AGRICULTURAL PRACTICES, BIODIVERSITY AND ECOSYSTEM SERVICES PROVIDED BY BATS IN MEDITERRANEAN CROPS

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Universitat de Girona

PhD Thesis

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Doctoral Programme in Environmental Sciences

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Sol·liciten el dipòsit de la present tesis doctoral, titulada:

Agricultural practices, biodiversity and ecosystem services provided by bats in Mediterranean crops,

El director, Dr. Constantí Stefanescu i Bonet,

El tutor, Dr. Pere Pons Ferran,

I l'autor, Xavier Puig Montserrat

Agraïments/acknowledgements

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List of publications

This thesis has been written as published peer reviewed articles compendium based on the specific regulations of the PhD program of the University of Girona.

Peer reviewed paper publications presented as chapters of this PhD is listed below, along with their Impact Factor (IF) from the 2019 update:

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General Index

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Summary

Agricultural intensification has allowed the world population to almost double in number during the last 50 years increasing only 10% the amount of cultured lands. The raise in production efficiency, mainly due to an agricultural intensification heavily relying on the use of chemical inputs in the crops, has severe detrimental effects on the ecosystems and significantly contributes to the global biodiversity crisis, often posing at risk both ecosystems' and human health. There's general consensus from both regional and international institutions within Europe (as in many other parts of the world) regarding the need for more sustainable farming practices. The supposedly biodiversity friendly farming approaches are diverse, ranging from integrated pest management systems, where the use of pesticides is minimized though allowed, to organic practices, where no synthetic inputs are allowed and where the natural regulatory ecosystem services are promoted as means to keep pests under control. Yet, the effect the different farming treatments have on biodiversity is not clear-cut, varying across taxa and along with structural and geographical factors beyond the crop limits. Regarding the regulatory ecosystem services, supposedly enhanced by organic farming practices, bats constitute an excellent case study in European crops since they have high metabolic rates and consume almost exclusively arthropods in the temperate regions of the planet, thus turning them into natural arthropod suppressors. The economic impact of bats as crop pest suppressors has been acknowledged to be over 3.5 billion dollars per year in the USA alone, yet nor local neither global estimates on their impact exist for Europe. This thesis focuses on the crop-biodiversity interactions and regulatory ecosystem services occurring in three major crops found in the Mediterranean basin, namely vineyards, olive groves and rice paddies, all of them occurring in Catalonia (NE Iberia), where all the field experiments have been conducted.

Mostly vineyards but also to some extent olive groves have seen a rapid transition to organic farming in the region, though the majority of them are still under conventional farming practices. Chapter 1 assesses the effect vineyard organic farming practices have on several taxa, and as expected, the sessile organisms (vascular plants) show a stronger response, their diversity being significantly higher in organically managed vineyards. Small sessile organism (butterflies) have a similar though less intense response, and the most mobile taxa analysed (birds) show no farming treatment related differences in terms of diversity or abundance. The second chapter explores how farming treatment conditions the richness and activity of bat communities in olive groves. Organic olive groves host significantly higher bat activity levels, and are preferred over conventional groves or forest plantations by some endangered bat species (*Rhinolophus spp.*), thus stressing their importance for the conservation of some species. The third and fourth chapters inquire on the regulatory ecosystems services bats exert in rice paddies, selected as case study because of the high density of both bats and arthropods occurring in that particular agroecosystem. Bats predate on both agricultural pests (moths and

midges) and disease-bearing pests (mosquitoes). The intensity with which they track pest movements to feed on them is possibly linked to the cost-benefit trade-off, more evidently shifting their hunting grounds when bigger pests (stripped rice borer moths) are available, and only slightly shifting in search of smaller and less profitable prey, such as mosquitoes. Their economic impact suppressing the stripped rice borer has been assessed in terms of the avoided pesticide cost per hectare of rice crop and year at around 50€. This ecosystem service that can be exerted if bat densities of 12 bats per hectare are reached. This constitutes the first economic valuation of the ecosystem services provided by bats in Europe.

Resumen

La intensificación agrícola ha permitido a la población mundial casi doblarse en número durante los últimos 50 años aumentando tan solo un 10% la cantidad de tierra cultivada. El incremento en la eficiencia de producción, básicamente debida a una intensificación agrícola fuertemente dependiente del uso de agroquímicos, tiene severos efectos perjudiciales sobre los ecosistemas y contribuye significativamente a la crisis global de biodiversidad, a menudo poniendo en riesgo tanto la salud de los ecosistemas como la humana. En Europa, como en otras partes del mundo, existe un consenso generalizado, tanto en administraciones regionales como internacionales, sobre la necesidad de adoptar prácticas agrícolas más sostenibles. Las prácticas supuestamente respetuosas con la biodiversidad son diversas, englobando desde los sistemas de manejo integrado, donde se minimiza el uso de agroquímicos aunque su uso está permitido, hasta el manejo ecológico, donde no se permite el uso de productos sintéticos o artificialmente modificados y donde se promueven los servicios ecosistémicos de regulación como método para mantener las plagas bajo control. Con todo, el efecto que las distintas prácticas tienen sobre la biodiversidad no es unívoco y varía entre grupos taxonómicos y debido a factores estructurales y geográficos que transcienden los límites de los cultivos. En cuanto a los servicios ecosistémicos de regulación, supuestamente fortalecidos por las prácticas ecológicas, los murciélagos constituyen un excelente caso de estudio en los cultivos de Europa dado que poseen una elevada tasa metabólica y que consumen artrópodos de forma casi exclusiva en todas las regiones temperadas del planeta. El impacto económico de los murciélagos como controladores de plagas agrícolas se ha estimado en más de 3.500 millones de dólares al año solo en EUA. Sin embargo, no existen estimaciones ni locales ni globales para el continente europeo. La presente tesis se centra en las interacciones entre cultivos y biodiversidad y en los servicios ecosistémicos de regulación que se dan en tres cultivos relevantes de la cuenca mediterránea, a saber, viñedos, olivares y arrozales, todos ellos presentes en Cataluña (NE Iberia), donde se han llevado a cabo todos los experimentos de campo.

Sobre todo los viñedos pero también los olivares están inmersos en una rápida transición al manejo ecológico en la región, aunque el manejo convencional es todavía la práctica mayoritaria. El primer capítulo evalúa el efecto que tiene el manejo ecológico de los viñedos sobre varios grupos taxonómicos. De acuerdo con la hipótesis inicial los organismos sésiles (plantas vasculares) muestran una respuesta más fuerte, siendo su diversidad significativamente mayor en viñedos ecológicos. Los organismos vágiles de reducido tamaño (mariposas diurnas) muestran una respuesta similar y significativa, aunque más moderada, y los organismos más móviles analizados (aves) no muestran diferencias entre prácticas agrícolas en cuanto a su riqueza ni abundancia. El segundo capítulo explora como las prácticas agrícolas condicionan la riqueza y abundancia de murciélagos en olivares. Los olivares ecológicos sustentan mayores valores de actividad de murciélagos y son seleccionados positivamente por encima de los convencionales o

de los pinares por parte de algunas especies amenazadas de murciélagos (*Rhinolophus spp.*), poniéndose así de manifiesto su importancia para la conservación de algunas poblaciones de murciélagos. Los capítulos tercero y cuarto indagan sobre los servicios ecosistémicos de regulación que los murciélagos ejercen en los arrozales, seleccionados como caso de estudio por la elevada densidad tanto de murciélagos como de artrópodos que albergan. Los murciélagos depredan tanto sobre plagas agrícolas (polillas y quironómidos) como sobre vectores de enfermedades humanas (mosquitos). La intensidad con la que siguen los movimientos de las plagas para alimentarse de ellas está posiblemente ligada al compromiso coste-beneficio, cambiando sus zonas de alimentación de forma evidente cuando presas de mayor tamaño (polilla del barrenador del arroz) están disponibles, y modificando solo levemente sus movimientos en pos de presas de menor talla y menos rentables, como los mosquitos. Su impacto económico en el control de la polilla del barrenador del arroz en cuanto a los costes evitados de pesticida per hectárea y año en el arrozal se ha estimado en unos 50€. Dicho servicio ecosistémico se puede ejercer si las densidades de murciélagos alcanzan los 12 individuos por hectárea. Esta constituye la primera valoración económica de los servicios ecosistémicos prestados en Europa por los murciélagos.

Resum

La intensificació agrícola ha permès a la població mundial quasi duplicar-se en nombre durant els darrers 50 anys augmentant tan sols un 10% la quantitat de terra cultivada. L'increment en l'eficiència de producció, bàsicament deguda a una intensificació agrícola fortament dependent de l'ús d'agroquímics, té severs efectes perjudicials sobre els ecosistemes i contribueix significativament a la crisi global de la biodiversitat, sovint posant en risc tant la salut dels ecosistemes com la humana. A Europa, com en altres regions del món, existeix un consens generalitzat, tant en administracions regionals com internacionals, sobre la necessitat d'adoptar pràctiques agrícoles més sostenibles. Les pràctiques suposadament respectuoses amb la biodiversitat són diverses, englobant des dels sistemes de gestió integrada, on es minimitza l'ús d'agroquímics malgrat el seu ús està permès, fins al maneig ecològic, on no es permet l'ús de productes sintètics o artificialment modificats i on es promouen els serveis ecosistèmics de regulació com a mètode per a mantenir les plagues sota control. Amb tot, l'efecte que les diferents pràctiques tenen sobre la biodiversitat no és unívoc i varia entre grups taxonòmics i degut a factors estructurals i geogràfics que transcendeixen els límits dels cultius. En quant als serveis ecosistèmics de regulació, suposadament enfortits per les pràctiques ecològiques, els ratpenats constitueixen un excel·lent cas d'estudi en els cultius d'Europa, atès que posseeixen una elevada taxa metabòlica i que consumeixen artròpodes de forma gairebé exclusiva a totes les regions temperades del planeta. L'impacte econòmic dels ratpenats com a controladors de plagues s'ha estimat en més de 3.500 milions de dòlars anuals només als EUA. Malgrat tot, no existeixen estimacions ni locals ni globals per al continent europeu. La present tesis es centra en les interaccions entre cultius i biodiversitat i en els serveis ecosistèmics de regulació que es donen en tres cultius rellevants de la conca mediterrània, a saber, vinyes, oliverars i arrossars, tots ells presents a Catalunya (NE Iberia), on s'han dut a terme tots els experiments de camp.

Sobretot les vinyes però també els oliverars es troben immersos en una ràpida transició cap al maneig ecològic a la regió, malgrat el maneig convencional es encara la pràctica majoritària. El primer capítol avalua l'efecte que té el maneig ecològic de la vinya sobre diversos grups taxonòmics. D'acord amb la hipòtesis inicial els organismes sèssils (plantes vasculars) mostren una resposta més forta, essent la seva diversitat significativament major en vinyes ecològiques. Els organismes vàgils de petita mida (papallones diürnes) mostren una resposta similar i significativa, encara que més moderada, i els organismes més mòbils analitzats (aus) no mostren diferencies entre pràctiques agrícoles en quant a la riquesa ni abundància. El segon capítol explora com les pràctiques agrícoles condicionen la riquesa i abundància de ratpenats en oliverars. Els oliverars ecològics sustenten majors valors d'activitat de ratpenats i són seleccionats positivament per sobre dels convencionals o de les pinedes per part d'algunes especies amenaçades de ratpenats (*Rhinolophus spp.*), posant-se així de manifest la seva importància per a la conservació d'algunes poblacions de ratpenats. Els capítols tercer i

quart indaguen sobre els serveis ecosistèmics de regulació que els ratpenats exerceixen en els arrossars, seleccionats com a cas d'estudi per l'elevada densitat tant de ratpenats com d'artròpodes que alberguen. Els ratpenats depreden tant sobre plagues agrícoles (arnes i quironòmids) como sobre vectors de malalties humanes (mosquits). La intensitat amb la que segueixen els moviments de les plagues per alimentar-se'n està possiblement lligada al compromís cost-benefici, canviant les seves zones d'alimentació de forma evident quan plagues de major mida (arna del barrinador de l'arròs) estan disponibles, i modificant només lleument els seus moviments a la cerca de preses de menor talla i menys rendibles, com els mosquits. El seu impacte econòmic en el control de l'arna del barrinador de l'arròs en quant als costos evitats de pesticida per hectàrea y any s'ha estimat en uns 50€. Aquest servei ecosistèmic es pot exercir si les densitats d ratpenats assoleixen els 12 individus per hectàrea. Aquesta constitueix la primera valoració econòmica dels serveis ecosistèmics prestats a Europa pels ratpenats.

General introduction

Organic farming and biodiversity

Although it has been focus of debate and controversy (Connor, 2008), organic farming has been suggested as a potential solution to protect wildlife and biodiversity, while fulfilling current increased food and energy demands from a growing world population (Gomiero *et al.*, 2011; Winqvist *et al.*, 2012). During the last two decades, organic agriculture has widely gained attention, from both consumers and producers (Bengtsson *et al.*, 2005; Hole *et al.*, 2005; Gomiero *et al.*, 2011; Winqvist *et al.*, 2012). This led local and regional institutions to design new adapted laws, policies and regulations such as the Protected Designations of Origin (DO) to certify market quality and distinguish organic from conventional products (Sanz Cañada and Macías Vázquez, 2005). In fact, the Food and Agriculture Organization of the United Nations (FAO) is currently working to increase integrated pest management and organic agriculture to enhance food security, rural development, sustainable livelihoods and environmental integrity in all member countries (FAO: COAG/2009/REP Para. 31). Although this is now a raising topic worldwide, these practices were actually born long ago, in the 1920s in Northern Europe (Germany) by the hand of enthusiast farmers attending Prof. Rudolf Steiner lectures (Gomiero *et al.*, 2011). The *organic* concept was first used by Walter Northourne in the 1940s in the UK, to describe an integrated group of farming approaches to increase biodiversity in agricultural lands, more recently reviewed by Bengtsson *et al.* (2005) and Hole *et al.* (2005).

Organic farming (OF) can be defined as these practices and systems where non-organic products are never used to control agricultural pests (e.g. chemical pesticides) or to increase productivity (e.g. chemical fertilizers or genetically modified organism lineages) (Paull, 2010; Willer *et al.*, 2010; Gomiero *et al.*, 2011). However, several recognized constrains of the organic farming are hindering its broad implementation: feasibility (many practices need specific environmental characteristics for their broad application), labor productivity (although these practices are energetically optimized, they require higher individual labor effort and working time), economic performance (balance between yields and inputs costs are not usually well assessed and while some studies defend their feasibility, others require a more comprehensive quantification), ecosystem services (agroecosystem services in ecological networks are not yet well-studied, compromising stronger support by local institutions) (Gomiero *et al.*, 2011).

Globally, both social pressure and legislation are encouraging the implementation of crop production systems that are more environmentally sustainable and respectful. The focus is no longer only on yields but also on the quality, health and environmental security of products and procedures. Implementation of the European Union's agri-environment programs has been compulsory for member states since 1992, although they are still voluntary for individual farmers (CEU, 1992). The agri-environmental measures these programs imply are aimed at enhancing environmental biodiversity in and around farmlands, and reward the farmers who put them into practice to enhance the ecological services their lands provide (Bradley et al., 2002). Organic farming is based mainly on the premise of favoring biodiversity and using only natural products, that is, no synthetic fertilizers, herbicides or pesticides, or genetically modified varieties are employed during the cultivation process. It also advocates that agroecosystems should have the potential to regulate basic services such as pollination and pest control if they are properly managed and biodiversity is preserved (Altieri and Farrell, 1995). The use of pesticides and herbicides, along with certain aggressive mechanical practices, can seriously harm the biodiversity of agroecosystems, a key component of their capacities to self-regulate and be self-sustainable (Köhler and Triebskorn, 2013). The large-scale agricultural production has promoted the monoculture (Kremen and Merenlender, 2018) and an uncontrolled and excessive use of pesticides which, at the same time, are increasing the environmental and water pollution (Köhler and Triebskorn, 2013; Ramankutty *et al.*, 2018; Sánchez-Bayo and Wyckhuys, 2019). Moreover, the ecosystem services provided by healthy agroecosystems transcend the boundaries of farms and stimulate a number of off-farm benefits ranging from protection from erosion and water flow regulation and purification, to pest control and carbon sequestration (Garbach *et al.*, 2014).

With the aim of providing a better understanding on the drivers underpinning biodiversity interactions in Mediterranean agroecosystems from NE Iberia, four experimental studies were carried out between years 2014 and 2019. On the one hand the studies estimate the effect of agricultural treatments (organic and conventional) to the diversity and abundance of different taxa, and on the other hand they deepen into the ecological interactions between bats (*Chiroptera*) and some major agricultural and disease-bearing pest species:

- **Chapter 1** explores the effect of the agricultural treatments of vineyards on a gradient of taxa ranging from sessile to increasingly more mobile species (vascular plants, butterflies, moths and birds).
- **Chapter 2** explores the effect of the agricultural treatments of olive groves on bat assemblages and on the conservation of endangered bat species, while assessing their interaction with the olive fruit fly (*Bactrocera oleae*), a major pest in the region.
- **Chapter 3** gauges the regulatory ecosystem service provided by bats in rice paddies as a result of the consumption they exert of the stripped rice borer moth (*Chilo suppressalis*), an invasive rice pest that causes important yield losses worldwide.

- **Chapter 4** studies the ecological interactions between bats and mosquitoes in rice paddies and further analyses the trophic interactions occurring between bats and other deleterious insects (*Chironomidae*).

Biodiversity in vineyards as a case study

Over the last two decades, OF practices have greatly expanded in Catalonia (NE Spain) and in the period 1995–2019 the amount of OF cultivation rose from just 4,936 to 229,609 ha (CCPAE, 2020). Of the crops responsible for this dramatic increase, vineyards are by far the most significant: in 2019, organic vineyards occupied 18,632 ha, 42% of all the organic crop surface area in Catalonia (excluding organic pastures, which are widespread in the Pyrenees). Despite the obvious economic importance of organic vineyards (their production in 2014 was estimated in Catalonia at €43.1 million – CCPAE, 2020–), debate still continues in the farming local community as to whether or not it is worthwhile following the strict criteria imposed by OF. One of the arguments put forward by the Catalan government to promote this type of farming is that it benefits biodiversity, which has been confirmed in a number of regional studies (see e.g. Chamorro *et al.* (2016) or Rollan *et al.* (2019)).

It could be argued that the lack of consensus about the benefits of OF in vineyards is also reflected in the contrasting conclusions reached by a limited number of studies. Bruggisser *et al.* (2010), working on Swiss vineyards and focusing on three trophic levels, did not find an increase of diversity in any of the groups studied (vascular plants, grasshoppers and spiders). In fact, grasshopper diversity was even lower in organic compared to conventional vineyards. This result was explained in the context of the intermediate disturbance hypothesis (Huston, 1979) considering that disturbance in OF vineyards was too low to be beneficial for biodiversity (i.e. only a few highly competitive species prevailed under this kind of management). On the other hand, research carried out in different Mediterranean wine producing areas has demonstrated a positive effect of OF in vineyards both in vascular plants (Nascimbene *et al.*, 2012), in some guilds of arthropod predators (Caprio *et al.*, 2015), and on the functional diversity of birds (Lourenço *et al.*, 2021). Moreover, Kehinde and Samways (2012) found an increase of monkey beetles (an important pollinator guild) but not of bees in OF compared to conventional South African vineyards. Likewise, Thomson and Hoffmann (2009) reported an increase in the abundance of natural enemies (including egg parasitoids) of an important local pest in Australian vineyards that included adjacent natural vegetation.

Therefore, although responses have proven to be idiosyncratic among taxonomic groups, results mostly suggest that OF in vineyards (and in many other crops) indeed contributes to promoting ecosystem services such as pollination and pest control.

Chapter 1 explores the response of both sessile (vascular plants) and vagile (butterflies, moths and birds) taxa to OF and conventional farming (CF) in vineyards of the Priorat county (Catalonia, NE Spain).

Regulatory ecosystem services in agroecosystems

Although there is no general agreement on how to define ecosystem services (Wallace, 2008), such services are generally regarded as ecosystem outcomes (e. g. use of less pesticides) that contribute to human well-being (Wallace, 2007; Fisher and Turner, 2008; Fisher *et al.*, 2009; Nelson *et al.*, 2009). Understanding the mechanisms that link ecological systems to human well-being is a fundamental task when studying ecosystem services (Haines-Young and Potschin, 2009). From a functional perspective, the human benefit (whether monetary or not) is the final outcome of a cascade process resulting in a service (Rollett *et al.*, 2008).

Agriculture intensification and large-scale production have led to an increase of monocultures with the subsequent biodiversity loss and simplification of ecosystems' complexity (Reich *et al.*, 2012). Since the Green Revolution, the use of pesticides has been the main strategy to overcome the increasingly severe and frequent insect pest outbreaks (Dhaliwal *et al.*, 2010). In recent years, Integrated Pest Management (IPM) techniques are being increasingly adopted by different countries in order to improve yields while reducing environmental impacts through sustainable practices (Parsa *et al.*, 2014), being biological control regarded as a sustainable and affordable solution to suppress agricultural pests (Nwilene *et al.*, 2013).

Bats and Integrated Pest Management in agroecosystems

Bats are one of the most biodiverse mammal assemblages on the planet and several ecosystem services have been attributed to this taxonomic group, from seed dispersal and pollination, to arthropod suppression both in natural environments and in agroecosystems, where some of the arthropods bats consume constitute pests (Jones *et al.*, 2009). With a high metabolic rate, bats consume 30%-80% of their body mass each night (Kurta *et al.*, 1989), and during the last decade several authors have drawn attention to the important contribution that bats make to insect pest control (Whitaker, 1995; Agosta and Morton, 2003; Lee and McCracken, 2005; Leelapaibul *et al.*, 2005; Cleveland *et al.*, 2006; Boyles *et al.*, 2011; Kunz *et al.*, 2011; Ghanem and Voigt, 2012; McCracken *et al.*, 2012).

The referred contribution has been assessed by the presence of pests in the diet of wild bat populations or by taking a step further and accounting for the economic value of such an ecosystem service. Given the natural complexity of ecological systems it is difficult to place a monetary value on the services provided by bats, a fact that restricts how their importance is understood by the public (Fisher and Turner, 2008). Cleveland et al. (2006) estimated the economic contribution of bats to the cotton dominated agroecosystems of southern Texas, USA, to be $12-$173 per acre each year. By extrapolating these figures to the whole country, Boyles et al. (2011) valued bats' economic contribution to the

USA's agroecosystems at between \$3.7 and \$53 billion/year. Even if the actual figures were lower, given the evidence gathered so far, the positive impact of bats on this aspect of the economy seems to be beyond dispute. This benefit can exceed the monetary value if the affected crop is a staple. For example, the pest control service provided by wrinkle-lipped bats' (*Tadarida plicata*) is responsible for securing the meals of 26,152 (± 15,817 SD) people each year in Thailand alone (Wanger *et al.*, 2014). All the aforementioned authors have stressed the consequent importance of protecting bat populations if the ecological service they provide is to be preserved. Chapter 3 assesses the economic impact of the regulatory ecosystem services (pest suppression) provided by bats in Mediterranean rice paddies. Using and avoided-cost approach we estimate the cost of pesticides that bats' activity prevent.

Over roughly the last decade, available technologies to survey bat activity (ultrasound recorders, GPS tags or radar systems among others) and to study their diet (e.g. metabarcoding techniques) have experienced important improvements and diminished their costs significantly, allowing the study of bat ensembles and their trophic ecology at unprecedented scale and detail (Horn and Kunz, 2008; Kerbiriou *et al.*, 2018; Torrent *et al.*, 2018; Conenna *et al.*, 2019; Vallejo *et al.*, 2019). Passive bat detectors can record bat activity autonomously for long periods of time and can be used to evaluate changes in populations and activity levels (Frick, 2013). Paired with the increase of commercial devices integrating this technology, a number of both commercial and open-source software solutions to detect and parametrize sound events and to identify them have been developed (Barré *et al.*, 2019) which, properly combined with manual validations, allow to efficiently and confidently analyze and identify large amounts of recordings otherwise difficult to handle (López-Baucells *et al.*, 2019). Molecular techniques have experienced a similar trend. During decades, diet studies were carried out by visual determination of pellets' content, detecting and visually identifying parts of the ingested prey (Whitaker, 1995; Kurta and Whitaker, 1998). Recent developments in genetics, and more particularly the raise of metabarcoding techniques, have allowed thorough dietary screenings up to species level using non-invasive methods (i.e. analyzing faeces) (Alberdi *et al.*, 2012; Vallejo *et al.*, 2019). These novel vocalization recording techniques have been used to assess bat activity in both olive groves (chapter 2) and rice paddies (chapter 4), to better understand either how bats relate to different farming treatments (organic v conventional olive groves) or to the presence of potential prey species (mosquito density in rice paddies). Metabarcoding has also been used to screen bats' diet in the rice paddies (chapter 4).

Olive groves treatment's interaction with bats

The Mediterranean Basin boasts the world's largest area of olive-groves (98% of total cover), as this is a perfect species for the marginal sub-humid and semi-arid lands (Delrio, 1985; Garcia-Mozo *et al.*, 2014). Olive oil sector represent an important facet of these countries' socioeconomic situation (Sanz Cañada and Macías Vázquez, 2005), as they allow the exploitation of natural resources in habitats that would otherwise be inappropriate for farming (especially due to extreme droughts, heat waves and strong winds) (Tanasijevic *et al.*, 2014). Olives crops also protect the soil because they avoid natural erosion and degradation and provide job opportunities and cultural values to local farmers (Delrio, 1985).

Although several insect pests are acknowledged to affect olive crops (over 255 according to the IOBC / WPRS), the major yield losses are due to the olive fruit fly (*Bactrocera olaea*) (Haniotakis, 2005). Because some olive plantations are usually found in wild rough environments, mechanized conventional protection is generally difficult to apply, which facilitates pest attacks. However, when pest management is practicable (generally in monoculture olive-groves) non-organic practices become predominant, including a massive use of pesticides. Old and traditional methods included bait sprays, molasses and sodium arsenate, all highly detrimental to the environment, from both the ground and the air (Haniotakis, 2005). Cheap pesticides spread by aircraft became common and widespread lately. Due to the obvious detrimental effects on ecosystem health, alternatives within the framework of Integrated Pest Management (IPM) such as the use of *Bacillus thuringiensis* have been developed during the last two decades and are now steadily being demanded by ecologists and conservationists (Rodriguez *et al.*, 2012).

More biodiverse agroecosystems may benefit from a higher predation pressure on the pests, and in turn may also act as biodiversity reservoirs for protected species. Such has been suggested to be the case of the traditional olive groves in the Mediterranean basin (Calabrese *et al.*, 2012). Since some protected bat species are known to use olive groves as feeding grounds (Flaquer *et al.*, 2008), it was plausible that this bidirectional benefit could occur. In chapter 2 we explore the interaction between farming treatment and conservation of endangered bat species in olive groves.

Bats and pest control in rice paddies

In the early 90's Heinrichs and Miller (1991) suggested that any improvement in the human condition in this planet must be concerned with rice. ln recent decades, both rice scientists and farmers have gained experience in the cultivation of rice and there has been a shift from a primarily unilateral approach to insect pest control, relying strongly on insecticides, to a multilateral approach involving a combination of control tactics (Heinrichs and Miller, 1991). Scientists throughout the world strive to develop and implement strategies to control rice pests more effectively and economically, to improve crop productivity and consequently the welfare of human populations. According to Food and Agriculture Organization of the United Nations (FAO) statistics, the global production of rice increased linearly from 216 million tons (mt) in 1961 to 722 mt in 2011 (FAO). New strategies to control insect pests include the use of one insecticide per one specific target species combined with biological methods (such as the use of parasitoids). However, the adoption of non-chemical approaches to pest control is not evenly distributed around the world and occurs mainly in those regions where legal constraints limit the number of approved chemical products (e.g. European Union). Over-use of pesticides is still an issue to be addressed (Peng *et al.*, 2009; Normile, 2013), particularly in those regions in which the vast majority of the world's rice production is concentrated, and the use of pesticides keeps growing (FAO).

Fifty percent of the insecticides used in rice fields in Asia target lepidopteran insects (Heong *et al.*, 1994). In 1991 it was estimated that an average global annual yield loss of 10 million tons was caused by just three moths: the striped rice borer (*Chilo suppressalis*), the yellow stem borer (*Scirpophaga incertulas*) and the leaf folder (*Cnaphalocrocis medinalis*) (Herdt, 1991). The striped rice borer is an invasive Asian moth currently present in paddies worldwide, and constitutes the main insect pest of the rice paddies of the study area. The adults lay their eggs on the stems and leaves of rice plants, and the larvae bore the stems to feed on the internal tissues, compromising both plant growth and productivity, sometimes fatally.

Moths constitute a habitual prey for many aerial hunting bats, and in the rice paddies of our study region the most common bat species is the Soprano pipistrelle (*Pipistrellus pygmaeus*) (Flaquer *et al.*, 2006), a common European bat species occurring from the British Isles through much of continental Europe East to Western Asia Minor, the Caucasus and Siberia (Dietz *et al.*, 2009). It is more abundant in lowland areas and is frequently associated with freshwater bodies (rivers, lakes, wetlands, etc.), being common in coastal wetlands, where most paddies in southern Europe occur, and where they can reach high population densities. The few unambiguous studies on diet indicate that they feed mainly on small diptera, though they include a wide array of small-sized aerial insects in their diet and is regarded as an opportunistic species (Vaughan, 1997; Bartonicka *et al.*, 2008), and hence with a potential to regulate the deleterious moths that affect the rice. Chapter 3 deepens in the ecological interaction between an abundant bat species in the rice paddies of NE Iberia (*Pipistrellus pygmaeus*) and a major pest moth (*Chilo suppressalis*), and provides and economical estimate of the pest suppression bats exert in that particular context.

Suppression of disease-vector insects by bats in rice paddies

Besides the agronomic considerations, rice plantations have a significant impact on human health. The prolonged irrigation cycle of the rice paddies turns this crop into an optimal breeding site for mosquitoes, which are one of the main disease vectors affecting human health (Reiter, 2001; Amusan *et al.*, 2005; Waterhouse *et al.*, 2007). Moreover, habitat destruction and temperature increase associated to the global change scenario are favouring opportunistic mosquitoes and other insects responsible for disease transmission outbreaks (Rosenzweig *et al.*, 2001) (e.g. West Nile Virus, malaria, dengue or Zika among many others) that put at stake human population in some regions (Hoover and Barker, 2016).

Despite bats are frequently portrayed as mosquito consumers and controllers (Pliny the Elder, on the 1st century BC, already mentions this in his *Naturalis Historia*), supporting evidence is scarce. Though a few papers report direct evidence of consumption (Swift *et al.*, 1985; Hoare, 1991; Beck, 1995; Barlow, 1997; Vaughan, 1997; Goiti *et al.*, 2003;

Biscardi *et al.*, 2007; Gonsalves *et al.*, 2013a; Wray *et al.*, 2018), and Nematocera are known to be a fundamental part of the pipistrelles (genus *Pipistrellus*) in Europe (Bartonicka *et al.*, 2008), more detailed ecological interactions between bats and mosquitoes have received almost no attention so far. Reiskind and Wund (2009), under enclosed conditions, found that the presence of the echolocating bat *Myotis septentrionalis* significantly reduced *Culex* mosquitoes oviposition. More recently, Gonsalves *et al.* (Gonsalves *et al.*, 2013c) radiotracked the small sized Australian insectivorous bat *Vespadellus vulturnus*, known to consume mosquitoes (Gonsalves *et al.*, 2013b), and found a shift in bat activity from open saltmarshes to coastal swamp forests relative to the changes in mosquito abundance. The available literature brings a similar scenario regarding *Chironomidae* with their presence in bat diet being reported in a number of papers (Swift *et al.*, 1985; Hoare, 1991; Beck, 1995; Whitaker, 1995; Barlow, 1997; Vaughan, 1997; Goiti *et al.*, 2003; Biscardi *et al.*, 2007; Ciechanowski and Zapart, 2012; Krüger *et al.*, 2014; Vesterinen *et al.*, 2016). Non-biting midges appear to be a significant resource for three European riverine trawling bat species, such as the endangered *Myotis capaccinii* (Biscardi *et al.*, 2007) or the more widespread *M. daubentonii* and *M. dasycneme* (Krüger *et al.*, 2014), which consume not only the flying adults but also the pupae and larvae, which they may pick up from the water surface. Nevertheless, the impact bats have on these dipterans' populations remains unknown.

In chapter 4 we provide new insights on the ecological interactions between bats and mosquitoes in rice paddies, and on the potential impact that enhancing bat populations could have on human health in that particular agroecosystem.

Objectives

Farming management and biodiversity

In order to assess the impact that the different agricultural treatments (i.e. OF versus CF) have on the biodiversity, we undertook a study aimed at testing for the first time the biodiversity effects of OF in vineyards in N Spain (chapter 1). We designed a multi-taxon approach, carried out in 2014–2015, for one of the main areas devoted to viticulture in Catalonia as a way of critically exploring and understanding how crop management (OF vs. CF) affects biodiversity across a range of taxa. The study focused on four different taxonomic groups (namely, plants, butterflies, moths and birds) with contrasting ecological attributes (e.g. mobility) that would enable us to gain some understanding of the effects of different types of farming systems at ecosystem level. We hypothesized that, firstly, differences in management practices would result in richer assemblages wherever no synthetic pesticides or herbicides are applied (OF) and secondly, that the magnitude of the response of a set of taxa would be linked to their mobility. We expected sessile organisms (i.e. plants) to be more affected than vagile ones (i.e. butterflies, moths and birds). However, birds and butterflies differ in terms of the spatial scale at which their biological processes occur (Seto *et al.*, 2004). Therefore, given that (i) most butterflies and moths have life cycles that are closely linked to local conditions and to specific host plants, and that (ii) birds, with greater mobility, are more generally affected by vegetation structure at larger spatial scales, we predicted a stronger response to different management treatments in Lepidoptera than in birds.

Farming treatment and bat conservation

We assessed the effects of different farming practices on olive crops on the foraging activity and diversity of bats, at both assemblage and species level (chapter 2). Specifically, we aimed to i) Compare bat richness and hunting activity on organic farming versus conventional olive groves and surrounding sparse coniferous forests; ii) Assess the effect of several environmental and weather factors (physical and environmental variables, wind and temperature) upon bat foraging activity on the different treatments; and iii) Identify if the presence of the Olive fruit fly (*Bactrocera olaea*) influences bat foraging activity in any of the sampled habitats, suggesting a potential pest control ecosystem services provided by them. These research questions have been addressed at both trophic guild and species level. We expected that all bat species, independently on their trophic guilds, would be favored by organic fields compared to conventional ones, as reported for many other taxa. Because gleaning bats are known to capture insects from the vegetation surface we predicted that this trophic guild would be the only possibly affected by the olive fruit fly abundance, since this pest flies at daytime and hence cannot be captured on the wing at night.

Rice paddies and pest suppression by bats

Provided that, to date no studies have been conducted to elucidate the ecological relations and the potential of bats as a biological control mechanism in rice paddies, our main study aims were (i) to determine whether soprano pipistrelles consume and regulate the stripe rice borer populations (chapter 3); (ii) to assess which mosquito and non-biting midges species are consumed in rice fields by the most common bat species (i.e. *Pipistrellus pygmaeus*) (chapter 3) and (iii) to evaluate the relation between mosquito density and other environmental variables (e.g. wind, temperature or precipitation) on bat foraging activity (chapter 3).

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1. Biodiversity in organic v conventional vineyards

Effects of organic and conventional crop management on vineyard biodiversity

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Effects of organic and conventional crop management on vineyard biodiversity

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Abstract

Although organic farming is rapidly expanding in the vineyards of southern Europe, conventional crop management, using treatments that require a number of chemical inputs to guarantee yields is still the most common approach to crop management. To gauge the effects of these management systems on biodiversity, communities of vascular plants, butterflies, moths and birds were studied in vineyards in the Priorat Appellation of Origin (Catalonia, NE Spain). Measurements inside plots (all four taxonomic groups) and in grass strips between crop lines (only butterflies and vascular plants) were taken in organically and non-organically treated vineyards. Crop treatment was found to have an important effect, stronger on the most sessile organisms. Organic farms hosted consistently richer communities of both vascular plants and butterflies, a trend that was also observed – albeit less significantly – in moths. The weaker response in this group was probably due to insufficient sampling. Birds, the most vagile of the surveyed taxa, showed no significant response to treatments. Grass strips acted in all cases as reservoirs of biodiversity and hosted richer assemblages. The current trend of placing vineyards on slopes without terracing should ensure the existence of uncultivated strips within the vineyards to enhance the biodiversity of these agroecosystems. As well, parameters such as altitude and urban surface area are important drivers of biodiversity in this region. Our results suggest that organic farming may contribute to halting the widespread decrease that is occurring in communities of butterflies and other insects in this region.

Keywords: vineyards biodiversity, organic farming, conventional farming, birds, vascular plants, *Lepidoptera*

Introduction

Globally, both social pressure and legislation are encouraging the implementation of crop production systems that are more environmentally sustainable and respectful. The focus is no longer only on yields but also on the quality, health and environmental security of products and procedures. Implementation of the European Union’s agri-environment programs has been compulsory for member states since 1992, although they are still voluntary for individual farmers (CEU, 1992). The agri-environmental measures these programs imply are aimed at enhancing environmental biodiversity in and around farmlands, and reward the farmers who put them into practice for the ecological services their lands provide (Bradley *et al.*, 2002). Organic farming (OF) is based mainly on the premise of enhancing biodiversity and using only natural products, that is, no synthetic fertilizers, herbicides or pesticides, or genetically modified varieties are employed during the cultivation process. It also advocates that agroecosystems should have the potential to regulate basic services such as pollination and pest control if they are properly managed and biodiversity is preserved (Altieri and Farrell, 1995). The use of pesticides and herbicides, along with certain aggressive mechanical practices, can seriously harm the biodiversity of agroecosystems, a key component of their capacities to self-regulate and be self-sustainable. Moreover, the ecosystem services provided by healthy agroecosystems transcend the boundaries of farms and stimulate a number of off-farm benefits ranging from protection from erosion and water flow regulation and purification, to pest control and carbon sequestration (Garbach *et al.,* 2014). Nevertheless, to our knowledge no estimates of their global impact exist.

Over the last two decades, OF practices have greatly expanded in Catalonia (NE Spain) and in the period 1995–2015 the amount of OF cultivation rose from just 4,936 to 142,024 ha (CCPAE, 2020). Of the crops responsible for this dramatic increase, vineyards are by far the most significant: in 2015, organic vineyards occupied 11,706 ha, 36% of all the organic crop surface area in Catalonia (excluding organic pastures, which are widespread in the Pyrenees). Despite the obvious economic importance of organic vineyards (their production in 2014 was estimated in Catalonia at €43.1 million – CCPAE, 2016 –), debate still continues in the farming local community as to whether or not it is worthwhile following the strict criteria imposed by OF. One of the arguments put forward by the Catalan government to promote this type of farming is that it benefits biodiversity; yet to date no studies have ever been carried out to test this assumption in this region.

It could be argued that the lack of consensus about the benefits of OF in vineyards is also reflected in the contrasting conclusions reached by a limited number of studies. Bruggisser *et al.* (2010), working on Swiss vineyards and focussing on three trophic levels, did not find an increase of diversity in any of the groups studied (vascular plants, grasshoppers and spiders). In fact, grasshopper diversity was even lower in organic compared to conventional vineyards. This result was explained in the context of the intermediate disturbance hypothesis (Huston, 1979) considering that disturbance in OF vineyards was too low to be beneficial for biodiversity (i.e. only a few highly competitive species prevailed under this kind of management). On the other hand, research carried out in N Italy has demonstrated a positive effect of OF in vineyards both in vascular plants (Nascimbene *et al.*, 2012) and in some guilds of arthropod predators (Caprio *et al.*, 2015), Moreover, Kehinde and Samways (2012) found an increase of monkey beetles (an important pollinator guild) but not of bees in OF compared to conventional South African vineyards. Likewise, Thomson and Hoffmann (2009) reported an increase in the abundance of natural enemies (including egg parasitoids) of an important local pest in Australian vineyards that included adjacent natural vegetation.

Therefore, although responses have proven to be idiosyncratic among taxonomic groups, results mostly suggest that OF in vineyards indeed contributes to promoting ecosystem services such as pollination and pest control.

Within this context, we undertook a study aimed at testing for the first time the biodiversity effects of OF in vineyards in N Spain. We designed a multi-taxon approach, carried out in 2014–2015, for one of the main areas devoted to viticulture in Catalonia as a way of critically exploring and understanding how crop management (OF vs. conventional farming – CF-) affects biodiversity across a range of taxa.

Our study focused on four different taxonomic groups (namely, plants, butterflies, moths and birds) with contrasting ecological attributes (e.g. mobility) that would enable us to gain some understanding of the effects of different types of farming systems at ecosystem level. We hypothesized that, firstly, differences in management practices would result in richer assemblages wherever no synthetic pesticides or herbicides are applied (OF) and secondly, that the magnitude of the response of a set of taxa would be linked to their mobility. We expected sessile organisms (i.e. plants) to be more affected than vagile ones (i.e. butterflies, moths and birds). However, birds and butterflies differ in terms of the spatial scale at which their biological processes occur (Seto *et al.*, 2004). Therefore, given that (i) most butterflies and moths have life cycles that are closely linked to local conditions and to specific host plants, and that (ii) birds, with greater mobility, are more generally affected by vegetation structure at larger spatial scales, we predicted a stronger response to different management treatments in Lepidoptera than in birds.

Material and methods

Study area

The study area consisted of the Priorat Appellation of Origin (DOQP in its original acronym), a wine-producing area located in the county of the same name in Catalonia (41° 8′ N, 0° 49′ E, see figure 1). It has a Mediterranean climate influenced by the proximity of the sea (mean annual precipitation around 600 mm). Topographically complex, the Priorat lies between two mountainous ranges and has an average altitude of 472±250 m a.s.l. It has a surface area of roughly 18,000 ha, of which 1,887 ha are covered by vineyards, the main economic activity in the area. Most of the vineyards are managed following CF procedures, although the presence of OF is increasing.

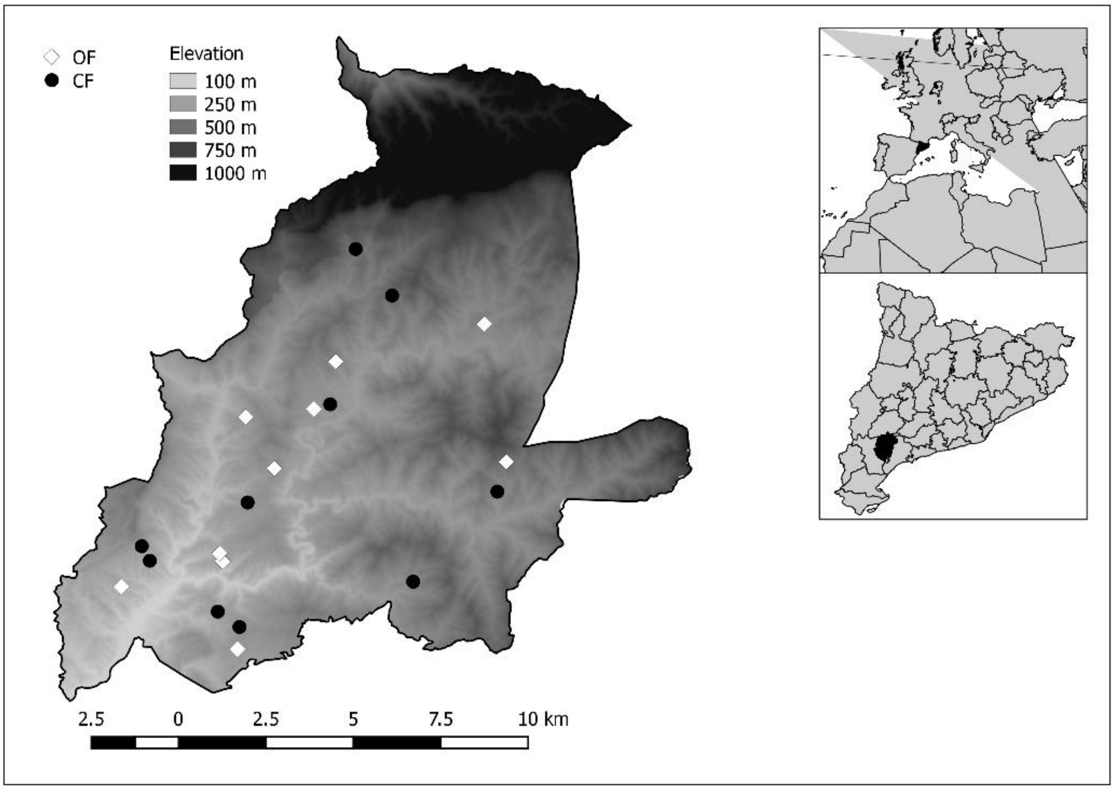

FIGURE 1-1 STUDY AREA. LOCATION OF THE SAMPLING LOCATIONS IN THE PRIORAT PDO (PROTECTED DESIGNATION OF ORIGIN). ORGANIC FARMING (OF) AND CONVENTIONAL FARMING (CF) PLOTS ARE SHOWN.

Plot selection and characterization

A total of 10 OF and 10 CF crop plots were selected for the study. Given that the region's vineyards vary greatly in size and are subject to a wide range of environmental conditions (e.g. exposure and altitude), a pre-selection of plots was made to minimize the effect of confounding factors.

To exclude the possible effect of agricultural parcel size, an up-to-date cadastral map of holdings was simplified by merging all neighbouring parcels (i.e. plots) into a number of production blocks. Of these, only blocks with surface areas within the 25th–75th percentile were selected (ranging between 0.6 and 2 ha). A digital elevation map of the region (ICGC, 2013) was used to estimate the altitude and exposure of each block. Since both insolation and altitude are factors that heavily influence community assemblages and are therefore prone to bias the results of surveys, only blocks between 150 and 550 m a.s.l. oriented predominantly southwards (between 135° and 230°) were selected. To prevent the influence of riparian habitats and neighbouring forests on biodiversity, buffers of 300 and 50 m were drawn around streams and forest patches, respectively, on the 0.25 m resolution land cover map (DMAH, 2005) and blocks falling within these buffers were excluded. From the remaining blocks, we also removed those containing a mixture of OF and other management practices. Finally, we selected 10 plots to represent OF management systems and 10 to represent CF management systems out of 20 blocks that shared uniform environmental and physical conditions. Information on the management practices of both OF and CF plots was provided by the DOQP bureau (Table 1). All geographic computations were conducted using QGIS (QGIS Development Team, 2013). To characterize each plot, a set of nine geographical variables was generated by taking into account the influence of physical conditions (altitude, roughness calculated as the standard deviation of elevation and slope) and habitat features (land covered by vineyards, shrub, forest, herbaceous vegetation and other habitat types, and the Shannon index of landscape diversity) around the vineyards. These variables were computed for three incremental buffer distances around the plots (500, 2000 and 4000 m). Physical variables were estimated from a digital elevation model with a resolution of 15x15 m (ICGC, 2013), while habitat features were taken from a land cover map of Catalonia (DMAH, 2005).

To check whether there were differences between treatments (OF and CF) in the nine variables in each buffer, Monte-Carlo tests were run using 1000 permutations of the t-student test. This test is more robust than conventional non-parametric tests and no particular distribution of the data needs to be assumed (Gotelli and Ellison, 2004). No significant differences were found between groups (OF vs. CF) when running the Monte-Carlo test for each independent variable ($/t/\leq 0.03$; $p>0.21$; 27 tests in total, corresponding to the nine geographical variables calculated at each of the three buffer distances; see results in Sup. Mat. 1). All variables were therefore found to be suitable for use in the model building process (see 'Species richness modelling').

All geo-processing and computation was performed with R (R Core Team, 2015). The Shannon index of landscape diversity was estimated with the package “vegan” for R (Oksanen *et al.*, 2015).

Sampling

The study was conducted in 2013 and 2014. Plants, butterflies and moths were sampled in both years but birds only in 2013. For vascular plants and butterflies two subsamples per plot were obtained: one in the crop lines and the other in the grass strips that exist within the vineyards (or in the boundaries between vineyards sharing the same management). For both moths and birds only one sample at plot level was obtained. Locations of survey plots, central line of vegetation survey quadrats, and butterfly transects are presented in the supplementary material (Sup. Mat. 2).

Vegetation

Vascular plants were sampled within randomly assigned quadrats of 16 m^2 (8 x 2 m) in April 2013 and May 2014 (i.e. the flowering period of most species). Each year, plots were surveyed twice on the same day (crop lines and grass strips): all vascular plants were identified and assigned a cover value ranging from 0 to 5 following Braun-Blanquet (1932). One of the plots lacked naturalized grass strips and hence two simultaneous surveys were conducted in the crop lines, resulting in a total of 21 surveys carried out in quadrats in the crop lines and 19 in grass strips.

Table 1-1. Summary of the main management treatments applied in the conventional and organic farming vineyards of the Priorat Apellation of Origin. Minimum and maximum number of annual treatments is shown.

		Conventional farming	**Organic farming**
Target	**Treatment**	Applications per year	Applications per year
Powdery mildew (*Uncinula necator*)	Sulfur based fungicides	4	3-4
Mildew	Copper based fungicides	0-2	0-2
European grapevine moth (*Lobesia botrana*) 2nd & 3rd generations	Bt Spinosad (products approved in organic agriculture)	0-2 (treatment prevented when sexual confusion is used)	0-2 (treatment prevented when sexual confusion is used)
European grapevine moth (*Lobesia botrana*) 2nd generation	Chlorpyrifos Methyl Chlorpyrifos	0-1 (treatment prevented when sexual confusion is used)	0
European grapevine moth (*Lobesia botrana*) 3rd generation	Fenoxicarb Tebufenocide	0-1 (treatment prevented when sexual confusion is used)	0
European grapevine moth (applied from may)	Sexual confusion	0-1	0-1
Weeds	Glyphosate	0-2	0
Weeds	Mowing	2-3	2-3
Weeds	Tillage	0-2	0-2

Butterflies

Counts of adult butterflies were made along fixed 100-m transects following the standard technique described in Van Swaay *et al.* (2008). Each year, plots were surveyed four times in April–August, a period that encompasses the flight periods of all species present in the area. Counts were undertaken only in sunny weather with no strong wind, between 11 a.m. and 4 p.m. In all, 9 plots of each treatment were sampled each year, and 288 transect counts were performed, i.e. eight counts (four within crop lines, four within grass strips) per plot per year.

Moths

Moths were surveyed from the end of April to early September using standard 6W actinic light Heath traps. Sampling nights were selected to coincide with the new moon to avoid moonlight interference and optimal weather conditions (i.e. absence of rain and of strong wind; Yela and Holyoak, 1997). On every sampling night two traps were operated simultaneously in the middle of one OF plot and one CF plot during the first 3–4 hours after sunset. In total, 18 plots were sampled in 2013 and 20 in 2014. Collected moths were anaesthetized with ethyl acetate and, after preliminary trials and counts, most of the macro moths were released *in situ*. The rest of the samples were carefully preserved by freezing for further study in the laboratory. Some of the sampled specimens were mounted and genitalia structures were studied for correct identification (especially for the microlepidoptera).

Birds

Acoustic and visual censuses of birds were conducted in each plot during the 2013 breeding season. Following the census periods established by the Catalan Common Bird Survey (Herrando *et al.*, 2008), the local version of the Pan-European Common Bird Monitoring Scheme, one visit was made between April 15 and May 15 and a second between May 15 and June 15. Data from both visits were pooled to estimate species richness. Plots were not resampled until at least 15 days after the first visit. Four sampling points were defined in each plot (at vertices if plots were rectangular or, alternatively, as far apart from each other as possible). Censuses consisted of 15-minute counts from each point, giving a one-hour effort per plot and sample. All birds seen or heard within the plots were identified and counted. All censuses were conducted between 08.00 and noon.

Species accumulation curves

Four data matrices with the number of individuals sampled (butterflies, moths and birds) or the presence/absence (vegetation) of the species in each focal group were created; species accumulation curves were used to estimate species richness (Gotelli and Colwell, 2001). The expected richness functions were calculated with EstimateSv. 9.1.0. (Colwell, 2013) after 100 randomizations (default option) of the observed number of species as accumulated samples. To ascertain the completeness of the inventory for each focal group, we used the Clench equation to adjust the species accumulation curves (Díaz-Francés and Soberon, 2005). To fit the Clench equation to the functions provided by EstimateS, we used the non-linear estimation module of Statistica v7.0 (Stat Soft Inc.) following the procedure outlined by Jiménez-Valverde and Hortal (2003). Asymptotic species density was calculated by dividing the intercept by the slope of the function fitted when a good fit between both curves was obtained ($r^2 > 0.99$). The completeness of the inventories was recorded as the ratio between the number of observed species and the number of expected species. Vegetation inventories were treated at qualitative level (presence/absence) and we used species density (i.e. the number of species detected per sampled plot) as a measure of diversity rather than species richness (i.e. the number of species related to the number of individuals sampled; Gotelli and Colwell, 2001).

Species richness modelling

We were interested in understanding how the species richness of different taxonomic groups in vineyards (dependent variable) was influenced by the chosen geographical factors and the two management treatments (OF and CF). Thus, we constructed generalized linear models with the nine geographical variables, the two types of management and for plants and butterflies their position in vineyards (crop lines or grass strips) as fixed factors.

Following the procedure recommended by Zuur et al. (2007), all independent variables were examined prior to model building (nine geographical variables for each of the three buffers, thereby 27 in total). Firstly, we tested for the existence of outliers and non-linear relationships between species richness and the independent variables (plotting and visual exploration). We explored the collinearity between variables using pair-wise correlation tests and rejected those with correlations of ±0.7 or greater, following Dormann *et al.* (2013), who suggest the $|r| > 0.7$ threshold on descriptive ecological studies. In addition, variance inflation tests (VIF) were made starting from a saturated model with all independent variables, followed by a step-wise exclusion of those variables with VIF>5 (Zuur *et al.*, 2007).

No apparent outliers were found and no consistent non-linear relationships emerged after visually examining the richness of the different taxa against the geographical variables. Therefore, no data transformation was performed. However, strong correlations (i.e. $/r/ \geq 0.7$) between some variables occurred at all buffer distances. The Shannon index of landscape diversity showed a strong correlation with herbaceous vegetation cover and altitude at all buffer distances, and was thus excluded from the models. Similarly, roughness and slope were correlated with altitude over distance and were also discarded. VIF values for all these variables were accordingly high, a further reason for their exclusion. The remaining six variables (altitude and vineyard, shrub, forest, herbaceous vegetation and other habitat type cover) had differential relationships at the various buffer distances, and a constant agreement between correlations and VIF values. The only exception was altitude in the 2000-m buffer, which had a VIF that was greater than the threshold of five but correlations at most of /0.7/ with the other non-collinear variables, and was eventually included in the models to preserve at least one physical variable. The final set of geographical variables and factor interactions used in the model building at the various buffer distances is given in Table 2. All these variables were standardized to improve the comparability of the coefficients and the convergence of the models. Standardization was done by subtracting the mean value, as implemented in the “scale” function in base R package (R Core Team, 2015).

Birds were the only taxonomic group with a single year of sampling and so, unlike the other groups, had no replications. Accordingly, general linear models (GLM) were constructed for birds and generalized linear mixed models (GLMM), which have the ability to deal with random factors and are thus better suited to replicated designs, for the rest of taxonomic groups. Separate models were built for each group and for each buffer distance, resulting in 12 sets of models.

GLMMs were built using sampling location nested with year of sampling as a random factor. The Laplace approximation was used to estimate parameter likelihood (Bolker *et al.*, 2009). Starting with the 12 initial saturated models, all possible models were run using the packages MuMIn (Barton, 2015) and bestglm (McLeod and Xu, 2014) for the GLMMs and the GLMs, respectively. We based model selection on the Akaike Information Criterion (AIC) and selected our best model as the one with the lowest AIC value. The models with their AIC differing by less than 2 from the AIC of the best model were considered as top-ranked models (statistically equivalent to the best model of the set). Goodness-of-fit was assessed using pseudo-R-squared. However, this interpretation needs to be taken with caution since it is not a straightforward measure of the explained variance as R^2 is in linear models, but rather a measure of the improvement of the selected model over the null model. Thus, values close to 1 indicate an excellent fit, while values close to 0 indicate a low or poor fit; no comparison is possible between models built from different datasets (UCLA, 2011).

TABLE 1-2. SETS OF VARIABLES, FACTORS, INTERACTIONS AND RANDOM FACTORS INCLUDED IN THE SATURATED MODEL FOR EACH CONSIDERED BUFFER AND ALL TAXONOMIC GROUPS (*VEG*: VEGETATION AND *BUT*: BUTTERFLIES).

		500-m buffer				**2000-m buffer**				**4000-m buffer**			
		Veg	**But**	**Moth**	**Bird**	**Veg**	**But**	**Moth**	**Bird**	**Veg**	**But**	**Moth**	**Bird**
Variable	**Type**	GLMM	GLMM	GLMM	GLM	GLMM	GLMM	GLMM	GLM	GLMM	GLMM	GLMM	GLM
Block nested within year	Random factor	●	●	●		●	●	●		●	●	●	
Treatment (OF or ICM)	Factor	●	●	●	●	●	●	●	●	●	●	●	●
Location (crop line or grass strip)	Factor	●	●			●	●			●	●		
Treatment x Location	Factor interaction	●	●			●	●			●	●		
Altitude (average)	Variable	●	●	●	●	●	●	●	●	●	●	●	●
Forest (cover)	Variable	●	●	●	●	●	●	●	●				
Vineyard (cover)	Variable	●	●	●	●								
Shrub (cover)	Variable					●	●	●	●	●	●	●	●
Herbaceous (cover)	Variable	●	●	●	●								
Other, mainly urban & infrastructures (cover)	Variable	●	●	●	●	●	●	●	●	●	●	●	●

Results

A large amount of convergent models were obtained (N=704), out of which a number of top-ranked models were selected for every taxa at each considered buffer distance (7.8 ± 4.0 top rank models per taxa and distance). Figure 2 summarizes these results showing the size of all significant standardized effects of the models. Hence, the relative weight of every considered factor and variable can be visually interpreted. To provide a more detailed outcome of the models, table 3 shows the results of a subset of the four best models for the three considered buffer distances. A comprehensive detailed list of models is given in the Sup. Mat. 3.

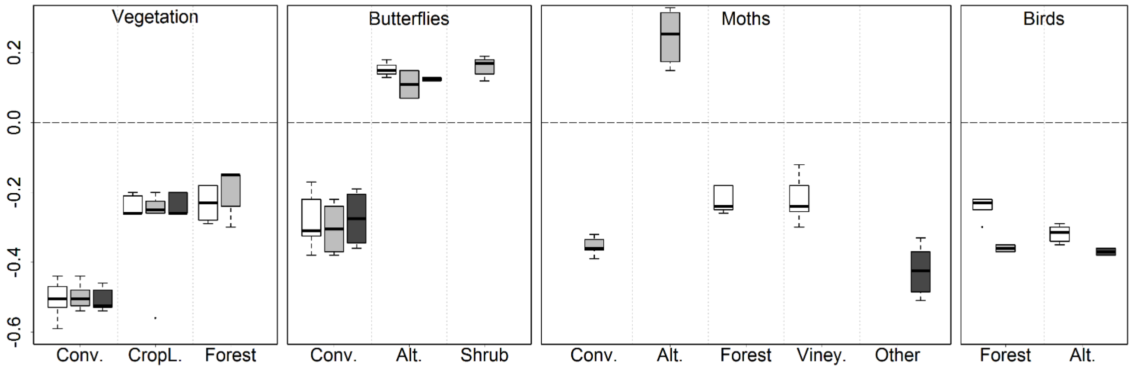

FIGURE 1-2 SIGNIFICANT EFFECT SIZES OF VARIABLES INCLUDED IN THE MODELS. STANDARDIZED EFFECTS, WHEN SIGNIFICANT, ARE SHOWN FOR THE THREE CONSIDERED DISTANCES. BOXPLOTS INCLUDE VALUES OF ALL TOP-RANKED MODELS WHEN AT LEAST THE EFFECT WAS SIGNIFICANT IN ONE OF THEM. WHITE BOXES REPRESENT 500 M BUFFERS, LIGHT GREY BOXES 2000 M BUFFERS AND DARK GREY BOXES 4000 M BUFFERS. WHERE CONV.: EFFECT OF CF AS COMPARED TO OF; CROPL: EFFECT OF SAMPLING IN THE CROP LINES AS COMPARED TO SAMPLING IN THE GRASS STRIPS; ALT.: EFFECT OF ALTITUDE; FOREST: EFFECT OF FOREST COVERAGE; VINEY: EFFECT OF VINEYARD COVERAGE; SHRUB: EFFECT OF SHRUB COVERAGE; OTHER: EFFECT OF OTHER LAND COVERS (SEE METHODS).

Vegetation

The vegetation inventories identified 168 species of vascular plants, 132 of which were found in OF plots and 125 in CF plots. The grass strips hosted a total of 143 species, whereas 123 species were found in the crop lines of the vineyards (Sup. Mat. 4.1).

Species accumulation curves revealed that vegetation species density was higher under OF than CF treatments (Sup. Mat. 5.a); greater differences were detected in vegetation communities in grass strips, where there was higher species density than within the crop lines (Sup. Mat. 5b). Vegetation inventories were still far from the asymptote (Table 4).

A total of 14, eight and six top ranked models were obtained for the 500, 2000 and 4000m buffers respectively. The estimate for the CF treatment had the highest absolute value (range from -0.54 to -0.44 ± 0.13–0.17) and was significant in all selected models ($P \leq 0.01$), with a negative relationship between vegetation species density and CF. The second strongest and most prevailing estimate was for the ‘crop line' sampling location (range from -0.26 to -0.20 ± 0.05–0.07), which was also significant in all models ($P \leq 0.01$) and was associated with a decrease in plant species density. However, no selected model showed any significant interaction between management treatment and location in the plot. Finally, at distances of 500 and 2000 m, forest coverage also had a negative effect on vegetation species density (range from -0.15 to -0.28 ± 0.07–0.11), an effect that was stronger at 500 m (present in all models) but weaker and less prevailing at 2000 m.

TABLE 1-3 MODEL RESULTS FOR RICHNESS CONSIDERING THE INFLUENCE OF LAND COVER AT A RADIUS OF 500 M AROUND THE VINEYARD PLOTS. THE FOUR BEST MODELS (INCREMENT OF AIC VALUE BELOW 2) ARE GIVEN, WITH THE TOTAL OF BEST MODELS INDICATED IN THE TABLE HEADINGS. STANDARDISED ESTIMATES ARE GIVEN FOR FIXED EFFECTS INCLUDED IN THE MODELS AND THE SD OF THE ESTIMATES IN PARENTHESIS. *P(Z)* INDICATES SIGNIFICANT ESTIMATES (* *P*< 0.001. ** *P*< 0.01. * *P*< 0.05). THE GREY SHADING INDICATES THE VARIABLES EXCLUDED FROM THE SATURATED MODELS. *TREAT-CONV*: CONVENTIONAL FARMING TREATMENT; *ALTITUDE, FOREST, VINEYARD, SHRUB, HERBACEOUS:* PERCENTAGE COVER AT THE SPECIFIED RADIUS; *OTHER:* PERCENTAGE COVER OF ALL REMAINING LAND COVERS AT THE SPECIFIED RADIUS; *LOC-CROPL*: LOCATIONS IN CROP LINES; *LOC-CROPL:TREAT-CONV:* INTERACTION BETWEEN CONVENTIONAL TREATMENT AND SAMPLING IN THE CROP LINES; *AIC*: AIKAIKE INFORMATION CRITERION; PSEUDO R-SQ: PSEUDO R-SQUARED VALUE.**

	Vegetation (14 models out of 160)				Butterflies (11 models out of 80)				Moths (12 models out of 64)				Birds (6 models out of 64)			
	Model1	**Model2**	**Model3**	**Model4**	**Model1**	**Model2**	**Model3**	**Model4**	**Model1**	**Model2**	**Model3**	**Model4**	**Model1**	**Model2**	**Model3**	**Model4**
Intercept	3.15 ***	3.16 ***	3.17 ***	3.12 ***	1.74 ***	1.68 ***	1.64 ***	1.76 ***	3.35 ***	3.34 ***	3.18 ***	3.19 ***	1.56 ***	1.55 ***	1.58 ***	1.51 ***
	(0.09)	(0.10)	(0.09)	(0.10)	(0.09)	(0.11)	(0.08)	(0.10)	(0.12)	(0.13)	(0.09)	(0.10)	(0.11)	(0.11)	(0.10)	(0.15)
Treat-conv	-0.50 ***	-0.53 ***	-0.55 ***	-0.44 **	-0.31 *	-0.17	-0.31 *	-0.34 **	-0.32	-0.29						0.09
	(0.13)	(0.13)	(0.13)	(0.14)	(0.12)	(0.15)	(0.12)	(0.13)	(0.18)	(0.19)						(0.20)
Altitude							0.15 *			0.16			-0.30 *	-0.34 **	-0.35 **	-0.31 *
							(0.06)			(0.09)			(0.12)	(0.12)	(0.12)	(0.12)
Forest	-0.18 *	-0.22 **	-0.28 **	-0.18 *	-0.13	-0.13	-0.13		-0.26 *		-0.25 *		-0.23	-0.30 *		-0.22
	(0.07)	(0.07)	(0.09)	(0.07)	(0.07)	(0.07)	(0.07)		(0.12)		(0.12)		(0.13)	(0.15)		(0.13)
Vineyard			-0.11						-0.27 *		-0.24 *					
			(0.08)						(0.11)		(0.11)					
Shrub	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Herbaceous	0.11			0.10	▨	▨	▨	▨	-0.18		-0.16					
	(0.07)			(0.07)	▨	▨	▨	▨	(0.09)		(0.10)					
Other																
Loc-CropL	-0.26 ***	-0.26 ***	-0.26 ***	-0.21 **	-0.21	-0.07		-0.21	▨	▨	▨	▨	▨	▨	▨	▨
	(0.05)	(0.05)	(0.05)	(0.07)	(0.11)	(0.15)		(0.11)	▨	▨	▨	▨	▨	▨	▨	▨
Loc-CropL:Treat-conv				-0.13		-0.31			▨	▨	▨	▨	▨	▨	▨	▨
				(0.11)		(0.22)			▨	▨	▨	▨	▨	▨	▨	▨
AIC	598.15	598.25	598.63	598.79	327.34	327.44	328.83	328.86	319.66	320.74	320.89	320.91	99.24	100.17	100.35	101.02
PseudoR-sq	0.945	0.943	0.944	0.946	0.239	0.259	0.202	0.201	0.992	0.991	0.991	0.99	0.473	0.501	0.385	0.479

Butterflies

A total of 47 species of butterflies were found in the vineyards, of which 40 species were detected in OF plots and 38 in CF plots (Sup. Mat. 4.2). Inside the vineyards 37 species were reported, while 40 were found on grass strips.

Species accumulation curves showed that the butterfly species density was higher under OF than CF treatment (Sup. Mat. 5.c); however, greater differences were detected in butterfly communities in grass strips, where there was higher species density than in crop lines (Sup. Mat. 5.d). The completeness of butterfly inventories was similar to that calculated for plants (Table 4).

A total of 11, 11 and 7 top ranked models were selected for the 500, 2000 and 4000m buffers respectively (Sup. Mat. 3.2).

Treatment consistently appeared as the most important factor (range from -0.17 to -0.38± 0.12), and was significant in 17 models ($P \leq 0.05$), above all at the smallest distances. Altitude often had a significant positive effect and species density increased with elevation. Finally, for the 2000-m buffer, shrub cover had a moderate positive effect in five of the 11 selected models.

TABLE 1-4 TOTAL SPECIES DENSITY, ASYMPTOTIC SPECIES DENSITY AND INVENTORY COMPLETENESS FOR THE FOUR TAXONOMIC GROUPS STUDIED FOR SAMPLED-BASED ACCUMULATION CURVES OF THE OBSERVED SPECIES (N = 20 SAMPLES). ALL POSSIBLE COMBINATIONS OF TREATMENTS (TWO FARMING LEVELS X TWO PLOT LOCATIONS) ARE PRESENTED WHEN AVAILABLE. THE ASYMPTOTIC NUMBER OF SPECIES WAS CALCULATED BY DIVIDING THE PARAMETERS OF THE CLENCH EQUATION (A/B) ADJUSTED TO THE OBSERVED SPECIES ACCUMULATION CURVE (SEE METHODS FOR DETAILS).

GROUP	TREATMENT	LOCATION	Total species density (mean ± SD)	Asymptotic species density	Inventory completeness (%)
Vegetation	OF	Grass strips	106.58 ± 2.23	134.69	77.96
		Crop lines	100.69 ± 2.91	131.96	78.06
	CF	Grass strips	105 ± 3.22	149.04	70.45
		Crop lines	77 ± 3.27	111.96	68.78
Butterflies	OF	Grass strips	33 ± 3.41	42.99	76.75
		Crop lines	30 ± 2.68	39.17	76.59
	CF	Grass strips	32 ± 2.35	44.41	72.05
		Crop lines	22 ± 3.20	34.57	63.64
Moths	OF		193.1 ± 7.83	282.18	68.40
	CF		190 ± 7.76	294.42	64.53
Birds	OF		33.51 ± 5.15	45.15	55.37
	CF		29.71 ± 3.99	40.67	59.01

Moths

In all, 2,163 moths belonging to 242 species were captured (Sup. Mat. 4.3), of which 185 species were identified in OF plots and 189 in CF plots.

The species accumulation curves show that moth species density was slightly higher in OF than in CF treatments (Sup. Mat. 5.e) but that differences were not significant. Moth inventories were somewhat less comprehensive than those of plants and butterflies. For this group, considerable further sampling would be required to approach the asymptotic value in species density (Table 4).

A total of 12, 9 and 8 top ranked models were selected from the 500, 2000 and 4000m buffers respectively (Sup. Mat. 3.3).

Results were not consistent over distances. Forest, herbaceous plant and vineyard cover had a significant ($P<0.05$) negative effect in three, one and four models, respectively. At the 2000-m buffer, altitude had a positive effect in five selected models; the CF treatment had a negative effect in one selected model ($P<0.05$). The strongest effects were found for the 4000-m buffer, where the cover of other habitat types (e.g. urban and infrastructures) negatively affected ($P<0.001$) all eight selected models, with no other variables or factors having a significant effect.

Birds

A total of 33 bird species were observed in the vineyards during the 2013 sampling season (Sup. Mat. 4.4), with 25 species being detected in the OF plots and 24 in the CF plots.

Species accumulation curves showed that bird species density was slightly higher under OF than CF treatments (Sup. Mat. 5.f). The bird inventories were the least comprehensive of the four studied groups, being the species density accumulation curve far from its asymptote (Table 4).

Only six, one and one top ranked models were selected (Sup. Mat. 3.4).

Bird species density was essentially influenced negatively by altitude (range from -0.38 to $-0.29 \pm 0.12–0.14$, $P \leq 0.05$). Forest cover also had a negative impact on bird richness; this variable was included in six models and was significant at 500- and 2000-m distances (range -0.37 to $-0.30 \pm 0.11–0.15$, $P \leq 0.05$). Finally, management treatment had no effect on this taxa.

Discussion

Differences in species richness and biodiversity between OF and CF has been the object of a number of studies that have consistently revealed the beneficial effects of the former in a wide range of taxa (see e.g. Bengtsson *et al.*, 2005; Hole *et al.*, 2005 for thorough reviews). Several works have confirmed this general trend in vineyards, although some exceptions have also been found (e.g. Brittain *et al.*, 2010; Bruggisser *et al.*, 2010). Our study makes a novel contribution in an area (N Spain) where, despite the enormous economic importance of the wine industry, no previous attempts had been made to evaluate the impact that OF in vineyards has on biodiversity.

Differences in species richness between these two types of managements emerged for both vascular plants and butterflies in our multi-taxon approach. In addition, although the treatment effects in the other two studied groups (moths and birds) were weak and non-significant (only in one model treatment had a significant effect in moths), the majority of the moth models showed a consistent positive relationship between species richness and OF. This result suggests that a similar interaction to that found for butterflies may also occur in moths.

Regarding local management, two major differences exist between OF and CF in our area: i) the use of herbicides (i.e. glyphosate), and ii) the use of synthetic insecticides (chlorpyrifos) and growth regulators (tebufenozide and fenoxycarb). Glyphosate is a broad-spectrum herbicide that affects both mono and dicotyledons, and hence can potentially have an important impact on vascular plant communities within the sprayed zone and within the reach of the spray drift (Marrs *et al.*, 1993). Regarding the insecticides used in the region, tebufenozide is specifically targeted to lepidopterans, and fenoxycarb and chlorpyrifos are wide-spectrum insecticides. Hence all three can negatively affect insect communities of the vineyards, and more acutely the lepidopteran communities. Most likely these factors accounted for most of the loss of species richness of both plants and butterflies in CF. A similar conclusion was reached by Nascimbene *et al.* (2012) in their study of Italian vineyards regarding the impact of herbicides.

Our results contrast with those found by Brittain *et al.* (2010) in vineyards of NE Italy, where OF did not have any effect on pollinator abundance and species richness, including butterflies. Brittain *et al.* (2010) concluded that this was because of the major impact of the surrounding landscape compared to the local management. However, their study area was located within an intensive agricultural landscape, dominated by large extensions of maize, soy and vine crops, that is, characterised by low heterogeneity and biodiversity (e.g. Benton *et al.*, 2003). On the other hand, our study area is found in a region where the predominant landscape is constituted by a mosaic of a variety of land uses and an overall high spatial heterogeneity. In any case, our data supported the general statement that OF has positive effects on pollinators (Holzschuh *et al.*, 2008).

An interesting but expected result was the greater effect that the type of farming treatment had on less mobile taxa (e.g. Fuller *et al.*, 2005). Thus, plants, which rely strictly on the conditions of the soil in which they grow, were the most affected by OF farming, while the weakest effect was recorded in birds, which generally have home ranges that are larger than the considered plots. For instance, one of the more common bird species in the area with the smallest territories, the Sardinian Warbler (*Sylvia melanocephala*), inhabits patches of around 2 ha (Bas *et al.*, 2005) and thus is very unlikely to occupy a single vineyard plot or to show clear responses at such a local scale. Although differences in avian richness and abundance between treatments in vineyards have been found by

Jedlicka *et al.* (2011), in these authors' study plots were roughly six-times larger than those in the present study, which further suggests that plot size could partially explain our results. The weak effect in moths could also be related to a greater mobility in this group, given that the sampling method (i.e. light traps) attracts individuals from a certain distance (Muirhead-Thompson, 2012), therefore reducing possible local habitat effects. Alternatively, the incompleteness of the moth surveys (Moreno and Halffter, 2001) could be partly responsible for this weaker effect, above all because some of the species that predictably would benefit the most from OF are rare specialists living at low population densities and are hence more difficult to detect than generalist species.

Our data also highlight important differences in biodiversity in microhabitats within plots. For plants and butterflies, a consistent pattern emerged of richer assemblages in grass strips compared to crop lines. Because in our design grass strips were located among fields and not closer to the natural vegetation surrounding the vineyards, the positive effect of this microhabitat was genuine and could not be explained merely as a gradient in biodiversity rise with increasing distance from the vineyards. In our case, grass strips were clearly associated with richer plant communities which, in turn, favoured richer butterfly communities by providing both an increase of larval host plants and nectar sources for adults. Our results provide further evidence on the importance as reservoirs of biodiversity of the naturalized strips within or along the margins of the crops, that has already been highlighted by other authors (e.g. Le Coeur *et al.*, 2002; Merckx *et al.*, 2012).

Grass strips within and between vineyards are a particular relevant feature of the study system since, given the complex topography of the region, most vineyards are characterised by their ranks of farmed terraces. Currently, there is a trend towards establishing vineyards directly on slopes, which benefits grapes by improving the natural air-flow and avoids mechanization and the soil erosion it can cause. However, this practice prevents the natural occurrence of grass strips within and between plots. To increase biodiversity and enhance natural ecosystem services, our results suggest that plots should be small and surrounded by non-cultivated strips.

Finally, our data also highlight the importance of other geographical and landscape factors that must be taken into consideration when trying to explain richness patterns in the taxa found in vineyards. For example, altitude had a strong influence on both birds and Lepidoptera — despite our attempt in our experimental design to restrict the altitudinal range of the plots. These two taxonomic groups had opposite responses to altitude, which agrees with previous knowledge of their ecology. The observed negative effect of both altitude and forest cover on bird richness resembles the effects detected by (Farina, 1997) in other Mediterranean agroecosystems and was expected given the regional scale of our sampling. On the other hand, altitude had a strong positive effect on butterflies, a result which is consistent with previous analyses showing a peak of diversity in this group at mid-mountain elevations in the study region (Stefanescu *et al.*, 2011).

Perhaps the most unexpected finding was the negative influence of urban cover on moth diversity that was detected in the 4000-m buffer. We believe that this response could indirectly indicate how light pollution negatively affects this group. The detrimental effects of outdoor lighting on moths and other insects are well known (e.g. Frank *et al.*, 2006), although to date no study has convincingly assessed its effects at community level. In our study area, small towns and villages probably act as light traps and negatively affect moth communities in vineyards over a considerable area (i.e. a few kilometres around settlements). Clearly, this possibility deserves further investigation and opens an interesting line for future research.

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2. Organic olive groves and bat conservation

Benefits of organic farming in olive crops for gleaning bats' conservation

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Benefits of organic olive farming for the conservation of gleaning bats

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Abstract

Current intensification and expansion of agricultural lands are some of the main anthropogenic processes driving the global decline of biodiversity. Organic farming is generally regarded as a better compromise between production and ecosystems and biodiversity preservation. However, while this practice is gaining popularity worldwide, conventional agriculture is still the main approach, hindering the conservation of many taxa. Bats are poorly studied and generally negatively affected by conventional farming. Their high mobility and long lifespan make them excellent ecological indicators in agroecosystems. We assessed the effect of different crop treatments (conventional and organic olive groves, and sparse coniferous forest as a control) on bat activity, at both guild and species level. In addition, we evaluated whether bat activity was influenced by the abundance of the olive fruit fly *Bactrocera oleae*, the major insect pest of olive groves worldwide. Bats were surveyed acoustically during autumn 2014 in all treatments using passive ultrasound detectors. In parallel, pheromone traps for *B. oleae* were used to monitor pest insect abundance. Our results show that aerial hunting bats were significantly more active in sparse coniferous forests compared to the other treatment areas. On the contrary, gleaning bats (those generally more threatened and vulnerable to habitat degradation) showed higher activity rates in organic olive groves. Due to their higher manoeuvrability and slow flight, gleaning species are generally well-adapted to forage in structurally complex and cluttered habitats such as olive groves. A significant negative relation was found between the density of the olive fruit fly and the gleaning bats activity, which were expected to prey on the pest and hence show some positive relation with its density. The reasons are unclear and further research with molecular techniques would be needed to better understand the ecological interaction, if any, between bats and the pest. Organic olive groves, usually more stratified than the conventional ones, are characterized by the presence of spontaneous herbaceous cover and higher diversity of arthropods (either beneficial or deleterious), which favours bat activity. Organic practices should be further prioritised in the agri-environment schemes of the European Union and those of its individual members. If organic farming is not widely implemented, agriculture intensification and the expansion of monocultures may put bat populations at stake, as well as compromise ecosystem quality and the conservation of biodiversity.

Keywords: Ecosystem services, bioacoustics, conventional agriculture, organic olive groves, bat conservation, pest management

Introduction

Agricultural intensification, monocultures and the widespread increase in the use of pesticides and fertilizers are consistently linked to declines of biodiversity all over the world (Groenendijk and van der Meulen, 2004; Thomas *et al.*, 2004; Franzén and Johannesson, 2007; Ellis, 2012). The large-scale agricultural production pressure has promoted monocultures and led to excessive use of pesticides worldwide, increasing environmental and water pollution and reducing biodiversity (Sánchez-Bayo and Wyckhuys, 2019; Topping *et al.*, 2020). These processes are almost always associated with cascading impacts on several taxa, such as birds and bats (Rosenberg *et al.*, 2019).

Organic farming has been suggested as a potential solution to protect wildlife and biodiversity (Connor, 2008), while fulfilling the increasing food and energy demands from a growing world population (Gomiero *et al.*, 2011; Winqvist *et al.*, 2012). During the last two decades, organic agriculture has gained broad attention from both consumers and producers (Crowder and Reganold, 2015; Reganold and Wachter, 2016; Seufert and Ramankutty, 2017). This has led product regulatory institutions to certify and distinguish organic from conventional products (Sanz Cañada and Macías Vázquez, 2005). In fact, the Food and Agriculture Organization of the United Nations (FAO) is currently working to increase Integrated Pest Management and organic agriculture to enhance food security, rural development, sustainable livelihoods and environmental integrity in all member countries (FAO).

Organic farming can be defined as a farming system based on the efficient use of local resources to maximize both soil fertility and productivity without the input of agrochemicals (Gomiero *et al.*, 2011). It is generally assumed, and has been repeatedly demonstrated, that organic systems increase local biodiversity in terms of both flora and fauna (Fuller *et al.*, 2005; Puig-Montserrat *et al.*, 2017). In fact, in the review by Bengtsson *et al.* (2005), positive effects on both species richness and abundance were reported for most of the taxonomical groups investigated. However, several recognised constraints of organic farming are hindering its broad implementation: limited feasibility (requires specific environmental characteristics), low labour productivity (high manual labour effort and time needed), unbalanced economic performance (lack of balance between assessed yields and inputs) and a deficient understanding of the practice (overall lack of agroecosystem services in ecological networks, compromising the ability to obtain stronger governmental support (Gomiero *et al.*, 2011)).

The Mediterranean basin boasts the world's highest production of olive-groves (98% of the crop worldwide), as the species has thrived in the marginal sub-humid and semi-arid Mediterranean lands (Delrio, 1985; Garcia-Mozo *et al.*, 2014). Olive oil production represents an important facet of the socio-cultural heritage of these countries (Sanz

Cañada and Macías Vázquez, 2005), allowing the exploitation of natural resources in habitats that would otherwise be unviable for farming due to extreme droughts, heatwaves and strong winds (Tanasijevic *et al.*, 2014). Although several insect pests are known to affect olive groves (over 255 according to the International Organisation for Biological and Integrated Control), the major yield losses are due to the olive fruit fly *Bactrocera olaea* (Haniotakis, 2005). These flies cause harvest losses ranging from 80-90% in untreated fields, compared to 5-40% in treated groves (Alonso-Muñoz and García-Marí, 2012).

Because some olive plantations are traditionally cultivated in rough terrains, conventional mechanised protection is sometimes difficult to apply. However, when pest management is feasible (generally in monoculture olive-groves), non-organic practices become predominant, including extensive use of pesticides. Conventional methods include the wide use of bait sprays (a mixture of attractant and pesticide) and conventional spraying (pesticide alone), both highly detrimental to the environment when applied from both the ground and the air (Haniotakis, 2005). Due to the obvious detrimental effects on ecosystem health, alternatives within the framework of Integrated Pest Management such as mass trapping have been developed during the last two decades and their use is being increasingly demanded by ecologists and conservationists (Rodriguez *et al.*, 2012). However, there is a general lack of scientific proof of the ability of organic practices to improve ecosystem health, and a paucity of quantitative studies of yield economic gains/losses (Tuck *et al.*, 2014). This lack of studies on the trade-off between biodiversity and crop yield delays social acceptance towards new eco-friendly practices, and thus, harmful old-fashioned techniques persist (Tuck *et al.*, 2014).

Insectivorous bats are among the most understudied and affected taxonomic groups by conventional agriculture. The decrease in insect availability, pesticide bioaccumulation and the loss of roosting and foraging sites are undeniably threatening their populations (Wickramasinghe *et al.*, 2003; Wickramasinghe *et al.*, 2004; Williams-Guillén and Perfecto, 2011; Herrera *et al.*, 2015). Due to their high mobility, as well as their sensitivity to environmental conditions and changes, these animals have been highlighted as important ecological indicators (Fenton, 1997; Jones *et al.*, 2009; López-Baucells *et al.*, 2017). However, only a few studies have evaluated bat assemblage responses to agricultural practices, especially in organic croplands. Many authors have supported the effectiveness of bats as controllers and suppressors of arthropod populations (Kunz *et al.*, 2011b; Mata *et al.*, 2016). They have been found predating on several important crop pests worldwide, such as the striped rice borer moth (the major rice pest spread across the world) (Puig-Montserrat *et al.*, 2015; Kemp *et al.*, 2019), the corn earworm (Maine and Boyles, 2015), the paddy swarming armyworm (Kemp et al., 2019), as well as the olive fruit fly *Bactrocera oleae* (Roswag et al., 2018) among many other diurnal dipterans. The fact that the olive fruit fly is a diurnal species does not prevent it to be consumed by bats: so far at least 372 species of dipterans (excluding the eminently nocturnal families *Culicidae* and *Chironomidae*) have been found in the diet of European bat species, with some diurnal families, like *Muscidae* being consumed by at least 19 species, including both gleaners (11 species) and aerial foragers (Puig-Montserrat et al. *in preparation*).

Foraging bat guilds are globally classified based on their hunting strategy, with gleaning bats (those that catch their prey directly from a surface) and aerial hunting bats (those that capture their prey in flight) comprising the most common guilds in Europe (Denzinger and Schnitzler, 2013). Their sensitivity to habitat structure (related to vegetation clutter) and habitat quality (related to prey diversity and availability) is strongly influenced by their hunting technique. In olive plantations, several bat species have been reported at activity levels similar to those of structurally similar habitats (Davy *et al.*, 2007; Flaquer *et al.*, 2008). However, the importance of organic and conventional olive orchards for gleaning and aerial hunter bats has not yet been assessed. Due to recent advances in acoustic methods and the availability of new affordable ultrasonic detectors, research questions about bat ecology and conservation can now be accessibly investigated in depth. The autonomy of acoustic detectors allows longer surveys in multiple study sites simultaneously (Flaquer et al., 2007), capable of recording elusive species which are otherwise hard to detect.

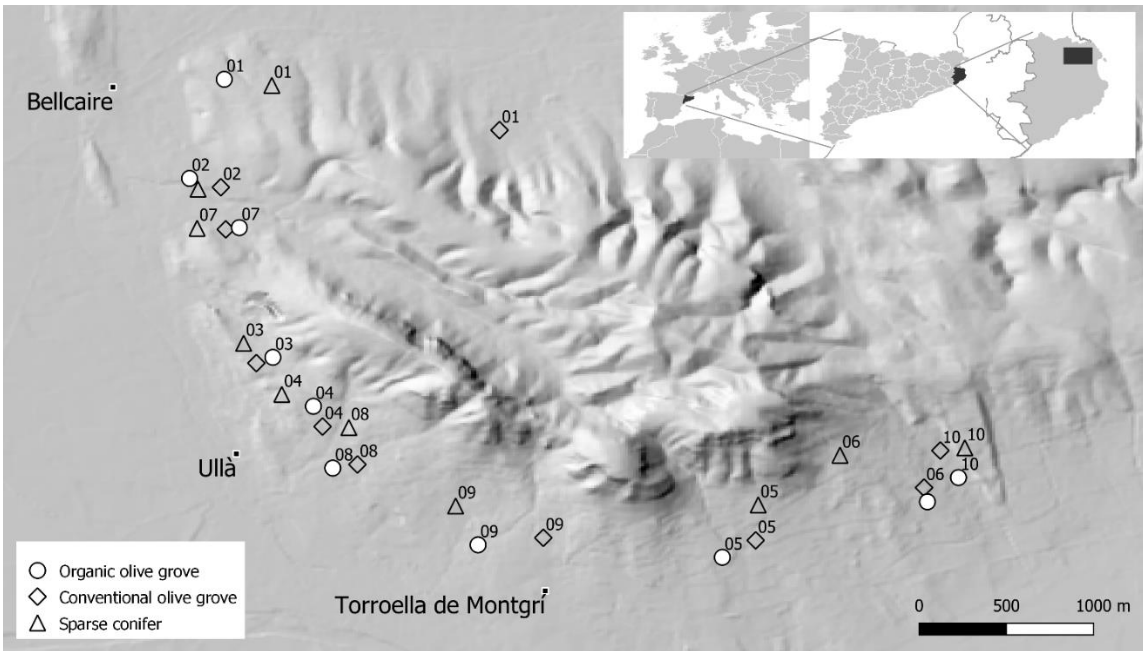
The main aim of this study was to assess the effects of different farming practices on the foraging activity and diversity of bats, at both assemblage and species-specific level using bioacoustics. Specifically, we aimed to i) ascertain the effect of olive groves management on bat activity; and, ii) determine whether the presence of the olive fruit fly (*Bactrocera oleae*) influenced bat foraging activity in any of the sampled habitats, suggesting a potential pest control ecosystem service provided by them. These research questions have been addressed at both guild and species level. We expected that all bat species, independently of their guilds, would be favoured by organic groves compared to conventional ones, as reported for many other taxa. Because gleaning bats are known to capture insects from vegetation surfaces, we predicted that the foraging activity of this guild would be the only one possibly affected by the olive fruit fly abundance.

Material and Methods

Study area

We conducted the study on the cultivated edges of the Montgrí massif, within the *Parc Natural del Montgrí, les Illes Medes i el Baix Ter*, Catalonia, Spain (3.15E, 42.05N), at an average altitude of 30 m.a.s.l.. The climate in the region is Mediterranean, with mild winters and summer droughts. Average annual rainfall amounts to 600-650 mm and the mean temperature is 15ºC. The region has been traditionally cultivated with drought-resistant rainfed crops, such as olive groves or cereals. Though many traditional crops have been replaced by more productive irrigated alternatives (such as maize or apples), the rim of the Montgrí massif, with drier conditions and rougher relief than the surrounding planes, is still dominated by olive groves, scattered pine plantations and scrublands. To account for the environmental differences that may exist along the massif's orientation gradient 30 sampling locations where placed along the rim of the massif, facing all possible orientations. The plots contained three treatments (10 plots per treatment), namely, organic olive groves, conventional olive groves and a control. The management of the olive groves was very even since they were managed by a small number of producers: all organic groves belonged to the same farmer, who would not mow the herbaceous vegetation until the reaping season (mid-November). The conventional crops were managed by three producers following the Integrated Production Management recommended by the local Plant Health Agency, with the herbaceous cover always kept at low densities, either by mechanical and chemical means, to prevent water competition with the trees.

FIGURE 2-1 STUDY AREA COMPRISING THE MONTGRÍ MASSIF AND ALL SAMPLED PLOTS. BLOCK NUMBER IS SHOWN ALONGSIDE EACH PLOT.



The control plots consisted of sparse conifer plantations, which constitute, after the olive groves, the second most common habitat with a developed vertical structure and a free growing herbaceous cover, similar to that of the organic groves in summer and autumn. Since the majority of the pine plots are regular grid plantations interspersed in the cultivated areas, and provided the roughness of the environment (low precipitation and a karstic lithology that rapidly drains the rain water), the pines grow to modest heights and remain structurally similar to the olive groves. Figure 1 depicts the distribution of the plots along the rim of the massif which was conditional to the presence of organic olive groves and ensured a minimum distance of 100 m between neighbouring plots.

Acoustic surveys

We conducted all surveys during October 2014, before the olive reaping, when the olive fruit fly (*Bactrocera oleae*) population peaks and causes most of the damage to the olives. Nine bat detectors were used simultaneously (SM2/SM3 bat detectors, Wildlife Acoustics, USA) in three randomly selected blocks during 3-4 days (covering all available plots), and subsequently relocated to 3 different plots throughout the whole study period. At the end of the project, each block had 3-4 replicates. We programmed the bat detectors to trigger at 8 kHz lower frequency threshold, and set 12 dB above sound to noise ratio (SNR) as the amplitude threshold. Recordings started 15 minutes before sunset and lasted four hours. According to the results obtained the previous year after 15 full nights of recordings at the study area, 80% of the nightly bat activity occurs in the first four hours of the night in the autumn.

We automatically classified the bat calls within the recordings using BatClassify (Scott, 2014) and SonoChiro (Biotope Research and Development, 2014) software. All automated identifications were manually validated using Avisoft SASLab Pro (Avisoft Bioacoustics, Berlin, Germany). We based species identification on standard sonogram measurements (Barataud, 2015) and available keys for European species present in the study area (Flaquer and Puig, 2012; Dietz and Kiefer, 2016). While some bat passes were classified at a species level, others that could not be confidently assigned to a particular species, due to the existing overlap in the echolocation call frequencies and shapes, were assigned to sonotypes (Tuneu-Corral *et al.*, 2020). Four gleaning (*Plecotus sp.; Myotis sp.; Rhinolophus hipposideros*; *Rhinolophus ferrumequinum*) and six aerial hunting (*Tadarida teniotis*; *Eptesicus serotinus/Nyctalus leisleri*; *Hypsugo savii*; *Pipistrellus kuhlii/nathusii*; *Pipistrellus pipistrellus*; *Pipistrellus pygmaeus/Miniopterus schreibersii*) species/sonotypes were identified.

Bat activity was used as a proxy of bat abundance (Hayes, 2000), and was estimated as the number of bat passes of each species/sonotype per sampling night. We defined a bat pass as any acoustic sequence with a minimum of two echolocation pulses of a particular species/sonotype within a five-second sound recording (Azam *et al.*, 2015; Millon *et al.*, 2015; Torrent *et al.*, 2018).

Insect surveys

Yellow sticky panel traps of 40x25 cm equipped with a specific pheromone for *D. oleae* (Dacunsex® Combi, Econex, S.L., Spain) were used. Alongside the bat detectors, we deployed one olive fruit fly trap in each sampling plot (9 per round) in the same 3-4 day scheduled round. Traps remained active during the whole plot sampling period and they were set a minimum of 30 meters apart from the bat detectors to prevent any cross-effects. We identified, counted and extracted all olive fruit flies from the panels *in situ* when removing the panels from the plots. The exact time of trap deployment and removal from each plot was recorded and used to estimate the daylight hours they had been operating since the olive fruit flies are mainly active during the day and the traps are mainly effective while there is daylight. *Bactrocera* density was then estimated as the average amount of captures per sampling hour.

Climatic and environmental variables

Meteorological data was provided by the Catalan Meteorological Service (*Servei Meteorològic de Catalunya* - http://en.meteocat.gencat.cat/xema) from the local government network of meteorological stations. The variables used were: average temperature, accumulated precipitation and maximum wind speed at 10 meters above ground. The original dataset contained one recording every 30 minutes for all variables. To estimate weather conditions during the sampling period, a single average temperature, accumulated rain and maximum wind speed were estimated for the first four hours of each survey night. To account for the spatial variation between plots several physical and land-cover related variables were averaged within a radius of 250 m around each plot. Shannon habitat diversity index was estimated with Vegan R package (Oksanen *et al.*, 2007) after reclassifying the available regional land cover map (CREAF, 2009) into 10 categories (olive groves, vineyards, other permanent crops, herbaceous crops, shrub, meadow, bare rock, coniferous forest, holm oak forest and urban). Raster R package (Hijmans *et al.*, 2013) was used to compute the average altitude, roughness, slope and orientation within the same radius.

Statistical analyses

To evaluate the effects of the plot management and the weather variables on both bat guild and olive fruit fly abundance, we performed generalised linear mixed models. Plot management, temperature, wind speed, precipitation and olive fruit fly density (when not used as dependent variable) were introduced as fixed factors and density (nightly count of bat passes or olive fruit fly captures) as a response variable with a negative binomial error distribution. Since sampling surveys were carried out during autumn (when there is a significant lowering of bat activity), the Julian day was also included as a fixed factor to account for the temporal variability. Prior to building the models, following Zuur (2009), the two sets of independent variables (climatic and physical) were tested for collinearity. All variables with a correlation value over |0.7| were discarded (Dormann *et al.*, 2013), and the Variance Inflation Factor (VIF) was estimated for the retained variables to ensure it was kept below the 5 threshold (Zuur, 2009). The distribution of all the response variables was explored with the function *descdist* from fitdistrplus R package (Delignette-Muller and Dutang, 2015), and by exploring the ratio between the mean and the variance. In all cases, a set of multiple models with all the potential combinations of explanatory variables was run with function *glmer.nb* in lme4 R package (Bates *et al.*, 2011) and compared using the *dredge* function in MuMIn R package (Barton, 2015). Models were compared based on the Akaike Information Criteria with a correction for small sample sizes (AICc), considering models as valid when their values were within the interval defined by the minimum AICc value ($AICc_m$) and $AICc_m + 2$ (Burnham and Anderson, 2004). Kruskal-Wallis was used to test for differences among plot management and specific/phonic type bat contacts. Finally, Monte-Carlo pair-wise comparisons between management and each bat species/sonotype group identified were run using the function *MC.test* in the R package Asbio (Aho and Aho, 2020) with 1000 permutations per run to ascertain whether there were significant habitat preferences.

All analyses were carried out using R v. 3.6.0. (R Core Team, 2015).

TABLE 2-1 MODEL RESULTS FOR THE BEST MIXED-EFFECT MODELS. THE BEST MODELS (INCREMENT OF AIC VALUE BELOW 2) ARE GIVEN, WITH THE TOTAL OF MODELS RUN INDICATED IN THE TABLE HEADINGS. STANDARDISED ESTIMATES ARE GIVEN FOR FIXED EFFECTS INCLUDED IN THE MODELS AND THE SD OF THE ESTIMATES IS GIVEN IN PARENTHESIS. P(Z) INDICATES SIGNIFICANT ESTIMATES (* P < 0.001; ** P < 0.01; * P < 0.05). THE GREY SHADING INDICATES VARIABLES NOT INCLUDED IN THE SATURATED MODELS. ONLY SIGNIFICANT EFFECTS ARE SHOWN. A MAXIMUM OF 6 MODELS PER TAXONOMIC GROUP ARE SHOWN (THE COMPLETE TABLE IS PROVIDED IN S2).**

	Bactrocera oleae			Aerial hunting bats						Gleaning bats					
	3 models out of 255			10 models out of 256						17 models out of 256					
	Md1	Md2	Md3	Md1	Md2	Md3	Md4	Md5	Md6	Md1	Md2	Md3	Md4	Md5	Md6
Intercept	3.15 *** (0.11)	3.14 *** (0.11)		24.06 *** (1.9)	24.33 *** (1.84)	23.95 *** (1.85)	24.22 *** (1.84)	24.71 *** (1.82)	24.28 *** (1.83)	16.81 *** (2.5)	15.84 *** (2.85)	19.46 *** (2.5)	17.13 *** (0.01)	17.34 *** (0.01)	15.51 *** (2.84)
Julian day				-0.07 *** (0.01)	-0.07 *** (0.01)	-0.07 *** (0.01)	-0.07 *** (0.01)	-0.07 *** (0.01)	-0.07 *** (0.01)	-0.05 *** (0.01)	-0.05 *** (0.01)	-0.06 *** (0.01)	-0.05 *** (0)	-0.05 *** (0)	-0.05 *** (0.01)
Altitude	0.18 * (0.07)	0.17 * (0.08)	0.17 * (0.07)									-0.42 * (0.21)			
Aspect										-0.56 * (0.22)	-0.57 * (0.23)	-0.57 * (0.22)			
Shannon										0.48 * (0.19)	0.46 * (0.2)	0.48 * (0.2)		0.27 *** (0.01)	
Org v Conv										0.85 * (0.38)	0.88 * (0.39)	0.87 * (0.38)			
Pine v Conv	-0.95 *** (0.16)	-0.91 *** (0.17)	-0.94 *** (0.16)	1.02 * (0.42)	0.89 * (0.41)	1.09 ** (0.41)	0.97 * (0.4)		0.94 * (0.39)	0.94 * (0.41)	1.13 ** (0.41)	0.96 * (0.42)			0.92 * (0.43)
Pine v Org	-0.91 *** (0.17)	-0.87 *** (0.18)	-0.91 *** (0.16)									0.08 *** (0.01)			
Temperature				0.29 *** (0.06)	0.29 *** (0.07)	0.29 *** (0.07)	0.28 *** (0.07)	0.28 *** (0.07)	0.28 *** (0.07)				0.17 *** (0.01)	0.17 *** (0.01)	
Wind				-0.27 *** (0.02)	-0.26 *** (0.06)	-0.27 *** (0.06)	-0.26 *** (0.06)	-0.26 *** (0.06)	-0.26 *** (0.06)						
Bactrocera	▨	▨	▨										-0.18 *** (0.01)	-0.19 *** (0.01)	
PseudoR-sq	0.339	0.322	0.343	0.97	0.97	0.971	0.97	0.97	0.971	0.681	0.678	0.677	0.664	0.667	0.667
AICc	721.3	721.5	723.1	2771.1	2771.3	2771.3	2771.3	2771.6	2772.4	1358.1	1358.3	1358.7	1358.8	1359	1359

Results

Bat guilds activity

We successfully accumulated 229 samples (complete 4 hours recording from sunset on), gathering a total of 918.29 hours of recordings distributed between the three treatments: 289 hours in organic olive groves, 332 hours in conventional olive groves and 297 hours in the control plots (sparse coniferous). Our results show differences between the response to each treatments by the two bat guilds (gleaner bats and aerial hunting bats)

(Fig 2, Fig 3 and Table 1). Gleaning bats were significantly more active in both organic groves and sparse coniferous compared to conventional groves.

However, while aerial hunting bats were significantly more abundant in sparse coniferous, their activity in the organic groves was no higher than in conventional groves. Regarding the considered environmental variables, the temperature had a positive effect on both guilds, while the wind had a negative influence only on aerial hunting bats, perhaps due to their higher exposure to the wind, and also because gleaning bats seem to avoid to some extent the higher altitudes and the northern rims of the massif (significant negative effect of the aspect, a variable estimated in degrees and therefore with maximum values in the northern and western slopes), more exposed to the dominant northern winds. Gleaning bats also showed a positive relation to more diverse habitat ensembles (Shannon index of landscape diversity). Contrary to the prediction of this study, gleaning bats show a significant negative relation with the olive fruit fly. Julian day had a negative effect on bat passes of both bat guilds (Fig. 3 and Table 1).

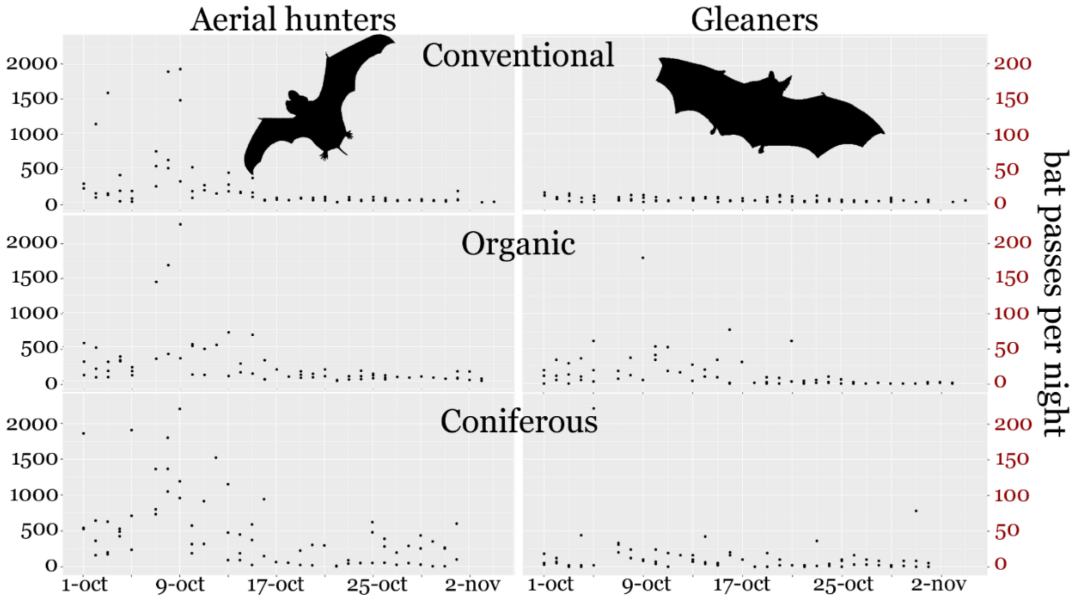


Figure 2-2 Bat passes per night for the aerial hunting and the gleaning bats on the two olive grove treatments (conventional and organic) and the control coniferous plots during the sampling period. Note the different scales for the two groups. Only the first four hours of night bat activity was recorded.

Olive fruit fly abundance

A total of 895 olive fruit flies were captured during the sampling - 428 in the conventional olive groves, 397 in the organic olive groves, and the remaining 70 in the spare coniferous. However, *B. oleae* abundance was significantly higher with altitude (Fig. 3 and Table 1). Olive groves were the most suitable habitat for *B. oleae*, appearing with slightly higher densities (non-significant differences) in the conventional ones.

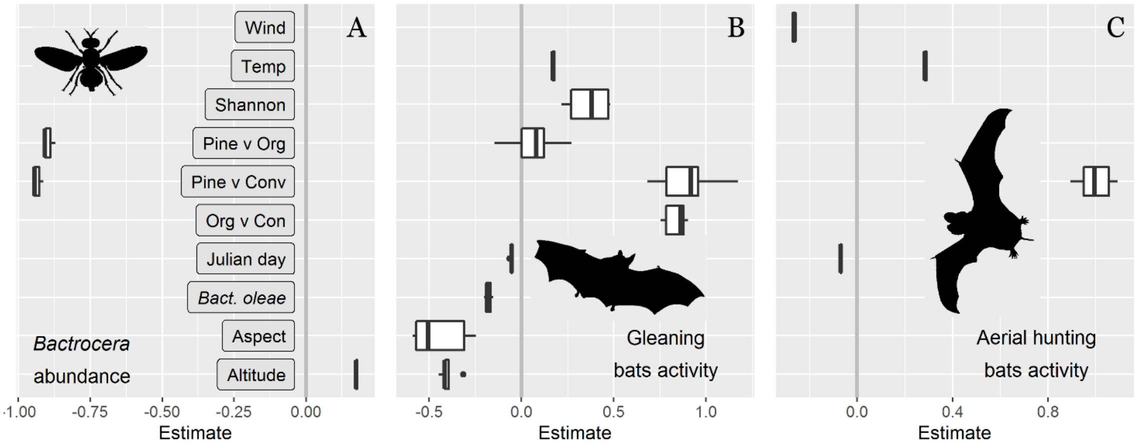


Figure 2-3 Significant standardized effect sizes of the environmental and climate variables upon olive fruit fly (*Bactrocera oleae*) abundance (A), gleaning bats (B) and aerial hunting bats (C) indices of activity. Modelled with GLMMs fitted with negative binomial distributions. Boxplots include values of all top-ranked models where at least the effect was significant in one of them. See Table 1 for the whole set of selected models. (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$)

Bat species activity

At the species level, our data show that both maximum and minimum activity levels of different species were found in sparse coniferous habitat, with *Rhinolophus spp*. showing the lowest activity and *Pipistrellus kuhlii* with the highest activity (Fig.4). Considering aerial hunters, the sparse coniferous habitat was the most suitable habitat for this guild. In contrast, organic groves were the least preferred habitat, except for the *P. pygmaeus/Miniopterus schreibersii* group, which is significantly more active in the organic olive groves (Fig. 4). For the gleaning bats, *Rhinolophus* species are strongly related to organic groves, and the sparse coniferous habitat is the treatment with their lowest activity. *Myotis sp.* is the only gleaner species group that presents no significant differences in activity between sparse coniferous forests and organic olive grove, though their activity is significantly lower in the conventional olive groves. *Tadarida teniotis*, *Pipistrellus pipistrellus* and *Plecotus sp.* showed no significant differences in activity between treatments.

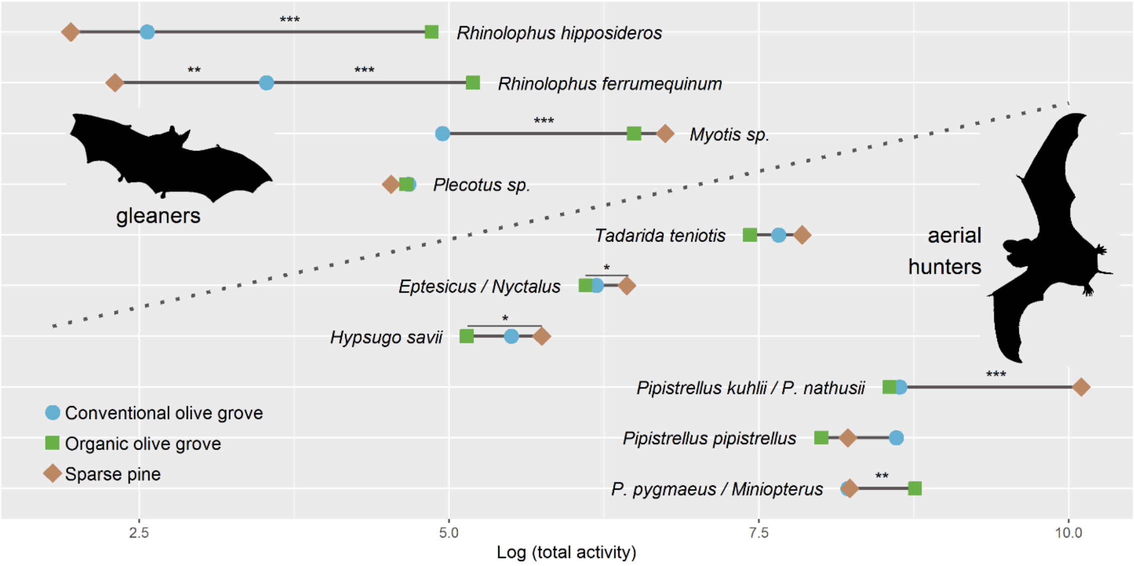


FIGURE 2-4 DIFFERENCES IN SPECIES-SPECIFIC BAT ACTIVITY BETWEEN TREATMENTS. BAT SPECIES ARE GROUPED BY GUILD, AND SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS ARE REPORTED INDICATING THEIR SIGNIFICANCE LEVELS (* P-VALUE < 0.05; ** P-VALUE < 0.01; ** P-VALUE < 0.001).

Discussion

A minimum of 14 bat species are known to occur in our study area of the Montgrí massif (NE Iberia) (Flaquer and Puig, 2012). However, while aerial hunting bats were significantly more active in sparse coniferous forests compared to the other treatments, gleaning bats (those generally more threatened and vulnerable to habitat degradation) showed high activity in organic olive groves. Contrary to the initial prediction the gleaning bats were negatively correlated to *B. oleae* abundance, the reasons being difficult to ascertain. Both horseshoe bat species present in the study area (*Rhinolophus ferrumequinum* and *R. hipposideros*) show a very significant preference for the organic olive groves over the conventional ones and the conifer forests, which implies that these traditional cultures might be relevant to their long-term preservation in the area.

We also provide further evidence that the activity of gleaner and aerial hunter bats was positively affected by temperature, and negatively affected by the wind at least for the latter, as is commonly documented in the literature (O'Donnell, 2000; Wolbert *et al.*, 2014; Wellig *et al.*, 2018).

Bat-guild level responses to olive groves management

However, gleaners showed similar activity levels in both organic olive groves and coniferous stands, aerial hunters showed higher activity levels in the coniferous stands, well above that of the two olive grove treatments. Differences in habitat selection between bat guilds might arise due to their evolutionary flight adaptations and dietary preferences. Gleaning bats have low aspect ratios and wing loading, enabling them to have a slow but highly manoeuvrable flight (Altringham, 2011) and allowing them to easily take-off from the ground, capture insects directly from surfaces and forage in cluttered environments.

On the contrary, aerial hunting bats have high aspect ratios and wing loading, necessary for their fast flight which enables them to capture fast-moving flying insects on the wing, especially in open and semi-open spaces (Altringham, 2011; Denzinger *et al.*, 2016). Gleaners are thus better suited to move within spatially complex environments like organic olive groves (Aldridge and Rautenbach, 1987; Sleep and Brigham, 2003). Also, organic olive groves are known to harbour richer communities of arthropods than more intensely managed ones (e.g. Ruano et al. 2004), potentially affecting bat foraging activity.

In a similar study in olive monocultures in the Mediterranean basin, Herrera et al. (2015) found a consistent decline in bat activity with the intensification of farming practices. However, the bat community of the olive groves they studied showed a remarkably low number of species compared with the regional species pool and extremely low activity levels overall. Therefore, the authors assumed that all olive groves within their study region (southern Portugal) were essentially used as commuting habitats. In our olive groves, both bat activity and richness was similar, or even higher, to that of the surrounding habitats, and all species/phonic types occurring in the region were detected. Since both habitat heterogeneity and crop size are known to influence biodiversity in agroecosystems, the drivers of such differences may be the higher landscape heterogeneity and the small size of the olive groves within our study area, (Belfrage *et al.*, 2015; Puig-Montserrat *et al.*, 2017).

Davy et al. (2007) found no differences in bat activity in a small Mediterranean island (Zakynthos, Greece) when comparing traditional organic olive groves with traditional non-organic ones. They also compared olive groves with the dominant forest habitats in the island (pines and oak woodlands), and found no significant differences in overall activity. Therefore, they concluded that olive groves provide foraging habitats comparable to the island forests. Contrary to our results, the gleaning greater horseshoe bat (*Rhinolophus ferrumequinum*) positively selected pine forests over olive groves, a difference that might be either related to ecological differences in the habitat structure between the study sites.

Bat-species/phonic group level response to olive grove management

In our study area, the *Myotis* phonic group is mainly represented by Geoffroy’s bat (Flaquer and Puig, 2012). Previous radiotracking studies conducted in the area proved that some of the common gleaning species present, such as Geoffroy’s bat (*Myotis emarginatus*), positively select both organic olive and pine groves at the edges of the Montgrí massif (Flaquer *et al.*, 2008), and spend most of their hunting time in these habitat areas. Their avoidance of the conventional olive groves might be related to their preference for herbaceous cover dependent prey species, such as orb weaving spiders (Brown, 1981). Analyses of the diet of Geoffroy’s bats based on faecal samples taken in late August 2014 in the Montgrí massif, when the olive fruit fly remains at low densities (S1), showed a strong dietary preference for orb-weaving spiders, which amounted to 60% of their diet, followed by *Diptera* and *Lepidoptera*, each of which comprising 20% of the prey content in the samples obtained (Vallejo *et al.*, 2019). Our results complement these findings and provide some insight into the fine-scale habitat selection of grove treatments by each species.

Horseshoe bats, represented in the study area by *Rhinolophus ferrumequinum* and *R. hipposideros,* feed on several dipterans and lepidopterans but are also capable of gleaning prey from the ground (Goiti *et al.*, 2004; Goiti *et al.*, 2008). Our results show a marked preference of the two species for organic olive groves, which were selected over conventional olive groves and pine forests. The underuse of the pine forests in the study area contradicts the current knowledge of both species, which are generally regarded as forest-dwelling species (Flanders and Jones, 2009; Reiter *et al.*, 2013). This might be explained by the dense understorey clutter of the lowland Mediterranean pine forests of the study area, compared to other European forest structures where the foraging behaviour of these horseshoe bats has been studied (UK and Austria in the cited examples).

The last gleaner phonic group (*Plecotus sp.*), which includes the Brown long-eared bat *Plecotus auritus* and the Grey long-eared bat *Plecotus austriacus*, showed no preference for organic or conventional olive crops. This suggests either a more opportunistic diet composition or the ability to hunt both in the air and from the ground and vegetation surfaces (Entwistle *et al.*, 1996).

Among the aerial hunters, the significantly predominant preference is for the sparse pine groves. Considering that aerial hunters generally fly high above the ground or the canopy (Aldridge and Rautenbach, 1987), the clutter level of the habitat should not hinder their ability to exploit it. This selection pattern was quite consistent across the whole guild and could be related to high aerial prey availability, although this was not assessed in the present study.

Bactrocera oleae and bat activity

The presence of *Bactrocera oleae* was weakly though negatively related to the activity of gleaning bats, *a priori* the most susceptible of capturing this pest species provided their ability to glean prey on the ground or other surfaces. Whether the negative relation is due to the strong avoidance of the conventional olive groves observed in two of the four reported gleaning species, or to other causes remains unclear. However, due to the drastic importance of this pest in olive plantations in the Mediterranean region, more robust acoustic and molecular data is still needed to determine if the species is regularly consumed by bats and to economically quantify the magnitude of the hypothetical ecosystem service they might provide. Insectivorous bats are able to eat over two-thirds of their body mass in insects per night (Kurta *et al.*, 1989). Their effectiveness as suppressors of insect pest populations is increasingly being recognised (Kunz *et al.*, 2011a; McCracken *et al.*, 2012; Puig-Montserrat *et al.*, 2015; Russo *et al.*, 2018), with ecosystem services estimated at a minimum of $3.7 billion per year in the United States (Boyles *et al.*, 2011) or more than $1.2 million each year in Thailand (Wanger *et al.*, 2014). However, an estimate of the economic gain of pest control associated with bats in olive groves is yet to be determined.

Conservation implications

Due to climate change, the cultivable area for olive groves may extend by 25% in the forthcoming years (Tanasijevic *et al.*, 2014). There is a current trend of shifting from conventional to organic farming and in Europe alone, the land devoted to organic farming has seen a 33% increase since 2012 (https://ec.europa.eu/eurostat), even though it still represents a small fraction of the cultivated land in Europe (7% on 2017 according to (Connaughton and Hughes (2018))). In this context, where environmentally friendly cultural practices are being encouraged by both civilians and governmental institutions, providing insight on the ecological interactions underpinning the biodiversity of agrosystems is key to ensure their sustainable development.

The guild of gleaning bats includes most of the endangered bat species in Spain, and the most sensitive species to environmental change. At least two gleaning bats in the area (out of the four species present) showed a strong reliance on organic olive groves. Therefore, preserving and promoting organic olive groves may be crucial to their conservation in the region. Thus, due to the current environmental emergency, we encourage land managers, politicians and practitioners to adopt these agricultural approaches. If these practices are not quickly and widely implemented, olive grove intensification and the expansion of monocultures may put local bat populations at stake.

With the aim of enhancing natural biodiversity, organic farming should be prioritised in the implementation of the European Union's agri-environment programs (Bradley, 2002), both at the continental and national level.

Finally, more effort is still needed to assess the biological control services provided by bats in olive groves, and to build bridges between the work of farmers and scientists and successfully share these findings to agroecosystem practitioners.

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3. Pest control provided by bats in rice paddies

Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions

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Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions

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Abstract

Pest control through integrated pest management systems stands as a very convenient sustainable hazard-free alternative to pesticides, which are a growing global concern if overused. The ability of the soprano pipistrelle bat (*Pipistrellus pygmaeus*) to control the rice borer moth (*Chilo supressalis*), which constitutes a major pest of rice around the world, was studied in the Ebre Delta, Northeastern Iberia. Evidence was found on the ability of this particular bat species to control borer infestations: a) the moth was consumed during at least the last two peaks of the moth activity, when most crop damage is done; b) the activity of bats significantly increased with moth abundance in the rice paddies; c) the pest levels have declined in the study area (Buda Island, Eastern Ebre Delta) after the deployment of bat boxes and their subsequent occupation by soprano pipistrelles. The value of the ecosystem service provided by bats was estimated at a minimum of 21€ per hectare, equivalent to the avoided pesticide expenditure alone. We suggest that this natural service can be enhanced by providing bat populations with artificial roosts in rice paddies were some key ecosystem features are present.

***Keywords**:* rice paddies, *Pipistrellus pygmaeus, Chilo supressalis,* ecosystem services, ecological functions, integrated pest management, biological control

Introduction

"*Any genuine improvement in the human condition on this planet must be concerned with rice*" (Heinrichs and Miller, 1991). In recent decades, both rice scientists and farmers have gained experience in the cultivation of rice and there has been a shift from a primarily unilateral approach to insect pest control, relying strongly on insecticides, to a multilateral approach involving a combination of control tactics (Heinrichs and Miller, 1991). Scientists throughout the world strive to develop and implement strategies to control rice pests more effectively and economically, to improve crop productivity and consequently the welfare of human populations. According to Food and Agriculture Organization of the United Nations (FAO) statistics, the global production of rice increased linearly from 216 million tons (mt) in 1961 to 722 mt in 2011 (FAO). New strategies to control insect pests include the use of one insecticide per one specific target species combined with biological methods (such as the use of parasitoids). However, the adoption of non-chemical approaches to pest control is not evenly distributed around the world and occurs mainly in those regions where legal constraints limit the number of approved chemical products (c. a. European Union). Over-use of pesticides is still an issue to be addressed (Normile, 2013; Peng et al., 2009), particularly in those regions in which the vast majority of the world's rice production is concentrated, and the use of pesticides keeps growing (FAO).

Fifty percent of the insecticides used in rice fields in Asia target lepidopteran insects (Heong et al., 1994). In 1991 it was estimated that an average global annual yield loss of 10 million tons was caused by just three moths: the striped rice borer (*Chilo suppressalis*), the yellow stem borer (*Scirpophaga incertulas*) and the leaf folder (*Cnaphalocrocis medinalis*) (Herdt, 1991). The striped rice borer is an Asian moth currently present in paddies worldwide. The adults lay their eggs on the stems and leaves of rice plants, and the larvae bore the stems to feed on the internal tissues, compromising both plant growth and productivity, sometimes fatally.

In Europe most bats are insectivorous. Bats may eat up to 80-100% of their body mass in insects on a nightly basis (Kurta et al., 1989),and during the last decade several authors have drawn attention to the important contribution that bats make to insect pest control (Agosta and Morton, 2003; Boyles et al., 2011; Cleveland et al., 2006; Ghanem and Voigt, 2012; Kunz et al., 2011; Lee and McCracken, 2005; Leelapaibul et al., 2005; McCracken et al., 2012; Whitaker, 1995). The referred contribution has been assessed by the presence of pests in the diet of wild bat populations or by taking a step further and accounting for the economic value of such an ecosystem service. Given the natural complexity of ecological systems it is difficult to place a monetary value on the services they provided by bats, a fact that restricts how their importance is understood by the public (Fisher and Turner, 2008). Cleveland et al. (2006) estimated the economic contribution of bats to the cotton dominated agroecosystems of southern Texas, USA, to be $12-$173 per acre each year. By extrapolating these figures to the whole country, Boyles et al. (2011) valued bats' economic contribution to the USA's agroecosystems at between $3.7 and $53 billion/year. Even if the actual figures were lower, given the evidence gathered so far, the positive impact of bats on this aspect of the economy seems to be beyond dispute. This benefit can exceed the monetary value if the affected crop is a staple. For example, the pest control service provided by wrinkle-lipped bats' (*Tadarida plicata*) is responsible for securing the meals of 26,152 (± 15,817 SD) people each year in Thailand alone (Wanger et al., 2014). All the aforementioned authors have stressed the consequent importance of protecting bat populations if the ecological service they provide is to be preserved.

Although there is no general agreement on how to define ecosystem services (Wallace, 2008), such services are generally regarded as ecosystem outcomes (e. g. use of less pesticides) that contribute to human well-being (Fisher and Turner, 2008; Fisher et al., 2009; Nelson et al., 2009; Wallace, 2007). Understanding the mechanisms that link ecological systems to human well-being is a fundamental task when studying ecosystem services (Haines-Young and Potschin, 2009). From a functional perspective, the human benefit (whether monetary or not) is the final outcome of a cascade process resulting in a service (Rollett et al., 2008).

FIGURE 3-1 A SOPRANO PIPISTRELLE (*PIPISTRELLUS PYGMAEUS*) HUNTING A STRIPED RICE BORER (*CHILO SUPRESSALIS*), IN THE EBRE DELTA. PHOTOGRAPHIC STROBOSCOPIC EFFECT (SERIES OF SHORT OR INSTANTANEOUS SAMPLES); COURTESY OF ORIOL MASSANA.

The soprano pipistrelle (*Pipistrellus pygmaeus*, Fig. 1) was separated taxonomically from the common pipistrelle bat (*Pipistrellus pipistrellus*) on 1997 (Barratt et al., 1997).Previous studies on diet contain mixed data on diet and distribution (e.g. Vaughan, 1997). Soprano pipistrelle is a common European bat species occurring from the British Isles through much of continental Europe (including the islands of Corsica and Sardinia) East to Western Asia Minor, the Caucasus and Siberia (Dietz et al., 2009). It is more abundant in lowland areas and is frequently associated with freshwater bodies (rivers, lakes, wetlands, etc.), being common in coastal wetlands, where most paddies in southern

Europe occur. The few unambiguous studies on diet indicate that they feed mainly on small diptera, though they include a wide array of small-sized aerial insects in their diet and is regarded as an opportunistic species (Bartonicka et al., 2008b; Vaughan, 1997). Following our success in improving soprano pipistrelle populations in Northeastern in a mixed landscape with both wetlands and paddies (Flaquer et al., 2006), we investigated the ability of the species to control striped rice borer populations. Having determined the potential of bats to control rice borer, we aimed to better understand under which environmental conditions soprano pipistrelles' populations could be enhanced to further promote their pest control ability. Since the experiment was conducted in a reduced area, we describe the conditions under which bat populations had been encouraged in the rice producing landscape of study, and emphasise the economic benefits they could provide. Although there is a long tradition of rice-fish culture aimed both at controlling pests and enhance food production (Xie et al., 2011), to our knowledge no other wild local vertebrate populations have been artificially manipulated for such a purpose and no previous research exists on enhancing bat populations as a pest biological control method in rice paddies.

Material and methods

Study area

We carried out the study within the Ebre Delta Natural Park (0º 50' E, 40º 42' N, Figure 2), Catalonia, NE Iberia, which is one of the biggest deltas in Europe (320 km^2). Approximately 65 km^2 are wetlands, 240 km^2 are crops (203 km^2 of which are paddies) and 16 km^2 are urban areas that host around 5,000 inhabitants. Mean annual temperatures range between 17 and 18ºC, mean annual precipitation between 500-550 mm, and mean annual evapotranspiration between 855-997 mm, in what is essentially a semi-arid Mediterranean climate, with a pronounced summerdrought.

Prior to this study, in 1999, 69 small wooden bat boxes, either with single or double compartment (Flaquer et al., 2006), were erected in Buda Island Natural Reserve (12.1 km^2, Figure 2), a deforested area lacking suitable vertical structures for the bats to roost in (Flaquer et al., 2005). Bat boxes were rapidly occupied (243 individuals were counted one year after boxes were deployed) by an increasing number of individuals, reaching a maximum of approximately 3,500 individuals in 2008 (Flaquer et al., 2006).

Pest control systems

Following Cleveland et al. (2006) we can estimate the value of the service provided by bats in two ways: 1/ assessing the economic expenses that were reduced/avoided due to bat predatory activity, and 2/ assessing the value of the crops that remained undamaged. The latter is harder to assess since some of the measurements required are difficult to gather in the wild (e.g. the total number of bats hunting per unit area of rice fields in a period of time). Thus, the conservative figure that is most easily calculated from the available local data is the expenditure on chemical treatments that was avoided in the 2.9 km^2 of paddies in Buda Island crops between 2006 and 2012.

Pest control procedures in the region are done by farmers in coordination with the Catalan Government that have created the Plant and Crop Protection Association in the Ebre Delta. These procedures consist of a permanent network of trapping devices lured with chemical attractants targeted at rice borer males. These are deployed at a rate of 4.5 traps per hectare all over the 203 km^2 of paddies, totalling approximately 91,300 traps at an estimated annual expenditure of 275,000 €. In addition to this non-hazardous biological method, a moth surveillance programme measuring the density of affected rice stems is done throughout the area during both the second and the third peak of the rice borer's activity. Aerial spraying with tebufenocide, at a cost of 21€/hectare, is applied in areas where the presence of rice borer eggs or caterpillars exceeds 0.85 rice stems/m^2 during the second peak of borer activity, or 2.15 rice stems/m^2 during the third and final peak. The effectiveness of the described treatments, measured as the reduction of striped rice borer moth density, is estimated to average around 70% for trapping and 35% for aerial spraying (ADV – Crop Defence Association).

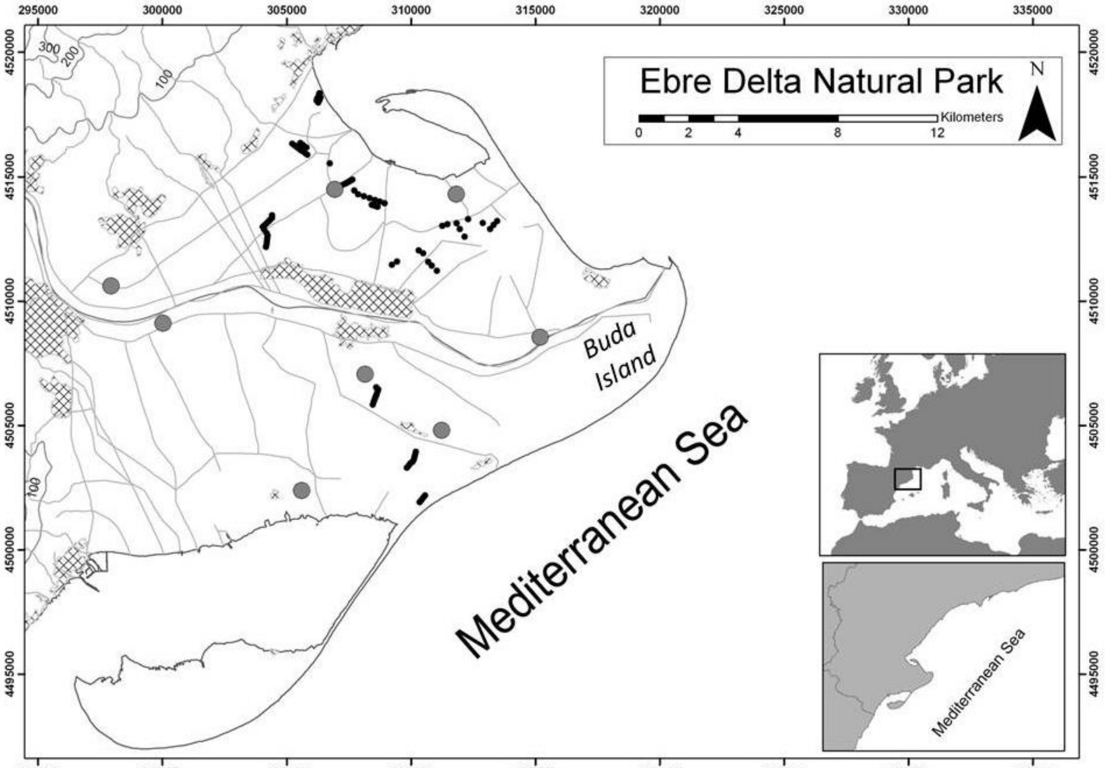

Figure 3-2 Situation of the study area and the sampling stations.Small black dots indicate the 87 independent bat detector stations (3-5 min duration) performed from July to September in 2007, 2009, and 2010, and large grey dots represent the location of the striped rice borer light-traps monitored from May to September 2007-2010. Shaded areas represent urban habitats.

Rice borer consumption by bats

Chilo suppressalis species – specific primers for 28S-D2 and mitochondrial COI genes have been designed in order to determine its presence in the soprano pipistrelles fecal DNA.

DNA extraction from rice borer, amplification and sequencing

The 5' region of *C. suppressalis* cytochrome oxidase I (COI) gene was recovered from GeneBank whilst the COI 3' region and the 28S-D2 sequences were not available. To obtain these sequences amplifications and sequencing of 28S-D2 and the 3' region of the COI gene were performed on rice borer DNA by using two different set of universal primers, ND2F/ND2Rev (Campbell et al., 1993) and C1-J- 2183/TL2-N-3014) (Simon et al., 1994) respectively.

DNA extraction from the borer was carried out by homogenizing the head, one forewing and one leg with a plastic pestle, and treating them with Chelex resin/proteinaseK (Vickerman et al., 2004).

The PCR cycling program for 28S-D2 primers pair was: 3min at 94°C, followed by 35 cycles of 45s at 94 °C, 1min at 52 °C, 2min at 72°C, and a final extension of 7min at 72°C. For the COI gene the PCR cycles were the same as described for 28S-D2 except that the annealing temperature was lowered to 48°C. All the amplifications were carried out in a 40µl reactions using 4µl of DNA template, 1x buffer (Promega), 0.2 mM of each dNTP, 10 pmol of each primer and 0,6 units of GoTaq DNA polymerase (Promega), and checked on 1% agarose gel stained with ethidium bromide.

The amplicons were directly sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready ReactionKit (PE Applied Biosystems), on the ABI PRISM 310 DNA Sequencer. Primers specific for *C. suppressalis* 28S and COI shorter internal portions were designed (see supplementary material for PCR primers, length of amplicons and amplifications conditions) with the software Primer-blast at NCBI (Rozen and Skaletsky, 2000). It uses Primer3 to design PCR primers and then submits them to BLAST search against user-selected database. In this study the database was made for all Lepidoptera other than *C. suppressalis* known sequences of the same regions. The results are then automatically analyzed to avoid primer pairs that can cause amplification of targets other than the input template.

Bat droppings collection

During 2008 and 2009 we analyzed 80 bat droppings (30 + 50 respectively) to determine whether the pest under study was being consumed by the soprano pipistrelles. All the droppings were

collected from 50 bat boxes in Buda Island Natural Reserve. In 2008, all samples were collected during June, thus corresponding to the second peak of the Rice borer, while in 2009 all droppings were collected in August, during the third and highest peak using the same method. In all cases the droppings were preserved in alcohol 70%.

DNA extraction from bat droppings

Droppings were air dried on filter paper to remove as much ethanol as possible, then placed into eppendorf tubes containing 150 µl of extraction buffer (10 mM tris HCl pH 8.0, 1 mM EDTA, 1% Nonidet P-40, 200 µg/ml Proteinase K) vortexed briefly, incubated overnight at 55°C, and 2 h at - 20°C. Samples were then subjected to a second round of lysis by adding 50 µl of extraction buffer, and incubation at 55°C for 2h. Proteinase K was inactivated heating the sample 10 minutes, and DNA recovered after centrifugation of 10 minutes at 14.000 rpm. The pellet containing the dropping debris was utilized for microscopy analysis. Each sample was split in two, and one of the portions was saved for later analysis. As positive control a small fragment of an antenna and a tarsum of the borer (size of fragments similar to that retrieved in bats' droppings) were added to two different faeces samples prior to perform DNA extraction (to be sure that there are not compounds inhibiting the PCR in the faeces). As negative control, DNA was extracted with the same protocol described above, from fragments retrieved in bat droppings and identified by microscopy analysis as "not Lepidoptera" specimens; they were two legs, a head, and head with antenna.

Morphological analysis of insects fragments found in bat droppings

Before running morphological analysis, two slides (one male and one female) of the borer were prepared following Noyes (1982) (mounting processes used for parasitoid insects) for identifying some key morphological characters that could help in identifying putative fragments in the bat droppings. Morphological analysis of the droppings was performed on all samples after DNA extraction, by crumbling gently the droppings in absolute ethanol and by examining at stereo- binocular (30x) the single fragments to look for those that could belong to *C. supressalis*. Putative fragments were slide mounted and compared with homolog parts on the slides (male and female) of C. *supressalis*.

Activity patterns

In southern European paddies, three generations of rice borer occur in a year, resulting in three moth peaks during the rice crop growing season from May to September (Figure 4). To ascertain the flight activity patterns of the striped rice borer populations, including both males and females, we distributed a total of eight light traps throughout the Ebre Delta, and monitored each light trap continuously from mid May to late September during four years (2007-2010). We assessed moth activity by counting the individuals caught in traps every 2-3 days. In order to ensure that data on rice borer moth activity was comparable to bat activity data, we pooled the counts in ten day intervals.

We quantified total bat activity as passes per minute by means of acoustic surveys and aimed to count *in situ* the number of bat passes around 53kHz (the fundamental frequency most used by soprano pipistrelles in the area) using the heterodyne system. We used Pettersson D240x bat detectors (Pettersson Elektronik AB, Sweden) with heterodyne and time expansion (x10) systems, and a digital recorder (Edirol R9) to randomly record time expanded samples of the echolocation and social calls to verify the field identifications in the laboratory (BatSound Software, Pettersson Elektronik). All the field work was undertaken by the same researcher to prevent differences in the identification skills of individuals to interfere in the results (Limpens, 2004). We collected data at randomly selected points in three different years; survey effort varied year to year according to the budget available. Each point (totally 87) has been surveyed only one year but in different occasions in order to have correlated data with moth activity from the same year. During 2007 we counted bat passes at 40 points (32 in rice paddies and 8 in reed beds *Phragmites spp,* as a control). Each point survey lasted five minutes and was located more than 100m apart from the nearest station to avoid pseudoreplication. In order to observe differences in bat foraging activity during the striped rice borer flight period, we surveyed each point eight times from early July to mid September every 10 days. During each survey we began the sampling process from a different point to avoid time biases, and all stations were surveyed on the same night within a three hour period. The same approach was performed in 2009 with 20 stations (17 in rice and 3 in reed beds) and four temporal replications (from late August to mid September), centring the sampling on the period when the third and highest moth peak occurs. Finally, in 2010 the acoustic survey consisted of three minute samples at 27 points (22 in rice and 5 in reed beds) with six temporal replications, from August to mid September. A total of 87 acoustic stations (71 in rice) produced 2,486 minutes of recordings (82% in rice paddies). All stations were sampled under similar climatic conditions, avoiding sampling in windy and/or rainy days.

Rice borer activity patterns between the four years were analysed by means of simple correlations, thus making all pair-wise comparisons for the average number of moths counted in the eight light-traps per sampling session (a total of 18 sessions from May to September).

We used differences between the mean activity of bats in rice paddies and in control areas (reed beds) to reveal the relationship between bat and rice borer moth activity. The former was considered to be the dependent variable that would respond to the rice borer population levels.

To examine bat activity in relation to moth activity while accounting for the effects of habitat, sampling location or time of survey, we used Generalized Linear Mixed Models (GLMMs, Bolker et al. (2009)). According to Gotelli and Ellison (2004) we assumed bat activity, which measures the number of occurrences of bat passes in a fixed interval of time, to be Poisson distributed. We introduced moth counts and month of sampling in the model as independent continuous variables. The model also accounted for the effects of habitat as an independent (fixed) factor. Since some sampling locations differed among years we introduced the former as a random effect nested within the sampling year.

Prey-predator associations alone constitute unreliable evidence of the ability of a particular predator to control its prey populations, since p.e. the predator may only feed on certain prey when the latter is very abundant, and thus the predatory pressure may not have any effect on its populations (Arditi and Dacorogna, 1988). With the aim of examining at which level of rice borer pest activity the bats were significantly attracted to the rice paddies, we arranged the data to meet the needs of an analysis of variance by generating two datasets: 1/ grouping moth activity into four categories using the quartiles of its density distribution, and 2/ grouping the same variable into two binary categories (Rice borer moths present or absent). Given the non-normality of the distribution of the dependent variable ($p<0.01$ in the Kolmogorov-Smirnov test of normality in both raw and log-transformed data), we used a non-parametric approach to test for differences in bat activity between the groups described. We used the Kruskal-Wallis by Ranks Test in the first case (four categories of the predictor available), and the Mann-Whitney U Test for the second one. We performed *post-hoc* multiple comparisons of mean ranks when results were significant to test for differences between all pairs of groups. Statistica 7.0 (StatSoft Inc., Tulsa, USA) and R 3.0.1 (R Development Core Team, 2014) were used to compute the analysis.

Habitat quantification within the study area

We used land cover maps produced by the Catalan Government (CREAF, 2009) to quantify the key habitats present within soprano pipistrelle territories around the occupied bat boxes in Buda Island. We reclassified land use into ecologically meaningful categories based on the habitat selection studies available for this species (Bartonicka et al., 2008a; Boughey et al., 2011; Davidson-Watts et al., 2006), maintaining those categories relevant to the present research: a) riparian forests; b) marshes and wetlands; c) river (water surface and unforested riversides); d) rice paddies; e) herbaceous vegetation; f) urban and suburban; and g) others. We calculated a 2500 metres buffer (19.63 km^2) around the area with the occupied bat boxes existing on Buda Island, as an approximation of the reported foraging areas for breeding colonies (Davidson-Watts et al., 2006; Nicholls and Racey, 2006). To test for differences in the available habitat between Buda Island and the rest of the Delta we estimated habitat composition around 69 points that were randomly distributed across the whole Ebre Delta and compared the mean coverage of each habitat category in the random sample with its mean coverage in the buffer around the 69 bat boxes with a Monte Carlo randomized analysis (Gotelli and Ellison, 2004). Geographical data extraction, analysis and representation was run under R 3.0.1 (R Development Core Team, 2014).

Service valuation and Minimum Safe Unit

We estimated the value of the service provided by bats using the avoided-cost approach, which accounts for the expenditure prevented by a service, following the method of Cleveland et al. (2006). We included only those figures that were readily measurable (cost of the avoided treatments) and avoided accounting for less measurable economical benefits (undamaged crops or avoided social cost of pesticides) that would increase the uncertainty of the result and the number of assumptions to be made.

Implementing the concept of Minimum Safe Unit (Luck et al., 2009), defined as the minimum amount of an element or set of elements of the ecosystem necessary to ensure a particular function that brings about a desired service, we estimated the number of soprano pipistrelles per hectare needed to maintain the striped rice borer populations below two different thresholds that are used locally by farmers to trigger the pest treatments: no treatment needed (<3,500 moths/ha) and aerial treatment needed (>8,500 moths/ha).

Results

Rice borer predation

During summer inspections of the roosts, bats were frequently observed hunting rice borer moths. In 2008, six out of thirty bat droppings (20%) were positive for the presence of striped rice borer during the second peak of borer activity (mid July). In 2009, the screening was conducted with samples from the third peak (last week of August to mid September) and resulted in 25 out of 50 positive results (50%).

Table 3-1 GLMM Poisson regression results for the relationship between bat activity and: density of the moth Chilo supressalis, habitat, and month of sampling (N = 543).

Effect	GLMM Estimate	Standard error	Z value	P value
Intercept	0.900	0.354	2.544	0.011
Density of rice borer	0.011	0.001	8.243	<0.0001
Habitat - reed	-0.942	0.222	-4.230	<0.0001
Month	-0.13511	0.042	0.048	0.962

Random effects

Sampling location/year estimated variance ± SD = 0.28 ± 0.53

Year estimated variance ± SD = 0.08 ± 0.29

Temporal distribution of the average number of moths trapped per every ten days in the eight light traps were significantly correlated among years (2007-2010, mean correlation coefficients for six pair-wise comparisons: $r = 0.76$, range 0.67-0.86, all $p < 0.05$; n = 18 sampling periods), suggesting that moth dynamics was similar among the study years, showing lows and peaks at similar dates.

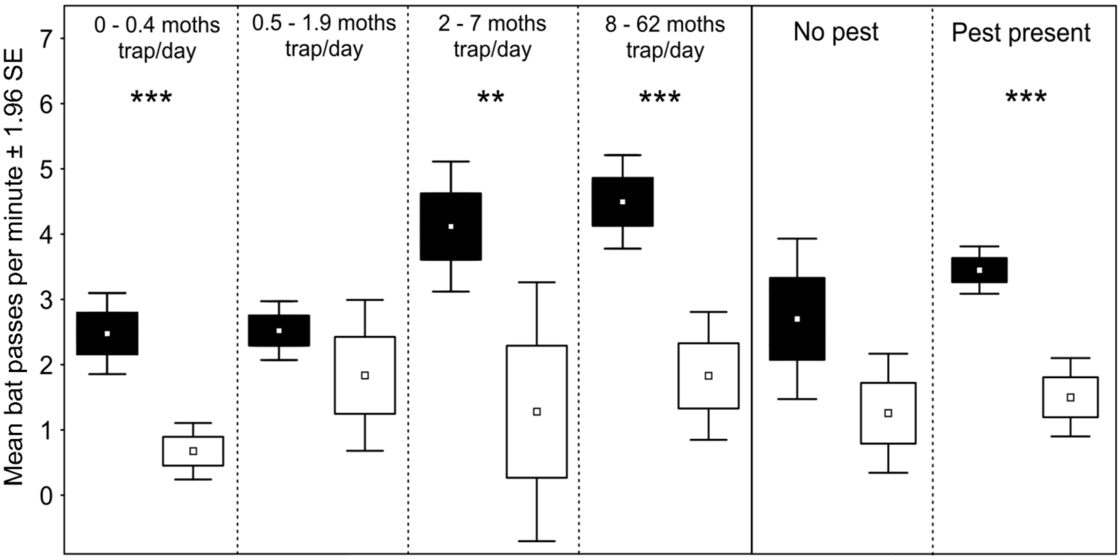

Figure 3-3 Relationship between bats and striped rice borer activity.Black boxes represent bat passes (± SD and 95% confidence interval) in rice paddies and white boxes bat passes in reed-beds considered as a control habitat (***: $p < 0.001$; **. $p < 0.01$). Rice borer activity was grouped according to the four quartiles of its abundance counted at the light-traps.

Bat activity was strongly associated to foraging events since bat passes were highly correlated with hunting buzzes in the three study years (year 2007: $r = 0.80$, $p < 0.0001$, n = 320; year 2009: $r = 0.90$, $p < 0.0001$, n = 80; year 2010: $r = 0.93$, $p < 0.0001$, n = 162). Between July and September (data pooled from the three years of sampling) the bat passes per minute in rice paddies averaged 2.9 SD±3.86 (range 0 to 22, n = 456). In the control areas, mean bat passes per minute were 1.18 SD±2.38 (range 0 to 11, n=106).

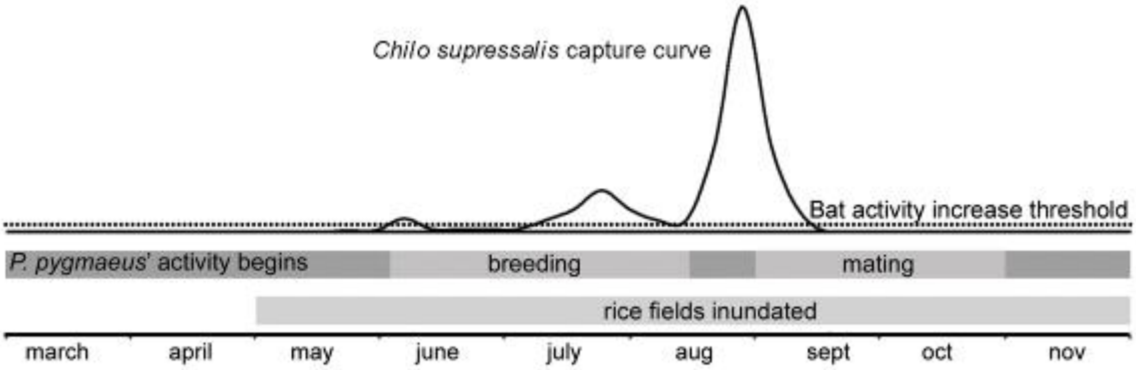

FIGURE 3-4 IDEALISED TIMING OF THE ACTIVITY CYCLE OF THE STRIPED RICE BORER MOTH (*CHILO SUPRESSALIS*). ACCORDING TO THE ADV DELTA DE L'EBRE - EBRE DELTA CROP DEFENCE ASSOCIATION (2005 TO 2013) AND ACTIVITY CYCLE OF THE SOPRANO PIPISTRELLE (*PIPISTRELLUS PYGMAEUS*).THE DOTTED LINE INDICATES THE 0.4 MOTHS PER TRAP THRESHOLD AT WHICH A SIGNIFICANT DIFFERENCE BETWEEN BAT ACTIVITY ON CONTROLS AND PADDIES APPEARS (AS SHOWN IN FIGURE 3). THE PERIOD DURING WHICH THE PADDIES ARE FLOODED IS ALSO SHOWN.

Bats significantly increased their activity when rice borer density increased (table 1, GLMM: $|z| = 8.243$, $p < 0.0001$), and showed a lower activity within the reed beds (table 1, GLMM: $|z| = 4.230$, $p < 0.0001$). Bat activity was significantly higher in the rice paddies than in the reed bed control plots provided the rice borer moths were present at any level (U =8450.0; $p < 0.001$, df = 541). Conversely no such difference was found when moths were absent from light trap catches (U =35.0, $p > 0.5$, df = 18; Figure 3). Bat activity did not vary significantly among the four quartiles of moth density in the reed beds ($K_{3,\ 80}$ =6.848, $p > 0.05$), however there was a significant increase in bat activity with rising levels of moth activity in the rice paddies ($H_{3,\ 463}$ =36.433, $p < 0.0001$). Bats increased significantly their activity in the rice fields when a threshold of two moths per light trap per day was reached ($z < 2.98$, $p < 0.05$) as revealed by the comparisons between groups (Figure 3). Figure 4 depicts the idealised yearly activity cycle of the rice borer and the threshold at which bats significantly increase their foraging activity in the rice fields: even the lowest first generation of the moth attracts bats.

Interestingly, the experiment of deploying bat boxes on Buda Island reported an important increase of the bat population in the area over a ten year period ($r = 0.95$, $p < 0.001$, n = 10, Figure 5). Bats started occupying boxes in the year 2000 (243 individuals were counted one year after bat box installation in 1999), and reached a maximum count of 3,500 ind. in 2007 and 2008 (the last years with a census). During the same period a significant decrease in the number of rice stems/m^2 infested by the rice borer was detected in the area (r = -0.73, $p < 0.01$, n = 10, Figure 5). The bat density in the boxes was negatively correlated to rice damage over a ten year period (r = -0.64, $p < 0.05$, n = 10).

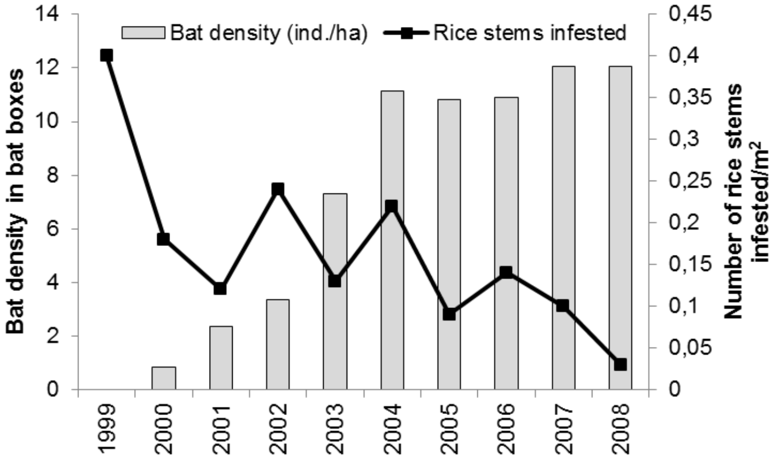

FIGURE 3-5 TEMPORAL PATTERNS OF STRIPED RICE BORER DAMAGE (RICE STEMS INFESTED/M2 DURING THE FIRST PEAK OF THE BORER: R = -0.73, P < 0.01, N = 10,) AND BAT DENSITIES (IND./HA: R = 0.95, P < 0.001, N = 10) ON BUDA ISLAND SINCE THE BAT BOXES WERE INSTALLED.

Habitat composition around bat boxes

Wetlands, rivers, riparian forests and herbaceous vegetation were significantly more available inside the 2.5 km buffer surrounding the Buda Island breeding colonies than around the random points that were scattered across the whole Ebre Delta ($|z| > 3{,}50$; $p < 0.001$), whereas there was no such difference regarding the coverage of cultivated land (rice paddies), urban structures or other land cover ($|z| > 3{,}50$; $p > 0.09$). Around the occupied bat box stations (n=69) the mean coverage of riparian forest was around 0.5%, rivers accounted for between 6-7% and urban structures ranged between 6-10% of the available area. Rice paddies were the dominant habitat, covering between 50-64% of the land, followed by marshes from 11% to 24%.

Service valuation

We estimated the cost of every chemical treatment is 21€ per hectare, i.e. roughly 6.000€ in less than 3km^2. According to our estimates (see Figure 6) between 9 and 16 bats per hectare are needed to reduce the number of stems affected by the striped rice borer during their second generation to less than 0.85 affected stems/m^2 (aerial treatment threshold). Between 42 and 67 bats per hectare would be needed to further lower the moth density below any treatment triggering threshold (0.35 affected stems/m^2). Assuming that the population of bats in Buda Island is represented by the colonized bat boxes (around 3,500 bats), we can consider that during the study period there were around 12 hunting bats per hectare in this area.

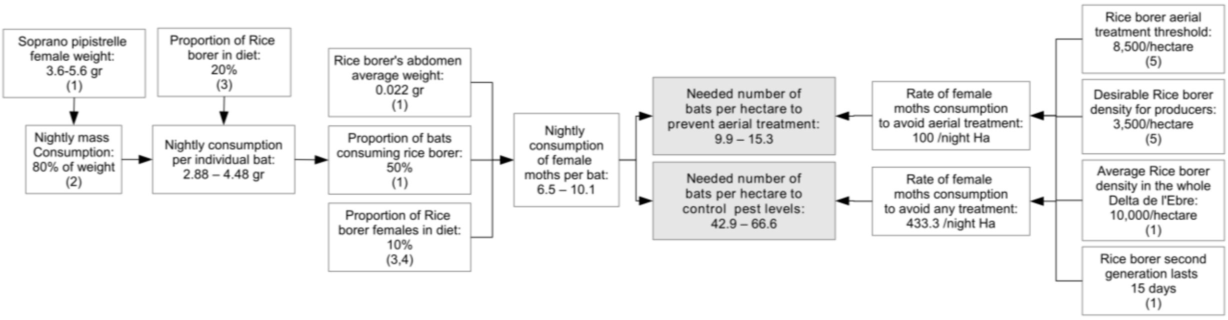

Figure 3-6 Estimate of the soprano pipistrelle Service Providing Unit required to keep pest density below the target threshold during the second peak of rice borer activity. The Service Providing Unit is expressed as the number of adult individuals per hectare. Where (1) Source: data collected by the authors during the study; (2) Lowest estimation from Kurta et al. (1989); (3) Conservative untested assumption (4) Female borer population is proportional to number of stems damaged since they lay the eggs on the rice stems, the effect of reducing the male borer population on the next generation of larvae is uncertain and therefore is not considered in this model; (5) Source: thresholds used by the ADV Delta de l'Ebre (Ebre Delta Crop Defence Association of 2013).

Discussion

Any biological pest control method capable of diminishing or even preventing the use of chemical treatments is to be encouraged where there is a concern for environmental and human health. Our results suggest that soprano pipistrelle bats provide natural control of a pest, the striped rice borer moth in the Ebre Delta. Evidence of this control is based on two related results: 1) soprano pipistrelle bats preyed upon striped rice borer moth, even during the second (lower) peak of borer activity; 2) activity of bats tracked the pest moth abundance in rice paddies. Besides, since 2006 borer density has been below the threshold for aerial spraying (*i.e.* below 0.85 stems hosting either larvae or eggs per square meter during the moths' second generation). A significant decline in the abundance of infested plants in the Buda Island could be partially explained by the presence of a bat population (in bat boxes) that is large enough to reduce rice borer populations, but this topic should be studied in depth.

Though larvae infestation surveys were conducted systematically over the whole Ebre Delta during the period covered in this study that trend was not reported anywhere else in the Ebre Delta (ADV –Crop Defense Association- data). The results obtained from this study provide further evidence that soprano pipistrelles habitually prey on the pest and move into the paddies even when few moths are available (Figure 3), responding to striped rice borer emergence by foraging more on the rice paddies when the aerial phase of the pest is present. Bats increase their activity on the rice fields well before the third and highest peak. This observed opportunistic feeding behaviour seems typical of this species (Bartonicka et al., 2008b).

From the evidence gathered, the ability of soprano pipistrelles to control rice borer population levels stands as the most parsimonious explanation for the decrease of the pest in the Buda Island rice paddies, where there are no less than 3,500 bats, corresponding to a density of 12 bats per hectare of paddies within the range of influence of bat boxes (Flaquer et al., 2006). So, we have tried to approximate the density of bats per hectare required to keep the stripped rice borer population below the threshold for aerial treatment in the study area (Fig. 4). When properly located (e.g. selecting the appropriate support and exposure, Flaquer et al. (2014), bat boxes are readily accepted as roosting places by bats in the area (Flaquer et al. 2006), and can be made at minimal cost by schoolchildren on environmental awareness programmes. Even when ready-made boxes are bought by farmers, each wooden roost costs around 25€ and lasts more than 10 years without any maintenance. In contrast, the cost of pheromone traps can be around 15€/ha per year (3€/trap x 5 traps/ha), while spraying one hectare once a year represents about 21€/ha per year. In practice, annual costs of chemical spraying are often higher because a second treatment is usually made if the third moth peak is severe. Therefore, the estimates on both chemical treatments are 6-8 fold higher than the installation of bat boxes accounting for a minimum of 12 bats per box.

In other areas of the world with dense human populations bat roosts are known to contribute to the reduction of pest damage on crops, e.g. in the North American continent (Boyles et al., 2011; Cleveland et al., 2006; McCracken et al., 2012) or Asia (Wanger et al., 2014). In both these areas the molossids (family *Molossidae*) involved in pest control gathered in huge colonies of up to millions of individuals, and foraged at a large regional scale. Molossids are fast flying species compared to many vespertilionids, including the pipistrelles, which fly at significantly lower speeds (Norberg and Rayner, 1987). Consequently the potential area for pest control by soprano pipistrelle colonies is significantly smaller, a fact that brings about new management challenges. Finding out which habitat features best encourage the presence of soprano pipistrelles would allow local management strategies to establish populations in target agroecosystems, to deliver the pest control ecosystem service.

Habitat availability around selected bat boxes of Buda Island is consistent with the preferences for the species described in other studies from central and eastern Europe (Bartonicka et al., 2008a; Boughey et al., 2011; Davidson-Watts et al., 2006), showing a reliance on flooded habitats (e.g. marshes and rivers) and broadleaved forests (e.g. riparian woodlands). The rapid colonisation of the bat boxes that were installed in the area in 1999 and the subsequent growth of the local bat population suggests that the limiting factor for this bat population was the existence of suitable roosts (Flaquer et al., 2006), given the lack of vertical structures that could provide shelter. The soprano pipistrelle is one of the most common bat species in southern Europe (Dietz et al., 2009), and can reach very high densities locally (Flaquer et al., 2006).

According to Wallace (2007) we should focus adequately managing ecosystem features to ensure the delivery of a service that will eventually improve our well-being. Remarkably, a simple management action, such as erecting bat boxes, can catalyse the performance of a desirable ecosystem service. Other examples of manipulating paddy habitats for enhancing ecosystem services can be found in Vietnam, where simple low-cost actions such as planting flowers and vegetables on the banks of the paddies have enhanced the abundance of the bees and wasps that parasitise and help control some of the insect pests (Normile, 2013). Laboratory studies of Indian meal moth (*Plodia interpunctella*), a pest moth belonging to the same super-family as the rice borer *Piraloidea* (Regier et al., 2012), have found that in the presence of ultrasound mating behaviour, spermatophore transfer, egg production and larval weight are significantly reduced (Huang and Subramanyam, 2004; Huang et al., 2003). Like the Indian Mealworm, rice borers possess tympanal organs and should therefore be capable of hearing foraging bats. If a similar response to ultrasound were observed in striped rice borer, it is possible that the presence of foraging pipistrelles in the fields could also stimulate lower reproductive rates in the moth, making a further contribution to its biological control. Though further research is necessary to test this theory, we hypothesise that several bat boxes distributed along the rice paddies could be more effective than huge artificial bat roosts.

In conclusion we have evidence that the soprano pipistrelle habitually and opportunistically preys on the striped rice borer moth in rice paddies and we have observed negative pest trends in an area with thousands of bats roosting in bat boxes (>12 bats per hectare).

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4. Bats and mosquitoes in rice paddies

Bats actively prey upon mosquitoes and other deleterious insects in rice paddies: Potential impact on human health and agriculture

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Oriol Massana i Valeriano

Bats actively prey upon mosquitoes and other deleterious insects in rice paddies: Potential impact on human health and agriculture

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Abstract

BACKGROUND: The fact that bats suppress agricultural pests has been measured for some particular dyads of predator and prey species both in economic and food security terms. The recent emergence of new molecular techniques allows for wide screenings of bat diet and provide a further evidence that bats consume an ample array of agricultural pest species. The main focus about the regulatory services that bats provide in agroecosystems has been on crop pests that cause yield losses. Rice paddies constitute a particular agronomic system with specific challenges, not only related to crop productivity but also to human health. Dipteran density in such ecosystems poses a serious threat to human wellbeing and hinders crop production. Mosquitoes cause direct harm to human populations transmitting a number of infectious diseases. Non-biting midges (*Chironomidae*) can consume and weaken rice seedlings and can cause major yield losses.

RESULTS: Mosquito populations and bat activity were assessed in rice paddies of *Montgrí, Medes i Baix Ter* Natural Park (NE Iberian Peninsula). Molecular analyses of bats faeces (6 weekly samples of 15 faeces each between mid-August and September) proved the presence of both mosquitoes and non-biting midges in all of the diet samples. Furthermore, bat activity at the sampling locations was related to adult mosquito density.

CONCLUSION: Our results suggest that bats actively exploit the emergence of adult mosquitoes and further prove that they prey on mosquitoes, non-biting midges and other deleterious insects. Promoting the presence of bats next to human settlements in such agroecosystems may constitute a biological control system with direct impact on both human health and crop yield.

Keywords: *Chiroptera*, bats, biological pest control, *Chironomidae, Culicidae*, mosquito-borne disease, rice, DNA metabarcoding

Introduction

Agricultural lands represent nearly 40% of the Earth's surface. The increase and improvement of agricultural techniques has accelerated the production and availability of food (Myers *et al.*, 2017). Even though, some regions such as Africa or Asia still have high prevalence of undernourishment (Roser and Ritchie, 2019). Rice ensures food access for almost half of the world's population (Long-ping, 2014), and in Asia this crop contributes to 50% of the daily human ingest (FAO, 2011).

Being one of the most important crops worldwide, rice plants are commonly affected by diseases such as bacterial infections and insect injury, being non-biting midges (*Chironomidae*) a major pest in temperate rice growing countries (Surakarn and Yano, 1995; Clampett *et al.*, 1999). Rice pests can cause significant harvest losses, as high as 40% of the harvested yield in tropical Asia (Savary *et al.*, 2012). Providing integrated sustainable pest management techniques is of vital importance to ensure food security in many regions in the world in the long term, and biodiversity and its associate ecosystem services are key to ensuring them (Savary *et al.*, 2012). Besides the agronomic considerations, rice plantations have a significant impact on human health. The prolonged irrigation cycle of the rice paddies turns this crop into an optimal breeding site for mosquitoes, which are one of the main disease vectors affecting human health (Reiter, 2001; Amusan *et al.*, 2005; Waterhouse *et al.*, 2007). Moreover, habitat destruction and temperature increase associated to the global change scenario are favouring opportunistic mosquitoes and other insects responsible for disease transmission outbreaks (Rosenzweig *et al.*, 2001) (e.g. West Nile Virus, malaria, dengue or Zika among many others) that put at stake human population in some regions (Hoover and Barker, 2016).

In addition, agriculture intensification and large-scale production have led to an increase of monocultures with the subsequent biodiversity loss and simplification of ecosystems' complexity (Reich *et al.*, 2012). Since the Green Revolution, the use of pesticides has been the main strategy to overcome the increasingly severe and frequent insect pest outbreaks (Dhaliwal *et al.*, 2010). The resulting unprecedented use of chemicals is leading to alarming levels of environmental and water pollution (Sánchez-Bayo and Wyckhuys, 2019), compromising not only global biodiversity conservation but also human health (Gilden *et al.*, 2010; Hernandez *et al.*, 2013; Kim *et al.*, 2017). In recent years, Integrated Pest Management (IPM) techniques are being increasingly adopted by different countries in order to improve yields while reducing environmental impacts through sustainable practices (Parsa *et al.*, 2014), being biological control regarded as a sustainable and affordable solution to suppress agricultural pests (Nwilene *et al.*, 2013).

Many authors have supported the efficiency of bats as pest suppressors, being increasingly relevant in the current biological pest control scenario (Williams-Guillen *et al.*, 2008; Wanger *et al.*, 2014; Maas *et al.*, 2015; Maine and Boyles, 2015; Puig-Montserrat *et al.*, 2015b). Bats are one of the most biodiverse mammal assemblages on the planet and several ecosystem services have been attributed to this taxonomic group, from seed dispersal and pollination, to arthropod suppression both in natural environments and in agroecosystems, where some of the arthropods bats consume constitute pests (Jones *et al.*, 2009). With a high metabolic rate, bats consume 30%-80% of their body mass each night (Kurta *et al.*, 1989). The ecosystem service bats provide on agroecosystems has been valued at an average of $22.9 billion per year in the United States (Boyles *et al.*, 2011) or at a yearly $1.2 million in rice plantations of Thailand (Wanger *et al.*, 2014). In Catalonia, we proved the role that soprano pipistrelles (*Pipistrellus pygmaeus*) play in suppressing the striped rice-borer moth (*Chilo supressalis*), a major rice pest found in rice paddies in Europe, Asia and Oceania (Puig-Montserrat *et al.*, 2015a). After the installation of bat boxes and their subsequent occupation by soprano pipistrelles (Flaquer *et al.*, 2006) a substantial decline in pest levels was detected in the area, rendering the rice borer below the chemical treatment threshold (Puig-Montserrat *et al.*, 2015b).

Over roughly the last decade, available technologies to survey bat activity (ultrasound recorders, GPS tags or radar systems among others) and to study their diet (e.g. metabarcoding techniques) have experienced important improvements and diminished their costs significantly, allowing the study of bat ensembles and their trophic ecology at unprecedented scale and detail (Horn and Kunz, 2008; Kerbiriou *et al.*, 2018; Torrent *et al.*, 2018; Conenna *et al.*, 2019; Vallejo *et al.*, 2019). Passive bat detectors can record bat activity autonomously for long periods of time and can be used to evaluate changes in populations and activity levels (Frick, 2013). Paired with the increase of commercial devices integrating this technology, a number of both commercial and open-source software solutions to detect and parametrise sound events and to identify them have been developed (Barré *et al.*, 2019) which, properly combined with manual validations, allow to efficiently and confidently analyse and identify large amounts of recordings otherwise difficult to handle (López-Baucells *et al.*, 2019). Molecular techniques have experienced a similar trend. During decades, diet studies were carried out by visual determination of pellets' content, detecting and visually identifying parts of the ingested prey (Whitaker, 1995; Kurta and Whitaker, 1998). Recent developments in genetics, and more particularly the raise of metabarcoding techniques, have allowed thorough dietary screenings up to species level using non-invasive methods (i.e. analysing faeces)(Alberdi *et al.*, 2012; Vallejo *et al.*, 2019).

Despite bats are frequently portrayed as mosquito consumers and controllers (Pliny the Elder, on the 1st century BC, already mentions this in his *Naturalis Historia*), supporting evidence is scarce: though a few papers report direct evidence of consumption (Swift *et al.*, 1985; Hoare, 1991; Beck, 1995; Barlow, 1997; Vaughan, 1997; Goiti *et al.*, 2003; Biscardi *et al.*, 2007; Gonsalves *et al.*, 2013a; Wray *et al.*, 2018), and *Nematocera* are known to be a fundamental part of the pipistrelles (genus *Pipistrellus*) in Europe (Bartonicka *et al.*, 2008), more detailed ecological interactions between bats and mosquitoes have received almost no attention so far. Reiskind & Wund (Reiskind and Wund, 2009), under enclosed conditions, found that the presence of the echolocating bat *Myotis septentrionalis* significantly reduced *Culex* mosquitoes oviposition. More recently, Gonsalves *et al.*(Gonsalves *et al.*, 2013c) radio tracked the small sized Australian insectivorous bat *Vespadellus vulturnus*, known to consume mosquitoes

(Gonsalves *et al.*, 2013b), and found a shift in bat activity from open saltmarshes to coastal swamp forests relative to the changes in mosquito abundance. The available literature brings a similar scenario regarding non-biting midges with their presence in bat diet being reported in a number of papers (Swift *et al.*, 1985; Hoare, 1991; Beck, 1995; Whitaker, 1995; Barlow, 1997; Vaughan, 1997; Goiti *et al.*, 2003; Biscardi *et al.*, 2007; Ciechanowski and Zapart, 2012; Krüger *et al.*, 2014; Vesterinen *et al.*, 2016). Non-biting midges appears to be a significant resource for three European riverine trawling bat species, such as the endangered *Myotis capaccinii* (Biscardi *et al.*, 2007) or the more widespread *M. daubentonii* and *M. dasycneme* (Krüger *et al.*, 2014), which consume not only the flying adults but also the pupae and larvae, which they may pick up from the water surface. Nevertheless, the impact bats have on these dipterans' populations remains unknown.

Rice paddies constitute excellent potential habitats for bats (Flaquer *et al.*, 2006), and host both mosquitoes and non-biting midges in high densities that are often regarded as problematic, either in terms of human health or crop productivity. Even though, to date no studies have been conducted to elucidate their ecological relations and the potential of bats as a biological control mechanism. Hence, this study aims (i) to assess which mosquito and non-biting midges species are consumed in rice fields by the most common bat species (i.e. *Pipistrellus pygmaeus*); (ii) to evaluate the relation between mosquito density and other environmental variables (e.g. wind, temperature or precipitation) on bat foraging activity, and (iii) to analyse how climatic variables affect mosquito abundances in our study area.

Material and methods

Study area

We carried out this study in the rice paddies embedded within *El Montgrí, les Illes Medes i el Baix Ter* Natural Park (3.15E, 41.98N), Catalonia, Spain. The Natural Park covers over 8,000 ha mostly composed by plains with a heterogeneous mosaic of habitats, including marshes, dunes, wetlands and extensive rice paddies, with interspersed riverine and pine forest. In terms of agriculture, while dry crops (e.g. vineyards and olive groves) can be found in non-irrigated areas, as on the foothills and slopes of the distant Montgrí massif, the plain and most riverbanks are cultivated with fruit tree orchards and irrigated crops (maize and rice). Sampling locations have been chosen together with the local farming association (Vegetal Defence Association or ADV), the local institution that provides guidance to the rice farmers regarding agricultural practices and pest treatments. We selected 15 sampling locations within rice paddies to carry out bat and insect surveys simultaneously (Fig. 1).

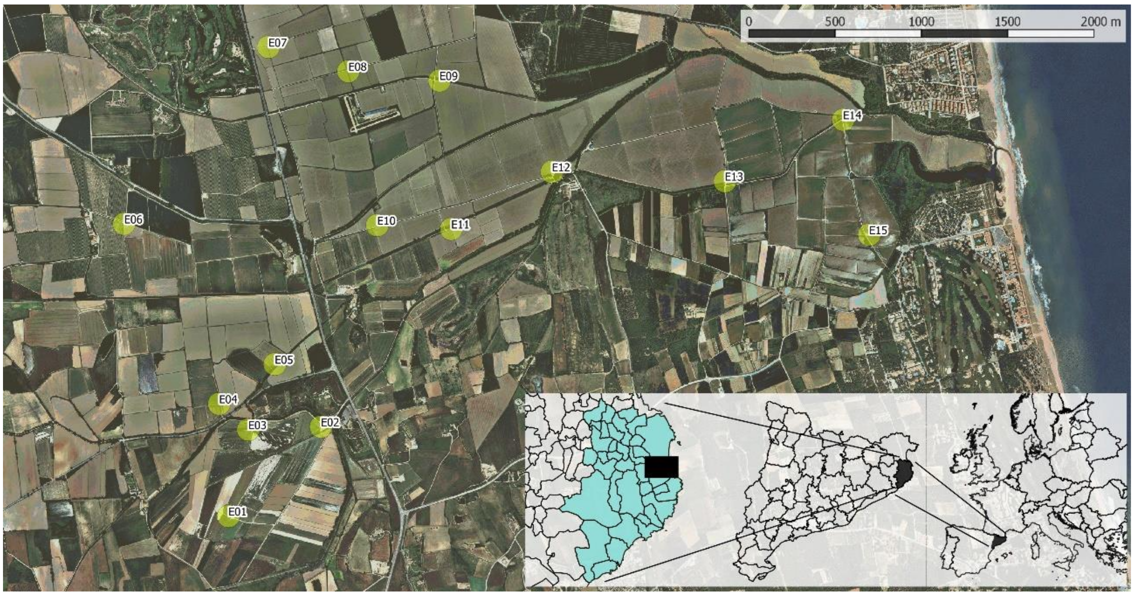

Figure 4-1 Study area

Bat surveys

We used four passive ultrasound detectors (SM2Bat+, Wildlife Acoustics, USA) in order to monitor nocturnal bat activity in all our sampling stations during the summers of 2017 (mid-June to early-November) and 2018 (August). During all sampling periods detectors were randomly shifted twice a week among the sampling locations. On each sampling location detectors were active for 3-4 successive nights, from 15 minutes before sunset to 15 minutes after the sunrise, using an 8 kHz low frequency threshold and a 12dB above sound-to-noise ratio (SNR) level for the triggering events. Sounds above both thresholds were automatically recorded. All recordings were lately automatically identified with Tadarida software (Bas *et al.*, 2017) and post-validated visually by an expert on bat bioacoustics using Avisoft Saslab Pro (Glienicke, Germany) as in Tuneu et al (Tuneu-Corral *et al.*, 2020). Species identifications were based on standard measurements (López-Baucells *et al.*, 2019) and available echolocation keys for Mediterranean species (Flaquer and Puig-Montserrat, 2012; Dietz and Kiefer, 2016). When overlap in echolocation frequencies and similar shapes between the sonograms of different species did not allow the identification of a recording to a specific level, we assigned the calls to sonotypes, which group several species with highly similar echolocation type (e.g. *Eptesicus/Nyctalus* or *Plecotus* sp.). Our analysis included a total of 9 species/phonic groups (see Table S1). For the following analyses and models, only *Pipistrellus* spp. were retained, as the most common and abundant bat species foraging in the area, therefore the only ones with potential to provide any ecosystem service on pest control at a landscape scale (Puig-Montserrat *et al.*, 2015b). This includes *P. pipistrellus*, *P. kuhlii* (the sibling *P. nathusii* is absent in the region)(Flaquer *et al.*, 2010; Flaquer and Puig, 2012) in the study area, and the phonic group *P. pygmaeus/Miniopterus schreibersii*. The majority of the calls in the later phonic group can be attributed to *P. pygmaeus* for several reasons: i) *M. schrebersii* is known to be rare in the region and has a single summer roost within a 12 km radius (Flaquer *et al.*, 2010; Flaquer and Puig, 2012), which hosts a total of 4000 individuals; ii) *M. schreibersii* is known to negatively select both water bodies and open areas in Southern France (Vincent *et al.*, 2010), which constitute the main landscape features of the study area; iii) P. pygmaeus is known to be the very abundant and the dominant species in other rice paddies of the region (Flaquer *et al.*, 2006; Puig-Montserrat *et al.*, 2015a).

Bat activity was used as a surrogate of bat feeding activity, as Pipistrelles' activity and feeding buzzes are known to be strongly correlated in the rice paddies of the region (Puig-Montserrat *et al.*, 2015a). We used the number of bat passes per sampling night as our response variable, and defined bat pass as any 5" long recording that had a minimum of two echolocation pulses of a certain species (Azam *et al.*, 2015; Millon *et al.*, 2015; Torrent *et al.*, 2018).

Mosquito surveys

We used a total of six BG Mosquitaire mosquito traps with BG-Lure attractant (Biogents AG, Germany), which mimics human scents. The traps were deployed along with the bat detectors, shifting randomly among the 15 sampling locations, thus ensuring a mosquito sampling paired with each bat ultrasounds 3-4 days sampling. Mosquito traps were connected to portable 12V and 7Ah batteries (SolarX 14 Xunzel) using an energy flow controller (CML12V5A) to ensure the correct functioning of the batteries and to prevent damaging them. Mosquito surveys were carried out during the summers of 2017 and 2018 (from June to September). Each trap was able to sample autonomously for a total of 40 hours, ensuring its capacity of sampling mosquitoes for one full day (24 hours) in the field. All mosquito samples were collected from the traps 24 hours after deployment, and kept frozen until they were identified. Specific identifications were carried out in the lab using magnification lenses and the MosKey Tool interactive identification key of mosquitoes of Euro-Mediterranean (Gunay *et al.*, 2017), with a previous training conducted by the regional Mosquito Control Service (*Servei de Control de Mosquits de la Badia de Roses i del Baix Ter*). Both the specific counts and the pooled total count per sampling location and date were recorded.

Diet analyses

With the aim of screening dipteran consumption by the most common bats foraging in rice paddies, faecal samples were collected from a colony of *Pipistrellus pygmaeus* established in a bat box deployed prior to the surveys within the sampling area. During 2018, samples of 15 faeces were weekly collected from mid-August to the end of September (N=6) using a plain tray collector under the colony covered with drying paper. Only fresh faeces (still with high level of moisture) were kept to ensure they could be assigned to the previous night with certainty. Once the samples were collected, we dried and froze them to avoid genetic material degradation.

DNA material was obtained from the faeces using the DNeasy PowerSoil DNA isolation kit (Qiagen) following instructions provided by the manufacturers. Cross-contamination was checked in all processes using blanks. DNA amplification and subsequent metabarcoding analyses using primers ZBJ-ArtF1c and ZBJ-ArtR2c, as described by

Zeale *et al* (2011) to amplify a fragment within the COI barcode region. These markers are known to be biased towards Lepidoptera and Diptera (Alberdi *et al.*, 2018), targeted insect orders in our study. The samples pool was sequenced in a MiSeq PE300 run (Illumina). Genetic analyses were carried out by AllGenetics & Biology S.L. (A Coruña, Spain).

Sequencing results were obtained in a FASTQ file, which was processed through bioinfomatic analyses using the software FastQC (Babraham Informatics, UK), FLASH2 (Magoč and Salzberg, 2011) (for reading) and CUTADAPT (Martin, 2011) (to remove sequences without the PCR primers). The sequences were quality filtered (only paired-end reads with Phred Quality Score > 20 were retained) and labelled using the script multiple split *libraries.py* implemented in Qiime (Caporaso *et al.*, 2010). The FASTA file was processed following Andújar et *al.* (Andújar *et al.*, 2018) and using VSEARCH (Rognes *et al.*, 2016). Sequences were dereplicated, clustered at a similarity threshold of 97%, and sorted. *De novo* chimaera detection was carried out using the UCHIME algorithm (Edgar *et al.*, 2011) implemented in VSEARCH. Then, sequences were trimmed by length using CUTADAPT with a maximum length of 160 bp. With the resulting list of MOTUs a quality filter was carried out by removing any sequence occurring at a frequency below 0.005% in the whole dataset (Bokulich *et al.*, 2013). To prevent mistagging, which may occur at different stages of the molecular pipeline, any MOTUs with less than 10 sequences in each sample were discarded (Esling *et al.*, 2015; Bartram *et al.*, 2016; Guardiola *et al.*, 2016).

Taxonomic matches of the sequences for each molecular operational taxonomic unit (MOTU) were retrieved from the Barcode of Life Data Systems (BOLD)(Ratnasingham and Hebert, 2007) and GenBank (Sayers *et al.*, 2018) databases, setting the minimum similarity criteria to 98% (Razgour *et al.*, 2011; Clare *et al.*, 2014). Using the combined results from both searches, we assigned the taxonomy following the criteria by Clare et *al* (2014): 1a = match to one species or several species in a genus with 100% similarity, most conservative taxonomy kept; 1b = same as 1a but match below 100% (>98%); 2 = match to more than one species (>98%), only one of which is present in the sampling range (that taxonomy kept); and 3 = close match to several species from different genera, or to a reference sequence lacking full taxonomic record (most conservative taxonomy kept). To ascertain whether a species was present in the study area (criteria 2) we used our own data from the Mosquito Control Service regarding *Culicidae* (presence and absence), data from regional pest control agencies regarding some Dipterans (presence only), and the available distributional data in the Global Biodiversity Information Facility (GBIF) for the rest of the taxa. Species with records in mid or southern Europe were regarded as possibly occurring in the study area (supplementary material S1).

Consume of each identified prey species was measured with both frequency of occurrence (FOO) and percentage of occurrence (POO).

Statistical analyses

We modelled the effect of weather conditions and prey availability (i.e. pooled count of mosquitoes) on Pipistrelle bat activity within rice paddies using generalized linear mixed models. Bat activity (number of bat passes per night) was the response variable (only nights when a paired sample of mosquitoes existed were included); mosquito abundance, night mean temperature, accumulated night rain, average night wind and mean nightly relative humidity were used as fixed factors; sampling station, year and detector as random factors. GLMM were run with *glmm.nb* function of the 'lmer' R package(Bates *et al.*, 2011) and fitted with a negative binomial data distribution. In order to better understand the dynamics of mosquito abundance within the rice paddies, their responses towards environmental conditions were also modelled using the same factors and adjusting the model to a *poisson* distribution (function *glmm* from the same package). All continuous fixed factors were previously scaled (function *scale* from 'base' R package) to ensure convergence of the models (Zuur, 2009).

In all cases all the potential combinations of fixed factors were modelled using the *dredge* function from 'MuMIn' R package. The best models were selected based on the Akaike Information Criteria (AIC), considering valid all the models with AIC values up to 2 units above the minimum obtained (Burnham and Anderson, 2004).

All analyses were carried out using R v. 3.6.0 (Team, 2019).

Results

Bat activity

We recorded a total of 760,246 bat passes during the two seasons, 646,151 in 2017 and 114,095 in 2018, the later with a shorter recording period (figure 2, table S2). Both years Pipistrelle bats (genus *Pipistrellus*) accounted for virtually all bat activity, with 98.5% of the overall recorded activity attributable to this genus phonotypes, 64.5% corresponding to the Soprano pipistrelle/*Miniopterus* phonotype (*Pipistrellus pygmaeus/Miniopterus schreibersii*), 20% to the common pipistrelle (*P. pipistrellus*) and 14% to the Kuhl's pipistrelle (*P. kuhlii*). Since the three species are generalist aerial hawkers of small size, and hence *a priori* equally susceptible to prey on both mosquitoes and non-biting midges, the subsequent analyses were run pooling their data.

Generalized Linear Mixed Models (GLMM) resulted in a single model selected (AIC = 33521.89) out of the 16 possible models. The selected model included all variables except for accumulated rain (Table 1). The variable with greater incidence on the model was temperature, which had a positive relation with bat activity, followed by mosquito abundance and relative humidity, still with positive effects, and by wind, which had a negative effect of a magnitude slightly higher than that of mosquito abundance and relative humidity (figure 3).

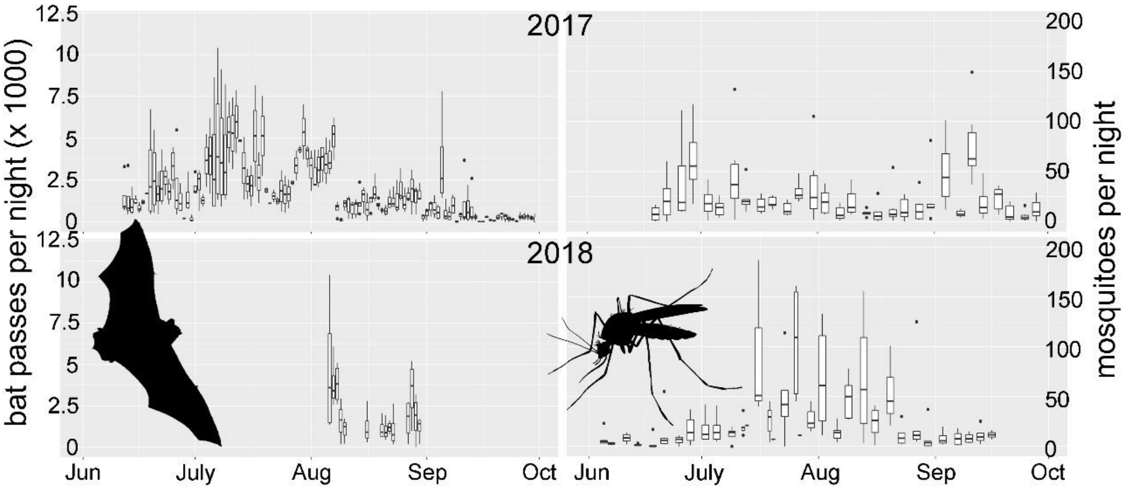

FIGURE 4-2 NIGHTLY RECORDS OF BAT PASSES PER NIGHT AND SAMPLING LOCATIONS (LEFT) AND OF MOSQUITO CAPTURES PER SAMPLING LOCATION (RIGHT). RESULTS FOR 2017 (TOP) AND 2018 (BOTTOM) ARE SHOWN.

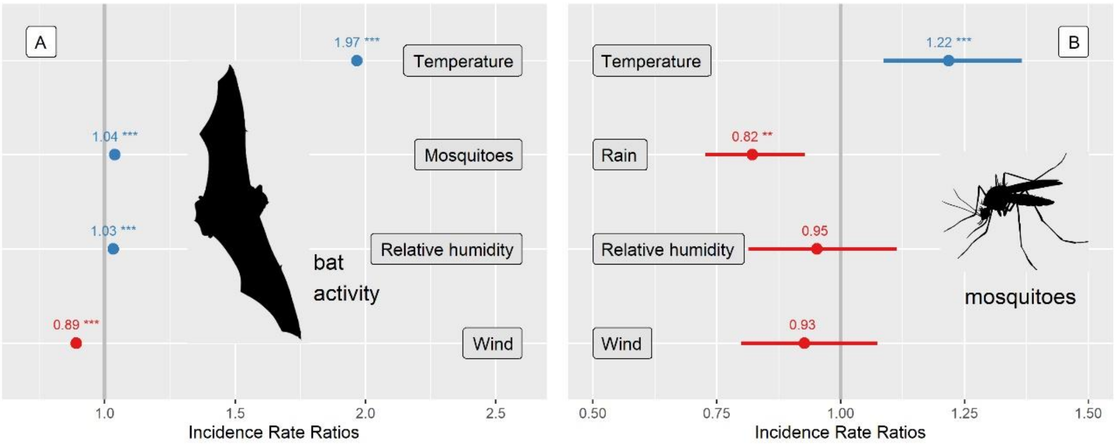

FIGURE 4-3 EFFECTS OF ENVIRONMENTAL VARIABLES UPON BAT (A) AND MOSQUITO (B) RELATIVE ABUNDANCE. MODELLED WITH GLMM MODELS FITTED WITH POISSON (BATS) AND NEGATIVE BINOMIAL (MOSQUITOES) DISTRIBUTIONS. SEE TABLE 2 FOR WHOLE SET OF SELECTED MODELS.

Mosquito activity

We captured a total of 8,825 mosquitoes, 4,004 in 2017 and 4,821 in 2018 (figure 2, table S3). We could not assign a genus to 55 of them, and we could identify 681 of the specimens to genus level only. The remaining 8,090 were identified to species level (see figure 4). The collected specimens were dominated by the genus *Culex*, which accounted for 99.08% of the captures. Three species of this genus were collected, with a clear predominance of *Culex modestus* (53.31%), followed by *Culex pipiens* (38.03%). Contrastingly, *Culex theileri* was found in a much lower proportion (0.25%). Other species captured in proportions below 1% included *Anopheles sp.*, *Ochlerotatus caspius*, *Aedes vexans* and *Culiseta longiareolata*.

Generalized Linear Mixed Models (GLMM) resulted in three models selected (AIC = 2925.2-2927.1) out of the 16 possible models. The selected models included all variables and showed all similar significances and effect sizes, though only temperature and accumulated rain had significant effects (table 2). The variable with greater incidence on the model was temperature, which had a positive relation with mosquito activity. Accumulated rain had a negative significant effect of less magnitude than temperature (figure 3).

Table 4-1 Model results for the best mixed-effect models for bats and mosquitoes abundance. The best models (increment of AIC value below 2) are given, with the total of run models indicated in the table headings. Standardised estimates are given for fixed effects included in the models and the SD of the estimates in parenthesis. *P*(z) indicates significant estimates (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). The grey shading indicates variables excluded from the saturated models. Only significant effects are shown.

	Bats (1 model out of 16)	**Mosquitoes (3 models out of 16)**		
Model type	**GLMM**	**GLMM**		
Predictor distribution	**Poisson**	**Negative binomial**		
Selected best models	**Model1**	**Model1**	**Model2**	**Model4**
Intercept	6.54 *** (0.41)	21.50*** (5.75)	21.49*** (5.69)	21.50*** (5.37)
Temperatures	0.68 *** (0.00)	1.21*** (0.13)	1.22*** (0.14)	1.21*** (0.13)
Mosquitoes	0.03 *** (0.00)			
Rain		0.83** (0.09)	0.83** (0.09)	0.83** (0.1)
Relative humidity	0.04 *** (0.00)			
Wind	-0.12 *** (0.00)			
AIC	33521.89	2925.2	2926.5	2927.1

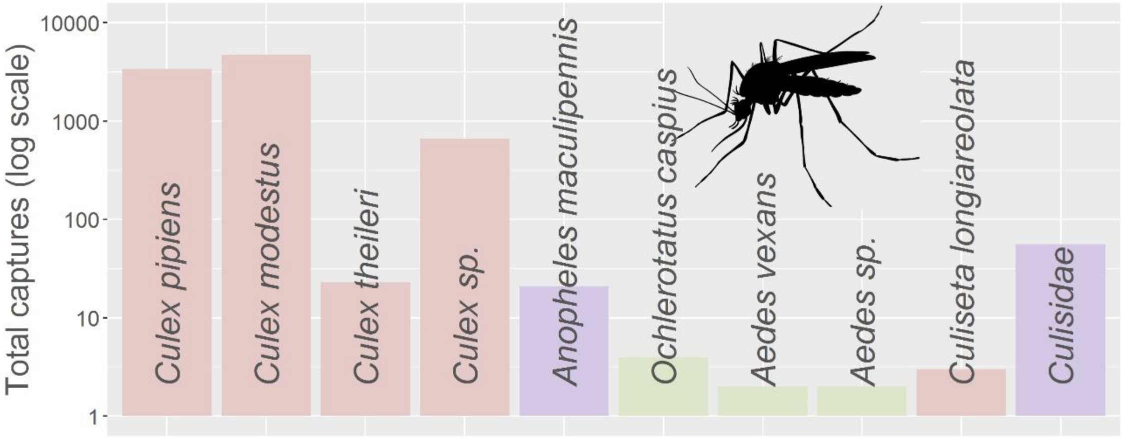

Figure 4-4 Relative abundance of captured mosquitoes in the rice paddies within the study area (pooled data for years 2017 and 2018). Colors indicate individuals of the same genera or taxonomic group. *Aedes sp.* might either be *A. vexans* or *Ochlerotatus (=Aedes) caspius*.

Diet analyses

Nematocerans were found in all faecal samples. Among the 30 taxa identified to species 17 were dipterans and 11 lepidopterans (table 2, table S4), accounting respectively for 75% and 18.8% of the occurrences (POO). The taxa with a highest frequency of appearance were the dipterans *Drosophila suzuki* (suborder *Brachycera*, family *Drosophilidae*), which appeared in all samples (FOO = 100%), and the genera *Procladius* (suborder *Nematocera*, family *Chironomidae*) and *Culex* (suborder *Nematocera*, family *Culicidae*), which appeared in 5 samples each (FOO = 83.3%). Three species of biting mosquitoes were identified: *Culex pipiens, C. theileri* and *Culiseta longiareolata.*

Table 4-2 Dietary composition found in the *Pipistrellus pygmaeus* faecal samples (N=6).Where FOO is the frequency of occurrence and POO is the percentage of occurrences.(Deagle *et al.*, 2019) The indices have been estimated at both family and species/genera level. Dipterans account for 75% of the presence of occurrence within the sample, followed by lepidopterans, which amount to 18.8% POO. Symbols: * other agricultural pest species; ** rice pest Chironomids; *** vectors of human diseases.

Order	Family	Family FOO	Family POO	Taxon	Taxon FOO	Taxon POO
Coleoptera	Curculionidae	16.7	3.1	*Curculio elephas* *	16.7	1.56
				Curculio glandium *	16.7	1.56
Diptera (Brachycera)	Anthomyiidae	33.3	3.1	*Delia platura* *	33.3	3.13
	Drosophilidae	100.0	9.4	*Drosophila suzukii* *	100.0	9.38
	Muscidae	33.3	3.1	*Lispe pygmaea*	33.3	3.13
	Tephritidae	16.7	1.6	*Acanthiophilus helianthi* *	16.7	1.56
Diptera (Nematocera)	Cecidomyiidae	50	4.7		50.0	4.69
	Ceratopogonidae		3.1	*Forcipomyia sp.*	33.3	3.13
	Chironomidae	83.3	26.6	*Chironomus aprilinus* **	16.7	1.56
				Cladopelma virescens	16.7	1.56
				Cricotopus bicinctus **	33.3	3.13
				Cricotopus sylvestris **	33.3	3.13
				Dicrotendipes nervosus	50.0	4.69
				Kiefferulus tendipediformis	50.0	4.69
				Procladius sp.	83.3	7.81
	Culicidae	83.3	15.6	*Culex pipiens* ***	50.0	4.69
				Culex theileri ***	16.7	1.56
				Culex sp.	83.3	7.81
				Culiseta longiareolata ***	16.7	1.56
	Limoniidae	33.3	3.1	*Dicranomyia ventralis*	33.3	3.13
	Psychodidae	33.3	3.1	*Clogmia albipunctata*	16.7	1.56
				Psychomora mycophila	16.7	1.56
	Sciaridae	16.7	1.6	*Bradysia tilicola*	16.7	1.56
Hemiptera	Miridae	16.7	3.1	*Lygus sp.*	33.3	3.13
Lepidoptera	Autostichidae	16.7	1.6	*Apatema baixerasi*	16.7	1.56
	Bedelliidae	16.7	1.6	*Bedellia somnulentella**	16.7	1.56
	Blastobasidae	33.3	3.1	*Blastobasis glandulella*	16.7	1.56
				Blastobasis phycidella	16.7	1.56
	Coleophoridae	16.7	1.6	*Coleophora texanella*	16.7	1.56
	Geometridae	16.7	1.6	*Rhodometra sacraria*	16.7	1.56
	Noctuidae	16.7	1.6	*Cryphia sp.*	16.7	1.56
	Plutellidae	16.7	1.6	*Plutella xylostella* *	16.7	1.56
	Pyralidae	16.7	1.6	*Isauria dilucidella*	16.7	1.56
	Tischeriidae	16.7	1.6	*Tischeria dodonaea*	16.7	1.56
	Tortricidae	16.7	3.1	*Cydia fagiglandana*	16.7	1.56
		16.7	3.1	*Cydia splendana*	16.7	1.56

Discussion

Our molecular results bring further evidence that bats consume both mosquitoes and non-biting midges, and the ultrasound recordings shed some light on the still poorly understood ecological interactions between bats and mosquitoes. Bats' activity is strongly conditioned in the study area by temperature, as can be expected in temperate regions (Davis and Reite, 1967; Erickson and West, 2002; Arbuthnott and Brigham, 2007), but it is likely to also be conditioned, to a lesser extent, by mosquito activity. Our results show that bats actively prey on mosquitoes, though they might not be their main energetic resource (they amount for 15.6% of the total prey occurrences in our sample), and the intensity of such predation remains unknown. Non-biting midges appear to be the most frequent family in the diet, amounting for 26.6% of the occurrences. Remarkably, 3 of the 7 identified *Chironomidae* species have been reported to be rice pests (i.e. *Chironomus aprilinus, Cricotopus bicinctus* and *C. sylvestris*). Furthermore, the dietary results prove the consumption by *Pipistrellus pygmaeus* of other important agricultural pests, unrelated to rice paddies, that had not been documented so far in their diet, like the diamondback moth (*Plutella xylostella*), a major pest with an annual worldwide control cost over US$1 billion (Zalucki *et al.*, 2012). The only species with a 100% of occurrence in the faecal sample is the spotted winged drosophila (*Drosophila suzukii*), a recently arrived invasive pest (Calabria *et al.*, 2012; Asplen *et al.*, 2015), which had been previously found in *P. pygmaeus* in France (Galan *et al.*, 2018).

The models show a response of nightly mosquitoes' activity to both temperature, with a strong positive impact, and rain, with a negative impact, which is consistent with the existing literature on mosquito ecology (Ogden *et al.*, 2019). Differences between mosquito species composition found in bat faeces and in the traps are unlikely due to a selective behaviour by pipistrelle bats, which are generally regarded as opportunistic (Swift *et al.*, 1985; Hoare, 1991; Bartonicka *et al.*, 2008). Some ethological differences may preclude bats from consuming mosquito species according to their overall availability. For instance, among the two dominant species (amounting to 91.32% of the captured mosquitoes) *C. modestus* is more crepuscular than *C. pipiens* (Veronesi *et al.*, 2012), which might contribute to its absence in the analysed faeces. Moreover, methodological biases in the molecular analyses may have contributed to the absence of *C. modestus*, namely, i) the detectability by the primers used (though the species has been found using the same primer in *Myotis daubentonii* in the Balkans) (Alberdi *et al.*, 2020); ii) the lower number of sequences available in the BOLD System and GenBank when compared to *C. pipiens* (409 sequences available for *C. modestus* versus 4065 for *C. pipiens*); iii) taxonomic assignment errors related to the limited number of pairs of bases used (N=160) in the faecal samples (Meusnier *et al.*, 2008). Furthermore, the lure used in the mosquito traps mimics human sweat odour, and is thus biased towards human or mammal-selective mosquitoes.

Our results are consistent with the previous dietary studies for *P. pipistrellus* and *P. pygmaeus* (Swift et al., 1985; Hoare, 1991; Beck, 1995; Barlow, 1997; Bartonicka et al., 2008). The two species were regarded as *P. pipistrellus* until 1999 (Jones and Barratt, 1999), thus the papers reporting dietary data published prior to the species split cannot be attributed to species with certainty (Swift *et al.*, 1985; Hoare, 1991; Beck, 1995). Both species are regarded as non-selective small flying nocturnal insect predators. Barlow et *al.* on 1997 reported differences in the preferred feeding grounds of the two phonotypes of the species, finding a preference of the 55 kHz phonic type (the one that was later described as *P. pygmaeus*)(Barlow and Jones, 1997) for aquatic habitats, and which harbour a greater proportion of non-biting midges and mosquitoes. On 2018, Bartonicka et *al.* specifically studied prey selection by *P. pygmaeus* in a floodplain of Western Europe, finding again a dominance of small *Nematocera*, and a high correlation between prey availability and consumption.

Regarding the ecological interactions between bats and mosquitoes, our findings are consistent with those by Gonsalves *et al.* (2013c) who concluded that Australian small-sized bat *Vespadellus vulturnus* slightly change its hunting grounds preferences according to the mosquito's availability. The same authors (Gonsalves *et al.*, 2013a), in a comparative study of several Australian bats, noted that mosquito consumption was conditioned by both bat echolocating strategy and size, being the bats with smaller body sizes (below 4.5 gr) and higher-pitched ultrasound signals (frequencies of maximum amplitude >50kHz) the ones that actually took advantage of mosquito outburst as an energetic resource. Under these circumstances, in order to meet their nightly metabolic rates these bats would need to consume around 3 times more mosquitoes than similar-sized moths, providing a rational ground as to why bats slightly shift their activity to track mosquitoes but mosquitoes are not the main driver of bat activity, as also our data seem to reveal in the particular context of the rice paddies. Interestingly the calls emitted the Australian *Vespadellus vulturnus* and the Pipistrelles (genus *Pipistrellus*) and bent-winged bats (genus *Miniopterus*) present in our study area are very similar not only in pitch but also in shape (Barlow and Jones, 1997; Law *et al.*, 2002), further suggesting that this could be a trait to consider when planning to promote bats as biological means to control mosquitoes. Characteristics of the echolocation calls used by bats could also explain why despite the efforts to control malaria-bearing mosquitoes in Texas (USA) in the early 20th century, no evidence was found of malaria decrease or mosquito consumption by the Brazilian free-tailed bats (*Tadarida brasiliensis*) (Howard, 1920). Massive house roosts for the mid-sized low pitched Brazilian free-tailed bat (a bat otherwise responsible for millionaire savings in the agricultural lands of the USA)(Cleveland *et al.*, 2006; Boyles *et al.*, 2011) were deployed and successfully colonized, but no traces of mosquitoes ever appeared in the faecal analyses conducted (Howard, 1920). Brazilian free-tailed bats weight on average 13gr and produce ultrasound signals with maximum amplitudes at around 27KHz (Gillam and McCracken, 2007), much lower than those of the studied pipistrelles, which may preclude small insects from their sensory niche (Safi and Siemers, 2009).

Because of their abundance, high energy demands (Kurta *et al.*, 1989) and the consequent predation pressure they can exert, common bat species have the potential to provide important biological control services (Boyles *et al.*, 2011; Puig-Montserrat *et al.*, 2015b). Provided bats usually start their foraging activity in the evening, overlapping with many mosquitoes and non-biting midges' activity peaks, they constitute excellent candidates to interact with these dipterans and to contribute to the control of their populations.

Health implications

Mosquitoes (*Culicidae*) are of global concern due to their impact on public health (Waterhouse *et al.*, 2007; Reiskind and Wund, 2009) linked to their role as disease vectors (e.g. transmission of West Nile Virus (Hoover and Barker, 2016), malaria (Rogers and Randolph, 2000), dengue (Vicente-Santos *et al.*, 2017), Zika (Diallo *et al.*, 2014), Tahyna (Li *et al.*, 2014), Tularemia (Petersen *et al.*, 2009), dog heartworm (Cancrini *et al.*, 2003), mixomatosis (Flowerdew *et al.*, 1992) or avian malaria (Atkinson *et al.*, 2000), which has considerable impacts on human societies and ecosystems. Moreover, in the current global change scenario the impact of mosquito-born diseases (MBD) is expected to increase both in frequency and intensity (Ogden *et al.*, 2019). Rice paddies are especially sensitive areas to control the spread of some of the mentioned infectious diseases, not only because they are particularly prone to host mosquitoes, but because the presence of avifauna might be important in the transmission cycle. It is the case of West Nile Virus, spread mainly through infected birds (Hoover and Barker, 2016). Birds can achieve considerable densities in the rice paddies and neighbouring marshes since these habitats are important stopover sites during bird migration, increasing the risk of developing high levels of this virus in their bloodstream and hence pass it to either other birds or to other vertebrates through infected biting mosquitoes (Hoover and Barker, 2016).

Bats have been long associated with mosquitoes and human health due to their insect-feeding habits, as already described in Howard back in 1920 (Howard, 1920). Although this is a matter of ancient knowledge that dates back to Roman times, it is currently gaining ground due to the relevance of MBDs worldwide. Bats have been observed and reported to actively hunt mosquitoes (see, for example, Rydell *et al.* 2002). Under lab conditions, with extreme mosquito densities, some bats species were reported to hunt them at a rate of 10 individuals per minute (Griffin *et al.*, 1960). Using these data Tuttle extrapolated a total of ~600 mosquitoes being potentially consumed per night (Tuttle, 2005). Although such estimates didn't take into consideration factors like the relative abundance of other prey or the calorific payoff of hunting mosquitoes as compared to other seemingly more profitable preys (e.g. moths). Using a different approach Reiskind and Wund (2009) found a 32% reduction in mosquito egg clutches laid by *Culex* spp. within enclosures where bats were kept for several days, providing further evidence that bats might affect mosquito populations. Our results add to the previous findings regarding the ecological interaction between bats and mosquitoes, confirming that mosquitoes are naturally present in bat diet and that bats actively hunt them. Thus, enhancing small-sized and high-pitched bat populations in and around areas where MBDs are a relevant issue might contribute to the mosquito biological control. Noticeably, populations of soprano pipistrelles have already been successfully enhanced in rice paddies by providing them with proper roosts (Flaquer *et al.*, 2006), which adds to their potential use as biological pest suppressors.

Agricultural pests

The role bats play in controlling agricultural pest has received an increasing attention over the last decade, providing a wealth of evidence on the magnitude of this functional ecosystem service both at local (Puig-Montserrat *et al.*, 2015b) and larger scale (Boyles *et al.*, 2011; Wanger *et al.*, 2014). Improvements in the genetic techniques occurred during the last years provide cost-effective means to comprehensively screen the diet of bats by non-invasive means, which has resulted in an increasing number of agricultural pests known to be present in the bats diet. The list of arthropods bats prey on includes not only insects but other sub-phyla like Arachnids, Diplopods or Chilopods. In some cases they can constitute a very significant portion of the diet, leading some authors to suggest that the right term to describe bats' trophic strategy should be *arthropodophagy* instead of the widely accepted *insectivory* (Segura-Trujillo, 2017). Despite the variety of their diet, insects have by far received the most attention when describing bat diet (Vaughan, 1997; Arrizabalaga-Escudero *et al.*, 2019).

Given the global economic impact of the Lepidoptera on agriculture (Boyles *et al.*, 2011; Maine and Boyles, 2015), it is this particular order, and more specifically the moths (*Heterocera*), that have so far received the most attention. In this regard, in spite of their impact on rice production (Surakarn and Yano, 1995; Stevens *et al.*, 2006), little is known on the ecological dynamics between bats and non-biting midges (*Chironomidae*). The larvae of non-biting midges cause significant losses in the rice crops, eating the roots or even the shoots of young stems and, though there are many different species, the majority of those regarded as pests belong to the genus *Chironomus* and *Cricotopus* (Stevens *et al.*, 2006).

Chironomids are known to be present in the diet of some riparian bat species in Europe (Reinhold *et al.*, 2000; Lilley, 2012; Vesterinen *et al.*, 2016), but to our knowledge no evaluations of the impact bats have on their populations have been conducted or published. Their prevalence in the dietary sample analysed in our study suggests that soprano pipistrelles (*P. pygmaeus*) consistently predate on them and may thus provide a regulatory ecosystem service still to be quantified but with potential economic impact in agriculture, and with health implications amounting to the chemicals' usage bats might prevent. This evidence adds value to already known regulatory services the soprano pipistrelles provide in rice paddies, a context where we already proved its value in controlling the striped rice-borer moth (*Chilo suppressalis*) (Puig-Montserrat *et al.*, 2015a), which constitutes a major agricultural pest in some rice producing areas.

Implications for conservation

- Some bat species can potentially act as mosquito suppressors in and around rice paddies and other marshy environments. Promoting their populations using bat boxes, a highly efficient method in the context of rice paddies (Flaquer *et al.*, 2006), to enhance mosquito biological control should minimize the distance from bat roosts to the main landscape features prone to host mosquito outburst and be oriented towards small-sized aerial hawkers with high-pitched calls.

- Education and dissemination are much needed if, as a society, we are to make the most of the available natural means to control insect pests. In many countries bats are still regarded with fear and persecuted. However, we should enhance the populations of insectivorous species where they can provide much needed and sustainable regulatory ecosystem services.

- Life cycle of bats should be taken into consideration when applying chemical treatments to reduce mosquito or non-biting midge populations, specially avoiding to the extent possible the lactation period, when pups are more vulnerable to the effects of pesticides (Gonsalves *et al.*, 2013c).

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General discussion and conclusions

Organic farming and biodiversity enhancement

Differences in species richness and biodiversity between OF and CF has been the subject of a number of studies that have consistently revealed the beneficial effects of the former in a wide range of taxa (see e.g. Bengtsson *et al.*, 2005; Hole *et al.*, 2005 for thorough reviews). Several works have confirmed this general trend in vineyards (Froidevaux *et al.*, 2017; Puig-Montserrat *et al.*, 2017; Rollan *et al.*, 2019), although some exceptions have also been found (e.g. Brittain *et al.*, 2010; Bruggisser *et al.*, 2010). Our study in the vineyards made a novel contribution in an area (Iberia) where, despite the enormous economic importance of the wine industry, only recently attempts have been made to evaluate the impact that OF in vineyards has on biodiversity (Rollan *et al.*, 2019; Lourenço *et al.*, 2021).

Differences in species richness between these two types of managements emerged for both vascular plants and butterflies in our multi-taxon approach. In addition, although the treatment effects in the other two studied groups (moths and birds) were weak and non-significant (only in one model treatment had a significant effect in moths), the majority of the moth models showed a consistent positive relationship between species richness and OF. This result suggests that a similar interaction to that found for butterflies may also occur in moths.

Regarding local management, two major differences exist between OF and CF in our area: i) the use of herbicides (i.e. glyphosate), and ii) the use of synthetic insecticides (chlorpyrifos) and growth regulators (tebufenozide and fenoxycarb). Glyphosate is a broad-spectrum herbicide that affects both mono and dicotyledons, and hence can potentially have an important impact on vascular plant communities within the sprayed zone and within the reach of the spray drift (Marrs *et al.*, 1993). Regarding the insecticides used in the region, tebufenozide is specifically targeted to lepidopterans, and fenoxycarb and chlorpyrifos are wide-spectrum insecticides. Hence all three can negatively affect insect communities of the vineyards, and more acutely the lepidopteran communities. Most likely these factors accounted for most of the loss of species richness of both plants and butterflies in CF. A similar conclusion was reached by Nascimbene *et al.* (2012) in their study of Italian vineyards regarding the impact of herbicides.

Our results contrast with those found by Brittain *et al.* (2010) in vineyards of NE Italy, where OF did not have any effect on pollinator abundance and species richness, including butterflies. Brittain *et al.* (2010) concluded that this was because of the major impact of the surrounding landscape compared to the local management. In this respect, their study area was located within an intensive agricultural landscape, dominated by large extensions of maize, soy and vine crops, that is, characterized by low heterogeneity and biodiversity (e.g. Benton *et al.*, 2003). On the other hand, our study area is found in a region where the predominant landscape is constituted by a mosaic of a variety of land uses and an overall high spatial heterogeneity. In any case, our data supported the general statement that OF has positive effects on pollinators (Holzschuh *et al.*, 2008).

An interesting though expected result was the greater effect that the type of farming treatment had on less mobile taxa (e.g. Fuller *et al.*, 2005). Thus, plants, which rely strictly on the conditions of the soil in which they grow, were the most affected by OF farming, while the weakest effect was recorded on birds, which generally have home ranges that are larger than the considered plots. For instance, one of the more common bird species in the area and also with the smallest territories, the Sardinian Warbler (*Sylvia melanocephala*), inhabits patches of around 2 ha (Bas *et al.*, 2005) and thus is very unlikely to occupy a single vineyard plot or to show clear responses at such a local scale. Although differences in avian richness and abundance between treatments in vineyards have been found by Jedlicka *et al.* (2011), in these authors' study plots were roughly six-times larger than those in the present study, which further suggests that plot size could partially explain our results. The weak effect found on moths could also be related to a greater mobility in this group, given that the sampling method (i.e. light traps) attracts individuals from a certain distance (Muirhead-Thompson, 2012), therefore reducing possible local habitat effects. Alternatively, the incompleteness of the moth surveys (Moreno and Halffter, 2001) could be partly responsible for this weaker effect, above all because some of the species that predictably would benefit the most from OF are rare specialists living at low population densities and are hence more difficult to detect than generalist species.

Our data also highlights important differences in biodiversity in microhabitats within plots. For plants and butterflies, a consistent pattern emerged of richer assemblages in grass strips compared to crop lines. Because in our design grass strips were located among fields and not closer to the natural vegetation surrounding the vineyards, the positive effect of this microhabitat was genuine and could not be explained merely as a gradient in biodiversity rise with increasing distance from the vineyards. In our case, grass strips were clearly associated with richer plant communities which, in turn, favored richer butterfly communities by providing both an increase of larval host plants and nectar sources for adults.

Our results provide further evidence on the importance as reservoirs of biodiversity of the naturalized strips within or along the margins of the crops, that has already been highlighted by other authors (e.g. Le Coeur *et al.*, 2002; Merckx *et al.*, 2012).

Finally, our data also highlight the importance of other geographical and landscape factors that must be taken into consideration when trying to explain richness patterns in the taxa found in vineyards. For example, altitude had a strong influence on both birds and Lepidoptera — despite our attempt in the experimental design to restrict the altitudinal range of the plots. These two taxonomic groups had opposite responses to altitude, which agrees with previous knowledge of their ecology. The observed negative effect of both altitude and forest cover on bird richness resembles the effects detected by Farina (1997) in other Mediterranean agroecosystems and was expected given the regional scale of our sampling. On the other hand, altitude had a strong positive effect on butterflies, a result which is consistent with previous analyses showing a peak of diversity in this group at mid-mountain elevations in the study region (Stefanescu *et al.*, 2011).

Organic farming and bat conservation

Farming treatment is known to have an impact on bat activity, for instance Wickramasinghe *et al.* (2003) found a significant overall higher activity of bats in the natural habitats within organic farms when comparing them to those within conventional farms. Nevertheless, the only comparative work conducted in olive groves (see Davy *et al.* (2007)) found no significant differences in overall bat activity when comparing the following four different habitats in a Greek island: organic and non-organic olive groves, and pine and oak woods. In our study area, the endangered Geoffroy's bats (*Myotis emarginatus*) were known to positively select olive groves and pine stands when foraging (Flaquer *et al.*, 2008) in the Montgrí massif (NE Iberia), where a minimum of 14 bat species are known to occur (Flaquer and Puig, 2012). However, while aerial hunting bats were significantly more active in sparse coniferous forests over the other treatments, gleaning bats (those generally more threatened and vulnerable to habitat degradation) showed high activity in organic olive groves. Contrary to the initial predictions, that assumed a positive relation between the density of *Bactrocera oleae* (active only during the day and hence out of reach of nocturnal aerial hunters) and gleaning bats, a significant negative relation was found between the two. This may point to a predatory effect though no further conclusions can be drawn until further research on their trophic interactions is made (comprehensive dietary analysis along the pest fly season). We also provide further evidence that gleaners and aerial hunters activity were positively affected by temperature and negatively affected by the wind, as it is commonly found in the literature (O'Donnell, 2000; Wolbert *et al.*, 2014; Wellig *et al.*, 2018).

Whereas gleaners showed similar activity levels on both organic olive groves and coniferous stands, aerial hunters showed higher activity levels on the coniferous stands, well above that of the two olive grove treatments. Differences on habitat selection between bat guilds might arise due to their evolutionary bat flight adaptations and diet preferences. Gleaning bats have low aspect ratios and wing loading, conferring them a slow but highly maneuverable flight (Altringham, 2011) and allowing them to take-off from the ground, capture insects directly from the surfaces and forage in cluttered environments. On the contrary, aerial hunting bats have high aspect ratios and wing loadings, necessary for their fast flights, which enable them to capture fast-moving flying insects on the wing, especially in open and semi-open spaces (Altringham, 2011; Denzinger *et al.*, 2016). Gleaners are thus better suited to move within spatially complex environments (Aldridge and Rautenbach, 1987; Sleep and Brigham, 2003). Also, organic olive crops are known to harbor richer communities of arthropods than more intensely managed ones (e.g. Ruano et al. 2004) potentially affecting bat foraging activity.

In a similar study in olive monocultures in the Mediterranean basin, Herrera et al. (2015) found a consistent decline in bat activity along with farming practices intensification.

However, the bat community present in the olive groves showed a remarkably low number of species, compared with the regional species pool, and extremely low activity levels. Therefore, the authors assumed that all olive groves within their study region (Southern Portugal) were essentially used as commuting habitats. In our study, both bat activity and richness was similar, or even higher, to that of the surrounding habitats, with all species/phonic types occurring in the region being detected within the olive crops. Since both habitat heterogeneity and crop size are known to favor biodiversity in agroecosystems, the drivers of such differences may be the higher landscape heterogeneity and the lower dimension of the olive crops within our study area, (Belfrage *et al.*, 2015).

Davy et al. (2007) in a small Mediterranean island (Zakynthos, Greece) found no differences in bat activity when comparing traditional organic olive groves with traditional non-organic ones. They also compared olive groves with the dominant forest habitats in the island (pines and oak woodlands), and found no significant differences in overall activity. Therefore, they concluded that olive groves provide foraging habitats comparable to the island forests. Contrary to our results, greater horseshoe bat (*Rhinolophus ferrumequinum*) positively selected pine forests over olive groves, a difference that might be either related to ecological differences between populations or to the habitat structural differences between the study sites.

Conservation implications

Due to climate change, the cultivable area for olive groves may extend around 25% in the forthcoming years (Tanasijevic *et al.*, 2014). There is a current trend of shifting from conventional to organic farming. In Europe, this supposes that the land devoted to organic farming has seen a 33% increase from 2012 (https://ec.europa.eu/eurostat). In this context, where environmentally friendly cultural practices are being encouraged by both the civil society and the governmental institutions, providing insight on the ecological interactions underpinning agroecosystems biodiversity are key to ensure their sustainable development.

Bat gleaners' guild includes most of the endangered bat species at the national level and the most sensitive species to environmental change. Contrarily, aerial insectivorous bats include mostly the commonest and more adaptable species. At least two gleaning bats in the area (out of the four species present) showed a strong reliance on organic olive crops. Thus, preserving and promoting organic olive crops may be crucial to their conservation in the region. Our results provide strong evidence of the positive effect of organic agriculture on bat conservation. Thus, due to the current nature emergency, we encourage land managers, politicians and practitioners to adopt these agricultural approaches. If these practices are not quickly and widely implemented, olive crop intensification and the expansion of monocultures may put at stake local bat populations.

Finally, more effort is still needed to assess the biological control services provided by bats in olive groves, and to build bridges between farmers and scientists and successfully communicate these findings to the practitioners.

Bats and pest control in rice paddies

Stripped rice borer moth

Our results (see chapter 3) suggest that soprano pipistrelle bats provide natural control of a pest, the striped rice borer moth in the Ebre Delta. Evidence of this control is based on two related results: 1) soprano pipistrelle bats preyed upon striped rice borer moth, even during the second (lower) peak of borer activity; 2) activity of bats tracked the pest moth abundance in rice paddies. Besides, since 2006 borer density has been below the threshold for aerial spraying (*i.e.* below 0.85 stems hosting either larvae or eggs per square meter during the moths' second generation). A significant decline in the abundance of infested plants in the Buda Island could be partially explained by the presence of a bat population (in bat boxes) that is large enough to reduce rice borer populations, but this topic should be studied in depth. Though larvae infestation surveys were conducted systematically over the whole Ebre Delta during the period covered in this study that trend was not reported anywhere else in the Ebre Delta (ADV –Crop Defense Association- data).

The results obtained from this study provide further evidence that soprano pipistrelles habitually prey on the pest and move into the paddies even when few moths are available (Fig. 3 on chapter 3), responding to striped rice borer emergence by foraging more on the rice paddies when the aerial phase of the pest is present. Bats increase their activity on the rice fields well before the third and highest peak of the pest. This observed opportunistic feeding behavior seems typical of this species (Bartonicka et al., 2008b).

From the evidence gathered, the ability of soprano pipistrelles to control rice borer population levels stands as the most parsimonious explanation for the decrease of the pest in the Buda Island rice paddies, where there are no less than 3,500 bats, corresponding to a density of 12 bats per hectare of paddies within the range of influence of bat boxes (Flaquer *et al.*, 2006). So, we have tried to approximate the density of bats per hectare required to keep the stripped rice borer population below the threshold for aerial treatment in the study area (Fig. 4 on chapter 3). When properly located (e.g. selecting the appropriate support and exposure (Flaquer *et al.*, 2014), bat boxes are readily accepted as roosting places by bats in the area (Flaquer et al. 2006), and can be made at minimal cost by schoolchildren on environmental awareness programmes. Even when ready-made boxes are bought by farmers, each wooden roost costs around 25€ and lasts more than 10 years without any maintenance. In contrast, the cost of pheromone traps can be around 15€/ha per year (3€/trap x 5 traps/ha), while spraying one hectare once a year represents about 21€/ha per year. In practice, annual costs of chemical spraying are often higher because a second treatment is usually made if the third moth peak is severe. Therefore, the estimates on both chemical treatments are 6-8 fold higher than the installation of bat boxes accounting for a minimum of 12 bats per box.

Habitat availability around selected bat boxes of Buda Island is consistent with the preferences for the species described in other studies from central and eastern Europe (Davidson-Watts *et al.*, 2006; Bartonicka *et al.*, 2008a; Boughey *et al.*, 2011), showing a reliance on flooded habitats (e.g. marshes and rivers) and broadleaved forests (e.g. riparian woodlands). The rapid colonization of the bat boxes that were installed in the area in 1999 and the subsequent growth of the local bat population suggests that the limiting factor for this bat population was the existence of suitable roosts (Flaquer *et al.*, 2006), given the lack of vertical structures that could provide shelter. The soprano pipistrelle is one of the most common bat species in southern Europe (Dietz *et al.*, 2009), and can reach very high densities locally (Flaquer *et al.*, 2006).

According to Wallace (2007) we should focus adequately when managing ecosystem features to ensure the delivery of a service that will eventually improve our well-being. Remarkably, a simple management action, such as erecting bat boxes, can catalyze the performance of a desirable ecosystem service. Other examples of manipulating paddy habitats for enhancing ecosystem services can be found in Vietnam, where simple low-cost actions such as planting flowers and vegetables on the banks of the paddies have enhanced the abundance of the bees and wasps that parasitize and help control some of the insect pests (Normile, 2013). Laboratory studies of Indian meal moth (*Plodia interpunctella*), a pest moth belonging to the same super-family *Pyraloidea* as the rice borer (Regier *et al.*, 2012), have found that in the presence of ultrasound the mating behavior, spermatophore transfer, egg production and larval weight are significantly reduced (Huang *et al.*, 2003; Huang and Subramanyam, 2004). Like the Indian Mealworm, rice borers possess tympanal organs and should therefore be capable of hearing foraging bats. If a similar response to ultrasound were observed in striped rice borer, it is possible that the presence of foraging pipistrelles in the fields could also stimulate lower reproductive rates in the moth, making a further contribution to its biological control. Though further research is necessary to test this hypothesis, we suggest that several bat boxes distributed along the rice paddies could be more effective than huge artificial bat roosts.

Mosquitoes and midges

Bats, and more specifically pipistrelle bats (*Pipistrellus spp.*) are known to prey on both mosquitoes (*Culicidae*) and midges (*Chironomidae*), as previously reported by several authors (Swift et al., 1985; Hoare, 1991; Beck, 1995; Barlow, 1997; Vaughan, 1997; Goiti et al., 2003; Biscardi et al., 2007; Gonsalves et al., 2013a; Wray et al., 2018). Even though, to date only few attempts have been done to ascertain the intensity of this trophic interaction (Gonsalves et al., 2013b) and its potential to suppress or control these deleterious insects populations (Reiskind and Wund, 2009).

The molecular results shown in chapter 4 (see table 2) bring further evidence that bats consume both mosquitoes and non-biting midges, and the ultrasound recordings shed some light on the still poorly understood ecological interactions between bats and mosquitoes. Bats' activity is strongly conditioned in the study area by temperature, as can be expected in temperate regions (Davis and Reite, 1967; Erickson and West, 2002; Arbuthnott and Brigham, 2007), but it is likely to also be conditioned, to a lesser extent, by mosquito activity. Our results show that bats actively prey on mosquitoes, though they might not be their main energetic resource (they amount for 15.6% of the total prey occurrences in our sample), and the intensity of such predation remains unknown. Non-biting midges appear to be the most frequent family in the diet, amounting for 26.6% of the occurrences. Remarkably, 3 of the 7 identified *Chironomidae* species have been reported to be rice pests (i.e. *Chironomus aprilinus*, *Cricotopus bicinctus* and *C. sylvestris*). Furthermore, the dietary results prove the consumption of other important agricultural pests, unrelated to rice paddies, that had not been documented so far in the diet of *Pipistrellus pygmaeus*, like the diamondback moth (*Plutella xylostella*), a major pest with an annual worldwide control cost over US$1 billion (Zalucki *et al.*, 2012). The only species with a 100% of occurrence in the faecal sample is the spotted winged drosophila (*Drosophila suzukii*), a recently arrived invasive pest (Calabria *et al.*, 2012; Asplen *et al.*, 2015), which had been previously found in *P. pygmaeus* in France (Galan *et al.,* 2018).

Regarding the ecological interactions between bats and mosquitoes, our findings are consistent with those by Gonsalves *et al.* (2013b) who concluded that Australian small-sized bat *Vespadellus vulturnus* slightly change its hunting grounds preferences according to the mosquitoes availability. The same authors (Gonsalves et al., 2013a), in a comparative study of several Australian bats, noted that mosquito consumption was conditioned by both bat echolocating strategy and size, being the bats with smaller body sizes (below 4.5 gr) and higher-pitched ultrasound signals (frequencies of maximum amplitude >50kHz) the ones that actually took advantage of mosquito outburst as an energetic resource. Under these circumstances, in order to meet their nightly metabolic rates, these bats would need to consume around 3 times more mosquitoes than similar-sized moths, providing a rational ground as to why bats only slightly shift their activity to track mosquitoes, which do not stand as a main driver of bat activity neither in our case study nor in the case described by Gonsalves *et al.* (2013b). Interestingly the calls emitted by the Australian *Vespadellus vulturnus* and the Pipistrelles (genus *Pipistrellus*) and bent-winged bats (genus *Miniopterus*) present in our study area are very similar not only in pitch but also in shape (Barlow and Jones, 1997; Law et al., 2002), further suggesting that this could be a trait to consider when planning to promote bats as biological means to control mosquitoes. Characteristics of the echolocation calls used by bats could also explain why despite the efforts to control malaria-bearing mosquitoes in Texas (USA) in the early 20th century, no evidence was found of malaria decrease or mosquito consumption by the Brazilian free-tailed bats (*Tadarida brasiliensis*)(Howard, 1920). Massive house roosts for the mid-sized low pitched Brazilian free-tailed bat (a bat otherwise responsible for millionaire savings in the agricultural lands of the USA (Cleveland et al., 2006; Boyles et al., 2011)) were deployed and successfully colonized, but no traces of mosquitoes ever appeared in the faecal analyses conducted (Howard, 1920). Brazilian free-tailed bats weight on average 13gr and produce ultrasound signals with maximum amplitudes at around 27KHz (Gillam and McCracken, 2007), much lower than those of the studied pipistrelles, which may preclude small insects from their sensory niche (Safi and Siemers, 2009).

Health implications

Mosquitoes (*Culicidae*) are of global concern due to their impact on public health (Waterhouse *et al.*, 2007; Reiskind and Wund, 2009) linked to their role as disease vectors (e.g. transmission of West Nile Virus (Hoover and Barker, 2016), malaria (Rogers and Randolph, 2000), dengue (Vicente-Santos *et al.*, 2017), Zika (Diallo *et al.*, 2014), Tahyna (Li *et al.*, 2014), Tularemia (Petersen *et al.*, 2009), dog heartworm (Cancrini *et al.*, 2003), mixomatosis (Flowerdew *et al.*, 1992) or avian malaria (Atkinson *et al.*, 2000)), which have considerable impacts on human societies and ecosystems. Moreover, in the current global change scenario the impact of mosquito-born diseases (MBD) is expected to increase both in frequency and intensity (Ogden *et al.*, 2019). Rice paddies are especially sensitive areas to control the spread of some of the mentioned infectious diseases, not only because they are particularly prone to host mosquitoes, but because the presence of avifauna might be important in the transmission cycle. It is the case of West Nile Virus, spread mainly through infected birds (Hoover and Barker, 2016). Birds can achieve considerable densities in the rice paddies and neighboring marshes since these habitats are important stopover sites during bird migration, increasing the risk of developing high levels of this virus in their bloodstream and hence pass it to either other birds or to other vertebrates through infected biting mosquitoes (Hoover and Barker, 2016).

Bats have been long associated with mosquitoes and human health due to their insect-feeding habits, as already described in Howard back in 1920 (Howard, 1920). Although this is a matter of ancient knowledge that dates back to Roman times, it is currently gaining ground due to the relevance of MBDs worldwide. Bats have been observed and reported to actively hunt mosquitoes (see, for example, Rydell et al. 2002). Under lab conditions, with extreme mosquito densities, some bats species were reported to hunt them at a rate of 10 individuals per minute (Griffin *et al.*, 1960). Using these data Tuttle extrapolated a total of ~600 mosquitoes being potentially consumed per night (Tuttle, 2005), although such estimates didn’t take into consideration factors like the relative abundance of other prey or the calorific payoff of hunting mosquitoes as compared to other seemingly more profitable preys (e.g. moths). Using a different approach, Reiskind and Wund (Reiskind and Wund, 2009) found a 32% reduction in mosquito egg clutches laid by *Culex* spp. within enclosures where bats were kept for several days, providing further evidence that bats might affect mosquito populations. Our results add to the previous findings regarding the ecological interaction between bats and mosquitoes, confirming that mosquitoes are naturally present in bat diet and that bats actively hunt them. Thus, enhancing small-sized and high-pitched bat populations in and around areas where MBDs are a relevant issue might contribute to the mosquito biological control. Noticeably, populations of soprano pipistrelles have already been successfully enhanced in rice paddies by providing them with proper roosts (Flaquer *et al.*, 2006), which adds to their potential use as biological pest suppressors.

Perspectives and open research questions

Biodiversity and farming in the region, within the EU framework

As it has been already exposed the meta-analyses available so far (Bengtsson *et al.*, 2005; Tuck *et al.*, 2014) show a predominantly positive correlation between organic farming and biodiversity; organically managed agroecosystems being on average 30% richer than those managed with conventional farming techniques. We here provide further evidence on the positive relations between organic farming and biodiversity conservation in a Mediterranean context (chapters 1 and 2), stress the importance of preserving hedges and vegetated rows between and within crops (chapter 1) and outline the functional benefits of promoting biodiversity (i.e. enhancing bat's populations) in rice paddies under conventional farming treatment (chapters 3 and 4).

According to the European Commission (2019) the main goals of the Common Agriculture Policy (CAP) include promoting the sustainable management of natural resources and the maintenance of rural landscapes across Europe, among others. Despite these general goals towards sustainability all agricultural treatments are currently supported by the CAP (organic, integrated and conventional) (Bradley *et al.,* 2002) regardless of their contribution to sustainability. Even though an approximate budget of €41 billion (roughly 25% of the EU total budget) is allocated on a yearly basis as direct income support to the farmers adhering to the guidelines of the CAP (European Commission, 2019), there are clear signs of a strong regression in the ecological health of the European agroecosystems linked to agricultural intensification, both at a continental and local scale within the studied region. The Farmland Birds Indicator, promoted by the European Commission along with private conservation organizations, using large-scale and long-term monitoring data on breeding populations across Europe shows a 57% decrease in common farmland breeding birds between 1980 and 2018, with a slightly less intense regional decrease in Southern Europe, amounting to 34% (PECBMS, 2020). At the regional scale of our study area (Catalonia), the available indicators show a similar trend for different taxa: the Catalan Living Planet Index (LPI-Cat) shows a 25% decrease on birds and butterflies linked to agroecosystems between 2002 and 2019 (Brotons *et al.,* 2020). Similarly, Chamorro *et al.* (2016) found a remarkable reduction of 47% in weed richness during the last 50 years in the region, linked to agricultural intensification. Meanwhile, the use of phytosanitaries has doubled in the region during the last 13 years (Brotons *et al.*, 2020). Chamorro *et al.* (2016) stresses the importance of organic farming to help partially recover the lost plant biodiversity.

On 2023 all EU countries should approve their new strategic plans for the implementation of the PAC, which in the context of the European Green Deal aims, among other environmentally friendly measures, at a 50% reduction in the use of pesticides by 2030. Achieving such goal, though desirable, seems unrealistic at least in our study region, since it would need a total reversion of the sign of the current trend, and an acute yearly decrease in pesticides use.

All the aforementioned trends illustrate how PAC's theoretical approaches towards sustainability have failed to preserve the biodiversity and ecological functionality of the European agroecosystems, and urges for a more integrative approach that must take into consideration scientific evidence and recommendations to draw a truly sustainable farming approach. It is crucial that the regional PAC strategic plans now being developed integrate the evidence based recommendations available in the scientific literature, including the ones in thesis and in the wealth of scientific literature available on the subject.

Utility value versus intrinsic value of biodiversity in agroecosystems: a practical approach.

The valuation of nature's ecosystem services, essentially in economic terms, have gained popularity within the academia and also among conservationists and policy makers (Matulis, 2014). Nevertheless, the debate as to whether nature should be monetized is an open one (McCauley, 2006; Kenner, 2018) and falls beyond the scope and intent of the present thesis to tackle it. Even though, decisions had to be made regarding the approach used to draw conclusions in the included chapters.

Chapter 1 and 2 focus mainly on the importance of organic farming practices as means to preserve biodiversity in vineyards and olive groves, hence placing the focus on the intrinsic value of biodiversity in crops where nature conservation and sustainability have already been ingrained in the culture schemes. Organic farming is experiencing an important increase in both crop types within the study region (CCPAE, 2020). Both studies were promoted and supported, among other stakeholders, by farmers, their main interest being how the organic treatment was conducive to healthier and more biodiverse landscapes. In these particular cases gathering local evidence on the ecosystem benefits of the organic treatments in use proved useful to acknowledge and reinforce the work already done by the organic farmers, and to encourage further conversions to organic farming by other producers.

Chapters 3 and 4 deal with the regulatory ecosystem services that bats provide in the rice paddies of the region, hence with their potential ability to suppress both crop's pests and mosquitos linked to human disease. In both cases the utilitarian, rather than the intrinsic, value of bats in terms of the direct benefit for the humans constitute the main outcome. Organic farming in the rice paddies of the study region is anecdotal, almost all the rice in the region being produced under integrated management schemes. Hence the focus was not placed on biodiversity but on productivity, and the used approached proved efficient in terms of conveying to the local farmers the importance of enhancing and preserving the bat populations in and around their rice fields. As a result bats (and possibly biodiversity in general) are perceived in a more friendly fashion by the local farmers, whom have deployed more than 500 bat boxes in the rice paddies of the Ebro Delta and over 150 in the rice paddies of the Empordà as a result of the findings.

Trophic interactions between bats and arthropods

Three of the chapters included in this thesis deal with the potential ecological interaction occurring between bat species and their prey, focusing on economic/human health relevant pest species (causing either crop losses or human health problems). The cumulative data gathered points to a complex predator-prey interaction that should be explored in more depths, and that is seemingly affected by a number of different factors, namely:

a) Pest density: Bats may track prey populations when the latter show high densities (e.g. the stripped rice borer – chapter 3-), resulting in a positive correlation, or may simply keep prey levels under certain thresholds due to their foraging pressure, and thus show a negative correlation (which could be the case with the olive fruit fly – chapter 2-). The relation between pest and prey density is therefore non-linear and the nature of the interactions should be better understood in order to ascertain to what extent bats suppress or control arthropods populations.

b) Pest size and metabolic rate of the predator: Which will affect the calorific payoff of tracking prey movements and population explosions. The evidence gathered in rice paddies, focusing on different prey species (chapters 3 and 4), suggest that the intensity with which bats will track pest populations is related not only to their abundance, but also to their size. Bats significantly shift their activity in the rice paddies to track relatively big prey, such as the stripped rice borer (chapter 3), but apparently only shift it subtly when the prey is less energetically profitable (mosquitos, chapter 4).

c) Bat’s sensory niche: Which is directly related to the frequency of the ultrasonic pulses emitted by bats. Our results (chapter 4, regarding the ability of small aerial hunters emitting at around 50 kHz to catch mosquitoes and other small dipterans) seemingly adds evidence to the fact that the sensory niche determines the prey size, probably precluding low pitched species (e.g. below 30 kHz, as it is the case with *Tadarida brasiliensis* –see Gillam and McCracken (2007)-) to hunt for small dipterans.

d) Bat’s hunting guild and habitat structure: Two main hunting strategies are found in the bats within the study area: aerial hunting and gleaning prey from the surfaces. Though some bat species can use both strategies, bats specialized to hunt on the wing have a faster and less maneuverable flight, which precludes them from hunting in cluttered environments. Gleaning bats have slower and more maneuverable flights, which allow them to pick up prey from the clutter, but precludes them from hunting fast flying insects on the wing. Our results show a differential use of the hunting grounds when comparing the two guilds, which results in a differential ability to suppress insect pest species, depending on the prey flying habits and habitat preferences (chapter 2), adding one more dimension of complexity to the trophic interactions between bats and their prey.

e) Presence of tympanate organs in the prey: Tympanate moths are known to respond to the presence of bat-like ultrasonic calls, resulting in a significantly lower reproductive success (smaller clutches sizes, reduced fertility, less time spent mating). Hence bats may not only negatively affect insect densities by direct consumption, but also indirectly due to the presence of their vocalizations. Though to our knowledge this deterring effect has not been evaluated in the field, it may prove relevant when assessing the real impact bats have on tympanate moths, many of which are relevant pest species worldwide (see chapter 3).

Hence, there still are relevant knowledge gaps to fill before we can fully understand the ecological interactions occurring between bats and their prey and model such interactions to get a more complete picture of its magnitude in different ecosystems, either agricultural or natural.

Conclusions

Organic farming and biodiversity

1. Biodiversity in the vineyards of the study area benefits from organic crop treatment, showing more diverse communities under this treatment. This effect is more apparent on the sessile taxa (i.e. plants), and decreases along with the mobility of the considered taxa, being non-significant on birds.
2. The current trend towards establishing vineyards directly on slopes prevents the natural occurrence of grass strips within and between experimental units and may hinder the biodiverse associated to the crops. Our results suggest that plots should be small and surrounded by non-cultivated strips to promote and benefit biodiversity.

Bats and agroecosystems

3. Gleaning bats, a guild constituted in the study region exclusively by protected species, benefits from the organic treatment of olive orchards. Some protected species, such as the horseshoe bats (*Rhinolophus spp.*) rely strongly on the presence of such habitats, which they favor even over the available pine plantations.
4. Our results evidence that the soprano pipistrelle habitually and opportunistically preys on the striped rice borer moth in rice paddies and we have observed negative pest trends in an area with thousands of bats roosting in bat boxes (>12 bats per hectare).
5. Bat populations can be easily enhanced in rice paddies by means of deploying artificial roosts, a practice that should be encouraged in the rice paddies of the Mediterranean basin, which share many commonalities with the ones in the study area.
6. Soprano pipistrelles (*Pipistrellus pygmaeus*) reach high densities in the rice paddies and wetlands of the region, and can potentially act as mosquito suppressors in those contexts. Their populations can be easily enhanced using bat boxes. Their deployment to enhance mosquito biological control should minimize the distance from bat roosts to the main landscape features prone to host mosquito outburst.
7. Education and dissemination are much needed if, as a society, we are to make the most of the available natural means to control insect pests. In many countries bats are still regarded with fear and persecuted. However, we should enhance bat populations where they can provide sustainable regulatory ecosystem services.

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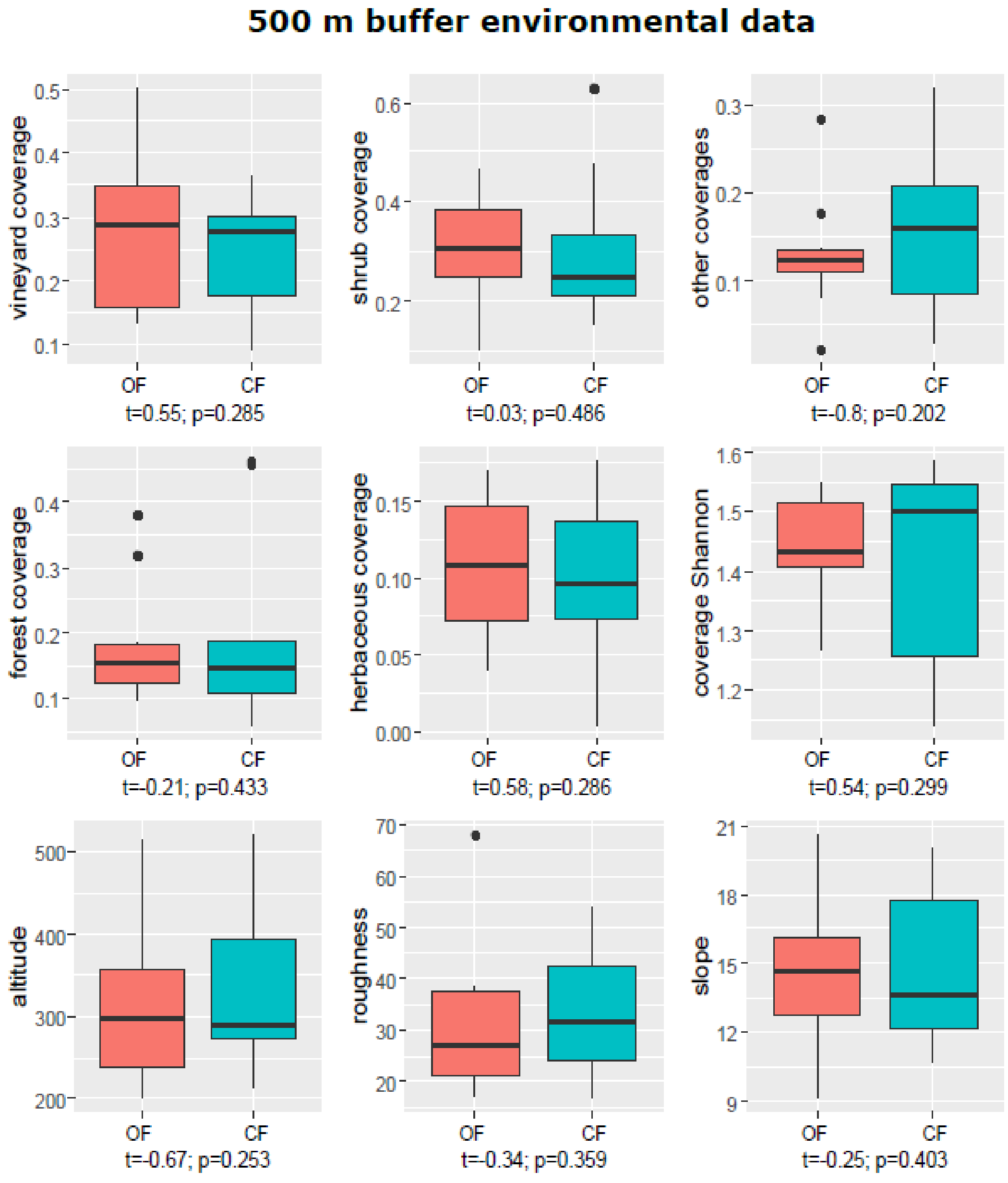
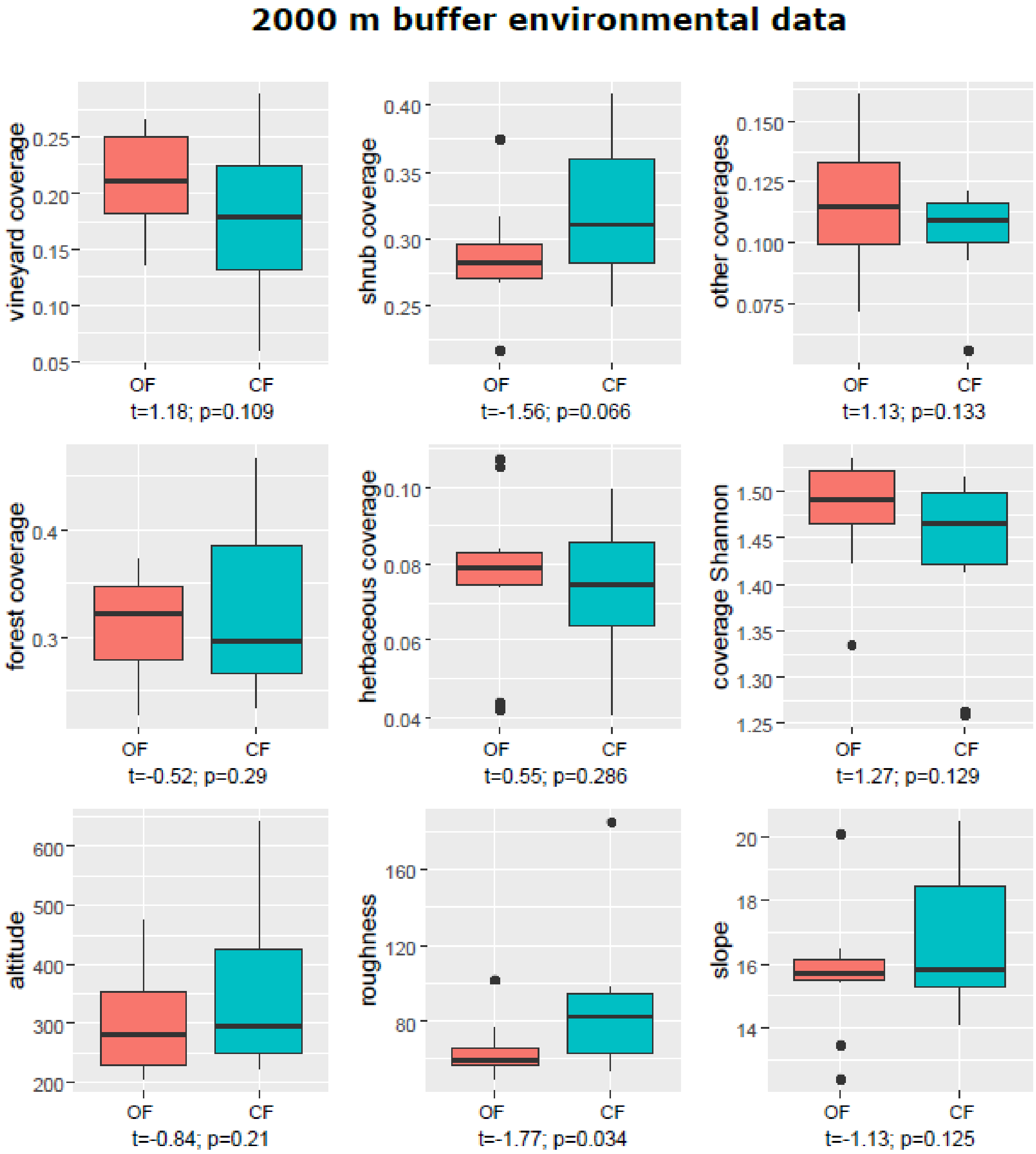
Supplementary material

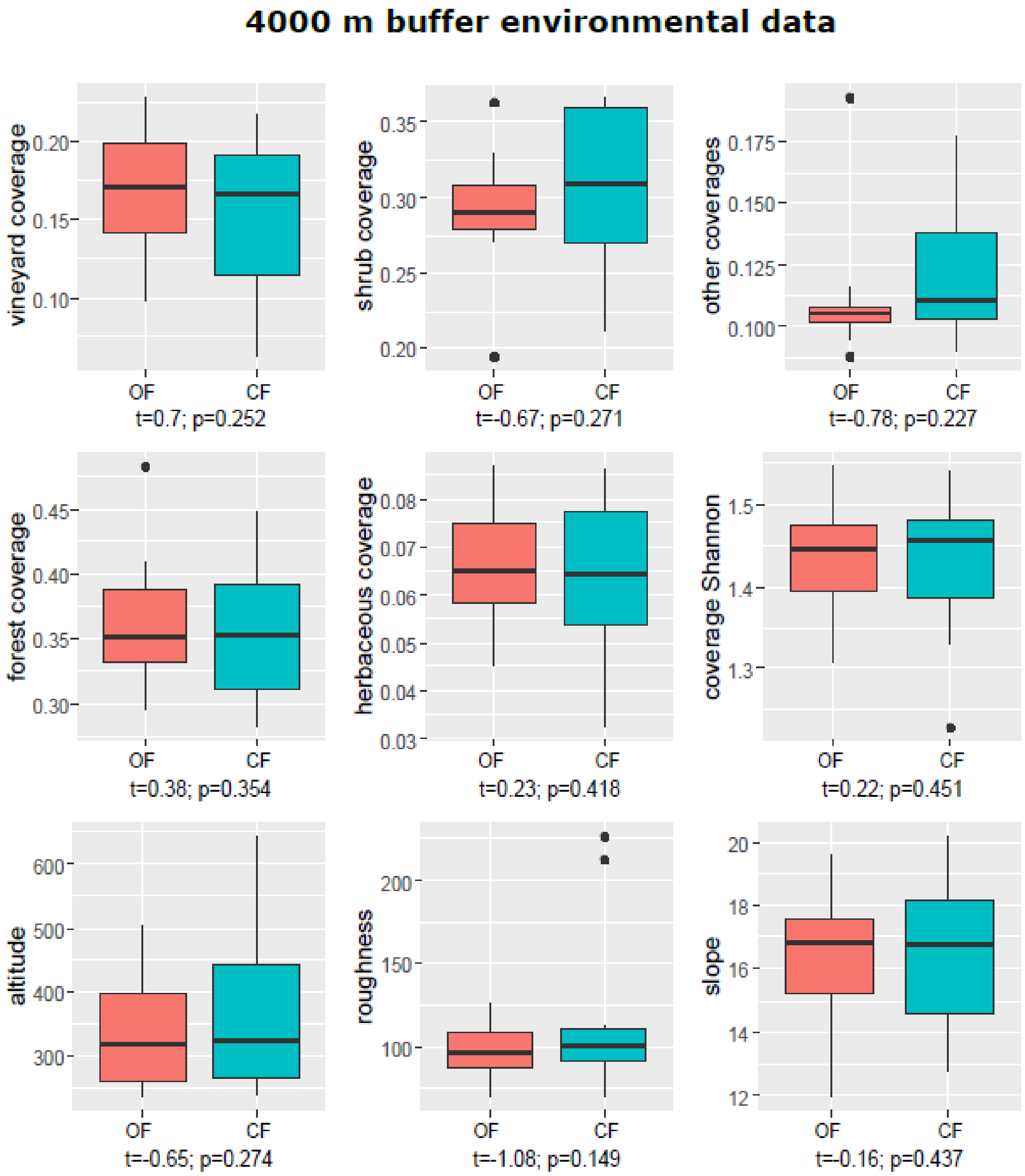
1. Biodiversity in organic v conventional vineyards

S1 - Environmental variables exploration: differences between treatments.

Results of the Monte Carlos permutations of the t-student comparing the values of land cover and physical attributes at a radius of 500 m around the plots of the two considered treatments (OF -organic farming- and CF -conventional farming-).
No significant differences in terms of environmental conditions were found between the two treatments.




2000 m buffer environmental data
vineyard coverage
0.25
0.20
0.15
0.10
0.05
OF
CF
t=1.18; p=0.109
shrub coverage
0.40
0.35
0.30
0.25
OF
CF
t=-1.56; p=0.066
other coverages
0.150
0.125
0.100
0.075
OF
CF
t=1.13; p=0.133
forest coverage
0.4
0.3
OF
CF
t=-0.52; p=0.29
herbaceous coverage
0.10
0.08
0.06
0.04
OF
CF
t=0.55; p=0.286
coverage Shannon
1.50
1.45
1.40
1.35
1.30
1.25
OF
CF
t=1.27; p=0.129
altitude
600
500
400
300
200
OF
CF
t=-0.84; p=0.21
roughness
160
120
80
OF
CF
t=-1.77; p=0.034
slope
20
18
16
14
OF
CF
t=-1.13; p=0.125


4000 m buffer environmental data
vineyard coverage
0.20
0.15
0.10
OF
CF
t=0.7; p=0.252
shrub coverage
0.35
0.30
0.25
0.20
OF
CF
t=-0.67; p=0.271
other coverages
0.175
0.150
0.125
0.100
OF
CF
t=-0.78; p=0.227
forest coverage
0.45
0.40
0.35
0.30
OF
CF
t=0.38; p=0.354
herbaceous coverage
0.08
0.07
0.06
0.05
0.04
0.03
OF
CF
t=0.23; p=0.418
coverage Shannon
1.5
1.4
1.3
OF
CF
t=0.22; p=0.451
altitude
600
500
400
300
OF
CF
t=-0.65; p=0.274
roughness
200
150
100
OF
CF
t=-1.08; p=0.149
slope
20
18
16
14
12
OF
CF
t=-0.16; p=0.437

S2 - Plots and transects location

See file txpm2de5.kmz

S3 - Summary of best models for all distance buffers

C.1. – GLMM results for vegetation richness considering influence of land cover at a radius of 500. 2000 and 4000 m around the vineyard plots. Best 5 models (increment of AIC value below 2) for each distance are shown. with total of best models indicated on table header. Standardised estimates are given for fixed effects included to the models. SD of the estimates in parenthesis. *P(z)* indicated on significant estimates (*** p < 0.001. ** p < 0.01. * p < 0.05). Shaded on grey those variables excluded from the saturated models for each buffer distance. Where *Treat-conv:* Conventional treatment; *Altitude*. *Forest. Vineyard*. *Shrub. Herbaceous:* percent coverage at the specified radius; *Other:* percent coverage of all remaining land covers at the specified radius; *Loc-CropL*: locations in crop lines; *Loc-CropL:Treat-conv:* interaction between Conventional treatment and sampling in the crop lines; *AIC*: Aikaike Information Criterion; *PseudoR-sq*: pseudo r-squared value.

	500 m buffer (14 models out of 160)					**2000 m buffer (8 models out of 80)**					**4000 m buffer (6 models out of 40)**				
	Model1	**Model2**	**Model3**	**Model4**	**Model5**	**Model1**	**Model2**	**Model3**	**Model4**	**Model5**	**Model1**	**Model2**	**Model3**	**Model4**	**Model5**
Intercept	3.15 ***	3.16 ***	3.17 ***	3.12 ***	3.13 ***	3.15 ***	3.12 ***	3.16	3.13 ***	3.17 ***	3.17 ***	3.14 ***	3.16 ***	3.16 ***	3.13 ***
	(0.09)	(0.10)	(0.09)	(0.10)	(0.10)	(0.11)	(0.11)	(0.10)	(0.10)	(0.11)	(0.11)	(0.12)	(0.11)	(0.11)	(0.12)
Treat-conv	-0.50 ***	-0.53 ***	-0.55 ***	-0.44 **	-0.46 **	-0.51 ***	-0.44 **	-0.53 ***	-0.47 **	-0.54 ***	-0.54 ***	-0.48 **	-0.52 **	-0.53 **	-0.46 **
	(0.13)	(0.13)	(0.13)	(0.14)	(0.14)	(0.15)	(0.16)	(0.14)	(0.15)	(0.16)	(0.16)	(0.17)	(0.16)	(0.16)	(0.17)
Altitude								0.12	0.12				-0.07		-0.07
								(0.11)	(0.10)				(0.08)		(0.08)
Forest	-0.18 *	-0.22 **	-0.28 **	-0.18 *	-0.22 **	-0.15	-0.15 *	-0.24 *	-0.24 *						
	(0.07)	(0.07)	(0.09)	(0.07)	(0.07)	(0.08)	(0.08)	(0.11)	(0.11)						
Vineyard			-0.11												
			(0.08)												
Shrub															
Herbaceous	0.11			0.10											
	(0.07)			(0.07)											
Other															
Loc-CropL	-0.26 ***	-0.26 ***	-0.26 ***	-0.21 **	-0.20 **	-0.25 ***	-0.20 **	-0.26 ***	-0.20 **	-0.26 ***	-0.26 ***	-0.20 **	-0.26 ***	-0.26 ***	-0.20 **
	(0.05)	(0.05)	(0.05)	(0.07)	(0.07)	(0.05)	(0.07)	(0.05)	(0.07)	(0.05)	(0.05)	(0.07)	(0.05)	(0.05)	(0.07)
Loc-CropL:Treat-conv				-0.13	-0.13		-0.14		-0.14			-0.14			-0.14
				(0.11)	(0.11)		(0.11)		(0.11)			(0.11)			(0.11)
AIC	598.15	598.25	598.63	598.79	598.82	602.76	603.16	603.46	603.90	604.33	604.33	604.83	605.68	606.14	606.14
PseudoR-sq	0.945	0.943	0.944	0.946	0.944	0.940	0.941	0.941	0.942	0.937	0.937	0.939	0.938	0.937	0.939

C.2. – GLMM results for *Rhopalocera* richness considering influence of land cover at a radius of 500. 2000 and 4000 m around the vineyard plots. Best 5 models (increment of AIC value below 2) for each distance are shown. with total of best models indicated on table header. Standardised estimates are given for fixed effects included to the models. SD of the estimates in parenthesis. *P(z)* indicated on significant estimates (*** p < 0.001. ** p < 0.01. * p < 0.05). Shaded on grey those variables excluded from the saturated models for each buffer distance. Where *Treat-conv:* Conventional treatment; *Altitude*. *Forest. Vineyard*. *Shrub.:* percent coverage at the specified radius; *Other:* percent coverage of all remaining land covers at the specified radius; *Loc-CropL*: locations in crop lines; *Loc-CropL:Treat-conv:* interaction between Conventional treatment and sampling in the crop lines; *AIC*: Aikaike Information Criterion; *PseudoR-sq*: pseudo r-squared value.

	500 m buffer (11 models out of 80)					**2000 m buffer (11 models out of 80)**					**4000 m buffer (7 models out of 40)**				
	Model1	**Model2**	**Model3**	**Model4**	**Model5**	**Model1**	**Model2**	**Model3**	**Model4**	**Model5**	**Model1**	**Model2**	**Model3**	**Model4**	**Model5**
Intercept	1.74 ***	1.68 ***	1.64 ***	1.76 ***	1.78 ***	1.79 ***	1.73 ***	1.77 ***	1.78 ***	1.71 ***	1.77 ***	1.71 ***	1.67 ***	1.76 ***	1.70 ***
	(0.09)	(0.11)	(0.08)	(0.10)	(0.09)	(0.10)	(0.11)	(0.10)	(0.10)	(0.11)	(0.10)	(0.11)	(0.08)	(0.10)	(0.11)
Treat-conv	-0.31 *	-0.17	-0.31 *	-0.34 **	-0.38 **	-0.37 **	-0.24	-0.36 **	-0.36 **	-0.23	-0.35 **	-0.21	-0.35 **	-0.33	-0.19
	(0.12)	(0.15)	(0.12)	(0.13)	(0.12)	(0.12)	(0.16)	(0.12)	(0.13)	(0.16)	(0.12)	(0.16)	(0.12)	(0.13)	(0.16)
Altitude			0.15 *		0.15 *	0.15 *		0.09	0.15 *		0.14 *	0.14 *	0.14 *	0.12	0.12
			(0.06)		(0.06)	(0.06)		(0.07)	(0.06)		(0.06)	(0.06)	(0.06)	(0.07)	(0.07)
Forest	-0.13	-0.13	-0.13								▨	▨	▨	▨	▨
	(0.07)	(0.07)	(0.07)												
Vineyard						▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Shrub	▨	▨	▨	▨	▨	0.17 **	0.17 **		0.19 **						
						(0.06)	(0.06)		(0.07)						
Other					0.10				0.06					-0.05	-0.05
					(0.07)				(0.07)					(0.07)	(0.07)
Loc-CropL	-0.21	-0.07		-0.21	-0.21	-0.21	-0.07	-0.21	-0.21	-0.07	-0.21	-0.07		-0.21	-0.07
	(0.11)	(0.15)		(0.11)	(0.11)	(0.11)	(0.15)	(0.11)	(0.11)	(0.15)	(0.11)	(0.15)		(0.11)	(0.15)
Loc-CropL:Treat-conv		-0.31					-0.31			-0.31		-0.31			-0.31
		(0.22)					(0.22)			(0.22)		(0.22)			(0.22)
AIC	327.34	327.44	328.83	328.86	328.90	326.40	326.50	327.47	327.56	327.57	327.80	327.90	329.29	329.32	329.42
PseudoR-sq	0.239	0.259	0.202	0.201	0.223	0.228	0.248	0.216	0.237	0.237	0.213	0.233	0.174	0.218	0.238

C.3. – GLMM results for *Heterocera* richness considering influence of land cover at a radius of 500. 2000 and 4000 m around the vineyard plots. Best 5 models (increment of AIC value below 2) for each distance are shown. with total of best models indicated on table header. Standardised estimates are given for fixed effects included to the models. SD of the estimates in parenthesis. *P(z)* indicated on significant estimates (*** p < 0.001. ** p < 0.01. * p < 0.05). Shaded on grey those variables excluded from the saturated models for each buffer distance. Where *Treat-conv:* Conventional treatment; *Altitude*. *Forest. Vineyard*. *Shrub. Herbaceous:* percent coverage at the specified radius; *Other:* percent coverage of all remaining land covers at the specified radius; *AIC*: Aikaike Information Criterion; *PseudoR-sq*: pseudo r-squared value.

	500 m buffer (12 models out of 64)					**2000 m buffer (9 models out of 32)**					**4000 m buffer (8 models out of 16)**				
	Model1	**Model2**	**Model3**	**Model4**	**Model5**	**Model1**	**Model2**	**Model3**	**Model4**	**Model5**	**Model1**	**Model2**	**Model3**	**Model4**	**Model5**
Intercept	3.35 ***	3.34 ***	3.18 ***	3.19 ***	3.19 ***	3.39 ***	3.35 ***	3.35 ***	3.18 ***	3.38 ***	3.20 ***	3.20 ***	3.20 ***	3.20 ***	3.26 ***
	(0.12)	(0.13)	(0.09)	(0.10)	(0.10)	(0.13)	(0.13)	(0.13)	(0.13)	(0.13)	(0.07)	(0.07)	(0.08)	(0.08)	(0.11)
Treat-conv	-0.32	-0.29				-0.39 *	-0.32	-0.32							-0.12
	(0.18)	(0.19)				(0.18)	(0.19)	(0.18)							(0.15)
Altitude		0.16				0.33 **	0.21 *	0.33 **	0.17	0.18		0.11		0.09	
		(0.09)				(0.12)	(0.09)	(0.13)	(0.09)	(0.10)		(0.08)		(0.08)	
Forest	-0.26 *		-0.25 *			-0.24		-0.18							
	(0.12)		(0.12)			(0.13)		(0.13)							
Vineyard	-0.27 *		-0.24 *												
	(0.11)		(0.11)												
Shrub											-0.16	-0.18			
											(0.11)	(0.11)			
Herbaceous	-0.18		-0.16												
	(0.09)		(0.10)												
Other						-0.15					-0.51 ***	-0.47 ***	-0.40 ***	-0.35 ***	-0.39 ***
						(0.10)					(0.11)	(0.11)	(0.08)	(0.09)	(0.08)
AIC	319.66	320.74	320.89	320.91	321.04	318.82	318.96	318.99	319.82	320.05	304.36	304.37	304.47	305.30	305.87
PseudoR-sq	0.992	0.991	0.991	0.99	0.99	0.992	0.991	0.992	0.99	0.991	0.994	0.994	0.994	0.994	0.994

C4. – GLM results for bird richness considering influence of land cover at a radius of 500. 2000 and 4000 m around the vineyard plots. Best 5 models (increment of AIC value below 2) for each distance are shown. with total of best models indicated on table header. Standardised estimates are given for fixed effects included to the models. SD of the estimates in parenthesis. *P(z)* indicated on significant estimates (*** p < 0.001. ** p < 0.01. * p < 0.05). Shaded on grey those variables excluded from the saturated models for each buffer distance. Where *Treat-conv:* Conventional treatment; *Altitude*. *Forest. Vineyard*. *Shrub. Herbaceous:* percent coverage at the specified radius; *Other:* percent coverage of all remaining land covers at the specified radius; *AIC*: Aikaike Information Criterion; *PseudoR-sq*: pseudo r-squared value.

	500 m buffer (6 models out of 64)						**2000 m buffer (1 models out of 32)**	**4000 m buffer (1 models out of 16)**
	Model1	**Model2**	**Model3**	**Model4**	**Model5**	**Model6**	**Model1**	**Model1**
Intercept	1.56 ***	1.55 ***	1.58 ***	1.51 ***	1.55 ***	1.56 ***	1.57 ***	1.57 ***
	(0.11)	(0.11)	(0.10)	(0.15)	(0.11)	(0.11)	(0.11)	(0.11)
Treat-conv				0.09				
				(0.20)				
Altitude	-0.30 *	-0.34 **	-0.35 **	-0.31 *	-0.32 *	-0.29 *		-0.38 **
	(0.12)	(0.12)	(0.12)	(0.12)	(0.13)	(0.14)		(0.12)
Forest	-0.23	-0.30 *		-0.22	-0.25	-0.22	-0.37 ***	▨
	(0.13)	(0.15)		(0.13)	(0.16)	(0.13)	(0.11)	▨
Vineyard					-0.03		▨	▨
					(0.15)		▨	▨
Shrub	▨	▨	▨	▨	▨	▨		
	▨	▨	▨	▨	▨	▨		
Herbaceous						0.03	▨	▨
						(0.13)	▨	▨
Other								
AIC	99.24	100.17	100.35	101.02	101.02	101.2	98.40	99.24
PseudoR-sq	0.473	0.501	0.385	0.479	0.475	0.475	0.442	0.418

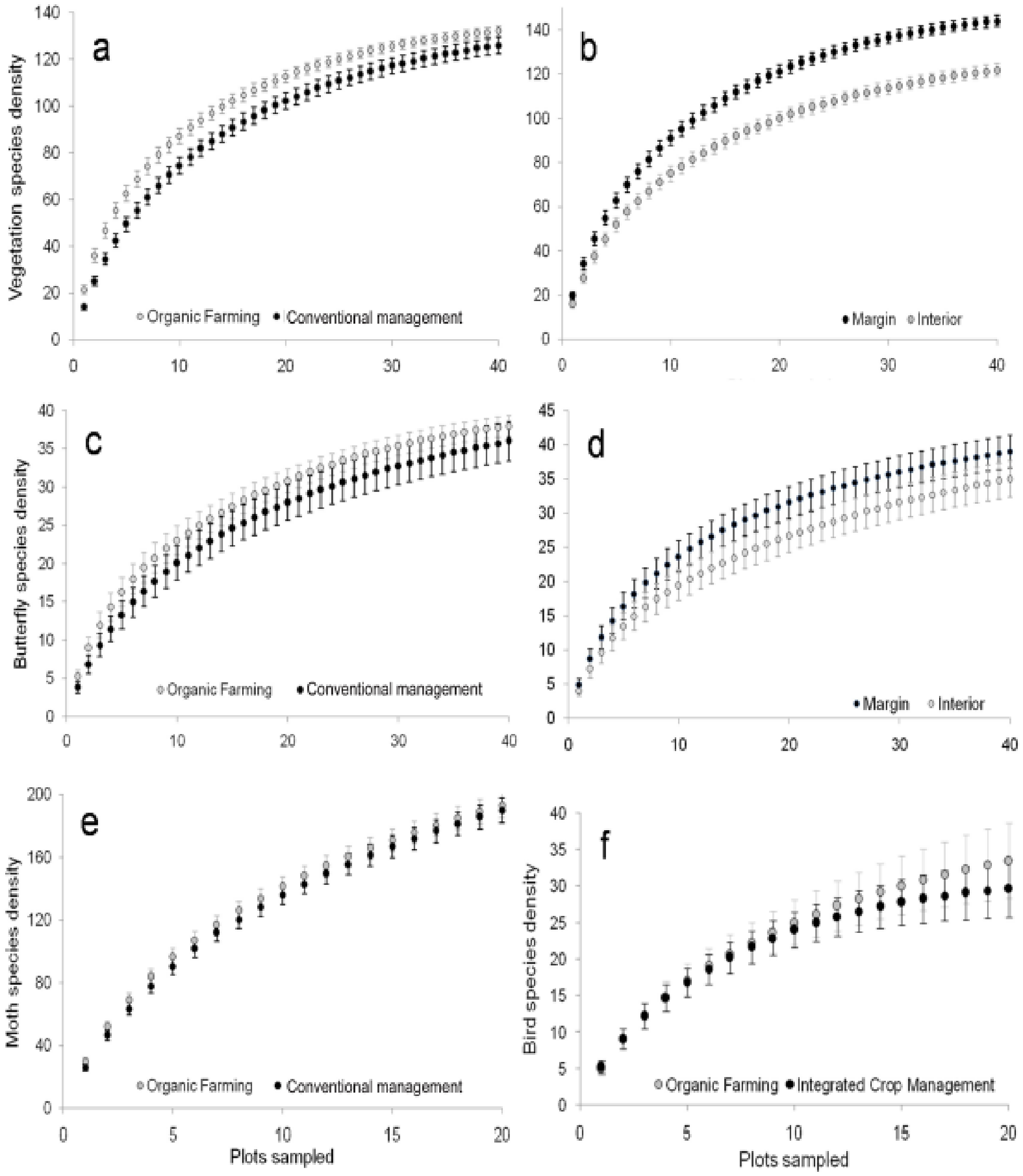
S4 - Data sets

See file txpm3de5.xslx

S5 - Species accumulation curves

Species accumulation curves

Sample-based species accumulation curves for vegetation (a.b),butterflies (c.d),moths (e) and birds (f), according to farming practices (Organic farming vs Conventional crop management) and plot situation (margin vs interior). Only farming practices were considered for moths and birds.

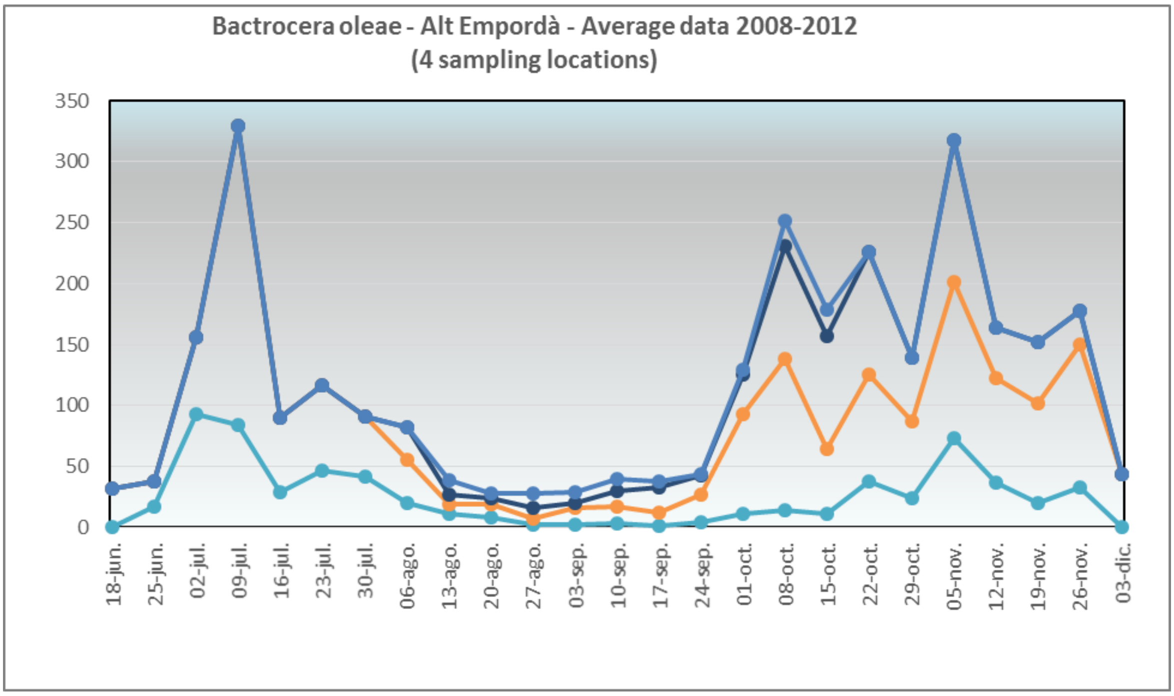


2. Organic olive groves and bat conservation

S1 - Bactrocera oleae activity patterns

Source:

Unitat de Sanitat Vegetal a Girona (*Crop Health Unit in Girona*)
Departament d'Agricultura, Ramaderia, Pesca i Alimentació
Generalitat de Catalunya



S2 - Best models from GLMMs

Model results for the best mixed-effect models. The best models (increment of AIC value below 2) are given, with the total of models run indicated in the table headings. Standardised estimates are given for fixed effects included in the models and the SD of the estimates is given in parenthesis. *P*(z) indicates significant estimates (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). The grey shading indicates variables not included in the saturated models. Only significant effects are shown.

	Bactrocera oleae			**Aerial hunting bats**									
	3 models out of 255			10 models out of 256									
	Md1	**Md2**	**Md3**	**Md1**	**Md2**	**Md3**	**Md4**	**Md5**	**Md6**	**Md7**	**Md8**	**Md9**	**Md10**
Intercept	3.15 ***	3.14 ***		24.06 ***	24.33 ***	23.95 ***	24.22 ***	24.71 ***	24.28 ***	24.66 ***	24.55 ***	24.02 ***	24.67 ***
	[0.11]	[0.11]		[1.9]	[1.84]	[1.85]	[1.84]	[1.82]	[1.83]	[1.82]	[1.84]	[1.84]	[1.83]
Julian day				-0.07 ***	-0.07 ***	-0.07 ***	-0.07 ***	-0.07 ***	-0.07 ***	-0.07 ***	-0.07 ***	-0.07 ***	-0.07 ***
				[0.01]	[0.01]	[0.01]	[0.01]	[0.01]	[0.01]	[0.01]	[0.01]	[0.01]	[0.01]
Altitude	0.18 *	0.17 *	0.17 *										
	[0.07]	[0.08]	[0.07]										
Pine v Conv	-0.95 ***	-0.91 ***	-0.94 ***	1.02 *	0.89 *	1.09 **	0.97 *		0.94 *			1.07 **	
	[0.16]	[0.17]	[0.16]	[0.42]	[0.41]	[0.41]	[0.4]		[0.39]			[0.4]	
Pine v Org	-0.91 ***	-0.87 ***	-0.91 ***										
	[0.17]	[0.18]	[0.16]										
Temperature				0.29 ***	0.29 ***	0.29 ***	0.28 ***	0.28 ***	0.28 ***	0.28 ***	0.29 ***	0.29 ***	0.28 ***
				[0.06]	[0.07]	[0.07]	[0.07]	[0.07]	[0.07]	[0.07]	[0.07]	[0.07]	[0.07]
Wind				-0.27 ***	-0.26 ***	-0.27 ***	-0.26 ***	-0.26 ***	-0.26 ***	-0.26 ***	-0.27 ***	-0.27 ***	-0.26 ***
				[0.02]	[0.06]	[0.06]	[0.06]	[0.06]	[0.06]	[0.06]	[0.06]	[0.06]	[0.06]
Bactrocera	▨	▨	▨										
PseudoR-sq	0.339	0.322	0.343	0.97	0.97	0.971	0.97	0.97	0.971	0.97	0.97	0.971	0.97
AICc	721.3	721.5	723.1	2771.1	2771.3	2771.3	2771.3	2771.6	2772.4	2772.4	2772.5	2772.5	2773

Gleaning bats

17 models out of 256

	Md1	Md2	Md3	Md4	Md5	Md6	Md7	Md8	Md9	Md10	Md11	Md12	Md13	Md14	Md15	Md16	Md17
Intercept	16.81 ***	15.84 ***	19.46 ***	17.13 ***	17.34 ***	15.51 ***	16.41 ***	16.51 ***	18.61 ***	15.46 ***	16.16 ***	16.69 ***	17.29 ***	15.69 ***	17.61 ***	16.31 ***	20.02 ***
	[2.5]	[2.85]	[2.5]	[0.01]	[0.01]	[2.84]	[2.14]	[2.22]	[0.02]	[2.84]	[2.33]	[2.21]	[2.63]	[2.62]	[0.01]	[2.7]	[0.01]
Julian day	-0.05 ***	-0.05 ***	-0.06 ***	-0.05 ***	-0.05 ***	-0.05 ***	-0.05 ***	-0.05 ***	-0.06 ***	-0.05 ***	-0.05 ***	-0.05 ***	-0.05 ***	-0.05 ***	-0.06 ***	-0.05 ***	-0.06 ***
	[0.01]	[0.01]	[0.01]	[0]	[0]	[0.01]	[0.01]	[0.01]	[0]	[0.01]	[0.01]	[0.01]	[0.01]	[0.01]	[0]	[0.01]	[0]
Altitude			-0.42 *						-0.45 ***						-0.31 ***		
			[0.21]						[0.02]						[0.01]		
Aspect	-0.56 *	-0.57 *	-0.57 *						-0.59 ***						-0.45 ***		
	[0.22]	[0.23]	[0.22]						[0.02]						[0.01]		
Shannon	0.48 *	0.46 *	0.48 *		0.27 ***				0.47 ***						0.48 ***		
	[0.19]	[0.2]	[0.2]		[0.01]				[0.02]						[0.01]		
Org v Conv	0.85 *	0.88 *	0.87 *					0.88 *	0.9 ***	0.9 *							
	[0.38]	[0.39]	[0.38]					[0.4]	[0.02]	[0.41]							
Pine v Conv	0.94 *	1.13 **	0.96 *			0.92 *			1.17 ***	0.92 *				0.86 *			
	[0.41]	[0.41]	[0.42]			[0.43]			[0.02]	[0.41]				[0.42]			
Pine v Org			0.08 ***						0.27 ***								
			[0.01]						[0.02]								
Temperature				0.17 ***	0.17 ***										0.16 ***		
				[0.01]	[0.01]										[0.01]		
Bactrocera				-0.18 ***	-0.19 ***										-0.2 ***		-0.19 ***
				[0.01]	[0.01]										[0.01]		[0.01]
PseudoR-sq	0.681	0.678	0.677	0.664	0.667	0.667	0.67	0.676	0.673	0.673	0.66	0.672	0.669	0.669	0.672	0.663	0.659
AICc	1358.1	1358.3	1358.7	1358.8	1359	1359	1359.2	1359.2	1359.2	1359.6	1359.6	1359.7	1359.7	1359.8	1359.9	1360	1360

3. Pest control provided by bats in rice paddies

S1 - PCR primers, length of amplicons and amplifications conditions

PCR amplification and screening

Primers specific for *C. suppressalis* 28S and COI were designed with the software Primer-blast at NCBI (Rozen and Skaletsky 2000).

The primers chosen are listed below:

On the 28S-D2 region

External primers

D2F/D2Rev (Campbell *et al*. 1993)

Inner primers

28S 333F (5’ ATC GAG GTC CTG CCT ATG TG 3’)

28S 434R (5’ GAA CTG ATC ATCGCA GAC AGA G 3’)

The length of the amplified product is 99bp

On the COI region both the couples of primers annealed in the first 5’ region. No region characteristic of *C. suppressalis* was found in the second half of this gene.

External primers

COI PPF1 (5’ AAT GGA GCT GGA ACA GGA TG 3’)

COI PPR1d (5’ GAA TTG GAT CTC CAC CAC CA 3’)

Inner primers

COI PPF2a (5’ GCT CAC GCT GGA AGT TCA G 3’)

COI PPR2b (5’ CAG CTA ATA CTG GTA GAG ATA GAA GT 3’)

The length of the amplified product is respectively 331bp, and 202bp.

With the external primer pairs the PCR conditions were the same for both couples of primers: the PCR cycling program was: 3min at 94 °C, followed by 30 cycles of 45s at 94 °C, 1min at 52 °C, 1min at 72°C, and a final extension of 7min at 72°C.

Nested PCR cycles were 3min at 94 °C, followed by 35 cycles of 45s at 94 °C, 1min at 55 °C, 45s at 72°C, and a final extension of 7min at 72°C.

All the amplifications were carried out in a 10μl reactions using 1μl of DNA template, 1x buffer (Promega), 0.2 mM of each dNTP, 10 pmol of each primer and 0,2 units of GoTaq DNA polymerase (Promega).

PCR products were run on a 2% agarose gel, stained with ethidium bromide. To validate the method a number of positive samples were included in the analysis, such as DNA from *C. suppressalis,* and the DNA from droppings with *C. suppressalis* antenna added.

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4. Bats and mosquitoes in rice paddies

S1 – Phonic types

Phonic types used in the bat recording analysis. Those retained for further analysis are indicated in the second column.

Phonic groups	**Retained in analyses**	**Notes**
Eptesicus serotinus / Nyctalus leisleri		
Hupsugo savii		
Myotis sp.		
Pipistrellus kuhlii / P. nathusii	Yes	*P. nathusii* is absent in the region, with no known records
Pipistrellus pipistrellus	Yes	
Pipistrellus pygmaeus / Miniopterus schreibersii	Yes	*P. pygmaeus* is the most frequently seen and captured species in the area, roosting in the nearby villages and scattered buildings, whereas only one *Miniopterus* roost exists in a radius of 12 km and the species is hence rare or even absent in the rice paddies
Plecotus sp.		
Rhinolophus ferrumequinum		
Tadarida teniotis		

S2 – Tables – Bat activity

Localities

Location	Lat	Long
E01	41.986318	3.142632
E02	41.990976	3.149114
E03	41.990829	3.144031
E04	41.992182	3.141993
E05	41.99424	3.145898
E06	42.001584	3.135373
E07	42.010795	3.145489
E08	42.009536	3.151077
E09	42.009041	3.157453
E10	42.00149	3.153075
E11	42.001285	3.158255
E12	42.004265	3.165302
E13	42.003761	3.1774
E14	42.006945	3.185681
E15	42.000944	3.187487

Bat activity

Location	Night	Detector	year	Pipkuh	Pippip	Pippyg	Bats	temp	RH	wind	rain
E01	19/06/2017	SM2-01	2017	21	297	390	708	19.92	92.67	2.46	0
E01	20/06/2017	SM2-01	2017	38	391	758	1187	21.68	88.67	2.72	0
E01	21/06/2017	SM2-01	2017	111	614	1122	1847	21.05	93.89	1.65	0
E01	29/06/2017	SM2-03	2017	115	644	604	1363	15.25	94.67	2.18	0
E01	30/06/2017	SM2-03	2017	13	15	19	47	13.94	95.72	1.84	1.3
E01	01/07/2017	SM2-03	2017	196	805	885	1886	15.74	94.33	1.62	0.2
E01	02/07/2017	SM2-03	2017	42	308	332	682	18.27	74.89	2.02	0
E01	27/07/2017	SM2-01	2017	277	793	1125	2195	19.5	95.37	1.62	0
E01	28/07/2017	SM2-01	2017	401	876	1197	2474	19.72	95.95	1.69	0
E01	29/07/2017	SM2-01	2017	815	1479	2251	4545	20.55	95.32	1.57	0
E01	30/07/2017	SM2-01	2017	699	2171	4147	7017	20.51	98.58	1.79	0
E01	03/08/2017	SM2-05	2017	258	379	1830	2467	21.37	98.32	1.38	0
E01	04/08/2017	SM2-05	2017	341	472	2523	3336	21.49	94.11	1.89	0
E01	05/08/2017	SM2-05	2017	651	918	3470	5039	23.46	80.74	1.7	0
E01	06/08/2017	SM2-05	2017	604	806	3506	4916	23.61	66.9	2.69	0
E01	21/08/2017	SM2-05	2017	85	69	364	518	15.33	83.05	1.97	0
E01	22/08/2017	SM2-05	2017	90	128	659	877	16.8	94.86	1.65	0
E01	23/08/2017	SM2-05	2017	75	148	681	904	19.08	95.19	1.83	0
E01	31/08/2017	SM2-02	2017	55	46	271	372	18.68	87.55	2.93	0.4
E01	01/09/2017	SM2-02	2017	141	107	420	668	18.3	70	3.09	0.1
E01	02/09/2017	SM2-02	2017	402	97	601	1100	18.67	61	2.71	1.3
E01	03/09/2017	SM2-02	2017	643	108	860	1611	18.15	80.91	1.44	0
E01	21/09/2017	SM2-03	2017	238	45	274	557	13.56	96.88	1.5	0
E01	22/09/2017	SM2-03	2017	245	24	95	364	14.28	97.58	1.56	0
E01	23/09/2017	SM2-03	2017	105	12	71	188	15.23	96.67	1.21	0
E01	24/09/2017	SM2-03	2017	100	16	65	181	16	97.83	2.03	0
E01	23/08/2018	SM2-05	2018	43	64	355	462	19.33	90	1.89	0
E02	22/06/2017	SM2-01	2017	235	1111	1277	2623	22.29	92.61	2.76	0
E02	23/06/2017	SM2-01	2017	112	1206	1147	2465	21.77	91.56	3.12	0
E02	24/06/2017	SM2-01	2017	128	989	1054	2171	22.07	86.83	2.51	0
E02	25/06/2017	SM2-01	2017	273	1591	2603	4467	21.31	91.67	2.31	0
E02	10/07/2017	SM2-02	2017	170	1895	1477	3542	19.82	94.11	1.84	0
E02	11/07/2017	SM2-02	2017	148	1662	1354	3164	19.29	96.67	1.57	0

E02	12/07/2017	SM2-02	2017	152	2450	1489	4091	20.08	95.17	1.62	0
E02	31/07/2017	SM2-01	2017	1942	692	1922	4556	21.89	97.42	1.81	0
E02	01/08/2017	SM2-01	2017	1784	588	1871	4243	22.24	97.89	2.31	0
E02	02/08/2017	SM2-01	2017	1213	848	2260	4321	21.11	98.16	1.98	0
E02	14/08/2017	SM2-02	2017	567	443	1365	2375	18.13	96.2	2.11	0
E02	15/08/2017	SM2-02	2017	507	438	1114	2059	19.33	97.15	1.68	0
E02	16/08/2017	SM2-02	2017	278	396	1060	1734	19.84	95.95	1.36	0
E02	24/08/2017	SM2-05	2017	153	113	619	885	21.23	92.52	2.5	0
E02	25/08/2017	SM2-05	2017	258	144	867	1269	22.04	95.9	2.05	0
E02	26/08/2017	SM2-05	2017	137	85	380	602	21.4	98.33	1.61	0
E02	27/08/2017	SM2-05	2017	319	117	810	1246	21.69	97.14	1.88	0
E02	23/08/2018	SM2-04	2018	28	30	121	179	19.33	90	1.89	0
E03	13/07/2017	SM2-01	2017	254	2490	2022	4766	21.11	91.94	3.66	0
E03	14/07/2017	SM2-01	2017	337	1886	2855	5078	23.63	55.94	3.71	0
E03	15/07/2017	SM2-01	2017	158	1476	1527	3161	24.11	49.33	3.43	0
E03	16/07/2017	SM2-01	2017	92	1559	1729	3380	20.25	74.72	1.66	0
E03	24/07/2017	SM2-01	2017	734	828	1536	3098	19.59	79.74	3.37	0
E03	25/07/2017	SM2-01	2017	725	773	1267	2765	21.31	63.16	4.13	0
E03	26/07/2017	SM2-01	2017	866	588	1065	2519	19.94	70.42	3.62	0.1
E03	07/08/2017	SM2-01	2017	4029	3398	5920	13347	22.71	84.2	3.73	0
E03	08/08/2017	SM2-01	2017	444	143	625	1212	21.19	80.25	4.23	0
E03	09/08/2017	SM2-01	2017	4	19	217	240	17.06	71.25	3.92	1.3
E03	08/09/2017	SM2-02	2017	1115	70	446	1631	16.14	85	2.35	0
E03	09/09/2017	SM2-02	2017	3	3	8	14	13.56	98	1.75	0.5
E03	10/09/2017	SM2-02	2017	412	12	90	514	14.4	79.27	2.16	0.1
E03	14/09/2017	SM2-03	2017	59	11	32	102	16.16	86.74	4.66	0.2
E03	15/09/2017	SM2-03	2017	1	0	0	1	13.08	92.3	3	5.9
E03	17/09/2017	SM2-03	2017	250	21	52	323	12.63	91.96	1.62	0
E03	06/08/2018	SM2-01	2018	1747	2728	5947	10422	22.98	93.7	1.78	0
E03	07/08/2018	SM2-01	2018	596	1564	3948	6108	23.87	90.6	2.96	0.4
E03	08/08/2018	SM2-01	2018	638	1041	3449	5128	23.48	89.35	3.26	0.5
E03	16/08/2018	SM2-04	2018	57	22	234	313	19.2	95.35	1.45	0
E03	30/08/2018	SM2-04	2018	20	16	161	197	20.14	91	2.07	1.5
E04	15/06/2017	SM2-02	2017	15	610	496	1121	21.21	85.83	1.89	0
E04	16/06/2017	SM2-02	2017	21	369	183	573	25.28	61.78	3.09	0
E04	17/06/2017	SM2-02	2017	24	484	530	1038	19.57	79.22	2.01	0

E04	18/06/2017	SM2-02	2017	31	770	890	1691	17.29	91.22	1.78	0
E04	22/06/2017	SM2-02	2017	24	553	471	1048	22.29	92.61	2.76	0
E04	23/06/2017	SM2-02	2017	79	735	769	1583	21.77	91.56	3.12	0
E04	24/06/2017	SM2-02	2017	49	476	523	1048	22.07	86.83	2.51	0
E04	25/06/2017	SM2-02	2017	120	668	1194	1982	21.31	91.67	2.31	0
E04	03/08/2017	SM2-01	2017	323	1166	2226	3715	21.37	98.32	1.38	0
E04	04/08/2017	SM2-01	2017	227	558	1609	2394	21.49	94.11	1.89	0
E04	05/08/2017	SM2-01	2017	296	983	1837	3116	23.46	80.74	1.7	0
E04	17/08/2017	SM2-05	2017	67	70	379	516	19.76	93.1	2.26	0
E04	18/08/2017	SM2-05	2017	154	179	911	1244	21.43	83.85	2.24	0
E04	19/08/2017	SM2-05	2017	69	107	542	718	22.66	65.85	4.78	0.1
E04	20/08/2017	SM2-05	2017	82	94	324	500	21.06	54.9	2.5	0
E04	04/09/2017	SM2-02	2017	414	112	475	1001	17.55	94.18	1.53	0.3
E04	05/09/2017	SM2-02	2017	594	688	2078	3360	18.94	92.45	1.92	0
E04	06/09/2017	SM2-02	2017	66	67	323	456	20.23	79.55	1.62	0
E04	07/09/2017	SM2-02	2017	81	75	336	492	17.33	76.77	1.95	0
E04	21/09/2017	SM2-02	2017	99	79	313	491	13.56	96.88	1.5	0
E04	22/09/2017	SM2-02	2017	98	49	369	516	14.28	97.58	1.56	0
E04	23/09/2017	SM2-02	2017	45	47	292	384	15.23	96.67	1.21	0
E04	24/09/2017	SM2-02	2017	79	37	111	227	16	97.83	2.03	0
E04	20/08/2018	SM2-05	2018	98	147	908	1153	22.16	68.85	2.3	0
E04	21/08/2018	SM2-05	2018	130	104	462	696	20.1	84.48	2.27	0
E04	22/08/2018	SM2-05	2018	184	123	730	1037	18.57	95.9	1.8	0
E04	30/08/2018	SM2-02	2018	86	219	944	1249	20.14	91	2.07	1.5
E05	19/06/2017	SM2-02	2017	151	3201	3389	6741	19.92	92.67	2.46	0
E05	20/06/2017	SM2-02	2017	202	2593	2707	5502	21.68	88.67	2.72	0
E05	21/06/2017	SM2-02	2017	178	1099	1726	3003	21.05	93.89	1.65	0
E05	26/06/2017	SM2-05	2017	1513	1245	2743	5501	20.53	95.39	1.63	0
E05	27/06/2017	SM2-05	2017	540	691	1084	2315	20.07	89.5	2.31	0
E05	03/07/2017	SM2-03	2017	77	190	653	920	17.34	92.11	2.18	0
E05	04/07/2017	SM2-03	2017	1118	1854	2278	5250	17.44	94.11	1.8	0
E05	05/07/2017	SM2-03	2017	1529	2494	2188	6211	18.11	97.89	1.89	0
E05	20/07/2017	SM2-02	2017	151	602	1330	2083	20.79	94.67	2.69	0
E05	21/07/2017	SM2-02	2017	81	410	658	1149	19.26	93.5	1.61	0
E05	22/07/2017	SM2-02	2017	94	520	574	1188	20.02	95.68	1.95	0
E05	23/07/2017	SM2-02	2017	113	402	496	1011	19.6	97.37	2.07	0

E05	03/08/2017	SM2-02	2017	301	694	1085	2080	21.37	98.32	1.38	0
E05	04/08/2017	SM2-02	2017	589	1420	2446	4455	21.49	94.11	1.89	0
E05	05/08/2017	SM2-02	2017	412	1200	2147	3759	23.46	80.74	1.7	0
E05	06/08/2017	SM2-02	2017	440	1227	1853	3520	23.61	66.9	2.69	0
E05	14/08/2017	SM2-05	2017	212	222	714	1148	18.13	96.2	2.11	0
E05	15/08/2017	SM2-05	2017	77	90	326	493	19.33	97.15	1.68	0
E05	16/08/2017	SM2-05	2017	18	9	38	65	19.84	95.95	1.36	0
E05	28/08/2017	SM2-05	2017	46	35	382	463	20.67	97.18	1.57	0
E05	29/08/2017	SM2-05	2017	79	27	354	460	20.75	97.32	1.96	0
E05	30/08/2017	SM2-05	2017	178	177	1038	1393	21.65	83.05	4.17	0
E05	14/09/2017	SM2-02	2017	2	27	231	260	16.16	86.74	4.66	0.2
E05	15/09/2017	SM2-02	2017	0	0	16	16	13.08	92.3	3	5.9
E05	16/09/2017	SM2-02	2017	4	3	3	10	10.59	98.04	2.04	8.4
E05	17/09/2017	SM2-02	2017	21	39	181	241	12.63	91.96	1.62	0
E05	25/09/2017	SM2-05	2017	3	3	16	22	15.47	96.83	1.53	1.2
E05	26/09/2017	SM2-05	2017	15	9	52	76	16.08	84.88	2	0
E05	27/09/2017	SM2-05	2017	15	25	140	180	14.25	97.29	1.25	0
E05	06/08/2018	SM2-02	2018	396	1696	3598	5690	22.98	93.7	1.78	0
E05	07/08/2018	SM2-02	2018	139	607	1808	2554	23.87	90.6	2.96	0.4
E05	08/08/2018	SM2-02	2018	426	1198	3029	4653	23.48	89.35	3.26	0.5
E05	23/08/2018	SM2-02	2018	196	191	650	1037	19.33	90	1.89	0
E06	12/06/2017	SM2-03	2017	14	442	241	697	18.85	89.33	1.92	0
E06	13/06/2017	SM2-03	2017	24	267	178	469	19.46	92.17	2.03	0
E06	14/06/2017	SM2-03	2017	12	261	187	460	19.42	91.89	1.67	0
E06	26/06/2017	SM2-03	2017	113	463	1074	1650	20.53	95.39	1.63	0
E06	27/06/2017	SM2-03	2017	72	332	287	691	20.07	89.5	2.31	0
E06	06/07/2017	SM2-03	2017	125	431	407	963	19.2	98.11	1.83	0
E06	07/07/2017	SM2-03	2017	180	883	748	1811	19.5	96.56	1.62	0
E06	08/07/2017	SM2-03	2017	274	673	920	1867	18.59	95.56	1.74	0
E06	09/07/2017	SM2-03	2017	224	555	549	1328	18.97	97.22	1.58	0
E06	13/07/2017	SM2-02	2017	356	2897	1690	4943	21.11	91.94	3.66	0
E06	14/07/2017	SM2-02	2017	108	783	467	1358	23.63	55.94	3.71	0
E06	15/07/2017	SM2-02	2017	255	542	572	1369	24.11	49.33	3.43	0
E06	16/07/2017	SM2-02	2017	240	345	355	940	20.25	74.72	1.66	0
E06	07/08/2017	SM2-03	2017	423	1040	2227	3690	22.71	84.2	3.73	0
E06	08/08/2017	SM2-03	2017	9	32	154	195	21.19	80.25	4.23	0

E06	09/08/2017	SM2-03	2017	0	3	12	15	17.06	71.25	3.92	1.3
E06	14/08/2017	SM2-01	2017	156	213	587	956	18.13	96.2	2.11	0
E06	15/08/2017	SM2-01	2017	274	416	1145	1835	19.33	97.15	1.68	0
E06	16/08/2017	SM2-01	2017	138	59	314	511	19.84	95.95	1.36	0
E06	31/08/2017	SM2-05	2017	1	5	33	39	18.68	87.55	2.93	0.4
E06	01/09/2017	SM2-05	2017	6	25	63	94	18.3	70	3.09	0.1
E06	02/09/2017	SM2-05	2017	18	46	265	329	18.67	61	2.71	1.3
E06	03/09/2017	SM2-05	2017	46	29	194	269	18.15	80.91	1.44	0
E06	11/09/2017	SM2-02	2017	43	66	263	372	16.11	83.27	1.78	0
E06	12/09/2017	SM2-02	2017	16	69	133	218	17.42	86.95	1.49	0
E06	13/09/2017	SM2-02	2017	47	41	223	311	16.25	94.57	2.47	0
E06	16/08/2018	SM2-02	2018	109	111	417	637	19.2	95.35	1.45	0
E06	27/08/2018	SM2-04	2018	25	12	146	183	17.65	94	1.54	0
E06	28/08/2018	SM2-04	2018	64	236	680	980	21.03	91.95	2.48	0
E06	29/08/2018	SM2-04	2018	3	1	8	12	21.07	95	3.11	1
E07	22/06/2017	SM2-05	2017	16	558	768	1342	22.29	92.61	2.76	0
E07	23/06/2017	SM2-05	2017	63	929	1988	2980	21.77	91.56	3.12	0
E07	24/06/2017	SM2-05	2017	16	635	792	1443	22.07	86.83	2.51	0
E07	25/06/2017	SM2-05	2017	109	821	1738	2668	21.31	91.67	2.31	0
E07	24/07/2017	SM2-02	2017	175	174	256	605	19.59	79.74	3.37	0
E07	25/07/2017	SM2-02	2017	242	122	178	542	21.31	63.16	4.13	0
E07	26/07/2017	SM2-02	2017	315	199	306	820	19.94	70.42	3.62	0.1
E07	10/08/2017	SM2-03	2017	283	154	242	679	17.48	76.8	2.03	1.6
E07	11/08/2017	SM2-03	2017	284	68	248	600	17	81.35	1.76	0.1
E07	12/08/2017	SM2-03	2017	182	58	254	494	18.45	77.95	1.79	0
E07	13/08/2017	SM2-03	2017	79	37	144	260	17.6	96.1	1.81	0
E07	24/08/2017	SM2-02	2017	56	137	402	595	21.23	92.52	2.5	0
E07	25/08/2017	SM2-02	2017	271	336	963	1570	22.04	95.9	2.05	0
E07	26/08/2017	SM2-02	2017	263	186	581	1030	21.4	98.33	1.61	0
E07	27/08/2017	SM2-02	2017	301	211	894	1406	21.69	97.14	1.88	0
E07	04/09/2017	SM2-03	2017	103	24	117	244	17.55	94.18	1.53	0.3
E07	05/09/2017	SM2-03	2017	449	303	1093	1845	18.94	92.45	1.92	0
E07	06/09/2017	SM2-03	2017	19	20	103	142	20.23	79.55	1.62	0
E07	07/09/2017	SM2-03	2017	28	13	113	154	17.33	76.77	1.95	0
E07	18/09/2017	SM2-02	2017	4	26	153	183	13.71	89.43	1.53	0.1
E07	19/09/2017	SM2-02	2017	4	18	55	77	14.39	70.38	2.13	0.1

E07	20/09/2017	SM2-02	2017	32	55	155	242	14.2	77.83	2.43	0
E07	25/09/2017	SM2-02	2017	2	12	60	74	15.47	96.83	1.53	1.2
E07	26/09/2017	SM2-02	2017	37	84	206	327	16.08	84.88	2	0
E07	27/09/2017	SM2-02	2017	60	76	213	349	14.25	97.29	1.25	0
E07	20/08/2018	SM2-02	2018	30	99	508	637	22.16	68.85	2.3	0
E07	21/08/2018	SM2-02	2018	314	131	633	1078	20.1	84.48	2.27	0
E07	22/08/2018	SM2-02	2018	436	155	769	1360	18.57	95.9	1.8	0
E08	15/06/2017	SM2-05	2017	6	972	640	1618	21.21	85.83	1.89	0
E08	16/06/2017	SM2-05	2017	7	495	281	783	25.28	61.78	3.09	0
E08	22/06/2017	SM2-03	2017	82	1138	3110	4330	22.29	92.61	2.76	0
E08	23/06/2017	SM2-03	2017	103	1561	1647	3311	21.77	91.56	3.12	0
E08	24/06/2017	SM2-03	2017	31	1043	1534	2608	22.07	86.83	2.51	0
E08	25/06/2017	SM2-03	2017	113	1894	2018	4025	21.31	91.67	2.31	0
E08	06/07/2017	SM2-05	2017	1672	3001	3956	8629	19.2	98.11	1.83	0
E08	07/07/2017	SM2-05	2017	1775	3346	5273	10394	19.5	96.56	1.62	0
E08	08/07/2017	SM2-05	2017	1144	3316	4675	9135	18.59	95.56	1.74	0
E08	09/07/2017	SM2-05	2017	945	2774	4497	8216	18.97	97.22	1.58	0
E08	03/08/2017	SM2-03	2017	870	937	2525	4332	21.37	98.32	1.38	0
E08	04/08/2017	SM2-03	2017	634	1461	2351	4446	21.49	94.11	1.89	0
E08	05/08/2017	SM2-03	2017	612	393	1272	2277	23.46	80.74	1.7	0
E08	06/08/2017	SM2-03	2017	623	688	1551	2862	23.61	66.9	2.69	0
E08	11/08/2017	SM2-03	2017	2	30	145	177	17	81.35	1.76	0.1
E08	12/08/2017	SM2-03	2017	7	73	110	190	18.45	77.95	1.79	0
E08	13/08/2017	SM2-03	2017	61	75	318	454	17.6	96.1	1.81	0
E08	14/08/2017	SM2-03	2017	116	61	307	484	18.13	96.2	2.11	0
E08	21/08/2017	SM2-01	2017	304	170	551	1025	15.33	83.05	1.97	0
E08	22/08/2017	SM2-01	2017	269	356	1317	1942	16.8	94.86	1.65	0
E08	23/08/2017	SM2-01	2017	591	394	1141	2126	19.08	95.19	1.83	0
E08	08/09/2017	SM2-03	2017	191	46	258	495	16.14	85	2.35	0
E08	10/09/2017	SM2-03	2017	6	14	86	106	14.4	79.27	2.16	0.1
E08	25/09/2017	SM2-01	2017	2	18	106	126	15.47	96.83	1.53	1.2
E08	26/09/2017	SM2-01	2017	62	172	354	588	16.08	84.88	2	0
E08	27/09/2017	SM2-01	2017	52	121	399	572	14.25	97.29	1.25	0
E08	09/08/2018	SM2-04	2018	8	18	153	179	21.8	91.55	3.45	0
E08	10/08/2018	SM2-04	2018	45	25	141	211	19.64	82.95	2.88	0
E08	16/08/2018	SM2-05	2018	191	296	699	1186	19.2	95.35	1.45	0

E08	30/08/2018	SM2-05	2018	176	190	1240	1606	20.14	91	2.07	1.5
E09	19/06/2017	SM2-05	2017	47	2166	1286	3499	19.92	92.67	2.46	0
E09	20/06/2017	SM2-05	2017	57	1415	2203	3675	21.68	88.67	2.72	0
E09	21/06/2017	SM2-05	2017	47	601	859	1507	21.05	93.89	1.65	0
E09	17/07/2017	SM2-02	2017	1291	2088	4812	8191	18.61	94.5	2.02	0
E09	18/07/2017	SM2-02	2017	278	995	2898	4171	22.09	90.78	4.28	0
E09	19/07/2017	SM2-02	2017	574	1851	5108	7533	23.11	90	3.5	0
E09	14/08/2017	SM2-03	2017	111	131	928	1170	18.13	96.2	2.11	0
E09	15/08/2017	SM2-03	2017	181	169	1119	1469	19.33	97.15	1.68	0
E09	16/08/2017	SM2-03	2017	151	144	672	967	19.84	95.95	1.36	0
E09	24/08/2017	SM2-01	2017	61	303	2143	2507	21.23	92.52	2.5	0
E09	25/08/2017	SM2-01	2017	333	431	2451	3215	22.04	95.9	2.05	0
E09	26/08/2017	SM2-01	2017	120	270	1661	2051	21.4	98.33	1.61	0
E09	27/08/2017	SM2-01	2017	159	264	1643	2066	21.69	97.14	1.88	0
E09	11/09/2017	SM2-03	2017	27	51	174	252	16.11	83.27	1.78	0
E09	12/09/2017	SM2-03	2017	38	28	881	947	17.42	86.95	1.49	0
E09	13/09/2017	SM2-03	2017	24	22	252	298	16.25	94.57	2.47	0
E09	18/09/2017	SM2-05	2017	2	3	24	29	13.71	89.43	1.53	0.1
E09	19/09/2017	SM2-05	2017	1	1	8	10	14.39	70.38	2.13	0.1
E09	20/09/2017	SM2-05	2017	4	15	125	144	14.2	77.83	2.43	0
E09	25/09/2017	SM2-03	2017	0	2	26	28	15.47	96.83	1.53	1.2
E09	26/09/2017	SM2-03	2017	50	30	217	297	16.08	84.88	2	0
E09	27/09/2017	SM2-03	2017	26	29	146	201	14.25	97.29	1.25	0
E09	20/08/2018	SM2-04	2018	16	59	580	655	22.16	68.85	2.3	0
E09	21/08/2018	SM2-04	2018	24	51	398	473	20.1	84.48	2.27	0
E09	22/08/2018	SM2-04	2018	44	57	658	759	18.57	95.9	1.8	0
E10	29/06/2017	SM2-05	2017	17	352	412	781	15.25	94.67	2.18	0
E10	01/07/2017	SM2-05	2017	30	648	1087	1765	15.74	94.33	1.62	0.2
E10	02/07/2017	SM2-05	2017	36	1211	1592	2839	18.27	74.89	2.02	0
E10	06/07/2017	SM2-02	2017	147	1801	2135	4083	19.2	98.11	1.83	0
E10	07/07/2017	SM2-02	2017	182	2659	3108	5949	19.5	96.56	1.62	0
E10	08/07/2017	SM2-02	2017	134	2475	2617	5226	18.59	95.56	1.74	0
E10	09/07/2017	SM2-02	2017	127	1241	2838	4206	18.97	97.22	1.58	0
E10	27/07/2017	SM2-02	2017	426	748	1341	2515	19.5	95.37	1.62	0
E10	28/07/2017	SM2-02	2017	980	1216	2086	4282	19.72	95.95	1.69	0
E10	29/07/2017	SM2-02	2017	902	1164	2019	4085	20.55	95.32	1.57	0

E10	30/07/2017	SM2-02	2017	412	1101	2223	3736	20.51	98.58	1.79	0
E10	17/08/2017	SM2-03	2017	577	139	736	1452	19.76	93.1	2.26	0
E10	18/08/2017	SM2-03	2017	440	202	856	1498	21.43	83.85	2.24	0
E10	19/08/2017	SM2-03	2017	160	100	754	1014	22.66	65.85	4.78	0.1
E10	20/08/2017	SM2-03	2017	242	77	543	862	21.06	54.9	2.5	0
E10	28/08/2017	SM2-01	2017	707	310	2096	3113	20.67	97.18	1.57	0
E10	29/08/2017	SM2-01	2017	747	198	1122	2067	20.75	97.32	1.96	0
E10	30/08/2017	SM2-01	2017	169	156	2008	2333	21.65	83.05	4.17	0
E10	08/09/2017	SM2-05	2017	381	42	970	1393	16.14	85	2.35	0
E10	09/09/2017	SM2-05	2017	0	0	14	14	13.56	98	1.75	0.5
E10	10/09/2017	SM2-05	2017	11	21	347	379	14.4	79.27	2.16	0.1
E10	14/09/2017	SM2-05	2017	13	36	454	503	16.16	86.74	4.66	0.2
E10	28/09/2017	SM2-05	2017	40	21	222	283	15.2	97.79	1.1	0
E10	29/09/2017	SM2-05	2017	5	32	220	257	14.8	97.75	1.07	0
E10	30/09/2017	SM2-05	2017	0	1	1	2	15.36	98.6	1.38	0
E10	01/10/2017	SM2-05	2017	18	15	72	105	15.99	96.4	1.39	0
E10	09/08/2018	SM2-01	2018	139	380	2424	2943	21.8	91.55	3.45	0
E10	10/08/2018	SM2-01	2018	532	150	557	1239	19.64	82.95	2.88	0
E10	16/08/2018	SM2-01	2018	529	345	1930	2804	19.2	95.35	1.45	0
E11	12/06/2017	SM2-02	2017	28	452	531	1011	18.85	89.33	1.92	0
E11	13/06/2017	SM2-02	2017	12	496	425	933	19.46	92.17	2.03	0
E11	14/06/2017	SM2-02	2017	22	650	477	1149	19.42	91.89	1.67	0
E11	26/06/2017	SM2-01	2017	65	463	400	928	20.53	95.39	1.63	0
E11	27/06/2017	SM2-01	2017	123	740	643	1506	20.07	89.5	2.31	0
E11	31/07/2017	SM2-02	2017	564	1264	2452	4280	21.89	97.42	1.81	0
E11	01/08/2017	SM2-02	2017	583	859	1958	3400	22.24	97.89	2.31	0
E11	02/08/2017	SM2-02	2017	311	456	1440	2207	21.11	98.16	1.98	0
E11	10/08/2017	SM2-01	2017	259	315	682	1256	17.48	76.8	2.03	1.6
E11	11/08/2017	SM2-01	2017	215	335	859	1409	17	81.35	1.76	0.1
E11	12/08/2017	SM2-01	2017	240	176	622	1038	18.45	77.95	1.79	0
E11	13/08/2017	SM2-01	2017	211	163	789	1163	17.6	96.1	1.81	0
E11	17/08/2017	SM2-02	2017	328	200	875	1403	19.76	93.1	2.26	0
E11	18/08/2017	SM2-02	2017	256	328	1218	1802	21.43	83.85	2.24	0
E11	19/08/2017	SM2-02	2017	138	216	661	1015	22.66	65.85	4.78	0.1
E11	20/08/2017	SM2-02	2017	138	151	405	694	21.06	54.9	2.5	0
E11	31/08/2017	SM2-01	2017	20	54	378	452	18.68	87.55	2.93	0.4

E11	01/09/2017	SM2-01	2017	166	131	518	815	18.3	70	3.09	0.1
E11	02/09/2017	SM2-01	2017	486	124	624	1234	18.67	61	2.71	1.3
E11	03/09/2017	SM2-01	2017	299	93	336	728	18.15	80.91	1.44	0
E11	11/09/2017	SM2-05	2017	19	11	67	97	16.11	83.27	1.78	0
E11	12/09/2017	SM2-05	2017	2	6	31	39	17.42	86.95	1.49	0
E11	13/09/2017	SM2-05	2017	7	16	42	65	16.25	94.57	2.47	0
E11	28/09/2017	SM2-03	2017	24	25	75	124	15.2	97.79	1.1	0
E11	29/09/2017	SM2-03	2017	3	20	62	85	14.8	97.75	1.07	0
E11	30/09/2017	SM2-03	2017	2	18	35	55	15.36	98.6	1.38	0
E11	01/10/2017	SM2-03	2017	20	19	88	127	15.99	96.4	1.39	0
E11	02/10/2017	SM2-03	2017	17	17	64	98	18.11	96.52	1.35	0.6
E11	06/08/2018	SM2-04	2018	316	305	742	1363	22.98	93.7	1.78	0
E11	07/08/2018	SM2-04	2018	795	689	2247	3731	23.87	90.6	2.96	0.4
E11	08/08/2018	SM2-04	2018	681	561	1743	2985	23.48	89.35	3.26	0.5
E11	27/08/2018	SM2-02	2018	595	192	1868	2655	17.65	94	1.54	0
E11	28/08/2018	SM2-02	2018	419	1834	2938	5191	21.03	91.95	2.48	0
E11	29/08/2018	SM2-02	2018	100	263	2177	2540	21.07	95	3.11	1
E12	12/06/2017	SM2-01	2017	57	497	308	862	18.85	89.33	1.92	0
E12	13/06/2017	SM2-01	2017	86	363	298	747	19.46	92.17	2.03	0
E12	14/06/2017	SM2-01	2017	35	307	277	619	19.42	91.89	1.67	0
E12	19/06/2017	SM2-03	2017	26	268	190	484	19.92	92.67	2.46	0
E12	20/06/2017	SM2-03	2017	95	283	176	554	21.68	88.67	2.72	0
E12	21/06/2017	SM2-03	2017	79	231	142	452	21.05	93.89	1.65	0
E12	26/06/2017	SM2-02	2017	110	214	150	474	20.53	95.39	1.63	0
E12	27/06/2017	SM2-02	2017	159	270	251	680	20.07	89.5	2.31	0
E12	28/06/2017	SM2-02	2017	51	95	69	215	17.99	89.17	1.88	0.1
E12	06/07/2017	SM2-01	2017	145	444	388	977	19.2	98.11	1.83	0
E12	07/07/2017	SM2-01	2017	194	658	495	1347	19.5	96.56	1.62	0
E12	08/07/2017	SM2-01	2017	162	426	331	919	18.59	95.56	1.74	0
E12	09/07/2017	SM2-01	2017	240	840	655	1735	18.97	97.22	1.58	0
E12	07/08/2017	SM2-02	2017	1705	1266	3279	6250	22.71	84.2	3.73	0
E12	08/08/2017	SM2-02	2017	181	151	619	951	21.19	80.25	4.23	0
E12	09/08/2017	SM2-02	2017	1	14	63	78	17.06	71.25	3.92	1.3
E12	10/08/2017	SM2-02	2017	457	134	410	1001	17.48	76.8	2.03	1.6
E12	11/08/2017	SM2-02	2017	535	160	670	1365	17	81.35	1.76	0.1
E12	12/08/2017	SM2-02	2017	479	132	588	1199	18.45	77.95	1.79	0

E12	13/08/2017	SM2-02	2017	574	147	637	1358	17.6	96.1	1.81	0
E12	21/08/2017	SM2-02	2017	277	111	366	754	15.33	83.05	1.97	0
E12	22/08/2017	SM2-02	2017	402	141	760	1303	16.8	94.86	1.65	0
E12	23/08/2017	SM2-02	2017	336	156	709	1201	19.08	95.19	1.83	0
E12	08/09/2017	SM2-01	2017	137	54	357	548	16.14	85	2.35	0
E12	09/09/2017	SM2-01	2017	5	1	91	97	13.56	98	1.75	0.5
E12	10/09/2017	SM2-01	2017	44	63	443	550	14.4	79.27	2.16	0.1
E12	21/09/2017	SM2-05	2017	16	14	75	105	13.56	96.88	1.5	0
E12	22/09/2017	SM2-05	2017	1	0	2	3	14.28	97.58	1.56	0
E12	23/09/2017	SM2-05	2017	0	0	3	3	15.23	96.67	1.21	0
E12	24/09/2017	SM2-05	2017	5	12	10	27	16	97.83	2.03	0
E12	28/09/2017	SM2-01	2017	106	65	306	477	15.2	97.79	1.1	0
E12	29/09/2017	SM2-01	2017	44	74	278	396	14.8	97.75	1.07	0
E12	30/09/2017	SM2-01	2017	27	42	451	520	15.36	98.6	1.38	0
E12	01/10/2017	SM2-01	2017	94	119	564	777	15.99	96.4	1.39	0
E12	02/10/2017	SM2-01	2017	161	68	321	550	18.11	96.52	1.35	0.6
E12	09/08/2018	SM2-02	2018	725	212	710	1647	21.8	91.55	3.45	0
E12	10/08/2018	SM2-02	2018	679	233	816	1728	19.64	82.95	2.88	0
E13	12/06/2017	SM2-05	2017	69	1612	1628	3309	18.85	89.33	1.92	0
E13	13/06/2017	SM2-05	2017	103	1437	1830	3370	19.46	92.17	2.03	0
E13	14/06/2017	SM2-05	2017	87	935	1077	2099	19.42	91.89	1.67	0
E13	03/07/2017	SM2-01	2017	192	820	635	1647	17.34	92.11	2.18	0
E13	04/07/2017	SM2-01	2017	217	812	1073	2102	17.44	94.11	1.8	0
E13	05/07/2017	SM2-01	2017	268	720	694	1682	18.11	97.89	1.89	0
E13	17/07/2017	SM2-01	2017	169	1008	926	2103	18.61	94.5	2.02	0
E13	18/07/2017	SM2-01	2017	75	546	511	1132	22.09	90.78	4.28	0
E13	19/07/2017	SM2-01	2017	286	1258	1218	2762	23.11	90	3.5	0
E13	31/07/2017	SM2-05	2017	847	664	1767	3278	21.89	97.42	1.81	0
E13	01/08/2017	SM2-05	2017	453	384	1028	1865	22.24	97.89	2.31	0
E13	02/08/2017	SM2-05	2017	525	427	1266	2218	21.11	98.16	1.98	0
E13	21/08/2017	SM2-03	2017	38	52	289	379	15.33	83.05	1.97	0
E13	22/08/2017	SM2-03	2017	68	118	667	853	16.8	94.86	1.65	0
E13	23/08/2017	SM2-03	2017	81	112	752	945	19.08	95.19	1.83	0
E13	28/08/2017	SM2-02	2017	228	222	1538	1988	20.67	97.18	1.57	0
E13	29/08/2017	SM2-02	2017	263	180	1397	1840	20.75	97.32	1.96	0
E13	30/08/2017	SM2-02	2017	74	142	1115	1331	21.65	83.05	4.17	0

E13	04/09/2017	SM2-01	2017	161	141	583	885	17.55	94.18	1.53	0.3
E13	05/09/2017	SM2-01	2017	758	589	6474	7821	18.94	92.45	1.92	0
E13	06/09/2017	SM2-01	2017	153	393	846	1392	20.23	79.55	1.62	0
E13	07/09/2017	SM2-01	2017	183	72	658	913	17.33	76.77	1.95	0
E13	06/08/2018	SM2-05	2018	191	370	960	1521	22.98	93.7	1.78	0
E13	07/08/2018	SM2-05	2018	412	574	2122	3108	23.87	90.6	2.96	0.4
E13	08/08/2018	SM2-05	2018	326	550	1716	2592	23.48	89.35	3.26	0.5
E13	20/08/2018	SM2-01	2018	161	338	1564	2063	22.16	68.85	2.3	0
E13	21/08/2018	SM2-01	2018	254	434	1236	1924	20.1	84.48	2.27	0
E13	22/08/2018	SM2-01	2018	292	221	1113	1626	18.57	95.9	1.8	0
E13	27/08/2018	SM2-05	2018	147	112	815	1074	17.65	94	1.54	0
E13	28/08/2018	SM2-05	2018	277	643	1954	2874	21.03	91.95	2.48	0
E13	29/08/2018	SM2-05	2018	234	148	981	1363	21.07	95	3.11	1
E14	15/06/2017	SM2-03	2017	63	510	548	1121	21.21	85.83	1.89	0
E14	16/06/2017	SM2-03	2017	106	575	497	1178	25.28	61.78	3.09	0
E14	17/06/2017	SM2-03	2017	80	404	876	1360	19.57	79.22	2.01	0
E14	29/06/2017	SM2-02	2017	297	861	1860	3018	15.25	94.67	2.18	0
E14	30/06/2017	SM2-02	2017	12	163	253	428	13.94	95.72	1.84	1.3
E14	01/07/2017	SM2-02	2017	168	1010	1818	2996	15.74	94.33	1.62	0.2
E14	02/07/2017	SM2-02	2017	217	1090	1786	3093	18.27	74.89	2.02	0
E14	20/07/2017	SM2-01	2017	194	591	906	1691	20.79	94.67	2.69	0
E14	21/07/2017	SM2-01	2017	97	358	615	1070	19.26	93.5	1.61	0
E14	22/07/2017	SM2-01	2017	203	551	1164	1918	20.02	95.68	1.95	0
E14	23/07/2017	SM2-01	2017	137	558	794	1489	19.6	97.37	2.07	0
E14	10/08/2017	SM2-05	2017	149	395	748	1292	17.48	76.8	2.03	1.6
E14	11/08/2017	SM2-05	2017	170	440	922	1532	17	81.35	1.76	0.1
E14	12/08/2017	SM2-05	2017	87	294	760	1141	18.45	77.95	1.79	0
E14	13/08/2017	SM2-05	2017	103	188	776	1067	17.6	96.1	1.81	0
E14	04/09/2017	SM2-05	2017	10	14	119	143	17.55	94.18	1.53	0.3
E14	05/09/2017	SM2-05	2017	138	160	1126	1424	18.94	92.45	1.92	0
E14	06/09/2017	SM2-05	2017	14	47	156	217	20.23	79.55	1.62	0
E14	07/09/2017	SM2-05	2017	9	11	109	129	17.33	76.77	1.95	0
E14	14/09/2017	SM2-01	2017	24	33	266	323	16.16	86.74	4.66	0.2
E14	15/09/2017	SM2-01	2017	8	5	31	44	13.08	92.3	3	5.9
E14	16/09/2017	SM2-01	2017	20	0	14	34	10.59	98.04	2.04	8.4
E14	17/09/2017	SM2-01	2017	6	31	331	368	12.63	91.96	1.62	0

E14	28/09/2017	SM2-02	2017	19	49	311	379	15.2	97.79	1.1	0
E14	23/08/2018	SM2-01	2018	200	456	1983	2639	19.33	90	1.89	0
E14	30/08/2018	SM2-01	2018	200	156	1335	1691	20.14	91	2.07	1.5
E15	29/06/2017	SM2-01	2017	74	387	429	890	15.25	94.67	2.18	0
E15	30/06/2017	SM2-01	2017	4	18	63	85	13.94	95.72	1.84	1.3
E15	01/07/2017	SM2-01	2017	64	771	887	1722	15.74	94.33	1.62	0.2
E15	02/07/2017	SM2-01	2017	89	1205	1026	2320	18.27	74.89	2.02	0
E15	10/07/2017	SM2-01	2017	644	2705	3852	7201	19.82	94.11	1.84	0
E15	11/07/2017	SM2-01	2017	345	2710	4384	7439	19.29	96.67	1.57	0
E15	12/07/2017	SM2-01	2017	475	2896	4507	7878	20.08	95.17	1.62	0
E15	07/08/2017	SM2-05	2017	1035	1183	3050	5268	22.71	84.2	3.73	0
E15	08/08/2017	SM2-05	2017	100	165	617	882	21.19	80.25	4.23	0
E15	09/08/2017	SM2-05	2017	14	7	171	192	17.06	71.25	3.92	1.3
E15	17/08/2017	SM2-01	2017	149	229	1119	1497	19.76	93.1	2.26	0
E15	18/08/2017	SM2-01	2017	398	398	2128	2924	21.43	83.85	2.24	0
E15	19/08/2017	SM2-01	2017	163	194	1077	1434	22.66	65.85	4.78	0.1
E15	20/08/2017	SM2-01	2017	83	116	551	750	21.06	54.9	2.5	0
E15	11/09/2017	SM2-01	2017	345	335	3015	3695	16.11	83.27	1.78	0
E15	12/09/2017	SM2-01	2017	197	221	1792	2210	17.42	86.95	1.49	0
E15	13/09/2017	SM2-01	2017	175	141	2289	2605	16.25	94.57	2.47	0
E15	18/09/2017	SM2-03	2017	11	9	104	124	13.71	89.43	1.53	0.1
E15	19/09/2017	SM2-03	2017	2	3	92	97	14.39	70.38	2.13	0.1
E15	20/09/2017	SM2-03	2017	24	28	434	486	14.2	77.83	2.43	0
E15	27/08/2018	SM2-01	2018	198	264	2242	2704	17.65	94	1.54	0
E15	28/08/2018	SM2-01	2018	678	961	2919	4558	21.03	91.95	2.48	0
E15	29/08/2018	SM2-01	2018	284	378	2536	3198	21.07	95	3.11	1

S3 – Tables – Mosquito sampling

S2 – Mosquitoes sampling

Localities

Location	Lat	Long
E01	41.986318	3.142632
E02	41.990976	3.149114
E03	41.990829	3.144031
E04	41.992182	3.141993
E05	41.99424	3.145898
E06	42.001584	3.135373
E07	42.010795	3.145489
E08	42.009536	3.151077
E09	42.009041	3.157453
E10	42.00149	3.153075
E11	42.001285	3.158255
E12	42.004265	3.165302
E13	42.003761	3.1774
E14	42.006945	3.185681
E15	42.000944	3.187487

Species codes

Code	**Species**
Culpip	*Culex pipiens*
Culmod	*Culex modestus*
Culthe	*Culex theileri*
Culsp	*Culex sp.*
Anomac	*Anopheles maculipennis*
Anosp	*Anopheles sp.*
Aedsp	*Aedes sp.*
Aedcas	*Aedes caspius*
Aedvex	*Aedes vexans*
Cullon	*Culiseta longiareolata*
Noid	Non identified

Mosquitoes activity

Sample	Location	Date	Culpip	Culmod	Culthe	Culsp	Anomac	Anosp	Aedsp	Aedcas	Aedvex	Cullon	Noid	Pooled
1	E09	19/06/2017	0	0	0	0	0	0	0	0	0	0	0	0
2	E13	19/06/2017	1	0	0	0	0	0	0	0	0	0	0	1
3	E12	19/06/2017	1	0	1	2	0	0	0	0	0	0	0	4
4	E01	19/06/2017	5	2	1	8	0	0	0	0	0	0	1	17
5	E05	19/06/2017	4	1	0	5	0	0	0	0	0	0	0	10
6	E06	19/06/2017	10	4	0	1	0	0	0	0	0	0	0	15
7	E03	22/06/2017	12	16	0	4	0	0	0	0	0	0	5	37
8	E08	22/06/2017	16	1	2	0	0	0	0	0	0	0	0	19
9	E14	22/06/2017	9	10	1	1	0	0	0	0	0	0	0	21
10	E02	22/06/2017	0	0	0	0	0	0	0	0	0	0	0	0
11	E07	22/06/2017	24	28	0	4	0	0	0	0	0	0	4	60
12	E04	22/06/2017	0	3	0	0	0	0	0	0	0	0	0	3
13	E05	26/06/2017	11	12	0	2	0	2	0	0	0	0	0	27
14	E06	26/06/2017	24	35	1	2	0	0	0	0	0	0	3	65
15	E09	26/06/2017	7	3	0	0	0	0	0	0	0	0	0	10
16	E12	26/06/2017	4	4	0	1	0	0	0	0	0	0	2	11
17	E03	26/06/2017	5	4	0	2	0	0	0	0	0	0	0	11
18	E01	26/06/2017	26	73	0	2	0	2	0	0	0	0	8	111
19	E14	29/06/2017	37	40	0	3	0	2	0	0	0	0	1	83
20	E07	29/06/2017	27	80	0	0	0	0	0	0	0	0	10	117
21	E01	29/06/2017	14	19	1	5	0	1	0	0	0	0	1	41
22	E15	29/06/2017	23	22	0	0	0	0	0	0	0	0	0	45
23	E08	29/06/2017	23	41	1	1	0	0	0	0	0	0	0	66
24	E10	29/06/2017	6	11	1	0	0	0	0	0	0	0	0	18
25	E04	03/07/2017	18	20	1	2	0	0	0	0	0	0	1	42
26	E03	03/07/7017	6	2	1	10	0	0	0	0	0	0	1	20
27	E02	03/07/2017	1	0	0	0	0	0	0	0	0	0	0	1
28	E11	03/07/2017	10	15	0	2	0	0	0	0	0	0	0	27
29	E13	03/07/2017	9	1	0	0	0	0	0	0	0	0	0	10
30	E05	03/07/2017	13	3	0	1	0	1	0	0	0	0	0	18
31	E12	06/07/2017	7	7	0	2	0	0	0	0	0	0	0	16
32	E08	06/07/2017	2	1	0	1	0	0	0	0	0	0	0	4
33	E14	06/07/2017	16	9	0	0	0	0	0	0	0	0	1	26
34	E06	06/07/2017	2	15	0	1	0	0	0	0	0	0	1	19
35	E01	06/07/2017	0	4	0	1	0	0	0	0	0	0	0	5
36	E10	06/07/2017	11	1	0	0	0	0	0	0	0	0	0	12
37	E09	10/07/2017	3	18	0	0	0	0	0	0	0	0	0	21

38	E04	10/07/2017	19	102	0	11	0	0	0	0	0	0	0	132
39	E07	10/07/2017	16	42	0	3	0	0	0	0	0	0	0	61
40	E15	10/07/2017	24	19	0	2	0	0	0	0	0	0	0	45
41	E02	10/07/2017	0	2	0	0	0	0	0	0	0	0	0	2
42	E11	10/07/2017	10	18	0	1	0	0	0	0	0	0	0	29
43	E06	13/07/2017	12	40	0	0	0	0	0	0	0	0	0	52
44	E05	13/07/2017	8	12	1	1	0	0	0	0	0	0	0	22
45	E08	13/07/2017	4	15	0	1	0	0	0	0	0	0	0	20
46	E10	13/07/2017	4	13	0	0	0	0	0	0	0	0	0	17
47	E01	13/07/2017	2	8	0	0	0	0	0	0	0	0	0	10
48	E03	13/07/2018	3	15	3	0	0	0	0	0	0	0	0	21
49	E09	17/07/2017	5	3	0	0	0	0	0	0	0	0	0	8
50	E13	17/07/2017	10	12	0	3	0	0	0	0	0	0	0	25
51	E12	17/07/2017	11	3	0	0	0	0	0	0	0	0	0	14
52	E04	17/07/2017	10	17	1	0	0	0	0	0	0	0	0	28
53	E07	17/07/2017	8	7	0	0	0	0	0	0	0	0	0	15
54	E15	17/07/2017	6	2	0	1	0	0	0	0	0	0	0	9
55	E14	20/07/2017	18	7	0	1	0	0	0	0	0	0	0	26
56	E02	20/07/2018	5	2	0	0	0	0	0	0	0	0	0	7
57	E11	20/07/2017	19	5	0	0	0	0	0	0	0	0	0	24
58	E05	20/07/2017	11	5	0	0	0	0	0	0	0	0	0	16
59	E10	20/07/2017	9	2	0	1	0	0	0	0	0	0	0	12
60	E01	20/07/2017	14	3	0	0	0	0	0	0	0	0	0	17
61	E07	24/07/2017	1	9	0	2	0	0	0	0	0	0	0	12
62	E15	24/07/2017	10	8	0	2	0	0	0	0	0	0	0	20
63	E08	24/07/2017	2	0	0	4	0	0	0	0	0	0	0	6
64	E09	24/07/2017	3	4	0	1	0	0	0	0	0	0	0	8
65	E04	24/07/2017	2	16	0	4	0	0	0	0	0	0	0	22
66	E03	24/07/2017	3	3	0	1	0	0	0	0	0	0	0	7
67	E05	27/07/2017	9	36	2	1	0	0	0	0	0	0	0	48
68	E10	27/07/2018	4	6	0	1	0	0	0	0	0	0	0	11
69	E12	27/07/2017	9	15	0	0	0	0	0	0	0	0	0	24
70	E01	27/07/2017	7	9	0	2	0	0	0	0	0	0	2	20
71	E06	27/07/2017	15	13	0	0	0	0	0	0	0	0	0	28
72	E09	31/07/2017	32	69	0	4	0	0	0	0	0	0	0	105
73	E13	31/07/2017	5	12	0	1	0	0	0	0	0	0	0	18
74	E02	31/07/2017	0	2	0	0	0	0	0	0	0	0	0	2
75	E15	31/07/2017	6	5	0	0	0	0	0	0	0	0	0	11
76	E11	31/07/2017	2	21	0	1	0	0	0	0	0	0	6	30
77	E03	31/07/2017	11	37	0	2	0	0	0	0	0	1	0	51
78	E05	03/08/2017	5	23	0	0	0	0	0	0	0	0	0	28

79	E08	03/08/2017	1	9	0	1	0	0	0	0	0	0	0	11
80	E01	03/08/2017	17	12	0	0	0	0	0	0	0	0	0	29
81	E04	03/08/2017	1	37	0	0	0	0	0	0	0	0	0	38
82	E14	03/08/2017	6	1	0	0	0	0	0	0	0	0	0	7
83	E07	03/08/2017	5	3	0	0	0	0	0	0	0	0	0	8
84	E02	07/08/2017	2	1	0	0	0	0	0	0	0	0	0	3
85	E15	07/08/2017	3	0	0	1	0	0	0	0	0	0	0	4
86	E06	07/08/2017	3	5	0	0	0	0	0	0	0	0	0	8
87	E03	07/08/2017	8	8	0	0	0	0	0	0	0	0	0	16
88	E09	07/08/2017	10	9	0	0	0	0	0	0	0	0	0	19
89	E10	07/08/2017	0	1	0	0	0	0	0	0	0	0	0	1
90	E11	10/08/2017	14	4	0	0	0	0	0	0	0	0	0	18
91	E13	10/08/2017	7	1	0	1	0	0	0	0	0	0	0	9
92	E07	10/08/2017	21	20	0	0	0	0	0	0	0	1	0	42
93	E14	10/08/2017	28	1	0	2	0	0	0	0	0	0	0	31
94	E12	10/08/2017	5	2	0	0	0	0	0	0	0	0	0	7
95	E08	10/08/2017	3	7	0	0	0	0	0	0	0	0	0	10
96	E06	14/08/2017	3	11	0	0	0	0	0	0	0	0	0	14
97	E03	14/08/2017	3	6	0	0	0	0	0	0	0	0	0	9
98	E05	14/08/2017	1	6	0	0	0	0	0	0	0	0	0	7
99	E09	14/08/2017	1	2	0	1	0	0	0	0	0	0	0	4
100	E01	14/08/2017	2	7	0	0	0	0	0	0	0	0	0	9
101	E02	14/08/2017	2	6	0	0	0	0	0	0	0	0	0	8
102	E10	17/08/2017	2	0	0	0	0	0	0	0	0	0	0	2
103	E11	17/08/2017	2	0	0	0	0	0	0	0	0	0	0	2
104	E07	17/08/2017	4	23	0	0	0	0	0	0	0	0	1	28
105	E15	17/08/2017	6	0	0	1	0	0	0	0	0	0	2	9
106	E14	17/08/2017	8	1	0	1	0	0	0	0	0	0	0	10
107	E04	17/08/2017	0	0	0	0	0	0	0	0	0	0	0	0
108	E01	21/08/2017	1	0	0	0	0	0	0	0	0	0	0	1
109	E05	21/08/2017	6	7	0	0	0	0	0	0	0	0	0	13
110	E06	21/08/2017	12	41	1	0	0	0	0	0	0	0	0	54
111	E12	21/08/2017	5	1	0	0	0	0	0	0	0	0	0	6
112	E08	21/08/2017	3	4	0	0	0	0	0	0	0	0	1	8
113	E13	21/08/2017	5	0	0	0	0	0	0	0	0	0	0	5
114	E14	24/08/2017	2	1	0	1	0	0	0	0	0	0	0	4
115	E09	24/08/2017	6	20	0	1	0	0	0	0	0	0	0	27
116	E11	24/08/2017	6	4	0	0	0	0	0	0	0	0	0	10
117	E15	24/08/2017	4	3	0	1	0	0	0	0	0	0	0	8
118	E02	24/08/2017	0	0	0	0	0	0	0	0	0	0	0	0
119	E07	24/08/2017	7	19	0	0	0	0	0	0	0	0	0	26

120	E13	28/08/2017	17	21	0	0	0	0	0	0	0	0	1	39
121	E12	28/08/2017	2	0	0	0	0	0	0	0	0	0	0	2
122	E05	28/08/2017	4	14	0	0	0	0	0	0	0	0	0	18
123	E04	28/08/2017	1	13	0	0	0	0	0	0	0	0	0	14
124	E03	28/08/2017	1	4	0	0	0	0	0	0	0	0	0	5
125	E10	28/08/2017	2	0	0	0	0	0	0	0	0	0	0	2
126	E08	31/08/2017	9	5	0	0	0	0	0	0	0	0	0	14
127	E11	31/08/2017	2	1	0	0	0	0	0	0	0	0	0	3
128	E02	31/08/2017	6	8	0	0	0	0	0	0	0	0	0	14
129	E01	31/08/2017	7	7	0	0	0	0	0	0	0	0	0	14
130	E09	31/08/2017	8	10	0	0	0	0	0	0	0	0	0	18
131	E06	31/08/2017	11	62	0	7	0	0	0	0	0	0	1	81
132	E04	04/09/2017	9	52	0	0	0	0	0	0	0	0	0	61
133	E14	04/09/2017	13	14	0	0	0	0	0	0	0	0	0	27
134	E07	04/09/2017	22	47	0	1	0	0	0	0	0	0	0	70
135	E15	04/09/2017	92	5	0	4	0	0	0	0	0	0	0	101
136	E13	04/09/2017	10	12	1	0	0	0	0	0	0	0	1	24
137	E05	04/09/2017	1	10	0	1	0	0	0	0	0	0	0	12
138	E02	08/09/2017	11	2	0	0	0	0	0	0	0	0	0	13
139	E08	08/09/2017	4	7	0	0	0	0	0	0	0	0	0	11
140	E10	08/09/2017	2	2	0	0	0	0	0	0	0	0	0	4
141	E12	08/09/2017	6	2	0	1	0	0	0	0	0	0	0	9
142	E03	08/09/2017	4	2	0	0	0	0	0	0	0	0	0	6
143	E01	08/09/2017	3	3	0	0	0	0	0	0	0	0	0	6
144	E04	11/09/2017	8	56	0	0	0	0	0	0	0	0	0	64
145	E09	11/09/2017	14	83	0	0	0	0	0	0	0	0	0	97
146	E13	11/09/2017	17	37	0	0	0	0	0	0	0	0	0	54
147	E06	11/09/2017	19	129	0	0	0	1	0	0	0	0	0	149
148	E15	11/09/2017	17	20	0	0	0	0	0	0	0	0	0	37
149	E11	11/09/2017	22	38	0	0	0	0	0	0	0	0	1	61
150	E14	14/09/2017	26	22	0	0	0	0	0	0	0	0	0	48
151	E08	14/09/2017	4	6	0	0	0	0	0	0	0	0	0	10
152	E12	14/09/2017	7	1	0	0	0	0	0	0	0	0	0	8
153	E03	14/09/2017	2	1	0	0	0	0	0	0	0	0	0	3
154	E05	14/09/2017	10	17	0	0	0	0	0	0	0	0	0	27
155	E10	14/09/2017	3	15	0	0	0	0	0	0	0	0	0	18
156	E13	18/09/2017	3	20	0	0	0	0	0	0	0	0	0	23
157	E06	18/09/2017	7	25	0	0	0	0	0	0	0	0	0	32
158	E09	18/09/2017	2	30	0	0	0	0	0	0	0	0	0	32
159	E11	18/09/2017	5	4	0	0	0	0	0	0	0	0	0	9
160	E15	18/09/2017	2	4	0	1	0	0	0	0	0	0	0	7

161	E07	18/09/2017	2	33	0	1	0	0	0	0	0	0	0	36
162	E03	21/09/2017	0	0	0	0	0	0	0	0	0	0	0	0
163	E10	21/09/2017	2	1	0	0	0	0	0	0	0	0	0	3
164	E12	21/09/2017	2	0	0	0	0	0	0	0	0	0	0	2
165	E01	21/09/2017	5	15	0	0	0	0	0	0	0	0	0	20
166	E02	21/09/2017	1	5	0	0	0	0	0	0	0	0	0	6
167	E04	21/09/2017	3	16	0	0	0	0	0	0	0	0	0	19
168	E15	25/09/2017	4	2	0	1	0	0	0	0	0	0	0	7
169	E13	25/09/2017	5	11	0	0	0	0	0	0	0	0	0	16
170	E05	25/09/2017	1	3	0	0	0	0	0	0	0	0	0	4
171	E09	25/09/2017	1	1	0	0	0	0	0	0	0	0	0	2
172	E08	25/09/2017	0	1	0	0	0	0	0	0	0	0	0	1
173	E07	25/09/2017	3	1	0	0	0	0	0	0	0	0	0	4
174	E14	28/09/2017	10	0	0	0	0	0	0	0	0	0	0	10
175	E10	28/09/2017	0	0	0	0	0	0	0	0	0	0	0	0
176	E04	28/09/2017	13	15	0	0	0	1	0	0	0	0	0	29
177	E11	28/09/2017	4	5	0	0	0	0	0	0	0	0	0	9
178	E06	28/09/2017	4	16	0	2	0	0	0	0	0	0	0	22
179	E12	28/09/2017	2	3	0	0	0	0	0	0	0	0	0	5
180	E07	05/06/2018	4	0	0	0	0	0	0	0	0	0	0	4
181	E14	05/06/2018	3	0	0	0	0	0	0	0	0	0	0	3
182	E13	05/06/2018	18	0	0	5	0	0	0	0	0	0	0	23
183	E05	05/06/2018	4	1	0	0	0	0	0	0	0	0	0	5
184	E04	05/06/2018	1	2	0	0	0	0	0	0	0	0	0	3
185	E15	05/06/2018	5	0	1	0	0	0	0	0	0	0	0	6
186	E12	07/06/2018	2	2	0	0	0	0	0	0	0	0	0	4
187	E08	07/06/2018	0	1	0	0	0	0	0	0	0	0	0	1
188	E02	07/06/2018	2	1	0	0	0	0	0	0	0	0	0	3
189	E03	07/06/2018	2	0	0	0	0	0	0	0	0	0	0	2
190	E10	07/06/2018	1	0	0	1	0	0	0	0	0	0	0	2
191	E01	07/06/2018	0	0	0	3	0	0	0	1	0	0	0	4
192	E15	11/06/2018	2	0	0	0	0	0	0	0	0	0	0	2
193	E11	11/06/2018	3	2	0	0	0	0	0	0	0	0	0	5
194	E09	11/06/2018	8	4	0	0	0	0	0	0	0	0	0	12
195	E06	11/06/2018	5	1	0	0	0	0	0	0	0	0	0	6
196	E13	11/06/2018	7	4	0	1	0	0	1	0	1	0	0	14
197	E04	11/06/2018	9	2	0	0	0	0	0	0	0	0	0	11
198	E12	14/06/2018	1	1	0	0	0	0	0	0	0	0	0	2
199	E14	14/06/2018	1	1	0	0	0	0	0	0	0	0	0	1
200	E05	14/06/2018	1	0	0	0	0	0	0	0	0	0	0	1
201	E03	14/06/2018	2	0	0	1	0	0	0	0	0	0	0	3

202	E10	14/06/2018	0	1	0	0	0	0	0	0	0	0	0	1
203	E08	14/06/2018	1	0	0	0	0	0	0	0	0	0	0	1
204	E06	18/06/2018	0	0	0	0	0	0	0	0	0	0	0	0
205	E07	18/06/2018	0	0	0	0	0	0	0	0	0	0	0	0
206	E13	18/06/2018	0	0	0	0	0	0	0	0	0	0	0	0
207	E09	18/06/2018	0	0	0	0	0	0	0	0	0	0	0	0
208	E15	18/06/2018	11	6	0	0	0	0	0	0	0	0	0	17
209	E11	18/06/2018	0	1	0	0	0	0	0	0	0	0	0	1
210	E04	21/06/2018	3	2	0	0	0	0	0	0	0	0	0	5
211	E03	21/06/2018	1	0	0	1	0	0	0	0	0	0	0	2
212	E01	21/06/2018	7	1	0	0	0	0	0	0	0	0	0	8
213	E12	21/06/2018	11	40	0	4	0	0	0	0	0	0	0	55
214	E02	21/06/2018	2	0	0	0	0	0	0	0	0	0	0	2
215	E10	21/06/2018	7	0	0	0	0	0	0	0	0	0	0	7
216	E09	25/06/2018	4	2	0	3	0	0	0	0	0	0	0	9
217	E01	25/06/2018	2	4	0	3	0	0	0	0	0	0	0	9
218	E03	25/06/2018	0	0	0	0	0	0	0	0	0	0	0	0
219	E02	25/06/2018	1	2	0	0	0	0	0	0	0	0	0	3
220	E05	25/06/2018	3	1	0	1	0	0	0	0	0	0	0	5
221	E06	25/06/2018	5	2	0	4	0	0	0	0	0	0	0	11
222	E15	28/06/2018	12	5	0	0	0	0	0	0	0	0	0	17
223	E14	28/06/2018	9	20	0	1	0	0	0	0	0	0	0	30
224	E11	28/06/2018	17	19	0	1	0	0	0	0	0	0	0	37
225	E10	28/06/2018	0	1	0	0	0	0	0	0	0	0	0	1
226	E04	28/06/2018	2	2	0	0	0	0	0	0	0	0	0	4
227	E07	28/06/2018	6	5	0	0	0	0	0	0	0	0	0	11
228	E06	02/07/2018	1	6	0	0	0	0	0	0	0	0	0	7
229	E01	02/07/2018	11	31	0	0	0	0	0	0	0	0	0	42
230	E05	02/07/2018	4	12	0	1	0	0	0	0	0	0	0	17
231	E12	02/07/2018	18	5	0	0	0	0	0	0	0	0	0	23
232	E08	02/07/2018	5	2	0	0	0	0	0	0	0	0	0	7
233	E13	02/07/2018	3	4	0	0	0	0	0	0	0	0	0	7
234	E15	05/07/2018	8	4	0	2	0	0	0	1	0	0	0	15
235	E11	05/07/2018	13	0	0	0	0	0	0	0	0	0	0	13
236	E07	05/07/2018	26	15	0	0	0	0	0	0	0	0	0	41
237	E09	05/07/2018	14	6	0	3	0	0	0	0	0	0	0	23
238	E02	05/07/2018	3	2	0	0	0	0	0	0	0	0	0	5
239	E14	05/07/2018	2	3	0	0	0	0	0	0	0	0	0	5
240	E05	09/07/2018	16	1	0	3	0	0	0	0	0	0	0	20
241	E04	09/07/2018	7	0	0	2	0	0	0	0	0	0	0	9
242	E13	09/07/2018	6	9	0	0	0	0	0	0	0	0	0	15

243	E03	09/07/2018	6	6	0	1	0	0	0	0	0	0	0	13
244	E12	09/07/2018	14	0	0	1	0	0	0	0	0	0	0	15
245	E10	09/07/2018	0	0	0	0	0	0	0	0	0	0	0	0
246	E11	12/07/2018	4	6	0	1	0	0	0	0	0	0	0	11
247	E02	12/07/2018	4	26	0	6	0	0	0	0	0	0	0	36
248	E09	12/07/2018	11	8	0	0	0	0	0	0	0	0	0	19
249	E01	12/07/2018	2	17	0	0	0	0	0	0	0	0	0	19
250	E08	12/07/2018	8	7	0	0	0	0	0	0	0	0	0	15
251	E06	12/07/2018	4	12	0	3	0	0	0	0	0	0	0	19
252	E03	16/07/2018	68	116	0	2	0	1	0	0	0	0	0	187
253	E15	16/07/2018	7	30	0	3	0	0	0	0	0	0	0	40
254	E04	16/07/2018	13	37	0	0	0	1	0	0	0	0	0	51
255	E06	19/07/2018	7	4	0	1	0	0	0	0	0	0	0	12
256	E14	19/07/2018	20	21	0	4	0	0	0	0	0	0	0	45
257	E10	19/07/2018	16	16	0	2	0	0	0	0	0	0	0	34
258	E12	19/07/2018	12	23	0	1	0	1	0	0	0	0	0	37
259	E05	19/07/2018	11	8	0	6	0	0	0	0	0	0	0	25
260	E01	19/07/2018	1	3	0	0	0	0	0	0	0	0	0	4
262	E02	23/07/2018	0	0	0	0	0	0	0	0	0	0	0	0
263	E13	23/07/2018	68	134	0	8	0	0	0	0	0	0	0	210
264	E11	23/07/2018	9	11	0	10	0	0	0	0	0	0	0	30
265	E09	23/07/2018	12	21	0	9	0	0	0	0	0	0	0	42
266	E15	23/07/2018	59	50	0	5	0	0	0	0	0	0	0	114
267	E03	23/07/2018	24	15	0	17	0	0	0	0	0	0	0	56
268	E01	26/07/2018	42	79	0	36	0	0	0	0	0	0	0	157
269	E07	26/07/2018	43	64	1	50	0	3	0	0	0	0	0	161
270	E14	26/07/2018	72	72	0	5	0	0	0	0	0	0	0	149
271	E05	26/07/2018	27	7	0	9	0	2	0	0	0	0	0	45
272	E04	26/07/2018	17	29	0	24	0	0	0	0	0	0	0	70
273	E08	26/07/2018	18	28	0	1	0	0	0	0	0	0	0	47
274	E10	30/07/2018	9	6	0	6	0	0	0	0	0	0	0	21
275	E06	30/07/2018	11	14	0	13	0	0	0	0	0	0	0	38
276	E03	30/07/2018	7	15	0	3	0	0	0	0	0	0	0	25
277	E09	30/07/2018	11	3	0	1	0	0	0	0	0	0	0	15
278	E15	30/07/2018	20	14	0	11	0	0	0	0	0	0	0	45
279	E02	30/07/2018	7	8	0	3	0	0	0	0	0	0	0	18
280	E14	02/08/2018	36	90	0	1	0	0	0	0	0	0	0	127
281	E13	02/08/2018	57	67	0	9	0	0	0	0	0	0	0	133
282	E11	02/08/2018	19	42	0	2	0	0	0	0	0	0	0	63
283	E08	02/08/2018	8	3	0	0	0	0	0	0	0	0	0	11
284	E07	02/08/2018	21	18	0	20	0	0	0	0	0	0	0	59

285	E12	02/08/2018	4	10	0	0	0	0	0	0	0	0	0	14
286	E04	06/08/2018	8	8	0	0	0	0	0	0	0	0	0	16
287	E03	06/08/2018	1	1	0	2	0	0	0	0	0	0	0	4
288	E11	06/08/2018	6	8	0	0	0	0	0	0	0	0	0	14
289	E13	06/08/2018	8	12	0	8	0	0	0	0	0	0	0	28
291	E05	06/08/2018	2	1	0	5	0	0	0	0	0	0	0	8
292	E14	09/08/2018	85	91	0	122	0	2	0	0	0	0	0	300
293	E06	09/08/2018	16	37	0	8	0	0	0	0	0	0	0	61
294	E12	09/08/2018	35	40	0	3	0	0	0	0	0	0	0	78
295	E08	09/08/2018	15	12	0	1	0	0	0	0	0	0	0	28
296	E10	09/08/2018	10	27	0	13	0	0	0	0	0	0	0	50
297	E01	09/08/2018	11	16	0	0	0	0	0	0	0	0	0	27
298	E15	13/08/2018	61	89	0	6	0	0	0	0	0	0	0	156
299	E04	13/08/2018	27	44	0	3	0	0	0	0	0	0	0	74
300	E07	13/08/2018	45	76	0	0	0	0	0	0	0	0	0	121
301	E02	13/08/2018	0	3	0	0	0	0	0	0	0	0	0	3
302	E09	13/08/2018	11	5	0	1	0	0	0	0	0	0	0	17
303	E11	13/08/2018	7	33	0	0	0	0	0	0	0	0	0	40
304	E03	16/08/2018	9	22	0	2	0	0	0	1	0	0	0	34
305	E05	16/08/2018	9	9	0	0	0	0	0	0	0	0	0	18
306	E06	16/08/2018	15	26	0	0	0	0	0	0	0	0	0	41
307	E10	16/08/2018	1	0	0	0	0	0	0	0	0	0	0	1
308	E08	16/09/2018	6	10	0	0	0	0	0	0	0	0	0	16
309	E01	16/09/2018	3	3	0	1	0	0	0	0	0	0	0	7
310	E04	20/08/2018	33	11	0	5	0	0	0	0	0	0	0	49
311	E12	20/08/2018	77	21	0	3	0	0	0	0	0	0	0	101
312	E13	20/08/2018	11	23	0	8	0	0	0	0	0	0	0	42
313	E15	20/08/2018	8	11	0	2	0	0	0	0	0	0	0	21
314	E07	20/08/2018	22	54	0	0	0	0	0	0	0	0	0	76
315	E09	20/08/2018	28	1	0	1	0	0	0	0	0	0	0	30
316	E05	23/08/2018	3	8	0	1	0	0	0	0	0	0	0	12
317	E01	23/08/2018	9	21	0	0	0	0	0	0	0	0	0	30
318	E14	23/08/2018	2	0	0	0	0	0	0	0	0	0	0	2
319	E10	23/08/2018	2	1	0	0	0	0	0	0	0	0	0	3
320	E02	23/08/2018	2	2	0	0	0	0	0	0	0	0	0	4
321	E11	23/08/2018	8	6	0	0	0	0	0	0	0	0	0	14
322	E13	27/08/2018	39	84	0	2	0	0	0	0	0	0	0	125
323	E15	27/08/2018	6	9	0	0	0	0	0	0	0	0	0	15
324	E12	27/08/2018	3	2	0	0	0	0	0	0	0	0	0	5
325	E11	27/08/2018	7	2	0	2	0	0	0	0	0	0	0	11
326	E06	27/08/2018	5	2	0	0	0	0	0	0	0	0	0	7

327	E03	30/08/2018	9	27	0	1	0	0	0	0	0	0	0	37
328	E04	30/08/2018	2	2	0	1	0	0	0	0	0	0	0	5
329	E08	30/08/2018	2	3	0	0	0	0	0	0	0	0	0	5
330	E14	30/08/2018	0	0	0	0	0	0	0	0	0	0	0	0
331	E10	30/08/2018	2	0	0	0	0	0	0	0	0	0	0	2
332	E02	30/08/2018	0	0	0	0	0	0	0	0	0	0	0	0
333	E13	03/09/2018	3	0	0	0	0	0	0	0	0	0	0	3
334	E09	03/09/2018	4	0	0	0	0	0	0	0	0	0	0	4
335	E06	03/09/2018	2	0	0	0	0	0	0	0	0	0	0	2
336	E01	03/09/2018	9	11	0	0	0	0	0	0	0	0	0	20
337	E12	03/09/2018	2	5	0	0	0	0	0	0	0	0	0	7
338	E05	03/09/2018	4	7	0	1	0	0	0	0	0	0	0	12
339	E08	07/09/2018	1	0	0	0	0	0	0	0	0	0	0	1
340	E07	07/09/2018	10	8	0	0	0	0	0	0	0	0	0	18
341	E04	07/09/2018	7	6	0	0	0	0	0	0	0	0	0	13
342	E02	07/09/2018	1	0	0	0	0	0	0	0	0	0	0	1
343	E14	07/09/2018	2	1	0	0	0	0	0	0	0	0	0	3
344	E03	07/09/2018	5	7	0	0	0	0	0	0	0	0	0	12
345	E11	10/09/2018	7	1	0	0	0	0	0	0	0	0	0	8
346	E06	10/09/2018	3	4	0	0	0	0	0	0	0	0	0	7
347	E05	10/09/2018	8	4	0	1	0	0	0	0	0	0	0	13
348	E09	10/09/2018	3	0	0	0	0	0	0	0	0	0	0	3
349	E12	10/09/2018	2	1	0	0	0	0	0	0	0	0	0	3
350	E13	10/09/2018	5	10	0	0	0	0	0	0	0	0	0	15
351	E14	13/09/2018	4	4	0	0	0	0	0	0	0	0	0	8
352	E01	13/09/2018	2	0	0	0	0	0	0	0	0	0	0	2
353	E07	13/09/2018	8	17	0	0	0	0	0	0	0	0	0	25
354	E10	13/09/2018	10	1	0	0	0	0	0	0	0	0	0	11
355	E08	13/09/2018	2	3	0	0	0	0	0	0	0	0	0	5
356	E15	13/09/2018	7	7	0	0	0	0	0	0	0	0	0	14

S4 – Tables – Molecular data

See file txpm4de5.xslx

S5 – Fasta: COI after filtering

See file txpm5de5.fasta