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The effect of landscape context on the biological control of *Sitobion avenae*: temporal partitioning response of natural enemy guilds

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Abstract Landscape contexts with high complexity may promote diversity of natural enemies, although the effect on biocontrol remains under discussion. Although biocontrol of *Sitobion avenae* is a well-studied system, little is known about the temporal effect of landscape context on the natural enemy assemblages. In a previous study, we showed a positive effect of predators in the decline of aphids; however, this effect had a temporal pattern responding to different landscape contexts. We study here two contrasting agricultural contexts, high landscape complexity with low intensification and low complexity with high intensification. Abundance and diversity of parasitoids was examined via a molecular approach, using a combination of diagnostic multiplex and singleplex PCR assays to test field-collected samples of *S. avenae* with genus- and species-specific parasitoid primer pairs. Temporal population dynamics were analyzed and differences related to these two contexts were observed. Parasitism rates were greater in the mid-sampling dates in high intensification simple landscapes, which were not observed for the low intensification complex landscapes. According to our results, we suggest that the greater landscape complexity in combination with a low agricultural intensification increase negative interactions for parasitoid population built-up; however, early predation by coccinellids was able to control the aphid populations. In contrast, under a simple landscape context with a high agricultural intensification, our results suggest an important role of parasitism with a complementary effect of late predation. We highlight the importance of different natural enemy guilds and their temporal dynamics under contrasting agricultural settings to further understand the relationship between functional diversity and biological control.

Key message

- Diagnostic PCR revealed differences in temporal dynamics of parasitism rates related to the agricultural landscape context.
- In simple contexts, parasitism rates were higher in the early to mid-season, with a population decrease by the end of the season.
- Early predation in complex contexts could negatively affect the parasitoid population build-up.
- Temporal partitioning of natural enemy guilds could occur in aphid exploitation between predators and parasitoid.
- Parasitoid assemblages have an important role in aphid population control in simple landscape contexts.

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Introduction

The main goal of biological control is to decrease pest population densities using living organisms. This has been historically performed mostly by releasing natural enemies using classical or augmentative/inundative methods. However, recent efforts have been made to understand how to improve the performance of already present natural enemies at the field level through conservation biological control (Landis et al. 2000). While diversity in general should favor pest suppression, weak and even negative effects have been observed of natural enemy biodiversity on the abundance of arthropod herbivores in terrestrial ecosystems (Letourneau et al. 2009). This biodiversity–function relationship in biological control has been discussed as a highly dependent relationship in the context where agriculture is being carried out (Tylianakis and Romo 2010), and perhaps this context dependency has determined that conservation biological control has not had the desired effect on pest control (Jonsson et al. 2010). Therefore, understanding how natural enemy assemblages work as a whole community should be the focus of "ecological engineering" in order to correctly carry out management decisions.

Agricultural systems are characterized by continuous spatial and temporal changes (Thies et al. 2005; Chaplin-Kramer et al. 2011) where the semi-natural vegetation could have an important beneficial effect providing more stability and resilience to the agroecosystem (Landis et al. 2000; Tscharntke et al. 2005; Woltz et al. 2012). Those systems are also characterized by the promotion of biological interactions between natural enemies and pests and within natural enemies/pests. Nevertheless, there is no clear evidence supporting the hypothesis that a higher diversity of natural enemies improves the biological control functioning per se (Chaplin-Kramer et al. 2011). Thus, some factors such as agricultural intensification of farming practices and habitat heterogeneity could contribute to this variability (Macfadyen et al. 2009a; Rusch et al. 2013).

In Chile, a total of 169 aphid species have been reported, 128 of them having been introduced and most of them constituting important agricultural pests (Nieto Nafría et al. 2016). Aphids and their natural enemies, such as parasitoids, can be influenced by the landscape complexity (Bianchi et al. 2006; Tscharntke et al. 2007). Studies have shown that surrounding semi-natural areas can provide important resources, which could improve the survival and fecundity of natural enemies (Starý et al. 1994; Tylianakis et al. 2004; Tscharntke et al. 2007; Straub et al. 2008). Nevertheless, functional redundancies and niche complementaries among natural enemy guilds should be studied in order to understand the effects on a given pest in agricultural systems, and the interactions between generalist/specialists' natural enemies as well as their temporal dynamics (Snyder and Ives 2003; Schellhorn and Andow 2005; Martin et al. 2013; Raymond et al. 2015).

In a previous study, we have shown that, although the pest population dynamics was similar in contrasting landscape contexts, the predator guild (mainly coccinellids and some carabid beetle species) revealed temporal differences in terms of predator abundance affecting the aphid pest populations. High abundance of predators occurred early in the season in complex landscapes, whereas in simple contexts a higher abundance and effect on aphid populations was reached at the end of the season (Raymond et al. 2015). Although differences in the temporal dynamics were found, the diversity of predators was not affected by the landscape context.

A rich assemblage of parasitoids co-occur in cereal systems (Zepeda-Paulo et al. 2013; Starý et al. 1994), potentially interacting with the predator guild (Raymond et al. 2015). Therefore, it is necessary to include the dynamics of the primary and secondary parasitoid species in order to obtain a full view of how pest population control is being achieved and to understand the functional redundancy or niche complementarity of these guilds in space and time. The English Grain Aphid [*Sitobion avenae* Fabricius, 1794 (Hemiptera: Aphididae)] is one of the most important cereal aphids in wheat fields distributed worldwide, generating great damage due to its direct feeding or as vector of disease (Carter et al. 1982). During the 190s, *S. avenae* arrived in Chile, becoming the most abundant and damaging aphid and constituting the main pest problem in cereal fields (Gerding et al. 1989; Figueroa et al. 2005). This drove the Chilean government to finance a biological control program introducing several parasitoid species (Starý 1995; Rojas 2005). The study of the parasitoid community, its dynamics and their interactions with other natural enemies of the aphid *S. avenae* in relation to landscape complexity could be a key to understand the biological control and how to enhance it. Nevertheless, many important aspects of parasitoids are still largely unknown (i.e. parasitoid–parasitoid interactions) which could affect the population dynamics and modify their relative abundances through the season.

In the field of trophic ecology, the use of DNA-based techniques has proven to be a successful methodology to study a wide range of trophic interactions, including parasitoid–host interactions. These novel approaches have been used extensively in the last years to analyze and evaluate the level of intra-guild predation, competition between natural enemies and also symbiosis with other organisms (Gariepy et al. 2007; Traugott and Symondson 2008; Roubinet et al. 2015; Staudacher et al. 2016). In order to determine the efficiency of biological control in

agricultural systems, DNA based methods allow the accurate identification of the immature stages (eggs, larvae and pupae) of the organisms at the lowest taxonomic level (Traugott et al. 2013; Gariepy et al. 2014; Gómez-Marco et al. 2015).

The aim of this study was to evaluate the diversity and abundance of natural enemies in 20 wheat fields in two contrasting landscape contexts across the season. As a prior study had already discussed the main predators, here we focused on the parasitoid assemblages and the differences in time compared to the predators. The effect of the landscape context on the parasitoid community and their interactions was examined in order to determine their potential as efficient biological control agents of the English Grain Aphid, using molecular analysis of the trophic interactions with diagnostic PCR.

We hypothesize that the parasitoid populations should be more abundant early in the season when predators are not present in simple landscape contexts. At the same time, some negative interactions, due to a greater diversity of parasitoids, should be occurring in complex landscape contexts, which would explain the lack of differences reported previously in aphid populations in both landscape contexts.

Materials and methods

Study system

Natural enemies of *S. avenae* include several parasitoid species (e.g., Hymenoptera: Braconidae) which can occur simultaneously (Quicke 2015) and several predatory species such as coccinellids and carabid beetles among others (Grez et al. 2011; Raymond et al. 2015). Parasitoids associated with cereal aphids in Chile are all the result of the introduction of nine species for biological control. Native species of Aphidiinae present in Chile are associated with natural forest systems (Starý et al. 2014). A total of nine species have been found attacking *S. avenae* with different relative prevalence; *Aphidius ervi* Haliday, 1834 (Hymenoptera: Braconidae) (38%), *Aphidius uzbekistanicus* Luzhetzki, 1960 (Hymenoptera: Braconidae) (28%), *Aphidius rhopalosiphi* De Stefani-Perez, 1902 (Hymenoptera: Braconidae) (12%), *Praon volucre* Haliday, 1833 (Hymenoptera: Braconidae) (9%), *Praon gallicum* Stary, 1971 (Hymenoptera: Braconidae) (6%), *Aphidius avenae* Haliday, 1834 (Hymenoptera: Braconidae) (3%), *Aphidius colemani* Viereck, 1912 (Hymenoptera: Braconidae) (3%), *Lysiphlebus testaceipes* Cresson, 1880 (Hymenoptera: Braconidae) (0.5%) and *Aphidius matricariae* Haliday, 1834 (Hymenoptera: Braconidae) (0.4%) (Zepeda-Paulo et al. 2013). Secondary parasitism, although reported for Chile, has not been thoroughly studied. The

species of secondary parasitoids reported for this region have been *Dendrocerus* sp. (Hymenoptera: Megaspilidae), *Alloxysta victrix* Westwood, 1833 (Hymenoptera: Charipidae), *Pachyneuron siphonophorae* Ashmead, 1886 (Hymenoptera: Pteromalidae), *Asaphes rufipes* Brues, 1908 (Hymenoptera: Pteromalidae) and *Asaphes vulgaris* Walker, 1834 (Hymenoptera: Pteromalidae) (Suzuki Sone and Vargas Mesina 1980; Guerra et al. 1998; Ferrer-Suay et al. 2013), nevertheless, the knowledge regarding their distribution, behavioral traits and repercussions for biological control are still unknown in Chile.

Field selection and landscape analyses

Sampling was carried out in two sets of 10 wheat fields. A 500-m-radius circular buffer around each selected field was analyzed according to the proportion of different components (including natural, semi-natural areas and both annual and perennial crops). One set was characterized by a simple surrounding landscape with a mean of 83% arable land in the surrounding buffer and a low value for semi-natural habitats (average 17%). The agricultural practices of these sites are characterized by a high input of mineral fertilizers, irrigation and periodic applications of phytosanitary chemicals for the simple context fields. This set of fields is called "simple set" from here on. The other set was characterized by a complex surrounding landscape with a mean of only 45% of arable land in the surrounding buffer and high value for semi-natural habitats (average 56%). Theses sites are characterized by having low agricultural intensification, with minimum (only during the sowing period) input of mineral fertilizers, with no irrigation (only received rainfall water) and without chemical phytosanitary applications. In this study, this set of fields was called "complex set". All sites were picked with a minimum of 30 km of distance between them. The elevation of the sampling fields in the simple and complex set comprised 185–270 and 60–135 m, respectively, and both areas are considered to have the same climatic and soil regimes (Santibañez and Uribe 1993). This study was performed in the Maule region in central Chile (35°58′S, 70°38′W). The climate is Mediterranean with an average annual rainfall of ∼650 mm concentrated in June–August (Espinoza et al. 2012). Landscape characteristics were determined using spatially explicit information on agricultural land-use analyzed with QGIS v.2.2 (QGIS Development Team 2009). The information obtained was analyzed in terms of the proportion of cultivated land and semi-natural areas. These analyses were calculated using R v.2.14 (R Core Team 2015) (for more information, see Raymond et al. 2015). Wheat growth stages did not differ in the two geographical areas (see table A.3 in Raymond et al. 2015). In order to explore possible block effects, a

reclassification analysis was carried out: two fields from the simple set with the higher amount of semi-natural habitats (respectively, fields S01 and S09, 41 and 27%) were considered as ''complex''; and the two fields from the complex set with the lower amount of semi-natural habitats (respectively, fields C05 and C12, 24 and 19%) were considered as ''simple''. As the reclassification analyses did not differ in terms of the results from the original landscape classification analyses, the results presented hereafter are the results of the analyses processed with the original landscape classification.

Sampling

Five sampling dates separated by 15-day intervals were performed over all fields for simple and complex sets during the main period of aphid colonization until the period of the aphid population breakdown. On each sampling date and in each field, three transects were chosen. Each transect was about 50 m long and located in parallel to one edge of the field and approximately 5 m from the border. Over each transect, live apterous *S. avenae* individuals (nymphs and adults morphs) were carefully collected by choosing at random 20 wheat plants (infested or not) by hand in a plot of 1 m² and removing all individual aphids using a small brush and counted. Only live individuals were collected and aphids containing parasitoid pupae (aphid mummies) were excluded in order to avoid misidentification because of the lack of morphological information of the aphid mummies. All specimens were then carried to the laboratory in single micro-centrifuge tubes containing 99% ethanol at 0 °C and then were taxonomically identified at species level using taxonomic keys (Blackman and Eastop 2000). A subset of the sample (maximum 10 individuals per sampling date and per field) were conserved in single 1.5-mL sterile micro-centrifuge tube containing 99% ethanol at −20 °C in order to diagnose endoparasitism occurring in the field. Also, coccinellid and carabid beetles were sampled alive at each sampling date using sweep-net strokes and pitfall traps, respectively (more details of the predator sampling are described in the separate publication, Raymond et al. 2015). All predators specimens were placed in a single 1.5-mL micro-centrifuge tube, counted and morphologically identified at the lowest level.

Diagnostic PCR evaluation of the collected aphids

Molecular approaches allow us to identify the presence of target species inside a single sample and describe the assemblage of primary and secondary parasitoids. A DNA-based protocol was used to diagnose the presence of fragments of DNA of different parasitoid species within each sampled aphid at the same time. Furthermore, in order to determine if each aphid sample could be parasitized, from the total aphid samples (collected from the 20 fields during the five sampling dates), 614 aphid specimens were chosen (369 and 272 specimens from the simple and complex set of fields, respectively). Then, DNA from the chosen specimens was extracted from the whole body and a diagnostic PCR screening (employing a multiplex/single-plex PCR protocol) was carried out using specific primer pairs for the parasitoid community described by Traugott et al. (2008).

DNA was extracted using the Salting Out protocol (Sunnucks and Hales 1996) by disrupting the tissue and homogenizing the specimen with a DNA-free pestle. Each sample was incubated with Proteinase K for 4 h at 58 °C, and finally the dried DNA pellets were eluted in 40 μL of double-distilled H_2O. As a second step, to check if the samples contained parasitoid DNA, a multiplex PCR and subsequently singleplex PCR system were carried out. In order to test for several parasitoid species within one reaction, the assay was performed using Traugott et al. (2008) methodology, which includes group-specific and species-specific primer pairs allowing the examination of seven parasitoid species (primary and secondary parasitoids). Primary tests with known specimens from previous collections in Chile indicated that the methodology published by Traugott et al. (2008) was able to detect all species of primary parasitoids except *A. colemani* and *A. matricariae*. A species-specific primer for *A. colemani* and *A. matricariae* was not developed as the prevalence of this parasitoid species in *S. avenae* is less than 3 and 0.4%, respectively (Zepeda-Paulo et al. 2013; Peñalver et al., unpublished). *A. victrix* and *Dendrocerus carpenteri* Curtis, 1829 (Hymenoptera: Megaspilidae) have been reported as the most abundant secondary parasitoids in comparison with others (Suzuki Sone and Vargas Mesina 1980; Guerra et al. 1998; Ferrer-Suay et al. 2013). All parasitoid species used in combination with multiplex and singleplex PCR by Traugott et al. (2008) have been reported for the aphid parasitoid fauna in Chile with the exception of *Toxares deltiger* Haliday, 1833 (Hymenoptera: Braconidae); nevertheless, as some taxonomical studies have been carried out for more than two decades, we decided to include all parasitoid species primer pairs in order to detect any possible new record of *T. deltiger* parasitoid species in Chile. Our modifications to the multiplex PCR system were as follows: 0.8 μL of buffer 5× (Promega), 0.1 μL of dNTPs (10 mM), 0.8 μL of $MgCl_2$ (25 mM; Promega) and 0.06 μL of Taq polymerase (5U/μL; Promega) were used. The primer pairs were added at the same concentration described by Traugott et al. (2008) for the multiplex PCR system (table 2 in Traugott et al. 2008). The cycling conditions were as follows: 15 min at 95 °C, 40 cycles of 30 s at

94 °C, 3 min at 62.5 °C, 1 min at 72 °C and a final elongation for 10 min at 72 °C. In the cases of the samples which tested positive with the *Aphidius* group primer pair in the multiplex PCR system, a second PCR reaction was carried out in order to identify four parasitoids at the species level using singleplex PCR. Those singleplex PCR protocols were performed covering the species, *A. avenae*, *A. ervi*, *A. uzbekistanicus* and *A. rhopalosiphi* using the same protocol described by Traugott et al. (2008) for the singleplex PCR systems. In order to avoid false-positive amplifications, a minimum of three negative controls were included in each batch of analyzed samples (double-distilled H_2O substituting for DNA extracts). All PCR reactions were carried out in a sterile laminar flow cabinet, free from insect DNA in order to avoid any possible cross-contamination. Samples of multiple parasitized aphids were sequenced and double-checked with singleplex PCR for each species to ensure any possible positives. PCR products were separated by electrophoresis with 3% agarose gels using 100 V for 120 min (multiplex PCR) and 90 min (singleplex PCRs).

Statistical analyses

All the data were analyzed using the statistical software R v.2.14 (R Core Team 2015). Normality of errors and homogeneity of variances were tested for the residuals of the models using Shapiro–Wilk and Barlett tests, respectively. In order to understand the temporal dynamics of *S. avenae* and its parasitism rates, differences in aphid relative abundances and parasitism rates were analyzed using generalized linear models (GLM) employing the *lme4* package (Bates et al. 2015) assuming a binomial error distribution. Parasitism rates were calculated as the number of aphids testing positive for parasitoid DNA (all species jointly and separated by species) in respect to the number of all screened aphids. Only samples with at least eight aphids were considered in this analysis in order to avoid artificial parasitism rates. This rule ensured a sample size of 542 aphid specimens 306 (from six replicates) and 236 (from five replicates) specimens from the simple and complex set of fields, respectively. Parasitism rates were more conserved compared with analyses including all samples from the field. However, significances in the interaction effect between the explanatory factors did not change when considering 7, 8 and 10 samples per date/field (data not shown). The landscape context (complex and simple sets of fields) and the sampling dates (five dates) were used as explanatory factors. All the GLMs were systematically checked for data over-dispersion and randomness of residuals, and the best suitable models were selected according to the value of the Akaike Information Criterion (AIC) of each model, using the *car* package for assessing significances between models in AIC values (Fox and Weisberg 2011).

Diversity indexes, as the specific richness, Shannon, and evenness of Pielou implemented in the package *vegan* (Oksanen et al. 2016) were calculated for the whole natural enemy assemblage in both sets of fields. The aphid predators: coccinellid and carabid beetles (details of this group are given in the separate publication, Raymond et al. 2015) jointly with the parasitoids assemblage were taken into account, as well as the parasitoid assemblage on its own. Each index was carried out considering each sampling date in order to understand any temporal effects, along the season and between both field sets. The interaction between both factors ("date" × "landscape") was also considered as an explanatory factor and the comparisons of these diversity indexes were carried out using ANOVAs.

Results

Landscape characteristics and the spatial explicit information on agricultural land-use are given in detail in Online Resource 1.

Abundance of aphids

As has been previously reported (Raymond et al. 2015), a total of 2150 aphid samples were collected. Among them, ∼85% were morphologically identified as *S. avenae* which corresponded to a total of 1819 individuals, considering the whole sampling period on both field sets. Over the five sampling dates, the mean *S. avenae* aphid relative abundance per field was only significantly different for the first sampling date (Z value = 2.31, $p = 0.02$) with an average of 62.7 ± 22.63 and 25.8 ± 8.39 aphids/field in the simple and complex set of fields, respectively (Fig. 1). For the remaining sampling dates, the temporal dynamics of the aphid population growth was similar and the relative abundance of *S. avenae* did not differ significantly between the two sets of fields (2nd date: Z value = 0.5, $p = 0.61$; 3rd date: Z value = −0.13, $p = 0.89$; 4th date: Z value = −0.06, $p = 0.95$; and 5th date: Z value = 0, $p = 1$) (Fig. 1) (details of the best suitable chosen model according to the AIC value are given in Online Resource 2).

Temporal dynamics of predator species

From all the sampled predator specimens over both field sets and the five dates, the four main observed coccinellid species were *Hippodamia variegata* Goeze, 1777 (Coleoptera: Coccinellidae) (50%), *Eriopis chilensis* Hofmann, 1970 (Coleoptera: Coccinellidae) (34%), *Hippodamia convergens*
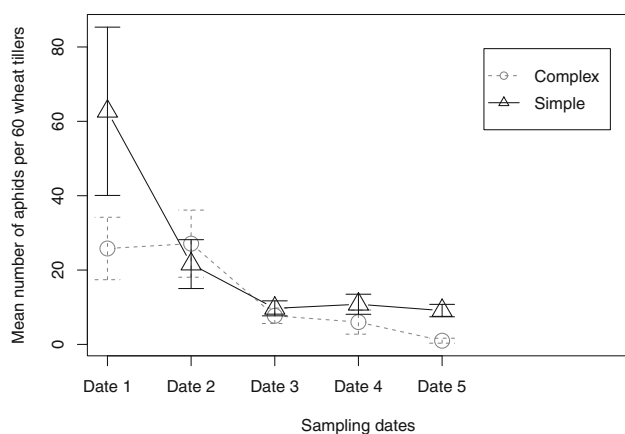
Fig. 1 Population growth of *S. avenae* at each sampling period (five dates) in each landscape context. Complex fields are in *gray* and simple fields are in *black*

Guérin-Méneville, 1842 (Coleoptera: Coccinellidae) (9%) and *Harmonia axyridis* Pallas 1773 (Coleoptera: Coccinellidae) (7%). According to the relative abundances of the coccinellid species, significant temporal differences occurred between the two field sets. A greater abundance was observed in the complex set during the 2nd, 3rd and 4th sampling dates, with a significantly lesser abundance on the 5th sampling date [previously reported in Raymond et al. (2015)]. Simple fields, in contrast, had higher abundances of coccinellids late in the season. Additionally, for all carabid beetle species collected, no significant differences were found between the two field sets. However, *Calosoma vagans* Dejean, 1831 (Coleoptera: Carabidae) was a dominating species which was mostly observed in the simple set of fields (previously reported in Raymond et al. 2015).

Temporal dynamics of parasitoid species

Over all the sampling period, in the case of the simple set, the number of samples which tested positive for one or more parasitoids was 138 (37.39%) and in the complex set, a total of 93 (34.19%) aphid samples tested positive for one or more parasitoids. From the ten parasitoid species which could be detected by both PCR protocols (multiplex and singleplex PCR), only five species of primary parasitoids were found in our study, which were: *A. ervi, A. avenae, A. rhopalosiphi, A. uzbekistanicus* and *P. gallicum. A. uzbekistanicus* was the most abundant species and was detected in 97 samples (70.28%) from the total of all parasitized samples of the simple set over all the sampling period. In the complex set, two primary parasitoid species were the most abundant: *A. ervi* and *A. uzbekistanicus* at a frequency of 52 (55.91%) and 50 (53.76%), respectively. Only one secondary parasitoid species, *D. carpenteri*, was detected in both the complex and simple sets.

Interestingly, from the total sample, the number of samples which contained more than one primary parasitoid species was very high, a total of 111 samples being observed as multiparasitized samples considering all sampling dates in both sets of fields, where 78 samples (61.41%) and 33 samples (55%) were considered as multiparasitized of the total of parasitized samples in the simple and complex set of fields, respectively.

The secondary parasitoid *D. carpenteri* was detected in 9 samples which contained more than one primary parasitoid (multiparasitized), otherwise the other remaining 27 aphid samples which contained *D. carpenteri* had only one primary parasitoid.

Regardless of the multiparasitism which could occur on each single aphid sample, the molecular approach revealed that, over all sampling dates and fields with eight or more aphids, the parasitism rates detected for each species for the simple set were: *A. uzbekistanicus* (63.06 ± 6.80%), *A. ervi* (40.77 ± 7.31%), *A. rhopalosiphi* (36.16 ± 6.51%), *A. avenae* (4.68 ± 2.76%) and *P. gallicum* (4.30 ± 3.36%). On the other hand, in the case of the complex set, the parasitism rates detected for each species were: *A. uzbekistanicus* (53.84 ± 8.74%), *A. ervi* (44.93 ± 9.04%), *A. rhopalosiphi* (36.82 ± 9.01%), *P. gallicum* (6.05 ± 4.72%) and *A. avenae* (0.65 ± 0.65%).

In terms of parasitism rates per landscape, in the simple set, the mean parasitism rate over all sampling dates was 41.07 ± 4.90%, which was similar to the complex set which was 34.89 ± 5.10% ($\chi^2 = 0.79$, $df = 1$, $p = 0.37$) over the whole sampling period (percentages refer to the rate in respect to the total number of the screened samples) (detailed parasitism rates per sampling date and field set are given as Online Resource 3). Nevertheless, the temporal dynamics of the parasitism rates were not equivalent between the two sets of fields. Particularly, in the simple set, the variability among sampling dates was higher. In terms of the proportion of parasitized aphids, significant differences were found (Table 1) at the 2nd and 3rd sampling dates (Z value = 2.19, $p = 0.02$; Z value = 3.00, $p < 0.01$, respectively), in comparison with the complex set which was less variable (Fig. 2).

The detection of *D. carpenteri* occurred only during the 1st and 2nd sampling dates and significant differences among both field sets and sampling dates were found, without interactions between both factors (Table 1). Independent of the sampling date, a significantly higher proportion of secondary parasitized samples were found in the complex set (1st sampling date: Z value = −2.32, $p = 0.02$; 2nd sampling date: Z value = −3.99, $p < 0.01$), with a similar temporal dynamics for both field sets (Fig. 3) (details of the best suitable chosen model according to the AIC value are given in Online Resource 2).

Table 1 Summary of the generalized linear mixed model of proportion of parasitized aphids and secondary parasitized aphids comparing the landscape context (*Landscape*) and the sampling date (*Date*) factors

	Factor	χ^2	df	p value
Parasitized aphids	Landscape	1.13	1	0.28
	Date	8.70	4	0.06
	Landscape × date	12.15	3	0.006*
Secondary parasitized aphids	Landscape	5.39	1	0.02*
	Date	15.92	4	0.003*

df degrees of freedom

*significant p value

Fig. 2 Mean proportion of parasitized aphids on each sampling period (five dates) in each landscape context. Complex fields are in *gray* and simple fields are in *black*
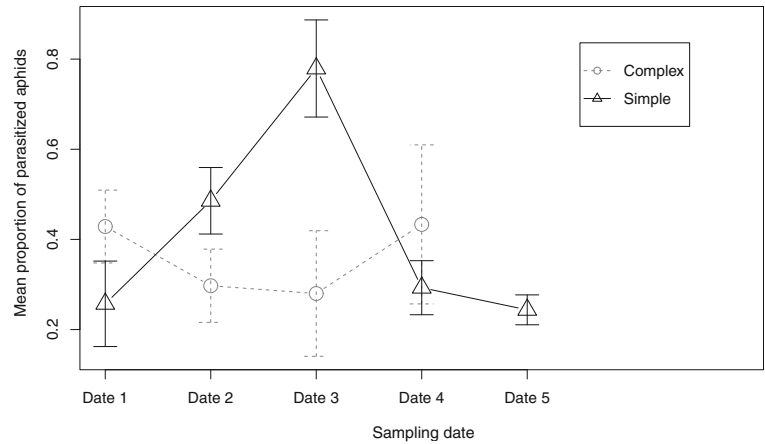
Fig. 3 Mean proportion of secondary parasitized aphids on each sampling period (five dates) in each landscape context. Complex fields are in *gray* and simple fields are in *black*
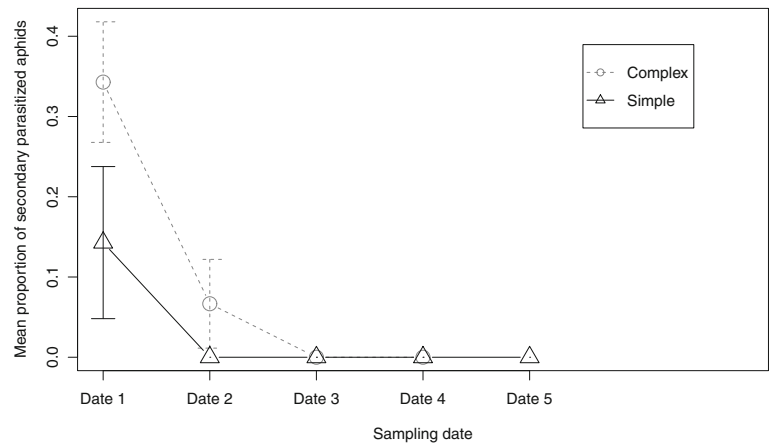
Diversity indexes of natural enemies

The details of the indexes for the whole natural enemy assemblage and for the parasitoid assemblage, according to the landscape complexity and sampling date, are given in Table 2.

In terms of the specific richness, the temporal dynamics of the whole assemblage of natural enemies (all sampled guilds) varies considerably between both sets of fields, as well as over time (interaction "date" × "landscape" F value = 5.85, $df = 4$, $p = 0.0003$). For the complex set, the greatest specific richness was found at the beginning of the season (1st sampling date: F value = 11.27, $df = 1$,

$p = 0.003$) with a steady decrease which was more evident in the last two sampling dates (Fig. 4a). Contrary to this, in the simple set, the richness increased slightly during the 4th sampling date, without presenting a decline by the end of the sampling period, significantly different to the dynamics of the complex set (5th sampling date: F value = 7.79, $df = 1$, $p = 0.01$) (Fig. 4a). When only the primary parasitoids assemblage is considered, we found differences depending on the sampling date and field set (interaction "date" × "landscape" F value = 4.31, $df = 4$, $p = 0.003$). Taking into account each sampling date, we found the greatest richness during the 1st and 3rd sampling

Table 2 Analysis of variance (ANOVA) of the influence of landscape complexity on the diversity indexes (specific richness index, Shannon index and evenness of Pielou) of the whole natural enemy assemblage and for the parasitoid assemblage alone over each sampling date

	Sampling date	df	Specific richness			Shannon			Evenness of Pielou		
			Mean sq	F value	p value	Mean sq	F value	p value	Mean sq	F value	p value
Whole natural enemy assemblage	Date 1	1	39.2	11.27	0.003*	1.95	12.01	0.002*	0.00	0.55	0.46
	Date 2	1	5.0	1.06	0.31	0.14	0.42	0.52	0.04	4.59	0.04*
	Date 3	1	3.2	0.93	0.34	0.03	0.15	0.7	0.05	6.59	0.01*
	Date 4	1	16.2	4.3	0.05	2.03	7.64	0.01*	0.08	4.75	0.04*
	Date 5	1	22.05	7.79	0.01*	1.97	11.5	0.003*	0.00	0.34	0.56
Parasitoid assemblage	Date 1	1	6.05	5.76	0.02*	0.71	4.94	0.03*	0.00	0.00	0.97
	Date 2	1	3.2	1.8	0.19	0.61	2.87	0.1	0.00	0.26	0.62
	Date 3	1	3.2	1.64	0.21	0.23	0.94	0.34	0.00	0.05	0.81
	Date 4	1	16.2	12.9	0.002*	1.87	11.8	0.002*	0.00	1.14	0.3
	Date 5	1	4.05	8.57	0.008*	0.08	1.09	0.31	0.00	1	0.42

df degrees of freedom

*significant p value
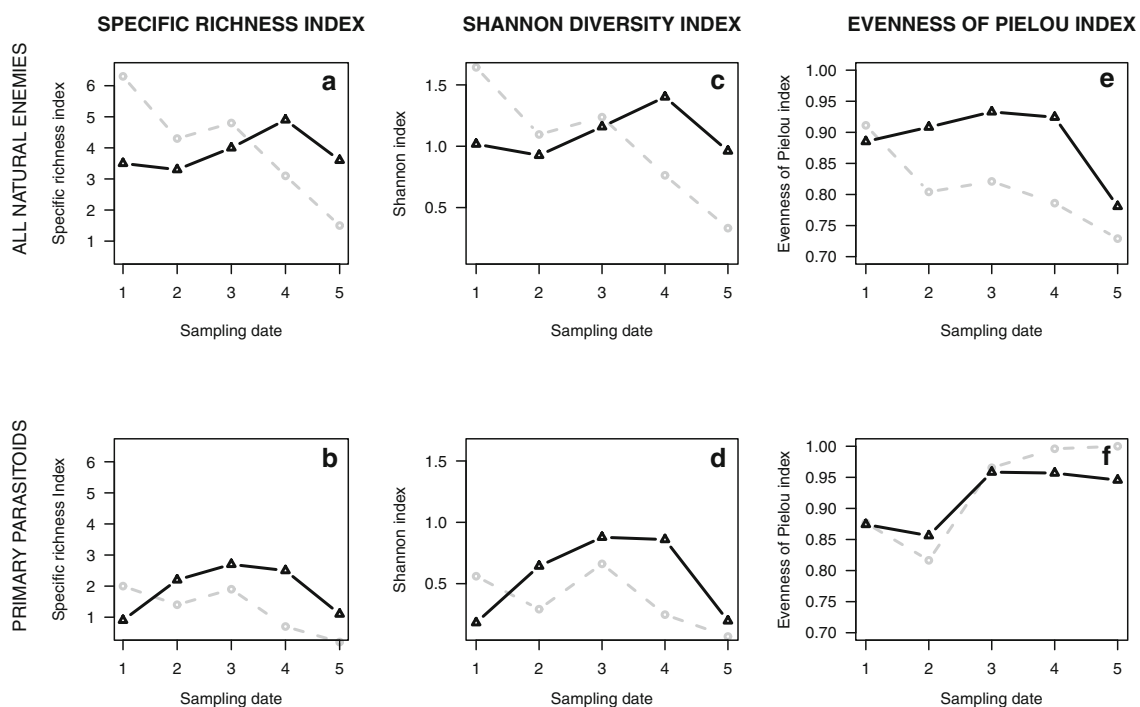
Fig. 4 Temporal dynamics of the diversity indexes for the whole natural enemy assemblage and for the parasitoid assemblage on each sampling period (five dates) in each landscape context. Complex fields are in *gray* and simple fields are in *black*

dates in the complex set; nevertheless, at the 4th and 5th sampling dates, a comparatively very low specific richness was found in this set of fields (Fig. 4b). For the simple set, a maximum richness occurred during the 3rd sampling date with a decline towards the 4th and 5th dates. Comparing both sets of fields, the specific richness is higher in the simple set at the 1st sampling date (F value = 5.76,

$df = 1$, $p = 0.02$) and then is lower at the last dates (4th sampling date: F value = 12.9, $df = 1$, $p = 0.02$ and 5th sampling date: F value = 8.57, $df = 1$, $p = 0.08$) (Fig. 4b).

Analyzing the temporal dynamics of the Shannon diversity indexes for the whole assemblage of natural enemies, we observed differences between both sets of fields and the

sampling date (interaction "date" × "landscape" F value = 6.53, df = 4, p = 0.0001). For each sampling period, the temporal dynamics of the diversity indexes was similar. A significantly higher Shanon index was observed for the complex set, decreasing along the sampling dates (1st sampling date: F value = 12.01, df = 1, p = 0.002). Significantly lower values of this index were found at the 4th and 5th sampling dates (4th sampling date: F value = 7.64, df = 1, p = 0.01 and 5th sampling date: F value = 11.5, df = 1, p = 0.003), whereas in the simple set the indexes were approximately the same (with an increase at the 4th sampling date) (Fig. 4c). In the case of the primary parasitoid assemblage, we also found a considerable effect of the sampling date and field set (interaction "date" × "landscape" F value = 3.96, df = 4, p = 0.005), and the temporal dynamics of this index was also very similar to the specific richness curve. For each sampling date, the Shannon index in the complex set was greater at the 1st and 3rd sampling dates (statistically different from the simple set in the 1st: F value = 4.94, df = 1, p = 0.03), declining from the 4th date [statistically different from the simple set (F value = 11.8, df = 1, p = 0.002)] and reaching their lowest value in the last sampling date (Fig. 4d). In contrast to this, the simple set exhibited a greater diversity of parasitoids from the 2nd date to the 4th, also reaching the lowest value at the last sampling date (Fig. 4d).

For the evenness of Pielou, the effect of the sampling date and landscape of the whole assemblage of natural enemies did not vary significantly (interaction "date" × "landscape" F value = 1.57, df = 4, p = 0.19). Despite this, the temporal dynamics varied depending on the sampling date. Differences between both field sets were found in the middle of the season (2nd sampling date: F value = 4.59, p = 0.04, 3rd sampling date: F value = 6.59, p = 0.01 and 4th sampling date: F value = 4.75, p = 0.04), the evenness in the simple set being greater than in the complex set (Fig. 4e). Considering only the assemblage of parasitoids, no differences were found in the field set over time (interaction "date" × "landscape" F value = 0.32, df = 4, p = 0.85) nor at each sampling date. Nevertheless, regardless of the field set, an increase of evenness was shown from the 3rd sampling date until the end of the season (Fig. 4f; Table 2).

Discussion

Sitobion avenae has been one of the most important cereal aphids for the wheat production in Chile; during the early 1970s, the damage produced important economical loses. This is why in 1975 the Chilean government financed an extensive biological control program, allowing the introduction of several parasitoid species. This successful program, which avoids the use of insecticides, has been successful against the introduced parasitoids (Rojas 2005). However, the role of native natural enemies has not been sufficiently studied.

In our previous study (Raymond et al. 2015), temporal differences in predator abundances were explained by the landscape context. Fields in complex contexts, with high landscape structural complexity and greater semi-natural areas with low input of agricultural practices, presented higher abundances of predators earlier in the season. In contrast, fields in simple contexts, with higher cropping areas and more intensive agricultural management, had low predator abundances early in the season, but with an increase at the end of the season. However, the role of the parasitoid assemblages in these two contexts was not addressed. By using molecular methods in this study, the host–parasitoid interactions could be analyzed in a cost- and time-effective manner in order to understand the influence of the landscape contexts on parasitism rates, contrasting these with the predatory data. The methods developed by Traugott et al. (2008) allowed us to check the frequency of the different species occurring in the two sets of fields, also addressing the interactions between parasitoid species, which are impossible to follow with classical approaches (Juen and Traugott 2005).

Abundance of aphids

In terms of landscape complexity, the difference between the simple and complex sets of fields in average arable land was only 38%. Therefore, the similar aphid abundances detected between sets of fields could be partially explained by this. Nevertheless, when comparing with studies conducted in Europe, the differences among simple and complex landscapes were in some cases less (20%; Plećaš et al. 2014) or more (46–51%; Gagic et al. 2011; Jonsson et al. 2015). Hence, these similarities in aphid abundance may not be completely attributable to these differences found between different landscape complexities.

As we observe in both landscapes, aphid abundance was high at the beginning of the season, especially in the simple set of fields with the peak population density around the first sampling dates and finally started to decrease towards the end of the season, with greater variability in simple fields which could be attributed in part to agricultural practices. A potential decrease in aphid abundances per field could also respond to the greater availability of crop area in the simple context, which may be supported by the dilution effect hypothesis (Tscharntke et al. 2012). In the case of the complex fields, a spurious increase in aphid populations could be a crowding effect, as complex landscapes have less arable land and therefore less host plants

on which to forage (Debinski and Holt 2000; Grez et al. 2004).

Temporal dynamics of predator species

Even though the difference in arable land between landscape contexts was only 38% and the aphid population densities, as mentioned above, were similar between the two sets of fields, the population temporal dynamics of the predators in these two systems were different. Predator dynamics observed in the complex contexts were characterized by the early arrival of individuals, as compared to the later arrival observed for the simple context fields, both driven mainly by coccinellid beetles and having a negative effect on aphid densities at a field level (GEEs results in table 1 in Raymond et al. 2015). This suggests that the biological control by predators is occurring in both sets of fields (Raymond et al. 2015).

Temporal dynamics of parasitoid species

In order to understand the total effect of the temporality observed for the predators and its effect on the aphid populations, the dynamics of other natural enemy guilds, such as the parasitoids, should be considered. Several studies have shown that parasitoid assemblages could be influenced by the landscape complexity (Bianchi et al. 2006; Tscharntke et al. 2007), as semi-natural areas provide important resources which improve the survival and fecundity of parasitoids, and also natural enemies are negatively affected by conventional (intensive) management, even more than other insects (Östman et al. 2001; Bengtsson et al. 2005). Nevertheless, as we report here, and similar to other studies (Vollhardt et al. 2008; Macfadyen et al. 2009b), arable fields in high-intensity simplified landscapes and structurally complex landscapes can support similar richness of cereal aphid parasitoids. The parasitoids *A. uzbekistanicus* and *A. ervi* were the most frequent species detected over both fields. Nevertheless, in the simple set, *A. uzbekistanicus* seems more frequent than *A. ervi*. As estimated here, the relative abundance based on the frequency of detection via molecular methods, without information on the final successful emergence of the adult parasitoid, means that we must be cautious in the interpretation of these frequencies. Indeed, 111 samples from both field sets and considering all dates had more than one parasitoid species. Compared to previous studies carried out in the same region, the relative frequency of more prevalent species does not change, with the exception of *A. uzbekistanicus*. Based on studies of Pons and Starý (2003), Plećaš et al. (2014) and our own (Zepeda-Paulo et al. 2013), *A. ervi* higher prevalences could be due to strong competition with *A. uzbekistanicus* (van Baaren, personal

communication), therefore the true relative importance could be lower (many samples collected from simple field had *A. uzbekistanicus* and *A. ervi* in the aphid as multi-parasitized samples, and the most probable emerging parasitoid would be *A. ervi*).

Strong differences in parasitism rates along the sampling dates were evidenced, suggesting that the parasitoid temporal dynamics is affected by the landscape context. In the case of the simple set of fields, aphid primary parasitism was greater between the second and third sampling dates, with a population decrease by the end of the season. This increase in the parasitism rates in simple landscapes contradicts the evidence of Roschewitz et al. (2005) and Plećaš et al. (2014) in long-term studies, where higher parasitism rates were found in complex landscapes. Proximity to alternative host-plants which can provide resources for adult parasitoids, such as other hosts, nectar and overwintering sites, should be increased in complex landscapes (Langer 2001; Tylianakis et al. 2004; Straub et al. 2008; Macfadyen et al. 2009a). However, greater complexity increases diversity which could increase negative interactions among natural enemies (e.g., the predation of natural enemies by general predators and more secondary parasitoids). Earlier predation in the complex fields could negatively affect the population build-up necessary for parasitoids. As we have shown previously, the greater abundance of predators occurring during the 2nd and 3rd sampling dates in the complex fields (Raymond et al. 2015) could have negatively affected the parasitoid populations. Preliminary analyses of the gut content of these predators have shown the frequent presence of Aphidinae parasitoids, as well as aphids and other coccinellid species (Ortiz-Martínez unpublished).

Effect on secondary parasitism

The low hyperparasitism rates found here could be related to the lack of information of other species, which were not included in this study. Future information about the ecology of aphid hyperparasitoids in Chile via taxonomical and molecular tools will be needed. Morphological identification of hyperparasitoids species have been reported for Chile (Suzuki Sone and Vargas Mesina 1980) suggesting the importance of *D. carpenteri* on the temporal dynamics of the primary parasitism at the field level. Secondary parasitoids could be more common in complex contexts, and indeed in our study *D. carpenteri* was detected only at the beginning of the season (1st and 2nd dates) and was more prevalent in the complex set of fields. Even though the main period of hyperparasitoid abundance in our study is in accordance with the Suzuki Sone and Vargas Mesina (1980) report, further research including mummies and other parasitoid species should be carried out. This partial

result, however, suggests that complex landscapes with more subsidiary resources could sustain more interactions and more trophic links, and possibly negatively affect the primary parasitoid populations (Rosenheim 1998; Tylianakis et al. 2004; Rand et al. 2012). Parasitoid food web studies have shown that in complex contexts the secondary parasitism rate increases with landscape complexity (Jonsson et al. 2009; Gagic et al. 2011). Here, the effect of *D. carpenteri* could be even greater than we have detected, as studies have shown that this parasitoid species prefers to lay eggs mostly in mummified aphids (Müller et al. 1999; Traugott et al. 2008), which we did not assess in this study.

Aphid use by different guilds of natural enemies

Some studies have suggested that generalist predators play an important role in cereal aphid suppression; however, the associated parasitoid assemblages had a greater effect on the pest populations in field exclusion tests (Snyder and Ives 2003; Schmidt et al. 2003). Our previous study shows a strong correlation between aphid population growth and predator increase at a regional scale, taking into account the temporal data using General Estimating Equations accounting for spatial autocorrelation. Landscape context significantl affectey the temporal dynamics between predators and aphids, thus showing that there is an early control in the complex context and a later control in the simple context (Raymond et al. 2015). When analyzing the parasitism rates, we found a higher parasitism rate occurring in the mid-sampling periods of the simple set of fields, but with a steady increase from the first sampling date (25.71% for the 1st sampling date), which could explain why, in these simple fields, aphids are controlled, although predator abundances and their effects were low (Fig. 2 in this study, and fig. 3 in Raymond et al. 2015). These results highlight the importance of parasitoids in simple landscapes with high agricultural intensification, even if these landscapes have many features, such as greater field sizes and regular perturbations, that have been shown to affect parasitoid populations (Holt et al. 1999; Thies et al. 2005). Therefore, temporal niche partitioning could occur for these two guilds resulting in a complementary impact on the aphid populations (Straub et al. 2008). Although, in the complex context fields, most of the control seems to occur as a result of predator abundances (Raymond et al. 2015), parasitism rates are steady through the season and reach moderate levels of parasitism. Therefore, in this case, predators may have a stronger effect on aphid populations than the parasitoids, possibly due to the effect of secondary parasitoids on the primary parasitoid populations at the beginning of the season. The greater diversity observed for all natural enemy guilds in the complex context (Fig. 4c) may promote some negative interactions. Aphid predators could cause mortality in the primary parasitoid populations via intra-guild predation. In this sense, it would be necessary to address the frequency in both sets of fields and the temporal dynamics of these predatory interactions within and between predators and parasitoids (Snyder and Ives 2003). As mentioned before, molecular approaches have been shown to be a reliable tool to study the frequency of these interactions (Juen and Traugott 2005; Traugott and Symondson 2008; Traugott et al. 2013), and is currently being used to assess the samples from this study.

We suggest an important effect of the parasitoid assemblages in the simple landscapes and a lower effect in the complex landscapes on the cereal aphid's biological control. Also, the presence of predators is an important factor which modulates the temporal dynamics of both groups of biological control agents. Furthermore, landscape context (structural complexity and agricultural management) is not the only factor which could alter the biological control of a given pest.

Author contributions

SO carried out the experiment design with BL, field sampling, laboratory and data analyses and manuscript preparation. BL carried out with SO experiment design and field selection, data analyses and manuscript preparation.

Compliance with ethical standards

Conflict of interest SO and BL declare that they have no conflict of interest. All authors contributed equally.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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