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Phytophthora Diversity in a Sentinel Arboretum and in a Nature Reserve Area †

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Abstract: Most soilborne *Phytophthora* species are invasive plant pathogens, and nursery plants for transplanting are considered a primary pathway for the introduction of exotic *Phytophthora* species into plant diversity conservation sites. As a preliminary contribution to the study of *Phytophthora* populations in plant conservation sites, we compared the diversity of *Phytophthora* in the protected natural area Complesso Speleologico Villasmundo S. Alfio Nature Reserve (NR) (Siracusa) and the botanical garden (BG) of the University of Catania, eastern Sicily (Italy). Samplings were carried out in spring 2019. Overall, 29 rhizosphere soil samples were collected, 17 from different types of vegetation in NR and 12 from different plant species in BG. *Phytophthora* species were recovered from soil samples by leaf baiting and isolation on a selective medium. Isolates were identified by combining morphological features with phylogenetic inferences from ITS-rDNA sequence analysis. Overall, 82 *Phytophthora* isolates, 30 from NR and 52 from BG, were characterized. Five *Phytophthora* species, *P. pseudocryptogea*, *P. cryptogea*, *P. bilorbang*, *P. plurivora* and *P. gonapodyides*, were recovered from NR, while only three species, *P. nicotianae*, *P. multivora* and *P. parvispora*, were found in BG. Factors contributing to shape *Phytophthora* populations of rhizosphere soil in these two vegetational contexts are discussed.

Keywords: nature reserve; botanical garden; leaf baiting; molecular analysis; ITS-rDNA; morphological characters; monitoring; oomycetes; botanical garden; arboretum

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1. Introduction

With more than 180 known species and several other informally described provisional taxa [1,2], plant pathogens of *Phytophthora* genus (Pythiaceae, Peronosporales, Oomycota, Chromista) represent one of the main threats for forests and other ecosystems [3–10]. The genus includes species with a well-known polyphagous attitude, including *P. nicotianae* [11–13] and *P. cryptogea* [14], as well as pathogens with a restricted host range, such as *P. prodigiosa* and *P. mekongensis* [15], two recently described species associated to Citrus in south-eastern Asia [16].

Several studies reported exotic *Phytophthora* species from non-native regions as invasive pathogens [17–26]. The introduction of these species in new environments may have destabilizing effects on ecosystems and may threaten the diversity of vegetation and the survival of rare plant species [27–30]. The movement of plants from nurseries to natural

and managed ecosystems represents one of the main pathways of spreading of non-native *Phytophthora* spp. as well as of their introduction in new environments [31–33]. In turn, the finding in forests and natural ecosystems of aggressive *Phytophthosa* spp., typically associated with cultivated plants, suggests plant diversity conservation sites may be reservoirs of *Phytophthora* inoculum for crops [29].

In this scenario, the circulation of non-native plants across naturalized and managed ecosystems represents the major means for the conveyance of exotic pests.

In Italy, the presence of some of the most destructive exotic *Phytophthora* species has been reported from various kinds of ecosystems [14,24,25,34–44]. Due to the polyphagy of these species and their ability to adapt to different environments, their communities in the wild show a great variability [24].

As a preliminary step toward understanding the ecological factors shaping the *Phytophthora* communities, we compared the diversity of populations of these oomycetes in two different types of plant diversity conservation sites, a nature reserve and a botanical garden in eastern Sicily (southern Italy) which can also be considered an arboretum.

2. Materials and Methods

2.1. Sampling Areas

Two sampling sites from the south east of Sicily, Italy, were selected for this study: (i) a natural reserve (Complesso Speleologico Villasmundo S. Alfio, Regional Natural Reserve—Melilli, Siracusa, Italy) and (ii) a managed semi-natural ecosystem represented by the botanical garden of Catania (Catania, Italy) (Figure 1). The botanical garden contains a living collections of trees partially destinated for scientific study; therefore, it can also be defined as arboretum. Sampling activities were carried out during the spring of 2019.

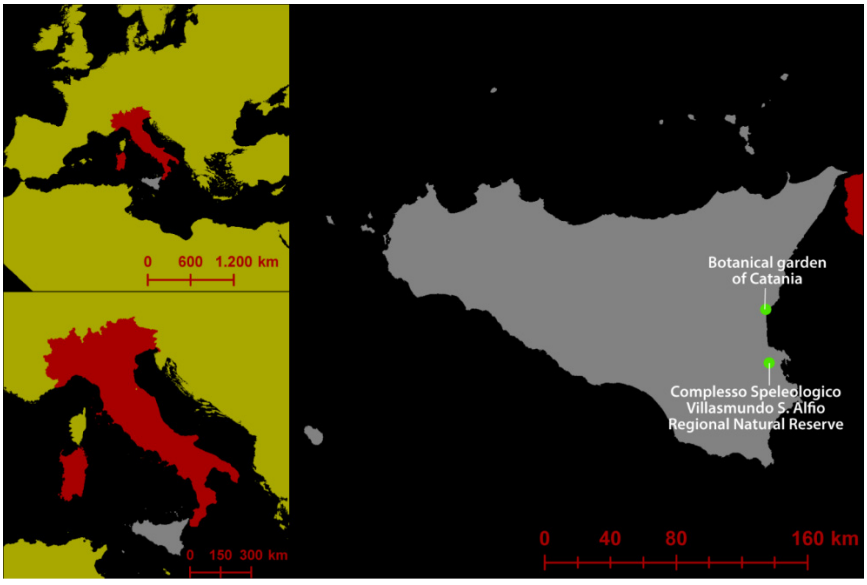

Figure 1. Geographical location of the surveyed areas included in this study.

2.2. Sampling and Phytophthora Isolation

In total, 29 rhizosphere soil samples were examined (Table 1), 17 from both mature trees and herbaceous plants of the main vegetational types present in the nature reserve (total area ca. 71.7 ha) and 12 collected from 12 diverse trees in the botanical garden (total area ca 1.6 ha). Nine out of these twelve plants were exotic. In both sites, plants were sampled irrespective of the presence of symptoms.

Soil sampling and isolation were performed in accordance with Jung et al. [45]: four soil cores were collected under each tree or shrub, 50–150 from the rhizosphere cm away from the stem base, and rhizosphere soil from all four cores were bulked together (volume of each sample about 1 L).

For each sample, subsamples of 400 mL were used for baiting tests that were performed in a walk-in growth chamber with 12 h natural daylight at 20 °C. Young leaves of *C. siliqua* and *Quercus* spp. floated over flooded soil were used as baits. After 24–48 h incubation, necrotic segments (2 × 2 mm) from symptomatic leaves were plated in Petri dishes onto selective PARPNH-agar medium [46]. Petri dishes were incubated at 20 °C in the dark. Outgrowing *Phytophthora* hyphae were transferred onto V8-juice agar (V8A) under the stereomicroscope. All the *Phytophthora* isolates were maintained on V8-agar in the dark at temperature of 6 °C.

2.3. Morphological Characterization of Isolates

Cultures of seven days, grown on V8A at 20 °C in the dark, were used to group all isolates into morphotypes on the basis of their colony growth patterns. For each host-plant and plant community, the different morphological types were labelled with progressive numbering (Roman numbering); then, isolates belonging to the same sampling hosts have been tagged with the relative type number.

Moreover, morphological features of chlamydospores, sporangia, oogonia, antheridia and hyphal swellings, were carefully analyzed and compared with species descriptions in the literature [3,20,25,47].

2.4. Molecular Identification of Isolates

The DNA of the pure cultures of isolates obtained from soil was extracted by using PowerPlant® Pro DNA isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's protocol. The DNA was preserved at −20 °C. The identification of *Phytophthora* species was performed by the analysis of Internal Transcribed Spacer (ITS) regions of ribosomal DNA (rDNA). DNA was amplified using forward primers ITS6 (5′-GAAGGTGAAGTCGTAACAAGG-3′) [48] and reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [49]. The PCR amplification mix and thermocycler conditions were in accordance with Cooke et al. [48]. All PCRs were carried out in a 25 µL reaction mix containing PCR Buffer (1x), dNTP mix (0.2 mM), $MgCl_2$ (1.5 mM), forward and reverse primers (0.5 mM each), Taq DNA Polymerase (1 U) and 100 ng of DNA. The thermocycler conditions were as it follows: 94 °C for 3 min; followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and then 72 °C for 10 min.

Amplicons were detected in 1% agarose gel and sequenced in both directions by an external service (Amsterdam, The Netherlands). Derived sequences were analyzed using FinchTV v.1.4.0 [50]. For species identification, blast searches in GenBank [51], in a local database containing sequences of ex-type or key isolates from published studies and in *Phytophthora* Database [52] were performed. Isolates were assigned to a species when their sequences were at least 99–100% identical to a reference isolate.

2.5. Soil Analysis and USDA Classification

Additional soil from the rhizosphere of the same plants sampled for the analysis of diversity of *Phytophthora* spp. was analyzed to determinate the following properties: pH-H2O, electrical conductivity at 25 °C, organic matter content, nitrates and soil texture (Table 2). The soil analyses were performed by an external service (Progetto Ambiente & C. s.a.s., Catania, Italy) following the "Official method of soil chemical analysis" in accordance with standard protocols defined by D.M. 13/09/1999, G.U. No. 248, 21/10/99 and D.M. 25/03/2002, G.U. No. 84, 10/04/2002.

The soil texture of each sample was determinated on the basis of the USDA classification method [53]. The percentage of each soil component (sand, clay and silt) has been used in order to assign each sample to a textural class.

Table 1. Geographic localization of the 29 soil samples in nature reserve and botanical garden and plant species sampled.

Sampling Site	Rhizosphere Soil Sample ID	Location—Municipality and Geographic Coordinates (DATUM WGS84)	Plant Species
Complesso Speleologico Villasmundo S. Alfio Regional Nature Reserve	NR_1903_S1	Melilli—37°13′17.54″ N; 15°6′19.52″ E	*Salix pedicellata*
	NR_1903_S2	Melilli—37°13′17.66″ N; 15°6′19.28″ E	*S. pedicellata*
	NR_1903_S3	Melilli—37°13′17.753″ N; 15°6′18.93″ E	*Platanus orientalis*
	NR_1903_S4	Melilli—37°13′17.86″ N; 15°6′18.81″ E	*P. orientalis*
	NR_1903_S5	Melilli—37°13′17.25″ N; 15°6′15.30″ E	*Euphorbia dendroides*
	NR_1903_S6	Melilli—37°13′17.48″ N; 15°6′15.31″ E	*Cynara cardunculus*
	NR_1903_S7	Melilli—37°13′17.60″ N; 15°6′15.30″ E	*Asphodelus* sp.
	NR_1903_S8	Melilli—37°13′11.75″N; 15° 6′1.20″ E	*Quercus ilex*
	NR_1903_S9	Melilli—37°13′11.00″ N; 15°5′59.69″ E	*Q. ilex*
	NR_1903_S10	Melilli—37°13′10.93″ N; 15°5′59.95″ E	*Q. ilex*
	NR_1903_S11	Melilli—37°13′11.788″ N; 15°6′0.547″ E	*Q. pubescens sensu latu*
	NR_1903_S12	Melilli—37°13′17.52″ N; 15°6′7.94″ E	*Sarcopoterium spinosum*
	NR_1903_S13	Melilli—37°13′17.50″ N; 15°6′8.57″ E	*S. spinosum*
	NR_1903_S14	Melilli—37°13′17.28″ N; 15°6′4.77″ E	*Pistacia lentiscus*
	NR_1903_S15	Melilli—37°13′17.50″ N; 15°6′5.13″ E	*P. lentiscus* + *Pyrus* sp., mixed sample
	NR_1903_S16	Melilli—37°13′16.94″ N; 15°6′7.66″ E	*P. lentiscus*
	NR_1903_S17	Melilli—37°13′16.93″ N; 15°6′6.24″ E	*P. lentiscus*
Botanical garden of Catania	BG_1903_S1	Catania—37°30′57.29″ N; 15°5′2.27″ E	*Araucaria cokii*
	BG_1903_S2	Catania—37°30′55.92″ N; 15°5′1.95″ E	*Phytolacca dioica*
	BG_1903_S3	Catania—37°30′55.08″ N; 15°4′59.75″ E	*Grevillea robusta*
	BG_1903_S4	Catania—37°30′57.56″ N; 15°5′1.47″ E	*Pistacia atlantica*
	BG_1903_S5	Catania—37°30′57.47″ N; 15°5′0.81″ E	*Sterculia diversifolia*
	BG_1903_S6	Catania—37°30′57.69″ N; 15°5′1.80″ E	*Eucalyptus citridora*
	BG_1903_S7	Catania—37°30′53.46″ N; 15°5′2.38″ E	*Zelkowa sicula*
	BG_1903_S8	Catania—37°30′53.35″ N; 15°5′1.89″ E	*Q. suber*
	BG_1903_S9	Catania—37°30′53.19″ N; 15°5′2.42″ E	*Olea europea*
	BG_1903_S10	Catania—37°30′53.34″ N; 15°5′2.40″ E	*Pistacia lentiscus*
	BG_1903_S11	Catania—37°30′57.92″ N; 15°5′0.74″ E	*Coffea arabica*
	BG_1903_S12	Catania—37°30′57.95″ N; 15°5′0.86″ E	*Mangifera indica*

Table 2. *Phytophthora* spp. recovered from plant rhizosphere and physico-chemical soil properties in samples collected in two different plant diversity conservation sites (Nature Reserve and Arboretum).

Sampling Site	Rhizosphere Soil Sample ID.	Host	Baited *Phytophthora* spp. [a]	Soil Properties				
				pH	Electrical Conductivity at 25 °C (µS/cm)	Soil Texture	Nitrates (mg/kg)	Organic Matter (%)
Complesso Speleologico Villasmundo S. Alfio Regional Nature Reserve	NR_1903_S1	*Salix pedicellata*	PSC	7.6 ± 0.1	1497.0 ± 49	Sandy clay loam	11.0 ± 1	6.5 ± 0.3
	NR_1903_S2	*S. pedicellata*	CRY	7.7 ± 0.1	938.0 ± 43	Sandy clay loam	1.6 ± 0.2	2.8 ± 0.1
	NR_1903_S3	*Platanus orientalis*	-	7.0 ± 0.1	913.0 ± 43	Sandy clay	7.1 ± 0.7	4.9 ± 0.2
	NR_1903_S4	*P. orientalis*	BIL	7.1 ± 0.1	1023.0 ± 44	Sandy clay loam	6.9 ± 0.7	5.4 ± 0.3
	NR_1903_S5	*Euphorbia dendroides*	-	7.3 ± 0.1	976.0 ± 44	Sandy clay	5.9 ± 0.6	7.1 ± 0.4
	NR_1903_S6	*Cynara cardunculus*	-	7.5 ± 0.1	822.0 ± 41	Sandy clay	7.3 ± 0.7	5.5 ± 0.3
	NR_1903_S7	*Asphodelus* sp.	-	7.5 ± 0.1	1122.0 ± 45	Sandy clay	7.2 ± 0.7	5.4 ± 0.3
	NR_1903_S8	*Quercus ilex*	GON	7.3 ± 0.1	1463.0 ± 48	Clay loam	13.0 ± 1	13.1 ± 0.7
	NR_1903_S9	*Q. ilex*	PLU	7.4 ± 0.1	1617.0 ± 53	Loamy sand	17.0 ± 2	21.0 ± 1
	NR_1903_S10	*Q. ilex*	-	7.6 ± 0.1	1397.0 ± 46	Sandy loam	11.0 ± 1	16.3 ± 0.8
	NR_1903_S11	*Q. pubescens* sensu latu	GON	7.2 ± 0.1	1174.0 ± 45	Clay loam	11.0 ± 1	11.4 ± 0.6
	NR_1903_S12	*Sarcopoterium spinosum*	-	7.2 ± 0.1	922.0 ± 42	Sandy clay	6.1 ± 0.5	5.1 ± 0.2
	NR_1903_S13	*S. spinosum*	-	7.3 ± 0.1	1102.0 ± 49	Sandy clay	7.1 ± 0.7	4.2 ± 0.1
	NR_1903_S14	*Pistacia lentiscus*	-	7.4 ± 0.1	831.0 ± 41	Sandy clay	6.7 ± 0.7	8.2 ± 0.4
	NR_1903_S15	P. lentiscus + *Pyrus* sp., mixed sample	-	7.2 ± 0.1	856.0 ± 43	sandy clay loam	5.3 ± 0.7	7.2 ± 0.2
	NR_1903_S16	*P. lentiscus*	-	7.3 ± 0.1	796.0 ± 41	Sandy clay	1.7 ± 0.2	7.7 ± 0.4
	NR_1903_S17	*P. lentiscus*	-	7.3 ± 0.1	1056.0 ± 44	Sandy clay	3.6 ± 0.4	8.7 ± 0.4
Botanical garden of Catania (Arboretum)	BG_1903_S1	*Eucalyptus citridora*	MUL, NIC	7.99 ± 0.1	877.5 ± 48	Loamy sand	145.3 ± 0.4	1.07 ± 0.2
	BG_1903_S2	*Araucaria cooki*	MUL, NIC	8.19 ± 0.1	3437.5 ± 46	Sandy loam	1210.9 ± 0.6	1.29 ± 0.1
	BG_1903_S3	*Gravillea robusta*	-	8.14 ± 0.1	852.5 ± 40	Loamy sand	145.3 ± 0.6	0.86 ± 0.1
	BG_1903_S4	*Phytolacca dioica*	-	8.26 ± 0.1	997.5 ± 43	Sandy clay loam	188.2 ± 0.7	1.01 ± 0.1
	BG_1903_S5	*Pistacia atlantica*	MUL	7.43 ± 0.1	3945.0 ± 45	Loamy sand	1076.6 ± 0.1	1.49 ± 0.1
	BG_1903_S6	*Quercus suber*	-	8.14 ± 0.1	765.0 ± 45	Loamy sand	45.3 ± 0.2	1.48 ± 0.2
	BG_1903_S7	*Zelkova sicula*	MUL	8.55 ± 0.1	970.0 ± 44	Sandy clay loam	103.9 ± 0.8	0.73 ± 0.1
	BG_1903_S8	*Sterculia diversifolia*	MUL, NIC	8.10 ± 0.1	1675.0 ± 48	Clay loam	1366.6 ± 1.0	0.11 ± 0.05
	BG_1903_S9	*Mangifera indica*	MUL, NIC	8.60 ± 0.1	765.0 ± 41	Clay loam	176.7 ± 0.4	1.0 ± 0.1
	BG_1903_S10	*Olea europaea*	-	7.10 ± 0.1	1540.0 ± 43	Sandy clay loam	55.3 ± 0.4	0.66 ± 0.1
	BG_1903_S11	*Pistacia lentiscus*	PAR	8.64 ± 0.1	867.5 ± 46	Sandy loam	148.2 ± 0.5	0.99 ± 0.1
	BG_1903_S12	*Coffea arabica*	-	8.40 ± 0.1	677.5 ± 50	Loamy sand	41.0 ± 0.3	0.82 ± 0.1

[a] BIL = *P. bilorbang*; CRY = *P. cryptogea*; GON = *P. gonapodyides*; MUL = *P. multivora*; NIC = *P. nicotianae*; PLU = *P. plurivora*; PSC = *P. pseudocryptogea*; PAR = *P. parvispora*.

3. Results

3.1. Composition of Phytophthora Communities

Thirteen out of 29 rhizosphere soil samples (six from the natural reserve and seven from the botanical garden) processed by baiting revealed the occurrence of *Phytophthora* species in both the surveyed sites. Overall, 82 *Phytophthora* isolates (30 from the natural reserve and 52 from the botanical garden) were obtained. Morphological and ITS sequence analyses made it possible to identify eight *Phytophthora* species (Table 2); in detail, *P. bilorbang*, *P. cryptogea*, *P. gonapodyides*, *P. plurivora* and *P. pseudocryptogea* were recovered in the nature reserve, while only three species, namely *P. nicotianae*, *P. multivora* and *P. parvispora*, were found in the botanical garden.

Baiting of rhizosphere soil samples from the natural reserve revealed the occurrence of 5 *Phytophthora* from six mature trees belonging to five plant species (Table 2). A single *Phytophthora* sp. was recovered from each sample. *Phytophthora pseudocryptogea* and *P. cryptogea* were the only species isolated from willow trees (*Salix pedicellata*), *P. bilorbang* was recorded from *Platanus orientalis* and *P. plurivora* from a mature specimen of evergreen oak (*Q. ilex*). Finally, *P. gonapodyides* was isolated both from *Q. ilex* and *Q. pubescens* s. l.

Overall, three *Phytophthora* species were isolated from seven out of 12 plant species from the botanical garden (Table 2). *Phytophthora nicotianae* and *P. multivora* occurred together from mature trees of *Araucaria cokii*, *Phytolacca dioica*, *Q. suber* and *O. europaea*. In addition, *P. multivora* was exclusively isolated from *Sterculia diversifolia* and from *Zelkowa sicula*. Finally, *P. parvispora* was isolated from *Coffea arabica*.

3.2. Analysis of Soil

Results of soil analysis are schematically summarized in Table 2.

In the natural reserve, values of soil pH ranged from 7.0 (*Platanus orientalis*—NR_1903_S3) to 7.7 (*Salix pedicellata*—NR_1903_S2); values of 7.5 were reported in soils from *Cynara cardunculus* (NR_1903_S6) and *Asphodelus* sp. (NR_1903_S7); with reference to the electrical conductivity at 25 °C obtained results showed an high variability, with values ranging from 796 ± 41 µS/cm (*Pistacia lentiscus*—NR_1903_S16) to 1617 ± 53 µS/cm (*Q. ilex*—NR_1903_S9). Five main soil textures were reported from the reserve: 1. sandy clay loam, from both sampled *S. pedicellata* (NR_1903_S1 and _S2), from *P. orientalis* (NR_1903_S4) and from the mixed soil sample of *Pistacia lentiscus* and *Pyrus* sp. (NR_1903_S15); 2. sandy clay, from *P. orientalis* (NR_1903_S3), *Euphorbia dendroides* (NR_1903_S5), *Cynara cardunculus* (NR_1903_S6), Asphodelus sp. (NR_1903_S7), Sarcopoterium spinosum (NR_1903_S12 and _S13) and *P. lentiscus* (NR_1903_S14, _S16 and S17); 3. clay loam, from *Q. ilex* (NR_1903_S8) and Quercus pubescens s. l. (NR_1903_S11); 4. sandy loam (*Q. ilex*—NR_1903_S10); 5. loamy sand (*Q. ilex*—NR_1903_S9). Similarly to the electrical conductivity, values of the content in nitrates had a high variability, ranging from 1.6 ± 0.2 mg/kg in soil of *S. pedicellata* (NR_1903_S2) to 17.0 ± 2 mg/kg in that of *Q. ilex* (NR_1903_S9). Finally, the content in organic matter was above the 25% in all the analyzed samples, with the highest value (21%) in the soil of one of the sampled *Q. ilex* (NR_1903_S9).

In the botanical garden, the values of soil pH in the majority of samples ranged from 7.99 (*Eucalyptus citridora*—BG_1903_S1) to 8.64 (*P. lentiscus*—BG_1903_S11); values under this range were observed only in soil from *Pistacia atlantica*—BG_1903_S5 (7.43) and Olea europaea—BG_1903_S10 (7.1). The electrical conductivity at 25 °C ranged mainly from 677.5 ± 50 µS/cm (*Coffea arabica*—BG_1903_S12) to 997.5 ± 43 µS/cm (*Phytolacca dioica*—BG_1903_S4); values in the range 1000–2000 µS/cm were recorded in the soil of *O. europaea* (BG_1903_S10) and *Sterculia diversifolia* (BG_1903_S8); finally, values above 3000 µS/cm were in the soil from *Araucaria cooki* (BG_1903_S2) and *P. atlantica* (BG_1903_S5). Four main soil textures were reported from the botanical garden: 1. loamy sand in soil from *Grevillea robusta* (BG_1903_S3), *P. atlantica* (BG_1903_S5), *Quercus suber* (BG_1903_S6) and *Coffea arabica* (BG_1903_S12); 2. sandy loam in soil from *A. cooki* (BG_1903_S2) and *P. lentiscus* (BG_1903_S11); 3. sandy clay loam in soil from *P. dioica* (BG_1903_S4), *Zelkova sicula* (BG_1903_S7) and *O. europaea* (BG_1903_S10); 4. clay loam in soil from *S. diversifolia*

(BG_1903_S8) and *Mangifera indica* (BG_1903_S9). The values of the content in nitrates were mainly in the range 41–188.2 mg/kg: values in the range 1000–1400 mg/kg were observed for *P. atlantica*, *A. cooki* and *S. diversifolia*. Finally, the content in organic matter was under the 1.5% in all the soil samples.

4. Discussion

The number of *Phytophthora* species recovered from the rhizosphere soil of vegetation in the nature reserve was higher than the number of species recovered from rhizosphere soil of eight exotic and four native woody plants grown in the botanical garden. This may have been due to the different extension of the two surveyed sites. Very probably in the botanical garden of the University of Catania the presence in a restricted area of different potential woody host-plants grown in close proximity to each other favored the spread and prevalence of invasive as well polyphagous *Phytophthora* species, such as *P. multivora* and *P. nicotianae*. In most cases, these two species were isolated together from the same sample. Conversely, in the nature reserve of Villasmundo the presence of different vegetational types and peculiar ecological niches may have favored the diversity of *Phytophthora* community even in a relatively restricted area. This is exemplified by *P. bilorbang*, a typically aquatic species that only occasionally behaves as an opportunistic plant pathogen [25]. In the Complesso Speleologico Villasmundo S. Alfio, Regional Nature Reserve, *P. bilorbang* was isolated from the riparian vegetation associated with a water course.

The widespread occurrence of *Phytophthora* species in soils with different physico-chemical characteristics in both surveyed sites confirms the ability of these oomycetes to adapt to different environments and thrive in a wide range of ecological conditions [2,26,27,36,54,55]. Previous studies of other Authors demonstrated a correlation between soil characteristics and the impact of *P. cinnamomi* on forest vegetation [56]. However, it would be more difficult to demonstrate the effects of soil characteristics and other environmental factors on complex *Phytophthora* communities composed by several species with diverse ecological requirements and behaviors. Several recent studies addressing the diversity of *Phytophthora* communities in natural and semi-natural ecosystems grouped *Phytophthora* species on the basis of their life style and habitat, distinguishing between soil-inhabitant and preferentially aquatic species [2,24,26,54,57,58]. In this study, both types were isolated from soil samples collected in the Complesso Speleologico Villasmundo S. Alfio, Regional Nature Reserve while only typically soil-borne species were recovered from the botanical garden of the University of Catania. The species recovered from the largest number of host-plants in this site was *P. multivora*, which is regarded as an emerging plant pathogen worldwide and in particular a major pathogen of woody plants in urban environments in Australia [59,60]. In recent years, much attention has been paid to *Phytophthora* communites in natural and semi-natural ecosystems worldwide. Conversely, only few studies addressed the problem of *Phytophthora* spp. in botanical gardens, including arboreta. Yet, the presence or introduction of *Phytophthora* spp. in these sites can be a threat for the survival of rare plant species and have serious implications for the in situ conservation of plant diversity. Moreover, it has been recently stressed the potential role of botanical gardens and arboreta as sentinel sites for early detection of new tree diseases [37,61–66].

5. Conclusions

This study revealed the presence of several *Phytophthora* species in soil of plant diversity conservation sites. Most of these species are aggressive plant pathogens and two of them in particular *P. multivora* and *P. nicotianae* are invasive and polyphagous. This may explain why they were so widespread and prevailed over other species in a site with uniform and conducive environmental conditions. However, it cannot be ruled out that the predominance of these species was due, at least in part to the isolation method. Methods based on leaf baiting have the advantage of recovering living and culturable isolates, but could have the disadvantage to isolate some *Phytophthora* species selectively or preferentially. This limit can be excluded using in parallel detection methods based

on next-generation sequencing (NGS) technology which are more sensitive to detecting *Phytophthora* species in environmental samples and are less influenced by environmental conditions [28,65]. NGS-based methods can help the fine tuning of studies aimed at exploring the complexity of *Phytophthora* communities in different ecosystems and the effects of ecological factors driving the diversity and the structure of these communities.

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Abbreviations

The following abbreviations are used in this manuscript: MDPI: Multidisciplinary Digital Publishing Institute; DOAJ: Directory of open access journals; TLA: Three letter acronym; LD: linear dichroism.

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