Long-term record of Argentine ant invasions reveals enduring ecological impacts

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Abstract. The ecological effects of species introductions can change in magnitude over time, but an understanding of how and why they do so remains incompletely understood. Clarifying this issue requires consideration of how temporal variation in invader traits affects invasion impacts (e.g., through differential effects on the diversity and composition of native species assemblages). We examine the temporal dynamics of Argentine ant invasions in northern California by resurveying 202 sites first sampled 30–40 yr ago. To test how invasion impacts change over time, we estimated native ant richness and species composition at 20 riparian woodland sites that span a 30-yr invasion chronosequence. We then use these data to test how variation in two invader traits (aggression and relative abundance) is related to time since invasion and invasion impact. Native ant assemblages along the chronosequence exhibited reduced native ant richness and altered species composition (compared to uninvaded control sites), but the magnitude of these impacts was independent of time since invasion. These results are corroborated by additional temporal comparisons of native ant assemblages at riparian sites sampled 20–30 yr ago. Our findings together illustrate that the impacts of invasions can persist undiminished over at least a 30-yr time frame and remain evident at regional scales. Although neither invader trait varied with time since invasion, native ant richness declined as the relative abundance of the Argentine ant increased. This latter result supports the hypothesis that factors reducing invader abundance at particular sites can decrease invasion impacts, but also that such changes may be due to site-specific factors (e.g., abiotic conditions) that affect invader abundance rather than time since invasion per se. Future studies should attempt to differentiate factors that are intrinsic to the process of invasion (e.g., changes in invader populations) from long-term environmental changes (e.g., climate change) that represent extrinsic influences on the dynamics of invasion.

Key words: California; Formicidae; historical resurvey; introduced species; invasion; Linepithema humile; *long-term studies; riparian ecosystems.*

Introduction

The ecological effects of biological invasions are widely acknowledged (Ricciardi et al. 2013, Simberloff et al. 2013). Introduced species can reduce native diversity, alter the composition of native species assemblages, and compromise ecosystem services (Ricciardi et al. 2013). While ecologists increasingly recognize that these types of invasion impacts can change in magnitude over time (Strayer 2012, Ricciardi et al. 2013), recent studies also illustrate that the effects of invasions can remain evident for many years (Sharpe et al. 2017). Understanding when and why invasion impacts change with time clearly deserves attention, but surprisingly few ecological data sets span sufficient temporal and spatial scales to provide definitive examples (Strayer et al. 2006, Strayer 2012, Doody et al. 2017). Moreover, only a handful of studies address how intrinsic, population-level changes in invader taxa act to alter invasion impacts as a function of time since invasion (Dostal et al. 2013, Yelenik and D'Antonio 2013, Flory and D'Antonio 2015).

Invasion impacts can increase or decrease in magnitude over time. Introduced species that exhibit lag times in population density between establishment and the achievement of peak abundance might cause minor ecological impacts when invader densities are low but more noticeable impacts as densities increase (Crooks 2005). Impacts that increase over time, however, do not necessarily require changes in invader populations. Environmental stressors (e.g., agents of disturbance) or environmental changes (e.g., physical conditions, nutrient availability), that favor introduced species over native species, for example, can result in a gradual but long-term ratcheting down of native diversity (Strayer et al. 2006, Yelenik and D'Antonio 2013). Invasion impacts may also diminish with time (Simberloff and Gibbons 2004, Strayer et al. 2006, Strayer 2012). Declines in impact could hypothetically result from (1) members of a recipient community that ecologically or evolutionarily respond to invasion, (2) invader populations that accumulate natural enemies (e.g., parasites, pathogens) or that undergo evolutionarily change in ways that reduce their ecological impact, or (3) changes in the abiotic environment that disproportionately and negatively affect introduced species compared to natives (Strayer et al. 2006, Ricciardi et al. 2013, Flory and D'Antonio 2015).

Ant invasions provide examples of introduced species that experience population declines over time (Lester and Gruber 2016). Recent studies, for example, have documented range contractions for some populations of introduced ants (Cooling et al. 2011, Cooling and Hoffmann 2015). Temporal

declines in invader abundance may also result in attenuated ecological effects. Morrison (2002), for example, resampled ant assemblages at a site in central Texas, USA, that was sampled 12 years earlier by Porter and Savignano (1990) and found that, in the interval of time separating the two surveys, densities of red imported fire ants decreased and native ant richness increased. Studies such as those of Morrison (2002) are sometimes interpreted (e.g., Strayer et al. 2006) as evidence that the effects of invasions tend to be greatest early on and then diminish with time as invader populations decline, but it should be noted that few such studies exist in general (Strayer 2012) and those that address ant invasions (Morrison 2002, Heller et al. 2008) were conducted at single locations. Although long-term studies conducted at one site contribute to an understanding of how invasion impacts can vary over time, larger scale studies are required to clarify whether or not observed changes represent geographically widespread phenomena.

We focus here on Argentine ant (*Linepithema humile*) invasions in northern California. Native to southern South America (Wild 2004), *L. humile* has become a widespread, abundant, and damaging invader over the past century (Holway et al. 2002). The Argentine ant was first found in California as early as 1905 (Smith 1936) and is now widely distributed in the state, especially along the coast and in the Sacramento Valley (Tremper 1976, Ward 1987, Suarez et al. 1998). Natural areas invaded by *L. humile* in California support fewer native ant species (Tremper 1976, Ward 1987, Human and Gordon 1996, Holway 1998a, b, Suarez et al. 1998, Sanders et al. 2001, Holway 2005, Menke and Holway 2006, Mitrovich et al. 2010, Hanna et al. 2015). Moreover, multi-year studies conducted in different parts of California document the active displacement of native ants as Argentine ant invasion fronts advance across formerly uninvaded areas (Erickson 1971, Holway 1998b, Sanders et al. 2001, Tillberg et al. 2007). Invader traits that contribute to the Argentine ant's ability to exclude native ants include numerical advantages and aggression. Argentine ant workers are smaller than workers of many of the native ant species that they displace, and field studies indicate that numerical advantages over natives increase multiple measures of competitive performance, such as resource discovery, recruitment potential, and interference ability (Human and Gordon 1996, 1999, Holway 1999). Lab studies further reveal evidence for increasing colony size enhancing interspecific aggression (Sagata and Lester 2009) and interference ability (Holway and Case 2001). Increases in Argentine ant abundance over time might thus result in decreased native ant diversity. The Argentine ant also fights with a variety of native ant species (Holway 1999, Human and Gordon 1999) and increases its level of aggression towards familiar adversaries (Thomas et al. 2005, 2007). One might thus expect aggression towards other ants to decrease with time since invasion if native ant abundance (and the likelihood of encountering heterospecifics) declines as a function of invasion history. Given the importance of these invader traits, site-level variation in Argentine ant aggression or relative abundance may be related to the magnitude of invasion impacts.

In this study, we combine regional-scale, historical resurvey data and replicated, contemporary sampling along an invasion chronosequence to test how and why ecological impacts associated with Argentine ant invasions change over a multi-decade time period. To quantify long-term changes in the distribution of *L. humile*, we resurveyed 202 sites in northern California first sampled 30–40 yr ago (Tremper 1976, Ward 1987) and then again about 20 yr ago (Holway 1995). Using this historical data set, we established an invasion chronosequence that consisted of 20 riparian woodland sites distributed across the Sacramento Valley (from Ward 1987) that have been invaded for different lengths of time over the past 30 yr. To determine how invasion impacts change over time, we sampled native ant assemblages along the invasion chronosequence to test whether or not sites invaded for different lengths of time differ with respect to richness and species composition. As an additional test of how invasion impacts vary with time, we compare the results of contemporary sampling with the results of previous studies (Ward 1987, Holway 1998a) on Argentine ant invasions conducted in the same riparian woodland ecosystems considered in this study. If invader impacts do vary with time, then changes in invader traits might underlie such variation. To clarify why invasion impacts might vary over time, we thus use the chronosequence data to test (1) how Argentine ant aggression and relative abundance change with time since invasion and (2) how variation in invader impact (native ant richness) relates to invader traits. Our study permits an unprecedented test of how and why multiple impacts of ant invasions change over a multi-decadal time frame, and whether or not such changes manifest themselves at a regional scale.

METHODS

Historical resurvey

We conducted a historical resurvey to quantify long-term changes in the distribution of the Argentine ant. In May and June of 2014, we resurveyed 86 riparian woodland sites in the Sacramento River valley (Fig. 1) first sampled in 1984–1985 (Ward 1987) and then again in 1993 (Holway 1995). In May and June of 2015, we resurveyed 116 sites in varied habitats along an east-west transect extending from the lower San Joaquin River to the coast south of San Francisco (Appendix S1) first sampled in 1974 (Tremper 1976) and then again in 1993 (Holway 1995). Geographic precision is an essential component of any historical resurvey (Tingley and Beissinger 2009), and the original surveys were conducted prior to the widespread use of GPS technology. Based on the location information and habitat descriptions in Tremper (1976) and details in our own field notes (P. S. Ward, D. A. Holway), however, we were able to locate 96% (194/202) of the sites without ambiguity. At all sites, we looked for ants in the immediate vicinity of each stated location. The precise location of eight sites remained unclear to within 100 m, and in these cases we checked for ants within a radius of ~100 m of where we believed each site was located. In all eight cases of location uncertainty, the invasion status (i.e., invaded or uninvaded) of each of the areas surveyed was consistent with that observed in the previous survey (Holway 1995) and did not vary within the area surveyed. During site visits in 2014 and 2015, we recorded GPS

coordinates and habitat type at all sites (Data S1-localities.csv). Careful inspection of field notes (of D. A. Holway) revealed a slight error in Holway (1995); that study reported 106 Argentine ant presences across total 201 sites, whereas the correct total should be 109 presences across 202 total sites. Data S1-localities.csv provides the correct information.

To determine the presence or absence of the Argentine ant, we emulated approaches used in earlier surveys (Tremper 1976, Ward 1987, Holway 1995), all of which employed similar methods. Appendix S2 provides excerpts from each of the three studies that describe methods used to determine Argentine ant presence or absence at individual sites. At each site we visually searched for ants by examining bare ground, vegetation (especially tree trunks), and under stones and logs. If the Argentine ant was detected, that site was classified as invaded. We classified sites as uninvaded if we did not detect the Argentine ant but did detect common native ant species (e.g., *Formica moki*, *Liometopum occidentale*, or *Tapinoma sessile*) that do not typically co-occur with *L. humile* (Ward 1987, Holway 1995, 1998a). For the small subset of sites where the Argentine ant was recorded in earlier surveys but not during our initial resurvey visits in 2014–2015, we conducted repeated surveys over a two-year period to clarify to the fullest extent possible whether or not the Argentine ant was indeed absent. Documentation of apparent absences represents a general challenge in historical resurveys, and repeated, site-level sampling serves as an objective and recommended method to cope with this challenge (Tingley and Beissinger 2009). Our follow-up surveys at each of these sites included baiting and visual surveys within ~100 m of the sites. In 2014 and 2015, we returned in April or May to conduct a second visual survey (i.e., a follow-up to the initial resurvey visit) and to bait ants in the vicinity of each site with 20–30 cotton balls soaked in a sucrose solution. These baits were placed >10 m apart in areas potentially attractive to the Argentine ant and left out for at least 4 h. If the Argentine ant was not detected during this second visit, we returned again in April or May of 2016 to conduct a third visual survey and to perform additional baiting. In 2016, we baited ants with 20 cookie (pecan sandies; Keebler(R) Sandies(R) Pecan Shortbread Cookies; Kellogg NA Co., Battle Creek, MI, USA) baits per site. Baits were placed >10 m apart in areas potentially attractive to the Argentine ant and left out for at least 2 h. If we failed to detect the Argentine ant during all follow-up visits, then we classified these sites as apparent losses.

Invasion chronosequence

We used the historical resurvey to establish an invasion chronosequence (see also Dostal et al. 2013) that consisted of 20 riparian woodland sites (from Ward 1987) distributed across the lower Sacramento Valley. Sites were distributed among five spatial blocks; each block was confined to an
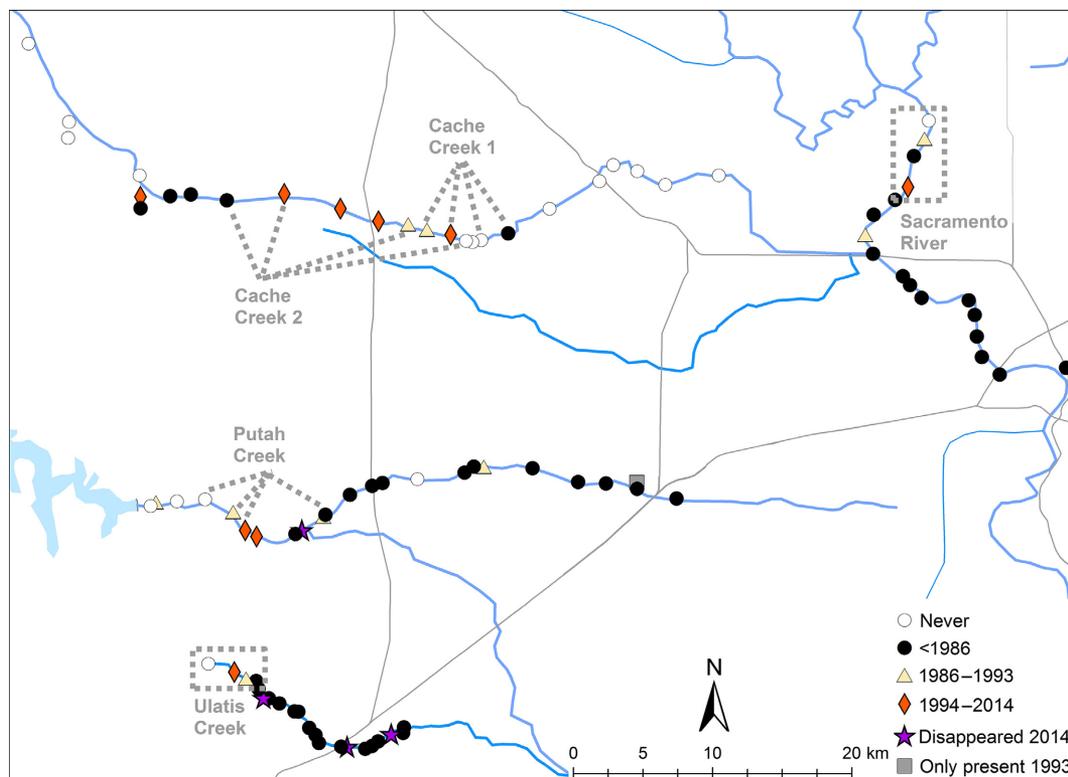
FIG. 1.   Map of the lower Sacramento River Valley, California, USA indicating major roads, watercourses, and the location of 86 riparian woodland sites sampled for Argentine ant presence/absence in 1984–1985 (Ward 1987), 1993 (Holway 1995), and 2014 (present study). Symbols indicate the following: open circles, Argentine ant never present; solid circles, sites invaded prior to 1986; triangles, sites invaded between 1986 and 1993; diamonds, sites invaded between 1994 and 2014; stars, Argentine ant present in earlier surveys but apparently absent in 2014; and squares, Argentine ant only detected in 1993. Dashed lines indicate spatial blocks that make up the invasion chronosequence.

individual watershed and included one site in each time-since-invasion category as well as one uninvaded control site (Fig. 1, Data S1-incidence.csv). The sites making up the chronosequence differ in their history of invasion as follows: sites invaded prior to 1986, sites invaded between 1986 and 1993, sites invaded between 1994 and 2014, and sites that were never invaded (Fig. 1). The validity of using an invasion chronosequence to test how and why invasion impacts change over time rests on the assumption that surveys conducted in 1984–1985, 1993, and 2014 capture sufficient information about the invasion history of each site. This assumption is based on published observations documenting (1) that independent colonization events in riparian woodlands appear to occur uncommonly (consistent with the inherent dispersal limitations of *L. humile*, whose queens do not fly) and (2) that the Argentine ant tends to persist in these habitats once established (Holway 1995, 1998b).

The chronosequence sites all support riparian woodlands composed of native trees: *Populus fremontii*, *Quercus lobata*, *Juglans hindsii*, *Aesculus californica*, and *Salix* spp. These woodland sites are all non-managed habitats that have experienced varying degrees of anthropogenic disturbance. Holway (1998b) qualitatively ranked riparian sites in these same watersheds with respect to six different measures of disturbance and found no relationship between native ant richness and the estimated level of disturbance (simple linear regression: $F_{1,18} = 0.71$, $P > 0.05$, $R^2 = 0.04$). This same study also found no relationship between the rate of Argentine ant spread and estimated level of disturbance (Holway 1998b).

We restricted our sampling to riparian woodlands for several reasons. First, compared to other habitat types in California, riparian woodlands most closely resemble the habitat preferred by *L. humile* in its native South America (LeBrun et al. 2007). Second, the Argentine ant invades a variety of non-managed habitats in California but attains exceptionally high abundance in riparian woodlands that border perennial watercourses, and past studies in this region have thus partly to entirely focused on the effects of ant invasions in this type of habitat (Tremper 1976, Ward 1987, Holway 1998a, b, see also Holway 2005). Lastly, confining our sampling to riparian woodlands controls for habitat-associated variation in the composition of native ant assemblages (Ward 1987) and to at least to some extent buffers our results from effects of drought.

Temporal variation in invasion impacts

To test the extent to which effects on native species have changed over time, we conducted standardized sampling at each site along our invasion chronosequence (Fig. 1) to estimate native ant richness and species composition. Sampling consisted of litter sampling, pitfall trapping, and vegetation beating. We used mini-Winkler extractors to sample leaf litter in early April 2016; at each site we collected 2 L of sifted leaf litter from four different areas (each separated by >10 m). We conducted pitfall trapping and vegetation beating in May 2016. At each site we deployed 20 pitfall traps; traps were separated by 10 m and placed in two parallel rows positioned roughly parallel to the riparian corridor. Pitfall traps consisted of a 50-mL centrifuge tubes (27 mm inner diameter) half-filled with soapy, salt water; traps were

left in the ground for 72 h. We also conducted vegetation beating at 20 trees or bushes at each site. We standardized this method by beating each tree or bush three times with a heavy stick into a beating net. Given that the configuration (e.g., width) of riparian woodlands varied considerably among the different rivers and streams in the chronosequence data set, we could not sample plots of fixed dimensions across all 20 sites although we were able to achieve this level of consistency for the sites within each block. Across all 20 sites, however, we sampled from very close to the same area of riparian habitat, with equal effort (time and sampling intensity), and at the same time of year.

These three field methods, used in combination, are considered effective in sampling ant assemblages (Bestelmeyer et al. 2000). To estimate native ant richness for each site, we pooled material obtained from the three sampling approaches. We used a one-way blocked ANOVA to test whether or not native ant richness depended on time since invasion. We also recorded the abundance of non-ant arthropods collected in pitfall traps at each site and conducted one-way blocked ANOVAs on beetle abundance, spider abundance, and total non-ant arthropod abundance to determine if numbers of these taxa change across the chronosequence. To estimate native ant species composition at each site, we considered the relative abundance of each native ant species to be equal to the proportion of pitfall traps in which that species was captured (Data S1-incidence.csv). Using PRIMER v6.1 (PRIMER-E Ltd, Plymouth, UK), we then conducted ordinations (non-metric multidimensional scaling [NMDS]) on untransformed proportions and used a one-way blocked PERMANOVA to compare the composition of native ant assemblages as a function of time since invasion. We used Euclidean distances in these ordinations because shared absences among invaded sites presumably result from the same underlying cause (Anderson et al. 2011), namely, native ant displacement by the Argentine ant.

In addition to analyzing the invasion chronosequence, we compared the results of contemporary sampling with those of previous studies conducted in the same riparian corridors. We first compared a subset of the pitfall trap data from the 2016 chronosequence data set (sites invaded between 1986 and 1993) to pitfall trap data from a different set of riparian woodland sites sampled in 1995 (Holway 1998a) that were also invaded between 1986 and 1993 (Ward 1987, Holway 1995). In both the 1995 and 2016 data sets, we used 20 pitfall traps to sample ants at five pairs of invaded and uninvaded sites in the month of May. Other sampling details differed. In 1995 each pitfall trap consisted of a glass test tube (18 mm inner diameter) about one-third filled with a 1:1 solution of water and antifreeze (mostly ethylene glycol) (Holway 1998a); at each site, traps were separated by 4 m and left open for two weeks. Moreover, sites sampled in 1995 were distributed along a 5-km stretch of Putah Creek between Lake Solano and Lake Berryessa (Holway 1998a) (Data S1-incidence.csv), whereas sites sampled in 2016 were distributed across a larger area within this same region (Fig. 1). The 1995 sites are in part represented in earlier surveys (Ward 1987, Holway 1995) and also include sites from Holway (1998a) that were recently invaded at the time of the latter study. Given methodological differences between the

two studies, we refrained from directly comparing estimates of species richness. Instead, we divided the number of native ant species present at each invaded site by the number present at the uninvaded site of that pair and then used a two-sample *t* test to compare these fractions across the two studies. To test whether or not native ant species composition differed as a function of time (1995 vs. 2016) or invasion status (invaded vs. uninvaded), we performed analyses similar to those used in the analyses of the chronosequence data.

As a second type of comparison, we used indicator species analysis (Dufrêne and Legendre 1997) to identify native ant taxa that were either positively or negatively associated with invaded sites. We performed separate analyses on three different data sets: (1) 22 sites sampled 1984–1985 from Ward (1987), (2) 10 sites sampled in 1995 from Holway (1998a), and 20 sites sampled in 2016 from the chronosequence data set (Data S1-occurence.csv). Methods used to sample ants differed in each study, but the universal goal was to document what native ant species occurred in riparian woodlands with and without the Argentine ant. These analyses were conducted in R (R Development Core Team 2016) using the *labdsv* package (Roberts 2016). For each of the three analyses, we pooled native ant species within genera for each site, assembled a community matrix made up of the presence or absence of individual genera at every site, and used the Holm correction to control for multiple comparisons. We conducted analyses at the genus level because members of several congeneric species pairs (e.g., *Formica moki* and *F. aerata*) tended to exhibit mutually exclusive distributions across sites. Analyses performed at the species level or on estimates of relative abundance (i.e., from pitfall captures) produced qualitatively similar results.

Variation in invader traits: relative abundance and aggression

Using the invasion chronosequence, we tested how relative invader abundance and aggression depend on time since invasion, and examined the extent to which variation in invader impact (native ant richness) depends on invader traits. For each invaded site we estimated relative Argentine ant abundance as the proportion of pitfall traps containing *L. humile*. We used a one-way blocked ANOVA to test how relative Argentine ant abundance (logit transformed) changes with time since invasion and a simple linear regression to test whether or not native ant richness depends on relative Argentine ant abundance.

To test for variation in the degree to which Argentine ant workers initiate aggression against native ant workers, we adapted published methods (Holway 1999, Roulston et al. 2003, Thomas et al. 2005, 2007). We sampled between 300 and 500 *L. humile* workers at invaded sites in our invasion chronosequence (Fig. 1) in May 2017. Subgroups of workers from these colony fragments were matched against workers of each of three, common native ant species (*F. moki*, *T. sessile*, and *L. occidentale*) collected from the uninvaded site associated with the Argentine ant sites in each block. Ants were always tested indoors, within several hours of collection, and at approximately 25°C. In each trial, we first introduced 10–20 Argentine ant workers into a small arena (a 10 × 10 cm plastic container with fluon-lined sides) by allowing them to walk onto a piece of paper placed in their collection container and then to walk off the paper after it was moved into the arena. We used groups of Argentine ant workers because Roulston et al. (2003) found that assays involving groups yielded less variable outcomes compared to assays that involved one-on-one interactions. After Argentine ant workers were given approximately three minutes to adjust to the arena, we introduced a single native ant worker. We scored all apparent mutual interactions between Argentine ant workers and native ant workers over the next 5 minutes as follows: neutral (workers appeared to detect one another, for example, through antennation but did not behave aggressively), Argentine ant workers initiated aggression (e.g., by chasing, lunging, grabbing with mandibles, or using chemical defenses), or native ant workers initiated aggression. For each block of sites, trials were conducted blind with respect to time-since-invasion category, and the last author (D.A. Holway) scored all behaviors. Trials were stopped before 5 minutes if the native ant was impaired or killed. Arenas were replaced after every trial.

Behavioral data were analyzed as follows. For each native ant species, we conducted five trials for each site and then summed all mutual interactions for that site. The response variable used in the analysis was the logit-transformed proportion of all interactions in a trial in which the Argentine ant initiated aggression. For each native ant species, we used a one-way blocked ANOVA to test for differences in the initiation of aggression as a function of time since invasion. We used correlations to test for associations between site-level native ant richness and the proportion of interactions in which the Argentine ant initiated aggression against native ants. These analyses do not include material from one site (Ulatis Creek; invaded between 1984–1993) due to a low abundance of Argentine ant workers at that site in 2017.

RESULTS

Historical resurvey

Resurveys conducted in 2014 and 2015 revealed that *L. humile* has undergone a slight, net expansion in its range over the past two decades (i.e., since the last resurvey [Holway 1995]). In the most recent resurvey, the Argentine ant was detected at 58% (118/202) of survey sites with new infestations at 17 sites and apparent losses at eight sites since 1993 (Fig. 1, Data S1), whereas the Argentine ant was present at 54% (109/202) of all sites in the 1993 resurvey. Per year colonization of sites not invaded in earlier surveys indicates a possible slowing of the rate that new sites become occupied: 23.0% (28/120) of sites over an approximately 8-yr period from Holway (1995) vs. 18.3% (17/93) of sites over an approximately 21-yr period in the present study. This apparent pattern, however, may simply be an artifact of the limited number of environmentally suitable sites in our data set that still lack the Argentine ant. Apparent losses ($n = 8$) in the present study were no more or less likely at sites first invaded in 1993 (Holway 1995) compared to sites first invaded in Tremper (1976) or Ward (1987) (Fisher exact test: $P = 0.57$). Half of apparent losses were at urban sites with the other half coming from a variety of non-urban environments (Appendix S3). In contrast, 16 of the 17 sites where the Argentine ant invaded since 1993 were in non-urban environments.

Temporal variation in invasion impacts

Sampling ants along the invasion chronosequence yielded 2,777 native ant workers representing 25 species. Data S1-incidence.csv provides a complete list of ant species by site. Comparisons of native ant richness and species composition at different points along the invasion chronosequence (Fig. 1) did not reveal any evidence that impacts of Argentine ant invasions increase or decrease over time. Invaded sites had 60% fewer native ant species compared to uninvaded sites (ANOVA, $F_{3,12} = 22.78$, $P < 0.001$; block $F_{4,12} = 1.33$, $P > 0.05$), but sites invaded for different lengths of time did not differ with respect to native ant species richness (Fig. 2A). We detected the same qualitative pattern in an analysis with incidence of native ants in pitfall traps as the response variable (ANOVA, $F_{3,12} = 5.41$, $P < 0.014$; block $F_{4,12} = 1.62$, $P > 0.05$). As with native ant species richness, the composition of native ant assemblages strongly differed between invaded and uninvaded sites (Fig. 3A; PERMANOVA, pseudo-$F_{3,12} = 2.41$, $P = 0.0006$; block pseudo-$F_{4,12} = 1.51$, $P = 0.043$), but invaded sites did not differ from one another with respect to time since invasion (pairwise PERMANOVA tests, <1986 vs. 1993, $t = 0.65$, $P > 0.05$; <1986 vs. 2014, $t = 0.77$, $P > 0.05$; 1993 vs. 2014, $t = 0.74$, $P > 0.05$). In contrast to patterns evident for native ant assemblages, non-ant arthropod abundance did not differ either between invaded and uninvaded sites or with respect to time since invasion: non-ant arthropods (ANOVA, $F_{3,12} = 0.65$, $P > 0.05$; block $F_{4,12} = 2.24$, $P > 0.05$), beetles (ANOVA, $F_{3,12} = 2.24$, $P > 0.05$; block $F_{4,12} = 3.66$, $P = 0.04$), and spiders (ANOVA: $F_{3,12} = 1.60$, $P > 0.05$; block $F_{4,12} = 0.87$, $P > 0.05$).

Comparisons of native ant assemblages from riparian woodland sites sampled in either 1995 or 2016 again did not reveal any evidence that invasion impacts change over time. The fraction of native ant species at paired invaded/uninvaded sites, for example, did not differ between sets of sites sampled 21 yr apart (Fig. 2B; two-sample t test, $t = 0.63$, df = 8, $P > 0.05$). The composition of these native ant assemblages strongly differed between invaded and uninvaded sites (Fig. 3B; PERMANOVA, pseudo-$F_{1,16} = 9.73$, $P = 0.0001$), but not between the two sampling years (PERMANOVA, pseudo-$F_{1,16} = 1.28$, $P > 0.05$).

Indicator species analysis revealed that compositional differences apparent between invaded and uninvaded sites (Fig. 3) appeared driven by a nested set of epigeic, aboveground foraging native ants, which were negatively associated with invaded sites (Table 1). With respect to *Crematogaster*, *Camponotus*, and *Lasius* in the 1984–1985 data set and *Lasius* in the 1995 data set, these genera were without exception restricted to uninvaded sites but were too sparse in their respective data sets to be identified as indicator taxa. Epigeic genera never identified as indicator taxa in any of the analyses include thermophiles (e.g., *Monomorium*, *Dorymyrmex*), winter-active taxa (e.g., *Prenolepis*), and rare taxa. Moreover, no hypogeic (i.e., belowground foraging) genera were ever associated with invaded (or uninvaded) sites. A separate indicator species analysis conducted only on the invasion chronosequence data set revealed that no native ant genera were associated with sites invaded for different lengths of time.
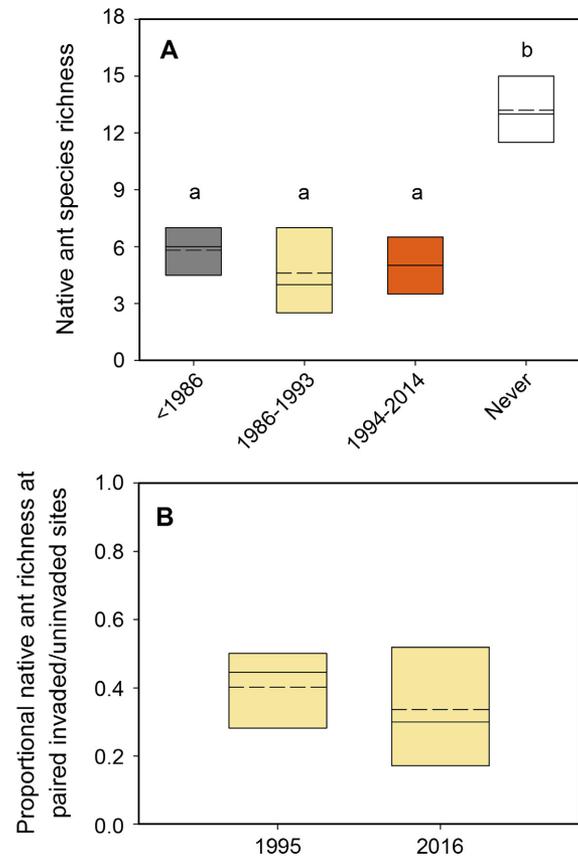
FIG. 2. (A) Box plots indicating native ant richness in riparian woodland sites that differ in their history of invasion along a chronosequence: sites invaded prior to 1986, sites invaded between 1986 and 1993, sites invaded between 1994 and 2014, and sites that were never invaded (Fig. 1). (B) Box plots indicating the fraction of native ant species observed at paired invaded/uninvaded riparian woodland sites that were sampled in either 1995 (Holway 1998a) or 2016 (present study). Boxes indicate the 25th and 75th percentiles. Solid lines equal medians; dashed lines equal means.

Variation in invader traits: relative abundance and aggression

In the chronosequence data set, there was no significant effect of time since invasion on either the relative abundance of the Argentine ant or on the proportion of mutual interactions in which Argentine ant workers initiated aggression against three different species of native ants (Table 2). Although not statistically significant, a potential trend toward slightly weaker aggression in older invasions appears evident for interactions between the Argentine ant and all three native ant species (Table 2). The proportion of interactions in which the Argentine ant initiated aggression ranged from 0.43 (against *T. sessile*) to 0.64 (against *F. moki*).

Tests of invasion impact vs. invader traits revealed a significant, negative relationship between native ant richness and Argentine ant relative abundance (simple linear regression, $F_{1,13} = 5.86$, $P = 0.03$, $R^2 = 0.31$). For each of the three focal species of native ants, no significant correlation existed between native ant richness and the proportion of mutual interactions in which Argentine ant workers initiated aggression (*F. moki* correlation, $r_{12} = -0.17$, $P > 0.05$; *L. occidentale* correlation, $r_{12} = -0.46$, $P > 0.05$; *T. sessile* correlation, $r_{12} = -0.13$, $P > 0.05$).
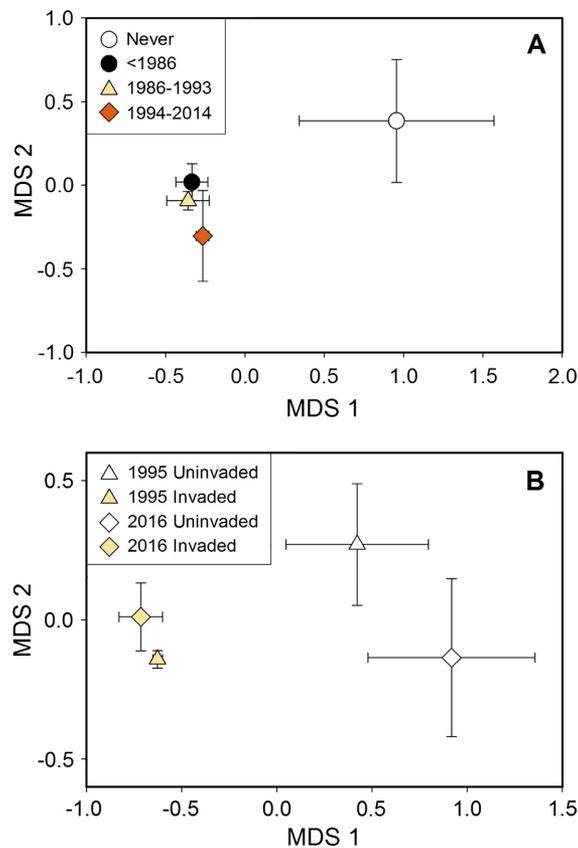
FIG. 3. Ordination based on nonmetric multidimensional scaling of native ant species composition in riparian woodland sites (A) that differ in their history of invasion along a chronosequence: sites invaded prior to 1986, sites invaded between 1986 and 1993, sites invaded between 1994 and 2014, and sites that were never invaded (Fig. 1) or (B) that were sampled in either 1995 (Holway 1998a) or 2016 (present study), but were invaded at about the same time (between 1986 and 1993). Symbols represent mean ± SE. The stress values for these ordinations are 0.09 (A) and 0.08 (B). See *Results* for PERMANOVA comparisons.

DISCUSSION

Sampling along a 30-yr chronosequence revealed that the ecological effects of ant invasions endure over at least a 30-yr time frame and remain evident at a regional scale. Native ant richness and species composition significantly differed

TABLE 1. Native ant genera negatively associated with sites invaded by the Argentine ant.

Genus	(1) 1984–1985	(2) 1995	(3) 2016
Liometopum	1.00***	1.00**	0.94***
Formica	0.83***	0.83*	0.94***
Tapinoma	0.75***	1.00**	0.88**
Crematogaster		0.80*	1.00***
Camponotus		0.80*	0.64*
Lasius			0.60*

Notes: Table entries are indicator values with their associated *P* values from indicator species analyses conducted on three different data sets: (1) 22 sites (with 19 genera) sampled in 1984–1985 from Ward (1987), (2) 10 sites (with 15 genera) sampled in 1995 from Holway (1998a), and (3) 20 sites (with 17 genera) sampled in 2016 (present study). Empty cells are non-significant. See Data S1-occurrence.csv for the site-level occurrence of genera used in each analysis.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

between invaded and uninvaded sites, but we found no evidence that these effects change over time. Comparative analyses provide further statistical evidence for a temporally consistent pattern of differential displacement of epigeic species (compared to hypogeic species) that was first reported by Ward (1987). Substantial overlap exists between epigeic genera that were negatively associated with invaded sites sampled at different times over a 30-yr period (in spite of the different methodological approaches employed in each study). In contrast, hypogeic genera never showed an association with invaded or uninvaded sites. Chronic impacts on epigeic native ants could affect the persistence of these species in riparian habitats and influence other arthropods (e.g., honey-producing Hemiptera and parasitoids) that interact strongly with these native ants.

Invaded sites in this study supported 60% fewer native ant species compared to uninvaded sites, and native richness at invaded sites continued to decline with increasing relative invader abundance. Although the relative abundance of the Argentine ant did not significantly differ among sites as a function of time since invasion, among-site variation in relative abundance was great enough to give rise to a significant negative relationship between invader abundance and invader impact (see also Suarez et al. 1998, Holway 2005, Hanna et al. 2015). Recent studies that document chronic invader impacts also point to a relationship between invader abundance and invasion impacts. In Lake Gatun, Panama, for

TABLE 2. Relationships between variation in invader traits and time since invasion.

Parameter	Time since invasion			Time since invasion	Block
	<1986	1986–1993	>1993		
Relative abundance†	0.44 ± 0.09	0.76 ± 0.10	0.62 ± 0.12	$F_{2,8} = 1.97$, $P > 0.05$	$F_{4,8} = 1.51$, $P > 0.05$
Initiation of aggression‡					
Formica moki	0.61 ± 0.053	0.63 ± 0.061	0.69 ± 0.060	$F_{2,7} = 2.15$, $P > 0.05$	$F_{4,7} = 6.35$, $P = 0.018$
Liometopum occidentale	0.42 ± 0.058	0.48 ± 0.040	0.53 ± 0.049	$F_{2,7} = 1.88$, $P > 0.05$	$F_{4,7} = 2.36$, $P > 0.05$
Tapinoma sessile	0.41 ± 0.033	0.41 ± 0.069	0.46 ± 0.087	$F_{2,7} = 0.32$, $P > 0.05$	$F_{4,7} = 3.82$, $P > 0.05$

†The relative abundance of the Argentine ant (mean ± 1 SE) proportion of pitfall traps capturing this species) as a function of time since invasion and one-way blocked ANOVAs testing if the relative abundance of the Argentine ant depends on time since invasion and spatial block.

‡The proportion (mean ± 1 SE) of all mutual interactions in which Argentine ant (*Linepithema humile*) workers initiated aggression against *Formica moki, Liometopum occidentale*, and *Tapinoma sessile* workers as a function of time since invasion and one-way blocked ANOVAs testing if initiation of aggression depends on time since invasion and spatial block.

example, Sharpe et al. (2017) found that the peacock bass, an introduced predatory fish, was the most abundant predator 45 yr after its introduction and that impacts associated with its introduction appeared comparable to those observed in the invasion's initial stages. Persistent effects of cane toad invasions in Australia also appear to be the result of consistently high invader populations (Doody et al. 2017).

Our results suggest that variation in relative invader abundance may be due more to site-specific factors than time since invasion. Such a pattern could result from changes in the abiotic environment that exhibit spatial heterogeneity and that disproportionately and negatively affect introduced species compared to natives (Flory and D'Antonio 2015). The drought affecting California between 2012 and 2015, for example, was the most severe in several centuries (Belmecheri et al. 2016) and presumaby reduced environmental favorability for the Argentine ant, which requires adequate levels of soil moisture to invade seasonally dry environments (Menke and Holway 2006). Site-level differences in drought-induced environmental stress provide a plausible explanation for the variation in relative Argentine ant abundance at our study sites.

Our study considered two types of invasion impacts: ecological effects on native species and geographic range (Parker et al. 1999). In terms of range size, resurveys at 202 sites first sampled 30 or 40 yr ago, reveal that the Argentine ant has undergone a net range expansion in the study region. This latter finding agrees with our earlier resurvey (Holway 1995) as well as with studies conducted in Bermuda where the Argentine ant has persisted at particular sites over an approximately 30-yr period (Haskins and Haskins 1965, 1988, Crowell 1968, Lieberburg et al. 1975). Given that the Argentine ant has declined in parts of New Zealand (Cooling et al. 2011), it would be of interest to identify environmental differences among introduced sites that might underlie apparent disparities in persistence. Such differences could include abiotic conditions, pathogens, pesticides, or other factors (Cooling et al. 2011, Gruber et al. 2017).

Although the impacts of Argentine ant invasions on native ants persist over at least a 30-yr time frame, these effects may not extend to assemblages of non-ant arthropods. Abundances of spiders, beetles, and all non-ant arthropods combined, for example, did not differ among invaded sites along the invasion chronosequence or between invaded and uninvaded sites (see also Holway 1998a, Hanna et al. 2015). A comprehensive understanding of the impacts of ant invasions on non-ant arthropods requires the identification of focal taxa to a level that allows for the separation of native and non-native species (Krushelnycky and Gillespie 2008, Hanna et al. 2015). Combining together non-ant arthropods into broad taxonomic categories or presumed functional groups carries the risk of pooling native and non-native taxa, thus making it impossible to isolate the effects of ant invasions on native species (Krushelnycky and Gillespie 2008). Misleading information seems especially likely to result from systems in which non-native arthropods themselves are reduced in abundance or diversity in the face of ant invasions (Porter and Savignano 1990, Hanna et al. 2015).

Identifying the underlying causes of spatiotemporal variation in invasion impact will require studies that separate factors that are intrinsic to the process of invasion (e.g.,

variation in invader traits) from long-term environmental changes (e.g., climate change) that represent extrinsic influences on the dynamics of invasion. Given that the ultimate goal in any study addressing temporal variation in invasion impacts will be to identify the mechanisms underlying such change (Strayer 2012), future studies should aim to uncover these underlying causes through the use of experiments and an informed understanding of site-level invasion history (Dostal et al. 2013, Yelenik and D'Antonio 2013).

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SUPPORTING INFORMATION

Long-term record of Argentine ant invasions reveals enduring ecological impacts. Sean B. Menke, Philip S. Ward, and David A. Holway. *Ecology.* **2018.**

Appendix S1

Fig. S1. Map of the southern San Francisco Bay region indicating the location of 116 sites

in varied habitats sampled for Argentine ant presence/absence in 1974 (Tremper 1976), 1993

(Holway 1995) and 2015 (present study).
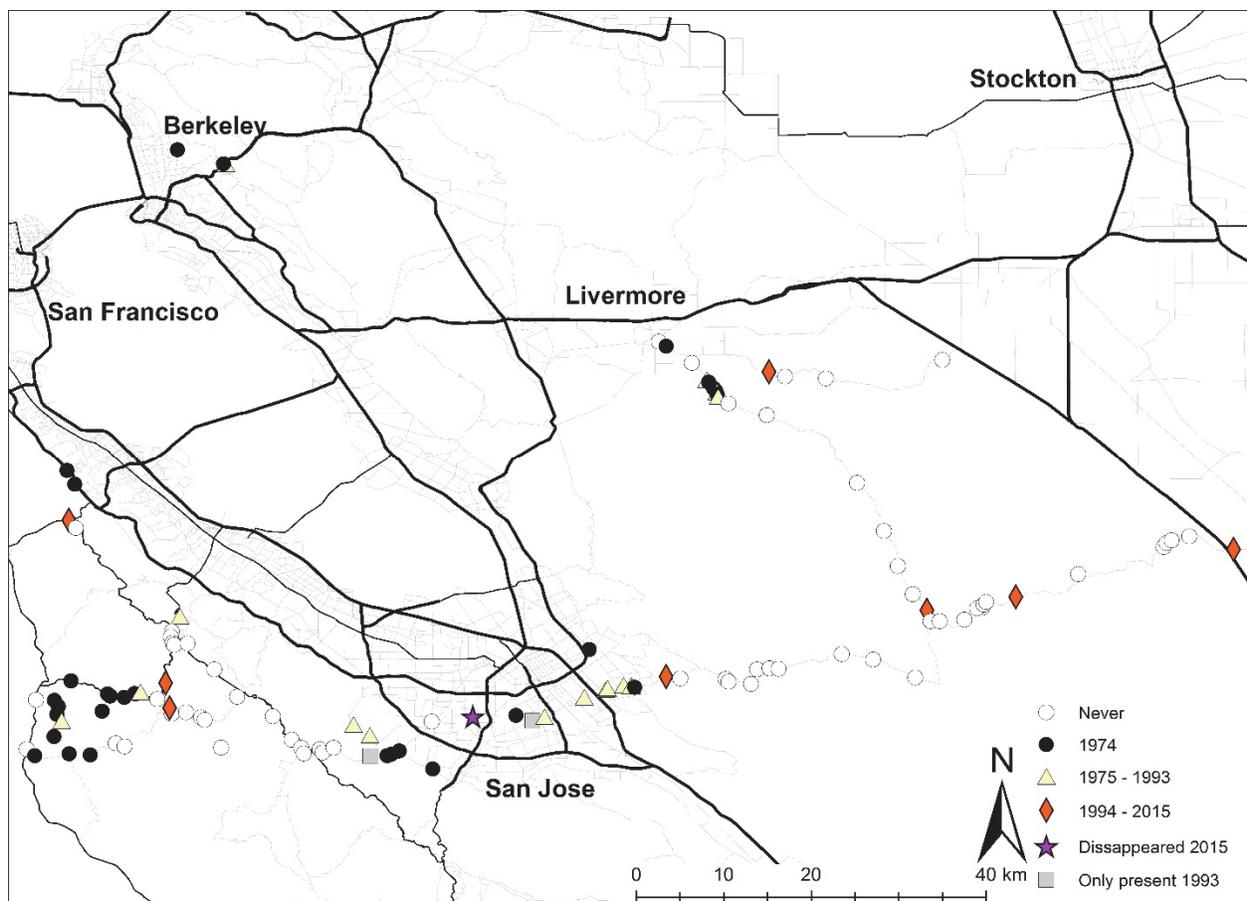
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Appendix S2. Excerpts from Tremper (1976), Ward (1987), and Holway (1995) that describe the methods used in each study to assess Argentine ant presence or absence at individual sites.

Tremper (1976): "Each locality was searched for ant nests by turning cover objects, raking through soil and litter, and pulling bark off logs; and searched for foragers by inspecting trees and the ground." (page 26)

Ward (1987): "… spot sampling for the presence of *Iridomyrmex humilis* and other epigaeic ants was carried out at 68 additional sites located along four valley riparian systems, and at 14 disturbed sites located along roadsides and irrigation ditches. These spot samples entailed about 30 minutes of general collecting per site." (page 5)

Holway (1995): "At each site I examined bare ground, tree trunks, and under stones and logs. If epigeic native ants (i.e., species known not to coexist with *L. humile*… were found, the site was scored as not having *L. humile* and the date, time, and native ants present were recorded. If Argentine ants were found, the site was scored as having *L. humile*." (page 1634)

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Appendix S3

Table S1. Sites where the Argentine ant was apparently absent in 2014-2016 but present in earlier surveys.

Transect	Site	Environment	Description
Tremper	51	Roadside Hwy 9	Riparian. Native ants common (85-100% of baits).
Tremper	57	Urban	Residential. Abundant *Nylanderia* and *Tetramorium* (70-100% of baits occupied).
Tremper	59	Urban	Residential. Abundant *Nylanderia* and *Tetramorium* (100% of baits occupied).
Ward	120	Urban	Residential. Native ants present (85-100% of baits occupied).
Ward	124	Urban	Residential. Native ants rare (35-50% of baits occupied).
Ward	133	Ulatis Creek; riparian corridor	Riparian. Abundant native ants (100% of baits occupied).
Ward	155	Putah Creek; oak savanna	Riparian. Abundant native ants (100% of baits occupied).
Ward	169	Putah Creek; riparian corridor	Riparian. Major soil removal (> 3m) along bank. Heavily disturbed. Rare pockets of *Monomorium ergatogyna* (3-5% of baits).