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## Surprising low diversity of the plant pathogen *Phytophthora* in Amazonian forests

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### Abstract

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The genus *Phytophthora* represents a group of plant pathogens with broad global distribution. The majority of them causes collar and root-rot of diverse plant species. Little is known about *Phytophthora* communities in forest ecosystems, especially in Neotropical forests where natural enemies could maintain the huge plant diversity via negative density dependence. We characterized the diversity of soil-borne *Phytophthora* communities in the North French Guiana rainforest and investigated how they are structured by host identity and environmental factors. In this little-explored habitat, 250 soil cores were sampled from ten plots hosting ten different plant families across three forest environments (Terra Firme, Seasonally Flooded and White Sand). *Phytophthora* diversity was studied using baiting approach and metabarcoding (High Throughput Sequencing) on environmental DNA extracted from both soil samples and baiting-leaves. These three approaches revealed very similar communities, characterized by an unexpected low diversity of *Phytophthora* species, with the dominance of two cryptic species close to *Phytophthora heveae*. As expected, *Phytophthora* community composition of French Guiana rainforest was significantly impacted by the host plant family and environment. However, these plant pathogen communities are very small and are dominated by generalist species, questioning their potential roles as drivers of plant diversity in these Amazonian forests.

**Key Words:** Biodiversity, Neotropical forests, Janzen-Connell, host specialization

### Introduction

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Neotropical forests are major diversity hotspots for plants (Jablonski *et al.*, 2006; Antonelli & Sanmartin, 2011; Ter Steege *et al.*, 2013). Amongst the multiple hypotheses proposed to explain how plant species coexistence is promoted in these especially diverse

forests (Wright, 2002), environmental filtering and negative density dependence of host-specific natural enemies have been highlighted (John *et al.*, 2007, Mangan *et al.*, 2010, Philipson *et al.*, 2012; Bever *et al.*, 2015). Indeed, the role of pathogens in the maintenance and structure of plant diversity has been a major topic in community ecology (Alexander, 2010; Keesing *et al.*, 2010), especially in Neotropical forests (Mangan *et al.*, 2010; Terborgh, 2012; Bagchi *et al.*, 2014). The Janzen-Connell hypothesis could explain the maintenance of plant diversity by an accumulation of host-specific natural enemies near reproductive adults that affects their offspring in the vicinity, thus limiting host plant species abundance and aggregation (Janzen, 1970; Schupp, 1992; Mills, 1998). This hypothesis requires that natural enemies have a significant degree of host specialization, even if they do not have to be strict species-level specialists (Bagchi *et al.*, 2014; Bever *et al.*, 2015). Alternatively, pathogens may be generalists and structured more by environmental filtering and biogeographic history than by host plant community structure (Bever *et al.*, 2015).

Because the genus *Phytophthora* contains important plant pathogen species, often *Phytophthora* species are often involved in seedling mortality, with a worldwide presence and an important economic impact (Hansen, 2015). These pathogens could represent a good target to study Janzen-Connell effects and host specialization in Neotropical forests. Indeed, *Phytophthora* species exhibit a large diversity of aggressiveness and host range (Erwin and Ribeiro, 1996). By 2018, the genus encompassed 173 described species and eight hybrids, with an estimation of 326 total species (Scott *et al.*, 2018), divided in twelve phylogenetic clades, corresponding to aquatic opportunists, foliar pathogens, or soil-borne pathogens (Hansen, 2015). However, *Phytophthora* communities have been understudied in Neotropical forests.

The few studies on *Phytophthora* in tropical forests have focused on characterizing *Phytophthora* diversity, without explicit focus on the factors shaping these communities. For example, Zeng *et al.*, 2009, found 14 species in the tropical island of Hainan, sampled from plant materials, soils or water at five locations, although only five species were found in forest. In their study across Australia, Burgess *et al.*, 2019, sampled 20 locations in the subtropical/tropical Queensland and found 13 *Phytophthora* species, among which the most frequent were *P. cinnamomi* and *P. heveae*. Wallace, 2015, showed that *Phytophthora* diversity could be relatively high in Neotropical forests of Costa Rica, with 21 species found in various forest locations. Some species such as *P. cinnamomi*, *P. heveae*, *P. castaneae* were common across these studies and appear to be widespread in tropical forests (Zeng *et al.*, 2009, Wallace, 2015, Burgess *et al.*, 2017, Jung *et al.*, 2017).

A traditional method for the study of soil-borne *Phytophthora* communities is the baiting technique. This approach involves use of plant tissues, usually leaves, as baits on which the targeted pathogens produce characteristic lesions. Isolation onto selective agar medium from the lesions enables detection and determination of species using molecular barcodes. More recently, high-throughput sequencing (HTS) methods were applied to study *Phytophthora* communities (Vettraino *et al.*, 2012; Coince *et al.*, 2013; Català *et al.*, 2015; Burgess *et al.*, 2017, Català *et al.*, 2017; Bose *et al.*, 2018). With this approach, DNA is directly extracted from environmental samples (e.g. soil), then DNA barcodes are amplified with *Phytophthora*-specific primers and sequenced for analysis (Scibetta *et al.*, 2012). These methods are less time-consuming and show greater sensitivity (Shokralla *et al.*, 2012), potentially circumventing biases such as seasonal variation of *Phytophthora* biomass or activity, and possible specificity of the leaves used as bait toward some *Phytophthora* species (Tsao & Ocaña, 1969). Several studies have compared these two approaches, (Vannini *et al.*, 2013; Català, 2017; Bose *et al.*, 2018; Khaliq *et al.*, 2018). The HTS approach generally enables detection of a much higher number of *Phytophthora* taxa. For example, in Bose *et al.*, 2018, five taxa were detected by baiting whereas 32 taxa were detected by metabarcoding.

In this study, we sampled soil at the stem collar of individuals from tree species belonging to ten different taxonomic families in each of three soil conditions at two different locations in French Guiana. We used baiting/isolation and metabarcoding methods to describe

*Phytophthora* communities in these different environments. Our aims were (1) to compare the methods (2) to estimate the diversity of these communities and compare it to diversity of other tropical and temperate studies, and (3) to unravel the primary factors that structure *Phytophthora* communities in the soils of these Neotropical forest sites. We hypothesized (i) that soil metabarcoding will provide greater precision in community estimates than traditional baiting techniques, (ii) that *Phytophthora* richness will be similar to other sampled moist tropical forests, higher than that found in temperate forests, and (iii) that host plant identity will play a stronger role than environmental filtering in structuring *Phytophthora* communities.

## Results

### *Phytophthora* diversity uncovered by baiting & isolation

Out of the 93 soil samples, 89 produced lesion spots on *Rhododendron* leaves, 92 on Avocado leaves and 91 on Cassava leaves. From these 272 bait leaves, 581 isolates were obtained from infection spots, out of which 366 were identified as *Phytophthora* species (Fig. 2). Among these *Phytophthora* isolates, 136 were found in TF soils, 128 in SF soils and 102 in WS soils. *Phytophthora* retrieval success depended on the bait and location (both  $p < 0.001$ ), but not on the environment ( $P = 0.83$ ) or host family ( $P = 0.42$ ). Fifty out of the 55 samples (91%) from the CSG site produced isolates that were identified as *Phytophthora* through Sanger sequencing, while only 24 out of the 38 samples (63 %) from the Laussat site produced isolates. *Phytophthora* strain recovery was high on *Rhododendron* leaves (167 isolates) and Avocado leaves (127 isolates), but lower on Cassava leaves (72 isolates).

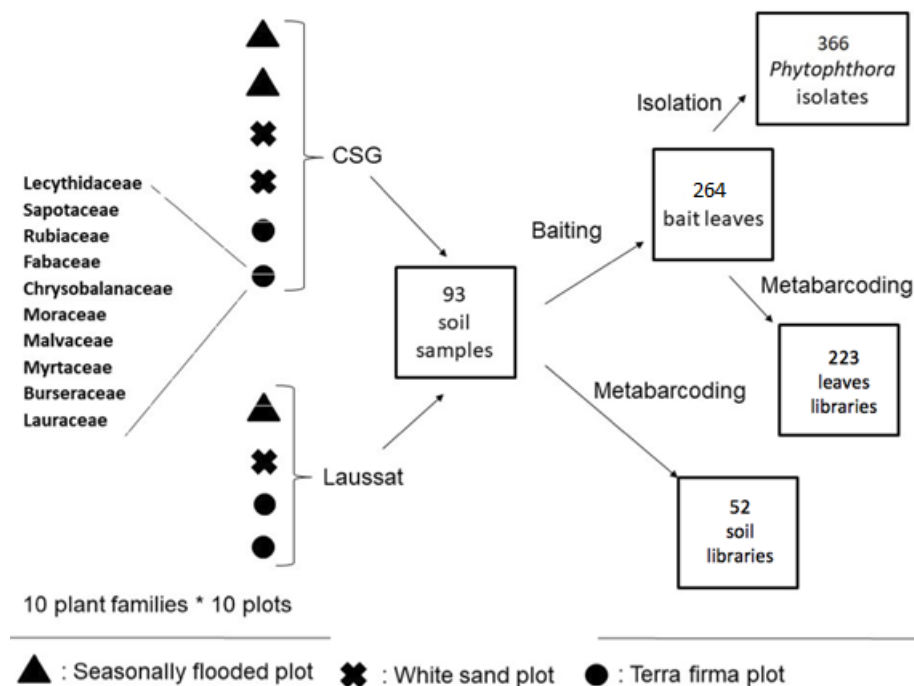


Figure 2. Experiment design with the number of samples at each step, from the sampling to the metabarcoding or isolation. Among 100 potential “tree families x plots” combinations, 93 individuals were sampled because some plant families could not be found in all plots.

The number of lesions per leaf weight was quantified on *Rhododendron* bait leaves as a measure of the presence of live *Phytophthora* in the baited soils. The number of lesions per leaf weight was not related to the location nor to the environment (Fig. 3). However, the number of spots was significantly different between host plant families with a strong “family x environment” interaction (both  $p < 0.001$ ). Figure 3 shows that while many families had higher lesion number on TF environments compared to WS or SF, other families (*Malvaceae*, *Moreceae*, *Myrtaceae*) showed limited differences between the environments.

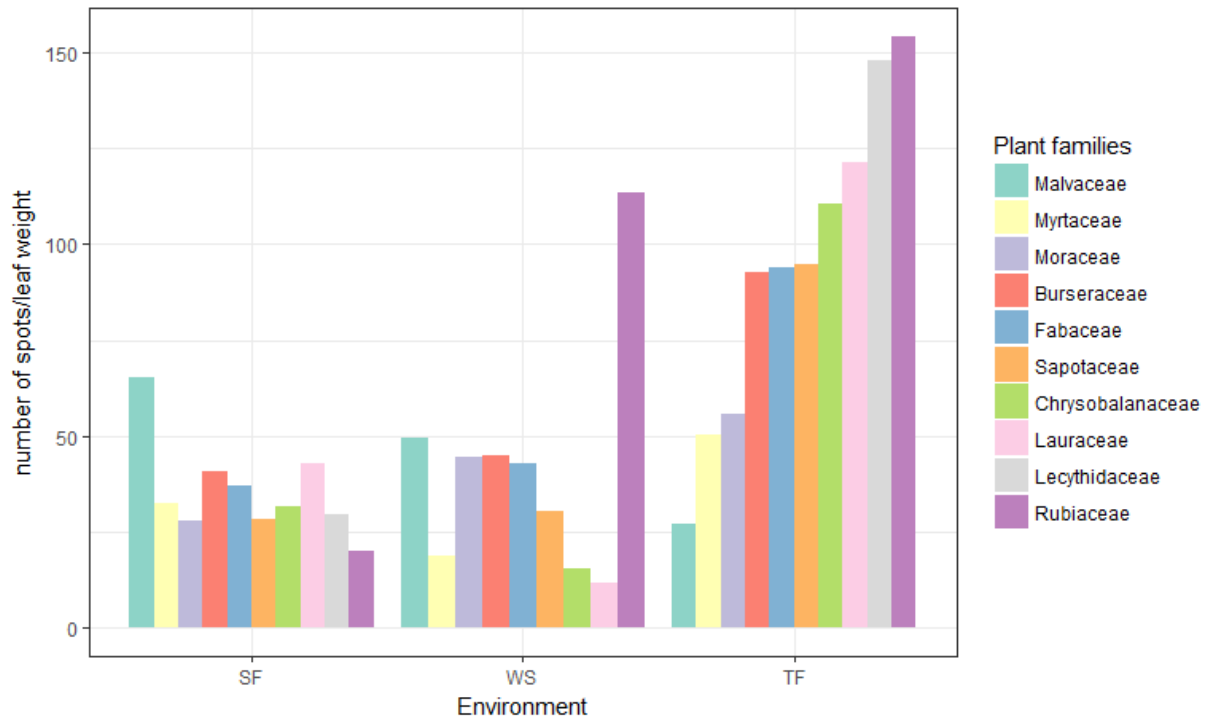


Figure 3. Number of *Phytophthora* infection spots on *Rhododendron* leaves per leaf weight. Three forest environments have been studied: TF (terra firma), SF (seasonally flooded), WS (white sand).

Sanger sequences of the ITS region of the *Phytophthora* isolates were approximately 800 bp in length (min 616 bp - max 876 bp), versus from 373 to 382 bp for *RAS-YPT* gene. Sequencing of the ITS region enabled us to identify five potential species: a species close to *P. heveae* (named here *P. heveae*-like, 304 isolates), a species close to *P. castaneae* (named here *P. castaneae*-like, 24 isolates), *P. cinnamomi* (23 isolates), *P. sp. cuyabensis* (6 isolates) and an undescribed species from clade 9, *P. sp. neb\_1543* (9 isolates). Isolates identified as *P. heveae*-like, *P. castaneae*-like and *P. sp. cuyabensis* showed some heterogeneity of ITS sequences, differing for up to 9 bases between *P. castaneae*-like isolates, up to 4 bases between *P. heveae*-like isolates and 2 bases between *P. sp. cuyabensis* isolates. ITS sequence alignments within either *P. cinnamomi* isolates or *P. sp. neb\_1543* isolates showed 100% homology. Clustering patterns and assignation from *RAS-Ypt* and ITS sequences were similar, although variability between *RAS-Ypt* sequences was lower (up to 3 bases between *P. heveae*-like isolates, up to 2 bases between *P. castaneae*-like isolates, and no variability among isolates of other species). Three plant species used as bait leaves enabled us to retrieve very similar *Phytophthora* communities, with one exception - *P. sp. cuyabensis*, the least frequent species, was not recovered from Cassava leaves.

Six *P. cinnamomi* isolates that were selected randomly among the 23 retrieved isolates, produced oospores after crossing with *P. cinnamomi* mating type A1, and none after crossing with mating type A2, suggesting that their mating type corresponded to A2.

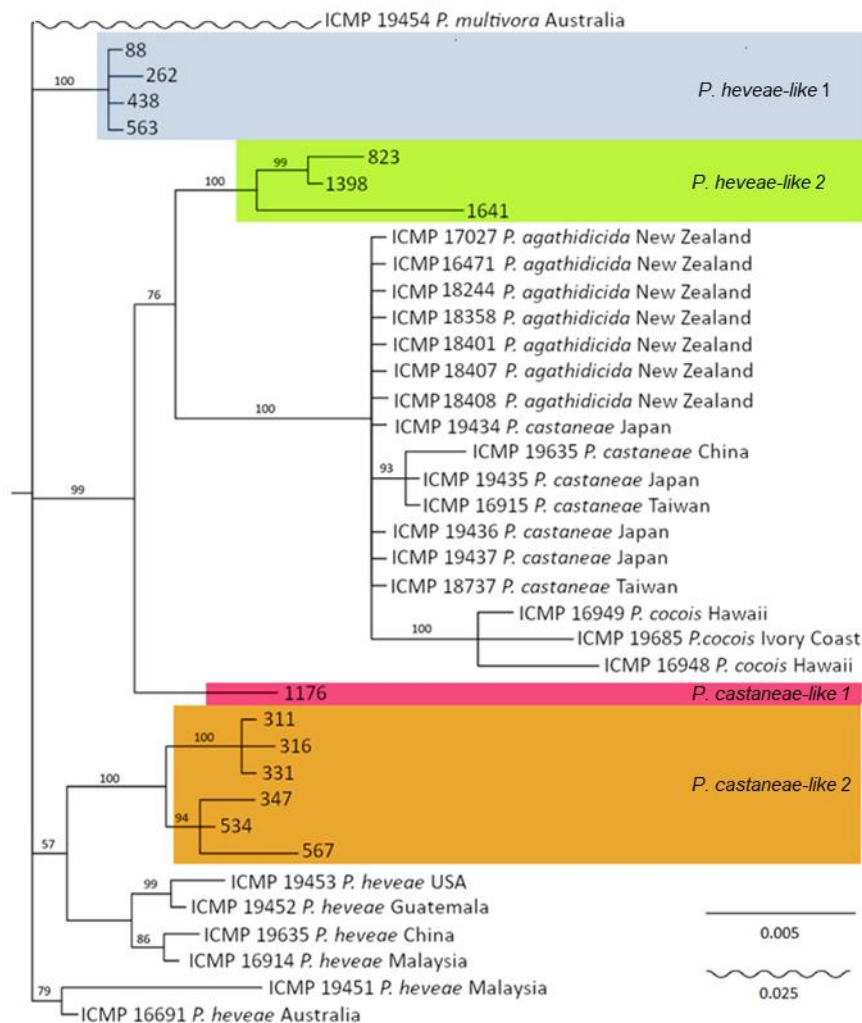


Figure 4: Bayesian inference phylogenetic trees of 14 *P. castaneae*-like and *P. heveae*-like isolates created by concatenation of nuclear genetic markers (ITS, *RAS-Ypt*, Enolase). Reference isolates taken from NCBI are designated by their accession numbers and origins in Table S1. Isolates from this study are designated by either *P. heveae*-like or *P. castaneae*-like. Monophyletic groups of isolates were clustered as groups which were used for further analysis. Distances are given in number of substitutions/site.

### Phylogenetic analysis of isolates

Figure 4 shows the phylogenetic tree built from the three nuclear markers (ITS, *RAS-Ypt*, Enolase), either sequenced from the studied set of clade-5 isolates or retrieved from GenBank (Table S1). The isolates retrieved from our study clustered into four separate groups. Two of them were close to *P. heveae*, and the two others were phylogenetically close to *P. castaneae*. Bayesian inference of these *Phytophthora* strains from nuclear data revealed that distance between respectively the two *P. heveae*-like groups and the two *P. castaneae*-like groups from French Guiana were equal or greater than between reference isolates from different species of clade-5: *P. castaneae*, *P. cocois*, *P. agathidicida* and *P. heveae* (Fig. 4). Thus, these clade-5 isolates of *Phytophthora* could potentially belong to new species and were treated as such for the rest of this study. These four *Phytophthora* groups from clade-5 could



be separated based on the ITS sequence alone, although the Enolase sequence was more discriminant. This enabled us to attribute each of the 304 *P. heveae*-like and 24 *P. castaneae*-like isolates retrieved from the baiting/isolation method to one of the 4 groups. On the other hand, for mitochondrial markers, all 15 isolates from clade-5 clustered with the reference *P. heveae* (Fig. S1); the mitochondrial phylogeny of the clade-5 isolates was thus not congruent with their nuclear phylogeny. While the two *P. heveae*-like groups could still be separated based on their mitochondrial markers, the two *P. castaneae*-like groups were merged with one of the *P. heveae*-like group.

The four *P. cinnamomi* isolates studied did not exhibit polymorphism for any of the studied markers.

Table 1: List of main *Phytophthora* species identified from environmental samples with the three methods, with the proportion (%) of samples containing each species.

Species	Clade	Method (% of samples containing the species)		
		Soil metabarcoding	Bait leaves metabarcoding	Isolation
<i>P. heveae</i> -like	5	100	100	92
<i>P. castaneae</i> -like	5	8	5	19
<i>P. cinnamomi</i>	7	11	20	18
<i>P. sp. cuyabensis</i>	9	0	0	3
<i>P. sp. neb_1543</i>	9	0	3	3
<i>P. sp. nov 10</i>	10	4	0	0

### Metabarcoding approach and comparison with baiting/isolation

The mean read number per sample was  $618 \pm 244$  for direct metabarcoding from soil samples, and  $805 \pm 146$  for leaf samples. The samples covering less than 72 reads were discarded. Indeed, a minimum depth of 72 reads was the compromise that allowed keeping most soil samples, while still describing all species of *Phytophthora* retrieved in the communities. Consequently, 52 soil sample libraries and 223 bait leaves libraries were used in the analysis. Both sets of data gave a mean proportion of 1% non-*Phytophthora* reads. *Phytophthora* reads from these samples were clustered in OTUs with a 99% similarity threshold, corresponding to five *Phytophthora* species only (Table 1). The R2 read length was around 400 nucleotides long in the metabarcoding libraries, while the Sanger ITS sequences previously discussed were c. 800 nucleotides long. As a consequence, ITS polymorphism of these shorter ITS fragments did not allow separation of the two *P. castaneae*-like groups, nor the two *P. heveae*-like groups, as could be done with the baiting/isolation results. Among the species retrieved, the *P. heveae* complex (*P. heveae* and/or the *P. heveae*-like groups) was present in every single sample and represented an average of 97% of the *Phytophthora* reads. Other detected species were *P. cinnamomi*, the *P. castaneae* complex, *Phytophthora. sp. neb\_1543* and a new putative species from clade 10, *Phytophthora sp. nov 10*.

The species communities obtained with the metabarcoding method differed from the one retrieved with baiting/isolation methods by one species only found by metabarcoding (*Phytophthora sp. nov 10*) and by one species only found by baiting/isolation (*P. sp. cuyabensis*). As a result, there was a significant difference in the qualitative composition of communities retrieved with each of the three methods previously described ( $p=0.001$ ), with the baiting / isolation method showing a lower frequency of the *P. heveae* complex and an increase in the rare species frequency (Table 1). There was no significant difference in the recovered *Phytophthora* communities between the bait leaf species (*Rhododendron*, Cassava, Avocado) ( $p=0.9$ ).

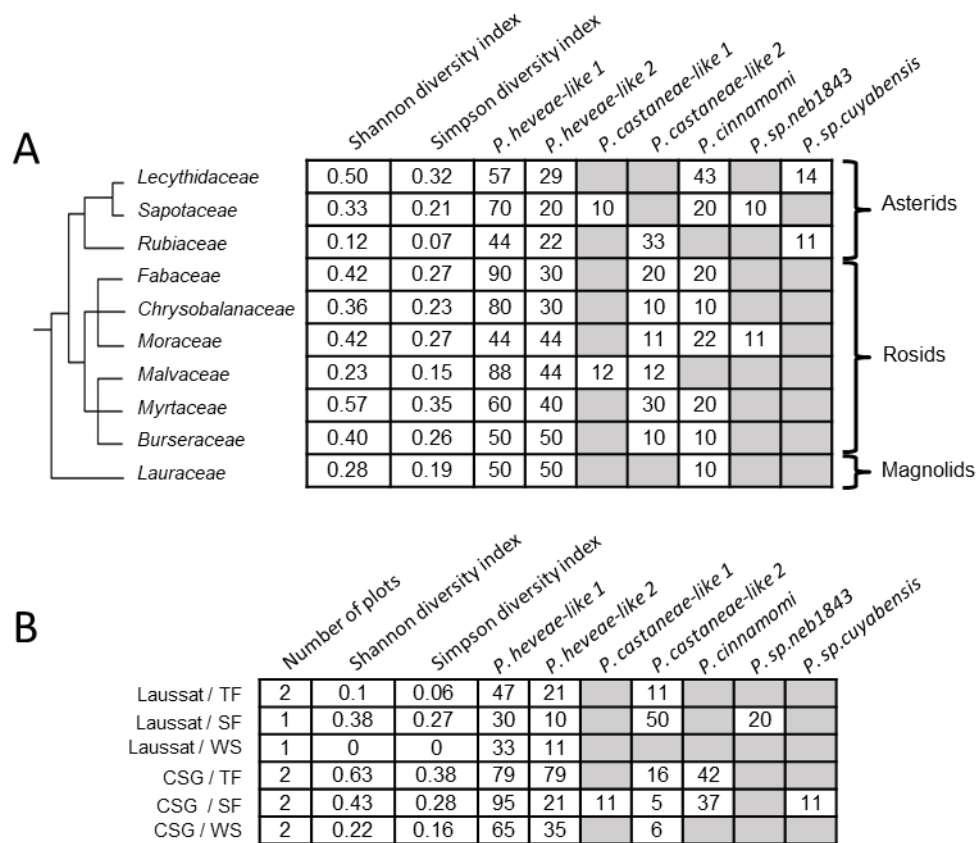


Figure 5. Proportion (%) of samples in which each of the *Phytophthora* taxa was retrieved by the baiting / isolation method, for (A) the ten sampled plant families and (B) the three environment types at both locations. One sample can contain several *Phytophthora* taxa. A: A significant difference was detected between families ( $p=0.004$ ) but not between higher clades ( $p=0.166$ ). B: There was a significant difference between the two locations Laussat and CSG ( $p=0.001$ ) and between environment types ( $p=0.017$ ). TF=Terra firma. SF=seasonally flooded. WS = white sand.

### Community structure analysis

The average number of species per plot was 1.4, with a dominance of the *P. heveae* complex as mentioned above. Taking into account the four new putative clade-5 species in the analysis of the *Phytophthora* communities described by the baiting and isolation method, we showed a strong location (CSG vs Laussat) effect ( $p=0.001$ ). Furthermore, a significant effect of forest environment (TF, SF and WS) ( $p=0.017$ ) was also detected (Fig. 5). The Shannon and Simpson diversity indices were higher in CSG than in Laussat and were higher in SF and TF environments compared to WS (Fig. 5). A significant effect of host plant family was detected ( $p=0.004$ ), with an interaction between the family and the location ( $p=0.029$ ). These results were congruent with analyses of Bray-Curtis similarity indices computed between communities (Table S2). Indeed, these similarity indices computed between the two locations (CSG vs Laussat) were low (0.31), while they were above 0.83 between environments. In addition, the Shannon and Simpson indices showed important variation between plant families (Fig. 5). However, at higher clade levels (Fig. 5A), no effect was detected between Asterids, Rosids and Magnoliids ( $p=0.166$ ).

To compare baiting/isolation results with metabarcoding results, we had to merge the two *P. heveae-like* species as well as the two *P. castaneae-like* species, as they could not be distinguished in the metabarcoding data. Combining the two approaches, no environment type effect was detected ( $p=0.165$ ). An effect of the host trees taxonomy was still detected at the



family level ( $p=0.017$ ), with an interaction with the method used ( $p=0.014$ ) (Fig. 6). A significant location effect between CSG and Laussat was detected with all methods ( $p=0.001$ ), with a strong interaction with the method used ( $p=0.002$ ).

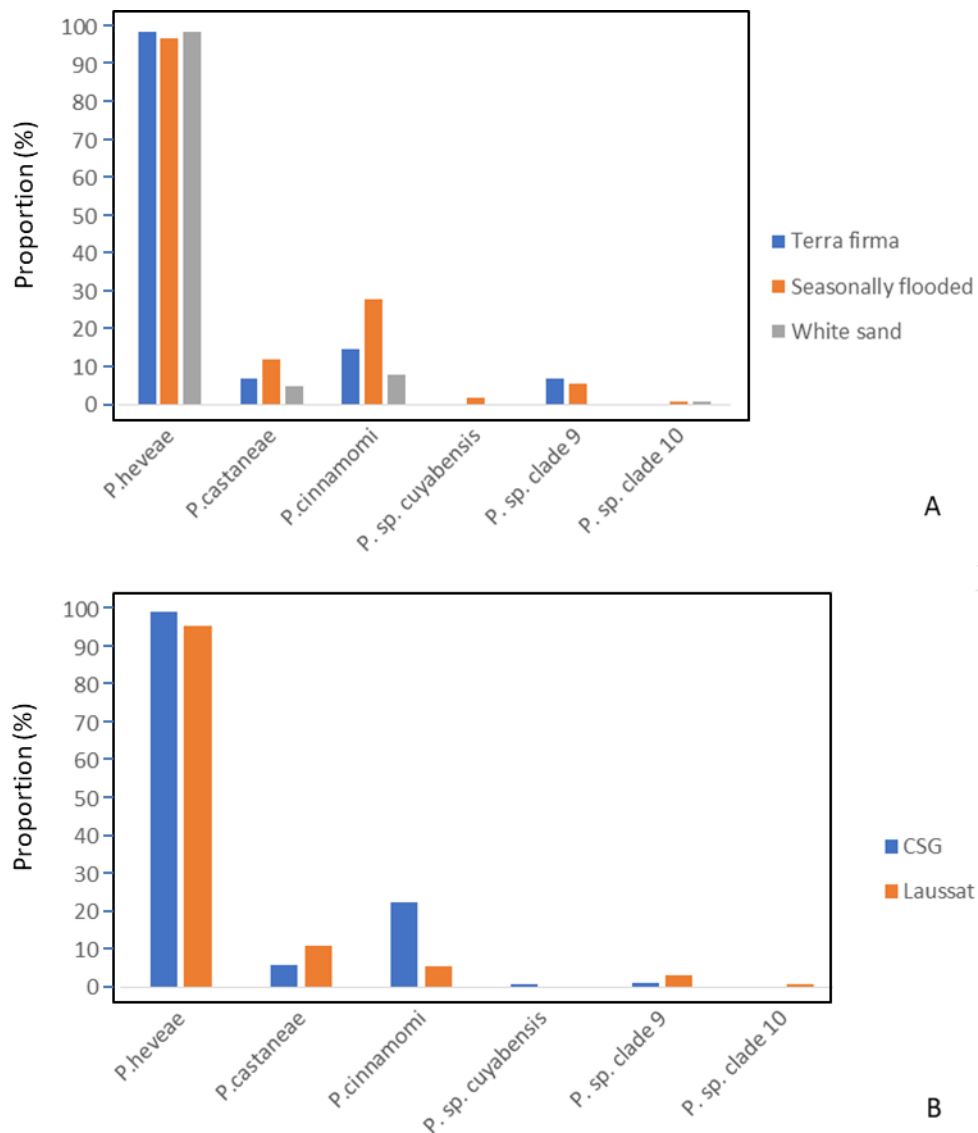


Figure 6. Proportion of samples containing the observed species of *Phytophthora*, grouped by environment type (A) and location (B). A: Significant difference between the different environment types was detected ( $p=0.003$ ), with seasonally flooded soils containing more rare species. B: Significant difference between the two locations was detected ( $p=0.001$ ).

## Discussion

Although the observed *Phytophthora* community structure depended on the approaches used, our results validated the benefits of HTS for large-scale surveys. Both HTS and baiting approaches uncovered a surprisingly low diversity of *Phytophthora* in the soils of these two French Guiana locations, with only six species retrieved and a strong dominance of the *P. heveae* complex in all samples. Moreover, no host specialist *Phytophthora* species were

identified. Despite this low diversity, the communities were to some degree structured by the plant hosts with a significant family effect, suggesting a certain degree of host preference.

### Comparison between baiting/isolation and metabarcoding

Baiting and molecular approaches have been described as complementary (Català *et al.*, 2015). Baiting techniques are time-consuming, and they may not detect low inoculum. Conversely, metabarcoding methods allow fast and massive pathogen detection. In our study, similar patterns were uncovered by the three approaches. Indeed, the three most abundant species were retrieved with all methods, and taxa of the *P. heveae* complex were largely dominant. However, the use of bait leaves followed by isolation increased the proportion of rare species compared to simple metabarcoding from soil or bait leaves. The isolate morphological observations in the initial step of the baiting/isolation method probably biased the selection toward rare species. Indeed, baiting could be advantageous to investigate communities from larger soil samples (200 ml versus about 0.5 ml). However, it did not enable us to detect additional diversity; rather, it proved to be a time-consuming additional step with little use in the metabarcoding approach. Our results therefore contrast with previous work in which metabarcoding methods were more efficient to describe *Phytophthora* community structure (Vannini *et al.* 2013; Català, 2017; Bose *et al.*, 2018; Khaliq *et al.*, 2018.). The suitability of the baiting/isolation method appears to depend largely on the *Phytophthora* community sampled, on sampling conditions, and on environmental factors. Indeed, baiting can be dependent on time of sampling, quantity and type of inoculum in the soil, species and size of the bait leaves, as well as soil parameters and origins. Timing of the sampling might for example explain the difference in *Phytophthora* recovery between the two sites we studied, which were sampled almost two months apart. One shortcoming of the metabarcoding method in this study is its inability to discriminate between the two putative *P. heveae*-like species in addition to the two putative *P. castaneae*-like species, because of the shorter length of the ITS fragment used. The baiting method does enable the isolation of living cultures for further characterisation; nevertheless, the less time-consuming metabarcoding from soil DNA was sufficient to obtain the bulk of the results in our study.

### Diversity of French Guiana communities of *Phytophthora*

The number of putative *Phytophthora* species observed in these French Guiana soils is lower than what is found in other forest ecosystems, even though our study sampled broadly across plant species from 10 different families representing the breadth of Angiosperm diversity, and across three very different environments. This result is surprising in one of the major biodiversity hotspots of the world and given the tendency of other organisms, including every studied fungal guild, to increase in diversity at lower latitudes (Tedersoo *et al.*, 2014; Willig *et al.*, 2003). Surveys in temperate, Mediterranean and tropical forests of several world regions have revealed a diversity of *Phytophthora* of 4-10 species per sampling location (Jung *et al.*, 2000; Balci *et al.*, 2007; Oh *et al.*, 2013; Scanu *et al.*, 2015; Sims *et al.*, 2015; Burgess *et al.*, 2017; Català *et al.*, 2017; Bose *et al.*, 2018; Redondo *et al.*, 2018; Mora-Sala *et al.* 2019). As an example, Burgess *et al.*, 2017, found a mean of 5 species per sampling location in a wide survey across Australia.

The low *Phytophthora* diversity we observed in French Guiana could be explained by several factors. First, we investigated two sites both located in the Guyana coastal range under similar climatic conditions of moist seasonal forests. Wallace, 2015, found a wider diversity of *Phytophthora* in Neotropical forests by investigating locations of differing hygrometry and altitude (plain rainforests vs. mountain cloud forests). In the same study, the author suggests that a greater diversity can be found in water streams. Also, in our study, we investigated soil adjacent to non-symptomatic juvenile trees irrespective of their proximity to conspecific adult trees, and host-specific *Phytophthora* species might not have accumulated on them if they were not adjacent to conspecific adults. Finally, even coastal forests in French Guiana receive

much less exposure to human disturbance than many other tropical and temperate forests. Many common *Phytophthora* species have been shown to be invasive and spread through worldwide trade (Santini *et al.*, 2013; Jung *et al.*, 2016), and human occupancy and history of trading could be decisive factors for predicting *Phytophthora* diversity. Indeed, *Phytophthora* diversity has been linked to the degree of anthropogenic disturbance (Dale, 2018; Redondo *et al.*, 2018), with the average number of *Phytophthora* species by location reaching nine in urban environments and only six in natural environments (Dale, 2018). In addition, Ruiz Gomez *et al.* (2019) find alpha-diversity of soil pathogenic Oomycetes was not correlated to the alpha-diversity of plants in Andalusian forests, but it would seem to be more related to the nature of the fungal community present in the soil. This calls for more standardized community descriptions in various parts of the world, especially tropical areas, which would allow future meta-analyses of *Phytophthora* diversity and composition, perhaps in link with other soil micro-organisms communities such as fungi.

Strikingly, the three dominant *Phytophthora* groups we observed in French Guiana, *P. heveae*, *P. castaneae* and *P. cinnamomi* are also the dominant *Phytophthora* groups in Hainan and Queensland soils (Zeng *et al.*, 2009; Burgess *et al.*, 2017). This suggests a similarity of the *Phytophthora* communities in tropical soils from South Asia to Australia and South America. For *P. heveae* and *P. castaneae*, this similarity could be spurious, as we found that they are likely closely related but different species. The low, but recurrent, nucleotide polymorphism of ITS sequences obtained from genotyped strains further suggests a potential species complex. The analysis of additional markers confirms that both putative species are heterogeneous and probably distinct from *P. heveae* and *P. castaneae*. The existence of isolates close but distinct from *P. heveae* and *P. castaneae* have already been reported by Zeng *et al.*, 2009, in Hainan. Clade 5 has therefore recently been revised (Weir *et al.*, 2015). However, part of the taxon of this *P. heveae* and *P. castaneae* species complex could be hybrid. Indeed, hybridization has been shown to be a common feature in natural populations of *Phytophthora* (Burgess *et al.*, 2015). Thus, the discrepancy between the nuclear and mitochondrial markers of the *P. heveae* and *P. castaneae* complexes could be an indicator of hybridization events. In addition, the ambiguity on topology of both phylogenetic trees could possibly be due to an introgressive hybridization of a *P. heveae*-like 2 mitochondrial genome in the two *P. castaneae*-like groups, as mitochondrial genes are inherited as a block during these events (Rubinoff & Holland 2005, Bachtrog *et al.* 2006).

*P. cinnamomi* is considered invasive in more than 70 countries (Erwin & Ribeiro, 1996), and the six isolates we tested showed 100% homology in the 6 tested loci. Furthermore, these six randomly selected isolates all belong to only one mating type, which does not suggest a native *P. cinnamomi* population (Erwin & Ribeiro, 1996). *P. cinnamomi* might therefore be an invasive species in French Guiana, although we cannot confirm this result without testing mating type of all the isolates. This opens the question whether the similarity of *Phytophthora* communities in the soils of tropical areas is due to cryptic speciation and/or to human-mediated invasions.

### Structure of the *Phytophthora* communities

The quantity of *Phytophthora* retrieved on bait leaves showed differences according to forest “environment x host family” interaction, with some families having especially high numbers of lesions on bait leaves in the TF environment. Owing to the large dominance of the *P. heveae* complex, this result probably refers mostly to *P. heveae*-like species. The fact that Oomycete mycelial growth is severely impaired in sandy soils compared to loamy soils (Lumsden, 1981) coupled with studies showing that many *Phytophthora* do not develop well in low pH soils (Jung *et al.*, 2000) could explain the low quantity in white sand soils for most families. However, it is more surprising that seasonally flooded soils were not more favourable, as high hygrometry is generally favourable to the multiplication of *Phytophthora* species (Erwin & Ribeiro, 1996), especially since higher *Phytophthora* diversity was observed in this environment. The sampling season is probably crucial: the soils were sampled in the two

studied locations during very wet periods and thus the situation was very favourable also in TF environment. Still, all sampling was achieved in a relatively short timeframe in each location, rendering unlikely any bias between environments induced by the climatic conditions under which samples were collected.

A host plant effect at the family level clearly shaped the composition of *Phytophthora* community. However, no *Phytophthora* species were confined to specific families, providing little evidence for host specialization in this forest. Instead, *Phytophthora* species displayed host preference rather than strict specificity. As illustrated by several studies (Arnold & Lutzoni 2007; Dickie, 2007; Ishida *et al.*, 2007; Tedersoo *et al.*, 2008; Schimann *et al.*, 2017), fungal communities may be influenced at different taxonomic levels of hosts. However, host taxonomic family effect has been shown to exist for two different fungal ecological groups: tropical epiphytic fungi (Kembel & Mueller, 2014) and temperate ectomycorrhizal fungi (Tedersoo *et al.*, 2013). Moreover, foliar plant pathogens do exhibit a phylogenetic signal in Neotropical forests (Gilbert & Webb, 2007).

However, because *Phytophthora* inoculum (motile zoospores) is known to be disseminated by stream and run-off from ground surfaces, sudden and heavy rainfall in tropical climates may be favourable to a rapid dissemination within both sites studied, therefore homogenising *Phytophthora* inoculum throughout local forests and blurring host taxonomy effects. Studying fine roots communities, instead of soil communities, could be an alternative method to detect host-specific *Phytophthora* species. Indeed, Khaliq *et al.*, 2018, retrieved 25 different *Phytophthora* species on fine roots in urban parks environment, compared to nearly half that amount (13) in soil.

The community we studied is dominated by large host spectrum species such as *P. heveae*-like, *P. cinnamomi* and *P. castaneae*-like. Although initially described in rubber tree plantations in south east Asia, *P. heveae* is mainly a known pathogen of fruits of avocado and cocoa (Turner, 1968; Ochoa-Ascensio, 2011) while *P. castaneae* has been described as infecting chestnut trees (Katsura, 1976). However, in natural environments, they are mostly described as present in soils without causing symptoms to neighbouring trees (Ko *et al.*, 2006; Zeng *et al.*, 2009; Jung *et al.*, 2017). The fact that generalists dominate fungal communities in Neotropical forests has already been described (Ferrer & Gilbert, 2003; Schimann *et al.*, 2017). Specialists may therefore be represented by very rare species.

Our study revealed that *Phytophthora* does not appear to be a promising agent of negative density dependence and Janzen-Connell effects in these French Guianan forests. However, only temporal demographic data, including long-term survival analysis of tropical seedlings, will ultimately arbitrate this result (Gilbert *et al.*, 2001). Alternatively, other groups of plant pathogens, such as fungi, could play the prominent role in these forests. We suggest that a general survey of plant pathogens across phyla and in different Neo-tropical forests, would represent an ambitious yet extremely meritorious initiative.

## Experimental Procedures

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### Sampling and location description

Soil was sampled in plots located in two regions, in coastal French Guiana, 137 km apart from each other (Fig. 1), Laussat region (5.48° N, 53.60° W, 4 plots) in mid-April 2015 and in the “Centre Spatial Guyanais” (abbreviated as CSG, 5.22° N, 52.84° W, 6 plots) in February and March 2015. These plots belonged to the three main forest environments in French Guyana: white sand (WS, 1 plot in Laussat and 2 in CSG), terra firme (TF, 2 plots in Laussat and 2 in CSG), and seasonally flooded forests (SF, 1 plot in Laussat and 2 in CSG). Each plot was 10.000 m<sup>2</sup>. These three types of environments are described in Randall, 2017. The sample locations were relatively undisturbed. In each plot, 50 to 60 saplings (2-3 m in height) belonging to ten taxonomic families were selected for sampling (two to twelve individuals per family). The sampled saplings did not present any visible symptoms of disease. The host families were



*Burseraceae*, *Chrysobalanaceae*, *Fabaceae*, *Lauraceae*, *Lecythydaceae*, *Malvaceae*, *Moraceae*, *Myrtaceae*, *Rubiaceae* and *Sapotaceae*. Focal families were selected for their relatively large distribution across Amazonian forests within the targeted environment types and the breadth of Angiosperm diversity they represent, as they belong to three major Angiosperms clades: the Asterids, Rosids and Magnoliids (see Fig. 5 for their repartition into these clades). Three soil samples 5-10 cm deep were collected at 15-40 cm from the stem collar after removing the litter at the base of each sapling. In each plot, soil samples collected from saplings belonging to the same family were then pooled. As all ten tree families could not be found in all ten plots, the number of pooled soil samples was 93.

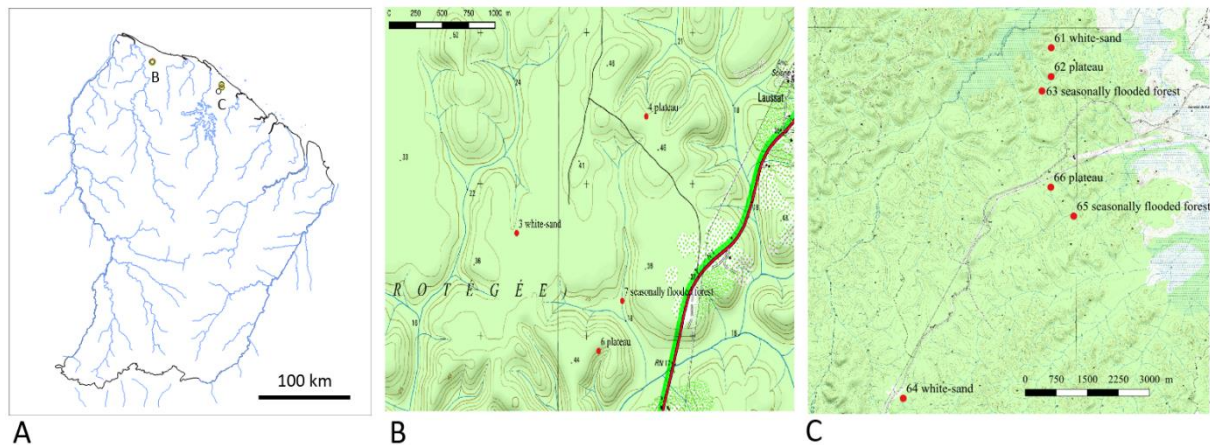


Figure 1. Sampling sites of the French Guiana experiment. **A**: Map of French Guiana showing the locations sampled for this study (B=Laussat ; C=CSG). **B** and **C** : Maps of Laussat (left) and CSG (right) showing the sampled plots as red dots. "Plateau" = Terra firma environment.

### Baiting and isolation

For each soil sample, two 1 l plastic containers filled with 200 mL of homogenised soil submerged by 500 mL of distilled water were prepared for baiting. In one container, three leaves of *Rhododendron* 'Cunningham's White' were placed floating upon the water, while the other received one leaf each of Cassava (*Manihot esculenta*) or Avocado (*Persea americana*). Care was taken to select flat leaves so to have homogeneous contact between the bait and the water. After three days of incubation at 20-22 °C, bait leaves were collected and weighed. The number of lesions was quantified on *Rhododendron* leaves only, because lesions became immediately too large and confluent on Cassava and Avocado leaves, preventing accurate quantification of infections. For each combination of bait leaf x soil sample, up to 10 lesions were cut and placed in Petri dishes containing a *Phytophthora* selective medium (V8 juice agar complemented with 10 mg L<sup>-1</sup> pimarinic acid, 250 mg L<sup>-1</sup> ampicilline, 10 mg L<sup>-1</sup> rifampicine, 67 mg L<sup>-1</sup> hymexazol, 15 mg L<sup>-1</sup> benomyl). After 48 hours incubation in the dark at 25°C, mycelium was transferred onto new Petri dishes containing the same medium previously described for up to 5 putative *Phytophthora* distinct colonies per soil sample per bait leaf combination, preferentially selecting isolates with different morphology. After an additional 48-72 hours period of incubation, the mycelium was collected for DNA extraction, using the DNeasy 96 Plant kit (Qiagen) according to the manufacturer's instruction. PCR products were amplified from this genomic DNA using the ITS6-ITS4 primer pair targeting the ITS region, using the PCR protocol from Cooke *et al.* (2000), and the YPh1F-YPh2R primer pair targeting *RAS-Ypt* gene, using the PCR protocol from Weir *et al.* (2015). Finally, 366 *Phytophthora* isolates were sequenced in both directions with the Sanger method. The different steps of the sampling, baiting, isolation and molecular identification process are reported in Fig. 2.

Additionally, as *P. cinnamomi* is a very important invasive species found worldwide, usually present as only one of the mating types (Erwin & Ribeiro, 1996), the *P. cinnamomi* isolates retrieved from our samples were further characterized by determining their mating type. They were paired with A1 and A2 mating types of *P. cinnamomi* isolates from a reference collection, using the method described by Zentmyer (1979).

### Phylogenetic analysis of isolates

As clade 5 isolates (phylogenetically close to *P. castaneae* and *P. heveae*) were the most frequent in our study, twenty of these isolates were selected for further investigation of their genetic diversity. We selected these isolates to maximize their ITS sequence diversity. We then sequenced for each one the *RAS-YPT* gene and three additional genetic markers, Cox, Enolase and NADH genes (see (Weir *et al.* 2015) for the method used). Multiple sequence alignments for each gene were made using Seaview 4.0 (Gouy *et al.* 2010). Bayesian inference (BI) was used to reconstruct the phylogenies using MrBayes v. 3.2.2 (Ronquist *et al.* 2012) for the two categories of sequences: first, a concatenation of the mitochondrial markers (Cox and NADH), and secondly another concatenation of the nuclear markers (ITS, *RAS-Ypt* and Enolase). Nuclear genes showed congruent phylogenies, and so did mitochondrial genes. However, the phylogenies were not congruent between nuclear and mitochondrial genes, so we did not create a tree based on all five genes. Sequences of clade-5 *Phytophthora* from Weir *et al.* were used as references, and in both trees, *P. multivora* was used as outgroup. Two *P. cinnamomi* isolates (clade-7) from Laussat and two from CSG were also randomly selected and sequenced for the same markers.

### Metabarcoding and Illumina Miseq sequencing

As detailed in Figure 1, two sets of DNA samples were created by extracting DNA from either (1) the 93 soil samples or (2) the 264 bait leaves corresponding to the three different bait species (Rhododendron, Avocado and Cassava, i.e. 93 x 3 bait leaves, minus 15 leaves showing no infection and discarded for further analysis). Soil samples were air-dried and sieved (2 mm mesh size). DNA extraction was performed using MP Biomedical Fast DNA™ SPIN Kit for soil on 500 mg of dried soil according to the manufacturer's instructions. Bait leaves were dried and ground using liquid nitrogen, and DNA of 500 mg leaf samples was extracted using DNeasy 96 plant kits.

PCR products were amplified from these 357 DNA samples using a nested PCR approach: the primer pair oom18SITS7 was used for the first round and the primer pair 18Ph2F/5.8-1R for the second round. The PCR protocol and the primer sequences are detailed in (Legeay *et al.*, 2019). In order to detect potential contaminants, fifteen control samples, consisting of pure distilled water added to the PCR mix, were included in the same PCR plates. The multiplexing and the Illumina MiSeq sequencing (half-run) were done by the GeT-PlaGe platform of Toulouse (<http://get.genotoul.fr>).

### Bioinformatics analysis

Sequences were sorted and trimmed according to their quality using Mothur (Schloss *et al.*, 2009) and Usearch (Edgar, 2010). Unfortunately, the quality of R1 and R2 reads was medium and the depth of sequencing was very heterogeneous between the samples. The R2 reads were finally retained because they covered the ITS1 nucleotide polymorphism region. The reads were then dereplicated to remove duplicated sequences and sorted by decreasing abundance, and the singletons were discarded using the `sortbysize` command of Usearch. Sequences were then clustered together to make consensus mOTUs with 99% similarity threshold, using `cluster_otus` command of Usearch. Generated mOTUs were taxonomically assigned using the BLAST algorithm against a database extracted from the NCBI database, containing all sequences assigned to Oomycetes with the "ITS" and "Internal Transcribed



Spacer” keywords in their definitions. OTUs assigned to the same species were merged according to their taxonomic identity at the species level. Given the heterogeneity of the sequencing depth, we defined a compromise between the maximum number of samples and the minimum number of reads in each sample to cover the optimal diversity of *Phytophthora*. In addition, because the Sanger sampling has much less depth than metabarcoding, and because *Phytophthora* diversity is known to be relatively low, we chose to submit the metabarcoding library to processes of decreasing random subsampling. As illustrated in Figure S2, we could have kept only a hundred samples (n=120) with a sequencing depth > 1000 reads (Fig. S2, insert D). However, compared to Sanger sequencing data, this depth of sequencing does not allow the identification of additional *Phytophthora* species. Consecutively, we chose to normalize the samples by subsampling 72 reads because this was the number of reads which maximized the quantity of rare species retrieved in all samples (Fig. S2, insert B). Indeed, this *Phytophthora* diversity was identical to that observed with 1000 reads per sample from 120 samples. Due to quantitative distortions using these amplification and sequencing methods (Legeay *et al.*, 2019), sequencing results were only analyzed qualitatively (i.e. presence or absence of species). ITS sequences were deposited in GenBank under the accession numbers MH938206 - MH938223. The sequences of RAS-Ypt, Enolase, Cox and NADH regions are available in the DRYAD database (<http://datadryad.org/>). DRYAD entry doi:XXXXX. The Miseq raw data were deposited in the Sequence Read Archive (<http://www.ncbi.nlm.nih.gov/sra>) as SUB5066167 (temporary access number).

## Data analysis

The efficiency of isolation from baits was studied by analysing the likelihood that a retrieved isolate was a *Phytophthora* species using a mixed logistic regression with a logit link, taking into account the bait leaf species, the environment, the host plant family and the location (glmer function of R). The plot was added as a random factor.

The number of lesions per bait leaves was analysed as a surrogate for the quantity of *Phytophthora* inoculum in soil. Effects of the different factors, host plant family, environment and location, on the number of lesions per bait leaf weight were tested with a generalized linear model using a Poisson distribution and a log link, with the medium leaf weight as offset (glmer function of R). The plot was added as a random factor.

Differences in the composition of *Phytophthora* communities between the different methods, environments, locations and host families were tested by Permutational Multivariate Analysis of Variance (PERMANOVA) using the *adonis* function (number of permutations = 500) in the vegan package of R (Oksanen *et al.*, 2016). As all information on *Phytophthora* species composition could not be used in the metabarcoding data, in particular on *P. heveae* and *P. castaneae* complexes, a second analysis was performed using only the data from the baiting/isolation study. To further characterize the diversity of communities in the different environment / families, we computed the Shannon diversity index for each host family and each “environment x location” level using *diversity* function from the vegan package.

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## References

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- Alexander HM (2010) Disease in Natural Plant Populations, Communities, and Ecosystems: Insights into Ecological and Evolutionary Processes. *Plant Disease* 94:492–503.
- Antonelli A, Sanmartin I (2011) Why are there so many plant species in the Neotropics? *Taxon* 60:403–414
- Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* 88:541–549.
- Bachtrog D, Thornton K, Clark A, Andolfatto P (2006) Extensive introgression of mitochondrial DNA relatives to nuclear genes in the *Drosophila Yakuba* species group. *Evolution* 60: 292–302
- Bagchi R, Gallery RE, Gripenberg S, et al (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* 506:85–88.
- Balci Y, Balci S, Eggers J, et al (2007) *Phytophthora* spp. Associated with Forest Soils in Eastern and North-Central U.S. Oak Ecosystems. *Plant Disease* 91:705–710.
- Bever JD, Mangan SA, Alexander HM (2015) Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics* 46:305–325.
- Bose T, Wingfield MJ, Roux J, Vivas M, Burgess TI (2018) Community composition and distribution of *Phytophthora* species across adjacent native and non-native forests of South Africa. *Fungal Ecology* 36: 17–25.
- Burgess, TI (2015). Molecular Characterization of Natural Hybrids formed between five Related Indigenous Clade 6 *Phytophthora* Species. Édité par Mark Gijzen. *Plos One* 10: e0134225.
- Burgess TI, White D, McDougall KM, et al (2017) Distribution and diversity of *Phytophthora* across Australia. *Pacific Conservation Biology* 23:150.
- Català S (2017) Development of DNA massive sequencing techniques and RealTime PCR for the detection, identification and quantitation of *Phytophthora* spp. in environmental samples. PhD Thesis, Universitat Politècnica de València.
- Català S, Berbegal M, Pérez-Sierra A, Abad-Campos P (2017) Metabarcoding and development of new real-time specific assays reveal *Phytophthora* species diversity in holm oak forests in eastern Spain. *Plant Pathology* 66:115–123.
- Català S, Pérez-Sierra A, Abad-Campos P (2015) The Use of Genus-Specific Amplicon Pyrosequencing to Assess *Phytophthora* Species Diversity Using eDNA from Soil and Water in Northern Spain. *PLOS ONE* 10:e0119311.
- Coince A, Caël O, Bach C, et al (2013) Below-ground fine-scale distribution and soil versus fine root detection of fungal and soil oomycete communities in a French beech forest. *Fungal Ecology* 6:223–235.
- Cooke DEL, Drenth A, Duncan JM, et al (2000) A Molecular Phylogeny of *Phytophthora* and Related Oomycetes. *Fungal Genetics and Biology* 30:17–32.
- Dale A (2018) Using genomic data to understand anthropogenic influences on Oomycete and *Phytophthora* communities, and the evolution of an alien invasive species responsible for sudden oak death, *Phytophthora ramorum*. PhD Thesis, University of British Columbia.
- Dickie IA (2007) Host preference, niches and fungal diversity. *New Phytologist* 174:230–233.
- Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461.
- Erwin DC, Ribeiro OK (1996) *Phytophthora* Diseases Worldwide. American Phytopathological Society Press, St Paul, MN, pp 269–280.
- Ferrer A, Gilbert GS (2003) Effect of tree host species on fungal community composition in a tropical rain forest in Panama: Fungal community composition in Panama. *Diversity and Distributions* 9:455–468.
- Gilbert GS, Harms KE, Hamill DN, Hubbell SP (2001). Effects of seedling size, El Nino drought, seedling density, and distance to nearest conspecific adult on 6-year survival of *Ocotea whitei* seedlings in Panama. *Oecologia*, 127: 509-516.
- Gilbert GS, Webb CO (2007) Phylogenetic signal in plant pathogen-host range. *Proceedings of the National Academy of Sciences* 104:4979–4983.

- Gouy M, Guindon S, Gascuel O (2010) SeaView Version 4: A Multiplatform Graphical User Interface for Sequence Alignment and Phylogenetic Tree Building. *Molecular Biology and Evolution* 27:221–224.
- Hansen EM (2015) *Phytophthora* Species Emerging as Pathogens of Forest Trees. *Current Forestry Reports* 1:16–24.
- Ishida TA, Nara K, Hogetsu T (2007) Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. *New Phytologist* 174:430–440.
- Jablonski D, Roy K, Valentine JW (2006) Out of the Tropics: Evolutionary Dynamics of the Latitudinal Diversity Gradient. *Science* 314:102–106.
- Janzen DH (1970) Herbivores and the Number of Tree Species in Tropical Forests. *The American Naturalist* 104:501–528.
- John R, Dalling JW, Harms KE, et al (2007) Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences* 104:864–869.
- Jung T, Chan, TT, Bakonyi J, Seres, D, Pérez-Sierra A, Yang X, Hong C, Scanu B, Fu CH, Hsueh KL, Maia C, Abad-Campos P, León M, Horta-Jung M, 2017. Diversity of *Phytophthora* species in natural ecosystems of Taiwan and association with disease symptoms. *Plant Pathology* 66, 194–211.
- Jung T, Blaschke H, Osswald W (2000) Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology* 49:706–718.
- Jung T, Orlikowski L, Henricot B, et al (2016) Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. *Forest Pathology* 46:134–163.
- Katsura K (1976) Two new species of *Phytophthora* causing damping-off of cucumber and trunk rot of chestnut. *Transaction of Mycological Society of Japan* 17 :238-242
- Keesing F, Belden LK, Daszak P, et al (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468:647–652.
- Kembel SW, Mueller RC (2014) Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany* 92:303–311.
- Khaliq, I., Hardy, G.E.S.J., White, D., Burgess, T.I., 2018. eDNA from roots: a robust tool for determining *Phytophthora* communities in natural ecosystems. *FEMS Microbiology Ecology* 94.
- Ko W-H, Wang SY, Ann PJ (2006) The possible origin and relation of *Phytophthora katsurae* and *P. heveae*, discovered in a protected natural forest in Taiwan. *Botanical Studies* 47:5
- Legeay J, Husson C, Cordier T, Vacher C, Marçais B, Buée M (2019) Comparison and validation of Oomycetes metabarcoding primers for *Phytophthora* high throughput sequencing. *Journal of Plant Pathology* 101: 743–748.
- Lumsden RD (1981) A Nylon Fabric Technique for Studying the Ecology of *Pythium aphanidermatum* and Other Fungi in Soil. *Phytopathology* 71:282.
- Mangan SA, Schnitzer SA, Herre EA, Mack KM, Valencia MC, Sanchez EI, Bever JD (2010) Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, 466: 752.
- Mills KE, Bever JD (1998) Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology* 79:1595–1601.
- Mora-Sala B, Gramaje D, Abad-Campos P, Berbegal M (2019) Diversity of *Phytophthora* Species Associated with *Quercus ilex* L. in Three Spanish Regions Evaluated by NGS. *Forests* 10: 979.
- Ochoa-Ascencio S, (2011) *Phytophthora heveae* causing basal rot of avocado fruit in Mexico, Proceedings VII World Avocado Congress 2011 (Actas VII Congreso Mundial del Aguacate 2011). Cairns, Australia. 5 – 9 September 2011
- Oh E, Gryzenhout M, Wingfield BD, et al (2013) Surveys of soil and water reveal a goldmine of *Phytophthora* diversity in South African natural ecosystems. *IMA Fungus* 4:123–131.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P R, O'hara R B, Wagner H (2016) vegan: community ecology package. R package version 2.0–7. 2013. URL <http://CRAN.R-project.org/package=vegan>.
- Philipson CD, Saner P, Marthews TR, et al (2012) Light-based Regeneration Niches: Evidence from 21 Dipterocarp Species using Size-specific RGRs. *Biotropica* 44:627–636.
- Randall WM (2017). A comparison of the forest soils in the Peruvian Amazon: Terra firme, palm, white sand and igap. *J Soil Sci Environ Manage.* 8(7):130-4.

- Redondo MA, Boberg J, Stenlid J, Oliva J (2018) Functional traits associated with the establishment of introduced *Phytophthora* spp. in Swedish forests. *Journal of Applied Ecology* 55:1538–1552.
- Ronquist F, Teslenko M, van der Mark P, et al (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology* 61:539–542.
- Rubinoff D, Holland BS (2005). Between Two Extremes: Mitochondrial DNA is neither the Panacea nor the Nemesis of Phylogenetic and Taxonomic Inference. *Systematic Biology* 54: 952–961.
- Ruiz Gómez FJ, Navarro-Cerrillo RM, Pérez-de-Luque A, Oßwald W, Vannini A, Morales-Rodríguez C (2019) Assessment of functional and structural changes of soil fungal and oomycete communities in holm oak declined dehesas through metabarcoding analysis. *Sci Rep* 9: 5315.
- Santini A, Ghelardini L, De Pace C, Desprez-Loustau M-L, Capretti P, Chandelier A, Cech T, Chira D, Diamandis S, Gaitniekis T, Hantula J, Holdenrieder O, Jankovsky L, Jung T, Jurc D, Kirisits T, Kunca A, Lygis V, Malecka M, Marçais B, Schmitz S, Schumacher J, Solheim H, Solla A, Szabò, I., Tsopelas, P., Vannini, A., Vettraiño, A- M., Webber, J., Woodward, S., Stenlid J (2013) Biogeographical patterns and determinants of invasion by forest pathogens in Europe. *New Phytologist* 197:238–250
- Scanu B, Linaldeddu BT, Deidda A, Jung T (2015) Diversity of *Phytophthora* Species from Declining Mediterranean Maquis Vegetation, including Two New Species, *Phytophthora crassamura* and *P. ornamentata* sp. nov. *PLoS ONE* 10, e0143234.
- Schimann H, Bach C, Lengelle J, et al (2017) Diversity and Structure of Fungal Communities in Neotropical Rainforest Soils: The Effect of Host Recurrence. *Microbial Ecology* 73:310–320.
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* 75, 7537–7541.
- Schupp EW (1992) The Janzen-Connell Model for Tropical Tree Diversity: Population Implications and the Importance of Spatial Scale. *The American Naturalist* 140:526–530.
- Scibetta S, Schena L, Chimento A, Cacciola SO, Cooke DE (2012). A molecular method to assess *Phytophthora* diversity in environmental samples. *Journal of microbiological methods* 88: 356-368.
- Scott P, Bader MK.-F, Burgess T, Hardy G, Williams N (2019) Global biogeography and invasion risk of the plant pathogen genus *Phytophthora*. *Environmental Science & Policy* 101: 175–182.
- Shokralla S, Spall JL, Gibson JF, Hajibabaei M (2012) Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology* 21:1794–1805.
- Sims LL, Sutton W, Reeser P, Hansen EM (2015) The *Phytophthora* species assemblage and diversity in riparian alder ecosystems of western Oregon, USA. *Mycologia* 107:889–902.
- Tedersoo L, Bahram M, Polme S, et al (2014) Global diversity and geography of soil fungi. *Science* 346:1256688–1256688.
- Tedersoo L, Jairus T, Horton BM, et al (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist* 180:479–490.
- Tedersoo L, Mett M, Ishida TA, Bahram M (2013) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytologist*
- ter Steege H, Pitman NCA, Sabatier D, et al (2013) Hyperdominance in the Amazonian Tree Flora. *Science* 342:1243092–1243092.
- Terborgh J (2012) Enemies Maintain Hyperdiverse Tropical Forests. *The American Naturalist* 179:303–314.
- Tsao PH, Ocaña G (1969) Selective Isolation of Species of *Phytophthora* from Natural Soils on an Improved Antibiotic Medium. *Nature* 223:636–638.
- Turner PD. (1968). Pod rot of cocoa in Malaysia caused by *Phytophthora heveae*. *FAO Plant Protection Bulletin* 16: 33-34.
- Vannini A, Bruni N, Tomassini A, et al (2013) Pyrosequencing of environmental soil samples reveals biodiversity of the *Phytophthora* resident community in chestnut forests. *FEMS Microbiology Ecology* 85:433–442.
- Vettraiño AM, Bonants P, Tomassini A, et al (2012) Pyrosequencing as a tool for the detection of *Phytophthora* species: error rate and risk of false Molecular Operational Taxonomic Units. *Letters in Applied Microbiology* 55:390–396.
- Wallace SF (2015) Diversity of *Phytophthora* Species in Costa Rica's Tropical Forest. PhD Thesis, Digital Repository at the University of Maryland. (<https://doi.org/10.13016/m2fw79>)

- Weir BS, Paderes EP, Anand N, et al (2015) A taxonomic revision of *Phytophthora* Clade 5 including two new species, *Phytophthora agathidicida* and *P. cocois*. *Phytotaxa* 205:21.
- Willig MR, Kaufman DM, Stevens RD (2003) Latitudinal Gradients of Biodiversity: Pattern, Process, Scale, and Synthesis. *Annual Review of Ecology, Evolution, and Systematics* 34:273–309.
- Wright JS (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* 130:1–14.
- Zeng H, Ho H, Zheng F-C (2009) A Survey of *Phytophthora* Species on Hainan Island of South China. *Journal of Phytopathology* 157:33–39.
- Zentmyer GA (1979). Stimulation of sexual reproduction in the A2 mating type of *Phytophthora cinnamomi* by a substance in avocado roots. *Phytopathology*, 69: 1129-1131.