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RAPID SPREAD OF *HARMONIA AXYRIDIS* IN CHILE AND ITS EFFECTS ON LOCAL COCCINELLID BIODIVERSITY

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ABSTRACT

Aim Biological invasions are one of the major threats to biodiversity. Usually highly disturbed anthropogenic habitats favours invasion by alien species such as the coccinellid *Harmonia axyridis*. The spread and impact of this species has been documented in Europe and North America, but no information exists for South America. The aims of this study were to: 1) document the process of invasion of *H. axyridis* in Chile, 2) compare the abundance of *H. axyridis* in different habitats with varying degrees of disturbance, and 3) assess change in the coccinellid assemblages in alfalfa fields over the six years following invasion.

Location Chile

Methods The spread of *H. axyridis* was estimated using information from citizen scientists alongside records from the National Pest Surveillance System. The abundance of *H. axyridis* in different habitat types and of all coccinellids in alfalfa fields was assessed using yellow sticky traps. In alfalfa, the variations in species richness, Shannon and Simpson diversity and equitability indices through time were compared.

Results *Harmonia axyridis* has rapidly increased in distribution: there have been 1875 records along 2863 km up to 2015 following the first observation in 2008 from Central Chile. The records span from sea level to 3200 m a.s.l. in the Andes. It has spread at an average rate of 184.8 km per year, preferentially colonizing disturbed habitats, but also invading native habitats. In alfalfa it is particularly abundant and has become the dominant species, with a concomitant decrease in species richness and diversity of co-occurring species.

Main conclusion Citizen science, alongside professional surveillance, has provided an effective method for studying invasion by *H. axyridis,* which is now well established and distributed across Chile. The rate of spread has been dramatic and the associated changes to the coccinellid community could disrupt the functioning, and ultimately resilience, of invaded ecosystems.

Keywords

Citizen science, Coccinellidae, Distribution, Harlequin ladybird, Invasion

INTRODUCTION

Biological invasions are among the greatest threats to global biodiversity (Lambertini *et al*., 2011). Usually, land use change from natural to highly disturbed anthropogenic habitats favours invasion by alien species (Gurevitch & Padilla, 2004; Didham *et al*., 2007).This is indeed the case within coccinellid assemblages, where the relative abundance of alien species increased significantly with increasing disturbance intensity (Grez *et al.,* 2013). Thus, the interaction between the processes of land use change and invasion may result in a decrease in biodiversity of local species assemblages (Didham *et al*., 2007).

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae), the multicoloured Asian lady beetle or harlequin ladybird, is a generalist predator native to central and eastern Asia that has been introduced accidentally, and intentionally for the biological control of aphids and other insects, to many countries in Europe, Africa, North and South America (Koch, 2003; Saini, 2004; Koch *et al*., 2006a; Brown *et al*., 2011a). Larvae and adults of this species prey on a variety of aphid species as well as other small insects (Hodek & Evans, 2012). The wide diet breadth, together with relatively large body size (5 – 8 mm long), high voracity and predation efficiency of *H. axyridis* coupled with ease of mass rearing, were considered good characteristics to provide successful biological control of many pests (Koch, 2003). Indeed, there is evidence that it has fulfilled this potential (Lucas *et al*., 2007; Soares *et al.*, 2007; Koch & Galvan, 2008; Evans *et al*., 2011). However following introduction, *H. axyridis* has spread rapidly and increased in abundance, with several impacts on agriculture, people and non-target species (Koch, 2003; Koch & Galvan, 2008). Today *H. axyridis* is considered as one of the most serious invasive alien species (IAS) around the globe, with potential adverse impacts that arguably outweigh the benefits of pest control (Majerus *et al*., 2006). Because of this, it is regarded a model species for understanding invasion (Roy & Wajnberg, 2008; Roy *et al*., 2016).

It has been widely acknowledged that *H. axyridis* is a threat to non-target arthropods, including native species. For example, Koch *et al*. (2006b) suggested *H. axyridis* to be a potential hazard to monarch butterflies, *Danaus plexippus* (L) (Lepidoptera: Nymphalidae) in the USA. More frequently, studies have shown decline in populations of existing coccinellids, particularly native species, after the establishment of *H. axyridis* in several countries around the world (e.g., Alyokhin & Sewell, 2004; Harmon *et al*., 2007 and Mizell, 2007 in USA; Brown *et al*., 2011b in England, Roy *et al*., 2012 in Europe). The decline in native aphidophagous coccinellids after the arrival of *H. axyridis* has been attributed to competition for prey or space, intraguild predation (IGP) of various life stages (eggs, larvae, and pupae), lower immunity to shared natural enemies, introduction of new pathogens or disrupted mating systems (Kenis *et al*., 2009; Brown *et al*., 2011b; Roy *et al*., 2012; Schmidtberg *et al*., 2013; Roy & Brown, 2015).

In South America, *H. axyridis* was introduced for biological control in the late 1980´s in several countries (Chile, Argentina, Brazil and Colombia), but wild populations has only been reported more recently in these countries and also in Peru, Paraguay, Uruguay, Ecuador and Venezuela (Saini, 2004; Grez *et al*., 2010a; Nedvěd & Krejčík, 2010; Amat-García *et al*., 2011; Brown *et al*., 2011a; González & Kondo, 2012; Kondo& González, 2013; Solano & Arcaya, 2014). Nevertheless, in most of these countries the negative impacts have not been assessed and so it has not been described as invasive. In central Chile (Quillota Province, 32º49’33’’ S, 71º13’38’’ W), a flightless strain was introduced from France in 1998 for the biological control of pest insects in greenhouses, but it did not establish. Later, in 2003, a first feral population was recorded 60km east of the previous location, associated with aphids in poplar trees (*Populus* sp.), without subsequent sightings until May 2008, when we collected one individual in an alfalfa field in Pirque (33º39’50’’ S, 70º35’31’’ W), South of Santiago (Grez *et al*., 2010a). Recently, genetic analyses have demonstrated that the Chilean invasive population originated from Eastern North America, independent of the previous introduction to Brazil and Argentina (Lombaert *et al*., 2014). Chile has a diverse landscape and is a fascinating place to study invasion phenomena, because it is geographically isolated, with the high Andes in the East, the Pacific Ocean in the West, the driest desert of the world in the North and the Antarctic in the South. It is 4,329 km from north to south, but exceptionally narrow (on average 180 km from east to west), with considerable variation in climatic conditions (Luebert & Pliscoff, 2006). Additionally, Chile has a diverse coccinellid assemblage, with 115 species (González, 2006), most of them natives but also including some very abundant alien species like *Hippodamia variegata* (Goeze), which was introduced from South Africa in the late 1970´s (Grez *et al*., 2012). The arrival of *H. axyridis* to Chile may alter the composition and abundance of these assemblages.

Coccinellids are popular insects with the public and many species are considered easy to identify, therefore citizen science approaches, involving non-expert volunteers in monitoring, is practical and achievable. There is increasing recognition of the value of volunteers in the collection of data, so called citizen science (Pocock *et al*., 2015). The role of citizen scientists in contributing to research on IAS has been demonstrated (Roy *et al*., 2015). The large-scale and long-term datasets required to study invasions would be difficult to achieve without the mass participation of citizens. The large number of observations on coccinellids collated through citizen science, particularly in the USA and UK (Gardiner *et al*., 2012, Roy *et al*., 2015), and now in Chile, highlight the potential of such an approach to monitoring the distribution and abundance of *H. axyridis*, and potentially other IAS. The development of a dedicated website with on-line recording for *H. axyridis* in Chile was initiated soon after the arrival of *H. axyridis* and has been hugely successfully both in terms of numbers of observations of this species, the accuracy of the records and people engaged with the research (Grez & Zaviezo, 2015).

Given that the populations of *H. axyridis* in Chile come from the invasive strain of eastern USA, we predict that in time its distribution in the country will continue to increase over time, it will colonize new habitat types and it will become the dominant species in coccinellid assemblages in disturbed anthropogenic disturbed habitats. Here, using observations from citizen science and professional surveys, we: 1) document the spread and distribution of *H. axyridis* in the Chile, 2) compare its abundance in different habitats with varying degrees of disturbance, and 3) assess the change in coccinellid assemblages in alfalfa fields, over the six year period following invasion.

This work represents the first systematic study of *H. axyridis* invasion in South America, and the first assessment of its impacts on the diversity of existing coccinellid assemblages.

METHODS

Analysing the distribution and spread of *H. axyridis* in the country:

Records of the occurrence of *H. axyridis* were obtained from 2008 to 2015 from our initial observations, from citizen science records and from professional surveys of the National Pest Surveillance System of the Chilean Phytosanitary Service (SAG). Citizen science records were gathered through a web site developed especially for this purpose (http://www.chinita-arlequin.uchile.cl/). In this case, each record represents a sighting of *H. axyridis* on a given date and location reported by one person, and could include one or more individual coccinellids observed at various life stages (larva, pupa or adult). Citizen science records were verified from photographs submitted alongside the record, to confirm the species and colour form. Records from SAG were derived from the yellow sticky traps and visual observations by trained personnel throughout Chile. To aid with the identification, a special factsheet with *H. axyridis* information was developed and distributed to SAG personnel. The dataset presented here comprises 1875 (90% from citizen science) validated and georeferenced records of *H. axyridis* in Chile over the time period May 2008 to May 2015. Over 95% of citizen science records corresponded to *H. axyridis* with a valid location.

Continental Chile was divided into 20 km^2 (4.47 x 4.47 km cells), similar to Adriaens *et al.* (2008) for Belgium, using an extension of ArcGIS 9.3 Hawth´s Analysis (Toolshttp://www.spatialecology.com/htools/tooldesc.php). Then, cells with *H. axyridis* occurrence were mapped for each time period (calendar year). To document the invasion process, we recorded the number of verified records per year and the number of new cells occupied per year. We also estimated the northward and southward annual spread from the 2008 observation near Santiago, and the cumulative distribution range (north to south) through the years. For this, in each year the northern and southern range margins of *H. axyridis* was defined as the mean latitude of the ten most northerly occupied cells or the ten most southerly occupied cells, respectively (Hickling *et al*., 2006; Brown *et al.*, 2008).The northward annual spread (km) was calculated as the difference between the northern range of a given year and the northernmost range recorded up to that year (Hickling *et al*., 2006; Brown *et al*., 2008). The southward annual spread was calculated similarly but considering the southernmost latitudes. We did not consider east-west spread because of the narrow shape of Chile, and the constraints to disperse in those directions (Andes mountain range and Pacific Ocean, respectively). The cumulative north to south range covered by *H. axyridis* through the years was calculated as the distance between the northernmost range and the southernmost range recorded up to that year.

Quantifying *H. axyridis* abundance across land cover types through time:

Harmonia axyridis was sampled in spring (September-October) and autumn (March-April) from 2010 to 2014, with the exception of 2013, in eight habitats (land cover types) present near Santiago, the region of our first record. The dominant land cover types, which have been shown to be favourable habitats for coccinellids (Grez *et al*., 2013; Grez *et al*., 2014), in the landscape were sampled: four agricultural crops: alfalfa, annual crops (wheat), vineyards and orchards (plums and walnuts); three semi-natural habitats (anthropogenic unmanaged habitats): blackberry strips, eucalyptus woodlands, tree hedgerows; and a natural habitat: sclerophyllous matorral. Each land cover type was replicated five to 13 times across the landscape, with replicates separated by at least 400 m. In each season, coccinellids were sampled for two weeks by placing five unbaited yellow sticky card traps (15 x 25 cm, two-faced) towards the centre of each patch, 80 cm above ground, and separated by at least 10 m. The sampling height was uniform in different habitats based on previous observations in arboreal vegetation in the same region, in which traps located at

0.8 m captured ladybirds while those at 9 m did not capture any (unpublished data). One-way ANOVAs were run to compare the abundance (log individuals/trap + 1) of *H. axyridis* across land cover types in each year using JUMP® Statistical Software 11.0 (SAS Institute Inc., Cary, NC, USA). Means were separated by Tukey HSD post hoc tests.

Estimating *H. axyridis* dominance, community indices and relationships with native coccinellid abundance in alfalfa fields through time:

Coccinellids were sampled in 10 to 18 commercial alfalfa fields near Santiago, in early spring from 2010 to 2014, using five yellow sticky card traps per field, as described above. Samples were obtained in early spring when coccinellids in alfalfa fields are abundant (Grez *et al*., 2010b). Alfalfa fields used were on average 4 ha, with the crop at least one year old and 30 cm height, with no pesticide application up to the moment of surveying. All adult coccinellids were counted and identified to species and separated by native or alien status. For each field, data from the traps were pooled and the proportion of *H. axyridis* calculated (individuals/total coccinellids). The following community indices were calculated: Species richness, Shannon and Simpson Diversity and Equitability using PAST software (Hammer *et al*., 2001).

One-way ANOVAs were run to compare these indices across years. Means were separated by Tukey HSD post hoc tests. Additionally, we compared the abundance of native coccinellids among years with a one-way ANOVA as above, and correlated the abundance of *H. axyridis* and the abundance of native coccinellids in each alfalfa field (individuals/trap/field/year) through time (2010 to 2014) with a linear regression analysis. All statistical analyses were done in Jump®.

RESULTS

Distribution and spread of *H. axyridis* in the country:

Since our single record in 2008 in Pirque, Central Chile, *H. axyridis* has rapidly increased its occurrence and spread throughout the country (Fig. 1, Table 1). The number of records per year increased from one in 2008 to 698 in 2015, and the number of new 20 km^2 cells occupied by at least one individual increased from 1 in 2008 to 211 in 2015. The total number of records to date is 1875 and the total number of cells occupied is 587, which is equivalent to an area of 11,740 km^{2}invaded. Initially, *H. axyridis* expanded its range to the north, but from 2012 on it expanded its range mostly to the south (Table 1). On average, since 2008, *H. axyridis* spread 54.6 km per year to the north and 129.8 km per year to the south, corresponding to a total annual spread of 184.8 km. Overall, in these seven years *H. axyridis* moved 382 km towards the north and 908.8 km towards the south (Table 1), but considering the extreme occurrences (Copiapó in the north and Punta Arenas in the south) it is now present along 2,863 km north to south (Fig. 1). Records indicate that *H. axyridis* is distributed from sea level to 3,200 m a.s.l. (the latter in the Andes). All individuals belong to the *succinea* colour morph type.

Harmonia axyridis **abundance across habitat types through time:**

Overall*, H. axyridis* had a three-fold increase in abundance from spring 2010 to spring 2014 (0.52±0.18 and 1.56±0.36, respectively). *Harmonia axyridis* has colonized preferentially more disturbed habitats, particularly alfalfa, but through time it has increased its abundance in the other habitat types, including the native sclerophyllous matorral (Fig. 2). In spring 2010, *H. axyridis* was only found in alfalfa, vineyards and hedgerows, but by autumn 2011 it was found in all other habitats except for the matorral, where it was first recorded in spring 2011. In all years, both in spring and autumn *H. axyridis* was significantly more abundant in alfalfa than in other habitats, with the exception of spring 2014, when it reached high abundances also in orchards and annual crops (Fig. 2). Although present in vineyards since spring 2010, it has never reached high abundances in this crop. In 2015, large aggregations of *H. axyridis* were found overwintering in natural habitats including the base of Chilean palm trees (*Jubaea chilensis*) in the sclerophyllous matorral, which demonstrate that this species is also invading native habitats (Fig. 3).

Harmonia axyridis **dominance, community indices and relationships with native coccinellid abundance in alfalfa fields through time:**

The coccinellid assemblages in alfalfa fields in spring in Central Chile comprised ten native and eight alien species, with alien species being much more abundant, representing over 90% of the individuals sampled (Table 2). The most abundant alien species were *H. axyridis* and *H. variegata* and the most abundant native was *Eriopis chilensis. Harmonia axyridis* doubled in abundance from 2010 to 2011, maintaining high abundances until 2014. Total coccinellid abundance varied through time, with the lowest populations in 2013 and the highest in 2014 (Table 2). The proportion of *H. axyridis* in coccinellid assemblages significantly increased on average from ~0.2 in 2010 to ~0.8 in 2013, with a decrease in spring 2014 (Fig. 4). Concomitantly, species richness decreased from 9 and 11 species on average in 2010 and 2011, respectively, to ~four species in 2012 and thereafter. Diversity (Shannon and Simpson indexes) also decreased from 2012, with the lowest values in the last three years of the study (Fig. 4). Equitability was lower in 2013 compared to 2010, but in general, it did not change through time.

In 2010, when *H. axyridis* had low total and relative abundances, coccinellid assemblages were more heterogeneous, with several species well represented in all alfalfa fields (see Figure S1 in Supporting Information). In 2011, when *H. axyridis* increased in abundance, it was also the dominant species in some alfalfa fields, with *H. variegata* being the other dominant species in the remaining fields (Table 2, see Figure S1). In 2012 and 2013, *H. axyridis* was the dominant species in almost all alfalfa fields, with several species being very rare or not present at all, consistent with the low species richness in these years (Fig. 4). In 2014, when *H. variegata* reached its highest abundance (two to 20 times higher than in the previous years; Table 2), *H. axyridis* again was only dominant in some alfalfa fields, like in 2011, but with a lower species richness and diversity (Shannon) (see Figure S1).

The abundance of native species (mean/trap/alfalfa field ± se) diminished from 1.66 ± 0.31 in 2010 to 0.19 ± 0.03 in 2013 ($F_{(4,55)} = 13.79$, $P < 0.001$; Fig. 5a, Table 2); increasing again in 2014. When relating abundance of native species to the abundance of *H. axyridis* in different years there was no significant relationship ($R^2 = 0.524$, $P = 0.167$; Fig. 5b).

DISCUSSION

Citizen science, coupled with professional surveys, has proved an excellent approach for monitoring the invasion of Chile by *H. axyridis*. Professional surveys using sticky traps enabled assessment of the early indications of local impact of *H. axyridis* on other species but the citizen science records were fundamental for determining the spread of the species across the country. The citizen science initiatives established in Chile are unique within South America but parallel those in North America and Europe. *Harmonia axyridis* is now established and distributed widely in Chile. It has spread rapidly from a very restricted area near Santiago in central Chile in 2008 to more than 2,863 km along the country in 2015. Additionally, although it has preferentially colonized alfalfa fields where it reaches high abundances, in more recent years *H. axyridis* has also colonized less disturbed habitats, including the native sclerophyllous matorral. Concurrently, in alfalfa fields, coccinellid assemblages have become significantly less diverse and native species less abundant since the arrival of *H. axyridis*.

Climate and habitat matching analyses comparing South America to the native range of *H. axyridis* in Asia suggested that much of South America has suitable conditions for its establishment (Koch *et al.*, 2006a, Poutsma *et al*., 2008, Bidinger *et al*., 2012). This has been confirmed by scattered records in almost all countries of South America, with the exception of Bolivia, Surinam, French Guiana and Guyanas (González, 2006). Koch *et al.* (2006a) highlighted northern Argentina and southern Brazil as suitable areas for *H. axyridis* and the species has established and spread in these regions, mainly in the last 15 years (summarised in Brown *et al*., 2011a). However, as far as we know, few comprehensive surveys in these regions have been carried out, so the proportion of these areas occupied by *H. axyridis*, and its abundance, are mostly unknown.

Here we provide empirical confirmation of the current status of *H. axyridis* in Chile, where the species has established widespread and abundant populations, and is considered an IAS by the Secretary of Environment of Chile. Moreover, Bidinger *et al.* (2012) predicted a higher probability of invading the southern part of Chile, which has also been confirmed by our records, with this species spreading further and faster to the south than to the north of the country. The rate of spread of *H. axyridis* in Chile has been extremely fast, with a spread of 185 km per year, similar to reports from Europe (Brown *et al*., 2011a), but less than estimates from South Africa (500 km per year) (Stals, 2010), or for eastern North America (442 km per year), although this latter rate might be an overestimation because it includes intentional releases at various locations (McCorquodale, 1998; Brown *et al.* 2011a). In Chile there have been no additional intentional releases after the unsuccessful one in 1998, but the spread of *H. axyridis* throughout the country has been probably mostly natural due to its high flight capacity (Jeffries *et al*., 2013), but also human assisted (e.g., (vegetable and fruit produce, movement with other plants - and accidental transport in cars, trains). The current distribution of the harlequin ladybird in Chile includes two extreme records: one in the north (Copiapó: 27°21´S, 70°19´W) and one in the far south (Punta Arenas: 53°09´S, 70°54´W) (Fig. 1), but these are single records and therefore establishment in these localities is uncertain. More likely, they represent isolated accidental introductions, perhaps as contamination of market produces. So far, our data show that there are big gaps between these extreme records and the core establishment area where *H. axyridis* is more frequently observed and continuously distributed in the country: from La Serena (29°54´S, 71°15´W) to Puerto Montt (41°23´S, 72°54´W) (Fig. 1), representing a considerable area along the country, of 1,283 km (linear distance). Chile has a marked north-south climate gradient, with an increase in precipitation and a decrease in temperature from north to south (di Castri & Hajek, 1976; Luebert & Pliscoff, 2006). The area where *H. axyridis* is most represented includes part of this gradient, with semi-arid environments in La Serena, Mediterranean environments near Santiago and temperate environments in Puerto Montt, with very different plant communities dominating these areas, from scrubby vegetation, sclerophyllous forest to temperate rainforests (Luebert & Pliscoff, 2006). Also, this is the region where most human populations reside and consequently anthropogenic activities are concentrated, including agriculture and alfalfa fields (accounting for over 80% of the alfalfa grown in Chile). This confirms that this species is eurytopic (Adriaens *et al*., 2008), occupying areas with very diverse geomorphologic, vegetational and climatic conditions. It is informative to compare and contrast the distribution of *H. axyridis* globally particularly reflecting on comparisons between climatically similar locations (Roy *et al*., 2016). In Chile, so far *H. axyridis* has not been observed in the arid regions in the north of the country, with desert and subtropical climates. This coincides with the predictions of Bidinger *et al.* (2012) and with observations worldwide, with *H. axyridis* very rarely documented in warm tropical and arid regions of the world (Roy *et al*., 2016). Despite the CLIMEX modelling of Poutsma *et al.* (2008) indicating the suitability of Mediterranean areas for *H. axyridis*, in Europe the distribution of the species is limited in these areas (for example, Spain, Portugal and Greece), while in Chile is widespread in regions with similar climates. This demonstrates the importance of extending conceptual models of invasion beyond climate to encompass other factors such as habitat features.

Invasion is a multi-step process, including introduction, establishment, population growth, spread and impact, with a characteristic lag phase between establishment and the population growth (Blackburn *et al*., 2011; Grarock *et al*., 2013). Since the first wild *H. axyridis* population in Chile was found in 2003, this multi-step process has been observed (Grez *et al*., 2010a). Between 2003 and 2008 no additional wild populations were observed, despite systematic surveys of coccinellids in central Chile where it was first recorded, representing the lag phase between establishment and population growth. By 2008 it was still very rare, with only one specimen in over

90,000 coccinellids sampled. But since then there has been an exponential increase in occurrence of this species and so its geographical range has increased very rapidly, indicating the spread phase. Some of the increase in occurrences may be explained by the increase in awareness of contributing citizens about this phenomenon through time (because most of our distribution data comes from records through the web page). However, only comparing 2014 to 2015, records almost doubled, suggesting that the invasion process is still in progress, and is probably already reaching the impact phase (Grarock *et al*., 2013).

Harmonia axyridis in other parts of the world is regarded as an arboreal species, occurring mostly in deciduous trees, apple orchards, and pine trees but also observed on herbaceous plants (e.g., nettle, maize, soybean) (Adriaens *et al*., 2008; Gardiner *et al*., 2009; Honek, 2012). On the contrary, in Chile from the onset of invasion *H. axyridis* has been shown to be consistently more abundant in alfalfa fields, and only rarely observed on trees such as eucalyptus, and in hedgerows or orchards. Our sampling method, with traps at low height, could have resulted in a bias towards more captures in herbaceous covers, nevertheless our personal observations as well as some studies in Europe (Howe *et al*., 2016) suggest that traps in the lower parts of the tree are well suited for estimated its abundance in arboreal vegetation. More recently, *H. axyridis* has increased in abundance in orchards and has also started to colonize other habitat types, suggesting that in time *H. axyridis* may colonize new habitats, including less disturbed ones such as the sclerophyllous matorral. This is similar to the situation in Quebec, Canada, where fifteen years after its arrival, *H. axyridis* became one of the dominant coccinellid species in an undisturbed wild meadow located in a protected area (Bélanger & Lucas, 2011). This may imply a threat to many native coccinellid species that thrive preferentially in these less disturbed habitats (Grez *et al*., 2013).

There are concerns over the impact of *H. axyridis* to the wine industry. This species is considered a pest of grapes in parts of the USA because it feeds on the grape clusters at harvest time, thus tainting wine (Hutchinson *et al*., 2010). However, *H. axyridis* has rarely been observed in Chilean vineyards, even in recent years, but monitoring this potentially high risk and economically important crop is essential given that in Chile there are 140,000 ha planted with grapes (SAG, 2012). Further societal impacts are predicted because the records gathered through the web page showed a high incidence of *H. axyridis* within houses in autumn, confirming that this species thrives in urbanized areas, impacting human populations (i.e. the overwintering aggregations may cause a nuisance to people because of the staining and odour of reflex blood that the coccinellids exude) (Koch, 2006; Roy *et al*., 2016).

The invasion of *H. axyridis* in many parts of the world has correlated with a decline in populations of existing coccinellids, including native species (Alyokhin & Sewell, 2004; Harmon *et al*., 2007; Mizell, 2007; Brown *et al*., 2011; Roy *et al*., 2012; Roy *et al*., 2016). This negative effect is likely to be the result of several ecological processes including resource competition, intra-guild predation and phenotypic plasticity (Majerus *et al*., 2006). Here we demonstrate reduction in species richness and diversity of coccinellid assemblages, and also the abundance of native coccinellids within alfalfa fields of Central Chile, since 2012, the year when *H. axyridis* became the dominant species (i.e., highest proportion of coccinellids) in alfalfa fields. Surprisingly, the dominance (but not the abundance, see Table 2) of *H. axyridis* diminished in 2014, a year when *H. variegata* reached very high abundance (Table 2) and was the dominant species in this crop. In this year, native species tended to increase in abundance to values similar to those in 2010 and 2011. Similar trends have been observed in Michigan where previous reports of declines of several species of native coccinellids are no longer observed (Balhai *et al*., 2015). Long-term monitoring is critical to derive robust distribution and population trends for coccinellids, and this in turn will enable assessment of when the loss of biodiversity in coccinellid assemblages may have serious consequences for biological control. The Insurance Hypothesis predicts that ecosystem services (e.g., biological control) in a fluctuating environment in the long term are better achieved by more diverse assemblages (Loreau *et al*., 2003). Thus, even though *H. axyridis* is considered an effective biocontrol agent, it can still threaten the pest control service provided by the coccinellid community, particularly in alfalfa, a crop that used to have a high diversity of coccinellid species, including many native species (see results; Grez *et al*., 2013).

Central Chile is one of the world's 35 biodiversity hotspots (Mittermeier et al., 2011), an important reservoir of biodiversity that is under threat from human activities. This is also the area where most native and endemic coccinellid species are distributed in Chile (González, 2006) which could be under threat by the invasion of *H. axyridis*. There are parallels with other regions of the world, for example in South Africa, where *H. axyridis* has also spread rapidly in various biomes, including to the Western Cape, part of which is another biodiversity hotspot (Stals, 2010). The current distribution and increasing populations of *H. axyridis* in central Chile suggests that negative impacts may be more severe in the near future, and thus mitigating strategies will be needed.

Conclusion

Harmonia axyridis is now well established and distributed widely in Chile. The associated changes to the coccinellid community could disrupt the functioning, and ultimately resilience, of invaded ecosystems. Continued long-term monitoring is essential to enhance understanding of the invasion process of *H. axyridis*, the impacts on ecosystem function, and ultimately knowledge for the management of this IAS and biodiversity conservation.

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Table 1. Number of verified records, number of new cells occupied, northward and southward annual spread (km), and cumulative range (km) North to South of *Harmonia axyridis* (Pallas) in Chile from May 2008 to May 2015. In each year, the northern and southern range margins of *H. axyridis* were estimated as the mean latitude of the 10 most northerly-occupied 20 km^2 cells or the 10 most southerly occupied 20 km^2 cells, respectively.

			Annual spread (km)		Cumulative range (km)
Year	No. verified records	No. of new cells occupied	Northward	Southward	North to South
2008	1	1	-	-	-
2009	4	4	108.5	0	108.5
2010	48	33	63.5	14.4	122.9
2011	130	50	56.0	51.9	294.3
2012	312	54	29.1	74.6	398.1
2013	315	91	0	264.1	662.2
2014	367	143	124.8	156.8	943.8
2015	698	211	0	347.0	1290.8
Total	1875	587	381.9	908.8	-
Mean	-	-	54.6	129.8	-

Table 2: Abundance (mean, SE) per alfalfa field of native and alien coccinellid species associated with alfalfa fields, in spring 2010 to spring 2014.

	2010	2011	2012	2013	2014
Natives					
Adalia angulifera Mulsant	0.35 ± 0.14	0.11 ± 0.05	0.02 ± 0.02	0.08 ± 0.03	0.12 ± 0.07
Adalia deficiens Mulsant	0.37 ± 0.13	0.17 ± 0.05	0 ± 0	0.01 ± 0.01	0.08 ± 0.06
Cycloneda sanguinea (L.)	0.50 ± 0.09	0.10 ± 0.04	0 ± 0	0.01 ± 0.01	0.02 ± 0.02
Cycloneda fulvipenis (Mulsant)	0.32 ± 0.13	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Eriopis chilensis Hofmann	0.11 ± 0.05	0.32 ± 0.05	0.17 ± 0.05	0.02 ± 0.02	0.40 ± 0.18
Eriopis eschscholtzi Mulsant	0 ± 0	0.05 ± 0.03	0.02 ± 0.02	0.02± 0.02	0.02± 0.02
Hyperaspis sphaeridioides (Mulsant)	0.01 ± 0.01	0.10 ± 0.07	0.07 ± 0.05	0.01 ± 0.01	0 ± 0
Scymnus bicolor (Germain)	0.01 ±0.01	0.04 ± 0.01	0 ± 0	0 ± 0	0.02± 0.02
Psyllobora picta (Germain)	0.01± 0.01	0.33 ± 0.12	0.10 ± 0.08	0.02± 0.02	0.24 ± 0.14
Parasidis porteris Brèthes	0 ± 0	0 ± 0	0 ± 0	0.01 ± 0.01	0 ± 0
Total natives	1.66 ± 0.31	1.20 ± 0.23	0.37 ± 0.11	0.19 ± 0.03	0.90 ± 0.27
Alien					
Adalia bipunctata (L.)	4.28 ± 1.50	0.83 ± 0.36	0.20 ± 0.15	0.08 ± 0.04	0 ± 0
Harmonia axyridis (Pallas)	2.01 ± 0.46	4.34 ± 1.12	5.83 ± 1.50	4.20 ± 0.71	5.00 ± 1.52
Hippodamia convergens (Guerin-Meneville)	0.59 ± 0.22	0.78 ± 0.21	0.12 ± 0.05	0.10 ± 0.04	0.22 ± 0.13
Hippodamia variegata (Goeze)	1.64 ± 0.31	5.24 ± 1.24	1.67 ± 0.25	0.56 ± 0.12	10.36 ± 4.72
Scymnus loewii Mulsant	0 ± 0	0.22 ± 0.07	0 ± 0	0.01± 0.01	0 ± 0
Olla v-nigrum (Mulsant)	0.81 ± 0.25	0.12 ± 0.03	0 ± 0	0.02 ± 0.02	0 ± 0
Coleomegilla quadrifasciata (Schöenherr)	0.01 ± 0.01	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Parastethorus histrio Chauzeau	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.06 ± 0.03
Total aliens	9.33 ± 2.16	11.51 ± 1.05	7.82 ±1.49	4.97 ± 0.73	15.64± 5.46
Total coccinellids	10.98± 2.30	12.71± 1.12	8.18± 1.49	5.16± 0.74	16.54± 5.55

Figure captions

Fig. 1. *Harmonia axyridis* (Pallas) occurrences in 20 km^2 (4.47 x 4.47 km cells) in Chile from May 2008 to May 2015. Extreme occurrences are shown in the inset.

Fig. 2. Abundance of *Harmonia axyridis* (Pallas) (mean individuals/trap ± se) in the different habitat types, from the least to the most disturbed according to Grez *et al*. (2013), in autumn and spring, from 2010 to 2014. Results of ANOVAs and Tukey HSD tests are reported for each season and year. Different letters above bars denote significant differences ($\alpha = 0.05$).

Fig. 3. *Harmonia axyridis* (Pallas) aggregation found in May 2015 at the base of the trunk of Chilean palm trees in the sclerophyllous matorral of Central Chile (Photo by Ronny Zúñiga).

Fig. 4. *Harmonia axyridis* (Pallas) proportion and community indices in alfalfa fields, in early spring from 2010 to 2014. Results of ANOVAs and Tukey HSD tests are reported for each index. Different letters above bars denote significant differences between years ($\alpha = 0.05$).

Fig. 5. a) Abundance of native coccinellids (mean individuals/trap/field ± se) in alfalfa fields from spring 2010 to spring 2014; b) Relationship between the abundances of *Harmonia axyridis* (Pallas) and native coccinellids in alfalfa fields through the years.

Fig. 1.

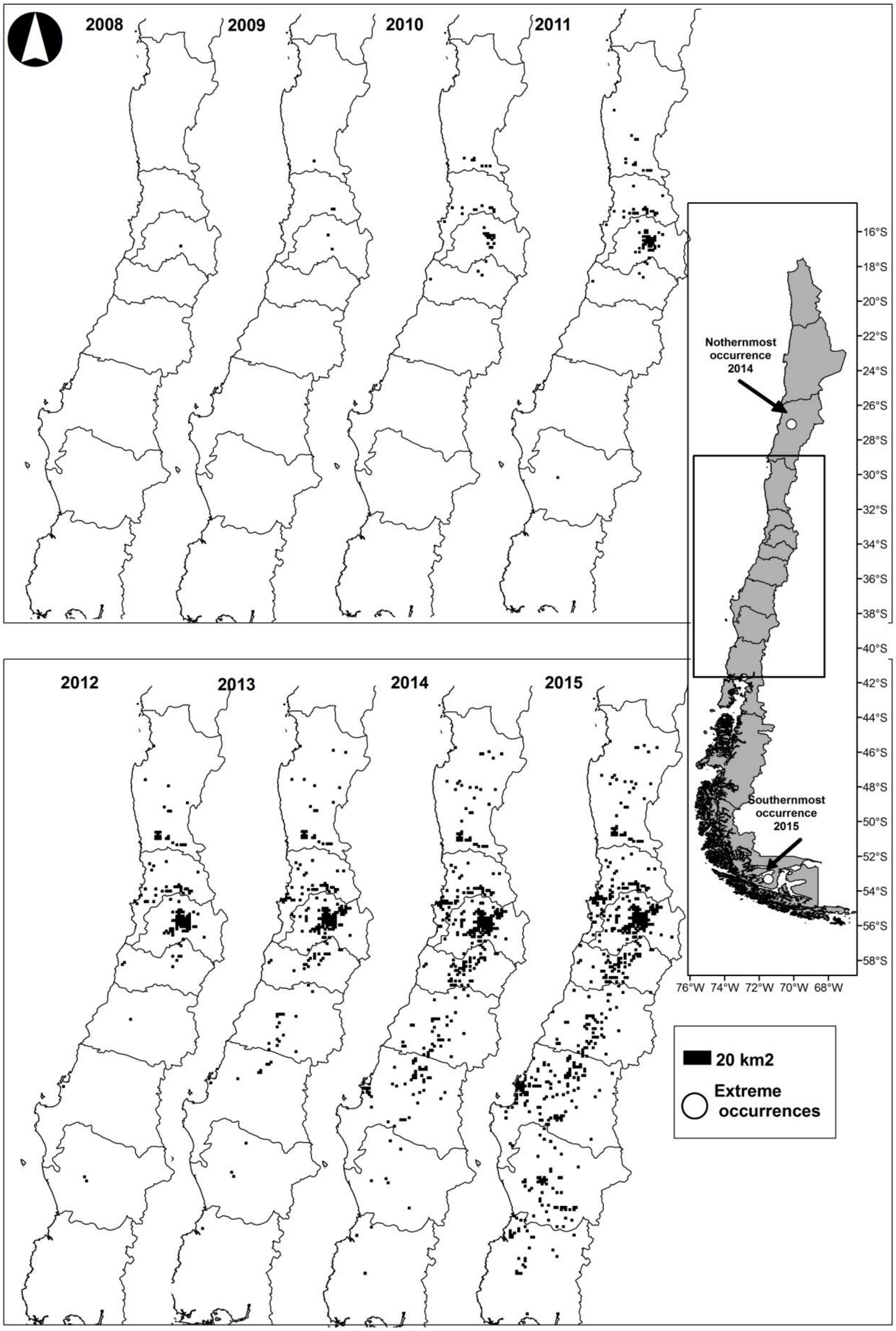
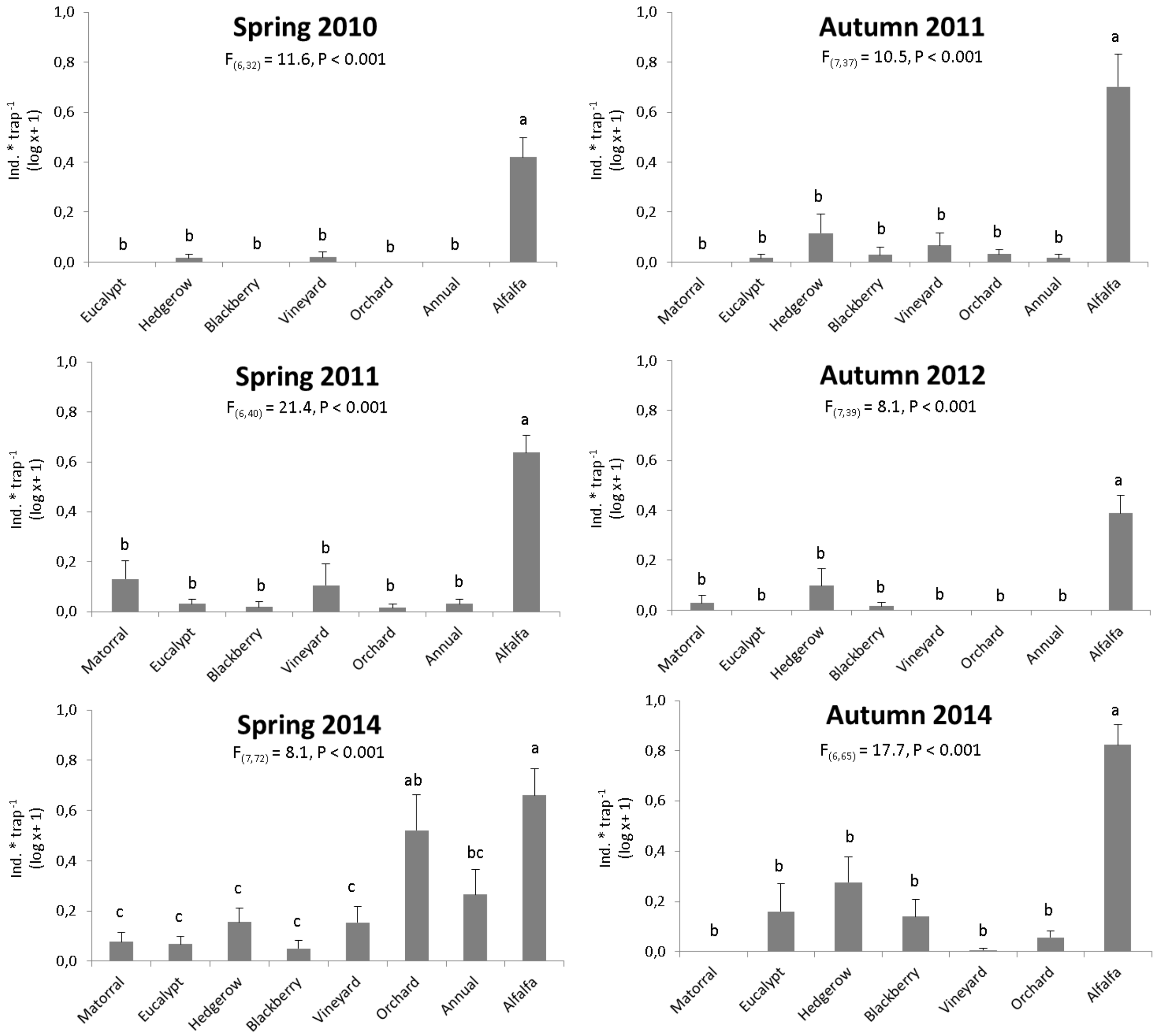

2008
2009
2010
2011
2012
2013
2014
2015
Nothernmost occurrence 2014
Southernmost occurrence 2015
-16°S
-18°S
-20°S
-22°S
-24°S
-26°S
-28°S
-30°S
-32°S
-34°S
-36°S
-38°S
-40°S
-42°S
-44°S
-46°S
-48°S
-50°S
-52°S
-54°S
-56°S
-58°S
76°W
74°W
72°W
70°W
68°W
20 km2
Extreme occurrences

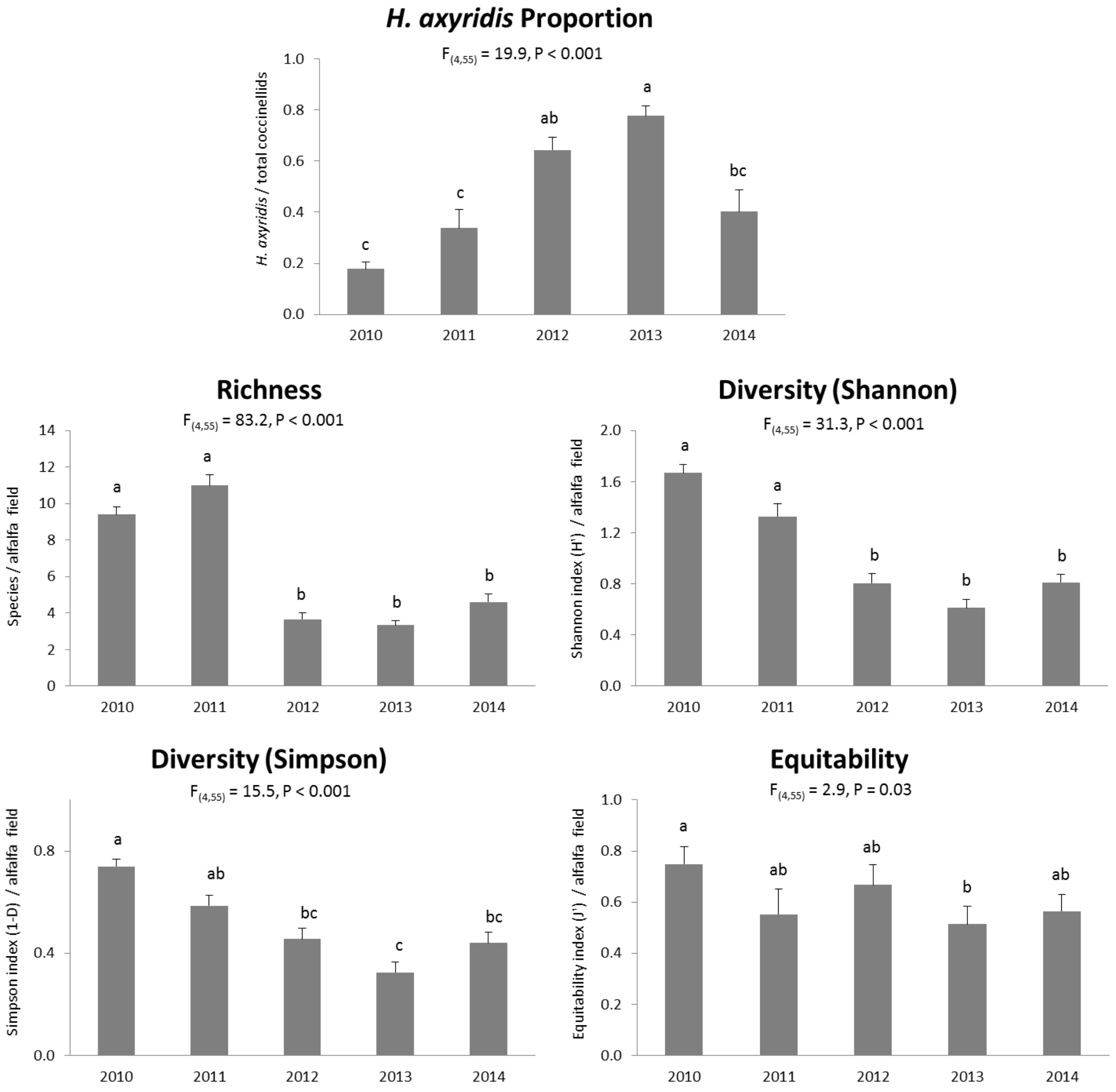
Fig. 2.



Spring 2010
F(6,32) = 11.6, P < 0.001
Ind. * trap-1 (log x+ 1)
1,0
0,8
0,6
0,4
0,2
0,0
b
b
b
b
b
b
a
Eucalypt
Hedgerow
Blackberry
Vineyard
Orchard
Annual
Alfalfa
Autumn 2011
F(7,37) = 10.5, P < 0.001
Ind. * trap-1 (log x+ 1)
b
b
b
b
b
b
b
a
Matorral
Eucalypt
Hedgerow
Blackberry
Vineyard
Orchard
Annual
Alfalfa
Spring 2011
F(6,40) = 21.4, P < 0.001
Ind. * trap-1 (log x+ 1)
b
b
b
b
b
b
a
Matorral
Eucalypt
Blackberry
Vineyard
Orchard
Annual
Alfalfa
Autumn 2012
F(7,39) = 8.1, P < 0.001
Ind. * trap-1 (log x+ 1)
b
b
b
b
b
b
b
a
Matorral
Eucalypt
Hedgerow
Blackberry
Vineyard
Orchard
Annual
Alfalfa
Spring 2014
F(7,72) = 8.1, P < 0.001
Ind. * trap-1 (log x+ 1)
c
c
c
c
c
ab
bc
a
Matorral
Eucalypt
Hedgerow
Blackberry
Vineyard
Orchard
Annual
Alfalfa
Autumn 2014
F(6,65) = 17.7, P < 0.001
Ind. * trap-1 (log x+ 1)
b
b
b
b
b
b
a
Matorral
Eucalypt
Hedgerow
Blackberry
Vineyard
Orchard
Alfalfa

Fig. 3

Fig. 4



H. axyridis Proportion

$F_{(4,55)}$ = 19.9, P < 0.001

Richness

$F_{(4,55)}$ = 83.2, P < 0.001

Diversity (Shannon)

$F_{(4,55)}$ = 31.3, P < 0.001

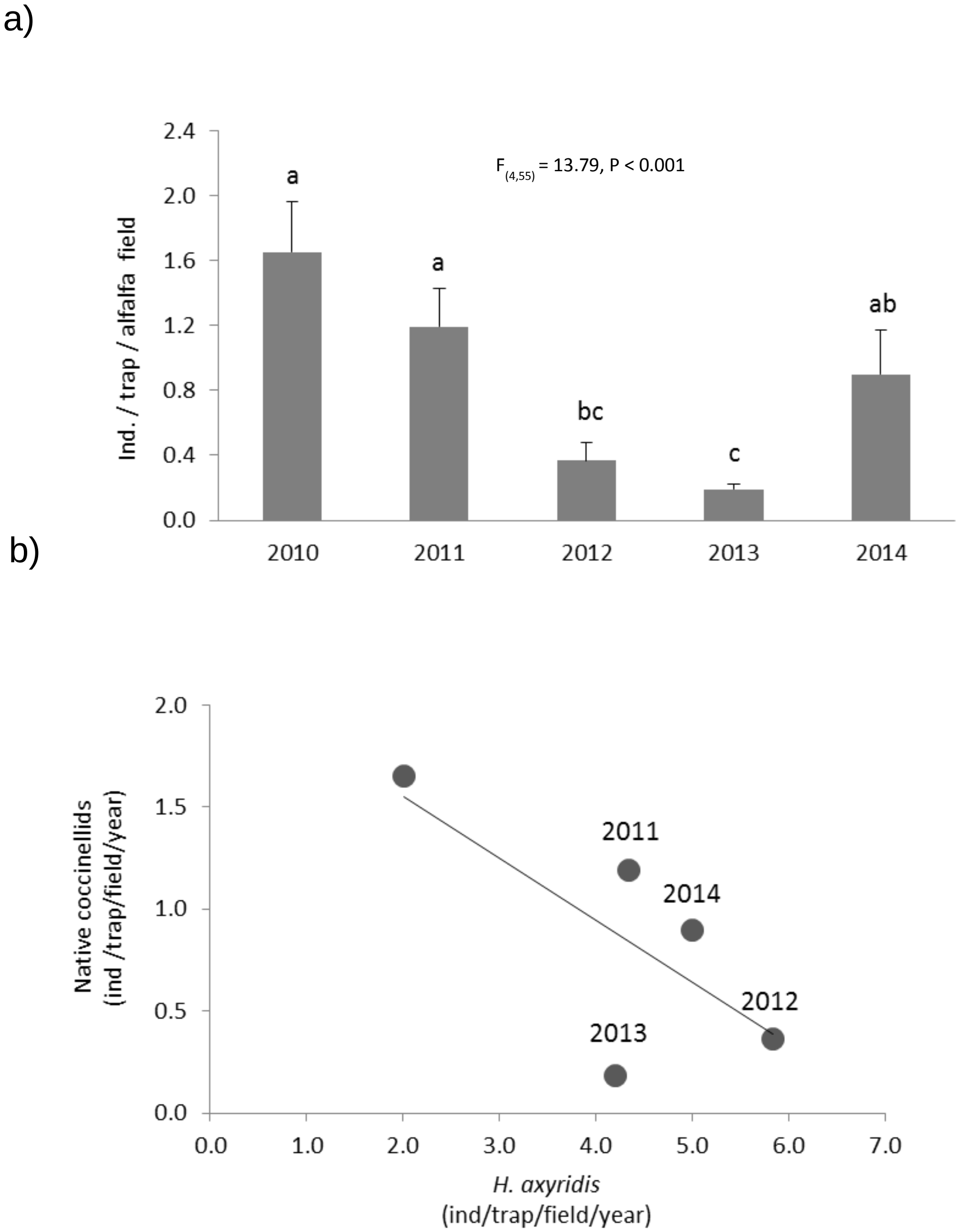
Diversity (Simpson)

$F_{(4,55)}$ = 15.5, P < 0.001

Equitability

$F_{(4,55)}$ = 2.9, P = 0.03

Fig. 5



a)
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Ind. / trap / alfalfa field
F(4,55) = 13.79, P < 0.001
a
a
bc
c
ab
2010
2011
2012
2013
2014
b)
2.0
1.5
1.0
0.5
0.0
Native coccinellids (ind /trap/field/year)
2011
2014
2012
2013
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
H. axyridis (ind/trap/field/year)

Supporting Information

Figure S1 {Relative abundance of coccinellid species per alfalfa fields and years}

Figure S1: Relative abundance of coccinellid species per alfalfa fields and years