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The Effects of Soils from Different Forest Types on the Growth of the Invasive Plant *Phytolacca americana*

Peng-Dong Chen [1], Yu-Ping Hou [1,*], Yan-Hui Zhuge [1], Wei Wei [1] and Qiao-Qiao Huang [2]

[1] College of Life Sciences, Ludong University, Yantai 264025, China; chen_pendong@163.com (P.D.C.); zhuge_yanhui@163.com (Y.H.Z.G.); zz8z00001@163.com (W.W.)

[2] Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China; huangqq@catas.cn

* Correspondence: hou_yuping@163.com; Tel.: +86-535-669-5003

Abstract: Due to increasing globalization and human disturbance, plant invasion has become a worldwide concern. Soil characteristics associated with the vegetation of recipient communities affect plant invasion success to a great extent. However, the relative importance of soil biotic and abiotic factors of different recipient communities in resisting plant invasion is not fully understood. We hypothesized that natural forest soils can better resist plant invasion than can plantation soils, that the allelopathic legacy of resident trees in soil plays a role in resisting invasive plants, and that late-successional soils have a strong effect. We examined the effects of soil and litter collected from four natural forests at successional stages and one *Robinia pseudoacacia* Linn. plantation in eastern China on the growth of *Phytolacca americana* L., which is a highly invasive species across China, and explored the individual effects of soil nutrients, allelochemicals, and soil microbes. We found that allelopathic activity of natural forest soils can effectively resist *P. americana* invasion, and that low level of nutrients, especially of phosphorus, in the soils might be potential limiting factors for the plant growth. The profound conditioning of soil resources by exotic *R. pseudoacacia* based on tree traits (including allelopathy) facilitated further *P. americana* invasion. Allelochemicals from forest litter inhibited the germination of *P. americana* seeds, but pH played a major role in *P. americana* growth when these substances entered the soil. However, we have no evidence that late-successional forest soils exhibit strong allelopathy toward *P. americana*. The present study will help to further our understanding of the mechanism of community resistance to invasion.

Keywords: plant invasion; community invasibility; forest soil; allelopathy; *Phytolacca americana*

1. Introduction

Due to increasing globalization and human disturbances, plant invasion has become a worldwide concern [1,2]. Understanding the factors that regulate community invasibility is critical for predicting and controlling plant invasions [3]. Prior studies have shown that successful plant invasions are related to the vegetation characteristics of recipient communities; these characteristics actually affect both the resource availability of habitats and biotic resistance [4–8]. For example, high species diversity sufficiently fills available niches and limits resource supply for invaders, while native species decrease establishment success of invader species that are similar to them in species traits (limiting similarity) [9–11]. Invaders become established only if propagules can survive while growing to maturity on the resources left unconsumed by resident species (resource-dependent establishment) [11]. Plant species shape their soil conditions, including the

biogeochemistry and biota, by governing, e.g., nutrient uptake processes, interactions with symbiotic mycorrhiza, and litter decomposition [12,13]. The scarcity of soil nutrients will decrease the probability that an invader propagule will survive and reach maturity, though soil resource pulses or disturbances will allow invaders to overcome this recruitment limitation [11,14]. However, soil nutrients often affect plant performance in combination with soil biotic factors rather than acting alone. For instance, soil pH and carbon content largely determine the soil microbial community, and soil microbe-linked nitrogen availability can impact invasion success [15–17]. Thus, the coupling of soil nutrients and biotic factors of associated vegetation may play a key role in determining community invasibility.

Allelopathy is the biological phenomenon of chemical interactions between living organisms in the ecosystem [18]. Plants release allelochemicals into the surrounding environment via volatilization, rain-induced leaching, litter decomposition and root exudation, and these allelochemicals can directly affect the growth of neighboring plants [18–21]. Furthermore, allelochemicals can cause changes in soil abiotic factors (e.g., soil nutrients) and biotic factors (e.g., soil microbes), resulting in a legacy effect and indirectly affecting the performance of subsequent plants [21–25]. Therefore, the soil-mediated transformation of allelochemicals is critical for plant performance. After an allelochemical reaches the soil, various mechanisms, such as microbe decomposition, surface adsorption, polymerization, and pH change, can reduce its direct toxicity toward plants or increase the toxicity of inert secondary metabolites [26–28]. Specifically, interactions between soil microbes and allelochemicals are crucial to invasion success [28,29]; for example, allelochemical-induced specific alterations in the soil microbial community may result in positive or negative feedback effects on plant growth [30–33]. Allelopathy has been extensively studied as one mechanism underlying the success of invaders [18,19,23,34]. However, some studies provide preliminary evidence that native plants exert allelopathic inhibitory activities against invasive plants, such as through the "Homeland Security" hypothesis and by designing and developing allelochemicals as highly sensitive and selective natural herbicides [35–39], although these processes have not been well studied. Additionally, studies have found that continuous accumulation of tree allelochemicals in soil drives the succession of forest communities, which simultaneously enhances the community resistance of a late-successional forest [39–41]. Currently, how allelopathy between native species and associated forest types resists invasive plants is poorly understood.

Phytolacca americana L. is a perennial herb in the family of Phytolaccaceae. This North American species, introduced in China in 1935, is now distributed across the country [42]. Studies have shown that *P. americana* is highly competitive with native plants due to its distinct characteristics, such as its large number of seeds [43], high photosynthetic capacity, morphological and physiological adaptability, and resource utilization efficiency [44,45]; it has severely invaded some coastal forests and poses a serious threat to biodiversity in those forests [46]. The China Forestry Administration listed *P. americana* as an invasive plant in 2009. *P. americana* has a broad distribution in *Robinia pseudoacacia* plantations, and individual plants can grow to adult height, though this infrequently occurs or poor growth is seen in other forests, such as *Pinus densiflora* Sieb. et Zucc. and *Quercus acutissima* Carruth. forests [46]. In this study, we selected four natural forests at different successional stages and an *R. pseudoacacia* plantation in the Kunyu Mountain National Nature Reserve to study the influence of soil and litter on the growth of *P. americana*. We hypothesized that (1) *P. americana* does not grow well in soils from natural forests but is better suited to an *R. pseudoacacia* plantation; (2) allelopathy inhibits *P. americana* growth in natural forests and promotes its growth at an *R. pseudoacacia* plantation; and (3) late-successional forest soils have stronger allelopathic inhibition to *P. americana* than do early-successional forest soils.

2. Materials and Methods

2.1. Study Area

The Kunyu Mountain range (121.37°–121.48° E, 37.10°–37.19° N) is located in eastern China, with a total area of 48 km^2 and maximum elevation of 923 m. The Kunyu Mountain has a warm-temperate monsoon and marine climate with an annual rainfall of 900–1000 mm and an annual average temperature of 11.8 °C. The area has four distinct seasons, summers are hot and winters are cold, with sufficient rainfall and a complex topography that provides suitable conditions for the survival and reproduction of various plants, resulting in high biodiversity. The mountain is a forest nature reserve with Chinese *P. densiflora* as the main protected species. We selected five types of forests in this mountain region as described by Wang and Zhou [47]. The information on the particular forest types is presented in Table 1.

Table 1. Information on the five types of forests.

Forest name	Forest type	Abbreviation	Dominant trees	Distribution
Pinus densiflora forest	Natural pine forest	F1	*Pinus densiflora*	Major distribution forest
Pinus densiflora+Quercus acutissima mixed forest	Natural pine-broadleaf mixed forest	F2	*Pinus densiflora* and *Quercus acutissima*	Major distribution forest
Quercus acutissima forest	Natural broadleaved forest	F3	*Quercus acutissima*	Major distribution forest
Deciduous broadleaved mixed forest	Natural secondary forest	F4	No obvious dominant species; common tree species: *Sorbus alnifolia* (Sieb. et Zucc.) K. Koch, *Ailanthus altissima* (Mill.) Swingle, *Kalopanax septemlobus* (Thunb.) Koidz., etc.	Distribution is relatively small
Robinia pseudoacacia plantation	Plantation	RP	*Robinia pseudoacacia*	Fragmented distribution on both sides of the roads

The *P. densiflora* forest (F1) is a coniferous forest in the early succession stage, and the *Q. acutissima* forest (F3) is a broadleaved forest in the late succession stage. The *P. densiflora+Q. acutissima* mixed forest (F2) is a transitional forest type that developed from the coniferous *P. densiflora* forest to the mixed pine-broad-leaved forest through natural colonization by the broadleaf tree species *Q. acutissima*. The deciduous broadleaved mixed forest (F4) is also a zonal vegetation type that developed through a secondary successional pathway. However, the successional status relationship between the deciduous broadleaved mixed forest and *Q. acutissima* forest is unclear [47].

The *R. pseudoacacia* plantation (RP) is dominated by the tree *R. pseudoacacia*, which is a naturalized tree native to North America. *R. pseudoacacia* was introduced into Shandong province in the late 19th century and planted on a large scale. *R. pseudoacacia* is considered a major invasive plant worldwide and has often invaded habitats including dry and semidry grasslands, open dry forests and shrubland [48]. Planting this nitrogen-fixing tree has posed a problem for nature conservation [49–51]. Using *R. pseudoacacia* for afforestation has resulted in its popularity as a widespread forest

tree, and it is an important part of the economy in some countries [48]. Negative ecological but positive socioeconomic impacts of *R. pseudoacacia* result in conflicts of interest between nature conservation, afforestation, and others. Although this species is well studied, most of the scientific papers address economic aspects. For control, nonforest (NF) soil was collected from natural undeveloped areas around the mountain with no trees and only sparse grass growth.

2.2. Material Collection and Soil Property Analysis

Samples of fresh litter and soil from depths of 0–20 cm were collected from the five forest types and nonforest mentioned above at 18 scattered sampling points across the mountain. All fresh (4 °C in a refrigerator) and air-dried (room temperature) soil samples were stored separately. Fresh samples were used to determine soil NH_4^+ and NO_3^- within a week from sampling, and air-dried samples were sieved through a 2-mm mesh to determine other soil properties.

To analyze soil properties, the 18 soil samples from each forest type were randomly grouped and mixed into 6 samples. The soil pH was measured using an electrode pH meter in a 1:2.5 (w/v) soil water suspension. The soil water content was determined by drying a 20-g subsample of fresh soil at 105 °C for 24 h. Soil total C and N were determined by dry combustion with an elemental analyzer. Ten-gram subsamples were extracted with 50 mL of 2 M KCl to determine the NO_3^- and NH_4^+ contents, and the extracted NO_3^- and NH_4^+ were measured using a spectrophotometer [52]. The NO_3^- and NH_4^+ contents are expressed based on the soil dry weight, and the available nitrogen contents in the soil are expressed by the sum of the NO_3^- and NH_4^+ contents. The total P and total K in the soil were first melted with NaOH [53]. Then, the total P concentration was determined using a spectrophotometer, and the K concentration was determined using a flame photometry detector. To determine the soil available P, five-gram soil samples were extracted with 25 mL of 0.05 M HCl-0.025 (½ M H_2SO_4), and the P concentration of the extract was determined using a spectrophotometer [54]. To obtain the soil available K content, five-gram soil samples were extracted with 50 mL of 1 M NH_4OAc, and the K concentration of the extract was determined using a flame photometry detector [53]. The soil total C:N is the ratio of total C to total N; total N:P is the ratio of total N to total P; and available N:P is the ratio of available N to available P.

2.3. Forest Soil Pot Experiments

A pot culture experiment was conducted in a greenhouse to study the effects of forest soil on *P. americana* growth in April 2017. All the 18 soil samples from each forest type were combined into one composite sample and homogenized by sieving through a 1-cm mesh [55] prior to potting and planting of *P. americana*. There were 3 soil treatments (original soil, activated carbon addition, and sterilization) for each soil type (5 forest types and a nonforest control). The original soils were used to test the overall effects of different forests on invasive *P. americana* growth. The allelopathic effect was studied by adding ¼ volume of activated carbon to the soil to adsorb any allelochemicals. The soil was sterilized to eliminate microbes. The sterilization process involved adding soil to cloth bags that were then placed in an autoclave for sterilization (121 °C, 0.105 MPa, 30 min) 3 times at 24-h intervals. After pretreatment, the soils were placed into small pots of the same size (6 cm in diameter and 8 cm in height). *P. americana* seeds (soaked in 15% H_2O_2 for 7 h to halt dormancy) were sown in a seedling bed to obtain similar-sized seedlings. When the seedlings were approximately 3 cm tall, uniform seedlings were selected and transplanted at one seedling per pot. There were 6 replicates for each treatment (6 soil types × 3 soil treatments = 18 treatments) in a completely randomized design for a total of 108 samples. Plants were watered every day and harvested after 30 days. The roots were rinsed gently with water to remove soil. The biomass of *P. americana* was dried for 72 h at 60 °C and then weighed.

2.4. Forest Litter Leachate Bioassay and Pot Experiments

A bioassay experiment was conducted to study the effects of forest litter on *P. americana* seed germination. Litter leachates from the 5 forest types were prepared before conducting the bioassay

experiments, and the process was as follows. All the 18 litter samples collected from each forest type were combined and homogenized after natural air drying in the laboratory. For each forest type, 400 g of the mixed litter was weighed and immersed in 4 L of distilled water for 24 h. The leachate was filtered with double gauze and used as a stock solution (0.1 g/mL), and then the solution was diluted (0.1 g/mL, 0.05 g/mL or 0.025 g/mL) with distilled water and used as three concentration treatments [56,57]. The pH of the leachate was measured using an electrode pH meter in triplicate. All leachates were stored in a refrigerator at 4 °C. After halting the dormancy of *P. americana* seeds, twenty seeds were placed in 9-cm-diameter Petri dishes containing 2 layers of filter paper. Initially, 5 mL of leachate was added to each Petri dish. Distilled water was used as the control. There were four replicates for each treatment (5 forest soil types × 3 leachates concentrations + 1 distilled water control = 16 treatments) for a total of 64 samples. All Petri dishes were placed in a light incubator with an alternating cycle of 28 °C/light (12 h) and 20 °C/dark (12 h). The percent germination was recorded each day, and seed germination was defined as a 1-mm-long radicle protrusion through the seed coat. The seeds barely germinated after the 11th day, and we stopped the experiment on the 14th day.

A pot experiment was conducted in a greenhouse to study the effects of forest litter from the five forest types on *P. americana* seedling growth in April 2017. Ninety-six pots (6 cm in diameter and 8 cm in height) were filled with soil collected from the nonforest site. *P. americana* seedling selection was completed per the forest soil pot experiment. On the third day after the seedlings were transplanted, 10 mL of the leachates described above were added to the pots each week according to the treatment. Distilled water was used as a control. There were 6 replicates for each treatment (5 forest types × 3 leachates concentrations + 1 distilled water control = 16 treatments) in a completely randomized design for a total of 96 samples. All pots were watered during the experiment to keep the soil moist. *P. americana* individuals were harvested after 30 days. The biomass harvest was completed per the forest soil pot experiment.

2.5. Statistical Analysis

Differences in soil properties among the different forests were compared using one-way ANOVA. Two-way ANOVA was conducted to test the effects of soil from all forest types, the soil treatments (original soil, activated carbon addition, and soil sterilization), and their interaction on the total *P. americana* biomass. A Student–Newman–Keuls multiple range test was used to identify statistically significant differences at $p < 0.05$.

We calculated the response index (RI) [28,58] to evaluate the allelopathic effects of forest litter on *P. americana* seed germination and seedling growth, as follows: (Variable$_{leachate}$ - Variable$_{water}$)/Variable$_{water}$, where Variable$_{leachate}$ was the individual value of replicates with leachate application and Variable$_{water}$ the average value of replicates with water addition. RI > 0 indicates a positive effect, RI < 0 indicates an inhibitory effect, and RI = 0 indicates no effect. The absolute value of the RI indicates the intensity of the effect.

Two-way ANOVA was conducted to assess differences in litter leachate pH values and the effect of litter leachate on the RI of germination rate and RI of total biomass of *P. americana* among different forest types and concentrations. Plant growth performance was explained using the litter leachate pH by constructing a regression model. All statistical analyses were performed using IBM SPSS statistics 24 (IBM Inc., Armonk, NY, USA). All plots were constructed using SigmaPlot 10 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Soil Properties

The forest types had overall effects on the soil properties (Figure 1). The soil pH (one-way ANOVA, $F = 16.145$, $p < 0.001$), water content ($F = 62.319$, $p < 0.001$), available N ($F = 3.324$, $p = 0.027$), available P ($F = 15.226$, $p < 0.001$), total K ($F = 13.538$, $p < 0.001$), available K ($F = 9.872$, $p < 0.001$) and available N:P ($F = 5.399$, $p = 0.003$) exhibited significant differences among the different forests, while

total C (*F* = 1.671, *p* = 0.193), total N (*F* = 1.657, *p* = 0.196), total P (*F* = 2.429, *p* = 0.075), total C:N (*F* = 1.087, *p* = 0.410) and total N:P (*F* = 1.170, *p* = 0.362) exhibited no significant differences (Figure 1). The total P (0.41 g/kg), available P (49.29 mg/kg) and available K (145.04 mg/kg) were highest in the RP soil. The water content (28.03%), total C (6.60 g/kg), total N (83.65 g/kg) and available N (127.74 mg/kg) were highest in the F4 soil. The soil available P was very low in the natural forest, but the soil N:P ratio was higher (F1–F4), particularly in F2 and F4.
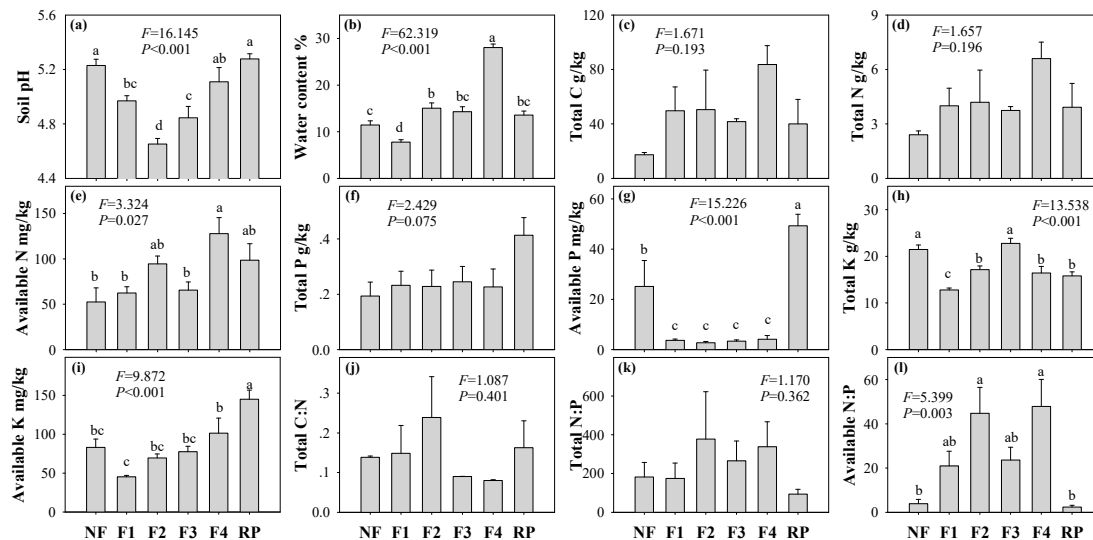

Figure 1. Soil properties of different forests. Statistically significant differences (*P* < 0.05) are denoted by different letters (NF: Nonforest, F1: *Pinus densiflora* forest, F2: *Pinus densiflora+Quercus acutissima* mixed forest, F3: *Quercus acutissima* forest, F4: Deciduous broadleaved mixed forest, and RP: *Robinia pseudoacacia* plantation).

3.2. Effects of Forest Soils on Plant Growth

Soils from different forests had different effects on the *P. americana* biomass (two-way ANOVA, *F* = 209.966, *p* < 0.001), while the soil treatments had no effect (*F* = 1.136, *p* = 0.326), though the forest type and soil treatments had a significant interactive effect (*F* = 18.378, *p* < 0.001) on the *P. americana* biomass (Appendix Table A1). Compared with NF soil, RP soil significantly promoted *P. americana* growth, while the four natural forest soils significantly inhibited its growth (Figure 2). After activated carbon was added to the soil, the biomasses of *P. americana* in the NF and RP soils decreased significantly relative to those in the original soils, indicating that the allelochemicals in both soils promoted *P. americana* growth (Figure 2). In contrast, the allelochemicals in the four natural forest soils inhibited *P. americana* growth, but the effect was not significant in F3 and F4 soils (Figure 2). After the soil was sterilized, the *P. americana* biomass in the NF soils increased significantly, indicating that the presence of soil microbes inhibited *P. americana* growth (Figure 2). Significant changes in the biomass of *P. americana* were not found between the original soil and the sterilized soil for any of the five forests (Figure 2). Moreover, the effects of allelopathy and microbes appear to oppose each other.
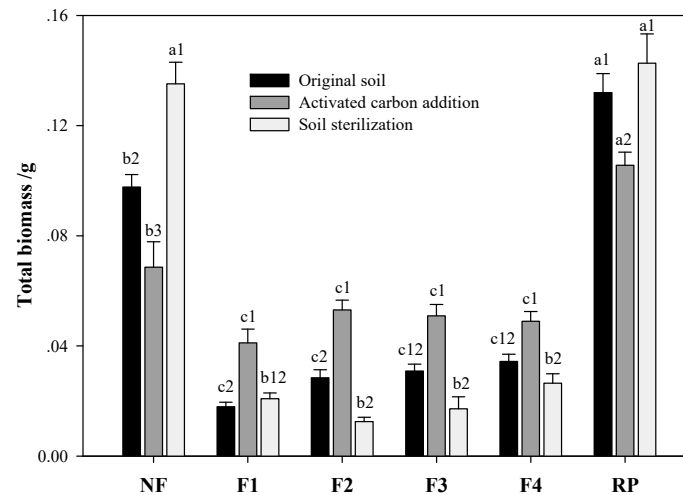
Figure 2. Effects of different forest soils on the growth of *Phytolacca americana*. Statistically significant differences (*p* < 0.05) between the six soils or three soil treatments are denoted by different letters and by different numerals, respectively (NF: Nonforest, F1: *Pinus densiflora* forest, F2: *Pinus densiflora+Quercus acutissima* mixed forest, F3: *Quercus acutissima* forest, F4: Deciduous broadleaved mixed forest, and RP: *Robinia pseudoacacia* plantation).

3.3. Effects of Forest Litter Leachate on Plant Performance

Both the forest type and concentration of litter leachate had significant effects on the leachate pH and significantly affected the seed germination rate and total biomass of *P. americana* seedlings (two-way ANOVA, *p* < 0.05; Appendix Table A2). The forest type and concentration had no significant interactive effects (two-way ANOVA, *p* < 0.05; Appendix Table A2). The litter leachate pH differed significantly among the forests, such that the pH from the RP was significantly higher than that from the four natural forests, litter leachate pH from F3 being significantly lower than that from the other forests (two-way ANOVA, *p* < 0.05; Figure 3c). Litter leachates from all the forest types inhibited *P. americana* seed germination, and that from F3 and F4 had significant inhibitory effects relative to that from F2 (two-way ANOVA, *p* < 0.05; Figure 3a). Litter leachates from the four natural forests inhibited *P. americana* seedling growth, and F3 was significantly lower than the other three (two-way ANOVA, *p* < 0.05; Figure 3b). Litter from RP promoted the growth of *P. americana* and had a significantly different effect relative to litter from the natural forests (two-way ANOVA, *p* < 0.05; Figure 3b).
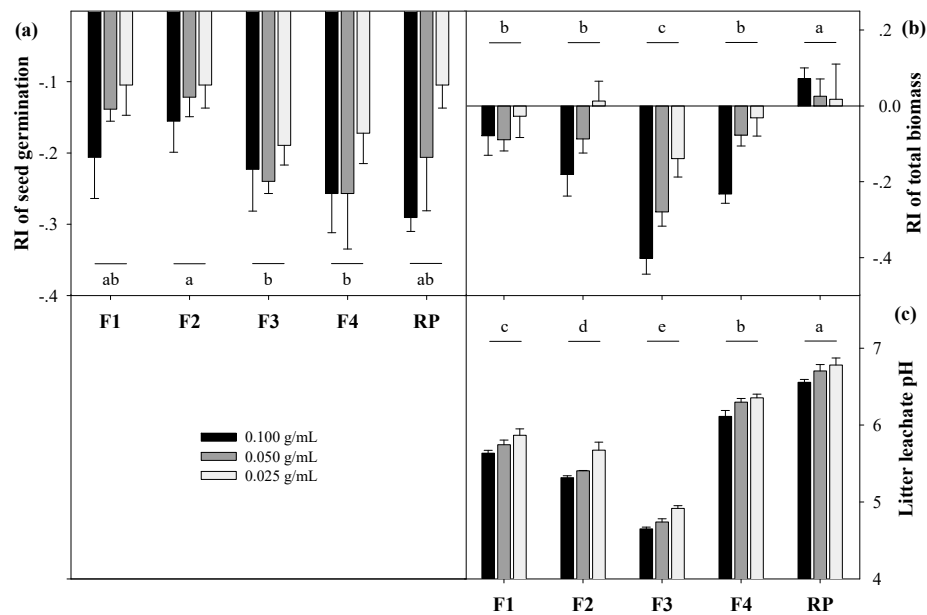
Figure 3. Effects of litter leachates on (**a**) seed germination, (**b**) biomass accumulation of *Phytolacca americana* and (**c**) the pH of the litter leachate. RI was used to evaluate the allelopathic effects of forest litter. RI > 0 indicates a positive effect, RI < 0 indicates an inhibitory effect, and RI = 0 indicates no effect. The absolute value of RI indicates the intensity of the effect. Statistically significant differences ($P < 0.05$) among 5 forest types (F1: *Pinus densiflora* forest, F2: *Pinus densiflora*+*Quercus acutissima* mixed forest, F3: *Quercus acutissima* forest, F4: Deciduous broadleaved mixed forest, and RP: *Robinia pseudoacacia* plantation) are denoted by different letters.

The pH of the litter leachate had no linear relationship with the *P. americana* seed germination rate in the bioassay experiment but did have a significant linear relationship with *P. americana* biomass in the pot experiment (linear regression, $p < 0.001$, $R^2 = 0.597$; Figure 4). Thus, the litter leachate pH was not a limiting factor for seed germination; however, the pH significantly affected the growth of seedlings in the soil.
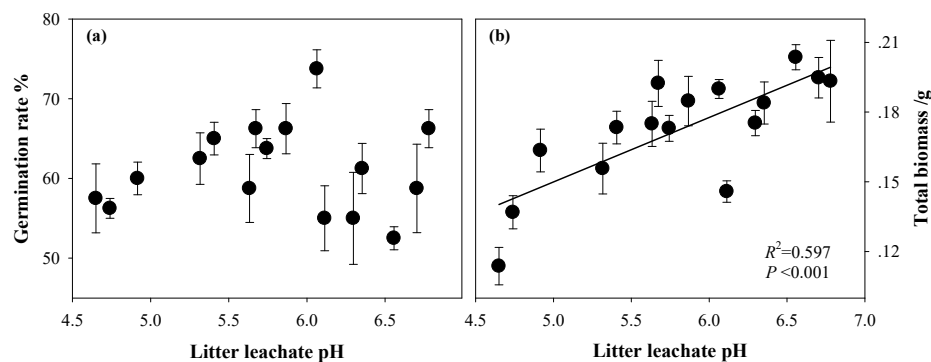

Figure 4. Relationships between the litter leachate pH from different forest types and *Phytolacca americana* (**a**) seed germination and (**b**) biomass accumulation. Bars indicate standard errors of several replicates.

4. Discussion

Research on forest soils may provide insights into the fundamental question of community invasibility: Why do some communities resist plant invasion while others do not? Our results show that the soil properties of the subjected forests differed: Four natural forest soils significantly

inhibited the growth of *P. americana*, while the RP soil significantly promoted its growth. This appears to confirm our first hypothesis that *P. americana* grows poorly in natural forest soils but is well-suited to the *R. pseudoacacia* plantation. Allelopathic effects partially explained the inhibiting phenomenon of natural forest soils, and low nutrient level, especially of phosphorus, in the soils might be additional limiting factors for *P. americana* growth. Litter leachates from different forests inhibited *P. americana* seed germination and influenced seedling growth to different degrees, with litter leachate pH as the potential limiting mechanism.

4.1. The Effects of Soil Nutrients on Plant Growth

Nutrient limitation in soil decreases the probability of invader recruitment in a highly competitive environment [11,59,60]. The available phosphorus content in soil is very low in the studied natural forests (Figure 1), and likely affected by low soil pH levels [61], to the extent that the growth of *P. americana* might be restricted. Furthermore, Yang et al. have shown that alterations in resource stoichiometry can change community resistance to invasion [62]. The N:P ratios varied among the different forest soils, as the four natural forest soils and especially the two mixed forests (F2 and F4) had higher N:P ratios than the RP soil (Figure 1). The limitation of soil phosphorus for biomass production will become more severe under increasing nitrogen [63], which explains, to a certain extent, the low accumulation of *P. americana* biomass under the subjected natural forest soil conditions. Phosphorus is an essential macromineral for plants, and the ability of a species to invade new communities is often influenced by the availability of phosphorus in the recipient community [64]. For example, *Centaurea diffusa*, an invader of North American grasslands, loses its competitive advantage at low phosphorus levels [60]. Therefore, these findings indicate that low phosphorus availability in natural forests might be a factor that enhances resistance to *P. americana* invasion.

The probability that an invader could survive, reach maturity and reproduce is primarily associated with traits of invaders and recipient communities (e.g., seed production) and trait differences between invaders and similar resident species [9]. The effects of *R. pseudoacacia* on plant communities and soil conditions, such as soil chemical properties and soil biota, as a result of nitrogen fixation are well known [50,51]. In particular, this species impacts nitrogen cycling associated with symbiotic rhizobia in root nodules and the rapid decay of nitrogen-rich leaves [49,51] and increases soil available phosphorus [65]. *P. americana* became established from propagules surviving and growing to maturity to profit from the sufficient soil resources of *R. pseudoacacia* but failed to establish itself in the four natural forest soils. Furthermore, the biomass of *P. americana* was significantly linearly related to the litter leachate pH when the leachate was added to the soil, which suggested litter-induced changes in soil acidity and fertility as the underlying mechanism, similar to findings from prior common garden experiments [12,13].

4.2. The Effects of Allelopathy on Plant Growth

Some evidence has suggested that allelopathic native plants play a role in inhibiting the growth of invaders [37–39]. We found that the allelopathic activity in natural forest soil and litter inhibited the growth of *P. americana* but that RP soil promoted its growth (Figures 2 and 3), which seems to confirm our second hypothesis: Allelopathy of natural forests inhibits *P. americana* growth and that of the *R. pseudoacacia* plantation promotes its growth. Similarly, Hou et al. [39] found that monsoon evergreen broadleaf forest soil has strong allelopathic activity towards invasive plants in lower subtropical China. Ning et al. [37] provided evidence that in grassland communities allelopathic native grasses increased community resistance to introduced plants. However, this study did not produce results for succession-associated allelopathy consistent with Hou et al. [39], who found that late-successional stage forest soils have stronger allelopathy than early-successional stages. Although litter leachate from F3 inhibited *P. americana* growth stronger than that of the other forests (Figure 3), the allelopathic effects of F3 and F4 soils on the growth of this plant were not significant (Figure 2). As a result, our third hypothesis, late-successional forest soils exhibit stronger allelopathic inhibition to *P. americana* than early successional soils, was not supported. Thus,

differences in the species composition of communities, which leads to species-specific allelopathic effects on invasive species, may be a more promising explanation [8,25,66] than succession.

The allelopathic effects of RP soil and litter were significantly different than those of the four natural forests. Prior studies have found that the allelopathy of legume trees is stronger than that of nonlegume trees, but that the species differ greatly [35], the effects of plant allelochemical on invasion success being species-specific [8]. Therefore, species-specific allelopathy and the conditioning of the soil based on the traits of *R. pseudoacacia* increased the probability of further community invasion by *P. americana*, which may hinder the recovery of native communities [67]. The "Invasional Meltdown" hypothesis suggests that the synergistic interactions among invaders contribute to further invasion and accelerate the impact on native ecosystems [68,69]. Unfortunately, *R. pseudoacacia* is widely distributed in the study area through planting and natural expansion. Similar to the invasive *P. americana*, correct management of this aggressive tree in temperate forests is urgently required [50].

Nonetheless, caution should be taken when interpreting the results of soil allelopathic analyses. Some studies have indicated that there may be potential side effects related to soil properties, microbes and plant growth when using activated carbon to adsorb allelochemicals in the soil [70,71]. Despite this concern, activated carbon, due to its effectiveness in absorption of allelochemicals, is still utilized by many researchers to neutralize allelochemicals in order to study the allelopathy of plants [21,36,37,39,72]. Additionally, caution should be exercised when using laboratory bioassays to simulate a complex field environment [66,73]. The now documented contrasting allelopathic effects between soils from natural forests and the plantation warrant further research to characterize the involved allelochemicals and their effects throughout the entire life span of invasive plants.

4.3. The Effects of Soil Microbes on Plant Growth

Prior studies have observed that soil microbes play an important role in determining allelopathic activity [28,74]. In the present study, the effects of allelopathy and soil microbes, the latter being explored by comparison of plant growth between sterilized and non-sterilized soil, showed opposing effects for *P. americana* growth (Figure 2). Rhizosphere microbes can counteract the negative influence of plant allelochemicals, and allelopathy affects microbial composition and activity as well, thereby indirectly affecting plant growth [17,29,32]. Almost all land plant species have a symbiotic relationship with mycorrhizal fungi [75], and absence of suitable mycorrhizal fungi can limit species establishment in a new range and act as one of multiple environmental filters slowing species spread [76]. Nuñez et al. [77] found that a lack of adequate ectomycorrhizal fungi hindered invasion by Pinaceae on Isla Victoria, Argentina, by reducing both the probability of establishment and the growth of invading individuals. In the current study, however, the growth of *P. americana* did not differ significantly between sterilized and non-sterilized soils in any of the forest types. Thus, the effects of soil microbes on *P. americana* invasion in the subjected forests may be negligible.

This study used short-term potted experiments in greenhouse to examine the effects of different forest soils and litters on *P. americana* growth. Care should be taken when considering the extrapolation of our results to complex field situations. For example, variable light conditions in the understory between different forest types and successional stages may aggravate or weaken the effects of soil factors via coupling effects, while such interactions do not apply to greenhouse experiments. In addition, plants have variable responses to soil factors at different growth stages. Our study focused on how the seed germination and seedling growth of *P. americana* were affected by the soil conditions among different forest types, seed germination, and seedling establishment being crucial stages for the invasion of exotic plants. The potential effects throughout the entire life span of plants need to be addressed in future studies.

5. Conclusions

We found that allelopathic properties of natural forest soils can effectively resist *P. americana* invasion, and that low nutrient levels, especially of phosphorus, in the soils might be limiting

factors for the plant growth. The profound conditioning of soil by exotic *R. pseudoacacia* based on tree traits (including allelopathy) facilitated further *P. americana* invasion in RP soil. Allelochemicals from forest litter inhibited the germination of *P. americana* seeds, litter leachate pH playing a major role in *P. americana* growth. We have no evidence that late-successional forest soils exhibited stronger allelopathy to *P. americana*. The present study will help to further our understanding of the mechanism of community resistance to invasion.

Author Contributions: Yu-Ping Hou conceived and designed the study. Peng-Dong Chen and Yan-Hui Zhuge performed the experiments and collected the data. Peng-Dong Chen led the writing of the manuscript. Wei Wei designed and performed the data analysis. Qiao-Qiao Huang and Yu-Ping Hou reviewed and edited the manuscript. All authors participated in data interpretation and revised the manuscript.

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Appendix

Table A1. The effects of forest type (5 forest types and 1 nonforest), soil treatments (original soil, activated carbon addition, and soil sterilization), and their interactions on the total biomass of *Phytolacca americana,* based on two-way ANOVA. Degrees of freedom are indicated as subscripted numbers in parentheses after the F-value. Statistically significant values ($p < 0.05$) are denoted by bold font.

Variables	$F_{(df)}$	p
Forests	**209.966$_{(5,90)}$**	**<0.001**
Treatments	1.136$_{(2,90)}$	0.326
Forests × Treatments	**18.378$_{(10,90)}$**	**<0.001**

Table A2. The effects of the forest type, litter leachate concentration (0.1 g/mL, 0.05 g/mL or 0.025 g/mL), and their interactions on the RI of the germination rate, RI of the total biomass of *Phytolacca americana* and litter leachate pH, based on two-way ANOVA. Degrees of freedom are indicated as subscripted numbers in parentheses after the F-value. Statistically significant values ($p < 0.05$) are denoted by bold font.

Variables	RI of germination rate		RI of total biomass		Litter leachate pH	
	$F_{(df)}$	P	$F_{(df)}$	P	$F_{(df)}$	P
Forest type (F)	**2.765$_{(4,45)}$**	**0.039**	**14.403$_{(4,75)}$**	**<0.001**	**446.407$_{(4,30)}$**	**<0.001**
Concentration (C)	**5.051$_{(2,45)}$**	**0.010**	**9.462$_{(2,75)}$**	**<0.001**	**24.066$_{(2,30)}$**	**<0.001**
F × C	0.522$_{(8,45)}$	0.834	1.846$_{(8,75)}$	0.083	0.539$_{(8,30)}$	0.817

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