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Article *in* Biological Invasions · November 2015

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Biological Invasions

ISSN 1387-3547
Volume 18
Number 2

Biol Invasions (2016) 18:517-532
DOI 10.1007/s10530-015-1024-y

Springer

Biol Invasions (2016) 18:517–532
DOI 10.1007/s10530-015-1024-y

ORIGINAL PAPER

Potential for exploitative competition, not intraguild predation, between invasive harlequin ladybirds and flowerbugs in urban parks

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Received: 31 December 2014 / Accepted: 19 November 2015 / Published online: 25 November 2015

Abstract In aphidophagous insect communities invaded by the harlequin ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), intraguild predation (IGP) is widely implicated in the displacement of native predators, however, indirect trophic interactions are rarely assessed. Using molecular gut-content analysis, we investigated the relative frequencies of IGP by *H. axyridis* on the predatory flowerbug *Anthocoris nemoralis* Fabricius (Heteroptera: Anthocoridae) and prey overlap for a shared prey, the lime aphid *Eucallipterus tiliae* L. (Hemiptera: Aphididae), in *Tilia* × europaea crowns in urban parks. The frequency of IGP by *H. axyridis* was low: 2.7 % of larvae and 3.4 % of adults tested positive for *A. nemoralis* DNA. The presence of lime aphid DNA in predators was higher: 56.5 and 47.9 % of *H. axyridis* larvae and adults, respectively, contained *E. tiliae* DNA, whereas 60.8 % of adult *A. nemoralis* tested positive for aphid DNA. Incorporating insect densities revealed that the density of *H. axyridis* larvae had a strong negative effect on the likelihood of detecting aphid DNA in *A. nemoralis*. Prey overlap for *E. tiliae* was widespread in space (2–13 m height in tree crowns) and time (May–October 2011) which suggests that interspecific exploitative competition, mediated by predator life-stage, more so than IGP, is an important indirect trophic interaction between co-occurring *H. axyridis* and *A. nemoralis*. Whether exploitative competition equates to displacement of *A. nemoralis* populations requires further investigation. Our results emphasize the need to incorporate indirect interactions in studies of insect communities following invasion, not least because they potentially affect more species than direct interactions alone.

Keywords Indirect effects · Molecular gut content · *Anthocoris nemoralis* · *Harmonia axyridis* · *Eucallipterus tiliae* · Predator–prey interactions

Electronic supplementary material The online version of this article (doi:10.1007/s10530-015-1024-y) contains supplementary material, which is available to authorized users.

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Introduction

Invasive generalist insect predators are typically involved in complex ecological interactions with

members of invaded communities, often resulting in negative impacts on resident native species (Snyder and Evans 2006; Crowder and Snyder 2010). As generalist invaders often achieve high densities in introduced communities, and given the relatively short time periods over which invasions occur, trophic interactions might be particularly vulnerable to change by invaders with potential effects extending beyond single trophic levels (Snyder and Evans 2006; Kenis et al. 2009). Within trophic communities species are linked through a myriad of direct and/or indirect trophic interactions, most of which are not readily perceptible (White et al. 2006; Tylianakis 2008). By way of their omnivorous diets, invasive generalist predators interact with multiple trophic levels in food webs; not only do they consume herbivorous prey, but also other predators, detritivores, plants and/or detritus may comprise parts of their diet (Polis and Strong 1996; Snyder and Evans 2006).

An invasive generalist predatory beetle implicated in disruption of trophic interactions is *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Originally introduced to North America as a biological control agent (BCA) against aphids and coccids (Chapin and Brou 1991; Brown et al. 2011b), long-term studies from North America have documented coccinellid declines in agroecosystems (Michaud 2002; Alyokhin and Sewell 2004; Harmon et al. 2007), while the spread of *H. axyridis* in Europe has impinged several native coccinellids whose niches overlap (Adriaens et al. 2008; Brown et al. 2011a; Roy et al. 2012). Intraguild predation (IGP) and resource competition by *H. axyridis* are suspected of contributing to native species declines (Michaud 2002; Lucas et al. 2007; Alhmedi et al. 2010; Roy et al. 2012). However, while studies of IGP by *H. axyridis* among native coccinellids in agroecosystems have received most attention, indirect trophic interactions such as exploitative and apparent competition have barely been explored in the field (Alhmedi et al. 2010; Roy and Handley 2012).

Intraguild predation occurs when predators sharing a common extraguild (EG) prey engage in predation of each other (Polis et al. 1989), and is often associated with native species displacement (Reitz and Trumble 2002). Laboratory studies confirm that *H. axyridis* is a strong asymmetric intraguild (IG) predator in encounters with other aphidophages, i.e. intraguild (IG) prey (for recent summary see Nedved et al. 2013). Field studies employing molecular gut-content techniques reveal IGP of native coccinellids by invasive *H. axyridis* is spatially widespread and relatively intense, providing support that this mechanism contributes to native species declines (Gagnon et al. 2011b; Brown et al. 2015).

Interspecific exploitative competition involves an indirect interaction between two species mediated through changes in abundance of a third species (Lawton and Hassell 1984; White et al. 2006). An invasive generalist competitor may be superior at utilising a limited shared prey through better harvesting ability and/or resource conversion efficiency, and reduced density of the limiting resource can result in indirect negative effects on native species, e.g. reduction in fecundity, growth or survival (Brown et al. 1994; Obrycki et al. 1998; Kasper et al. 2004; Evans et al. 2011). Resource competition among aphidophages is likely to be widespread due to the ephemeral distribution of aphids in space and time (Hironori and Katsuhiro 1997; Dixon 1998; Osawa 2000; Ware et al. 2009). Predator niche overlap for shared prey involving *H. axyridis* populations in invaded communities has received limited attention (Pell et al. 2008; Kenis et al. 2010), despite that niche overlap can contribute to native species displacement (Kasper et al. 2004). On the other hand, spatial and temporal niche partitioning of competitors may reduce effects of interspecific interactions involving invasive generalist predators, thereby contributing to coexistence (Amarasekare 2003; Crowder and Snyder 2010).

The predatory insect *Anthocoris nemoralis* F. (Heteroptera: Anthocoridae) is common throughout Western Europe (Péricart 1996), and tracks prey on leaf surfaces of deciduous trees (Anderson 1962; Lattin 1999; Sigsgaard 2010). Both *H. axyridis* and *A. nemoralis* are found on common lime *Tilia* × europaea L., Malvaceae (Anderson 1962; Adriaens et al. 2003; Brown et al. 2008), which also hosts the monophagous aphid and dominant herbivore *Eucallipterus tiliae* L. (Hemiptera: Aphididae; Dixon 1971a). *Eucallipterus tiliae* (EG prey; Fig. 1) is shared prey for *H. axyridis* (IG predator; Fig. 1) and *A. nemoralis* (IG prey; Fig. 1), and important for pre-overwintering reproduction in *A. nemoralis* (Anderson 1962; Tomov et al. 2009).

Laboratory investigations of interspecific interactions between *H. axyridis* and *A. nemoralis* revealed a

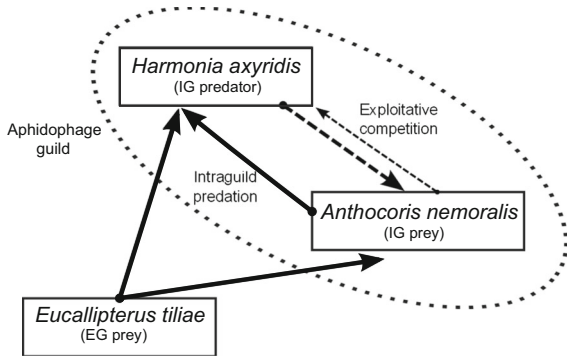

Fig. 1 Conceptual diagram of species and trophic interactions in *Tilia* × europaea crowns in urban parks in Copenhagen, Denmark. The aphidophage guild comprises invasive *Harmonia axyridis* [intraguild (IG) predator] and *A. nemoralis* [intraguild (IG) prey], with *E. tiliae* their shared [extraguild (EG) prey]. Direct trophic interactions *solid lines*; indirect interaction *dashed lines*

low level of IGP by coccinellids in potted *Tilia cordata* Mill. (Malvaceae) microcosms, and a negative effect on *A. nemoralis* weight gain through competition for *E. tiliae* (Howe et al. 2015). We were interested in assessing the relative strengths of trophic interactions under field conditions and whether predator niche overlap could explain interaction strengths. We hypothesised (1) that IGP of *A. nemoralis* by *H. axyridis* is limited on *T.* × europaea in urban parks, but occurs more frequently as *E. tiliae* populations decline. Furthermore, as predators on *T.* × europaea share a limiting aphid prey, (2) that interspecific competition is potentially a more important trophic interaction than IGP as spatiotemporal niche overlap for prey occurs in *T.* × europaea crowns. By sampling in tree crowns we hoped to increase the spatial area in which to detect trophic interactions, while also illuminating the spatial distribution of arboreal species.

Materials and methods

Field collection and assessment of insect density

In 2009, *Tilia* × europaea was sampled in seven urban parks and green spaces on six dates from October 2 to 22 [green spaces are situated between 55°47′16.6″N 12°29′39.1″E and 55°40′08.5″N 12°25′32.8″E; see Electronic supplementary material (ESM) for further descriptions]. This period coincided with high densities of *H. axyridis* and low *E. tiliae* densities. Only *H. axyridis* adults and larvae (4th instar) were collected for DNA-gut analysis during 2009. In 2011, five *T.* × europaea in 5 urban parks were sampled 1–3 times per park, between May and October 2011, in total 10 sampling dates (Table 2), which covered predators' active period. Both insect community densities and trophic interactions were assessed in tree crowns in 2011. As there was at least a month between repeated collections from the same parks, samples were considered to be independent.

Bottom-up sampling was conducted to assure that insects higher up in tree crowns were not disturbed by sampling at lower heights. Within trees, insects were sampled at three heights, low (up to 2.5 m), mid (5–7 m) and high (10–13 m), although on three dates only two heights were sampled (Table 2). Collection heights were based on tree architecture and limitations of the access technique (single rope technique) relating to responsible, safe and non-destructive tree climbing.

Densities of insects other than aphids were assessed with a white fabric beating-tray (75 cm × 75 cm) mounted to a telescopic aluminium pole (Ronstan Battle stick-tiller extender, Australia) which allowed 4 m extension, adequate to obtain samples from the outer crown in trees. Ten branches at different orientations in crowns at each height (in 2009 only at low height), were hit 5 times per branch with a light-weight, rubber-ended metal pole (2.5 m long). Insects other than aphids were counted and sorted immediately, within crowns, for subsequent identification/analysis. Aphids were sampled by inspecting 50 leaves at each height, and wherever possible, leaves in the outer crown were included by bending branches to within a safe working distance. Adults included only winged individuals (i.e. parthenogenetic virginoparae and males), while nymphs were apterous, but could have included ovipare wingless females (Dixon 1971b).

On occasions when no predators were recovered using the aforementioned beating procedure, extra beating was employed to attain predators for subsequent DNA gut-content analysis. Predators to be used for gut-content analysis were immediately placed individually into 1.5 mL Eppendorf tubes filled with chilled 85 % EtOH (in 2009 insects were stored dry). Individuals were either scooped from the net directly into Eppendorf tubes to minimise potential contamination between samples, or were transferred by pooter

for fast-moving species. After collection in trees, specimens were transferred to an iced cooler box (5–8 °C) and shaded in the field for 3–6 h before being frozen at −20 °C, until subsequent morphological identification and DNA analysis. Previously, *H. axyridis* in Japan has been shown to feed between 1000 and 1600 hours (Miura and Nishimura 1980). In 2009, collection adhered to this period; during 2011 the period was extended from 1000 to 1800 hours to allow for the time demands associated with tree climbing.

Molecular DNA-gut analysis

A PCR-based detection system was developed for primer pairs specific for *A. nemoralis* and *E. tiliae* mitochondrial DNA (mtDNA) from the cytochrome oxidase subunit I (COI) gene. We followed the procedure described by Aebi et al. (2011, Fig. 1 therein) to test primer specificity and post-feeding detection times. See ESM for details of primer specificity tests.

DNA extraction

All stages of *H. axyridis*, adult *Anthocoris* and reference insect species for specificity tests (see ESM Table 1) were briefly dried of EtOH on paper towel, before being transferred individually to a new 1.5 mL Eppendorf tube containing 180 µL of PBS buffer, and homogenised using a sterile plastic micro-pestle. DNA was extracted using a DNEasy Blood & Tissue Kit (Qiagen, Denmark) following the manufacturer's specifications.

The success of all extractions at all stages of the study was tested using the LepF/LepR primer pair (Foottit et al. 2008) which amplifies an approximately 700 bp fragment of mtDNA from the COI gene. Each PCR (25 µL) contained 1.25 U of Promega Taq polymerase (0.125 µL, Promega, Sweden), 5× Green Flexi buffer (5 µL, Promega), 200 µM of each dNTP (0.5 µL), 2 mM $MgCl_2$ (2 µL), 0.14 µM each primer (0.35 µL) and 14.675 µL MilliQ H_2O and 2 µL of extracted DNA. Thermocycling conditions followed Hebert et al. (2003). PCR was verified with electrophoresis of 7 µL of product in 1.5 % agarose in 0.5× TBE buffer run at 65 V for up to 90 min. DNA was visualised with GelRed (Biotium, USA) and photographed on a UV transilluminator. Only samples testing positive for successful extraction were used for the assays.

Primer design

Following morphological identification of insects collected from lime trees (Table 1, ESM Table 1) with keys (Southwood and Leston 1959) and validation by a national Heteroptera expert, DNA was extracted from 1 to 2 individuals of each species (as for DNA extraction). COI sequences were amplified with the LepF/LepR primer pair (PCR conditions as for DNA extraction) and shipped to Macrogen (the Netherlands, Europe) for purification and sequencing in both directions using the same primer pair.

Target primers were designed for mtDNA because thousands of mtDNA copies in an invertebrate cell enhance sensitivity and probability of amplifying DNA from a target prey species (Hoy 1994; King et al. 2008). Primers were designed to amplify amplicons <150-bp in order to optimise detectability of semi-digested DNA and to contain as high a GC content as possible (King et al. 2008; Aebi et al. 2011). We used Geneious (version 5.5, Biomatters, 2011) to edit sequences, Bioedit for sequence alignment (http://www.mbio.ncsu.edu/bioedit/bioedit.html), designed primers by sight based on sequence differences in alignments, while Primer 3 was used to assess primer properties (Koressaar and Remm 2007; Untergasser et al. 2012). Morphological identification of species was verified using the BOLD identification system for COI (Ratnasingham and Hebert 2007).

PCR amplification with target primers

PCR reactions (25 µL) contained 5 µL 5× Promega Green Flexi Buffer, 0.5 µL of dNTPs (200 µM each), 2 µL of 2 mM $MgCl_2$, 0.35 µL of primer (0.14 µM forward/reverse), 1.25 U Promega Taq-polymerase, and 14.675 µL MilliQ H_20 in addition to 2 µL of raw DNA. For *A. nemoralis* primers, 5 µL Q-solution (Qiagen, Denmark) was added per reaction with H_20 reduced accordingly. The optimal primer specific thermocycle times were: for *A. nemoralis* (primer pair Ans-F2/R2), 95 °C for 90 s initial denaturation was followed by 20 touchdown cycles of 95 °C for 30 s, 61–51 °C for 60 s (0.5 °C reduction per cycle), 72 °C for 30 s; followed by 20 cycles at 95 °C for 30 s, 51 °C for 60 s and 72 °C for 30 s and concluded with 72 °C for 10 min; for *E. tiliae*

Table 1 Densities of non-aphid insects (per 50 hits) and *E. tiliae* (per 50 leaves), sampled over 10 dates during 2011 in 5 *Tilia* × europaea crowns in inner city parks in Copenhagen, Denmark

Family	Species	Height in canopy (m)						No. dates species was sampled (10 max.)
		2.5		5–6		10–13		
		Mean	SE	Mean	SE	Mean	SE	
Aphididae	*Eucallipterus tiliae* L.	17.30	8.19	3.88	1.11	2.87	0.67	10
	E. tiliae (nymph)	82.3	38.41	54.33	24.70	29.62	11.24	10
Anthocoridae	*Anthocoris nemoralis* Fabricius	1.8	0.74	2	1.56	0.62	0.50	7
Coccinellidae	*Harmonia axyridis* Pallas	3.40	0.67	2.88	0.63	4.62	2.01	9
	H. axyridis (larva)	1.4	0.64	3.22	1.05	0.50	0.38	9
	Adalia bipunctata (L.)	0.2	0.2			0.37	0.26	3
	A. decempunctata (L.)	0.3	0.21	0.11	0.11			3
	Calvia quattuordecimguttata (L.)	0.1	0.1			0.25	0.25	2
	Exochomus quadripustulatus (L.)	0.1	0.1					1
	Native coccinellids (total)	0.70	0.40	0.11	0.11	0.62	0.32	
Miridae	*Blepharidopterus angulatus* (Fallen)	0.10	0.10	0.55	0.55			2
	Neolygus viridis (Fallen)	0.9	0.55			0.62	0.62	3
	Deraeocoris flavilinea (Costa)	0.50	0.40	1.22	0.66	0.25	0.25	3
	D. lutescens (Schilling)	0.50	0.50					1
	Phytocoris tiliae (F.)	0.20	0.13	0.11	0.11			2
	Mirids (total)	2.2	0.68	1.89	0.77	0.87	0.64	

Number of collections per height: up to 2.5 m (10), 5–6 m (9), 10–13 m (8). Numbers denote adult densities unless stated otherwise

(primer pair Eti-F5.1/R2), 95 °C for 90 s was followed by 45 cycles of 95 °C for 30 s, 54 °C for 60 s and 72 °C for 30 s prior to final elongation at 72 °C for 10 min. For both pairs of primers 35 cycles did not produced sufficient amplification of target DNA and the number of cycles was therefore increased. Four controls were included in all PCR reactions: a negative control of the specific laboratory raised predator (*H. axyridis* or *A. nemoralis*) fed *Ephestia kuehniella* (Zeller); a negative blank with MilliQ H_2O substituting DNA; and two positive target prey DNA controls in 1:1 and 1:100 dilution (*E. tiliae*: predator) with the appropriate predator. PCR was verified as for DNA extractions success, using a 100 bp DNA ladder.

Post-feeding detection period of prey

To assess prey detectability times the following four predator–prey combinations were investigated: 4th instar *H. axyridis* larvae ingesting one 2nd instar *A. nemoralis* nymph; 4th instar *H. axyridis* larvae ingesting one *E. tiliae* adult; *H. axyridis* adults ingesting one *E. tiliae* adult and; *A. nemoralis* adults ingesting one *E. tiliae* adult (see ESM for starvation times of predators prior to being offered prey).

Predators were offered prey in 1.5 mL Eppendorf tubes and observed for an hour until ingestion which was noted to the nearest quarter hour. Six to eight *H. axyridis* were frozen as soon as ingestion was complete (time = 0), whereas for *A. nemoralis* ingestion lasted up to an hour (time = 1). Thereafter all predators were transferred to Petri dishes kept in climate chambers (20 ± 1 °C, 16:8 L:D and RH 50 ± 20 %). Initially, *H. axyridis* adults offered *E. tiliae* were given up to 24 h digestion time (following Gagnon et al. 2011a), which was not long enough to calculate a prey detectability half-life; unfortunately we were unable to extend digestion times beyond 24 h for this combination. However, remaining combinations were given 2, 4, 6, 8, 24 and 36 h to digest prey (see ESM Table 2). At each time interval 6 live individuals were transferred to a freezer (−20 °C), until PCR of six *H. axyridis* from each digestion time tested for prey DNA digestion. Six *A. nemoralis* were tested as for ladybirds, except at times t = 1 ($n = 5$), t = 6 ($n = 5$), t = 8 ($n = 5$)

and t = 36 (n = 4). Despite starving *A. nemoralis* for 48 h, we were unable to achieve greater sample sizes due to their reluctance to reliably ingest prey and the constraints this places on available resources. See ESM for further details of post-feeding detection periods and cross-reactivity specimens (n = 26; ESM Table 1).

Data analysis

Prey detectability half-lives and weighting prey detectability

Prey detectability half-lives (DS_{50}, i.e. the time post-feeding at which prey remains are detected in half the assayed predators (reviewed by Greenstone et al. 2014) were calculated using probit regression model parameters (i.e. −b0/b1) from individual predator–prey digestion periods (following Chen et al. 2000). As mentioned, DS_{50} value for *H. axyridis* adults fed *E. tiliae* was not calculated; for an overview of DS_{50} values (see Table 3, ESM Table 2). Standard errors of DS_{50} values were estimated using the delta method with the msm package in R (Jackson 2011), while predator DS_{50} values were compared using Pearson's Chi square statistic with Yates continuity correction. To account for potential differences in prey detectability between predators which may lead to biased interpretations of raw qualitative gut-content data (Greenstone et al. 2014), we calculated weighted DS_{50} values and subsequently corrected field-based IGP and aphid predation values.

Likelihood of detecting E. tiliae DNA in predators

In 2011, only five positive IGP events occurred in 2011 which precluded statistical analysis. In contrast, predator gut content data (presence/absence *E. tiliae* DNA) reflecting prey overlap were analysed separately for each predator. The likelihood of detecting *E. tiliae* DNA was analysed by logistic regression with random effects (GLMM). In both models, raw response variables (presence/absence *E. tiliae* DNA) were analysed, because DS_{50} values for digestion of *E. tiliae* did not reveal differences between predators. However, as we did not calculate a DS_{50} value for *H. axyridis* adults digesting *E. tiliae*, we analysed the likelihood of detecting aphid DNA for *H. axyridis* life stages combined (i.e. larvae and adults) and for larvae alone to account for potentially different DS_{50} values (between life stages and predators).

For *A. nemoralis*, we analysed the explanatory fixed effects: collection height (3 levels), densities of *E. tiliae* (adults and nymphs separately), total density of predatory insects (i.e. potential competitors: mirids, coccinellids, *A. nemoralis*, *H. axyridis* larvae and adults), densities of *H. axyridis* larvae and adults (separately), and the sex of adult *A. nemoralis*. For *H. axyridis*, explanatory fixed effects included: collection height, densities of *E. tiliae* (adults and nymphs separately), densities of mirids, coccinellids and *A. nemoralis*, total density of predatory insects, and the life stage of *H. axyridis* (two levels, larva/adult); the latter removed in the larvae only model.

Random effects were location and location at a given date to account for heterogeneity between sampling locations. Model reduction was based on backwards elimination with a 5 % cut-off value. P values correspond to likelihood ratio tests (LR). Effects are recorded as odds ratios accompanied by 95 % confidence intervals in brackets.

Correlations between aphid, *H. axyridis* and *A. nemoralis* densities were investigated using Pearson correlation coefficients on raw data, while poisson regression accompanied by post hoc pairwise comparisons (adjusted for multiple testing with the single-step method) assessed whether species' densities differed with height.

In 2009, no explanatory variables were recorded; therefore only positive detection and weighted values of IGP and *E. tiliae* DNA in *H. axyridis* is reported (data not analysed).

Statistical analysis was conducted using R, version 3.0.0 (R Core Team 2013). GLMMs were fitted using the glmer function in the lme4 package (Bates et al. 2014), multiple comparisons with the multcomp package (Hothorn et al. 2008), and figures were produced with ggplot2 (Wickham 2009).

Results

Predatory insects and aphids in tree crowns

Within inner city parks, *Tilia* × europaea crowns to 13 m contained a host of predatory insects, including several members of the aphidophage guild

(Table 1). Native coccinellids were not abundant with only 4 species found, while 5 species of mirids were caught. *Harmonia axyridis* (larvae and adults) accounted for 59 % of all predators caught, while *A. nemoralis* was the next most abundant predator (17 %). In tree crowns, *A. nemoralis* and *H. axyridis* were caught simultaneously on the same date and height, but not after 20 September (Table 2). Densities of predators were similar between heights, suggesting homogenous distribution throughout tree crowns (pairwise comparisons between *H. axyridis* adults and larvae, *A. nemoralis*, coccinellids and mirids: $P > 0.05$).

Aphid nymph and adult densities varied across sites, dates and heights in trees (Table 1; Fig. 2). Aphid nymph densities ranged between 0.09 and 6.20 aphids/leaf (mean ± SE: 1.38 ± 0.59 aphids/leaf), while aphid adults densities ranged from 0.02 to 1.74 aphids/leaf (mean ± SE: 0.52 ± 0.16). Both aphid adult and nymph densities generally decreased with height (pairwise comparisons: $P < 0.01$, except adults which had similar densities at mid and high positions: $P > 0.05$), although this could reflect the physical difficulties associated with counting aphids on leaves in the outer upper crown, more so than the actual distribution of aphids.

There was a marginal significant positive correlation for densities of *E. tiliae* nymphs and *H. axyridis* larvae ($r = 0.39$, $P = 0.04$). Stronger positive linear relationships were revealed for *E. tiliae* nymphs and *A. nemoralis* ($r = 0.654$, $P = 0.0002$), and densities of *A. nemoralis* and *H. axyridis* larvae ($r = 0.519$, $P = 0.005$).

Detectability half-lives and weighting prey detectability of field-caught predators

The primer pair Ans-F2 (5′-GAATGACAGGAGTTATTTTAGC-3′)/Ans-R2 (5′-GTGGAAGTGTGCTACTACG-3′) was specific for a 79 bp sequence of *Anthocoris nemoralis* mtDNA, while aphid predation was detected using the primer pair Eti-F5 (5′-TTTCTTATTAATAATGGTACAGG-3′)/Eti-R2.1 (5′-TGAGATTCCTGCTAAATGTAGC-3′), specific for a 134 bp amplicon of *E. tiliae* mtDNA. Primer pairs were specific for target DNA when tested against 26 insect species occurring on *Tilia* spp. (ESM Table 1).

Detectability half-lives decreased significantly with time post-digestion for all combinations except *H. axyridis* adults fed *E. tiliae* which could not be calculated (Table 3, ESM Table 2). The DS_{50} for detection of *A. nemoralis* in *H. axyridis* larvae (5.6 h)

Table 2 Number of *H. axyridis* and *A. nemoralis* testing positive for *E. tiliae* DNA in 2011 at 3 heights (low: up to 2.5 m; mid: 5–7 m; high: 10–13 m), in five trees (*Tilia* × europaea) in five urban parks in Copenhagen, Denmark: a = Churchillparken, b = Ørstedsparken, c = Genforeningspladsen, d = Bispebjerg Parkallé, e = Østre Anlæg

Height/date	*H. axyridis* (larva)			*H. axyridis* (adult)			*A. nemoralis* (adult)		
	Low	Mid	High	Low	Mid	High	Low	Mid	High
31/05[a]	0 (2)	3 (7)	0 (0)	1 (2)	1 (2)	0 (0)	2 (2)	0 (0)	0 (0)
06/06[b]	8 (13)	3 (6)	–	0 (1)	2 (2)*	–	1 (2)	0 (0)	–
20/06[c]	1 (1)	–	0 (0)	1 (2)	–	0 (0)	5 (5)	–	0 (0)
22/06[b]	2 (2)	7 (11)	–	3 (5)	2 (3)	–	3 (6)	8 (11)	–
30/06[a]	0 (0)	1 (1)	0 (0)	5 (6)	3 (4)	8 (8)*	6 (8)	2 (4)	1 (4)
20/07[d]	1 (3)	2 (3)*	3 (5)	1 (1)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)
22/08[e]	0 (0)	0 (0)	0 (0)	1 (2)	1 (5)	3 (7)	1 (1)	0 (0)	0 (1)
20/09[b]	1 (5)	1 (5)*	0 (0)	1 (6)	1 (1)	2 (2)	0 (0)	0 (0)	1 (1)
26/09[a]	0 (0)	3 (4)	1 (1)	3 (6)	1 (2)*	0 (7)	0 (0)	0 (0)	0 (0)
03/10[c]	1 (1)	2 (3)	0 (0)	0 (4)	0 (6)	0 (3)	0 (0)	0 (0)	0 (0)
Total	14 (27)	22 (40)	4 (6)	16 (35)	11 (25)	13 (27)	18 (26)	10 (15)	2 (6)

Number in parenthesis: total no. tested individuals

– no sample

* Locations and heights intraguild predation was detected in *H. axyridis*

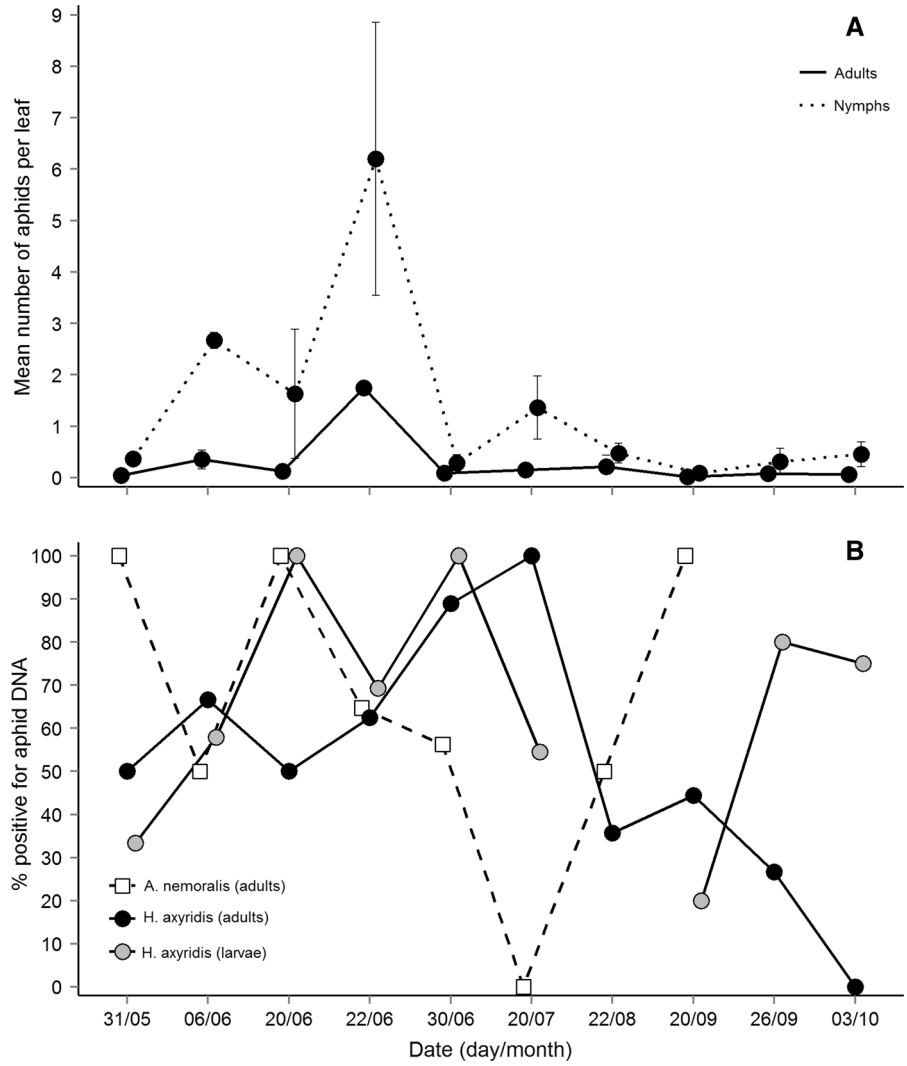

Fig. 2 **a** Mean number of *E. tiliae* per leaf on sampling dates (SD is based on 2–3 pooled heights). **b** Percentage of *H. axridis* adults/larvae and *A. nemoralis* screening positive for *E. tiliae* on sampling dates (2011). Each date represents results for pooled heights

was significantly shorter than DS_{50} values for *E. tiliae* digested by *H. axyridis* larvae (32.4 h; d.f. = 1, $\chi^2 = 10.322$, $P = 0.001$) and *A. nemoralis* (24.8 h; d.f. = 1, $\chi^2 = 8.095$, $P = 0.004$). However, DS_{50} values for *E. tiliae* in predators did not differ (d.f. = 1, $\chi^2 < 0.0001$, $P > 0.05$; Table 3, ESM Table 2).

In 2009, seventy *H. axyridis* larvae (4th instar) collected in October were screened for prey DNA. A single IGP event (1.4 %) was detected (caught 19/10), whereas *E. tiliae* DNA was detected in 32 individuals (45.7 %).

In 2011, 160 *H. axyridis* were collected from tree crowns. Seventy-three *H. axyridis* larvae (3rd and 4th instars) were screened for prey DNA in which IGP was detected in only two 4th instar larvae (2.7 %; collected 20/7, 20/9). In contrast, 40 larvae screened positive for aphid DNA (54.8 %; Fig. 2; Table 3). Similarly for adult *H. axyridis* ($n = 87$: 45 females, 42 males), 3 adults revealed IGP events (3.4 %; collected: 6/6, 30/6, 26/9) and 45.9 % or 40 individuals (19 males, 21 females) tested positive for *E. tiliae* (Fig. 2; Table 3). All *H. axyridis* adults, but only one larva, testing positive for *A. nemoralis* DNA, also tested positive for *E. tiliae* DNA.

Forty-seven *A. nemoralis* (28 females, 19 males) were collected in 2011. Thirty individuals (9 males, 21 females) screened positive for *E. tiliae* DNA (63.8 %; Fig. 2; Table 3).

Applying the DS_{50} correction revealed that the relative importance of IGP was between 5.7 and 6.4 times less than aphid consumption by *H. axyridis*

Table 3 Calculations of weighted trophic interactions based on detectability half-life assays and field-collected predators among *H. axyridis* [intraguild (IG) predator], *A. nemoralis* [intraguild (IG) prey] and the aphid *E. tiliae* [extraguild (EG) prey] on *Tilia* × europaea

Predator–prey	IGP (%)	Aphid DNA (%)	$DS_{50} \pm SE$ (h)	$DS_{50weighted}$*	$IGP^{+}_{weighted}$	Aphid $DNA^{+}_{weighted}$
H. axyridis (larva)–*A. nemoralis*	1.4†/2.7	–	5.6 ± 0.6^{a}	1.00	0.014†/0.027	–
H. axyridis (adult)–*A. nemoralis*	3.4	–	DNA decay not tested	–	–	–
A. nemoralis (adult)–*E. tiliae*	–	63.8	24.8 ± 3.4^{b}	0.22	–	0.14
H. axyridis (larva)–*E. tiliae*	–	45.7†/54.8	32.4 ± 6.8^{b}	0.17	–	0.08†/0.09
H. axyridis (adult)–*E. tiliae*	–	45.9	Not calculated††	–	–	–

DS_{50} values followed by a different letter are significantly different

* $DS_{50weighted}$ derived as: the shortest DS_{50} was assigned a value of 1.00, other $DS_{50weighted}$ values attained by dividing shortest DS_{50} (numerator) with relevant DS_{50} (denominator)

+ Obtained by multiplying proportion of positive DNA by $DS_{50weighted}$ values

† Trophic interactions from specimens collected in 2009, otherwise 2011

†† Post-digestion times did not extend long enough to evaluate DS_{50}

larvae (Table 3). Corrected values for aphid consumption were markedly less for both predators compared with raw data, but comparatively, relatively more anthocorids tested positive for aphid DNA (Table 3). Furthermore, aphid consumption by *H. axyridis* larvae was similar in 2009 and 2011 (0.08 and 0.09, respectively) compared to proportions of raw data testing positive for aphid DNA (Table 3).

Interestingly, aphid DNA was detected in predators collected at all heights, however, IGP events in 2011 were detected in coccinellids collected above 2.5 m (Table 2).

Factors affecting the likelihood of detecting *E. tiliae* DNA in predators

Four variables significantly influenced the likelihood of aphid ingestion by *A. nemoralis* (Table 4). The abundance of *H. axyridis* larvae had a strong negative effect on the likelihood of detecting aphid DNA in *A. nemoralis*. The density of aphid nymphs had a weak positive influence, while a stronger albeit negative influence of aphid adult density was revealed. Finally, the sex of *A. nemoralis* was highly influential with the likelihood of aphid ingestion decreasing markedly for male bugs (Table 4). Other variables were not influential (LR: height, $P = 0.18$; *H. axyridis* adults, $P = 0.84$; competitors, $P = 0.18$).

Collection height did not influence the likelihood of aphid ingestion in either predator, suggesting similar aphid predation levels at all heights. None of the remaining variables contributed to explaining the likelihood of detecting *E. tiliae* in *H. axyridis* guts compared to null models (both life stages, LR: $P = 0.12$; larvae alone, LR: $P = 0.29$).

Discussion

IGP and interspecific competition for *E. tiliae* in urban parks

DNA gut-content analysis revealed low levels of IGP by *H. axyridis*, but higher levels of prey overlap between co-occurring predators in tree crowns (Fig. 2; Tables 2, 3). While adjusting raw gut-content data with the DS_{50} correction values reduced the relative proportions of predators testing positive for prey DNA, the weighted values did not alter the relative importance of trophic interactions in this system. Furthermore, positive correlations of predator density through time, and a negative effect of *H. axyridis* larvae density on aphid predation by *A. nemoralis*, are indicative that spatiotemporal niche overlap and exploitation competition with *H. axyridis* occur in these invaded urban habitats.

Table 4 Summary of final model with variables influencing the likelihood of detecting *E. tiliae* DNA in *A. nemoralis*

Variable	Comparison	Odds ratio (95 % CI)	Variable effect on odds	*P* value
No. of aphid adults	One unit change	0.853 (0.754–0.964)	Negative	0.01
No. of aphid nymphs	One unit change	1.033 (1.006–1.062)	Positive	0.01
No. of *H. axyridis* larvae	One unit change	0.495 (0.267–0.531)	Negative	0.03
Sex	Male versus female	0.083 (0.013–0.531)	Negative	0.01

P values correspond to the likelihood ratio test of the effect of a variable in the presence of the other variables in the model. Odds ratios (Wald CI) are adjusted for other variables in the model

The frequency of intraguild predation by *H. axyridis* usually increases concurrent with declines in extraguild prey densities (Yasuda et al. 2004; Noia et al. 2008; Gagnon and Brodeur 2014). In this system, aphid densities peaked in late June 2011 and declined thereafter. Indeed, three of the five IGP events in 2011 took place after aphid populations had peaked in late June (Table 2; Fig. 2). However, even though predators co-occurred temporally and often spatially in trees, IGP on *A. nemoralis* was a minor trophic interaction (1.4–3.4 %; $IGP_{weighted} = 0.014$), which supports our first hypothesis (Tables 2, 3).

Recent studies detected traces of native coccinellid in 12.2 and 20.5 % of sampled *H. axyridis* larvae collected on *Tilia* spp. (Hautier et al. 2011; Thomas et al. 2013). In *H. axyridis* sampled predominantly from *T* x europaea across Europe, Brown et al. (2015) report 0–13.7 % of larvae contain *A. decempunctata* L. DNA and 0–11.4 % contain remains of *A. bipunctata* L. While these results reveal comparatively higher IGP levels against European coccinellids, Rondoni et al. (2014) report lower IGP levels (1.5 and 5.0 %) against two native coccinellids in Italy. In comparison, *A. nemoralis* in this study apparently suffers less direct negative effects (e.g. IGP) than native coccinellids in similar habitats. Noteworthy, as IGP was detected in individuals collected above 2.5 m in tree crowns, it is possible we would have missed these events had we only sampled trees from the ground. We propose that IGP contributes little to mortality of *A. nemoralis* populations in *Tilia* × europaea, and probably elsewhere these species co-occur. Mechanisms underlying low IGP levels require investigation, but could be related to escape behaviour afforded by high mobility of large *A. nemoralis* nymphs and adults, dropping behaviour upon encounters, and utilisation of leaf axils and stipules as refuge from predation (Dixon and Russel 1972; Lucas 2005; Howe et al. 2015).

We hypothesised that interspecific competition is potentially a more important interaction than IGP between predators, as it may occur over longer temporal scales. Potential for prey shortage was reflected in decreasing aphid densities from late June and a decreased proportion of *H. axyridis* adults testing positive for *E. tiliae* following the aphid population peak in late June (Fig. 2). Concurrently, detection of aphid DNA in predators remained vastly higher throughout the year relative to IGP levels, revealing prey overlap and potential for interspecific (but also intraspecific) competition for a limited EG prey (Fig. 2; Table 3). Rondoni et al. (2014) recently reveal vastly higher levels of *E. tiliae* predation by *H. axyridis* larvae (0.28, derived from DS_{50} data in Rondoni et al. 2014) relative to IGP of native coccinellids (*A. bipunctata*: 0.015; *Oenopia conglobata* L.: 0.012) over a 2-month sampling period on *Tilia* spp. Despite these authors did not test the extent of prey (*E. tiliae*) overlap with native coccinellids, their results demonstrate IGP is a much weaker trophic interaction compared to EG predation, which lends support to the findings of the present study in *T.* × europaea.

The strength of competition may be ameliorated through niche partitioning in space and time, thereby fostering co-existence (Hutchinson 1959; Armstrong and Mcgehee 1980; Pell et al. 2008; Crowder and Snyder 2010). However, by exploiting multiple resources invasive generalist species reduce the extent of niche partitioning in invaded habitats, which may impinge on native species (Snyder and Evans 2006). Our results suggest temporal co-occurrence, as predators were simultaneously collected from tree crowns until late September in 2011 (Fig. 2; Table 2). In

northern Europe, these predators are bivoltine, becoming active in May, *H. axyridis* remains active through to November, whereas *A. nemoralis* begins hibernation between August and October (Anderson 1962; Adriaens et al. 2008; Sigsgaard 2010). Spatial niche overlap was further supported by significant positive correlations of predator densities, predator and aphid nymph densities, and similar densities of predators between heights. Moreover, detection of aphid DNA was not influenced by collection height, lending further support that these predators forage for prey at similar spatial scales in *T.* × europaea crowns.

Different prey preferences contribute to niche partitioning in aphid/predator systems (Losey and Denno 1998; Pell et al. 2008), and may occur to an extent between *A. nemoralis* and *H. axyridis*. Due to their relatively small body size, anthocorids are ineffective at capturing large aphid nymphs/adults (Dixon and Russel 1972). Our analysis showed a positive influence of aphid nymph and negative influence of aphid adult densities on aphid predation by *A. nemoralis*, which could indicate that anthocorids prefer small prey. In contrast, none of the investigated variables contributed to explaining aphid predation by *H. axyridis* (independent of the ladybird's life stage), suggesting the ladybird does not discriminate between aphid sizes. These results could imply that niche partitioning based on prey size is curtailed (Snyder and Evans 2006). This effect is potentially amplified for *A. nemoralis* apterous nymph stages, due to their small size relative to adults, but also as emigration in response to dwindling aphid densities in late summer is restricted (Dixon 1971b). Furthermore, second generation nymphs are likely to contend for a shortage of aphids at local scales (Dixon 1971b), subsequently increasing the intensity of exploitative competition and concurrently the risk of intraguild predation among aphidophages (Obrycki et al. 1998; Ware et al. 2009).

Anthocorid males were significantly less likely to contain aphid DNA than females (Table 4). This may not necessarily equate to a distinct disadvantage for males, as *A. nemoralis* can utilise various prey (Anderson 1962). However, as *E. tiliae* is important for *A. nemoralis* reproduction (Anderson 1962), female anthocorids are potentially more dependent on *E. tiliae*, and thereby more vulnerable to interspecific exploitation competition by *H. axyridis*.

Invasive insect generalist predators typically achieve higher densities than the native predators they replace (Snyder and Evans 2006; Crowder and Snyder 2010). Comparable to European studies reporting coccinellid densities (Hautier et al. 2011; Brown et al. 2011a) we found *H. axyridis* densities 2.5–8 times greater than *A. nemoralis* (Table 2). The density of *H. axyridis* larvae had a strong negative effect on aphid predation by *A. nemoralis* suggesting that *H. axyridis* life stage may mediate interspecific competition between these predators. Furthermore, although weighted proportions revealed relatively higher interactions between anthocorids and *E. tiliae* compared to *H. axyridis* larvae and aphids, the values do not take differences in predator voracity into account. For example, adult *H. axyridis* may eat between 15 and 65 aphids per day (ref. in Koch 2003). In contrast, adult *A. nemoralis* eat between 3.6 and 12.7 aphids per day (Meyling et al. 2003). While we draw attention to the small sample size ($n = 47$), the negative effect of larvae density underscores that the extent and strength of interspecific competition may be exacerbated by superabundant densities of voracious *H. axyridis*.

The strength of IGP and resource competition is influenced by habitat structure (Hoogendoorn and Heimpel 2004; Crowder and Snyder 2010). Complex structured habitats may reduce encounter rates, impede predator perception, and provide spatial refuge for intraguild prey compared to simple structures (Persson and Eklov 1995; Lucas et al. 2000; Raak-van Den Berg et al. 2012). However, when an IG prey is an inferior competitor, the benefit of reduced IGP may be outweighed by competition for shared prey (Janssen et al. 2007). For example, dramatic declines of predator assemblages including anthocorids (*Orius insidious* Say) on pecan trees (12–15 m high) and ornamental crape myrtle (2–4 m high) were shown following invasion by *H. axyridis*, presumably through resource competition (Mizell 2007). However, competition with *H. axyridis* in a structurally complex habitat can be alleviated when co-occurring predators adjust their distribution in response to *H. axyridis* (Hoogendoorn and Heimpel 2004). In our study, complex *T.* × europaea crowns may be a factor contributing to low IGP levels, but given the high level of spatiotemporal niche overlap of predators, competitive abilities of these species are more likely to drive outcomes of interspecific interactions in tree crowns.

Known characteristics of *H. axyridis* conferring competitive advantage over native coccinellids include superior foraging behaviour, e.g. prey searching and attack efficiency, interspecific aggression (Yasuda et al. 2004; Labrie et al. 2006), while recent laboratory microcosm studies reveal *H. axyridis* superior competition for *E. tiliae* compared to *A. nemoralis* (Howe et al. 2015).

Limitations of this study

The limited spatial and temporal scales of this study precluded assessment of whether resource competition has negative consequences on *A. nemoralis* populations, or contributes to its competitive displacement. We had a priori knowledge that parks contained dense populations of *H. axyridis*, which may inflate trophic interactions quantified here. Assessment of interactions where invasive *H. axyridis* is less abundant would provide a more nuanced picture of interaction strengths (Kenis et al. 2009). In addition, although *E. tiliae* is the numerically dominant herbivore on *Tilia* spp., other insects are found on *Tilia* spp., and are potentially exploited as alternate resources by polyphagous predators (Lattin 1999; Alford 2002; Vandereycken et al. 2012; Table 1). Such resources can weaken competition (MacArthur and Levins 1967; Tilman 1982), which has potential to alter the strength of interactions demonstrated in this study.

Detection of aphid predation in this study may not precisely reflect prey overlap in sampled tree crowns, as we cannot discount that winged adults ingested aphids in nearby *Tilia* spp. Dispersal between trees, or at larger scales between parks, may contribute toward reducing resource competition (Amarasekare 2003); future studies could reduce this potential bias by focusing on immature life stages of predators. Furthermore, testing primer specificity against a greater number of species/families for cross-reactivity (i.e. >26 species) would reduce the potential for interpretation of false-positives as a result of amplification of non-target prey. This is applicable not only to non-target species within *Tilia* spp. crowns, but also species ingested by focal (generalist) predators dispersing from other locations/plants to focal study sites. This may be particularly relevant in cities compared to agroecosystems, due to the heterogeneity of urban green spaces within a relatively small area, e.g. diverse assemblages of vegetation types supporting different insect communities, which may inflate the potential for bias through cross-reactivity.

Secondary predation and scavenging events are indistinguishable using DNA-based methods which may distort detected interaction strengths by yielding false positives (Sheppard and Harwood 2005; Foltan et al. 2005; Hosseini et al. 2008). Laboratory feeding trials with *H. axyridis* and *A. nemoralis* did not reveal scavenging (Howe et al. 2015), but the breadth of scavenging by both species in natural settings requires more attention, and could improve knowledge of trophic pathways in general (Lattin 1999; Gagnon et al. 2011a). In addition, IGP potentially overestimated prey overlap in cases where prey DNA detected in *H. axyridis* was actually ingested by *A. nemoralis*. While this does little to alter our prey overlap results (aphid DNA detected in 4 of the 5 IGP events), it may have been a confounding factor were IGP levels higher. Similarly, aphid DNA in either predator could be a result of IGP of non-focal predators, but given densities of other predators were relatively low in tree crowns this effect is probably negligible (Table 1).

Finally, a range of factors influence the detection period of target DNA following ingestion including the effect of temperature on enzyme activity during digesting, time since ingestion, length of the target DNA sequence, predator species, activity levels, starvation and sample storage, e.g. ethanol versus dry (Hoogendoorn and Heimpel 2001; King et al. 2008; Gagnon et al. 2011a).

Aphid DNA decayed at similar rates in predators and as such the respective trophic interactions between predators and *E. tiliae* are comparable (Gagnon et al. 2011a; Table 3, ESM Table 2). Calculation of DS_{50} for *H. axyridis* adults ingesting *E. tiliae* obviously requires digestion times beyond 24 h, but assuming adults share a DS_{50} similar to larvae, weighted trophic interactions probably reflect values for *H. axyridis* adults, which were similar to larvae, at least, for raw gut-content data. The relatively short detection period for *A. nemoralis* DNA likely reflects IGP taking place on the day samples were collected (i.e. up 6 h after an IGP event), although a slightly longer detection period would enhance detection of a rare event. Our sampling time extended 2 h beyond *H. axyridis* primary feeding time (due to setup of tree climbing equipment), which may equate to underestimation of prey ingestion because individuals had longer time to digest meals in crowns. However, collection height did not affect

the likelihood of aphid detection (high positions were sampled latest during a collection day), so this potential error was likely negligible. Furthermore, trophic interactions in this study are a "snapshot in time" in that we do not precisely know when or how many individuals were ingested (Chen et al. 2000), which could be addressed by developing a qPCR approach (King et al. 2008). Finally, digestion times were performed using *A. nemoralis* reared for biocontrol, whose populations (origins from Germany and Denmark) may differ from those in Copenhagen parks.

Conclusion

A consequence of invasive generalist insect predators is the competitive displacement of related or functionally similar native predators (Snyder and Evans 2006; Crowder and Snyder 2010). A combination of mechanisms typically contribute to competitive displacement (Reitz and Trumble 2002), and specifically for *H. axyridis* asymmetrical IGP is identified as a driver of native coccinellid displacement (Koch and Galvan 2008; Roy et al. 2012). Our results reveal resource competition for *E. tiliae* is a stronger trophic interaction relative to IGP between *A. nemoralis* and *H. axyridis* in *Tilia* x europaea crowns. As both larvae and adult *H. axyridis* exploit *E. tiliae* over multiple, overlapping generations throughout the year, competition for *E. tiliae* is likely to be intense, especially where *H. axyridis* achieves high densities. Additionally, the negative effect of *H. axyridis* larvae density on detection of aphid ingestion by *A. nemoralis* suggests that interspecific exploitative competition may be life stage-mediated, i.e. most intense when *H. axyridis* are larvae.

Although interactions between *H. axyridis* and coccinellids have received most attention to date, this study revealed numerous non-coccinellid aphidophages co-occur with invasive *H. axyridis* in *T.* × europaea. Findings here are indicative that for the assemblage of native aphidophages sharing resources with, yet avoiding IGP by invasive *H. axyridis*, indirect trophic interactions are likely to play an important role in shaping invaded insect communities. Given *H. axyridis* eurytopic and polyphagous nature, this has implications for many native insect species, especially those whose life stages overlap with *H. axyridis* 3rd and 4th larval stages. Whether ecosystem services remain intact when functional diversity is altered by *H. axyridis* remains a pressing area of research (Pell et al. 2008; Roy et al. 2012); while *H. axyridis* is a rare case of a BCA becoming invasive, environmental risk assessment of future BCAs can benefit from post-introduction studies elucidating effects of BCAs on trophic interactions in invaded food webs (Aebi et al. 2011).

Acknowledgments We are grateful to Lars Skipper (www.miridae.dk) for verifying the identity of mirids and EWH BioProduction ApS for provision of *A. nemoralis*; Gitte Jensen (DK), Renate Zindel and Mario Waldburger (Agroscope ART, CH) for technical assistance; and Peter Brown (UK) and Brecht Ingels (BEL) for collection of specificity samples. We also thank three anonymous reviewers for their insightful comments. HPR was financed by Villum Fonden, AGH was supported by a PhD grant from University of Copenhagen.

References

Adriaens T, Branquart E, Maes D (2003) The multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? Belg J Zool 133:195–196

Adriaens T, San M, Maes D (2008) Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. Biocontrol 53:69–88

Aebi A, Brown PM, De Clercq P, Hautier L, Howe A, Ingels B, Ravn HP, Sloggett JJ, Zindel R, Thomas A (2011) Detecting arthropod intraguild predation in the field. Biocontrol 56:429–440

Alford DV (2002) Pests of ornamental trees, shrubs and flowers. Manson Publishing Ltd., London

Alhmedi A, Haubruge E, Francis F (2010) Intraguild interactions implicating invasive species: *Harmonia axyridis* as a model species. Biotechnol Agron Soc Environ 14:187–201

Alyokhin A, Sewell G (2004) Changes in a lady beetle community following the establishment of three alien species. Biol Invasions 6:463–471

Amarasekare P (2003) Competitive coexistence in spatially structured environments: a synthesis. Ecol Lett 6:1109–1122

Anderson NH (1962) Bionomics of six species of *Anthocoris* (Heteroptera: Anthocoridae) in England. Trans R Entomol Soc Lond 114:67–95

Armstrong RA, Mcgehee R (1980) Competitive-exclusion. Am Nat 115:151–170

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.0-6

Brown JS, Kotler BP, Mitchell WA (1994) Foraging theory, patch use, and the structure of a Negev Desert granivore community. Ecology 75:2286–2300

Brown PMJ, Roy HE, Rothery P, Roy DB, Ware RL, Majerus MEN (2008) *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid. Biocontrol 53:55–67

Brown PMJ, Frost R, Doberski J, Sparks T, Harrington R, Roy HE (2011a) Decline in native ladybirds in response to the arrival of *Harmonia axyridis*: early evidence from England. Ecol Entomol 36:231–240

Brown PMJ, Thomas CE, Lombaert E, Jeffries DL, Estoup A, Handley LJL (2011b) The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. Biocontrol 56:623–641

Brown PMJ, Ingels B, Wheatley A, Rhule EL, de Clercq P, van Leeuwen T, Thomas A (2015) Intraguild predation by *Harmonia axyridis* (Coleoptera: Coccinellidae) on native insects in Europe: molecular detection from field samples. Entomol Sci 18:130–133

Chapin JB, Brou VA (1991) *Harmonia axyridis* (Pallas), the third species of the genus to be found in the United States (Coleoptera: Coccinellidae). Proc Entomol Soc Wash 93:630–635

Chen Y, Giles KL, Payton ME, Greenstone MH (2000) Identifying key cereal aphid predators by molecular gut analysis. Mol Ecol 9:1887–1898

Crowder DW, Snyder WE (2010) Eating their way to the top? Mechanisms underlying the success of invasive insect generalist predators. Biol Invasions 12:2857–2876

Dixon AFG (1971a) Role of aphids in wood formation. 2. Effect of lime aphid, *Eucallipterus tiliae* L. (Aphididae), on growth of lime, *Tilia* x vulgaris Hayne. J Appl Ecol 8:393

Dixon AFG (1971b) Role of intra-specific mechanisms and predation in regulating numbers of lime aphid, *Eucallipterus tiliae* L. Oecologia 8:179

Dixon AFG (1998) Aphid ecology: an optimization approach. Chapman & Hall, London

Dixon AFG, Russel RJ (1972) Effectiveness of *Anthocoris nemorum* and *A. Confusus* (Hemiptera: Anthocoridae) as predators of sycamore aphid, *Drepanosiphum platanoides*. 2. Searching behavior and incidence of predation in field. Entomol Exp Appl 15:35

Evans EW, Soares AO, Yasuda H (2011) Invasions by ladybugs, ladybirds, and other predatory beetles. Biocontrol 56: 597–611

Foltan P, Sheppard S, Konvicka M, Symondson WOC (2005) The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. Mol Ecol 14:4147–4158

Foottit RG, Maw HEL, Von Dohlen CD, Hebert PDN (2008) Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. Mol Ecol Resour 8:1189–1201

Gagnon AE, Brodeur J (2014) Impact of plant architecture and extraguild prey density on intraguild predation in an agroecosystem. Entomol Exp Appl 152:165–173

Gagnon AE, Doyon J, Heimpel GE, Brodeur J (2011a) Prey DNA detection success following digestion by intraguild predators: influence of prey and predator species. Mol Ecol Resour 11:1022–1032

Gagnon AE, Heimpel GE, Brodeur J (2011b) The ubiquity of intraguild predation among predatory arthropods. Plos One 6(11):e28061

Greenstone MH, Payton ME, Weber DC, Simmons AM (2014) The detectability half-life in arthropod predator–prey research: what it is, why we need it, how to measure it, and how to use it. Mol Ecol 23:3799–3813

Harmon JP, Stephens E, Losey J (2007) The decline of native coccinellids (Coleoptera: Coccinellidae) in the United States and Canada. J Insect Conserv 11:85–94

Hautier L, Martin GS, Callier P, de Biseau JC, Gregoire JC (2011) Alkaloids provide evidence of intraguild predation on native coccinellids by *Harmonia axyridis* in the field. Biol Invasions 13:1805–1814

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological Identifications through DNA Barcodes. Proceedings: Biological Sciences: 270(1512):313–321

Hironori Y, Katsuhiro S (1997) Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. Entomophaga 42:153–163

Hoogendoorn M, Heimpel GE (2001) PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Mol Ecol 10:2059–2067

Hoogendoorn M, Heimpel GE (2004) Competitive interactions between an exotic and a native ladybeetle: a field cage study. Entomol Exp Appl 111:19–28

Hosseini R, Schmidt O, Keller MA (2008) Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. Entomol Exp Appl 126:194–202

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363

Howe AG, Ransijn J, Ravn HP (2015) A sublethal effect on native *Anthocoris nemoralis* through competitive interactions with *Harmonia axyridis*. Ecol Entomol. 40:639–649

Hoy MA (1994) Insect molecular genetics: an introduction to principals and applications. Academic Press, San Diego

Hutchinson GE (1959) Homage to Santa-Rosalia or why are there so many kinds of animals. Am Nat 93:145–159

Jackson CH (2011) Multi-state models for panel data: the msm package for R. J Stat Softw 38(8):1–29

Janssen A, Sabelis MW, Magalhaes S, Montserrat M, Van der Hammen T (2007) Habitat structure affects intraguild predation. Ecology 88:2713–2719

Kasper ML, Reeson AF, Cooper SJB, Perry KD, Austin AD (2004) Assessment of prey overlap between a native (*Polistes humilis*) and an introduced (*Vespula germanica*) social wasp using morphology and phylogenetic analyses of 16S rDNA. Mol Ecol 13:2037–2048

Kenis M, Auger-Rozenberg MA, Roques A, Timms L, Pere C, Cock M, Settele J, Augustin S, Lopez-Vaamonde C (2009) Ecological effects of invasive alien insects. Biol Invasions 11:21–45

Kenis M, Adriaens T, Brown P, Katsanis A Van, Vlaenderen J, Eschen R, Golaz L, Zindel R San, Martin y Gomez G, Babendreier D, Ware R (2010) Impact of *Harmonia axyridis* on European ladybirds: which species are most at risk? IOBC/WPRS Bull 58:57–59

King RA, Read DS, Traugott M, Symondson WOC (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol Ecol 17:947–963

Koch RL (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. J Insect Sci 3:32

Koch RL, Galvan TL (2008) Bad side of a good beetle: the North American experience with *Harmonia axyridis*. Biocontrol 53:23–35

Koressaar T, Remm M (2007) Enhancements and modifications of primer design program Primer3. Bioinformatics 23:1289–1291

Labrie G, Lucas E, Coderre D (2006) Can developmental and behavioral characteristics of the multicolored Asian lady beetle *Harmonia axyridis* explain its invasive success? Biol Invasions 8:743–754

Lattin JD (1999) Bionomics of the Anthocoridae. Annu Rev Entomol 44:207–231

Lawton JH, Hassell MP (1984) Interspecific competition in insects. In: Huffaker CB, Rabb RL (eds) Ecological entomology. Wiley, New York, pp 451–495

Losey JE, Denno RF (1998) Positive predator–predator interactions: enhanced predation rates and synergistic suppression of aphid populations. Ecology 79:2143–2152

Lucas E (2005) Intraguild predation among aphidophagous predators. Eur J Entomol 102:351–363

Lucas E, Coderre D, Brodeur J (2000) Selection of molting and pupation sites by *Coleomegilla maculata* (Coleoptera: Coccinellidae): avoidance of intraguild predation. Environ Entomol 29:454–459

Lucas E, Labrie G, Lazarovits G (2007) The multicolour Asian ladybird beetle: beneficial or nuisance organism? In: Vincent C, Goettel MS, Lazarovits G (eds) Biological control: a global perspective. CABI Publishing, Wallingford, pp 38–52

MacArthur R, Levins R (1967) Limiting similarity convergence and divergence of coexisting species. Am Nat 101:377

Meyling NV, Enkegaard A, Brødsgaard H (2003) Two *Anthocoris* bugs as predators of glasshouse aphids—voracity and prey preference. Entomol Exp Appl 108:59–70

Michaud JP (2002) Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. Environ Entomol 31:827–835

Miura T, Nishimura S (1980) The larval period and predacious activity of an aphidophagous coccinellid, *Harmonia axyridis* PALLAS. Bull Fac Agric Shimane Univ 14:144–148

Mizell RF (2007) Impact of *Harmonia axyridis* (Coleoptera: Coccinellidae) on native arthropod predators in pecan and crape myrtle. Fla Entomol 90:524–536

Nedved O, Fois X, Ungerova D, Kalushkov P (2013) Alien vs. predator—the native lacewing *Chrysoperla carnea* is the superior intraguild predator in trials against the invasive ladybird *Harmonia axyridis*. Bull Insectol 66(1):73–78

Noia M, Borges I, Soares AO (2008) Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): the role of intra and extraguild prey densities. Biol Control 46:140–146

Obrycki JJ, Giles KL, Ormord AM (1998) Interactions between an introduced and indigenous coccinellid species at different prey densities. Oecologia 117:279–285

Osawa N (2000) Population field studies on the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): resource tracking and population characteristics. Popul Ecol 42:115–127

Pell JK, Baverstock J, Roy HE, Ware RL, Majerus MEN (2008) Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. Biocontrol 53:147–168

Péricart J (1996) Family Anthocoridae Fieber. 1836—flower bugs, minute pirate bugs. In: Aukema B, Rieger C (eds) Catalogue of the Heteroptera. Ponsen and Looijen, Wageningen, pp 108–140

Persson L, Eklov P (1995) Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology 76:70–81

Polis GA, Strong DR (1996) Food web complexity and community dynamics. Am Nat 147:813–846

Polis GA, Myers CA, Holt RD (1989) The ecology and evolution of intraguild predation—potential competitors that eat each other. Annu Rev Ecol Syst 20:297–330

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing

Raak-van Den Berg CL, De Lange HJ, Van Lenteren JC (2012) Intraguild predation behaviour of ladybirds in semi-field experiments explains invasion success of *Harmonia axyridis*. Plos One 7(7):e40681

Ratnasingham S, Hebert PDN (2007) BOLD: the barcode of life data system (www.barcodinglife.org). Mol Ecol Notes 7:355–364

Reitz SR, Trumble JT (2002) Competitive displacement among insects and arachnids. Annu Rev Entomol 47:435–465

Rondoni G, Athey KJ, Harwood JD, Conti E, Ricci R, Obrycki JJ (2014) Development and application of molecular gut-content analysis to detect aphid and coccinellid predation by *Harmonia axyridis* (Coleoptera: Coccinellidae) in Italy. Insect Sci 00:1–12. doi:10.1111/1744-7917.12165

Roy HE, Handley LJL (2012) Networking: a community approach to invaders and their parasites. Funct Ecol 26:1238–1248

Roy HE, Adriaens T, Isaac NJB, Kenis M, Onkelinx T, San Martin G, Brown PMJ, Hautier L, Poland R, Roy DB, Comont R, Eschen R, Frost R, Zindel R, Van Vlaenderen J, Nedved O, Ravn HP, Gregoire JC, de Biseau JC, Maes D (2012) Invasive alien predator causes rapid declines of native European ladybirds. Divers Distrib 18:717–725

Sheppard SK, Harwood JD (2005) Advances in molecular ecology: tracking trophic links through predator–prey food-webs. Funct Ecol 19:751–762

Sigsgaard L (2010) Habitat and prey preferences of the two predatory bugs *Anthocoris nemorum* (L.) and *A. nemoralis* (Fabricius) (Anthocoridae: Hemiptera-Heteroptera). Biol Control 53:46–54

Snyder WE, Evans EW (2006) Ecological effects of invasive arthropod generalist predators. Annu Rev Ecol Evol Syst 37:95–122

Southwood TRE, Leston D (1959) Land and water bugs of the British Isles (CD-ROM version). Pisces Conservation Limited, Lymington

Thomas AP, Trotman J, Wheatley A, Aebi A, Zindel R, Brown PMJ (2013) Predation of native coccinellids by the invasive alien *Harmonia axyridis* (Coleoptera: Coccinellidae): detection in Britain by PCR-based gut analysis. Insect Conserv Divers 6:20–27

Tilman D (1982) Resource competition and structure. Princeton University Press, Princeton

Tomov R, Trencheva K, Trenchev G, Kenis M (2009) The multicolored invasive Asian ladybird *Harmonia axyridis* (PALLAS, 1773) (Coleoptera: Coccinellidae) new to the fauna of Bulgaria. Acta Zool Bulg 61:307–311

Tylianakis JM (2008) Understanding the web of life: the birds, the bees, and sex with aliens. PLoS Biol 6:224–228

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3-new capabilities and interfaces. Nucleic Acids Res 40(15):e115

Vandereycken A, Durieux D, Joie É, Haubruge É, Verheggen FJ (2012) Habitat diversity of the multicolored Asian ladybeetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in agricultural and arboreal ecosystems: a review. Biotechnol Agron Soc Environ 16(4):553–563

Ware R, Yguel B, Majerus M (2009) Effects of competition, cannibalism and intra-guild predation on larval development of the European coccinellid *Adalia bipunctata* and the invasive species *Harmonia axyridis*. Ecol Entomol 34:12–19

White EM, Wilson JC, Clarke AR (2006) Biotic indirect effects: a neglected concept in invasion biology. Divers Distrib 12:443–455

Wickham H (2009) Ggplot2: elegant graphics for data analysis. Springer, New York

Yasuda H, Evans EW, Kajita Y, Urakawa K, Takizawa T (2004) Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141:722–731