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Decline of European Beech in Austria: Involvement of *Phytophthora* spp. and Contributing Biotic and Abiotic Factors

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Abstract: A severe decline and dieback of European beech (*Fagus sylvatica* L.) stands have been observed in Austria in recent decades. From 2008 to 2010, the distribution and diversity of *Phytophthora* species and pathogenic fungi and pests were surveyed in 34 beech forest stands in Lower Austria, and analyses performed to assess the relationships between *Phytophthora* presence and various parameters, i.e. root condition, crown damage, ectomycorrhizal abundance and site conditions. In total, 6464 trees were surveyed, and *Phytophthora*-associated collar rot and aerial bark cankers were detected on 133 trees (2.1%) in 25 stands (73.5%). Isolations tests were performed from 103 trees in 27 stands and seven *Phytophthora* species were isolated from bleeding bark cankers and/or from the rhizosphere soil of 49 trees (47.6%) in 25 stands (92.6%). The most common species were *P.* ×*cambivora* (16 stands) followed by *P. plurivora* (eight stands) and *P. cactorum* (four stands), while *P. gonapodyides*, *P. syringae*, *P. psychrophila* and *P. tubulina* were each found in only one stand. Geological substrate had a significant effect on the distribution of *P.* ×*cambivora* and *P. plurivora* while *P. cactorum* showed no site preferences. In addition, 21 fungal species were identified on beech bark, of which 19 and five species were associated with collar rot and aerial bark cankers, respectively. Four tested fine root parameters showed differences between declining and non-declining beech trees in both *Phytophthora*-infested and *Phytophthora*-free stands. In both stand categories, ectomycorrhizal frequency of fine root tips was significantly higher in non-declining than in declining trees. This study confirmed the involvement of *Phytophthora* species in European beech decline and underlines the need of more research on the root condition of beech stands and other biotic and abiotic factors interacting with *Phytophthora* infections or causing beech decline in absence of *Phytophthora*.

Keywords: *Phytophthora* ×*cambivora*; *Phytophthora plurivora*; root rot; bark canker; ectomycorrhiza

1. Introduction

European beech (*Fagus sylvatica* L.) is a dominant broadleaved tree species of temperate forests with a distribution of 17 million hectares across Europe [1–3]. Populations of beech are exposed to biotic and abiotic stressors, which are predicted to increase in intensity and frequency. Predicted climatic trends with increasing temperature and drought will affect the habitat suitability of European beech [2–4]. These climatic extremes are expected to restrict its xeric boundaries in Central and Southern

Europe [5,6]. During the last century, beech forests have suffered from repeated outbreaks of pests such as the limantrid moth (*Dasychira pudibunda* L.; syn. *Calliteara pudibunda* L.) and the beech scale insect (*Cryptococcus fagisuga*) [7,8], which occurred particularly often in Central and Northern Europe. Cankers caused by *Neonectria ditissima* and *N. coccinea* do not pose a serious risk to beech trees, but in combination with beech scale infestations they can trigger the complex "Beech Bark Disease" resulting in severe mortality [9–12]. The main biotic threat to European beech is posed by pathogens from the oomycete genus *Phytophthora*. First outbreaks of severe decline and mortality of beech forests caused by *Phytophthora* spp. were reported during the 1930s from the UK [13,14]. In recent decades, numerous studies have reported decline and dieback of beech stands caused by a diverse range of *Phytophthora* spp. in 16 European countries and in the USA [15–38]. Currently, 17 *Phytophthora* species are known to be associated with European beech forests [20,39]. In Central Europe, after the drought and heatwave of summer 2003, a dramatic increase in declining beech trees, showing crown defoliation, fine root destructions, collar rot and aerial cankers and eventually mortality, was observed in *Phytophthora* infested areas [19,30,40]. The predicted climatic changes and the increasing spread of non-native invasive *Phytophthora* spp. with infested nursery stock into the wider environment are expected to exacerbate the situation and further destabilize the European forests of beech, oaks and other tree species [20–22,30,41–47].

Site conditions have a strong effect on water availability, and hence, the physiological and health status of trees, and influence the spread and infections of *Phytophthora* species via sporangia and zoospores. In general, *Phytophthora* species have a preference for a soil pH higher than 3.5 and sandy-loamy to clayey soil texture, but the ecological amplitudes of individual *Phytophthora* species can differ substantially from each other [20,22,30,45,48]. Therefore, more field research is needed to establish significant associations between site conditions and specific *Phytophthora* species.

Most trees depend on their mutualistic associations with ectomycorrhizal fungi to acquire nutrients such as nitrogen or phosphorus and, in exchange, provide the fungal partners with carbohydrates [49]. Long-term infected declining trees have decreased photosynthetic rates and carbon storage, negatively affecting ectomycorrhizal abundance [50,51]. On the other hand, ectomycorrhizal symbiosis can play an important role in the interaction between trees and *Phytophthora* root pathogens by suppressing *Phytophthora* infections [52].

Since two decades, in the forests of Eastern Austria, increasing numbers of declining beech trees with *Phytophthora*-typical bleeding bark cankers and root rot symptoms, secondary attacks by insects and root, bark and wood infecting fungi have been observed [20,24]. In the present study, in 34 beech forest stands in Lower Austria, the presence of bleeding bark cankers, the occurrence of *Phytophthora* and fungal pathogens in the rhizosphere and the bark of beech trees and site conditions were assessed. In addition, in 10 of these stands, crown transparency, fine root parameters and ectomycorrhizal abundance were analyzed in order to develop a better understanding of the possible relationships between the different factors.

2. Material and Methods

2.1. Study Sites and Field Assessments

The selected 34 forest sites cover the full spectrum of beech forest types and sites occurring across Lower Austria, comprising the hilly areas of the "Leithagebirge" and "Rosaliengebirge" at the border to the province of Burgenland, the Vienna Forest, the mountainous Lower Austrian Prealps and the adjacent hilly areas of the "Waldviertel" and "Weinviertel", both regions bordering the Czech Republic, as well as the region "Bucklige Welt" along the border to the Austrian province of Styria (Figure 1; Tables 1 and 2). Selection of sites was supported by local forest authorities and private forest owners reporting observations of crown thinning and/or *Phytophthora*-typical bleeding bark cankers. Selected sites varied in size from 1 to 8.6 ha and comprised only mature beech stands (>60 years old) with distinct crown thinning of varying degree.

Ten of the 34 sites were selected for detailed investigations, hereafter named intensive survey, while the remaining 24 sites were subjected to a more extensive survey. From 2008 to 2010, in total, 6464 beech trees in the 34 forest sites (50–500 trees per site) were visually assessed for the presence of *Phytophthora*-typical bleeding bark lesions on stems, collars or surface roots, for the presence of fungal fruiting bodies and symptoms caused by pathogenic fungi and for the presence and symptoms of pests, in particular the exit holes and breeding galleries of bark beetles (Figures 2 and 3). Since fungal species were only macroscopically identified several fungi could only be identified to genus level, i.e., *Neonectria*, *Armillaria* and *Hypoxylon*. A differential diagnosis was performed for the trees with bark lesions to clarify whether the lesions were caused by biotic or abiotic agents or mechanical injuries. For each site, general data like geographical coordinates, altitude and geological substrate, were collected (Tables 1 and 2, Table S1 (Supplementary Materials)).

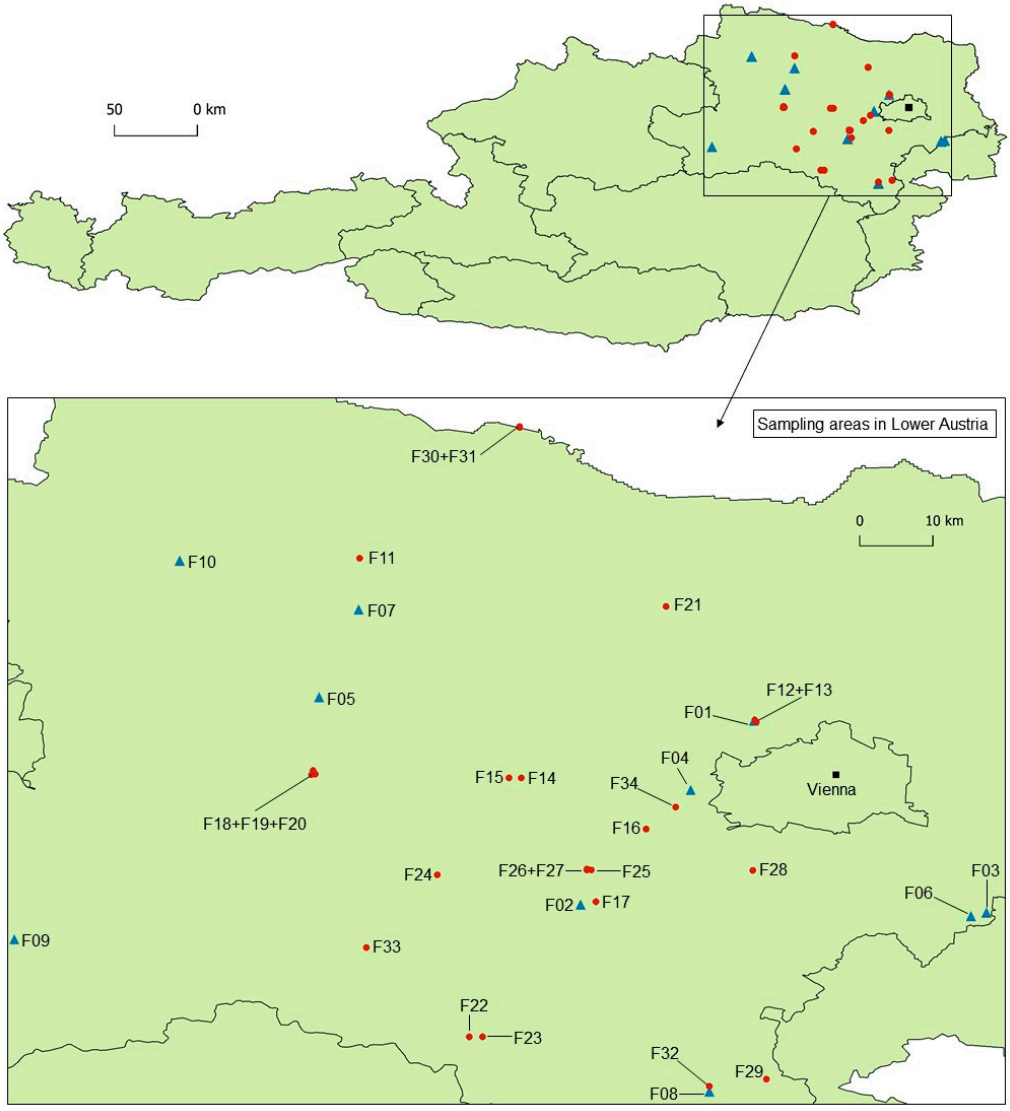

Figure 1. Location of Lower Austria within Austria (small rectangle) and the detailed location of the 10 intensively surveyed (F01–F10; blue triangles) and the 24 extensively surveyed (F11–F34; red dots) beech forest stands included in the *Phytophthora* survey in Lower Austria. For GPS coordinates, see Tables 1 and 2.

Table 1. Altitude, geological substrate, soil texture, mean pH and concentrations ($g \times Kg^{-1}$) of phosphorous, nitrogen and organic carbon in 10 intensively surveyed beech stands in Lower Austria, and *Phytophthora* species isolated from rhizosphere soil and bleeding bark cankers of declining and non-declining mature beech trees (n = 6–9 per stand).

Forest Stand		Altitude (m. a.s.l.)	Geological Substrate	Soil Texture	pH ($CaCl_2$)	P	N	Corg	*Phytophthora* spp. Isolated from	
no.	Name								Rhizosphere Soil (no. of Trees) [a]	Collar Rot and Aerial Cankers (no. of Trees) [a]
F01	Hadersfeld 1	351	Sandstone	Sandy loam	3.9	0.3	1.1	13.0	CAM (1) [b,c]	
F02	Hocheck 1	992	Limestone	Sandy loam	7.4	1.7	9.1	113.0		
F03	Kaiserstein-bruch	237	Limestone	Clay	7.2	0.3	2.8	39.5	PLU (1), SYR (1) [b]	PLU (1) [d]
F04	Purkersdorf	406	Claystone and sandstone	Loamy sand	4.2	0.3	1	13.8	CAM (2) [b]	
F05	Rossatz	614	Gneiss	Clayey sand	3.7	0.3	1.1	22.8	PLU (1) [b]	
F06	Sommerein	308	Schist	Loamy silt	4.4	0.2	1.3	20.0	CAC (2), PSY (1) [bc]	CAC (1) [e]
F07	Stackelberg	527	Gneiss	Clayey sand	3.8	1.0	1.0	17.0	CAM (1) [b,c]	
F08	Thernberg 2	560	Limestone	Sandy silt	7.5	0.6	3.6	57.5	PLU (1)	PLU (1) [e]
F09	Ybbsitz	1061	Limestone	Clayey loam	7.2	0.9	5.9	82.3	[b]	
F10	Zwettl	519	Granodiorite	Clayey sand	4.2	2.7	3.2	36.0	CAM (2) [b]	CAM (1) [e]

[a] CAC = *P. cactorum*, CAM = *P.* ×*cambivora*, PLU = *P. plurivora*, PSY = *P. psychrophila*, SYR = *P. syringae*. [b] *Globisporangium* sp. also isolated. [c] *Saprolegnia* sp. also isolated. [d] Aerial bleeding canker. [e] Bleeding collar rot lesion.

Table 2. Isolation of *Phytophthora* species from rhizosphere soil samples and bleeding bark cankers of declining and non-declining mature beech trees in 24 extensively surveyed forest stands in Lower Austria.

Forest Stand		Altitude (m a.s.l.)	Geological Substrate	*Phytophthora* spp. Isolated from	
no.	Name			Rhizosphere Soil (no. of Trees) [a]	Collar rot and Aerial Cankers (no. of Trees) [a]
F11	Fuglau	420	Schist	n.a.	CAM (1) [b]
F12	Hadersfeld 2	380	Sandstone	n.a.	CAM (4) [b]
F13	Hadersfeld 3	313	Sandstone	n.a.	n.a.
F14	Haspelwald 1	363	Claystone	CAM (1)	n.a.
F15	Haspelwald 2	353	Claystone	n.a.	CAM (1) [b]
F16	Hengstlberg	530	Claystone	n.a.	CAM (6) [b,c]
F17	Hocheck 2	630	Limestone	n.a.	PLU (2) [b,d], negative (1) [b]
F18	Hohenegg 1	390	Gneiss	n.a.	CAM (2) [b]
F19	Hohenegg 2	397	Gneiss	CAM (1), GON (1), PLU (1), TUB (1)	n.a.
F20	Hohenegg 3	481	Gneiss	n.a.	n.a.
F21	Hollabrunn	309	Alluvial deposits	n.a.	n.a.
F22	Höllental-Schwarza	562	Limestone	CAC (1) [e]	n.a.
F23	Höllental-Weichtalklamm	968	Limestone	n.a.	n.a.
F24	Kerschenbach	434	Claystone and sandstone	CAM (1) [e]	negative (1) [b]
F25	Kleinmariazell 1	531	Claystone	n.a.	n.a.
F26	Kleinmariazell 2	581	Claystone	n.a.	n.a.
F27	Kleinmariazell 3	637	Claystone	n.a.	CAM (1), negative (1) [b]
F28	Mödling Richardhof	457	Limestone	PLU (2) [f]	n.a.
F29	Rosalia		Gneiss	n.a.	CAM (1) [b]
F30	Thayatal 1	360	Limestone	CAC (1), PLU (1) [e]	n.a.
F31	Thayatal 2	330	Gneiss	CAM (1)	n.a.
F32	Thernberg 1	394	Schist	n.a.	PLU (1) [b]
F33	Türnitz	1255	Limestone	n.a.	n.a.
F34	Wienerwaldsee	338	Claystone	CAC (1)	CAM (1)

[a] CAC = *P. cactorum*, CAM = *P.* ×*cambivora*, GON = *P. gonapodyides*, PLU = *P. plurivora*, TUB = *P. tubulina*. [b] Bleeding collar rot lesions. [c] Bleeding bark lesions on surface roots. [d] Aerial bleeding canker. [e] *Globisporangium* sp. also isolated. [f] Both trees with inactive aerial cankers. n.a. = isolation test not attempted.

Declining beech trees show deteriorations of the crown structure characterized by long-term stunted growth of shoots, clustering of lateral branches around the major branches and at the ends of branches leading to brush- and claw-like structures, excessive losses of lateral twigs and small branches and as a result, crown transparency >25% (Jung 2009). In 2008, in each of the 10 intensively surveyed stands, three declining beech trees showing the aforementioned crown deteriorations and crown transparency ≥30% and three healthy trees with crown transparency ≤20% were selected. The 60 sample trees had no bleeding bark cankers. The crown transparency of the 60 trees was recorded in summer 2008 according to [53].

2.2. Sampling and Isolation Procedures, Species Identification

The assessment of *Phytophthora* presence was performed in 27 of the 34 beech stands between 2008 and 2010 by sampling rhizosphere soil and bleeding bark lesions according to [30,44].

2.2.1. Soil Sampling and Baiting

At the 10 sites selected for the intensive survey, rhizosphere soil samples were taken in 2008 from each three healthy and declining beech trees per site, and in 2010, in stands F05 and F10 from three additional declining trees with collar rot lesions (in total 63 trees). In eight of the other 24 stands, soil samples were taken between 2008 and 2010 from 13 trees with inactive, dry dark-brown *Phytophthora*-typical bark lesions, from which direct isolation attempts are usually not promising [30]. Three monoliths (ca. 30 × 30 × 30 cm) of rhizosphere soil were excavated around each sample tree at 50–100 cm distance from the stem. After the removal of the upper organic soil layer (ca. 5–10 cm) which is usually not infested by *Phytophthora* [21], mineral soil was taken from all the monoliths and mixed

into a bulked sample of ca. 2 l per tree. For the baiting tests, each soil sample was carefully mixed and flooded with distilled water so that the soil was covered by ca. 3 cm of water. Young soft leaves from European beech as well as from cork oak (*Quercus suber*), pedunculate oak (*Q. robur*) and Turkey oak (*Q. cerris*) were used as baits, floating on the water surface for 3–7 days [21,44]. Leaves developing necrotic areas were checked under the light microscope at ×80 magnification for *Phytophthora* sporangia and then plated onto selective PARPNH-agar [44] and incubated at 20 °C in the dark.

2.2.2. Isolations from Bleeding Collar Rot and Aerial Bark Lesions

Isolations were performed from bleeding bark lesions of 27 beech trees in 15 stands (Tables 1 and 2). Approximately 10–15 cm long bark samples, including phloem and cambium, were taken from the upper lesion margins using a hammer and a chisel, immediately placed in distilled water and taken to the laboratory. As a first attempt to detect the presence of *Phytophthora*, a quick test consisting of a lateral flow-device (Pocket Diagnostic Kit *Phytophthora*, CSL now FERA, Sand Hutton, York YO41 1LZ) was performed using a small amount of the inner bark lesions. In case of a positive result, the remaining bark sample was incubated in distilled water at 16–18 °C for 2–3 days. The distilled water was replaced three times per day in order to remove the polyphenols released by the bark. Then, in the case of active lesions with an orange-brown, flamed appearance of the inner bark, 3–5 mm pieces of inner bark were blotted dry on filter paper and plated onto selective PARPNH and incubated at 20 °C in the dark [30,54]. Inactive, dry dark-brown lesions were shredded and the small pieces flooded with distilled water and baited with oak and beech leaflets (Figure 3d). The water was replaced daily in order to remove excess polyphenols and decrease bacterial populations [30,54]. Isolations from necrotic baiting leaves were performed as described for soil baitings. With one inactive bark lesion in stand F17 both direct plating and baiting were used in parallel.

Beginning at 12h after plating, all PARPNH plates with plated baiting leaves or bark tissues were regularly checked under the stereomicroscope at ×20 for developing *Phytophthora* colonies which were transferred onto V8 juice agar (V8A) [44] and after 3 weeks growth at 20 °C, they were stored at 8 °C in the fridge.

Phytophthora species were identified by examining the morphological characters of sporangia, oogonia, antheridia, chlamydospores and hyphal swellings under the light microscope at ×320 magnification, and comparing them together with colony growth patterns on V8A and carrot agar (CA) [55] and cardinal temperatures of growth on V8A with descriptions in the Literature [48,56–59]. Sporangia were produced by cutting discs (15 mm diam) from the growing edge of a 5–7 days old culture grown on V8A at 20 °C in the dark, and immersing them in non-sterile soil extract water [44]. In case the classical species identification was ambiguous, the isolates were identified using ITS DNA sequence analysis according to [60,61]. ITS sequences from representative isolates of all *Phytophthora* species and *cox1* sequences of representative isolates of new species obtained in this study were deposited at GenBank and accession numbers are given in Supplementary Table S2 (Supplementary Materials).

2.3. Root Biometry

Biometrical analyses of roots were performed in the 10 intensively surveyed sites following the method described by [45]. At each site, in 2008, four of the six selected trees (two declining and two healthy trees) were sampled. All the roots with diameters ≤5 mm were collected from the three soil–root monoliths sampled per tree for the soil baitings (see Section 2.2.1) and mixed to one sample per tree. The root samples were transported in sealed plastic zip bags to the laboratory where they were immersed in water for 12–18 h. Then, the roots were washed thoroughly in running tap water and subsequently cleaned of adhering soil particles in an ultrasonic cleaning box for 10 min. After the removal of roots from other plant species, the roots were divided into five sub-samples per beech tree which were then immersed in a water tub and scanned in a root scanner (Epson Transparency Unit, model EU-22, Meerbusch, Germany).

The scans were analyzed using the program WINRHIZO 2004a (Regent Instruments INC., Ch Saint-foy, QC, Canada). The roots were separated into fine roots (≤2 mm diameter) and coarse roots (2–5 mm diameter) and, according to [45], the following parameters were measured: total fine root length (FRL); total coarse root length (CRL); total fine root surface (FRS); total coarse root surface (CRS); total number of fine root tips (FRT). These parameters were used to calculate the root ratio parameters FRL/CRL, FRS/CRL, FRT/CRL and FRT/CRS. In order to allow the comparisons between different stands, the influence of different site and climatic conditions was reduced by calculating relative root ratio parameters (FRL/CRL_{rel}, FRS/CRL_{rel}, FRT/CRL_{rel}, FRT/FRL_{rel}, FRT/CRS_{rel}) by using the site-specific mean values of the non-declining sample trees from the respective sites as reference values (100%) [45].

2.4. Ectomycorrhizal Frequency

Following the root scanning, the ectomycorrhizal frequency of fine root tips in the 40 declining and non-declining sample trees of the 10 intensively surveyed stands was assessed. The water tubs with the immersed roots of a subsample were underlaid by a grid of 2 × 2 cm, and 502 × 2 cm units per subsample were randomly selected. In each unit, the number of ectomycorrhized root tips (MT) with a turgid mantle and a shiny appearance and non-mycorrhized root tips (NMT) [62] was assessed under the stereomicroscope at ×40, and the ratio MT/NMT was calculated for each tree. Then, analogous to the root ratio parameters (see Section 2.3), the relative MT/NMT_{rel} ratios were calculated using the mean MT/NMT ratio of the non-declining trees from the respective site as a site-specific reference value (100%).

2.5. Climate, Geological Substrate and Soil Analysis

For the 10 intensively surveyed stands, the long-term mean precipitation and temperature values for 30 years from the meteorological stations closest to the surveyed sites were provided by the Hydrographischer Dienst Österreich, Abteilung I/3-Wasserhaushalt (HZB; Vienna, Austria). For eight stands, the mean annual precipitation (522–779 mm) and mean monthly precipitation during the vegetation period April–September (60.8–76.7 mm) was comparable to each other whereas the two high altitude sites F02 Hocheck 1 (1650.5 and 163.9 mm, respectively) and F09 Ybbsitz (1450 and 139.2 mm, respectively) received considerably higher precipitation (Table S4 (Supplementary Materials)). Mean annual temperatures and mean temperature values for the vegetation period of the 10 stands ranged from 6.4 to 9.6 °C and from 12.5 to 16.4 °C, respectively, with site F02 Hocheck 1 being the coldest and sites F03 Kaisersteinbruch 1 and F06 Sommerein being the warmest sites (Table S3 (Supplementary Materials)).

Data on the geological substrates of the 34 surveyed beech forests were obtained from detailed geological maps (Geological Maps 1:50,000 and 1:200,000, Geological Survey of Austria, Geologische Bundesanstalt, Vienna, Austria). The sites showed a wide range of geological substrates representative for the distribution of beech forests in Lower Austria (Tables 1 and 2).

A detailed analysis of soil physical and chemical properties was performed for the 10 intensively surveyed stands F01–F10 (Table 1). Soil parameters included soil texture, i.e. percentages of sand, clay and silt, pH (measured in calcium chloride = pH_{CaCl2}), and the total contents of N, P and organic carbon (C_{org}). The soil samples were homogenized, sieved (2 mm mesh) and air-dried. Soil dry weight was determined by thermogravimetry (Moisture Analyzer HR73, Mettler Toledo, Columbus, OH, USA). Total soil carbon (C_t) and total nitrogen (N_t) contents were determined by dry combustion using a LECO C/N TruMAC Analyzer (LECO, Saint Joseph, MI, USA). Carbonate ($CaCO_3$) content was measured using a Scheibler calcimeter. Total contents of C_{org} were calculated as the difference between the contents of C_t and $CaCO_3$. Total P was determined using an acid-assisted (HNO_3) microwave leaching plus ICP-OES analysis (Perkin Elmar Optima 8300 (Perkin Elmar Inc., Waltham, MA, USA).

2.6. Statistical Analysis

Statistical analyses were performed in R software statistical package 3.6.2 (R Core Team, 2019; https://www.r-project.org/) and CANOCO 5 [63]. For all tests, the significance level $\alpha = 0.05$ was used.

The relationship between the geological substrate and *Phytophthora* spp. assemblages was studied by multivariate statistical methods. As the gradient length tested by detrended correspondence analysis (DCA) showed the unimodal nature of the data, the canonical correspondence analysis (CCA) was used to assess the links between the individual *Phytophthora* species and the geological substrate. To eliminate the distorting effect of rare species, which is an issue for all unimodal methods, rare species were down weighted using the preset function of the software.

The effect of the geological substrate on *Phytophthora* spp. occurrence was tested by two different tests: ANOSIM (analysis of similarities) and PERMANOVA (permutational multivariate analysis of variance), both implemented in the R package vegan. Statistical significance was based on 9999 permutations. *Phytophthora* species contributing most to the dissimilarity between individual geological substrates, i.e. being most responsible for the differences in *Phytophthora* species assemblages between geological substrates, were identified by similarity percentage breakdown (SIMPER).

The relationship between tree decline, root parameters and ectomycorrhizal frequency was analyzed by means of generalized linear mixed models (GLMM) with Gamma distribution and inverse link function implemented in the R package lme4 and follow-up type III likelihood-ratio test. Multiple comparisons were carried out using the Tuckey's post hoc test. Correlations between the root parameters, ectomycorrhizal frequency and crown transparency were assessed by Kendall's Tau.

3. Results

3.1. Phytophthora-Related Disease Symptoms

In beech stands infested by *Phytophthora* species, in particular *P.* ×*cambivora* and *P. plurivora*, individual trees and groups of trees showed thinning and dieback of crowns (Figure 2a). Bleeding bark cankers on surface roots (Figure 2b) were found on individual trees while open callusing lesions on coarse roots and small woody roots (Figure 2c) and extensive losses of lateral roots and fine roots (Figure 2d) were widespread. In total, 6464 trees were surveyed in the 34 beech stands. *Phytophthora*-typical collar rot (Figure 2e) and aerial bleeding bark cankers (Figure 2f) were detected on 133 trees (2.1%) in 25 stands (73.5%) (Table S1 (Supplementary Materials)). Collar rot cankers were observed in 87 trees (1.3%) in 24 stands (70.6%) including, seven of the 10 intensively surveyed stands and 17 of the 24 extensively surveyed stands. Aerial cankers were found in 46 trees (0.7%) in 13 stands (38.2%) including four and nine intensively and extensively surveyed stands, respectively (Table S1). Collar rots and aerial bleeding cankers were characterized by tongue-shaped necrosis of the inner bark and the cambium, with orange-brown discoloration in active cankers and dark-brown discoloration in inactive cankers, and tarry or rusty spots on the surface of the bark (Figure 2e,f). Collar rots usually extended 1–2 m from the stem base (Figure 2e), but in some cases, could reach up to 5 m. Aerial bleeding cankers were observed along the stems up into the canopy, had no particular orientation and were often located above collar rots and below stem forks (Figure 2f). In three beech stands on temporarily waterlogged sites with high clay contents (F04 Purkersdorf, F16 Hengstlberg, F26 Kleinmariazell 2) and in stand F08 Thernberg 2, individual trees and groups of trees were recently wind-thrown (Figure 2g). Most of these trees showed severe destructions of the root system with extensive losses of lateral and fine roots and numerous lesions on woody roots (Figure 2c,d). Often beech trees with collar rots were concentrated along forest roads (Figure 3a,b). In several stands, the breeding of bark beetles, in particular *Taphrorychus bicolor*, in active *Phytophthora* lesions could be observed (Figure 3c).

Figure 2. Decline and dieback symptoms caused by *Phytophthora* spp. on *Fagus sylvatica* in Lower Austria; (**a**) severe dieback, chlorosis and mortality due to root and collar rot caused by *Phytophthora* ×*cambivora* in stand F01 Hadersfeld 1; (**b**) bleeding bark lesions caused by *P.* ×*cambivora* on surface roots in stand F16 Hengstlberg; (**c**) coarse roots of wind-thrown beech trees in stand F16 Hengstlberg, with severe losses of lateral roots and open callusing lesions caused by *P.* ×*cambivora*; (**d**) small woody roots with extensive losses of lateral roots and fine roots caused by *P. plurivora* and *P. syringae* in stand F03 Kaisersteinbruch; (**e**) bleeding collar rot lesion caused by *P.* ×*cambivora* in stand F34 Wienerwaldsee; (**f**) aerial bleeding canker caused by *P. plurivora* in stand F19 Hohenegg 2; (**g**) wind-thrown beech trees in stand F16 Hengstlberg due to severe root losses and root lesions caused by *P.* ×*cambivora*.

Figure 3. (**a**,**b**) Bleeding collar rot lesions caused by *Phytophthora ×cambivora* on *Fagus sylvatica* trees along forest roads in stand F15 Haspelwald 2; (**c**) surface root of a beech tree in stand F16 Hengstlberg with exit holes of the bark beetle *Taphrorychus bicolor* being mostly connected to the bleeding bark lesions caused by *P. ×cambivora*; (**d**) baiting test from an inactive aerial canker of a beech tree in stand F17 Hocheck 2 with lesions on *F. sylvatica* baiting leaves caused by *P. plurivora*.

3.2. Distribution of Phytophthora Species and Their Association with Disease Symptoms

Phytophthora isolation tests from bark cankers and rhizosphere soil using baiting and direct isolation methods were performed with 103 beech trees in 27 stands, and *Phytophthora* species were isolated from 49 trees (47.6%) in 25 stands (92.6%) (Tables 1 and 2). The most common species was *P. ×cambivora,* which was obtained from 28 trees in 16 stands, followed by *P. plurivora* (12 trees in eight stands) and *P. cactorum* (six trees in four stands). *Phytophthora gonapodyides*, *P. syringae*, *P. psychrophila* and *P. tubulina* were each isolated from only one stand (Tables 1 and 2).

From bleeding collar rot and aerial bark lesions, isolations were attempted from 27 trees in 15 stands (Tables 1 and 2) and three *Phytophthora* species were isolated from 24 trees (88.9%) in 14 stands (93.3%). *Phytophthora ×cambivora* was the most common species isolated from 19 trees in nine stands, whereas *P. plurivora* and *P. cactorum* were recovered from four trees in four stands and one tree in one stand, respectively. In one stand (F17 Hocheck 2), *P. plurivora* could be baited from an inactive aerial bark lesion (Figure 3d) while the direct plating of necrotic canker tissue on PARPNH-agar failed.

Rhizosphere soil was collected from 63 beech trees in the 10 intensively surveyed stands and from 13 beech trees with inactive dry bark cankers and/or severe crown dieback in eight of the 24 extensively surveyed stands. Phytophthoras were present in the rhizosphere soil from 25 trees (32.9%) in 16 stands (88.9%; each eight intensively and extensively surveyed stands; Tables 1 and 2, Table S4 (Supplementary Materials)). The most common species were *P. ×cambivora* with 10 trees in eight stands and *P. plurivora* with seven trees in six stands. *Phytophthora cactorum* was found in five trees in four stands while *P. gonapodyides*, *P. syringae*, *P. psychrophila* and *P. tubulina* were each isolated only once.

3.3. Influence of Geological Substrate, Soil Texture and Soil pH on Phytophthora Distribution

The 34 beech stands were growing on six main geological substrates, including limestone (10 stands), claystone (nine stands), gneiss/granodiorite (eight stands), sandstone (five stands), schist

(three stands) and alluvial deposits (one stand) (Tables 1 and 2). In the 10 intensively surveyed stands, *P.* ×*cambivora* was present at four sites with acidic soil reaction (pH_{CaCl2} 3.8–4.2) and clayey and sandy texture of the soils which were derived from claystones, gneiss, granodiorite and sandstones (Table 1). Additionally, in the 24 extensively surveyed stands, *P.* ×*cambivora* was only found on geological substrates which form acidic soils with high contents of clay and sand, including claystones, gneiss, sandstones and schist (12 stands; Table 2). *Phytophthora* ×*cambivora* was not recovered from any of the 10 limestone sites. *Phytophthora plurivora* showed a different ecological amplitude occurring in three intensively surveyed stands with soils of different textures (clayey sand, clay and sandy silt) and pH_{CaCl2} values of 3.7, 7.2 and 7.5, respectively (Table 1). Over all 34 stands, the eight sites from which *P. plurivora* was recovered were situated on gneiss (two sites), limestone (five sites) and schist (Tables 1 and 2). The four stands with presence of *P. cactorum* were located on claystone, limestone (two sites) and schist. Since *P. gonapodyides*, *P. psychrophila*, *P. syringae* and *P. tubulina* were each detected in only one stand, their association with site conditions remains unclear.

The CCA revealed that geological substrate explained 29.9% of the total variability in *Phytophthora* distribution. There is a clear association between *P.* ×*cambivora* and sandstone and between *P. plurivora* and limestone, while *P. cactorum* shows no evident substrate preference (Figure 4). The results of ANOSIM (R = 0.3316; $p \leq 0.001$) and PERMANOVA (F = 7.505; $p \leq 0.001$) confirm that the geological substrate has a significant effect on *Phytophthora* species distribution. Pairwise PERMANOVA revealed significant differences in *Phytophthora* species occurrence between claystone and limestone (F = 34.138; R^2 = 0.7563; p = 0.007), gneiss/granodiorite and limestone (F = 12.208; R^2 = 0.5260; p = 0.02) and sandstone and limestone (F = 22.788; R^2 = 0.7341; p = 0.02), respectively. SIMPER results further specify that for all these substrate pairs, the differences in *Phytophthora* distribution are given by the presence/absence of *P.* ×*cambivora* and *P. plurivora*, i.e. these two species are responsible for 84.5%, 83.7% and 86.1%, respectively, of the pairwise differences in *Phytophthora* assemblages between these substrates.

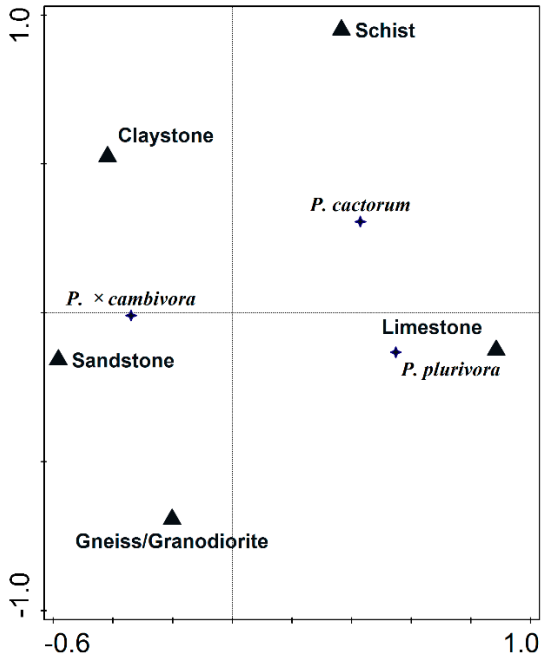

Figure 4. Canonical correspondence analysis (CCA) of *Phytophthora* community structure (CAC = *P. cactorum*, CAM = *P.* ×*cambivora*, PLU = *P. plurivora*) according to the geological substrate in 34 beech stands in Lower Austria.

3.4. Fungal Pathogens and Pests

In 21 of the 34 beech stands, a total of 21 fungal species were recorded on various types of bark damages and on dead wood (Table S5 (Supplementary Materials)). In 19 stands, 18 fungal species were associated with collar rot cankers, in many cases as secondary invaders of lesions where a *Phytophthora* species was isolated from the active lesion margins (Figure 5). With 12 stands, *Armillaria* spp. which were not identified to the species level, were most common, followed by *Fomes fomentarius* (10 stands), *Trametes* spp. and *Hypoxylon* sp. (each eight stands), *Schizophyllum commune* (six stands) and *Ustulina deusta* (four stands). Other aggressive secondary bark pathogens like *Bjerkandera adusta* and *Neonectria* sp./*Cylindrocarpon* sp. were more rare (Table S5 (Supplementary Materials); Figure 5). Eight of the 19 fungal invaders of primary *Phytophthora* collar rot lesions were also colonizing mechanical injuries on buttroots and stems (Table S5 (Supplementary Materials)). In three stands, *F. fomentarius*, *Inonotus nidus-pici*, *Oudemansiella mucida* and *Polyporus squamosus* were secondary invaders of aerial *Phytophthora* bark cankers. *Neonectria ditissima* was locally widespread in the stands in Hohenegg, causing in twigs, branches and in rare cases, also stems, typical perennial cankers which were not related to primary *Phytophthora* infections. The wood decay fungus *S. commune* was typically associated with sunburn injuries in five stands.

Figure 5. Secondary fungal bark infections on *Fagus sylvatica* trees infected by *Phytophthora* spp. in Lower Austria: (**a**) bleeding collar rot lesion (white arrows) caused by *P.* ×*cambivora* in stand F16 Hengstlberg with secondary infection by *Cylindrocarpon* sp./*Neonectria* sp. (red arrows); (**b**) white pycnidia of *C. candidum* (red arrows) and red perithecia of *N. coccinea* in stand F16 Hengstlberg; (**c**) bleeding collar rot lesion (white arrow) caused by *P.* ×*cambivora* in stand F12 Hadersfeld 2 with fruiting bodies of *Bjerkandera adusta*; (**d**,**e**) aerial bleeding cankers (white arrows) caused by *P. cactorum* in stand F06 Sommerein with fruiting bodies of the secondary invaders *Oudemansiella mucida* (red arrows) and *Fomes fomentarius* (yellow arrows); (**f**) fruiting bodies of the secondary invader *Fomitopsis pinicola* above a collar rot lesion caused by *P.* ×*cambivora* (not shown) on a beech tree in stand F04 Purkersdorf.

In five stands (F01 Hadersfeld 1, F04 Purkersdorf, F07 Stackelberg, F12 Hadersfeld 2, F16 Hengstlberg), in 26 of the 33 trees with *Phytopthora*-collar rot lesions, secondary attacks by

the opportunistic bark beetle *Taphrorychus bicolor* were detected. In the stands F04 Purkersdorf and F16 Hengstlberg, in three trees, numerous exit holes of *T. bicolor* were detected in actively bleeding collar rot lesions from which *P. ×cambivora* could be isolated (Figure 3c).

3.5. Relationship between Root Parameters, Ectomycorrhizal Frequency, Crown Transparency and Phytophthora Infestation in Ten Intensively Surveyed Beech Stands

In the eight *Phytophthora*-infested sites, declining sample trees had 38% higher crown transparency than non-declining sample trees. In the two sites without *Phytophthora* infestation, the difference was 23.4%. In both stand categories, this difference was statistically significant ($p \leq 0.001$; Table 3).

In the eight *Phytophthora*-infested stands, the fine root status of the non-declining trees was better than in the declining trees. The relative root ratio parameters FRL/CRL_{rel}, FRS/CRS_{rel}, FRT/CRL_{rel}, FRT/FRL_{rel}, FRT/CRS_{rel} of the 16 non-declining trees were 7.8–13.4% higher compared to the 16 declining trees. For the parameters FRL/CRL_{rel} and FRS/CRS_{rel}, the differences between the declining and non-declining trees were statistically significant ($p \leq 0.05$) and non-significant ($p \leq 0.1$), respectively. However, due to low sample numbers and relatively high variation for the parameters FRT/CRL_{rel} and FRT/CRS_{rel}, the differences between declining and non-declining trees showed no statistical significance (Table 3). In the two *Phytophthora*-free high-altitude stands, the differences of all relative root ratio parameters between the four non-declining and the four declining trees were even higher (21.4–27.1%) than in the *Phytophthora*-infested stands (Table 3).

In both the *Phytophthora*-infested and *Phytophthora*-free stands, the non-declining trees showed 57.3% and 64.3% higher ectomycorrhizal frequency of fine root tips than the declining trees. However, in both stand categories, the differences in the relative ratio mycorrhized/non-mycorrhized fine root tips (MT/NMT_{rel}) between non-declining and declining trees were statistically not significant (Table 3). In both stand categories, the ectomycorrhizal frequency (MT/NMT) was not correlated with any of the root parameters.

Table 3. Crown transparency and relative values of root parameters and ectomycorrhizal frequency of healthy and declining beech trees in eight *Phytophthora*-infested and two non-infested forest stands in Lower Austria, and the significance of differences (Type III likelihood-ratio test).

Forest Stands	*Phytophthora* spp. [a]	Crown Transparency (%)		FRL/CRL$_{rel}$ [b]		FRS/CRS$_{rel}$ [b]		FRT/CRL$_{rel}$ [b]		FRT/CRS$_{rel}$ [b]		MT/NMT$_{rel}$ [b]	
8 *Phytophthora*-Infested Stands (16 Healthy and 16 Declining Beech Trees)													
		H	**D**	**H**	**D**	**H**	**D**	**H**	**D**	**H**	**D**	**H**	**D**
F01 Hadersfeld 1	CAM	10.0	37.5	100.0	118.1	100.0	142.9	100.0	111.7	100.0	129.0	100.0	26.0
F03 Kaisersteinbruch 1	PLU, SYR	10.0	50.0	100.0	112.1	100.0	97.5	100.0	153.1	100.0	147.0	100.0	19.7
F04 Purkersdorf	CAM	10.0	42.5	100.0	91.6	100.0	98.4	100.0	70.0	100.0	69.7	100.0	43.5
F05 Rossatz	PLU	10.0	62.5	100.0	116.1	100.0	132.2	100.0	113.3	100.0	119.8	100.0	52.5
F06 Sommerein	CAC, PSY	12.5	47.5	100.0	96.4	100.0	88.1	100.0	134.6	100.0	122.1	100.0	79.1
F07 Stackelberg	CAM	10.0	30.0	100.0	30.5	100.0	35.4	100.0	30.2	100.0	28.1	100.0	58.6
F08 Thernberg 2	PLU	10.0	37.5	100.0	89.7	100.0	94.4	100.0	89.3	100.0	88.2	100.0	24.8
F10 Zwettl	CAM	12.5	32.5	100.0	38.7	100.0	36.2	100.0	35.3	100.0	32.2	100.0	37.7
Mean of 8 infested stands		**10.6**	**34.0**	**100.0**	**86.6**	**100.0**	**90.6**	**100.0**	**92.2**	**100.0**	**92.0**	**100.0**	**42.7**
Significance [c]		***		*				**n.s.**		**n.s.**		**n.s.**	
2 *Phytophthora*-Free Stands (4 Healthy and 4 Declining Beech Trees)													
		H	**D**	**H**	**D**	**H**	**D**	**H**	**D**	**H**	**D**	**H**	**D**
F02 Hocheck 1	-	10.0	55.0	100.0	78.6	100.0	71.3	100.0	85.6	100.0	82.3	100.0	23.2
F09 Ybbsitz	-	5.0	37.5	100.0	77.8	100.0	82.3	100.0	71.6	100.0	63.6	100.0	48.1
Mean of 2 non-infested stands		**7.5**	**46.3**	**100.0**	**78.2**	**100.0**	**76.8**	**100.0**	**78.6**	**100.0**	**72.9**	**100.0**	**35.7**
Significance [c]		***		**n.s.**		**n.s.**		**n.s.**		**n.s.**		**n.s.**	

[a] CAC = Phytophthora cactorum, CAM = P. ×cambivora, PLU = P. plurivora, PSY = P. psychrophila, SYR = P. syringae. [b] FRL = fine root length, CRL = coarse root length, FRS = fine root surface, CRS = coarse root surface, FRT = number of fine root tips, MT = mycorrhized root tips, NMT = non-mycorrhized root tips; for detailed explanations of root parameters, see Material and Methods. [c] $p \leq 0.1$, * = $p \leq 0.05$, *** = $p \leq 0.001$, n.s. = non-significant.

4. Discussion

This study in 34 beech forests across Lower Austria demonstrated the widespread occurrence of *Phytophthora*-typical disease symptoms and soilborne *Phytophthora* species. In total, seven *Phytophthora* species were isolated from rhizosphere soil and from bleeding bark lesions of 48% of sample trees in 25 of the 27 stands from which isolation tests were performed. Considering the size of Lower Austria of 19.186 km^2, *Phytophthora* diversity in the 34 surveyed beech stands was relatively high in comparison to surveys in beech stands of other European countries. In Bavaria, southern Germany, where 134 beech stands were surveyed between 2003 and 2007, across an area three times the size of Lower Austria, nine *Phytophthora* species were recorded from 81% of the sample trees in 93% of the stands [20,30]. In several separate surveys in Italy, 10 *Phytophthora* species have been detected in 13 beech stands [17,20,23,28,36]. In contrast, in 13 other European countries, only between one and six *Phytophthora* species were recorded from beech stands [16,18–22,25–27,29,31,32,35,37,38]. Across Europe, 17 *Phytophthora* species are currently known to occur in European beech forests [20–23,36,39]. As in other European countries, in Lower Austria, *P.* ×*cambivora*, *P. plurivora* and *P. cactorum*, all considered in Europe as introduced invasive pathogens [20–22,39,59], were the most common *Phytophthora* species in beech forests, whereas *P. syringae* and the putatively native *P. gonapodyides*, *P. psychrophila* and *P. tubulina* [21,39] showed only rare occurrence. A population genetic study demonstrated that the genetic diversity of *P. plurivora* is higher in Europe than in North America and a European origin of this pathogen was proposed [64]. However, in this study, no Asian isolates were included and recent findings of *P. plurivora* in remote healthy forests in Nepal, Yunnan and Taiwan [61,65,66] and the ubiquitous distribution of *P. plurivora* in healthy forest ecosystems and streams across Japan (T. Jung, C.M. Brasier and K. Kageyama, unpublished results) clearly indicate an Asian origin for this pathogen. This is also supported by the fact that the diversity of both known and yet undescribed species from phylogenetic *Phytophthora* Clade 2c, to which also *P. plurivora* belongs, is extremely high in Asia [61,67]. In addition, the high aggressiveness of *P. plurivora* to major European forest tree species like beech, *Tilia cordata*, *Acer* spp. and *Quercus* spp., and the widespread association of *P. plurivora* with the decline, dieback and mortality of forests across Europe [20–23,30,33,35,44,45,54,56,57,59] demonstrate a lack of long-term co-evolution, whereas in Asia, *P. plurivora* is associated with healthy ecosystems [61,65,66]. Interestingly, *P. tubulina*, a new species closely related to *P. quercina,* which is one of the main drivers of chronic European oak decline [16,20,22,39,45,68–70], has never been found anywhere else before. *Phytophthora pseudosyringae*, commonly associated with beech and oak forests in Germany and Italy, [17,19,20,22,25,30,57] was not isolated from beech forests in Lower Austria. Results from recent surveys in Taiwan and Vietnam suggest that in the absence of invasive *Phytophthora* species, native *Phytophthora* species are widespread and common in natural and semi-natural forests [61,67]. It appears, hence, that multiple invasive *Phytophthora* species are outcompeting native *Phytophthora* species in the soils of European beech stands, possibly due to their higher aggressiveness to native tree species and their higher cardinal temperatures, which provide them with a selective advantage over native low-temperature *Phytophthora* species in times of rising annual and winter temperatures. This was also recently suggested for the invasive aggressive *P. cinnamomi* and potentially native *Phytophthora* species in the Valdivian rainforests of Chile [71]. However, more surveys and long-term field studies are needed to confirm this hypothesis.

This study demonstrated the site preferences of *P.* ×*cambivora* and *P. plurivora* which were in agreement with the results from previous surveys in beech and oak forests in other countries [19,20,22,25,30–32,37,45,59]. *Phytophthora* ×*cambivora* was exclusively found on geological substrates forming acidic soils with high contents of clay and sand and a tendency for temporary waterlogging, including claystones, gneiss, granodiorite, sandstones and schist. In contrast, *P. plurivora* was mainly, though not exclusively, found in limestone sites. Like in Bavarian beech stands, *P. cactorum* did not show clear site preferences [30]. The geological substrate alone explains 30% of the *Phytophthora* species distribution in the 25 *Phytophthora*-infested beech stands in Lower Austria. In comparison to other ecological studies, this is a remarkably high value for a single ecological factor [72]. The upper altitudinal limits

of *P.* ×*cambivora*, *P. plurivora* and *P. cactorum* in Lower Austria were slightly lower than in Bavaria (637, 614 and 562 m a.s.l. versus 750, 870 and 600 m a.s.l. [30]).

The question arises how exotic invasive *Phytophthora* species were introduced to the mature beech forests in Lower Austria. Almost ubiquitous infestations of nursery fields and young planting sites of beech and numerous other tree species across Europe, including Austria, with *P.* ×*cambivora*, *P. plurivora*, *P. cactorum,* and more than 50 other *Phytophthora* species demonstrated, beyond any reasonable doubt, that the planting of infested nursery stock is the major pathway of non-native *Phytophthora* pathogens into forest ecosystems [21]. In addition, in several forest stands in Lower Austria beech trees with bleeding cankers caused by *P.* ×*cambivora* or *P. plurivora* were concentrated along forest roads, suggesting the relatively recent introduction and spread of these invasive pathogens with infested road-building materials and/or infested soil particles adhering to vehicles and hikers boots, as previously shown for *P. cinnamomi* in Western Australia and *P. lateralis* in Oregon [73,74].

Phytophthora ×*cambivora*, *P. plurivora* and *P. cactorum* are the most common *Phytophthora* pathogens associated with bark cankers and the decline of beech stands in other European countries [16,19–23,25–32,35,37,59]. Across Europe, all three pathogens are also involved in the widespread decline of oak forests, the ink disease of *Castanea sativa* and the declines of numerous other broadleaved tree species including *Aesculus hippocastanum*, *Acer* spp., *Fraxinus excelsior*, *Populus* spp. and *Tilia* spp. [15,16,20–22,29,44,45,59,65,74–78]. In pathogenicity trials, *P.* ×*cambivora*, *P. plurivora* and *P. cactorum* demonstrated high aggressiveness to roots and the bark of *F. sylvatica*, several European oak species and *C. sativa* while *P. gonapodyides*, *P. psychrophila* and *P. tubulina* were only moderately aggressive [15,28,39,44,56–58,66,79–81]. *Phytophthora* ×*cambivora*, *P. plurivora* and *P. cactorum* also showed high aggressiveness to several poplar clones commonly used in riparian plantations in Europe [78]. In addition, in pathogenicity trials, *P. plurivora* and *P. cactorum* caused considerable bark lesions on *F. excelsior* and *Alnus glutinosa* while *P.* ×*cambivora* was highly aggressive to *Prunus laurocerasus*, a rare understorey species in Southern European beech forests [82–84].

In Lower Austria, *Phytophthora*-typical bleeding bark cankers were found in 74% of the surveyed 34 beech stands with 65% of these cankers in 71% of the stands located at the collar while 35% of the cankers in 38% of stands had no connection to the roots (aerial cankers). Similar results were reported from a survey in 134 beech stands in Bavaria with collar rot and aerial bleeding cankers occurring in 74 and 36% of surveyed stands, respectively [30]. It is noteworthy that no *Phytophthora*-typical bleeding cankers were observed in the four high-altitude stands (F02, F09, F23, F33) supporting the altitudinal limits of *Phytophthora* spp. in Lower Austria suggested by the isolation records. As in previous surveys in Bavaria, Lower Saxony (Northern Germany), Belgium, Norway and Sicily [20,21,23,25,30,31,37], *P.* ×*cambivora* was the most common *Phytophthora* species isolated from necrotic beech bark in Lower Austria. However, while *P.* ×*cambivora* was only detected in collar rot lesions, *P. plurivora* could be isolated from both collar rot and aerial cankers. Also in Bavaria, *P. plurivora* was the most common species recovered from aerial cankers in beech trees, but other *Phytophthora* species like *P.* ×*cambivora*, *P. cactorum* and *P. gonapodyides* were infrequently isolated, too [30]. *Phytophthora plurivora*, like most other soilborne *Phytophthora* species, lacks caducous sporangia for aerial spread and its movement to higher stem heights was demonstrated to be achieved via the passive transport inside non-symptomatic xylem vessels of beech trees resulting in isolated aerial bark cankers along the stems [26]. Snails sucking on the exudates of bleeding cankers were also suggested to act as *Phytophthora* vectors along beech stems [30].

Like in other European countries and the USA [19,20,22,23,25,30–33,37,59], in Lower Austria, the beech trees affected by *Phytophthora* cankers were usually showing a severe decline and dieback. However, apart from the stands F12, F27 and F31 with 14–20% canker incidence, the proportions of beech trees affected by bark cankers were in most of the 25 *Phytophthora*-infested beech stands too low to explain the observed decline of beech trees in groups or at stand level. Also in Bavaria, in 87% of the surveyed 134 beech stands, the distribution of beech trees with bleeding bark lesions was scattered, and similar to oak decline, the *Phytophthora*-related destruction of the root system was suggested as main driver of beech decline [19,20,22,30,44,45]. In the present study, the fine root conditions of each

two healthy and declining beech trees were assessed in 10 beech stands in Lower Austria. In the eight *Phytophthora*-infested stands, the four relative root ratio parameters, FRL/CRL_{rel}, FRS/CRS_{rel}, FRT/CRL_{rel}, FRT/FRL_{rel}, FRT/CRS_{rel} were between 7.8 and 13.4% higher in the 16 non-declining compared to the 16 declining trees. However, due to the low sample numbers, these differences were statistically only significant or almost significant for two root parameters (FRL/CRL_{rel} and FRS/CRS_{rel}). In a comparable study of 19 *Phytophthora*-infested oak stands in Bavaria, the parameters FRL/CRL_{rel} and FRT/CRL_{rel} for the 59 healthy oak trees were on average 21.6 and 35.4% higher than in the 65 declining trees [45]. Several factors might explain the smaller differences in the root parameters between healthy and declining trees in beech as compared to oaks. Trees are in a functional equilibrium between the water absorbing fine root system and the transpiring and producing leaf area [85,86]. The threshold for fine root losses leading to the onset of crown thinning and eventually decline is most likely considerably lower in the drought-sensitive *F. sylvatica* than in drought-tolerant oak species [87–89]. Furthermore, oak decline in temperate Central Europe is mainly driven by *P. quercina*, an oak-specific specialized fine root pathogen [16,20,22,44,45,64–66], whereas the most common species involved in beech decline, *P.* ×*cambivora*, *P. cactorum* and *P. plurivora*, are both fine root and bark pathogens causing bleeding lesions on stems and woody roots. Like in Bavaria [30], the root systems of wind-thrown beech trees in the three beech stands on temporarily waterlogged sites with high clay contents (F04 Purkersdorf, F16 Hengstlberg, F26 Kleinmariazell 2) and in the limestone stand F08 Thernberg 2 showed, besides extensive losses of lateral and fine roots, also numerous *Phytophthora*-like lesions on woody roots. These lesions certainly reduce both the supply of distant parts of the root system with carbohydrates and the transport of water to the crowns. As demonstrated in the present study, bark lesions on roots and stems can be colonized by a range of secondary fungal pathogens like *Armillaria* spp., *Ustulina deusta*, *Neonectria coccinea*, *Bjerkandera adusta*, *Fomes fomentarius*, *Oudemansiella mucida* etc., exacerbating the primary bark damages and colonizing the underlying xylem leading to a reduced water supply. Furthermore, this study showed that *Phytophthora* bark lesions are weakening affected beech trees and predisposing them to attacks by opportunistic bark beetles. The observation of active breeding galleries in fresh collar lesions caused by *P.* ×*cambivora* raises the question whether, similar to ant species [90], bark beetles might act as *Phytophthora* vectors. In Hungary, *T. bicolor,* together with other bark beetles, was involved in the mass mortality of beech forests [91]. Unfortunately, in Hungary, a possible involvement of *Phytophthora* species has not been investigated. Additional studies of declining and non-declining beech trees are needed to assess the extent of fine root losses and of bark lesions on woody roots, their effect on the health status of beech trees and the synergistic interaction between primary *Phytophthora* infections, secondary fungal infections and bark beetle colonization of beech bark.

The ectomycorrhizal frequency of fine root tips (MT/NMT_{rel}) was considerably higher, though not statistically significant, in non-declining versus declining beech trees and seemed to be a good indicator of beech vitality. In the 10 intensively studied beech stands in Lower Austria, no consistent ecological factor was found which would explain the selective reduction of the ectomycorrhizal frequency of individual trees within a relatively homogeneous forest stand. Ectomycorrhizal symbiosis relies on a solid bidirectional exchange of carbohydrates and nutrients between plant and fungal partners [49]. The reduced mycorrhizal frequency of declining beech trees might have been caused by a reduced supply of the fungal partners with carbohydrates due to reduced tree vitality. The reduced mycorrhization of fine roots results in reduced nutrient uptake and in turn further decreases tree vitality. *Phytophthora* pathogens are known to alter ectomycorrhizal symbioses [52]. In the eight *Phytophthora*-infested beech stands in Lower Austria, the observed losses of fine roots and necrotic lesions on the woody roots of declining trees could certainly have negative impacts on the ectomycorrhiza by reducing both the available number of fine root tips and the fine root length for mycorrhizal colonization, and also the transport of carbohydrates to the fungal partners. Also in *Phytophthora*-infested oak forests in Europe, declining trees were associated with a decrease in ectomycorrhizal frequency [50,92]. In contrast, in a chestnut stand in Italy severely affected by ink disease caused by *P.* ×*cambivora*

ectomycorrhizal frequency, and species richness, were both higher compared to a healthy chestnut forest [93]. Studies of Swiss needle cast of Douglas-fir caused by *Phaeocryptopus gaeumannii* in Oregon demonstrated that ectomycorrhizal frequency did not vary between sites with different disease incidences. However, ectomycorrhizal density and species richness were positively correlated with needle retention [94]. Unfortunately, in the present work, ectomycorrhizal species richness had not been examined. Additional research in beech stands is needed on the relationship between *Phytophthora* presence, root parameters, tree vitality and ectomycorrhizal frequency and species richness.

In Bavaria, *Phytophthora*-related decline and dieback of beech forests reached an epidemic extent during summer and autumn 2003 and in 2004 as a consequence of the exceptionally wet year 2002, followed by the severe drought during the summer of 2003 [30,40]. In Lower Austria, according to the data from the meteorological stations of the HZB, similar successions of abnormally wet and dry periods occurred which might have triggered beech decline. In almost all 10 intensively surveyed beech stands, from 2004 to 2006, precipitation in late winter and spring exceeded the long-term average, while summer 2004 and autumn 2006 were drier than the long-term average (data not shown). Climatic models predict for Europe continuously rising annual temperatures and a significant increase in the frequency and duration of both heavy summer rains and prolonged summer droughts [6,46,95]. These climatic changes will most likely trigger more severe *Phytophthora* disease epidemics in forest ecosystems including beech forests, and favor the spread and establishment of invasive high-temperature *Phytophthora* species like *P.* ×*cambivora*, *P. cactorum*, *P. cinnamomi*, *P. niederhauserii*, *P. multivora* and *P. plurivora* and provide them with a selective advantage over European native low-temperature species like *P. castanetorum*, *P. psychrophila*, *P. pseudosyringae*, *P. tubulina* and *P. vulcanica* [20,22,30,41,42,44,45,47].

5. Conclusions

Our study demonstrated the widespread occurrence of soilborne *Phytophthora* species, in particular the introduced invasive *P.* ×*cambivora*, *P. cactorum* and *P. plurivora*, in beech forests of Lower Austria, and their site preferences and their involvement in beech decline. Primary *Phytophthora* collar rot and aerial bark cankers on beech stems were found to be widespread, but in the majority of stands, their distribution was scattered. The analyses of several root parameters showed that the fine root status of declining beech trees was reduced compared to healthy trees. The reduced water uptake due to the fine root losses in combination with reduced water transport due to *Phytophthora* lesions on woody roots most likely triggered crown thinning, eventually leading to decline. However, additional studies of declining and non-declining beech trees in a statistically representative number of both *Phytophthora*-free and *Phytophthora*-infested stands are needed to quantify fine root losses, ectomycorrhizal frequency and the extent of bark lesions on woody roots, and assess their effect on the health status of beech trees and the synergistic interaction between primary *Phytophthora* infections and secondary fungal infections and bark beetle colonization of beech bark.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/8/895/s1, Table S1: Location and altitude of 34 forest stands of *Fagus sylvatica* in Lower Austria, the areas and numbers of trees surveyed, and the numbers of trees with *Phytophthora*-typical collar rot and aerial bark cankers, Table S2: GenBank accession numbers of ITS and partial *cox1* sequences generated in this study for representative *Phytophthora* isolates from Lower Austrian beech forests, Table S3: Long-term (1961–1991) climatic data of climatic stations closest and most comparable in altitude to ten intensively surveyed beech forest stands in Lower Austria, provided by the Hydrographischer Dienst Österreich (HZB; Vienna, Austria), Table S4: Crown condition of 60 beech trees in the 10 intensively surveyed mature beech stands in 2008 and the occurrence of *Phytophthora* spp, Table S5: Diversity of fungi associated with different symptoms and injuries in 21 beech stands in Lower Austria.

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