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Effects of Fipronil on Non-target Ants and Other Invertebrates in a Program for Eradication of the Argentine Ant, *Linepithema humile*

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Abstract

Pesticides are frequently used to eradicate invasive ant species, but pose ecological harm. Previous studies assessed non-target effects only in terms of the increase or decrease of abundance or species richness after pesticide applications. Positive effects of the release from pressure caused by invasive ant species have not been considered so far. To more accurately assess pesticide effects in the field, the non-target effects of pesticides should be considered separately from the positive effects of such releases. Here, we used monitoring data of ants and other invertebrates collected in a program for the eradication of the Argentine ant, *Linepithema humile* (Mayr), using fipronil. First, we separately assessed the effects of *L. humile* abundance and fipronil exposure on non-target ants and other invertebrates using generalized linear models. The abundance of *L. humile* and the number of pesticide treatments were negatively associated with the total number of non-target individuals and taxonomic richness. We also noted negative relationships between the number of individuals of some ant species and other invertebrate taxonomic groups. The *L. humile* × pesticide interaction was significant, suggesting that the abundance of *L. humile* affected the level of impact of pesticide treatment on non-target fauna. Second, we evaluated the dynamics of non-target ant communities for 3 years using principal response curve analyses. Non-target ant communities treated with fipronil continuously for 3 years recovered little, whereas those treated for 1 year recovered to the level of the untreated and non-invaded environment.

Introduction

Biological invasion is a global environmental problem that harms biodiversity and ecosystem function (Clavero & Garcia-Berthou, 2005; Mack et al., 2000). Ants are some of the most successful invasive taxa in the world, with invasive ant species having become established on almost every continent (Suarez et al., 2010). The success of these species is related to a suite of characteristics that favor interactions with humans (Hoffmann et al., 2016; Holway et al., 2002; McGlynn, 1999). These tramp species cause serious harm to the environment, agricultural productivity, human health, and the economy (Holway et al., 2002; Williams, 1994), as reflected by the fact that five ant species are listed among the world's 100 worst invasive alien species (IUCN ISSG, 2013).

Invasive ants are typically controlled with pesticides, such as in bait carriers (Rabitsch, 2011; Williams, 1994). Pesticides have been used successfully in dozens of eradication programs targeting ant species, such as the little fire ant, *Wasmannia auropunctata*, on Santa Fe Island in the Galápagos (Abedrabbo, 1994; Causton et al., 2005), the African big-headed ant, *Pheidole megacephala*, and the tropical fire ant, *Solenopsis geminata*, within Kakadu National Park, Australia (Hoffmann & O'Connor, 2004), and the Argentine ant, *Linepithema humile*, on landfill islands in Japan (Sakamoto et al., 2017). However, the use of pesticides also harms non-target species (Pisa et

al., 2015; Prasifka et al., 2005), as toxic baits can attract non-target ants and other arthropods. Fipronil, hydramethylnon, pyriproxyfen, and methoprene, commonly used in invasive ant eradication programs, pose risks (Hoffmann et al., 2016).

The impacts of these pesticides on non-target species in the field have been assessed only in terms of increases or decreases in the abundance or taxonomic richness of non-target species after invasive ant control. Plentovich et al. (2010) reported that hydramethylnon can be used to control *S. geminata* and *Tetramorium bicarinatum* but also noted its negative effects on non-target ants, cockroaches, and crickets. By contrast, Hoffmann (2010) documented the eradication of a small population of *P. megacephala* using hydramethylnon and the recovery of native ant abundance and species richness within the treated area. Inoue et al. (2015) reported the short-term recovery of non-target communities after application of fipronil to control *L. humile* and observed no non-target effects. However, the positive effects of release from pressure caused by invasive ant species have not been considered in previous research. To more accurately evaluate the effects of pesticides in the field, the non-target effects of pesticides must be considered separately from the positive effects of such releases.

Linepithema humile, a native of South America, is one of the most significant pest ant species worldwide (Passera, 1994). In Japan, it was first discovered in 1993 (Sugiyama, 2000) and has since spread to 12 prefectures (National Institute of Environmental Studies, 2014). Since 2011, our group has conducted an eradication program using toxic baits containing fipronil (Inoue et al., 2015), and we have successfully eradicated two Tokyo populations on landfill islands (Sakamoto et al., 2017). The aim of the eradication program is to protect the indigenous invertebrate communities from the invasive alien ant species. Thus, non-target effects cannot be ignored when the method is applied to delicate natural areas.

In this study, we evaluated the non-target effects of applying fipronil to eradicate *L. humile* in the two Tokyo populations. First, we separately assessed the effects of the pesticide and *L. humile* on non-target ant and non-ant invertebrates using generalized linear models (GLMs). Next, we used principal response curve (PRC) analyses to evaluate the dynamics of the non-target communities after successful eradication and cessation of the pesticide applications. Our findings will be useful for minimizing the risks to indigenous fauna as the eradication program moves to other areas.

Materials and methods

Study sites

The Tokai site is on a landfill island 370 m west of the Oi Container Terminal, one of the largest international shipping ports in Japan, where 8.5 ha was invaded by *L. humile* (Fig 1a). The Jonan site is on the landfill island Jonan-jima, 1750 m southwest of the terminal, where 16 ha was invaded (Fig 1a).

Pesticide

Fipronil is a phenyl pyrazole insecticide and a potent disrupter of the arthropod central nervous system via interference through the chloride channel regulated by γ-aminobutyric acid (Rhône Poulenc, 1996). Fipronil acts slowly, allowing the pesticide in baits to be transferred from insect to insect (including queen and brood among social insects) by trophallaxis or contact (Vail et al., 2003), resulting in reproductive inhibition in colonies. Fipronil is effective at controlling invasive ant species, especially *L. humile* (Klotz et al., 2007).

Pesticide treatment

The eradication program began in April 2011 (Inoue et al., 2015). To evaluate the effects of the program, we established three monitoring plots each at Tokai (Plots i–iii; Fig 1b) and Jonan (Plots I–III; Fig 1c). We applied paste baits and sprays once a month (Fig 2). The paste bait, Aruzenchin Ari Ultra Sugoto-taiji (50 mg L⁻¹ fipronil; Fumakilla, Ltd., Hiroshima, Japan), was placed every 5 to 10 m along the streets and buildings. The bait was applied in a given month only if *L. humile* had been found in the same plot at any time during the previous 6 months. If we found brood or queens in vegetation or under pavement during bait application, we sprayed them with a solution of 50 mg L⁻¹ fipronil (Aruzenchin Ari Sugoto-taij Ekizai, Fumakilla, Ltd.). The estimated total rate of active ingredient applied was 137 mg/ha at Tokai and 1045 mg/ha at Jonan over the 3 years. Plot I at Jonan was not treated in the first year for comparison with treated areas.
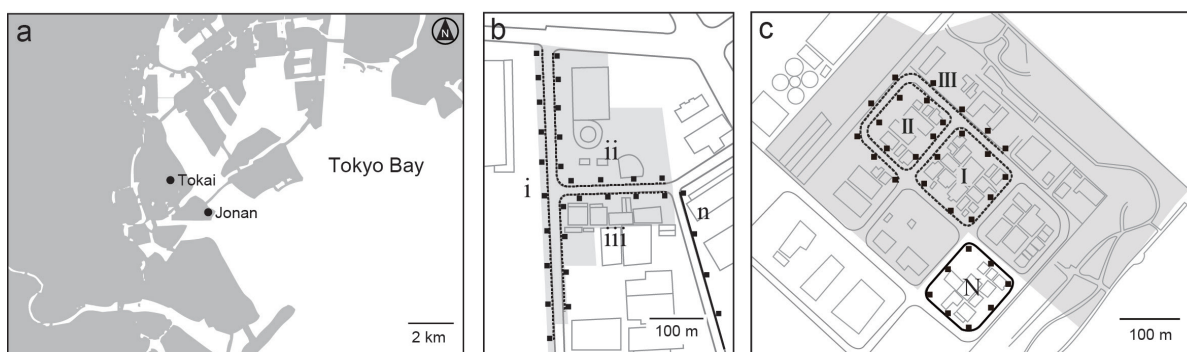

Fig 1. Maps of (a) Tokyo Bay area and monitoring plots at (b) Tokai and (c) Jonan. Solid line indicates transects in non-invaded plots (n, N); dashed line indicates transects in plots invaded by *Linepithema humile* (i–iii, I–III). Squares indicate locations of sticky traps.
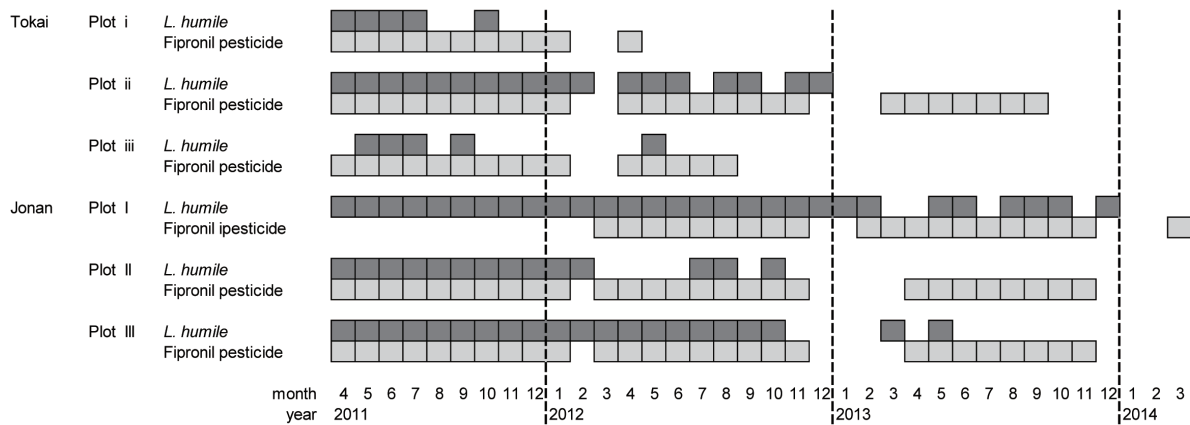
Fig 2. Presence of *Linepithema humile* and fipronil pesticide use history in each plot at Tokai and Jonan over 3 years.

Sampling of ant species and non-ant invertebrates

To monitor the abundance of ant species and non-ant invertebrates, sticky traps (8.8 cm × 19.5 cm × 2.2 cm; Monitoring PP Trap #J, Kankyokiki Co., Ltd., Osaka, Japan) were placed every 50 m or so along the perimeter of invaded plots (Plots i–iii and I–III) and non-invaded, untreated plots (Plot n in Tokai and Plot N in Jonan; Fig 1b, c). The traps were laid once a month from April 2011 to March 2014 in invaded plots and from April (Jonan) or May (Tokai) 2013 to March 2014 in non-invaded plots and were collected after 3 days. Up to 108 trap points were set per month. Captured ants and non-ant invertebrates were then identified and counted in the laboratory. Ants were identified to species. The other invertebrates were identified to order, except for Myriapoda and land snails, and Coleoptera were identified to superfamily because of the variety of beetle feeding habits. We define all species except for *L. humile* as "non-target".

Numerical and statistical analyses

The effects of *L. humile* invasion and pesticide application on ants and non-ant invertebrates were statistically examined by two approaches. All analyses were conducted in R v. 3.1.1 software (R Development Core Team, 2013).

First, we used GLMs to examine the relationship of *L. humile* and pesticide treatment, and their interaction, with the total number of individuals and species or taxonomic richness (number of species or taxonomic groups) of non-targets captured by each trap (McCullagh & Nelder, 1989). We created models in which the response variables were the $\log_{10}(x + 1)$-transformed total number of individuals of non-target ant species or invertebrate taxonomic groups per trap or the integral number of non-target ant species or invertebrate taxonomic groups per trap. These models assumed a Gaussian distribution in the response variable and used an identity-link function. The explanatory variables were the $\log_{10}$(number of *L. humile* per trap), number of pesticide treatments in the past 6 months, their interaction, and site (dummy variable). We were not interested in seasonal change, and so we selected

the month with the largest number of individuals to avoid the effect of season. We therefore used the datasets of August 2011 and August 2013 in invaded plots (Plots i–iii and I–III) and that of August 2013 in non-invaded plots (Plots n and N). We previously confirmed that the number of *L. humile* workers was not correlated with the number of pesticide treatments ($R^2 = 0.06$). We also analyzed the relationships of the explanatory variables with the number of individuals of each non-target ant species or each invertebrate taxonomic group because those explanatory variables were associated with the total number of individuals and species richness in the above analyses. Zero-inflated Poisson regression models with the function *zeroinfl* from the *pscl* package (Jackman, 2017) were used to analyze the relationships, because count data of the number of individuals of each species often include many zero observations. The explanatory variables and dataset were the same as above. We then tested whether the zero-inflated Poisson regression model fit the data better than an ordinary Poisson regression model by applying the Vuong test (using the function *vuong* from the *pscl* package). We do not present results that could not be calculated owing to small sample sizes of species or taxonomic groups. We did not use Bonferroni's correction for multiple analyses because this would inflate the likelihood of a type II error. Instead, we used $p < 0.025$ for significance to decrease the likelihood of a type I error.

Second, to analyze the temporal dynamics of ant and non-ant invertebrate communities under pesticide treatment, we conducted PRC analyses (Van den Brink & Ter Braak, 1999) using the *vegan* package (Oksanen, 2013) of R. The PRC method, which is based on the redundancy analysis ordination technique, can compare the temporal dynamics of treated communities with an arbitrarily prescribed "control" community (Van den Brink & Ter Braak, 1999). We performed the analyses of non-target ant and invertebrate community dynamics in Plots i and ii in Tokai from April 2011 to March 2014 with data from Plot n (never invaded) as a control. In Plot i the pesticide was discontinued after about 1 year, whereas in Plot ii it was used for almost 3 years (Fig 2). Species abundance data were ln ($10x + 1$)-transformed to down-

weight high abundance values (Lepš & Šmilauer, 2003). The significance of the overall treatment effect was tested using 1000 permutations and the first eigenvalue. The resulting PRC diagram displays the regression coefficient (C_{dt}, left axis) of the first principal component in the community pattern at each site d at each time t compared with the control, whose C_{dt} is always zero by definition. An advantage of PRC analysis is that it can detect taxon-level effects. The right axis indicates the species (or taxon) weight (b_k). For a quantitative evaluation of PRC, the quotient $\exp (C_{dt} \times b_k)$ can be calculated for each species k at each site and each time. If the quotient is positive, species k is more abundant in the community than in the control. If it is negative, species k is less abundant. Therefore, species k is more abundant if b_k is on the same side of C_{dt} on the vertical axis and is less abundant if b_k is on the opposite side of C_{dt}. The greater the value of the quotient, the more different the abundance of species k is between treatment and control.

Results

The fauna

Table 1 shows the total numbers of ants and non-ant invertebrates caught by traps. In total, we collected 51,307 ants belonging to 35 species, including *L. humile*, and 41,324 non-target invertebrates. The last observations of *L. humile* were in December 2012 in Tokai and December 2013 in Jonan. In the treated plots (Plots i, ii, iii, I, II, and III), *L. humile* was eradicated by the pesticide, as demonstrated with a statistical model (Sakamoto et al., 2017). Although the initial density of *L. humile* and number of fipronil treatments differed among plots (Fig 2), the number of non-target ant individuals also decreased in the first year but started to recover after fipronil treatment ceased (Fig 3).

Table 1. Total numbers of each ant species and non-ant invertebrate taxonomic groups collected in monitoring traps at the Tokai and Jonan sites.

Taxa	Total	Tokai plots[1]				Jonan plots[1]			
		i	ii	iii	n	I	II	III	N
Ant species									
Dolichoderinae									
Linepithema humile	18628	74	1372	21	0	14626	218	2317	0
Ochetellus glaber	330	237	63	0	15	2	1	12	0
Technomyrmex gibbosus	8	0	5	0	0	2	0	1	0
Formicinae									
Camponotus japonicus	562	122	85	88	241	12	3	11	0
Camponotus vitiosus	212	105	26	17	3	26	6	21	8
Formica japonica	1750	546	227	137	180	13	5	216	426
Lasius japonicus	50	6	9	2	1	9	7	4	12
Lasius fuji	1	0	0	0	0	1	0	0	0
Lasius productus	13	0	1	0	0	0	12	0	0
Lasius sakagamii	18	4	0	7	0	4	1	2	0
Lasius umbratus	1	0	0	0	1	0	0	0	0
Nylanderia amia	284	108	3	37	1	17	3	2	113
Nylanderia flavipes	35	5	1	8	10	5	6	0	0
Paraparatrechina sakurae	1911	11	465	471	47	311	259	339	8
Paratrechina longicornis	8	0	8	0	0	0	0	0	0
Ponerinae									
Brachyponera chinensis	1565	320	16	234	12	10	914	39	20
Hypoponera opaciceps	1	0	0	0	0	0	0	1	0
Myrmicinae									
Aphaenogaster osimensis	2	2	0	0	0	0	0	0	0
Crematogaster matsumurai	2145	685	31	1185	156	4	6	38	40
Crematogaster osakensis	84	0	2	0	66	13	3	0	0
Crematogaster teranishii	5	0	0	0	5	0	0	0	0
Crematogaster vagula	7	0	0	0	7	0	0	0	0
Myrmica kotokui	7	1	6	0	0	0	0	0	0
Monomorium chinense	26	5	21	0	0	0	0	0	0
Pheidole indica	1	1	0	0	0	0	0	0	0

Table 1. Total numbers of each ant species and non-ant invertebrate taxonomic groups collected in monitoring traps at the Tokai and Jonan sites. (Continuation)

Taxa	Total	Tokai plots[1]				Jonan plots[1]			
		i	ii	iii	n	I	II	III	N
Myrmicinae									
Pheidole noda	1135	31	209	0	889	2	1	0	3
Pristomyrmex punctatus	3339	41	246	112	173	32	3	2704	28
Pyramica membranifera	1	0	1	0	0	0	0	0	0
Solenopsis japonica	32	0	18	7	0	1	1	5	0
Strumigenys lewisi	7	0	0	6	0	1	0	0	0
Temnothorax anira	1	1	0	0	0	0	0	0	0
Temnothorax congruus	190	66	45	24	0	14	13	25	3
Temnothorax spinosior	5	3	2	0	0	0	0	0	0
Tetramorium bicarinatum	4	0	0	0	0	0	4	0	0
Tetramorium tsushimae	18939	1558	3974	2286	401	568	2151	6215	1786
Total	51307	3932	6836	4642	2208	15673	3617	11952	2447
Non-ant invertebrates (common name[2])									
Isopoda (sowbugs)	33389	1452	9084	9669	507	5442	1571	5600	64
Myriapoda (centipedes/millipedes)	1343	171	155	581	8	103	122	184	19
Araneae (spiders)	1242	193	198	297	24	169	181	165	15
Orthoptera (grasshoppers)	239	25	35	28	9	73	13	44	12
Dermaptera (earwigs)	1708	338	78	355	149	121	315	340	12
Blattodea (cockroaches)	113	31	23	8	13	14	7	4	13
Mantodea (mantis)	1	0	0	0	0	1	0	0	0
Hemiptera (bugs)	820	89	96	306	19	94	100	107	9
Coleoptera (beetles)									
Byrrhoidea (pill beetles)	13	2	0	1	0	1	9	0	0
Cantharoidea (soldier beetles)	3	0	1	0	0	0	1	1	0
Caraboidea (ground beetles)	750	70	51	106	7	106	343	61	6
Chrysomeloidea (longhorn beetles)	62	7	10	19	0	4	10	9	3
Cucujoidea (darkling beetles)	326	67	84	81	7	11	50	24	2
Curculionoidea (weevils)	618	63	89	107	6	61	70	212	10
Dermestoidea (carpet beetles)	8	1	1	0	0	2	1	3	0
Elateroidea (click beetles)	107	10	11	50	5	4	16	9	2
Scarabaeoidea (gold beetles)	258	61	50	30	14	14	37	48	4
Staphylinoidea (rove beetles)	80	18	6	11	0	2	14	27	2
Land snails	244	5	74	56	25	14	21	46	3
Total	41324	2603	10046	11705	793	6236	2881	6884	176

[1] Plots i–iii and I–III were invaded by *Linepithema humile, and plots n and N were untreated, non-invaded plots.* [2] Representative example.

Effects of *L. humile* and pesticide on non-target species

Both *L. humile* abundance and fipronil treatment had negative associations with total number of ant individuals, ant species richness, total number of non-ant invertebrates, and non-ant invertebrate taxonomic richness (Table 2). The *L. humile* × pesticide treatment interaction was significantly associated with total number of ant individuals. Total number of non-ant invertebrates and taxonomic richness were affected by sites

Table 3 shows the relationships between *L. humile* abundance and fipronil treatment and the number of individuals in each ant species or each non-ant taxonomic group. Modeling the data with zero-inflated Poisson regression fit significantly better than ($p < 0.025$) or did not differ from the ordinary Poisson regression model. In the majority of the species/ taxonomic groups, both *L. humile* and pesticide treatment were not significant in the zero-inflated part of the model. In the Poisson part, the abundance of *L. humile* was negatively associated with the number of *Pristomyrmex punctatus* and
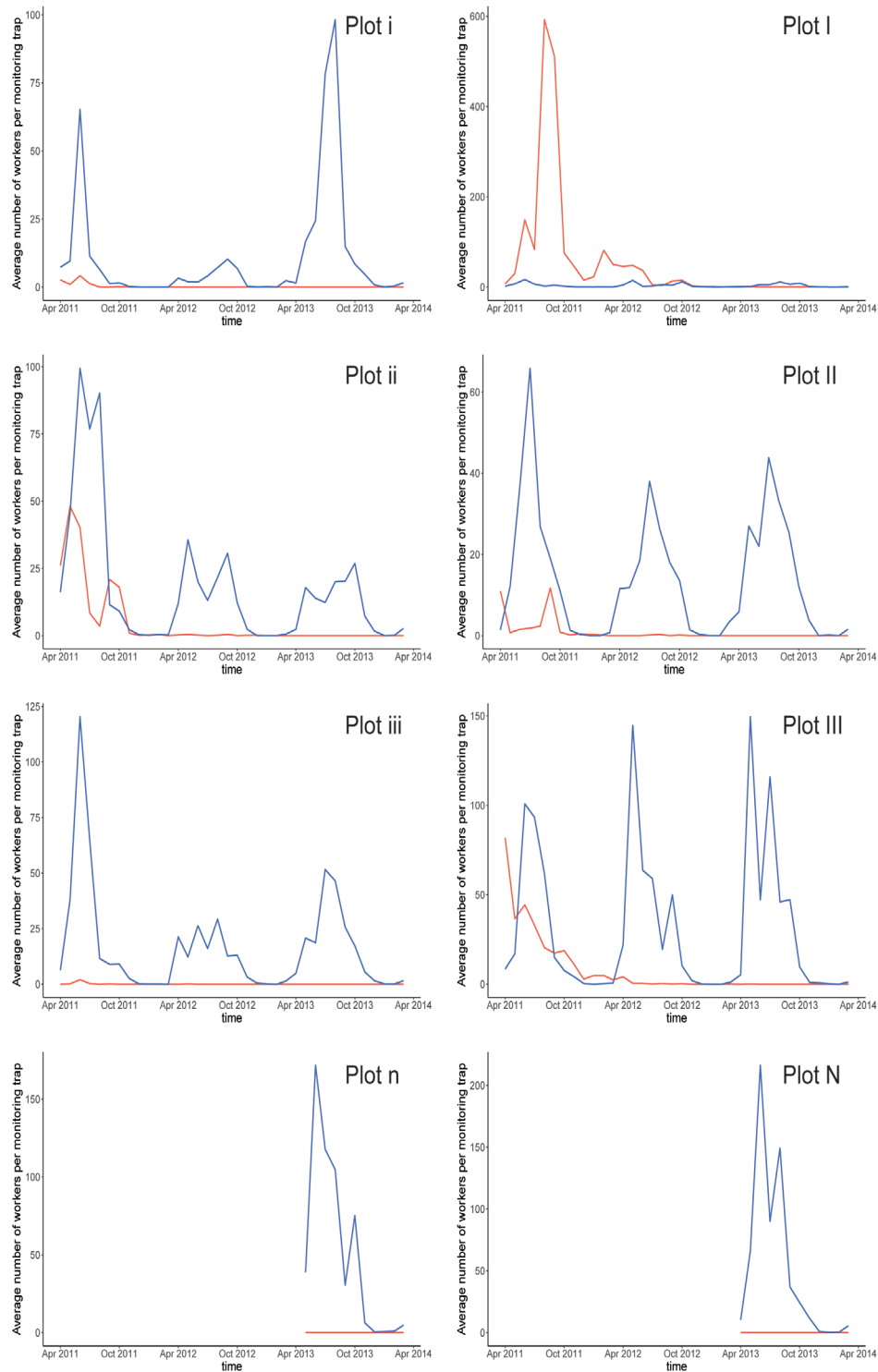
Fig 3. Mean total number of *Linepithema humile* (red line) and non-target ants (blue line) per trap by month in plots invaded by *L. humile* (i–iii, I–III) and untreated, non-invaded plots (n, N).

Tetramorium tsushimae individuals, whereas the number of pesticide treatments was negatively associated with the number *Formica japonica*, *Paraparatrechina sakurae*, *Pheidole noda*, *P. punctatus*, and *T. tsushimae* individuals. The interaction between the two variables was significantly associated with the number of *P. punctatus* and *T. tsushimae*. Among the non-ant invertebrate groups, Isopoda had negative relationships with both *L. humile* and fipronil treatment and

Blattodea had a negative relationship with pesticide treatment. Some species and taxonomic groups were affected by site.

Community dynamics during chemical control and after eradication of L. humile

The dynamics of non-target ant (Fig 4) and non-ant invertebrate communities (Fig 5) differed between pesticide usage histories. The deviations from the control were larger

from spring to fall (March–November) and smaller in winter (December–February). The structures of non-target ant communities in Plots i and ii clearly deviated from that in the non-invaded (control) plot (Fig 4). The ant community structure in 3-year-treated Plot ii responded to the treatment, in which the deviation from the control was larger in the second and third years than in the first year (Fig 4a), whereas that in 1-year-treated Plot i initially deviated from that in the non-invaded plot but was more similar in the third year (Fig 4b). These results indicate that the ant community structure recovered about a year after the eradication program ended. The structures of non-ant invertebrate communities in Plots i and ii did not deviate so clearly from that in the non-invaded (control) plot. The community structure in the 3-year-treated plot changed unidirectionally (i.e., Isopoda decreased) with time (Fig 5a), whereas that in the 1-year-treated plot did not respond clearly (Fig 5b).
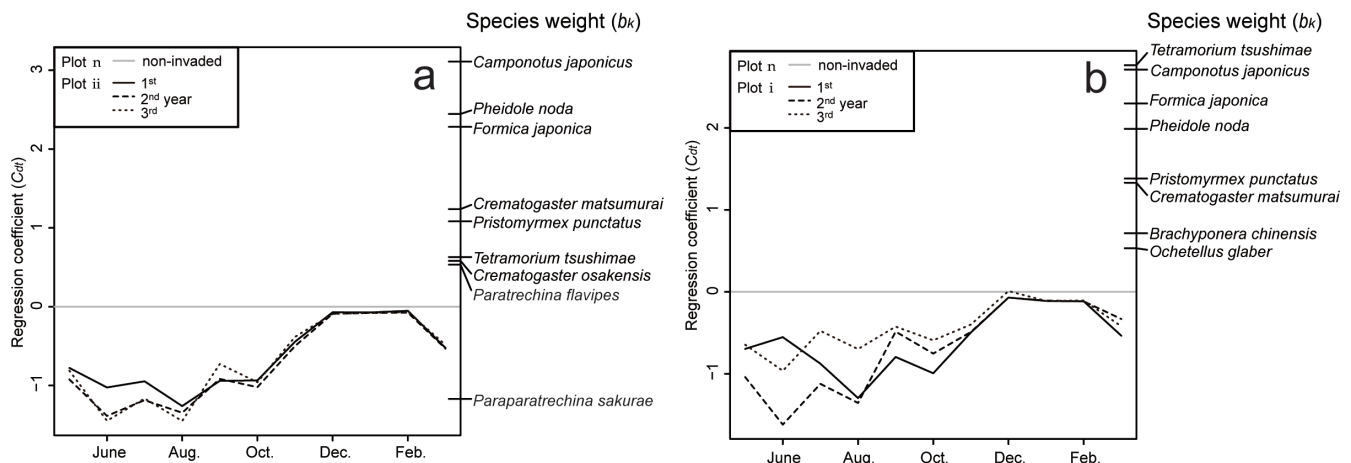

Fig 4. Principal response curve diagrams illustrating the shift of non-target ant communities over 3 years. (a) Plot ii, where no pesticide was applied after October 2013, and (b) Plot i, where no pesticide was applied after May 2012, relative to the control (untreated and non-invaded) plot over time. Left axis, regression coefficient; right axis, species weights (only species with a score of >0.5 or <−0.5 are shown). The first canonical axis explains 56.63% ($p < 0.001$) of the total variation in Plot ii and 50.96% ($p < 0.001$) in Plot i.
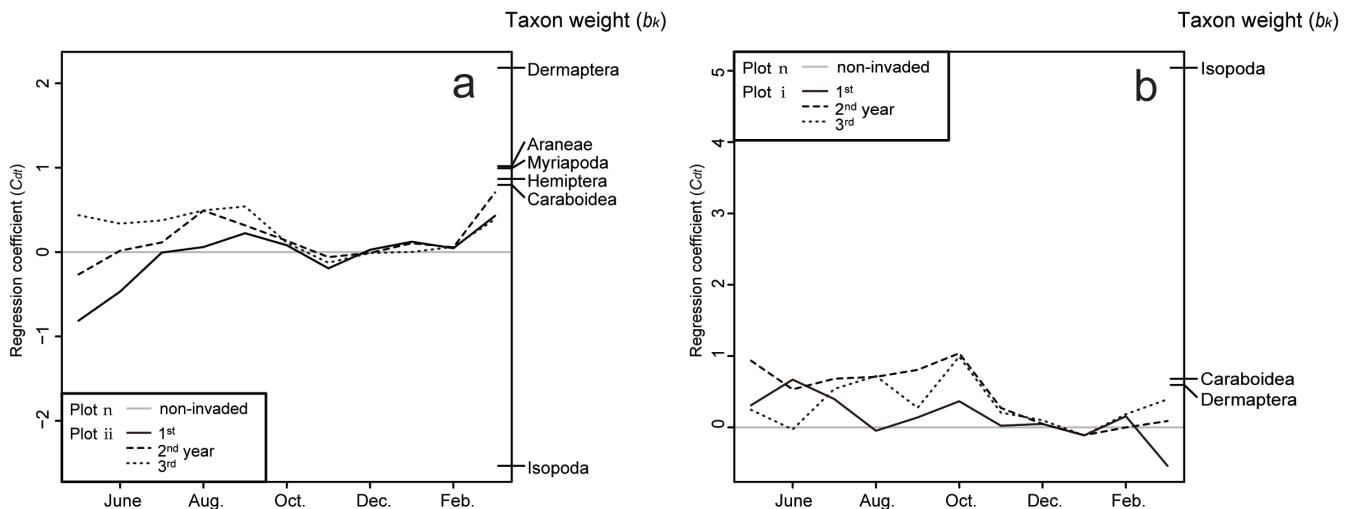

Fig 5. Principal response curve diagrams illustrating the shift of non-ant invertebrate communities over 3 years. (a) Plot ii, where no pesticide was applied after October 2013, and (b) Plot i, where no pesticide was applied after May 2012, relative to the control (untreated and non-invaded) plot over time. Left axis, regression coefficient; right axis, taxon weights (only taxa with a score of >0.5 or <−0.5 are shown). The first canonical axis explains 29.72% ($p < 0.001$) of the total variation in Plot ii and 62.97% ($p < 0.001$) in Plot i.

Discussion

The number of *L. humile* was negatively associated with the total number of non-target ant individuals and species richness. As reported elsewhere in Japan (Miyake et al., 2002), in the USA (Heller, 2004; Suarez et al., 1998), and in Europe (Oliveras et al., 2005), *L. humile* reduces the diversity of indigenous ants. Therefore, it is reasonable to conclude that the invasion by *L. humile* harmed non-target ants in our study area as well. Our GLM results for each ant species indicated decreases in *P. punctatus* and *T. tsushimae* abundance. The incidence of *T. tsushimae* was also reported to be clearly lower where *L. humile* had increased over time in several parks in Japan (Park et al., 2014). Two mechanisms have been proposed to explain the displacement of indigenous ant fauna by *L. humile* invasion: exploitative competition and interference

Table 2. Results of generalized linear models examining the effects of *Linepithema humile*, pesticide treatment, their interaction, and site on total number of individuals and species or taxonomic richness per trap.

Variable	Estimate	SE	z-value	p-value	
Ant species					
Total number of individuals					
Intercept	1.911				
Linepithema humile	−0.651	0.101	−6.443	<0.001	*
Pesticide treatment	−0.127	0.027	−4.650	<0.001	*
LH × PT[1]	0.137	0.036	3.850	<0.001	*
Site	−0.262	0.126	−2.080	0.040	
Species richness					
Intercept	4.155				
Linepithema humile	−1.205	0.259	−4.654	<0.001	*
Pesticide treatment	−0.326	0.070	−4.640	<0.001	*
LH × PT[1]	0.161	0.091	1.762	0.081	
Site	0.300	0.324	0.927	0.356	
Non-ant invertebrates					
Total number of individuals					
Intercept	2.202				
Linepithema humile	−0.499	0.080	−6.205	<0.001	*
Pesticide treatment	−0.099	0.022	−4.539	<0.001	*
LH × PT[1]	0.071	0.028	2.513	0.014	*
Site	−0.333	0.101	−3.307	0.001	*
Taxonomic richness					
Intercept	5.892				
Linepithema humile	−1.143	0.283	−4.044	<0.001	*
Pesticide treatment	−0.182	0.077	−2.367	0.020	*
LH × PT[1]	0.022	0.100	0.216	0.829	
Site	−1.724	0.353	−4.881	<0.001	*

[1] *Linepithema humile × pesticide treatment interaction.* $* p < 0.025$

competition (Holway, 1999; Human & Gordon, 1996). However, although negative associations were reported between several other ant species (i.e., *F. japonica* and *Crematogaster matsumurai*) and *L. humile* in previous studies (Miyake et al., 2002; Park et al., 2014), no associations were observed between them in this study. The difference in results may be due in part to seasonal or temporal factors and/or small sample sizes.

Likewise, the number of *L. humile* was negatively associated with the total number of non-ant individuals and taxonomic richness. The analysis for each taxonomic group, however, showed that *L. humile* had negative associations with abundance of only isopods. Almost all isopods we found were *Armadillidium vulgare*, which can reproduce in urban areas (Hornung et al., 2007). *Linepithema humile* has been reported to cause both significant decreases (Stanley & Ward, 2012) and increases in isopod abundance (Cole et al., 1992; Human & Gordon, 1997; Walters & Mackay, 2003). The impacts of

L. humile on other invertebrate taxonomic groups are also not universal, and various studies have reported negative, positive, or no relationship. Factors underlying negative relationships may include direct feeding by *L. humile* on adult or immature organisms or spatial competition for limited habitats (Cole et al., 1992; Dreistadt et al., 1986), while factors underlying positive relationships may include feeding by invertebrates on dead and immature *L. humile* individuals or on the remains of prey items brought to the nest area by foraging *L. humile* (Cole et al., 1992).

Negative effects of pesticide treatment were found in ants as well as in non-ant invertebrates. The greatest factor underlying the negative effects is that toxic baits are typically attractive to a wide range of non-target species (Buczkowski, 2017). Our GLM analyses showed that the *L. humile* × pesticide treatment interaction also affected the total number of ants and non-ant invertebrates. This result can be interpreted in two ways, based on different biological scenarios. First, the effect of *L. humile* on indigenous invertebrates decreases when there is pesticide treatment, which kills the invasive ants. Second, the effect of pesticide treatment on non-target fauna may decrease when the *L. humile* population is large and it may increase when the population is small. This idea reflects our observations and those of previous studies (Abedrabbo, 1994; Hoffmann & O'Connor, 2004) that the invasive alien ants were eradicated first and non-target ants were not eradicated. This likely occurred because non-target fauna was deprived of opportunities to eat the bait because of greater consumption by *L. humile* (Buczkowski & Bennett, 2008; Hoffmann, 2010; Holway, 1999; Human & Gordon, 1996).

We compared the PRC results for 3 years in this study with those for only the first year in the previous study (Inoue et al., 2015) in the same eradication program. It should be noted that the plots with low-density *L. humile* were selected for PRC analyses and the control plot was untreated and never invaded in this study, whereas the plots with high-density *L. humile* were selected and the control plot was untreated but invaded in the previous study (Inoue et al., 2015). Inoue et al. (2015) concluded that non-target populations recovered within the first year of pesticide treatment, and they found no non-target effects of pesticide in the first year, we suspect because the number of *L. humile* was large. By contrast, our PRC results showed that the non-target community structure recovered about a year after the eradication program ended. That is, until *L. humile* was eradicated, the negative effects of pesticides on non-targets increased as *L. humile* decreased, suggesting that non-target effects cannot be ignored.

Ensuring that indigenous ants and other invertebrates remain after pesticide treatment is crucial for ecosystem recovery after the eradication of invasive species. In fact, the invertebrate community recovered to a similar structure as in the non-invaded plot in this study. Such recovery eventually can be achieved by the continuous recruitment of immigrants of indigenous species from non-invaded sites (Holway, 1998).

However, our data revealed temporary negative effects of pesticides on non-target communities. It is also important to reduce the impacts on indigenous communities, because their restoration inhibits successful re-invasion into the ecological gap by invasive alien species (Hoffmann, 2010; Hoffmann & O'Connor, 2004; Plentovich et al., 2009; Tschinkel & King, 2017). When an eradication program comes close to achieving success, the end should be judged by using a statistical model (Sakamoto et al., 2017), for example, so as to avoid the unnecessary prolongation of pesticide treatment. Moreover, in delicate infested habitats and in the presence of sensitive wildlife, traditional eradication methods with toxic baits may be inappropriate. To reduce the impacts on non-target fauna, target-specific approaches should be developed, such as using ribonucleic acid interference (Campbell et al., 2015; Gould, 2008) or prey-baiting (Buczkowski, 2017).

Table 3. Results of zero-inflated Poisson regression models examining the effects of *Linepithema humile*, pesticide treatment, their interaction, and site on the number of individuals of each ant species or non-ant invertebrate taxonomic group per trap.

Species/Taxonomic group[1]	Variable	Poisson model					Zero-inflated model				
		Estimate	SE	z-value	p-value		Estimate	SE	z-value	p-value	
Ants											
Formica japonica	*Linepithema humile*	−38.02	NA	NA	NA		−89.79	448.9	−0.200	0.842	
	Pesticide treatment	−0.226	0.023	−10.03	<0.001	*	0.253	0.107	2.377	0.018	*
	LH × PT[3]	7.462	NA	NA	NA		18.20	89.78	0.203	0.839	
	Site	−0.665	0.107	−6.194	<0.001	*	−0.33		−0.687	0.492	
Paraparatrechina sakurae	*Linepithema humile*	−24.22	NA	NA	NA		−108.3	379.9	−0.285	0.775	
	Pesticide treatment	−0.195	0.050	−3.885	<0.001	*	−0.308	0.126	−2.443	0.015	*
	LH × PT[3]	4.96	NA	NA	NA		21.43	75.97	0.282	0.778	
	Site	0.02	0.230	0.099	0.921		−0.34	0.51	−0.655	0.513	
Pheidole noda	*Linepithema humile*	−30.00	443.9	−0.068	0.946		−235.9	541.5	−0.436	0.663	
	Pesticide treatment	−1.419	0.143	−9.92	<0.001	*	−18.84	NA	NA	NA	
	LH × PT[3]	9.967	88.77	0.112	0.911		107.7	NA	NA	NA	
	Site	−62.84	995.6	−0.063	0.950		−520.2	1201	−0.433	0.665	
Pristomyrmex punctatus	*Linepithema humile*	−2.333	0.825	−2.827	0.005	*	−1.4755	1.608	−0.918	0.359	
	Pesticide treatment	−0.769	0.234	−3.284	0.001	*	−0.404	0.482	−0.838	0.402	
	LH × PT[3]	0.757	0.193	3.929	<0.001	*	0.493	0.365	1.352	0.176	
	Site	−3.100	1.197	−2.591	<0.001	*	−2.233	2.507	−0.891	0.373	
Solenopsis japonica	*Linepithema humile*	−50.56	6945	−0.007	0.994		58.39	1001000	0.000	1.000	
	Pesticide treatment	−9.585	387.2	−0.025	0.980		−7.570	NA	NA	NA	
	LH × PT[3]	10.50	1389	0.008	0.994		−11.82	200100	0.000	1.000	
	Site	4.128	0.985	4.192	<0.001	*	7.438	19	0.383	0.702	
Tetramorium tsushimae	*Linepithema humile*	−1.240	0.155	−8.022	<0.001	*	1.502	0.524	2.866	0.004	*
	Pesticide treatment	−0.116	0.008	−13.91	<0.001	*	0.164	0.135	1.214	0.225	
	LH × PT[3]	0.303	0.032	9.509	<0.001	*	−0.298	0.166	−1.797	0.072	
	Site	−0.480	0.042	−11.54	<0.001	*	0.568	0.569	0.999	0.318	
Non-ant invertebrates											
Isopoda (sowbugs[2])	*Linepithema humile*	−0.996	0.082	−12.09	<0.001	*	0.637	0.575	1.109	0.268	
	Pesticide treatment	−0.140	0.010	−13.63	<0.001	*	0.274	0.159	1.729	0.084	
	LH × PT[3]	−0.081	0.031	−2.581	0.010	*	0.059	0.175	0.340	0.734	
	Site	−0.292	0.054	−5.447	<0.001	*	1.497	0.657	2.279	0.023	*
Araneae (spiders[2])	*Linepithema humile*	−0.355	0.985	−0.360	0.719		2.042	1.690	1.208	0.227	
	Pesticide treatment	0.155	0.080	1.949	0.051		0.242	0.381	0.634	0.526	
	LH × PT[3]	0.055	0.200	0.275	0.784		−0.359	0.351	−1.023	0.306	
	Site	−0.084	0.252	−0.333	0.739		1.953	1.048	1.864	0.062	
Orthoptera (grasshoppers[2])	*Linepithema humile*	0.070	0.280	0.249	0.804		−10.785	47406	0.000	1.000	
	Pesticide treatment	−0.092	0.109	−0.839	0.401		0.336	0.198	1.699	0.089	
	LH × PT[3]	1.068	0.648	1.648	0.099		22.274	142.6	0.156	0.876	
	Site	1.016	0.572	1.775	0.076		31.430	14269	0.002	0.998	

Table 3. Results of zero-inflated Poisson regression models examining the effects of *Linepithema humile*, pesticide treatment, their interaction, and site on the number of individuals of each ant species or non-ant invertebrate taxonomic group per trap. (Continuation)

Species/Taxonomic group[1]	Variable	Poisson model					Zero-inflated model			
		Estimate	SE	z-value	p-value		Estimate	SE	z-value	p-value
Blattodea (cockroaches[2])	*Linepithema humile*	–0.496	0.376	–1.318	0.188		–51.85	2490	–0.021	0.983
	Pesticide treatment	–0.353	0.141	–2.508	0.012	*	–0.054	0.276	–0.197	0.844
	LH × PT[3]	1.744	0.707	2.467	0.014	*	23.71	146.9	0.161	0.872
	Site	0.842	0.653	1.290	0.197		20.75	716.3	0.029	0.977
Hemiptera (bugs[2])	*Linepithema humile*	–0.687	0.379	–1.813	0.070		0.215	1.095	0.196	0.845
	Pesticide treatment	–0.042	0.045	–0.925	0.355		0.187	0.187	1.001	0.317
	LH × PT[3]	–0.051	0.115	–0.442	0.658		–0.034	0.289	–0.117	0.907
	Site	–0.896	0.241	–3.723	<0.001	*	–0.596	0.891	–0.670	0.503
Caraboidea (ground beetles[2])	*Linepithema humile*	0.053	0.268	0.196	0.844		–1.927	3502	–0.001	1.000
	Pesticide treatment	–0.071	0.100	–0.708	0.479		0.190	0.200	0.948	0.343
	LH × PT[3]	–0.305	0.186	–1.642	0.101		–0.323	700.4	0.000	1.000
	Site	0.802	0.499	1.608	0.108		14.747	617.5	0.024	0.981
Cucujoidea (darkling beetles[2])	*Linepithema humile*	–0.595	0.616	–0.965	0.335		2.921	4624	0.001	0.999
	Pesticide treatment	0.287	0.183	1.563	0.118		10.39	116.8	0.089	0.929
	LH × PT[3]	0.100	0.166	0.604	0.546		–0.710	924.8	–0.001	0.999
	Site	–2.167	1.270	–1.706	0.088		0.616	2.0	0.308	0.758
Curculionoidea (weevils[2])	*Linepithema humile*	0.787	0.575	1.370	0.171		2.122	1.842	1.152	0.249
	Pesticide treatment	0.002	0.184	0.013	0.989		0.05	0.35	0.13	0.896
	LH × PT[3]	–0.327	0.211	–1.550	0.121		–8.343	117.7	–0.071	0.943
	Site	0.808	0.721	1.120	0.263		5.612	4.35	1.291	0.197
Elateroidea (click beetles[2])	*Linepithema humile*	–15.68	NA	NA	NA		–0.442	25990	0.000	1.000
	Pesticide treatment	41.77	NA	NA	NA		12.12	393	0.031	0.975
	LH × PT[3]	3.650	NA	NA	NA		0.490	5198	0.000	1.000
	Site	–3.188	0.920	–3.464	0.001	*	–30.93	1661000	0.000	1.000
Scarabaeoidea (gold beetles[2])	*Linepithema humile*	0.143	0.336	0.426	0.670		0.050	0.723	0.069	0.945
	Pesticide treatment	–0.030	0.168	–0.178	0.858		–0.037	0.391	–0.096	0.924
	LH × PT[3]	–0.207	0.173	–1.195	0.232		–0.416	0.639	–0.650	0.515
	Site	–0.763	0.769	–0.993	0.321		–1.932	2.128	–0.908	0.364

[1] Species or taxonomic groups that could not be calculated because of insufficient data are not shown.

[2] Representative example

[3] *Linepithema humile* × pesticide treatment interaction

* *p < 0.025*

Acknowledgments

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