strips. Egg-laden strips were harvested after 24 h and stored at 4 °C for a maximum of four days until exposure. Strips contained 45 ± 16 eggs on average. To determine predation rates, baits were attached to randomly selected one-year-old branches and were exposed there for 72 h. We exposed five baits per plot between May and September. Sampling frequency differed between study years, resulting in two sampling dates in 2015 and 2017, four sampling dates in 2016 and 2019, and five sampling dates in 2018. The eggs were counted before and after exposition using stereomicroscopes (Zeiss, Jena, Germany). We stored eggs that remained on the baits in a climate chamber at 70% RH and 21 °C for two weeks to check for parasitism, but did not find any parasitised eggs.

Data analysis

All statistical analyses were done in R version 3.6.3 (R Core Team 2023). To account for the different sampling intensities, data were combined for each year. Densities of mites, thrips and leafhoppers on leaves were averaged over all sampling dates of each year to obtain one observation per year per plot. Numbers of sampled arthropods by beat-sheet were averaged to obtain one observation per year per ten vines shaken per plot. To obtain a general predator abundance, abundances of spiders, earwigs, ants, lacewings, harvestmen and ladybirds were summed. Percentages of predated *L. botrana* eggs were averaged to obtain one observation per year per plot.

The distribution of response variables was checked visually using the qqp() function (R package car; Fox and Weisberg 2019). Accordingly, all variables were analyzed with negative binomial distribution using generalized linear models fitted with the function glm.nb() and a log link (R package MASS; Venables and Ripley 2002). Models contained pruning (two categories), spraying frequency (continuous), year (five categories) and variety (four categories) as well as the interactions pruning×year, spraying frequency year, and pruning×spraying frequency as explanatory variables. No further model simplification was done. Post-hoc tests were conducted if there was a significant effect of the variables 'year', 'variety' and the interaction 'year×pruning' using the function emmeans() (R package emmeans). *P*-values were adjusted with the Tukey method. Effects of densities of the two beneficial mite families (Phytoseiidae, Tydeidae) on densities of phytophagous mites (*Cal. vitis, Co. vitis*), thrips and *Empoasca* sp. were analyzed using generalized linear models fitted with the function glmer.nb() and a log link (R package MASS; Venables and Ripley 2002). Models contained Phytoseiidae and Tydeidae as explanatory variables and year as a random factor. Cook's distance was used to check for outliers. Assumptions were checked for all models using graphical validation procedures (Zuur et al. 2009).

Results

Climatic conditions varied greatly over the five studied years. Highest sums of cumulative precipitation during the study period occurred in 2017 with more than 272 mm. Average leaf moisture was highest in 2016 (41%). Average temperatures varied between 18.3 °C in 2017 and 19.8 °C in 2018, while highest temperature maxima occurred in 2015 (39.8 °C). Similarly, pathogen pressure varied over the five studied years. Downy mildew occurred in 2016 only, with lowest incidences under full fungicide applications and in trellis system (Kraus et al. 2018). Powdery mildew mostly occurred in 2019 with lowest incidences under full fungicide applications and in trellis system (Kraus, personal communication).

Arthropod abundances, mite densities, and predation rates differed over the study period, with no clear overall temporal trend (Figs. 1, 2, and 3; supplementary Table S3). For instance, phytoseiid density was significantly higher in 2018 and 2019 compared to 2015–2017, while densities of *Cal. vitis* differed in all years except for 2017 and 2018 (supplementary Table S4). Furthermore, seven arthropod taxa were affected by the grape variety with no clear overall trend. Densities of the leaf mesofauna were similar in the varieties Villaris and Gf 84–58-988 but differed in the other two varieties. Cicadellid abundances were

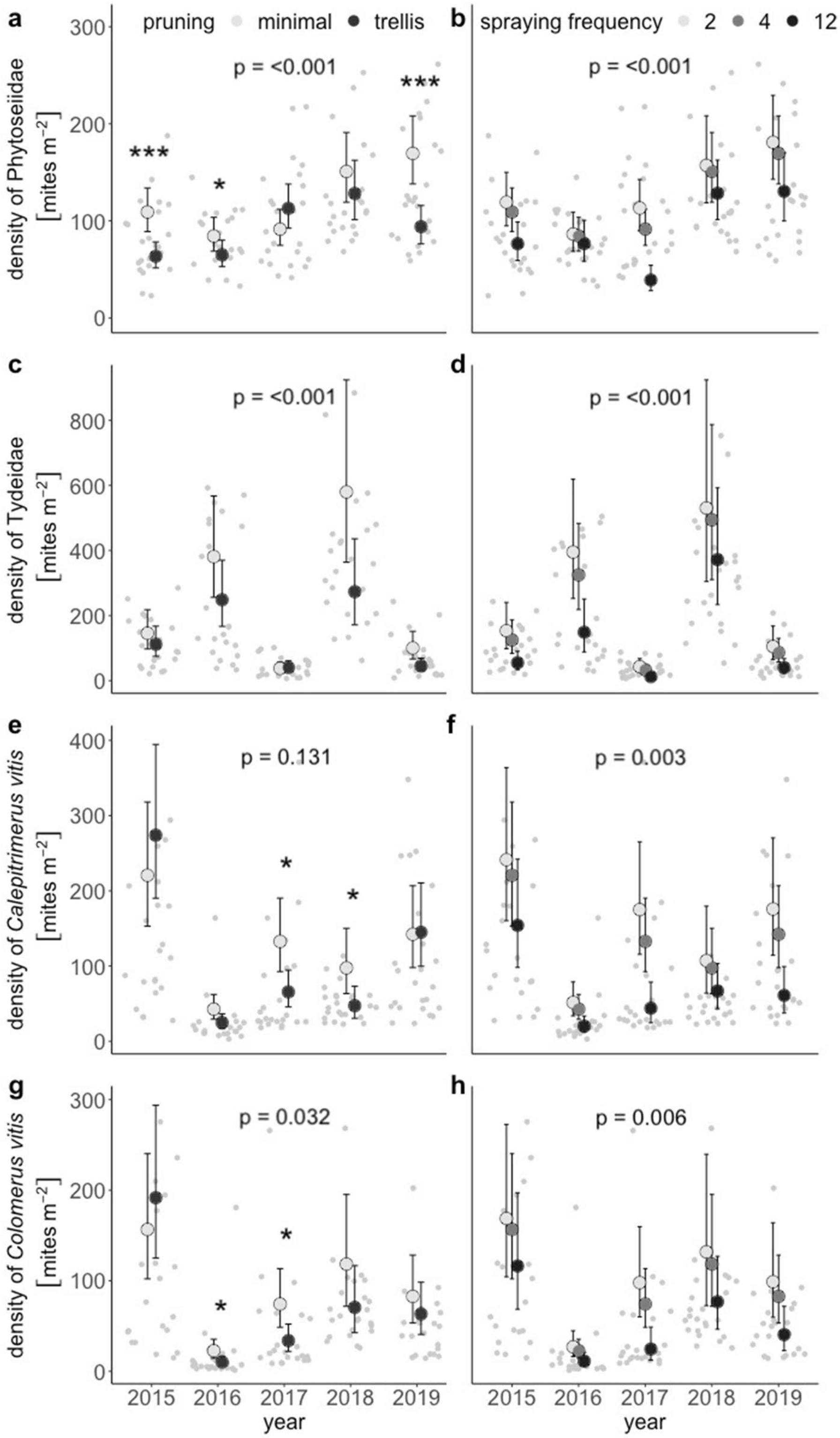

Fig. 1 Mite densities of Phytoseiidae (**a**, **b**), Tydeidae (**c**, **d**), and the two eriophyid mites *Cal. vitis* (**e**, **f**) and *Col. vitis* (**g**, **h**) in five consecutive years with respect to pruning system (**a**, **c**, **e**, **g**) and spraying frequency of fungicides (**b**, **d**, **f**, **h**). Larger dots show model predicted means with respective error bars (95% confidence interval), while smaller grey dots represent individual plots of the experimental vineyard. *P*-values for the impact of pruning and spraying frequency are indicated in the upper middle of the panels, respectively. Differences between the pruning systems are indicated with asterisks ($p<0.001$ '***'; $p<0.05$ '*') for the respective year when overall interactive effects between pruning system and year occurred. The significance of the differences between spraying frequencies are not displayed since no interactive effects between spraying frequency and year occurred

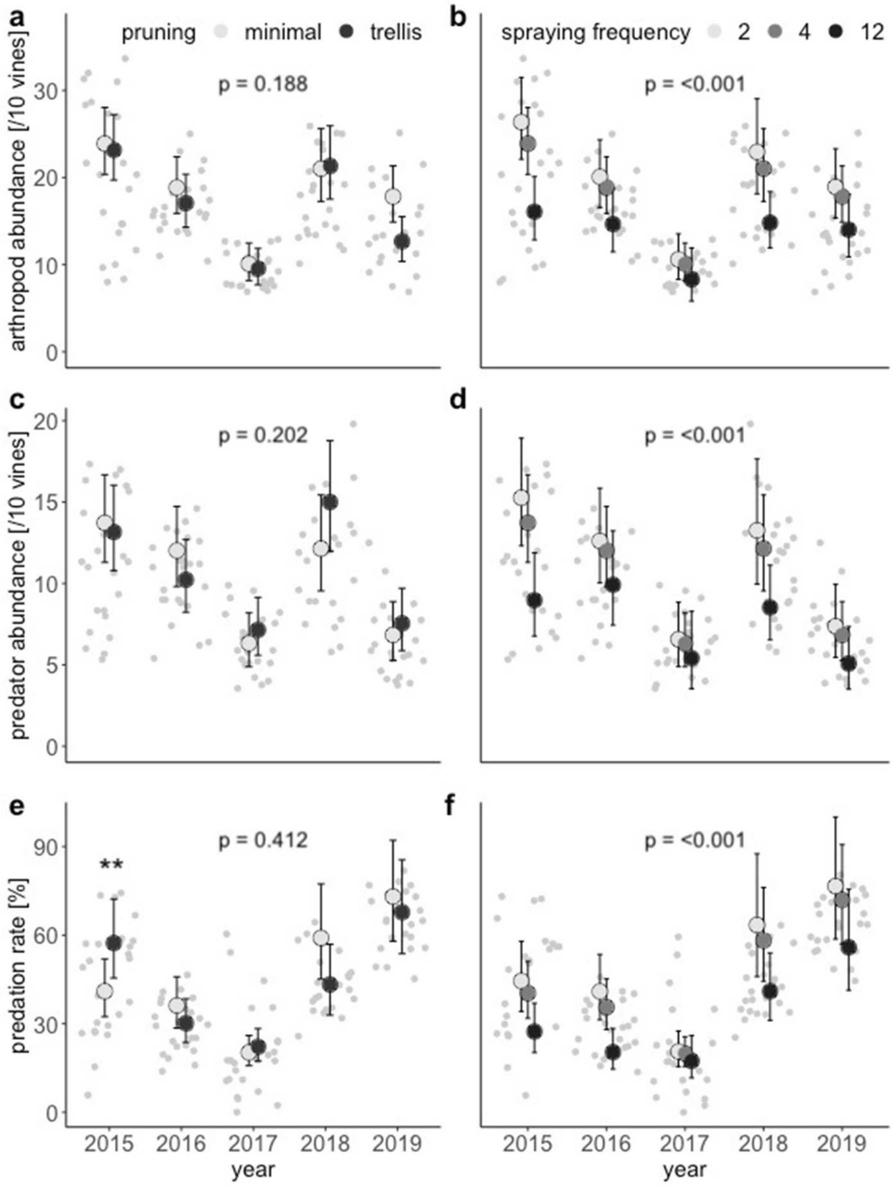

Fig. 2 Arthropod abundance (**a**, **b**), predator abundances (**c**, **d**) and predation rates of *L. botrana* eggs (**e**, **f**) in five consecutive years with respect to pruning system (**a**, **c**, **e**) and spraying frequency of fungicides (**b**, **d**, **f**). Larger dots show model predicted means with respective error bars (95% confidence interval), while smaller grey dots represent individual plots of the experimental vineyard. *P*-values for the impact of pruning and spraying frequency are indicated in the upper middle of the panels, respectively. Differences between the pruning systems are indicated with asterisks ($p < 0.01$ '**') for the respective year when overall interactive effects between pruning system and year occurred. The significance of the differences between spraying frequencies are not displayed since no interactive effects between spraying frequency and year occurred

higher in red than in white varieties (supplementary Table S4).

Leaf mesofauna

The densities of the two beneficial mite families, Phytoseiidae and Tydeidae, as well as the two phytophagous mites, *Co. vitis* and *Cal. vitis*, were higher in reduced fungicide sprayings (Table 1, supplementary Table S3, Fig. 1). Phytoseiid and tydeid mite densities were 37% and 45% higher under minimal compared to standard sprayings, respectively. These effects were strongest in 2017 for Phytoseiidae (68% increase under minimal sprayings). Smallest effects of reduced sprayings were found in 2016 for Phytoseiidae (17% increase under minimal sprayings). *Co. vitis* and *Cal. vitis* densities were 26% and 27% higher, respectively, under minimal compared to standard sprayings.

Both beneficial mite families as well as *Co. vitis* benefitted from minimal pruning (Table 1, supplementary Table S3, Fig. 1). Phytoseiid mite densities

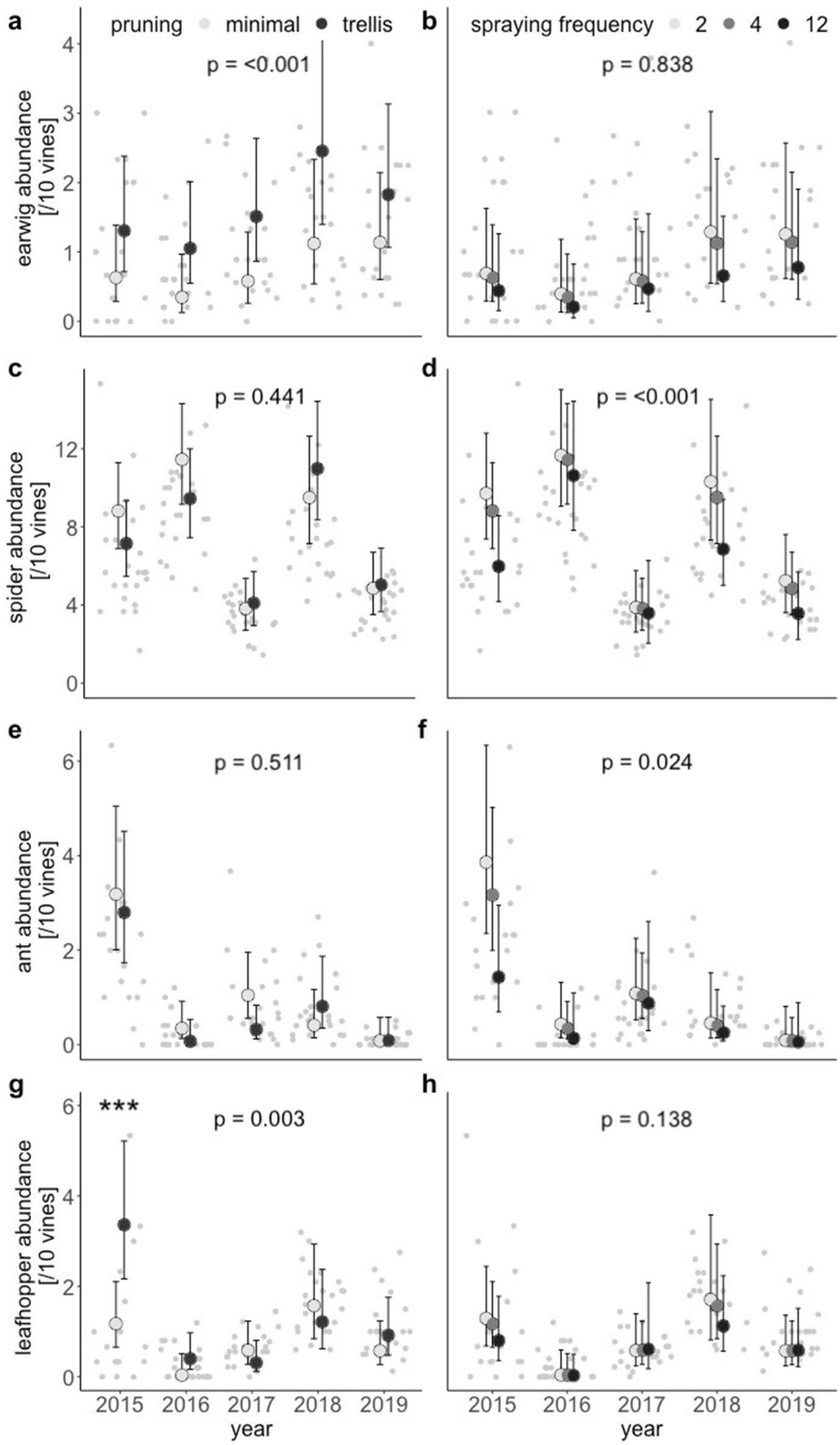

Fig. 3 Abundances of spiders (**a**, **b**), earwigs (**c**, **d**), ants (**e**, **f**) and cicadellid leafhoppers (**g**, **h**) in five consecutive years with respect to pruning system (**a**, **c**, **e**, **g**) and spraying frequency of fungicides (**b**, **d**, **f**, **h**). Larger dots show model predicted means with respective error bars (95% confidence interval), while smaller grey dots represent individual plots of the experimental vineyard. *P*-values for the impact of pruning and spraying frequency are indicated in the upper middle of the panels, respectively. Differences between the pruning systems are indicated with asterisks ($p < 0.001$ '***') for the respective year when overall interactive effects between pruning system and year occurred. The significance of the differences between spraying frequencies are not displayed since no interactive effects between spraying frequency and year occurred

Table 1 Effects of minimal pruning and reduced fungicide spraying on arthropod densities and predation rates

	Pruning (df=1)		spraying frequency (df=1)		Year (df=4)		Variety (df=3)		Interaction (year-pruning) (df=4)		Interaction (yearspraying frequency) (df=4)		Interaction (spraying frequency×pruning) (df=1)	
	X^2	p	X^2	p	X^2	p	X^2	p	X^2	p	X^2	p	X^2	p
Leaf mesofauna														
Phytoseiidae	30.07	**< 0.001**	44.42	**< 0.001**	82.16	**< 0.001**	17.09	**< 0.001**	27.15	**< 0.001**	18.27	**0.001**	1.26	0.263
Tydeidae	11.20	**< 0.001**	30.23	**< 0.001**	259.55	**< 0.001**	7.01	0.072	8.03	0.091	4.16	0.385	0.99	0.321
Cal. vitis	2.28	0.131	8.67	**0.003**	185.90	**< 0.001**	89.04	**< 0.001**	15.06	**0.005**	7.59	0.108	10.34	**0.001**
Co. vitis	4.61	**0.032**	7.68	**0.006**	175.05	**< 0.001**	98.03	**< 0.001**	9.70	**0.046**	4.93	0.295	4.66	**0.031**
Empoasca sp.	0.65	0.419	1.21	0.271	136.65	**< 0.001**	2.23	0.527	7.80	0.099	0.73	0.948	0.53	0.467
Thrips	31.83	**< 0.001**	0.14	0.707	213.28	**< 0.001**	18.18	**< 0.001**	5.03	0.284	2.71	0.607	1.14	0.286
Macrofauna														
Total abundance	1.73	0.188	27.67	**< 0.001**	123.57	**< 0.001**	5.84	0.120	6.15	0.188	2.31	0.679	1.43	0.232
Predators	1.63	0.202	14.90	**< 0.001**	84.50	**< 0.001**	1.83	0.608	5.29	0.259	2.88	0.578	1.74	0.187
Spiders	0.59	0.441	11.42	**< 0.001**	96.69	**< 0.001**	7.44	0.059	4.19	0.381	3.48	0.481	0.02	0.881
Earwigs	30.23	**< 0.001**	0.04	0.838	13.74	**0.008**	10.85	**0.013**	1.28	0.864	0.46	0.977	2.68	0.101
Ants	0.43	0.511	5.08	**0.024**	137.79	**< 0.001**	13.12	**0.004**	9.29	0.054	1.52	0.823	2.60	0.107
Leafhoppers	8.61	**0.003**	2.20	0.138	77.71	**< 0.001**	26.95	**< 0.001**	17.15	**0.002**	1.06	0.901	0.06	0.809
Predation rates														
L. botrana eggs	0.67	0.412	10.89	**< 0.001**	156.84	**< 0.001**	2.23	0.526	12.32	**0.015**	4.15	0.386	5.30	**0.021**

Significant *P*-values (< 0.05) are displayed in bold were 25% higher in minimal pruning, with strongest effects in 2019 (46% increase in minimal pruning). Tydeid mite densities were 41% higher in minimal pruning. Densities of *Co. vitis* were 10% higher in minimal pruning, with strongest effects in 2017 (53% increase in minimal pruning). Although densities of *Cal. vitis* were significantly higher in 2017 and 2018 (37–49% increase in minimal pruning) there was no clear temporal trend. Densities of both phytophagous mites were higher in reduced fungicide sprayings under minimal pruning. In contrast, *Co. vitis* and *Cal. vitis* densities were higher under intensive fungicide sprayings in trellis system (Table 1, supplementary Table S3).

Thrips densities were 38% higher in trellis system. Densities of Phytoseiidae and Tydeidae were not related to thrips densities (supplementary Table S5). Densities of *Empoasca* sp. were neither affected by pruning system nor by spraying frequency, but decreased with increasing phytoseiid mite densities (Table 1, supplementary Tables S3, S5). Densities of both phytophagous mite species were higher when tydeid mite densities were low (supplementary Table S5).

Macrofauna and predation rates

In five years, we identified a total of 12,590 arthropods that belonged to 20 orders. Highest arthropod abundances were observed in 2015 (21.2 ± 8.8 individuals per ten vines) and lowest in 2017 (9.4 ± 2.0). Predation rates of *L. botrana* eggs were highest in 2019 (67.6 ± 10.7%) and lowest in 2017 (42.3 ± 15.6%). Reduced fungicide sprayings enhanced total arthropod abundance, predator abundance, and predation rates (Table 1, supplementary Table S3, Fig. 2). However, no clear effects of the pruning system could be observed. Total arthropod abundance was 27% higher under minimal compared to standard sprayings. Likewise predator abundance was 24% higher. Predation rates were 19% higher under reduced sprayings. Further, effects of trellis system on predation rates were highest in 2015 (34% increase in trellis system; Table 1), but with no clear temporal trend (Fig. 2 e).

Abundances of spiders and ants were 26 and 35% higher, respectively, under fungicide reduction, while earwig and cicadellid leafhopper abundances were not affected (Table 1, supplementary Table S3, Fig. 3). In contrast, earwig and cicadellid leafhopper abundances were 59% and 37% higher in trellis system, respectively (Table 1, supplementary Table S3, Fig. 3). Nevertheless, the effects of trellis system on leafhopper abundances varied significantly over time with strongest effects of trellis system in 2015 (90% increase; Table 1, Fig. 3g).

Discussion

Effects of reduced fungicide applications

In agreement with our hypothesis, we found strong effects of reduced fungicide applications on most of the studied arthropods every year. Significant variation of the fungicide effect over time was found for only one taxon, the Phytoseiidae. We conclude that the impacts of fungicide sprayings are consistent even under strong variation of environmental conditions. In spite of the high number of applications, fungicide use was restricted to three months per year. Non-target effects of pesticides are strongest shortly after spraying (Schindler et al. 2022). Thus, we assume that arthropod populations levelled out between our plots over the non-sprayed period of the year due to population recovery and movement between plots, making cumulative effects of fungicides unlikely to appear.

Beneficial mites as well as pest mites benefitted from reduced sprayings. Both Phytoseiidae and Tydeidae are susceptible to numerous fungicides and strongly affected by frequent sprayings (Peverieri et al. 2009; Pozzebon et al. 2010; Gadino et al. 2011; Möth et al. 2021; Reiff et al. 2021a). Furthermore, sulphur is used to control pest mites (Duso et al. 2012). Thus, adverse effects of sulfur-based fungicides on eriophyid mites were expected. We also found strong effects of reduced fungicide treatments on total arthropod abundances as well as on predators and predation rates. Spiders are the most abundant predators in vineyards (Costello and Daane 2005). Unlike previous studies on canopy dwelling spiders in vineyards (Nash et al. 2010; Pennington et al. 2019) we found clear benefits of reduced fungicide sprayings on spider abundance. Ants are susceptible to insecticides but less so to other pesticides and particularly sulphur (Chong et al. 2007; Olotu et al. 2013; Masoni et al. 2017). However, we found small but consistent effects of reduced fungicide applications on ants, which might be attributed to the copper content of the sprayings (Migula and Głowacka 1996; Diehl et al. 2004). Since most ants live in colonies in the soil, the catches in the grapevine canopy are, however, not representative of ant densities (Schlick-Steiner et al. 2006). By contrast, densities of *Empoasca* sp. and thrips, as well as the abundance of earwigs were not affected by fungicide intensities. James et al. (2002) already reported a certain tolerance of thrips towards fungicides. Although earwigs are susceptible to insecticides, fungicides appear to have no or low effects on them (Huth et al. 2011; Malagnoux et al. 2015; Orpet et al. 2019). The experimental vineyard was not treated with insecticides, which could thus explain why earwigs were not affected by reduced sprayings. Similar results were also observed in vineyards of self-marketing wineries in the same study region (Reiff et al. 2023).

Effects of minimal pruning

In contrast to the consistent effects of fungicides, effects of minimal pruning were less strong, and restricted to the leaf mesofauna, earwigs, and leafhoppers. Contrarily, total arthropod abundance and, particularly, predator abundance were not affected by minimal pruning. This contrasts findings of Langellotto and Denno (2004) highlighting that increased architectural complexity of plants promotes natural enemies. However, the effects of the different pruning systems were strongly modulated by the studied years and presumably affected by weather conditions. Beneficial mites as well as *Co. vitis* densities were higher in minimal pruning. Both pest and beneficial mites overwinter on wooded parts of the grapevine, e.g., in bark fissures and under grape bud scales (Kinn and Doutt 1972; Duso and de Lillo 1996). The higher amounts of wood and increased abundance of buds in minimal pruning might thus have already increased overwintering populations. Further, Duso and de Lillo (1996) describe a favourable development of *Co. vitis* inside leaf patches with high RH. Similarly, high humidity and leaf moisture favours phystoseiid development (Nakai et al. 2021). In agreement with this, the densities of *Co. vitis* and Phytoseiidae were higher in minimal pruning with increased humidity in the grapevine canopy (Kraus et al. 2018). By contrast, thrips densities were higher in trellis system despite their development being also favoured by humidity (Omkar 2021). This indicates increased natural pest control in minimal pruning. However, the abundance of omnivorous earwigs was higher in trellis system. Huth (2011) found more earwigs in tight compared to loose grape clusters. Since minimal pruned vines have less compact grape clusters than vines in trellis system (Intrieri et al. 2011) we assume that earwigs find more shelter in trellis systems despite the higher wood proportion in minimal pruning. Despite possible differential effects of altered canopy architecture and resulting microclimatic variation on some spider families (Entling et al. 2007; Herrmann et al. 2010), overall spider abundance remained unaffected by minimal pruning. Pennington et al. (2019) describe opposing effects of minimal pruning for some spider families, but detected no overall effect on spider abundance either.

Implications for pest control

Lobesia botrana is consumed by a wide range of predators, including spiders, earwigs, and ants (Marchesini and Dalla Montà, 1994; Reiff et al. 2021b). Both predator abundance and predation rates on *L. botrana* eggs increased in reduced fungicide sprayings. These findings are in line with studies in vineyards of self-marketing wineries in Austria and Germany (Reiff et al. 2021b, 2023). Furthermore, reduced pesticide input fostered predator abundances and pest control also in other viticultural regions (Gaigher and Samways 2010; Caprio et al. 2015; Muneret et al. 2019a, 2019b), but it remains unclear to which extent this effect results from the reduction of fungicides, herbicides and/or insecticides. While predator abundance decreased in 2019, predation rates were still high. On the one hand, predators could have been more effective. For instance, a single individual of *Tettigonia viridissima* (Orthoptera:Tettigoniidae) predated five pupae of *L. botrana* in one night of camera-surveyed sentinel card exposition (Reiff et al. 2021b). On the other hand, predator communities with high biomass species (like earwigs) may have higher intraguild predation and, thus, reduced effects on pest populations (Ostandie et al. 2021).

In the study region, Typhlocibynae such as *Empoasca* sp. were the most abundant leafhoppers in beat-sheet samples (Reiff et al. 2023). Despite being susceptible to organic spraying regimes with copper and sulphur (Pavan 1994) we found no effects of reduced sprayings on leafhopper abundance. Nevertheless, regarding the overall low number of leafhoppers we assume that generally high numbers of predators kept leafhopper densities below economic relevance.

All four pest taxa of the leaf mesofauna occurred in relatively low densities. For instance, economic thresholds of 280 mites per leaf of *Cal. vitis* (Hluchý and Pospíŝil 1992) were undercut by almost a factor of ten in our study. Similarly, even the highest thrips densities in our study undercut the economic threshold by a factor of eight (James et al. 2002). Infestations with *Empoasca* sp. in vineyards can cause severe damage to quality and yield (Bosco et al. 1997; Olivier et al. 2012). However, even in 2018 their density undercut the economic threshold by almost a factor of three (Popa and Roşca 2011). Furthermore, it is assumed that the infestation with *Co. vitis* does not cause severe damages (Duso and de Lillo 1996). Both eriophyid mites responded negatively to increased densities of Tydeidae, and *Empoasca* sp. appeared to be negatively affected by increasing densities of Phytoseiidae. Both Phytoseiidae and Tydeidae can feed on several pests such as eriophyid mites and thrips (Schruft 1972; Engel and Ohnesorge 1994). We thus assume that natural pest control was effective throughout the study period, particularly in the treatments with reduced fungicide applications.

Limitations and future perspectives

Experimental vineyards allow to investigate effects in a standardized way. However, upscaling to display real-world conditions must be done with caution. Given the small plot size in our studied vineyard and the high mobility of certain taxa, arthropod movement between plots could have dampened the results. Furthermore, grape varieties affected several taxa without a clear pattern which fails to fit with morphological characteristics (e.g., leaf hairiness). Despite these limitations, similar impacts of fungicides were found in single-year studies across multiple vineyards of the same study region under production conditions of commercial vineries with multiple grape varieties (Reiff et al. 2021a, 2023). Taken together, the long-term study in the experimental vineyard and the observations in the commercial vineries suggest that most of the observed effects can be generalized in our study region.

Arthropods in general and especially phytoseiid mites are directly affected by weather conditions, such as heat and drought, but also indirectly by plant growth (habitat, food, shelter) and pathogen pressure (Yarwood 1943; Cerdá et al. 1998; Duso et al. 2005; Pozzebon and Duso 2008; Gadino and Walton 2012; Orpet et al. 2019; Fricke et al. 2022; Kaczmarek et al. 2022). However, given the large number of variables likely to affect arthropod abundances, we cannot adequately address inter-annual variability. Hence, it underlines once more the persistent benefits of reduced fungicide sprayings. In the studied varieties disease incidence was low in all years even under the strongest fungicide reduction. We conclude that fungicide applications can be strongly reduced in fungus-resistant varieties while maintaining healthy grapes.

With generally high numbers of predators (Shapira et al. 2018; Retallack et al. 2019; Sáenz-Romo et al. 2019; Reiff et al. 2023), vineyards are habitats with high potential for natural pest control. Our study revealed that natural pest control can be fostered even more by reducing fungicide sprayings. Despite altered climatic conditions in the five consecutive years of the study and arthropods of different trophic levels being studied, we found consistently strong benefits of reduced fungicide sprayings. However, effects of minimal pruning were less constant over the studied years. We conclude that short-term studies may be sufficient to predict the effect of strong-impact variables like fungicide sprayings. Nevertheless, long-term studies remain important to display effects of other predictor variables. Moreover, with increasing effects of habitat disruption and climate change, short-term studies may fail to predict the direction of shift. In this sense, the benefits of altered microclimatic conditions in minimal pruned grapevine canopies may become more prominent with climate change.

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Author contributions MHE, CH, TP, SK and JMR conceived the ideas and designed methodology; JMR, TP, SK, KT, EA, PM, RS, ME performed the experiment; JMR led the data analysis and writing. All authors contributed critically to the drafts and gave final approval for publication.

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Declarations

Conflict of interest The authors have no financial or proprietary interests in any material discussed in this article.

Research involving human and animal rights The study did not involve human participants and/or vertebrate animals.

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