



Review

Food webs and biological control: A review of molecular tools used to reveal trophic interactions in agricultural systems



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ABSTRACT

Modern monocultural agro-ecosystems can be perceived as a simplification of natural ecosystems, where a single plant species is usually grown over vast areas. In these systems, it has historically been assumed that the concept of a *food chain* can describe the relationships between an insect pest and a single biocontrol agent. In reality, multiple potentially complex ecological interactions are involved, and these comprise food webs. However, identifying, analysing and quantifying the relative strengths of these multi-trophic interactions are very difficult using orthodox methods such as dissection and subsequent visual gut-content identification. An emerging field of study using molecular tools to analyse prey DNA in predators as well as parasitoid DNA within their hosts can now begin to address these impediments and help to better understand multi-trophic dynamics and improve biological control. In this article, we review the scientific literature published between 2000 and 2015 related to the use of molecular tools to analyse trophic interactions in agroecosystems in the context of biological control, using the ISI Web of Science search engine. A total of 213 articles were found and a steady increase in the volume of this literature occurred over the period studied. Based on the analysis of those publications, we propose future avenues in which advanced molecular tools can contribute to a mechanistic understanding of biological control, suggesting how this approach could help design agricultural systems based on agroecological techniques.

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1. Introduction

By 2050, it is expected that the human population will reach over 9 billion people (Godfray et al., 2010). This presents an extreme challenge

to modern agriculture, especially considering the potential consequences of climate change and biodiversity loss, leading to declines in ecosystem functions (Steffen, 2010; Steffen et al., 2011). In this context, a recent report from the United Nations highlighted agro-ecology as the only option for sustainable farming practices to produce enough food without having major harmful effects on the environment (De Schutter, 2010). The term agro-ecology was first used in 1928, and in

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general terms, it refers to “the integrative study of the ecology of the entire food systems, encompassing ecological, economic and social dimensions” or “the ecology of food systems” (Francis et al., 2003). Agroecological practices that enhance biodiversity can influence the provision of several ecosystem services, such as pollination, water provision and purity, nutrient regulation, biological control of pests, weeds and diseases, resilience to extreme weather events, carbon sequestration, rural development, aesthetics and human well-being (Altieri, 1999; Cardinale et al., 2012; Dallimer et al., 2012; Kremen and Miles, 2012; Wratten et al., 2012, 2013). As a result, particular attention has been given to the effect of different agricultural practices on biodiversity. However, despite the current realization of the importance of biodiversity in ecosystem services, this framework has not been historically used for biological control. Given that the history of biological control covers more than 2000 years, Gurr et al. (2000), presented a chronology where the main achievements in this science are highlighted. They proposed four main eras; Pre-scientific, from ancient China to 1880; Classical, from 1880 to 1939; Chemical, from 1939 to 1962 and the Integrated era, from 1962 to 2000. Since the early days of biological control, it has been implicitly assumed that the biocontrol agent acts alone, regardless of its surrounding biotic and abiotic environment. During the late Classical era, mathematical theory was introduced to explore stability in parasitoid/host systems (Nicholson and Bailey, 1935). This approach continued for many years, overlapping with the Integrated era but these models were inherently unstable, leading to several authors pursuing how density-dependence could stabilise them (e.g., Beddington et al., 1975; Hassell and May, 1973; Mills and Getz, 1996). However, in agroecosystems this stability rarely occurs, partly because the environment changes and partly because many predator/parasitoid models' assumptions are unrealistic in terms of ecological complexity and dynamics (Putman and Wratten, 1984). It is therefore not surprising that classical biological control programmes which sought population stability based on these assumptions were often not successful (Gurr and Wratten, 2000). In some cases, they also had non-target effects on biodiversity (Louda et al., 2003). Contrasting with the “Nicholsonian” approach, a simulation modelling school arose (e.g., Gilbert et al., 1976). This attempted to capture and explore a wide range of variables to simulate real biocontrol dynamics. However, this approach no longer plays a major role in biocontrol research. This seems to be because such models cannot easily be interrogated, their generality has been questioned and the assumptions needed to deliver realistic outputs were not always sound (Legaspi et al., 1996). For those reasons, here we highlight the importance of understanding the often short-term trophic interactions present in inherently unstable agricultural ecosystems, to understand and promote agro-ecological practices that produce a range of ecosystem services. For example, aspects of conservation biological control comprises the enhancement of the natural enemy communities already present in agroecosystems by understanding the resources needed by natural enemies other than their prey/host. These can be summarized as SNAP, which is an acronym for shelter, nectar alternative food and pollen (Barnes et al., 2010). The overall contribution of natural enemy communities to pest reduction has been reviewed, suggesting that in 75% of the cases evaluated, natural enemies contributed to reduced pest numbers (Symondson et al., 2002). Although the importance of the natural enemy communities in pest control, and how habitat manipulation can protect and enhance their populations is now largely recognised (Gurr et al., 2016; Jonsson et al., 2008; Landis et al., 2000; Zehnder et al., 2007), quantifying the interactions between pest and natural enemy communities in agricultural food webs has historically been difficult. In a predator–prey system, these interactions can be revealed by visual estimation of insect gut contents after abdominal dissections (Pompanon et al., 2012). Unfortunately, this technique is biased because usually only hard remnants of insect bodies can be detected in insect predators, making it very difficult to identify soft-bodied prey (Furlong, 2015). In parasitoid–host systems, rearing parasitized or mummified hosts can help identify trophic

interactions (Plečáček et al., 2014; Tylianakis et al., 2007). Despite the valuable contribution of that type of work to the understanding of food web structure, rearing hosts to evaluate parasitoid emergence is a time-consuming task, and the outputs could be biased by the occurrence of multi-trophic level interactions, such as hyperparasitism, that could affect our understanding of the contribution of a certain parasitoid species to the success of biological control.

The use of molecular tools has revolutionised ecological research and a sub-discipline focusing on the study of food-web interactions using molecular methods has emerged in the late twentieth century (Symondson, 2002) and recently it has grown exponentially (Symondson and Harwood, 2014). A number of workers have proposed the use of high-throughput DNA sequencing to analyse predators' gut content for prey DNA (Furlong, 2015; King et al., 2011; Pompanon et al., 2012) and parasitoid DNA within hosts (Garipey et al., 2007; Hřček and Godfray, 2015; Varennes et al., 2014), as an alternative to traditional methods to better unravel multi-trophic interactions. These tools have led to valuable advances in agricultural sciences, which from a biological control perspective can help in the selection of species as candidates for biological control, including predators, such as carabids against slugs (e.g., Bohan et al., 2000), spiders against aphids (e.g., Harwood et al., 2005); parasitoids attacking aphids (e.g., Zhou et al., 2014), as well as alternative prey items involved in natural enemy community dynamics in the absence of pest prey species (e.g., Agustí et al., 2003). In addition, molecular tools could contribute to a better understanding of the existing food web leading to better-designed biological control programmes. However, when classical biological control programmes need to be implemented for different reasons, such as a wide-scale invading non-native pest, molecular tools can help assess the potential risk of such introduced biological control agents (Garipey et al., 2014). Despite the recent advances in the use of molecular tools for studying agricultural food webs for biological control, the contribution of these methods to the success of biological control programmes remains unclear. Therefore, the overall aim of this article is to analyse research trends in the use of molecular approaches to study agricultural food webs in the context of better understanding the dynamics and mechanisms behind biological control. This analysis focuses on the type of molecular method used, the type of research performed (see “Categories” below), number of species involved in the analysed food web and quantification of biological control. Based on these analyses, future avenues of research are suggested in which these techniques can improve the understanding of invertebrate community interactions on agricultural food webs.

2. Literature review process

As one of the objectives of this study was to analyse recent developments in the use of molecular tools in agricultural food webs for biological control, the literature review was performed on publications dated between 2000 and 2015, using all databases available in ISI Web of Science search engine. The initial search was performed to identify articles in which molecular methods were used using a series of keywords. The keywords used to find the articles, as well as the corresponding code (i.e., combination of keywords) are available in Appendix A. Then, relevant references cited in the articles retained, such as reviews, were also checked and incorporated to the study if appropriate. A total of 831 articles were found using the keywords and an additional 35 by the review of the references they contained, adding up to a total of 866 publications. Every article that was not related to agroecosystems was then removed from this initial pre-selection, which reduced the number from 866 to 304. Finally, only articles that were related to the use of molecular tools for the study of agricultural food webs in the context of biological control were retained, further reducing the number of articles, from 304 to 213 (Table 1 in the online version at <http://dx.doi.org/10.1016/j.fooweb.2016.04.003>).

3. Criteria used

From the final group ($n = 213$), information on the molecular method used, types of articles, number of species analysed in food webs and effects on biological control were obtained. These criteria are described as follow:

- Types of articles were categorised as reviews, methods or experimental. *Reviews* papers were those in which the review of one or several molecular tools used in foods web studies were presented (e.g., [Garipey et al., 2007](#); [Symondson, 2002](#)). *Methods* papers were all research in which some development/improvement of the methodologies such as PCR protocol improvement, primers set development and/or sample collection methods related to molecular tools in agricultural food webs for biological control were considered (e.g., [Chapman et al., 2010](#); [Hagler, 2011](#)). Finally, *Experimental* articles were those only in which experiments were performed, at field or laboratory level, in which the composition of invertebrate food web were assessed by molecular tools. This last category included research related to pest-predator population dynamics (e.g., [de Roince et al., 2012](#)), pest population control by natural enemies (e.g., [Traugott et al., 2012](#)), but also other research questions relevant for biological control, such as the importance of non-crop vegetation in parasitoid dispersal to cropland (e.g., [Derocles et al., 2014](#)).
- Number of species analysed in *Methods* and/or *Experimental* papers were categorized as Two species (Two sp), more than two species (>Two spp) and tri-trophic level interactions (TTW). Articles in which the effect of one species on predation or parasitism of another were grouped as *Two sp*. (e.g., [Hoogendoorn and Heimpel, 2001](#)). When more than two species were studied, we categorized those articles as >*Two spp*. (e.g., [Kuusk and Ekbom, 2012](#); [Thomas et al., 2009](#)). In some cases, tri-trophic level interactions were observed, in which more than two species were interacting, but each of them at a different trophic level, such as an aphid–parasitoid–hyperparasitoid system (e.g., [Varennnes et al., 2014](#)) or in the case of a whitefly–predator–parasitoid (e.g., [Moreno-Ripoll et al., 2012](#)). These articles were grouped as *TTW*. Intra- and inter-guild predation articles were considered as >*Two spp*. or *TTW* depending on each case.
- Effects on pest population were categorized as population densities (PDs) and biological control (BC). These categories were based on the effect of predators and/or parasitoids on any target pest, reported in *Experimental* papers (see description above). The concept of *PDs* was used here to categorize articles in which population densities of both the prey/host and predator/parasitoid were measured at the same time and/or location (e.g., [Harwood et al., 2007](#); [Hatteland et al., 2013](#)). On the other hand, all articles in which the actual effect of an organism or group of organisms on the target pest was evaluated, quantifying the pest population reduction by the action of natural enemies was grouped here as *BC* (e.g., [Lundgren and Fergen, 2011](#); [Schmidt et al., 2014](#)).

3.1. Trends in the use of molecular tools in agricultural food webs for biological control

The molecular methods used in each article are presented in Table 1 in the online version at <http://dx.doi.org/10.1016/j.fooweb.2016.04.003>. Many papers used more than one such method to study trophic interactions, so the number of references presented in Table 1 in the online version at <http://dx.doi.org/10.1016/j.fooweb.2016.04.003> is much larger ($n = 305$) than the actual number of articles used in this review ($n = 213$). The same issue occurred when the articles were grouped by type of research, species considered and effects on biological control. For example, some articles developed methods and performed experiments, thereby falling in both, *Methods* and *Experimental* categories, which increased the number of references from 213 to 295. Based on

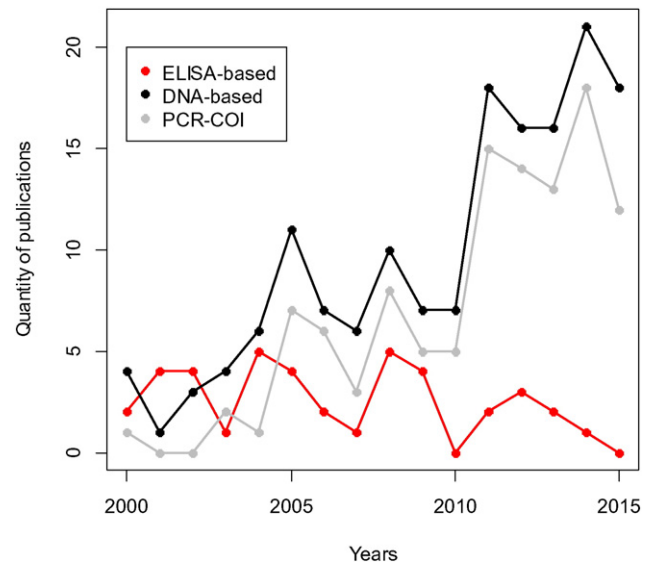


Fig. 1. Trends in the number of scientific publications from 2000 to 2015 using molecular tools for the study of agricultural food webs. The number of DNA-based studies are presented in black, with studies using PCR-COI in grey, while ELISA-based studies are presented in red.

Table 1 in the online version at <http://dx.doi.org/10.1016/j.fooweb.2016.04.003>, the most common molecular method used for the evaluation of food webs in agriculture was polymerase chain reaction (PCR) with subsequent amplification of the cytochrome oxidase I region (COI), with 50% of the reviewed articles ($n = 213$). The second most used protocol was monoclonal antibody enzyme-linked immune assay (ELISA-mab) with 15% of the reviewed articles. Considering that the most widely used methods were PCR-COI and ELISA-mab, and many of the different methods listed in Table 1 in the online version at <http://dx.doi.org/10.1016/j.fooweb.2016.04.003> were used only few times in the literature analysed here, we further grouped all the articles that used PCR techniques (including next generation sequencing) under the label *DNA-based* research. *ELISA-based* research was grouped similarly (see Fig. 1). As expected from advances in PCR-based approaches over several scientific disciplines, the use of these tools for the understanding of food webs in agriculture for biological control has increased since 2002, with annual fluctuations between 6 and 11 articles during the 2004–2010 period, and an average of 18 articles per year between 2011 and 2015. Although DNA-based research showed a rapid increase in the last 15 years, ELISA-based tests have been used regularly, with one to five articles between 2000 and 2009, showing a slow decrease lately, varying from 0 to three articles between 2010 and 2015. This dominance of PCR-COI based articles could be related to the easiness to use this method, associated with low costs of development and the availability of well-developed primers for a number of agricultural pests ([Fournier et al., 2008](#); [Greenstone et al., 2014](#); [Symondson, 2002](#)). On the other hand, ELISA analyses are based on the production of antibodies (monoclonal in most cases, due to their specificity in prey detection), which are time-consuming and difficult to develop ([Fournier et al., 2008](#)). However, after the antibodies are produced, the cost of ELISA is cheaper and faster than PCR ([Sheppard and Harwood, 2005](#)), especially when large sample sizes are taken into account ([Fournier et al., 2008](#)). With this regard, the high cost and time spent to produce the antibody (e.g., US\$12,000 and one year of work ([Fournier et al., 2008](#))), could be one of the reasons for the increase in DNA-based techniques. Another could be the implementation of multiplex PCR techniques, in which the DNA of multiple target species is amplified simultaneously from a single sample. This can reveal trophic

interactions between multiple pests species and their associated predator community (e.g., King et al., 2011), thereby improving our understanding of the effects of natural enemy communities on pest control. However, ELISA-based tests are still useful when studying the interactions between one predator and its prey in studies with a large sample size, although in more complex food webs, DNA-based analyses are better at describing community effects on pest populations (Pompanon et al., 2012; Sheppard and Harwood, 2005).

3.1.1. Types of research conducted using molecular tools

When all articles were considered ($n = 213$), regardless of the molecular method used, a steady increase was observed from 2002, with on average 11 articles published per year between 2003 and 2010 (Fig. 2). Between 2011 and 2015, an average of 21 articles was published each year. *Experimental* articles were the most common during the evaluated period, followed by *Methods* and *Reviews*, with 64%, 29% and 7% of all reviewed articles, respectively (Fig. 2). The increase in the number of *Experimental* articles in the recent years probably reflects the development of specific molecular tools for the analysis of agricultural food webs. Considering that these methods are relatively new in the field of agricultural biological control, it is not surprising that an increase in publications of *Experimental* papers has occurred (Fig. 2).

3.1.2. Number of species considered in trophic interactions and number of biological control publications

In relation to the number of species studied, 49% ($n = 141$) of the publications used for this review considered more than two species (>Two spp), while 16% ($n = 48$) studied only two species (Two spp). Tri-trophic food webs (TTW) corresponded to 3% ($n = 9$) of all reviewed articles (Fig. 3). Publications focusing on more than two species have prevailed since 2002 and represented 95% and 93% of those published in 2014 and 2015, respectively. At the same time, publications focusing on only two species have oscillated between one and five publications per year. This change from research evaluating two species interactions into studies considering more than two species interactions could

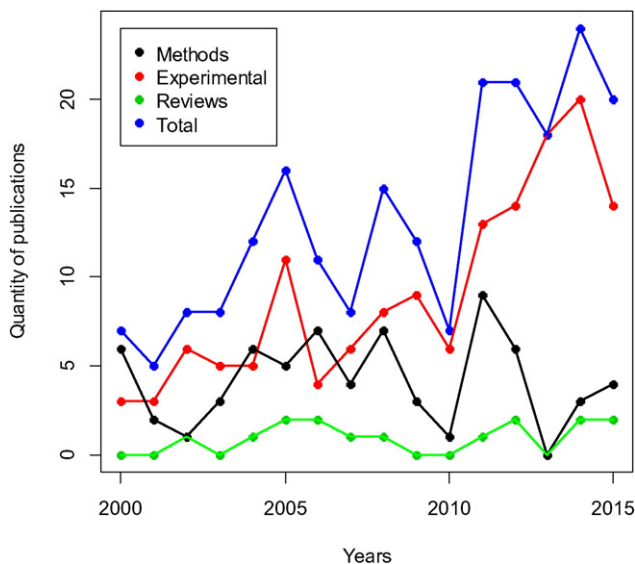


Fig. 2. Trends in the number of scientific publications from 2000 to 2015 using molecular tools for the study of agricultural food webs. Total number of publications are presented in blue. Here, different categories were used for a better understanding of the observed trends (see text for description). The evolution of *Experimental*, *Methods*, and *Review* articles are shown in red, black and green, respectively.

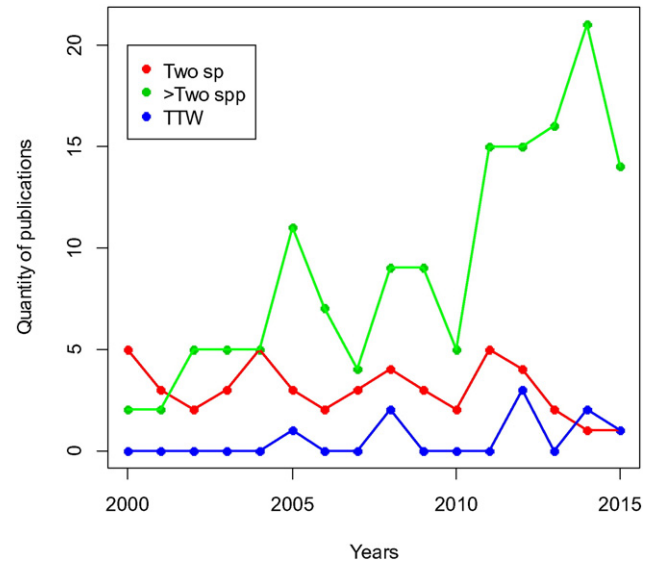


Fig. 3. Trends in the number of scientific publications from 2000 to 2015 considering different number of species interacting using molecular tools. In red, two species interactions were studied (Two spp), in green, more than two species were studied (>Two spp), and in blue the articles in which tri-trophic level interactions were considered (TTW).

reflect a change in the biological control paradigm (at least in the publications considered in this review); from *food chain* to *food webs*, which highlights the importance of generalist predators and their interactions with other insect species on pest control (Symondson et al., 2002). Notwithstanding the above, the specificity of a control agent for the target pest remains essential when designing a classical biological control programme (Hassell and May, 1986). In this sense, research able to discover the interactions between a host and its associated parasitoid species, is valuable to improve our understanding of potential non-target effects on native invertebrate community associated with the release of biocontrol agents (Barratt et al., 2010) and in reducing the potential risk associated with exotic species introductions (Garipey et al., 2014). This change of paradigm from *food chain* to *food webs*, can greatly impact some agroecological practices, such as mulching, planting flowering strips, establishment of hedgerows or the preservation of non-crop vegetation, for the enhancement of local natural enemy communities (Landis et al., 2000; Wratten et al., 2012; Zehnder et al., 2007), and therefore could help to reduce pests in agricultural landscapes (Jonsson et al., 2015) without the use of pesticides. In this review, the articles studying the effects of arthropods on biological control accounted for 10% ($n = 30$) of all reviewed articles, but unfortunately none of them investigated the relationship between the effectiveness of biological control and its effects on crop yield.

Pest and natural enemy population densities were measured in 21% ($n = 63$) of the publications (categorised here as PDs), and in half of those, the population density of natural enemy population was related to reductions of the pest population, being considered here as biological control publications (BC). The evolution in the number of these articles is shown in Fig. 4. The number of PDs and BC publications increased since 2008, with eight PDs publications on 2014, and six of those considered as BC here (Fig. 4). These numbers remain low compared to the number of publications in which the diet of more than two species were studied on year 2014 ($n = 21$) indicating that the use of molecular tools for measuring biological control is still in its infancy. Nevertheless, the recent increase in publications measuring biological control since 2008, suggests an increasing understanding of trophic dynamics in agricultural food webs, with practical applications for biological control.

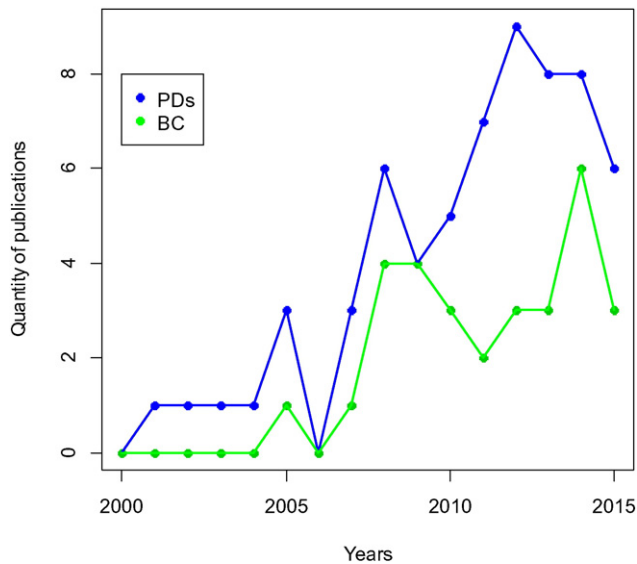


Fig. 4. Trends in the number of scientific publications from 2000 to 2015 evaluating biological control by using molecular tools. In blue, the number of articles measuring both, the pest and biological control agent populations are shown (PDs). In green, the number of articles measuring a reduction in pest numbers by natural enemies are presented (BC). See text for a description of these categories.

4. Future challenges

4.1. Implications of molecular tools in assessing the effects of farm managements in the success of biological control

In this review we addressed scientific publications related to the use of molecular tools to study agricultural food webs for biological control, from year 2000 until 2015. Despite the increase in the number of publications over that period (Fig. 2), few articles used molecular techniques to evaluate the effects of the natural enemy community on the control of agricultural pests. In this regard, the presence of alternative food sources is crucial in the performance of natural enemies (Landis et al., 2000; Zehnder et al., 2007). For example, Harwood et al. (2005), found that spiders can act as important biological control agents of aphids before the pest's population establishment. Such early-season predation can be critically important for maintaining pests below outbreak levels and improve yield (Athey et al., 2016). However, the presence of alternative food sources, such as collembolan species may impact spiders' predation on aphids (Harwood et al., 2004; Kuusk and Ekbom, 2010), although these alternative sources of food are likely to promote the survival of natural enemy communities during pest-free periods (Agustí et al., 2003; Kuusk and Ekbom, 2010). It has recently been suggested that a decrease in aphid populations on canola, due to climatic changes or predation, can lead to an overall reduction in the abundance of generalist natural enemies (Layman and Lundgren, 2015). Such a reduction in natural enemies might provide a temporal opportunity for *Pieris rapae* L. (Lepidoptera: Pieridae) to increase its numbers, resulting in a pest outbreak due the lack of early predation pressure on this insect (Layman and Lundgren, 2015).

Another factor that must be considered in the success of biological control is the impact of intra-guild predation on pest control (Gagnon et al., 2011). Recently, it has been reported that adult parasitoid populations can be affected by carabid predation on parasitized aphids in winter wheat (Traugott et al., 2012). Because mummified aphids are immobile, they are likely to be more predated by carabids than non-parasitized aphids. Therefore, the short-term positive effect of carabid predation on aphids, may lead to a long term negative effect on parasitoid populations resulting in an overall reduction of biological control

(Snyder and Ives, 2001). Despite the negative effects that intra-guild predation can exert over pests, contrasting results had been reported in the literature, highlighting the potential for a number of confounding effects related to predator traits, such as life history, foraging behaviour and relative predator size (Straub et al., 2008). Notwithstanding the ubiquity of intra-guild predation (Gagnon et al., 2011), the success of conservation biological control depends largely on the farm managements and landscape complexity around the farm (Jonsson et al., 2012, 2015). Unfortunately, few articles have used molecular methods to investigate changes in diet or the effect of farm management on host preferences by natural enemies, and no article assessed landscape complexity effects on biological control success. Nevertheless, the effect of herbicides in a cabbage field in the USA affected predator populations by the removal of refuges provided by an oat cover crop (Szendrei et al., 2014). In that study, the reduction in population on *P. rapae* was related to the herbicide application that changed the pest's behaviour, rather than predation rates affected by the removal of the cover crop. A similar result was found in a potato field in USA, where straw-mulch applications reduced aphid numbers, despite higher pest consumption in plots without mulch application (Szendrei et al., 2010). These results suggest that management practices can alter pest population dynamics by themselves, without having an apparent effect on the composition of the predator community but simply through changes in pest and/or predators behaviours due to temporal and spatial variation of their refuges and/or prey availability. The latter statement was recently tested in banana plantations in Martinique (French West Indies) where the predator community changed its consumption preferences in relation to the Banana root borer *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae), increasing predation in some species, and decreasing predation in others under different cover crops regimes (Mollet et al., 2014). Lundgren and Fergen (2011) also reported that cover crops enhanced natural enemy communities, negatively affecting the population of Western corn rootworm *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae). Furthermore, Schmidt et al. (2014) showed how molecular tools can help to improve biological control of squash bug *Anasa tristis* De Geer (Hemiptera: Coreidae) by determining the optimal time during crop development when row-cover needs to be replaced for maximum pest reduction by natural enemy communities. These studies, relative to the impact of cover crops and row-covers on pest control, highlight the existence of complex food webs in agricultural systems and the importance of identifying these interactions to understand how management can affect biological control.

Despite the importance of habitat manipulation (as a management strategy) to increase biodiversity for biological control (Kremen and Miles, 2012; Landis et al., 2000; Zehnder et al., 2007), the landscape complexity seems crucial for the successful adoption of agroecological techniques for pest control (Batáry et al., 2011; Bianchi et al., 2013; Gagic et al., 2011; Jonsson et al., 2012, 2015). In this regard, the use of molecular tools could also help to better understand the effects of landscape complexity on biological control success by assessing the contribution of the whole community of natural enemies (predators and parasitoids) to pest control. So far, it seems that only parasitoid and hyperparasitoids have been studied in this landscape context.

4.2. Next generation sequencing and its advantages in the study of agricultural food webs

In this review, only six publications were found using next generation sequencing (NGS) methods for analysing biological control in agriculture (Table 1 in the online version at <http://dx.doi.org/10.1016/j.fooweb.2016.04.003>), showing the early stage that the application of these techniques has reached in the understanding of agricultural food webs for biological control. This can be explained by the fact that in most cases, the pest species and its biological control are well known and molecular tools are used to measure to what extent a particular pest is predated or parasitized by a particular predator or parasitoid.

To detect these predation events, conventional PCR using species-specific primers is sufficient. When multiple predators or multiple prey are investigated, a multiplex PCR approach can be used. The latter consists of using a combination of several primer pairs, each of which is specific to a particular species. Because the number of species investigated usually remains limited and both pests and potential biological agents are well known, PCR and multiplex PCR have been the tool of choice in biological control studies (e.g., Hatteland et al., 2013; King et al., 2011). However, these methods cannot identify unsuspected trophic interactions (Sheppard and Harwood, 2005). With increasing evidence that alternative food sources and intra-guild predation are ubiquitous in agricultural systems, NGS will become necessary to fully appreciate the fact that food webs are more complex than previously thought (Wirta et al., 2014), helping in the design of efficient biological control programmes. One of the main advantages of this technology is that it amplifies all the DNA present in a mixed DNA sample, using so-called “universal” primers, thereby avoiding the necessity of producing specific primers for each of the species assumed to be present (Boyer et al., 2012; Pompanon et al., 2012). Although DNA of all predated species in predator guts or parasitoid species within a host can be detected and amplified at once (Pompanon et al., 2012), it is necessary to have a reference library to which DNA sequences produced by NGS can be compared to identify the species involved. Therefore, one limitation of using NGS in food web analyses is that species for which no reference sequence exists, cannot be identified solely with their DNA sequence (Garipey et al., 2014). Despite the advantages of using so-called “universal” primers, caution is required when NGS data are interpreted from field samples because some dietary components could be underestimated by such primers, as in the case of seed DNA present in the regurgitates of *Harpalus rufipes* De Geer (Coleoptera: Carabidae) (Wallinger et al., 2015).

Because NGS methods can amplify small amounts of DNA present in a mixed sample (Boyer et al., 2013), this can help to reveal rare or cryptic interactions in agricultural food webs. PCR-based techniques, including NGS methods, can help to establish trophic relationship, but they can be very biased when it comes to quantifying the number or amount of prey actually consumed by a predator (Deagle et al., 2013). In that sense, most studies provide only qualitative results by recording the proportion of predators tested positive for a particular prey. For animals which completely consume their prey (or host), the simple occurrence of a prey species' DNA in their gut or faeces corresponds to at least one individual prey item eaten, which provides a minimum estimate of predation (Boyer et al., 2015). However, this provides at best only semi-quantitative results. Alternatively, real-time PCR is one of the rare tools that provides a relative quantitative estimation of predation by measuring the number of prey DNA copies in predators' guts or faeces (Pompanon et al., 2012). A combination of qualitative methods (prey occurrence in diet) with quantitative ones (prey abundance in diet), has been recently used in a study aiming at quantifying the predation of the aphid *Nasonovia ribisnigri* Mosley (Hemiptera: Aphididae) and the thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) in lettuce agroecosystems (Gomez-Polo et al., 2015). In this study, real-time PCR was used to measure the contribution of different prey to the diet of syrphid larvae, while NGS analysis revealed intra-guild predation in this system, emphasizing the complementarity between both methods. The use of these complementary molecular approaches could help evaluate the effects of habitat manipulation on agricultural food webs, with emphasis on biological control (Gomez-Polo et al., 2015). It is however important to note that the rate of decay of dietary items in predators' guts can be prey/predator species-specific, therefore a detectability “half-life” approach combined with comparative weighting of prey species (as described recently by Greenstone et al., 2007, 2014; Pérez-Sayas et al., 2015) are required for accurate quantification of predation.

Another important limitation lies in the inability of PCR-based techniques and NGS to distinguish between direct and secondary predation

(Sheppard et al., 2005). Because the latter can arise from intra-guild predation, it may lead to an overestimate of biological control. Similarly, when a predator engages in cannibalism or scavenging (consuming dead prey instead of live ones), the proportion of predators tested positive for a given pest is not a true reflection of biological control services provided by that predator. One way to differentiate between predation compared to scavenging is to apply different protein markers (such as rabbit IgG or chicken IgG) on live and dead prey (Mansfield and Hagler, 2016). Although this method is efficient in an experimental or manipulated environment, it may not be easily applied to a natural ecosystem. For a better understanding of complex food webs, research in biological control must embrace new molecular tools and in particular the use of NGS, whilst remaining aware of their limitations.

4.3. The potential contribution of molecular techniques to agro-ecology

As mentioned before, the fact that biodiversity has an impact on biological control at farm and landscape level is well documented (Altieri, 1999; Cardinale et al., 2012; Kremen and Miles, 2012; Wratten et al., 2012). However, how pest reduction by natural enemies impacts on plant yield is less clear, and it appears that only few articles in the scientific literature have dealt with this issue in well replicated conditions (Jonsson et al., 2008; Kremen and Miles, 2012). Only one of the article selected in this review showed how a “rich” community of natural enemies decreased the population density of the pest *D. virgifera*, which in turn reduced damage to maize roots, although, the effects on corn yield were not evaluated (Lundgren and Fergen, 2011). This type of analysis is crucial for the adoption of appropriate sustainable agricultural techniques worldwide. Agro-ecology with the enhancement of multiple ecosystem services is considered by many as the best approach to feed the increasing world population without harnessing the environment (De Schutter, 2010). However, there are still concerns regarding the low yields that sustainable agriculture (e.g., organic agriculture) can achieve. A general figure that is often cited in the literature is that organic agriculture produces 21% less than conventional farming (de Ponti et al., 2012). Although, these somewhat low yields are crop and cultural context dependent (Seufert et al., 2012), it is likely that a better understanding of agricultural food webs and their role on biological control through the use of molecular tools could contribute to closing the production gap between conventional and sustainable agriculture.

5. Conclusions

The number of scientific publications using molecular techniques to reveal the mechanisms contributing to biological control by predators or parasitoids has increased in the recent years. A PCR approach targeting the COI gene was used in a large majority of the studies with either specific primers targeting particular prey species or non-specific primers amplifying the DNA of all predated species. Most of the publications used in this review considered the interactions between more than two species, suggesting a change of paradigm in biological control: from *food chain* to *food webs*. Despite being marginally used over the studied period (2000–2015), NGS methods have the potential to greatly enhance our understanding of complex food webs, which is essential to design efficient biological control programmes, particularly in a context of conservation biological control. With the advantages of NGS technology, the effects of landscape complexity over the whole community of natural enemies could be assessed, thereby allowing the development of regional plans for conservation biological control. Such plans are pivotal to achieve optimal agricultural productivity and biodiversity conservation goals at regional scale, but can be deployed only with a full understanding of the mechanisms underpinning pest control by predation or parasitism, and how their magnitude is affected by farm management and landscape complexity. Potentially, a broad understanding of these population dynamics will help to promote the adoption of agroecological based strategies for pest management, which

has the potential to reduce the gap between sustainable and conventional agriculture yields.

Historically, biological control has essentially focussed on the effect of one species on another, in order to reduce damage in agricultural crops. The use of molecular tools has the potential to reveal much more complex interactions that usually exist between natural enemies and their prey. However, in this review only a few articles (10%) applied molecular tools to evaluate the effects of natural enemy communities on the target pest, categorized here, as “biological control papers”, suggesting that the use of molecular tools to study trophic interactions in agricultural systems is still in its infancy. Certainly, the evolution of biological control science from “food chains” to “food webs” revealed through this review is strongly contributing to our understanding of understanding the role of food webs for biological control in agricultural systems. This suggests that we have entered a new “era” in biological control science, in which the interactions between the biocontrol agents, their hosts/prey and their communities are key components for sustainable agriculture. However, further field studies focussing on the mechanistic processes behind biological control, and on how farm management and landscape complexity can affect its outcomes are necessary to promote the further development of sustainable agriculture.

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Appendix A. Combination of keywords used in ISI Web of Science search engine to collect the articles considered in this review

(“predat*” OR “parasitoid*”) AND (“food web*” OR “agriculture” OR “generalist predators” OR “predator–prey” OR “gut analysis” OR “intraguild” OR “diet” OR “gut content” OR “dietary” OR “arthropod” OR “soil food web*”) AND (“molecular” OR “molecular gut analysis” OR “PCR based” OR “DNA based” OR “NGS” OR “molecular markers” OR “gut*” OR “immunological” OR “ELISA” OR “PCR” OR “multiplex” OR “DNA”) AND (“arthropod*” OR “predators” OR “parasitoids” OR “host*”) NOT “isotop*” NOT “marine” NOT “aqua*” NOT “forest*” NOT “alga*” NOT “model*” NOT “snakes” NOT “bats” NOT “mammals” NOT “fish*” NOT “vertebrates”.

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