Grape moth density in Bordeaux vineyards depends on local habitat management despite effects of landscape heterogeneity on their biological control

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Summary

1. Biological control of crop pests is a major ecosystem service affected by several variables acting at multiple spatial scales. Among these variables, heterogeneity at the habitat and landscape scales are known key drivers of trophic interactions and pest density in agroecosystems. However, studies that try to disentangle their relative effects in perennial cropping systems are scarce and nothing is known about their impacts on insect pest density and pesticide applications.

2. We examined the effect of heterogeneity at these two scales on grape moths, one of the most damaging insect pests in European vineyards, and their biological control in 20 vineyards during three consecutive years. We used local vegetation management and the proportion of semi-natural habitats in the surrounding landscape as proxies of heterogeneity at the habitat and landscape scales. Grape moth density was measured over time, as well as biological control services provided by different groups: birds, invertebrate predators, parasitoids and entomopathogenic fungi.

3. Over the 3 years, grape moth density was mainly determined by local heterogeneity, with significantly fewer larvae of the first generation established in vineyards with full compared to partial grass cover.

4. Despite these effects, biological control of grape moths was not primarily affected by local vegetation management but by landscape heterogeneity, and the direction of this effect varied over time. Notably, predation by birds increased with landscape heterogeneity in spring, depending on local vegetation management, while attacks by pathogenic fungi decreased with landscape heterogeneity during winter.

5. *Synthesis and applications.* Our results suggest that bottom-up processes related to habitat heterogeneity drive grape moth occurrence much more than top-down processes. These results have important implications for the ecological intensification of vineyard landscapes. We found that maintaining full grass cover within vineyards reduced grape moth density to a level below common intervention thresholds. Landscape heterogeneity in the close vicinity of vineyards contributed to improved biological pest control by birds, but depended on local vegetation management. Moreover, opposing effects of landscape management on biological pest control services over time revealed that strategies based only on manipulating landscape heterogeneity might not be the optimal option to limit grape moth density in vineyards.

Key-words: agroecology, ecosystem services, *Eupoecilia ambiguella*, landscape, *Lobesia botrana*, natural pest control, parasitoid, predator, *Vitis vinifera*

Introduction

Agricultural intensification has been successful in increasing food production. However, several negative impacts on the environment and human health have become evident (Matson *et al.* 1997; Tilman *et al.* 2001; Moss 2008). Ecological intensification of farming systems has been proposed as a way to reduce these impacts by including regulating and supporting ecosystem services in agricultural practices (Bommarco, Kleijn & Potts 2013).

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Biological control of crop pests by their natural enemies is a major ecosystem service delivered by natural enemies such as predators or parasitoids that may contribute to more sustainable pest management in agroecosystems (Losey & Vaughan 2006). The integration of biological control strategies into farming systems could be greatly improved if we better understand mechanisms driving their delivery as well as the scales at which they operate (Chaplin-Kramer *et al.* 2011; Bommarco, Kleijn & Potts 2013). The factors that determine the strength of trophic interactions and success of biological control measures across spatial scales from the plants to the landscape are not well-known (Rusch *et al.* 2010). It remains therefore unknown which management options farmers should use to enhance pest control and reduce pest damage, particularly in perennial agroecosystems.

A large body of evidence exists concerning the positive effect of habitat heterogeneity on natural enemies, herbivores and the strength of trophic interactions (Langellotto & Denno 2004). Several studies have demonstrated that more complex habitats exhibit lower herbivore populations and reduced plant damage (Langellotto & Denno 2004; Letourneau *et al.* 2010). This effect is attributed to two non-exclusive hypotheses: the natural enemy and the resource concentration hypotheses (Root 1973; Rusch *et al.* 2010). The first hypothesis states that fewer herbivores and reduced damage occur in more complex habitats owing to more abundant and/or diverse predators and parasitoids (Langellotto & Denno 2004). This hypothesis attributes the effect to a higher attractiveness of complex habitats for predators because of increased resource availability. The second hypothesis states that smaller herbivore populations and reduced plant damage are found in more complex or diverse habitats owing to a lower probability of herbivores finding host plants and feeding or reproducing in these habitats (Root 1973; Andow 1991). This effect is attributed to chemical or physical confusion as well as change in plant physiological status due to interspecific competition.

Higher landscape heterogeneity (e.g. higher proportion of semi-natural habitats in the landscape) may promote the abundance and diversity of natural enemies and increases the level of biological control in agroecosystems (Chaplin-Kramer *et al.* 2011; Rusch *et al.* 2016). Moreover, it has been hypothesized that landscape heterogeneity may interact with local management to modify levels of biodiversity and ecosystem service delivery (Tscharntke *et al.* 2005). According to this hypothesis, the benefits of local management on ecosystem services are expected to be smaller in extremely simple or complex landscapes compared with landscapes of intermediate heterogeneity. Such effects are expected because levels of biodiversity and services are assumed to be high in complex landscapes, whereas the species pool in extremely simplified landscapes may not be sufficient enough to result in positive effects of local management on biological control. However, at an intermediate level of heterogeneity, biodiversity is expected to be sufficient to reveal the benefit of local management on services. This hypothesis, however, lacks empirical validation and the relative effect of heterogeneity at both local and landscape scales on pest populations in agroecosystems remains poorly understood (but see Birkhofer *et al.* 2015).

Most studies focussed on effects of landscape heterogeneity on natural enemies have been conducted in annual cropping systems and almost nothing is known concerning perennial cropping systems. Such systems greatly differ from annual cropping systems in terms of temporal dynamics and disturbance regimes (Bruggisser, Schmidt-Entling & Bacher 2010; Rusch *et al.* 2015). These dynamics could therefore modify the effect of landscape composition on natural enemy and pest communities as well as on biological control services. Moreover, a large majority of studies on biological control have quantified the effect of landscape heterogeneity on predation or parasitism rates at a given period within the season and very little is known about the temporal variability in biological control (but see Costamagna, Venables & Schellhorn 2015). Consequently, it is not known if landscape heterogeneity positively affects pest control over time or if antagonistic relationships between guilds emerge over time leading to neutral effects of landscape heterogeneity. Exploring how landscape heterogeneity affects natural pest control services at different time period and delivered by multiple natural enemies is therefore a major challenge from an applied perspective.

Grape moths (Lepidoptera, Tortricidae) are among the most damaging insects in vineyards (Thiéry 2008). However, mechanisms explaining their distribution pattern on large scales and effects of natural enemies on populations are almost unknown (but see Rusch *et al.* 2015). In the present study, we used management of local vegetation in vineyards and the proportion of semi-natural habitats in the landscape to analyse the relative contribution of heterogeneity at the local and landscape scale on grape moth density and their biological control for different periods. We examined biological control services delivered by a wide range of natural enemy groups ranging from entomopathogenic fungi to birds across a 3-year period. We hypothesized that levels of pest control are higher in more complex habitats (H1) as well as in more complex landscapes (H2) and that these effects jointly lead to lower pest density (H3). We also expected that local heterogeneity affects the level of biological control depending on landscape heterogeneity, with higher effects of local heterogeneity at intermediate levels of landscape heterogeneity (H4). By including biological pest control services delivered by different functional groups at different periods we expected to highlight potential synergies or trade-offs between pest control services in agroecosystems.

Materials and methods

STUDY SITES AND DESIGN

We selected field sites in Bordeaux vineyards in southwestern France that allowed testing for the effects of heterogeneity at the local and the landscape scale on biological pest control services by multiple natural enemy groups on the main insect pests of grapevine. This wine production area is about 138 000 ha of vineyards, approximately 20% of the French vineyard area, and receives between 13 and 16 treatments (including herbicide, fungicide and insecticide) a year per unit area (Agreste, 2013). At the national level, approximately 15% of the total volume of pesticides applied is dedicated to vineyards (Butault *et al.* 2010). There is therefore a strong need for alternative ways to manage pest populations in vineyards and reduce pesticide use.

Our study design consisted of 20 independent vineyards that differed in local vegetation management and that were selected along a landscape heterogeneity gradient. Among the study sites, local heterogeneity differed in the management of the vegetation between grape rows. Our design initially consisted of nine fields with partial grass cover due to soil tillage in half of the inter-rows and 11 fields with full grass cover due to no tillage in the inter-rows. Landscape heterogeneity was calculated as the proportion of semi-natural habitats in a 1-km radius around each vineyard (Westphal, Steffan-Dewenter & Tscharntke 2003). This proportion ranged from 0% to 68%. Semi-natural habitats mainly consisted of woodland, grasslands, hedgerows and shrubs. All vineyards were planted with the same cultivar (Merlot) and vine stock density did not differ between surveyed vineyards (approximately 5000 vine stocks ha^{-1}). Information about fungicide and insecticide active ingredients used in the studied vineyards is provided in Table S1, Supporting Information.

ECOLOGY OF GRAPE MOTHS IN GRAPEVINE

Four grape moth species are found in most European vineyards and are distributed according to their climatic preferences: the European grapevine moth *Lobesia botrana* (Denis & Schiffermüller), the grape berry moth *Eupoecilia ambiguella* (Hübner) and the grape tortrix *Argyrotaenia ljungiana* (Thunberg) are polyvoltine species, while the leaf-rolling tortrix *Sparganothis pilleriana* (Denis & Schiffermüller) is univoltine. These species are the major grapevine pests in Europe, and larvae naturally develop on most grapevine cultivars (Thiéry & Moreau 2005; Thiéry, Monceau & Moreau 2014). In our study region, the major species are *L. botrana* and *E. ambiguella*. Females oviposit on grape clusters and larvae are polyphagous and feed on flowers or berries. Although the larvae are polyphagous, *Vitis vinifera* is their main host in areas dominated by vineyards (Maher & Thiéry 2006). These species complete three generations and two complete life cycles per year in the study area and therefore have high potential to show strong dynamics in population size over the growing season within the year. Both species mostly overwinter as pupae under the bark and occasionally in the soil.

MEASURES OF GRAPE MOTH DENSITY

In each vineyard, we measured density of grape moth larvae for the first (end of May–early June) and second generations (end of July–early August) during three consecutive years from 2013 to 2015. Because larvae build nests with silk, we monitored nest occurrence along transects of 100 independent grape clusters per field to assess grape moth density. All larval nests were collected and returned to the laboratory. They were maintained in small individual boxes with fresh parts of bunch (collected on the same sampling plot) to provide additional larval food until the end of their development (Moreau *et al.* 2009). Larval populations were checked until pupation, upon which pupae were removed from the flower buds and individualized in glass tubes and stored at 22 °C, 60% relative humidity and 16/8 h of light/dark. Adult moths were identified after emergence.

QUANTIFICATION OF BIOLOGICAL PEST CONTROL SERVICES

We measured biological pest control services of grape moths at different key phenological stages of vineyards. Our assessment covered the biological control services delivered by entomopathogenic fungi, parasitoids, arthropod predators and birds during winter, spring and summer. Parasitism rates in spring were assessed each year using larval populations collected as described previously. Parasitoid species were identified after emergence using the taxonomic key in Sentenac (2011).

During 2013–2015, avian insectivory was assessed using plasticine caterpillars that imitate natural prey of insectivorous birds (Maas *et al.* 2015). These plasticine caterpillars (light brown, inodorous) were 1 cm long to simulate caterpillars of *L. botrana* and *E. ambiguella* (third–fourth larval instars), and were positioned on grape inflorescences using metal wires (diameter of 0·5 mm). Each year, in May, 30 plasticine caterpillars were exposed to predation on six vine stocks in each vineyard. Ten days after the placement, the plasticine caterpillars were collected and checked for predation marks. Based on typical and distinguishable marks, we classified predation marks using the following groups: birds, mall rodents, reptiles, arthropods or unidentifiable predators (Barbaro *et al.* 2012). We only kept marks left by insectivorous birds in further analyses as frequency of observation of the other groups were very low.

During the summer (i.e. the second grape moth generation), biological control of eggs and larvae by invertebrates was assessed using sentinel cards located in the centre of each vineyard in 2014 and 2015. These measures took place during the summer between vine stages 73 and 75 (BBCH scale) (Baillod & Baggiolini 1993). The development stages correspond to the summer generation of the grape moths in vineyards in our study area. We used eggs and larvae of laboratory strains of *L. botrana* and *E. ambiguella* as described in Thiéry & Moreau 2005. In each vineyard, 100 freshly laid eggs (10 cards of 10 eggs each, with each a 2 ×5 cm plastic card) of *L. botrana* were exposed to predation and parasitism. Eggs were directly laid at the same time during one night on a greaseproof white paper (Cenpac©, Roissy-En-France, France, 45 g m^{-2}) in breeder laying cages. The laying paper was cut in batches of 10 eggs under binocular microscope and then glued on a plastic card. All cards were placed on bunches using wires. After 5 days, the cards were collected and the number of removed or parasitized eggs was assessed. We also exposed 50 pinned larvae in each vineyard (10 cards of 5 larvae each, with each a 5 ×10 cm cardboard card). The number of removed larvae was assessed after a 24-h exposure period.

Predation, parasitism and mortality in winter were assessed using overwintering pupae in 2013, 2014 and 2015. In each vineyard, 10 cards each supporting 10 overwintering pupae of *L. botrana* were exposed starting from the end of September and during winter on trunks. In early March of the following year, cards were collected and the number of intact pupae as well as the number of pupae with marks of parasitism, predation or infestation with pathogenic fungi was assessed for each card. All collected and intact pupae were then individualized in glass tubes until emergence.

STATISTICAL ANALYSES

We used generalized linear mixed effect models (GLMM) to analyse the effect of local vegetation management and landscape heterogeneity (at a specific spatial scale) on variables characterizing either pest density or biological pest control. Depending on the nature of the response variables we fitted models with a binomial distribution (proportional data) or a Poisson distribution (count data). We used the number of larval nests as the response variable to analyse grape moth density. Models examining biological pest control services used the following response variables independently: the proportion of parasitized larvae, the proportion of predated eggs, the proportion of predated larvae, the proportion of plasticine caterpillars with avian predation marks or the proportion of overwintering pupae with marks of predation, parasitism or pathogenic fungi. In all models, fixed effects were the proportion of semi-natural habitat at a given scale and the type of local vegetation management (full or partial grass cover). Year and field site were fitted as crossed random effects to control for repeated samplings within and between years when needed. We did not include data from 2014 when analysing larval parasitism of the first generation owing to extremely low grape moth populations in this year. GLMM were checked for overdispersion and an individual-level random effect was added when overdispersion was present. Table 1 summarizes all the response data available and the types of models used to analyse the different data sets. Models were simplified by backward selection based on the Akaike information criterion (AIC) value (drop1 function). Significance of fixed effects and their interactions were tested by comparing models with a

likelihood-ratio test (i.e. χ^2 test). We fitted separate models for each spatial scale (i.e., 250, 500, 750, 1000 m) and used the AIC to compare individual models. We performed graphical validations of model assumptions and the assumptions were not violated.

In addition, to verify that there was no cofounding effect between overall insecticide applications in our study sites and grass cover management or the landscape heterogeneity gradient, we collected the name of the insecticide used, the number of applications, the rate of applications and the area treated. Based on these data we calculated the Treatment Frequency Index (TFI) that is an indicator commonly used in Europe to estimate the cropping system dependence on pesticides (OECD 2001). The index was calculated for 2013 (17 fields on 20) and 2014 (18 fields on 20) as we could only have access to these data (see Appendix S1 for further details on index calculations). For each year, we build a linear model using the TFI for insecticide as a response variable and the grass cover management and the landscape heterogeneity gradient (and their interaction) as explanatory variables. No effect of these variables was found for each year (see Appendix S1).

All the analyses were performed using R, version 2.15 (R Core Team, 2015) and the package "lme4" (Bates *et al.* 2015).

Results

GRAPE MOTH DENSITY

Grape moth density and species composition of both generations varied between the three consecutive years (Table 2). Larval nest density was the highest in 2013 for both generations while the lowest numbers were recorded in 2014. Vegetation management significantly affected the number of larval nests for the first generation ($\chi^2 = 6.42$, $P = 0.01$), with more larval nests in partially covered fields than in fields with full grass cover (Fig. 1a and Table 3). No effect of landscape heterogeneity was detected on larval nest density for the first generation. There was no effect of local vegetation management or

Table 1. Characteristics of the full models used for each response variable concerning pest density and biological control services

Response variables	Sampled year	Model structure	Error distribution
Number of larval nest (first and second generation)	2013, 2014, 2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Year) + (1|Field)	Poisson
Larval parasitism rates (first generation)	2013, 2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Year) + (1|Field)	Binomial
Avian predation (first generation)	2013, 2014, 2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Year) + (1|Field)	Binomial
Egg predation (second generation)	2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Field)	Binomial
Larval predation (second generation)	2014, 2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Year) + (1|Field)	Binomial
Overwintering predation	2013, 2014, 2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Year) + (1|Field)	Binomial
Overwintering parasitism rates	2013, 2014, 2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Year) + (1|Field)	Binomial
Overwintering pathogenic fungi	2013, 2014, 2015	Y ~ Local vegetation management * proportion of semi-natural habitats at a given scale + (1|Year) + (1|Field)	Binomial

Table 2. Summary of the mean number of grape moth larval nests per field (±SD), moth species composition and parasitism rates for the first and second moth generations in 2013, 2014 and 2015

Year	Mean number of larval nests per field	Moth species composition (%)		Parasitism rates (%)	
		Lobesia botrana	*Eupoecilia ambiguella*	*Lobesia botrana*	*Eupoecilia ambiguella*
1st generation (Spring)					
2013	11·7 ± 17·8	70	30	17·1 ± 27·3	6·6 ± 16·4
2014	3·8 ± 10·1	47	52	2·3 ± 7	16·9 ± 35·2
2015	7·1 ± 7·2	52	47	1·4 ± 3·8	10·6 ± 11·2

Year	Mean number of larval nests per field	Species composition from emerged moths (%)		Parasitism rates (%)	
		Lobesia botrana	*Eupoecilia ambiguella*	*Lobesia botrana*	*Eupoecilia ambiguella*
2nd generation (Summer)					
2013	18·4 ± 30·4	97	3	2·2 ± 6	0
2014	0	–	–	–	–
2015	7·4 ± 11·6	97	3	0	0

landscape heterogeneity over the 3 years for the second generation (Fig. 1b).

BIOLOGICAL PEST CONTROL DURING SPRING

Parasitism rates

Mean larval parasitism rate of the first generation per field was highly variable between fields and years (Table 2). The mean parasitism rate of the first generation of *L. botrana* was the highest in 2013 (17·1 ± 27·3%), whereas for the first generation of *E. ambiguella* the highest was in 2014 (16·9 ± 35·2%). Local vegetation management or landscape heterogeneity at any spatial scale did not affect parasitism rates of the first generation of both moth species.

Avian predation

The mean proportion of plasticine caterpillars per field with avian predation marks was generally low. We found a mean proportion of plasticine caterpillars with avian predation marks of 15·5 ± 10% per field in 2013, 4·3 ± 5·3% in 2014 and 9 ± 5·7% in 2015.

The model using the proportion of semi-natural habitats in the 250 m radius had the lowest AIC among all models. We found a significant effect of the interaction between local vegetation management and landscape heterogeneity on the proportion of attacked plasticine caterpillars considering landscape heterogeneity at this scale ($\chi^2 = 5.00$, $P = 0.02$) (Fig. 2 and Table 3). We found a positive effect of landscape heterogeneity on avian predation only in vineyards with partial grass cover. In the models that considered landscape heterogeneity at 1000, 750 or 500 m, the landscape heterogeneity did not significantly affect avian predation.

BIOLOGICAL PEST CONTROL DURING SUMMER

Egg predation, larval parasitism and predation

The mean predation rate of eggs from the second generation per field was 30·6 ± 13·6 in 2015. Mean larval parasitism rate per field was extremely low or non-existent for the second generation and were thus not analysed (Table 2). Mean predation rates on larvae per field were 77·2 ± 19·3% in 2014 and 84·5 ± 21·9% in 2015. Local vegetation management or landscape heterogeneity (at any spatial scale) did not affect egg predation or larval predation.

BIOLOGICAL PEST CONTROL DURING WINTER

Parasitism rates

The mean parasitism rate for overwintering pupae per field was 1·9 ± 4·9% in 2013, 12·2 ± 9·0% in 2014 and 7·2 ± 7·4% in 2015. Local vegetation management or landscape heterogeneity at any spatial scale did not significantly affect parasitism rates of overwintering pupae.

Predation rates

The mean predation rate on overwintering pupae per field was 26·4 ± 16·3% in 2013, 16·1 ± 8·5% in 2014 and 34·8 ± 16·9% in 2015. Local vegetation management or landscape heterogeneity at any spatial scale did not significantly affect predation rates of overwintering pupae.

Pathogenic fungi

The mean proportion of pupae attacked by pathogenic fungi per field was 18 ± 11·5% in 2013, 22·4 ± 12·2% in 2014 and 8 ± 5·8% in 2015.
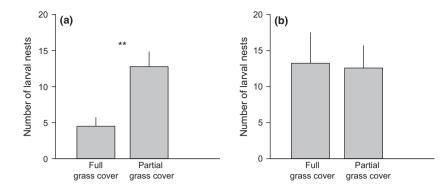
Fig. 1. Mean number of larval nests per 100 grape clusters (±SEM) over the three sampled years depending on the type of local vegetation management (full vs. partial grass cover) for the (a) first and (b) second moth generations. **$P = 0.01$.

Table 3. Summary of the final generalized linear mixed-effects models explaining the number of grape moth larval nests in the spring, the rates of moth predation by birds or the proportion of overwintering moth attacks by pathogenic fungi. For the number of moth larval nest model: marginal $R^2 = 0.15$ and conditional $R^2 = 0.26$; for the avian predation rates model: marginal $R^2 = 0.02$ and conditional $R^2 = 0.09$; for the pathogenic fungi model: marginal $R^2 = 0.02$ and conditional $R^2 = 0.15$

Response variables	Explanatory variables selected in the final model	Estimate	Standard error	z value	P value
Number of moth larval nest (first generation)	Local vegetation mgmt (:partial grass cover)	1·64	0·55	2·93	0·003
Avian predation rates (first generation)	Local vegetation mgmt (:partial grass cover)	−0·20	0·33	−0·60	0·54
	% of semi-natural habitats at 250 m	−0·009	0·008	−1·07	0·28
	Local vegetation mgmt × % of semi-natural habitats at 250 m	0·03	0·0164	2·31	0·02
Pathogenic fungi (during winter)	% of semi-natural habitats at 500 m	−0·017	0·006	−2·66	0·007

Landscape heterogeneity was always retained in the final model as the only significant explanatory variables (250 m: $\chi^2 = 3.75$, $P = 0.05$; 500 m: $\chi^2 = 6.02$, $P = 0.01$; 750 m: $\chi^2 = 4.35$, $P = 0.03$; and 1000 m: $\chi^2 = 3.5$, $P = 0.06$). Models revealed that landscape heterogeneity negatively affected the proportion of pupae attacked by pathogenic fungi at all spatial scales (Fig. 3 and Table 3). The model including the proportion of semi-natural habitats in the 500 m radius had the lowest AIC. There was
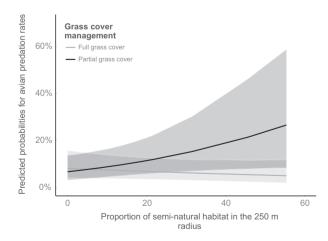

Fig. 2. Interactive effect of local vegetation management and the proportion of semi-natural habitats in the 250 m radius on the proportion of plasticine caterpillars with typical predation marks left by birds. Lines represent model predictions with 95% confidence intervals, grey line represents vineyards with full grass cover, black line represents vineyards with partial grass cover.

no effect of local vegetation management on the proportion of pupae attacked by pathogenic fungi.

Discussion

Biological control of grape moths was not affected by local vegetation management but mainly by landscape heterogeneity and the direction of this effect varied over time. Despite this effect, the larval nest density of the first moth generation was only affected by local vegetation management with a significantly lower number of larval nests per field in vineyards with full compared with partial grass cover. These results suggest that a bottom-up process related to the resource concentrations hypothesis, much more than a top-down process related to the natural enemy hypothesis, determined grape moth density in the vineyards. Our findings provide valuable information for winegrowers and practitioners to develop ecological intensification in vineyards by indicating that management of within-field vegetation is a key option to potentially reduce grape moth density early in the season.

LOCAL HETEROGENEITY AFFECTS GRAPE MOTH ESTABLISHMENT

Our results suggest that grape moth occurrence was reduced in vineyards with full compared with partial grass cover. This result is consistent with the large body of literature demonstrating lower herbivore populations and damage in complex habitats (Langellotto & Denno 2004).
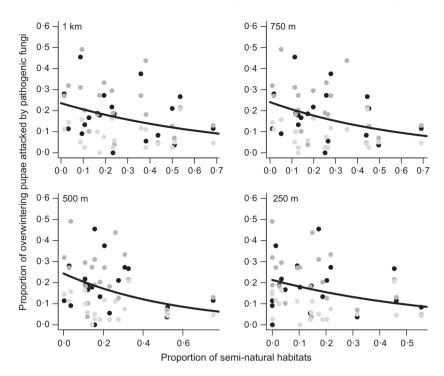
Fig. 3. Mean level of overwintering pupae attacked by entomopathogenic fungi in relation to the proportion of semi-natural habitats in the 1-km, 750-m, 500-m and 250-m radius around fields. Dot colours (black, grey and light grey) represent the 3 sampled years (2013, 2014 and 2015, respectively). The lines represent model predictions.

Our results suggest that this effect is mainly due to bottom-up processes related to the resource concentration hypothesis and not to top-down effects such as for the natural enemy hypothesis. We did not find any effect of local vegetation management on predation or parasitism rates of the pests (eggs, larvae and pupae) that would support a strong top-down effect for any season.

The resource concentration hypothesis states that insect herbivores are more likely to find and remain on their host plants in simple habitats such as monocultures, where their host is concentrated (Root 1973). This effect can be attributed to a host dilution effect, chemical and physical disruption or camouflage during the process of finding the host plant, leading to lower egg deposition and higher emigration rates of females (Thiery & Visser 1986; Finch & Collier 2000; Rusch *et al.* 2010). For instance, Finch & Collier (2000) found significantly fewer eggs laid by eight insect pest species on cabbage plants if grown with clover compared to monoculture. Finding appropriate sites for oviposition is a challenging task for grape moth (Gabel & Thiéry 1996) and females use a combination of visual, mechanical and chemical volatile and non-volatile information to locate and select the host plant for oviposition (Tasin *et al.* 2005; Anfora *et al.* 2009). Among these cues, olfaction plays an important role as shown by the well-developed antennal lobes of *L. botrana* and help in understanding the resource concentration hypothesis. Such an effect may be supplemented by a plant vigour effect on pest density attributed to different levels of competition among plants owing to grass cover management (Price 1991). This hypothesis states that herbivores preferentially feed on vigorous plants because they provide a better food source owing to changes in plant growth and physiological status (Price 1991; Waring & Cobb 1992). This hypothesis has been validated over a large range of insect species (Waring & Cobb 1992). In grapes, a recent study demonstrated that soil management affects vigour and thus fungal disease development (Valdés-Gómez *et al.* 2011). Our results support the idea that vegetation management and reduced soil tillage between rows is a promising management option correlated with reduced pest pressure in vineyards.

LANDSCAPE HETEROGENEITY AFFECTS BIOLOGICAL PEST CONTROL SERVICES OVER TIME

Our results showed that landscape heterogeneity was the main variable affecting biological pest control service in vineyards and that the direction of this effect varied over time, ranging from negative to positive.

We found an interactive effect between the proportion of semi-natural habitats in the surrounding landscape and local vegetation management on predation of larvae by birds.

Predation of larvae in spring increased with the proportion of semi-natural habitats around vineyards with partial grass cover. There was no effect of landscape heterogeneity on predation by birds in vineyards with full grass cover. The fact that insect predation by birds increased with the proximity of semi-natural habitats is consistent with recent studies of bird predation on arthropod pests (Karp *et al.* 2013; Maas *et al.* 2015). That we only detected an effect of semi-natural habitats in vineyards with partial grass cover might have its origin in effects of grass cover on bird community composition or from higher prey density in those fields. Crop structure

and management affects bird communities in agricultural landscapes and therefore the potential for pest control (Wilson, Whittingham & Bradbury 2005; Jedlicka, Greenberg & Letourneau 2011). Moreover, prey densities in vineyards with full grass cover may not have been sufficient to lead to a functional response of bird communities (Salamolard *et al.* 2000). That the positive effect of semi-natural habitats was only detected at the 250 m spatial scale suggests that spillover of birds between semi-natural habitats and vineyards operated at relatively small scales.

Landscape heterogeneity did not affect predation or parasitism rates of sentinel eggs and larvae by arthropods during spring and summer. This result contradicts recent meta-analyses on the effect of landscape heterogeneity on biological pest control services in agroecosystems (Chaplin-Kramer *et al.* 2011; Rusch *et al.* 2016). It should, however, be noted that the large majority of these studies considered annual cropping systems. Perennial crops such as vineyards greatly differ from annual crops in terms of disturbance regimes and resource availability over time (Bruggisser, Schmidt-Entling & Bacher 2010; Rusch *et al.* 2015). Perennial systems usually receive high amounts of agrochemicals that may limit biological pest control services (Rusch *et al.* 2015). Moreover, perennial crops have higher resource continuity over time compared with annual crops and this may limit spill-over of species and ecosystem services from semi-natural habitats (Rand, Tylianakis & Tscharntke 2006; Schellhorn, Gagic & Bommarco 2015). We could not test the effect of pesticide use *per se* in the landscape on pest density and biological control services as such data were not available for our study area. Jonsson *et al.* (2012) recently found that landscape-context effects on host–parasitoid interactions were mainly driven by habitat disturbance and frequency of insecticide application. Thus, we cannot exclude the fact that pesticide might have hindered the detection of landscape heterogeneity effects on predation or parasitism rates.

Landscape heterogeneity negatively affected the rate of attacks by pathogenic fungi during winter. This indicates that, in winter, simple landscapes have higher levels of pest control potential than more complex landscapes. This result stands in contrast to the large body of literature about biological pest control along landscape heterogeneity gradients (Chaplin-Kramer *et al.* 2011). However, to our knowledge, no study has quantified biological pest control during winter or by pathogenic fungi. The negative effect of landscape heterogeneity on the proportion of pupae attacked by pathogenic fungi suggests that, in vineyard landscapes, arable soils may be the main source of fungal inoculum compared with semi-natural habitats. Entomopathogenic fungi are widely distributed in soils of arable land and semi-natural habitats, but knowledge about the ecology of entomopathogenic fungi in agricultural landscapes remains limited (but see Keller, Kessler & Schweizer 2003; Meyling & Eilenberg 2007). However, several studies demonstrated that reduced or no-tillage systems increase the density of several key pathogenic fungi such as *Beauveria bassiana* or *Metarhizium anisopliae* (Meyling & Eilenberg 2007). All vineyards in our area are subject to reduced or no soil tillage and this could explain why the number of pupae attacked by pathogenic fungi increased with the proportion of arable land in the landscape. This effect was detected at all spatial scales, which suggests passive dispersal at a relatively large scale (Aylor 2003).

We illustrate here that trade-offs between individual biological pest control services can occur when considering several time periods, as shown by the negative effect of landscape heterogeneity on predation rates and attacks by pathogenic fungi during winter and the positive effect of landscape heterogeneity on avian predation during spring. Such trade-offs may explain why the strong effect of landscape heterogeneity on natural enemy communities and pest control services revealed by several previous studies does not necessarily lead to effective control of pest populations in agroecosystems (Chaplin-Kramer *et al.* 2011; Rusch *et al.* 2016). This result highlights the importance of considering temporal variation for designing agricultural landscapes that optimize biological control services (Schellhorn, Gagic & Bommarco 2015).

Conclusions and management implications

Ecological intensification of vineyards is urgently needed as wine growers usually apply significant amounts of pesticides, especially in the study area where the mean number of pesticide treatments is around 16 treatments a year per unit area (Agreste, 2013). Our results have important implications for the ecological intensification of agricultural landscapes and pest management in vineyards. The usual intervention threshold in southwestern France is one insecticide treatment against grape moth during the second generation when the number of larval nests during the first generation exceed five per 100 grape clusters (see Thiery 2005; for a thresholds review). Our results show that maintaining complete grass cover can maintain larval nest density of grape moths below this threshold, while partial grass cover implies the application of one insecticide treatment during the second generation. Moreover, maintaining partial grass cover within vineyards usually implies the use of herbicide (or soil tillage), which may be avoided if complete grass cover is maintained. Local vegetation management is therefore a valid habitat management option to limit pest density and pesticide use in vineyards of southwestern France. Moreover, we show that the presence of semi-natural habitats in the close vicinity of vineyards may improve the biological pest control by birds depending on local vegetation management even if landscape heterogeneity also reduces the attacks rates by entomopathogens. Further research is now needed to demonstrate which mechanisms related to the resource concentration and plant vigour hypotheses cause the local effect of vegetation heterogeneity observed in this study.

Authors' contributions

A.R. and D.T. conceived the ideas and designed the experiment; A.R. and L.D. collected the data; A.R. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Data for grape moth density, biological control services and habitat heterogeneity are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.85096 (Rusch *et al.* 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Summary of active ingredients (fungicide and insecticide) used in the 20 study fields.

Appendix S1. Calculation and statistical analyses on the Treatment Frequency Index.