A Test for Pre-Adapted Phenotypic Plasticity in the Invasive Tree Acer negundo L.

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Abstract

Phenotypic plasticity is a key mechanism associated with the spread of exotic plants and previous studies have found that invasive species are generally more plastic than co-occurring species. Comparatively, the evolution of phenotypic plasticity in plant invasion has received less attention, and in particular, the genetic basis of plasticity is largely unexamined. Native from North America, Acer negundo L. is aggressively impacting the riparian forests of southern and eastern Europe thanks to higher plasticity relative to co-occurring native species. We therefore tested here whether invasive populations have evolved increased plasticity since introduction. The performance of 1152 seedlings from 8 native and 8 invasive populations was compared in response to nutrient availability. Irrespective of nutrients, invasive populations had higher growth and greater allocation to above-ground biomass relative to their native conspecifics. More importantly, invasive genotypes did not show increased plasticity in any of the 20 traits examined. This result suggests that the high magnitude of plasticity to nutrient variation of invasive seedlings might be pre-adapted in the native range. Invasiveness of A. negundo could be explained by higher mean values of traits due to genetic differentiation rather than by evolution of increased plasticity.

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Introduction

Phenotypic plasticity has been widely recognized as an important feature for plants to cope with environmental changes [1,2]. Numerous studies have shown that plants are plastic for a large array of traits related to structure, development, metabolic activity, morphology, physiology, phenology, and reproduction [3–10]. Phenotypic plasticity has also been classified as a major determinant of the success of invasive species by increasing fitness relative to native species in recipient communities [11,12,13]. Broader distributions of alien species are correlated with higher levels of plasticity in response to increasing resource availability [14], and on artificial gradients, invasive species are also more plastic than co-occurring native or non-invasive species [15–20]. Davidson et al. [21] recently synthesized this work via a meta-analysis of 75 pairs of invasive/native species concluding that invasive species do express greater phenotypic plasticity than native species irrespective of the response traits measured. However, there are instances that did not support this pattern [22–25], and it has been suggested that the success and fitness advantage of invasive species can be mediated by the expression of constant higher mean trait values across different environments and not necessarily by the plasticity of these traits [26–29]. For instance, invasive species frequently possess higher trait values for growth rate [30,31,32], lower leaf mass per area [30,33,34], and advanced leaf unfolding and flowering periods [35,36,37]. Phenotypic plasticity should therefore be considered in combination with mean trait values when testing for plant invasiveness.

Higher plasticity levels of invasive species are generally hypothesized to be related to post-introduction evolution of phenotypic plasticity [13,30,39]. In this regard, intraspecific contrasts across environmental gradients have been analyzed in the following cases: (i) between populations from native and invasive ranges [40,41] and (ii) between populations within the invasive range [12,42,43]. Overall, no general pattern has emerged to date. Invasive populations of Senecio inaequidens were for instance more plastic than their native conspecifics in response to fertilization [44] while no difference was observed between native and invasive populations of Microstegium vimineum across a large array of environments [45]. Variation in light and soil moisture availability induced differences in plasticity for above-ground biomass and leaf mass per area among invasive populations of Microstegium vimineum but not for reproduction-related traits among invasive populations of Polygonum cuspidatum [42,43]. Pre-adapted plasticity might therefore be a common feature of several invasive plants, and it should now be more explicitly tested. A total of 357 tree species has been reported to be invasive worldwide disrupting major native ecosystem structure and functioning [46,47,48]. Invasive trees are thus appropriate models to evaluate the role of ecological and evolutionary processes in invasion given their large impacts, frequency, and longevity [49–
Table 1. The 16 source populations sampled to examine phenotypic plasticity of invasive tree species Acer negundo L.

| Province/Department | Collection site | River | Latitude | Longitude | Distance to the nearest population (km) |
|---------------------|-----------------|-------|----------|-----------|---------------------------------------|
| Native range        |                 |       |          |           |                                       |
| Ontario             | Paris           | Grand | 43°12’27”N | 80°21’58”W | 65                                    |
|                     | Fergus          | Grand | 43°41’53”N | 80°22’50”W | 65                                    |
|                     | Nicolston       | Nottawasaga | 44°10’40”N | 79°49’02”W | 18                                    |
|                     | Angus           | Nottawasaga | 44°18’59”N | 79°53’08”W | 18                                    |
|                     | Toronto Home Smith park | Humber | 43°39’06”N | 79°29’44”W | 26                                    |
|                     | Toronto Serena Gundy park | Don | 43°43’05”N | 79°21’15”W | 26                                    |
| Quebec              | Sherbrooke      | Saint-François | 45°23’44”N | 71°52’50”W | 24                                    |
|                     | Windsor         | Saint-François | 45°34’04”N | 72°00’23”W | 24                                    |
| Invasive range      |                 |       |          |           |                                       |
| Landes              | Saubusse        | Adour | 43°39’22”N | 01°11’13”W | 10                                    |
|                     | Riviere-Saas-et-Gourby | Adour | 43°40’29”N | 01°08’06”W | 10                                    |
|                     | Pontonx-sur-l’Adour | Adour | 43°47’03”N | 00°55’30”W | 35                                    |
| Gironde             | Cestas          | Eau Bourde | 44°45’20”N | 00°40’49”W | 30                                    |
|                     | Bruges          | Les Jalles | 44°54’13”N | 00°36’16”W | 30                                    |
|                     | Moulon          | Dordogne | 44°51’30”N | 00°13’10”W | 19                                    |
|                     | Castillon-la-Bataille | Dordogne | 44°51’04”N | 00°02’16”W | 19                                    |
|                     | St-Denis-la-Pile | Isle | 44°59’34”N | 00°12’29”W | 22                                    |

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53]. To date, most studies examining the importance of phenotypic plasticity in tree invasion compared invasive vs. native tree species [15,39,54,53]. With the exception of Melaleuca quinquenervia and Triadica sebifera [39,56], the genetic differences in plasticity between native and invasive populations of exotic trees are unexamined.

Porté et al. [57] recently found that the invasive tree Acer negundo significantly expressed higher magnitude of phenotypic plasticity than its co-occurring native species with increasing environmental resources, and particularly nutrient availability. The purpose of this study was therefore to examine the genetic basis of plasticity in A. negundo, i.e. to determine whether higher plasticity of invasive populations is due to post-introduction evolution or pre-adaptation in the native range. The performance of native and invasive populations of A. negundo was compared across a gradient of nutrient availability. Life-history traits related to growth, physiology, leaf morphology and biomass and known to promote plant invasiveness [31,32] were measured. We hypothesize that invasive populations possess greater plasticity in growth and associated traits relative to populations from the native range. These findings would support the idea that plasticity could have evolved in the introduced range. In contrast, the absence of difference in plasticity between populations from native and invasive ranges would indicate that higher plasticity of invasive populations of A. negundo could be due to pre-adaptation in the native range.

Materials and Methods

Studied Species

Acer negundo L. (Box Elder or Manitoba maple) is a widely distributed mid-successional species native to North America. Its distribution range extends from southern Alberta and central Manitoba to Mexico and Guatemala southward and from central Montana to New England states and central Florida eastward [58,59,60]. This species is frequently found in floodplains and riparian habitats but can also occur in dry coniferous forests, oak savannas, and grasslands [61,62]. A. negundo was intentionally introduced in the Old Continent at the end of the seventeenth century, i.e. in 1688 in England, and in France in 1749 [60,63]. Currently, A. negundo is highly invasive throughout southern, central and eastern Europe [60,64]. It frequently occurs not only in riparian habitats characterized by high rate of flood disturbance and high soil nutrient level [65,66] but also under drier conditions along roadsides, industrial wastelands, and dry ruderal sites [52,67].

Experimental Design

Seeds of A. negundo were harvested between September and November 2009 from eight native populations sampled in Ontario and Quebec, Canada and from eight invasive populations located in the Landes and Gironde departments of Aquitaine region, Southern France (Table 1). No specific permissions were required for these locations that are not part of protected areas and do not involve endangered species. All native and invasive populations were sampled from riparian forests. Populations in the invasive range were distributed within the Adour-Garonne river basin. Seeds came from 9 to 12 maternal trees in each source populations with maternal trees randomly selected and at least 10 m apart. In February 2010, 30 seeds per maternal tree were subjected to a cold treatment (14 weeks at 5°C in a cold chamber) at the INRA research station of Pierroton, France (44°44’N, 0°46’W). In spring 2010, 27 seeds per maternal tree were sown into 4 L (15×15×17.7 cm) pots filled with a commercial sphagnum peat soil mixture (organic matter 80%, pH = 6). We first sowed three seeds per pot until germination and then kept one seedling in each pot thereby generating a total of 90 seedlings per source population. Pots were then placed under a greenhouse that was side-opened to permit wind and insects to enter. We did not
control light and temperature that approximated ambient conditions. Seedlings were watered twice a week to saturation.

A split-plot design was used with nutrient level as the fixed main effect and range of *A. negundo* populations (native or invasive) as the fixed sub-effect with all native and invasive populations subjected to three nutrient levels. We selected 72 seedlings from 8 to 10 families (i.e. maternal trees) per population for a total of 1152 seedlings structured as follows: 6 blocks × 3 nutrient levels × 2 ranges × 8 populations × 4 individuals. The experiment was initiated on February 17th, 2011 and lasted 147 days. Nutrients were applied on the 25th, 53rd, 81st and 109th days of the experiment. The nutrient treatment corresponded to the addition

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**Figure 1.** Mean ± SE of life-history traits for native and invasive seedlings of *Acer negundo*. Differences in growth (A,B), physiology (C,D), leaf morphology (E), biomass (F,G) and biomass allocation (H) were calculated across nutrient levels. n = 576 (height and diameter), 96 (A\textsubscript{area}) and 144 (N\textsubscript{area}, LMA, W\textsubscript{r} and A\textsubscript{l}) per range. See text for definition of terms. * P<0.05, ** P<0.01, *** P<0.001.

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of the complete slow release 16-7-15 (NPK plus micronutrients) fertiliser Floranid Permanent (Compo France SAS, Levallois-Perret, France). In the low nutrient level (N0), seedlings did not receive any additional fertilizer. In the medium and high nutrient levels (N1 and N2, respectively), seedlings received four fertilizer doses equivalent to 0.125 g and 0.500 g N each, for a total of 0.500 g N. During the pre-adaptation phase, seedlings were grown in a greenhouse in pre-adaptation chambers with a 16-h light period 600 μmol m−2 s−1 PPFD, a temperature of 22 °C and a relative humidity of 80%±10% of ambient. Growth was measured by recording the total length of each seedling using a camera and image analysis software. A pre-adaptation period was used in order to allow the seedlings to acclimate to the different nutrient levels before the main experiment started. This was necessary due to the high variability in growth potential among seedlings from different cuttings. Seedlings were pre-adapted for 6 weeks.

Gas Exchange
Photosynthetic rate measurements were performed on 192 seedlings. In each treatment, four individuals from different families and blocks were randomly sampled per source population. The measurements were done on sunny days between June 20th and July 7th. Leaf gas exchange measurements were carried out with a portable steady-state flow-through chamber (PLC6) connected to an infrared gas analyser (CIRAS-2, PP Systems, Hitchin, UK) equipped with temperature, humidity, light and CO2 control modules. Net gas exchanges were measured within a 600 μmol PAR m−2 s−1 standard cuvette of 2.5 cm2, with an air CO2 concentration of 380±5 ppm, a temperature of 22±0.5°C and a relative humidity of 80%±10% of ambient, controlled by regulating the flow diverted through a desiccant. To obtain the maximum assimilation rate per unit leaf area (Aarea μmol CO2 m−2 s−1) at ambient CO2, leaves were illuminated with a red-blue light source attached to the gas exchange system and maintained at saturated light (PPFD = 1500 μmol PAR m−2 s−1). Prior to the measurements, the gas analyser was calibrated in the laboratory using 400 ppm standard gas, while full CO2 and H2O zero and differential calibrations were performed in the field after each set of six measurements. Up to three measurements were carried out on each sampled individual, and data were recorded when assimilation curves remained stable for more than 20 s. All measurements were taken between 8:00 and 11:00 solar time on fully expanded and sun-exposed leaves to avoid midday stomatal closure.
Leaf Morphology and Biochemistry

Leaf nitrogen content and morphological traits were measured on 288 seedlings representing six individuals per population and per treatment (including those used for gas exchange measurements). Leaves were sampled on the same days as the photosynthetic rate measurements. Three to five leaves were collected per sampled individual. Leaf surface area was measured with a planimeter (Light Box model, Gatehouse, Scientific Instruments LTD, Norfolk, UK) and the average leaf size ($L_s$, cm$^2$) was calculated. Leaves were then placed in an oven at 65°C until constant dry weight and leaf dry mass was later weighed with an electronic weighing scale (Explorer Pro, EP 114 model, Ohaus Corporation, Pine Brook, NJ, USA). Leaf mass per area index (LMA, g leaf m$^{-2}$ leaf) was calculated as the ratio of leaf weight by leaf area. Finally, leaf samples were crushed to a powder with a ball mill (MM 200, Fisher Bioblock Scientific, France) and leaf nitrogen content ($N_{mass}$, %) was determined using an elementary analyser Eager 300 CHNOS (FlashEA 1112, ThermoElectron).

![Figure 2: Plasticity of native and invasive seedlings of Acer negundo to nutrient availability.](https://www.plosone.org/figure/2)

*Figure 2. Plasticity of native and invasive seedlings of Acer negundo to nutrient availability.* Means ± SE of traits related to growth (A,B), physiology (C,D), leaf morphology (E), biomass (F,G) and biomass allocation (H) are represented. n = 192 (height and diameter), 32 ($A_{area}$) and 48 ($N_{area}$, LMA, $W_t$ and $A_t$) per range and nutrient level. See text for definition of terms.

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leaf biomass were separated from soil and washed. Biomass was oven-dried at 80°C until constant dry weight and further weighed using an electronic weighing scale (Explorer Pro, EP 114 model, Ohaus Corporation, Pine Brook, NJ, USA). The following traits were measured: total biomass (W₂, g), stem biomass (Wₛ, g), root biomass (Wᵣ, g), total leaf area (Aₐ, m²), leaf nitrogen content per leaf area (Narea, g N m⁻²), leaf nitrogen content per leaf dry mass (Nmass, g N g⁻¹), leaf nitrogen content per leaf mass (Nmass, g N g⁻¹), stem weight ratio (SWR, g stem g⁻¹ plant), root weight ratio (RWR, g root g⁻¹ plant) and leaf area ratio (LAR, m² leaf g⁻¹ leaf).

### Growth and Biomass

A total of seven individuals died during the course of the experiment and therefore, final height and stem collar diameter of 1145 seedlings were recorded on July 4th. A graduated pole to 0.12 m accuracy was used to record heights, and diameters were measured with an electronic calliper to 0.01 mm accuracy. The 288 individuals previously used for morphological measurements were harvested on July 14th after 147 days of growth. Above-ground biomass was separated into stems and leaves, and roots were separated from soil and washed. Biomass was oven-dried at 65°C until constant dry weight and further weighed using an electronic weighing scale (Explorer Pro, EP 114 model, Ohaus Corporation, Pine Brook, NJ, USA). The following traits were calculated: total biomass (W₂, g), above-ground biomass (Wᵢ, g), leaf biomass (Wᵢ, g), stem biomass (Wₛ, g), root biomass (Wᵣ, g), total leaf area (Aₐ, m²), root shoot ratio (RSR, g g⁻¹), leaf weight ratio (LWR, g leaf g⁻¹ plant), stem weight ratio (SWR, g stem g⁻¹ plant), root weight ratio (RWR, g root g⁻¹ plant) and leaf area ratio (LAR, m² leaf g⁻¹ leaf).

### Statistical Analyses

Differences in traits were tested with a generalized linear mixed model that was fit to a split-plot design (procedure MIXED, REML method in SAS, version 9.2, SAS Institute, Cary, NC, USA) [70]. We used nutrient level, range, and the interaction of nutrient level × range as fixed factors whilst block, block × range, population nested within range, and the interaction of nutrient level × population nested within range were treated as random factors. To account for the influence of plant size on biomass allocation [71], we used total biomass as a covariate when we tested the following traits: Aₐ, RSR, LWR, SWR, RWR and LAR. Type III sums of squares were used for the calculation of F statistics. Random effects were further evaluated using a log likelihood ratio (LLR) test from the full and reduced models. All factors were identified significant at alpha <0.05. A significant range effect for a given trait indicated an overall genetic differentiation between seedlings from native and invasive populations. Moreover, phenotypic plasticity was examined here at the population-level [13,72]. A significant effect of nutrient level indicated plasticity for a given trait. The difference in plasticity of a given trait between seedlings from native and invasive populations

## Table 3. Relative Distance Plasticity Index (RDPI) along a nutrient gradient for populations of Acorn negundo from the native and invasive ranges.

| Traits               | RDPI low-to-medium nutrient levels | RDPI medium-to-high nutrient levels |
|----------------------|-----------------------------------|------------------------------------|
|                      | Invasive  | Native               | Invasive  | Native               |
| **Growth**           |          |                      |          |                      |
| Height               | 0.12±0.02 | 0.10±0.02           | 0.04±0.01 | 0.05±0.01           |
| Diameter             | 0.08±0.01 | 0.07±0.01           | 0.04±0.01 | 0.04±0.01           |
| **Leaf traits**      |          |                      |          |                      |
| Aarea                | 0.29±0.04 | 0.28±0.04           | 0.19±0.03 | 0.14±0.04           |
| Amass                | 0.24±0.04 | 0.25±0.06           | 0.15±0.03 | 0.11±0.03           |
| Narea                | 0.24±0.03 | 0.22±0.02           | 0.27±0.02 | 0.28±0.04           |
| Nmass                | 0.20±0.04 | 0.19±0.03           | 0.27±0.02 | 0.25±0.03           |
| PNUE                 | 0.11±0.04 | 0.14±0.04           | 0.21±0.05 | 0.22±0.04           |
| LMA                  | 0.06±0.02 | 0.09±0.02           | 0.03±0.01 | 0.10±0.02*          |
| Ls                   | 0.11±0.01 | 0.07±0.03           | 0.11±0.03 | 0.08±0.02           |
| **Biomass**          |          |                      |          |                      |
| W₁                   | 0.23±0.04 | 0.26±0.06           | 0.08±0.02 | 0.14±0.04           |
| Wₛ                   | 0.24±0.04 | 0.28±0.07           | 0.09±0.03 | 0.15±0.05           |
| Wᵣ                   | 0.28±0.06 | 0.31±0.08           | 0.12±0.04 | 0.15±0.04           |
| Wᵢ                   | 0.23±0.04 | 0.28±0.07           | 0.09±0.02 | 0.16±0.05           |
| Wᵣ                   | 0.21±0.05 | 0.20±0.05           | 0.09±0.04 | 0.11±0.03           |
| Aᵢ                   | 0.27±0.06 | 0.28±0.08           | 0.11±0.03 | 0.10±0.02           |
| **Biomass allocation** |        |                      |          |                      |
| RSR                  | 0.15±0.03 | 0.12±0.04           | 0.09±0.03 | 0.10±0.01           |
| LWR                  | 0.11±0.04 | 0.09±0.02           | 0.08±0.04 | 0.06±0.04           |
| SWR                  | 0.04±0.01 | 0.04±0.01           | 0.02±0.01 | 0.03±0.01           |
| RWR                  | 0.11±0.02 | 0.09±0.03           | 0.07±0.02 | 0.07±0.01           |
| LAR                  | 0.13±0.04 | 0.13±0.03           | 0.12±0.03 | 0.16±0.03           |

Comparisons of RDPI using a Generalized Linear Mixed Model with range as a fixed factor and population nested within range as a random factor. Significant difference between ranges (P<0.05) denoted by an asterisk. See text for definition of terms. doi:10.1371/journal.pone.0074239.t003

Pre-Adaptation of Plasticity in Acer negundo.
was reported when the interaction of nutrient level \( \times \) range was significant. The variation of trait of native and invasive seedlings was also reported as follows: \((1 - \text{trait}_{\text{env2}}/\text{trait}_{\text{env1}})\times 100\). Lastly, we calculated the Relative Distance Plasticity Index (RDPI) [73], and the Plasticity Index (PI) [5] for two experimental nutrient level changes, low-to-medium and medium-to-high, as follows:

\[
\text{RDPI} = \frac{\text{mean(env1)} - \text{mean(env2)}}{\text{mean(env1)} + \text{mean(env2)}}
\]

\[
\text{PI} = \frac{\text{mean(env1)} - \text{mean(env2)}}{\max(\text{mean(env1)}, \text{mean(env2)})}
\]

For each trait, the two indexes were calculated for each population using mean values in each treatment (i.e. nutrient level). The difference in RDPI and PI between native and invasive ranges was examined using a generalized linear mixed model with range as a fixed factor and population nested within range as a random factor.

### Results

#### Overall Trends

Irrespective of nutrients, individuals of \( A. \ negundo \) from invasive populations expressed significantly greater heights and smaller diameters than their native conspecifics (significant range effect; Table 2; Fig. 1A, B; see Table S1 for means per treatment). There was no significant difference in maximum assimilation rates \( (\text{A}_{\text{area}} \text{ and } \text{A}_{\text{mass}}; \text{Fig. 1C}) \), and invasive seedlings had lower leaf nitrogen contents \( (\text{N}_{\text{area}} \text{ and } \text{N}_{\text{mass}}; \text{Fig. 1D}) \) and greater PNUE (Table 2). Invasive seedlings also had lower average leaf size and LMA (Table 2; Fig. 1E). There were no statistical differences in total and aboveground biomass (Table 2; Fig. 1F). Seedlings from invasive populations however allocated more resources to foliage than to roots, displaying greater \( \text{A}_r \), LWR, SWR and LAR, and lower \( \text{W}_r \), RSR and RWR compared to seedlings from native populations (Table 2; Fig. 1G, H). Significant genetic variations were found in diameter among invasive populations (within invasive range: LLR = 5.6, \( P = 0.018 \); within native range: LLR = 0.6, \( P = 0.44 \)) and in height among native populations (within native range: LLR = 14.1, \( P = 0.0002 \); within invasive range: LLR = 0.5, \( P = 0.44 \)).

### Table 4. Plasticity Index (PI; Valladares et al. 2000) along a nutrient gradient for populations of \( A. \ negundo \) from the native and invasive ranges.

| Traits | PI low-to-medium nutrient levels | PI medium-to-high nutrient levels |
|--------|---------------------------------|----------------------------------|
|        | Invasive | Native | Invasive | Native |
| **Growth** | | | | |
| Height | −0.22±0.03 | −0.17±0.03 | −0.04±0.03 | −0.01±0.04 |
| Diameter | −0.14±0.02 | −0.13±0.02 | −0.06±0.03 | −0.07±0.02 |
| **Leaf traits** | | | | |
| \( A_{\text{area}} \) | −0.44±0.04 | −0.43±0.05 | −0.14±0.11 | −0.14±0.09 |
| \( A_{\text{mass}} \) | −0.38±0.04 | −0.38±0.09 | −0.13±0.10 | −0.07±0.08 |
| \( N_{\text{area}} \) | −0.38±0.03 | −0.35±0.03 | −0.43±0.02 | −0.43±0.05 |
| \( N_{\text{mass}} \) | −0.31±0.07 | −0.32±0.05 | −0.43±0.02 | −0.39±0.04 |
| PNUE | −0.14±0.07 | −0.17±0.09 | 0.33±0.07 | 0.35±0.06 |
| LMA | −0.06±0.05 | −0.02±0.07 | 0.01±0.03 | −0.02±0.08 |
| \( L_s \) | −0.12±0.06 | −0.08±0.07 | −0.17±0.05 | −0.09±0.05 |
| **Biomass** | | | | |
| \( W_t \) | −0.30±0.09 | −0.35±0.10 | −0.07±0.06 | −0.07±0.10 |
| \( W_v \) | −0.32±0.10 | −0.39±0.09 | −0.10±0.06 | −0.10±0.11 |
| \( W_a \) | −0.40±0.08 | −0.43±0.08 | −0.21±0.06 | −0.13±0.10 |
| \( W_r \) | −0.29±0.10 | −0.37±0.10 | −0.07±0.06 | −0.09±0.12 |
| \( W_f \) | −0.23±0.11 | −0.28±0.09 | −0.01±0.08 | 0.00±0.08 |
| \( A_t \) | −0.40±0.08 | −0.39±0.09 | −0.18±0.06 | −0.13±0.05 |
| **Biomass allocation** | | | | |
| RSR | 0.14±0.09 | 0.20±0.06 | 0.09±0.06 | 0.11±0.06 |
| LWR | −0.18±0.06 | −0.17±0.03 | −0.13±0.04 | −0.06±0.04 |
| SWR | 0.00±0.03 | −0.05±0.03 | 0.01±0.02 | −0.02±0.03 |
| RWR | 0.12±0.07 | 0.14±0.05 | 0.06±0.05 | 0.07±0.04 |
| LAR | −0.12±0.09 | −0.12±0.08 | −0.15±0.07 | −0.03±0.11 |

Comparisons of PI using a Generalized Linear Mixed Model with range as a fixed factor and population nested within range as a random factor. See text for definition of terms.
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 Trait Plasticity

 Seedlings of _A. negundo_ responded significantly to increases in nutrients (significant nutrient effect for all traits but LMA and SWR; Table 2; Fig. 2) with increased growth, maximum assimilation rate, total biomass, and above-ground allocation (AGB, TLA, LWR, LAR) and decreased below-ground allocation (RWR, RWR). The change from low-to-medium nutrient conditions had a stronger effect on seedling trait values than the change from medium-to-high nutrient conditions. Individuals of _A. negundo_ respectively showed a 19%, 44% and 35% increase in height, maximum assimilation rate and total biomass from low-to-medium nutrient conditions but a 2%, 18% and 9% increase from medium-to-high nutrient conditions (Fig. 2; see Tables 3 and 4 for trait RDPI and PI values). Across all populations, traits such as SWR and LMA showed low plasticity along the nutrient availability gradient (mean RDPI\_SWR = 0.03, mean RDPI\_LMA = 0.07) while W\_t, A\_area, and N\_area exhibited larger changes (mean RDPI\_W\_t = 0.21, mean RDPI\_A\_area = 0.22, mean RDPI\_N\_area = 0.25). There were no significant differences in plasticity between seedlings from native and invasive populations for any traits (non-significant nutrient x range effect; Table 2; Fig. 2). There was also no difference in RDPI or PI for any traits but the RDPI\_LMA, between medium and high nutrient levels did differ (Tables 3 and 4; across the whole gradient, mean trait RDPI = 0.15 and 0.14 and mean trait PI = 0.14 and 0.15 for native and invasive populations, respectively). The magnitude of plasticity differed at the population level for height, maximum assimilation rate, and SWR (significant nutrient x population effect; Table 2).

 Discussion

 Higher magnitudes of plasticity relative to native species are common in invasive plants, particularly in invasive trees [15,21,74]. Nevertheless, these differences are not necessarily a product of post-introduction evolution and can also be explained by innate characteristics. This null hypothesis was tested and supported in this study using the highly invasive tree species _Acer negundo_. Although increased nutrient availability is a key component of tree recruitment dynamics [75,76], this artificial gradient tested here did not elicit differences in plasticity between native and invasive seedlings. Pre-adapted plasticity to nutrient availability is thus a reasonable explanation for the successful spread of this species, at least at this early stage of development.

 The evolution of plasticity in invasive species is relatively infrequent and no consensus has been reached in the literature so far (Table S2). Variation in resource conditions lead to differences in plasticity between seedlings from native and invasive populations for perennials _Centauraea stoebe_ and _Taraxacum officinale_ and trees _Melaleuca quinquenervia_ and _Triadica sebifera_ also outperformed native congeners [39,36,85]. Interestingly, invasive seedlings of _A. negundo_ did not achieve greater height growth via physiological advantages but only via a preferential allocation to foliage. Significant lower leaf nitrogen content and similar maximum assimilation rate were found here. This contradicts recent studies on the genetically-based difference of functional traits in invasive plant species that showed higher values of physiological traits for invasive genotypes [86,87,88]. These divergences might be due to the rapid adaptation of _A. negundo_ in its introduced range reflecting a change in adaptive strategy. Whilst plasticity may not have evolved _de novo_, it is possible that most of the traits conferring faster growth (such as greater allocation to above-ground biomass) may have done so to provide a competitive advantage over native species of recipient communities.

 Multi-species comparisons in the native range of exotic plant species showed that invasive aliens differed in traits but not in plasticity from their non-invasive alien congeners [26,28], and pre-adaptation of plasticity in invasive plant species might finally be more common than expected. Phenotypic plasticity is a common denominator for invasive plant species but tolerance of invasive genotypes across a broad range of conditions might rely more on a combination of life-history traits rather than on evolved plasticity in the introduced range. This would be the case for _A. negundo_ since the species occupies wide and similar ranges of habitats such as wet-rich and dry-poor nutrient riparian forests both in North America and in Europe [52,61,89]. Furthermore, various mechanisms such as founder effects, multiple introductions, and selective pressures can drive genetic differentiation between native and invasive populations. Molecular analyses using neutral markers over large areas sampled including whole native and invasive ranges would thus be necessary to fully understand the role of these factors [90]. Given that there was no consistent variation in traits amongst populations from the invasive range, genetic data would provide valuable information on the origin of those populations sampled in French riparian areas, e.g. whether they have all undergone similar selective pressures or come from the same pool of native populations which were not sampled in this study (i.e. founder effects).

 Conclusions

 The origin of increased plasticity in invasive plant species is an important and relatively understudied set of hypotheses. Given the geographical scope of the populations we were able to sample herein, pre-adaptation is a more viable explanation for the high magnitude of plasticity of invasive _A. negundo_ seedlings to variation in nutrient availability. Future studies should however test in the native range the response of native and invasive genotypes sampled at broader scales to a combination of abiotic factors in order to test more effectively both the importance of evolved versus
Acer negundo

of allocation of eight native and eight invasive populations

exchange and leaf morphology, biomass and biomass

comparing phenotypic plasticity between native and invasive

Summary of studies

References

Table S1

Table S2

Table S2 Intraspacific comparisons of phenotypic plasticity in invasive plant species. Summary of studies comparing phenotypic plasticity between native and invasive populations of exotic plant species in response to variation in environmental conditions. Plasticity was reported for various traits related to biomass (B), defense to herbivory (D), growth (G), leaf morphology (M), phenology (P), physiology (P) and reproduction (R).

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Author Contributions

Conceived and designed the experiments: LJL, AJP SD. Performed the experiments: LJL, CEJBL. Analyzed the data: LJL, AJP CJL SD. Wrote the paper: LJL AJP CJL SD.

Pre-Adaptation of Plasticity in A. negundo

pre-adapted plasticity and increases in competitive ability of invasive species.

Supporting Information

Table S1 Mean ± SE for traits related to growth, gas exchange and leaf morphology, biomass and biomass allocation of eight native and eight invasive populations of Acer negundo grown along a nutrient gradient. Sample sizes are \( n = 24 \) for growth traits, \( n = 4 \) for physiology traits and \( n = 6 \) for leaf morphology and biomass related traits. See text for definition of terms.

Table S2 Intraspacific comparisons of phenotypic plasticity in invasive plant species.

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