Characterising plant pathogen communities and their environmental drivers at a national scale

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General abstract

Plant pathogens play a critical role for global food security, conservation of natural ecosystems and future resilience and sustainability of ecosystem services in general. Thus, it is crucial to understand the large-scale processes that shape plant pathogen communities. The recent drop in DNA sequencing costs offers, for the first time, the opportunity to study multiple plant pathogens simultaneously in their naturally occurring environment effectively at large scale. In this thesis, my aims were (1) to employ next-generation sequencing (NGS) based metabarcoding for the detection and identification of plant pathogens at the ecosystem scale in New Zealand, (2) to characterise plant pathogen communities, and (3) to determine the environmental drivers of these communities.

First, I investigated the suitability of NGS for the detection, identification and quantification of plant pathogens using rust fungi as a model system. I compared two fundamentally different metabarcoding methods along with traditional cloning approaches. I found a phylogenetic bias driven by metabarcoding primer design, but no effect of sequencing method per se. This result supports the usage of metabarcoding for large scale detection and quantification of plant pathogens. At the same time it underpins the importance of the primer choice for metabarcoding, which can result in the failure to detect particular plant pathogens.

After confirming the semi-quantitative nature of metabarcoding for the large scale detection of rust fungi, I expanded the approach to fungi, oomycete and bacteria plant pathogens across a wide range of different land use types, sampling soil, roots and leaf substrates. I found a higher species richness of plant pathogens in agricultural than in natural systems across substrate and pathogen taxa. In contrast, there was almost no variation in composition among plant pathogen communities from site-to-site, suggesting a similar species turnover within land uses. I detected plant pathogen groups in the substrate types and land use categories as expected based on known ecology or literature. This strongly indicates that the metabarcoding approach worked well for the overwhelming majority of fungi, oomycete and bacteria plant pathogens.

Next, I quantified the relative importance of environmental drivers for plant pathogen communities and richness. The composition of plant species (plant community at site) could generally explain most of the variance in pathogen community and richness, even after accounting for other environmental parameters such as geomorphology, climate, land use and soil. These results suggest an unequal relationship among plant pathogen, plant and environment, and furthermore that any changes in plant pathogen communities as well as richness will mostly be related to changes in plant communities, rather than direct effects of the abiotic environment.

Lastly, I applied network analysis in order to identify non-random and predictable co-occurrence patterns of plant pathogens. I demonstrated that the overwhelming complexity of above and belowground plant pathogens across different ecosystems can be reduced into distinct plant pathogen communities which in turn can be more easily studied than the vast number of individual plant pathogens. The taxonomic identity of the pathogen seemed to play a far greater role in the formation of these plant pathogen communities than the substrate. How these plant pathogen communities will shift in a changing world remains subject to future research. However, predictable and defined plant pathogen communities will greatly help us anticipate future impacts on food and ecosystem production.

The overall results of this thesis showed that NGS metabarcoding and network theory can successfully be applied to gain new insights about plant pathogens at an ecosystem scale. NGS metabarcoding emerged as an appropriate tool particularly for studying and predicting entire plant pathogen communities. The ecological community approach to studying plant pathogens has the potential to bring us one step closer to sustainable solutions to global food security and ecosystem services in the immediate future.

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Chapter 1

General introduction

1.1 Background: Arising threats from plant pathogens

Since the dawn of agriculture, farmers have been faced with multitudes of harmful organisms which diminish yields, collectively called pests (Flood, 2010; Bebber *et al.*, 2013). These pests are comprised of weeds (i.e. competitive plants), animal pests and plant pathogens (fungi, oomycetes, bacteria, viruses and viroids) (Oerke, 2006). Each of them account for losses of the global harvest (Bullock *et al.*, 2017). Weeds and animal pests are often the most easily observed pests. However, history has shown that some of the most severe economic, ecological and social effects have been driven by plant pathogens (Money, 2006).

For example, the oomycete *Phytophthora infestans* (causing potato blight) struck Europe unexpectedly in the 1840s and was a major culprit in Ireland's 'Great Famine' forcing millions of Irish to emigrate and causing social upheaval (Goss *et al.*, 2014). The pathogen became especially virulent due to (i) lack of resistance in the host plant, a varietal white potato called the 'Irish Lumper', (ii) high dependency of the Irish on this potato variety for sustenance which lead to potato monocultures, (iii) and conducive climate for the oomycete. Still today plant pathogens challenge global food security, where fungal and oomycete plant pathogens have the biggest impact causing together up to 20% loss of the harvest around the world (Bebber & Gurr, 2015).

Islands are particularly susceptible to plant disease outbreaks (Simberloff, 1995; Wikelski *et al.*, 2004). As an isolated landmass which has only been inhabited for about 750 years (Wilmshurst *et al.*, 2008; Thomson, 2011), New Zealand has been subject to various severe plant pathogen invasions (Pennycook *et al.*, 1989; Ridley *et al.*, 2002; McKenzie, 2004). By the turn of the 20th century, yellow leaf disease caused by a phytoplasma bacterium had destroyed New Zealand's flax (*Phormium*) export industry and leaf rust *Puccinia recondita* had made the growing of wheat uneconomic (Dingley, 1969; Liefting *et al.*, 2007). Due to the gall forming rust *Uromycladium notabile*, *Acacia* plantations grown to produce tannins for the leather industry,

were abandoned by 1930 (McKenzie, 2004). Kiwifruit canker caused by the bacteria *Pseudomonas syringae* pv. *actinidiae* (PSA) had a devastating impact on the kiwifruit industry immediately after its arrival in 2010 (Everett *et al.*, 2011; Vanneste, 2012, 2017) and still costs New Zealand between NZ$310 million and NZ$410 million per year, with annual costs predicted to double over the next 15 years (Greer & Saunders, 2012). Root rotting fungi of the *Armillaria* genus (including the introduced *Armillaria limonea* and *Armillaria hinnulea*) had a high destructiveness on *Pinus radiata* plantations and are still challenging New Zealand's pine forestry industry (Wargo *et al.*, 1985; Chou, 1991; Brockerhoff & Bulman, 2014).

The impact of pathogens on yield has always been of interest and therefore subject of studies in New Zealand (Carpenter *et al.*, 1999; Madden *et al.*, 2000; Tate *et al.*, 2000; Beresford & Mackay, 2012; Beresford *et al.*, 2012) and around the world (Abawi & Widmer, 2000; Pinkerton *et al.*, 2000; Oerke, 2006). However, their influence on less economically important species is still poorly understood, and we are only beginning to grasp the manner in which pathogens can affect the structure of whole ecosystems (Ellison *et al.*, 2005; Van Der Heijden *et al.*, 2008; Loo, 2009; Fisher *et al.*, 2012; Burgess *et al.*, 2017a,b). The oomycete *Phytophthora agathidicida*, for example, is responsible for the dieback of New Zealand's iconic kauri tree (Beever *et al.*, 2009; Waipara *et al.*, 2013; Bassett *et al.*, 2017). Exotic rust fungi are reported from a wide range of New Zealand's indigenous trees placing its unique forests in jeopardy (Ridley *et al.*, 2000). A recent example of such an arrival is myrtle rust (*Austropuccinia psidii*). Its impact on New Zealand's native flora is still difficult to estimate (Fernandez Winzer *et al.*, 2018). However, the impact of introduced myrtle rust on a wide range of members of the Myrtaceae around the world has been devastating, for example on *Eugenia koolauensis* in Hawai'i (Uchida *et al.*, 2006; Glen *et al.*, 2007; Giblin, 2013; Carnegie *et al.*, 2016; McTaggart *et al.*, 2016; Fernandez Winzer *et al.*, 2018). Not only in New Zealand but worldwide, the impacts of plant pathogens on natural ecosystems are yet largely unexplored (Desprez-Loustau *et al.*, 2007b; Gilbert, 2002; Helfer, 2014; Mitchell *et al.*, 2014).

Comprehending the drivers of plant pathogen success is crucial for choosing appropriate measures in order to preserve economically and ecologically valuable species, and secure the provision of future ecosystem services. A central question here is what is the relative importance of environmental drivers for plant pathogens at large scale. Solving this question requires prior knowledge about how we can detect plant pathogens at large scale and how plant pathogen diversity patterns change with different landscapes. Moreover, we need to find appropriate tools

that can capture and simplify the complexity of plant pathogens and their ecosystem interactions. Narrowing down the overwhelming complexity to key elements that can be studied is essential to eventually supply general solutions for future challenges such as food security or the provision of ecosystem services.

1.2 The "next-generation" detection of plant pathogens

The detection of plant pathogens has traditionally been a problem due to their cryptic nature (Atkins & Clark, 2004; Newton *et al.*, 2010). Classical detection of plant pathogens based on morphological criteria of the plant pathogen or the interpretation of visual symptoms of the diseased plant is time consuming and biased by the skills and taxonomic knowledge of the researcher (McCartney *et al.*, 2003). In contrast, molecular based detection methods (despite having their own drawbacks (Ari & Arikan, 2016)) allow rapid and standardised results (Schaad & Frederick, 2002; Thynne *et al.*, 2015).

There is a range of diverse molecular techniques suited to identify and survey multiple different co-occurring plant pathogen species at large scale (Rastogi & Sani, 2011; Jones *et al.*, 2009; Vanwonterghem *et al.*, 2014). Two complementary molecular methods most commonly practised are (1) the construction of clone libraries followed by 'first-generation sequencing' methods such as Sanger sequencing (Sanger *et al.*, 1977) and (2) 'next-generation sequencing' (NGS) such as 454 pyrosequencing (Jones *et al.*, 2009), Ion Torrent (Brown *et al.*, 2013) or Illumina sequencing (Siddique & Unterseher, 2016). The clone library method enables the generation of long (several kbp) DNA sequence reads and thus provides high quality species community data when costs are not limiting (Taylor *et al.*, 2014). However, because cloning is labour, time and cost intense, many researchers are increasingly relying on NGS which has the potential to allow much greater replication and sequencing depth at lower costs.

NGS describes a number of different modern sequencing approaches of which 'Ion Torrent' and 'Illumina' are currently two of the most available platforms (Goodwin *et al.*, 2016; Levy & Myers, 2016). These NGS platforms, (1) do not involve bacterial cloning of target DNA fragments with plasmid vectors but use NGS libraries in a cell free system instead, and (2) produce, in lieu of hundreds, many millions of sequencing reactions in parallel (Van Dijk *et al.*, 2014). The accuracy obtained by NGS is generally lower than clone libraries due to shorter NGS reads, higher error rates and, paradoxically, the accumulation of errors in large data sets (Dickie, 2010). However, NGS provides a labour, cost and time effective sequencing method based on

the high-throughput of sequences (Goodwin *et al.*, 2016). The lower cost of NGS compared to earlier techniques offers a wide range of novel applications, of which one subset is termed metabarcoding (Holdaway *et al.*, 2017b). Metabarcoding combines DNA based identification with high-throughput sequencing and thereby allows the consideration of vast species numbers within a sample (Taberlet *et al.*, 2012b; Cristescu, 2014). The typical procedure is to mass-amplify short barcode genes with universal PCR primers from a mixed DNA sample. The PCR product is then sequenced with high-throughput DNA sequencing. The large numbers of obtained amplicons (i.e. NGS raw data output) are assigned to so called operational taxonomic units (OTUs) by an algorithm, either based on similar sequences to reference OTUs, or on clustering sequences based on a pre-defined similarity threshold. The representative sequence of each OTU is checked for a match in a reference library resulting in a large list of species names from the original mixed DNA sample (Lindahl *et al.*, 2013). By allowing the identification of vast species numbers, the application of metabarcoding facilitates the survey of communities and further helps understand ecosystem functioning (Kress *et al.*, 2015; Wood *et al.*, 2017).

Metabarcoding has helped assess the diversity of animals, plants, zooplankton, bacteria and fungi, and their community response to changing environmental conditions (Schmidt *et al.*, 2013; Tu *et al.*, 2015; Cordier *et al.*, 2017; Deiner *et al.*, 2017; Djurhuus *et al.*, 2018; Laroche *et al.*, 2018). With respect to plant pathogens, DNA metabarcoding already helps in identifying invasive plant pathogen species. Comtet *et al.* (2015) highlight metabarcoding as a promising tool for the early detection of known and unknown plant pathogens and as a complementary method which ameliorates early warning systems and biosecurity. Moreover, metabarcoding has the potential to reveal possible biocontrol agents (Poudel *et al.*, 2016). A big advantage of metabarcoding is its ability to overcome the difficulties in detecting low abundances of plant pathogen species (Comtet *et al.*, 2015; Nicolaisen *et al.*, 2017). Although the detection of DNA does not necessarily mean the detection of living organisms, metabarcoding has proven to facilitate the study of plant pathogens in a considerable number of cases. However, metabarcoding data of plant pathogens at large scales (regional, national) remains a significant challenge (Geisen *et al.*, 2015; Prigigallo *et al.*, 2016; Burgess *et al.*, 2017b). While metabarcoding holds promising potential for the detection and the monitoring of plant pathogens in their natural environment, it is important to better understand the strengths and possible limitations of this new approach.

1.3 Revealing large scale diversity patterns of plant pathogens

Once the advantages and pitfalls of the "next-generation" detection of plant pathogens are understood, applying the new approach across a wide range of environments can shed light on general diversity patterns of plant pathogens, which hitherto was simply not feasible to study with conventional methods (mainly due to labour and costs). Furthermore, the so obtained sequencing data can serve as a "baseline data bank" when dealing with future pathogen incursions and outbreaks.

Mounting evidence suggests that plant pathogens in nature are organised in communities, in which they are bound together by a shared environment and have the possibility to interact with each other (Singer *et al.*, 2016; Tollenaere *et al.*, 2016; Burgess *et al.*, 2017b). Thus, plant pathogen diversity and composition is most likely to change with pathogen taxa (Dung *et al.*, 2014; Lamichhane & Venturi, 2015; Susi *et al.*, 2015; Stopnisek *et al.*, 2016; Jung *et al.*, 2018) and along environmental gradients, including vegetation (Castello *et al.*, 1995; Lin, 2011; Piepenbring *et al.*, 2011; Mangelsdorff *et al.*, 2012; Hantsch *et al.*, 2013, 2014; García-Guzmán & Heil, 2014; Latz *et al.*, 2016) and land use (Datnoff *et al.*, 2007; Mangelsdorff *et al.*, 2012; Palti, 2012; Gossner *et al.*, 2016; Zhang *et al.*, 2017). However, knowledge about how plant pathogen diversity, such as richness and composition, changes belowground and aboveground, across different land use types and with different pathogen taxa is extremely scarce.

The "next-generation" detection of plant pathogens offers a chance to understand how plant pathogen communities change with different land uses and vegetation on broad scale. This will improve our general knowledge about pathogens, and is a crucial step in sustainably managing natural and productive ecosystems.

1.4 Identifying the relative importance of environmental drivers for plant pathogens

A further key prerequisite for choosing appropriate measures to manage plant pathogens, besides the understanding of how plant pathogen diversity and composition change at large scale, is the understanding of what drives those changes. Geomorphology, climate, land use, soil and plant communities are closely intertwined with each other and plant pathogens (Foley *et al.*, 2005; Boulangeat *et al.*, 2014; Bever *et al.*, 2015; Garrett *et al.*, 2015; Jantz *et al.*, 2015) and are

therefore able to shape plant pathogen communities. However, the contribution of the individual drivers remains unclear.

In particular, climate as driver of plant pathogens has recently gained public interest due to climate change (Garrett *et al.*, 2015; Bernreiter, 2016; Jones, 2016; Hulme, 2017). As mounting evidence shows, individual plant pathogen species are affected by warmer temperatures, higher levels of CO_2 and ozone, decreased rainfall and increased rainfall variability (Juroszek & Von Tiedemann, 2011; Luck *et al.*, 2011; Barbetti *et al.*, 2012; Ghini *et al.*, 2012; Siebold & Von Tiedemann, 2012; Tu *et al.*, 2015). Hot and dry summers, for example, favour the persistence of necrotrophic fungi (Sivasithamparam *et al.*, 2005). In contrast, several rust fungi reduce their urediniospore germination rate above 25 °C (Tapsoba & Wilson, 1997; Gilles & Kennedy, 2003; Johnson & Cummings, 2013), similar to the production of sporangia and chlamydospores in *Phytophthora ramorum* (Englander *et al.*, 2006). Moreover, warmer temperatures negatively influence plant resistance genes (Wright *et al.*, 2000). Water stress can increase plant susceptibility to pathogens (Mayek-Pérez *et al.*, 2002) or stimulate drought tolerance by the production of substances like sugars, prolines, putrescines, and antioxidants in infected plants (Xu *et al.*, 2008). Some models show wet environments to favour general pathogen-caused damage (Spear *et al.*, 2015). Climate parameters can even affect whole plant pathogenic groups. Models suggest populations of fungi, oomycetes and bacteria move polewards in a warming world, whereas viruses are not expected to move (Bebber *et al.*, 2013).

Plant diversity, encompassing the diversity of plant hosts, is another central driver of plant pathogens. Reductions in plant diversity and species richness are frequently associated with increased disease transmissions and outbreaks (Keesing *et al.*, 2010; Haas *et al.*, 2011; Cardinale *et al.*, 2012), while high plant diversity is hypothesised to buffer plant populations against widespread pathogen epidemics (Altizer *et al.*, 2003; Cardinale *et al.*, 2012). There are also reciprocal effects of plant pathogens on plant diversity, suggested by expanding the "Janzen-Connell hypothesis" (Janzen, 1970; Packer & Clay, 2000; Mangan *et al.*, 2010; Bever *et al.*, 2015; Teste *et al.*, 2017). Land use and geomorphology mainly impacts plant pathogens through interactions with environmental factors, such as altering microclimate and soil functions (Foley *et al.*, 2005), or by changing host plant communities and the plant diversity, for instance, by establishment of monocultures. Therefore, the effect of land use, soil and and geomorphology on plant pathogens is believed to be rather indirect.

One paradigm combining the relationship of the drivers of plant pathogens described

above is the classical disease triangle (Stevens, 1960). The three variables, pathogen, host and environment, are proposed to have the same influence and are therefore displayed in an equilateral triangle. However, recent studies increasingly highlight changing environmental conditions as more important (Francl, 2001; Scholthof, 2007; Grulke, 2011). Testing this long-standing general plant pathogen-host-environment relationship requires multi-host, multi-pathogen systems and a large scale approach including a wide range of environmental variables.

1.5 Quantifying and simplifying the complexity of plant pathogens and their ecosystems

Where which plant pathogens occur, how often, and why is of central importance to understanding plant pathogen community ecology and a sustainable provision of ecosystem services. In studying these questions, the greatest challenge today lies in capturing and simplifying the complexity of plant pathogens and their ecosystems. Such complexity includes not only abiotic but also biotic interactions with the environment.

While traditional models of plant pathology such as the Henle-Koch postulates assume plant diseases to be triggered by a single organism (Evans, 1976), this may capture only a minority of the interactions taking place and excludes interactions between plant pathogenic species or groups. Plant pathogens may affect each other reciprocally, resulting in positively or negatively correlated occurrence (Le May *et al.*, 2009; Dung *et al.*, 2014; Lamichhane & Venturi, 2015; Susi *et al.*, 2015; Stopnisek *et al.*, 2016; Abdullah *et al.*, 2017; Jung *et al.*, 2018), with interactions varying depending on the individual host-pathogen system and environment. Another layer of complexity is added by biotic interactions with other microorganisms that have been shown to significantly affect the occurrence of plant pathogens. For example, fungi from *Epicoccum* and *Trichoderma* are well known to contain not just plant pathogens but also endophytes that can have negative effects on plant pathogen through antibiosis and mycoparasitism (Harman *et al.*, 2004; Favaro *et al.*, 2012; Qian *et al.*, 2013).

This complexity of plant pathogen ecosystems, including the outcome of all biotic and abiotic interactions with the environment, can be described in co-occurrence networks, which can visualize potential relationships and shared environments between organisms (Jakuschkin *et al.*, 2016; Derocles *et al.*, 2018). While the application of co-occurrence networks in ecology have recently gained a lot of traction (Araújo *et al.*, 2011; Barberán *et al.*, 2012; Chagnon *et al.*, 2012;

Mougi & Kondoh, 2012; Bennett *et al.*, 2013; Heleno *et al.*, 2014; Menezes *et al.*, 2015; Coux *et al.*, 2016; Creamer *et al.*, 2016; Delgado-Baquerizo *et al.*, 2018), co-occurrence networks of plant pathogens have received less attention. Correlated co-occurrences do not mean causation, however, when used to describe the outcome of all processes involved, co-occurrence networks of plant pathogens have great potential to provide new insights and a better understanding of the ecological rules guiding plant pathogen community composition.

1.6 Thesis aim and research objectives

The goal of the present thesis was to find *novel tools to study the complexity of plant pathogens at the ecosystem scale*, then apply these tools to achieve *insights into the relative importance of environmental drivers for plant pathogen communities at ecosystem scale.* The gained knowledge about the new methods and the drivers will help improve plant pathogen management measures necessary for the future supply of global ecosystem services, particularly considering changes in climate and land use (Ghini *et al.*, 2012; Elad & Pertot, 2014; Matthews *et al.*, 2014; Garrett *et al.*, 2015; Bernreiter, 2016; Newbery *et al.*, 2016). The objectives of the present thesis were as follows, each of them dedicated a full chapter:

Objective 1: Investigate possible differences in detection and abundance of plant pathogens between new metabarcoding and traditional cloning approaches using rust fungi as a model system (Chapter 2).

Objective 2: Apply the metabarcoding approach for the detection of plant pathogens at large scale in order to reveal commonalities and differences in diversity patterns of fungi, oomycete and bacteria plant pathogens across land uses (Chapter 3).

Objective 3: Test the equilateral disease triangle by quantifying the relative importance of environmental factors for plant pathogen communities and richness at large scale (Chapter 4).

Objective 4: Identify non-random and predictable co-occurrence patterns of plant pathogen communities, and apply network analysis in order to simplify the overwhelming complexity of above and belowground plant pathogens across different ecosystems (Chapter 5).

Chapter 2

Biases in metabarcoding technologies of plant pathogens: Rust fungi as a model system

Notes

The results of this chapter are intended to be submitted to Molecular Ecology Resources.

Abstract

Plant pathogens such as rust fungi (Pucciniales) are of global economic and ecological importance. As such, reliably and cost effectively detecting, identifying and monitoring these fungi at large scales is important to industry, government and research institutions. Metabarcoding is one approach, based on next-generation sequencing, that could be used for this purpose. However, metabarcoding has not been widely applied for the monitoring of rust fungi. This is in part due to uncertainties of the new approach, mainly concerning the ability to detect rare species and reliability of measuring species abundances (quantification). Furthermore, outcomes might differ between sequencing platforms, although this aspect has not been well examined. In this chapter, I investigated possible differences in (1) detection ability and (2) quantification of rust fungi from environmental DNA (eDNA) samples between two metabarcoding approaches and traditional DNA cloning. I then analysed (3) the causes of these differences. I used two fundamentally different next-generation sequencing technologies (Illumina MiSeq and Ion Torrent), and cloning followed by Sanger sequencing. I found strong biases between observed and expected numbers of shared rust fungal operational taxonomic units (OTUs) across different methods. However, there was no significant difference in abundance of OTUs that all methods were capable of detecting. Therefore, differences between the methods were mainly driven by the method's ability to detect specific OTUs. The difference in detection could be explained by a phylogenetic bias of the methods. This bias was very likely caused by base pair mismatches of the metabarcoding

primer to some *Puccinia* species. These results highlight the importance of the primer choice for metabarcoding studies. Metabarcoding studies should therefore carefully examine *in silico* what taxa their primers might discriminate against and incorporate this knowledge into interpreting their results. Furthermore, the detection ability did not seem to be influenced by sequence length differences across methods, the bioinformatic pipeline used for each method, or differing ability to detect rare species. As primers per se are independent of the method, the congruity among three independent methods demonstrates the promising potential of DNA metabarcoding for tracking important taxa such as rust fungi from within larger metabarcoding communities. Altogether, these results support the use of metabarcoding for large scale detection and quantification of plant pathogens and oppose its use for confirming absence of species.

Keywords

environmental DNA, next-generation sequencing, Pucciniales, Illumina, Ion Torrent, cloning

2.1 Introduction

Plant pathogens can be critical threats to global food security (Oerke, 2006), conservation of natural ecosystems and future resilience and sustainability of ecosystem services (Van der Putten *et al.*, 1993; Mitchell *et al.*, 2002; Rottstock *et al.*, 2014; Bever *et al.*, 2015). Fungi are an important group of plant pathogens (Van Alfen, 2001; Dean *et al.*, 2012) and are responsible for a high percentage of diseases in plants (Anderson *et al.*, 2004). For example, with about 7800 described species, rust fungi (Pucciniales) constitute one of the largest group of known plant pathogens today (Savile, 1971; Smith *et al.*, 2004; Aime, 2006; Helfer, 2014). Because they occur in all vegetation zones around the world, rust fungi play an important regulatory role in shaping plant communities and composition globally (Dobson & Crawley, 1994). Certain rust species have large economic and ecological impacts. For example, myrtle rust (*Puccinia psidii*) is currently decimating a wide range of Myrtaceae around the world (Glen *et al.*, 2007; McTaggart *et al.*, 2016), such as the endemic *Eugenia koolauensis* in Hawai'i (Carnegie *et al.*, 2016). Coffee leaf rust (*Hemileia vastatrix*) is substantially damaging *Coffea* plantations worldwide (McCook, 2006; Talhinhas *et al.*, 2017). Wheat leaf rusts like *Puccinia triticina*, *Puccinia recondita* and *Puccinia striiformis* are causing serious production losses for one of the world's biggest food crops raising concerns for global food security (McCallum *et al.*, 2012). While many studies focus on rust fungi as perceived pests, they constitute a vital component of global biodiversity and natural ecosystem functioning. In contrast to agroecosystems, rusts in their natural ecosystems are less well studied and some species are actually threatened by extinction due to global change (Desprez-Loustau *et al.*, 2007b; Helfer, 2014). Their disappearance would have unknown consequences on ecosystem functions around the world. Because of the economic and ecological importance of plant pathogens, like rust fungi, new, reliable and cost-effective tools are needed to monitor them at large scales.

Metabarcoding is one approach that could be used for this purpose. It integrates DNA based species identification (barcoding) with next-generation sequencing. Metabarcoding has the potential to develop into an effective method for the molecular identification of multiple plant pathogens out of environmental DNA (eDNA) samples (Andersen *et al.*, 2012; Taberlet *et al.*, 2012a). Metabarcoding seems especially auspicious for the monitoring of plant pathogens, as it bypasses the need for cultivation and isolation of species. Furthermore, metabarcoding, like all DNA based techniques, is able to detect plant pathogens when they occur asymptomatically or at hardly discernible levels. While metabarcoding holds great potential for detecting and

monitoring fungi in their environment (Bellemain *et al.*, 2013; Schmidt *et al.*, 2013; Miller *et al.*, 2016; Durand *et al.*, 2017), it has not yet been widely applied to pathogens specifically. It is therefore crucial to understand the potential and possible limitations of this new approach.

Two limitations which frequently arise in metabarcoding studies are the accuracy of taxa abundances (Fierer & Jackson, 2006; Murray *et al.*, 2011; Keller *et al.*, 2015; Kraaijeveld *et al.*, 2015; de la Cuesta-Zuluaga & Escobar, 2016), and false positives/negatives in detection introduced by PCR amplification, library preparation and sequencing (Coissac *et al.*, 2012). The present chapter addresses these two possible limitations of metabarcoding using the group of rust fungi as model system.

I investigated possible differences in detection and abundance of rust fungal species between metabarcoding and more traditional cloning approaches. Furthermore, I investigated what caused these differences. For the metabarcoding approach I used two different sequencing technologies (Illumina MiSeq and Ion Torrent) and fungal metabarcoding primers to detect rust fungi from within a larger fungal community. I compared these results to a cloning approach, targeting the same gene region but focussing cloning on rust fungi using a rust fungal specific primer pair.

I hypothesised that the three methods (cloning, Illumina and Ion Torrent)

(1) differ in their detection of rust species, i.e. observed from expected number of detected rust species.

(2) differ in their ability to quantify relative abundances of rust fungal species on a plot level.

Given that hypothesis one was supported, I then tested hypotheses for the mechanisms driving differences between methods. Specifically, I hypothesised that differences among methods are due to

(a) sequence length differences across methods.

(b) differences in the most appropriate bioinformatic pipelines for each method.

(c) a phylogenetic bias of the methods.

(d) different abilities to detect rare species.

2.2 Methods and materials

2.2.1 Study sites and sampling

As part of the "Next-generation biodiversity assessment" (NGBA) project [1] we sampled a total of 30, 20 m × 20 m grassland plots. The plots were based on the national 8 km × 8 km grid that is used extensively for national biodiversity monitoring in New Zealand (Allen *et al.*, 2003). The plots were selected across all of New Zealand based on the output of the Geographic Information System and stratified random sampling (see map of plots Fig. 2.1, full plot location details are stored along with all vegetation data in New Zealand's National Vegetation Survey repository, NVS [2]). The positioning of the plots was obtained by following the standardised protocol of Hurst & Allen (2007). All sampling was carried out under dry weather conditions between November 2014 and March 2015. At each plot, samples were collected using a sterilised leaf puncher within a 64 min period of time (4 min for each of 16, 5 m × 5 m subplots) to ensure balanced sampling of the whole plot. Every identifiable plant part (e.g. healthy leaves, leaves with lesions, bryophytes, grass stems, lichens, bark, seeds etc.), including healthy as well as diseased plant material, was sampled to get all variants and maximize rust fungal diversity. Since most of these samples represent above-ground herbaceous material, mainly leaves, I hereafter refer to these samples simply as "leaf samples". The leaf samples were immediately pooled by plot, stored in a 50 ml Falcon Tube containing autoclave-sterilised DMSO-NaCl solution (20% DMSO, 0.25 M disodium-EDTA, and NaCl to saturation, pH 7.5), sealed with Parafilm M and kept at 4 °C until laboratory processing.

[1] http://www.landcareresearch.co.nz/science/portfolios/enhancing-biodiversity/next-generation-biodiversity-assessment (accessed 01 January, 2018)

[2] https://nvs.landcareresearch.co.nz/ (accessed 01 January, 2018)

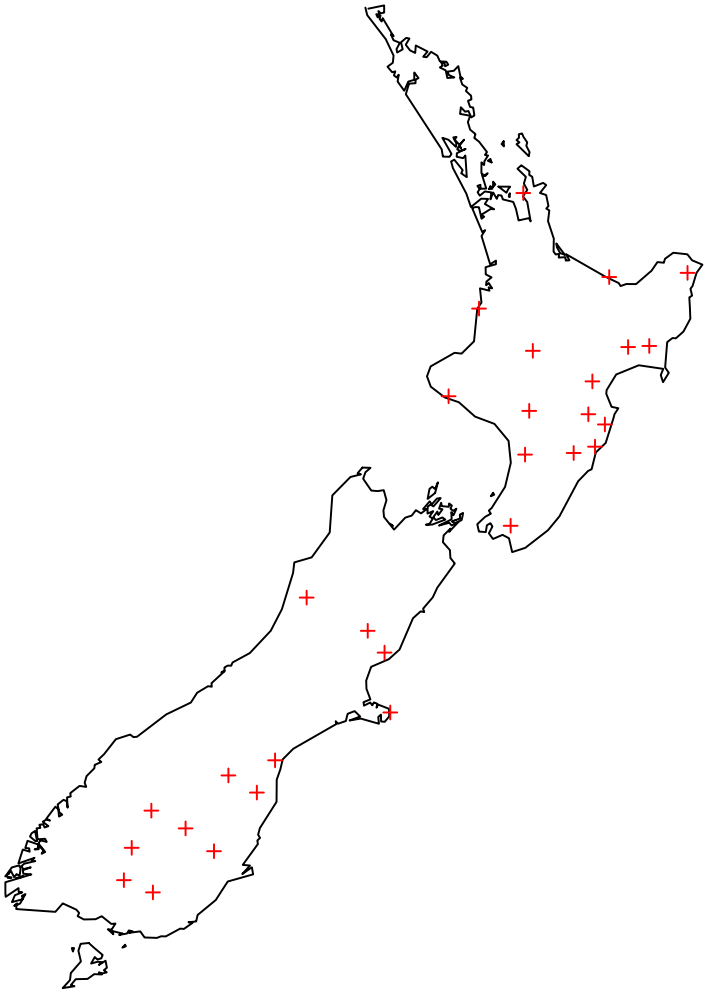

Figure 2.1: Map of the 30 grassland study sites across New Zealand. Plot location details are stored in New Zealand's National Vegetation Survey repository (NVS).

2.2.2 DNA extraction

eDNA extraction from the pooled leaf samples of each plot was carried out using the Macherey-Nagel NucleoSpin 96 Plant II kit (robot extraction) following the manufacturer's protocol. I used both provided lysis buffers separately (cetrimonium bromide (CTAB) lysis buffer PL1 and a sodium dodecyl sulphate (SDS) based lysis buffer PL2) to enhance the amount of extracted DNA. I checked the success of the DNA extraction. Five µl of product was quantified using a Qubit 2.0 fluorometer (Life Technologies) and the broad-range assay kit following the manufacturer's protocol before equally pooling the extracts from the same plot.

2.2.3 Preparation of next-generation sequencing libraries

I prepared NGS libraries in a one-step PCR (Immolase MoTASP Protocol) to avoid the risk of contamination, following Clarke *et al.* (2014). I used the fungal primers fITS7: GTGART-CATCGAATCTTTG (Ihrmark *et al.*, 2012) and ITS4: TCCTCCGCTTATTGATATGC (White *et al.*, 1990) amplifying the highly variable internal transcribed spacer region 2 (ITS2) with universal linker sequences at the 5' end for fITS7: TCGTCGGCAGCGTC and for ITS4: GTCTCGTGGGCTCGG. Illumina adapter sequences with index sequences and complementary linker sequences were:

F: AATGATACGGCGACCACCGAGATCTACAG-8nt index-TCGTCGGCAGCGTC,

R: CAAGCAGAAGACGGCATACGAGAT-8nt index-GTCTCGTGGGCTCGG.

The universal fITS7 primer has been noted to exclude certain Ascomycota (*Penicillium*, Orbiliales), and most Mucorales (Ihrmark *et al.*, 2012), but was chosen because it is more fungal specific compared to other universal primers (e.g. fITS9 or gITS7, which match some plants because they are degenerated at two positions, potentially overwhelming any fungal signal in leaf substrates). Moreover, the primer pair fITS7 and ITS4 is believed to capture most of the Basidiomycetes, including rust fungi, is well represented in the UNITE database, and its amplicon lengths are well suited for next-generation sequencing (average of 258.5 bp ± 27.3 bp for Ascomycota, and 309.8 bp ± 35.6 bp for Basidiomycota) (Ihrmark *et al.*, 2012; Bokulich & Mills, 2013). Purification and size selection (280 bp - 520 bp) was performed using a PippenPrep system to exclude primer dimers and high molecular weight DNA, before paired-end sequencing the samples at the Australian Genome Research Facility Ltd, Melbourne, Australia.

2.2.4 Preparation of clone libraries

To focus the cloning procedure on Pucciniales and to get to species resolution I amplified an approximately 1400 bp target region with the rust fungal specific forward primer Rust2inv: GATGAAGAACACAGTGAAA (Aime, 2006) and reverse primer LR6: CGCCAGTTCTGCT-TACC; (Vilgalys & Hester, 1990), starting in the 5.8S subunit and spanning the highly variable ITS2 region and the three most divergent domains (D1, D2, D3) of the large subunit (LSU, 28S). I performed PCRs for the two DNA extracts of each plot using the TaKaRa Ex Taq DNA Polymerase kit (25 µl reaction volumes, containing 2.5 µl 10X Ex Taq Buffer, 2 µl dNTP Mixture (2.5 mM each), 5 µl 10 µg/ml rabbit serum albumin (RSA), 0.6 µl 10 µM of each upstream and downstream primer, 0.125 µl TaKaRa Ex Taq, 1 µl DNA template and 13.175 µl of sterilized

distilled water). PCR conditions consisted of an initial denaturation step of 2 min at 94 °C, 35 cycles of 30 s at 94 °C, 1 min at 57 °C, and 1.5 min at 72 °C, and a final extension of 7 min at 72 °C, as initially described by Aime (2006). I pooled 1 µl of PCR product originating from the CTAB and 1µl from the SDS based lysis buffer DNA extractions per plot and cloned using the Strataclone PCR cloning kit (Agilent, Stratagene), following the manufacturer's protocol. I performed blue-white screening of colonies using X-gal. I chose 48 white colonies of each plot for colony PCR based on a preliminary restriction fragment length polymorphism (RFLP) screen of the plots using the Hinf I restriction enzyme and the probability of detecting the rarest patterns/species with a probability of 91.47% (rarest pattern occurred at least five times out of 100 clones). I picked a total of 1440 colonies and performed colony PCRs with the plasmid specific primer pair M13–20: GTAAAACGACGGCCAG and M13RSP: CAGGAAACAGCTATGACCAT (Wood *et al.*, 2012), using the TaKaRa Ex Taq DNA Polymerase kit (15 µl reaction volumes, containing 1.5 µl 10X Ex Taq Buffer, 1.2 µl dNTP Mixture (2.5 mM each), 0.6 µl 10 µg/ml rabbit serum albumin (RSA), 0.24 µl 10 µM of each upstream and downstream primer, 0.075 µl TaKaRa Ex Taq, colony DNA template and 10.15 µl of sterilized distilled water). PCR conditions consisted of an initial denaturation step of 12 min at 94 °C, 35 cycles of 20 s at 94 °C, 10 s at 55 °C and 1.5 min at 65 °C, and a final extension of 10 min at 65 °C, following the method of Wood *et al.* (2012). After a gel visualisation, sequencing of colony PCR products in the forward direction was conducted with the Rust2inv primer at the Bio-Protection Sequencing facility, Lincoln University, New Zealand. Reverse sequencing was not conducted as the gene regions of interest (ITS2, D1, D2, D3) lie within the first 750 bp of the forward sequencing read.

2.2.5 Bioinformatics

I quality filtered clone sequences manually using FinchTV v1.4.0 (Geospiza Inc., 2004-2006) and Chromatogram Explorer v4.0.0 (Heracle BioSoft SRL, 2011). I trimmed off low quality bases at the sequence beginnings and ends, and removed primer and vector sequences. I aligned the sequences using the MUSCLE algorithm (Edgar, 2004) and trimmed the beginning so they start at the same point of the gene region as the sequences from Ion Torrent and Illumina using the fITS7 primer. One hundred percent similar sequences were dereplicated and N-padded to the same length. N-padding, that is adding Ns (which represent any nucleotide) to the end of each sequence until they have the same lengths, was needed because the clustering algorithm that was used considers terminal gaps to be absolute differences. I clustered the N-padded sequences

to a 97% similarity threshold without using singletons using UPARSE algorithm (Edgar, 2010). This threshold represents the ITS barcode gap for the overwhelming majority of fungal species including Pucciniomycotina (Schoch *et al.*, 2012).

The forward and reverse Illumina reads were merged and sequences with more than one expected error and less than 175 bp were removed. Ion Torrent sequences were only used if the forward and the reverse primer complement could be found within the sequence and if the sequence was at least 175 bp long. I discarded Ion Torrent sequences with more than two expected errors (EE). I set a higher EE threshold because the mean expected error rate of the Ion Torrent runs at the sequence length of 300 bp was two errors. I trimmed off non-biological sequences allowing 10% bp mismatch using the Python tool "cutadapt" version 1.13 (Martin, 2011), if the forward primer or the reverse primer complement could be found at the sequence ends. One hundred percent similar sequences were dereplicated. Illumina and Ion Torrent data was independently clustered to 97% similarity threshold without using singletons using UPARSE complete-linkage clustering algorithm (Edgar, 2013).

I constructed a reference database from UNITE and INSD (accessed 20.11.2016), and matched the representative sequence of each OTU to this database using BLAST version 2.5.0+ (Altschul *et al.*, 1997). I considered an OTU to represent the order Pucciniales if it matched Pucciniales sequences in the database >80% identity over at least 150 bp. Extraction blanks, positive and negative controls were checked for contamination. Tag jumping, which are false combinations of used tags and hence cause incorrect assignment of sequences to samples (Schnell *et al.*, 2015) was accounted for by using a regression of the abundance of contaminants versus the maximum of total abundances in all other samples. The coefficient estimate for the 90th quantile regression was then used to subtract that many sequences from all OTUs. Hence, this tag-jumping correction takes into account that more abundant OTUs are more likely to do tag-jumping. Additionally to this filtering step, one OTU was removed from the Illumina data. The reason for this was that this OTU matched a *Coleosporium* species and was present with only two sequences in one plot. *Coleosporium* needle rust of pine would not have been expected to be present in a grassland plot with no pine species (and no pine species in the surrounding area of the grassland plot). Closer examination showed that these two sequences were most likely to be contamination from a planted pine forest plot where this species was highly abundant. The pine forest plot was part of the overarching NGBA project and thus sequenced in the same Illumina run. I blasted OTUs obtained from the three different methods against each other and

considered them to be the same OTU if they matched at >98.5% similarity which corresponds to approximately 3% clustering of the NGS data using distance-based greedy clustering UPARSE algorithm (Edgar, 2013), but allows different sequence lengths as opposed to matching with USEARCH (Altschul *et al.*, 1997; Edgar, 2010, 2013).

2.2.6 Statistical analysis

I used R version 3.4.1 (R Core Team, 2017) for conducting analyses and creating graphs if not stated otherwise. Plot locations were visualised using the R package "maps" (Ray *et al.*, 2017). To test whether a method detected more or less shared/unique rust fungal OTUs than expected by chance, I used the "permatswap" function of the R package "vegan" version 2.0-7 (Oksanen *et al.*, 2017) to create a null expectation. The simulated community matrices are based on Monte Carlo iterations, whereby total number of OTUs per plot and total abundance within OTU were kept constant. I tested for differences in OTU abundances between methods using a generalized additive model (GAM) of the package "mgcv" version 1.8-18 (Wood, 2001). A GAM was selected because (1) it allows beta distribution for the response variable, which in this case was the appropriate distribution for the proportional abundance of each OTU found within a plot (to account for different sequencing depths). (2) Furthermore, the approach allowed the test for OTU and plot as random effects, and interaction between method and OTU. Data was rescaled to exclude zeros and ones, as suggested by Smithson & Verkuilen (2006). Wald test was used to test the significance of each parametric and smooth term (Bolker *et al.*, 2009; Wood, 2012). To see if perceived rust fungi communities differ between methods, I converted the obtained community data into Jaccard distance matrices, using Wisconsin double standardization. Four plots with zero OTUs, as well as unique communities, had to be discarded because of infinite dissimilarity. I displayed the dissimilarities with non-linear multidimensional scaling and tested for significance between the configurations using procrustes rotation and the "protest" function part of the "vegan" package, and the "mantel.test" function of the "ape" package (Paradis *et al.*, 2004). I tested if a bias between methods was caused by different sequence lengths or bioinformatic pipelines, applying the same sequence length (248 bp) and/or an identical bioinformatic pipeline to all methods. To look for a phylogenetic bias in detection of the different methods I constructed a neighbour-net phylogeny (Bryant & Moulton, 2004) using Splitstree 4.0 (Huson *et al.*, 2008) and used χ^2 test to test if phyogenetic clusters are independent from methods. I tested if a possible difference is due to detection of rare and dominant OTUs by rerunning all tests using the top

and lower 50% of the rank abundance of each method. Species identities are based on the best BLAST match and were displayed as networks using the "igraph" package version 1.0.1 (Csardi & Nepusz, 2006) with edge width representing relative species abundance within method.

2.3 Results

2.3.1 Differences between methods in detection of OTUs

There were seven rust fungal operational taxonomic units (OTUs) shared across the three methods, which was much less than would be expected, by random sampling (17.05 ± 0.33). The difference was driven by OTUs uniquely detected by a single method (Fig. 2.2), i.e. Illumina (1 OTU) and Ion Torrent (2 OTUs), and especially through cloning (10 OTUs). The three methods (i.e. cloning, Illumina and Ion Torrent) hence differed in detection of rust fungal OTUs.

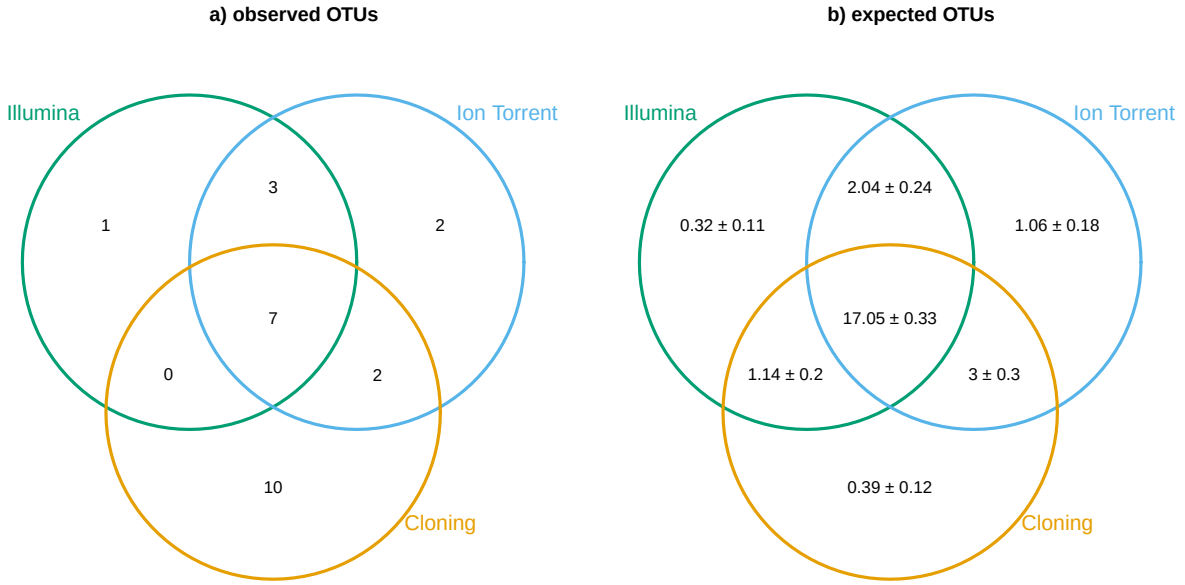

Figure 2.2: (a) Observed and (b) expected number of rust fungal operational taxonomic units (OTUs) per method. OTUs were considered to be identical between methods when >98.5% BLAST similarity. Expectations were based on Monte Carlo random sampling (100 iterations) and displayed with 95% confidence intervals.

2.3.2 No differences between methods in relative abundances of shared OTUs and in perceived community composition

There was no evidence of differences in quantification of relative abundances across the three methods (i.e. cloning, Illumina and Ion Torrent) among OTUs that all methods were capable of detecting. Wald and likelihood ratio test between models with and without the interaction (method:OTU) was not significant (Wald χ^2=7.62, df=12, P= 0.81).

In general, rust communities perceived by the three methods did not result in largely different patterns as visualised by the overlap of the communities in the multidimensional scalings

(Fig. 2.3). Mantel test and Procrustes analysis suggest significantly similar patterns ($P < 0.05$) for Ion Torrent/cloning (abundance data), and Ion Torrent/cloning and Illumina/Ion Torrent (presence/absence data).

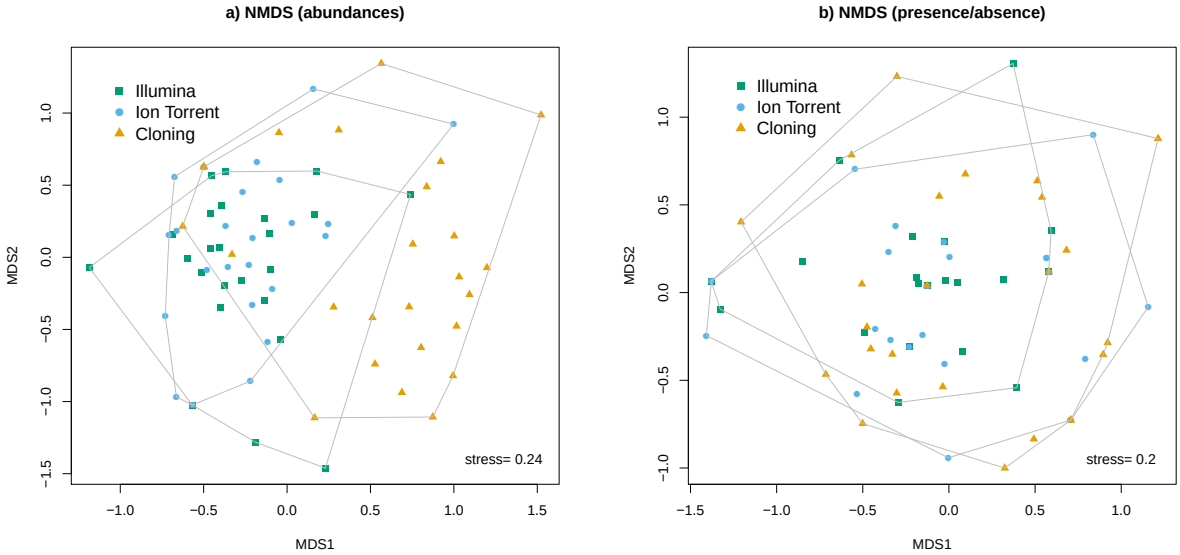

Figure 2.3: Multidimensional scaling of rust communities (using abundance and presence/absence data) as perceived by three different methods. Illumina (green, squares), Ion Torrent (blue, circles), cloning (orange, triangles). Four plots were dropped because of lack of any detected rust communities in these plots.

2.3.3 Mechanisms driving OTU detection differences between methods

Differences in detection among methods seemed not to be due to sequence length differences across methods. After trimming all sequences to the same length (248 bp), which is the shortest common sequence of all methods, and rerunning the analysis, the number of observed (7 OTUs) from randomly expected (17 OTUs) shared rust OTUs stayed unchanged.

Differences in detection among methods also seemed not to be due to differences in the most appropriate bioinformatic pipelines for each method. Using a completely identical bioinformatic pipeline for all methods made differences even more extreme, with only 4 OTUs shared across methods, compared to 7 (with the most appropriate pipelines) or 17 (expected).

Differences in detection among methods were due to a phylogenetic bias of the methods. Neighbour-net phylogeny (Fig. 2.4) indicates three phylogenetic clusters. Cluster 1 could equally be detected by all methods. Cluster 2 was only detected using Illumina. Cluster 3 was only

detected using cloning. The Chi squared test for independence was significant (χ^2=17.536, df=4, $P < 0.01$) and confirmed that clusters were not equally formed by the different methods.

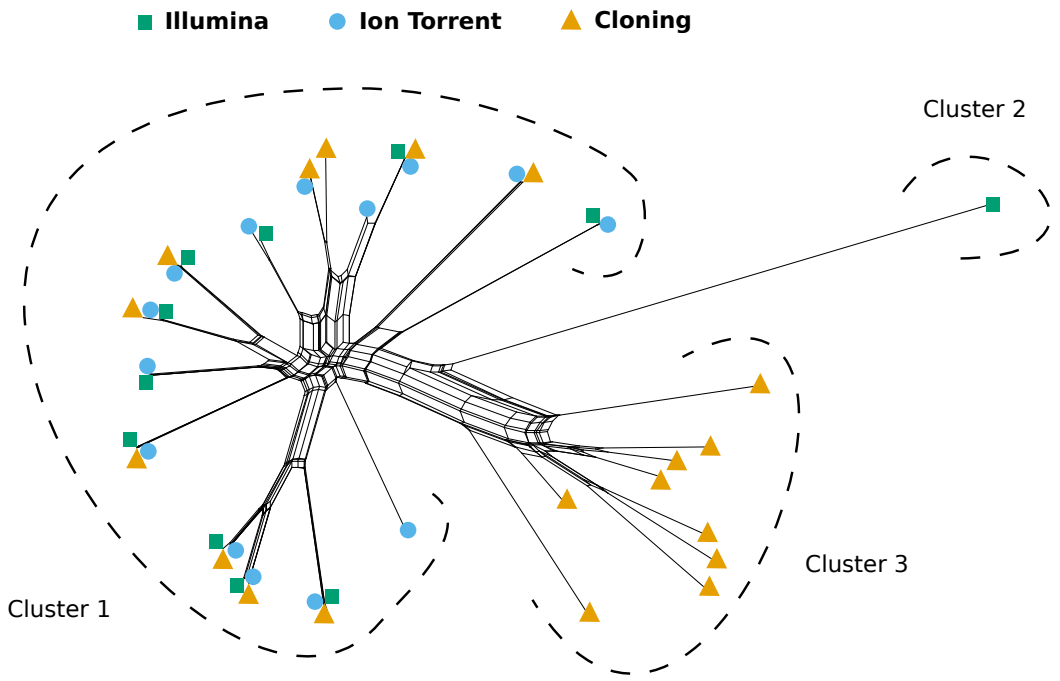

Figure 2.4: Neighbour-net phylogeny of rust fungal operational taxonomic units (OTUs) detected by methods. Illumina (squares), Ion Torrent (circles), Cloning (triangles).

Species identities of cluster 3 (i.e. uniquely detected by cloning) and cluster 2 (i.e. uniquely detected by Illumina) were displayed in a co-occurrence network (Fig. 2.5). While Illumina's uniquely detected species is from the genus *Kuehneola*, uniquely detected species from cloning and Ion Torrent are from the genus *Puccinia*.

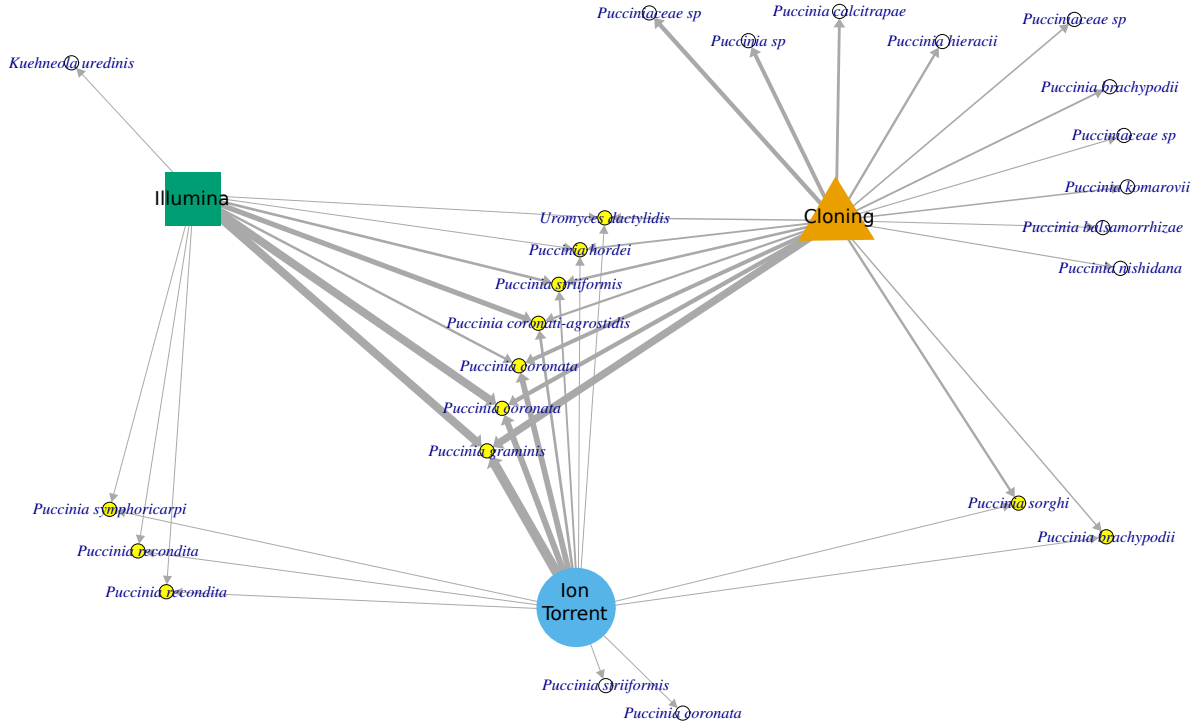

Figure 2.5: Network representing shared and unique rust fungal operational taxonomic units (OTUs) between methods. Edge width represents proportional abundance of an OTU within method. Species identities are based on their best BLAST match. OTUs found in each method are considered to be identical when >98.5% sequence similarity.

The phylogenetic bias seemed not to be driven by poor detection of rare OTUs. The same clusters occur when only considering the upper 50% of rank abundance, hereafter called dominant OTUs (Fig. 2.6 and Fig. 2.7); and when only considering the lower 50% of rank abundance, hereafter rare OTUs (Fig. 2.8 and Fig. 2.9). The number of observed shared dominant OTUs (6 OTUs) and rare OTUs (2 OTU) still differs significantly from randomly expected (11.08 $\pm$ 0.36 OTUs) shared rust OTUs. This difference in observed from expected is still mainly due to the uniquely detected OTUs from cloning (cluster 2 of Fig. 2.6 and cluster 3 of Fig. 2.8).

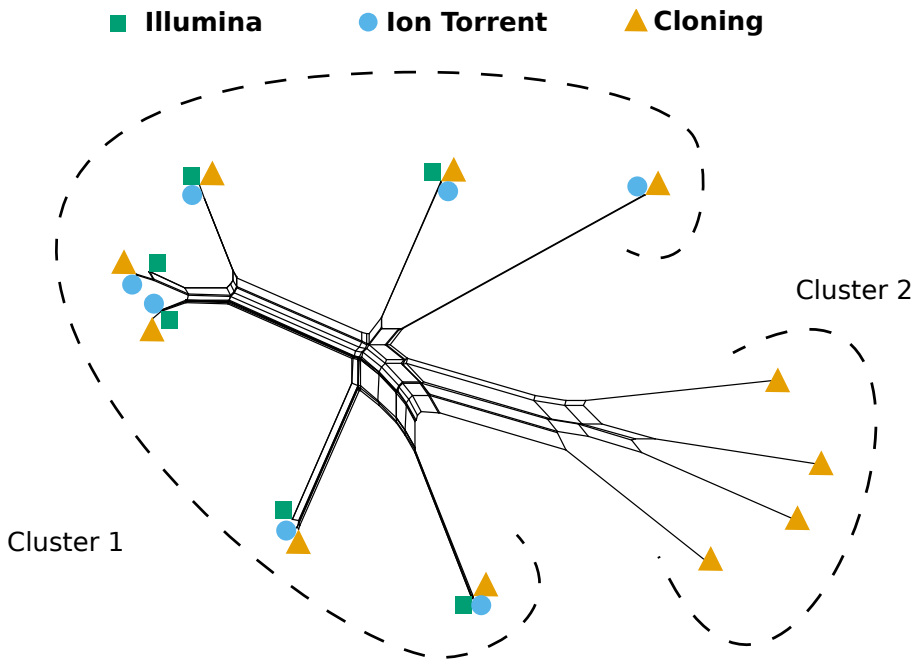

Figure 2.6: Neighbour-net phylogeny of dominant (upper 50% of rank abundance) rust fungal operational taxonomic units (OTUs) detected by three methods. Illumina (squares), Ion Torrent (circles), Cloning (triangles).

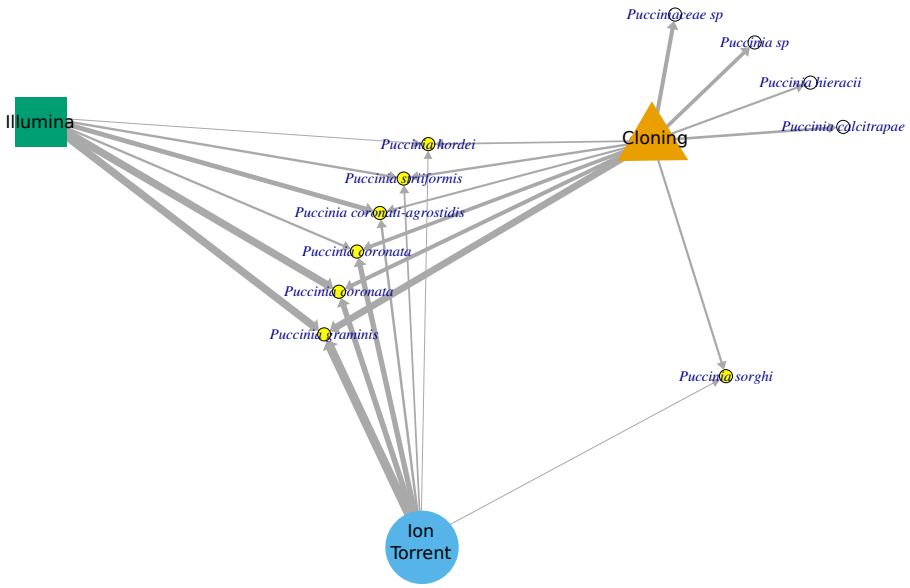

Figure 2.7: Network representing shared and unique dominant (upper 50% of rank abundance) rust fungal operational taxonomic units (OTUs) between the three methods. Edge width represents proportional abundance of an OTU within method. Species identities are the best BLAST match. OTUs found in each method are considered to be the same >98.5% sequence similarity.

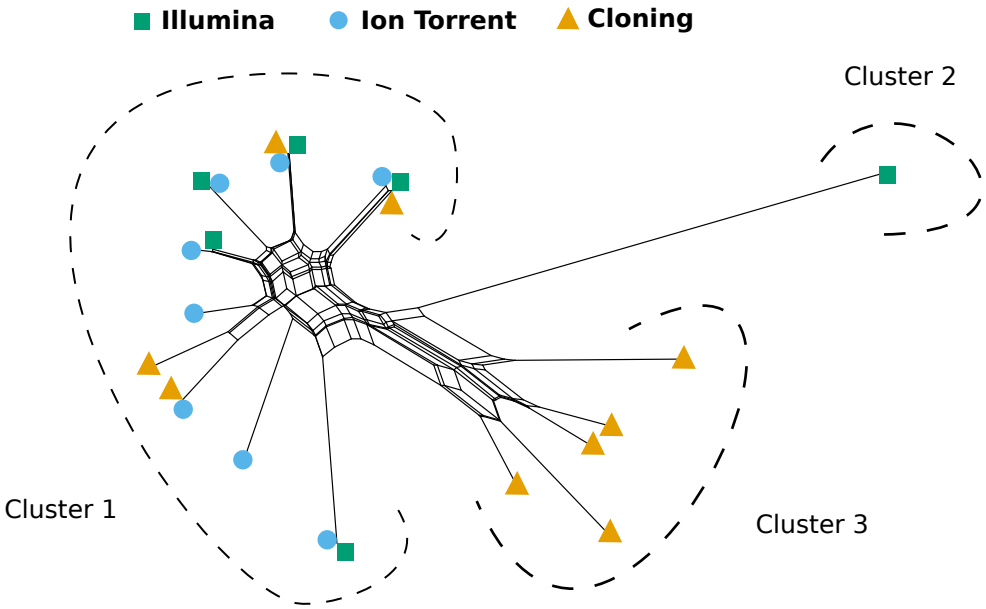

Figure 2.8: Neighbour-net phylogeny of rare (lower 50% of rank abundance) rust fungal operational taxonomic units (OTUs) detected by methods. Illumina (squares), Ion Torrent (circles), Cloning (triangles).

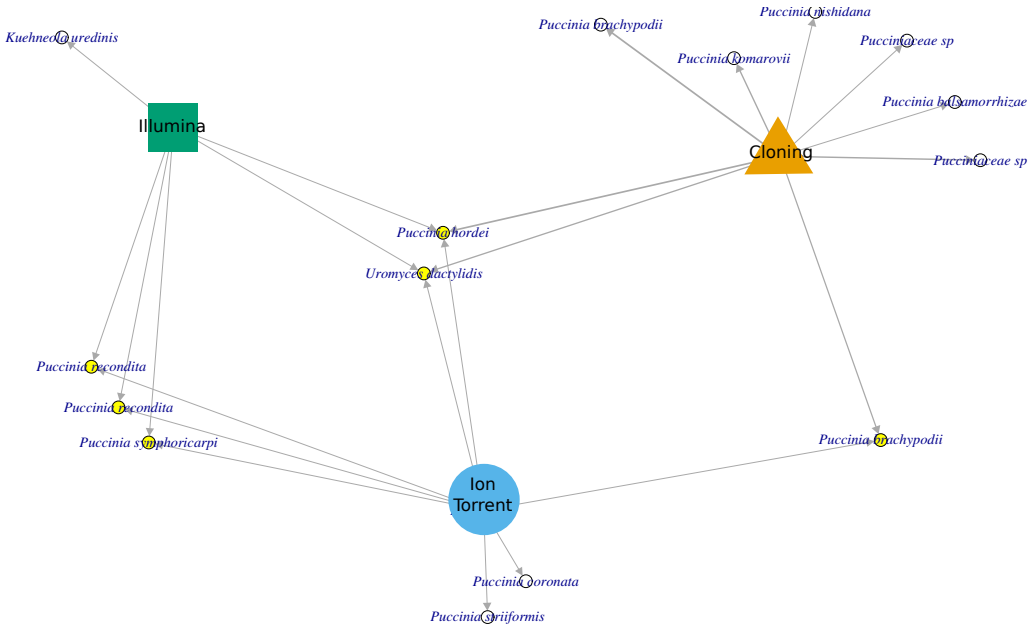

Figure 2.9: Network representing shared and unique rare (lower 50% of rank abundance) rust fungal operational taxonomic units (OTUs) between methods. Edge width represents proportional abundance of an OTU within method. Species identities are the best BLAST match. OTUs found in each method are considered to be the same >98.5% sequence similarity.

2.4 Discussion

This study demonstrates that environmental DNA (eDNA) metabarcoding is an effective technique for large-scale detection of rust fungi plant pathogens, but that phylogenetic biases due to primer selection are a potential limitation. I found differences in detection of rust fungal species between Illumina, Ion Torrent platforms, and cloning followed by Sanger sequencing. However, I found no significant difference in the relative abundances of rust fungal species that all methods were capable of detecting between methods. The mechanism driving detection differences between methods seemed to be due to a phylogenetic bias which was very likely caused by base pair mismatches of the metabarcoding primer pair to some *Puccinia* species. Otherwise, the consistency among fundamentally different and independent methods shows that metabarcoding and cloning are on par. Altogether the results support the application of metabarcoding for large scale detection of plant pathogens (presences), and oppose its application for inferring absence of species. These findings are important to future metabarcoding studies, because they highlight the main source of difference between methods, and rule out several mechanisms that could drive differences.

The main difference between the methods, metabarcoding and cloning, was due to their biases in species detection, not quantification. This suggests that previous problems when using quantitative next-generation sequencing data (Elbrecht & Leese, 2015; Piñol *et al.*, 2015) were likely induced by PCR, and not by the method or sequencing platform per se. Furthermore, this is in line with the finding that the difference in detection between metabarcoding and cloning shows a phylogenetic bias. Both the metabarcoding and the cloning primers have either a perfect match or only a maximum of two base pair mismatches to all detected rust fungi in this study. Moreover, the metabarcoding primers were thought to capture most of the Basidiomycetes (White *et al.*, 1990; Ihrmark *et al.*, 2012), including rust fungi. Consequently, the metabarcoding and the cloning primers would be expected to detect a similar assemblage of rust fungi. However, the base pair mismatches of the metabarcoding primer occur to species that are only detected by cloning; and the cloning primer has no mismatches to these species. See Table 2.1 for examples of these mismatches. The lower specificity of the "universal" metabarcoding primer is therefore more likely to discriminate against the amplification of those species when exposed to 100% matching other fungal sequence templates (Bellemain *et al.*, 2010). Although phylogenetically clustered, the *Puccinia* species with the base pair mismatch of the metabarcoding primer seemed not to fall into a known taxonomic cluster, like a subgenus (Van der Merwe *et al.*, 2007). This shows again that differences between methods are likely induced by PCR.

Table 2.1: Metabarcoding primer mismatches to selected species that were detected by cloning but not by metabarcoding. Mismatches are highlighted (bold and underlined). Sequences were selected from NCBI to cover the gene region of cloning and metabarcoding primers when possible. Dot indicates no entry of base pair in the database. Accession numbers are given as footnotes.

Species	**Metabarcoding primers and mismatches** **5'-fITS7 (forward primer)**	**3'-ITS4 (reverse primer)**
	GTGARTCATCGAATCTTTG	GCATATCAATAAGCGGAGGA
Puccinia calcitrapae[1]	GTGAATCAT**T**GAATCTTTG	GCATATCAATAAGC**A**GAGGA
Puccinia nishidana[2]	. . . . .ATCAT**T**GAATCTTTG	GCATATCAATAAGC**A**GAGGA
Puccinia balsamorrhizae[3]	. . . . . .CAT**T**GAATCTTTG	GCATATCAATAAGC**A**GAGGA
Puccinia komarovii[4]	GTGAATCAT**T**GAATCTTTG	GCATATCAATAAGC**A**GAGGA
Puccinia hieracii[5]	. . . . . .CATCGAATCTTTG	GCATATCAATAAGC**A**GAGGA

Accession numbers: [1]JN204183.1, [2]HM022141.1, [3]JN204182.1, [4]KC466553.1, [5]HQ317515.1

There are numerous metabarcoding studies that point out that metabarcoding primers can discriminate against certain taxa (Bellemain *et al.*, 2010; Schmidt *et al.*, 2013; Clarke *et al.*, 2014; Cowart *et al.*, 2015; Elbrecht & Leese, 2015; Shaw *et al.*, 2016). Some studies have tried to limit this bias to some extent by using quantitative PCR and correction factors (Pawluczyk *et al.*, 2015; Thomas *et al.*, 2016), primer mixes (Tedersoo *et al.*, 2015), or blocking oligonucleotides to non-target DNA (Piñol *et al.*, 2015). Ficetola *et al.* (2010) proposed an "electronic PCR" application to measure barcode coverage and specificity. This *in silicio* approach has proven useful to identify the appropriate barcode gene regions and when comparing different primers for fungi (Bellemain *et al.*, 2010) and vertebrates (Valentini *et al.*, 2016). The results from this study and from the literature, taken together, highlight the importance of primer choice for metabarcoding studies. Metabarcoding studies should therefore carefully examine *in silicio* what taxa their primers might discriminate against in order to select appropriate metabarcoding markers and aid interpretation of results.

Moreover, this study ruled out several mechanisms that could possibly drive detection differences between metabarcoding and cloning. I found no evidence that sequence length, bioinformatic pipeline, or ability to detect rare species caused any differences across methods.

I found that shortening all sequences to the length of the shortest sequence (248 bp) did not change the interpretation of the overall results and resulting phylogeny. Min & Hickey (2007) and Han *et al.* (2013) showed that reducing sequence length can have effects on the accuracy of phylogenies when DNA barcoding fungi. They also showed that despite some loss of phylogenetic signal, shorter sequences can still resolve the terminal nodes of the phylogeny quite efficiently in most cases. Current next-generation sequencing technologies still require amplification of short sequences, and some barcode regions, for example, the ITS region for fungi, can lack the necessary

resolution for particular fungal taxa (Gazis *et al.*, 2011). Despite these challenges, short sequences provide enough resolution at a genus and often within-genus level for the majority of fungi (Blaalid *et al.*, 2013). While short sequences have been repeatedly shown to be sufficient for genus or even species level identifications (Hebert *et al.*, 2003, 2004; Hajibabaei *et al.*, 2006; Jørgensen *et al.*, 2012; Blaalid *et al.*, 2013; Quéméré *et al.*, 2013; Bokulich & Mills, 2013; Sickel *et al.*, 2015; Richardson *et al.*, 2015; Zaiko *et al.*, 2015), future next-generation sequencing technologies should be able to overcome the current length limitations and provide the field of metabarcoding with even better species delimitations (Goodwin *et al.*, 2016).

Bioinformatic pipelines can have profound effects on the outcome of metabarcoding studies (Mahé *et al.*, 2014; Flynn *et al.*, 2015). In this study, the error rate heavily differed between Illumina, Ion Torrent and Sanger sequencing runs. Using an identical bioinformatic pipeline, such as identical quality filtering and clustering, resulted in a much lower number of shared OTUs between the methods. These results justify using the most appropriate bioinformatic pipeline for each method.

Moreover, I did not find any effect of rare species on detection ability across methods. The same phylogenetic bias between the methods occurred when only looking at the dominant or only looking at the rare OTUs. Rare OTUs in metabarcoding data are generally more prone to errors due to the accumulation of errors (Dickie, 2010; Kunin *et al.*, 2010; Bachy *et al.*, 2013), tag jumping (Schnell *et al.*, 2015), chimera formation (Nilsson *et al.*, 2010; Edgar *et al.*, 2011), or false positive/negatives (Ficetola *et al.*, 2015). However, previous studies have shown that if these problems associated with rare OTUs are overcome, the ability of metabarcoding to detect rare species is equal or exceeds non-molecular methods (Zhan *et al.*, 2013; Meier *et al.*, 2016; Valentini *et al.*, 2016).

Metabarcoding seems appropriate for large scale detection of rust fungi, and less appropriate for inferring absence of species for a couple of reasons. This emerged from this study because, regardless of the method, it was easy to miss certain species. For example, the species *Puccinia sorghi* was initially present in the raw data of all three methods. However, only two sequences of this species were present in the Illumina raw data. These two sequences exhibited a point mutation or a possible sequencing error in their reverse sequence read, and got treated as being unique sequences (singletons) after merging. Hence, although initially present in the Illumina raw data, these two sequences could not form an OTU. This phenomenon of species getting lost during merging of paired-end sequencing has been noted earlier by Nguyen *et al.* (2015), and was

mostly caused by the usually poorer quality of reverse sequencing reads of the Illumina MiSeq platform. The problem of missing extremely rare species, however, is not method specific, as the case of *Kuehneola uredinis* demonstrates. This rare species had a total of 47 sequences in the Illumina data and was initially present as a single sequence in the raw data of the clone libraries. Because singletons got discarded regardless of the method, *Kuehneola uredinis* got discarded from the clone data. The fact that the cloning primer pair had a perfect match to *Kuehneola uredinis* and that this species got picked up once, clearly shows that the detection of rare species does not rely on the applied method but rather on sequencing depth and bioinformatic assumptions. Picking a greater number of clones would likely have resulted in at least another sequence of *Kuehneola uredinis* and finally in the detection of this species. Despite failing to detect two rare species by some methods, other rare species, such as *Uromyces dactylidis* and *Puccinia hordei*, could be detected regardless of the method.

Another way of easily missing species when merging paired end sequencing reads is to lose "too long" sequences since these would not overlap. This can be simply tested by not merging the reads and using forward and reverse read separately. In this study, I found no rust fungus species getting lost during merging as a result of "too long" sequences. The actual Illumina sequencing process, however, is known for discriminating against longer amplicons (Allen *et al.*, 2016). Although less likely than, for instance, a primer mismatch, the *Puccinia* species that could not be detected by metabarcoding but could by cloning, could possibly have been gotten missed during the next-generation sequencing process due to slightly longer amplicons.

I did not compare abundance data to a field survey or biomass, but found no significant difference in relative abundances of OTUs on plot level across metabarcoding and cloning. This suggests that any biases in quantification are not method dependent. Several studies do show metabarcoding to be successful for quantitative abundance estimation of, for example, feather mite communities in birds (Diaz-Real *et al.*, 2015), fish and amphibians in freshwater ecosystems (Evans *et al.*, 2016), plant-pollinator interactions (Pornon *et al.*, 2016), the biomass of macroinvertebrates (Elbrecht & Leese, 2015), and fungi (Taylor *et al.*, 2016). These studies suggest that if obstacles associated with PCR biases (Aird *et al.*, 2011) can be overcome, metabarcoding holds promising potential not only for the detection, but also for the quantification of species. Moreover, PCR-free techniques may remedy primer and amplification biases in the future.

Environmental DNA metabarcoding has been increasingly recognised as a promising tool for biomonitoring species and complex communities (Hänfling *et al.*, 2016; Ishige *et al.*, 2017;

Hatzenbuhler *et al.*, 2017; Klymus *et al.*, 2017). In a few cases it has been applied for measuring the diversity of plant pathogens, such as *Phytophthora* (Prigigallo *et al.*, 2016; Burgess *et al.*, 2017b; Català *et al.*, 2017). This study proposes to extend the use of eDNA metabarcoding for detecting and monitoring plant pathogenic groups, like rust fungi, from within larger metabarcoding communities.

Chapter 3

Diversity patterns of plant pathogens across land uses

Notes

The results of this chapter are intended to be submitted to Molecular Ecology or Fungal Ecology.

Abstract

Little is known about diversity patterns of plant pathogens at large scale. This study employed next-generation sequencing and metabarcoding to describe diversity and composition of putative plant pathogen communities in soil, roots and leaves across five major land uses at a national scale in New Zealand. Almost all plant pathogen communities (fungi, oomycetes and bacteria) showed strong responses to land use and substrate type. Land use category could explain up to 24% of the variance between communities. Alpha-diversity (richness) of plant pathogenic OTUs was consistently lower in natural forests than in agricultural systems. While there was a general low pathogen richness in soil and roots from forests, richness in leaves of planted forests was among the highest. Differences in within-land use β-diversity of plant pathogens (the variation in composition among plant pathogen communities from site-to-site) were subtle compared to changes in α-diversity. Most plant pathogen communities did not differ in β-diversity between land uses, indicating a similar turnover rate. The relative sequence abundance of pathogen taxa known to be specific to substrate or land use category followed expectations based on known ecology. For example, known leaf pathogens were only present in leaf extracts, while not present in roots and soil extracts. Forest pathogens were only present at forest sites, while not present at grasslands. These compositional changes demonstrate niche differentiation and host preference of certain plant pathogen groups. Most plant pathogen operational taxonomic units (OTUs) were fungi, followed by oomycetes and only few bacteria. Of all OTUs roughly 2% were of known plant pathogenic origin. These results are in keeping with the non-metabarcoding literature and suggest that the metabarcoding approach worked well for fungi, oomycete and bacteria plant pathogens.

This is to the best of my knowledge the first large-scale study combining metabarcoding and next-generation sequencing to investigate plant pathogen diversity from all three groups, fungi, oomycete and bacteria. The new approach could help answering questions about large scale patterns and distributions of plant pathogens which were hitherto not feasible with conventional survey methods.

Keywords

Next-generation sequencing, metabarcoding, plant pathogen communities, Illumina

3.1 Introduction

Plant pathogens play a critical role in ecosystems around the world. In natural ecosystems they are known to enhance the diversity of forests and grasslands (the Janzen-Connell and the dilution effect hypotheses) (Mitchell *et al.*, 2002; Rottstock *et al.*, 2014; Bever *et al.*, 2015; Albornoz *et al.*, 2017) and shape the succession of vegetation (Van der Putten *et al.*, 1993; Kardol *et al.*, 2006; Dickie *et al.*, 2017b). Outside their natural range invasive pathogens can have large effects on agricultural systems (Oerke, 2006) and alter entire ecosystems (Desprez-Loustau *et al.*, 2007a; O'Brien & Hardy, 2014; Dickie *et al.*, 2017a). When studying plant pathogens, very often the focus lies on individual host-pathogen systems (Ma *et al.*, 2013; Tomioka *et al.*, 2013; Whitelaw-Weckert *et al.*, 2013; Barros *et al.*, 2014; Freeman *et al.*, 2014; Kuzdraliński *et al.*, 2014; Papavasileiou *et al.*, 2015). However, plant pathogens in nature do not exist in isolation but are part of complex communities (Tollenaere *et al.*, 2016). To date, very little is known about plant pathogen communities and how their diversity and community composition change at large scales (Singer *et al.*, 2016; Burgess *et al.*, 2017b).

Plant pathogen diversity and composition is tightly connected to plant communities (Castello *et al.*, 1995; Lin, 2011; Piepenbring *et al.*, 2011; Mangelsdorff *et al.*, 2012; Hantsch *et al.*, 2013, 2014; Latz *et al.*, 2016). Hence, plant pathogen communities are strongly linked with land use, which drives vegetative community composition, diversity, and disturbance frequency (Zechmeister *et al.*, 2003; Holdaway *et al.*, 2017a).

For example, plant pathogen species richness, i.e. α-diversity (Whittaker, 1972), has been found to be especially high on hosts with a history of agricultural use (Mitchell & Power, 2003; Mitchell *et al.*, 2010). Moreover, many agricultural plants have a wide geographical range size, which is one attribute commonly associated with higher pathogen richness in a wide range of hosts including plants, animals and fungi (Mitchell *et al.*, 2010; Kamiya *et al.*, 2014). Certain agricultural practises, such as crop rotation and mono-cultures, can have an effect on plant pathogen α-diversity. For example, plant pathogen species can accumulate due to increasing residence time of a plant since establishment and with plant density which contributes to pathogen spread (Torchin & Mitchell, 2004; Perkins *et al.*, 2008; Diez *et al.*, 2010; Mitchell *et al.*, 2010; Kamiya *et al.*, 2014). Moreover, plant pathogen α-diversity could also be positively influenced by plant richness, since a wider niche space (i.e., more host plants) should support more pathogen species (Bond & Chase, 2002). For example, additional plant species can be important for completing the life cycles of certain plant pathogens (Mundt *et al.*, 2011).

Another component of plant pathogen diversity is the variation in pathogen community composition from site-to-site within a given land use, i.e. β-diversity (Whittaker, 1972). For example, it has been proposed that β-diversity of fungal plant pathogens decreases with land use intensification as a result of reduction of plant diversity (Gossner *et al.*, 2016). The loss of plant pathogen β-diversity could be due to the loss of specialised species as a result of biotic homogenisation. Thus, plant pathogen specialists are believed to be more sensitive to plant species richness, while plant pathogen generalists are believed to be more sensitive to plant species composition (Agrios, 2005).

Plant pathogen α- and β-diversity may not necessarily be a measure of pathogen load on plants, as noted by Torchin & Mitchell (2004). Nevertheless, pathogen diversity and pathogen load are closely linked. For example, Hantsch *et al.* (2014) found a similar response of plant pathogen richness and load for a wide range of possible drivers, such as functional tree diversity, the presence of particular disease-prone tree species, leaf traits, the range size of the host species, phenolic and tannin content of the leaves. Similarly, an excess of fertilization (Anderson, 2002; Datnoff *et al.*, 2007) usually results in both higher plant pathogen load and richness, e.g. as observed in organic and conventional farming practises (Letourneau & van Bruggen, 2006; Hartmann *et al.*, 2015; Talhinhas *et al.*, 2017). On the other hand, there could be an opposing effect of plant pathogen richness and load as suggested by Rottstock *et al.* (2014). If plant communities drive plant pathogens, then plant community diversity can promote pathogen diversity while at the same time reducing pathogen load and spread. Regardless of effect, both plant pathogen diversity and load are frequently linked to disturbance frequency (Mangelsdorff *et al.*, 2012; Zhang *et al.*, 2017), plant diversity (Mitchell *et al.*, 2002; Pautasso *et al.*, 2005; Haas *et al.*, 2011), biodiversity (Keesing *et al.*, 2010), the plant's life strategy and physical size (Van der Putten *et al.*, 1993; García-Guzmán & Heil, 2014), fertiliser input (Anderson, 2002; Datnoff *et al.*, 2007) and irrigation (Hong & Moorman, 2005; Palti, 2012). Because land use encompasses many of these attributes, it should strongly impact plant pathogen diversity patterns.

Plant pathogen community composition is strongly tied to vegetation (Gilbert & Webb, 2007) both within and as part of land use. Changes in plant pathogen composition with vegetation are best known from successional studies (Van der Putten *et al.*, 1993; Packer & Clay, 2000, 2003; Dickie *et al.*, 2017b; Dighton & White, 2017). These studies suggest that certain pathogen groups are adapted either to r-selected or k-selected plant species. As a result one would expect major changes in plant pathogen composition between grassland, shurb, and forest vegetations.

For example, Piepenbring *et al.* (2011) compared data on plant parasitic fungi and oomycetes across different regions but certain pathogens like Albuginales, Erysiphales, Peronosporales, and Taphrinales were almost never present in forests regardless of region.

The composition of plant pathogens is furthermore expected to greatly vary with plant substrate tested and inhabited niche due to different pathogen life strategies (Jones & Dangl, 2006). Micro-environments, such as soil, roots and above ground plant material, harbour a wide spectrum of diverse plant pathogens (Berg *et al.*, 2014). Many of which are adapted to colonize specific plant parts or endure in hostile environments (Hopkins, 1989; Jones & Dangl, 2006; Morris *et al.*, 2007; Underwood *et al.*, 2007). Although major compositional changes of plant pathogens are expected across different micro-environments, the nature of these changes remains poorly understood for the majority of plant pathogens.

In order to gain insights into the diversity and compositional patterns of plant pathogens, it is important to study where plant pathogenic organisms occur, rather than where they are simply apparent (e.g., as disease symptoms, or pathogen load) (Cunnington, 2015). Many plant pathogens can be asymptomatic or 'cryptic' at times (Jacobson *et al.*, 1998; Malcolm *et al.*, 2013; Stergiopoulos & Gordon, 2014) and persist in adverse environmental conditions or at hardly discernible levels (Stanosz *et al.*, 1997, 2007; Ploch & Thines, 2011; Crone *et al.*, 2013). Such pathogens often remain largely concealed until the next disease outbreak. However, these pathogens can be detected using DNA-based approaches such as metabarcoding (see Chapter 2). Hence, DNA-based approaches allow a holistic picture of plant pathogen communities, even where the organisms are not causing symptoms. Furthermore, the metabarcoding approach bypasses the need for cultivation and isolation of possibly non-culturable pathogens. One limitation of metabarcoding is that it cannot differentiate between an organism's behaviour as a pathogen or not. While this does not pose a problem for obligate plant pathogens (Helfer, 2014; Kamoun *et al.*, 2015; Kemen *et al.*, 2015), it could for facultative plant pathogens (Barton *et al.*, 2018). Facultative plant pathogenic organisms would, however, still represent plant pathogen potential. Thus, I hereafter refer to "*organisms which match to a known plant pathogen database to a pre-defined identity*", simply as plant pathogens, while recognizing that the presence of these organisms may not indicate actual pathogen load.

In this study I used metabarcoding to reveal large-scale patterns of plant pathogen diversity and community composition. I amplified barcode genes from known groups of plant pathogens, including fungi (Dean *et al.*, 2012), oomycetes (Kamoun *et al.*, 2015) and bacteria (Mansfield

et al., 2012), from soil, roots and aboveground plant substrate on a plot level across five major land uses across New Zealand.

Specifically, I asked:
(1) How does plant pathogen (alpha and beta) diversity in soil, roots and leaves change across different land uses?

2) How does the community composition of plant pathogen fungi, oomycetes and bacteria change in soil, roots, and leaves, and across different land uses?

3.2 Material and methods

3.2.1 Study sites and sampling

As part of the 'Next-generation biodiversity assessment' (NGBA) project we sampled a total of 75, 20 m × 20 m plots. The plots were based on the national 8 km × 8 km grid that is used extensively for national biodiversity monitoring in New Zealand (Allen *et al.*, 2003). We limited our sampling to plots located at altitudes < 1000 m in order to avoid alpine communities and ensure comparability amongst land uses. We further limited our sampling to comprise 15 plots of each of 5 different land uses (natural forest, planted forest, low-producing grassland, high-producing grassland, and perennial cropland) based on the output of the Geographic Information System and random sampling (see map of all plots Fig. 3.1, full plot location details are stored along with all vegetation data in New Zealand's National Vegetation Survey repository, NVS). We obtained the positioning of the plots by following the standardised protocol of Hurst & Allen (2007). We carried out all sampling under dry weather conditions between November 2014 and March 2015. We collected soil samples (15 cm deep and 4.75 cm in diameter) with sterilised soil sampling equipment (soil corer (AMS Inc. Idaho, USA)) following the protocol described by Orwin *et al.* (2016), and with the permission of the landowners and regional council as appropriate. Surface litter layer was removed prior to coring. Briefly, 24 sub-samples per plot were taken and pooled per plot, representing the total of 75 soil samples. All soil samples were kept cool at 4°C until laboratory processing.

We also collected pooled leaf samples with sterilised leaf punchers at each plot within a 64 min period of time (4 min for each of 16, 5 m × 5 m subplots). This procedure ensured balanced sampling of the whole plot, and consistent sampling effort across plots. All leaf samples of one plot were stored together in a vial (Falcon Tube) with autoclave-sterilised DMSO-NaCl solution (20% DMSO, 0.25 M disodium EDTA, and NaCl to saturation, pH 7.5). Vials were sealed with Parafilm M and kept cool at 4°C until laboratory processing. For more information about the leaf sampling see methods of Chapter 2.

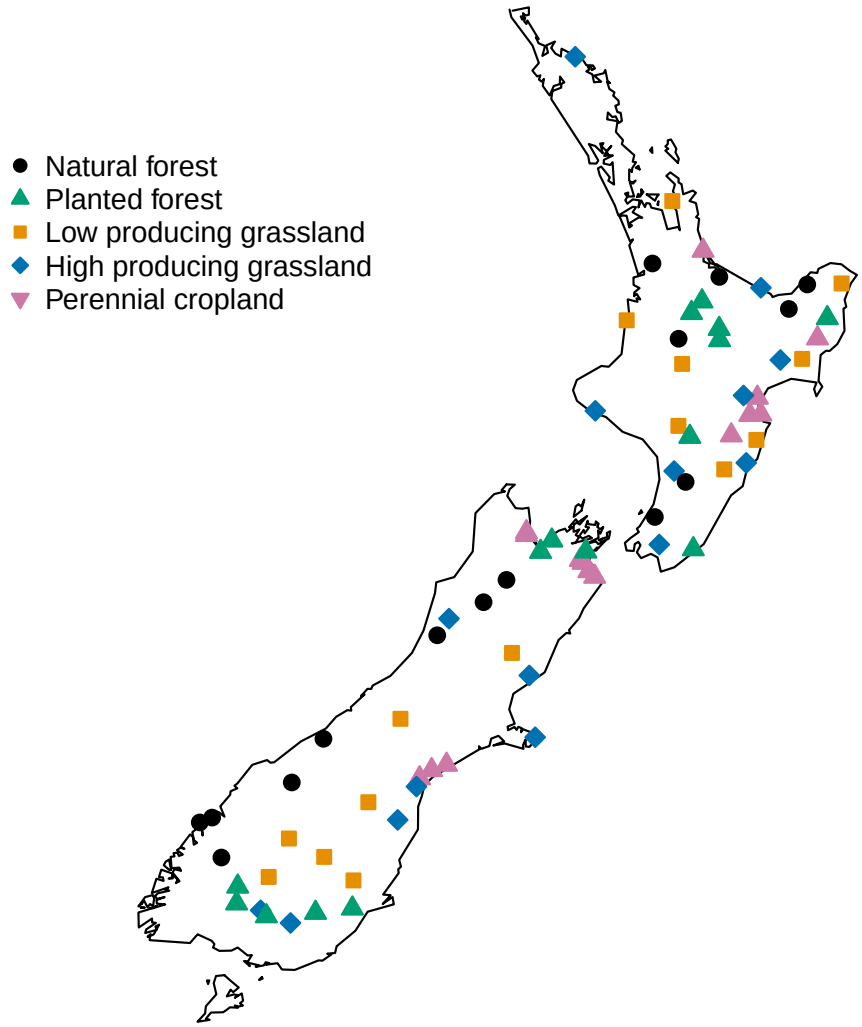

Figure 3.1: Map of all 75 study sites across New Zealand. Plot location details are stored in New Zealand's National Vegetation Survey repository, NVS.

3.2.2 DNA extraction

Soil and leaf samples were processed within the first 4 days after collection. We isolated genomic DNA from a total of four sources for each plot, i.e. two bulk soil samples, roots and leaf samples. Bulk soil DNA was isolated in two separate steps. First, we isolated DNA from 5 g of bulk soil using the PowerMax DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). Second, we isolated DNA from 250 g of bulk soil using 15 mL of phosphate buffer added to solution C2 (step 7 of the manufacturer's protocol, MoBio PowerMax soil kit) as described in Taberlet *et al.*

(2012c). Roots were sieved from the soil cores. Root sample DNA was isolated from 20 mg of dried milled roots. Root material was ground using 2 stainless steel balls (3 mm diameter) in a bead mill (Retsch MM301) for 3 min at 25 Hz. The content of a MoBio PowerBead Tube (from PowerSoil DNA Isolation Kit) was added to the root samples, along with 60 µL of Solution C1, and vortexed for 5 - 10 seconds. The tube was then incubated on a thermomixer at 60 °C for 10 min at 450 rpm, before being vortexed at maximum speed for 10 min using the MoBIO Vortex Adapter tube holder. We then followed the manufacturer's protocol of the MoBio PowerSoil DNA Isolation Kit. Leaf sample DNA was extracted using the Macherey-Nagel Soil NucleoSpin 96 well plate kit as described in Chapter 2. We checked the success of the DNA extraction with a PCR. Five µl of product was quantified using a Qubit 2.0 fluorometer (Life Technologies) and the broad-range assay kit following the manufacturer's protocol before equally pooling the two bulk soil extracts per plot.

3.2.3 Preparation of next-generation sequencing libraries

For NGS library preparation we used three PCR product pools, i.e. soil, roots and leaves, for each of the 75 plots. To help reduce contamination, we prepared NGS libraries in a one-step PCR (Immolase MoTASP Protocol), following Clarke *et al.* (2014). Taxon-specific primers (and gene region amplified) were: Bacteria (16S V4 region) F515 and R806 (Caporaso *et al.*, 2011); fungi (ITS2 region) fITS7 (Ihrmark *et al.*, 2012) and ITS4 (White *et al.*, 1990); oomycetes (ITS1 region) OOMUP18Sc (Lievens *et al.*, 2004) and ITS2P (Znajda *et al.*, 2002). Universal linker sequences at the 5' end were: TCGTCGGCAGCGTC - target sequence and GTCTCGTGGGCTCGG - target sequence. Illumina adapter sequences with barcode sequences and complementary linker sequences were: AATGATACGGCGACCACCGAGATCTACAG - 8nt barcode - TCGTCGGCAGCGTC and CAAGCAGAAGACGGCATACGAGAT - 8nt barcode - GTCTCGTGGGCTCGG. Purification and size selection (fungi/bacteria: 280 bp - 520 bp, oomycetes: 170 bp - 950 bp) was performed using a PippenPrep system to exclude primer dimers and high molecular weight DNA. I selected a larger size for oomycete amplicons because a few oomycetes, such as some *Peronospora*, were reported to have a longer ITS region. PCR products were paired-end sequenced using the Illumina MiSeq platform at the Australian Genome Research Facility Ltd, Australia.

3.2.4 Bioinformatics and statistical analysis

I merged forward and reverse Illumina reads for each taxa using USEARCH version 9.0.2132 (Edgar, 2010). I trimmed off non-biological sequences allowing 10% bp mismatch using the python tool "cutadapt" version 1.13 (Martin, 2011), if the forward primer or the reverse primer complement could be found at the sequence ends. I removed sequences with more than one expected error and less than 175 bp using VSEARCH version 2.3.0 (Rognes *et al.*, 2016). VSEARCH was selected for quality filtering because it is freely available as 64-bit version and is open-source. Moreover, USEARCH's and VSEARCH's quality filtering produced identical results. One hundred percent similar sequences were dereplicated and non-singleton sequences were clustered to 97% similarity threshold using UPARSE complete-linkage clustering algorithm (Edgar, 2013). I used BLAST version 2.5.0+ (Altschul *et al.*, 1997) to match the representative sequence of each OTU to the following reference databases. Fungi: UNITE and INSD (International Nucleotide Sequence Database, accessed 20.11.2016). Oomycetes: DNA Databank of Japan (DDBJ), a morror of INSD, using the following search ("ITS1" OR "ITS 1" OR "internal transcribed spacer 1" OR "internal transcribed spacer1") NOT ("unverified" OR "uncultured"), lineage: Oomycetes, sequence length: 100 - 5000 bp, (accessed 20.02.2017). Bacteria: Greengenes 16S unaligned 08.2013. Plant pathogen reference names were gathered from multiple sources, including FUNGuild (accessed 27.07.2017) (Nguyen *et al.*, 2016), published lists of plant pathogenic bacteria names and their synonyms (Bull *et al.*, 2010, 2012, 2014), and taxa where the majority of species have been recorded to be plant pathogens. For the lists of gathered plant pathogen names and synonyms see supplementary material A. OTUs were considered to be plant pathogens when their representative sequence matched to >93% identity over 175 bp for fungi and 150 bp for oomycetes, representing genus level; plant pathogenic bacteria had to match to >97% identity over 150 bp, representing species level. These decisions were based on 97% pre-clustered Greengenes database and suggestions from Robideau *et al.* (2011); Schoch *et al.* (2012) and Nguyen *et al.* (2016). Extraction blanks, positive and negative controls were checked for contamination. Tag jumping (Schnell *et al.*, 2015) was accounted for by using a regression of the abundance of contaminants versus the maximum of total abundances in all other samples; the coefficient estimate for the 90th quantile regression was then used to subtract that many sequences from all OTUs. This tag-jumping correction takes into account that more abundant OTUs are more likely to do tag-jumping. I used R version 3.4.1 (R Core Team, 2017) for conducting analyses and creating graphs. To calculate pathogen richness, I randomly sampled species from the entire community

to the minimum size per sample and then used the sum of the plant pathogen OTUs present in the rarefied community. This approach allowed equalisation for different sequencing depths between the samples even when very low plant pathogen OTUs were present. I used ANOVA and TukeyHSD post-hoc test to test for differences in plant pathogen richness. I visualised plant pathogen communities as non-metric multidimensional scalings based on Raup-Crick distance matrices. The Raup-Crick method applies a probabilistic null model to control for differences in α-diversity. This method was preferred over other methods as large differences in α-diversity could have biased β-diversity calculation (Anderson *et al.*, 2011; Chase *et al.*, 2011). I used PERMANOVA for distance matrices to test for differences between plant pathogen communities and land use. Beta diversity was calculated on the Raup-Crick dissimilarities with the "betadisper" function part of the "vegan" package (Oksanen *et al.*, 2017). I used ANOVA like permutation test and TukeyHSD post-hoc test to test for differences in plant pathogen beta diversity.

3.3 Results

3.3.1 General results

A total of 804 plant pathogen OTUs were obtained across all plots. Fungi represented the majority of plant pathogens identified in this study (527 OTUs), followed by oomycetes (233 OTUs), and bacteria (44 OTUs). The percentage of plant pathogen OTUs out of all fungal, oomycete and bacteria OTUs was 2.33%. A large number of oomycete OTUs (2953) had to be discarded as they matched poorly in length and identity to any database. Hence, they could not clearly be identified as plant pathogens. Their best BLAST match to NCBI, however, suggests Stramenopiles/oomycete origin. Their best BLAST match to the oomycete database suggests Leptomitales origin (Figs. A.5, A.6 and A.7 in Supplement material A).

3.3.2 Plant pathogen diversity across land uses

Almost all plant pathogen communities across substrate types showed strong responses to land use (Fig. 3.2). Plant pathogen fungal communities were significantly different between land uses in soil ($F_{1,4}$=2.99, R^2=0.15, P<0.001), roots ($F_{1,4}$=2.77, R^2=0.14, P<0.001), and leaves ($F_{1,4}$=5.17, R^2=0.23, P<0.001). Plant pathogen oomycete communities were significantly different between land uses in soil ($F_{1,4}$=2.04, R^2=0.11, P<0.001) and roots ($F_{1,4}$= 1.75, R^2=0.11, P<0.001), but not in leaves ($F_{1,4}$= 1.01, R^2=0.094, P=0.431). Plant pathogen bacteria communities were significantly different between land uses in soil ($F_{1,4}$=3.09, R^2=0.16, P<0.001), roots ($F_{1,4}$= 2.71, R^2=0.14, P<0.001), and leaves ($F_{1,4}$=5.40, R^2=0.24, P<0.001).

Land use explained between 10% and 24% of the variance between different plant pathogen communities. With the exception of oomycetes, land use could explain the highest variance in leaf pathogen communities compared to soil or root pathogen communities.

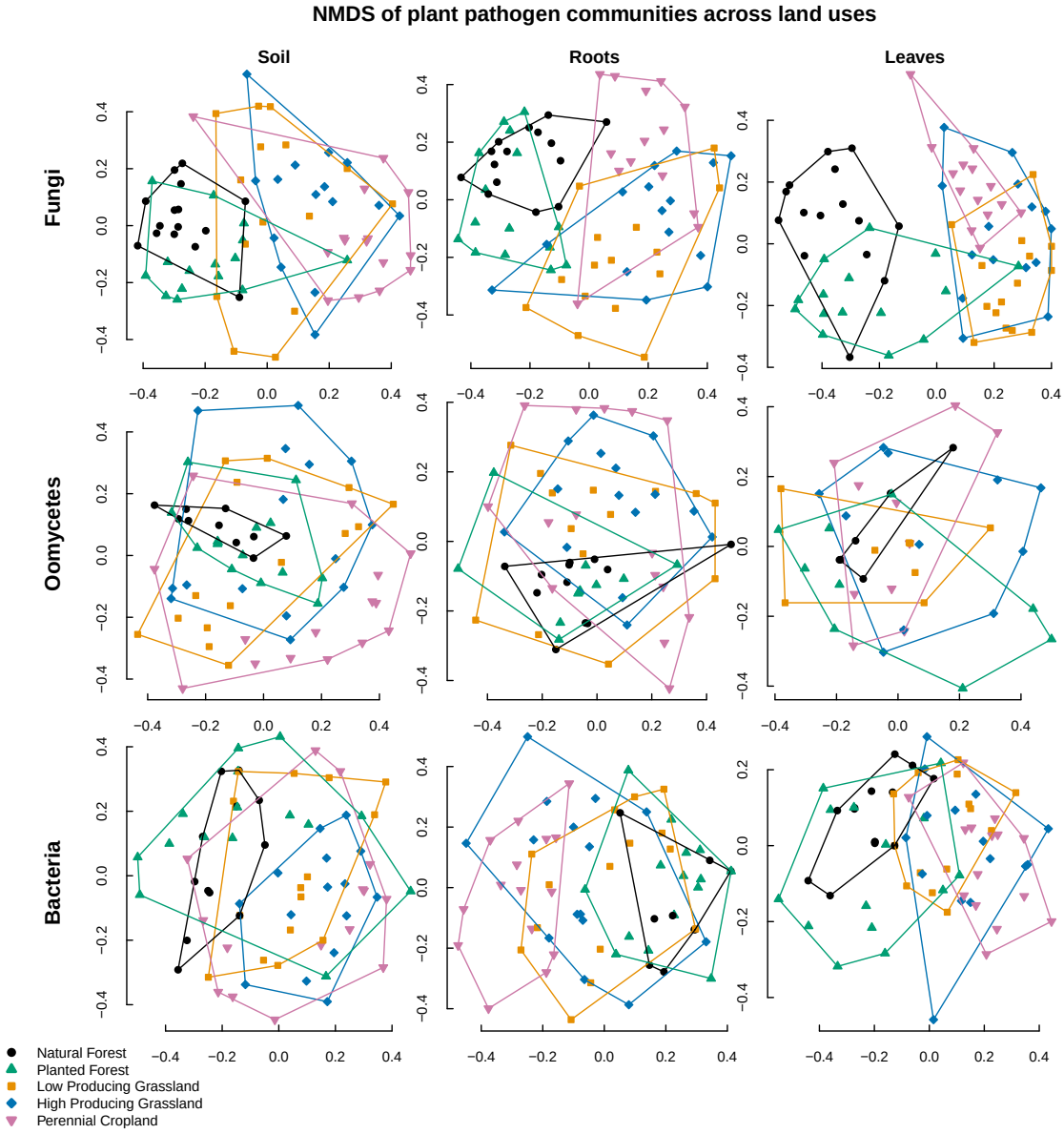

Figure 3.2: Plant pathogen communities as non-metric multidimensional scalings (NMDS) in different extracts across land uses. Dissimilarities are based on Raup-Crick. Plots with zero pathogen presence were discarded.

3.3.2.1 α- and β-diversity of plant pathogens across land uses

Plant pathogen species accumulation curves indicated a consistently lower pathogen richness (α-diversity) in natural forests across pathogen groups and substrates, while agricultural systems such as perennial croplands had a higher richness (Fig. 3.3). While there were few plant pathogen species in soil and roots of planted forests, plant pathogen species richness in leaves of planted

forests was among the highest compared to other land uses. Plant pathogen richness was usually similar among extracts with the exception of a much lower richness of oomycetes in leaves compared to soil and roots. The results of the pathogen accumulation curves were supported by rarefied community pathogen richness (Figs. 3.4 - 3.6). Whereas the slopes of the pathogen accumulation curves between land uses (Fig. 3.3) often differed, these results were not supported by Raup-Crick's α-diversity-independent measurement of β-diversity (Figs. 3.4 - 3.6). In general, β-diversity of plant pathogen communities was not significantly different between land uses (Figs. 3.4 - 3.6).

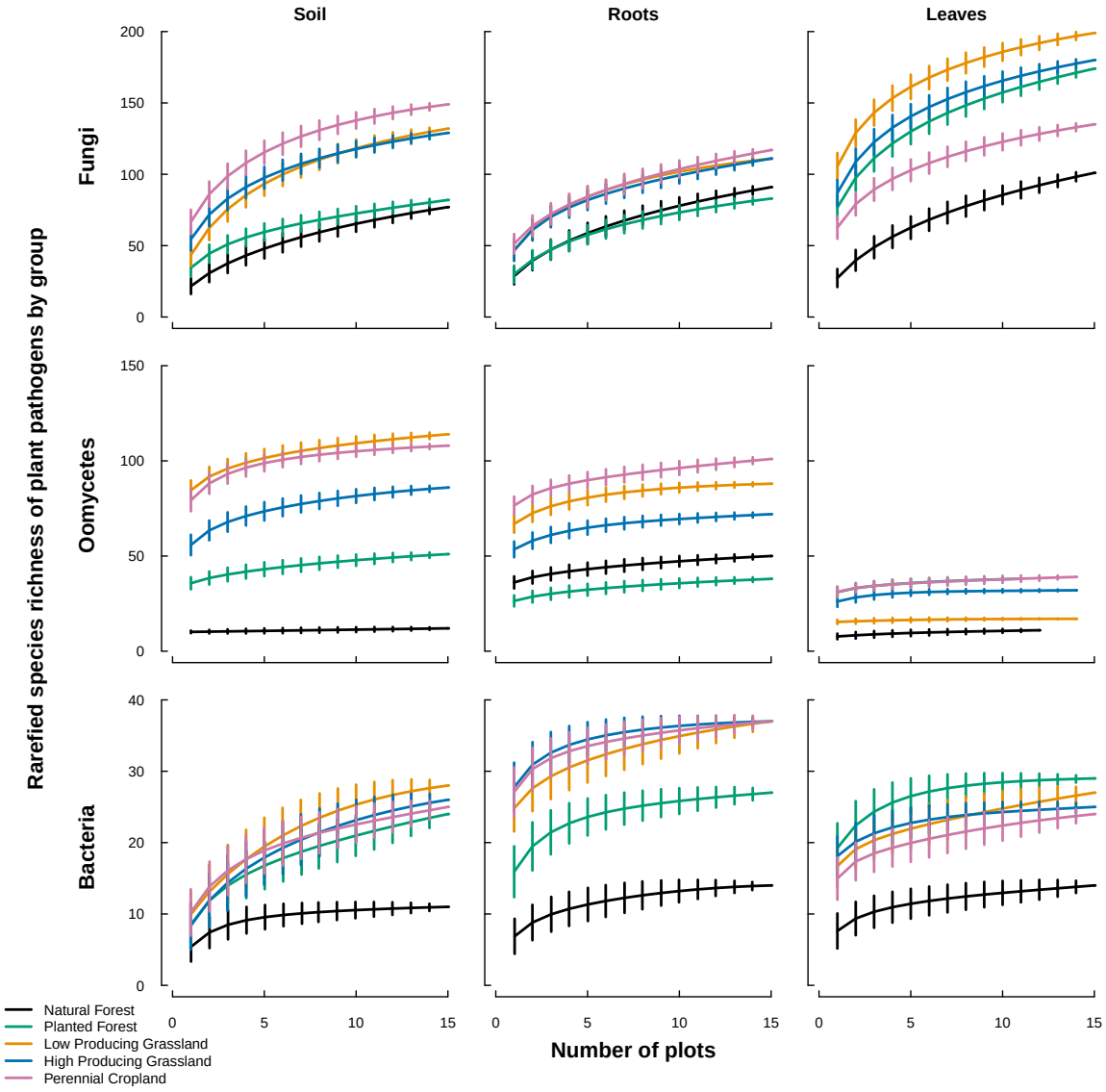

Figure 3.3: Rarefied richness of plant pathogens with 95% confidence interval in different substrates across land uses.

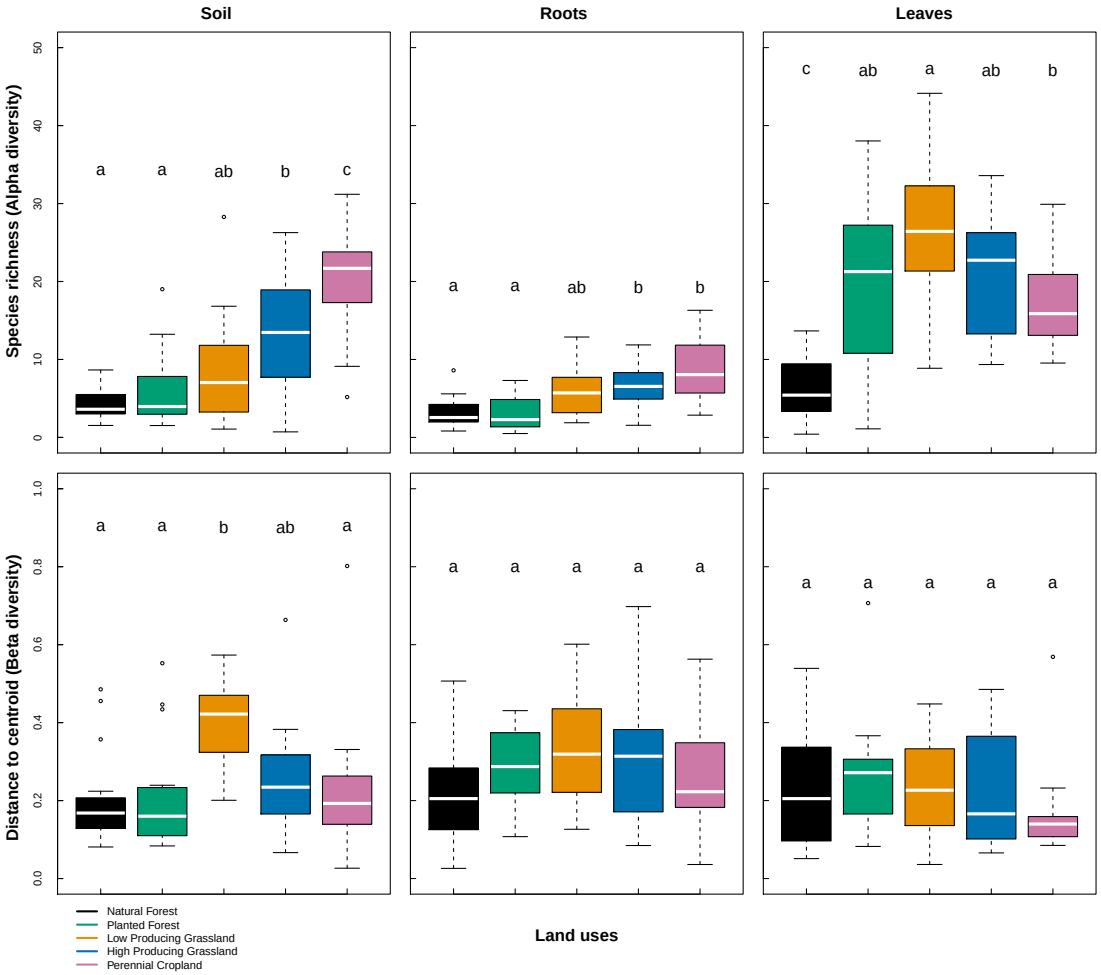

Figure 3.4: Plant pathogen fungi α- and β-diversity in different extracts across land uses. Boxes span the interquartile range (the first quartile to the third quartile). The segment inside the rectangle (white) shows the median. Whiskers represent 1.5 times the interquartile range of the data. Unfilled circles represent any data points that fall outside this range. The results of Tukey HSD (honest significant difference) post-hoc test are displayed as letters with different letters representing a significant ($P < 0.05$) difference between the means.

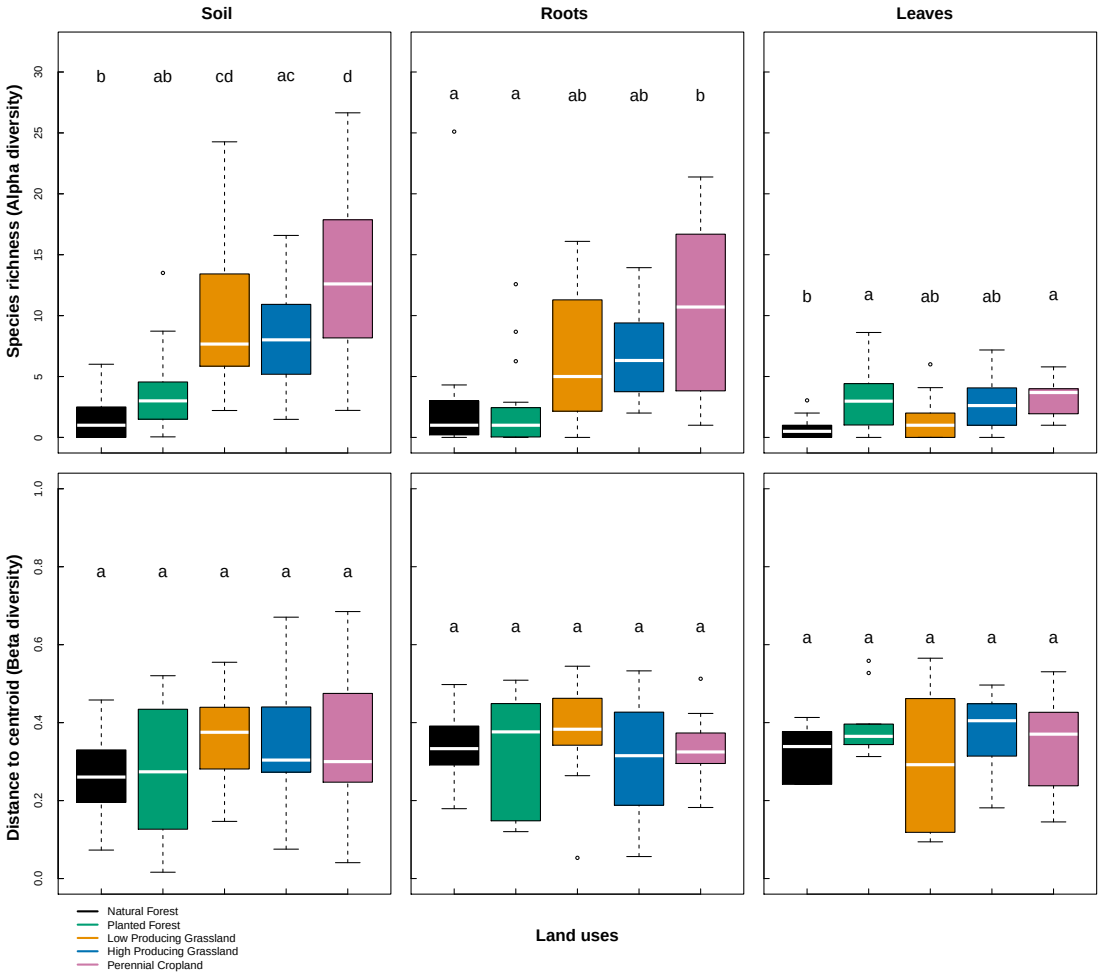

Figure 3.5: Plant pathogen oomycete α- and β-diversity in different extracts across land uses. Boxes span the interquartile range (the first quartile to the third quartile). The segment inside the rectangle (white) shows the median. Whiskers represent 1.5 times the interquartile range of the data. Unfilled circles represent any data points that fall outside this range. The results of Tukey HSD (honest significant difference) post-hoc test are displayed as letters with different letters representing a significant ($P < 0.05$) difference between the means.

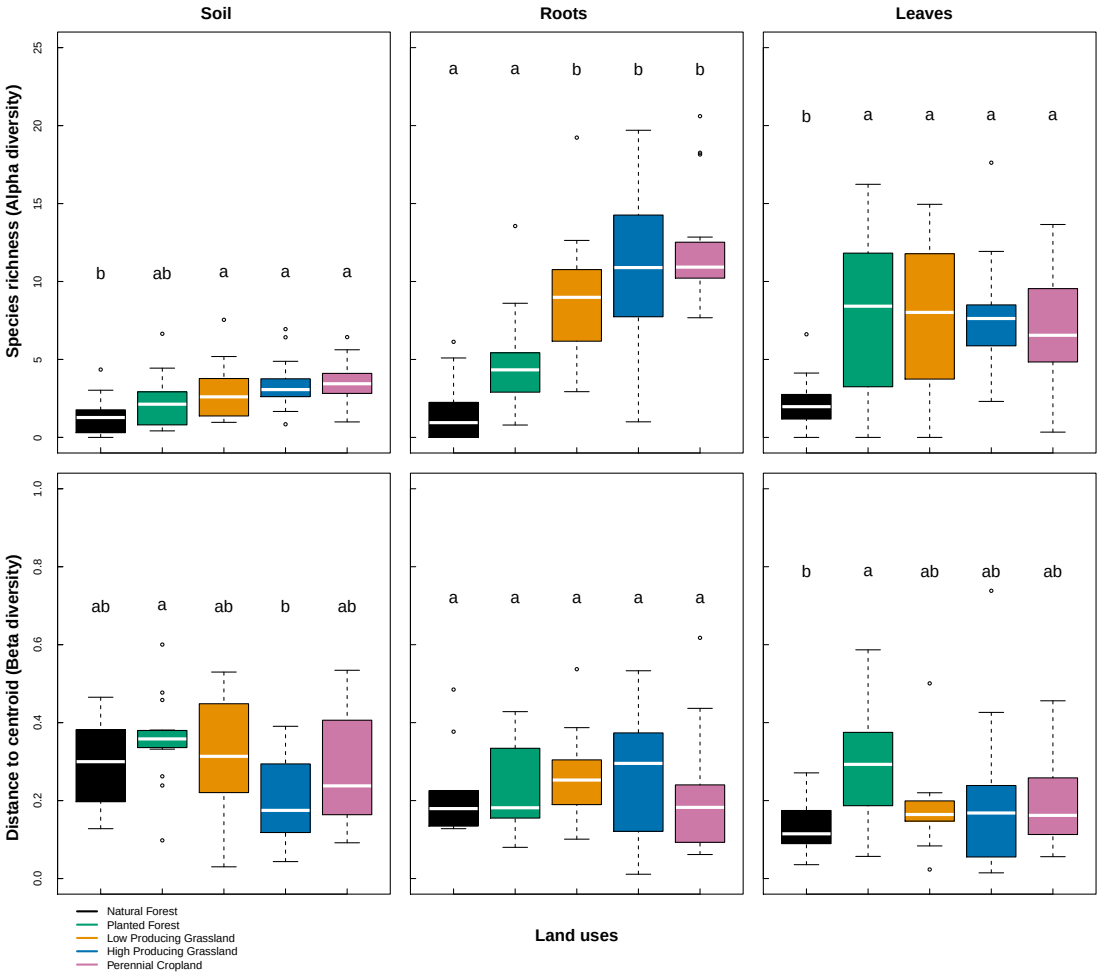

Figure 3.6: Plant pathogen bacteria α- and β-diversity in different extracts across land uses. Boxes span the interquartile range (the first quartile to the third quartile). The segment inside the rectangle (white) shows the median. Whiskers represent 1.5 times the interquartile range of the data. Unfilled circles represent any data points that fall outside this range. The results of Tukey HSD (honest significant difference) post-hoc test are displayed as letters with different letters representing a significant ($P < 0.05$) difference between the means.

3.3.3 Composition of plant pathogens

3.3.3.1 Composition of plant pathogens in soil, roots and leaves

Pathogen communities were diverse and different genera dominated in different substrate types (Figs. 3.7, 3.8 and 3.9, for fungi, oomycetes, and bacteria; respectively). For example, known leaf pathogens from the genera *Puccinia, Aureobasidium, Exobasidium, Monochaetia, Corynelia, Peronospora, Albugo, Methylobacterium* and *Curtobacterium* dominated the leaf substrate but could hardly be detected in the soil or root substrates. Similarly, soil and root pathogens like *Alternaria, Truncatella, Fusarium, Pezicula, Trichoderma, Globisporangium, Phytophthora, Ralstonia, Acidovorax, Streptomyces, Burkholderia, Herbaspirillum, Ralstonia* and *Sphingobium* could hardly be detected in the leaf substrate. *Agrobacterium, Xanthomonas* and *Pantoea* were dominantly detected in roots and leaf substrate but almost not present in soil.

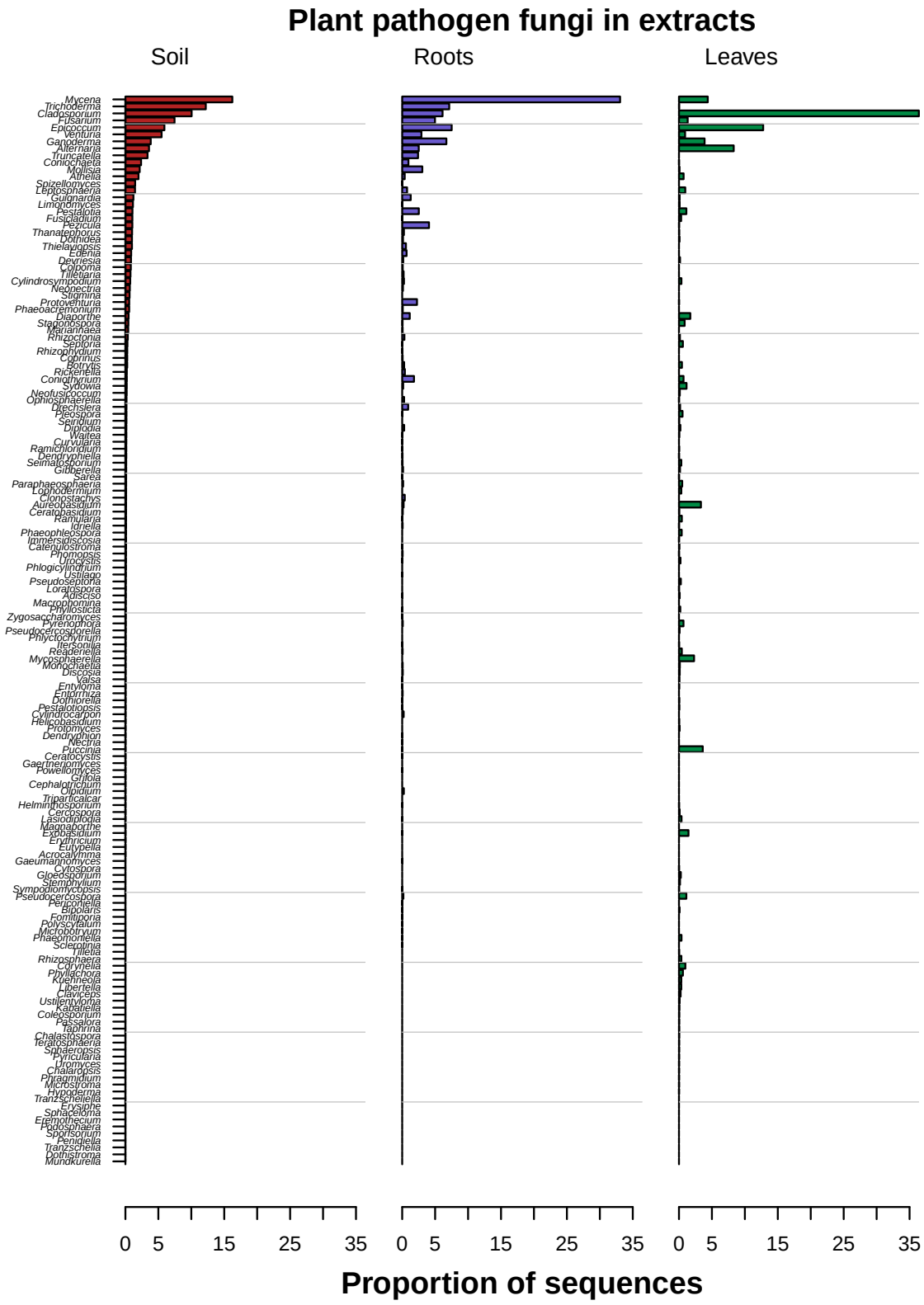

Figure 3.7: Composition of plant pathogen fungi genera detected in soil, roots and leaves. Plant pathogen identification is based on species and genus, but only genus is displayed.

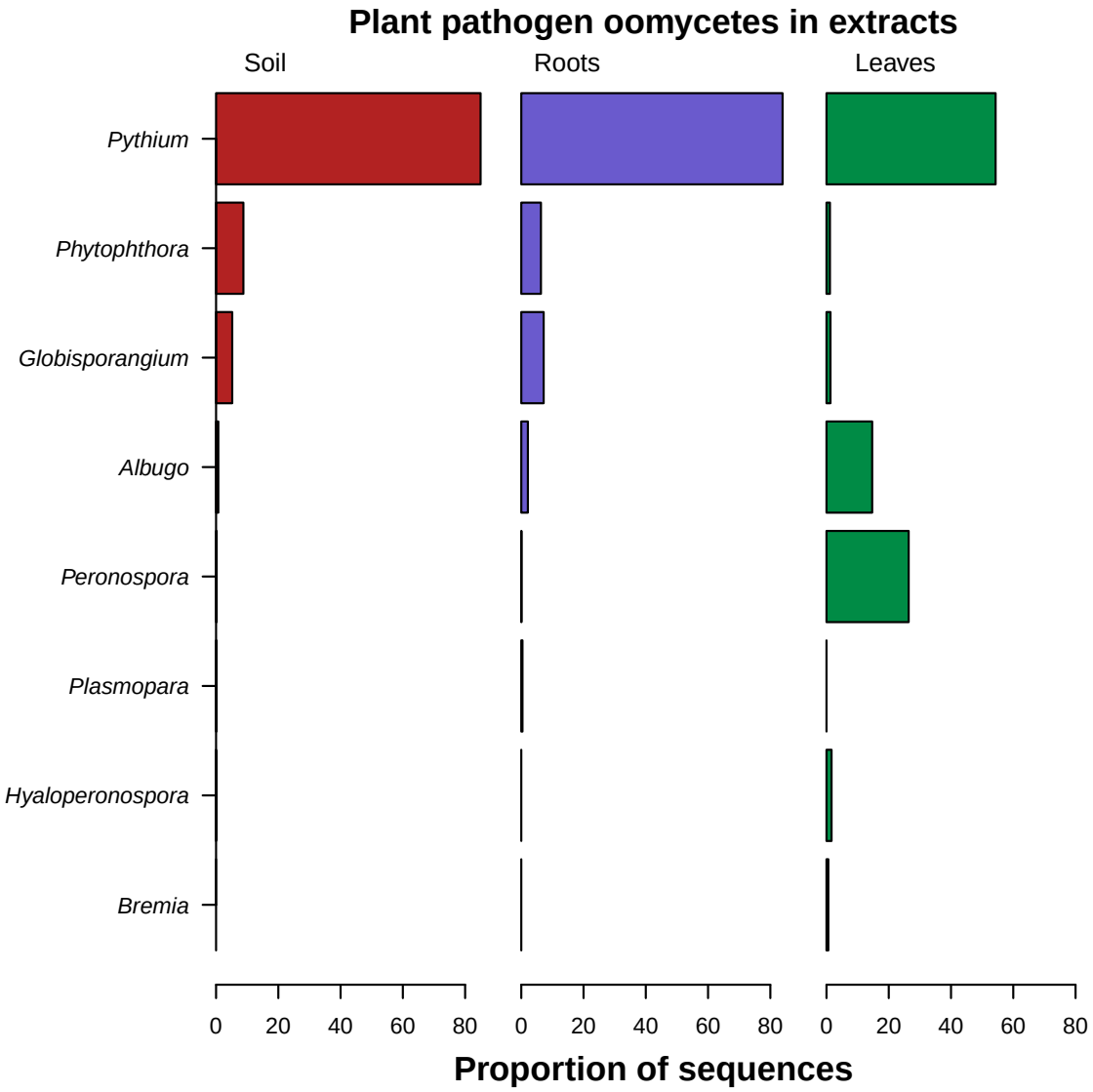

Figure 3.8: Composition of plant pathogen oomycete genera detected in soil, roots and leaves. Plant pathogen identification is based on species and genus, 97% and 93% identification, respectively. Only genus is displayed.

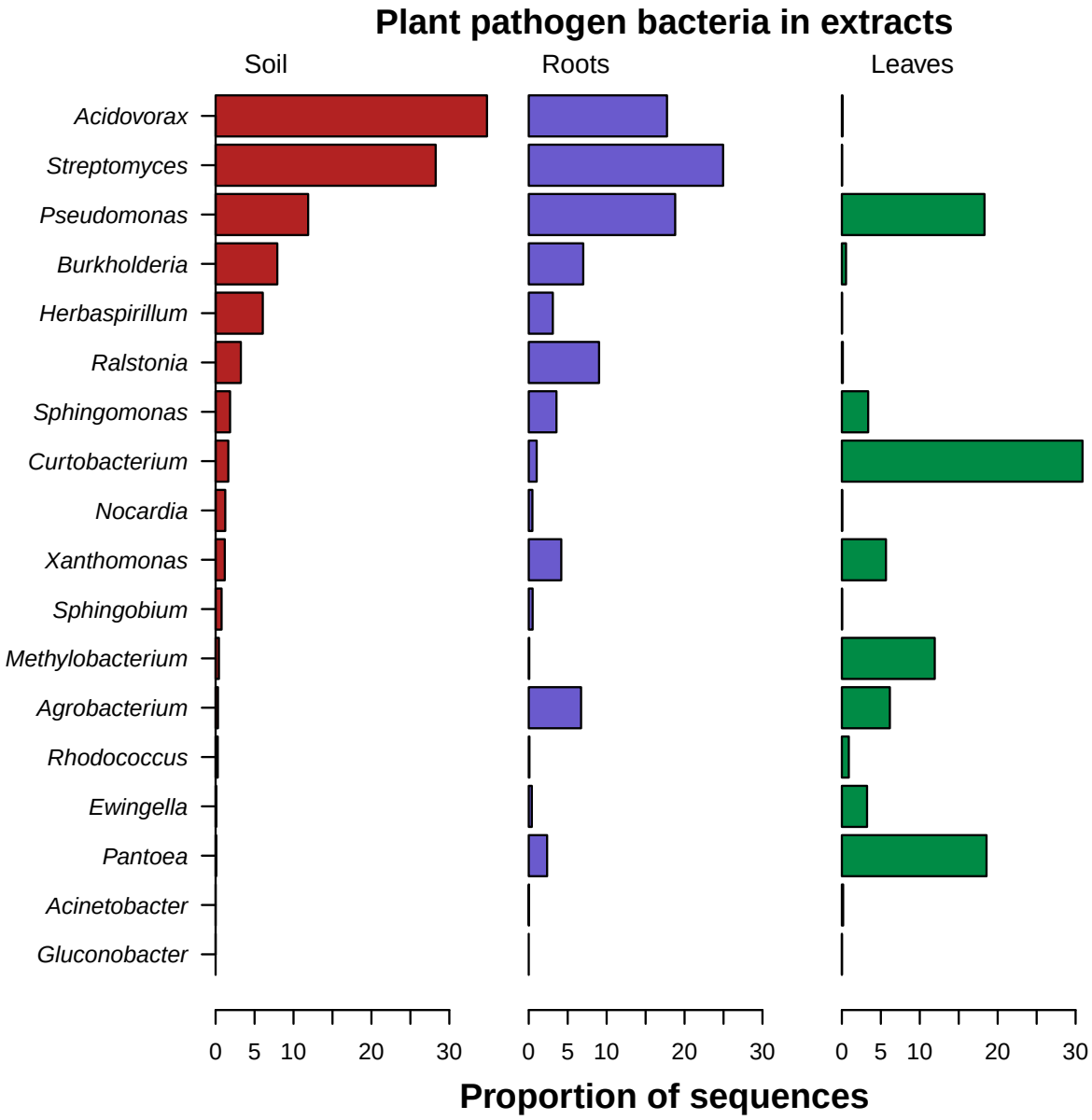

Figure 3.9: Composition of plant pathogen bacteria genera detected in soil, roots and leaves. Plant pathogen identification is based on 97% identification. Only genus is displayed.

3.3.3.2 Composition of plant pathogen fungi in different land use types

Fungal plant pathogen composition changed with land use type (Figs. 3.10 - 3.12). While some genera like *Pestalotia, Venturia* and *Ganoderma* dominated in forests, others like *Thielaviopsis, Truncatella, Epicoccum* and *Fusarium* dominated in grasslands and croplands regardless of substrate type.

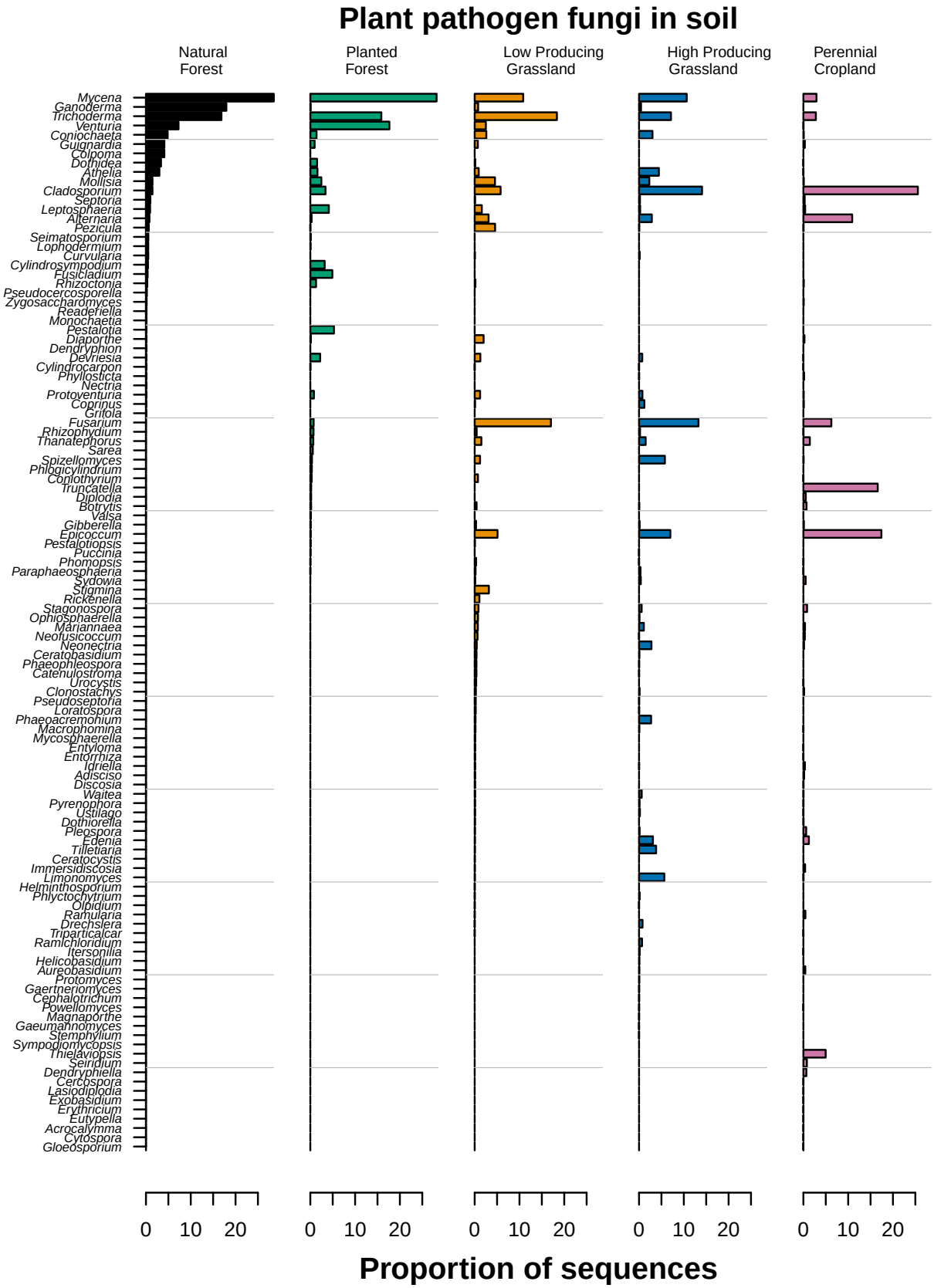

Figure 3.10: Composition of plant pathogen fungi genera detected in soil across different land uses.

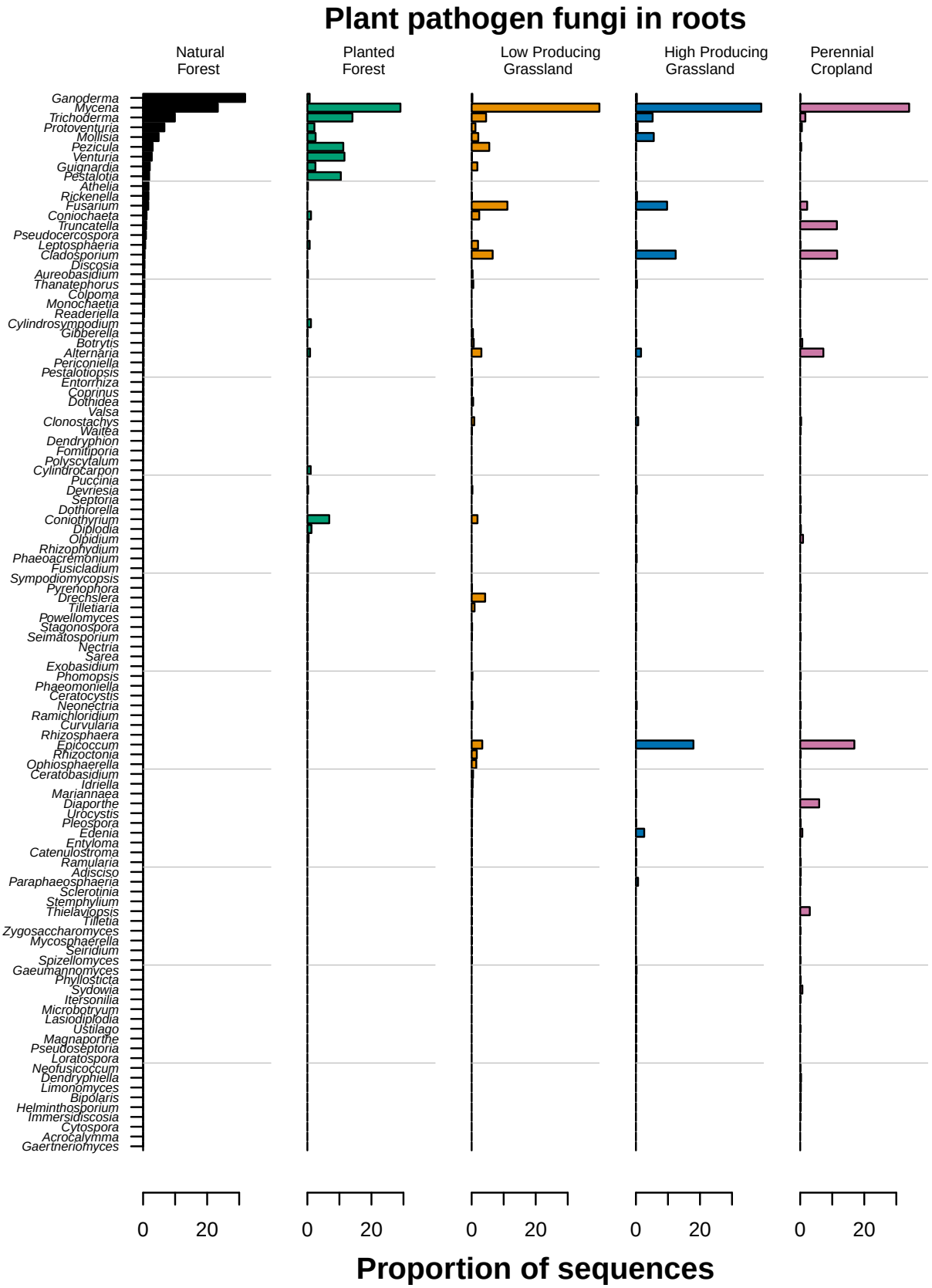

Figure 3.11: Composition of plant pathogen fungi genera detected in roots across different land uses.

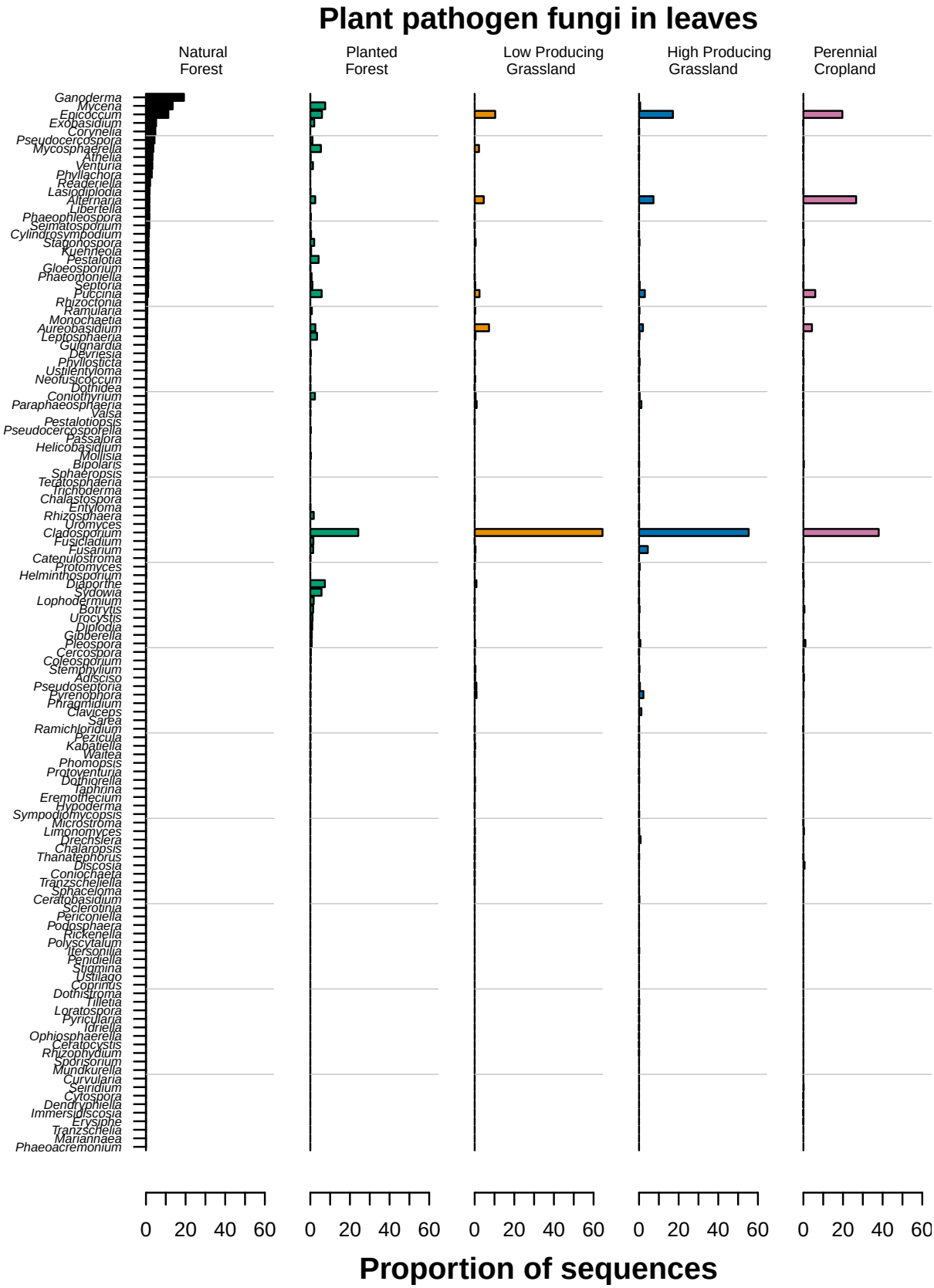

Figure 3.12: Composition of plant pathogen fungi genera detected in leaves across different land uses.

3.3.3.3 Composition of plant pathogen oomycetes in different land use types

Oomycete plant pathogen composition changed with land use type (Figs. 3.13 - 3.15). *Pythium* was the dominant plant pathogen oomycete found in all land use types and substrates. The sequence abundance of *Phytophthora* was highest in natural forests and lowest in agricultural systems, especially in soil and root substrate. Hence, for soil and root substrate the relative abundance of *Phytophthora* to *Pythium* was highest in forests.

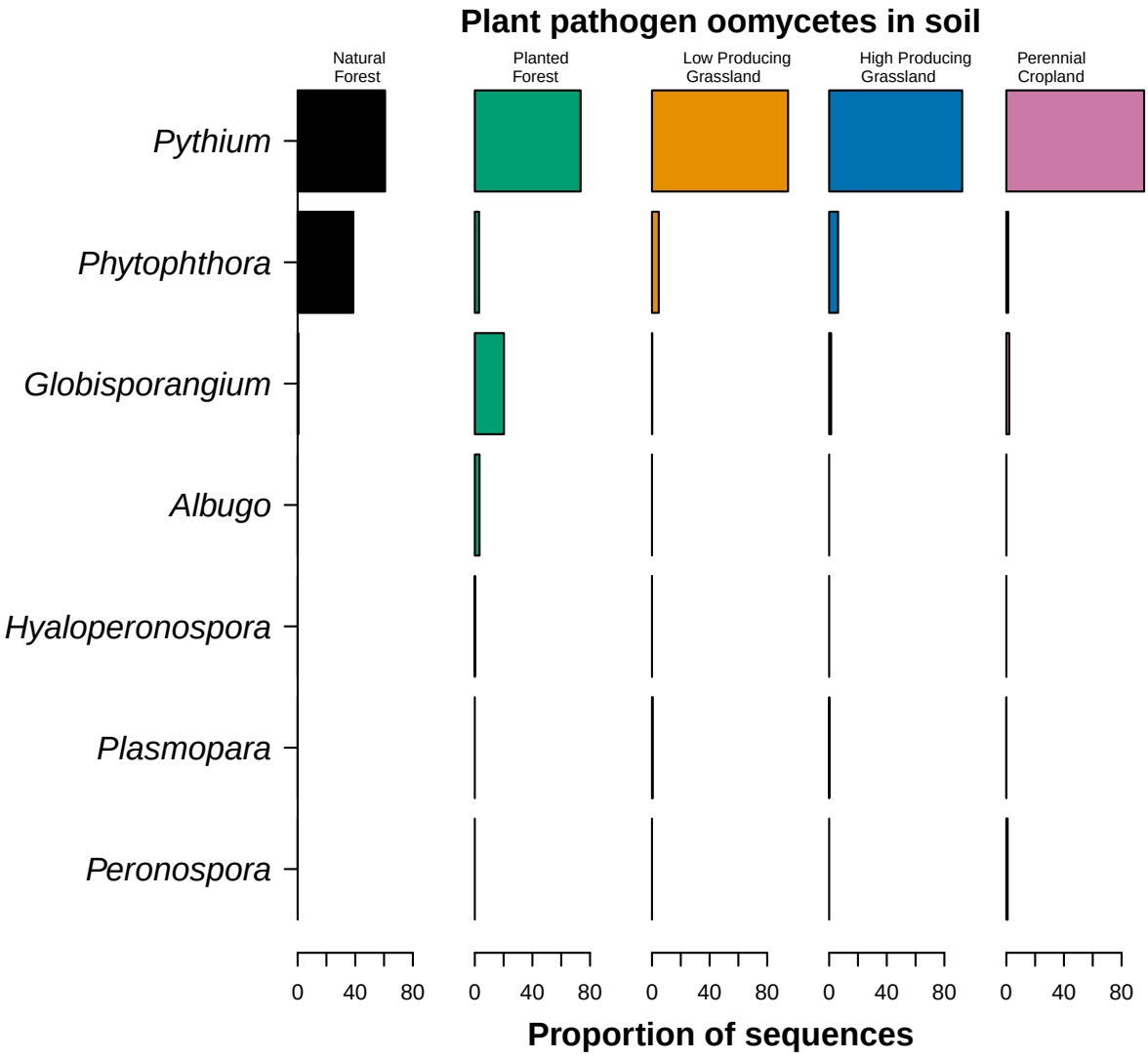

Figure 3.13: Composition of plant pathogen oomycete genera detected in soil across different land uses.

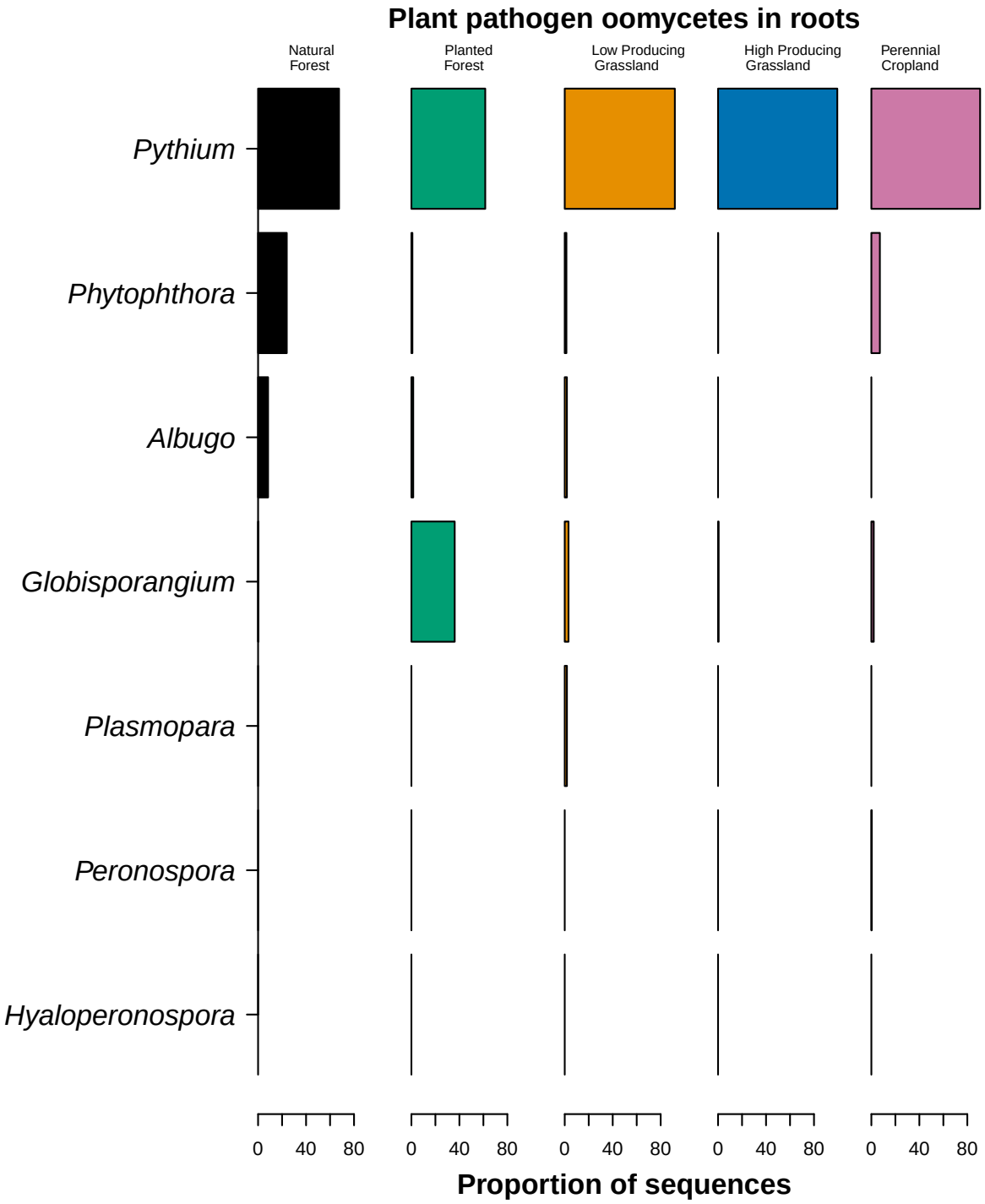

Figure 3.14: Composition of plant pathogen oomycete genera detected in roots across different land uses.

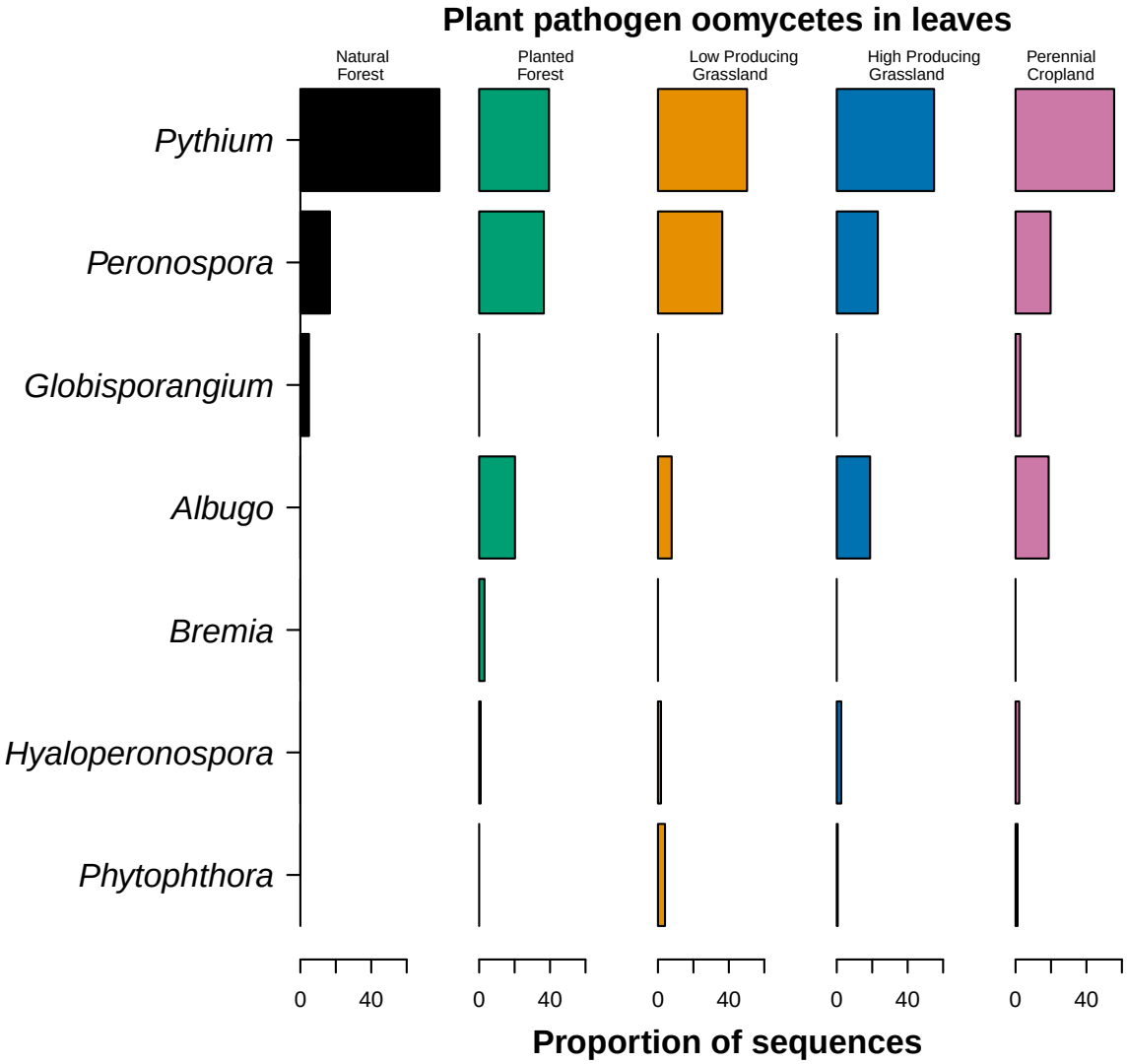

Figure 3.15: Composition of plant pathogen oomycete genera detected in leaves across different land uses.

3.3.3.4 Composition of plant pathogen bacteria in different land use types

The composition of pathogen bacteria also varied with land use (Figs. 3.16 - 3.18). For example, *Agrobacterium* and *Pantoea* were never detected in natural forests. There was a major compositional change in the plant pathogen bacteria in soil-roots and in leaves. *Acidovorax, Pseudomonas, Burkholderia* and *Herbaspirillum* dominated soil and roots in natural forests. In contrast, *Curtobacterium* dominated leaves in natural forest with roughly 70% of plant pathogenic bacterial sequences.

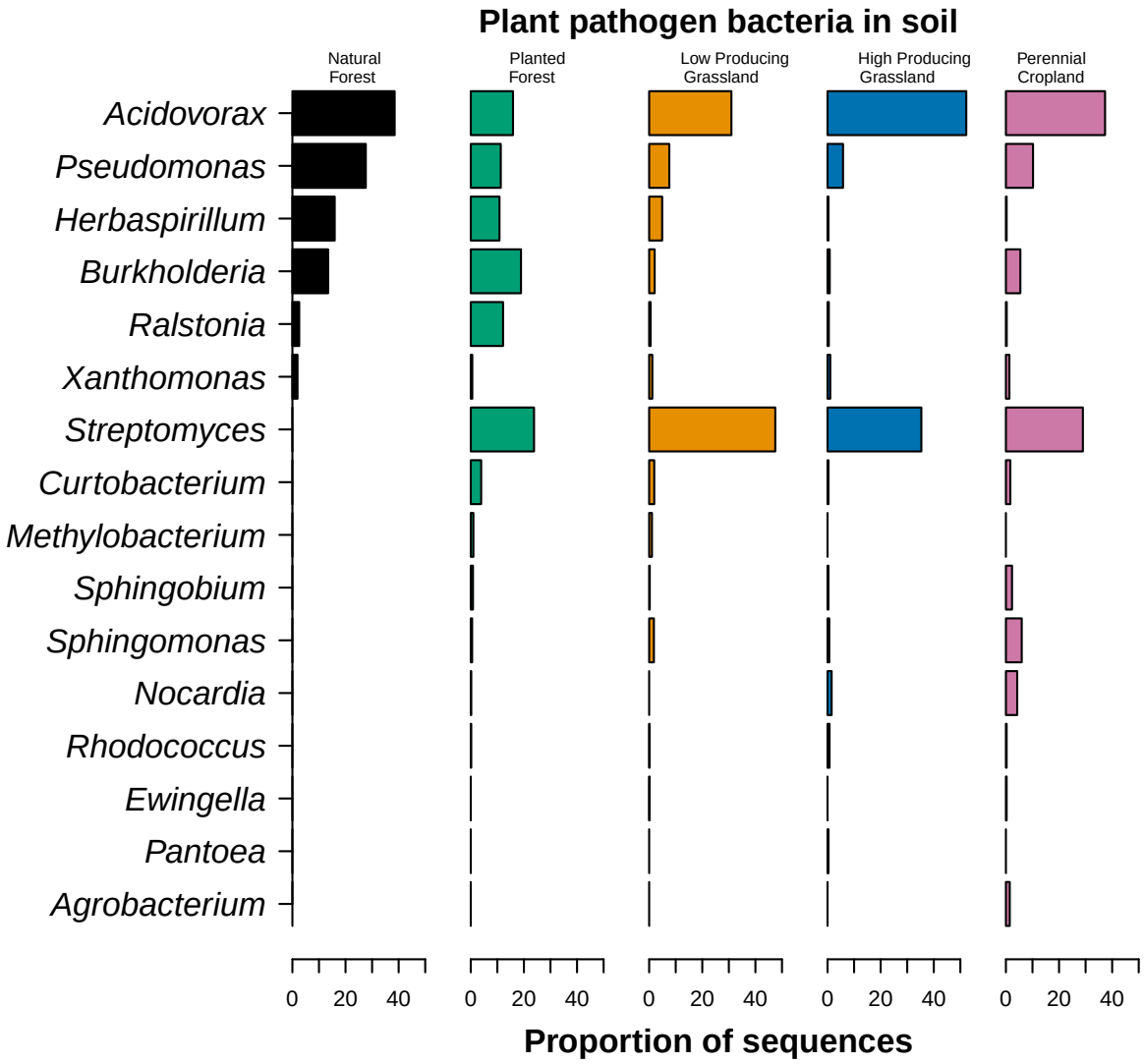

Figure 3.16: Composition of plant pathogen bacteria genera detected in soil across different land uses.

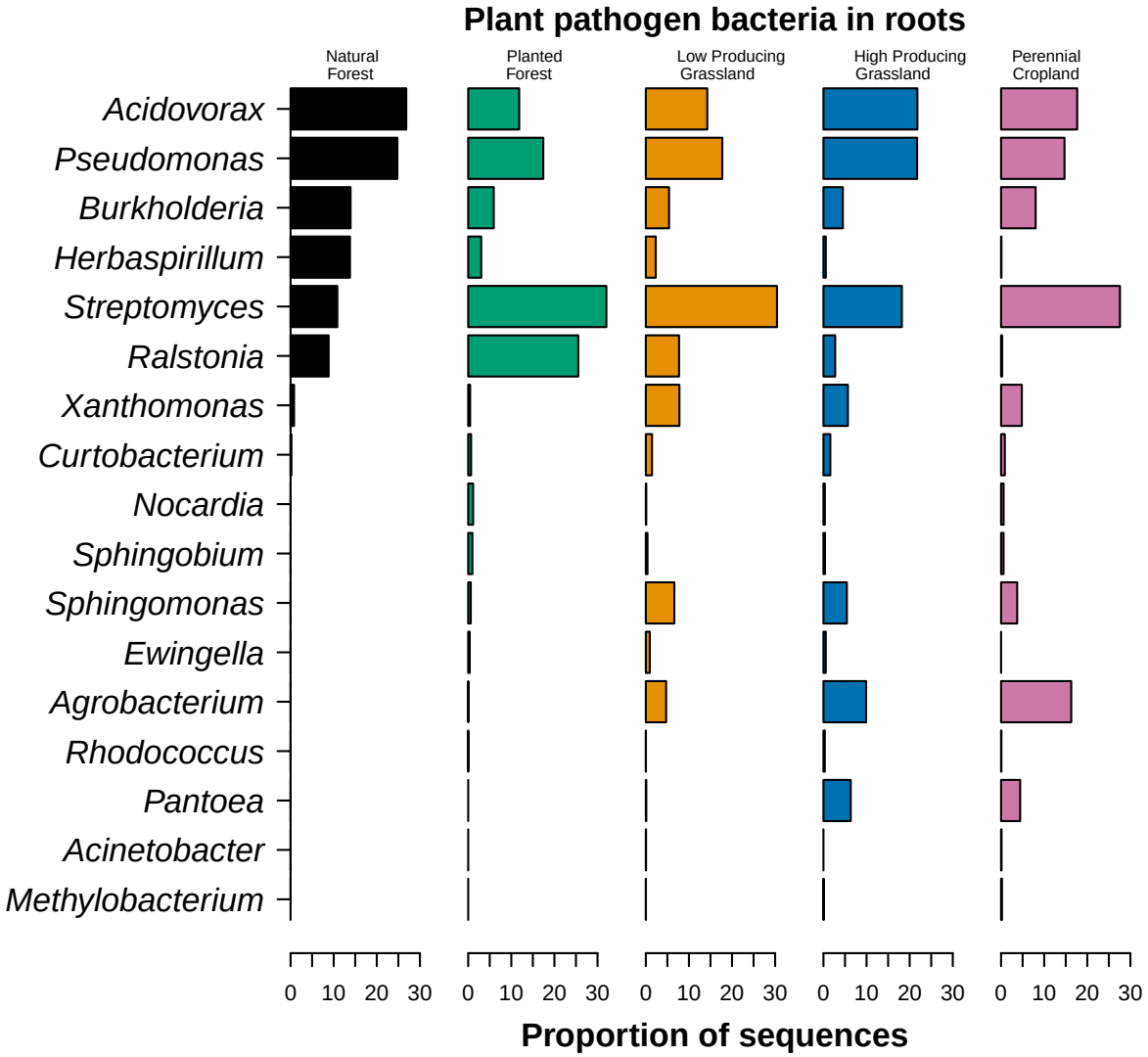

Figure 3.17: Composition of plant pathogen bacteria genera detected in roots across different land uses.

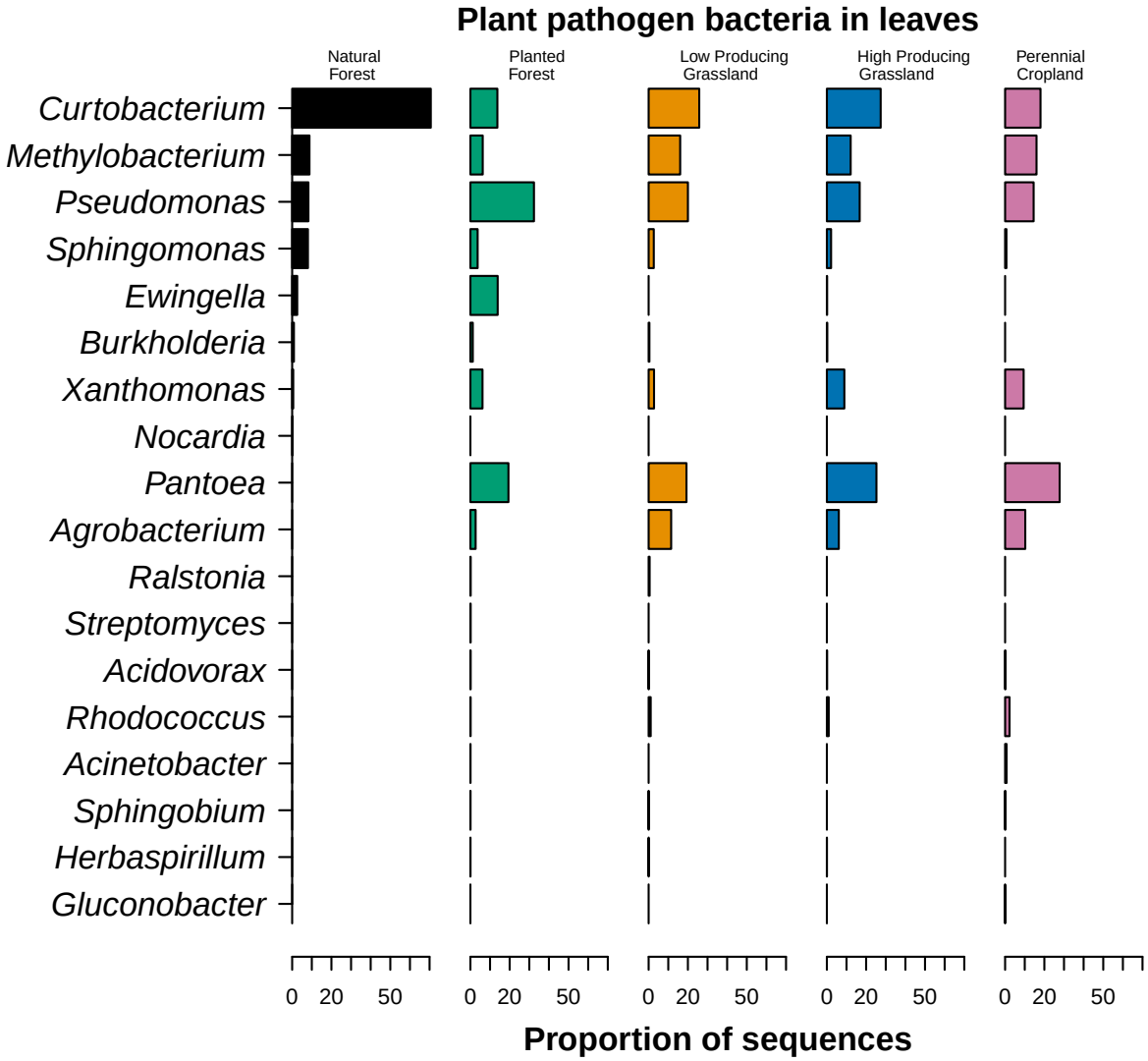

Figure 3.18: Composition of plant pathogen bacteria genera detected in leaves across different land uses.

3.4 Discussion

Plant pathogens are a vital part of ecosystems. Understanding their diversity and community patterns at large scale is crucial for choosing appropriate prevention and management strategies, and for a sustainable provision of future ecosystem services. Although several studies have investigated the effect of land use and vegetation on specific plant pathogens or severity of disease, there is an apparent lack of information about broad scale diversity patterns of plant pathogens. This study combined next-generation sequencing and metabarcoding to reveal insights into the richness and compositional changes, of plant pathogen fungi, oomycetes and bacteria across five major land uses.

3.4.1 The impact of land use on plant pathogen diversity

Land use type was an important habitat factor for plant pathogen diversity in this study. For example, plant pathogen richness (α-diversity) was very often higher in agricultural systems than it was at natural forest sites and especially high at perennial cropland sites. While pathogen richness in planted forest was low for soil and root substrate, leaf substrate showed a high pathogen richness. The results were consistent across all investigated plant pathogen groups (fungi, oomycete and bacteria) and across all substrate types (soil, roots and leaves).

Several factors may contribute to the often observed high pathogen richness in agricultural systems in this study. Firstly, plant life strategy has been proposed to play a major role explaining levels of pathogen richness. For example, early successional ephemeral plant species such as grasses and forbs encounter only low levels of pathogens in the absence of conspecific host plants (Van der Putten *et al.*, 1993; Packer & Clay, 2000, 2003; Kardol *et al.*, 2006; Mangan *et al.*, 2010). Hence, their investment in growth is usually higher than in defence (Coley *et al.*, 1985; Endara & Coley, 2011). This makes plants with rapid growth more susceptible to plant pathogens (Endara & Coley, 2011) and usually results in a high pathogen richness (Blumenthal *et al.*, 2009). For example, a review of García-Guzmán & Heil (2014) showed far more reports on diseases of annual than perennial, and light-demanding than shade-tolerant plant species. Forests and agricultural grasslands represented two extreme points in this study with two opposing plant dispersal and survival strategies (k-/r-selected). Hence, plant life strategy could be one of the attributes explaining the low observed plant pathogen richness in natural forests were only few grasses and forbs were present.

On the other hand, plant pathogen richness in leaves of planted forests was among the highest in this study. The different pattern for leaf pathogens could have been caused by forbs and exotic understory plants such as blackberry, that were often found at the planted forest sites. Planted forests could also have legacy from previous land use (such as exotic grassland in most cases). Exotic grassland tends to persist under pines for the first 10 - 15 years after planting and is well known for a steep increase of the abundance of exotic species in the early years of subsequent pine rotations (Brockerhoff *et al.*, 2003). Similarly, Mangla & Callaway (2008) reported that introduced plants can accumulate pathogens. Why soil and root pathogens in planted forests had an equally low pathogen richness as natural forests deserves further investigation. Especially examining shared biotic and abiotic factors between natural and planted forest soils could give further insights.

Such shared abiotic factors that were common between all forest sites were, for instance, the absence of irrigation and no or relatively limited fertilizer input (Smaill & Clinton, 2016). Studies looking at what environments are especially beneficial to plant pathogens have often found the cause to be an excess of irrigation (Hong & Moorman, 2005; Palti, 2012) and fertilizer input (Letourneau & van Bruggen, 2006; Hartmann *et al.*, 2015). While the right amount of nutrients supplied to plants can strengthen plants' immunity, excess of fertilizer or unbalanced nutrient supply usually benefits plant pathogens (Anderson, 2002; Kursar & Coley, 2003; Datnoff *et al.*, 2007; Wright *et al.*, 2010). This effect is known to be especially enhanced for nitrogen, a major part of sheep, beef and cattle farming in low- and high-producing grasslands and also frequently applied as fertilizer in perennial croplands (Mitchell *et al.*, 2003; Liu *et al.*, 2017). Thus, irrigation and fertilizer input could explain the higher pathogen richness in these land uses.

Lastly, disturbance frequency has been suggested to support a higher plant pathogen richness. For example, Mangelsdorff *et al.* (2012) and Zhang *et al.* (2017) found a higher diversity and richness of fungal pathogens at disturbed than undisturbed sites. Disturbance, manifested by intensification of land use has been shown to suppress plant beneficial micro-organisms and thereby promoting plant pathogen richness (Letourneau & van Bruggen, 2006; Hartmann *et al.*, 2015; Talhinhas *et al.*, 2017). Furthermore, this is in line with a higher plant pathogen richness observed in conventional than in organic farming practises (Letourneau & van Bruggen, 2006; Hartmann *et al.*, 2015; Talhinhas *et al.*, 2017). Hence, the land use types with higher disturbance frequency could explain the higher pathogen richness found in this study.

Land use type seems to represent an important factor in explaining especially plant pathogen

α-diversity. In contrast, β-diversity showed little response to land use. An exception was fungal soil pathogens which had a higher β-diversity in low-producing grasslands than in forests and perennial croplands. However, the general absence of significant differences in β-diversity suggest that the land use categories described the majority of pathogen communities well. Contrary to Gossner *et al.* (2016), who suggested that land use intensification reduced pathogen β-diversity as a result of a reduction of plant and biodiversity, I found that most of the difference between plant pathogen communities and land use was driven by different α-diversity. Plant diversity and biodiversity in general have been shown multiple times to be important components of land uses and for plant pathogens (Dupouey *et al.*, 2002; Mitchell *et al.*, 2002; Pautasso *et al.*, 2005; Van Der Heijden *et al.*, 2008; Keesing *et al.*, 2010; Laliberte *et al.*, 2010). Incorporating measurements of plant evenness, richness, functional traits and abiotic factors could provide further information explaining the drivers behind plant pathogen diversity. General species richness (the richness of all fungi, oomycetes and bacteria), however, seemed not to show a consistent pattern explaining α- and β-diversity of plant pathogens in this study (Fig. A.1 in Supplement material A).

3.4.2 Compositional changes of plant pathogens

I observed major compositional changes of plant pathogen groups across different substrate types and land uses. This result supports different ecological niches for plant pathogens. For example, leaf and stem plant pathogens such as from the genera *Puccinia*, *Aureobasidium*, *Exobasidium*, *Monochaetia*, *Corynelia*, *Peronospora*, *Albugo*, *Methylobacterium* and *Curtobacterium* were almost exclusively detected in the aboveground substrates in this study and could hardly be detected in the soil or root substrates. These genera are known to spent most of their life cycle aboveground inside their hosts (Luttrell, 1974; Petersen, 1974; Alexander & Burdon, 1984; Beattie & Lindow, 1995; Butin & Kehr, 1995; Chou *et al.*, 2000; Andrews *et al.*, 2002; Ragazzi *et al.*, 2003; Agrios, 2005; Punyasiri *et al.*, 2005; Gangadevi *et al.*, 2008). Similarly, soil and root pathogens such as from the genera *Alternaria*, *Truncatella*, *Fusarium*, *Pezicula*, *Trichoderma*, *Globisporangium*, *Phytophthora*, *Ralstonia, Acidovorax, Streptomyces, Burkholderia, Herbaspirillum* and *Sphingobium* could hardly be detected in the leaf substrates but were found in soil and root substrates where they were expected (Rotem *et al.*, 1994; Newsham *et al.*, 1995a; Jung *et al.*, 2000; Pryor *et al.*, 2002; Haas & Défago, 2005; Desjardins *et al.*, 2006; Li Destri Nicosia *et al.*, 2015; Sugawara *et al.*, 2015). Finding genera in the substrate types where they would be expected is not, in of itself, novel but supports the metabarcoding approach. Not finding, for example, leaf pathogens in soil and

root substrates, can serve a similar purpose as a negative control. Moreover, these observed compositional changes suggest different ecological niches of plant pathogens and adaptations to different plant parts.

Further evidence for niche differentiation of plant pathogens comes from compositional changes in plant pathogen communities between land uses, such as between forest and grassland in this study. This implies host-preference and specialisation of plant pathogens to certain plant types. For example, genera like *Pestalotia*, *Venturia* and *Ganoderma* were recorded in natural and planted forests. Because these pathogens were almost completely absent in grassland plots, this demonstrates their preference for forest habitat and forest plant species (Sivanesan, 1977; Arrhenius & Langenheim, 1986; Sankaran *et al.*, 2005; Holeski *et al.*, 2009; Agustini *et al.*, 2014; Hidayati *et al.*, 2014). Others including *Thielaviopsis*, *Truncatella*, *Epicoccum* and *Fusarium* were mostly recorded in grasslands, showing a different preference (Newsham *et al.*, 1995b; Caretta *et al.*, 1999; Wilberforce *et al.*, 2003; Rodinkova *et al.*, 2015; Wingfield *et al.*, 2015). A prime example of host preference and, hence, a further example of niche differentiation was found in perennial cropland plots, in particular vineyards. The species *Botrytis cinerea* with a wide host range of over 200 hosts (Williamson *et al.*, 2007) and *Plasmopara viticola* which is a rather specialised pathogen of grapevine, were both predominantly detected in vineyards plots. Grapevine is known to be especially susceptible to these two pathogens (Aziz *et al.*, 2003). Another interesting compositional change between natural forests and agricultural systems was detected in oomycete communities, possibly indicating host preference on genus level. In soil and root substrates the relative abundance of *Phytophthora* to *Pythium* was highest in natural forests, whereas it was lowest in agricultural systems. *Phytophthora* and *Pythium* are both known for preventing re-establishment of plants, and hence contributing to plant species turnover in early succession (Van der Putten *et al.*, 1993; Packer & Clay, 2000, 2003; Augspurger & Wilkinson, 2007) and maintaining plant diversity in mature ecosystems (Bagchi *et al.*, 2010). However, a big change in their abundance ratios as observed in this study implies an especially important role of *Phytophthora* in mature ecosystems. In summary, these compositional changes of plant pathogen species and groups indicate different roles and positions in their environment. Substrates, land use and host preference of certain plant pathogens demonstrate distinct ecological niches.

3.4.3 Relative importance of plant pathogens

Only a very small percentage (∼2%) of operational taxonomic units (OTUs) was found to be of plant pathogenic origin in this study. While similar low numbers have been reported for pathogens in general such as animal, human and plant pathogens (Woolhouse & Gowtage-Sequeria, 2005; Moore *et al.*, 2011), the number of plant pathogens that we failed to identify as such remains unknown. The overwhelming majority of plant pathogen OTUs observed in this study were fungal and oomycete. This is accord with the current literature and underpins the role of fungi and oomycete as important plant pathogens. For example, 70-85% of all known plant diseases are fungal and oomycete (Carris *et al.*, 2012; Kumar *et al.*, 2016). Interestingly, the proportion of detected fungi and oomycete to bacteria plant pathogens in this study is consistent with estimates of non-metabarcoding studies. For example, a survey estimated that the American State of Ohio had about one thousand diseases of plants caused by fungi and fungal-like organisms, and only about fifty due to bacteria (Moore *et al.*, 2011). This is in line with 760 plant pathogenic fungi and oomycete OTUs compared to 44 plant pathogenic bacterial OTUs in this study. The significant role of fungi and oomycete as plant pathogens is further supported by lists of recorded plant species extinctions (Fisher *et al.*, 2012). While there are about seven plant species extinctions due to fungi and oomycetes, there has not been a single record of a plant species extinction due to bacteria, viruses or helminths (Fisher *et al.*, 2012).

3.4.4 Possible limitations of the approach

Using metabarcoding to describe plant pathogen communities is a new concept. Hence, it is crucial to understand possible limitations of the new approach, although the composition results of this study suggest that the approach worked well in comparison to more traditional methods.

One limitation of metabarcoding is that the approach heavily relies on reference databases. Incomplete or erroneous databases still pose a major challenge (Coissac *et al.*, 2012; Abad *et al.*, 2016). In the case of this study I tried to limit false positives/negatives by using curated databases and only published names of plant pathogens. Limiting fungi and oomycetes to genus level allowed to a certain degree room for new species identification within a genus. However, undescribed genera of plant pathogens would be very likely missed. It is conceivable that there is less knowledge about plant pathogens outside of agricultural systems. Thus, an incomplete reference database and undescribed plant pathogens could have driven the lower plant pathogen richness observed in natural forests. However, my data opposes this idea. The largest proportion

of OTUs that had to be discarded due to low quality matches to the reference database occurred in the oomycete data. Although there were some Peronosporales pathogens missed in the leaf substrates (suggested by A.7 in Supplement material A), the overwhelming majority of discarded OTUs in all substrates belonged to the Leptomitales or closely related species (Figs. A.5 and A.6 in Supplement material A). Evidence suggests that Leptomitales-related oomycetes are not plant pathogenic (Diéguez-Uribeondo *et al.*, 2009; Blackwell *et al.*, 2015). Even though the discarded poor quality matches to the database could have been undescribed plant pathogens, they were evenly spread across land uses. Thus, it would likely not have changed the overall richness results of this study. The fact that some undescribed Peronosporales pathogens might have been missed in the leaves, could explain why plant pathogenic oomycete communities in the leave samples were not significantly separated by land use. However, no amplification of any or only few oomycete DNA in the leaf samples of some natural forest plots again suggests that the observed lower oomycete richness in leaves of natural forests is real.

Another possible limitation of the approach is that some organisms can be both, plant pathogens and mutualists. *Fusarium* is a prime example of a genus that can contain non pathogenic species (Validov *et al.*, 2011). Similarly, *Trichoderma* or *Pseudomonas* strains have been reported as being plant pathogenic (Harman *et al.*, 2004; Xin & He, 2013; Li Destri Nicosia *et al.*, 2015) as well as in suppressing other plant pathogens (O'Sullivan & O'Gara, 1992). Species with possibly both, parasitic and mutualistic behaviour, however, represented only a very small minority of the data in this study. Furthermore, these opportunistic pathogens are rarely included in plant pathogen databases simply because they usually do not cause significant economic losses (Charkowski, 2016). Even if opportunistic pathogens were included in this study, they still can represent pathogen potential. Thus, the overwhelming signal explaining the richness and compositional patterns in this study was mainly obtained from primary plant pathogens.

3.4.5 Conclusions

This study described broad scale plant pathogen diversity and community patterns. Although a wide range of plant pathogens was included, the patterns of pathogen richness across different land uses were surprisingly consistent. A higher plant pathogen richness in agricultural systems suggests a better environment for plant pathogens in these systems in general. Moreover, detected compositional changes in plant pathogen communities across soil, roots and leaves and land uses support the metabarcoding approach and demonstrate niche differentiation and host preference

of certain plant pathogen groups. At the same time, this study revealed an apparent lack of knowledge about oomycetes in general and suggests potential for further discoveries. Several new species (Amal *et al.*, 2006; Paul, 2006; Ke *et al.*, 2009) and even a new family of oomycetes (Hulvey *et al.*, 2010) have only recently been discovered. Some of the most abundant oomycetes in this study had low quality matches to the *Saprolegnia-Achlya* clade (Figs. A.2 - A.7 in Supplement material A). Although it is known that these oomycetes can be very abundant (Dighton & White, 2017), their precise role in terrestrial ecosystems is yet unclear and remains an exciting future research subject.

Metabarcoding has proven to be a useful tool that could reveal diversity patterns and compositional changes of a wide range of different plant pathogens in this study. Thus, it could be applied to answer other important large scale questions about plant pathogens including, for example, temporal questions such as seasonal fluctuations in their abundances (Wilberforce *et al.*, 2003) or causal questions about their drivers. The here collected plant pathogen sequence data is a valuable baseline against which future changes or pathogen incursions can be measured.

Chapter 4

The relative importance of environmental factors for plant pathogens: Testing the disease triangle

Notes

The results of this chapter are intended to be submitted to New Phytologist.

Abstract

Although interactions between plant pathogens, the environment, land use, and plants have been described many times, the individual contribution of the different parameters remains largely unknown. This study employed metabarcoding of fungi, oomycete and bacteria plant pathogens at a national scale across New Zealand to investigate the relative importance of environmental factors, including geomorphology, climate, land use, soil and plant communities (the composition of plant species), for plant pathogen communities and richness. Plant pathogen communities as well as richness showed a unified strong response towards plant communities which generally could explain most of the variance after accounting for all other parameters. In face of future changes in climate and land use, these results suggest that changes in plant pathogen communities as well as richness will mostly be mediated through changes in plant communities, rather than direct effects of climate or soils.

Keywords

metabarcoding, next-generation sequencing, multiple regression on matrices

4.1 Introduction

Interrelations among plant pathogens, the environment, and plants have long been recognised. For example, over 100 years ago, Duggar (1909) wrote about fungal plant diseases and their connection with "climatological factors". Later, Stevens (1960) introduced the equilateral disease triangle, putting equal weight on each of the three factors. Since then many different versions of the disease triangle have emerged by adding one or more parameters and giving certain parameters more weight than others which resulted in varieties such as disease tetrahedrons, prisms, and cones (Agrios, 2005; Chappelka & Grulke, 2016). While the relationship between plant pathogens, environment, and plants is well accepted, there is still uncertainty about the relative importance of the individual factors.

Some of the main factors that are frequently associated with plant pathogens include climate, geomorphology, land use, soil, and plant communities (the composition of plant species) (Agrios, 2005; Bever *et al.*, 2015). These factors can influence plant pathogens directly and indirectly, some of which might be more important than others (Fig. 4.1).

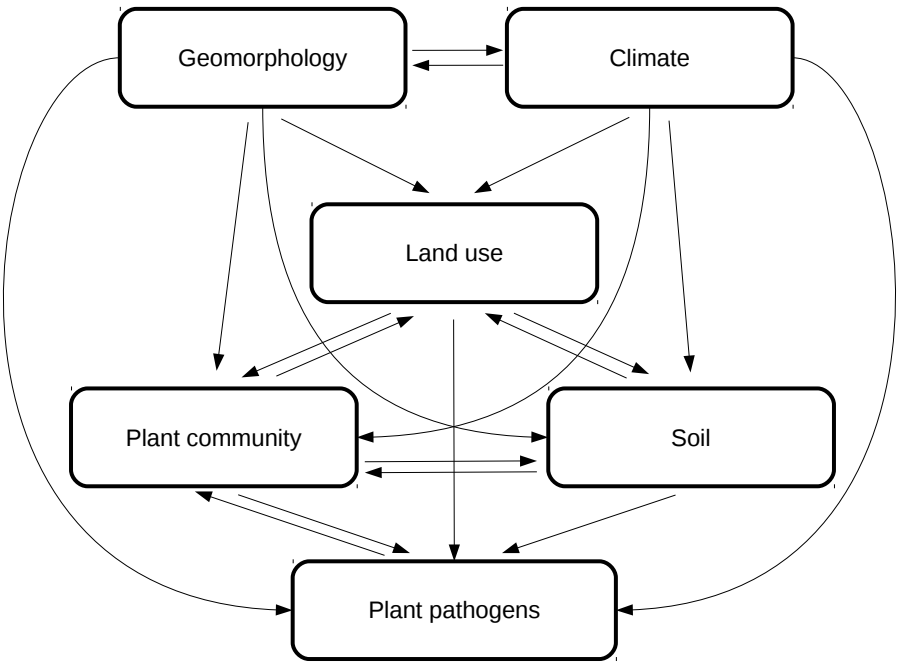

Figure 4.1: Conceptual framework of interrelationships between plant pathogens, their host community (plants) and potentially important environmental factors.

Within the framework of Fig. 4.1, climate and geomorphology could be especially important for explaining plant pathogens since they can be an external driver of all other parameters (Veldkamp & Lambin, 2001; Guo & Gifford, 2002; Yamaura *et al.*, 2011). The direct effects of climate on individual pathogen species and groups have been extensively studied, reflecting concerns over future climate change potentially increasing pathogen outbreaks and altering pathogen life cycles and distributions (Prestidge & Pottinger, 1990; Tapsoba & Wilson, 1997; Mayek-Pérez *et al.*, 2002; Gilles & Kennedy, 2003; Sivasithamparam *et al.*, 2005; Englander *et al.*, 2006; Chakraborty & Newton, 2011; Eastburn *et al.*, 2011; Juroszek & Von Tiedemann, 2011; Luck *et al.*, 2011; Barbetti *et al.*, 2012; Fisher *et al.*, 2012; Ghini *et al.*, 2012; Siebold & Von Tiedemann, 2012; Bebber *et al.*, 2013; Johnson & Cummings, 2013; Spear *et al.*, 2015).

Moreover, land use has been shown to be a reliable predictor of plant pathogen communities and richness in Chapter 3. This may reflect the fact that land use integrates climate and geomorphology, and reciprocal effects on plant communities and soil properties (see Fig. 4.1, and (Lauber *et al.*, 2008; Orwin *et al.*, 2015; Tyler *et al.*, 2017; Wood *et al.*, 2017)). Additionally, land use encompasses other attributes frequently linked to plant pathogens, such as fertilization, irrigation, and disturbance (Anderson, 2002; Hong & Moorman, 2005; Datnoff *et al.*, 2007; Mangelsdorff *et al.*, 2012; Palti, 2012; Zhang *et al.*, 2017),

Furthermore, soil can be a direct and indirect regulator of plant pathogens. For example, soil properties, like pH, water holding capacity and cation exchange capacity, can directly affect soil pathogens (Bittner, 2016). Other soil properties, particularly macro-nutrients like nitrate, potassium, and plant available phosphorus, affect plant pathogens indirectly through increasing or decreasing host plant susceptibility (Anderson, 2002; Datnoff *et al.*, 2007).

Finally, the plant community per se could represent a strong predictor of plant pathogens. The plant community as a whole comprises not only α- and β-diversity (Bond & Chase, 2002; Mitchell *et al.*, 2002; Pautasso *et al.*, 2005; Keesing *et al.*, 2010; Haas *et al.*, 2011), but also many other attributes that have been shown to affect plant pathogens. Such attributes include, for example, the plant's life strategy (Van der Putten *et al.*, 1993; Packer & Clay, 2000, 2003; Dickie *et al.*, 2017b; Dighton & White, 2017), maximum physical size (García-Guzmán & Heil, 2014), geographic region and range (Mitchell *et al.*, 2010; Piepenbring *et al.*, 2011; Kamiya *et al.*, 2014), agricultural history (Mitchell & Power, 2003; Mitchell *et al.*, 2010), phylogenetic signals from co-evolution (Gilbert & Webb, 2007), different functional traits and the presence of particular disease-prone plant species (Hantsch *et al.*, 2014).

Plant pathogens, climate, geomorphology, land use, soil and plant community are closely intertwined with each other. Despite the fact that these drivers are well known to affect plant pathogens, they have been mostly studied in isolation (Mitchell *et al.*, 2002, 2003, 2010; Bever *et al.*, 2015; Gossner *et al.*, 2016). Previous studies have generally been confined to drivers within particular land uses, and hence fail to account for how land use change could impact pathogen communities. Furthermore, as opposed to direct effects of climate on plant pathogens, the indirect effects of climate via modifying land use or plant communities have received less attention.

In order to investigate the relationship and relative importance of different environmental parameters for plant pathogen communities and richness, this study employed metabarcoding of fungi, oomycete and bacteria plant pathogens across a wide range of environmental variables and land use types at a national scale across New Zealand. Considering the recent attention that plant pathogens received in light of climate change (Garrett *et al.*, 2015; Newbery *et al.*, 2016; Fones & Gurr, 2017; Lukas *et al.*, 2017), I was particularly interested in the relative importance of climate on plant pathogens. I hypothesized that

1. Variance in plant pathogen community composition is explained more by climate than land use, geopmorphology or plant community.

2. Variance in plant pathogen richness is also explained more by climate than land use, geopmorphology or plant community.

4.2 Material and methods

A detailed description of the methods including study sites, sampling, DNA extraction, preparation of next-generation sequencing libraries and bioinformatics can be found in Chapter 3 under the section material and methods 3.2.1.

4.2.1 Environmental variables

I tested the relative importance of five environmental parameters for plant pathogens: climate, geomorphology, land use, soil and plant community (the composition of plant species).

Climate variables consisted of mean annual rainfall and mean annual temperature. Both variables were obtained from Land Environments of New Zealand (Leathwick *et al.*, 2003) and have a resolution of 25 metres which roughly corresponds with the 20 m x 20 m plots used for sampling.

Geomorphology variables consisted of slope and altitude. Slope was measured in the field using a clinometer (the average slope from the plot center to up-slope and down-slope 20 x 20 m plot edge). Altitude was taken from topographic maps.

Land use type was classified as under section 3.2.1 in Chapter 3, and distinguished between five land uses: (1) Natural and (2) planted forest, (3) low- and (4) high-producing grassland and (5) perennial cropland (Leathwick *et al.*, 2003).

Soil variables consisted of pH, water holding capacity (WHC), soil carbon (C), nitrogen (N), C/N ratio, olsen phosphorus, nitrate, total phosphorus, calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), cation exchange capacity (CEC), and base saturation. All soil variables were measured following the procedure described in Orwin *et al.* (2016) with the exception of pH. I measured the soil pH per plot, following the protocol as used by Landcare Research (suspension in H_20) with two modifications. Firstly, instead of taking the pH value after 30 seconds, I took the value after the last value had not changed for at least 30 seconds in order to obtain a similar saturation point. Secondly, I used 4 g of dried soil in lieu of 8 g fresh soil because the soil samples were dried for storage.

The mean and the standard error of all soil, climate, and geomorphology variables in each land use category can be found in Supplement B, Table B.1.

Plant species were identified by a botanist in the field. Site-level percentage cover scores were calculated for each plant species as a measure of plant species abundance, using the geometric mean for each cover class, averaged across all height classes and plots within a site.

4.2.2 Statistical analysis

I examined the relationship of plant pathogen communities and richness to environmental variables through multiple regression on distance matrices (MRM; (Lichstein, 2007)) using the "ecodist" package (Goslee & Urban, 2007) in R version 3.4.1 (R Core Team, 2017). A path modelling framework to disentangle the direction of the complex relationships among plant pathogens, plants and environment could not be employed due to multiple mutual relationships between the variables which did not represent an acyclical causal graph (see Fig. 4.1, (Shipley, 2009)). Furthermore, collinearity between the closely related variables would have made any causal inference impossible. To investigate the relative importance of different environmental variables, MRM was preferred over other methods, such as a partial Mantel test, because it is highly flexible and allows to regress a response matrix on multiple explanatory matrices (Lichstein, 2007).

Response matrices were calculated as follows. To quantify dissimilarity in plant pathogen communities across sites, dissimilarity matrices based on Bray-Curtis dissimilarities among plots were used. To quantify dissimilarity in plant pathogen richness across sites, I compiled a dissimilarity matrix using the absolute log response ratio (Barnes *et al.*, 2016) between the plant pathogen richness values obtained from the rarefied community (see Chapter 3, material and methods 3.2.1).

I used five explanatory matrices: Climate, geomorphology, land use, plant community and soil. To quantify dissimilarity in geomorphology, climate and soil across sites and to avoid multicollinearity, I calculated Mahalanobis dissimilarities (Mahalanobis, 1936) from the standardized variables (see section 4.2.1). I checked the variance inflation factors (VIF) using the "imcdiag" function of the R package "mctest" (Ullah & Aslam, 2017). The land use dissimilarity matrix was a factorial matrix, where the dissimilarity between plots from the same land use category was zero (no dissimilarity) and plots from a different land use category was one (most dissimilarity). To quantify dissimilarity in plant communities across sites, dissimilarity matrices based on Bray-Curtis dissimilarities among plots were used.

I obtained variances (R^2 values) and associated P-values from the multiple regressions using Legendre *et al.* (1994)'s permutation test. The null hypothesis, that the regression coefficient is equal to zero (i.e. no effect), was rejected for P-values below 0.0083 (Bonferroni correction).

4.3 Results

4.3.1 Plant pathogen communities

Almost all plant pathogen communities across substrate types showed strong responses to land use, climate, soil, geomorphology and plant community, with the sole exception of oomycetes in leaf extracts (Fig. 4.2; and for a detailed model summary Supplement material B Table B.2). Most of the variance of the plant pathogen communities could be explained by the plant community present at site after accounting for other predictors, such as climate (Figs. 4.2 - 4.5). Strong collinearity between the predictors indicated that the individual effects of land use, climate, soil, geomorphology and plants could not be separated fully.

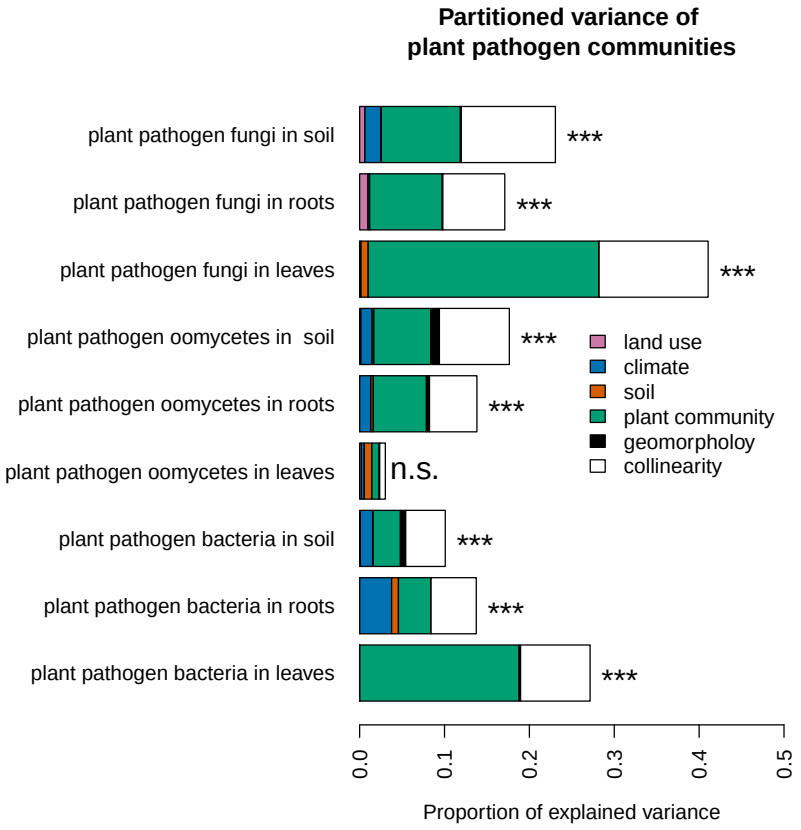

Figure 4.2: Explained variance of plant pathogen communities and environmental parameters using multiple regression on matrices (MRM). Input matrices with variables: Land use (natural forest, planted forest, low-/high-producing grassland, perennial cropland), climate (mean annual temperature, mean annual rainfall), soil (pH, water holding capacity, carbon, nitrogen, carbon-nitrogen-ratio, Olsen phosphorus, total phosphorus, calcium, magnesium, potassium, sodium, cation exchange capacity, base saturation), geomorphology (altitude, slope), and plant community (all plant species).

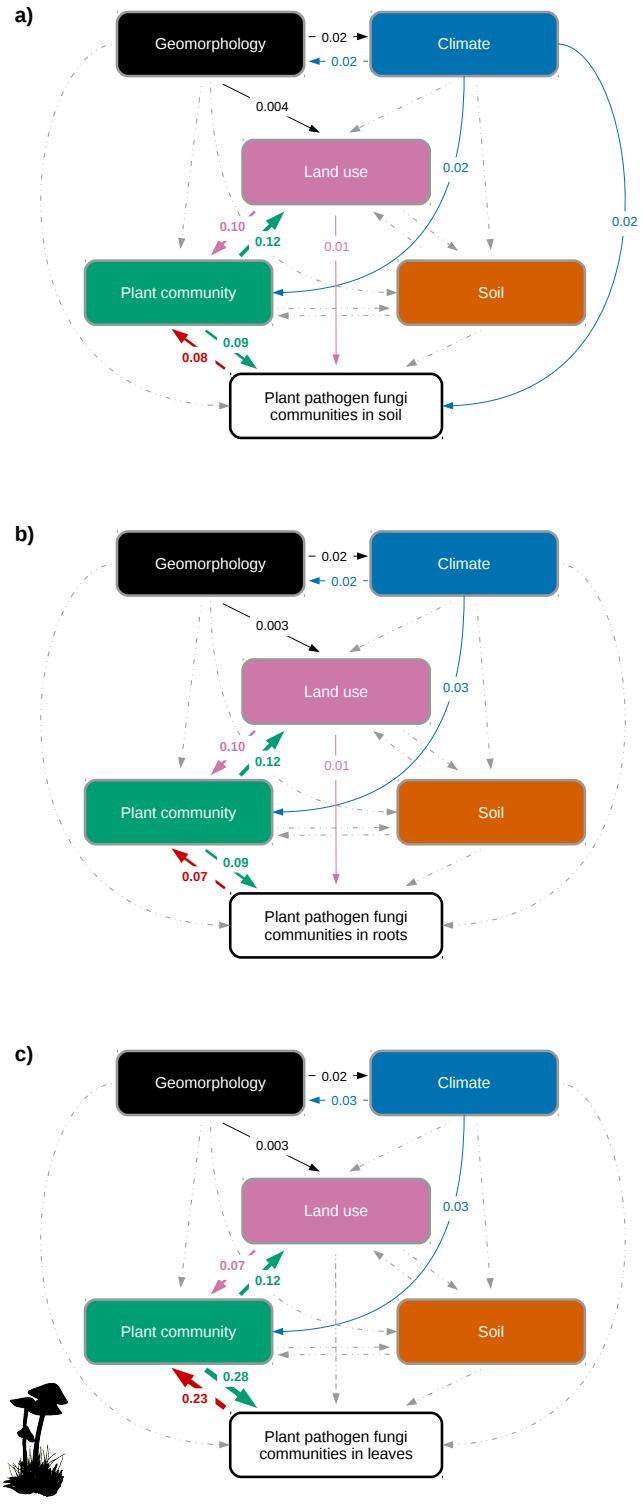

Figure 4.3: Correlations of fungi plant pathogen communities and their environmental variables in a) soil, b) roots, and c) leaves. Dotted lines indicate non significant correlations. Thickness of lines is proportional to explained variance. R^2 values (explained variance) of the multiple regressions are displayed on the arrows indicating the strength of the relationship.

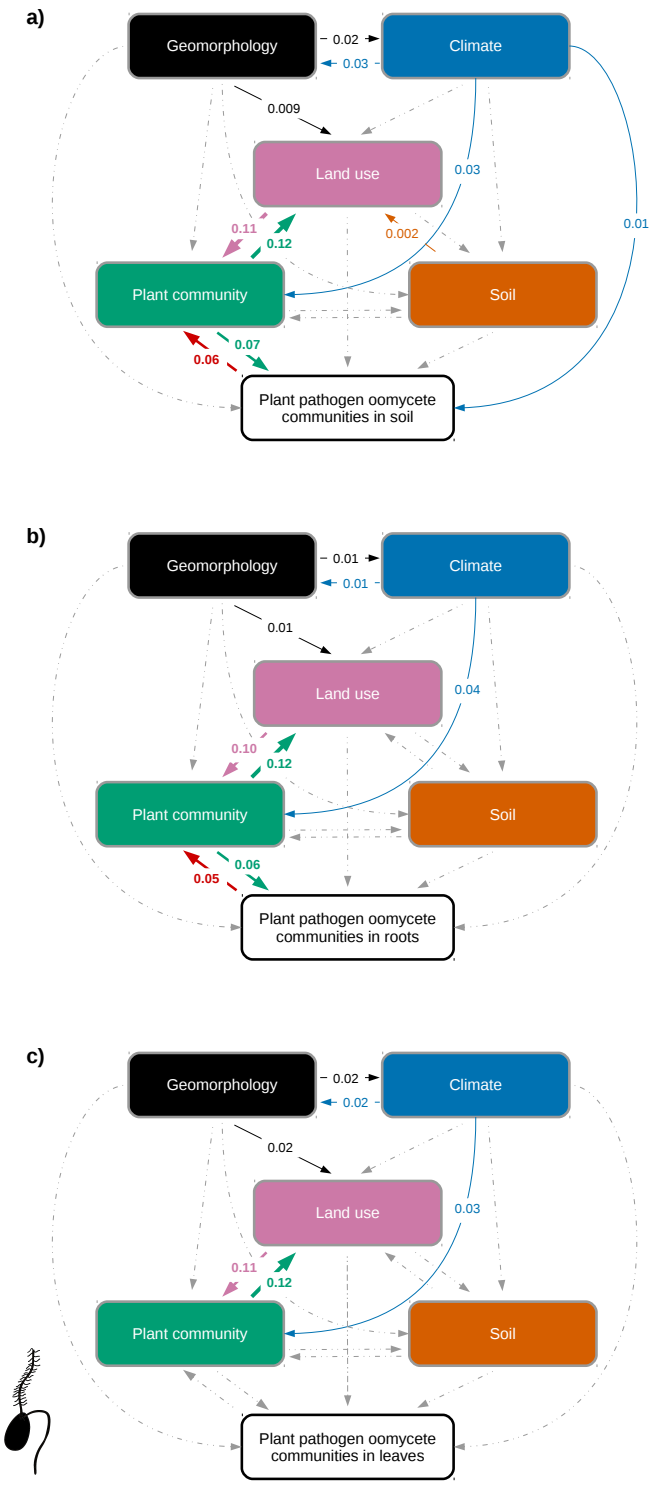

Figure 4.4: Correlations of oomycete plant pathogen communities and their environmental variables in a) soil, b) roots, and c) leaves. Dotted lines indicate non significant correlations. Thickness of lines is proportional to explained variance. R^2 values (explained variance) of the multiple regressions are displayed on the arrows indicating the strength of the relationship.

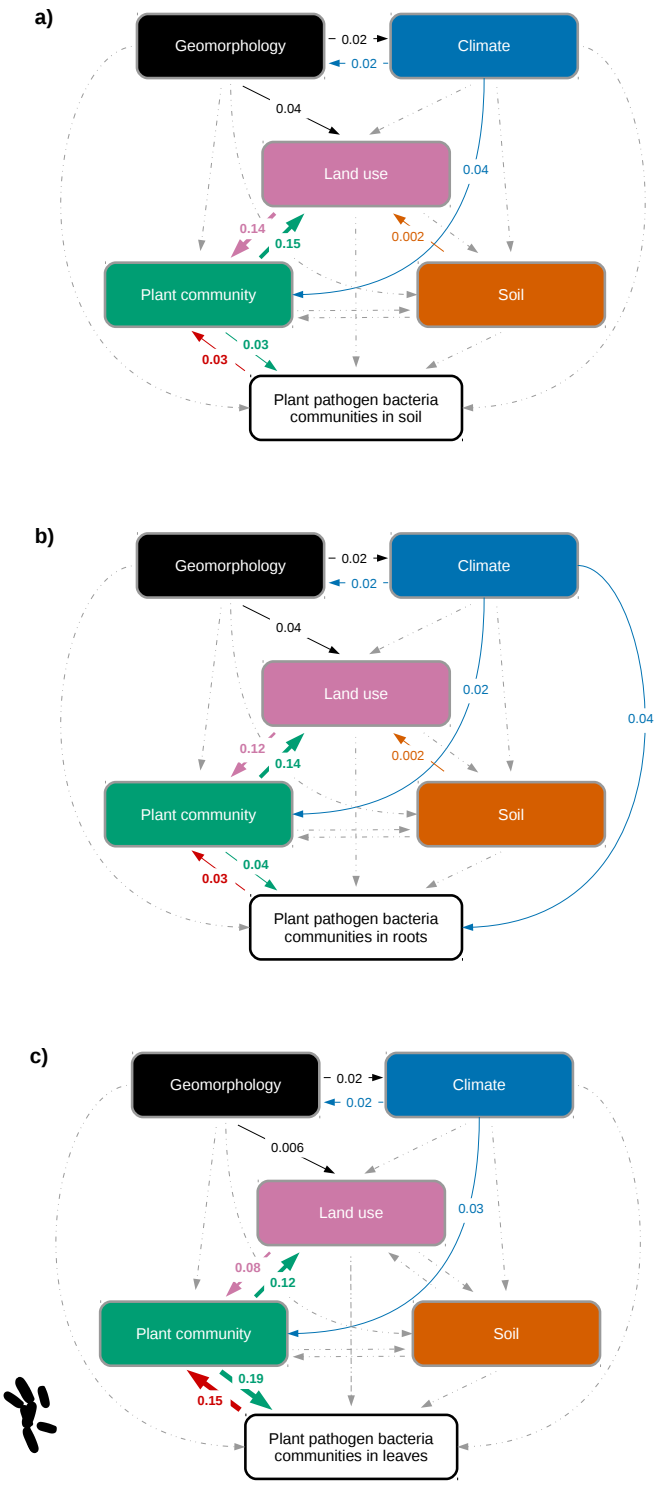

Figure 4.5: Correlations of bacteria plant pathogen communities and their environmental variables in a) soil, b) roots, and c) leaves. Dotted lines indicate non significant correlations. Thickness of lines is proportional to explained variance. R^2 values (explained variance) of the multiple regressions are displayed on the arrows indicating the strength of the relationship.

4.3.2 Plant pathogen richness

Plant pathogen richness across substrate types showed mostly strong responses to land use, climate, soil, geomorphology and plant community, with the sole exception of oomycetes in leaf extracts (Fig. 4.6; and for a detailed model summary Supplement material B Table B.3). Most of the variance of plant pathogen richness could be explained by the plant community present at site after accounting for other predictors (Figs. 4.6 - 4.9), with the exception of plant pathogen bacteria in soil which seem to show their strongest response to climate (Figs. 4.6 and 4.9a).

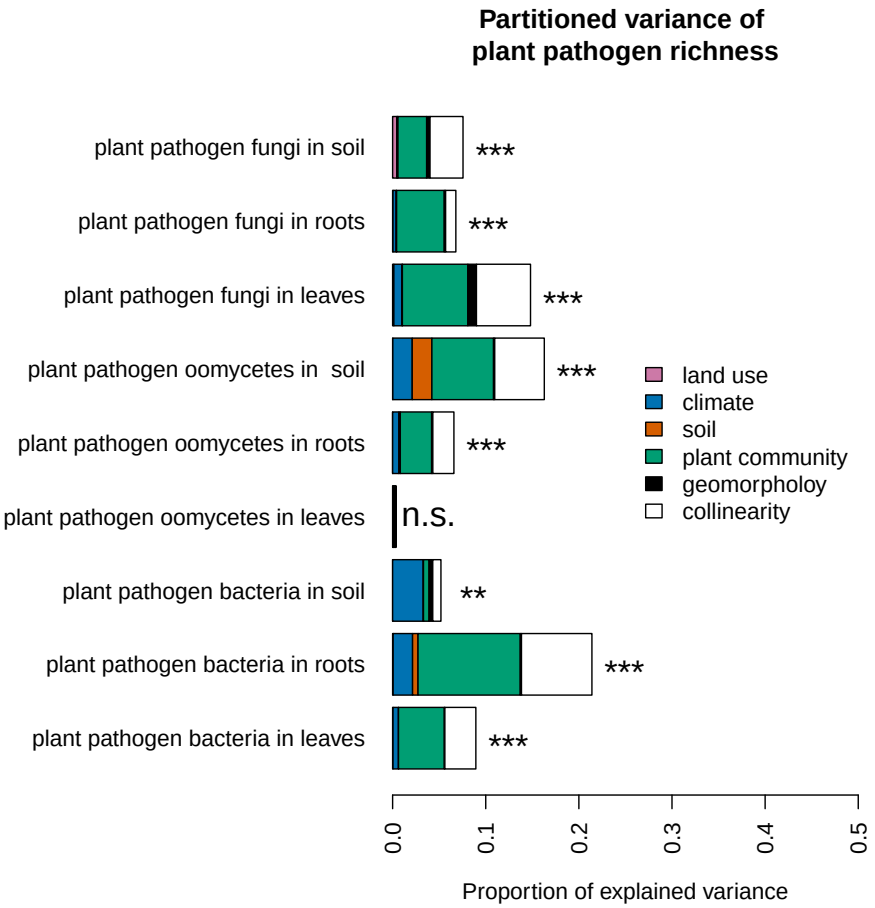

Figure 4.6: Explained variance of plant pathogen richness and environmental parameters using multiple regression on matrices (MRM). Input matrices with variables: Land use (natural forest, planted forest, low-/high-producing grassland, perennial cropland), climate (mean annual temperature, mean annual rainfall), soil (pH, water holding capacity, carbon, nitrogen, carbon-nitrogen-ratio, Olsen phosphorus, total phosphorus, calcium, magnesium, potassium, sodium, cation exchange capacity, base saturation), geomorphology (altitude, slope), and plant community (all plant species).

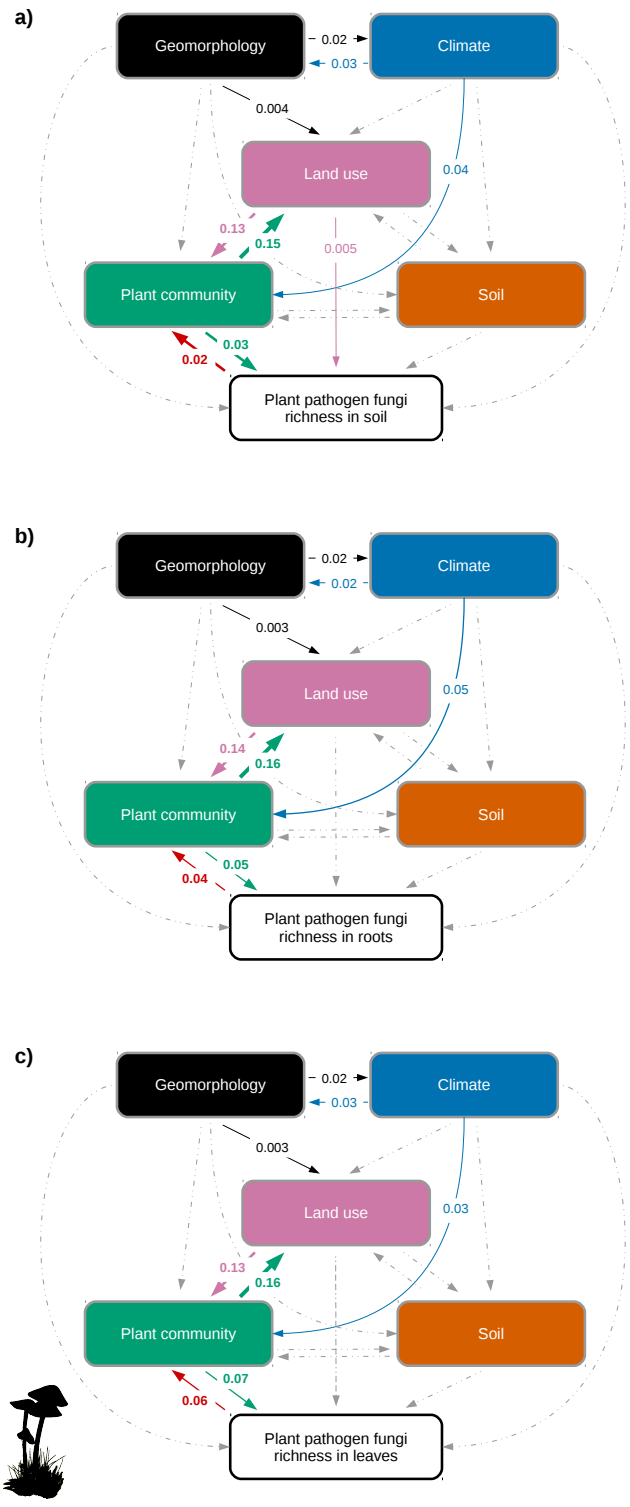

Figure 4.7: Correlations of fungi plant pathogen richness and environmental variables in a) soil, b) roots, and c) leaves. Dotted lines indicate non significant correlations. Thickness of lines is proportional to explained variance. R^2 values (explained variance) of the multiple regressions are displayed on the arrows indicating the strength of the relationship.

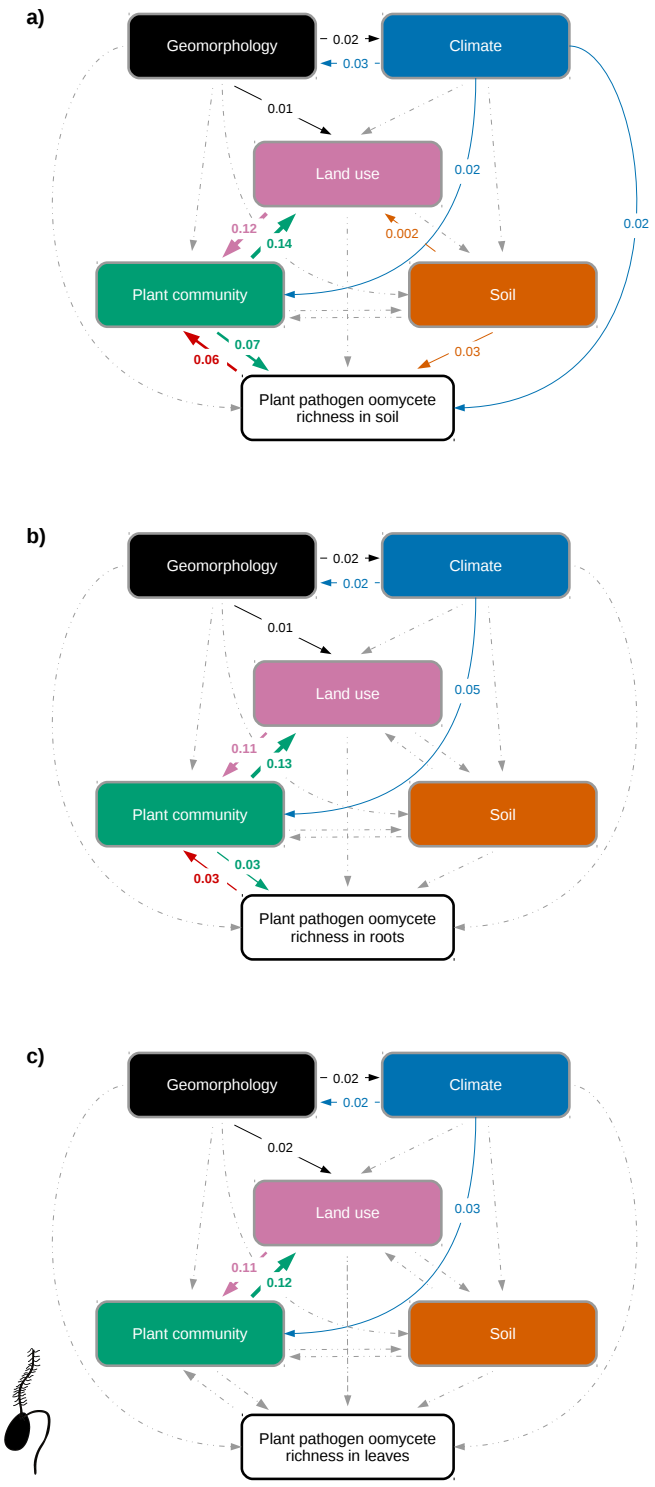

Figure 4.8: Correlations of oomycete plant pathogen richness and environmental variables in a) soil, b) roots, and c) leaves. Dotted lines indicate non significant correlations. Thickness of lines is proportional to explained variance. R^2 values (explained variance) of the multiple regressions are displayed on the arrows indicating the strength of the relationship.

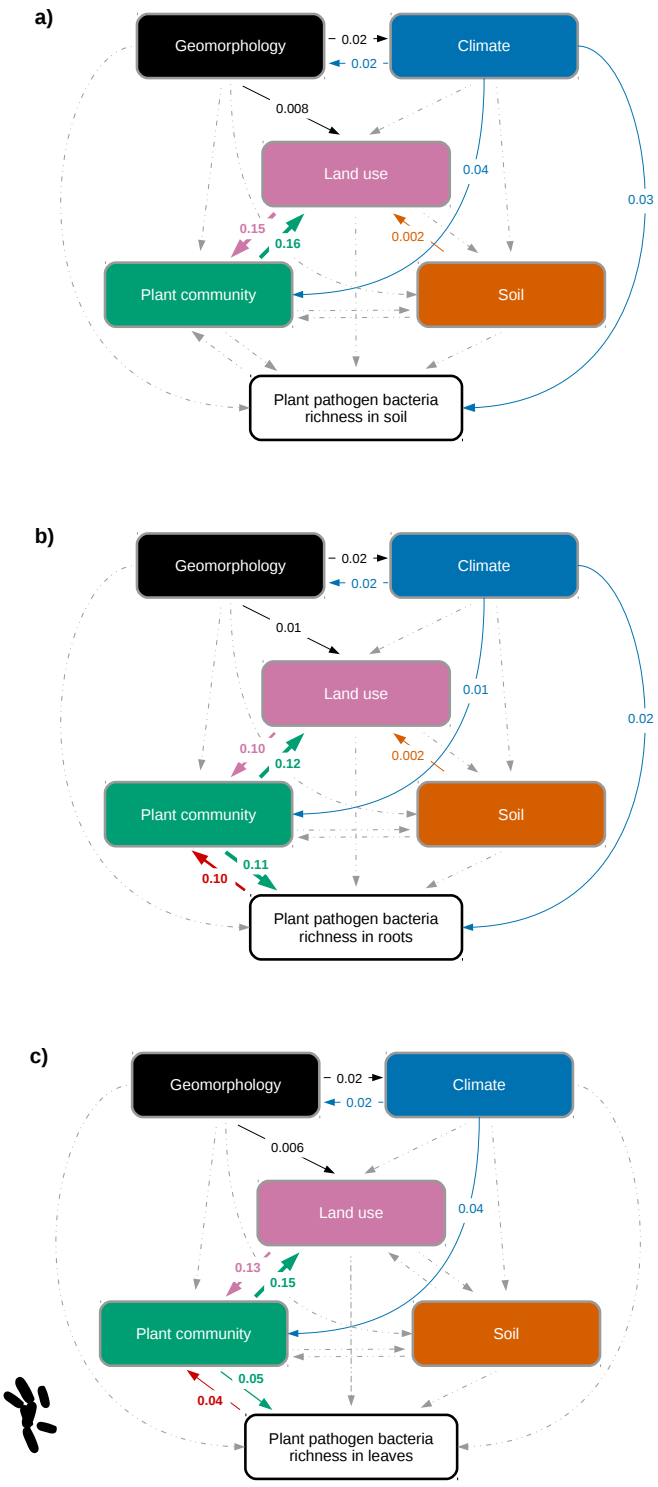

Figure 4.9: Correlations of bacteria plant pathogen richness and environmental variables in a) soil, b) roots, and c) leaves. Dotted lines indicate non significant correlations. Thickness of lines is proportional to explained variance. R^2 values (explained variance) of the multiple regressions are displayed on the arrows indicating the strength of the relationship.

4.4 Discussion

Both of the study's hypotheses, that climate could explain more variance of plant pathogen communities and richness, after accounting for land use, soil, geomorphology and plant community, had to be rejected. Instead, I found a unified strong response of most plant pathogen communities and richness towards plant communities. This contrasts with studies which found a direct effect of climate on plant pathogens (Harvell *et al.*, 2002; Gilles & Kennedy, 2003; Eastburn *et al.*, 2011; Barbetti *et al.*, 2012; Siebold & Von Tiedemann, 2012; Bebber *et al.*, 2013; Johnson & Cummings, 2013; Gouache *et al.*, 2015; Spear *et al.*, 2015; Bebber *et al.*, 2016; Newbery *et al.*, 2016). However, these effects are often pathosystem-specific and contrasting individual effects of climate on plant pathogen species make general statements challenging (Garrett *et al.*, 2006; West *et al.*, 2012). Furthermore, relatively few studies have (1) taken into account multiple competing environmental variables, (2) included multiple pathogen groups, and (3) most studies were conducted within a single land use type, making it impossible to test for effects across land uses. For example, Mitchell *et al.* (2002, 2003) investigated some environmental variables such as the effects of plant species diversity, elevated CO_2 and nitrogen addition but restricted their analysis only on foliar fungal pathogens within grasslands and did not include climate. Similarly, Gossner *et al.* (2016) constrained their analysis of plant pathogen diversity within grasslands and did not test for the effect of climate. In Chapter 3, I found a significant effect of land use for most plant pathogen communities and richness, but did not consider other, correlated environmental variables.

The present study demonstrates how important it is to cover multiple environmental variables to obtain a holistic picture of the drivers of plant pathogens. For example, the analysis in this chapter showed strong correlations of land use with plant communities, as would be expected. This very likely explains the significant effect of land use on plant pathogen communities and richness that was found in Chapter 3. Moreover, it shows that the significant effect of land use was likely caused by the plant community, rather than, for example, changes in soil properties. Similarly, this study showed correlations between climate and plant communities. However, leaf pathogen communities and richness were never correlated with climate after accounting for all other variables, and only few and weak correlations were found between climate and soil and root plant pathogens communities and richness. Together these results show that the plant community present at site is relatively more important for plant pathogen communities and richness than other environmental variables including climate. Hence, this study supports the view of indirect

climatic effects on plant pathogens through plant communities rather than a strong direct effect of climate on plant pathogens in general (West *et al.*, 2012; Hulme, 2017).

Alternative explanations of why the effect of climate on plant pathogens was not the most important with respect to the other environmental factors in this study could be due to micro-climate and collinearity.

Micro-climatic differences within a plot were not measured and could have played an important role for plant pathogens (Matson *et al.*, 1997; Fuhrer, 2003; Lin, 2011). Although, the climate on plot level in this study ranged from 6.8°C - 15.6°C mean annual temperature, and 453 mm - 5660 mm mean annual precipitation (Supplement B, Table B.1), there could have been temperature fluctuations within a plot affecting plant pathogen communities and richness.

Furthermore, relatively strong collinearity between the environmental predictors indicated that the variance of climate (or any other environmental variable) could not be partitioned entirely. Considering that climate, geomorphology, land use, soil and plant communities had close interrelations, collinearity was not surprising. Although non-orthogonal predictors could have hidden the effect of climate, the relative importance of climate for plant pathogen communities and richness would be unlikely to have changed. This is due to the fact that most of the observed collinearity in this study was caused by plant communities and land use.

One limitation of the study is that interactions were not considered. Including interactions between the plant community, environmental stressors and other micro-organisms might explain more variance of plant pathogen communities and richness (Francl, 2001; Pérez-de Luque *et al.*, 2017). Here, I did not include interaction terms because I was (1) explicitly interested in the effect of climate on plant pathogen communities and richness, and (2) including interaction terms would have resulted in an overfitted model due to too many variables. Nevertheless, interactions represent an exciting area for future research and could reveal additional insights into the ecology of plant pathogen communities. A more focused, hypothesis-driven measurement could overcome the problem of overfitting in future work. My results indicate that the plant community would likely be a key part of such interactions.

The observed strong relationship between plant pathogens and plant communities in this study after accounting for environmental variables furthermore suggests that the plant community itself very likely is the dominant component for controlling plant pathogens. Thus, future research should focus on what within the plant community drives plant pathogens. This includes moving beyond general descriptions of plant diversity (richness and evenness), and towards specific plant

community attributes such as plant (functional) traits (Mangelsdorff *et al.*, 2012; Hantsch *et al.*, 2013; García-Guzmán & Heil, 2014; Hantsch *et al.*, 2014; Schuldt *et al.*, 2017), certain disease prone species (Mitchell *et al.*, 2003; Hantsch *et al.*, 2013), or possible interactions of the biotic community associated with the plant community (Borowicz, 2001; Ma *et al.*, 2013; Dung *et al.*, 2014; Latz *et al.*, 2016).

4.4.1 Conclusions

Man made climate change and its consequences on the provision of ecosystem services are one of the central challenges facing our planet during the next century (Feulner, 2017). The growing unpredictability of the effects of plant pathogens on ecosystem services with forthcoming changes in climate and land use have raised major concerns (Anderson *et al.*, 2004; Cheatham *et al.*, 2009; Chakraborty & Newton, 2011; Luck *et al.*, 2011; Fisher *et al.*, 2012; Ghini *et al.*, 2012; Elad & Pertot, 2014; Godfray & Garnett, 2014).

The present study showed an unequal relationship among the pathogen-plant-environment triangle. The plant community itself was relatively more important for plant pathogen communities as well as richness, than the abiotic environment. In light of forthcoming changes in climate and land use, these results indicate that any changes in plant pathogen communities and richness will largely be mediated through changes in plant communities, rather than direct effects of climate, geomorphology, land use, or soils.

Chapter 5

Co-occurrence patterns in plant pathogen communities

Notes

The results of this chapter are intended to be submitted to ISME Journal: Multidisciplinary Journal of Microbial Ecology.

Abstract

Very little is known about the structure of plant pathogen communities, despite their importance to ecosystems. In this chapter I integrated environmental (eDNA) metabarcoding with network analysis and investigated co-occurrence patterns of fungi, oomycete and bacteria plant pathogen communities at a plot level using a national next-generation sequencing dataset. Specifically, I was interested in the question if plant pathogens co-occurred (1) non-randomly and (2) predictably. Modularity analysis revealed distinct plant pathogen communities across and within plant pathogen taxa (fungi, oomycetes, and bacteria) and substrates (soil, roots, and leaves). Contingency table analyses showed that the formation of plant pathogen communities always depended on taxonomic group, and in few cases, on substrate, as well. Despite their complex structure, plant pathogen communities could predict the occurrence of other plant pathogen communities from different taxa and substrates, positively and negatively. Here I demonstrated that the information derived from plant pathogen co-occurrence networks can reduce the overwhelming complexity of plant pathogen species into distinct communities. These communities and their responses to the environment can be studied more easily than characterising the immense amount of individual plant pathogens. Moreover, predictable plant pathogen communities will help us anticipate how plant pathogen communities will shift in a changing world and, more importantly, understand their impact on future food and ecosystem production.

Keywords

Network analysis, modularity, next-generation sequencing, metabarcoding, community detection

5.1 Introduction

In "The Origin of Species" Darwin used the "entangled bank" as a metaphor to illustrate the non-randomness and complexity by which individual organisms can co-occur (Darwin, 1859). It is now widely accepted that the co-occurrence of species is likely the product of many factors including chance, history, speciation, phylogenetic distance, migration, extinction, dispersal, abiotic environmental factors, and biotic interactions (Cody & Diamond, 1975; Connor & Simberloff, 1979; Gotelli, 1999; Stone & Roberts, 1990, 1992; Webb, 2000; Hubbell, 2001). However, the interplay of these factors and the complexity of ecological systems still represents a significant challenge for studying co-occurrence patterns (Sanderson *et al.*, 2009; Mayfield & Levine, 2010; Connor *et al.*, 2013).

One way of describing the complexity of ecological systems is through network analysis, which has gained increasing attention in the recent years. For example, co-occurrence network properties have illuminated community structure, function and underlying ecological mechanisms (Montoya *et al.*, 2006; Zhou *et al.*, 2010; Araújo *et al.*, 2011; Barberán *et al.*, 2012; Chagnon *et al.*, 2012; Mougi & Kondoh, 2012; Bennett *et al.*, 2013; Coux *et al.*, 2016; Creamer *et al.*, 2016). Such co-occurrence networks have been inferred for a range of organisms and habitats and, since the rise of next-generation sequencing technologies, increasingly for microbes (Falkowski *et al.*, 2008; Fuhrman, 2009; Qin *et al.*, 2010; Zhou *et al.*, 2010; Arumugam *et al.*, 2011; Steele *et al.*, 2011; Barberán *et al.*, 2012; Gilbert *et al.*, 2012; Consortium *et al.*, 2012; Boutin *et al.*, 2013; Menezes *et al.*, 2015; Delgado-Baquerizo *et al.*, 2018). However, plant pathogen co-occurrence networks have received less attention in this process. As a result the community structure of plant pathogens is still poorly understood, despite their importance to ecosystems (Oerke, 2006; Cheatham *et al.*, 2009; Bever *et al.*, 2015; Albornoz *et al.*, 2017; Dickie *et al.*, 2017a).

Two plausible causes driving the community structure of plant pathogens that have been frequently discussed in literature are (1) habitat (Weller *et al.*, 2002; Mangelsdorff *et al.*, 2012; Palti, 2012; Bittner, 2016; Zhang *et al.*, 2017) and (2) mutualistic and antagonistic relationships between pathogen taxa (Le May *et al.*, 2009; Lamichhane & Venturi, 2015; Tollenaere *et al.*, 2016). For example, plant pathogens can co-occur when they share the same habitat, and aggregate when there is a mutualistic relationship between them (Le May *et al.*, 2009; Lamichhane & Venturi, 2015; Tollenaere *et al.*, 2016; Deveau *et al.*, 2018). On the other hand, two plant pathogens can simply not co-occur due to different habitats, or they could have the same habitat and exclude each other competitively. Unequal habitat preferences of plant pathogens between soil, roots

and leaves were shown in Chapter 3. Hence, non-random co-occurrences and the structure of plant pathogen communities could be a result of habitat. Furthermore, the results of Chapter 3 revealed that plant pathogen taxa responded differently to the same habitat, indicating that co-occurrences might depend on pathogen taxa, as well.

Multiple cases from literature demonstrate non-random co-occurrences across and within plant pathogen taxa (Dung *et al.*, 2014; Lamichhane & Venturi, 2015; Susi *et al.*, 2015; Stopnisek *et al.*, 2016; Abdullah *et al.*, 2017; Jung *et al.*, 2018). One example of positive co-occurrence across plant pathogenic groups is *Panax notoginseng* root rot disease, which is reported to occur with many common plant pathogenic fungi, oomycetes and bacteria, such as species from *Alternaria, Cylindrocarpon, Fusarium, Phytophthora, Phoma, Rhizoctonia, Pseudomonas* and *Ralstonia*)(Ma *et al.*, 2013). Within taxa positive co-occurrence is also well documented (Tomioka *et al.*, 2013; Whitelaw-Weckert *et al.*, 2013; Barros *et al.*, 2014; Freeman *et al.*, 2014; Kuzdraliński *et al.*, 2014), for example, within the genus *Monilinia* (Papavasileiou *et al.*, 2015). It has been proposed that suppression of host plant immunity could be one mechanism that could explain that some plant pathogens favour the co-occurrence with plant pathogens across and within taxa, as recently noticed for *Albugo* in some Brassicaceae (Cooper *et al.*, 2008). In contrast, it has been suggested that some plant pathogens could exclude each other resulting in negative co-occurrence when they compete for the same resources (Syller, 2012). Indeed, trophic competition between fungi and bacteria is well documented, including competition of saprotrophs for carbon (Mille-Lindblom *et al.*, 2006), e.g. facultative plant pathogens *Alternaria* (Tsuge *et al.*, 2016) and *Rhizoctonia* (Nawrocka *et al.*, 2017). Co-occurrences across and within plant pathogen taxa as well as habitats have mostly been documented on an individual host-pathogen level (Dung *et al.*, 2014; Lamichhane & Venturi, 2015; Susi *et al.*, 2015). At large scales plant pathogen communities are as yet poorly understood, as is the structures of plant pathogen communities.

In order to gain insights into the community structure of plant pathogens, I used a national metabarcoding dataset (as used in chapters 3 an 4) from which I constructed co-occurrence networks of fungi, oomycete and bacteria plant pathogens in soil, roots and leaves. Specifically, I was interested in if there was structure to co-occurring plant pathogens, and how possible structure was affected by habitat and taxa. To address these questions I made use of modularity analysis, which can measure the strength of division of a network into communities (Blondel *et al.*, 2008). Highly positive modularity indicates dense connections within such communities and at the same time sparse connections (separation) between each community (Blondel *et al.*,

2008). Thus, modularity analysis can reveal communities of plant pathogens across habitat or taxa. It can further indicate what is more likely to structure these communities when modularity changes with habitat and taxa.

First, I hypothesised that

(1) fungi, oomycete and bacteria plant pathogens have positive modularity in co-occurrence patterns across soil, roots and leaves (substrates) across taxa.

Given that the hypothesis was supported, I then tested each plant pathogen community for deviations from independence in taxa and substrate. Moreover, I tested for positive modularity

(1a) within substrate after controlling for taxa,

(1b) within taxa after controlling for substrate,

(1c) within both substrate and taxa.

Second, I hypothesised that

(2) plant pathogen communities from one taxa (and substrate) can predict positive and negative co-occurrence of plant pathogen communities from a different taxa (and substrate).

5.2 Material and methods

5.2.1 Study sites and sampling

A detailed description of the methods including study sites, sampling, DNA extraction, preparation of next-generation sequencing libraries and bioinformatics can be found in Chapter 3 under the section material and methods 3.2.1.

5.2.2 Statistical analysis

I used R version 3.4.1 (R Core Team, 2017) for conducting analyses and plotting graphs. Network graphs were created using gephi version 0.9.1 (Bastian *et al.*, 2009) and the "igraph" package version 1.0.1 (Csardi & Nepusz, 2006).

For network inference, I calculated all possible Spearman's rank correlations between plant pathogenic OTUs within each extraction type using the R package "Hmisc" (Harrell Jr *et al.*, 2017). I considered a valid co-occurrence event for a P-value of less than 0.001, which equates to a Spearman's rho of less than -0.38 or greater than 0.38 (Fig. C.1 in supplement C). In this constructed network, nodes represent plant pathogen OTUs, whereas the edges correspond to a strong and significant correlation (co-occurrence).

I used several network properties in order to examine the structure of plant pathogen networks. I used the Louvain Method for plant pathogen community (modules) detection, and calculated modularity across and within different taxa and substrates (Blondel *et al.*, 2008). Modularity is the number of edges within community exceeding the number expected on the basis of chance. Thus, positive and high modularity can indicate distinct plant pathogen communities. I compared the average degree, the diameter (greatest distance between any pair of vertices), the average path length, and the density of the co-occurrence networks to find variation in structure (complexity, stability) of plant pathogen communities among pathogen group (fungi, oomycetes, bacteria) and substrate (soil, roots, leaves). Network topology and properties were extracted from gephi version 0.9.1 (Bastian *et al.*, 2009).

A three-dimensional contingency table (community $\times$ taxa $\times$ substrate) was used to test for deviations in taxa and substrates to form communities equally, following Zar *et al.* (1999). After overall test of independence (to control overall α), I tested for independence of taxa and substrate within each community using the χ^2 test statistics.

For multiple comparison of correlated communities, P-values were adjusted according to Benjamini *et al.* (2006) using a two-stage step-up false discovery controlling procedure in the R package "multtest" (Pollard *et al.*, 2005).

5.3 Results

5.3.1 Non-random plant pathogen communities

Plant pathogens across taxonomic groups (fungi, oomycetes, and bacteria) and across substrates (soil, roots, leaves) co-occured non-randomly, as shown by positive modularity (Table 5.1). Network properties confirmed a non-random structure and indicate a sparse, complex network (Table 5.1). Module clustering suggested 11 distinct pathogen communities of mixed taxonomic groups (Fig. 5.1). 0.79% of all co-occurrences were significantly negative (red edges in Fig. 5.1).

Table 5.1: Network properties across fungi, oomycete and bacteria plant pathogen networks across soil, roots and leaves.

Number of plant pathogen modules	11
Modularity	0.567
Average Degree	19.633
Network Diameter	7
Graph Density	0.025
Average Path Length	3.044
Number of connected components	1

The frequency of plant pathogens was significantly different between (1) communities, (2) taxa and (3) substrate (contingency table analysis; χ^2=726.3, df=84, $P < 0.001$). Moreover, the compositions of the 11 communities of plant pathogens were driven by differences between substrate and taxa (contingency table analysis; χ^2=68.69, df=4, P<0.001; Fig. 5.2). Whereas the compositions of the 11 communities of plant pathogens were always driven by differences between fungi, oomycetes and bacteria, only the compositions of communities four, eight and eleven were additionally driven by differences between soil, roots and leaves (P-values in Fig. 5.2). Bacteria predominantly occurred in community one, but equally between substrates (Fig. 5.2). Similarly, oomycetes predominantly occurred in community nine, but equally between substrates. Generally, plant pathogen communities in leaves seemed to be dominated by fungi, with the exception of community nine.

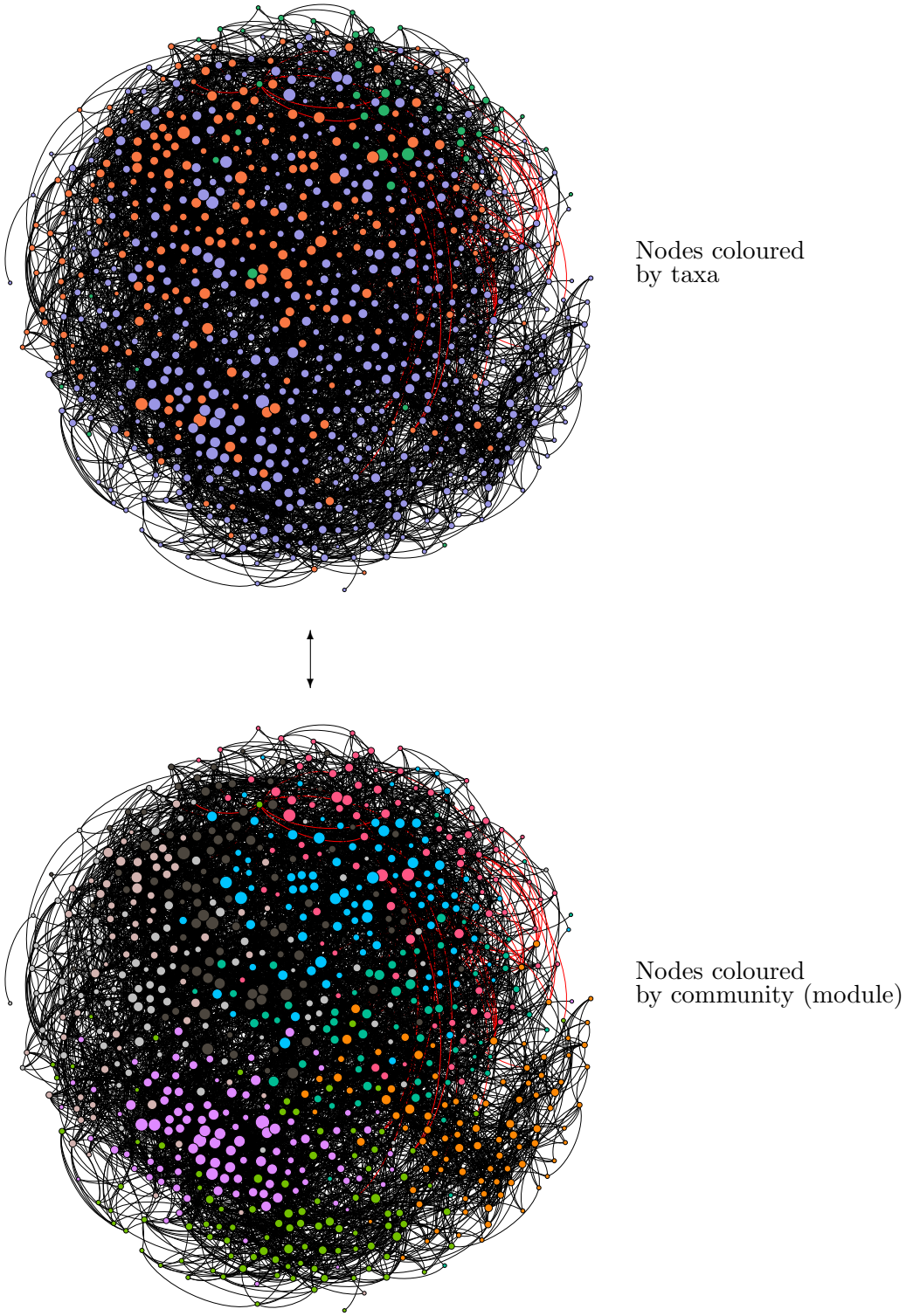

Figure 5.1: Co-occurrence network of plant pathogens across taxonomic groups (fungi, oomycetes, and bacteria) across substrates (soil, roots and leaves). Top: Co-occurring nodes (OTUs) coloured by taxa (fungi=blue, oomycete=red, bacteria=green). Bottom: Co-occurring nodes (OTUs) coloured by modules (communities). Number of modules (n=11) and other network properties are described in Table 5.1. Edges represent significant co-occurrence (positive=black, negative=red), and size of OTU is proportional to its degree (number of connections).

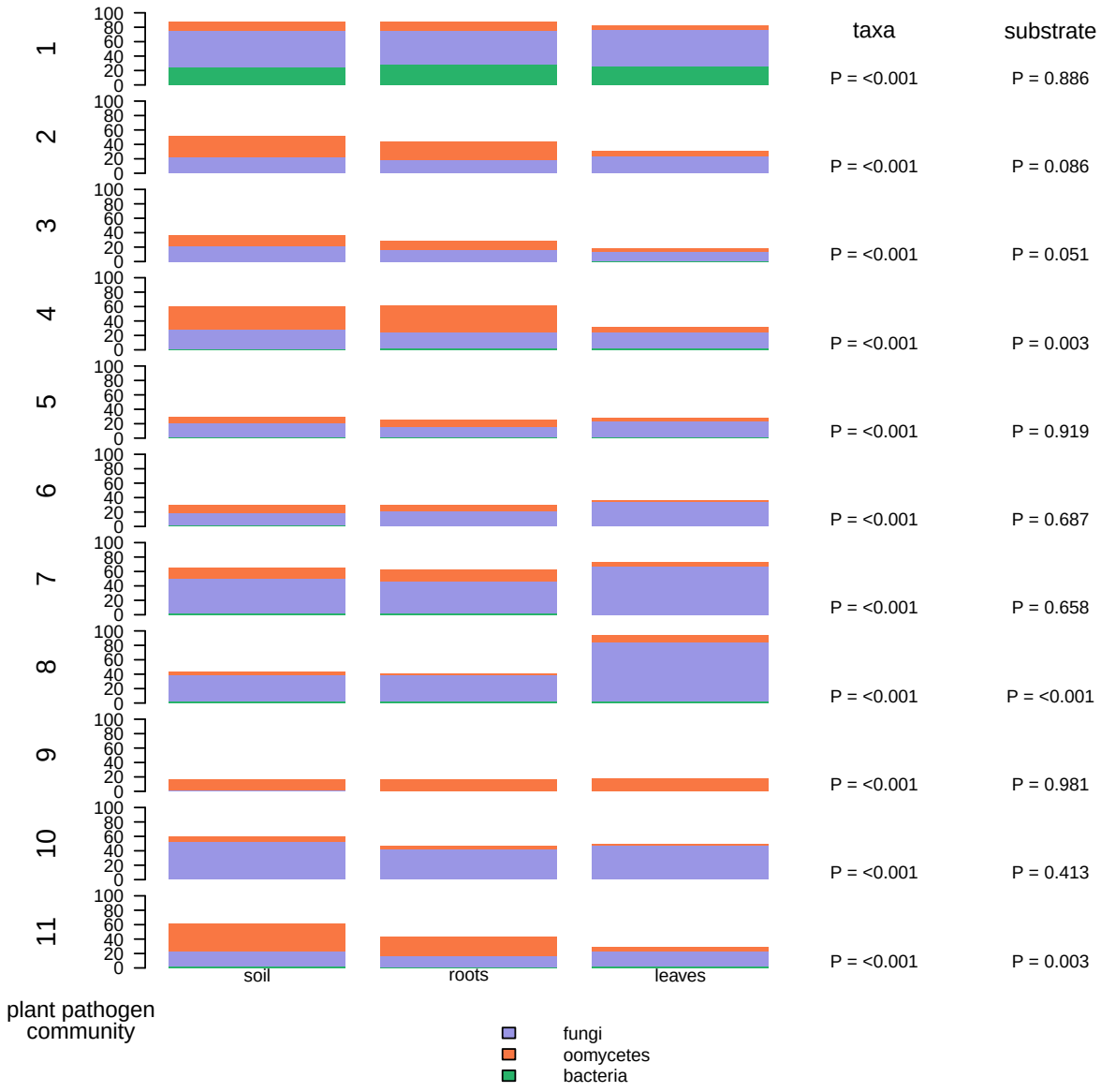

Figure 5.2: Frequencies of fungi (blue), oomycetes (red) and bacteria (green) plant pathogens in the eleven communities identified with module clustering (Fig. 5.1). There was an overall significant deviation from expected frequencies (χ^2=726.3, df=84, $P < 0.001$). P-values show if composition of the community was driven by differences between substrate or taxa.

Additionally, high modularity was observed within substrate (Table 5.2), within taxa (Table 5.3), and within taxa within substrate (Table 5.4), demonstrating non-random co-occurrence of plant pathogens and distinct pathogen communities.

Table 5.2: Network properties across fungi, oomycete and bacteria plant pathogen networks within soil, roots and leaves. The networks after controlling for the effect of taxa are shown in Supplement C Fig. C.2.

Extraction	**Soil**	**Roots**	**Leaves**
Number of plant pathogen modules	10	11	10
Modularity	0.535	0.528	0.591
Average Degree	19.089	15.674	14.625
Network Diameter	7	8	7
Graph Density	0.035	0.032	0.030
Average Path Length	3.06	3.179	3.185
Number of connected components	1	1	1

While pathogen communities are very distinct within substrate (Table 5.2) and within fungi and oomycete plant pathogens (Table 5.3), modularity within bacteria plant pathogens is relatively low (Table 5.3), showing a weak separation within bacteria plant pathogens. This is further supported by a higher observed density within bacteria plant pathogen communities, a lower average path length and a smaller network diameter compared to the other taxa (Table 5.3).

Table 5.3: Network properties within fungi, oomycete and bacteria plant pathogen across substrates. The networks after controlling for the effect of substrate are shown in Supplement C Fig. C.3.

Extraction	**Fungi**	**Oomycetes**	**Bacteria**
Number of plant pathogen modules	8	8	5
Modularity	0.59	0.595	0.313
Average Degree	13.322	11.214	6.882
Network Diameter	7	8	5
Graph Density	0.025	0.049	0.209
Average Path Length	3.243	3.142	2.175
Number of connected components	1	1	2

Although plant pathogen communities within taxa and substrate co-occured non-randomly, shown by the network properties in Table 5.4, plant pathogen oomycetes in leaves as well as bacteria in roots showed a less complex structure and less modularity than other taxa. In contrast to plant pathogen communities across taxa (Table 5.2), the plant pathogen communities within taxa and substrate (Table 5.4) never showed highest modularity in leaves.

Table 5.4: General network properties within fungi, oomycete and bacteria plant pathogens within different substrates. The networks after controlling for the effect of taxa and substrate are shown in Supplement C Fig. C.4.

	Fungi			**Oomycetes**			**Bacteria**		
Extraction	Soil	Roots	Leaves	Soil	Roots	Leaves	Soil	Roots	Leaves
Nr. of plant pathogen modules	10	10	9	8	8	8	6	4	5
Modularity	0.588	0.619	0.600	0.575	0.532	0.363	0.696	0.374	0.536
Average Degree	11.083	7.673	13.211	10.746	10.735	7.471	1.826	7.188	4.722
Network Diameter	8	9	7	11	9	11	10	5	6
Graph Density	0.035	0.028	0.035	0.058	0.065	0.112	0.083	0.232	0.135
Average Path Length	3.43	3.788	3.2	3.312	2.955	3.962	3.802	2.183	2.837
Nr. of connected components	1	1	1	1	2	4	4	1	1

5.3.2 Predictable plant pathogen communities

The occurrences of some plant pathogen communities independently clustered within taxa and substrate was correlated (Fig. 5.3). Thus, the occurrence of plant pathogens from one taxonomic group could predict the occurrence of plant pathogens from a different taxonomic group. Similarly, the occurrence of plant pathogens from one substrate could predict the occurrence of plant pathogens from a different substrate.

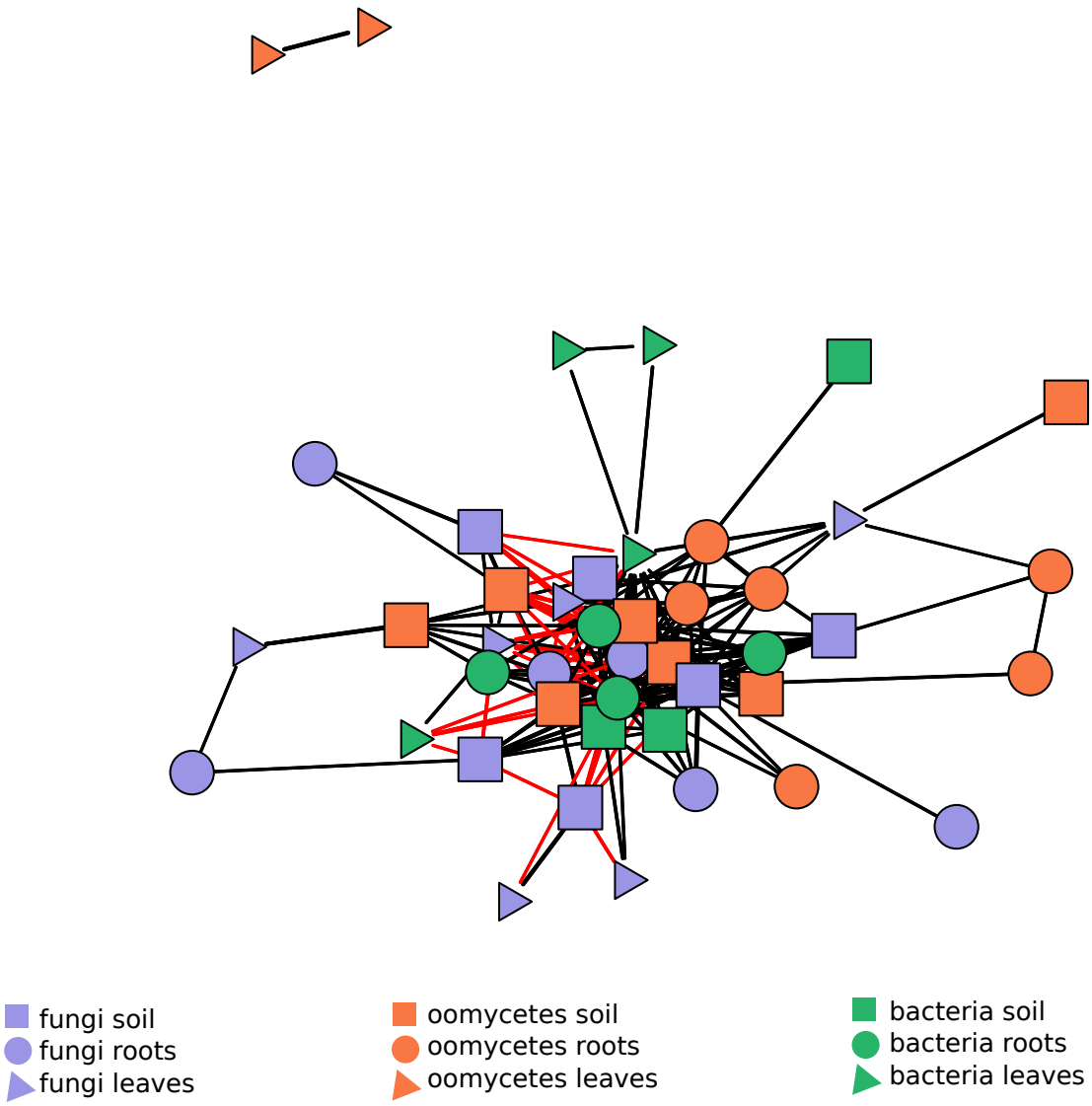

Figure 5.3: Positively (black) and negatively (red) correlated plant pathogen module frequencies (modules < 9 OTUs were dropped), after false discovery rate controlling procedure. Fungi=blue, oomycete=red, bacteria=green. Soil=square, roots=circle, leaves=triangle.

5.4 Discussion

In this chapter, I present a novel framework for studying plant pathogens. The main aspect of this proof of concept study was to show that network theory and metabarcoding are complementary and can reduce the overwhelming complexity of plant pathogen species. Here, I showed that plant pathogens across different taxa and substrates co-occurred as (1) non-random and furthermore (2) predictable communities.

5.4.1 The value of finding plant pathogen communities

The topology of plant pathogen co-occurrence networks in this study was typical for complex networks and as expected in a real world system, i.e. neither purely regular nor purely random (Boccaletti *et al.*, 2006). Reducing this complexity and the immense amount of plant pathogen species into distinct communities is the first step for investigating the mechanisms that structure plant pathogens, and a prerequisite in order to assess potential impacts on future food and ecosystem production. More broadly speaking, the existence of definable plant pathogen communities is very promising because it allows us to study group responses and shared drivers of pathogens, in similar fashion to, for example, plant communities (Walker *et al.*, 1999).

Studying communities and the factors that influence diversity, distribution, spread and abundance of species, and the structure of communities, including interactions with the abiotic and biotic world has been useful to many fields, such as ecosystem restoration (Palmer *et al.*, 1997), biological invasions (Klironomos, 2002; Shea & Chesson, 2002), conservation (Angelstam, 1992; Laurance & Bierregaard, 1997; Naiman *et al.*, 2010; Thomas, 2011), global change ecology (Zak *et al.*, 2003; Tylianakis *et al.*, 2008), functional trait (McGill *et al.*, 2006; Agler *et al.*, 2016; Delgado-Baquerizo *et al.*, 2018) and evolutionary biology (Webb *et al.*, 2002). These are just a few examples of research areas, addressing pressing issues that can often only be solved when investigating naturally occurring communities. To date the field of plant pathology has mainly focused on autecology, the study of individual species (Agrios, 2005; Lamichhane & Venturi, 2015). Only few studies have addressed plant pathogen communities so far, and even less have been conducted across a large gradient of environments in order to address the big picture (Nelson & Karp, 2013; Burgess *et al.*, 2017b; Nicolaisen *et al.*, 2017).

Moving from plant pathogen species to communities has the potential to revolutionise the field of plant pathology because it will allow us to simplify, categorise and generalise responses for pathogen groups. This will result in a deeper understanding of general pathogen dynamics

and ecology, but also enable us to address questions that were hitherto simply not possible to answer when focused on individual pathosystems. The latter includes, for example, responses to plant community change and climate (Elad & Pertot, 2014).

5.4.2 Co-occurrence of plant pathogens

Generally, we would expect plant pathogens to follow co-occurrence patterns of plants, considering that plant pathogen community structure is strongly linked to plant communities (Chapter 4, and Piepenbring *et al.* (2011); Mangelsdorff *et al.* (2012); Hantsch *et al.* (2013, 2014); Latz *et al.* (2016)).

A meta-analyses from Götzenberger *et al.* (2012) proposed that non-random co-occurrence of plant species was not a widespread phenomenon. As a result, a similar response could have been concluded for plant pathogens. However, in the present study I observed strong non-random occurrences of plant pathogens. These results support the opposing view of non-random co-occurrence of plant species as suggested by, for example, Clements (1916); Wolek (1997); Dieleman *et al.* (2015); Wood *et al.* (2017) and Delalandre & Montesinos-Navarro (2018). Furthermore, the non-random co-occurrence of plant pathogens as observed in this study is in line with other research on organisms which are known to form strong relationships with plants. For example, non-random co-occurrences have been demonstrated for arbuscular mycorrhizal fungi (Bennett *et al.*, 2013), pollinators (Rezende *et al.*, 2007; Burkle *et al.*, 2013; Coux *et al.*, 2016), bacteria (King *et al.*, 2012; Cardinale *et al.*, 2015), and leafhoppers (Trivellone *et al.*, 2017).

While co-occurrence can be due to many factors (Cody & Diamond, 1975; Connor & Simberloff, 1979; Gotelli, 1999; Stone & Roberts, 1990, 1992; Webb, 2000; Hubbell, 2001; Sanderson *et al.*, 2009; Mayfield & Levine, 2010; Connor *et al.*, 2013), the results of the present study showed that the formation of plant pathogen communities at least strongly depended on taxa (11 out of 11 communities), whereas substrate seemed to play a less important role (three out of 11 communities).

The fact that modularity was largely structured by taxa but less by substrate mainly has two implications. First, it indicates that these plant pathogen communities likely respond similarly, regardless of substrate, i.e. in the present case between belowground (soil, roots) and aboveground (leaves). This strong link between above and belowground plant pathogens strongly points towards an environment, such as host plants, which is more important for structuring pathogen communities than a possible differences between substrates, like leaves and soil (Chapter

4). Thus, it might very well be possible to only sample the more easily measurable aboveground plant pathogen communities, and expect a similar response for belowground plant pathogen communities. Secondly, a different taxonomic composition of plant pathogen communities regardless of substrate implies cross-taxa commonalities of substrates and potential cross-taxa interactions within such communities, which seem likely when habitats are shared. Although this study did not set out to measure such possible interactions, other recent co-occurrence analyses showed significant associations resulting in interactions between plant pathogens from different taxa, for example, between bacteria and fungi (Stopnisek *et al.*, 2016; Deveau *et al.*, 2018; Jung *et al.*, 2018).

Furthermore, the contingency table results are in line with the modularity analysis which showed similarly high modularity within the substrates soil, roots and leaves, but a distinct lower modularity within the bacteria taxa as opposed to within fungi and oomycetes. A lower modularity across substrates, as for example observed within bacteria, suggests that these pathogens form less distinct communities than other pathogens. It remains to be seen if this pattern is real, as it implies that either such pathogens were more widespread than others (Barrett *et al.*, 2009; Bäumler & Fang, 2013), or were of relative low abundance. That these plant pathogens were widespread was not supported by a "number of sites" x "mean abundance" plot (not shown) and seems further less likely since widespread organisms are generally associated with a generalist life mode (Barberán *et al.*, 2012). Moreover, this would be in sharp contrast to what is known for bacteria in general (Monard *et al.*, 2016) and would challenge the view of an equal level of resource exploitation between generalist and specialist plant pathogens (Peers *et al.*, 2012). A larger, global study would be needed to reveal generalist and specialist plant pathogens. Such a study would be useful because it could identify plant pathogens which are (i.e. specialist) and are not (generalist) affected by climate change (Agrios, 2005; Gough *et al.*, 2015).

A more plausible explanation of the low observed modularity in bacteria plant pathogens could be of methodological nature. Modularity optimization can fail to identify communities smaller than a certain scale, the so-called resolution limit problem (Newman, 2006; Fortunato & Barthelemy, 2007; Waltman & Van Eck, 2013; Dinh *et al.*, 2015; Nicolini & Bifone, 2016). Because bacteria plant pathogens were generally less abundant, as were oomycetes in leaves (see results of Chapter 3), this could have caused the observed low modularity in this study. However, the algorithm of Blondel *et al.* (2008) which was used in the present study should have been less prone to this type of bias, despite the fact that it was specifically designed for large and complex

networks.

Finally, a low observed modularity could simply be due to the size of the sampled organism. Fungi, oomycetes and bacteria are often considered as "micro-organisms" (Buée *et al.*, 2009; Marulanda *et al.*, 2009; Talaro & Chess, 2018). However, the largest recorded plant pathogen fungi *Armillaria ostoyae* can grow over several square kilometres (Casselman, 2007), whereas plant pathogen bacteria (and bacteria in general) change their composition within milli- to centimetres (Kuske *et al.*, 2002). Thus, the sampling effort in this study (24 soil samples per 20 m x 20 m) might just have been more appropriate for fungi and oomycete than for bacteria pathogens.

5.4.3 Towards predicting plant pathogen occurrence in the near future

Plant pathogen communities of different taxa and substrates were generally strongly correlated and could predict each others co-occurrence, positively and negatively. Two oomycete modules in leaves could predict each other's co-occurrence positively, however, were not correlated in their occurrence to any other plant pathogen module. Although their isolation raises questions, the general results are encouraging, as they suggest strong application potential for predicting plant pathogen occurrences in the future. Predicting belowground from aboveground plant pathogens as well as predicting pathogens from different taxa, will help us improve agricultural and natural ecosystems, and better anticipate the outcome of changing ecosystems.

Consequently, future research should make use of these new tools and expand them. For example, on a more individual level keystone plant pathogen species could be identified for typical ecosystems and pathogen communities, using topological network features such as degree (Araújo *et al.*, 2011; Berry & Widder, 2014). Moreover, interactions between plant pathogens, and other microbes could be revealed when accounted for habitat filtering correctly. Hence, metabarcoding and network theory could help discover potential biological control agents (Poudel *et al.*, 2016). Increasing data availability may also provide the opportunity to model movements of whole plant pathogen communities, in lieu of individual pathogen species, under climate change scenarios and enhance our understanding of their impact on future food and ecosystem production (Bebber *et al.*, 2013).

Thus, finding non-random and predictable plant pathogen communities is especially valuable because they can help us answer big picture questions and bring us one step closer to unravelling the complexity of ecological systems.

Chapter 6

Concluding discussion

6.1 Key findings

This PhD thesis represents a body of research work which successfully applied novel tools such as next-generation sequencing (NGS), metabarcoding and network theory to study plant pathogens at an ecosystem scale. Here I referred to organisms which *matched to a known plant pathogen database to a pre-defined identity* simply as plant pathogens because the results of all chapters indicated that the metabarcoding approach was appropriate for the detection and quantification of organisms with a known plant pathogen status. Furthermore, mounting evidence from literature suggests a strong positive correlation of visual disease assessments and relative read abundances in NGS data (Sapkota *et al.*, 2015; Jakuschkin *et al.*, 2016). At the same time I recognise that the metabarcoding approach may not indicate actual pathogen load in a few cases, such as for some facultative plant pathogens. Nevertheless, the following results strongly encourage the utilisation of metabarcoding for large scale detection of plant pathogens.

In Chapter 2, I investigated possible differences in detection and abundance of plant pathogens between new metabarcoding and traditional cloning approaches using rust fungi as a model system. Besides a primer bias, I found otherwise strong congruity among all methods such as in relative abundance of detected rust fungi. This chapter demonstrated the promising potential of DNA metabarcoding for tracking important taxa such as plant pathogens from within larger metabarcoding communities. The main conclusion here was that metabarcoding seemed appropriate for large scale detection and quantification of plant pathogens, however, seemed less suited for confirming absence of species due to, for example, metabarcoding primer biases. Confirming the semi-quantitative nature of next-generation sequencing for the large scale detection of plant pathogens laid the foundation of this thesis and is highly encouraging for future researchers.

In Chapter 3, I applied the metabarcoding approach for the detection of plant pathogens

at large scale in order to reveal commonalities and differences in diversity patterns of fungi, oomycete and bacteria plant pathogens across land uses. The results showed a consistent pattern of a higher α-diversity (richness) of plant pathogenic OTUs in agricultural than in natural systems across substrate and pathogen taxa. In contrast, differences in β-diversity of plant pathogens (the variation in composition among plant pathogen communities from site-to-site within land use) were subtle, indicating a similar turnover within land uses. Plant pathogen groups were detected in the substrate types and land use categories as expected based on known ecology or literature, suggesting that the metabarcoding approach worked well for fungi, oomycete and bacteria plant pathogens.

In Chapter 4, I tested the equilateral disease triangle by quantifying the relative importance of environmental factors for plant pathogen communities and richness at large scale. Plant pathogen communities as well as richness showed a unified strong response towards plant communities (the composition of plant species) which generally could explain most of the variance after accounting for other environmental parameters such as geomorphology, climate, land use and soil. These results suggest an unequal relationship among the pathogen-plant-environment triangle. In light of forthcoming changes in climate and land use, these results furthermore suggest that changes in plant pathogen communities as well as richness will mostly be mediated through changes in plant communities, rather than direct effects of climate or soils.

In Chapter 5, I identified non-random and predictable co-occurrence patterns of plant pathogen communities, and applied network analysis in order to simplify the overwhelming complexity of above and belowground plant pathogens across different ecosystems. Modularity analysis revealed distinct plant pathogen communities across and within plant pathogen taxa (fungi, oomycetes, and bacteria) and substrates (soil, roots, and leaves). I found that the formation of plant pathogen communities always depended on taxonomic group, but only in a few cases, on substrate, as well. The results of this chapter are very encouraging as they showed that the overwhelming complexity of plant pathogen species can be reduced into defined communities. These in turn can be more easily studied than characterising the immense amount of individual plant pathogens. Furthermore, predictable and defined plant pathogen communities will help us more easily anticipate future impacts on food and ecosystem production.

In summary, the results of these four chapters encourage the utilisation of metabarcoding as an important tool for elucidating large scale processes of plant pathogens.

6.2 From metabarcoding data towards plant pathogen status

Inferring functional traits such as plant pathogen status from metabarcoding data comes with a range of unique challenges (Nguyen *et al.*, 2015; Dickie & St John, 2016; Ficetola *et al.*, 2016; Nguyen *et al.*, 2016). Hence, it is important to discuss the key findings with respect to the methodological bottlenecks that had to be overcome during the course of this PhD, and to identify future research needs. There were some major considerations which helped to infer plant pathogen status for an operational taxonomic unit (OTU) and to study plant pathogens at large scale, leading through the full process from field and laboratory work to bioinformatics and data analysis.

6.2.1 Field controls

In this study the separate sampling of different substrate types such as soil, below- and above-ground plant material and different land use and vegetation types was an indispensable field control.

For example, I found well known plant pathogens such as rust fungi only in the above ground plant substrates, or known soil and root pathogens only in soil and belowground plant substrates (Chapter 3). Moreover, specialised plant pathogens that were only detected together with their known host served as a further field control, such as *Plasmopara viticola* a grapevine pathogen which was only detected in vineyards (Chapter 3). The already known ecology of whole plant pathogen groups could be used as positive field control, too. For example, as expected I detected *Fusarium* in grasslands (Wilberforce *et al.*, 2003) whereas it was hardly present in forests. This procedure is comparable to Nguyen *et al.* (2015), who sampled monodominant plots containing either ectomycorrhizal or arbuscular mycorrhizal hosts as a field control.

Such natural field controls are an effective way to confirm that the entire metabarcoding approach worked, and give confidence in the results.

6.2.2 The benefit of sampling "everything"

Including healthy as well as diseased plant material in the sampling had some major benefits - especially when employing a metabarcoding approach for the detection of plant pathogens.

By including healthy as well as diseased plant material, I was able to tap the full potential of metabarcoding for the detection of plant pathogens. While using metabarcoding comes at the cost of not demonstrating disease causing ability and detecting DNA from non-living pathogens, it

has several advantages such as detecting asymptomatic infections (Jacobson *et al.*, 1998; Malcolm *et al.*, 2013; Stergiopoulos & Gordon, 2014), plant pathogens at hardly discernible levels (Stanosz *et al.*, 1997, 2007; Ploch & Thines, 2011; Crone *et al.*, 2013), and as-yet undescribed pathogens that are closely related to pathogens with a database entry. Moreover, metabarcoding can detect unculturable micro-organisms which constitute the overwhelming majority of organisms, including plant pathogens (Colwell & Grimes, 2000; Schloss & Handelsman, 2005).

To better exploit these benefits of metabarcoding, all possible habitats of plant pathogens were sampled including asymptomatic or healthy hosts and environments, thereby ensuring the detection of (1) asymptomatic plant pathogens that would have been concealed until the next disease outbreak, and (2) an unknown number of as-yet undescribed and (3) unculturable plant pathogens.

6.2.3 Minimising biases

Metabarcoding is prone to a range of biases (Nguyen *et al.*, 2015; Schnell *et al.*, 2015; Aivelo & Medlar, 2017). Minimising such biases is especially important when the organisms of interest are low in abundance. In this thesis plant pathogens represented less than 3% of the whole metabarcoding dataset and had a relatively low abundance when present. Thus, it was important to minimise possible biases through, for example, multiple DNA extractions, *a priori* primer testing, and controlling for contamination.

Some DNA extraction kits are well known to perform better for certain substrate types and for certain taxa (Lear *et al.*, 2018). Although studies increasingly try to find the optimal extraction method in order to minimise such biases in DNA extractions (Fouhy *et al.*, 2016; Hermans *et al.*, 2018; Lear *et al.*, 2018), this has not been done for all taxa yet. For example, in Chapter 2 I minimised extraction biases for rust fungal DNA in plant material by using different lysis buffers separately and equally pooling the extracts before performing PCRs.

A further - and probably the biggest - consideration when it comes to metabarcoding plant pathogens should be the choice of primers. The main conclusion of Chapter 2 was that differences in plant pathogen detection were not caused by different methods such as next-generation sequencing technologies or a cloning approach but overwhelmingly by primer base pair mismatches. In fact, this is a well known problem of "universal" metabarcoding primers deliberately designed to have some mismatches to amplify a wider range of species (Bellemain *et al.*, 2010; Schmidt *et al.*, 2013; Clarke *et al.*, 2014; Cowart *et al.*, 2015; Elbrecht & Leese,

2015; Shaw *et al.*, 2016). When metabarcoding plant pathogens it is worth analysing beforehand what and how many plant pathogens could be missed due to primer base pair mismatches. I tested several oomycete primer combinations *in silico* and validated them in the laboratory using mock communities, because there had not been well established oomycete specific metabarcoding primers at the start of this PhD. The primer pair OOMUP18Sc (Lievens *et al.*, 2004) and ITS2P (Znajda *et al.*, 2002) was found to be highly oomycete specific (no non-target amplification of e.g. plant material), amplifying the right length for a paired-end MiSeq Illumina run (about 250 bp), and the right gene region (intra-specific gene variability to differentiate most plant pathogen oomycete species and an established reference data bank). Thus, it has an advantage over other recently published metabarcoding primers for amplification of oomycete DNA (Riit *et al.*, 2016), which amplify a too long gene region. Although the OOMUP18Sc/ITS2P primer combination had never been used for metabarcoding before this thesis, the *a priori* testing ensured amplification of all major groups of oomycete plant pathogens and resulted in a successful recovery of those in the study.

Metabarcoding data can be skewed through contamination and tag jumps (Schnell *et al.*, 2015). I minimised the risk of contamination, for example, by minimising the number of necessary PCRs (Clarke *et al.*, 2014). I also used positive and negative controls which helped discover possible contamination, and furthermore allowed an estimate of tag-jumping which could then be corrected for. As a later step, the biological interpretation of the results can help discover possible sources of contamination. For example, in this study a pine-specific rust fungi (*Coleosporium*) was discovered in a grassland plot, with no pine trees present and no history of pine trees. Further investigation revealed a physical contamination with a DNA sample from a pine forest plot, which could then be resolved.

6.2.4 Bioinformatics

The choice of bioinformatic pipeline can have a profound impact on the detection of a plant pathogen. During the course of this PhD multiple bioinformatic pipelines and programs were assessed including combinations of usearch (Edgar, 2010), vsearch (Rognes *et al.*, 2016), swarm (Mahé *et al.*, 2014), qiime (Caporaso *et al.*, 2010), and mothur (Schloss *et al.*, 2009). Each step such as different quality of forward and reverse reads, trimming primers and merging was carefully evaluated in the context of the whole pipeline.

For example, I assumed some oomycete species would get filtered out during the process of merging forward and reverse Illumina reads due to a few cases of a longer than 400 bp reported gene region. I tested this using only the forward read and comparing the outcome with the merged data. Thereby I could confirm that no species were lost in this dataset because of merging.

In order to assess if a 3% clustering threshold was appropriate to represent species level of plant pathogens, I ran clustering thresholds from 90% to 100% in 0.1% steps for some major plant pathogen groups and looked for a crossover between over- and undersplit OTUs (over- and under-representation of species names for each OTU). An example of this relationship using the major plant pathogen group of rust fungi can be found in Supplement D, Fig. D.1. While this ensured finding an approximation for an optimal clustering threshold, the optimal values did not differ more than 1% from the widely used 3% threshold for the majority of plant pathogen taxa. Although I had no reason to deviate from the widely applied 3% sequence similarity to represent plant pathogen species level, such a procedure can help to decide what percentage level might be appropriate in future studies, or when dealing with specific plant pathogen groups which have a higher or lower barcode gene variability in order to represent optimal species level resolution.

Inferring plant pathogen status for OTUs was a major step of this thesis. For this reason I mainly used curated reference sequence databases. Furthermore, the use of curated reference sequence databases allowed to lower the species level identity for obligate pathogen genera such as *Puccinia*, and hence allowing the detection of not recorded plant pathogen species out of these genera. For example, in the case of *Puccinia*, a 95% match to a known *Puccinia* species would still place the not recorded species within the *Puccinia* genus and thus identify the species as likely plant pathogen.

Similarly, plant pathogen reference names were gathered only from published sources. I included synonyms because in some cases outdated taxonomic names were found in curated databases, and taxonomy changes faster than databases. This collection of plant pathogen names can be found in supplementary material 3 of this thesis and represents a valuable contribution which will facilitate future metabarcoding studies of plant pathogens.

6.2.5 Data analysis

The data analysis in this thesis had some unique challenges, with the solutions I found able to be applied to the analysis of any metabarcoding dataset.

The widely used sequence standardisation such as general rarefaction (Heck *et al.*, 1975) or extrapolation (Schmidt *et al.*, 2013; Chao *et al.*, 2015) could not be applied to the plant pathogen metabarcoding dataset in this thesis. The main reason was that these techniques cannot deal with extremely low sequences in samples. However, sequence standardisation between samples is absolutely necessary for an appropriate species richness estimate when dealing with unequal sequencing depths (sampling effort) in NGS datasets (Leray & Knowlton, 2015). For example, Tedersoo *et al.* (2014) standardised by using the residuals of OTU richness in relation to the square root of the number of obtained sequences. In the case of this thesis several richness models were explored, too, such as a square root, a quadratic, a Michaelis Menten, and a logistic model. However, these models were not satisfying as they described the richness poorly. For example the best fitted model was a log-model ($F_{1,73}$=126.99, R^2=0.63, P<0.001, Supplement D, Fig. D.2) and could only describe 63% of the data. I circumvented this problem in Chapter 2 by using the proportional abundance of each OTU found within a plot in order to standardise abundances between different sequencing datasets. Possible differences between the datasets could then be tested using a generalised additive model which allowed the consideration of beta distribution, random effects, and interactions. Another way of dealing with low abundances in metabarcoding datasets was introduced in Chapter 3. Here I randomly sampled species from the entire community to the minimum size per sample and then used the sum of the plant pathogen OTUs present in the rarefied community. This approach allowed equalisation for different sequencing depths between the samples even when very low plant pathogen OTUs were present. This is an elegant solution of standardising between samples and can be applied to any metabarcoding dataset with a particular interest in low abundance or rare species.

The utility of metabarcoding is especially huge for investigating community responses. However, multivariate analysis of community data can be a convoluted terrain which is often about finding "the most appropriate approach given the question and the data" (McCune *et al.*, 2002). In Chapter 4, I employed multiple regression on distance matrices (MRM), as a robust way of retaining the whole information of the plant pathogen community and at the same time including all the variation from environmental matrices, without multicollinearity issues or the usual problems associated with variable selection and reducing information (George, 2000). The same robust approach can also be used to analyse species richness when converting species richness into a dissimilarity matrix using the absolute log response ratio (similar to effect sizes), as demonstrated by Barnes *et al.* (2016) and in Chapter 4. Although more widely applied in

other fields such as, for example, landscape ecology and genetics (Zapala & Schork, 2006; Wang, 2013), the flexibility and robustness of the MRM approach in combination with metabarcoding is a useful addition to the toolbox of community ecologists (Legendre *et al.*, 2005; Lichstein, 2007). Network analysis is another useful tool which allows the study of plant pathogen communities and to move beyond the basic inventory of diversity- and barplots. The use of network analysis in Chapter 5 could simplify the complexity of a large number of plant pathogens and showed promising potential for predicting the occurrence of whole plant pathogen groups. In this thesis, network analysis was used to describe co-occurrences or associations between plant pathogens, which are generally the result of many factors such as chance, history, speciation, phylogenetic distance, migration, extinction, dispersal, abiotic environmental factors, and biotic interactions (Cody & Diamond, 1975; Connor & Simberloff, 1979; Gotelli, 1999; Stone & Roberts, 1990, 1992; Webb, 2000; Hubbell, 2001). While current methods of network inference are yet more robust in describing these associations than retrieving individual signals such as interactions from these complex ecological systems (Weiss *et al.*, 2016; Sander *et al.*, 2017), we might be able to untangle the individual factors of co-occurrence in the near future. For example, Bohan *et al.* (2011) maximised the likelihood of learning a predation interaction in a food web using logic-based machine learning and co-occurrence networks. Combining novel developments in the field of artificial intelligence with large networks derived from metabarcoding data sets (Bohan *et al.*, 2017) is one example where new methods could be used in the near future to deepen our understanding of plant pathogen interactions with their environment.

From field and laboratory work to bioinformatics and the introduced tools for analysing plant pathogen metabarcoding data - herein is provided considerations and research which will aid all researchers who want to employ a metabarcoding approach for investigating plant pathogens. Furthermore, as the field of metabarcoding progresses methods for analysing NGS data will inevitably continue to improve.

6.3 Final remarks

There is a need for a deeper understanding of plant pathogen community ecology and their role in maintaining multiple ecosystem processes and services, particularly facing imminent changes in our ecosystems. Moving from plant pathogen species to communities will allow us to simplify, categorise and generalise responses for pathogen groups. This will potentially revolutionise the field of plant pathology since a plant pathogen community approach will enlarge our knowledge

of general pathogen dynamics and ecology, but also enable us to address questions that were hitherto simply not feasible to answer when focused on individual pathosystems. The latter includes pressing issues such as general plant pathogen responses to climate change (Elad & Pertot, 2014).

Metabarcoding is a promising tool which can be employed for this purpose. However, only when considered as a whole, from field and laboratory work, to bioinformatics and data analysis the challenge of inferring plant pathogen status from metabarcoding data can be overcome. The results of this thesis suggest that, already with current knowledge, biases can be minimised to a level that allows us to integrate and interpret traits such as plant pathogen status in metabarcoding data.

Thus, the overall findings of this PhD thesis encourage moving beyond the ongoing debate about the ecological relevance of sequence abundances in metabarcoding data, and whether or not plant pathogen status (or any functional trait) can be inferred from metabarcoding data sets. Instead we should focus on the increasing number of cases where metabarcoding is successfully applied to study plant pathogens (Sapkota *et al.*, 2015; Jakuschkin *et al.*, 2016; Singer *et al.*, 2016; Burgess *et al.*, 2017b) - a logical and necessary step in order to push the field of metabarcoding forward and provide sustainable solutions to global food security and ecosystem services in the near future.

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Appendix A

Supplement Chapter 3

A.1 Names considered to be potential plant pathogenic fungi, from Nguyen *et al.* (2016)

Acrocalymma medicaginis, Acrodontium simplex, Acrophialophora fusispora, Acrosporium tingitaninum, Aecidium, Aecidium aechmantherae, Aecidium amaryllidis, Aecidium breyniae, Aecidium campanulastri, Aecidium cannabis, Aecidium cantensis, Aecidium caspicum, Aecidium foeniculi, Aecidium narcissi, Ahmadiago, Albonectria rigidiuscula, Allodus podophylli, Alternaria, Alternaria alternata, Alternaria black spot of canola, Alternaria brassicae, Alternaria brassicicola, Alternaria carthami, Alternaria cinerariae, Alternaria dauci, Alternaria dianthi, Alternaria dianthicola, Alternaria euphorbiicola, Alternaria helianthi, Alternaria helianthicola, Alternaria japonica, Alternaria leucanthemi, Alternaria linicola, Alternaria padwickii, Alternaria panax, Alternaria radicina, Alternaria raphani, Alternaria saponariae, Alternaria senecionis, Alternaria solani, Alternaria tenuissima, Alternaria triticina, Alternaria zinniae, Amazonia, Amphobotrys ricini, Anguillosporella vermiformis, Anthostomella pullulans, Antrodia albida, Antrodia serialiformis, Antrodia serialis, Apiospora montagnei, Appendiculella, Armillaria heimii, Armillaria sinapina, Armillaria socialis, Armillaria tabescens, Arthrocladiella, Arthuriomyces peckianus, Ascochyta asparagina, Ascochyta caricae, Ascochyta diseases of pea, Ascochyta doronici, Ascochyta fabae, Ascochyta fabae f.sp. lentis, Ascochyta graminea, Ascochyta hordei, Ascochyta humuli, Ascochyta medicaginicola, Ascochyta pisi, Ascochyta prasadii, Ascochyta sorghi, Ascochyta spinaciae, Ascochyta tarda, Ascochyta tritici, Ascospora ruborum, Aspergillus aculeatus, Aspergillus fischerianus, Aspergillus niger, Asperisporium caricae, Asteridiella, Asteroma caryae, Athelia arachnoidea, Athelia rolfsii, Aurantiporus fissilis, Aureobasidium pullulans, Bambusiomyces, Bayoud disease, Beniowskia sphaeroidea, Bionectria ochroleuca, Bipolaris, Bipolaris cactivora, Bipolaris cookei, Bipolaris incurvata, Bipolaris sacchari, Biscogniauxia capnodes, Biscogniauxia marginata, Bjerkandera adusta, Blakeslea trispora, Blumeria graminis, Boeremia lycopersici,

Botryodiplodia oncidii, Botryodiplodia ulmicola, Botryosphaeria cocogena, Botryosphaeria dothidea, Botryosphaeria marconii, Botryosphaeria obtusa, Botryosphaeria rhodina, Botryosphaeria ribis, Botryosphaeria stevensii, Botryosporium pulchrum, Botryotinia, Botryotinia fuckeliana, Botryotinia polyblastis, Botrytis allii, Botrytis anthophila, Botrytis cinerea, Botrytis fabae, Botrytis narcissicola, Botrytis squamosa, Brachybasidiaceae, Brasiliomyces, Brasiliomyces malachrae, Briosia ampelophaga, Buckeye rot of tomato, Bulbomicrosphaera, Cadophora malorum, Caespitotheca, Calonectria, Calonectria ilicicola, Calonectria indusiata, Calonectria kyotensis, Calonectria pyrochroa, Calonectria quinqueseptata, Camarotella acrocomiae, Camarotella costaricensis, Canna rust, Capitorostrum cocoes, Capnodium footii, Capnodium mangiferum, Capnodium ramosum, Capnodium theae, Cephalosporium gramineum, Ceratobasidium cereale, Ceratobasidium cornigerum, Ceratobasidium noxium, Ceratobasidium ramicola, Ceratobasidium setariae, Ceratobasidium stevensii, Ceratocystis adiposa, Ceratocystis coerulescens, Ceratocystis fimbriata, Ceratocystis moniliformis, Ceratocystis oblonga, Ceratocystis obpyriformis, Ceratocystis paradoxa, Ceratocystis pilifera, Ceratocystis pluriannulata, Ceratocystis polyconidia, Ceratocystis tanganyicensis, Ceratocystis zombamontana, Ceratorhiza hydrophila, Ceratospermopsis, Cercoseptoria ocellata, Cercospora, Cercospora apii, Cercospora apii f.sp. clerodendri, Cercospora apiicola, Cercospora arachidicola, Cercospora asparagi, Cercospora atrofiliformis, Cercospora beticola, Cercospora brachypus, Cercospora brassicicola, Cercospora brunkii, Cercospora cannabis, Cercospora cantuariensis, Cercospora capsici, Cercospora carotae, Cercospora corylina, Cercospora fuchsiae, Cercospora fusca, Cercospora fusimaculans, Cercospora gerberae, Cercospora halstedii, Cercospora handelii, Cercospora hayi, Cercospora hydrangeae, Cercospora kikuchii, Cercospora lentis, Cercospora liquidambaris, Cercospora longipes, Cercospora longissima, Cercospora mamaonis, Cercospora mangiferae, Cercospora medicaginis, Cercospora melongenae, Cercospora minuta, Cercospora nicotianae, Cercospora odontoglossi, Cercospora papayae, Cercospora penniseti, Cercospora pisa-sativae, Cercospora platanicola, Cercospora puderii, Cercospora pulcherrima, Cercospora rhapidicola, Cercospora rosicola, Cercospora sojina, Cercospora solani, Cercospora solani-tuberosi, Cercospora sorghi, Cercospora theae, Cercospora tuberculans, Cercospora vexans, Cercospora vicosae, Cercospora zeae-maydis, Cercospora zebrina, Cercospora zonata, Cercosporella rubi, Ceriporia spissa, Ceriporia xylostromatoides, Cerrena unicolor, Ceuthospora lauri, Choanephora, Choanephora cucurbitarum, Choanephora infundibulifera, Chrysanthemum white rust, Chrysomyxa cassandrae, Chrysomyxa himalensis, Chrysomyxa ledi, Chrysomyxa ledi var. rhododendri, Chrysomyxa ledicola, Chrysomyxa nagodhii, Chrysomyxa neoglandu-

losi, Chrysomyxa piperiana, Chrysomyxa pirolata, Chrysomyxa pyrolae, Chrysomyxa reticulata, Chrysomyxa roanensis, Chrysomyxa succinea, Cladosporium, Cladosporium arthropodii, Cladosporium cladosporioides, Cladosporium cladosporioides f.sp. pisicola, Cladosporium cucumerinum, Cladosporium herbarum, Cladosporium musae, Cladosporium oncobae, Claviceps fusiformis, Claviceps purpurea, Claviceps sorghi, Claviceps zizaniae, Climacodon pulcherrimus, Climacodon septentrionalis, Clitocybe parasitica, Clonostachys rosea f. rosea, Clypeoporthe iliau, Cochliobolus, Cochliobolus carbonum, Cochliobolus cymbopogonis, Cochliobolus hawaiiensis, Cochliobolus heterostrophus, Cochliobolus lunatus, Cochliobolus miyabeanus, Cochliobolus ravenelii, Cochliobolus sativus, Cochliobolus setariae, Cochliobolus spicifer, Cochliobolus stenospilus, Cochliobolus tuberculatus, Cochliobolus victoriae, Coleosporium helianthi, Coleosporium ipomoeae, Coleosporium madiae, Coleosporium pacificum, Coleosporium tussilaginis, Colletotrichum acutatum, Colletotrichum arachidis, Colletotrichum capsici, Colletotrichum cereale, Colletotrichum crassipes, Colletotrichum dematium, Colletotrichum dematium f. spinaciae, Colletotrichum derridis, Colletotrichum destructivum, Colletotrichum glycines, Colletotrichum gossypii, Colletotrichum higginsianum, Colletotrichum kahawae, Colletotrichum lindemuthianum, Colletotrichum lini, Colletotrichum mangenotii, Colletotrichum musae, Colletotrichum nigrum, Colletotrichum orbiculare, Colletotrichum pisi, Colletotrichum sublineolum, Colletotrichum trichellum, Colletotrichum trifolii, Colletotrichum truncatum, Conidiosporomyces, Coniella castaneicola, Coniella diplodiella, Coniella fragariae, Coniothecium chomatosporum, Coniothyrium celtidis-australis, Coniothyrium henriquesii, Coniothyrium rosarum, Coniothyrium wernsdorffiae, Coprinopsis psychromorbida, Cordana johnstonii, Cordana musae, Coriolopsis floccosa, Corticium invisum, Corticium penicillatum, Corticium theae, Coryneopsis rubi, Corynespora cassiicola, Coryneum rhododendri, Crinipellis sarmentosa, Cristulariella depraedans, Cronartium ribicola, Cryphonectriaceae, Cryptobasidiaceae, Cryptocline cyclaminis, Cryptomeliola, Cryptosporella umbrina, Cryptosporiopsis tarraconensis, Cryptosporium minimum, Curvularia caricae-papayae, Curvularia penniseti, Curvularia senegalensis, Curvularia trifolii, Cyclaneusma needle cast, Cylindrocarpon ianthothele var. ianthothele, Cylindrocarpon magnusianum, Cylindrocarpon musae, Cylindrocladiella camelliae, Cylindrocladiella parva, Cylindrocladium clavatum, Cylindrocladium lanceolatum, Cylindrocladium peruvianum, Cylindrocladium pteridis, Cylindrosporium cannabinum, Cylindrosporium juglandis, Cylindrosporium rubi, Cymadothea trifolii, Cytospora, Cytospora palmarum, Cytospora personata, Cytospora sacchari, Cytospora sacculus, Cytospora terebinthi, Cytosporina ludibunda, Dactuliophora elongata, Davidiella dianthi, Davidiella tassiana, Deightoniella papuana, Deightoniella

torulosa, Dendrophoma marconii, Dendrophora erumpens, Denticularia mangiferae, Dermea pseudotsugae, Diaporthaceae, Diaporthe, Diaporthe arctii, Diaporthe beilharziae, Diaporthe dulcamarae, Diaporthe eres, Diaporthe fraxini-angustifoliae, Diaporthe helianthi, Diaporthe lagunensis, Diaporthe litchicola, Diaporthe lokoyae, Diaporthe melonis, Diaporthe nothofagi, Diaporthe orthoceras, Diaporthe pascoei, Diaporthe perniciosa, Diaporthe phaseolorum, Diaporthe phaseolorum var. caulivora, Diaporthe phaseolorum var. phaseolorum, Diaporthe phaseolorum var. sojae, Diaporthe rudis, Diaporthe salicicola, Diaporthe tanakae, Diaporthe toxica, Dicarpella dryina, Didymella arachidicola, Didymella bryoniae, Didymella pinodella, Didymella pinodes, Didymosphaeria taiwanensis, Dilophospora alopecuri, Dimeriella sacchari, Diplocarpon mespili, Diplocarpon rosae, Diplodia allocellula, Diplodia laelio-cattleyae, Diplodia manihoti, Diplodia paraphysaria, Diplodia seriata, Diplodia theae-sinensis, Discosia artocreas, Discostroma corticola, Distocercospora, Distocercospora livistonae, Dothideomycetes, Dothiorella brevicollis, Dothiorella dominicana, Dothiorella dulcispinae, Dothiorella gregaria, Drechslera avenacea, Drechslera campanulata, Drechslera dematioidea, Drechslera gigantea, Drechslera glycines, Drechslera musae-sapientium, Drechslera teres f. maculata, Drechslera wirreganensis, Eballistra lineata, Eballistra oryzae, Eballistraceae, Echinodontium ryvardenii, Echinodontium tinctorium, Ectendomeliola, Elsinoë ampelina, Elsinoë batatas, Elsinoë brasiliensis, Elsinoë leucospila, Elsinoë randii, Elsinoë rosarum, Elsinoë sacchari, Elsinoë theae, Elsinoë veneta, Endomeliola, Endothia radicalis, Endothiella gyrosa, Entorrhizomycetes, Entyloma, Entyloma ageratinae, Entyloma dahliae, Entyloma ellisii, Epicoccum nigrum, Eremothecium coryli, Eremothecium gossypii, Erysiphales, Erysiphe, Erysiphe betae, Erysiphe brunneopunctata, Erysiphe cichoracearum, Erysiphe cruciferarum, Erysiphe graminis f. sp. avenae, Erysiphe graminis f.sp. tritici, Erysiphe heraclei, Erysiphe pisi, Exobasidiaceae, Exobasidium burtii, Exobasidium reticulatum, Exobasidium vaccinii var. japonicum, Exobasidium vaccinii-uliginosi, Exobasidium vexans, Exophiala, Foamy bark canker, Fomes lamaënsis, Fomitopsis rosea, Fusarium, Fusarium affine, Fusarium arthrosporioides, Fusarium crookwellense, Fusarium culmorum, Fusarium ear blight, Fusarium incarnatum, Fusarium solani, Fusarium langsethiae, Fusarium mangiferae, Fusarium oxysporum f.sp. albedinis, Fusarium oxysporum f.sp. asparagi, Fusarium oxysporum f.sp. batatas, Fusarium oxysporum f.sp. betae, Fusarium oxysporum f.sp. cannabis, Fusarium oxysporum f.sp. carthami, Fusarium oxysporum f.sp. cattleyae, Fusarium oxysporum f.sp. ciceris, Fusarium oxysporum f.sp. coffea, Fusarium oxysporum f.sp. cubense, Fusarium oxysporum f.sp. cyclaminis, Fusarium oxysporum f.sp. dianthi, Fusarium oxysporum f.sp. lentis, Fusarium oxysporum f.sp. lini, Fusar-

ium oxysporum f.sp. lycopersici, Fusarium oxysporum f.sp. medicaginis, Fusarium oxysporum f.sp. pisi, Fusarium oxysporum f.sp. radicis-lycopersici, Fusarium oxysporum f.sp. spinacia, Fusarium oxysporum f.sp. vasinfectum, Fusarium pallidoroseum, Fusarium patch, Fusarium proliferatum, Fusarium crown rot of wheat, Fusarium redolens, Fusarium sacchari, Fusarium solani f.sp. pisi, Fusarium subglutinans, Fusarium sulphureum, Fusarium verticillioides, Fusarium wilt, Fusicladium pisicola, Fusicoccum aesculi, Fusicoccum amygdali, Fusicoccum quercus, Gaeumannomyces graminis var. avenae, Gaeumannomyces graminis var. graminis, Galactomyces candidum, Ganoderma brownii, Ganoderma lobatum, Ganoderma megaloma, Ganoderma meredithiae, Ganoderma orbiforme, Ganoderma philippii, Ganoderma sessile, Ganoderma tornatum, Ganoderma zonatum, Geastrumia polystigmatis, Georgefischeriaceae, Georgefischeriales, Geotrichum, Geotrichum candidum, Geotrichum klebahnii, Gibberella, Gibberella acuminata, Gibberella avenacea, Gibberella baccata, Gibberella cyanogena, Gibberella fujikuroi, Gibberella intricans, Gibberella pulicaris, Gibberella stilboides, Gibberella tricincta, Gibberella xylarioides, Gibberella zeae, Gibellina cerealis, Gilbertella persicaria, Gjaerumiaceae, Gliocladiopsis tenuis, Gliocladium vermoeseni, Gloeocercospora sorghi, Gloeocystidiellum porosum, Gloeophyllum mexicanum, Gloeophyllum trabeum, Gloeosporium cattleyae, Gloeosporium theae-sinensis, Glomerella cingulata, Glomerella graminicola, Glomerella tucumanensis, Gnomonia caryae, Gnomonia comari, Gnomonia dispora, Gnomonia iliau, Gnomonia rubi, Golovinomyces cichoracearum var. latisporus, Graphiola phoenicis, Graphiolaceae, Graphium rigidum, Graphium rubrum, Graphyllium pentamerum, Grovesinia pyramidalis, Guignardia bidwellii f. muscadinii, Guignardia camelliae, Guignardia fulvida, Guignardia mangiferae, Guignardia musae, Guignardia philoprina, Gummy stem blight, Gymnoconia nitens, Gymnopus dryophilus, Gymnosporangium kernianum, Gymnosporangium libocedri, Gymnosporangium nelsonii, Gymnosporangium yamadae, Haematonectria haematococca, Hansenula subpelliculosa, Hapalosphaeria deformans, Haplobasidion musae, Helicobasidium compactum, Helicobasidium longisporum, Helicobasidium purpureum, Helicoma muelleri, Helminthosporium cookei, Helminthosporium solani, Hendersonia creberrima, Hendersonia theicola, Hericium coralloides, Heterobasidion irregulare, Heterobasidion occidentale, Hexagonia hydnoides, Hymenula affinis, Hyphodermella corrugata, Hyphodontia aspera, Hyphodontia sambuci, Hypoxylon canker of shade trees, Hypoxylon tinctor, Inonotus arizonicus, Inonotus cuticularis, Inonotus dryophilus, Inonotus hispidus, Inonotus ludovicianus, Irenopsis, Irpex destruens, Irpex lacteus, Kabatiella caulivora, Karnal bunt, Kretzschmaria zonata, Kuehneola uredinis, Kutilakesa pironii, Laetiporus ailaoshanensis, Laetiporus baudonii,

Laetiporus caribensis, Laetiporus conifericola, Laetiporus cremeiporus, Laetiporus gilbertsonii, Laetiporus huroniensis, Laetiporus montanus, Laetiporus portentosus, Laetiporus zonatus, Laxitextum bicolor, Leandria momordicae, Lentinus tigrinus, Lenzites betulina, Lenzites elegans, Leohumicola atra, Leohumicola incrustata, Leohumicola levissima, Leotiomycetes, Leptodontidium elatius var. elatius, Leptographium microsporum, Leptosphaeria acuta, Leptosphaeria cannabina, Leptosphaeria coniothyrium, Leptosphaeria libanotis, Leptosphaeria lindquistii, Leptosphaeria maculans, Leptosphaeria musarum, Leptosphaeria pratensis, Leptosphaeria sacchari, Leptosphaeria woroninii, Leptosphaerulina crassiasca, Leptosphaerulina trifolii, Leptothyrium nervisedum, Leptotrochila medicaginis, Leucocytospora leucostoma, Leucostoma auerswaldii, Leucostoma canker, Leucostoma kunzei, Leucostoma persoonii, Leveillula compositarum f. helianthi, Leveillula leguminosarum f. lentis, Leveillula taurica, Limacinula tenuis, Linochora graminis, Lopharia crassa, Lophodermium, Lophodermium aucupariae, Lophodermium schweinitzii, Macrophoma mangiferae, Macrophoma theicola, Macrosporium cocos, Magnaporthe, Magnaporthe grisea, Magnaporthe salvinii, Magnaporthiopsis, Mamianiella coryli, Marasmiellus cocophilus, Marasmiellus stenophyllus, Marasmius crinis-equi, Marasmius sacchari, Marasmius semiustus, Marasmius stenophyllus, Marasmius tenuissimus, Massarina walkeri, Mauginiella scaettae, Melampsora, Melampsora lini var. lini, Melampsora occidentalis, Melanconis carthusiana, Melanconium juglandinum, Meliola, Meliola mangiferae, Meliola zangii, Meliolaceae, Meruliopsis ambigua, Microascus brevicaulis, Microbotryum silenes-dioicae, Microbotryum violaceum, Microbotryum violaceum infection of Silene latifolia, Microdochium bolleyi, Microdochium dimerum, Microdochium panattonianum, Microdochium phragmitis, Microsphaera, Microsphaera coryli, Microsphaera diffusa, Microsphaera ellisii, Microsphaera euphorbiae, Microsphaera hommae, Microsphaera penicillata var. vaccinii, Microsphaera vaccinii, Microsphaera verruculosa, Microstroma juglandis, Moesziomyces bullatus, Monilinia azaleae, Monilinia fructicola, Monilinia fructigena, Monilinia laxa, Monilinia oxycocci, Moniliophthora roreri, Monilochaetes infuscans, Monochaetia coryli, Monochaetia mali, Monographella albescens, Monographella cucumerina, Monographella nivalis var. neglecta, Monographella nivalis var. nivalis, Monosporascus cannonballus, Monosporascus eutypoides, Monostichella coryli, Mucor circinelloides, Mucor hiemalis, Mucor hiemalis f. silvaticus, Mucor mucedo, Mucor paronychius, Mucor piriformis, Mucor racemosus, Mycena citricolor, Mycocentrospora acerina, Mycoleptodiscus terrestris, Mycosphaerella arachidis, Mycosphaerella areola, Mycosphaerella berkeleyi, Mycosphaerella bolleana, Mycosphaerella brassicicola, Mycosphaerella caricae, Mycosphaerella caryigena, Mycosphaerella cerasella, Mycosphaerella

coffeicola, Mycosphaerella confusa, Mycosphaerella cruenta, Mycosphaerella dendroides, Mycosphaerella eumusae, Mycosphaerella gossypina, Mycosphaerella graminicola, Mycosphaerella henningsii, Mycosphaerella horii, Mycosphaerella juglandis, Mycosphaerella lageniformis, Mycosphaerella linicola, Mycosphaerella louisianae, Mycosphaerella musae, Mycosphaerella musicola, Mycosphaerella palmicola, Mycosphaerella pistaciarum, Mycosphaerella pistacina, Mycosphaerella platanifolia, Mycosphaerella polymorpha, Mycosphaerella pomi, Mycosphaerella punctiformis, Mycosphaerella pyri, Didymella rabiei, Mycosphaerella recutita, Mycosphaerella rosicola, Mycosphaerella rubi, Mycosphaerella stigmina-platani, Mycosphaerella striatiformans, Mycovellosiella concors, Passalora fulva, Mycovellosiella koepkei, Mycovellosiella vaginae, Myriogenospora aciculispora, Myrothecium roridum, Myrothecium verrucaria, Naevala perexigua, Naohidemyces vaccinii, Nectria cinnabarina, Nectria ditissima, Nectria foliicola, Nectria mammoidea var. rubi, Nectria peziza, Nectria pseudotrichia, Nectria radicicola, Nectria ramulariae, Nectriella pironii, Nemania diffusa, Nemania serpens var. serpens, Neocosmospora vasinfecta, Neodeightonia phoenicum, Neoerysiphe, Neofabraea perennans, Northern corn leaf blight, Oidiopsis gossypii, Oidium, Oidium arachidis, Oidium caricae-papayae, Oidium indicum, Oidium mangiferae, Oidium manihotis, Olpidium brassicae, Omphalia tralucida, Ophiobolus anguillides, Ophiobolus cannabinus, Ophioirenina, Ovulinia azaleae, Ovulitis azaleae, Oxyporus corticola, Ozonium texanum var. parasiticum, Pauahia, Peach scab, Peltaster fructicola, Penicillium expansum, Penicillium funiculosum, Peniophora, Periconia circinata, Periconiella cocoes, Peridermium californicum, Pestalosphaeria concentrica, Pestalotia longiseta, Pestalotia rhododendri, Pestalotiopsis, Pestalotiopsis adusta, Pestalotiopsis arachidis, Pestalotiopsis disseminata, Pestalotiopsis guepini, Pestalotiopsis leprogena, Pestalotiopsis longiseta, Pestalotiopsis mangiferae, Pestalotiopsis palmarum, Pestalotiopsis sydowiana, Pestalotiopsis theae, Peyronellaea, Peyronellaea curtisii, Phacidiopycnis padwickii, Phaeochoropsis mucosa, Phaeocytostroma iliau, Phaeocytostroma sacchari, Phaeoisariopsis bataticola, Phaeoramularia heterospora, Phaeoramularia indica, Phaeoramularia manihotis, Phaeoseptoria musae, Phaeosphaerella mangiferae, Phaeosphaerella theae, Phaeosphaeria avenaria f.sp. avenaria, Phaeosphaeria avenaria f.sp. triticae, Phaeosphaeria herpotrichoides, Phaeosphaeria microscopica, Phaeosphaeria nodorum, Phaeosphaeriopsis obtusispora, Phaeotrichoconis crotalariae, Phakopsora gossypii, Phakopsora pachyrhizi, Phialophora asteris, Phialophora cinerescens, Phialophora gregata, Phialophora tracheiphila, Phoma, Phoma clematidina, Phoma costaricensis, Phoma cucurbitacearum, Phoma destructiva, Phoma draconis, Phoma exigua, Phoma exigua var. exigua, Phoma exigua var. foveata, Phoma exigua var. linicola,

Phoma glomerata, Phoma glycinicola, Phoma herbarum, Phoma insidiosa, Phoma microspora, Phoma narcissi, Phoma nebulosa, Phoma oncidii-sphacelati, Phoma sclerotioides, Phoma strasseri, Phomopsis asparagi, Phomopsis asparagicola, Phomopsis cannabina, Phomopsis coffeae, Phomopsis ganjae, Phomopsis javanica, Phomopsis longicolla, Phomopsis mangiferae, Phomopsis prunorum, Phomopsis sclerotioides, Phomopsis theae, Phragmidium, Phragmidium mucronatum, Phragmidium rosae-pimpinellifoliae, Phragmidium rubi-idaei, Phragmidium violaceum, Phyllachora banksiae, Phyllachora cannabis, Phyllachora graminis var. graminis, Phyllachora gratissima, Phyllachora musicola, Phyllachora pomigena, Phyllachora sacchari, Phyllactinia, Phyllosticta, Phyllosticta alliariaefoliae, Phyllosticta arachidis-hypogaeae, Phyllosticta batatas, Phyllosticta capitalensis, Phyllosticta carpogena, Phyllosticta coffeicola, Phyllosticta concentrica, Phyllosticta coryli, Phyllosticta cucurbitacearum, Phyllosticta cyclaminella, Phyllosticta erratica, Phyllosticta hawaiiensis, Phyllosticta lentisci, Phyllosticta manihotis, Phyllosticta micropuncta, Phyllosticta minima, Phyllosticta mortonii, Phyllosticta nicotianae, Phyllosticta palmetto, Phyllosticta penicillariae, Phyllosticta perseae, Phyllosticta pseudocapsici, Phyllosticta sojaecola, Phyllosticta theae, Phyllosticta theicola, Phymatotrichopsis omnivora, Physalospora disrupta, Physalospora perseae, Physoderma alfalfae, Physoderma leproides, Physoderma trifolii, Physopella ampelopsidis, Pileolaria terebinthi, Pine-pine gall rust, Pineapple black rot, Piricaudiopsis punicae, Piricaudiopsis rhaphidophorae, Piricaudiopsis rosae, Plenodomus destruens, Plenodomus meliloti, Pleochaeta, Pleosphaerulina sojicola, Pleospora alfalfae, Pleospora betae, Pleospora herbarum, Pleospora lycopersici, Pleospora tarda, Pleospora theae, Pleuroceras, Podosphaera, Podosphaera fuliginea, Podosphaera fusca, Podosphaera leucotricha, Podosphaera macularis, Podosphaera pannosa, Polyscytalum pustulans, Poria hypobrunnea, Postia tephroleuca, Powdery mildew, Pseudocercospora, Pseudocercospora arecacearum, Pseudocercospora cannabina, Pseudocercospora fuligena, Pseudocercospora gunnerae, Pseudocercospora pandoreae, Pseudocercospora puderi, Pseudocercospora rhapisicola, Pseudocercospora theae, Pseudocercospora vitis, Pseudocercosporella capsellae, Pseudocochliobolus eragrostidis, Pseudoepicoccum cocos, Pseudopeziza jonesii, Pseudopeziza medicaginis, Pseudopeziza trifolii, Pseudoseptoria donacis, Puccinia, Puccinia angustata, Puccinia arachidis, Puccinia aristidae, Puccinia asparagi, Puccinia cacabata, Puccinia campanulae, Puccinia carthami, Puccinia coronata, Puccinia coronata var. hordei, Puccinia dioicae, Puccinia erianthi, Puccinia extensicola var. hieraciata, Puccinia helianthi, Puccinia hordei, Puccinia jaceae var. solstitialis, Puccinia kuehnii, Puccinia libanotidis, Puccinia malvacearum, Puccinia mariae-wilsoniae, Puccinia melanocephala, Puccinia menthae, Puccinia oxalidis, Puccinia

pelargonii-zonalis, Puccinia pittieriana, Puccinia poarum, Puccinia purpurea, Puccinia recondita, Puccinia schedonnardii, Puccinia sessilis, Puccinia striiformis f. sp. hordei, Puccinia striiformis var. striiformis, Puccinia subnitens, Puccinia substriata var. indica, Puccinia verruca, Puccinia xanthii, Pucciniaceae, Pucciniastrum, Pucciniastrum americanum, Pucciniastrum arcticum, Pucciniastrum epilobii, Pucciniastrum hydrangeae, Pycnostysanus azaleae, Pyrenochaeta lycopersici, Pyrenochaeta terrestris, Pyrenopeziza brassicae, Pyrenophora, Pyrenophora avenae, Pyrenophora chaetomioides, Pyrenophora graminea, Pyrenophora seminiperda, Pyrenophora teres, Pyrenophora teres f. maculata, Pyrenophora teres f. teres, Pyrenophora tritici-repentis, Ramichloridium musae, Ramularia, Ramularia beticola, Ramularia coryli, Ramularia cyclaminicola, Ramularia macrospora, Ramularia menthicola, Ramularia necator, Ramularia primulae, Ramularia spinaciae, Ramularia subtilis, Ramularia tenella, Ramularia vallisumbrosae, Ramulispora sorghi, Ramulispora sorghicola, Raspberry leaf spot, Raspberry spur blight, Red thread disease, Rhinocladium corticola, Rhizoctonia, Rhizoctonia leguminicola, Rhizoctonia rubi, Rhizoctonia solani, Rhizophydium graminis, Rhizopus arrhizus, Rhizopus circinans, Rhizopus microsporus, Rhizopus oryzae, Rhizopus soft rot, Rhynchosporium, Rhynchosporium secalis, Rhytisma punctatum, Rhytisma vitis, Rigidoporus vinctus, Rosellinia arcuata, Rosellinia bunodes, Rosellinia necatrix, Rosellinia pepo, Saccharicola taiwanensis, Sarocladium oryzae, Sawadaea, Schiffnerula cannabis, Schizophyllum commune, Schizopora flavipora, Schizothyrium pomi, Sclerotinia borealis, Sclerotinia bulborum, Sclerotinia minor, Sclerotinia ricini, Sclerotinia sclerotiorum, Sclerotinia spermophila, Sclerotinia trifoliorum, Sclerotium, Sclerotium cinnamomi, Sclerotium delphinii, Scytinostroma galactinum, Seimatosporium, Seimatosporium mariae, Seimatosporium rhododendri, Selenophoma linicola, Septobasidium, Septobasidium bogoriense, Septobasidium euryae-groffii, Septobasidium gaoligongense, Septobasidium pilosum, Septobasidium polygoni, Septobasidium pseudopedicellatum, Septobasidium theae, Septocyta ruborum, Septoria, Septoria ampelina, Septoria azaleae, Septoria bataticola, Septoria campanulae, Septoria cannabis, Septoria cucurbitacearum, Septoria darrowii, Septoria dianthi, Septoria eumusae, Septoria glycines, Septoria helianthi, Septoria humuli, Septoria hydrangeae, Septoria lactucae, Septoria lycopersici, Septoria lycopersici var. malagutii, Septoria menthae, Septoria passerinii, Septoria pisi, Septoria rhododendri, Septoria secalis, Septoria selenophomoides, Setosphaeria rostrata, Setosphaeria turcica, Spencermartinsia pretoriensis, Sphaceloma, Sphaceloma arachidis, Sphaceloma menthae, Sphaceloma perseae, Sphaceloma poinsettiae, Sphaceloma sacchari, Sphaceloma theae, Sphacelotheca reiliana, Sphaerotheca castagnei, Sphaerulina oryzina, Sphaerulina rehmiana, Sphaerulina rubi,

Sphenospora kevorkianii, Spilocaea oleaginea, Sporisorium, Sporisorium cruentum, Sporisorium ehrenbergii, Sporisorium scitamineum, Sporisorium sorghi, Sporonema phacidioides, Stagonospora avenae f.sp. triticae, Stagonospora meliloti, Stagonospora recedens, Stagonospora sacchari, Stagonospora tainanensis, Stagonosporopsis, Stagonosporopsis trachelii, Stegocintractia junci, Stemphylium, Stemphylium alfalfae, Stemphylium bolickii, Stemphylium cannabinum, Stemphylium globuliferum, Stemphylium lycopersici, Stemphylium sarciniforme, Stemphylium solani, Stemphylium vesicarium, Stenella, Stenella anthuriicola, Stigmatomycosis, Stigmina, Stigmina carpophila, Stigmina palmivora, Stigmina platani-racemosae, Stromatinia cepivora, Sydowiella depressula, Sydowiellaceae, Synchytrium endobioticum, Tapesia acuformis, Tapesia yallundae, Taphrina coryli, Taphrina potentillae, Thanatephorus cucumeris, Thecaphora solani, Thielaviopsis, Thielaviopsis basicola, Thielaviopsis ceramica, Thyrostroma compactum, Tiarosporella urbis-rosarum, Tilletia barclayana, Tilletia caries, Tilletia controversa, Tilletia laevis, Tilletia tritici, Tilletia walkeri, Tilletiariaceae, Togniniaceae, Tomato leaf mold, Tranzschelia pruni-spinosae var. discolor, Trichoderma koningii, Trichoderma paucisporum, Trichoderma songyi, Trichoderma theobromicola, Trichoderma viride, Truncatella, Tubercularia lateritia, Tunstallia aculeata, Typhula blight, Typhula idahoensis, Typhula incarnata, Typhula ishikariensis, Typhula variabilis, Typhulochaeta, Ulocladium consortiale, Uncinula, Uredo behnickiana, Uredo kriegeriana, Uredo musae, Uredo nigropuncta, Uredo rangelii, Urocystis, Urocystis agropyri, Urocystis brassicae, Urocystis occulta, Uromyces, Uromyces apiosporus, Uromyces beticola, Uromyces ciceris-arietini, Uromyces dianthi, Uromyces euphorbiae, Uromyces graminis, Uromyces inconspicuus, Uromyces lineolatus subsp. nearcticus, Uromyces musae, Uromyces oblongus, Uromyces pisi-sativi, Uromyces proëminens var. poinsettiae, Uromyces medicaginis, Uromyces trifolii-repentis var. fallens, Uromyces viciae-fabae var. viciae-fabae, Urophlyctis leproides, Urophlyctis trifolii, Ustilaginales, Ustilaginoidea virens, Ustilaginomycetes, Ustilago, Ustilago avenae, Ustilago esculenta, Ustilago hordei, Ustilago maydis, Ustilago nigra, Ustilago nuda, Ustilago scitaminea, Ustilago tritici, Vankya ornithogali, Venturia, Venturia carpophila, Veronaea musae, Verticillium, Verticillium albo-atrum, Verticillium albo-atrum var. menthae, Verticillium alfalfae, Verticillium dahliae, Verticillium isaacii, Verticillium klebahnii, Verticillium longisporum, Verticillium nonalfalfae, Verticillium theobromae, Verticillium wilt, Verticillium zaregamsianum, Waitea circinata, Westea, Wuestneiopsis georgiana, Xenodidymella applanata, Xeromphalina fraxinophila, Zopfia rhizophila, Zygosaccharomyces bailii, Zygosaccharomyces florentinus, Zythiostroma, Xanthomonas campestris, Xanthomonas juglandis, Waitea, Websdanea, Wentiomyces, Whetzelinia, Whetzelinia sclero-

tiorum, Wilsonomyces, Wilsonomyces carpophilus, Woronichina, Xanthomonas, Xanthomonas axonopodis, Typhula, Uleiella, Uleodothis, Ulocladium, Uncinula macrospora, Uncinula necator, Uncinula parvula, Uncinuliella, Uncinuliella flexuosa, Uncol, Undifilum, Uraecium, Uredendo, Uredinopsis, Uredo, Uredo cajani, Uredo glumarum, Uredopeltis, Uredostilbe, Urocystis cepulae, Urocystis colchici, Urocystis magica, Uromyces appendiculatus, Uromyces betae, Uromyces cicerisarietini, Uromyces craccae, Uromyces dactylidis, Uromyces fabae, Uromyces junci, Uromyces lineolatus, Uromyces proeminens, Uromyces scirpi, Uromyces striatus, Uromyces terebinthi, Uromyces trifolii-repentis, Uromyces trifoliirepentis, Uromyces viciae-fabae, Uromyces viciaefabae, Uromycladium, Urophlyctis, Urophlyctis alfalfae, Uropyxis, Ursicollum, Urupe, Ustacystis, Ustanciosporium, Ustilaginoidea, Ustilago kolleri, Ustilago maydis-maize, Ustilago maydismaize, Ustilago segetum, Ustilago striiformis, Ustilago violacea, Ustilago zeae, Ustilentyloma, Ustulina deusta, Ustulina zonata, Utrechtiana, Valdensia, Valdensinia, Valsa, Valsa abietis, Valsa ambiens, Valsa auerswaldii, Valsa ceratosperma, Valsa sordida, Valsella, Valseutypella, Vankya, Venturia compacta, Venturia inaequalis, Venturia pirina, Vermicularia, Vermicularia circinans, Veronaea, Verrucobotrys, Verticillium alboatrum, Verticillium lecanii, Vialaea, Viegasella, Virgella, Viscum, Viscum album, Vitreostroma, Vladracula, Volkartia, Volutella, Volutella colletotrichoides, Volvocisporium, Soleella, Solutoparies, Sonderhenia, Sorataea, Sorosporium, Spaceloma, Spaceloma glycines, Sparassiella, Sparassis, Sparrasis, Sparrasis crispa, Sparrowia, Spathulospora, Spencermartinsia, Spermospora, Spermospora ciliata, Spermospora lolii, Spermospora poagena, Spermosporina, Sphacelia segetum, Sphacelia sorghi, Sphaceloma ampelinum, Sphaceloma batatas, Sphaceloma coryli, Sphaceloma fawcettii, Sphaceloma mangiferae, Sphaceloma manihoticola, Sphaceloma necator, Sphaceloma pirinum, Sphaceloma randii, Sphaceloma rosarum, Sphacelotheca, Sphacelotheca cruenta, Sphacelotheca sorghi, Sphaerella, Sphaerella erysiphina, Sphaerella platanifolia, Sphaerella punctiformis, Sphaeria erysiphina, Sphaerodothella, Sphaerodothis, Sphaerodothis acrocomiola, Sphaerophragmium, Sphaeropsis, Sphaeropsis malorum, Sphaeropsis tumefaciens, Sphaeropsis ulmicola, Sphaerostilbe, Sphaerostilbe repens, Sphaerotheca, Sphaerotheca fuliginea, Sphaerotheca fusca, Sphaerotheca humuli, Sphaerotheca macularis, Sphaerotheca pannosa, Sphaerotheca xanthii, Sphaerulina maydis, Sphenospora, Spilocaea, Spilocaea pomi, Spilodochium, Spilosticta, Spiniger meineckellum, Spizellomyces, Splanchonema, Splanchonema platan, Spongospora, Spongospora subterranea, Sporendocladia, Sporisorium holci-sorghi, Sporisorium holcisorghi, Sporomega, Sporonema oxycocci, Sporophlyctidium, Sporophlyctis, Sporotrichum, Sporotrichum versisporum, Spumula, Stagonospora, Stagonospora arenaria, Stagonospora ave-

nae, Stagonospora nodorum, Stagonospora paspali, Stagonospora taiwanensis, Stagonosporopsis hortensis, Stakmania, Steccherinum ochraceum, Stegocintractia, Stegophora ulmea, Stellosetifera, Stemphyliopsis, Stemphylium botryosum, Stemphylium consortiale, Stemphylium floridanum, Stemphylium herbarum, Stemphylium radicinum, Stemphylium ramulosa, Stenella citri-grisea, Stenella citrigrisea, Stenocarpella, Stenocarpella macrospora, Stenocarpella maydis, Stephanotheca, Stereostratum, Stigmatula, Stigmella, Stigmella platani, Stigmella platani-racemosae, Stigmella plataniracemosae, Stigmina compacta, Stigmina liquidambaris, Stigmina mangiferae, Stigmina platani, Stigmina plataniracemosae, Stigmochora, Stilbum flavidum, Stollia, Stomatogene, Strasseria, Strasseria geniculata, Strasseria oxycocci, Strelitziana, Streptobotrys, Streptomyces, Streptomyces ipomoeae, Streptopodium, Streptotinia, Striatoidium, Stromaster, Stromatinia, Stylina, Sydowia polyspora, Symphaeophyma, Sympodiomycopsis, Synchronoblastia, Synchronoblastia crypta, Synchytrium, Synchytrium aureum, Synchytrium fragariae, Synchytrium liquidambaris, Synchytrium macrosporum, Synchytrium phaseoli-radiati, Synchytrium phaseoliradiati, Synchytrium umbilicatum, Synchytrium vaccinii, Syspastospora parasitica, Takamatsuella, Talbotiomyces, Tapesia, Taphridium, Taphrina, Taphrina deformans, Taphrina wiesneri, Telimena, Telimenella, Telimenochora, Telomapea, Teratosphaeria, Teretispora, Terriera, Testicularia, Thallomyces, Thanatephorus, Thecaphora, Thecaspora, Thekopsora, Thekopsora minima, Thermomyces, Thermomyces lanuginosus, Therrya, Thielaviopisis, Thielaviopisis paradoxa, Thielaviopsis paradoxa, Thirumalachariella, Thoreauomyces, Thrauste, Thyrospora, Thyrospora lycopersici, Thyrostroma, Ticomyces, Tilletia, Tilletia foetida, Tilletia indica, Tilletiacaries, Tilletiacaries foetida, Tilletiacaries laevis, Tilletiacaries tritici, Tilletiaria, Tilletiaria anomala, Tilletiella, Togninia, Tolyposporella, Tolyposporium, Tomophagus, Toroa, Torula, Torula caligans, Torula herbarum, Tothiella, Trachyspora, Tracya, Trames, Trames hirsuta, Tranzschelia, Tranzschelia discolor, Tranzschelia pruni-spinosae, Tranzschelia prunispinosae, Tranzscheliella, Tremateia, Tretospora, Triblidiopsis, Trichocintractia, Trichoderma harzianum, Trichoderma lignorum, Trichoderma longibrachiatum, Trichodothella, Trichodothis, Trichopsora, Trichothecium, Trichothecium roseum, Tridens, Triodiomyces, Triparticalcar, Triphragmiopsis, Triphragmium, Tripospermum acerinum, Tripospora, Truncatella angustata, Tryblidiopsis, Tryblidiopycnis, Tubakia, Tuberculariа, Tuberculariа ulmea, Tuberculariа vulgaris, Tuberculina, Tubeufia pezizula, Tubisorus, Tubakia dryina, Tuburcinia, Tunicopsora, Langdonia, Laocoön, Lasiobotrys, Lasiodiplodia, Lasiodiplodia theoboomae, Lasiodiplodia theobromae, Lasiodiplodia triflorae, Lasiostemma, Latruncellus, Laurilia, Laurobasidium, Laxitectum, Laxitectum bicolor, Laxitectum

sulcata, Leandria, Lecanosticta, Lectera, Leptascospora, Lepteutypa, Lepteutypa cupressi, Leptodiscus, Leptodiscus terrestris, Leptographium, Leptographium wageneri, Leptoguignardia, Leptomeliola, Leptoperidia, Leptosphaeria, Leptosphaeria herpotrichoides, Leptosphaeria korrae, Leptosphaeria maydis, Leptosphaeria microscopica, Leptosphaeria narmari, Leptosphaeria nodorum, Leptosphaeria obtusispora, Leptosphaeria taiwanensis, Leptosphaerulina australis, Leptostroma, Leptothyrium, Leptothyrium nerviseda, Leptothyrium theae, Leptothyrium zeae, Leptotrochila, Leucocintractia, Leucocytospora, Leucocytospora cincta, Leucodiaporthe, Leucostoma, Leucostoma cincta, Leucostoma nivea, Leucotelium, Leveillula, Leveillula compositarum, Leveillula leguminosarum, Lewia, Libertella, Libertella blepharis, Ligniera, Ligniera pilorum, Limacinula, Limonomyces, Limonomyces roseipellis, Lineostroma, Linochora, Lipocystis, Liroa, Lirula, Lixa, Lixa grande, Lixa pequena, Lizonia, Loborhiza, Lohwagia, Lophodermella, Lophodermium hypophyllum, Lophodermium oxycocci, Lophomerum, Lophophacidium, Loratospora, Lundquistia, Macabuna, Macalpinomyces, Macrochytrium, Macroderma, Macrodiplodiopsis, Macrodiplodiopsis dasmazieresii, Macrophoma, Macrophoma zeae, Macrophomina, Macrophomina phaseoli, Macrophomina phaseolina, Macrophomopsis, Macrospora, Macrosporium, Macruropyxis, Magnaporthe oryzae, Magnaporthe poae, Malthomyces, Malupa, Mamianiella, Maramiellus, Maramiellus scandems, Marasasiomyces, Marasmiellus inoderma, Marasmius crinisequi, Marasmius equicrinus, Marasmius perniciosus, Marasmius sheath, Maravalia, Marchalia, Mariannaea elegans, Marielliottia, Marielliottia triseptata, Marssonina, Marssonina coronaria, Marssonina fragariae, Marssonina juglandis, Marssonina panattoniana, Marssonina potentillae, Marssonina rosae, Marthamyces, Martininia, Massaria platan, Massariella, Mastigosporium, Mastigosporium kitzebergense, Mastigosporium rubricosum, Mauginiella, Mazzantia, Medeolaria, Medusosphaera, Megachytrium, Mehtamyces, Melampsora lini, Melampsora medusae, Melampsorella, Melampsoridium, Melanconis, Melanconis chartusiana, Melanconium, Melanconium fuligineum, Melanconium sacchari, Melaniella, Melanodothis, Melanops, Melanopsichium, Melanospora cannabis, Melanospora parasitica, Melanospora zamiae, Melanotaenium, Melanotus phillipsii, Melanoxa, Melanustilospora, Melasmia, Meliola camelliae, Meloderma, Meloidogyne, Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica, Meria, Meripilus, Meruliopsis ambiguus, Mesochytrium, Metacoleroa, Metasteridium, Microascus cinereus, Microbotryum, Microcyclus, Microdiplodia, Microdochium, Microdochium nivale, Microdochium oryzae, Microdochium tabacinum, Microgloeum, Microidium, Micromyces, Micronectriella, Micronectriella nivalis, Microsphaera alni, Microsphaera extensa, Microsphaera penicillata, Microstroma, Microthia, Mic-

ularia, Microxyphium, Mikronegeria, Milesia, Milesina, Mimema, Mintera, Mitteriella, Miyagia, Moesziomyces, Moesziomyces penicillariae, Molleriella, Mollisia, Mollisia cinerea, Monascus purpureus, Monascus ruber, Monilia, Monilia fructigena, Monilinia, Monilinia mali, Moniliophthora, Moniliophthora perniciosa, Monochaetia, Monographella, Monographella nivale, Monographella nivalis, Monosporascus, Monosporidium, Monostichella, Montagnellina, Moreaua, Morispora, Moserella, Moutoniella, Mucoharknessia, Mucor hiematis, Mucor paronychia, Muelleromyces, Mundkurella, Munkiella, Muribasidiospora, Muscinupta, Musicillium, Mycaureola, Mycena, Mycena citricola, Mycocentrospora, Mycocentrospora cantuariensis, Mycocoscoma, Mycohypallage, Mycoleptodiscus, Mycopappus, Mycosphaerella, Mycosphaerella allii-cepae, Mycosphaerella alliicepae, Mycosphaerella angulata, Mycosphaerella arachidicola, Mycosphaerella capsellae, Mycosphaerella carinthiaca, Mycosphaerella citri, Mycosphaerella dianthi, Mycosphaerella erysiphina, Mycosphaerella fijiensis, Mycosphaerella fragariae, Mycosphaerella melonis, Mycosphaerella nigromaculans, Mycosphaerella personata, Mycosphaerella pinodes, Mycosphaerella platanicola, Mycosphaerella rabiei, Mycosphaerella sentina, Mycosphaerella stigminaplatani, Mycosphaerella tassiana, Mycosphaerella usoenskajae, Mycosphaerella zeae-maydis, Mycosphaerella zeaemaydis, Mycosyrinx, Mycovellosiella, Mycovellosiella biformis, Mycovellosiella cajani, Mycovellosiella phaseoli, Myrioconium, Myriophacidium, Myriosclerotinia, Myriosclerotinia borealis, Myrothecium gramineum, Naevia, Naevia oxycocci, Naiadella, Nakataea, Nakataea sigmoidae, Nannfeldtiomyces, Naohidemyces, Napicladium, Narasimhania, Nattrassia, Nattrassia mangiferae, Nectria, Nectria coccinea, Nectria galligena, Nectria haematococca, Nectria mammoidea, Nectria mauritiicola, Nectria ochroleuca, Nectria rigidiuscula, Nectricladiella, Nemataspora, Nemataspora coryli, Nematococcomyces, Nematospora, Nematospora gossypii, Nematostigma, Nematostoma, Nematothecium, Neococcomyces, Neocoleroa, Neodeightonia, Neofabrae, Neofabrae perennans, Neofabraea, Neofusicoccum, Neofusicoccum mangiferae, Neokarlingia, Neonectria, Neoparodia, Neoscytalidium, Neotyphodium coenophialum, Neotyphodium lolii, Neotyphodium typhinum, Neovossia, Neovossia horrida, Neovossia indica, Nervostroma, Neurospora crassa, Newinia, Nigrospora oryzae, Nimbya, Nothoravenelia, Nothorhytisma, Nothostrasseria, Nowakowskia, Nowakowskiella, Nummularia discreta, Nymanomyces, Nyssopsora, Obelidium, Oberwinkleria, Ochrocladosporium elatum, Ochropsora, Octagoidium, Oculimacula, Oidiopsis, Oidiopsis sicula, Oidiopsis taurica, Oidium asteris-punicei, Oidium asterispunicei, Oidium balsamii, Oidium caricae, Oidium ericinum, Oidium indica, Oidium latisporum, Oidium leucoconium, Oidium monilioides, Oidium passerinii, Oidium tingitaninum, Oidium tuckeri, Olivea, Olpidium, Omphalia flavida,

Omphalia pigmentata, Oncobasidium, Oncobasidium theobromae, Operculella, Operculella padwickii, Ophiobolus anguillidus, Ophiociliomyces, Ophiodiaporthe, Ophiodothella, Ophiomeliola, Ophioparodia, Ophiosphaerella agrostis, Ophiosphaerella herpotricha, Ophiosphaerella korrae, Ophiosphaerella narmari, Ophiostoma, Ophiostoma novo-ulmi, Ophiostoma novoulmi, Ophiostoma piliferum, Ophiostoma pluriannulatum, Ophiostoma ulmi, Orphanomyces, Orphnodactylis, Orphnodactylus, Oswaldina, Otthia, Ovularia, Ovularia pulchella, Ovularia pusilla, Ovulariopsis, Ovulariopsis ellipsospora, Ovulariopsis papayae, Ovulinia, Ovulitis, Oxodeora, Oxycoccus, Oxycoccus macrocarpus, Ozonium omnivorum, Ozonium texanum, Pachypatella, Paecilomyces fulvus, Paepalopsis, Palawaniella, Paracercospora fijiensis, Paradendryphiella, Parapenidiella, Paraphaeosphaeria michotii, Paraphaeosphaeria obtusispora, Paraphysoderma, Parasteridium, Parastigmatea, Parauncinula, Paravalsa, Parberya, Parenglerula, Parmulariopsella, Parmulariopsis, Parmulina, Parodiella, Parodiellina, Parvacoccum, Parvulago, Passalora, Patellaria theae, Patellina, Patellina fragariae, Pellicularia, Pellicularia filamentosa, Pellicularia koleroga, Pellicularia rolfsii, Pellicularia sasakii, Peltaster, Penicillium aurantiogriseum, Penicillium chrysogenum, Penicillium citrinum, Penicillium corymbiferum, Penicillium cyclopium, Penicillium digitatum, Penicillium frequentans, Penicillium glabrum, Penicillium hirsutum, Penicillium italicum, Penicillium oxalicum, Penicillium purpurogenum, Penicillium ulaiense, Penidiella, Peniophora cinerea, Peniophora sacrata, Pericladium, Periconia, Periconia byssoides, Periconia digitata, Periconia hispidula, Periconia prolifica, Periconiella, Periconiella musae, Peridermium, Peridiopsora, Peridipes, Perischizon, Perisporiopsis, Peroneutypa, Peronoplasmopara, Peronoplasmopara humuli, Pesotum, Pesotum ulmi, Pestalopezia, Pestalosphaeria, Pestalotia adusta, Pestalotia guepini, Pestalotia laurocerasi, Pestalotia longisetula, Pestalotia mangiferae, Pestalotia palmarum, Pestalotia sydowiana, Pestalotia theae, Pestalotia vaccinii, Pestalotia versicolor, Petersonia, Pezicula, Pezicula malicorticis, Pezicula malicorticus, Phacellium, Phacidiella, Phacidiella coniferarum, Phacidium, Phacidium abietis, Phacidium coniferarum, Phacidium infestans, Phacidium infestans var. abietis, Phacidium lunatum, Phaeoacremonium, Phaeobotryon, Phaeobotryosphaeria, Phaeochora, Phaeochorella, Phaeochoropsis, Phaeocryptopus, Phaeocryptopus gaeumannii, Phaeocytosporella, Phaeocytosporella zeae, Phaeocytostroma, Phaeocytostroma ambiguum, Phaeodimeriella, Phaeoisariopsis, Phaeoisariopsis griseola, Phaeoisariopsis personata, Phaeomoniella, Phaeophleospora, Phaeoramularia, Phaeoramularia angolensis, Phaeoramularia dissiliens, Phaeoramularia fusimaculans, Phaeosclerotinia, Phaeosphaerella, Phaeosphaeria avenaria, Phaeosphaeria maydis, Phaeostigme, Phaeotrichoconis, Phakopsora, Phakopsora meibomiae,

Phellinus, Phialophora malorum, Phialophoropsis, Phloeospora, Phloeospora azaleae, Phloeospora humuli, Phloeospora multimaculans, Phloeospora pistaciae, Phloeosporella, Phlogicylindrium, Phlyctaena, Phlyctema, Phlyctochytrium, Phlyctorhiza, Phoma aliena, Phoma andigena, Phoma apiicola, Phoma arachidicola, Phoma betae, Phoma cajani, Phoma caricae-papayae, Phoma caricaepapayae, Phoma chrysanthemi, Phoma chrysanthemicola, Phoma conidiogena, Phoma costarricensis, Phoma diversispora, Phoma foveata, Phoma lingam, Phoma lycopersici, Phoma macdonaldii, Phoma medicaginis, Phoma meliloti, Phoma oleracea, Phoma oncidiisphacelati, Phoma pinodella, Phoma rostrupii, Phoma solanicola, Phoma sorghina, Phoma subglomerata, Phoma theicola, Phoma tracheiphila, Phoma trifolii, Phoma tropica, Phomopsis achilleae, Phomopsis amygdali, Phomopsis anacardii, Phomopsis arnoldiae, Phomopsis caricae-papayae, Phomopsis caricaepapayae, Phomopsis casuarinae, Phomopsis citri, Phomopsis cucurbitae, Phomopsis elaeagni, Phomopsis helianthi, Phomopsis ipomeae-batatas, Phomopsis ipomeaebatatas, Phomopsis leptostromiformis, Phomopsis ligustri-vulgaris, Phomopsis lokoyae, Phomopsis mali, Phomopsis manilkarae, Phomopsis oblonga, Phomopsis obscurans, Phomopsis orchidophila, Phomopsis perseae, Phomopsis phaseoli, Phomopsis pseudotsugae, Phomopsis scabra, Phomopsis sclerotiodes, Phomopsis sojae, Phomopsis tanakae, Phomopsis vaccinii, Phomopsis viticola, Phragmeriella, Phragmidiella, Phragmidium imitans, Phragmidium potentillae, Phragmidium rosaepimpinellifoliae, Phragmidium rubiidaei, Phragmogibbera, Phragmopyxis, Phragmotaenium, Phragmotelium, Phyllachora, Phyllachora cannabidis, Phyllachora cynodontis, Phyllachora dactylidis, Phyllachora graminis, Phyllachora maydis, Phyllachora minutissima, Phyllachora pennisetina, Phyllachora sylvatica, Phyllachora torrendiella, Phyllactinia angulata, Phyllactinia corylea, Phyllactinia guttata, Phyllactinia rhododendri, Phyllactinia suffulta, Phylleutypa, Phyllocrea, Phyllosticta alliariifoliae, Phyllosticta allii, Phyllosticta ampelicida, Phyllosticta anacardiacearum, Phyllosticta arachidishypogaea, Phyllosticta arachidishypogaea, Phyllosticta bractearum, Phyllosticta brassicae, Phyllosticta brassicina, Phyllosticta cajani, Phyllosticta caricae-papayae, Phyllosticta caricaepapayae, Phyllosticta circumscissa, Phyllosticta citricarpa, Phyllosticta colocasiicola, Phyllosticta cunninghamii, Phyllosticta cyclaminicola, Phyllosticta cyclaminis, Phyllosticta deckdua, Phyllosticta draconis, Phyllosticta dulcamarae, Phyllosticta dusana, Phyllosticta elongata, Phyllosticta humuli, Phyllosticta hydrangeae, Phyllosticta lupulina, Phyllosticta manihotae, Phyllosticta manihoticola, Phyllosticta maydis, Phyllosticta musarum, Phyllosticta nicotiana, Phyllosticta phaseolina, Phyllosticta platani, Phyllosticta rhododendri, Phyllosticta sapotae, Phyllosticta sojicola, Phyllosticta solitaria, Phyllosticta theifolia, Phyllosticta vaccinii, Phymatotrichopsis, Phymatotrichopsis om-

nivorum, Phymatotrichum, Phymatotrichum omnivorum, Physalospora abdita, Physalospora fusca, Physalospora glandicola, Physalospora malorum, Physalospora obtusa, Physalospora piricola, Physalospora rhodina, Physalospora tucumanesis, Physalospora vaccinii, Physalospora zeae, Physalospora zeicola, Physalosporina, Physarum, Physarum cinereum, Physocladia, Physoderma, Physoderma graminis, Physoderma maydis, Physoderma paspali, Physonema, Physopella, Physopella pallescens, Physopella zeae, Physorhizophidium, Pichia membranifaciens, Pichia subpelliculosa, Piggotia, Piggotia coryli, Pileolaria, Pilgeriella, Pilidiella, Pilidiella quercicola, Pilidium, Pilocintractia, Piptarthron, Piptarthron macrosporum, Piptoporus, Piricularia, Pithomyces, Pithomyces maydicus, Pithya, Placomelan, Planetella, Planistroma, Planistromella, Plasmodiophora, Plasmodiophora brassicae, Platycarpa, Platychora, Platyspora, Platyspora pentamera, Platysporoides, Plectosphaera, Plectosphaerella, Plectosporium, Plectosporium tabacinum, Plenodomas, Plenodomas destruens, Pleocyta, Pleocyta sacchari, Pleomeliola, Pleomerium, Pleospora, Pleospora allii, Pleurobotrya, Pleurotus dryinus, Pleurotus ostreatus, Ploioderma, Podisoma, Podochytrium, Pododimeria, Podosphaera clandestina, Podosphaera tridactyla, Podosphaera xanthii, Polioma, Poliomopsis, Pollaccia, Polychytrium, Polycyclina, Polycyclus, Polyphagus, Polyphlyctis, Polyporopsis, Polyrhizon, Polyscytalum, Polystigma, Polystigma ochraceum, Polystigmina, Polystomella, Polythrincium trifolii, Pomatomyces, Porodisculus, Porotenus, Portalia, Potebniamyces pyri, Potriphila, Powellomyces, Prataprajella, Pratylenchus, Pratylenchus brachyurus, Pratylenchus coffeae, Pratylenchus goodeyi, Pratylenchus reniformia, Proliferobasidium, Propolis, Prosopidicola, Prospodium, Protomyces, Protomyces inouyei, Protomycopsis, Protoscypha, Protothyrium, Protoventuria, Protoventuria myrtilli, Pseudobotrytis, Pseudobotrytis terrestris, Pseudocercosoporella, Pseudocercosoporella herpotrichoides, Pseudocercospora calospilea, Pseudocercospora cruenta, Pseudocercospora fusco-virens, Pseudocercospora fuscovirens, Pseudocercospora handelii, Pseudocercospora mali, Pseudocercospora musae, Pseudocercospora punicae, Pseudocercospora purpurea, Pseudocercospora rubi, Pseudocercospora subsessilis, Pseudocercospora tamonae, Pseudocercosporella, Pseudocercosporella albida, Pseudocercosporella caryigena, Pseudocercosporella herpotrichoides, Pseudocercosporidium, Pseudociboria, Pseudocladosporium, Pseudocochliobolus, Pseudocochliobolus pallescens, Pseudodermatosorus, Pseudodoassansia, Pseudoepicoccum, Pseudofabraea, Pseudofusicoccum, Pseudoidium, Pseudolembosia, Pseudolizonia, Pseudomeliola, Pseudomonas, Pseudomonas cichorii, Pseudomonas solanacearum, Pseudoparodia, Pseudoparodiella, Pseudoperonospora, Pseudoperonospora cannabina, Pseudoperonospora cubensis, Pseudoperonospora humuli, Pseudopezicula, Pseudopezicula tetraspora, Pseudopez-

icula tracheiphila, Pseudopeziza, Pseudopeziza tracheiphila, Pseudophaeolus, Pseudophaeolus baudonii, Pseudopileum, Pseudoplagiostoma, Pseudorhytisma, Pseudoseptoria, Pseudoseptoria everhartii, Pseudoseptoria stomaticola, Pseudothiella, Pseudothiopsella, Pseudotracya, Pseudoyuconia, Ptechetelium, Pterosporidium, Puccinia allii, Puccinia brachypodii, Puccinia calcitrapae, Puccinia cynodontis, Puccinia extensicola, Puccinia graminis, Puccinia horiana, Puccinia pelargoniizonalis, Puccinia polysora, Puccinia schedonnardi, Puccinia scleriae, Puccinia scleriicola, Puccinia sorghi, Puccinia stenotaphricola, Puccinia striiformis, Puccinia substriata, Puccinia tanaceti, Puccinia tritici-duri, Puccinia triticiduri, Puccinia triticina, Puccinia zoysiae, Pucciniastrum coryli, Pucciniastrum myrtilli, Pucciniastrum pustulatum, Pucciniastrum vaccinii, Pucciniosira, Pucciniostele, Pureke, Pycnochytrium, Pycnopeziza, Pyrenobotrys, Pyrenobotrys compacta, Pyrenochaeta glycines, Pyrenopeziza, Pyrenopeziza medicaginis, Pyrenophora dictyoides, Pyrenophora lolii, Pyrenophora phaeocomes, Pyrenophora semeniperda, Pyrenophora trichostoma, Pyrenophora triticirepentis, Pyricularia, Pyricularia grisea, Pyricularia oryzae, Pyriculariopsis, Pyriculariopsis parasitica, Quambalaria, Quasiphloeospora, Quaternaria, Questieriella, Raciborskiomyces, Racospermyces, Radopholus, Radopholus similis, Radulum, Ragnhildiana, Ramakrishnania, Ramichloridium, Ramichloridium apiculatum, Ramichloridium biverticillatum, Ramularia brunnea, Ramularia coryi, Ramularia gossypii, Ramularia nigromaculans, Ramularia phaseoli, Ramularia pulchella, Ramularia pusilla, Ramulaspera, Ramulaspera holci-lanati, Ramulaspera holcilanati, Ramulispora, Ramulispora acuformis, Ramulispora herpotrichoides, Ravenelia, Readeriella, Redheadia, Rehmiodothis, Resinicium bicolor, Restiosporium, Retroa, Retrostium, Rhabdocline, Rhabdocline pseudotsugae, Rhabdocline weirii, Rhabdogloeum, Rhabdospora ramealis, Rhagadolobium, Rhamphospora, Rhexocercosporidium, Rhinocladium, Rhinocladium corticum, Rhipidocarpon, Rhizidium, Rhizina undulata, Rhizoclosmatium, Rhizoctonia bataticola, Rhizoctonia carotae, Rhizoctonia cerealis, Rhizoctonia fragariae, Rhizoctonia microsclerotia, Rhizoctonia oryzae, Rhizoctonia oryzae-sativae, Rhizoctonia oryzaesativae, Rhizoctonia ramicola, Rhizoctonia zeae, Rhizogene, Rhizomorpha subcorticalis, Rhizophydium, Rhizopus, Rhizopus circinana, Rhizopus nigricans, Rhizopus nodosus, Rhizopus stolonifer, Rhizopycnis, Rhizopycnis vagum, Rhizosphaera, Rhizotexis, Rhodosticta, Rhodotorula hordea, Rhombiella, Rhopalophlyctis, Rhopographus, Rhopographus zeae, Rhynchosporium orthosporum, Rhynchosporium oryzae, Rhytidenglerula, Rhytidhysteron rufulum, Rhytisma, Rickenella, Rikatlia, Roesleria, Roesleria hypogaea, Roesleria subterranea, Roeslerina, Roestelia, Romellia, Rosellina, Rosellina necatrix, Rosellinia subiculata, Rosenscheldiella, Roseodiscus, Rostraureum, Rostronitschkia, Rostrupia,

Saccardinula, Saccharata, Saccomyces, Saccopodium, Sakireeta, Salmacisia, Salmonia, Salmonia malachrae, Sarcinella, Sarea resinae, Savulescuella, Sawadaia, Scalarispora, Scherffeliomyces, Scherffeliomycopsis, Schiffnerula, Schizochora, Schizonella, Schizoparme, Schizoparme straminea, Schizothyrium perexiguum, Schizoxylon microsporum, Schroeteriaster, Scirrhia, Scleroconidioma, Sclerocrana, Sclerophoma eustomis, Sclerophoma pythiophila, Sclerophoma semenospora, Sclerospora, Sclerospora graminicola, Sclerospora macrospora, Sclerospora maydis, Sclerospora miscanthi, Sclerospora philippinensis, Sclerospora sacchari, Sclerospora sorghi, Sclerospora spontanea, Sclerotinia, Sclerotinia homoeocarpa, Sclerotinia libertiana, Sclerotinia oxycocci, Sclerotinia rolfsii, Sclerotium bataticola, Sclerotium cepivorum, Sclerotium cinnomomi, Sclerotium hydrophilum, Sclerotium oryzae, Sclerotium rhizodes, Sclerotium rolfsii, Scolicotrichum graminis, Scolionema, Scopulariopsis brevicaulis, Scopulariopsis brumptii, Scutelliformis, Seaverinia, Seimatosporium lichenicola, Seiridium, Seiridium unicorne, Selenophoma donacis, Selenophoma everhartii, Septochytrium, Septocyta, Septogloeum, Septogloeum potentillae, Septoidium, Septoria aciculosa, Septoria agropyrina, Septoria anacardii, Septoria apicola, Septoria avenae, Septoria calamagrostidis, Septoria cannabina, Septoria caryae, Septoria chrysanthemi, Septoria citri, Septoria cynodontis, Septoria divergens, Septoria elymi, Septoria fragariae, Septoria fragariaecola, Septoria fructigena, Septoria humulina, Septoria linicola, Septoria liquidambaris, Septoria loligena, Septoria lupulina, Septoria macropoda, Septoria macrospora, Septoria nodorum, Septoria ostryae, Septoria oudemansii, Septoria passerinni, Septoria passiflorae, Septoria passifloricola, Septoria pistaciae, Septoria pistaciarum, Septoria pistacina, Septoria platanifolia, Septoria pyricola, Septoria rosae, Septoria rubi, Septoria tenella, Septoria theae, Septoria triseti, Septoria tritici, Septoria zeae, Septoria zeicola, Septoria zeina, Septosperma, Septotinia, Septotis, Serenomyces, Setameliola, Setoidium, Setosphaeria, Setosphaeria pedicellata, Setosphaeria prolata, Shivasia, Siphonaria, Sirosporium, Sirosporium diffusum, Sivanesania, Sivanesaniella, Skierka, Alysidiella, Acantharia, Acanthorhynchus, Acanthorhynchus vaccinii, Acarosporium, Acerviclypeatus, Achlya, Achlya conspicua, Achlya klebsiana, Achroomyces, Achrotelium, Acidomyces, Acremoniella, Acremonium, Acremonium crotocinigenum, Acremonium strictum, Acrocylindrium, Acrocylindrium oryzae, Acrodontium, Acrophialophora, Acrosporium, Adisciso, Aecidiconium, Aecidiolum, Aecidium clematidis, Aeciure, Aizoago, Aldona, Aldonata, Alina, Allantophomoides, Allantophomopsiella, Allantophomopsiella pseudotsugae, Allantophomopsis, Allantophomopsis cytisporea, Allantophomopsis lycopodina, Allantophomopsis pseudotsugae, Allantoporthe, Allewia, Allodus, Allotelium, Alternaria arachidis, Alternaria citri, Alternaria cucumerina, Alternaria

gerberae, Alternaria humuli, Alternaria limicola, Alternaria longipes, Alternaria macrospora, Alternaria mali, Alternaria passiflorae, Alternaria porri, Alternaria protenta, Alternaria ramulosa, Alternaria sonchi, Alternaria tenuis, Alternaria tomato, Alternariaster, Alveolaria, Amauroderma, Ambrosiella, Amerosporium, Amoebochytrium, Amphicypellus, Amphilogia, Amphobotrys, Angiopsora, Angiopsora zeae, Angiosorus, Angiosorus solani, Anguillosporella, Anisogramma, Anisogramma anomala, Anisomyces, Anomalomyces, Antennaria, Antennularia, Antherospora, Anthomycetella, Anthostoma, Anthracocystis, Anthracoidea, Anthrinium, Anthrinium sacchari, Anungitea, Anungitopsis, Aphanostigme, Apiognomonia veneta, Apioporthe, Apioporthella, Apiosphaeria, Apiospora, Apiosporina, Apiosporina morbosa, Aplopsora, Aplosporella, Apodothina, Aporhytisma, Aporia, Apostrasseria lunata, Apra, Arcticomyces, Arkaya, Arkoola, Arkoola nigra, Armatella, Armillaria bulbosa, Armillaria fuscipes, Armillaria luteobubalina, Armillaria mellea, Armillaria ostoyae, Armillaria solidipes, Armillariella, Armillariella mellea, Armillariella tabescens, Arthuria, Arthuriomyces, Ascocalyx, Ascochta, Ascochta tarda, Ascochyta, Ascochyta adzamethica, Ascochyta avenae, Ascochyta bohemica, Ascochyta boltshauseri, Ascochyta carica, Ascochyta caricae-papayae, Ascochyta caricaepapayae, Ascochyta chrysanthemi, Ascochyta desmazieresii, Ascochyta gossypii, Ascochyta hordeicola, Ascochyta humuliphila, Ascochyta ischaemi, Ascochyta lentis, Ascochyta leptospora, Ascochyta maydis, Ascochyta paspali, Ascochyta phaseolorum, Ascochyta phleina, Ascochyta pinodella, Ascochyta pinodes, Ascochyta poagena, Ascochyta rabiei, Ascochyta zeicola, Ascocoma, Ascospora, Ashbya, Ashbya gossypi, Ashbya gossypii, Aspergillus flavus, Aspergillus glaucus, Aspergillus parasiticus, Asperisporium minutulum, Asteridiella perseae, Asterocalyx, Asteroma, Asteroma coryli, Asteroma inconspicuum, Asteroma ulmeum, Asteromella, Asteromella brassica, Asteromella brassicae, Asterula, Atelocauda, Atopospora, Auerswaldiella, Aulacostroma, Aurantiosporium, Aurapex, Aureobasidium bolleyi, Aureobasidium caulivorum, Aureobasidium lini, Aureobasidium zeae, Aurifilum, Austrobasidium, Baeodromus, Bagcheea, Bagnisiopsis, Balladyna, Balladynocallia, Balladynopsis, Barriopsis, Basavamyces, Batcheloromyces, Batistopsora, Bauerago, Beelia, Beniowskia, Bibulocystis, Bicornispora, Bifusella, Bifusepta, Bipolaris australiensis, Bipolaris buchloes, Bipolaris cynodontis, Bipolaris hawaiiensis, Bipolaris maydis, Bipolaris micropus, Bipolaris oryzae, Bipolaris papendorfii, Bipolaris ravenelii, Bipolaris setaria, Bipolaris setariae, Bipolaris sorokiniana, Bipolaris spicifera, Bipolaris stenospila, Bipolaris victoriae, Bipolaris zeicola, Bisbyopeltis, Bitrimonospora, Bitrimonospora indica, Bivallum, Blastospora, Blumeria, Blumeriella, Blumeriella jaapii, Blyttiomyces, Boeremia, Boeremia exigua, Borde, Borde blanco, Bothrodiscus, Botryobambusa, Botry-

oconis, Botryodiplodia, Botryodiplodia gossypii, Botryodiplodia hydrodermia, Botryodiplodia phaseoli, Botryodiplodia quercuum, Botryodiplodia rhodina, Botryodiplodia theobromae, Botryorhiza, Botryosphaeria, Botryosphaeria berengeriana, Botryosphaeria disrupta, Botryosphaeria festucae, Botryosphaeria quercuum, Botryosphaeria theobromae, Botryosphaeria vaccinii, Botryosphaeria xanthocephala, Botryosphaeria zeae, Botryosporium, Botryostroma, Botryotinia allii, Botryotinia porri, Botryotinia ricini, Botryotinia squamosa, Botrytis aclada, Botrytis byssoidea, Botrytis porri, Botrytis ricini, Botrytis vulgaris, Brachybasidium, Briosia, Briosia azaleae, Brobdingnagia, Brunchorstia, Brunneosphaerella, Bryochiton, Bryomyces, Bryoscyphus, Buerenia, Bulgaria inquinans, Burenia, Burrillia, Bursaphelenchus, Bursaphelenchus cocophilus, Butlerelfia eustacei, Butleria, Byssochlamys fulva, Caeoma, Caeomurus, Caetea, Calacogloea, Caliciopsis, Calidion, Calonectria colhounii, Calonectria crotalariae, Calonectria keyotensis, Calonectria nivalis, Calonectria rigidiuscula, Calonectria theae, Caloscypha, Calostilbe, Calostilbella, Camarosporium, Camarosporium pistaciae, Campanulospora, Campoa, Canasta, Canavirgella, Candelobrochaete, Candelobrochaete langioisii, Capitorostrum, Capnodiastrum, Capnodium citri, Capnodium coffeae, Capnodium mangiferae, Caproventuria, Carpenterella, Catacauma, Catacauma mucosum, Catenolaria, Catenulostroma, Celoporthe, Centrolepidosporium, Centrospora, Centrospora acerina, Cephaleuros, Cephaleuros parasiticus, Cephaleuros virescens, Cephalosporium, Cephalosporium acremonium, Cephalosporium crotocinigenum, Cephalosporium gregatum, Cephalosporium maydis, Cephalosporium proliferatum, Cephalosporium sacchari, Cephalothecium, Cephalothecium roseum, Cephalotrichum stemonitis, Ceraceopsora, Ceratelium, Ceratobasidium koleroga, Ceratobasidium oryzae-sativae, Ceratobasidium oryzaesativae, Ceratocoma, Ceratocystis, Ceratocystis ulmi, Ceratophacidium, Ceratorhiza, Ceratorhiza ramicola, Ceratostomella fimbriata, Cercoseptoria, Cercoseptoria cajanicola, Cercoseptoria theae, Cercospora agrostidis, Cercospora albida, Cercospora angraeci, Cercospora bataticola, Cercospora biformis, Cercospora cajani, Cercospora calospilea, Cercospora canescens, Cercospora caracallae, Cercospora castellanii, Cercospora chrysanthemi, Cercospora circumscissa, Cercospora citrullina, Cercospora coffeicola, Cercospora concors, Cercospora coryli, Cercospora cruenta, Cercospora duddiae, Cercospora eustomae, Cercospora festucae, Cercospora fragariae, Cercospora frogeye, Cercospora fuligena, Cercospora fusco-virens, Cercospora fuscovirens, Cercospora gossypina, Cercospora granadillae, Cercospora indica, Cercospora instabilis, Cercospora ipomoeae, Cercospora janseana, Cercospora koepkei, Cercospora lensii, Cercospora melonis, Cercospora minima, Cercospora oryzae, Cercospora passiflorae, Cercospora passifloricola, Cercospora phaseoli, Cercospora pisasativae, Cercospora puderi,

Cercospora pulcherrimae, Cercospora regalis, Cercospora rhapisicola, Cercospora rubi, Cercospora rubrotincta, Cercospora seminalis, Cercospora solanituberosi, Cercospora thirumalacharii, Cercospora truncatella, Cercospora vaginae, Cercospora vanderysti, Cercospora vignicola, Cercospora zeaemaydis, Cercosporella, Cercosporella brassicae, Cercosporella gossypii, Cercosporella poagena, Cercosporidium, Cercosporidium graminis, Cercosporidium henningsii, Cercosporidium personatum, Cercosporina, Cercosporina regalis, Cerebella, Cerion, Ceriospora dubyi, Ceropsora, Cerotelium, Cerradoa, Ceuthospora, Ceuthospora lunata, Chaconia, Chaetoseptoria, Chaetoseptoria wellmanii, Chaetosticta, Chailletia, Chalara, Chalara elegans, Chalara fraxinea, Chalara paradoxa, Chalara thielavioides, Chalaropsis, Chalaropsis thielavioides, Chalastospora, Chardoniella, Chevalieropsis, Chloridium phaeosporum, Chlosyne, Chlosyne ehrenbergii, Chmelia, Choanephora trispora, Chondrostereum, Chondrostereum pupureum, Chrysella, Chrysocelis, Chrysocrypta, Chrysocyclus, Chrysomyxa, Chrysoporthe, Chrysoporthella, Chrysopsora, Chytridium, Cibiessia, Ciboria, Ciborinia, Cintractia, Cintractiella, Cionothrix, Cistingophora, Cladochytrium, Cladosporium allii-cepae, Cladosporium alliicepae, Cladosporium britannicum, Cladosporium carpophilum, Cladosporium echinulatum, Cladosporium elatum, Cladosporium fulvum, Cladosporium maracuja, Cladosporium orchidis, Cladosporium oxycocci, Cladosporium oxysporum, Cladosporium phlei, Cladosporium pisicola, Cladosporium tenuissimum, Cladosporium viticola, Clathrospora, Clathrospora pentamera, Claviceps, Claviceps gigantea, Cleistosphaera, Cleptomyces, Clethridium, Clethridium corticola, Clinoconidium, Clintamra, Clitocybe tabescens, Clonostachys, Clypeoporthe, Clypeoporthella, Coccodiella, Coccodiella miconiae, Coccodinium, Coccodothis, Coccoidella, Coccoidea, Coccomyces, Cocconia, Coccophacidium, Coccostroma, Coccostroma palmicola, Cochliobolus australiensis, Cochliobolus cynodontis, Cochliobolus eragrostidis, Cochliobolus geniculatus, Cochliobolus intermedia, Cochliobolus intermedius, Cochliobolus lunata, Cochliobolus pallescens, Cochliobolus penniseti, Cochliobolus protuberata, Cochliobolus sativa, Cochliobolus senegalensis, Cochliobolus trifolii, Cocoicola, Coleophoma empetri, Coleopuccinia, Coleopucciniella, Coleosporium, Coleroa, Colleotrichum, Colleotrichum gloeosporioides, Colleotrichum kahawae, Colletogloeopsis, Colletogloeum, Colletotrichum, Colletotrichum ananas, Colletotrichum atramentarium, Colletotrichum cajani, Colletotrichum camelliae, Colletotrichum circinans, Colletotrichum coccodes, Colletotrichum falcatum, Colletotrichum fragariae, Colletotrichum gloeosporiodes, Colletotrichum gloeosporioides, Colletotrichum graminicola, Colletotrichum lagenarium, Colletotrichum mangenoti, Colletotrichum prunicola, Colletotrichum spadix, Colletotrichum spinaciae, Colletotrichum theae-sinensis, Colletotrichum theaesinensis, Collophora, Collybia,

Collybia dryophila, Colpoma, Coma, Completoria, Coniella, Coniodictyum, Coniothecium, Coniothecium chromatosporum, Coniothyrium, Coniothyrium celtidisaustralis, Coniothyrium fragariae, Coniothyrium fuckelii, Conostroma, Cophinforma, Coprinus, Coprinus kubickae, Coprinus psychromorbidus, Corbulopsora, Corynelia, Coryneliopsis, Coryneliospora, Corynespora cassicola, Crandallia, Criella, Crinipellis perniciosa, Cristulariella, Cristulariella moricola, Cristulariella pyrimidalis, Crivellia, Cronartium, Crossopsora, Crotalia, Crotone, Cryocaligula, Cryphonectria, Cryptascoma, Cryptocline, Cryptodiaporthe, Cryptometrion, Cryptomyces, Cryptosphaeria, Cryptosporella, Cryptosporiopsis curvispora, Cryptosporiopsis perennans, Cryptosporium, Cryptostistis, Cryptostistis mariae, Cudoniopsis, Cumminsiella, Cumminsina, Curvicladiella, Curvularia, Curvularia carica-papayae, Curvularia caricapapayae, Curvularia clavata, Curvularia cymbopogonis, Curvularia eragrostidis, Curvularia geniculata, Curvularia inaequalis, Curvularia intermedia, Curvularia lunata, Curvularia maculans, Curvularia pallescens, Curvularia spicifera, Curvularia tuberculata, Cuscuta, Cuscuta hyalina, Cycloschizon, Cyclostomella, Cylindrocarpon, Cylindrocarpon candidum, Cylindrocarpon destructans, Cylindrocarpon ehrenbergii, Cylindrocarpon heteronemum, Cylindrocarpon ianthothele, Cylindrocarpon radicicola, Cylindrocarpon tenue, Cylindrocarpon willkommii, Cylindrochytridium, Cylindrocladiella, Cylindrocladiella peruviana, Cylindrocladiopsis, Cylindrocladium, Cylindrocladium camelliae, Cylindrocladium colhounii, Cylindrocladium crotalariae, Cylindrocladium floridanum, Cylindrocladium ilicicola, Cylindrocladium parvum, Cylindrocladium petiole, Cylindrocladium scoparium, Cylindrocladium scoparum, Cylindrocladium spathiphylli, Cylindrocladium theae, Cylindrosporium, Cylindrosporium chrysanthemi, Cylindrosporium concentricum, Cylindrosporium humuli, Cylindrosporium pomi, Cylindrosympodium, Cymadothea, Cystomyces, Cystopsora, Cystopus, Cystopus tragopogonis, Cystotheca, Cytosphaera, Cytosphaera mangiferae, Cytospora abietis, Cytospora ambiens, Cytospora chrysosperma, Cytospora cincta, Cytospora eucalypticola, Cytospora kunzei, Cytospora leucosperma, Cytospora leucostoma, Cytospora nivea, Cytospora platani, Cytosporina, Cyttaria, Cyttariella, Dactuliochaeta, Dactuliochaeta glycines, Dactuliophora, Dactuliophora glycines, Dactylaria, Dactylaria humicola, Daedaleopsis confragosa, Daldinia eschscholzii, Dangeardia, Dangeardiana, Darkera, Dasturella, Dasyspora, Davisomycella, Davisoniella, Decorospora, Deightoniella, Dematophora necatrix, Dendrophoma, Dendrophoma obscurans, Dendrophora albabadia, Dendryphiella, Dendryphion, Dennisiella, Dermatosorus, Dermea, Deshpandiella, Desmella, Desmellopsis, Desmosorus, Deuterophoma, Deuterophoma tracheiphila, Devriesia, Diabole, Diabolidium, Diachora, Diachorella, Diadema, Dianesea, Diaphanopellis, Diapleella,

Diapleella coniothyrium, Diaporthe batatatis, Diaporthe citri, Diaporthe meduseae, Diaporthe perexigua, Diaporthe umbrina, Diaporthe vaccinii, Diaporthella, Diaporthopsis, Diatrype, Diatrypella, Dibotryon, Dicarpella georgiana, Dicellomyces, Dicheirinia, Dichomera, Dictochaeta, Dictochaeta fertilis, Dictyochaeta triseptata, Dictyocyclus, Dictyodochium, Didymaria, Didymascella, Didymella, Didymella applanata, Didymella exitalis, Didymella ligulicola, Didymella lycopersici, Didymellina, Didymopsora, Didymopsorella, Didymosphaeria arachidicola, Didymosphaeria igniaria, Dietelia, Dilophospora, Dimeriella, Dimerina, Dimerium, Dimerosporis, Diorchidiella, Diorchidium, Diplocarpon, Diplocarpon earlianum, Diplocarpon mali, Diplochytridium, Diplodia, Diplodia cacaoicola, Diplodia cajani, Diplodia frumenti, Diplodia gossypina, Diplodia gummosis, Diplodia laeliocattleyae, Diplodia macrospora, Diplodia manihotis, Diplodia maydis, Diplodia mutila, Diplodia natalensis, Diplodia phoenicum, Diplodia pinea, Diplodia theaesinensis, Diplodia theobromae, Diplodia zeae, Diplodina lycopersici, Dipyxis, Discocainia, Discochora, Discochora philoprina, Discohainesia, Discohainesia oenotherae, Discosia, Discostroma, Discula platani, Discula umbrinella, Doassansia, Doassansiella, Doassansiopsis, Doassinga, Doratomyces stemonitis, Dothidasteroma, Dothidea, Dothidea sambuci, Dothidella, Dothidella ulmi, Dothideovalsa, Dothidotthia, Dothiorella, Dothiorella aromatica, Dothiorella mangiferae, Dothiorella ulmi, Dothistroma, Drechslera, Drechslera avenae, Drechslera catenaria, Drechslera dictyoides, Drechslera erythrospila, Drechslera graminea, Drechslera halodes, Drechslera musae-sapientum, Drechslera musaesapientum, Drechslera nobleae, Drechslera phlei, Drechslera poae, Drechslera prolata, Drechslera siccans, Drechslera spicifera, Drechslera teres, Drechslera tritici-repentis, Drechslera triticirepentis, Drepanoconis, Drepanopeziza, Dreschlera hawaiiensis, Dreschslera, Dreschslera gigantea, Dumontinia, Duplicaria, Duplicariella, Durandiella, Durandiella pseudotsugae, Dysrhynchis, Eballistra, Echinodontium, Echinomyces, Edenia, Edythea, Elateraecium, Elliottinia, Elsinoe, Elsinoe ampelina, Elsinoe australis, Elsinoe batatas, Elsinoe brasiliensis, Elsinoe fawcettii, Elsinoe leucospila, Elsinoe mangiferae, Elsinoe phaseoli, Elsinoe piri, Elsinoe randii, Elsinoe rosarum, Elsinoe sacchari, Elsinoe theae, Elsinoe veneta, Elytroderma, Embellisia, Embellisia allii, Endobasidium, Endoconidiophora, Endoconidiophora fimbriata, Endocronartium, Endocronartium harknessii, Endodesmidium, Endomelanconiopsis, Endophyllum, Endoraecium, Endostigme, Endothia, Endothia gyrosa, Endothiella, Endothlaspis, Endoxylina, Englerodothis, Englerula, Entomosporium, Entomosporium mespili, Entorrhiza, Entyloma dactylidis, Entyloma lineatum, Entyloma oryzae, Entyloma petuniae, Entyloma vignae, Entylomaster, Entylomella, Eocronartium, Epibryon, Epichloe, Epichloe typhina, Epicoccum, Epicoccum pur-

purascens, Epipolaeum, Episphaerella, Eremothecium, Eremothecium cymbalariae, Erikssonia, Eriocaulago, Eriocercospora, Eriocortex, Eriomoeszia, Eriosporium, Erratomyces, Eruptio, Erwinia, Erwinia carotovora, Erwinia chrysanthemi, Erysibe, Erysiphe azaleae, Erysiphe communis, Erysiphe digitata, Erysiphe graminis, Erysiphe izuensis, Erysiphe polygoni, Erysiphe rhododendri, Erysiphe umbelliferaum, Erysiphe vaccinii, Erythricium, Erythricium salmonicolor, Esalque, Eudimeriolum, Eumela, Eupropolella, Eupropolella oxycocci, Eutiarosporella, Eutypa, Eutypa armeniacae, Eutypa heteracantha, Eutypa lata, Eutypella, Eutypella parasitica, Eutypella scorparia, Exoascus, Exobasidium, Exobasidium azaleae, Exobasidium oxycocci, Exobasidium perenne, Exobasidium rostrupii, Exobasidium vaccinii, Exobasidium vacciniiuliginosi, Exoteliospora, Exserohilum, Exserohilum pedicellatum, Exserohilum prolatum, Exserohilum rostratum, Exserohilum turcicum, Extrawettsteinina, Fabraea, Fabraea maculata, Falciformispora, Farmanomyces, Farysia, Faryspo-rium, Fassia, Ferrarisia, Fibroidium, Fimicolochytrium, Fistulina, Fitzpatrickella, Flamingomyces, Flaminia, Flammulina velutipes, Floromyces, Foliocryphia, Fomitiporia, Fomitiporia mediterranea, Fomitopsis pinicola, Franzpetrakia, Fremitomyces, Frommea, Frommea obtusa, Frommeella, Fugomyces, Fulvia, Fulvia fulva, Fulvisporium, Fusarium acuminatum, Fusarium anthophilum, Fusarium aquaeductuum, Fusarium avenaceum, Fusarium bulbicola, Fusarium chlamydosporum, Fusarium decemcellare, Fusarium decemcellulare, Fusarium episphaeria, Fusarium equiseti, Fusarium eumartii, Fusarium eylarioides, Fusarium gibbosum, Fusarium graminearum, Fusarium heterosporum, Fusarium lateritium, Fusarium merismoides, Fusarium moniliforme, Fusarium nivale, Fusarium oxysporum, Fusarium poae, Fusarium polyphialidicum, Fusarium pseudograminearum, Fusarium rimosum, Fusarium roseum, Fusarium sambucinum, Fusarium scirpi, Fusarium seedling, Fusarium semitectum, Fusarium sporotrichiodes, Fusarium sporotrichioides, Fusarium stilboides, Fusarium tabacinum, Fusarium tricinctum, Fusarium udum, Fusculina, Fusicladium, Fusicladium britannicum, Fusicladium carpophilum, Fusicladium effusum, Fusicladium pyrorum, Fusicladosporium, Fusicoccum, Fusicoccum cajani, Fusicoccum putrefaciens, Gabarnaudia, Gaertneriomyces, Gaeumannomyces, Gaeumannomyces graminis, Gaeumannomyces incrustans, Gaeumannomyces wongoonoo, Galactomyces, Galactomyces citriaurantii, Galactomyces geotrichum, Gallowaya, Gambleola, Ganoderma, Geastrumia, Gelatinosporium, Geminago, Geminispora, Geniculodendron, Georgefischeria, Gerlachia, Gerlachia nivalis, Gerwasia, Gibbago, Gibbera, Gibbera compacta, Gibbera myrtilli, Gibberella canker, Gibberella coronicola, Gibberella gordonia, Gibberella indica, Gibberella moniliformis, Gibberella subglutinans, Gibberella saubinett, Gibellina, Gibellulopsis, Giberella, Giberella pulicaris, Gjaerumia, Gliocladium roseum, Gloeocercospora,

Gloeodes, Gloeodes pomigena, Gloeosporidiella, Gloeosporium, Gloeosporium bolleyi, Gloeosporium coryli, Gloeosporium inconspicuum, Gloeosporium minus, Gloeosporium olivarum, Gloeosporium theaesinensis, Gloeosporium ulmeum, Gloeosporium ulmicola, Glomerella, Glomerella falcatum, Glomerella glycines, Glomerella gossypii, Glomerella lagenarium, Glomerella lindemuthiana, Glomerella singulata, Glomopsis, Glomospora, Glomosporium, Gnomonia, Gnomonia depressula, Gnomonia errabunda, Gnomonia fragariae, Gnomonia gnomon, Gnomonia leptostyla, Gnomonia nerviseda, Gnomonia petiolorum, Gnomonia ulmea, Gnomonia veneta, Godronia cassandrae, Golovinomyces, Gonatobotrys simplex, Gondwanamyces, Goosia, Goplana, Graciloidium, Grandinia, Grandinia granulosa, Graphiola, Graphium penicillioides, Graphium ulmi, Greeneria uvicola, Gremmenia, Gremmenia abietis, Gremmenia infestans, Gremmenia pini-cembrae, Grifola, Grifola frondosa, Grifola sordulenta, Grimmicola, Grosmannia, Grovesinia, Guignardia, Guignardia bidwellii, Guignardia citricarpa, Guignardia cocoicola, Guignardia endophyllicola, Guignardia miconiae, Guignardia vaccinii, Gymnocintractia, Gymnoconia, Gymnopilus pampeanus, Gymnosporangium, Gymnosporangium clavipes, Gymnosporangium fuscum, Gymnosporangium globosum, Gymnosporangium juniperi-virginianae, Gymnosporangium juniperivirginianae, Gyroflexus, Haddowia, Haematonectria, Hainesia, Hainesia lythri, Halobyssus, Hamaspora, Hansenula, Hapalophragmium, Hapalosphaeria, Haplaria, Haplobasidion, Haplosporella, Haraea, Helicobasidium, Helicobasidium brebissonii, Helicobasidium mompa, Helicotylenchus, Helicotylenchus dihystera, Helicotylenchus multicinctus, Helminthosporium, Helminthosporium allii, Helminthosporium australiense, Helminthosporium avenaceum, Helminthosporium avenae, Helminthosporium cactivorum, Helminthosporium carbonum, Helminthosporium cassiicola, Helminthosporium chlorophorae, Helminthosporium helianthi, Helminthosporium maydis, Helminthosporium papulosum, Helminthosporium pedicellatum, Helminthosporium rostratum, Helminthosporium sacchari, Helminthosporium sativum, Helminthosporium setariae, Helminthosporium sorokinianum, Helminthosporium tritici-repentis, Helminthosporium triticirepentis, Helminthosporium turcicum, Helminthosporium victoriae, Helminthosporium vignae, Helminthosporium vignicola, Helochora, Hemigrapha, Hemileia, Hemileia coffeicola, Hemileia vastatrix, Hemimyriangium, Hemiphacidium, Hendersonula, Hendersonula toruloidea, Hennenia, Henningsia, Hericium erinaceous, Herpobasidium, Heterodoassansia, Heterotolyposporium, Hinomyces, Hiratsukaia, Hiratsukamyces, Holocryphia, Hormodendrum, Hormodendrum cladosporioides, Hortaea, Humphreya, Hyalomeliolina, Hyalopsora, Hyalotheles, Hyalothyridium, Hyalothyridium maydis, Hydnopolyporus, Hygrochroma, Hymenochaete agglutinans, Hymenula, Hymenula cerealis, Hypasteridium,

Hypochnicium vellereum, Hypochnus, Hypochnus centrifugus, Hypochnus ochroleucus, Hypoderma, Hypodermella, Hypohelion, Hypoxylon fragiforme, Hypoxylon fuscum, Hypoxylon stygium, Hysterodiscula, Hysterostomella, Idriella lunata, Ilyonectria, Ilyonectria radicicola, Imazekia, Immersidiscosia, Ingoldiomyces, Inocyclus, Insolibasidium, Intrapes, Iola, Irene, Irene perseae, Isaria fuciformis, Isariopsis, Isariopsis clavispora, Isothea, Isthmiella, Itersonilia, Itersonilia perplexans, Jamesdicksonia, Joerstadia, Johncouchia mangiferae, Johnkarlingia, Juliohirschhornia, Kabatiella, Kabatiella caulivorum, Kabatiella zeae, Kallistoskypha, Karakulinia, Karlingiomyces, Kellermania, Kernella, Kernkampella, Khuskia oryzae, Kiehlia, Kimuromyces, Kirramyces, Kochiomyces, Kochmania, Kohninia, Kordyana, Korunomyces, Korunomyces prostratus, Kretzschmaria deusta, Kriegeriella, Kuehneola, Kunkelia, Kuntzeomyces, Kutilakesa, Kweilingia, Labrella, Labrella coryli, Lacustromyces, Laeticorticium, Laeticorticium roseum, Laetisaria, Laetisaria fuciformis, Laeviomeliola, Lagena, Lagena radicicola, Lagenulopsis, Lambertellinia, Myriogenospora, Myrothecium verracaria, Nigrospora sphaerica, Pestalotia, Phoma eupyrena, Phoma terrestris, Phomopsis, Rhizoctonia crocorum, Trichoderma, Ulocladium atrum, Xenodiella, Xenodium, Xenodochus, Xenophacidiella, Xenostele, Xenostigme, Xenotypa, Xerula, Xyloschizon, Yelsemia, Yenia, Ypsilospora, Zaghouania, Zeloasperisporium, Zeta, Zeus, Zimmermaniella, Zimmermaniella trispora, Zimmermanniella, Zundeliomyces, Zygophiala jamaicensis, Zygorhizidium, Zygosaccharomyces, Zygosporium, Zygosporium echinosporum, Zygosporium gibbum, Zythia, Zythia fragariae, Epichloë, Epichloë typhina, Aureobasidium, Athelia, Apiognomonia errabunda, Arthrinium arundinis, Acervuloseptoria ziziphicola, Acervuloseptoria, Acervuloseptoria capensis, Acicuseptoria, Acicuseptoria rumicis, Albonectria, Apiognomonia, Coniochaeta, Aequabiliella effusa, Aequabiliella, Phaeomoniella effusa, Phaeomoniella prunicola, Phaeomoniella dura, Phaeomoniella tardicola, Collophora africana, Collophora capensis, Collophora paarla, Collophora pallida, Collophora rubra, Coniochaeta africana, Coniochaeta prunicola, Allantonectria, Allantonectria miltina, Nectria australiensis, Nectria berberidicola, Nectria canadensis, Nectria eustromatica, Nectria hoheriae, Nectria lateritia, Nectria mariae, Nectria novaezelandiae, Nectria paraguayensis, Nectria pyriformis, Nectria noackiana, Coniosporium, Sirodesmium, Sirodesmium indicum, Coryneum beijerinckii, Coryneum, Discula, Harknessia, Sirococcus clavigignenti-juglandacearum, Sirococcus, Ophiognomonia leptostyla, Ophiognomonia, Marssoniella juglandis, Marssoniella, Avachytrium, Knufia, Allophoma, Amphisphaeria, Amycosphaerella, Anguillospora, Angulomyces, Apoharknessia, Aquamyces, Arecophila, Aristastoma, Arxiella, Ascobotryozyma, Aurantioporthe, Aurantiosacculus, Auratiopycnidiella, Bambusicularia,

Barretomyces

A.2 Names considered to be potential plant pathogenic oomycetes

Pythium, Phytophthora, Plasmopara, Halophytophthora, Hyaloperonospora, Phytopythium, Peronospora, Bremia, Albugo, Trachysphaera, Globisporangium, Pustula, Wilsoniana, Peronosclerospora, Pseudoperonospora, Sclerophthora, Peronophytophthora, Diasporangium, Aphanomyces cladoganus, Aphanomyces cochlioides, Aphanomyces euteiches, Aphanomyces raphani

A.3 Names considered to be potential plant pathogenic bacteria, from Bull *et al.* (2010, 2012, 2014)

Acetobacter aceti, Acetobacter pasteurianus, Acidovorax anthurii, Acidovorax avenae, Acidovorax avenae subsp. avenae, Acidovorax avenae subsp. cattleyae, Acidovorax avenae subsp. citrulli, Acidovorax cattleyae, Acidovorax citrulli, Acidovorax konjaci, Acidovorax oryzae, Acidovorax valerianellae, Actinomyces candidus, Agrobacterium larrymoorei, Agrobacterium radiobacter, Agrobacterium rhizogenes, Agrobacterium rubi, Agrobacterium tumefaciens, Agrobacterium vitis, Arthrobacter ilicis, Bacillus megaterium, Bacillus megaterium pv. cerealis, Bacillus pumilus, Brenneria alni, Brenneria goodwinii, Brenneria nigrifluens, Brenneria paradisiaca, Brenneria quercina, Brenneria quercina pv. lupinicola, Brenneria quercina pv. quercina, Brenneria rubrifaciens, Brenneria salicis, Burkholderia andropogonis, Burkholderia caryophylli, Burkholderia cepacia, Burkholderia gladioli, Burkholderia gladioli pv. agaricicola, Burkholderia gladioli pv. alliicola, Burkholderia gladioli pv. gladioli, Burkholderia glumae, Burkholderia plantarii, Burkholderia solanacearum, Candidatus Liberibacter, Candidatus Liberibacter africanus, Candidatus Liberibacter africanus subsp. capensis, Candidatus Liberibacter americanus, Candidatus Liberibacter asiaticus, Candidatus Liberibacter psyllaurous, Candidatus Liberibacter solanacearum, Candidatus Phlomobacter, Candidatus Phlomobacter fragariae, Candidatus Phytoplasma, Candidatus Phytoplasma allocasuarinae, Candidatus Phytoplasma americanum, Candidatus Phytoplasma asteris, Candidatus Phytoplasma aurantifolia, Candidatus Phytoplasma australasia, Candidatus Phytoplasma australiense, Candidatus Phytoplasma brasiliense, Candidatus Phytoplasma caricae, Candidatus Phytoplasma castaneae, Candidatus Phytoplasma convolvuli, Candidatus Phytoplasma

costaricanum, Candidatus Phytoplasma cynodontis, Candidatus Phytoplasma fragariae, Candidatus Phytoplasma fraxini, Candidatus Phytoplasma graminis, Candidatus Phytoplasma japonicum, Candidatus Phytoplasma lycopersici, Candidatus Phytoplasma mali, Candidatus Phytoplasma omanense, Candidatus Phytoplasma oryzae, Candidatus Phytoplasma phoenicium, Candidatus Phytoplasma pini, Candidatus Phytoplasma prunorum, Candidatus Phytoplasma pyri, Candidatus Phytoplasma rhamni, Candidatus Phytoplasma rubi, Candidatus Phytoplasma spartii, Candidatus Phytoplasma sudamericanum, Candidatus Phytoplasma tamaricis, Candidatus Phytoplasma trifolii, Candidatus Phytoplasma ulmi, Candidatus Phytoplasma ziziphi, Clavibacter iranicus, Clavibacter michiganensis, Clavibacter michiganensis subsp. insidiosus, Clavibacter michiganensis subsp. michiganensis, Clavibacter michiganensis subsp. nebraskensis, Clavibacter michiganensis subsp. sepedonicus, Clavibacter michiganensis subsp. tessellarius, Clavibacter rathayi, Clavibacter toxicus, Clavibacter tritici, Clavibacter xyli, Clavibacter xyli subsp. cynodontis, Clavibacter xyli subsp. xyli, Clostridium puniceum, Corynebacterium betae, Corynebacterium beticola, Corynebacterium fascians, Corynebacterium flaccumfaciens, Corynebacterium flaccumfaciens pv. betae, Corynebacterium flaccumfaciens pv. flaccumfaciens, Corynebacterium flaccumfaciens pv. oortii, Corynebacterium flaccumfaciens pv. poinsettiae, Corynebacterium flaccumfaciens subsp. betae, Corynebacterium flaccumfaciens subsp. flaccumfaciens, Corynebacterium flaccumfaciens subsp. oortii, Corynebacterium flaccumfaciens subsp. poinsettiae, Corynebacterium ilicis, Corynebacterium insidiosum, Corynebacterium iranicum, Corynebacterium iranicus, Corynebacterium michiganense, Corynebacterium michiganense pv. insidiosum, Corynebacterium michiganense pv. iranicum, Corynebacterium michiganense pv. michiganense, Corynebacterium michiganense pv. nebraskense, Corynebacterium michiganense pv. rathayi, Corynebacterium michiganense pv. sepedonicum, Corynebacterium michiganense pv. tritici, Corynebacterium michiganense subsp. insidiosum, Corynebacterium michiganense subsp. michiganense, Corynebacterium michiganense subsp. nebraskense, Corynebacterium michiganense subsp. sepedonicum, Corynebacterium michiganense subsp. tessellarius, Corynebacterium michiganensis pv. insidiosus, Corynebacterium michiganensis pv. iranicum, Corynebacterium nebraskense, Corynebacterium oortii, Corynebacterium poinsettiae, Corynebacterium rathayi, Corynebacterium sepedonicum, Corynebacterium tritici, Curtobacterium flaccumfaciens, Curtobacterium flaccumfaciens pv. betae, Curtobacterium flaccumfaciens pv. flaccumfaciens, Curtobacterium flaccumfaciens pv. ilicis, Curtobacterium flaccumfaciens pv. Ilicis, Curtobacterium flaccumfaciens pv. oortii, Curtobacterium flaccumfaciens pv. poinsettiae, Dickeya chrysanthemi, Dickeya chrysanthemi pv. chrysanthemi, Dickeya

chrysanthemi pv. parthenii, Dickeya dadantii, Dickeya dadantii subsp. dadantii, Dickeya dadantii subsp. dieffenbachiae, Dickeya dianthicola, Dickeya dieffenbachiae, Dickeya paradisiaca, Dickeya zeae, Enterobacter agglomerans, Enterobacter agglomerans pv. millettiae, Enterobacter cancerogenus, Enterobacter cloacae, Enterobacter cloacae subsp. dissolvens, Enterobacter dissolvens, Enterobacter mori, Enterobacter nimipressuralis, Enterobacter pyrinus, Erwinia alni, Erwinia amylovora, Erwinia amylovora pv. pyri, Erwinia ananas, Erwinia ananas pv ananatis, Erwinia ananas pv. uredovora, Erwinia ananatis, Erwinia ananatis pv. ananatis, Erwinia cacticida, Erwinia cancerogena, Erwinia carnegiana, Erwinia carnegieana, Erwinia carotovora, Erwinia carotovora pv. atroseptica, Erwinia carotovora pv. carotovora, Erwinia carotovora subsp. atroseptica, Erwinia carotovora subsp. betavasculorum, Erwinia carotovora subsp. brasiliensis, Erwinia carotovora subsp. carotovora, Erwinia carotovora subsp. odorifera, Erwinia carotovora subsp. wasabiae, Erwinia chrysanthemi, Erwinia chrysanthemi pv. chrysanthemi, Erwinia chrysanthemi pv. dianthi, Erwinia chrysanthemi pv. dianthicola, Erwinia chrysanthemi pv. dieffenbachiae, Erwinia chrysanthemi pv. paradisiaca, Erwinia chrysanthemi pv. parthenii, Erwinia chrysanthemi pv. zeae, Erwinia cypripedii, Erwinia dieffenbachiae, Erwinia dissolvens, Erwinia herbicola, Erwinia herbicola f. sp. gypsophilae, Erwinia herbicola f.sp. gypsophilae, Erwinia herbicola pv. gypsophilae, Erwinia herbicola pv. millettiae, Erwinia mallotivora, Erwinia millettiae, Erwinia nigrifluens, Erwinia nimipressuralis, Erwinia nulandii, Erwinia papayae, Erwinia paradisiaca, Erwinia persicina, Erwinia piriflorinigrans, Erwinia pirina, Erwinia proteamaculans, Erwinia psidii, Erwinia pyrifoliae, Erwinia quercina, Erwinia rhapontici, Erwinia rubrifaciens, Erwinia salicis, Erwinia stewartii, Erwinia tracheiphila, Erwinia uredovora, Erwinia uzenensis, Ewingella americana, Gibbsiella quercinecans, Gluconobacter oxydans, Herbaspirillum rubrisubalbicans, Janthinobacterium agaricidamnosum, Klebsiella pneumoniae, Leifsonia cynodontis, Leifsonia xyli, Leifsonia xyli subsp. cynodontis, Leifsonia xyli subsp. xyli, Lonsdalea quercina, Lonsdalea quercina subsp. britannica, Lonsdalea quercina subsp. iberica, Lonsdalea quercina subsp. quercina, Nocardia vaccinii, Pantoea agglomerans, Pantoea agglomerans pv. gypsophilae, Pantoea agglomerans pv. millettiae, Pantoea allii, Pantoea ananatis, Pantoea ananatis pv. ananatis, Pantoea ananatis pv. uredovora, Pantoea citrea, Pantoea cypripedii, Pantoea stewartii, Pantoea stewartii subsp. indologenes, Pantoea stewartii subsp. stewartii, Pectobacterium atrosepticum, Pectobacterium betavasculorum, Pectobacterium cacticida, Pectobacterium carnegieana, Pectobacterium carotovorum, Pectobacterium carotovorum subsp. atrosepticum, Pectobacterium carotovorum subsp. betavasculorum, Pectobacterium carotovorum subsp. brasiliensis, Pectobacterium caro-

tovorum subsp. carotovorum, Pectobacterium carotovorum subsp. odoriferum, Pectobacterium carotovorum subsp. wasabiae, Pectobacterium chrysanthemi, Pectobacterium chrysanthemi pv. chrysanthemi, Pectobacterium chrysanthemi pv. dianthicola, Pectobacterium chrysanthemi pv. dieffenbachiae, Pectobacterium chrysanthemi pv. parthenii, Pectobacterium chrysanthemi pv. zeae, Pectobacterium cypripedii, Pectobacterium rhapontici, Pectobacterium wasabiae, Pseudomonas adzukicola, Pseudomonas agarici, Pseudomonas amygdali, Pseudomonas andropogonis, Pseudomonas andropogonis pv. andropogonis, Pseudomonas andropogonis pv. sojae, Pseudomonas andropogonis pv. stizolobii, Pseudomonas asplenii, Pseudomonas avellanae, Pseudomonas avenae, Pseudomonas avenae subsp. avenae, Pseudomonas avenae subsp. citrulli, Pseudomonas avenae subsp. konjaci, Pseudomonas beteli, Pseudomonas blatchfordae, Pseudomonas cannabina, Pseudomonas cannabina pv. alisalensis, Pseudomonas cannabina pv. cannabina, Pseudomonas caricapapayae, Pseudomonas caryophylli, Pseudomonas cattleyae, Pseudomonas cepacia, Pseudomonas cichorii, Pseudomonas cissicola, Pseudomonas coronafaciens, Pseudomonas corrugata, Pseudomonas costantinii, Pseudomonas dodoneae, Pseudomonas fabae, Pseudomonas ficuserectae, Pseudomonas flectens, Pseudomonas fuscovaginae, Pseudomonas gardneri, Pseudomonas gingeri, Pseudomonas gladioli, Pseudomonas gladioli pv. agaricicola, Pseudomonas gladioli pv. alliicola, Pseudomonas gladioli pv. gladioli, Pseudomonas glumae, Pseudomonas hibiscicola, Pseudomonas marginalis, Pseudomonas marginalis pv. alfalfae, Pseudomonas marginalis pv. marginalis, Pseudomonas marginalis pv. pastinacae, Pseudomonas mediterranea, Pseudomonas meliae, Pseudomonas morsprunorum f.sp. persicae, Pseudomonas palleroniana, Pseudomonas pallidae, Pseudomonas plantarii, Pseudomonas pomi, Pseudomonas pseudoalcaligenes subsp. citrulli, Pseudomonas pseudoalcaligenes subsp. konjaci, Pseudomonas rubrilineans, Pseudomonas rubrisubalbicans, Pseudomonas salomonii, Pseudomonas savastanoi, Pseudomonas savastanoi pv. fraxini, Pseudomonas savastanoi pv. glycinea, Pseudomonas savastanoi pv. nerii, Pseudomonas savastanoi pv. phaseolicola, Pseudomonas savastanoi pv. retacarpa, Pseudomonas savastanoi pv. savastanoi, Pseudomonas solanacearum, Pseudomonas syingae pv. averrhoi, Pseudomonas syringae, Pseudomonas syringae pv. aceris, Pseudomonas syringae pv. actinidiae, Pseudomonas syringae pv. aesculi, Pseudomonas syringae pv. alisalensis, Pseudomonas syringae pv. antirrhini, Pseudomonas syringae pv. apii, Pseudomonas syringae pv. aptata, Pseudomonas syringae pv. atrofaciens, Pseudomonas syringae pv. atropurpurea, Pseudomonas syringae pv. avellanae, Pseudomonas syringae pv. avii, Pseudomonas syringae pv. berberidis, Pseudomonas syringae pv. broussonetiae, Pseudomonas syringae pv. cannabina, Pseudomonas syringae pv. castaneae,

Pseudomonas syringae pv. cerasicola, Pseudomonas syringae pv. ciccaronei, Pseudomonas syringae pv. coriandricola, Pseudomonas syringae pv. coronafaciens, Pseudomonas syringae pv. coryli, Pseudomonas syringae pv. cunninghamiae, Pseudomonas syringae pv. daphniphylli, Pseudomonas syringae pv. delphinii, Pseudomonas syringae pv. dendropanacis, Pseudomonas syringae pv. dysoxyli, Pseudomonas syringae pv. eriobotryae, Pseudomonas syringae pv. fici, Pseudomonas syringae pv. garcae, Pseudomonas syringae pv. glycinea, Pseudomonas syringae pv. helianthi, Pseudomonas syringae pv. hibisci, Pseudomonas syringae pv. japonica, Pseudomonas syringae pv. lachrymans, Pseudomonas syringae pv. lapsa, Pseudomonas syringae pv. maculicola, Pseudomonas syringae pv. mellea, Pseudomonas syringae pv. mori, Pseudomonas syringae pv. morsprunorum, Pseudomonas syringae pv. myricae, Pseudomonas syringae pv. oryzae, Pseudomonas syringae pv. panici, Pseudomonas syringae pv. papulans, Pseudomonas syringae pv. passiflorae, Pseudomonas syringae pv. persicae, Pseudomonas syringae pv. phaseolicola, Pseudomonas syringae pv. philadelphi, Pseudomonas syringae pv. photiniae, Pseudomonas syringae pv. pisi, Pseudomonas syringae pv. porri, Pseudomonas syringae pv. primulae, Pseudomonas syringae pv. proteae, Pseudomonas syringae pv. rhaphiolepidis, Pseudomonas syringae pv. ribicola, Pseudomonas syringae pv. ricini, Pseudomonas syringae pv. savastanoi, Pseudomonas syringae pv. sesami, Pseudomonas syringae pv. solidagae, Pseudomonas syringae pv. spinaceae, Pseudomonas syringae pv. striafaciens, Pseudomonas syringae pv. syringae, Pseudomonas syringae pv. tabaci, Pseudomonas syringae pv. tagetis, Pseudomonas syringae pv. theae, Pseudomonas syringae pv. tomato, Pseudomonas syringae pv. tremae, Pseudomonas syringae pv. ulmi, Pseudomonas syringae pv. viburni, Pseudomonas syringae pv. zizaniae, Pseudomonas syringae subsp. savastanoi, Pseudomonas syringae subsp. savastanoi pv. fraxini, Pseudomonas syringae subsp. savastanoi pv. myricae, Pseudomonas syringae subsp. savastanoi pv. nerii, Pseudomonas syringae subsp. savastanoi pv. oleae, Pseudomonas syzygii, Pseudomonas tolaasii, Pseudomonas tremae, Pseudomonas viridiflava, Pseudomonas woodsii, Ralstonia solanacearum, Ralstonia syzygii, Rathayibacter iranicus, Rathayibacter rathayi, Rathayibacter toxicus, Rathayibacter tritici, Rhizobacter dauci, Rhizobium larrymoorei, Rhizobium nepotum, Rhizobium radiobacter, Rhizobium rhizogenes, Rhizobium rubi, Rhizobium skierniewicense, Rhizobium vitis, Rhizomonas suberifaciens, Rhodococcus fascians, Samsonia erythrinae, Serratia marcescens, Serratia proteamaculans, Sphingomonas melonis, Sphingomonas suberifaciens, Spiroplasma citri, Spiroplasma kunkelii, Spiroplasma phoeniceum, Streptomyces acidiscabies, Streptomyces albidoflavus, Streptomyces candidus, Streptomyces caviscabies, Strepto-

myces collinus, Streptomyces europaeiscabiei, Streptomyces intermedius, Streptomyces ipomoeae, Streptomyces luridiscabiei, Streptomyces niveiscabiei, Streptomyces puniciscabiei, Streptomyces reticuliscabei, Streptomyces scabiei, Streptomyces setonii, Streptomyces steliiscabiei, Streptomyces turgidiscabies, Streptomyces wedmorensis, Tatumella citrea, Tatumella morbirosei, Tatumella ptyseos, Xanthomonas albilineans, Xanthomonas alfalfae, Xanthomonas alfalfae subsp. alfalfae, Xanthomonas alfalfae subsp. citrumelonis, Xanthomonas ampelina, Xanthomonas arboricola, Xanthomonas arboricola pv. celebensis, Xanthomonas arboricola pv. corylina, Xanthomonas arboricola pv. fragariae, Xanthomonas arboricola pv. juglandis, Xanthomonas arboricola pv. poinsettiicola, Xanthomonas arboricola pv. populi, Xanthomonas arboricola pv. pruni, Xanthomonas axonopodis, Xanthomonas axonopodis pv. alfalfae, Xanthomonas axonopodis pv. allii, Xanthomonas axonopodis pv. anacardii, Xanthomonas axonopodis pv. aurantifolii, Xanthomonas axonopodis pv. axonopodis, Xanthomonas axonopodis pv. bauhiniae, Xanthomonas axonopodis pv. begoniae, Xanthomonas axonopodis pv. betlicola, Xanthomonas axonopodis pv. biophyti, Xanthomonas axonopodis pv. cajani, Xanthomonas axonopodis pv. cassavae, Xanthomonas axonopodis pv. cassiae, Xanthomonas axonopodis pv. citri, Xanthomonas axonopodis pv. citrumelo, Xanthomonas axonopodis pv. clitoriae, Xanthomonas axonopodis pv. coracanae, Xanthomonas axonopodis pv. cyamopsidis, Xanthomonas axonopodis pv. desmodii, Xanthomonas axonopodis pv. desmodiigangetici, Xanthomonas axonopodis pv. desmodiilaxiflori, Xanthomonas axonopodis pv. desmodiirotundifolii, Xanthomonas axonopodis pv. dieffenbachiae, Xanthomonas axonopodis pv. erythrinae, Xanthomonas axonopodis pv. fascicularis, Xanthomonas axonopodis pv. glycines, Xanthomonas axonopodis pv. khayae, Xanthomonas axonopodis pv. lespedezae, Xanthomonas axonopodis pv. maculifoliigardeniae, Xanthomonas axonopodis pv. malvacearum, Xanthomonas axonopodis pv. mangiferaeindicae, Xanthomonas axonopodis pv. Mangiferaeindicae, Xanthomonas axonopodis pv. manihotis, Xanthomonas axonopodis pv. martyniicola, Xanthomonas axonopodis pv. melhusii, Xanthomonas axonopodis pv. nakataecorchori, Xanthomonas axonopodis pv. passiflorae, Xanthomonas axonopodis pv. patelii, Xanthomonas axonopodis pv. pedalii, Xanthomonas axonopodis pv. phaseoli, Xanthomonas axonopodis pv. phaseoli var. fuscans, Xanthomonas axonopodis pv. phyllanthi, Xanthomonas axonopodis pv. physalidicola, Xanthomonas axonopodis pv. poinsettiicola, Xanthomonas axonopodis pv. punicae, Xanthomonas axonopodis pv. rhynchosiae, Xanthomonas axonopodis pv. ricini, Xanthomonas axonopodis pv. sesbaniae, Xanthomonas axonopodis pv. spondiae, Xanthomonas axonopodis pv. tamarindi, Xanthomonas axonopodis pv. vasculorum, Xanthomonas axonopodis pv. vesicatoria, Xanthomonas axonopodis pv. vi-

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A.4 Rarefied richness of all fungi, oomycetes and bacteria

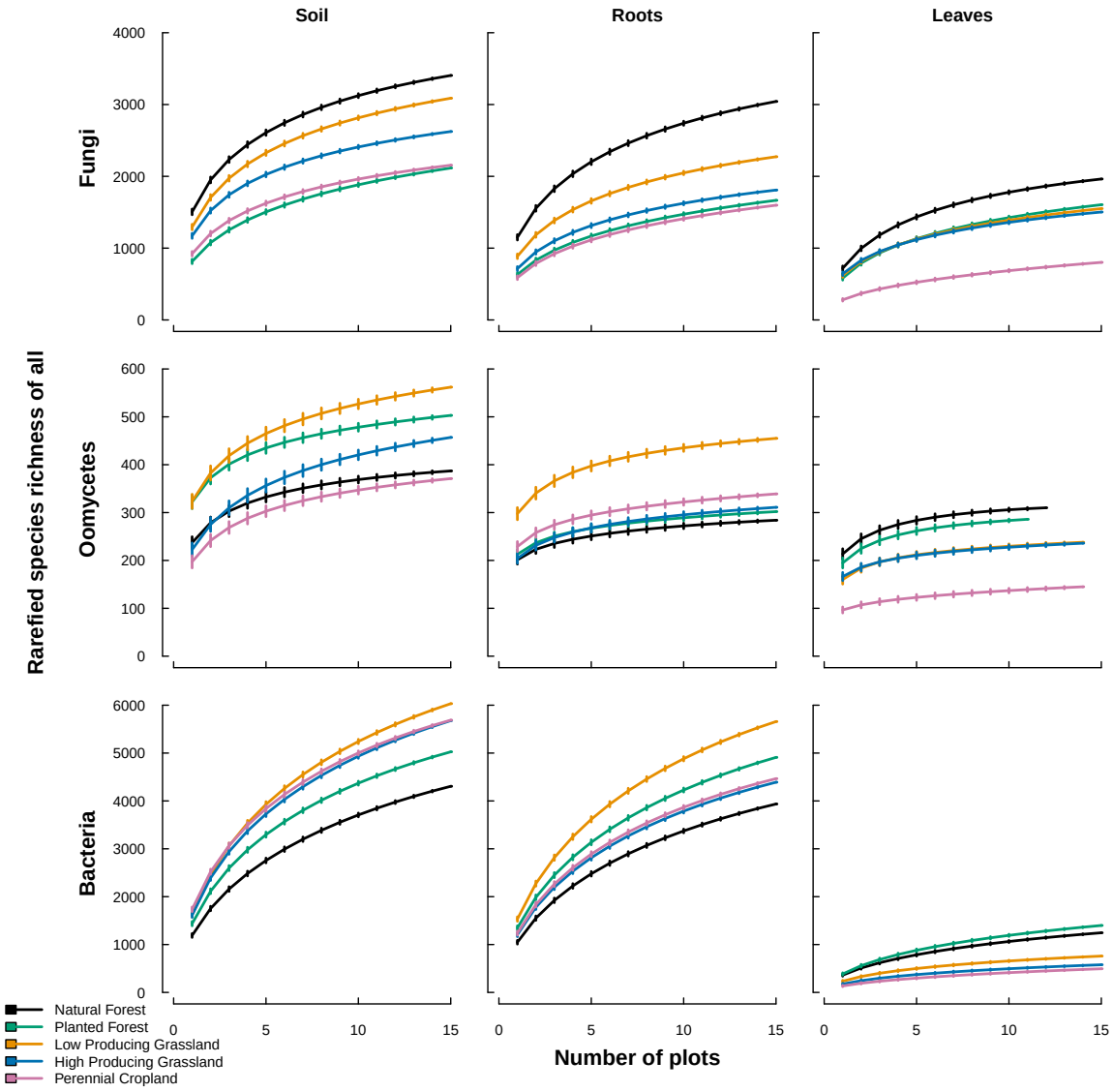

Figure A.1: Rarefied richness of all fungi, oomycetes and bacteria with 95% confidence interval in different substrates across land uses.

A.5 Composition of all oomycetes across land uses and substrate types

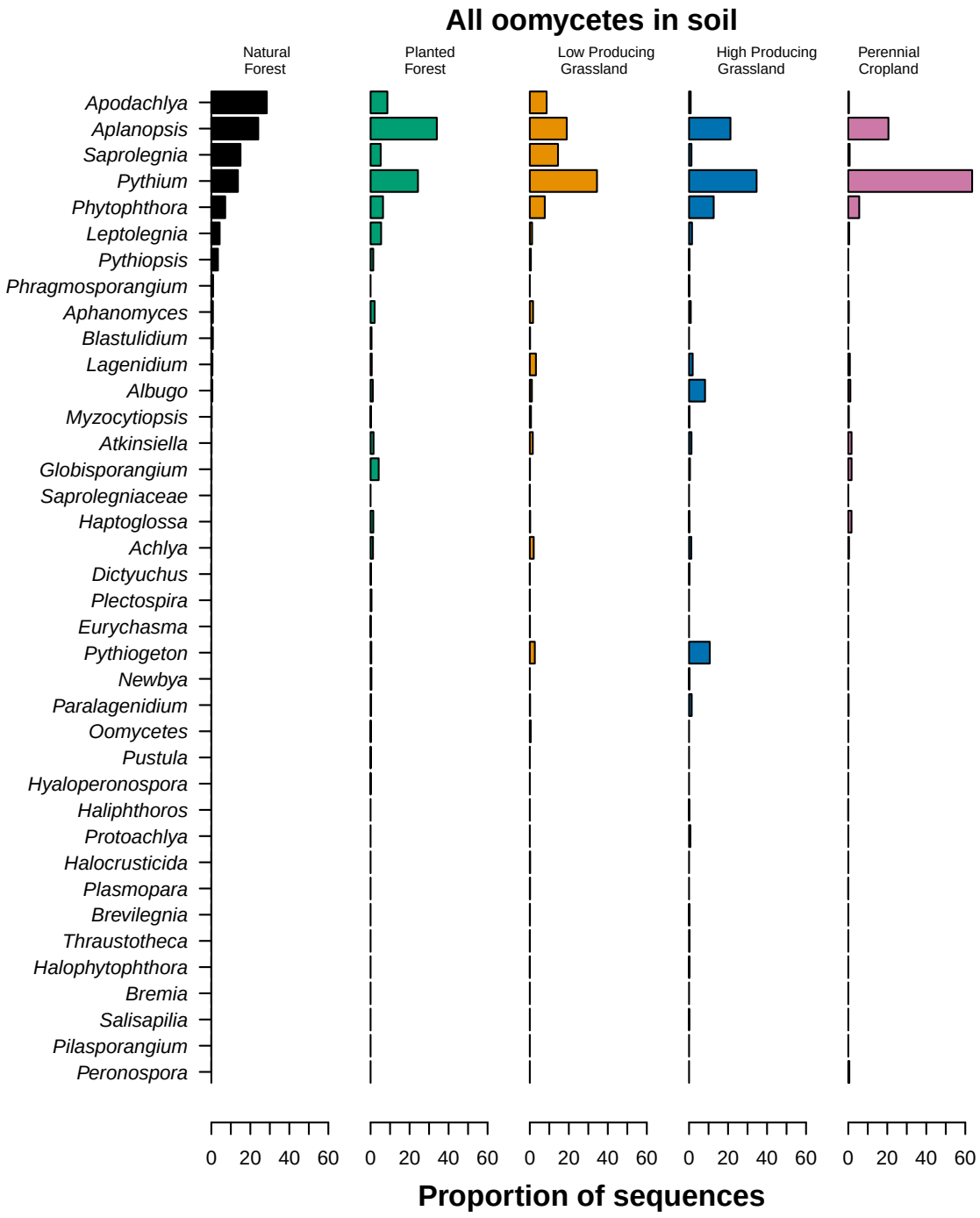

Figure A.2: Composition of all oomycetes in soils across land uses, including low quality matches.

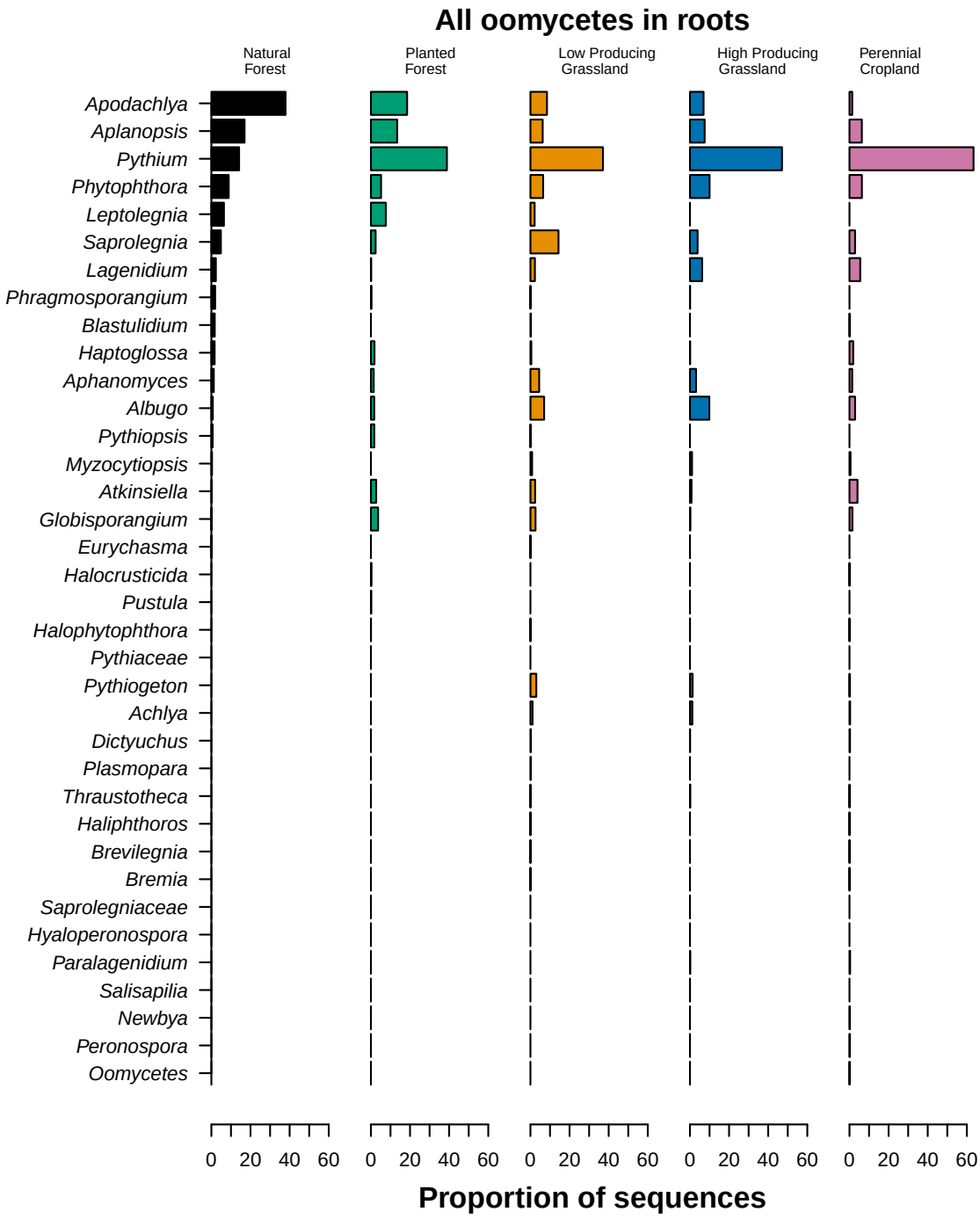

Figure A.3: Composition of all oomycetes in roots across land uses, including low quality matches.

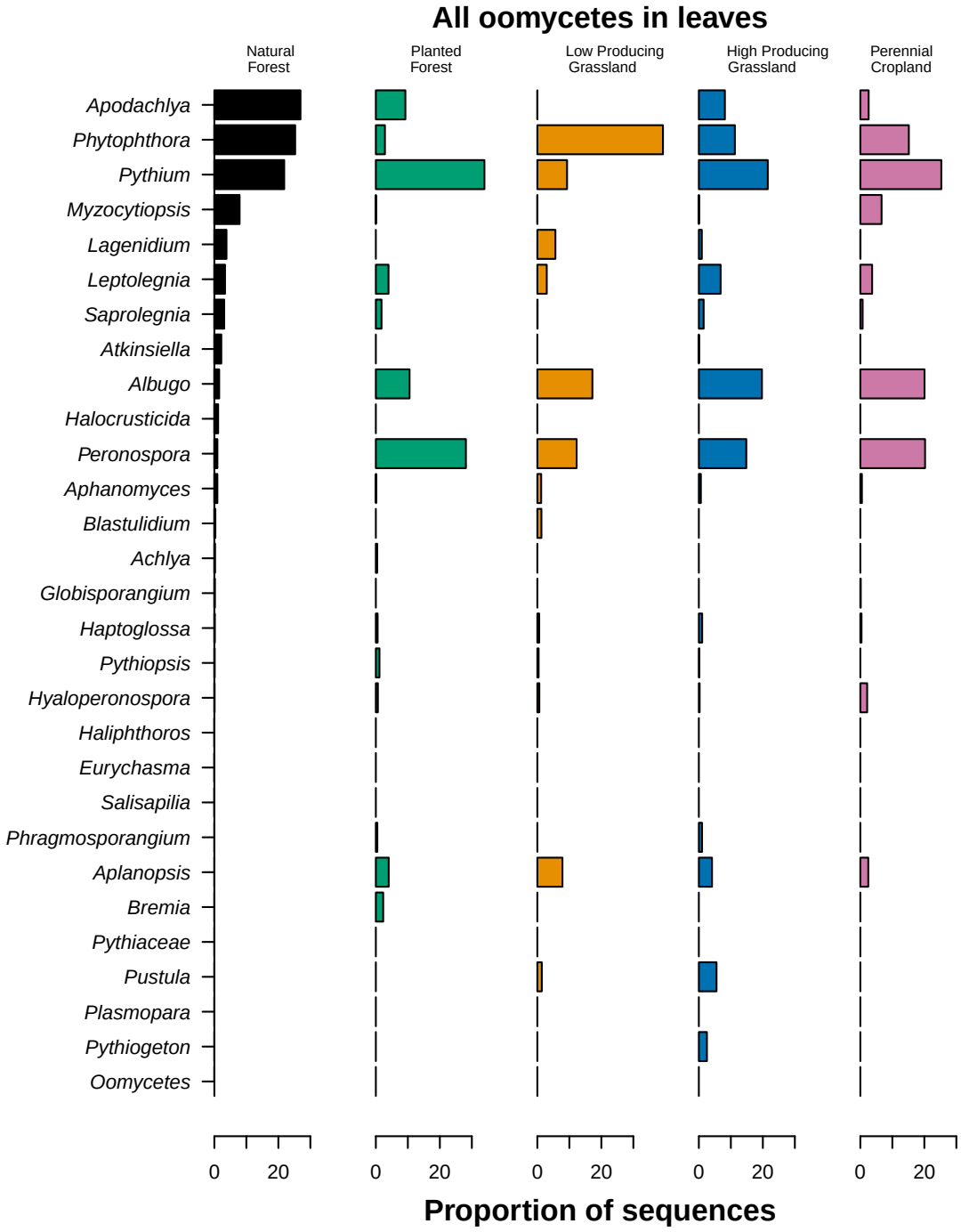

Figure A.4: Composition of all oomycetes in leaves across land uses, including low quality matches.

A.6 Operational taxonomic unit quality of oomycetes

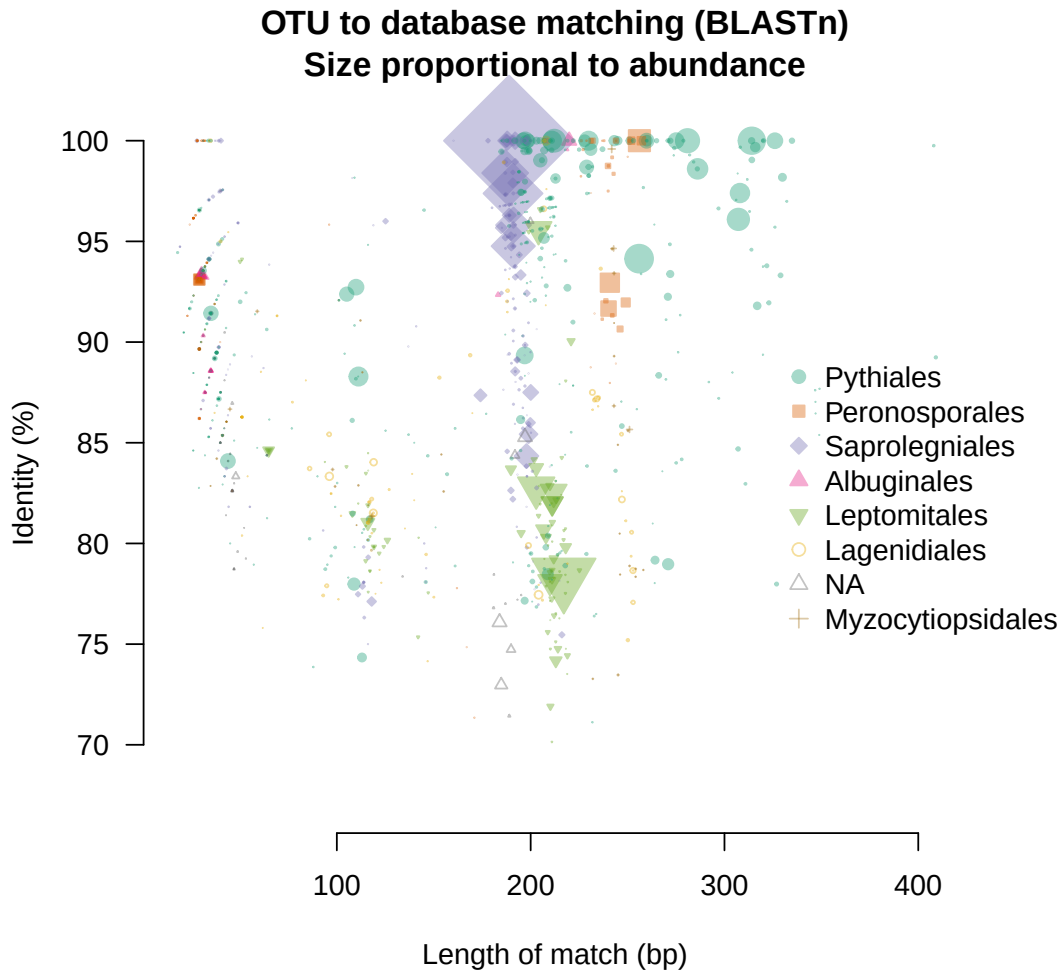

Figure A.5: Operational taxonomic unit length and identity matches of all oomycetes in soil substrates to database.

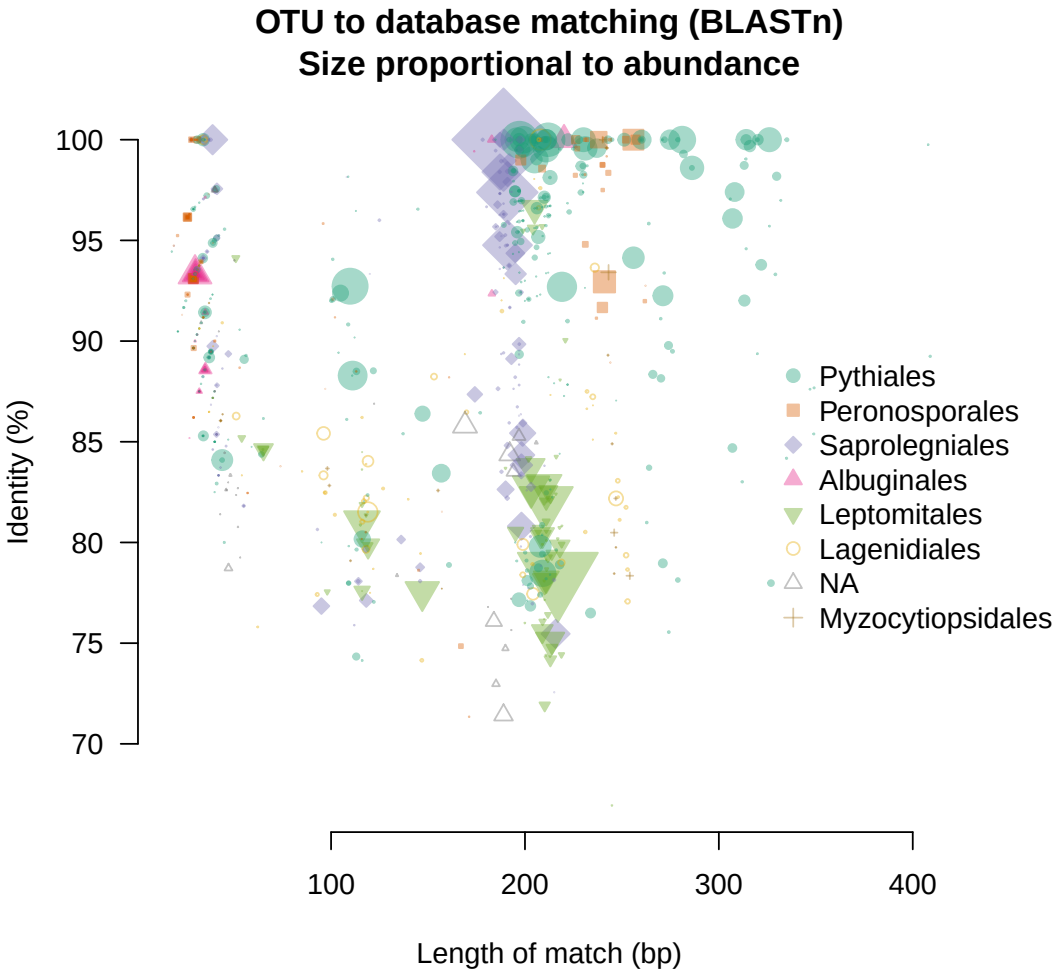

Figure A.6: Operational taxonomic unit length and identity matches of all oomycetes in root substrates to database.

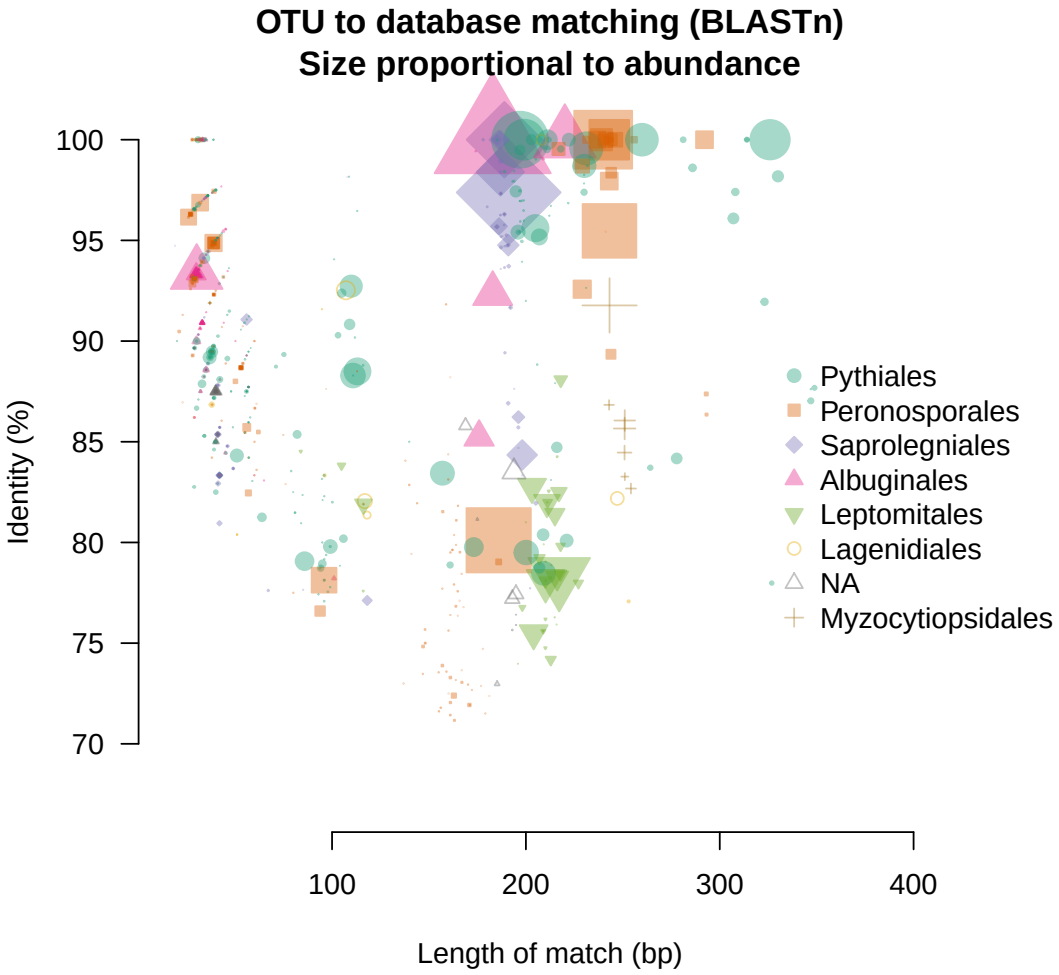

Figure A.7: Operational taxonomic unit length and identity matches of all oomycetes in leaf substrates to database.

Appendix B

Supplement Chapter 4

B.1 Environmental variables

The environmental variables in Table B.1 demonstrate the differences between land use categories and the diverse environments in this study.

Table B.1: Environmental variables (pH, WHC=water holding capacity (% moisture content on a dry weight basis), C=organic carbon (%), N=total nitrogen (%), C/N=carbon nitrogen ratio, Olsen P (mg/kg), nitrate (mg/kg), total P (mg/kg), Ca (cmol(+)/kg), Mg (cmol(+)/kg), K (cmol(+)/kg), Na (cmol(+)/kg), CEC=cation exchange capacity (cmol(+)/kg), Base sat=base saturation (%), mar=mean annual rainfall (mm), mat=mean annual temperature (°C), altitude (m), slope (°)) and their mean with standard error (SE) in each land use category.

	Natural forest		**Planted forest**		**Low-producing grassland**		**High-producing grassland**		**Perennial cropland**	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
pH	4.40	0.13	4.96	0.09	5.40	0.13	5.54	0.08	5.98	0.07
WHC	167.82	17.00	100.48	8.17	89.20	5.40	86.06	4.07	69.11	4.95
C	9.64	1.40	5.53	0.49	4.70	0.30	4.79	0.49	3.33	0.36
N	0.43	0.04	0.32	0.02	0.40	0.03	0.41	0.04	0.31	0.03
C/N	22.40	1.72	17.40	0.86	11.93	0.48	11.53	0.62	10.60	0.21
Olsen P	7.27	1.04	11.33	2.35	15.53	3.07	22.87	3.20	35.80	6.77
nitrate	6.05	3.71	10.21	1.76	14.53	5.02	28.17	6.27	22.68	4.02
total P	473.13	50.38	503.33	53.02	765.27	57.40	1061.60	183.16	1085.00	159.86
Ca	3.43	0.67	6.76	0.80	11.71	1.97	11.69	1.85	15.54	2.29
Mg	1.55	0.29	2.08	0.33	2.69	0.45	1.86	0.24	1.91	0.27
K	0.36	0.07	0.59	0.06	0.64	0.09	0.56	0.09	0.86	0.14
Na	0.25	0.04	0.23	0.03	0.24	0.05	0.27	0.05	0.18	0.03
CEC	30.42	3.42	26.27	1.99	23.28	1.99	20.74	1.71	20.83	2.45
Base sat	19.20	3.00	38.13	4.00	63.27	6.91	69.60	6.35	86.73	2.86
mar	2716.53	365.47	1300.93	88.56	1303.73	153.15	1140.93	90.68	913.13	82.38
mat	10.24	0.422	10.760	0.337	10.780	0.575	11.900	0.486	12.573	0.283
altitude	442.00	70.66	344.67	40.18	387.60	60.02	152.13	34.82	48.40	14.68
slope	20.07	3.80	19.07	3.17	19.80	2.82	7.60	2.14	0.07	0.07

B.2 Full multiple regression models of plant pathogen communities

Table B.2: Results of the multiple regression models showing significance and variance explained in the different plant pathogen communities as response matrices and land use, climate, soil, geomorphology and plant community as predictor matrices.

Plant pathogen community	**F-test**	**R^2**	**p-value**	**Significance**
fungi in soil	166.02	0.23	<0.001	***
fungi in roots	111.19	0.17	<0.001	***
fungi in leaves	375.50	0.41	<0.001	***
oomycetes in soil	103.19	0.18	<0.001	***
oomycetes in roots	70.73	0.14	<0.001	***
oomycetes in leaves	6.42	0.03	0.169	n.s.
bacteria in soil	57.22	0.10	<0.001	***
bacteria in roots	74.56	0.14	<0.001	***
bacteria in leaves	190.00	0.27	<0.001	***

B.3 Full multiple regression models of plant pathogen richness

Table B.3: Results of the multiple regression models showing significance and explained variance of plant pathogen richness as response matrices and land use, climate, soil, geomorphology and plant community as predictor matrices.

Plant pathogen community	**F-test**	**R^2**	**p-value**	**Significance**
fungi in soil	45.29	0.08	<0.001	***
fungi in roots	39.25	0.07	<0.001	***
fungi in leaves	93.69	0.15	<0.001	***
oomycetes in soil	93.74	0.16	<0.001	***
oomycetes in roots	31.07	0.07	<0.001	***
oomycetes in leaves	0.77	<0.01	0.820	n.s.
bacteria in soil	27.92	0.05	<0.01	**
bacteria in roots	127.43	0.21	<0.001	***
bacteria in leaves	50.06	0.09	<0.001	***

Appendix C

Supplement Chapter 5

C.1 Relationship between Spearman's rank correlations and p-values

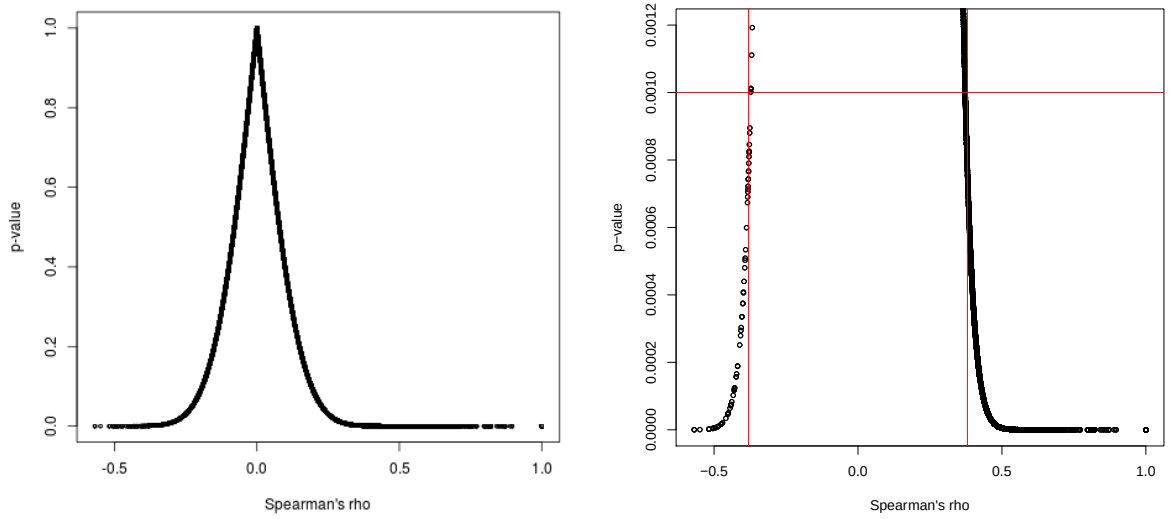

Figure C.1: Relationship between Spearman's rank correlations and p-values, indicating a significant co-occurrence below and above Spearman's rho of -0.38 and 0.38, respectively (red line).

C.2 Co-occurrence network of plant pathogens within substrates

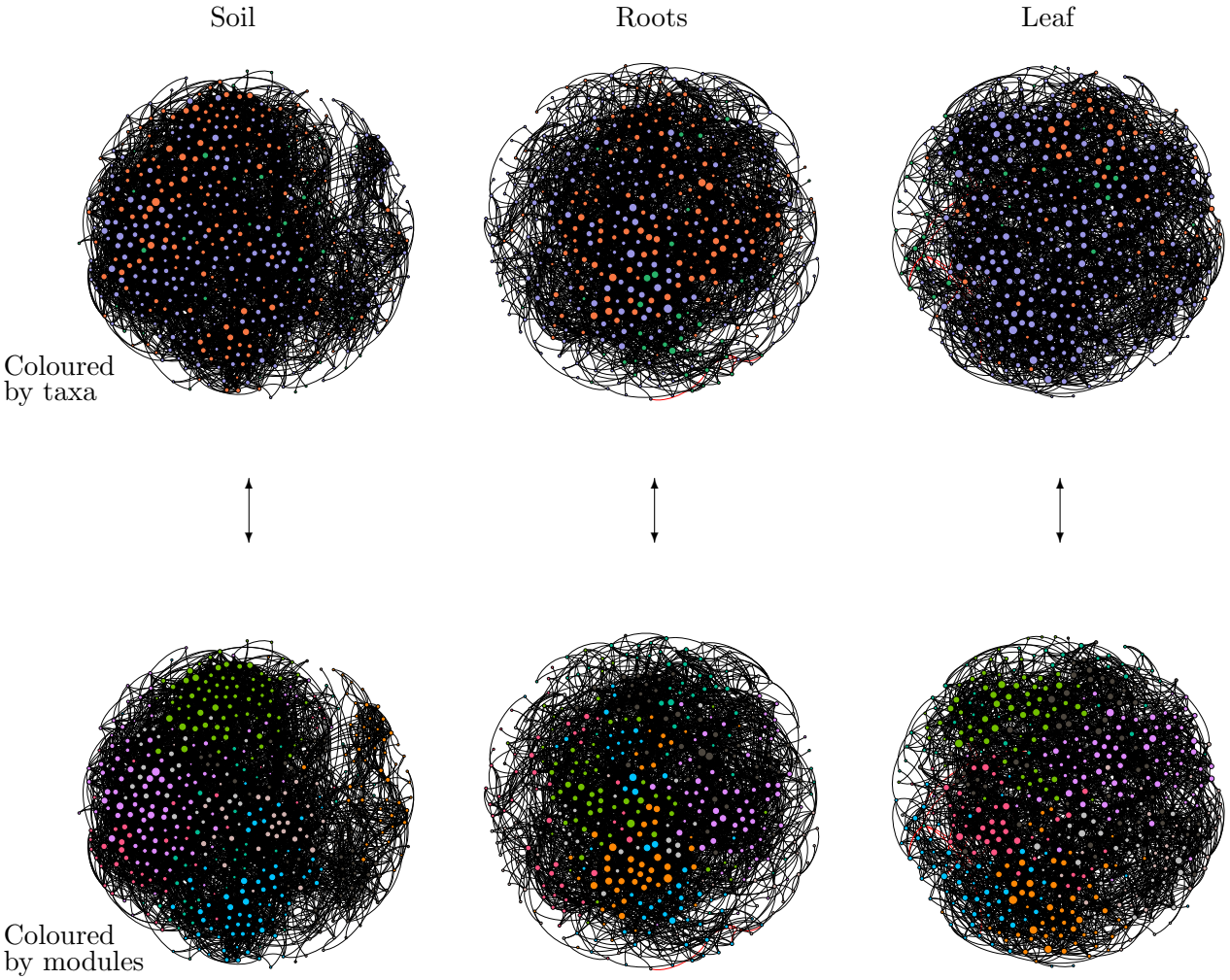

Figure C.2: Co-occurrence networks of plant pathogens across taxonomic groups for each extraction type: Soil (left), roots (centre), leaf (right). Top: Co-occurring OTUs coloured by taxa (fungi=blue, oomycete=red, bacteria=green). Bottom: Co-occurring OTUs coloured by modules. For the number of modules and other network properties see Table 5.2. Edges represent significant co-occurrence (positive=black, negative=red), and size of OTU is proportional to its degree (number of connections).

C.3 Co-occurrence network of plant pathogens within taxa

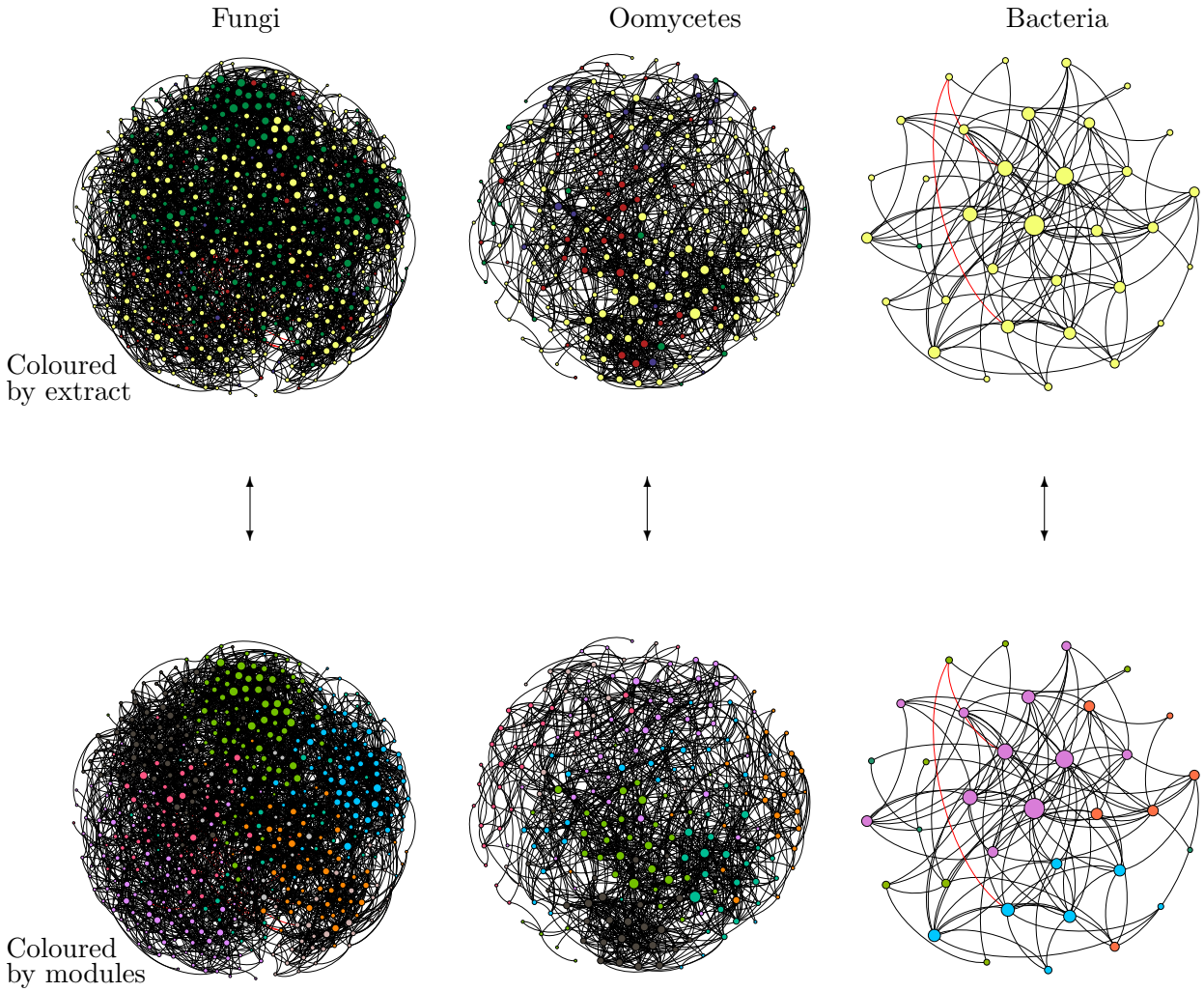

Figure C.3: Co-occurrence networks of plant pathogens within taxonomic groups across substrates. Top: Co-occurring OTUs coloured by substrate (soil=blue, roots=red, leaves=green, detected in more than one substrate=yellow). Bottom: Co-occurring OTUs coloured by modules. For the number of modules and other network properties see Table 5.3. Edges represent significant co-occurrence (positive=black, negative=red), and size of OTU is proportional to its degree (number of connections).

C.4 Co-occurrence network of plant pathogens within taxa and substrate

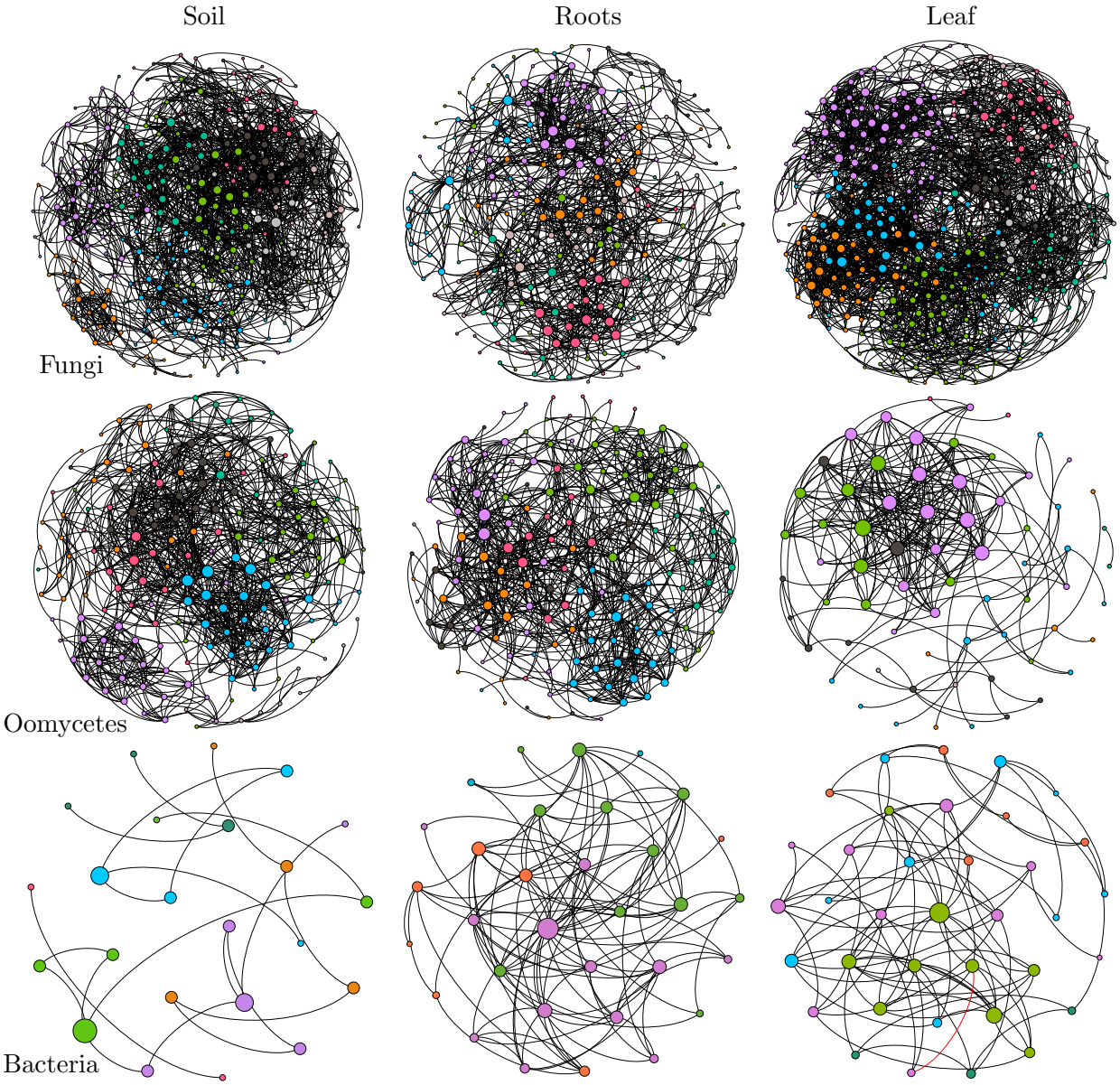

Figure C.4: Co-occurrence networks of plant pathogens within taxonomic groups for each extraction type: Soil (left), roots (centre), leaf (right). Top: Fungi. Middle: Oomycetes. Bottom: Bacteria. Co-occurring OTUs coloured by modules. For the number of modules and other network properties see Table 5.4. Edges represent significant co-occurrence (positive=black, negative=red), and size of OTU is proportional to its degree (number of connections).

Appendix D

Supplement Chapter 6

D.1 Example of the relationship between over- and undersplit operational taxonomic units (OTUs)

The proportion of over- and undersplit OTUs (over- and under-representation of species names for each OTU) can be very useful and help to determine an approximation of an appropriate OTU clustering threshold, here shown using rust fungal OTUs as an example.

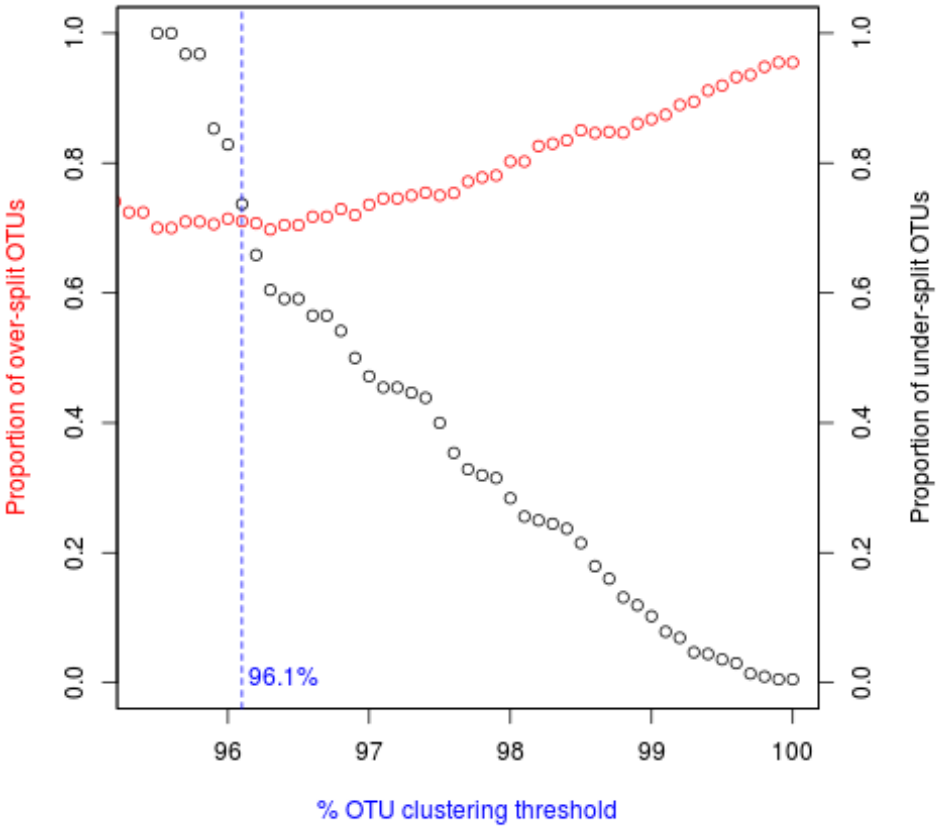

Figure D.1: Relationship and crossover between over- and undersplit OTUs (over- and under-representation of species names for each OTU), using rust fungal OTUs as an example.

D.2 Logistic richness model

Standardisation of different sampling effort or sequencing depths can sometimes be achieved by using the residuals of a fitted model. In this thesis several richness models were explored, such as a square root, a quadratic, a Michaelis Menten, and a logistic model. The best fitted model was a log-model ($F_{1,73}$=126.99, R^2=0.63, P<0.001, Fig. D.2). However, only about 63% of the data could be described well enough.

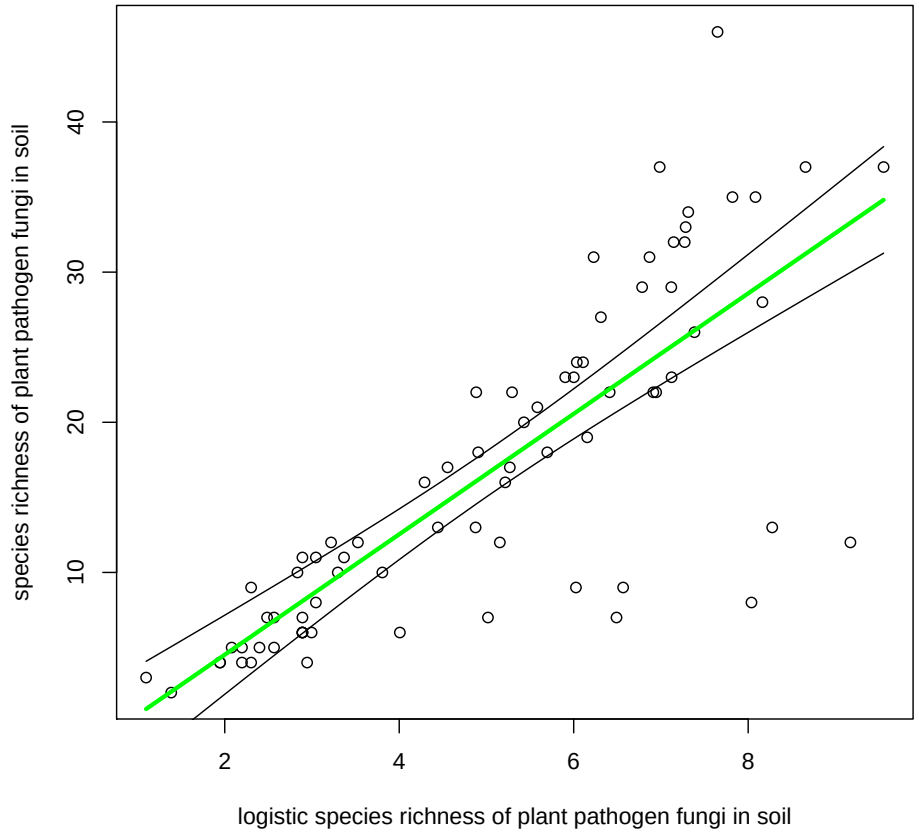

Figure D.2: Relationship between log-transformed richness and actual richness with 95% confidence intervals, using soil fungal OTUs as an example.