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Differential impacts of soil microbes on native and co-occurring invasive tree species

Aleksandra Wróbel,[1,]† Elizabeth E. Crone,[2] and Rafał Zwolak[1]

[1]*Department of Systematic Zoology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland*
[2]*Department of Biology, Tufts University, Medford, Massachusetts, USA*

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Abstract. The Janzen-Connell effect is often generated by interactions between trees and soil microbes while the enemy release hypothesis states that invasive species are released from regulation by natural enemies. Thus, the strength of the Janzen-Connell effect could differ between native vs. nonnative plants. We tested this hypothesis with congeneric pairs of invasive and native tree species in Europe: boxelder (*Acer negundo*) vs. Norway maple (*A. platanoides*), and Northern red oak (*Quercus rubra*) vs. pedunculate oak (*Q. robur*). We conducted greenhouse experiments using soil sterilization treatments, field experiments on distance-dependent germination, and field surveys of early life stages of the focal species. Greenhouse and field experiments demonstrated patterns consistent with Janzen-Connell effect that is most likely caused by negative distance dependence in seed germination and stem growth which was found in both genera of the native, but not the invasive trees. Soil sterilization experiments suggested that these effects are driven by interactions with soil biota. Field surveys revealed the Janzen-Connell pattern in the distribution of seedlings and saplings of the native, but not the invasive *Acer* species. Our findings indicate that weakened Janzen-Connell effect might contribute to successful invasions of certain nonnative plant species.

Key words: congeneric comparison; distance dependence; enemy release hypothesis; invasion; Janzen-Connell hypothesis; plant–soil biota interactions.

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† **E-mail:** wrobel_a1@wp.pl

INTRODUCTION

Species invasions are a source of agricultural and economic losses worldwide and represent a major threat for biodiversity (Vitousek et al. 1997, Mack et al. 2000). However, despite considerable research effort, our understanding of the factors that allow certain species to dominate novel communities has not led to high predictive certainty. Although certain factors have been shown to be important in particular instances, these appear to be idiosyncratic among systems (Hawkes 2007, Moles 2012). It may be that we need to reframe this question as, "How often is a particular mechanism important?" rather than "Is a particular mechanism important?" (cf. Moles 2012). In this paper, we evaluate the role of plant–soil feedbacks in determining differences in distribution and performance of native and invasive tree seedlings. We use these data to examine three hypotheses: (1) native plants experience more negative soil feedback than invasive congeners (Reinhart and Callaway 2004, Vilá and Weiner 2004, Meiners 2005), (2) negative soil feedback is stronger near adult conspecific plants (Packer and Clay 2000, Bever 2003, Comita 2014), and (3) these feedbacks lead to different patterns of seedling distribution in native and invasive plants in the field.

In general, one hypothesis for the success of invasive species is that they are released from regulation by natural enemies (the enemy release hypothesis; Blossey and Nötzold 1995, Keane and Crawley 2002, Heger and Jeschke 2014), particularly soil microbes (Packer and Clay 2000, Agrawal et al. 2005, Reinhart and Callaway 2006, Gundale et al. 2014). Negative soil community feedbacks mediate the outcome of competitive plant–plant interactions (Van der Heijden 1998, Bever 2003, Bever et al. 2015, Albornoz et al. 2017, Bachelot et al. 2017), shape local distribution of plant species (Packer and Clay 2000, Klironomos 2002, Reinhart and Callaway 2006, Bachelot et al. 2017), provide distance-dependent regulation of expanding populations (Bever 1994, Reinhart et al. 2003, Mordecai 2011, Comita 2014), and help to maintain plant species diversity (Augspurger and Kelly 1984, Van der Putten et al. 1993, Mills and Bever 1998, Klironomos 2002, Bever 2003, Gundale et al. 2014, Bever et al. 2015). In some cases, invasive plants have been shown to avoid the regulation by soil organisms since interactions of alien species with other organisms are often thought to be weakened in nonnative range (Packer and Clay 2000, Callaway et al. 2001, 2004, Agrawal et al. 2005, Reinhart and Callaway 2006, Gundale et al. 2014).

The enemy release hypothesis is in many ways the inverse of the longer-standing Janzen-Connell hypothesis, one of the most widely accepted mechanisms of maintaining forest diversity and determining plant distribution (Comita 2014). The Janzen-Connell hypothesis proposes that diversity of plant communities in natural ecosystems is promoted by host-specific enemies that reduce survival of seeds or seedlings in a density- and/or distance-dependent manner (Janzen 1970, Connell 1971). Seeds or seedlings close to or in areas with high density of conspecific adults suffer higher mortality due to increased activity of seed predators, herbivores, and pathogens. In this case, individuals have a greater negative impact on conspecifics than on heterospecifics. By preventing conspecific trees from creating clumped distributions, this mechanism contributes to the formation of mixed communities. Recent studies have demonstrated that the Janzen-Connell effect is often generated by interactions with soil organisms (Van der Heijden 1998, Mordecai 2011, Gundale et al. 2014, Bachelot et al. 2017, Deniau et al. 2018), which is consistent with the notion that release from natural enemies for invasive species may disrupt coexistence mechanisms and allow one species (the invasive one) to dominate a community.

Thus, the invasiveness of tree species may be driven by differential impact of natural enemies responsible for the Janzen-Connell effect on native and invasive trees. Invaders probably encounter non-adapted and therefore less damaging enemies that differ in density, species composition, and diversity relative to their native ranges. If the Janzen-Connell effect is an important mechanism of coexistence in natural communities (Bever 2003, Comita 2014), and if it is caused by interactions with soil pathogens that act as natural enemies, the absence of this effect in alien species may partially explain their advantage over native species. However, soil organisms that interact with plants include not only pathogens, but also important mutualists (Van der Heijden 1998, Paudel et al. 2014, Bachelot et al. 2017). One of the generalities that emerge from current research on invasive plants is that plants in their nonnative ranges tend to be facilitated by soil biota, while native plant species tend to be negatively affected by the accumulation of host-specific portions of the soil biota (Reinhart and Callaway 2004, Agrawal et al. 2005, Gundale et al. 2014, Bardgett and van der Putten 2014). A tree species whose abundance is limited by soil pathogens in its native range may become locally more abundant in its nonnative range where control by the soil community is absent or facilitation is stronger (Packer and Clay 2000, Reinhart et al. 2003, Gundale et al. 2014, Bever et al. 2015). This may lead to high potential for invasiveness of plants in new regions, where such feedback does not occur.

In this study, we tested whether native and invasive tree species are influenced by the Janzen-Connell effect caused by soil-borne organisms. As model species, we used congeneric pairs consisting of species nonnative vs. native to Europe, that is, the boxelder (*Acer negundo*) vs. the Norway maple (*A. platanoides*), and the Northern red oak (*Quercus rubra*) vs. the pedunculate oak (*Q. robur*). Past work with the two *Acer* species (Reinhart and Callaway 2004, Saccone et al. 2010, Porté et al. 2011) has shown that soil feedbacks are an important contributor to invasiveness in their respective native ranges.

For this species pair, the questions in this study are whether these effects take the distance-dependent form hypothesized by the Janzen-Connell hypothesis and, to a lesser extent, whether the same patterns hold in different regions of Europe and North America than the original studies. For the *Quercus* species, we did not yet know at the outset of this study whether soil communities were an important component of invasiveness or coexistence in their native ranges (but see Reinhart et al. 2012). Both boxelder and the Northern red oak have been introduced to Europe from North America at the turn of XVIII and XIX centuries for planting in gardens and to enrich impoverished forest stands. However, multiple contemporary studies have demonstrated a negative impact of these trees on diversity and abundance of native vascular plant species (Maeglin and Ohmann 1973, Woziwoda 2002, Saccone et al. 2010, Woziwoda et al. 2014). Thus, the boxelder and the Northern red oak are considered highly invasive in Europe.

We conducted field surveys of early life stages of the focal species, greenhouse experiments using soil inoculation treatments, and field experiments on distance-dependent germination probabilities to test the hypothesis that negative plant–soil feedbacks are stronger in the native than in the invasive trees. Based on the enemy release hypothesis, we expected that in greenhouse conditions, seed germination and seedling development (measured by biomass, stem height, and root length) would be negatively affected by soil collected under adult conspecifics in native, but not in invasive species (Prediction 1). Based on the Janzen-Connell hypothesis, we expected that germination probability of seeds sown in the field would increase with distance to conspecifics in the native, but not in the invasive tree species (Prediction 2). Based on the net effects of both processes, we predicted that the average distance of seedlings and saplings from adult conspecifics would be lower in the invasive than in the native species (Prediction 3).

METHODS

Study sites

Fieldwork was conducted in Puszcza Zielonka (52°30′N, 17°82′E; 78 m a.s.l.) and Wielkopolski National Park (52°16′N 16°48′E; 65 m a.s.l.), both located in Greater Poland Voivodeship, Poland. This region is characterized by mild temperate climate and mostly flat topography. The average air temperature ranges from −2.5°C in January to 18.2°C in July, and the annual precipitation averages 520 mm. We established study sites in managed 50- to 70-yr-old stands. Forest at our plots was transformed by former forest management, replacing mixed and broadleaved forests with monocultures of Scots pine (*Pinus sylvestris*) before the 50s of XX century (Nowak et al. 2000). After that time, Wielkopolski National Park (a national park since 1957) and some parts of Puszcza Zielonka (a landscape park since 1993) have become protected areas with enhanced natural regeneration. The two areas are characterized by the abundant occurrence of numerous species of alien woody plants (Gazda and Szwagrzyk 2016). For both *Acer* species, study sites included mixed forests dominated by *Populus* spp. and Scots pine, while for both *Quercus* species, mixed mesic forests dominated by common hornbeam (*Carpinus betulus*) and Scots pine. Understorey and the ground flora were poorly developed at our stands and included mostly the common wood sorrel (*Oxalis acetosella* L.), *Carex* spp., and the male fern (*Dryopteris filix-mas* L.).

Observations and experiments

To test Prediction 1 (germination and seedling development are negatively affected by soil collected close to adult conspecifics in native, but not in invasive species), we conducted a greenhouse experiment in spring and summer 2016 and 2017 at the Faculty of Biology of Adam Mickiewicz University in Poznań. We collected soil samples near the tree trunk (1 m; hereafter "under conspecifics") and at greater distance (13 m; hereafter "away from conspecifics") from 10 randomly chosen adult trees of each study species that grew at our field sites. The samples were placed in plastic bags separately and kept in a refrigerator no longer than two weeks (Reinhart and Callaway 2004). The substrate used for sowing seeds was obtained by mixing 25 mL of either non-sterilized or sterilized soil (collected either close to or far from the tree trunk) + ~2.5 L sterilized garden soil. This method helps to avoid differences in the bioavailability of minerals due to sterilization, which otherwise could confound the results (Troelstra et al. 2001). Thus, the experiment had a 2 × 2 factorial design: 2 distances (under and away from conspecifics) × 2 soil types (non-sterilized or sterilized). The sterilization was conducted by autoclaving the soil for three hours in 121°C. We did not mix soil collected under different trees of the same species because such a procedure leads to falsely precise effect estimates (Reinhart and Rinella 2016). For the greenhouse experiment, we purchased all seeds from a local nursery. Before performing the experiment, the seeds were cleaned up of potential pathogens by placing them in 5% bleach solution for 10 min, and then rinsing with deionized water. We used 16 seeds per each soil sample (eight for non-sterilized and eight for sterilized soil type), and thus, we planted 80 seeds per each type of substrate in each tree species (10 trees × 2 distances × 2 soil treatments × 8 seeds = 320 seeds per species per year). All seeds were put out in both soil types in individual plastic pots (5 × 5 × 5 cm) in 32 greenhouses (100 × 40 × 50 cm) randomly, and regularly watered. Each greenhouse contained pots with soil collected either close to or far from conspecifics, but from both sterilization treatments, with sterilized and non-sterilized soil pots located at least 20 cm apart. The data were collected during four months after planting seeds in the experimental substrate. We counted germinated seeds weekly. Stem height, root length, and total fresh biomass were measured at the end of the experiment.

To test Prediction 2 (on distance-dependent germination in native, but not in invasive species), we conducted an experimental field study in autumn 2015 and 2016. We randomly chose several adult (≥50 cm in diameter at breast height) individuals of each species (14 for *A. negundo*, 14 for *A. platanoides*, 13 for *Q. rubra*, and 12 for *Q. robur*). We created three transects, each with sampling points at four distances from a tree: 1, 5, 9, and 13 m (see Packer and Clay 2000, Reinhart and Callaway 2004, Yamazaki et al. 2008 for similar approach). The neighborhood of all experimental points was also chosen randomly because the distance between heterogenous trees was shorter than the length of our transects. At each point, we set up stations marked with wooden stakes where we planted seeds of the focal species. Seeds for planting were collected at study plots outside the observational transects. In 2015, there were insufficient numbers of *Q. robur* acorns on study sites, and thus, we purchased seeds from a local forest nursery. We sowed five seeds at each experimental station (5 seeds × 4 distances × 3 transects = 60 seeds per each tree). The number of seedlings that emerged at each point was determined next spring.

To test Prediction 3 (shorter distances from adult conspecifics in invasive than native species), we conducted an observational field study in spring and early summer in 2016 and 2017. We used the same adult trees as in the experimental field study described above. However, during the study, we excluded one *Q. robur* tree because its surroundings were disturbed by logging. We counted conspecific seedlings (≤0.5 m height) and saplings (0.5–3 m height) at three transects (length 13 m; width 1 m) originating from each adult individual.

Data analyses

Statistical analyses were conducted in R with glmmADMB and lme4 packages (Fournier et al. 2012, R Development Core Team 2017, Bates et al. 2015, Skaug et al. 2016), using generalized linear mixed models and linear mixed models fitted by maximum likelihood using a Laplace approximation. Testing for statistical significance was conducted with Wald chi-square tests (package car, Fox and Weisberg 2011, 1 degree of freedom in each test). In the greenhouse experiments (Prediction 1), we analyzed whether the distance from the nearest conspecific adult and soil sterilization affect (1) the probability of germination, (2) biomass, (3) stem height, and (4) root length in all tested species. In analysis (1), we used binomial error distribution (logit link); in analyses (2–4), we used Gaussian error distribution (identity link). Explanatory variables consisted of the location (soil collected close or far from adult tree), sterilization type (soil sterilized or non-sterilized), and their interaction, while source tree and greenhouse were included as random effects. In the field experimental study (Prediction 2), we analyzed whether (5) the distance from the nearest conspecific adult affects the probability of germination in all species (binomial error distribution, logit link). In the field observational study (Prediction 3), we analyzed whether (6) native vs. nonnative congenerics differ in the average distance of recruits (both seedlings and saplings) to the nearest conspecific adult (Gaussian error distribution, identity link). In both the field observational and experimental studies, distance to the nearest conspecific was entered as fixed effect (continuous in the field studies and binary in the greenhouse study), while site, tree, and transect were included as nested random effects. When needed, we log-transformed response variables to improve the distribution of residuals.

RESULTS

Seed germination and properties of seedling emerged in greenhouse experiments

In support of Prediction 1, germination of the native, but not of the invasive species, was higher in sterilized than unsterilized soil from under adult conspecifics (marginally significant Sterilization × Distance effect in Table 1, Figs. 1, 2). In addition, the probability of germination was higher in unsterilized soil samples collected away from adult conspecifics than in samples collected under adults in *A. platanoides* and *Q. robur* (distance effect in Table 1, Fig. 1).

We found a positive effect of soil sterilization on the biomass of *A. negundo* and *Q. robur* (although in the latter the effect was slightly above the nominal significance threshold: sterilization effect in Table 1, Fig. 2), and a negative effect of soil from further distance on biomass of *Q. rubra* (distance effect in Table 1, Fig. 2). Yet, in contrast to our predictions, the effects of sterilization on biomass did not depend on soil origin (non-significant Sterilization × Distance effects in Table 1, Figs. 1, 2).

The average stem height increased with sterilization in both *Acer* species and *Q. robur* (Table 1, Figs. 1, 2). Moreover, the effect of sterilization was affected by distance from the conspecific trees in both native (Fig. 1) and invasive (Fig. 2) trees (Table 1), supporting Prediction 1.

We did not find any significant effects of sterilization, distance, or interaction between them on root length of focal species (Table 1).

Seed germination in field experiments

Seed germination did not depend on the distance from the adult conspecifics in invasive species (*A. negundo*: $\chi^2 = 0.036$, $P = 0.85$; *Q. rubra*: $\chi^2 = 0.473$, $P = 0.49$; Fig. 3). However, the probability of seed germination increased with the distance in native species (from 25% at 1 m to 38% at 13 m in *A. platanoides*: $\chi^2 = 4.809$, $P = 0.03$, and from 29% at 1 m to 40% at 13 m in *Q. robur*: $\chi^2 = 6.195$, $P = 0.05$; Fig. 3). Thus, Prediction 2 was supported for both *Acer* and *Quercus*.

Field surveys of early life stages

Seedlings and saplings occurred significantly further from adult conspecifics in *A. platanoides* than in *A. negundo* (mean ± SE: *A. platanoides*:

Table 1. Effect of treatments on germination and seedling establishment in the greenhouse experiment.

		Invasive species				Native species			
		A. negundo		*Q. rubra*		*A. platanoides*		*Q. robur*	
Dependent variables	Fixed effects	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Germination	Sterilization	0.171	0.679	0.034	0.853	4.075	0.936	0.014	0.906
	Distance	0.119	0.730	0.000	0.998	0.065	**0.044**	1.300	0.254
	Sterilization × Distance	0.954	0.329	0.022	0.881	3.786	**0.052**	3.402	0.065
Biomass	Sterilization	5.632	**0.018**	0.397	0.529	1.688	0.194	3.459	0.063
	Distance	1.054	0.305	5.156	**0.023**	2.054	0.152	0.913	0.339
	Sterilization × Distance	0.281	0.596	0.154	0.695	2.172	0.141	0.606	0.436
Stem height	Sterilization	15.455	**<0.001**	0.020	0.887	13.555	**<0.001**	5.170	**0.023**
	Distance	0.046	0.829	0.046	0.831	0.030	0.862	0.283	0.594
	Sterilization × Distance	0.682	0.409	2.240	0.134	8.848	**0.003**	7.449	**0.006**
Root length	Sterilization	0.370	0.543	1.178	0.278	0.490	0.484	1.435	0.231
	Distance	2.829	0.093	2.521	0.112	0.008	0.930	0.782	0.376
	Sterilization × Distance	0.082	0.775	1.154	0.283	1.108	0.292	0.064	0.800

Notes: Data were fitted with a general linear mixed model and tested with the likelihood ratio statistic. Root length of *Acer negundo*, *Quercu robur*, and *Quercu rubra* was log-transformed. Each statistical test was conducted on 1 degree of freedom. Significant results are shown in boldface type.

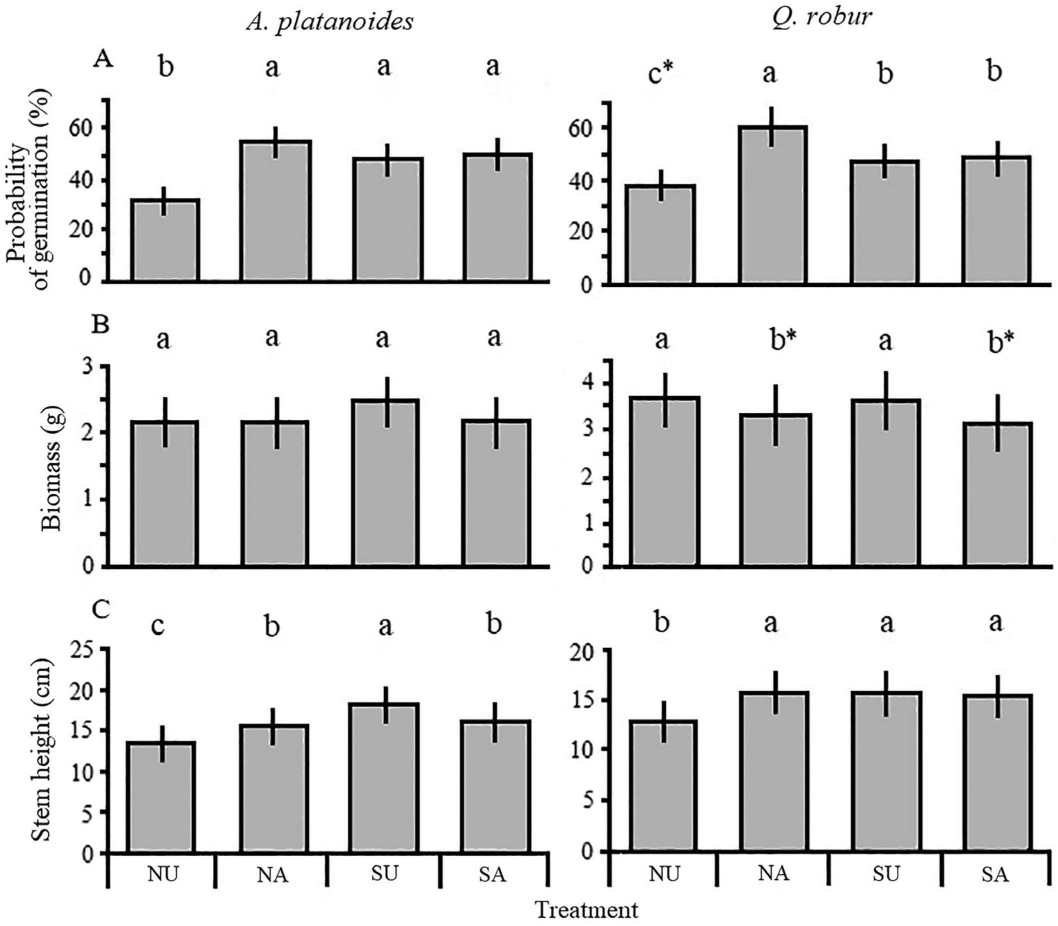

Fig. 1. Estimated (A) probability of germination, (B) biomass, and (C) stem height of native tree species with regard to treatment in the greenhouse experiment (NU, non-sterilized soil collected under conspecifics; NA, non-sterilized soil collected away from conspecifics; SU, sterilized soil collected under conspecifics; SA, sterilized soil collected away from conspecifics). Different letters indicate differences significant at $P < 0.05$, and letters with asterisk differences are marginally significant ($P < 0.10$).

7.92 ± 0. 12 m, N = 973; *A. negundo*: 5.22 ± 0.19 m, N = 440; $\chi^2 = 13.395$, $P < 0.001$; Fig. 4), supporting Prediction 3. However, we did not find any difference in this regard between the two oaks (*Q. robur:* 5.61 ± 0.50 m, N = 40; *Q. rubra*: 6.77 m ± 0.62 m, N = 68; $\chi^2 = 0.336$, $P = 0.56$; Fig 3).

DISCUSSION

Our results support the notion that invasive species are affected less by negative interactions with soil biota (consistent with the enemy release hypothesis) and that these effects decline with distance from the parent plant (consistent with the Janzen-Connell hypothesis). However, these effects led to the expected patterns of seedling distributions in *Acer* but not in *Quercus* (see Table 2 for a summary of our predictions vs. findings). Our findings are consistent with previous studies in similar systems consisting of *Acer* species (Reinhart and Callaway 2004, Saccone et al. 2010, Porté et al. 2011, Lamarque et al. 2012). Responses of *Quercus* sp. seem to be less generalizable (Vansteenkiste et al. 2005, Reinhart et al. 2012, Sheffer et al. 2013, Bogdziewicz et al. 2018*b*, *c*). Our results for *Quercus* also echo Deniau et al. (2017), who detected

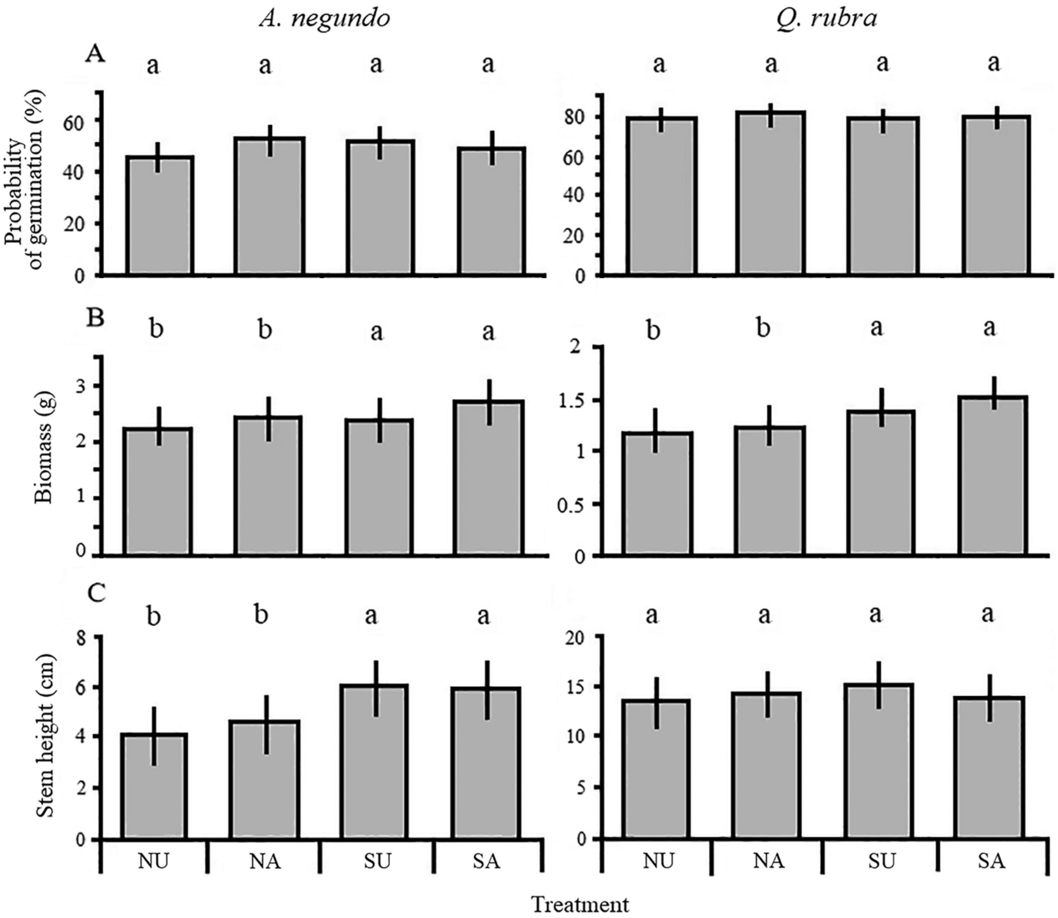

Fig. 2. Estimated (A) probability of germination, (B) biomass, and (C) stem height of invasive tree species with regard to treatment in the greenhouse experiment (NU, non-sterilized soil collected under conspecifics; NA, non-sterilized soil collected away from conspecifics; SU, sterilized soil collected under conspecifics; SA, sterilized soil collected away from conspecifics). Different letters indicate differences significant at $P < 0.05$, and letters with asterisk differences are marginally significant ($P < 0.10$).

Janzen-Connell-like patterns of tree seedling performance in native European oaks, but attribute these to a wider variety of positive and negative feedbacks between heterospecific and conspecific individuals. In *Acers*, distribution patterns in the field are broadly consistent with the Janzen-Connell hypothesis driven by distance dependence in natives but not in invasives. In some ways, this result reinforces a past study, in which both boxelder (invasive in Europe) and Norway maple (invasive in North America) grew better on soils from invaded than from native range (Reinhart and Callaway 2004). However, our results differed from theirs in that their distance-dependent effects reflected stronger positive feedback in the nonnative range, whereas we detected only negative feedback. In fact, contrary to several previous studies (Klironomos 2002, Reinhart and Callaway 2004, Saccone et al. 2010, Paudel et al. 2014, Gundale et al. 2014, Badalamenti et al. 2015, but see Beckstead and Parker 2003), we found only negative effects of soil biota across all four tested species. In previous experiments conducted on *Acers*, transplanted juveniles were used (Reinhart and Callaway 2004, Saccone et al. 2010, Porté et al. 2011, but see Meiners 2005) while our study was focused on germination and development directly after germination.

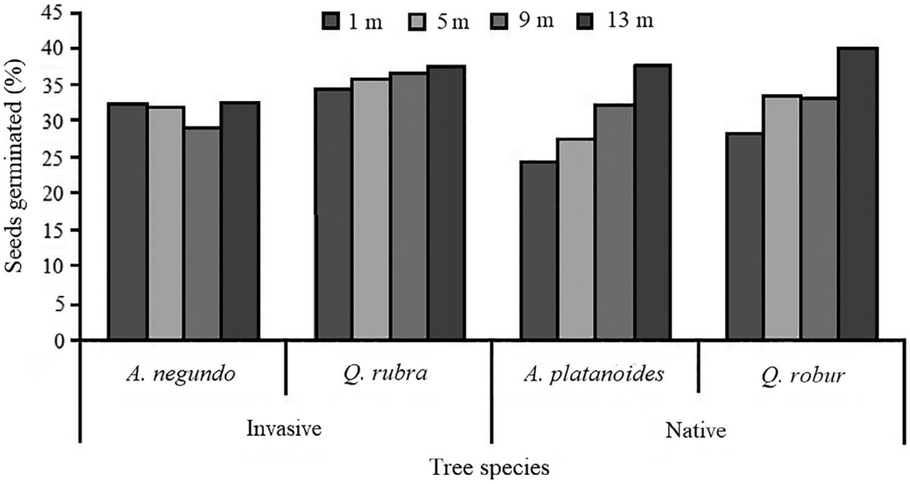

Fig. 3. Germination of seeds sown in the field at different distances to adult conspecifics.

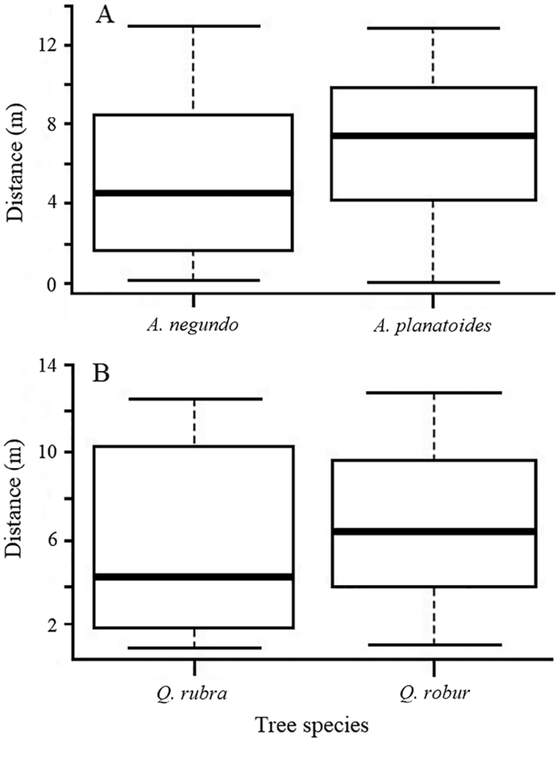

Fig. 4. Distance of recruits from adult conspecifics in (A) *Acer* and (B) *Quercus* species. Boxes denote 25th, 50th, and 75th percentiles; whiskers denote the farthest data points within 1.5 interquartile range; and the open circles denote data points beyond the 1.5 interquartile range.

Thus, this difference could reflect differential effects on different life stages as well as possible geographic variation between the specific regions of other studies.

In contrast, *Quercus* seedlings did not show patterns consistent with release from Janzen-Connell effects (even though invasive seedlings were less inhibited by soil biota than native ones in mechanistic experiments). In their native range, red oaks are often unable to establish under their own canopy (Vansteenkiste et al. 2005, Sheffer et al. 2013, but see Reinhart et al. 2012). This inhibitory effect seems to be absent in the invaded range (this study, Bogdziewicz et al. 2018*b*). However, lack of Janzen-Connell pattern in natural regeneration of both oak species might indicate that soil microbes have the potential to limit native oak recruitment as predicted by the Janzen-Connell hypothesis (and demonstrated in our greenhouse and field experiments), but other factors suppress this pattern in the field. As an example, probability of rodent seed predation on *Q. rubra* and *Q. petraea* was lower rather than higher close to adult trees (thus distance had negative rather than positive effect on seed survival) (Bogdziewicz et al. 2018*b*), potentially counteracting the pathogen effect. Moreover, *Q. rubra* in the nonnative range is rarely attacked by pre-dispersal seed predators when compared with both the co-occurring *Q. petraea* and with conspecifics in the native range (Bogdziewicz et al. 2018*a, c*). Furthermore, it is less often

Table 2. Summary of tested predictions and research findings.

Predictions	Response variables	Results
(1) Seed germination and seedling development (measured by biomass, stem height, and root length) will be negatively affected by soil collected under adult conspecifics in native, but not in invasive species	Germination	Confirmed in both *Acer* and *Quercus* (results marginally significant)
	Biomass	Not confirmed
	Stem height	Confirmed in both *Acer* and *Quercus*
	Root length	Not confirmed
(2) Germination probability will increase with distance to conspecifics in the native, but not in the invasive tree species	Germination	Confirmed in both *Acer* and *Quercus*
(3) The average distance of seedlings and saplings from adult conspecifics will be lower in invasive than in native species	Distance	Confirmed in *Acer*

consumed by rodents than the native oak, but also less readily dispersed by mice (*Apodemus flavicollis*) and jays (*Garrulus glandarius*; Wróbel *unpublished manuscript*, Myczko et al. 2014, Bogdziewicz et al. 2018*b, c*). We should also note that seedlings and saplings of both oaks were rather rare on our transects, limiting our power to detect patterns in their distribution.

Our results suggest that at least part of the invasive plant advantage might result from the differential impact of soil biota on native and invasive plants, but that these effects need to be interpreted in a broader context than any single experimental result. Even for *Acer*, not all of our findings unequivocally support our hypothesis of differential impacts of soil microbes on the invasive and the native species. The differences in germination probability and stem growth demonstrated that invasive trees may have an advantage over natives when recruiting close to conspecifics, but seedling biomass and root length were unaffected by soil treatments. In similar studies, seedling establishment was enhanced both under greenhouse conditions (Reinhart et al. 2003) and in the field (Sun et al. 2015). Moreover, stem height and biomass were increased in seedlings grown in soils from nonnative ranges in comparison with native ranges both under greenhouse conditions (Blossey and Nötzold 1995, Reinhart et al. 2003, Reinhart and Callaway 2004) and in field surveys (Jakobs et al. 2004). These contrasts emphasize the importance of considering multiple aspects of individual performance. They also point to the importance of studies such as ours that compare experimental results to field patterns. An even stronger approach would be to incorporate the effects of multiple aspects of species interactions and affected life stages into models that calculate their relative contributions to overall invasiveness (e.g., using matrix population models: Elwood et al. 2018). Although it is beyond the scope of this study, this approach would be a valuable direction for future research.

Past tests of the enemy release hypothesis have taken two approaches (Hawkes 2007): comparing performance of conspecifics in the native and invasive range, and comparing native and invasive congeners in the same location. These approaches test two different ecological hypotheses. The first tests whether invasive plants experience fewer negative interactions than they did in their native ranges, possibly leading to higher abundance in the native than in the invasive range. The second (our approach) tests whether they experience fewer negative interactions than similar native species, which could allow them to reach higher abundance than native species in the invasive range. Few studies have tested for both phenomena (but see Reinhart and Callaway 2004).

While there is a general tendency to consider whether or not a certain mechanism is involved in invasiveness (Reinhart et al. 2003, Colautti et al. 2004, Schultheis et al. 2015, Martínez-García et al. 2016), we find it important to recognize either frequency or strength of such mechanisms. Research on enemy release in invasive species to date has led to inconsistent findings either supporting the hypothesis (Wolfe 2002, Mitchell and Power 2003, Reinhart et al. 2003, Gundale et al. 2014, Correia et al. 2016) or not (Blaney and Kotanen 2001, Beckstead and Parker 2003, Schultheis et al. 2015). Thus, differential impacts of soil biota on native and nonnative plants are likely to provide a mechanism behind some, but not all, successful plant invasions (Reinhart and Callaway 2006). Moreover, release from pathogens might act synergistically with other forms of enemy release, contributing to the improved performance of invasive plants (Mitchell 2006).

As a caveat, we did not identify soil microorganisms that were responsible for the observed effects. Consequently, we cannot distinguish between direct and indirect effects of soil microbes. For example, in some cases microbes mediate the effects of autotoxicity—they break down root exudates into substances that are toxic to seeds or seedlings (Huang et al. 2013). While our study did not examine the exact mechanism of the harmful effects, it nonetheless supports the notion that soil microbes associated with adult plants harm conspecific seeds and seedlings in native, but not in invasive species. Although mechanisms of coexistence of various species and causes of invasiveness are often considered separately, they may be indeed inevitably linked. The Janzen-Connell effect has been invoked to explain coexistence in plant communities and patterns of tree abundance and rarity (Janzen 1970, Connell 1971, Klironomos 2002, Bever 2003, Freckleton and Lewis 2006, Comita 2014, Kempel et al. 2018) while this phenomenon can also play an important role in plant invasions with recognized differential impacts of enemies. Our results indicate that the strength of Janzen-Connell effect differs between native and invasive tree species, providing mechanism that could contribute to the establishment and spread of alien plants. However, there is probably multitude of factors involved in this process. An important next step would be to identify soil pathogens that cause Janzen-Connell effect in the native species, examine their virulence to *A. negundo* and *Q. rubra*, and evaluate the potential for host-switching to the invasive trees. This could eventually lead to designing effective strategies of invasive plant control and management.

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