Invasion biology of forest *Phytophthora* species in Sweden

Pathways, traits, climate, and host adaptation

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Cover: Alders at the shore of Kävlinge river at Gårdstånga
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Invasion biology of forest *Phytophthora* species in Sweden. Pathways, traits, climate, and host adaptation.

Abstract

New introductions of *Phytophthora* species pose a threat to forest ecosystems globally. This thesis aims to increase our understanding of the invasion process of forest *Phytophthora* species, and of the long term consequences of these invasions on host populations. *Phytophthora* species were obtained from nurseries, rivers, and forests, by isolation and by a newly developed metabarcoding approach to investigate the factors involved in *Phytophthora* introduction, establishment and spread. *In vitro* inoculations were performed on progenies of alder trees from invaded and uninvaded sites to study whether alder populations have the potential to adapt to species of the *P. alni* complex.

Five *Phytophthora* species were widespread in Sweden, namely *P. plurivora*, *P. cambivora*, *P. cactorum*, *P.* x *alni,* and *P. uniformis* and they were considered invasive. The occurrence of three of these invasive *Phytophthora* species and the alpha diversity of *Phytophthora* communities were higher in urban settlements than in natural forests, pointing at human activities as pathways during invasion. Both the distribution of single *Phytophthora* species, and the diversity of *Phytophthora* communities were associated with climatic factors. The cold sensitive *P.* x *alni* was restricted to the southern areas of Sweden with milder winters, whereas the more cold tolerant *P. uniformis* was found across the studied region. The diversity of communities containing species that develop most of their life cycle in soil was associated with total annual precipitation, whereas the diversity of communities containing species mostly developing their life cycle in water was associated with mean annual temperature. The functional diversity of communities revealed a convergence of traits in areas with low temperature and precipitation, where species able to create survival structures and displaying low cardinal temperatures dominated the communities. Adverse climatic conditions seemed to act as an environmental filtering on 20% of the terrestrial *Phytophthora* communities, although this effect was only of 3% for aquatic communities. *In vitro* inoculations on progenies of alders invaded by *P. uniformis* revealed a lower susceptibility to the pathogen than uninvaded populations, pointing to an effect of natural selection. By contrast, no signs of natural selection were observed in *P.* x *alni* invaded populations. The broad sense heritability of resistance against *P. uniformis* was higher than against *P.* x *alni*, suggesting that low genetic variation in resistance might slow natural selection, and therefore adaptation.

Keywords: adaptation, climate change, dispersal pathways, forest pathogens, functional traits, human activities, invasion biology.

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Invasionbiologi av Phytophthora-arter i sverige. Spridningsvägar, egenskaper, klimat och värdanpassning.

Abstract

Nya introduktioner av *Phytophthora*-arter utgör ett hot mot skogsekosystem världen över. Denna avhandling syftar till att öka vår kunskap om invasioner av *Phytophthora*-arter i skog och den långsiktiga påverkan på trädpopulationer. För att undersöka de faktorer som påverkar introduktionen, etableringen och spridningen av *Phytophthora*-arter så studerades deras förekomst i plantskolor, vattendrag och skogsbestånd, dels genom isolering och dels med hjälp av en nyutvecklad metodik för så kallad metabarcoding. För att studera om populationen av al har potential att anpassa sig till patogener tillhörande *P. alni*-komplexet så utfördes inokuleringar *in vitro* på avkommor av träd från alpopulationer som invaderats och från populationer som inte tidigare exponerats för patogenerna. Fem *Phytophthora*-arter hittades utbredda i Sverige, nämligen *P. plurivora*, *P. cambivora*, *P. cactorum*, *P. x alni* och *P. uniformis* och samtliga kunde klassas som invasiva arter. Förekomsten av tre av dessa arter samt alfa-diversiteten av de studerade *Phytophthora*-samhällena var högre i urbana miljöer jämfört med i naturliga skogar. Detta indikerar att antropogena aktiviteter utgör viktiga spridningsvägar under invasionen. Klimatfaktorer påverkade både enskilda arters utbredning och *Phytophthora*-samhällenas artsammansättning. Den köldkänsliga *P. x alni* var begränsad till de södra delarna av Sverige med mildare vintrar, medan den mer köldtoleranta *P. uniformis* hittades utbredd över hela det studerade området. På samhällsnivå var alfa-diversiteten av arter som till större del utvecklar sin livscykel i jord associerad till den genomsnittliga årsnederbörden, medan alfa-diversiteten av arter som mestadels utvecklar sin livscykel i vatten var associerad med den genomsnittliga årstemperaturen. Den funktionella diversiteten hos *Phytophthora*-samhällena visade en konvergens av egenskaper i områden med låga temperaturer och låg nederbörd. Här dominerade arter som kan skapa överlevnadsstrukturer och arter med låg karinaltemperatur. Ogynnsamma klimatförhållanden verkar påverka runt 20% av de jordlevande *Phytophthora*-samhällena, en effekt kallad miljöfiltrering. Denna effekt sågs endast hos 3% av de vattenlevande samhällena. Inokuleringarna av al-plantor visade att alpopulationer som tidigare invaderats av *P. uniformis* uppvisade en större motståndskraft mot patogenen än populationer som tidigare inte exponerats vilket tyder på att det pågår ett naturligt urval för motståndskraft mot patogenen. Däremot återfanns inte detta mönster hos al-populationer som invaderats av *P. x alni*. Vidare analyser visade att resistens hos al mot *P. uniformis* har en större generell heritabilitet än resistens mot *P. x alni.*

Keywords: anpassning, klimatförändringar, spridningsvägar, skogspatogener, funktionella egenskaper, mänskliga aktiviteter, invasionbiologi.

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Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Redondo, M.A.,** Boberg, J., Stenlid, J. & Oliva, J. (2017). Functional traits associated with the establishment of introduced *Phytophthora* spp. in northern forests. *Journal of Applied Ecology*. Doi: 10.1111/1365-2664.13068.

II **Redondo, M.A.,** Boberg, J., Olsson, C.H.B. & Oliva, J. (2015). Winter conditions correlate with *Phytophthora alni* subspecies distribution in Southern Sweden. *Phytopathology* (105), 1191–1197.

III **Redondo, M.A.,** Boberg, J., Stenlid, J. & Oliva, J. Contrasting distribution patterns between aquatic and terrestrial *Phytophthora* species along a climatic gradient are linked to functional traits. (Manuscript).

IV **Redondo, M.A.,** Stenlid, J. & Oliva, J. Differential genetic resistance in host explains the strength of natural selection against closely related invasive forest pathogens. (Manuscript).

Additional publications that are not part of this thesis:

Redondo, M.A., Thomsen, I.M., Oliva, J. (2016). First report of *Phytophthora uniformis* and *P. plurivora* causing stem cankers on *Alnus glutinosa* in Denmark. Plant Disease, (101), 512.

Redondo, M.A. & Oliva, J. (2016). First report of *Phytophthora pseudosyringae* causing stem canker on *Fagus sylvatica* in Spain. *Plant Disease*, (100), 1508.

Redondo, M.A., Pérez-Sierra, A., Abad-Campos, P., Torres, L., Solla, A., Reig-Armiñana, J. & García-Breijo, F. (2015) Histology of *Quercus ilex* roots during infection by *Phytophthora cinnamomi*. *Trees*, (29), 1943–1957.

Redondo, M.A., Boberg, J., Stenlid, J. & Oliva, J. (2015). First report of *Phytophthora pseudosyringae* causing basal cankers on horse chestnut in Sweden. *Plant Disease*, (100), 1024.

The contribution of Miguel Ángel Redondo to the papers included in this thesis was as follows:

I Performed the survey, analysed all the data and was main responsible for the writing and correspondence with the journal.

II Performed the survey, contributed to the analysis of data and was main responsible for the writing and correspondence with the journal.

III Performed the survey, analysed all the data and was main responsible for the writing.

IV Contributed to the design of the experiment, performed the survey, carried out the experiment, analysed all the data and was main responsible for the writing.

Abbreviations

HTS	High throughput sequencing
ITS	Internal Transcribed Spacer
OTU	Operational taxonomic unit
PacBio	Pacific BioSciences

1 Background

During the last decades, living organisms have increasingly spread beyond their native habitats (Vitousek et al., 1997; Ricciardi, 2007; Hulme, 2009; Fisher et al., 2012). International trade and human movements have contributed to the introduction of forest pathogens in new areas, triggering novel ecological interactions (Fisher et al., 2012; Liebhold et al., 2012; Santini et al., 2013). Introductions of plant pathogenic *Phytophthora* species are being reported around the globe (Brasier & Webber, 2010; Burgess et al., 2016; Jung et al., 2016), thus calling for studies addressing these invasions. This thesis aims at studying the invasion process of forest *Phytophthora* species and the long-term impact of these invasions on host populations.

1.1 The genus *Phytophthora*

1.1.1 *Phytophthora* can pose a threat to forest health

The name *Phytophthora* is derived from the Greek and it literally means plant (*phyto*-) destroyer (*-phthora*). Fungal-like organisms of the genus *Phytophthora* belong to the phylum Oomycetes, placed in the Stramenopila kingdom within the eukaryotes (Katz & Grant, 2015). The genus *Phytophthora* comprises more than 140 species, divided into 10 phylogenetic clades, containing both saprophytic and plant pathogenic species (Yang, Tyler & Hong, 2017). For instance, with some exceptions such as *P. pinifolia* (Durán et al., 2008) and some species of the clade 6a (Burgess et al., 2018), many of the species of the phylogenetic clade 6 and 9 could be considered saprotrophs (Jung et al., 2011; Hansen, Reeser & Sutton, 2012; Marano et al., 2016). By contrast, other species belonging to clade 1 such as *P. infestans*, or clade 7 and 8, such as the *P. alni* complex, *P. cinnamomi* or *P. ramorum* are responsible for large agricultural and forest losses. *P. infestans* has caused losses on potato and other solanaceous

crops worldwide since the middle of 19th century (Erwin & Ribeiro, 1996). In Europe, species of the *P. alni* complex have decimated the populations of alders (*Alnus* spp.) across riverbank ecosystems (Jung & Blaschke, 2004; Aguayo et al., 2014). Other examples include *P. ramorum*, the causal agent of sudden oak and larch death (Rizzo & Garbelotto, 2003; Brasier & Webber, 2010), and *P. cinnamomi*, that caused extensive mortality on several *Eucalyptus* spp. and many other species in Western Australia (Shearer, Crane & Cochrane, 2004). For being a major plant pathogen (Burgess et al., 2016), *P. cinnamomi* has been included in the list of the 100 world´s worst invasive species (Lowe et al., 2000).

1.1.2 Dispersal pathways of *Phytophthora*

In the life cycle of *Phytophthora*, there is a sexual and an asexual phase. During the sexual phase, homothallic (self-fertile) and heterothallic (self-sterile) species can produce oospores that can persist inside hosts or in soil (Erwin & Ribeiro, 1996). In heterothallic species, oospores are produced when both mating types grow together. During the asexual phase, sporangia are produced, and swimming asexual zoospores are released in the presence of water (Erwin & Ribeiro, 1996). For soil-borne *Phytophthora* species, dispersal usually takes place after periods of heavy rain or floods, when zoospores are released and swim chemotactically attracted by plant roots, where they encyst, and penetrate the host (Erwin & Ribeiro, 1996). For air-borne *Phytophthora* species, zoospores are released under favourable moisture conditions, and they disperse by water splashes or carried by wind in small droplets (Erwin & Ribeiro, 1996). The zoospores of both soil and air-borne species can be washed out into rivers and disperse downstream (Jules et al., 2002). For some *Phytophthora* species (mostly belonging to clade 6 and 9), rivers might constitute not only a dispersal pathway, but also the environment where they develop most of their life cycle (Jung et al., 2011). For some indigenous *Phytophthora* species of clade 6, rivers constituted the environment where natural hybridisation occurred (Nagel et al., 2013; Burgess, 2015).

Phytophthora can also be dispersed long distances by animals or humans moving infested soil (Cushman & Meentemeyer, 2008; Webber & Rose, 2008). Some *Phytophthora* species are able to create sexual and/or asexual resistant structures, such as oospores, chlamydospores or hyphal aggregations, that allow them to survive unfavourable conditions and also allow them to survive during transport in soil (Crone et al., 2013; Jung, Colquhoun & Hardy, 2013). Transport of infected plants constitute another long-distance dispersal pathway of *Phytophthora* (Jung et al., 2016). Nurseries can concentrate infected stock within their facilities (Schwingle, Smith & Blanchette, 2007; Simamora et al., 2017).

Phytophthora species can also be introduced in nursery facilities by using *Phytophthora*-infected water in irrigation systems (Hong & Moorman, 2005; Rytkönen et al., 2008; Ghimire et al., 2011). Following the introduction in nurseries, the pathways of dispersal into forests are not always clear. Recently, some studies suggested that populated areas might receive high amount of *Phytophthora* inoculum, owing to the recurrent out-planting of infected ornamental plants and the arrival of inoculum carried by humans (Dale et al., 2017; Hulbert et al., 2017). Yet, further studies are needed to confirm whether human activities could be a dispersal pathway of *Phytophthora,* and if populated areas are more likely to harbor more *Phytophthora* species than natural areas.

1.1.3 Climate and the global distribution of *Phytophthora*

The life cycle of *Phytophthora* seems to be affected by temperature and moisture (Erwin & Ribeiro, 1996; Sturrock et al., 2011). Indeed, species of *Phytophthora* require water for the release and dispersal of asexual zoospores (Erwin & Ribeiro, 1996), whose infectivity also appear to be affected by temperature (Granke & Hausbeck, 2009; Shelley et al., 2017). For some *Phytophthora* species, such as *P.* x *alni*, or *P. ramorum*, laboratory experiments have shown that low temperatures and long frost periods can reduce their survival (Schumacher et al., 2006; Tooley, Browning & Berner, 2008; Černý, Filipová & Strnadová, 2012). By contrast, other species, such as *P. uniformis*, seems to have a higher tolerance to cold, as suggested by the species widespread distribution in Alaska (Adams, Catal & Trummer, 2010).

The effect of low temperatures on the survival of *Phytophthora* under laboratory conditions is one of the factors utilized to estimate the distribution range of certain species, such as *P. ramorum*, *P.* x *alni*, or *P. cinnamomi* (Ireland, Hardy & Kriticos, 2013; Aguayo et al., 2014; Burgess et al., 2016). For instance, the distribution of *P. ramorum* seems to be restricted by dry conditions in mid-latitudes, and cold conditions in northern latitudes (Ireland, Hardy & Kriticos, 2013). Similarly, cold temperatures also seem to constitute a barrier for the presence of *P. cinnamomi* and *P.* x *alni* and, within Sweden, only the Southern region seems to be climatically suitable for their occurrence (Aguayo et al., 2014; Burgess et al., 2016). Studies exploring the association between climate and the distribution of *Phytophthora* species might contribute to the predictions of the establishment of certain *Phytophthora* species under changing climatic conditions (Ireland, Hardy & Kriticos, 2013; Aguayo et al., 2014; Burgess et al., 2016).

1.1.4 The adaptation of tree hosts to *Phytophthora*

The outbreaks of invasive *Phytophthora* species can have different long-term impacts on host populations. Because of the exotic origin of invasive *Phytophthora* species, and therefore their lack of co-evolution with the hosts, they can cause high mortality within the invaded populations (Hayden et al., 2011). For example, Hayden et al. (2011) showed that tanoak trees (*Notholithocarpus densiflorus*) at all their study sites were susceptible to *P. ramorum*, and therefore the long-term survival of the host populations seemed to be compromised. However, in other cases, hosts affected by *Phytophthora* species, such as *Quercus agrifolia* or *Eucalyptus marginata* (affected by *P. ramorum*, and *P. cinnamomi*, respectively) displayed varying levels of genetic resistance (Stukely & Crane, 1994; Dodd et al., 2005), potentially providing the gene pool for natural adaptation (McKinney et al., 2014).

In theory, for hosts to evolve and adapt to invasive *Phytophthora* species, the criteria of natural selection should be fulfilled: (i) the pathogen must have an effect on the host fitness, (ii) the effects on the host fitness must be not random, and (iii) the host offspring must inherit the selected trait (Strauss, Lau & Carroll, 2006) (Fig. 1).

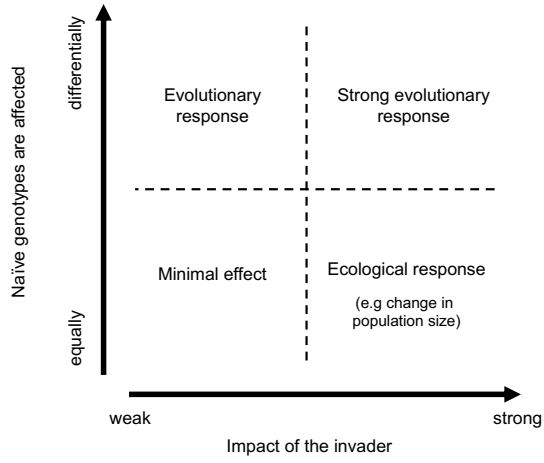

Figure 1. The potential for evolution of host species depends both on the pathogen and on the naïve host populations. Naïve populations should have a size and genetic variability large enough for the selection to act. When an invader has an impact on the population, and differentially affects naïve genotypes, evolutionary responses such as adaptation, are expected to occur (Adapted from Strauss et al. 2006. Ecology letters 9: 357-374).

Similar to other outbreaks caused by forest pathogens, such as *Hymenoscyphus fraxineus,* or *Ophiostoma novo-ulmi* (Solla et al., 2005; Kjær et

al., 2012), studies aiming to predict whether hosts will be able to adapt to invasive *Phytophthora* species are critical to assess the long-term impact of new introductions on host populations (Budde et al., 2016).

1.1.5 Current methods to study *Phytophthora* communities

Because of the impacts of certain *Phytophthora* species on forest health, many studies have focused on single species (e.g., Jules et al., 2002; Cushman & Meentemeyer, 2008; Ellis, Václavík & Meentemeyer, 2010; Schoebel et al., 2014). However, *Phytophthora*-induced diseases are usually linked to more than one species (Pérez-Sierra et al., 2013; Scanu et al., 2015; González et al., 2017), as suggested by several studies with a community approach (Sims et al., 2015; Dunstan et al., 2016). The study of *Phytophthora* communities was traditionally done by isolation from a certain substrate: plant, soil or river water (Jung & Blaschke, 2004; Jung, 2009; Hüberli et al., 2013). Nevertheless, isolation procedures are usually time consuming and can be biased, e.g., *P. cinnamomi* is seldom isolated from water streams (Burgess et al., 2016; Dunstan et al., 2016), creating the risk of false negative results. The use of high throughput sequencing (HTS) techniques can represent an alternative to assess diversity of *Phytophthora* communities more efficiently than isolation (Coince et al., 2013; Vannini et al., 2013; Català, Pérez-Sierra & Abad-Campos, 2015; Burgess et al., 2016, 2017). However, HTS also has limitations when it comes to taxonomical classification. The primers used so far in HTS studies targeted the region 1 of the Internal Transcriber Spacer (ITS) (Coince et al., 2013; Vannini et al., 2013; Català, Pérez-Sierra & Abad-Campos, 2015; Burgess et al., 2016, 2017). Català, Pérez-Sierra & Abad-Campos (2015) argued in the discussion of their study that the ITS 1 region has limitations for taxonomic identification of species of clade 1, 2, and clade 6. Due to the low sequence variability, it was not possible for the authors to separate 10% of the species from their database. Studies improving these current HTS methods might be needed in order to obtain a better resolution for species identification.

1.2 Studying *Phytophthora* from an invasion biology approach

1.2.1 The field of invasion biology

The invasion biology field describes aspects of introduction, establishment and spread of organisms in new locations, as well as the interactions of these

organisms with the native communities that they invade (Richardson & Pyšek, 2008; Richardson & Ricciardi, 2013). Studies addressing these processes are relevant, because they provide concepts to understand, communicate, and manage invasions (Stenlid et al., 2011; Richardson & Ricciardi, 2013). Not all introduced species have the same impact, therefore, different management strategies are implemented depending on the status of the introduced species (Blackburn et al., 2011). Studies with an invasion biology approach can also improve the methods for risk assessment by evaluating the likelihood of establishment of certain alien organisms, or by assessing which species will disrupt the invaded ecosystem, contributing to the allocation of resources towards species that constitute a real threat (Leung et al., 2012; Gilbert & Levine, 2013; Roy et al., 2018). In the case of *Phytophthora*, studies applying an invasion biology approach could shed light on dispersal pathways, enable predictions of the likelihood of establishment of certain species, and contribute to the assessment of long-term impacts of invasions on host populations.

1.2.2 A unified framework for biological invasions

During the development of the invasion biology as a field of research, several inconsistencies regarding the use of terms emerged (Heger et al., 2013). In an attempt to clarify concepts, Blackburn et al. (2011) proposed a unified framework for biological invasions. Their aim was to integrate previous invasions frameworks into a single one that could accommodate all human-mediated invasions. For this reason, the framework of Blackburn et al. (2011) was used as the reference in this thesis when applying the concepts of the field of invasion biology to the invasion process of *Phytophthora*. The framework of Blackburn et al. (2011) describes the invasion process as a series of stages separated by barriers that introduced organisms need to overcome in order to establish and spread in the new environment, subsequently becoming invasive. Blackburn et al. (2011) provides a terminology for species across the invasion process based on how far along the framework they have reached (Fig. 2). One important aspect of Blackburn´s classification is that the impacts are not considered when defining the status of an alien species. Thus, following Blackburn´s definition, an invasive *Phytophthora* would be defined as a non-native species able to establish and spread across a range of different habitats, regardless of its impact. The main reason is that impacts can vary and occur irrespective of the stage of invasion. For example, a given *Phytophthora* species can be introduced into a city park, establish and locally damage trees. However, if that species does not spread and establish across a range of habitats, it would not be considered invasive according to Blackburn et al. (2011). Another reason

to not include the impact when defining an invasive *Phytophthora* is that impact of fungi and fungal-like organisms is sometimes difficult to assess (Desprez-Loustau et al., 2007), particularly when they have indirect impacts on the invaded ecosystems (Bjelke et al., 2016).

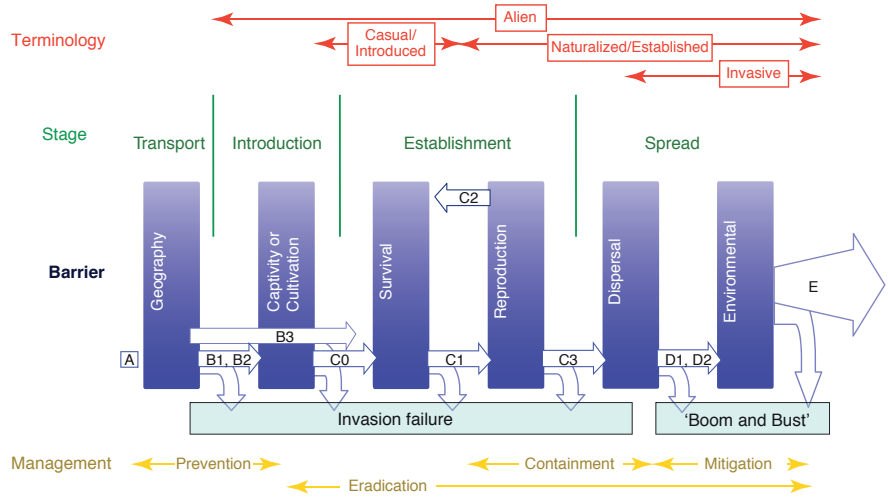

Figure 2. The proposed unified framework for biological invasions of Blackburn et al. (2011). In the framework, organisms are classified based on how far along the invasion process they have reached. The alphanumeric codes correspond to the classification of species with respect to the invasion process. Species are first transported beyond their native limits (A) and either contained at the arrival point (B1, B2), or directly released into the novel environment (B3), becoming introduced species. Some of these introduced species cannot survive in the novel area (C0), whereas others succeed in surviving and reproducing without further spreading (C1, C2, C3) and they are therefore considered established. Some of the established organisms are able to disperse and survive a significant distance from the original point of introduction (D1, D2), and further establish across a different range of habitats (E), becoming invasive. Blackburn et al. 2011. *Trends in Ecology and Evolution* 26: 333-339. Reproduced with the permission of the publisher.

Blackburn et al. (2011) assumed that barriers can affect the life cycle of the invader, e.g., barrier for survival and reproduction. In the case of *Phytophthora*, the presence/absence of susceptible hosts (particularly for host-specific *Phytophthora* species) or adverse climatic conditions, can constitute barriers along the invasion process (Ireland, Hardy & Kriticos, 2013; Aguayo et al., 2014; Burgess et al., 2016). The presence of barriers during invasion opens the possibility to study characteristics of the location, such as the type of hosts present or climatic factors, together with traits of the *Phytophthora* species present, such as host range or climatic suitability. This approach might be needed in order to assess the effect of environmental filtering hindering the

establishment of certain *Phytophthora* species, once they have been introduced in a new area.

1.3 The study of functional traits and functional diversity

Scientists can look at traits rather than species to understand the structure of natural communities (Tilman et al., 1997; Asner et al., 2017; Cernansky, 2017; Schneider et al., 2017). This functional trait approach has allowed ecologists to predict the distribution of macroorganisms based on their traits (Bodegom, Douma & Verheijen, 2014; Stahl, Reu & Wirth, 2014; Bäcklund, 2016; Costa-Saura et al., 2016; Frainer et al., 2017). A functional trait is defined as a measurable property of organisms that influences their performance (McGill et al., 2006). Based on information of traits for a set of species, one can calculate different parameters of functional diversity, which is defined as the distribution of functional traits among organisms (Violle et al., 2014; Asner et al., 2017). The most common functional diversity indices used are functional richness, functional dispersion, functional divergence, and functional evenness. They provide information of how the species are distributed across the multivariate space defined by the set of traits. For instance, functional richness represents the amount of multivariate space filled by the community (Villéger, Mason & Mouillot, 2008). Functional dispersion is an indication of the spread of the species within the multivariate space (Laliberté & Legendre, 2010). Functional divergence and functional evenness describe how species are distributed within the community niche. Functional divergence measures whether species are clustered towards the center of gravity or the edge of the multivariate space (Villéger, Mason & Mouillot, 2008; Clark et al., 2012), whereas functional evenness measures how evenly the functional space is filled (Villéger, Mason & Mouillot, 2008; Clark et al., 2012). Information about traits can be measured *in situ* or compiled from databases for macroorganisms. However, it is not always easy to do the same for microorganisms, because of the lack of species descriptions for many species. In the case of *Phytophthora*, information about traits is available in monographs (Erwin & Ribeiro, 1996), species descriptions or reviews of the genus (Kroon et al., 2011). Therefore, a functional-trait approach when studying *Phytophthora* communities could potentially increase our understanding of the patterns of distribution and assembly of *Phytophthora* communities.

1.4 Functional traits and environmental filtering

Environmental filtering is believed to be one of the major mechanisms structuring natural communities in which the abiotic factors select against certain functional traits, and therefore against some species (Cadotte & Tucker, 2017). Nevertheless, the absence of species from a community can be a consequence of other ecological processes, such as dispersal limitation or competitive exclusion (Kraft et al., 2015; Cadotte & Tucker, 2017). In fact, biotic and abiotic filters commonly occur together, and observational field studies usually cannot disentangle the effects between them (Kraft et al., 2015). One of the methodologies used to assess the effect of the environmental filtering on natural communities is to evaluate the clustering level of communities based on the similarity of their traits, and compare it with a null expectation based on random sampling from the species pool (Cornwell, Schwilk & Ackerly, 2006; Lamanna et al., 2014; Cadotte & Tucker, 2017). Theory predicts that environmental filtering would constrain the type of traits in a certain environment, and therefore communities will show higher clustering of traits than expected from the pool of species (Lamanna et al. 2014). In contrast, competitive exclusion would cause divergence in traits, and therefore lower clustering than the null expectation (Cornwell, Schwilk & Ackerly, 2006; Lamanna et al., 2014). Recent developments in coexistence theory have shown that certain trait values might confer species a stronger average fitness, and therefore competitive exclusion can also result in a higher clustering than expected from the pool of species (Kraft et al., 2015).

Kraft and colleagues (2015), suggested researchers to re-evaluate their use of the environmental filtering concept, by re-defining it (*sensu stricto*) as the process in which abiotic factors hinder the establishment of some species in the absence of biotic competition. According to Kraft et al. (2015) two lines of evidence would support an effect of environmental filtering (*sensu stricto*): (i) a certain species has the ability to arrive to a certain site (i.e., excluding dispersal limitation), and (ii) this species is not able to tolerate the environmental conditions of the site in absence of competitors. This latter information could be obtained by experimental manipulation or by laboratory-based measures of physiological tolerance. Mainly in response to Kraft et al. (2015), Cadotte & Tucker (2017) argued that the use of the environmental filtering concept is valid as long as researchers provide information regarding trait-environment correlations. Thus, Cadotte & Tucker (2017) suggested three lines of evidence supporting an effect of environmental filtering: (i) there must be evidence of clustering of species compared to null expectation from the pool of species and traits, (ii) there must be a demonstrable environmental gradient, and (iii) environmental conditions must be correlated with species traits or phylogenies.

Studying the effect of the environmental filtering on *Phytophthora* communities could be useful to understand which abiotic filters might affect the establishment of certain species.

1.5 What is known about *Phytophthora* in Northern Europe and what remains to be understood?

Before the start of this thesis in 2014, relatively little information was available about *Phytophthora* in Northern Europe in general, and Sweden in particular. In Finland, *P. cactorum* was reported in the ´90s on strawberry plants (Lilja et al., 1998), and more recently this and other species have been found in nurseries (Rytkönen et al., 2008, 2012, 2013). Similarly, *P. cactorum* was reported in the ´90s affecting strawberry crops in Norway (Stensvand A., Herrero M. L. & Talgø V., 2008). More recently, other *Phytophthora* species have been found in nurseries, parks, and beech forests in the same country (Herrero et al., 2006; Herrero, Toppe & Brurberg, 2010; Telfer K. H. et al., 2015). In Sweden, early reports of *Phytophthora* species affecting forest trees came from a forest nursery (Molin, Persson & Persson, 1961). Years later, studies explored which *Phytophthora* species were involved in alder and oak decline (Olsson, 1999; Jönsson et al., 2003, 2005, Jönsson, 2004, 2006). In parallel with this thesis, recent studies have revealed a diversity of *Phytophthora* species infecting trees and woody plants in both urban and natural forests (e.g., Cleary et al., 2016; Blomquist, 2017). In line with these previous studies, some aspects remained to be understood. For instance, of the species that are present in Sweden, which ones could be considered invasive? Given that *Phytophthora* species seem to be introduced with imports of plants, how are they further dispersed? Once these species are introduced and dispersed, can perhaps climate prevent the establishment of some of them? The *Phytophthora* species that do establish, do they have certain traits in common? And also, could tree populations adapt to these new invaders? In this thesis, these questions are addressed in Sweden, expecting that the findings here could be used to answer similar questions in other regions.

2 Objectives and outline of the methods

The aim of this thesis was to investigate the factors involved in the introduction, establishment and spread of forest *Phytophthora* species, and study the long-term impacts of these invasions on host populations. The specific objectives were:

- To determine which *Phytophthora* species could be considered invasive in Sweden, and what may be the dispersal pathways during invasion (**paper I**).
- To investigate the association between climatic factors and the distribution of *Phytophthora* at species (**paper II**) and community level (**paper III**).
- To evaluate the potential of functional traits to explain patterns of distribution and community assembly processes (**paper I, III**).
- To assess whether alder populations have the potential to adapt to species of the *Phytophthora alni* complex (**paper IV**).

To determine which *Phytophthora* species are invasive in Sweden and what their possible dispersal pathways are, a survey was conducted in eight Swedish nurseries, six urban forests, two livestock grazing areas, six natural forests, and 16 river streams in **paper I**. It was assumed that nurseries, urban forests, grazing areas, and natural forests could represent different stages of the invasion process, according to Blackburn et al. (2011). By using traditional *Phytophthora*-isolation techniques, the composition of *Phytophthora* communities in each of the environments was obtained. The definition of an invasive species from Blackburn et al. (2011) was applied to determine which *Phytophthora* species could be considered invasive in Sweden. The diversity of *Phytophthora* communities was compared between nurseries, urban forests, and natural forests to test whether human activities may be dispersal pathways during *Phytophthora* invasion.

To determine the role of climatic factors on the distribution of *Phytophthora*, a systematic survey was conducted in 16 rivers and riverside-alder stands distributed across Southern Sweden along a climatic gradient. In this survey, the focus was first placed on the distribution of two species of the *P. alni* complex that display different tolerance to low temperatures in laboratory conditions. By isolating *Phytophthora* from infected alder stem, it was assessed if differences in cold tolerance between *P.* x *alni* and *P. uniformis* could reflect their distribution. Secondly, in **paper III**, the composition of *Phytophthora* communities was obtained along the climatic gradient by a new metabarcoding setup based on PacBio sequencing of river filtrates and the primers developed by Drenth et al. (2006). It was assumed that rivers harbour both *Phytophthora* species washed out from the surrounding ecosystems (terrestrial *Phytophthora* spp.) and *Phytophthora* species developing their life cycle in water bodies (aquatic *Phytophthora* spp.). The association between alpha diversity and climatic factors was analysed for both terrestrial aquatic communities.

To test if functional traits can help to explain patterns of distribution and community assembly of *Phytophthora*, information about nine life-history traits for each of the *Phytophthora* species obtained in **paper I** and **paper III** were compiled. In **Paper I**, life-history traits of *Phytophthora* communities were assessed across the stages of the invasion process. In **paper III**, the functional diversity of *Phytophthora* communities, and the distribution of traits at community and species level was assessed along the climatic gradient. By comparing the clustering level of the communities with the one expected from the regional pool of species and traits, the effect of the environmental filtering on terrestrial and aquatic *Phytophthora* communities was analysed.

To investigate if alder trees could adapt to *P. alni* species, signs of natural selection exerted by species of the *P. alni* complex on alder populations were explored. Since *P. alni* species are mainly restricted to riverbank ecosystems, alder stands far away from the river shore and without any symptoms of *P. alni* induced decline (uninvaded areas) could presumably reflect the genetic composition of the naïve alder population before the invasion occurred in the early 1990. By contrast, invaded sites were riverbank-stands from which species of *P. alni* were recovered in the survey of **paper II**. In **paper IV**, we compared the susceptibility of progenies of trees obtained from *P.* x *alni* and *P. uniformis* invaded and uninvaded sites by performing *in vitro* inoculations. The broad sense heritability of resistance against *P. uniformis* and *P.* x *alni* was obtained to test the association between levels of genetic variation in resistance and the strength of natural selection.

3 Invasive *Phytophthora* species and pathways of dispersal

The first objective of this thesis was to determine which *Phytophthora* species could be considered invasive in Sweden, based on the definition of Blackburn et al. (2011), and what may be the dispersal pathways of *Phytophthora* during invasion. Although it has been shown that *Phytophthora* species are mainly introduced in new regions through nursery stock (Schwingle, Smith & Blanchette, 2007; Liebhold et al., 2012; Jung et al., 2016), there is less knowledge on how these *Phytophthora* species are further spread within the invaded area.

3.1 Which *Phytophthora* species are invasive?

In **paper I**, a survey across nurseries and forests was conducted. It was assumed that these environments represent each of the stages of the invasion process described by Blackburn et al. (2011): "transport-introduction" (nurseries), "introduction-establishment" (anthropogenic forests) and "spread" (natural forests) (Fig. 3). Nurseries were considered as a pre-establishment environment corresponding to the stage of transport-introduction based on strong evidence of nursery stock as a major pathway for dispersal of *Phytophthora* between regions (Jung et al., 2016). As long as *Phytophthora* species remain in nursery facilities, they could be considered transported-introduced in Sweden, but not yet established, because they had not shown their ability to survive under natural environmental conditions. In contrast to nurseries, *Phytophthora* species surviving in forests had undergone natural environmental conditions, and could be considered introduced-established, according to Blackburn et al. (2011).

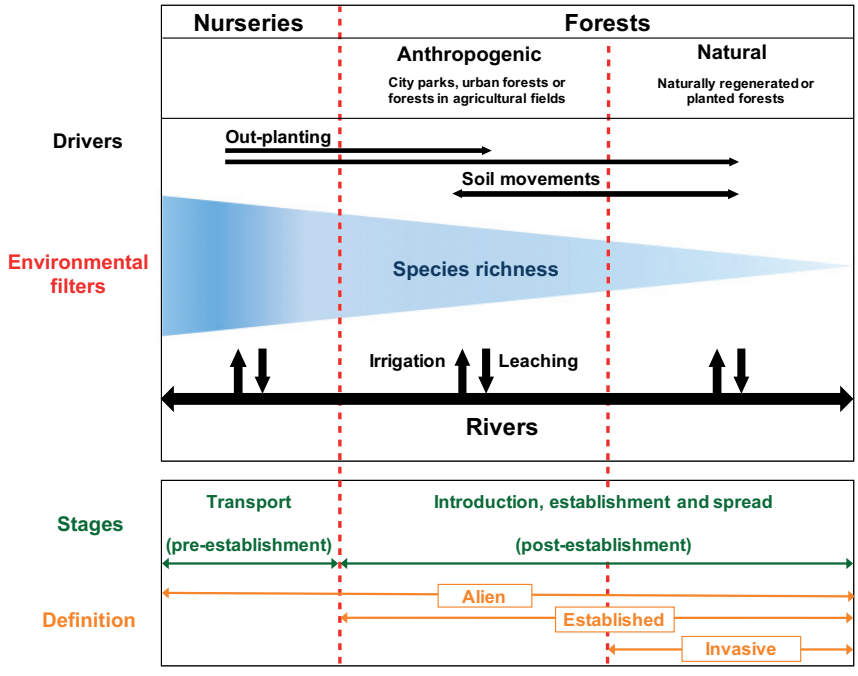

Figure 3. Diagram of the hypothesized *Phytophthora* invasion process in Sweden presented in **paper I**, based on Blackburn et al. (2011). We assumed that nurseries constitute the main entry point for alien *Phytophthora* species. Once *Phytophthora* species are introduced, establishment mainly occurs in locations where nursery material is recurrently planted (anthropogenic forests). From there, *Phytophthora* species can be further dispersed into natural forest or other urban areas by humans/animals moving infested soil. During the invasion process, a decrease of species richness across stages of invasion is expected to occur as a result of environmental filters. The criteria for defining an invasive species in **paper I** was based on the observation of *Phytophthora* species across different type of environments where they manage to establish, regardless of their ecological or economic impact, following Blackburn et al. (2011). Therefore, those *Phytophthora* species suspected to be alien in Sweden, and found in nurseries, anthropogenic forests, and natural forests, regardless of their impact, were classified as invasive.

Forests can differ in terms of the amount of *Phytophthora* inoculum that they receive from nurseries, and therefore in the number and type of *Phytophthora* species that they harbour (Dale et al., 2017; Hulbert et al., 2017). Hence, forests were further divided into anthropogenic and natural forests. Anthropogenic forests consisted of urban forests or grazing woodlands areas. It was assumed that anthropogenic forests receive higher loads of inoculum from nurseries than natural forests (Blomquist, 2017; Hulbert et al., 2017). The presence of a *Phytophthora* species in anthropogenic forests might be an indication of its ability to survive and reproduce in the new environment, but not yet to disperse further. Therefore, species found only in this type of forests would be considered

naturalized or established, but not yet invasive according to Blackburn et al (2011). Natural forests consisted of coniferous and deciduous forests in remote areas with low levels of out-planting activities and human disturbances. It was assumed that these environments receive the lowest amount of *Phytophthora* from nursery stock, (Jung *et al.* 2016), and that the species found would mainly have been spread from anthropogenic forests (Cushman & Meentemeyer, 2008; Webber & Rose, 2008). Those *Phytophthora* species that are suspected to be non-native, and that are found in nurseries and in both anthropogenic and natural forests, would be classified as invasive.

The results of **paper I** showed that three *Phytophthora* species, namely *P. plurivora*, *P. cambivora*, and *P. cactorum*, were found in nurseries and both type of forests. In order to be defined as invasive, these species must be non-native to Sweden. Defining a species as non-native is difficult when dealing with microorganisms because their origin is often unknown (Santini et al., 2013). Studies of genetic population structure can provide information about the origin of some *Phytophthora* species. The indirect criteria from Jung et al. (2016) could also help determining the alien origin of *Phytophthora* species based on (i) their aggressiveness to native trees, (ii) their occurrence in healthy ecosystems on other continents, (iii) the low genetic variation of their populations, and/or (iv) their close phylogenetic relatedness to non-native species. Based on these criteria, *P. plurivora*, *P. cambivora*, and *P. cactorum* have been considered alien to Europe (Jung et al. 2016). For a heterothallic species such as *P. cambivora*, the finding that nearly all European isolates belong to the A2 mating type (Vettraino et al., 2005) might also indicate that the species was introduced to Europe. If *P. cambivora* was native in Europe, both mating types would be in a 1:1 ratio, as reported for *P. infestans* in its centre of origin (Goss et al., 2014). *P. cambivora*, *P. plurivora*, and *P. cactorum* seem to be alien to Europe, and they could therefore be considered invasive in Sweden.

The definition of invasive *Phytophthora* that we used in this thesis was mainly used to classify species found in **paper I**. However, this concept could be utilized to classify other *Phytophthora* species as long as they fulfil the same criteria: alien to Sweden, and established across a different range of habitats from nurseries to forests. For instance, in **paper II** we performed a systematic survey of alder stands in riverbanks located in both anthropogenic and natural forests. We found both *P.* x *alni* and *P. uniformis* established across both type of forests in 15 river systems. These two species are presumably alien to Sweden: *P. uniformis* seem to be alien to Europe based on its population structure (Aguayo et al., 2013), and *P.* x *alni* seem to be originated by a recent hybridization event in nurseries (Brasier et al., 2004). It could be that these two species have been introduced with nursery stock; *P. uniformis* was found in

nurseries of **paper I**, and *P.* x *alni* was widely found across European nurseries (Jung et al., 2016). Therefore, the two species of the *Phytophthora alni*-complex that were found in **paper II** could also be considered invasive in Sweden in accordance to the definition of Blackburn et al. (2011).

Several other species were found during the survey of **paper I** in different stages of invasion, and their status remains open for discussion. For instance, *P. quercina* was found in **paper I** in both anthropogenic and natural forests, but not in nurseries. These results support previous findings of *P. quercina* across oak stands in Southern Sweden (Jönsson et al., 2003). Assuming its alien origin based on Jung et al (2016), and according to the classification used here and based of Blackburn et al. (2011), *P. quercina* could be considered established/naturalised in Sweden. The status of other species such as *P. gonapodyides* remains difficult to determine. Although its establishment in Sweden is supported by its records in nurseries and rivers in **paper I,** and in anthropogenic forests in Southern Sweden (Cleary et al., 2016), its cryptogenic origin (Santini et al., 2013; Jung et al., 2016) makes it difficult to classify it as introduce/alien species. It is possible that other species have been missed during the survey of **paper I**, and therefore future surveys in Sweden will clarify the invasive status of other *Phytophthora* species.

3.2 Human activities as a pathway for *Phytophthora* invasion

Human trade and movements seem to be important pathways of dispersion of forest pathogens worldwide (Fisher et al., 2012; Liebhold et al., 2012; Santini et al., 2013, 2018). In the case of *Phytophthora ramorum,* for instance, Cushman & Meentemeyer (2008) showed that this species occurred more commonly in trails used by humans than in off-trail areas, and that the likelihood of *P. ramorum* occurrence increased with increasing population density. Urban environments and populated areas seem to be a port-of-entry for *Phytophthora* species (Hulbert et al., 2017), as supported by recent studies showing greater diversity of *Phytophthora* in urban forests compared to natural forests (Dale et al., 2017). In accordance with these studies, the results of **paper I** showed that species richness in nurseries and anthropogenic forests was higher than in natural forests. This could be due to the recurrence of ornamental import and out-planting on populated areas (Jung et al., 2016; Blomquist, 2017; Hulbert et al., 2017).

The baiting performed in rivers flowing through anthropogenic and natural forests in **paper I**, revealed that three of the species that were defined as invasive (*P. plurivora*, *P. cambivora*, and *P. cactorum*) tended to appear together, and

their occurrence was significantly higher in urban locations than in forests. These three species were frequently found in Swedish nurseries (**paper I**), and reported in 40% of the nurseries inspected by Jung et al. (2016). It seems plausible that these invasive species share a common dispersal pathway, namely out-planting of nursery stock, which is expected to occur more often in anthropogenic forests than in natural forests. How these invasive *Phytophthora* species dispersed from anthropogenic to natural forests is still unclear. All natural forests sampled in **paper I** were crossed by roads, and some of them were close to natural parks frequently visited by humans. It is possible that the inoculum of these species has been introduced in natural forests via human-mediated propagule movements, as reported for other *Phytophthora* species (Cushman & Meentemeyer, 2008; Webber & Rose, 2008).

It should be kept in mind that sampling one year and using isolation procedures could have affected the number and type of *Phytophthora* species found in **paper I**. Nevertheless, the species reported here were in accordance with those obtained from other surveys in Sweden over several years (Cleary et al., 2016; Blomquist, 2017). Similarly, all the *Phytophthora* species found by Jönsson et al. (2003) in their sampling of oak stands were also found in **paper I**.

4 Climate and the distribution of *Phytophthora*

The second objective of this thesis was to investigate the association between climatic factors and the distribution of *Phytophthora*. The reason to address this aspect is that, although *Phytophthora* species are introduced in new regions, not all of them may establish and spread further. Certain climatic conditions, such as low temperature and low moisture, can affect their survival (Browning et al., 2008; Černý, Filipová & Strnadová, 2012), and they may constitute barriers for establishment. Studying whether climate affects the distribution of *Phytophthora* at species and community level could contribute to our ability to predict the establishment of specific *Phytophthora* species under changing climate conditions.

4.1 Temperature and the distribution of species belonging to the *P. alni* complex

It has been shown that *P.* x *alni* cannot survive long frost periods (Černý, Filipová & Strnadová, 2012), while *P. uniformis* seems to be able tolerate cold temperatures given its widespread distribution in Alaska (Adams, Catal & Trummer, 2010; Jung, pers. comm.). Sweden may constitute the northern limit of the distribution for these species in Europe, providing the opportunity to test if climate is limiting the distribution area of the cold sensitive *P.* x *alni*. **Paper II** aimed at testing if the differences in cold tolerance between *P. uniformis* and *P.* x *alni* were also reflected in their distribution in Sweden. By conducting a systematic survey in riverbank alder stands across 16 rivers, it was observed that *P.* x *alni* was located in areas of Sweden with the warmest temperatures in February (the coldest month of the year), whereas *P. uniformis* was found widespread all over the surveyed area (Fig. 4).

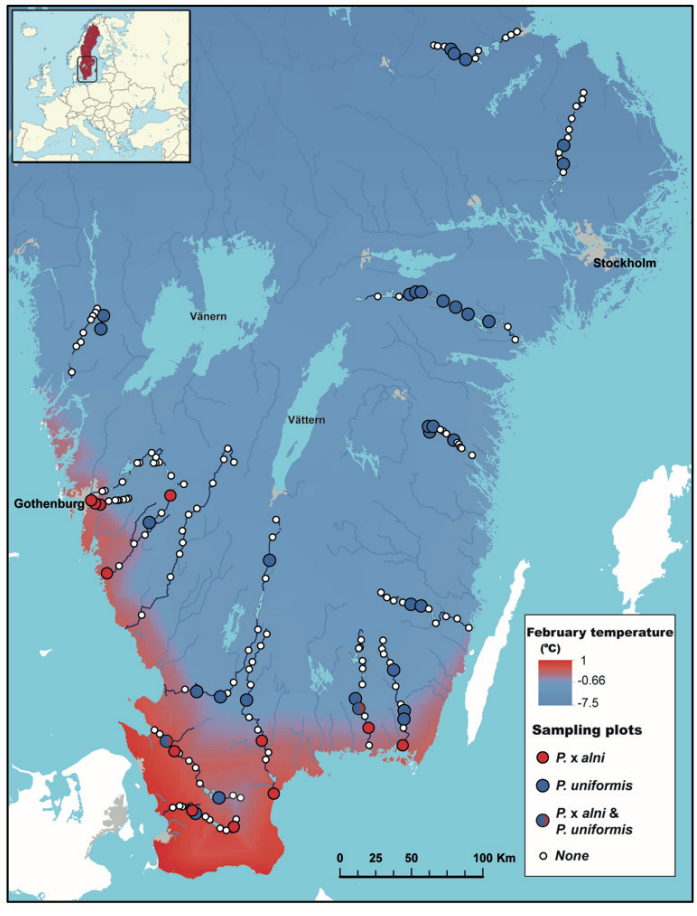

Figure 4. Distribution of species of the *P. alni* complex isolated from alder tissue in **paper II**. Shown is the average temperature of February (the coldest month of the year) of the last 20 years. "None" indicates no detection *Phytophthora* species.

These results are consistent with predictions of occurrence of *P.* x *alni* in Europe, which suggested that Southern Sweden would be the northernmost distribution limit of *P.* x *alni* in the field (Aguayo et al., 2014; Marcais pers. comm). Contrarily to our findings, *P.* x *alni* has been reported in the Czech Republic under colder average winter temperatures as Southern Sweden (Černý, Filipová & Strnadová, 2012). However, the survival of *P.* x *alni* is not only affected by low temperatures, but also by long frost periods (i.e. consecutive days with temperature < -5°C) (Černý, Filipová & Strnadová, 2012). **Paper II** showed that for the same value of average monthly temperature, the average

frost period was twice as long in Sweden compared to Czech Republic. Similar to predictions made for other *Phytophthora* species (Ireland, Hardy & Kriticos, 2013; Burgess et al., 2016), a warmer climate might allow *P.* x *alni* to expand its area of distribution northwards (Aguayo et al., 2014).

When studying the effect of the climate on the establishment of *Phytophthora* species, it should be considered that they may still be expanding geographically. This fact was pointed by Václavík & Meentemeyer (2012) when they suggested that predicting the distribution range of species that are in early stages of their invasion could be misleading. Nevertheless, this may not be the case of *P.* x *alni*, because its absence in cold regions seems to be supported experimentally (Černý, Filipová & Strnadová, 2012).

4.2 Climate and the diversity of *Phytophthora* communities

4.2.1 A novel methodology to study *Phytophthora* communities

In **paper III**, we developed a novel metabarcoding setup that could improve some of the limitations of the current high throughput sequencing (HTS) approaches used to study *Phytophthora* communities. This novel methodology was based on Pacific BioSciences (PacBio) sequencing technology and a modified version of the *Phytophthora*-specific primers developed by Drenth et al. (2006). The primers designed by Drenth and colleagues (2006) targeted the whole ITS region (ITS1, ITS2, and the 5.8 subunit of the ribosomal RNA gene (5.8S)). Nevertheless, these original primers could have missed some species that were not described at the time, such as *P. gallica* (Jung & Nechwatal, 2008). The validity of these primers was tested on a curated database of *Phytophthora* ITS provided by Jung and Horta, and two extra forward primers were developed to correct for the mismatches on *P. gallica* and *P. pseudosyringae* (**paper III**). With these adapted primers, it was possible to amplify fragments of 750-900 bp containing both ITS1 and ITS2 regions, and the 5.8S, allowing for a higher resolution for taxonomical identification than the previous metabarcoding approaches used. The sequencing of these long amplicons was possible owing to the longer read lengths and lower size-based bias of the PacBio sequencing technology compared to other platforms such as Illumina (Rhoads & Au, 2015; Tedersoo, Tooming-Klunderud & Anslan, 2018).

Sampling in rivers has been used to monitor *Phytophthora*, because both *Phytophthora* species present in the water (mostly belonging to clades 6 and 9), as well as species washed out from inland areas located upstream could be

obtained (Frankel, 2008; Sutton et al., 2009; Hüberli et al., 2013; Sims et al., 2015; Aghighi et al., 2016; Stamler et al., 2016). In **paper III**, *Phytophthora* communities were studied in 96 sites distributed across 16 rivers by using the previously described PacBio-based approach. A total of 228 913 PacBio sequence reads were obtained, and they were clustered at 99.5% similarity in Operational Taxonomical Units (OTU) by using the SCATA pipeline (https://scata.mykopat.slu.se). The most common sequence of the OTU was blasted in Genbank (https://www.ncbi.nlm.nih.gov/genbank) and Phytophthora-ID (www.phytophthora-id.org), to identify OTUs at species level (i.e. 98% identity with reference sequences). For those OTUs that could not be identified at species level, a maximum-likelihood tree was built with the most common sequence of that OTU and the database of ITS sequences obtained from Phytophthora-ID, to assign that OTU to one of the 10 clades of the genus *Phytophthora*. From the total number of sequence reads, 74.2% were assigned to the *Phytophthora* genus (884 *Phytophthora* reads per sample) belonging to 64 OTUs. The specificity for the genus *Phytophthora* of the primers used in **paper III** was lower than the one reported by Català, Pérez-Sierra & Abad-Campos (2015) for water samples in their study (96.9%).

In **paper III**, 96 plots were sampled and ca. 884 reads of *Phytophthora* per plot were obtained. A site rarefaction curve showed that increasing sample depth would have possibly not rendered a higher number of *Phytophthora* OTUs. By contrast, sampling more sites would have possibly increased the number of OTUs, although the sampling was done close to the asymptotic phase also for sites (Fig. 5).

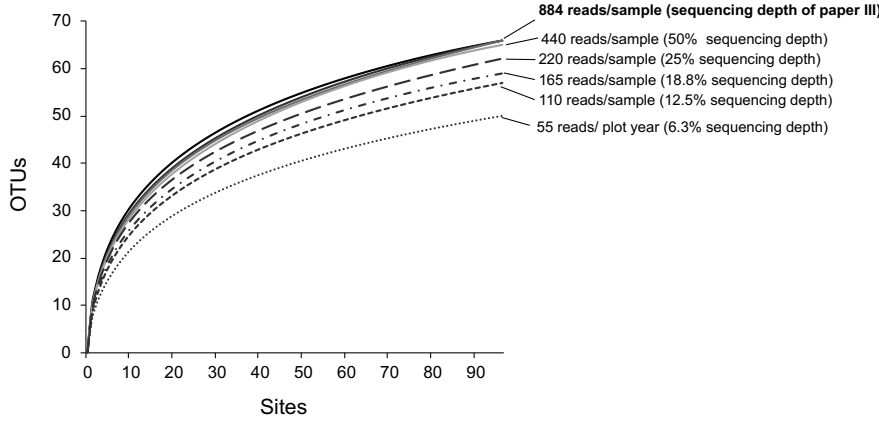

Figure 5. Site-based rarefaction curves of the reads obtained in **paper III**, for different simulated sequencing depths obtained by random subsampling. The graph shows that even obtaining half of the amount of reads that we obtained in **paper III**, we would have not decreased the number of

OTUs. In addition, the gain of OTUs by sequencing deeper stops at a certain threshold, after which the gain of OTUs is larger by increasing sampling sites than by sequencing deeper.

To compare the efficiency of the metabarcoding approach to traditional baiting, the river water was filtered simultaneously to baiting procedures reported in **paper I**. Considering that OTUs would correspond to species, we obtained three times the number of species by PacBio sequencing than by traditional baiting (36 species by PacBio sequencing vs 12 species by baiting). A similar comparison was made by Vanini et al. (2012) showing that they could detect 67% more species by pyrosequencing than by traditional baiting (15 species by pyrosequencing vs nine species by baiting). These results support the potential of HTS methodologies to study *Phytophthora* communities in a more efficient way than traditional isolation techniques. Nevertheless, HTS can also have some drawbacks. For instance, some species such as *P. plurivora* or *P. cambivora* were baited in certain sites of **paper I,** but were not detected in the same site by PacBio sequencing in **paper III**. This could be due to the baits remaining in the water for at least 48 hours, in contrast to water filtering, which is done once and only using ca. 10 litres of water, and thus sampling a much lower water volume.

4.2.2 Diversity patterns of *Phytophthora* communities are associated with temperature and precipitation

The distribution of terrestrial and aquatic organisms seems to follow climatic gradients (Roy et al., 1998; Francis & Currie, 2003; Fuhrman et al., 2008; Tedersoo et al., 2014; Zhou et al., 2016). For instance, temperature is associated with diversity for marine bacteria (Fuhrman et al., 2008), whereas both temperature and precipitation correlate with diversity of soil microorganisms (Tedersoo et al., 2014; Zhou et al., 2016). Tedersoo et al. (2014) found that precipitation is positively correlated with fungal diversity, while Zhao et al. (2016) found a positive correlation between temperature and soil fungal diversity. In **paper III**, we studied the composition of *Phytophthora* communities in 16 rivers distributed along a climatic gradient over two consecutive years. Mean annual temperature (°C) and total annual precipitation (mm) were positively correlated. Still, precipitation accounted for more variation of alpha diversity of terrestrial *Phytophthora* species (i.e., species that do not belong to clades 6 or 9) than temperature, in accordance with the results of Tedersoo et al. (2014) for soil fungi. For terrestrial communities, the species richness and parameters of alpha diversity increased with increasing total annual precipitation. These findings are consistent with the life cycle of *Phytophthora*,

in which the presence of water is important for the release of zoospores and the infection of new plant hosts (Erwin & Ribeiro, 1996).

In contrast to terrestrial *Phytophthora* species, patterns of diversity of aquatic *Phytophthora* species (i.e., species belonging to clade 6 or 9) were more associated with mean annual temperature, and with the type of the environment surrounding the river site (city, agricultural field, or forest) than with total annual precipitation. In the second year of the survey of **paper III**, water samples were taken to determine their pH, conductivity, total organic carbon, and total nitrogen. The total nitrogen and pH were positively correlated with the species richness and Shannon index of alpha diversity, respectively, whereas conductivity was negatively correlated with Shannon and Simpson indices. The chemistry of the water changed between environments, probably explaining why aquatic communities differed between river sites located in cities, agricultural fields, and forests. In aquatic environments, where water does not constitute a limitation for dispersal, it seems reasonable that temperature and water chemistry are stronger drivers than precipitation, in accordance with other studies showing an association between temperature and aquatic organisms (Roy et al., 1998; Fuhrman et al., 2008).

Taken together, the results suggest that the association between climatic factors (temperature and precipitation) and the diversity patterns of *Phytophthora* communities differs depending on where *Phytophthora* species develop most of their life cycle (water or soil). These results support previous studies suggesting that the diversity of terrestrial and aquatic organisms are likely driven by different factors (Bhatt, Manish & Pandit, 2012). Some aspects of **paper III** could be further discussed**.** For instance, as the sampling was conducted in rivers, it could be that some of the terrestrial *Phytophthora* species may be underrepresented. Some studies showed that certain terrestrial species such as *P. cinnamomi* are seldom recovered from rivers by baiting (Dunstan et al., 2016). Other studies performing both baiting in rivers and riverbank soils found varying amounts of terrestrial *Phytophthora* species in rivers; from none (Hüberli et al., 2013), to 25% (Aghighi et al., 2016), or more than 60% (Sims et al., 2015). Perhaps, these low levels of terrestrial *Phytophthora* species could be due to the limitations of baiting or a low sampling effort, given that Català and colleagues (2015) showed that 90% of the species found in terrestrial soils were also found in water by using HTS techniques.

5 Functional traits and distribution of *Phytophthora*

The third objective of this thesis was to study if functional traits could explain patterns of distribution and assembly processes of *Phytophthora* communities. Functional traits have been used to study the distribution of macroorganisms along gradients, and in this thesis, it is suggested that the same approach can be applied to *Phytophthora* communities. By analyzing the functional diversity and trait distribution of *Phytophthora* communities, (i) the traits of *Phytophthora* that were associated with their establishment and distribution in Sweden (**paper I**), and (ii) the role of environmental filtering shaping *Phytophthora* communities (**paper III**) was explored.

5.1 Functional traits of *Phytophthora*

Information about functional traits of *Phytophthora* can be found in monographs (Erwin & Ribeiro, 1996), species description papers or reviews (Kroon et al., 2011). In **paper I** and **III**, information about nine *Phytophthora* traits, belonging to three categories (life-history, environmental tolerance, and specialization), was compiled. These categories were related to dispersal mode, production of survival structures, cardinal temperatures for growth, and host colonization ability (Table 1). Functional diversity analyses based on these traits were thus performed on the *Phytophthora* communities obtained in both **paper I** and **III**.

Table 1. *Phytophthora traits used in **paper I** and **paper III** for the functional diversity calculations.*

Category	Trait	Type	Source
Life history	Clade	Factor	Kroon et al. 2011
	Reproductive mode	Factor	Kroon et al. 2011
	Persistence of sporangia	Factor	Kroon et al. 2011
	Asexual survival structures	Factor	Erwin & Ribeiro 1996, Species description papers, and expert knowledge
Environmental tolerance	Minimum temperature	Numeric	Erwin & Ribeiro 1996, Species description papers and expert knowledge
	Optimum temperature	Numeric	Species description papers
	Maximum temperature	Numeric	Species description papers
Specialization	Host range	Factor	Kroon et al. 2011, and expert knowledge
	Infected tissue	Factor	Kroon et al. 2011, and expert knowledge

5.2 Functional traits associated with the establishment and distribution of *Phytophthora*

Several studies have shown changes in functional traits of macroorganisms along environmental gradients (Bodegom, Douma & Verheijen, 2014; Stahl, Reu & Wirth, 2014; Costa-Saura et al., 2016; Frainer et al., 2017). For instance, Costa-Saura et al. (2016) showed that, in the Mediterranean region, a single trait such as the Specific Leaf Area (SLA) could explain differences in the distribution of plant species across an aridity gradient. A similar study performed across North America, showed that trees with high values of wood density or high seed mass do not colonize regions with annual temperatures < 0°C (Stahl et al. 2014). Studies of this type are not common for microorganisms yet, besides attempts to connect traits with life history of arbuscular mycorrhizal fungi (Chagnon et al., 2013).

In **paper I**, the trait composition of *Phytophthora* communities between pre- and post-establishment environments (nurseries and both types of forests, respectively) was compared. In forests, there was a higher proportion of species in the community with the ability to create asexual survival structures compared

to nurseries, suggesting that species of *Phytophthora* that are able to create asexual survival structures are more likely to establish in Sweden.

In **paper III**, changes in the functional diversity of *Phytophthora* communities were studied along a climatic gradient. Functional richness and functional evenness of terrestrial *Phytophthora* communities (i.e., not belonging to clade 6 or 9) declined with decreasing temperature and precipitation, pointing to a convergence of traits in communities located in cold and dry areas. Moreover, the trait analysis of **paper III** showed that species that dominated the communities in areas with low temperature and precipitation, had the ability to create asexual structures and exhibited a low optimum growth temperature, traits that seemed to be related to the climatic suitability (Jung et al., 2013; Crone et al., 2013). Studies of mycorrhizal communities in boreal forests have shown that the most abundant root-associated ascomycetes in boreal forests have thick melanized cells allowing them to survive adverse environmental conditions (Robinson, 2001; Clemmensen et al., 2015). It seems that surviving structures could be an important trait for microorganisms to establish in northern latitudes, irrespective of the trophic interaction between the microorganisms and the plant.

The dominance of specific functional traits should be interpreted with care, particularly for uneven communities. For this reason, **paper III** also analysed whether the traits of the most dominant species were also displayed by the other species of the community. Thus, the association between functional traits and distribution of each terrestrial *Phytophthora* species was analysed. It was observed that the optimum temperature for growth and the ability to create asexual survival structures were significantly associated with the distribution of single *Phytophthora* species along the climatic gradient, regardless of their dominance in the community. Species with low optimum temperature were present in dry and cold regions (Fig. 6), and species unable to produce asexual resistant structures were located in areas with temperature and precipitation levels above the average of the surveyed area (**paper III**). These results open the door to the possibility of making predictions regarding the establishment and distribution of individual *Phytophthora* species based on traits, as has already been done for plants (Stahl, Reu & Wirth, 2014; Costa-Saura et al., 2016).

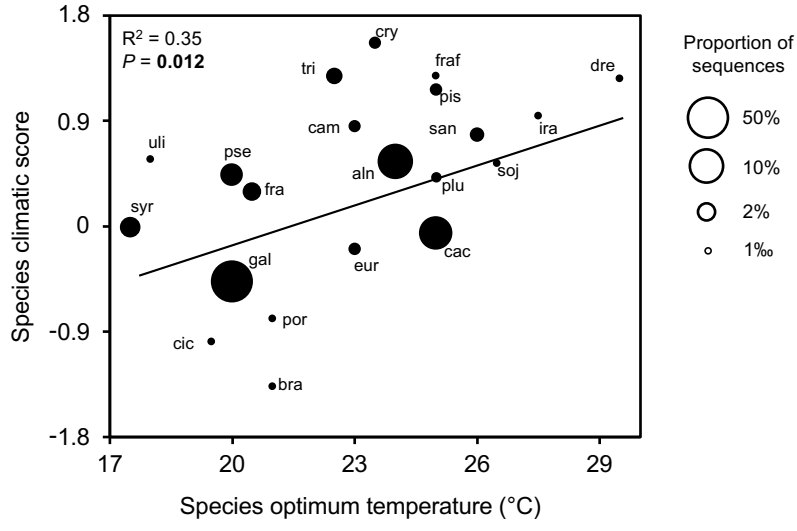

Figure 6. Association between the optimum temperature of each species and the species climatic score. The species climatic score was calculated as the climatic score of the sites where the species was present, weighted by the proportion of sequences of the species at that site. The climatic score was obtained from the first axis of a Principal Component Analysis that includes the temperature and precipitation of all sampling sites (**paper III**). A climatic score with a value of 0 corresponds to the average temperature and precipitation of all sampling sites. The R^2 and the *P*-value (*P*) is based on a linear regression weighted by the proportion of total reads for each of the species obtained in **paper III**. Only terrestrial species (not belonging to phylogenetic clades 6 or 9) were included in this analysis. bra, *P. brassicae*; cic, *P. cichorii*; por, *P. porri*; gal, *P. gallica*; eur, *P. europaea*; syr, *P. syringae*; fra, *P. fragariae*; plu, *P. plurivora*; pse, *P. pseudosyringae*; soj, *P. sojae*; aln, *P. alni* (species complex); uli, *P. uliginosa*; san, *P. sansomeana*; cam, *P. cambivora*; ira, *P. iranica*; pis, *P. pisi*; dre, *P. drechsleri*; fraf, *P. fragariaefolia*; tri, *P. trifolii*; cry, *P. cryptogea*.

5.3 Environmental filtering shaping *Phytophthora* communities

In accordance with the criteria of Cadotte & Tucker (2017), the functional-trait analysis of *Phytophthora* communities performed in **paper III**, could provide information on the role environmental filtering in shaping *Phytophthora* communities. In **paper III**, the values of the functional richness of the observed terrestrial communities were compared with a null expectation obtained from all the pool of species and traits obtained along the gradient, as performed in Lamanna et al. (2014). The results showed that 20% of the terrestrial *Phytophthora* communities displayed a higher clustering level than expected by the null expectation, and most (64%) of these communities were composed by only three species, and tended to appear in dry and cold areas. These results

suggest that there may be an effect of environmental filtering in 20% of the terrestrial *Phytophthora* communities within the studied climatic gradient. This proportion of communities affected by environmental filtering seems consistent with the results of Lamanna *et al.* (2014), who observed that environmental filtering decreased among plant communities with increasing latitude (70% versus 40% in tropical and temperate forests, respectively). Nevertheless, there might be other processes besides environmental filtering shaping *Phytophthora* communities. In fact, we observed low relative abundances, rather than absences, of some species not creating asexual structures in cold and dry regions. It could therefore be speculated that, in some cases, species displaying asexual resistant structures or low optimum temperatures can outcompete other species not displaying such traits.

The results of **paper III** seem to suggest a partial effect of the environmental filtering as defined by Cadotte & Tucker (2017) on *Phytophthora* communities. Supporting these findings, the results of **paper II** suggest an effect of environmental filtering (*sensu stricto*) (Kraft et al., 2015) on single *Phytophthora* species. As shown in **paper II**, *P.* x *alni* could not colonize areas with low temperatures, presumably owing to its low physiological tolerance to long frost periods. Assuming that *P.* x *alni* has the same dispersal potential as *P. uniformis*, **paper II** may provide an example of low temperatures acting as an environmental filter (*sensu stricto*) against the establishment of *P.* x *alni* in cold areas of Sweden.

Some other aspects of the environmental filtering are still open for discussion. Recently, scientists have questioned the possibility to infer the "pure" effect of environmental filtering on macroorganisms such as plants or animals, because they never interact with the environment alone, but together with the microorganisms that they harbour (Aguilar-Trigueros, Rillig & Ballhausen, 2017). In the case of pathogenic *Phytophthora* species, the same caveat may apply, because their interaction with the abiotic environment usually occurs mediated by a plant host. Consequently, free living microorganisms might be the only case where a pure environmental filtering could be observed (Aguilar-Trigueros, Rillig & Ballhausen, 2017). In the case of *Phytophthora*, free living organisms would perhaps correspond to saprophytic *Phytophthora* species, that usually belong to clade 6 or 9 (Marano et al., 2016). Future studies may clarify to which extent there is a "pure" effect of environmental filtering on assemblies of *Phytophthora*, and which may be the abiotic factors shaping these communities.

6 The potential of alders to adapt to *P. alni*

The fourth objective of this thesis was to assess the long-term impact of invasive *Phytophthora* species on host populations. To address this objective, the *P. alni*-*Alnus glutinosa* pathosystem was used to study whether alder trees have the potential to adapt to species of the *P. alni* complex. This could provide insights into the long-term sustainability of alder populations in face of increasing *Phytophthora* arrivals.

6.1 Signs of natural selection in alder populations invaded by *P. alni*

Species of the *P. alni* complex were first reported in Sweden in the 1990s (Olsson, 1999). Although both *P.* x *alni* and *P. uniformis* were reported almost simultaneously, it is still unknown if they arrived to Sweden at the same time. Currently, *P.* x *alni* and *P. uniformis* are widespread causing mortality across numerous riverbank alder-stands in Southern Sweden (hereinafter invaded sites) (**paper II**), in which healthy looking trees were observed nearby dead individuals. The *P. alni*-alder pathosystem could be a good model to study signs of natural selection exerted by invasive pathogens. Species of the *P. alni* complex are mainly restricted to riverbanks, and therefore alders growing far away from rivers (hereinafter uninvaded sites) might reflect the genetic composition of the populations before the invasion. In **Paper IV**, the susceptibility of progenies of trees from *P.* x *alni* and *P. uniformis* invaded and uninvaded sites was compared by performing *in vitro* inoculations. The results of the inoculation experiment showed that the survival of progenies from *P. uniformis* invaded sites was significantly higher than the survival of progenies from uninvaded sites. By contrast, there was not a difference in survival between *P.* x *alni* invaded and uninvaded sites (Fig. 7).

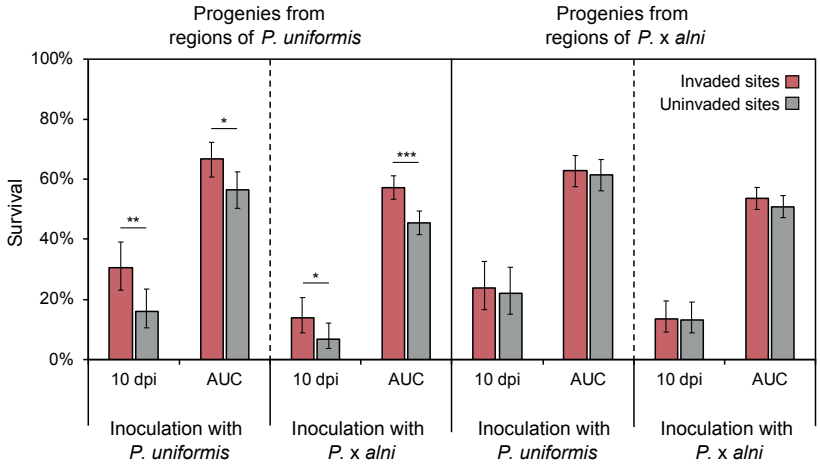

Figure 7. Results of the inoculation experiment of **paper IV**. Survival rate 10 days post inoculation (dpi) and survival area under the curve (AUC) for progenies obtained from invaded and uninvaded sites, after being inoculated with either *P.* x *alni* or *P. uniformis*. Bars represent 95% confident intervals. Asterisks represent significant differences, "***" *P*-value < 0.001, "**" *P*-value < 0.01, "*" *P*-value < 0.05.

The differences in survival between *P. uniformis*-invaded and uninvaded sites pointed to an effect of natural selection. It could be that the most susceptible genotypes have been already eliminated, thus surviving trees in invaded sites have higher resistance than uninvaded populations. To explore if resistance against *P. uniformis* had a genetic component, the broad sense heritability (H^2) of the resistance against *P. uniformis* was obtained for families coming from invaded and uninvaded sites. The broad sense heritability could be defined as the portion of the variation of a phenotypic trait (e.g., resistance) attributable to genetic factors. In theory, uninvaded populations where resistant and susceptible genotypes coexist, would display higher genetic variation associated with resistance (i.e., higher H^2) than invaded populations, where susceptible genotypes might have been already eliminated. Mousseau & Roff (1987) revealed in their study that traits related to fitness, presumably under strong selection pressure, display lower heritability that other traits, because selection has already acted on them. **Paper IV** showed that the broad sense heritability of the genetic resistance against *P. uniformis* was significantly lower in invaded sites than in uninvaded, again suggesting that natural selection might have occurred in sites invaded by *P. uniformis*. By contrast, this difference in broad sense heritability was not found between *P.* x *alni* invaded and uninvaded progenies (Fig. 8). In addition, the broad sense heritability of resistance against

P. uniformis was significantly higher than against *P.* x *alni* in uninvaded populations (Fig. 8). It could be speculated that, because of low genetic variation in resistance, most of the mortality caused by *P.* x *alni* would happen at random between naïve genotypes, perhaps explaining the lack of natural selection in *P.* x *alni* invaded sites.

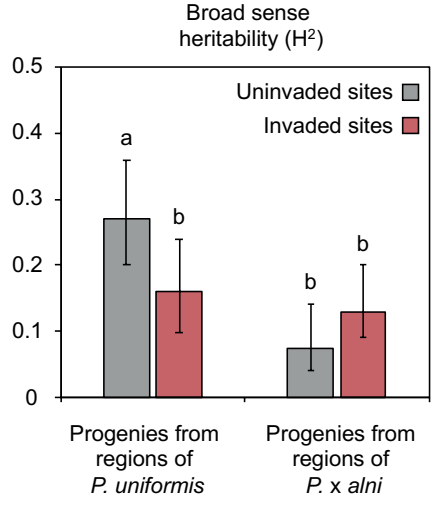

Figure 8. Differences in broad sense heritability (H^2) of progenies of alder trees from *P. uniformis* and *P.* x *alni* invaded and uninvaded sites. For progenies of each region, the broad sense heritability of resistance against the pathogen present in that region was calculated. Bars represent the 90% quantile. The mean and 90% quantile were obtained after randomizing the progenies in invaded and uninvaded sites by subsampling with replacement 100 000 times. After calculating the differences of H^2 between invaded and uninvaded populations, H^2 was considered significant when the "0" value was not included in the lower 5% quantile ($P < 0.05$). Significant differences were represented by different letters.

6.2 What is needed for adaptation? Genetic variation in resistance or low pathogenicity?

Paper IV showed that *P.* x *alni* was more aggressive than *P. uniformis*, in accordance with previous studies (Brasier & Kirk, 2001). It was then striking that natural selection was observed in *P. uniformis* invaded sites, but not in *P.* x *alni* invaded sites, because higher aggressiveness is usually connected with stronger selection pressure (Parker & Gilbert, 2004). However, aggressive pathogens such as *P.* x *alni* might produce short duration infections, decreasing the number of infectious trees and therefore the transmission rate in the population, a phenomenon explained by the virulence trade-off hypothesis (Alizon et al., 2009). Therefore, low genetic variation in resistance, and decrease

of transmission owing to high aggressiveness, could constitute two non-exclusive processes explaining the absence of natural selection exerted by *P.* x *alni*.

In **paper IV**, these two processes were tested by creating simulations in which the (i) randomness of the selection, as a consequence of genetic variation in resistance, and (ii) the transmission rate, as a result of the virulence trade-off hypothesis were manipulated. The simulation parameters were obtained from the results of the *in vitro* inoculations. The results of the simulations in which only the randomness of selection was considered showed a 12% higher gain of resistance after invasion of *P. uniformis* than after invasion of *P.* x *alni.* This simulation showed that to obtain a similar gain for *P.* x *alni* than for *P. uniformis*, a 3.4 times longer time lapse would be needed. When the decrease of transmission rate of *P.* x *alni* was also included in the simulation, the gain of resistance following a *P. uniformis* invasion was 13.9% higher than following a *P.* x *alni* invasion. Naïve populations would require a 4 times longer time lapse to obtain a similar gain to *P.* x *alni* than to *P. uniformis*.

Taken altogether, the results of the simulations suggest that low genetic variation in resistance of alders to *P.* x *alni* could slow natural selection. The fact that *P.* x *alni* is more pathogenic than *P. uniformis*, and therefore it may be less efficient in dispersing, may have slowed the selection, but this mechanism seems to be of a lesser importance than low genetic variation in resistance of hosts. The presence of genetic variation in resistance against *P. uniformis* suggests that alder trees could potentially adapt to this species (Strauss, Lau & Carroll, 2006). Altogether, the findings of **paper IV** are consistent with the theoretical predictions suggesting that varying levels of resistance are needed for the success of natural selection, and therefore for evolution (Altizer, Harvell & Friedle, 2003; Parker & Gilbert, 2004; Strauss, Lau & Carroll, 2006; Ennos, 2015; Budde et al., 2016).

7 Conclusions and future perspectives

This thesis aimed at studying the introduction, establishment and spread of *Phytophthora*, as well as the long-term impact of invasive *Phytophthora* species on host populations. The main findings of this thesis were:

1. Five *Phytophthora* species were considered invasive in Sweden according to the definition of Blackburn et al (2011), namely *P. plurivora*, *P. cambivora*, and *P. cactorum, P.* x *alni, and P. uniformis* (**paper I** and **II**).
2. Several species of *Phytophthora* were reported for the first time in Sweden (**paper I**).
3. The occurrence of three of the invasive *Phytophthora* species and the diversity of *Phytophthora* communities were significantly higher in urban locations than in natural forests (**paper I**), pointing at human activities as pathways during invasion.
4. The distribution of species of the *P. alni* complex was associated with temperature. The cold susceptible *P.* x *alni* was found in the areas of the country with mildest winters, whereas *P. uniformis* was found all across the survey area (**paper II**).
5. The metabarcoding setup based on PacBio sequencing developed in this thesis provided a higher resolution than traditional baiting, and allowed a longer read length than previous DNA-based setups (**paper III**).
6. The diversity patterns of terrestrial *Phytophthora* communities were associated with total annual precipitation, whereas variation on diversity of aquatic communities was associated with mean annual temperature and water chemistry, suggesting that the environment where *Phytophthora* develop most of their live cycle may modulate climate constrains differently (**paper III**).

7. The ability to create asexual survival structures and low cardinal temperatures for growth were traits associated with the establishment of *Phytophthora* in Sweden (**paper I**).
8. The *Phytophthora* communities located in areas with low mean annual temperature and low total annual precipitation were dominated by species able to create asexual survival structures and with low optimum growth temperatures (**paper III**).
9. The effect of environmental filtering was observed in 20% of terrestrial communities, which displayed a higher trait clustering level than expected by random. By contrast, environmental filtering was only observed in 3% of aquatic communities (**paper III**).
10. Heritability of resistance against *P. uniformis* was higher than against *P.* x *alni*, suggesting that alders might have enough genetic background to adapt faster to *P. uniformis* than to *P.* x *alni* (**paper IV**).
11. Alder populations invaded by *P. uniformis* displayed lower susceptibility and lower broad sense heritability than uninvaded populations, pointing to an effect of natural selection. By contrast, this pattern was not observed in *P.* x *alni* invaded sites (**paper IV**).
12. The putative lack of selection exerted by *P.* x *alni* seemed to be more associated to a low of genetic variation in resistance (low heritability) than to a decrease in transmission rate owing to its high aggressiveness (virulence trade-off hypothesis) (**paper IV**).

These results could constitute the baseline of future studies of forest *Phytophthora* species. For instance, the definition of invasive species that we used in this thesis, could be applied when defining invasive *Phytophthora* species in other regions. This could contribute to a standardization of concepts when studying *Phytophthora* invasions across regions.

The role of humans as pathways during invasion suggests that the focus of surveys, performed both by researchers and citizens, should be targeted towards populated areas (Hulbert et al., 2017). In fact, such as initiative has recently been developed in Sweden by Witzell and Cleary (www.phytophthora.se). By sampling in these areas, *Phytophthora* could be detected in the early stages of invasion.

The metabarcoding setup developed in **paper III**, could be used when studying *Phytophthora* communities in water in other regions. In the near future, this setup should be validated in soil samples, and could be used to study *Phytophthora* communities in soil and water. By doing so, it could be confirmed whether diversity of terrestrial and aquatic *Phytophthora* species is associated with different climatic factors, as suggested in **paper III**. The metabarcoding

approach developed in **paper III** can also be used to study the diversity of *Phytophthora* communities along a larger gradient than the one of **paper III**.

The use of functional traits of *Phytophthora* could be used to study the distribution of species globally. This could enable predictions of establishment of certain species based on traits. Given the relatively low amount of described species, and the availability of physiological traits on species descriptions, a trait database could be created for all *Phytophthora* species, and this database could be used to study the dominant traits of *Phytophthora* communities in different latitudes.

The inoculation method used in **paper IV** could be used in other studies when screening the resistance of alder or other hosts to *Phytophthora* species. Within the genotypes that were tested in **paper IV**, those with highest levels of resistance could be perhaps used as the pool for regeneration in areas with high mortality.

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Popular science summary

The genus *Phytophthora* contains a large array of species of microorganisms, many of which cause diseases on economically or aesthetically important plants around the world. During the last decades, some species of *Phytophthora* have been reported killing forest trees in Southern Sweden, such as alders, oaks, and beeches. Because of the potential impact these species may have on Swedish forest health, this thesis studied their pathways of introduction and spread, their patterns of distribution, and their long-term impact on tree populations.

Scientists have suggested that *Phytophthora* species are dispersed between and within countries with the trade and transport of plants. If so, we would expect a higher number of *Phytophthora* species in environments heavily impacted by plant logistics. To investigate this, a survey was conducted in nurseries, city forests, grazing areas, and natural forests. We observed the largest amount of species in nurseries, supporting previous scientific findings. Moreover, forests located in cities had more *Phytophthora* species than natural forests, suggesting that human activities that occurs in populated areas, such as out-planting of infected plants, can contribute to the dispersion of *Phytophthora*. Three *Phytophthora* species identified in the survey are not native in Sweden, but were found in every investigated habitat: *P. plurivora*, *P. cambivora*, and *P. cactorum*. Since these species have been able to enter, establish, proliferate and spread across a variety of environments to which they would normally be alien, they can be considered "invasive species", and might require special management practices to be controlled.

Although many *Phytophthora* species seem to be dispersed with the help of humans, not all of them are fit to establish permanently in the Swedish environment. We wanted to discern how climatic variations impact the number of present species. To this end, we studied the distribution of *Phytophthora* species along a climatic gradient, from the southern, warmer parts of Sweden to areas located more than 60° latitude north. It was found that colder areas of the country had less *Phytophthora* species than the warmer areas, suggesting that

there might be some species not able to tolerate cold temperatures. Moreover, those species that were commonly present in cold areas had two particular features: first, they were able to create a morphological structure in their mycelia that might allow them to survive low temperatures, and second, they had low optimal temperatures for growth when cultivated in the laboratory. These findings could be used to predict which species are likely to establish in Sweden currently, and which ones will be able to establish in the future when temperatures raise as a consequence of climate change.

Once *Phytophthora* species have been introduced and established, they can compromise the sustainability of some tree populations. It is important to know the actual long-term impact of *Phytophthora* on trees, so that resources can be allocated towards those *Phytophthora* species that are a real threat. Thus, we wanted to know whether Swedish trees are able to adapt to newly introduced *Phytophthora* species; in other words, whether natural selection for resistance occurs on invaded tree populations. To address this, seeds were collected from alder trees that grew in areas invaded by each of two alder-specific *Phytophthora* species (*P. uniformis* or *P.* x *alni*), and from trees in areas without the pathogens. These uninvaded areas reflect how the population of alder trees looked before the first arrival of alder-*Phytophthora*, and invaded areas reflect a stage in which the natural selection has already occurred; i.e., if the trees from the invaded areas display a stronger resistance towards the pathogen, it is assumed natural selection has removed the weaker genotypes. The seedlings were grown and inoculated with each of the pathogens. It was observed that the seedlings from sites invaded by *P. uniformis* were more resistant to the pathogen than progenies from uninvaded sites, but this pattern was not observed on trees coming from *P.* x *alni* sites. Thus, it appears that there is a natural selection for resistance to *P. uniformis*: the most vulnerable genotypes have already been killed, and the surviving trees are on average more resistant against the pathogen than uninvaded trees. Further analysis supported this observation by showing that the resistance of alder trees against *P. uniformis* had a stronger genetic component than against *P.* x *alni*. Therefore, it seems possible that alder trees will be able to adapt to *P. uniformis* in the long run, but perhaps not to *P.* x *alni*.

In summary, the results of this thesis suggest that many *Phytophthora* species seem to be introduced in Sweden with the import of plants, and likely further dispersed in new forests by out-planting of infected nursery material. Once introduced, the species able to create resilient mycelial structures and tolerate cold temperatures are more likely to establish in Sweden. If the pathogens are able to spread across different tree populations, the long term sustainability of hosts will depend on whether parts of this resistance is controlled by genetic factors, allowing for natural selection for less susceptible individuals.

Populärvetenskaplig sammanfattning

Släktet *Phytophthora* består av en lång rad arter av mikroorganismer, varav många orsakar sjukdomar på växter viktiga för jordbruket, handeln eller med andra estetiska eller kulturella värden. Under de senaste årtiondena har *Phytophthora*-arter rapporterats angripa träd i södra Sverige, som al, ek och bok. Dessa patogener befaras få omfattande inverkan på svensk skogshälsa. I denna avhandling studeras därför deras introduktions- och angreppsvägar, spridningsmöster, och långsiktiga effekt på svenska trädpopulationer.

Det har antagits att *Phytophthora*-arter sprids mellan och inom länder via handel och transport av växter. Om så vore fallet, skulle vi förvänta oss ett större antal sådana arter i områden som på olika sätt påverkats av växtlogistik. För att undersöka det, studerade vi artrikedomen i plantskolor, stadsskogar, beteshagar, floder och naturliga skogar. Vi noterade flest arter i plantskolor, vilket stöder tidigare observationer. Vi såg också att stadsskogar hade fler *Phytophthora*-arter än naturliga skogar, vilket också indikerar att människans handhavande av växtmaterial bidrar till spridningen av patogenerna. I undersökningen hittades tre *Phytophthora*-arter som inte är naturligt förekommande i Sverige, men ändå påträffades i samtliga miljöer; *P. plurivora*, *P. cambivora*, och *P. cactorum*. Eftersom dessa arter introducerats och etablerats i Sverige, och därefter dessutom kunnat fortplantas och spridas över olika ekosystem till vilka de vanligtvis skulle anses främmande, kan de beskrivas som invasiva arter, och kan komma att kräva särskilda skötselplaner för att kontrolleras i framtiden.

Även om många *Phytophthora*-arter kan introduceras och spridas med mänsklig hjälp, är inte alla kapabla att permanent etablera sig i den svenska miljön. Vi ville utreda om klimatvariationer påverkade antalet närvarande arter. För att åstadkomma detta studerade vi spridningen av *Phytophthora* längs en klimatgradient, från södra Sverige upp till norr om den 60 breddgraden. Vi kunde se att kallare delar av landet hade färre arter, vilket indikerar att vissa arter inte klarar av låga temperaturer. Dessutom såg vi att de arter som var vanligt förekommande i kalla områden hade två gemensamma egenskaper: de kan bilda

en morfologisk mycelstruktur som möjliggör för dem att överleva i kyla, och de hade låga temperaturer för optimal tillväxt under växtförsök på laboratoriet. Dessa upptäckter kan användas för att förutse vilka arter som har bäst förutsättningar för att etablera sig i Sverige i dag, och vilka som kan komma att göra det i framtiden när klimatförändringarna orsakat varmare temperaturer.

När väl *Phytophthora*-arter etablerats i en trädpopulation kan de orsaka omfattande skador. Det är viktigt att känna till de individuella arternas långsiktiga påverkan på träden, så att resurser kan riktas mot de som utgör allvarligast hot. Därför ville vi veta om de svenska träden är kapabla att anpassa sig till nyintroducerade arter; d.v.s., om det pågår ett naturligt urval för motståndskraft i invaderade trädpopulationer. För att ta reda på det samlade vi in frön från alar i områden som invaderats av de al-specifika *Phytophthora*-arterna *P. uniformis* och *P. x alni,* samt från träd i områden som inte exponerats för patogenerna. Dessa icke invaderade områden motsvarar hur alpopulationerna såg ut innan *Phytophthora*-arterna introducerades, och de invaderade områdena motsvarar hur de ser ut när det naturliga urvalet verkat. Om träden från de invaderade områdena har större motståndskraft, förmodar vi således att det naturliga urvalet sållat bort de mer känsliga genotyperna. Fröna planterades ut och de resulterande plantorna inokulerades med respektive patogen. Vi noterade att plantorna från områden som invaderats av *P. uniformis* var mer motståndskraftiga mot patogenen än de från icke invaderade områden, men också att detta mönster inte återfanns i träd från *P. x alni*-områden. Därför verkar det som att det pågår ett naturligt urval för motståndskraft mot *P. uniformis*, där de svagare individerna redan dött och de överlevande i genomsnitt är starkare än individer från icke invaderade områden. Vidare analyser stöder denna observation genom att visa att motståndskraften mot *P. uniformis* har en större genetisk komponent än den mot *P. x alni*. Därför verkar det troligt att alen kommer att kunna anpassa sig till *P. uniformis* på lång sikt, men inte till *P. x alni.*

Sammanfattningsvis visar resultaten från denna avhandling att många *Phytophthora*-arter verkar introduceras i Sverige genom importen av växter, och sannolikt sprids vidare i nya skogar genom att infekterat plantskolematerial planteras ut i skogen. När de väl introducerats, har arter som kan bilda härdiga mycelstrukturer och växer bra vid låga temperaturer bättre förutsättningar för att etableras i Sverige. Om patogenerna kan spridas över olika trädpopulationer, kommer värdarnas långsiktiga överlevnad bero på deras naturliga motståndskraft, och huruvida denna kontrolleras av genetiska faktorer som möjliggör naturligt urval för mindre mottagliga individer.

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There are always more reasons to be thankful, so I will leave this page without a full stop