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# The Effect of Regulating Soil pH on the Control of Pine Wilt Disease in a Black Pine Forest

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**Abstract:** Pine wilt disease (PWD) is a highly destructive disease in forest ecosystems, resulting in extensive forest decline and substantial economic losses. As soil pH plays a critical role in soil microbial activity and significantly impacts the prevalence and severity of diseases, we conducted an experiment to regulate soil pH for alleviating PWD in a black pine (*Pinus thunbergii*) forest. The result reveals that: (1) The pH of the soil under a *P. thunbergii* forest was 5.19 ± 0.40, which was significantly lower than that of soils under other vegetation types at 8.53 ± 0.44. (2) Finely ground shell powder (F-SP) was the optimal size for long-term and efficient regulation, but quicklime (QL) exhibited the strongest efficacy in raising soil pH, followed by F-SP and plant ash. The regulation effect strengthened with the dosage amount. (3) In the situ experiments, part of symptomatic black pine in F-SP or QL plots were apparently improved and converted to asymptomatic trees separately by 15.9% and 5.4%. Applying F-SP can alleviate PWD in a sustainable way. This paper presents the first investigation to assess the effects of regulating soil pH for controlling PWD. It holds significant practical value for the rational planning and the sustainable development of artificial forests in coastal regions.

**Keywords:** *Bursaphelenchus xylophilus*; *Pinus thunbergii*; acidic soil amelioration; nematode; infection rate



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## 1. Introduction

Pine wilt disease (PWD) is one of the most destructive diseases in forest ecosystems, leading to large-scale forest decline and devastating economic losses, especially in Asia and Europe [1]. In 1905, PWD was first investigated in Japan and subsequently gained traction, disseminating to other Asian countries, including Korea and China, during the 1980s [2]. In China, the initial documentation of this phenomenon originated in Nanjing in 1982 [3]. Afterward PWD has progressively extended to 726 counties across 18 provinces over 40 years, impacting over 1.892 million hectares of forest, leading to substantial economic losses and ecological challenges [4,5]. The causal agent of PWD is the nematode species *Bursaphelenchus xylophilus*, commonly known as the pine wood nematode (PWN) [6]. *B. xylophilus* feeds on the cellular tissue surrounding the resin ducts, leading to the occurrence of "tracheid cavitation", characterized by the formation of air pockets within the water transport system [7]. Eventually, the tree cannot transport water upwards and withers to death, showing symptoms of wilting from the bottom up.

PWN can kill the infected pines within 40 days [8]. Consequently, several measures have been implemented to mitigate the spread of PWD, including monitoring, quarantine and the removal of dead trees. The susceptible pine species vary in different countries or regions [8]. In detail, most infected pines are *Pinus pinaster* in Spain and Portugal, while the main affected species are *P. densiflora* and *P. thunbergii* in the United States. In Canada, in addition to pines, firs such as *Abies balsamea*, *Picea glauca*, and *Picea mariana* are also infected. In Asia, *P. thunbergii* and *P. densiflora* are susceptible to PWN, which has caused widespread pine dieback in China, Japan and Korea. High temperatures and drought are considered to be the main factors behind the onset of PWD [8]. Specifically, PWD occurred rarely under 10 °C, sporadically between 10 and 12 °C, epidemically over 12 °C, and explosively over 14 °C [8], creating a difference in incidence between North China and South China. In detail, PWD in China is severe in the south while mild in the north, with early onset in the south andlate onset in the north. However, it is worth noting that China's soil is typically acidic in the south but alkaline in the north.

Soil pH plays a critical role in soil microbial activity and significantly impacts the prevalence and severity of diseases, especially those pathogens that primarily spread through root systems [9]. Soil pH is also important in maintaining soil fertility and facilitating nutrient availability, and the altered nutrition caused by soil acidity can weaken the plant host, making it more susceptible to diseases [10]. Studies conducted in strawberry fields have revealed a negative correlation between soil pH and disease severity, indicating that elevating soil pH could potentially serve as a strategy for managing strawberry diseases [11]. In agricultural production, it was found that the disease caused by *Meloidogyne* occurred easily when the soil pH was 3–5 but rarely occurred when the soil pH was 6–7 [12]. Similarly, when wood is inspected and quarantined, wood infected with PWN usually turns yellow in response to bromophenol blue, indicating its acidity [13]. Based on the above analysis, we speculate that the soil and the trunk of pines with PWD are acidic, which provides favorable conditions for the growth and reproduction of PWN. With this assumption, this study conducted an experiment to regulate soil pH to alleviate PWD.

These findings offer innovative ideas and a new direction for PWN control. We selected Changdao as a research area, which was the earliest epidemic area of PWD in North China [2]. *P. thunbergii*. forest degradation poses a major threat to the local ecology and environment. Therefore, the government urgently needs efficient and environmentally friendly methods to combat PWD besides common measures. This paper presents the first investigation to determine the impact of regulating soil pH on controlling PWD in a sustainable way. Hence, it has important practical value and provides fundamental theoretical support for the rational planning and sustainable development of artificial forests in coastal areas.

## 2. Materials and Methods

### *2.1. Research Area*

The experiment was conducted in Changdao, Shandong Province (120.73° E, 37.92° N). It has a warm temperate monsoon climate with an average annual temperature of 11.0–12.0 °C and an average annual precipitation of 537 mm. The most important soil types are brown soils (57.2%), cinnamon soils (41.8%) and some fluvo–aquic soils (1%). The vegetation coverage of this island is up to 72.6%, most of which consists of *P. thunbergii* (black pine) forest planted around the 1960s. In the early 1990s, Changdao became the earliest and only endemic area of PWD in the north of China [2]. Despite the measures taken to clear dead wood, black pines infected with PWD still exist today. From 2018 to 2021, the number of dead black pine was about 85,000, 190,000, 140,000 and 133,000 each year, accounting for 19.57% of the total black pine. This has undoubtedly become a major trap and potential opposition to the construction of a national park in Changdao.

### *2.2. Classification of Black Pines*

Currently, common methods for detecting PWN include morphological, molecular, chemical, and physical methods. In the field experiment, we have to identify each black pine in multiple quadrats, which is quite a large number. A previous study proved that more nematodes were extracted from the seedlings with more developed symptoms [14]. Therefore, the quantity and severity of symptoms can reflect the degree of internal nematode infection. Based on the field identification methods mentioned in the literature and combined with the practical experience of the forestry department, we summarize the following identification criteria. These criteria should be considered objectively in combination with the growth condition of each tree. It also requires trained, experienced personnel familiar with the local forest's appearance and the symptoms of plant diseases and insect pests to make a good assessment.

In the early stage of PWD, *P. thunbergii* often show the following features [15]: (1) lack of resin exudation of bark wounds (e.g., with a knife); (2) the needles gradually lose their luster and appear yellowish in part from the tips; (3) beetle oviposition sites are often found on the bark; (4) rather short new shoots and sparse needles; (5) discoloration starts in the old needles on the underside and then spreads to newer needles on the upper side; and (6), several branches shed their needles completely. Black pines at the early stage of PWD are considered symptomatic trees (STs). In the late stage of PWD, it is easy to recognize because the entire tree turns yellow or presents with red-brown needles. At this stage, the needles have completely withered and cannot be easily revived. Therefore, at this stage, black pines are considered dead trees (DTs). Other black pines are considered asymptomatic trees (ATs).

### *2.3. Soil pH Survey*

In order to find out the soil pH under the black pine forest and the pH difference under divergent types of vegetation. A total of 48 accessible sampling sites were selected every 1 km within the vegetation area (Figure 1). Soil samples were collected from the top 20 cm after removing litter with three replicates. In principle, these sample areas covered all types of vegetation and all types of soil. In addition, a total of 8 plots in the black pine forest were randomly selected. In each plot, 0–20 cm soil samples underneath six ATs and all STs were separately taken from each plot with three replicates.

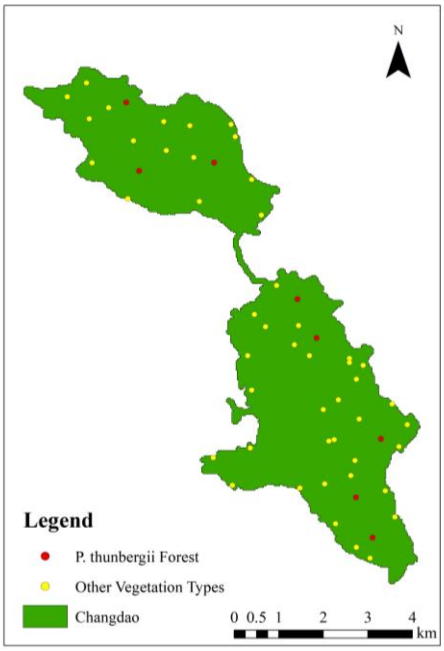


**Figure 1.** The location of sample sites in soil pH survey.

Subsequently, the collected soil samples were air-dried for 2 weeks and then passed through a 2 mm mesh sieve to remove debris in the soil and thoroughly mixed before use. Soil pH was measured with a glass electrode (Leici PHS-2F, INESA, Shanghai, China) in the supernatant suspension of a 1:2.5 mixture of air-dried soil (5 g) and deionized water (12.5 g).

### *2.4. Regulate Soil pH in Pot*

The pot experiment is mainly used to determine the appropriate dosage of quicklime (QL), plant ash (PA) and shell powder (SP) for soil pH regulation, as well as the optimal size of SP, which is the most suitable for long-term and efficient regulation of soil pH to conduct subsequent in situ experiments. QL and PA are both easily available and affordable soil pH conditioners. Shell is also rich in islands and coastal zones. The soil used in all experiments in this study was sandy loam (28.1% sand, 68.5% silt, 3.4% clay). 0–20 cm of soil under a black pine forest was collected and mixed, and then 2.5 kg of soil was added to each pot. The inner diameter of the pot is 20 cm. Dosage and pH monitoring intervals were adapted from a prior study on oyster SPs' impact on soil pH [16]. In the first experiment, the shell was ground to three sizes, separately as fine (F-SP, <0.02 mm), medium (M-SP, 0.02–2 mm), and coarse (C-SP, >2 mm). The dosage of F-SP, M-SP and C-SP was separately set as 0.5 g, 1 g, 2 g and 4 g in each pot with three replicates. In the second experiment, soil pH was adjusted by separately adding fine QL, SP or PL and mixing thoroughly to produce the following treatments with three replicates: (1). 0.5 g added; (2). 1 g added; (3). 2 g added; (4). 4 g added. The control group was adjusted to 0 g with three replicates. The soil in the pot was well mixed and watered regularly after placing the ameliorants. The soil moisture was maintained at 15% as the average soil moisture of the black pine forest by distilled water. Subsequently, the soil pH in each pot was measured on the 10th, 20th, 40th and 80th days thereafter. The experiment was repeated on the 81st day and lasted for two consecutive 80 days.

### *2.5. Regulate Soil pH In Situ Experiment*

Generally, *P. thunbergii* becomes infected with PWN in the spring, and the infected trees will rapidly die in autumn in Southern China [8]. While in Northern China, they usually die within the next year or a few years later [8]. This gives us a long period to control PWD in North China. The optimal size and appropriate dosage were selected through the pot experiment and applied to an in situ experiment from April to September 2022 for two consecutive 80 days in order to maintain the regulatory effect of ameliorants on soil pH. In detail, 80 kg of fine QL and SP were sprayed in each 25 × 25 m quadrat with three repetitions each. A control group (CG) was also set up. The black pines were numbered and recorded in all quadrats, and no trees were felled during the experimental period. We calculated the number of AT, ST, and DT in each plot before treatment and counted again after PWD began in October. Because the total number of trees varied between quadrats, the data was converted to percentages for analysis. Infection rate (IR) was compared to determine the effect of regulation of soil pH on mitigating PWD symptoms of *P. thunbergii*. IR is calculated as follows:

$$\mathrm{IR}(\%) = \frac{\mathrm{ST} + \mathrm{DT}}{\mathrm{AT} + \mathrm{ST} + \mathrm{DT}} \times 100\% \tag{1}$$

ANOVA and *t*-tests were performed to compare group means with SPSS 26.0 (IBM Corporation, Armonk, NY, USA, 2021) for differences in soil pH. A least significant difference (LSD test) was used to perform comparisons between group means. The percentages of ST, AT, DT, and IR were analyzed using non-parametric statistical methods (Kruskal–Wallis test) in SPSS 26.0. Figures were generated in Origin (OriginLab Corporation, Northampton, MA, USA, 2017) and Arcgis 10.8 (ESRI Inc., Redlands, CA, USA, 2020). All statistical analyses were performed with a $p = 0.05$ confidence level.

## **3. Results**

### *3.1. Soil pH Characteristics*

The pH of the soil under *P. thunbergii* forest was 5.19 ± 0.40, which was significantly lower than that of soils under other vegetation types at 8.53 ± 0.44 (Figure 2a, $p < 0.001$). Other vegetation types included almost all kinds of grasses, shrubs and trees in Changdao. The pH of the soil below them ranged from 6.96 to 9.45, mostly belonging to alkaline soil. Several sites of others also contained *P. thunbergii*, but they were individual trees planted along the road or a small area of seedlings. In these seedlings or solitary trees of *P. thunbergii*, the diameter at the breast height of the trees was about 7–8 cm and there were less than 30 trees in each patch. The mean soil pH of these sites was 8.50 ± 0.79. In the *P. thunbergii* forest, the soil pH of AT was slightly lower than that of ST (Figure 2b, $p = 0.258$). The soil pH under AT was 5.13 ± 0.37 and ranged from 4.30 to 6.08. While soil pH under ST was 5.27 ± 0.43, ranging from 4.66 to 6.34. More detailed pH data can be found in the Supplementary Materials (Tables S1 and S2).

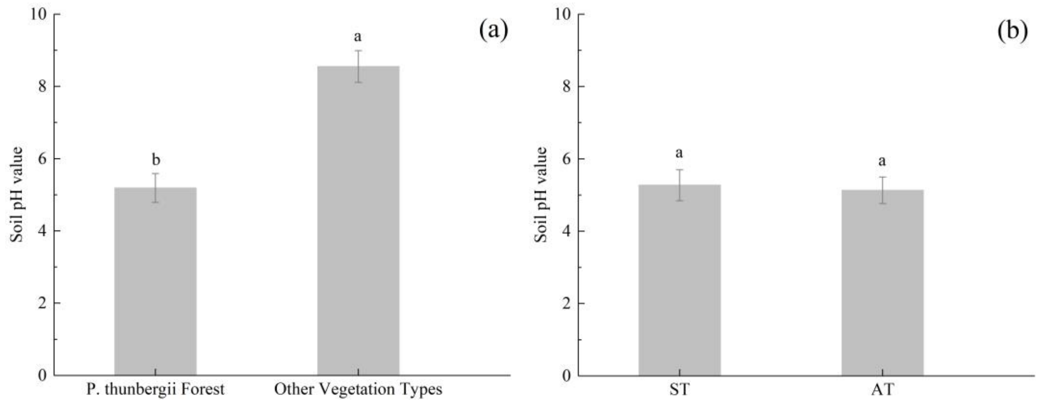


**Figure 2.** Characteristics of soil pH. (**a**) Soil pH value under *P. thunbergii* forest and other vegetation types; (**b**) soil pH value under symptomatic tree (ST) and asymptomatic tree (AT) in *P. thunbergii* forest. Different letters (a, b) over the columns indicate significant differences between the two groups in terms of soil pH value (*t*-test, $p < 0.05$).

### 3.2. The Effect of SP with Different Particle Sizes on Regulating Soil pH

The result of two consecutive 80-day experiments with F-SP, M-SP and C-SP in the pot is presented in Figure 3. The dotted line is the average pH of all pots before treatment as 5.22. The result showed that: (1) Soil pH increased the most after treatment with F-SP, but groups except M-SP4 showed no obvious changes; (2) In F-SP group, soil pH increased with dosage amount and could be adjusted to 6.00–6.50; (3) Soil acidification would occur after the peak, and soil pH was lowest in 80 d in both experiments; (4) In F-SP and M-SP, soil pH was higher in the second 80 d than in the first 80 d. Therefore, considering that M-SP and C-SP showed neither obvious regulation of soil pH nor expected long-lasting effects, F-SP was selected to conduct the following experiment.

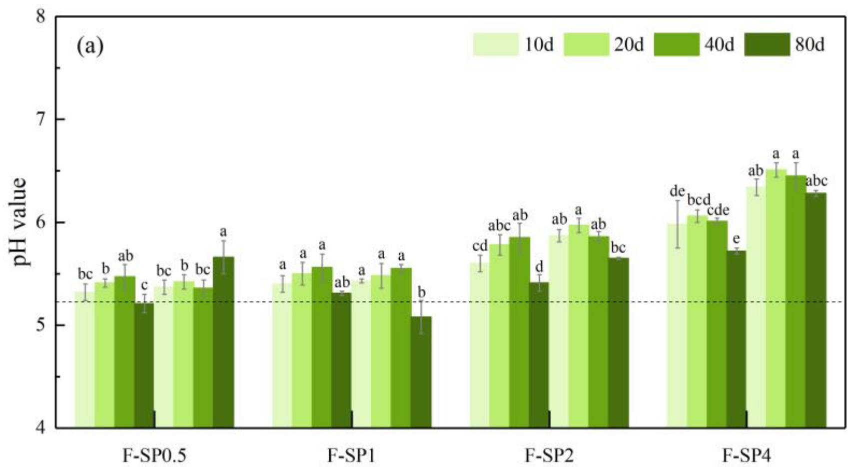


**Figure 3.** *Cont*.

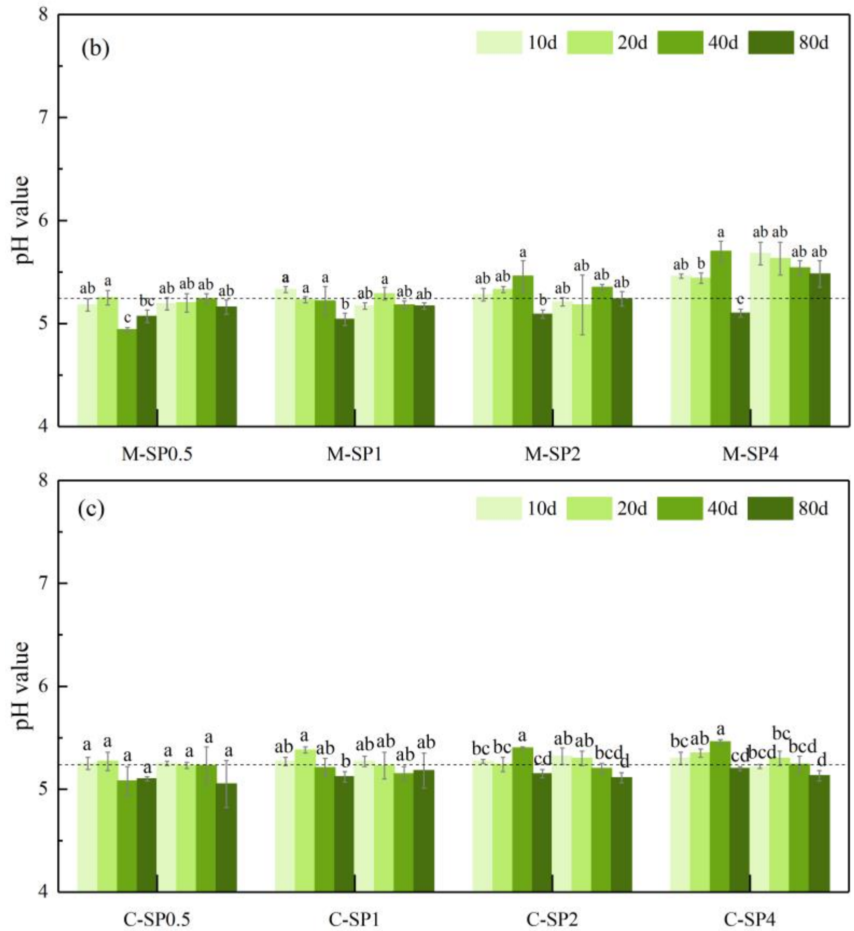


**Figure 3.** The soil pH changes after being treated with different sizes of shell powder (two consecutive 80-day experiments). (**a**) fine shell powder, F-SP; (**b**) medium shell powder, M-SP; (**c**) coarse shell power, C-SP. The horizontal scale line was the dividing mark of the two experiments. Different letters over the columns indicate significant variations at different experimental periods within the same group in terms of soil pH value (LSD test, $p < 0.05$).

### *3.3. The Effect of Different Ameliorants on Regulating Soil pH*

The result of two consecutive 80-day experiments with QL, PA and F-SP in pot is shown in Figure 4. The dotted line is the average pH of all pots before treatment as 5.22. The results showed that: (1) PA had little effect on increasing soil pH or even making the soil more acidic after 80 days, which was not suitable for subsequent experiments; (2) QL had the best effect of increasing soil pH, it could increase to 6.00–6.50 after the first treatment, and further increase by about 15% after the second treatment, and the effect strengthened with the dosage amount.

### *3.4. The Result of In Situ Experiment*

Based on the above results, we applied QL and F-SP as soil ameliorants in a *P. thunbergii* forest. Each 25 × 25 m plot was sown with 80 kg of QL or F-SP onto the surface soil at the rate of 4 g/pot. The dotted line is the average pH of all pots before treatment as 4.88 (Figure 5). The result revealed that the effects of QL and F-SP on soil pH were similar and did not differ as much as that in the pot experiment (Figure 4). Soil pH could reach 5.50 after the first adjustment and could reach 6.00 after the second adjustment. Soil pH was highest in the 40 d of the second experiment. The effect of QL was slightly better. However, the error bar of the in situ experiment was much larger than that in the pot experiment. In the field test, soil acidification would also occur at 80 d after the peak.

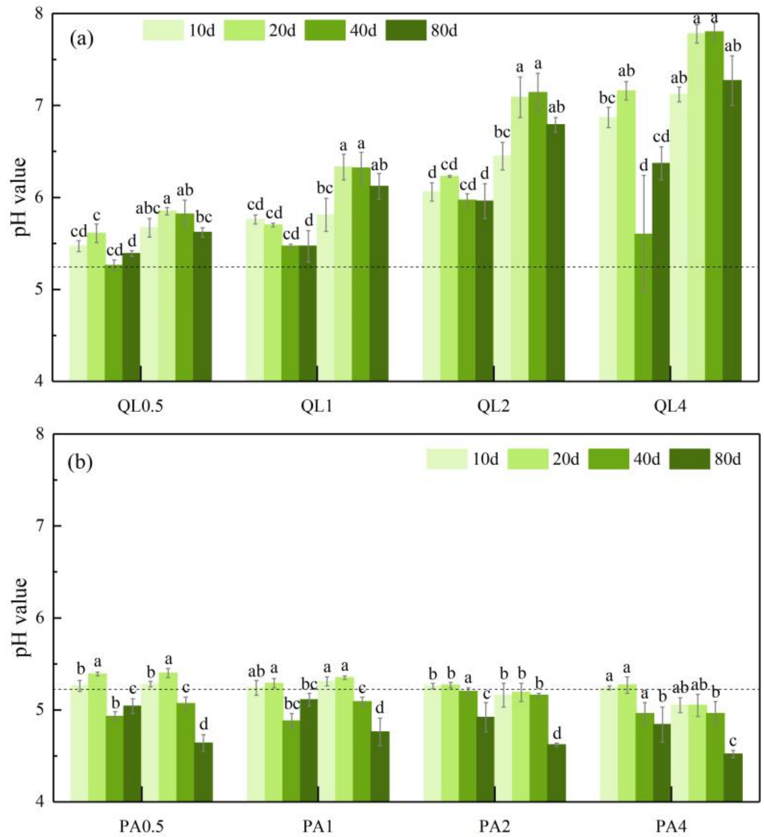


**Figure 4.** The soil pH changes after treated with QL and PA (two consecutive 80-day experiments). (**a**) quicklime, QL; (**b**) plant ash, PA. The horizontal scale line was the dividing mark of the two experiments. Different letters over the columns indicate significant variations at different experimental period within the same group in terms of soil pH value (LSD test, $p < 0.05$).

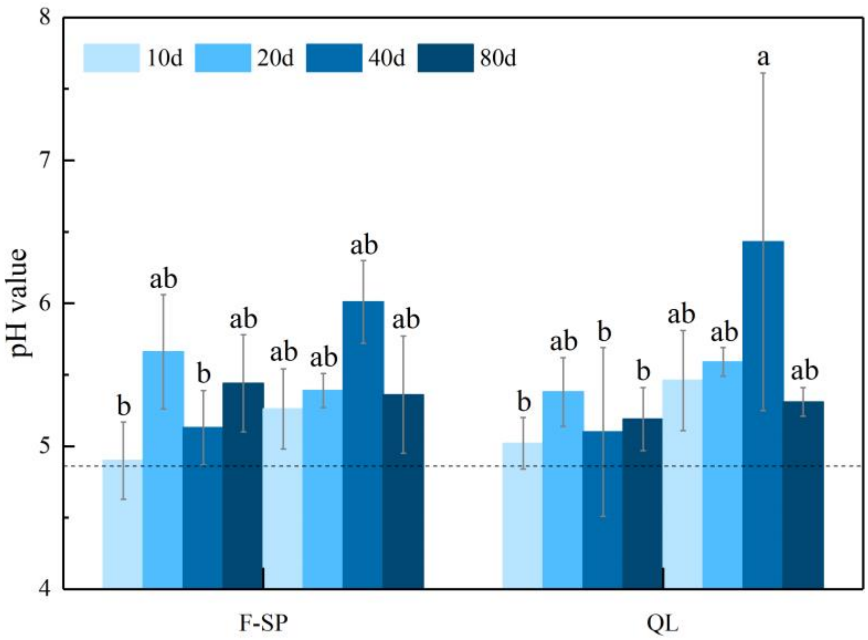


**Figure 5.** Regulation effect of F-SP and QL on soil pH in situ experiment (two consecutive 80-day experiments). The horizontal scale line was the dividing mark of the two experiments. Different letters over the columns indicate significant variations at different experimental periods within the same group in terms of soil pH value (LSD test, $p < 0.05$).

The percentages of asymptomatic, symptomatic and dead trees were calculated before and after the in situ experiment (Table 1). In the control group, the proportion of AT decreased by 11.1%, while the proportion of ST increased by 10.1%. In contrast, ST in F-SP or QL plots were apparently improved and converted to AT based on the PWD discrimination standard. The percentage of ST decreased by 15.9% and 5.4% in F-SP and QL plots. The percentage of AT increased separately by 18.1% and 4.4% in F-SP and QL, while IR decreased by the same amount. The proportion of DT increased by 1% in QL and CG, while it decreased by 2.2% in the F-SP plot. Furthermore, based on the results of the Kruskal–Wallis test (Table 1), *p*-values were greater than 0.05 except $ST_{after}$ and $DT_{before}$, indicating that there was no significant difference among different sample plots under the same pine status, but $ST_{after}$ and $DT_{before}$ had significant difference within group. Therefore, the application of soil pH ameliorants has resulted in a transition from non-significant to significant differences in ST proportion, while DT showed the opposite trend, which confirms the effect of ameliorants in mitigating PWD symptoms.

**Table 1.** The percentage of AT, ST, and DT in all sites.

| Pine Status / Ameliorant | AT | | ST | | DT | | IR | |
|---|---|---|---|---|---|---|---|---|
| | Before% | After% | Before% | After% | Before% | After% | Before% | After% |
| F-SP | 50.8 | 68.9 | 33.9 | 18.0 | 15.3 | 13.1 | 49.2 | 31.1 |
| QL | 45.6 | 50.0 | 43.5 | 38.1 | 10.9 | 11.9 | 54.4 | 50.0 |
| CG | 61.8 | 50.7 | 32.4 | 42.5 | 5.8 | 6.8 | 38.2 | 49.3 |
| *p* (Kruskal–Wallis) | 0.051 | 0.066 | 0.066 | 0.039 | 0.027 | 0.058 | 0.051 | 0.066 |

Note: AT, ST, DT, and IR separately represent asymptomatic tree, symptomatic tree, dead tree and infection rate.

## 4. Discussion

### *4.1. Where Does H+ Come from in P. thunbergii Forest?*

In Changdao, the pH of surface soil under infected black pine forest ranged from 4.66 to 6.34, which was significantly lower than that under farmland, orchard, treelawn, and forests of other plant species, as 6.96–9.45. H+ comes from various sources such as humus decomposition, acid rain, root secretion and so on [17–19]. In detail, dead leaves and twigs decomposed humic acid, resulting in a low pH of surface soil. In natural forests, the surface mineral soils exhibited a decrease rate of soil pH between 0.01 and 0.03 units per year, whereas plantation forests showed a decrease rate between 0.03 and 0.05 units per year [19]. For this reason, the soil under the seedlings or individual trees of *P. thunbergii* planted in recent years was neutral to alkaline but acidic under the *P. thunbergii* forest created by large-scale afforestation at least 40 years age. In addition, coniferous tree litter produces much organic acid in the decomposition process, so *P. thunbergii* is more likely to cause soil acidification than other tree species. Furthermore, it is worth noting that atmospheric deposition can contribute significantly to the H+ in temperate forests [18]. The large and dense canopies of forest trees tended to hold large amounts of acids, such as acid rain, which worsened the acidification of the soil. Acid rain is a prevalent issue in many industrialized countries, and it is considered one of the contributing factors to forest decline in European and North American regions [17]. We also collected samples of 40 rainfalls in Changdao for a year, the average pH of which was 6.20. Six of them were below 5.60, which is attributed to acid rain. Moreover, it is important to highlight that the main source of H+ contributing to forest soil acidification is the net excretion of H+ from plant roots [18]. Conifer species can promote soil acidification by secreting organic acids and absorbing basic cations through their mycorrhizal associations [20,21]. Previous studies revealed that the mean pH for PWD-infected forests was 5.3, and for uninfected forests was 4.9 [22], consistent with our experimental results. The biological effect of mycorrhiza ceased after infection, and the pH in diseased soils increased significantly [23]. This could be the reason to explain the slight increase in soil pH in ST (Figure 2b).

*4.2. The Internal Mechanism between Soil pH and PWD*

The in situ experiment proved that PWD can be mitigated by increasing soil pH. It could also be identified by the differences between *P. thunbergii* forest and solitary or seedling *P. thunbergii* Soil pH under AT of *P. thunbergii* forest was 5.13 $\pm$ 0.37 but was much higher under solitary or seedling of *P. thunbergii* as 8.50 $\pm$ 0.79. Previous studies had already found that the PWD infection rate was related to tree age in Changdao [24]. Of all trees infected with PWD, 11- to 20-year-old black pines accounted for 63.5%, over 20-year-old 31.7%, and other young trees under 10-year-old, only 4.8%. As we discussed in Section 4.1, soil pH gradually decreased with forest age due to the accumulation of acids in the soil. Therefore, these varying infection rates might be directly related to soil pH. In this scenario, young trees in a neutral-to-alkaline soil environment favored pines' resistance to PWN and vice versa.

Although the dispersal of *B. xylophilus* between different trees depends on its insect vector (*Monochamus alternatus*) from trunk to trunk, its survival and propagation are still related to the underlying soil. Soil pH has a correlation with certain root diseases and influences the accessibility of vital nutrients, thereby impacting the occurrence and severity of leaf diseases. Furthermore, PWN moved slowly and only downward during the initial phase of PWD, then the movement accelerates with increasing population and becomes bidirectional [25,26]. Therefore, the roots of infected pines were rich in PWN.

Soil pH could be coupled with fungi, nematodes, bacteria and acid rain to influence PWN. On the contrary, PWD could also alter soil biotic and nutrient properties. In detail, acidic soil limited soil fertility and nutrient availability, which weakened the plant host through altered nutrition and increased disease susceptibility [10]. Previous studies have indicated that stands disturbed by PWD exhibit lower soil hyphal density, pH, P and K content but higher levels of organic matter and total N ($p < 0.05$) in comparison to undisturbed stands [27]. PWD disturbances have also been associated with notable increases in dissolved organic carbon, N and $NO_3$-N levels when compared to the undisturbed stand [28].

Plant roots are inhabited by a variety of fungi from different functional groups, including plant pathogens, facultative saprotrophic fungi and mycorrhizal fungi, etc., which affect plant health and nutrition [29]. Following PWN infection, a decline in host plant resistance has been observed, accompanied by a reduction in the community composition, species richness, and species diversity of endophytic and rhizospheric fungi [30–32]. In detail, resistant pines (*P. elliottii*, *P. caribaea*, *P. taeda*) and healthy *P. massoniana* had a higher fungal abundance of *Cladosporium* and class Eurotiomycetes but a lower abundance of *Graphilbum*, *Sporothrix*, *Geosmithia* and *Cryptoporus* than wilted *P. massoniana* [32]. For *P. massoniana*, fungi *Penicillifer*, *Zygoascus*, *Kirschsteiniothelia*, etc., were greater in infected pines than in healthy ones [31]. These differences in fungal community might be associated with pathogen resistance of the pines to PWN [31]. Hence, these root-associated fungi exert significant effects on plant growth and play a crucial role in enhancing host plant resistance to pathogens and various abiotic stresses [33]. Research has shown that the product of the serine protease gene in PWN-trapping fungus was not transcribed at pH 4, while this gene was transcribed well under alkaline conditions such as pH 8 and 9 [34]. Therefore, the acidic soil environment was not conducive to root-associated fungi and further affected the resistance of pines.

*B. xylophilus* usually co-inhabits with several other nematode species, such as *Seinura wuae*, *B. mucronatus*, *B. aberrans*, etc. [35]. These free-living nematodes have been recognized for their potential as biological control agents and their significant value in managing PWN infestations. For example. *Parasitorhabditis* sp. significantly reduced PWN population densities both in the laboratory and in the field due to competition for space and resources [26]. The predatory *Seinura* species is considered a potential biocontrol agent for PWN [35]. Generally, these free-living nematodes were significantly more abundant in limed forest soil than in unlimed forest soil [36]; therefore, PWN was probably suppressed in neutral or alkaline soils.

Some bacteria also play an important role in PWD, as phytotoxins secreted by PWN-associated bacteria may be involved in the pathogenesis of PWD by damaging plant cells [25]. Nematodes and bacteria mutually enhanced proliferation in an infected tree and also on pine callus tissue [25]. Both PWNs and bacteria increased in number, especially the bacteria *Pseudomonas fluorescens*, *Pantoea* sp. and *Sphimgomenas pancimobilis* [37]. Since some bacteria like *P. fluorescens* prefer a slightly acidic environment around pH 5.5 than pH 6 or 6.3 [38], as well as the bacterial community in infected pines was also linked to soil microbiota [39]. Therefore, acidic soil and trunk could create a better growth environment for bacteria and PWN.

Acid rain could also promote PWD by reducing pine resistance to pathogens [40]. The wilting rate of infected black pine seedlings was found to be significantly accelerated when subjected to simulated acid rain compared to those treated with distilled water [14,17]. Additionally, the mortality rate increased with the acidity and the initial population density of the inoculated PWN [41]. The mortality rate was highest when acidic water was irrigated directly onto the rhizosphere soil. With the same initial population density of PWN, spraying acidic water on soil could lead to a more significant increase in PWN than on needles, which is about six times higher than spraying distilled water [41]. Although healthy seedlings were not killed by acid rain or distilled water, more needles significantly fell off when exposed to acid rain than distilled water [14,42]. The daily sap flow amount of pine trees exposed to acid rain dropped to zero 30 days after inoculation, which was 10 days earlier than those exposed to distilled water [17]. Root growth of *P. thunbergii* was also inhibited under simulated acid rain at pH 2 [17]. As we observed, 15% of precipitation belonged to acid rain. Therefore, acid rain coupling with soil pH accelerated wilting rate and increased the mortality rate of black pines in Changdao.

### *4.3. The Common Method in Deal with PWD*

Controlling and preventing *B. xylophilus* poses significant challenges due to its ability to cause severe damage, rapid mobility, wide distribution, and strong drug resistance to conventional treatments [43]. Multiple strategies are employed from different perspectives to control PWD, such as (1) control PWN themselves, (2) control insect vectors, and (3) increase resistance to PWN. Based on the above three directions, several chemical controls, physical controls and biotic controls have been invented [2]. In detail, (1) chemical controls have been widely used as a major strategy for eradicating and preventing the spread of PWD. Current control methods are mainly based on nematocides or pesticides (abamectin, fluopyram, etc.) via chemical fumigation, spraying or trunk injection. Regrettably, the employment of chemical controls in PWD management is accompanied by the unavoidable detriment to the surrounding regions, thus posing potential risks to human health and the ecological environment. Moreover, although several chemical nematocides are commercially available, their widespread application is impeded by their exorbitant cost and toxicity. (2) Physical control methods have been proven to be highly effective in managing PWD. Various tactics, such as felling, crushing, and burning infected pine trees, as well as the establishment of isolation belts, can be utilized for large-scale treatments. (3) Biotic control is generally environmentally friendly, increasingly favored and widely used by humans. Biocontrol harnesses the natural capabilities of organisms such as predators, parasites, entomopathogenic microorganisms, fungi, and entomophilic nematodes to effectively manage the disease. Biotic control mainly includes biotic pesticides (piperine, antibiotics, matrine, etc.), biological competition (free-living nematodes, etc.) [26], biocontrol agents (*Esteya vermicola*, etc.) and quarantine.

### *4.4. Successful Cases of Controlling Plant Diseases by Regulating Soil pH*

In addition to pines, soil pH strongly influenced the severity of Fusarium wilt on strawberry plants. In strawberry fields, areas with a high incidence of Fusarium wilt typically exhibit a soil pH of 4.7 to 5.4, whereas low-abundance areas tend to have a soil pH of 6.3 to 6.7 [11]. Another study demonstrated that the severity of the disease was most pronounced in highly acidic soil, characterized by a pH of 5.2. Conversely, the disease exhibited the least severity in neutral soil with a pH of 6.7, where it had the least impact on the growth of strawberry plants [10]. Field studies have demonstrated successful control of Fusarium wilt on strawberry plants through the regulation of soil pH. The results showed that the best growth of strawberries occurred at pH 6.7, followed by pH 7.5, 5.8, and 5.2 [10]. Therefore, raising soil pH has been proposed as a method for managing Fusarium wilt in various crops, including tomatoes, cotton, melons, and bananas, due to the observed relationship between higher soil pH and lower levels of the disease [9,44]. Similar to Fusarium wilt, diseases like club root of *Brassicas* and bacterial canker of peaches are aggravated by the acidification of alkaline soils or decreased by liming acid soil [45], and PWD in our study. However, some other diseases are reduced due to the acidification of alkaline soils or increased by liming acid soils, such as scab of potato, take-all in wheat, *Phytophthora* root rots, *Pythium* root rots of pines, and *Vericillium* wilt of sunflowers [45]. Therefore, manipulating soil pH holds significant potential for effectively managing plant diseases in a sustainable manner without the current reliance on chemicals.

### *4.5. Soil pH Regulation with SP and Its Prospects*

Soil pH regulation is a method that combines chemical and biotic control. Since some of the above control strategies may cause environmental issues or high costs, SP could be used as an excellent alternative. In Northern China, plenty of *P. thunbergii* are planted along the coast to protect the coastal zone, where various shells such as oysters, scallops and clams are readily available. If not used, a large number of shells could just be discarded as garbage every year. Applying SP to regulate soil pH can convert waste into resources. After simple washing to remove salt, drying and grinding, all shells can be applied with low cost and simple operation and are conducive to promotion.

In addition, the excessive use of QL leads to the decomposition of soil organic matter, damage to soil structure and a reduction in the availability of iron, manganese, copper, zinc and phosphorus in the soil. SP is a natural substance mainly composed of $CaCO_3$ but also contains copper, magnesium, potassium, molybdenum, phosphorus, manganese, iron, zinc, etc. Although the pH increase in the SP group was lower than that in the QL group, the decrease in IR was higher. It is possible that these medium elements and microelements promote plant growth. In previous investigations, we found that the soil of Changdao was slightly deficient in Fe (15.4 mg/kg) and Mo (0.06 mg/kg), and its content of Mn (9.9 mg/kg) and Zn (1.7 mg/kg) and Ca (8.7 g/kg) was lower than the average content of Northern China. The deficiency of various elements would affect the growth of black pines, such as chlorophyll synthesis, yellowing of new leaves and cell division. After improving these processes, the resistance of PWN will be enhanced, and then pines will be restored from infection. Thus, these elements contained in SP are also a good supplement to the local soil.

## 5. Conclusions

This study reveals the possibility of soil pH regulation to alleviate PWD. Consistent with our hypothesis, the soil pH of black pine forests is generally acidic, even under asymptomatic trees. In the pot experiments, finely ground shell powder is the optimal size for long-term and efficient regulation. The best regulation ameliorant on soil pH is QL, followed by F-SP and PA. In the in situ experiments, the effect of QL was slightly better than F-SP in terms of increasing soil pH. Whereas the symptomatic trees decreased separately by 15.9% and 5.4% when treated with F-SP and QL. Therefore, treatment with F-SP was better than with QL for alleviating PWD. Soil pH has a complex mechanism in terms of manipulating PWN, which could be coupled with fungi, nematodes, bacteria, and acid rain to influence pine resistance. Applying SP to regulate soil pH can convert waste into resources. By increasing soil pH and supplementing nutrient elements, the resistance of pine trees can be comprehensively enhanced. Therefore, manipulating soil pH by SP holds significant potential and practical value for effectively managing PWD in a sustainable manner.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14081583/s1, Table S1. The soil pH under *Pinus thunbergii* forest; Table S2. The soil pH under other vegetation types.

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