

Article

# *Phytophthora cinnamomi* Colonized Reclaimed Surface Mined Sites in Eastern Kentucky: Implications for the Restoration of Susceptible Species

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**Abstract:** Appalachian forests are threatened by a number of factors, especially introduced pests and pathogens. Among these is *Phytophthora cinnamomi*, a soil-borne oomycete pathogen known to cause root rot in American chestnut, shortleaf pine, and other native tree species. This study was initiated to characterize the incidence of *P. cinnamomi* on surface mined lands in eastern Kentucky, USA, representing a range of time since reclamation (10, 12, 15, and 20 years since reclamation). Incidence of *P. cinnamomi* was correlated to soil properties including overall soil development, as indicated by a variety of measured soil physical and chemical parameters, especially the accumulation of soil organic carbon. *P. cinnamomi* was detected in only two of the four sites studied, aged 15 and 20 years since reclamation. These sites were generally characterized by higher organic matter accumulation than the younger sites in which *P. cinnamomi* was not detected. These results demonstrate that *P. cinnamomi* is capable of colonizing reclaimed mine sites in Appalachia; additional research is necessary to determine the impact of *P. cinnamomi* on susceptible tree species at these sites.

**Keywords:** forest health; mine reclamation; Forestry Reclamation Approach; *Phytophthora*; ink disease; American chestnut

## 1. Introduction

Appalachian forests are threatened by many stressors, including climate change [1,2], land use change [3–5], and invasive pests and pathogens [6,7]. Forest restoration and management efforts must be informed by a clear understanding of these and other impacts to ensure forest health and resilience in the future [8].

The American chestnut (*Castanea dentata* (Marsh.) Borkh.) story is a well-known example of the dramatic effects of invasive pathogens. American chestnut was once a dominant canopy species throughout the Appalachian region, which includes the states of West Virginia and parts of Alabama, Georgia, Kentucky, Maryland, Mississippi, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, and Virginia. Composing 50% or more of the forest canopy over much of its range, American chestnut was functionally eliminated from eastern forests in only a few decades in the early 1900's by the introduced fungal pathogen causing chestnut blight, *Cryphonectria parasitica* (Murr.) Barr [7,9,10]. Thanks to breeding targeted at introducing resistance genes from blight-resistant

Chinese chestnut (*Castanea mollissima* Blume) into American chestnut, American chestnut varieties with reasonable levels of blight resistance are becoming available for use in restoration plantings [11].

Unfortunately, even before the introduction of chestnut blight, *Phytophthora cinnamomi* Rands, a pathogen causing ink disease in American chestnut, had been introduced in the southeastern US and had been slowly causing American chestnut decline in the mid-late 1800s [12,13]. Thought to have originated from Papua New Guinea or Taiwan (but since distributed globally) [14], *P. cinnamomi* is a soil-borne oomycete pathogen with >2000 susceptible species [15], with disease symptoms including black exudate staining infected roots, chlorotic leaves, and thinning crowns [7]. The pathogen continued its slow march northward, overshadowed by the much more dramatic activity of chestnut blight [7]. Unfortunately, genes conferring resistance to chestnut blight do not necessarily also confer resistance to *P. cinnamomi*; thus, some of the early blight-resistant American chestnut varieties were killed by *P. cinnamomi* when planted [16,17]. These early observations have led researchers to focus on identifying genes conferring resistance to *P. cinnamomi* and developing strategies for improving blight-resistant varieties to also be *P. cinnamomi* resistant [18,19]. In addition to the genetic improvement of susceptible hosts, such as American chestnut, a recent review recommended the construction of a more robust dataset on *P. cinnamomi* distribution in eastern US forests [20].

*P. cinnamomi* is broadly distributed globally. At very small spatial scales (i.e., ~1 m<sup>2</sup>), the pathogen appears to be somewhat randomly distributed; although it may be present within a given square meter soil patch, it is likely to be detected in only a fraction of samples collected within that patch [21–23]. Pathogen propagules are also capable of moving vertically within the soil profile, retreating to depth to survive inclement conditions [21,24]. Across broader spatial scales, *P. cinnamomi* is thought to be associated with moist soils at low topographic positions, such as drainages. Conversely, the pathogen is generally thought to be absent from higher and drier soils [22,23,25,26]. However, *P. cinnamomi* has been isolated from dry ridgetop soils in Australia [27] and eastern Kentucky [28], suggesting that environmental conditions limiting *P. cinnamomi* and its distribution are complex. *P. cinnamomi* has been present in the southeastern US for over 150 years [12], and is known to be widely distributed in the Appalachian region [29,30], but topographic and soil factors controlling its distribution at smaller spatial scales (e.g., within a watershed) have not been elucidated in any detail. These patterns must be well-understood before informed decisions about the restoration of susceptible tree species can be made.

In addition to introductions of non-native pests and pathogens, Appalachian forests have been degraded by a legacy of surface mining for coal. An estimated 600,000 ha of formerly forested land in Appalachia have been converted to novel grassland systems, characterized by high soil compaction and vegetative competition, and generally unfavorable for colonization by native trees [31–33]. These grassland patches perpetuate negative impacts of surface mining into the future, increasing forest fragmentation, increasing species invasion opportunity, and inhibiting site productivity and carbon storage [3,33–35].

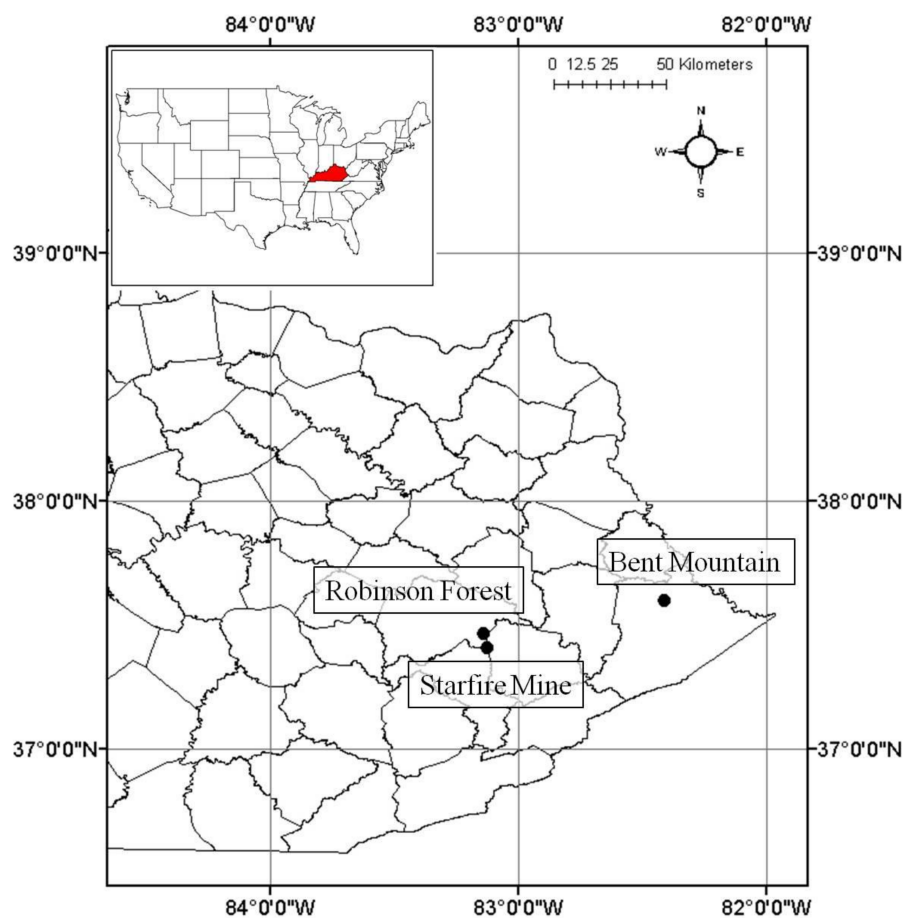
This situation prompted researchers, regulators, and industry practitioners to develop a set of recommendations for reforestation at surface mined sites, termed the Forestry Reclamation Approach (FRA, [36]). These recommendations encourage minimal spoil compaction and reduced vegetative competition, improving the growth and survival of native trees over traditional reclamation practices [37–39]. These mine spoils, while initially devoid of organic matter, can support rapid tree growth and organic matter accumulation, and are considered soils after reclamation [34]. Yellow poplar (*Liriodendron tulipifera* L.) and white oak (*Quercus alba* L.) planted in FRA plots exhibited growth rates similar to the growth of these species in naturally regenerating clear cuts [40]. A chronosequence study in West Virginia found that soil organic carbon (SOC) accumulation in mine soils could be predicted by a logarithmic model, with 75% of 50-year SOC stock (13.3 Mg ha<sup>-1</sup>) accumulating in the first ten years [41]. Other studies have found even higher SOC accumulation—stocks of 19.2 Mg ha<sup>-1</sup> 13 years after reclamation and 38 Mg ha<sup>-1</sup> 25 years after reclamation at sites in Ohio [34,42], and 16.8 Mg ha<sup>-1</sup> 16 years after reclamation at sites in Kentucky [34]. Together with SOC accumulation, above-ground biomass and litter accumulation contribute to overall ecosystem C

sequestration, projected at 140.8–162.3 Mg ha<sup>-1</sup> after 60 years in forest reclamation sites from Kentucky, Ohio, Indiana, and Illinois [34].

Because these mine soils are typically not reclaimed with native topsoil, *P. cinnamomi* and other soil-borne pathogens are generally thought to be initially absent from these sites [43–46]. FRA sites may also be unfavorable for *P. cinnamomi* for some time after reclamation due to high infiltration rates, low moisture retention, and relatively high temperatures associated with low shade, low organic matter, and little to no clay content [47–49]. *P. cinnamomi* was not detected in mine reforestation plots in southeastern Kentucky one and three years after reclamation [50], and was also not detected in a number of sites 3–20 years after reclamation in Ohio [46]. However, a recent chronosequence study in eastern Kentucky suggests that rates of microbial activity (assayed by dehydrogenase activity), microbial biomass C and N, litter decomposition rates, and CO<sub>2</sub> efflux in mined sites eight years after reclamation were similar to native forests 12 years after clear-cutting [51]. It is possible that *P. cinnamomi* can colonize these sites over time, along with other soil microorganisms, as soil development progresses. This study was initiated to evaluate whether *P. cinnamomi* colonizes FRA sites representing a chronosequence of time since reclamation in eastern Kentucky, and relate this incidence to soil development parameters, especially SOC accumulation.

## 2. Methods and Materials

Eight reclaimed surface mined sites of varying ages (two sites each reclaimed in 1997, 2003, 2005, and 2007) in eastern Kentucky were selected for screening for the presence of *P. cinnamomi* (Figure 1).



**Figure 1.** Location of reclaimed mine sites and unmined forest control, Breathitt (Robinson Forest), Perry (Starfire Mine), and Pike (Bent Mountain) Counties, Eastern Kentucky, USA.

Sites reclaimed in 1997 and 2003 were located at Starfire Mine (Breathitt County, Kentucky, 37.40939° N, −83.1229° W), and sites reclaimed in 2005 and 2007 were located at Bent Mountain Mine (Pike County, Kentucky, 37.60023 N, −82.40848 W). Climate in this region is temperate humid continental, with an average temperature of 13.3 °C [52]. Average annual precipitation in Robinson Forest (adjacent to the Starfire Mine site) is 117.5 cm [53], and average annual precipitation in Pike County is 114 cm [47]. Typical temperatures range from 18–30 °C in summer and −5–6 °C in winter at Robinson Forest, and from 18–32 °C in summer and −4–7 °C in winter in Pike County [47]. Sites were constructed using low-compaction spoil placement techniques [36], with overburden sourced from the Breathitt formation, which is dominated by sandstones and shale [54]. For site details, see [40] for the 1997 site, [51] for the 2003 site, [55] for the 2005 site, and [56] for the 2007 site. Similar tree species were planted at each of these sites during reclamation, and white oak was present at all sites at the time of sampling. Because these sites were reclaimed using similar techniques with spoil from the same geologic formation, and are subject to similar weather conditions, we employed a site-for-time substitution (chronosequence approach) to evaluate trends over time [51].

Twenty soil samples were collected underneath white oak at each site in October–November 2017, for a total of 160 samples, and all 160 samples were screened independently. Samples for *P. cinnamomi* screening were collected in 50 mL tubes from the top 5 cm of soil. Samples were screened using a rapid screening approach developed in our laboratory [28]. Briefly, ~40 mL soil samples were flooded with sterile water in 50 mL tubes, and baited with six ~6 mm diameter rhododendron leaf discs for five to seven days. Baits were then removed and frozen in 1.5 mL tubes for subsequent DNA extraction. DNA was extracted from baits using the DNeasy UltraClean Microbial DNA Extraction Kit [Qiagen]. Amplifiable DNA was confirmed for each DNA extraction using universal ITS1–ITS4 primers [57]. DNA was screened for *P. cinnamomi* using a conventional PCR assay with primers Ycin3F and Ycin4R targeting the Ypt gene recommended for *P. cinnamomi*-specificity [58,59]. Samples were screened in duplicate with positive controls, *P. cinnamomi* isolate RF5 (isolated from Robinson Forest, GenBank Accession #MF966152) limit of detection  $1.5 \times 10^{-2}$  ng per PCR, and no template negative controls. Primer annealing temperatures were 55 °C and 58 °C for the ITS and Ypt PCRs, respectively, with PCR amplicons visualized using agarose gel electrophoresis, 1.5% (*m/v*). *P. cinnamomi* incidence was assessed as % of total samples (20 samples screened per site) screened as positive.

In addition to screening for *P. cinnamomi*, a number of soil physical and chemical parameters were assessed at these reclaimed mine sites, as well as a mature white oak stand in Robinson Forest (Breathitt and Perry Counties, Kentucky) selected as an unmined forested control. Three soil samples per site age (sampled to 10 cm using a soil probe) were collected for chemical and physical analyses, and data were averaged by site age (10, 12, 15, and 20 years since reclamation), to permit direct comparison to data reported by previous studies [60]. Particle size was assessed by quantifying the mineral grain size distribution, and sand, silt, and clay fractions as defined by the Wentworth Scale [61]. These samples were dried at ~75 °C for 24–48 h, gently disaggregated, wet-sieved through 2 and 0.5 mm sieves, and treated with dilute H<sub>2</sub>O<sub>2</sub> to destroy organic binding agents [62,63]. Samples were then analyzed using a Malvern Mastersizer S-2000, a laser-optical particle size characterization instrument capable of accurately resolving particles over a size range of 0.02 to 2000 µm.

Concentrations of Al, Mn, Fe, Mg, K, Ca, and Na were assessed by inductively coupled plasma mass spectrometry (ICPMS) after samples were completely dissolved using concentrated acids (HF, HCl, and HNO<sub>3</sub>) over heat. Bulk density was assessed by the excavation method [64], and SOC was measured using an LECO CHN 2000 analyzer after an acid pretreatment (HCl). Although conventional SOC assessment (using a LECO analyzer) follows an acid pretreatment to remove inorganic carbon (e.g., carbonate minerals), this step has been known to incompletely eliminate carbonates from carbonate-rich mine soils, and can overestimate SOC [60,65]. Thus, soil organic matter (SOM) was evaluated by the thermogravimetric method—this method more accurately differentiates “new organic carbon” contributed by biomass from “old organic carbon” contributed by coal fragments or inorganic carbon contributed by carbonate minerals [60,65].  $\delta^{13}\text{C}$  (‰) was measured after HCl

pretreatment (and was thus reflective of organic C only) on a Thermo-Finnigan Delta XP Isotope Ratio Mass Spectrometer.

Soil physical and chemical data (means by site age) were analyzed by regression, together with data from previous studies at these sites where available [40,49–51,55,56,60], with years since reclamation as the main effect (PROC GLM, SAS 9.3). Both linear and quadratic relationships were tested for each variable; data were interpreted using a quadratic regression if the quadratic factor was significant ( $p < 0.05$ ), but were interpreted using a linear regression if the quadratic factor was insignificant ( $p > 0.05$ ). Sand and silt data were available from these sites at times representing 0, 1, 2, 3, and 8 years after reclamation [40,49–51,55,56,60]. SOM, SOC, and  $\delta^{13}\text{C}$  data were available for these sites at times representing 0, 2, 3, and 8 years since reclamation [51,60]. *P. cinnamomi* incidence was interpreted in light of trends over time in soil physical or chemical parameters to provide insight into *P. cinnamomi* colonization of these sites.

### 3. Results

Soil particle size distribution was significantly correlated ( $p < 0.05$ ) with time since reclamation for % sand and % silt (Figure 2). Sand decreased from 70% in new mine soils to <40% in mine soils 12–20 years after reclamation. In contrast, silt increased from 20% in new mine soils to 55% in mine soils 12–20 years after reclamation. This shift in particle size distribution toward a dominance of silt may be related to increasing trends in concentrations of some of the metal analytes evaluated in this study (e.g., Al, Fe, Mg, and Ca, Table 1). Decreased soil particle size corresponds to dramatically increased soil surface area and reactivity, which very likely increases the sorption of these and other cations [66,67].

**Table 1.** Soil physical and chemical characteristics.

|          | Years Since Reclamation |               |               |               | Robinson Forest |
|----------|-------------------------|---------------|---------------|---------------|-----------------|
|          | 10                      | 12            | 15            | 20            |                 |
| Al (ppm) | 36,800 ± 640            | 36,800 ± 1200 | 46,600 ± 7200 | 48,100 ± 9800 | 50,800 ± 5000   |
| Mn (ppm) | 184 ± 8.5               | 316 ± 25      | 321 ± 38      | 259 ± 39      | 997 ± 150       |
| Fe (ppm) | 9770 ± 140              | 17,800 ± 960  | 17,400 ± 2500 | 19,800 ± 3400 | 25,600 ± 2000   |
| Mg (ppm) | 1420 ± 37               | 2280 ± 63     | 2700 ± 550    | 3300 ± 680    | 1980 ± 230      |
| K (ppm)  | 13,500 ± 400            | 12,400 ± 800  | 15,400 ± 2500 | 14,600 ± 2700 | 12,900 ± 1400   |
| Ca (ppm) | 489 ± 36                | 1040 ± 130    | 1540 ± 440    | 1220 ± 62     | 455 ± 110       |
| Na (ppm) | 3880 ± 31               | 2740 ± 120    | 2650 ± 220    | 1800 ± 590    | 833 ± 57        |
| % Sand   | 60.6 ± 3.3              | 37.5 ± 1.9    | 48.4 ± 5.9    | 38.1 ± 2.1    | 27.3 ± 3.7      |
| % Silt   | 35.9 ± 3.0              | 56.4 ± 2.2    | 47.1 ± 5.8    | 55.1 ± 2.0    | 62.6 ± 3.6      |
| % Clay   | 3.45 ± 0.38             | 6.07 ± 0.48   | 4.53 ± 0.33   | 6.80 ± 1.6    | 10.1 ± 0.28     |

Total SOC, determined using an LECO analyzer, also demonstrated a significant correlation with time since reclamation, increasing from very low levels in new mine soils (0.1–0.2% in two to three year old soils) to >3.0% in 15–20 year old soils, nearing the SOC levels in unmined forest soils (Figure 3). Similarly, “new organic carbon” represented by SOM measured by the thermogravimetric method increased from 0.03–0.10% in zero to two year old mine soils to 1.5–2.2% in 15–20 year old mine soils, nearing SOM levels in unmined forest soils (Figure 4). SOC concentrations measured by the LECO analyzer tended to be higher than SOM measured by thermogravimetry (Figure 5).

While organic carbon concentrations increased with time since reclamation,  $\delta^{13}\text{C}$  values decreased with time since reclamation, approximating  $\delta^{13}\text{C}$  values in unmined forest soils (Figure 6). This relationship between  $\delta^{13}\text{C}$  and SOM is negative and quadratic, with lower  $\delta^{13}\text{C}$  values and higher SOM concentrations in the older mine soils, nearing levels in unmined forest soils (Figure 7). Total SOM stocks ( $\text{Mg C ha}^{-1}$ ) exhibited different trends across sites, suggesting some differences in site quality, likely related to differences in regional geology and site construction (Figure 8) [60]. However, at both sites, SOM stocks approached  $20 \text{ Mg ha}^{-1}$  by eight to 12 years after reclamation.

*P. cinnamomi* was detected at all four Starfire sites (15 and 20 years after reclamation), but was not detected in any of the Bent Mountain sites (10 and 12 years after reclamation, Figure 9). Of the samples screened at the Starfire sites, 27.5% of samples (range: 5–50%) at the 15-year old sites were positive, and 12.5% (range: 10–15%) of samples at the 20-year old sites were positive. These sites tended to have lower % sand and higher % silt, as well as increased SOC and SOM stocks, compared to the younger Bent Mountain sites.

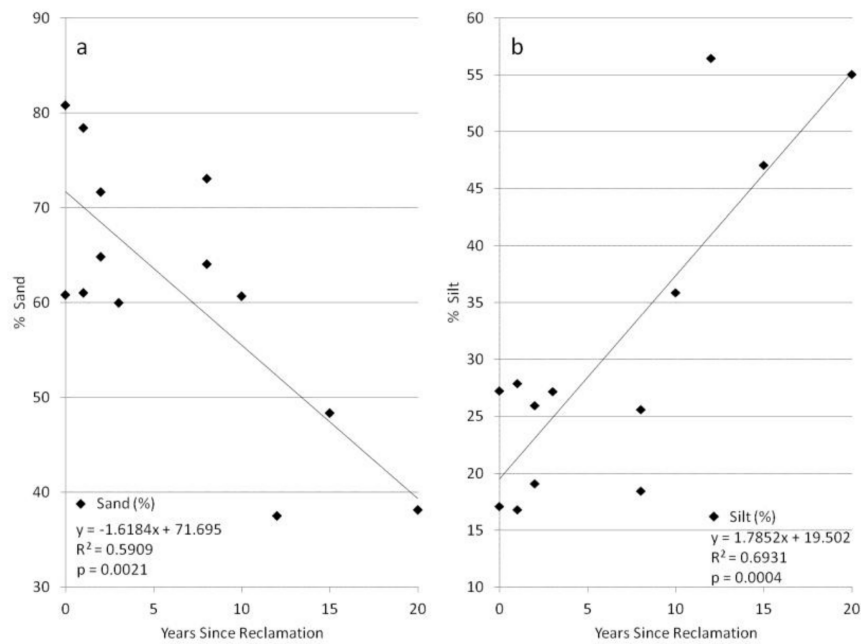


Figure 2. Changes in (a) % sand and (b) % silt in mine soils over time since reclamation.

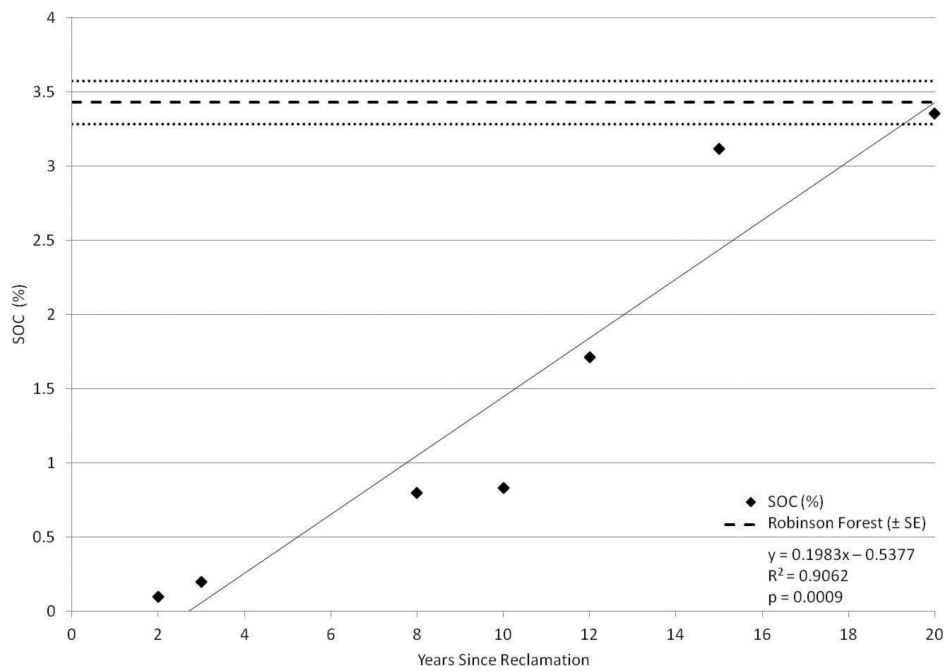
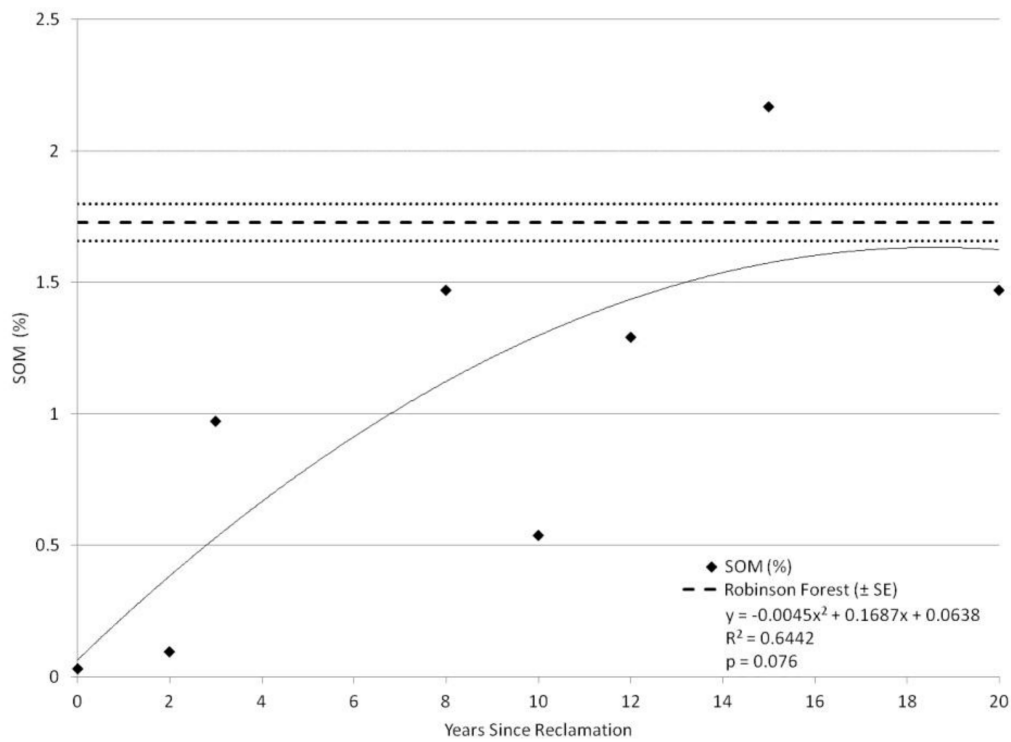
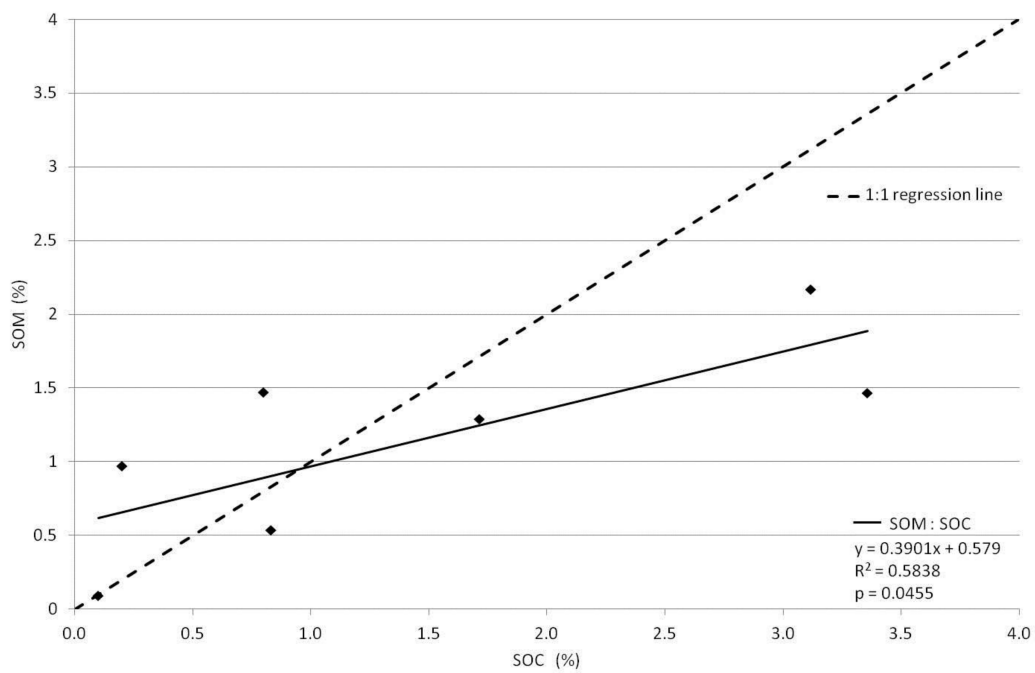


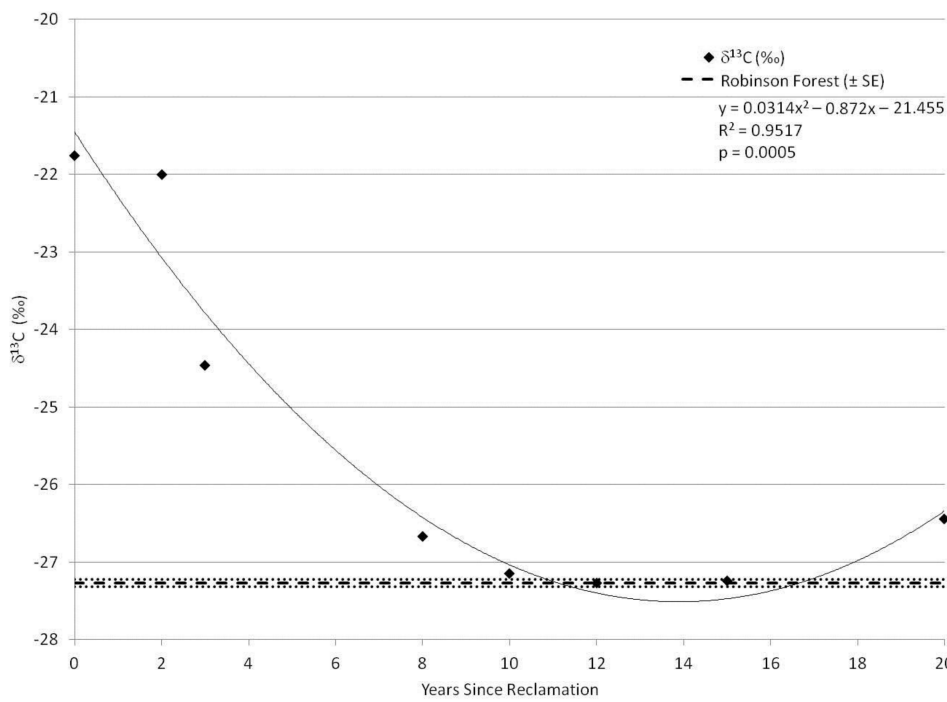
Figure 3. Changes in soil organic carbon (SOC; LECO analysis) in mine soils over time since reclamation, with mean % SOC ( $\pm$ SE) for Robinson Forest plotted for reference.



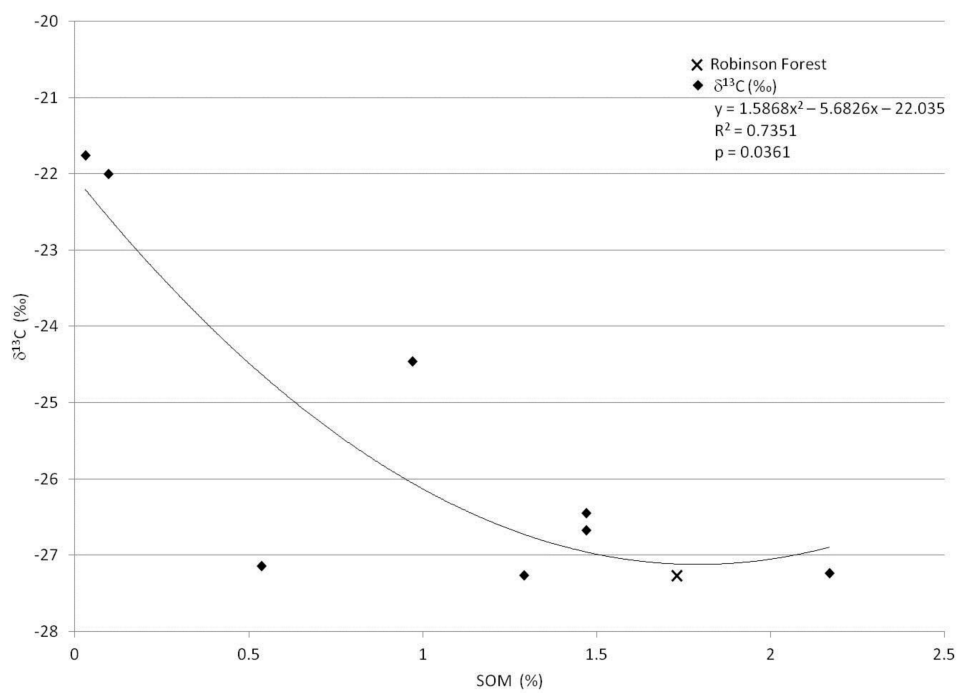
**Figure 4.** Changes in soil organic matter (SOM; thermogravimetric analysis) in mine soils over time since reclamation, with mean % SOM ( $\pm$ SE) for Robinson Forest plotted for reference.



**Figure 5.** Correlation of SOC (LECO analysis) and SOM (thermogravimetric analysis), compared to a 1:1 reference line.

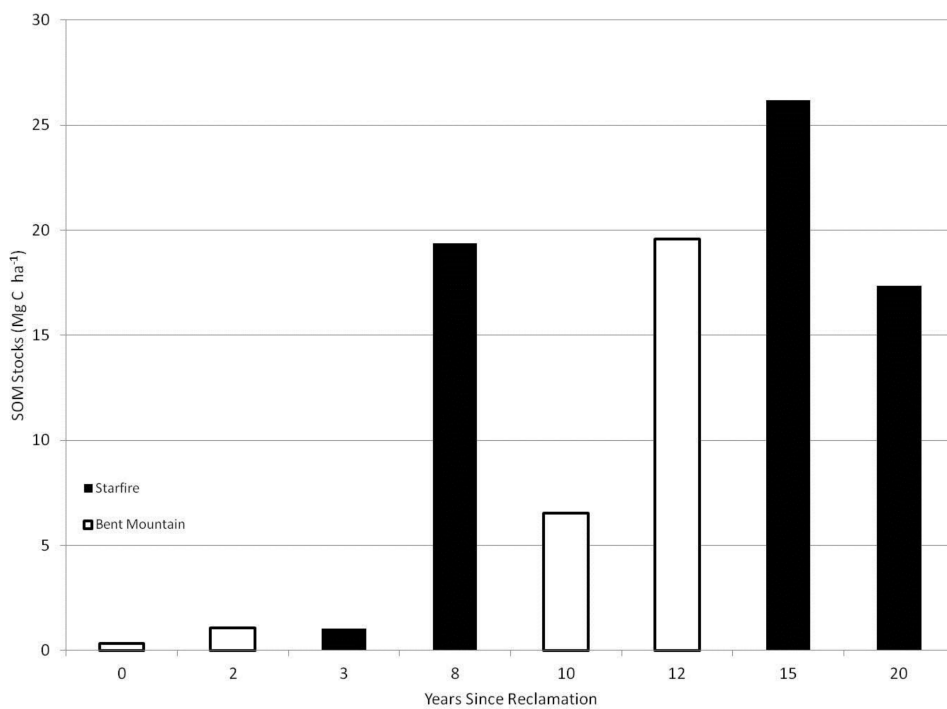


**Figure 6.** Changes in  $\delta^{13}\text{C}$  (‰) in mine soils over time since reclamation, with mean  $\delta^{13}\text{C}$  ( $\pm\text{SE}$ ) for soil from a mature white oak stand in Robinson Forest plotted for reference.

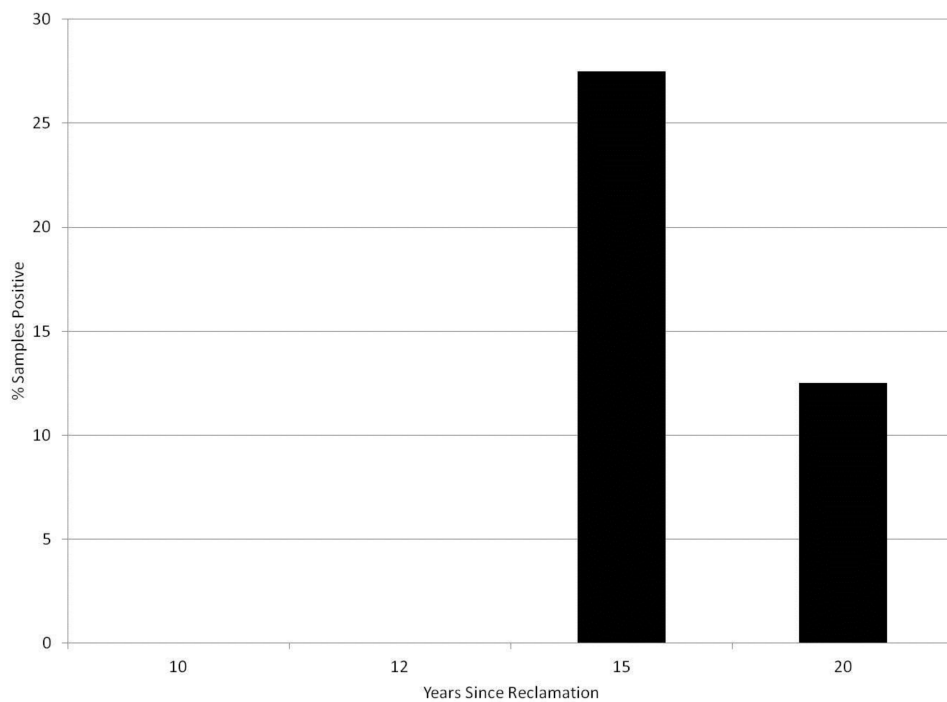


**Figure 7.** Correlation between % SOM (thermogravimetric analysis) and  $\delta^{13}\text{C}$  (‰) in mine soils representing a range of time since reclamation, with Robinson Forest plotted for reference.





**Figure 8.** SOM stocks in mine soils over time since reclamation. Rates of accumulation of SOM in Bent Mountain sites appeared to lag slightly behind those of Starfire Mine sites.



**Figure 9.** Incidence of *Phytophthora cinnamomi* in mine soils over time since reclamation.

#### 4. Discussion

Soil particle size shifts are consistent with those observed by previous studies at these sites, generally reporting increased fines and decreased coarse fractions with time [39,56]. Over time, this shift in particle size is certain to increase soil reactivity, reflected in the increasing concentrations of metals

seen in this study [66,67]. Increased SOC and SOM concentrations with time since reclamation are also consistent with other studies investigating mine soil development [34,41,60]. Values of SOC (LECO analyzer) tended to be higher than values of SOM (thermogravimetric method) in this study. Acid (HCl) pretreatment followed by measurement of SOC on a LECO analyzer can inadequately remove inorganic carbon by incompletely dissolving carbonate minerals in carbonate-rich mine soils. LECO analysis also does not distinguish “old organic carbon” in coal fragments from “new organic carbon” in biomass, which can also lead to the overestimation of SOC [60,65]. The thermogravimetric method used in this study to assess SOM more accurately differentiates “new organic carbon” in biomass from inorganic carbon (e.g., carbonate minerals) and “old organic carbon” (e.g., coal fragments); thus, values of SOM (thermogravimetric method) are expected to be lower than those of SOC (LECO analyzer) [60,65]. SOM stocks at the sites included in this study (20 Mg ha<sup>-1</sup> by eight to 12 years after reclamation) were similar to those reported by other studies on reclaimed sites in Appalachia, ranging from 13.3 Mg ha<sup>-1</sup> over 10 years in West Virginia [41], to 26 Mg ha<sup>-1</sup> after 10 years in Ohio [34,42].

*Phytophthora cinnamomi* was only detected at sites 15 and 20 years after reclamation, demonstrating that *P. cinnamomi* is capable of colonizing FRA reclaimed sites over time. The older sites where *P. cinnamomi* was detected were located on Starfire Mine (Perry County), while the younger sites where *P. cinnamomi* was not detected were located at Bent Mountain (Pike County). While some differences in site quality are suggested by observed differences in SOM accumulation, *P. cinnamomi* has been documented in areas adjacent to both mine sites, isolated from soils in Robinson Forest (adjacent to Starfire Mine, Breathitt County) [17,50] and from a dead American chestnut seedling at a nearby reforestation plot on Bent Mountain (Pike County) [68]—suggesting that sources of *P. cinnamomi* are available for colonization at all sites. A parallel study in Robinson Forest detected *P. cinnamomi* in 45% of screened samples [28], suggesting that the incidence of *P. cinnamomi* in unmined forest may be higher than the frequencies reported for reclaimed mined land in this study. Future surveys will clarify whether *P. cinnamomi* becomes more prolific in these sites over time.

Infected seedlings may be an important source of contamination on these sites—a California study documented the introduction of *P. cinnamomi* to previously uninfested sites via seedlings used in restoration plantings [69]. As mentioned above, *P. cinnamomi* was detected on a dead American chestnut in a restoration planting on Bent Mountain; it is unknown whether this seedling was infected before planting (i.e., with propagules from the nursery at which it was grown) or after planting (i.e., with propagules already present at the site) [68]. Nurseries supplying seedlings for the restoration of these surface mined areas should be screened for the presence of *P. cinnamomi* to reduce the risk of contaminating restoration sites.

To our knowledge, this is the first study reporting *P. cinnamomi* incidence at FRA sites. In a previous study, *P. cinnamomi* was not detected at a site in eastern Kentucky (the 10 year old site in this study) during the first season after spoil placement [50]. *P. cinnamomi* was also not detected at a series of reclaimed sites in Ohio ranging from three to 20 years since reclamation [46]. These researchers did not report surveys of adjacent forest soils for *P. cinnamomi*; thus, while *P. cinnamomi* had been previously reported in more southerly regions of Ohio [70], it is unknown whether *P. cinnamomi* is present in unmined forests in their study area.

More generally, a recent study in eastern Kentucky found that some microbial community metrics, such as microbial biomass C and N, and microbial activity (assessed by dehydrogenase activity), in mine soils eight years after reclamation, were similar to regenerating clear cut soils in unmined forests [51]. The current study supports observations that microbial community development occurs over time since reclamation, alongside plant community and soil development, including the recruitment of individual plant pathogens such as *P. cinnamomi*.

In previous studies on FRA-reclaimed sites, such as one of the Bent Mountain sites referenced here, forest development occurred rapidly on favorable mine soils, with planted trees achieving partial canopy closure after only nine growing seasons [39]. Alongside tree growth and canopy closure, these researchers also reported the development of a litter layer and recruitment of shade-tolerant

understory species [39]. Over time, shading provided by canopy closure and moisture storage provided by accumulating litter are expected to regulate soil moisture conditions, increasing site favorability for the recruitment of soil microbes. *P. cinnamomi* is thought to prefer moist soils—development of conditions increasing soil moisture may improve site quality for *P. cinnamomi* [71]. However, the presence of *P. cinnamomi* alone is not sufficient to cause disease in some susceptible hosts. For example, although *P. cinnamomi* was widespread at reclaimed bauxite mine sites in Western Australia, root rot in susceptible jarrah (*Eucalyptus marginata*) was related to high moisture conditions in poorly drained sites with ponding rainwater [72]. In these systems, researchers recommended intentional site preparation (e.g., deep tillage to improve drainage) to reduce ponding and reduce *P. cinnamomi* infection risk [73]. FRA sites are constructed with low-compaction spoil placement techniques, and are characterized by high infiltration and low runoff rates [36,47–49]. Although *P. cinnamomi* was detected on 15- and 20-year old FRA sites, no above ground symptoms of Phytophthora root rot were observed in the chestnuts and white oak growing at these sites; additional studies on roots of these species will be necessary to definitively document infection status at these sites. Although *P. cinnamomi* is present, it is unclear whether or not conditions at these sites are conducive for the development of Phytophthora root rot in susceptible species. Also, follow-up studies will be required to assess whether *P. cinnamomi* will eventually colonize the 10- and 12-year old sites screened in this study in which *P. cinnamomi* was not detected.

## 5. Conclusions

These data suggest that site quality at FRA-reclaimed mine sites is sufficient by 15 years after reclamation for colonization by *P. cinnamomi*. To our knowledge, this is the first study documenting *P. cinnamomi* colonization of FRA-reclaimed mine sites, and demonstrates that these sites do not remain “Phytophthora-free” over time. Additional research will be necessary to clarify the impact of *P. cinnamomi* on susceptible hosts at these sites. While *P. cinnamomi* was detected at these sites, it is unclear whether or not environmental conditions are conducive to the development of *P. cinnamomi*-related disease in susceptible hosts (such as white oak). Finally, potential routes of invasion of *P. cinnamomi* onto reclaimed mine sites should be assessed—especially distinguishing whether *P. cinnamomi* is more likely to colonize sites via infected seedlings used in plantings, or by the transport of propagules from adjacent infested forest sites.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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