Natural enemies and the diversity of plant communities

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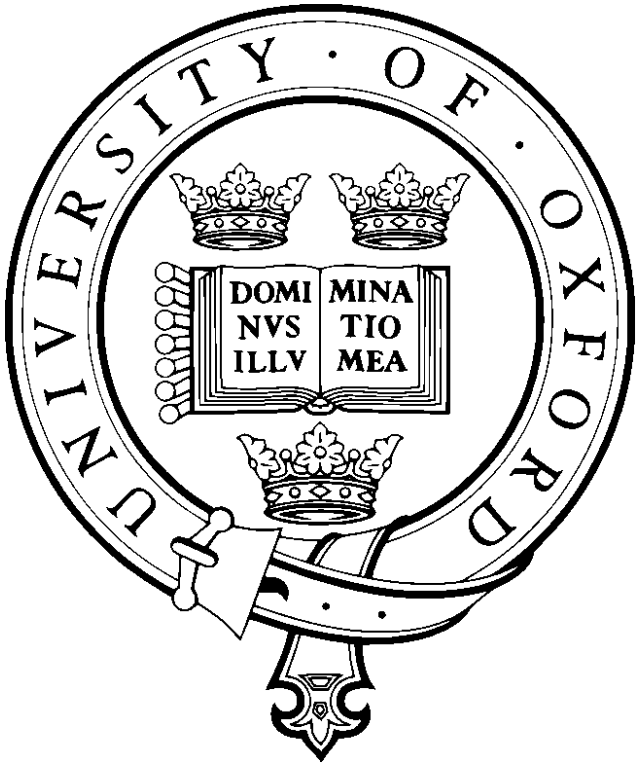

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Abstract

The processes that determine the structure of plant communities are of considerable practical and theoretical interest. Natural enemies such as herbivores, seed predators and pathogens provide one potentially important influence on plant diversity. I investigated the effects of natural enemies on plant diversity in two contrasting, species-rich plant communities (tropical forests in Panama and temperate grasslands in the UK), focusing on pre-dispersal seed predation by insects, and the mortality of seeds and seedlings caused by soil fungi. In Panama I found that pre-dispersal insect seed predators generate significant levels of mortality in multiple tropical tree species, with high heterogeneity in predation rates among individuals and at different forest sites. Insect seed predators were highly host-specific, consistent with a role in enhancing plant diversity. At Upper Seeds, a calcareous grassland site in the UK, I used manipulative experiments to show that soil fungi increase the diversity of plants propagating from soil seed banks. A parallel experiment in Panama, mimicking germination under light gap conditions, revealed differential effects of fungi among sites, with fungicide treatment appearing to increase the diversity of propagated seedlings at some sites but reducing it at others. These results suggest that the influence of soil fungi on pre-emergence mortality can alter plant diversity, even when post-emergence mortality from fungal pathogens is limited. In Panama, I also tested whether enemy-mediated mortality increases with rainfall, potentially contributing to the positive regional correlations widely observed between precipitation and plant diversity. In contrast to predictions, neither pre-dispersal insect seed predation nor the influence of soil fungi on seedling recruitment were affected significantly by site humidity, or (for soil fungi) with experimentally manipulated soil moisture levels. Overall, my results provide evidence that pre-dispersal seed predators and soil fungi can affect plant recruitment and diversity at early life stages, with potential consequences for the community structure of adult plants.

Dedications and acknowledgements

To my Dad, who I wish could have joined me

To my Gran, who supported and encouraged me

And foremost to my Mum, who is always there for me

To Laura, who has put up with a lot and still hasn't run away

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To all CERO members and residents of office E41 past and present, for sharing their knowledge and tolerating numerous monologues (both relevant and irrelevant to this thesis)

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Declaration of author contributions

Collaborating authors in this thesis are Clive Hambler (CH), Joe Wright (JW), Lars Markesteijn (LM), Owen Lewis (OL), Patrick Kennedy (PK), Phoebe Griffith (PG), and Simon Mortimer (SM), Sofia Gripenberg (SG), and Stephen Harris (SH).

Chapter 2: Idea formulation and experimental design were assisted by SG, OL, and JW. Chapter construction was assisted by SG and OL.

Chapter 3: Idea formulation and experimental design were assisted by SG, OL, LM and PK. Data collection was conducted primarily by PK and PG, and assisted by OL. Chapter construction was assisted by SG and OL.

Chapter 4: Idea formulation and experimental design was assisted by OL and CH. Plant identification was conducted by SH. Plant community quadrat data was provided by SM. Chapter construction was assisted by OL and CH.

Chapter 5: Idea formulation and experimental design were assisted by OL and LM. Data collection was assisted by LM. Chapter construction was assisted by LM and OL.

Valuable additional contributions to each chapter are listed in corresponding 'Acknowledgements' sections.

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General introduction

Christopher T. Jeffs

General introduction

In this brief introduction I provide context for the chapters within my thesis investigating the importance of natural enemies – such as herbivores, seed predators and pathogens – in influencing plant community diversity. I first highlight the significance of understanding the processes structuring plant diversity and outline how mechanisms delaying or preventing competitive exclusion enhance and maintain diversity. I go on to highlight how the effects of natural enemies on plant survival, particularly at early life stages, have great consequences for plant community structure. I proceed to review key research demonstrating the effects of natural enemies attacking seed and seedling stages on plant recruitment and diversity, and focus on key contexts in which natural enemies are likely to have great importance. Finally, I outline how each chapter of my thesis aims to address gaps in our knowledge within two key contexts; pre-dispersal insect seed predation in tropical forests and the influence of fungal pathogens within the soil seed bank.

1.1 The value of plant diversity

A central aim of community ecology is to elucidate the processes determining plant community structure i.e. species composition, richness and relative abundance (Crawley & May 1987; Crawley 1997; Wright 2002). As plants form the basis of terrestrial food chains, understanding how plant communities are structured is integral for understanding patterns of diversity in associated higher trophic levels. Protecting and promoting diverse plant communities is an important aim for conservation for reasons of stewardship, for their intrinsic and recreational value, their economic value, and also to maintain the beneficial functions and services that species provide (e.g. nutrient cycling, water retention and carbon storage; Diaz & Cabido 1997) (Crawley

1997). Levels of ecosystem functioning are known to increase with plant diversity (Hector & Bagchi 2007), and thus understanding the processes that enhance and maintain diverse plant communities are relevant to maintaining healthy, functioning ecosystems. With greater understanding of the processes determining plant composition and diversity, we can also improve our ability to forecast how anthropogenic climate change and habitat degradation may influence plant communities and the ecosystem functions they provide (reviewed by Chapin et al. 2000; Hooper et al. 2012).

1.2 Mechanisms influencing plant community diversity

1.2.1 Background

Physical and physiological traits determine the range of abiotic conditions in which an individual can survive and reproduce (Crawley 1997). However, whether a species can persist under certain abiotic conditions is influenced by competition for resources with other coexisting species (i.e. the fundamental vs the realised niche). Plant competitive ability is therefore determined by both the abiotic and biotic environment (Tilman 1997).

Generally in a homogeneous environment, e.g. with uniform soil and climate conditions, the species best able to obtain and utilise available resources will exclude the weaker competitor from that space (Hardin 1960) thus lowering species richness in the community. Processes that prevent or delay competitive exclusion therefore enhance diversity. Numerous mechanisms can facilitate coexistence between competing species by reducing opportunities for direct competition. For instance, spatial and temporal variation in environmental conditions (Ricklefs 1977; Chesson 1985), periodic disturbance creating regeneration sites for weaker competitors (Grubb 1977), and trade-offs between dispersal/fecundity and competitive ability (Levins & Culver 1971; Levin 1974; Slatkin 1974; Crawley & May 1987) can provide refuges allowing the persistence of

weaker competitors. However in addition to direct interactions between plant species, interactions with non-plant mutualists and antagonists can also determine the ability of plant species to coexist. These interactions and their consequences are the focus of my thesis.

1.2.2 Effects of natural enemies on plant recruitment and diversity

Plant species are subject to attack from a wide range of natural enemies primarily grouped into three broad categories; invertebrates (e.g. arthropods, molluscs and nematodes), vertebrates (e.g. mammals and birds), and microbial pathogens (e.g. fungi, oomycetes, bacteria and viruses). Attack by an enemy directly influences the growth and survival of a plant individual with potential population and community level consequences (Crawley 1997). Whilst natural enemy attacks on adults can affect community structure (Bakker et al. 2006; Allan et al. 2010; Pautasso et al. 2013), a greater prevalence of processes influencing plant performance non-randomly in respect to species identity occur at early life stages (Green et al. 2014). Survival at early life stages has therefore been identified as being highly influential in determining plant population dynamics and community structure (Webb & Peart 1999; Harms et al. 2000; Bagchi et al. 2014). The majority of research investigating the biotic determinants of plant community diversity has therefore concentrated on the effects of natural enemies on survival at the seed and seedling stages.

When natural enemies differentially affect the performance of individual species, we see changes in both species evenness and richness, the components determining measures of community diversity (Magurran & McGill 2011). Ultimately, biotic interactions reducing the relative abundance of dominant species can promote diversity, while those reducing the relative abundance of rare species can decrease diversity. Natural enemies may also affect diversity if their action causes the extirpation, or the establishment and persistence of a species within the community.

Diversity enhancing effects may be particularly strong if host-specific natural enemies generate

negatively density-dependent survival, limiting the abundance of potentially dominant species. The Janzen-Connell (JC) hypothesis proposes that host-specific natural enemies can enhance plant diversity by driving negative density-dependent and positive distance-dependent survival (Figure 1.1), giving rare species an advantage and preventing the exclusion of weaker competitors (Janzen 1970; Connell 1971). Conversely, positively density-dependent survival can also occur where a larger proportion of individuals survive at greater conspecific densities. Positive density-dependent survival facilitates an increase in the dominance of a species with a corresponding reduction in community diversity unless other factors regulate population densities. However, Crawley (2013) highlights that even when mortality is density-independent, spatial and temporal variation in mortality within and between species can also enable coexistence of competing species by creating refuges for persistence of the weaker competitor.

The intensity of mortality caused by a natural enemy species, its host-specificity, and its response to spatially varying resource densities are factors particularly likely to determine the impact of an enemy on recruitment success (Janzen 1970; Lewis & Gripenberg 2008; Crawley 2013). For the remainder of this introduction, I provide an overview of these important factors and how they determine the likely impacts of natural enemies on plant recruitment and community diversity. I focus on four key early life-stages: a) pre-dispersal seeds, b) post-dispersal seeds above ground, c) post-dispersal seeds below ground, and ultimately d) seedlings. Due to the large scope of the subject area, below I highlight key contexts in which mortality at each of these critical early life stages is likely to be important in determining the diversity of plant communities.

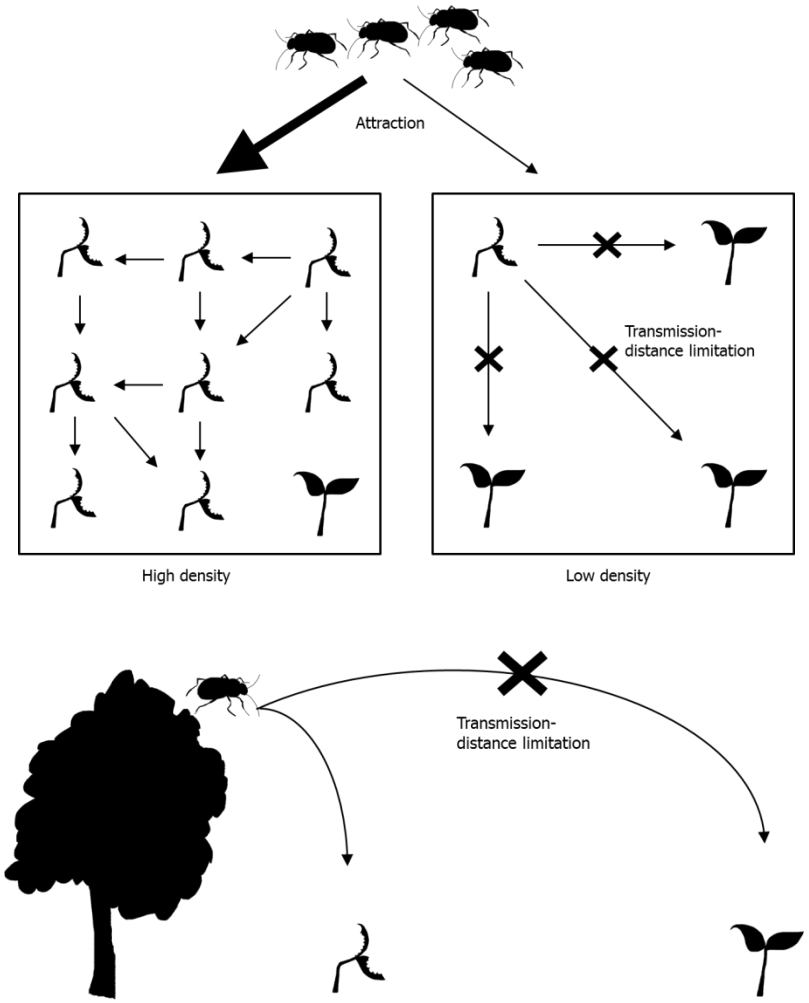

Figure 1.1. Examples of enemy-mediated mechanisms enhancing plant diversity. Above: Negative density-dependent survival. Animal enemies can be more strongly attracted to higher densities of resources (e.g. due to higher chemical attractant concentrations) resulting in greater proportional mortality than for low density plots attracting fewer enemy individuals. Additionally, a greater proportion of plant individuals may be killed at higher densities as the transmission of enemies between individuals is more effective (e.g. via contact and splash transmission of pathogens) relative to lower densities where transmission-distances may be too great. Below: Positive distance-dependent survival. Parent plants and conspecifics act as a source of enemies able to disperse to and attack juvenile conspecifics. The probability of survival increases with distance of a juvenile from its parent due to the reduced probability of transmission of dispersal-limited enemies.

1.3 Life stages

1.3.1 Pre-dispersal seed mortality

A diverse range of enemy groups can destroy seeds before they are released from the parent plant. Vertebrates such as mammals (primates, bats, and rodents) and birds are important seed predators removing seeds from parent plants but can also act as seed dispersers (Dennis et al. 2005; see below). Insects that develop within seeds or fruits are also a particularly important source of mortality at this stage (Vander Wall et al. 2005; Kolb et al. 2007; Lewis & Gripenberg 2008). Particular attention has been given to those attacking herbaceous species in agroecosystems due to their potential use as biological control agents of weeds (e.g. Westerman et al. 2008; Delerue et al. 2014). Microbial enemies can also be a source of seed mortality at the pre-dispersal stage with most examples focussing on fungi (Beckman & Muller-Landau 2011), although this group is far less frequently studied than animal seed mortality agents.

Excluding species that reproduce vegetatively, spermatophytes depend on the production of seeds for reproduction. Enemy mediated seed mortality reduces the number of viable seeds produced by an individual, even reversing interspecific differences in fecundity when pre-dispersal seed mortality rates differ. Pre-dispersal seed mortality therefore has potential consequences for seedling recruitment, the population dynamics of strongly competing species (Green & Palmbald 1975), and ultimately for community diversity (Greig 1992; Turnbull et al. 2000; Nathan & Muller-Landau 2000; Wright et al. 2005; Svenning & Wright 2005; Kolb et al. 2007).

1.3.1.1 The intensity of pre-dispersal seed mortality and effects on recruitment:

Insect pre-dispersal seed predators can frequently generate high mortality, killing >90% of seeds within a crop, although low and modest predation rates are potentially more typical (Janzen 1971; Hulme & Benkman 2002; Fenner & Thompson 2005; Kolb et al. 2007; Crawley 2013). Seed addition experiments suggest that most plant species are seed-limited at some level, i.e. greater

seed production results in increased population size (Turnbull et al. 2000; Svenning & Wright 2005; Clark et al. 2007). Therefore when seed limitation occurs, even small reductions in viable seed production such as through seed predation can lower seedling recruitment (Louda et al. 1990; Louda & Potvin 1995; Svenning & Wright 2005). Louda (1982) applied insecticide to the shrub *Haplopappus squarrosus* during flowering which decreased pre-dispersal insect seed predation from 94% to 41%. The reduction in seed predator-mediated mortality resulted in 23-fold increase in the number of recruited seedlings, and to increases in flowering adult densities. It is suggested that such effects may be typical of short-lived perennial species relying on regeneration from current seeds and transient seed banks (Louda & Potvin 1995). Such examples from forest ecosystems, especially in the tropics, are notably limited and even typical rates of pre-dispersal seed predation across species are poorly known.

However, high predation rates do not necessarily translate into large reductions in population densities in seed limited species when density-dependent compensatory mortality occurs at later life stages (Kolb et al. 2007; Fenner & Thompson 2005; Crawley 2013). Similarly, high predation rates may not affect recruitment when the abundance of suitable germination sites is limiting, i.e. when a species is microsite, not seed, limited (Crawley 2013).

1.3.1.2 Tropical forest diversity – host-specificity and density-dependent responses:

Seed limitation has been documented to be common and strong in tropical tree species (Svenning & Wright 2005; Dennis et al. 2005; Clark et al. 2007), and pre-dispersal insect seed predators have been implicated as key enemies potentially enhancing tropical forest diversity (Janzen 1970; Lewis & Gripenberg 2008). Janzen (1970) suggested that pre-dispersal insect seed predators were particularly likely to generate diversity enhancing negatively density-dependent survival due to their high host-specificity relative to vertebrates (Janzen 1971; Hammond & Brown 1998; Silvius 2005) and their expected aggregative and demographic responses to changing resource densities (Hammond & Brown 1998). However despite evidence of high host-specificity, demonstrations of pre-dispersal insect seed predators generating negatively density-dependent survival are

extremely rare (but see Jones & Comita 2010). In addition, positive density-dependent survival may also be common at the pre-dispersal seed stage (Crawley 1989; Wright et al. 2005; Crawley 2013) due to 'satiation' occurring at the highest seed abundances. Satiation favours clumped distributions and a lowering of community diversity (Fenner & Thompson 2005; Hulme & Kollman 2005), and may mask negative density-dependence at lower resource abundances (e.g. Jones & Comita 2010). Currently, the relative prevalence of seed predator-mediated negative and positive density-dependent survival is unknown in tropical forests. Further research is therefore clearly needed to test this oft-cited diversity enhancing role of pre-dispersal insect seed predators (Janzen 1970; Lewis & Gripenberg 2008; Jones & Comita 2010).

1.3.1.3 Summary

The intensity of enemy mediated pre-dispersal seed mortality is highly variable between plant species. The effects of pre-dispersal seed predation on recruitment will be strongest in seed limited species relying on the current year's seed production. Diversity enhancing effects of seed predators will therefore be greatest where seed limitation is common among species and when generating negatively density-dependent survival. Conversely, seed predation will have minor effects on recruitment when plants regenerate primarily by vegetative propagation or from a persistent seedbank, when regeneration is micro-site rather than seed limited, and when seed predators are commonly satiated at high resource densities. Despite the potentially important ecological role of this enemy guild, demonstrations of the effects of enemy mediated pre-dispersal seed mortality on long term population dynamics and community diversity are conspicuously scarce.

1.3.2 Above ground post-dispersal seed mortality

The species responsible for mortality pre-dispersal are typically distinct from those acting at the post-dispersal stage of the same plant species (Fenner & Thompson 2005). Common guilds of post-dispersal mortality agents are foragers such as ungulates, rodents, birds, carabid beetles and

ants, and internal feeders and parasites such as weevils and lygaeid bugs (Andersen 1987; Manson et al. 1998; Vander Wall et al. 2005; Hulme & Kollman 2005; Velho et al. 2012) whilst fungi are also seed mortality agents (Gilbert 2002; Tomita et al. 2002). As with pre-dispersal mortality, post-dispersal seed predation can be very high with cases >90% (Dennis et al. 2005; Crawley 2013) but with lower rates likely to be typical (Hulme & Benkman 2002). Whilst invertebrates can generate high intensities of mortality post-dispersal (Crawley 2013), numerous studies have found vertebrates to generate a far greater intensity of seed mortality than invertebrates in tropical palms (Nottman & Villegas 2005) and temperate plant species (Crawley 1989). Seed predation levels by vertebrates can be very high in tropical forests (Dennis et al. 2005), and in temperate forests a small number of rodent species may drive the majority of seed predation (Hulme & Kollman 2005).

However, as with pre-dispersal losses even high post-dispersal seed mortality may not alter recruitment if plant species are microsite rather than seed limited. For instance, experiments combining seed addition and predator exclusion suggest that post-dispersal seed predation by rodents in herbaceous grasslands may limit recruitment rates of some species, but others experience compensating density-dependent seedling mortality at later life stages (e.g. due to plant competition or seedling herbivory) nullifying consequences of seed predation on plant population dynamics (Edwards & Crawley 1999). Still, examples where post-dispersal seed predators reduce plant recruitment are present in numerous ecosystems (e.g. ants in temperate woodlands, Anderson 1987; rodents in tropical rainforests Paine & Beck 2007). For instance, Silman et al. (2003) demonstrated that the loss of white lipped peccaries from a Peruvian rainforest due to poaching resulted in greater seedling densities in a species of palm. In tropical forests the literature on above ground post-dispersal seed predation is dominated by studies on palm species (Arecaceae) (Beck 2005), suggesting further research is required to test the generality of these patterns in other plant families and ecosystems.

1.3.2.1 Effects of post-dispersal seed predation on the spatial patterns of recruitment:

Spatial variation in enemy-mediated mortality can modify plant distributions between the seed, seedling, and resultant adult life stages (Hutchings 1997) in a way reflecting their seed searching strategy and efficiency (Lewis & Gripenberg 2008). Post-dispersal seed predation can therefore directly alter the degree of conspecific aggregation (Hutchings 1997) with consequences for community diversity. Such effects will be particularly strong when survival is density- or distance-dependent. For instance, small mammals have been documented to enhance seedling diversity in a Peruvian rainforest through generating negatively density-dependent survival, disproportionately consuming abundant species (Paine & Beck 2007). Seed dispersal typically results in greater seed densities at shorter distances from the parent resulting in spatially 'clumped' recruitment (Janzen 1970). However, positive distance-dependent survival (figure 1.1) can enhance diversity by creating a more regular spacing of recruited conspecifics (e.g. Wyatt & Silman 2004). Greater mortality closer to the parent plant creates space in which heterospecific species not sharing the same natural enemies can recruit (Janzen 1970; Connell 1971).

Mobile seed predators may generate distance-dependence when using parent plants as cues to locate resources (Crawley 1997) or when being attracted to high seed densities closer to parent trees (Watkinson 1989; Nottman & Villegas 2005). However, as with pre-dispersal seed predators, satiation appears to be common at the post-dispersal seed stage (Anderson 1987; Fenner & Thompson 2005; Wright et al. 2005; Bachi et al. 2011; Crawley 2013). Satiation at high seed abundances can even mask positive distance- and negative density-dependent survival at lower abundances, resulting in the spatial aggregation of recruits (e.g. Anderson 1987). Whilst generalists (typically vertebrates) may be less vulnerable to satiation (Crawley & Long 1995), host-specific enemies (typically internally feeding invertebrates) are more likely to generate frequency stabilising negative density-dependence (Janzen 1970; Hammond & Brown 1998). Invertebrates are also suggested to have more restricted foraging ranges, favouring the generation of distance-dependent survival (Hammond & Brown 1998; Vander Wall et al. 2005). Therefore whilst vertebrates have been shown to generate distance-dependent seed predation (Wyatt &

Silman 2004) and enhance seedling community diversity through density-dependent predation (Paine & Beck 2007), invertebrates may be more likely to generate these effects (Nottman & Villegas 2005).

1.3.2.2 A dual-role: seed-predator and seed-disperser

Many vertebrate frugivores and granivores disperse and deposit seeds widely through the environment in a viable condition (e.g. via spitting after pulp removal or when defecated after ingestion; Lambert & Chapman 2005) (Dennis et al. 2005; Fenner & Thompson 2005). This dual-role of post-dispersal seed predators is likely to be particularly important in determining spatial patterns of seedling recruitment in two non-mutually exclusive contexts. Firstly, when suitable microsites are abundant but plants rely on animal dispersal for their seeds to reach them. Secondly, when dispersal facilitates the escape of seeds and seedlings from distance- and density-dependent mortality agents (Janzen 1970; Dennis et al. 2005). For instance, the African tree species *Balanites wilsoniana* depends entirely on elephants for seed dispersal. In forests where elephant poaching leads to local extinction, the lack of dispersal results in intense distance-dependent seedling mortality and lower overall recruitment threatening population persistence (Babweteera et al. 2007). Scatterhoarding and caching seed predators can also facilitate escape from distance or density-dependent enemies, reduce intraspecific competition at later life stages, and result in a more evenly distributed (i.e. non-aggregated) spatial pattern of recruits (Smith & Follmer 1972; Hutchings 1997). Scatterhoarding animals may even be essential for regeneration of large seeded mast fruiting trees (Hulme & Kollman 2005; Crawley 2013).

1.3.2.3 Summary:

Post-dispersal seed predators can cause significant mortality in many plant species with consequences for plant recruitment. Enemy mediated positive distance-dependent and negative density-dependent survival may reduce the spatial aggregation of recruits, enhancing plant community diversity. The dual-role of animals as seed predators and dispersers may further alter the spatial distribution of recruitment, enhancing recruitment especially in microsite limited

species and those suffering distance- and density-dependent mortality.

1.3.3 Below ground post-dispersal seed mortality

Smaller seeds are more likely to be incorporated into the soil and form a dense seed bank than larger seeds (Thompson et al. 1993; Dalling 2005), and are typically overlooked by animal seed predators post-dispersal (e.g. seeds <3mg in temperate species, Thompson 1987; <100mg in tropical species, Dalling 2005). Although insects and earthworms can generate substantial seed mortality within the soil (Thompson 1987; Alvarez-Buyulla & Martinez-Ramos 1990; Dalling 2005), dormancy for extended time periods within the soil greatly increases a seed's vulnerability to pathogens (Alvarez-Buyulla & Martinez-Ramos 1990; Dalling, Swaine & Garwood 1998; Schafer & Kotanen 2004). Fungicide addition experiments in both temperate and tropical ecosystems demonstrate that the effects of fungal pathogens (fungi and fungus-like oomycetes) on survival are highly plant species-specific and dependent on the time since burial (reviewed by Wagner & Mitschunas 2008) eventually reaching or approaching 100% mortality (Dalling et al. 1997). Greater soil moisture levels can also enhance fungal-mediated seed mortality (e.g. Schafer & Kotanen 2003), potentially creating spatial and regional variation in the influence of pathogens on recruitment and diversity.

1.3.3.1 Importance of below ground enemy mediated mortality in the regeneration niche:

The seed bank is particularly important to the maintenance of plant diversity with many species depending on their 'regeneration niche' for persistence, escaping from stronger competitors and recruiting even after being absent from the aboveground flora for years (Grubb 1977; Wellstein et al. 2007). In temperate grasslands, soil disturbance creating areas of bare ground (e.g. following grazing, vertebrate digging, wind or frost upheaval) is essential for propagation and recolonisation from the seed bank (Kalamees & Zobel 2002; Fenner & Thompson 2005), whilst in more arid grassland fires can stimulate germination (Snyman 2005). In tropical forests 10 - 20% of tree species are classified as dependent on propagation from the seed bank when tree falls occur creating light gaps and upturned soils (Dalling 2005). Garwood (1983) demonstrated that early

germination from the seed bank is particularly important in determining the outcome of competition in tropical light gaps. Enemy mediated mortality affecting the density and diversity of viable seeds within the seed bank is therefore likely to have key implications for recruitment, establishment, and resultant community diversity in gap environments.

It is typical for a very small number of species to contribute the vast majority of germinating seedlings from tropical seed banks (Dalling et al. 2002). Disproportionately high pathogen-mediated mortality in numerically dominant species can cause seasonal increases in seed bank evenness and diversity (e.g. Dalling et al. 1997). Fungicide addition experiments also demonstrate that fungal pathogens can generate positive distance-dependent survival within the seed bank under parent trees (Dalling, Swaine & Garwood 1998). Thus, even when the density of seeds for a given species is greater nearer to the parent tree, pathogen-mediated distance-dependent mortality can enhance the local diversity of seed banks and potentially of seedlings germinating from the seed bank. How important enemy mediated mortality within the seed bank is for seedling recruitment within tropical and temperate ecosystems is currently unknown. Few species have been tested and rarely have such effects been related to the subsequent diversity of seedlings propagating or recruits establishing. Seed-banks comprise mostly *r*-selected 'ruderal' species (Grime 1977; Grubb 1977) whose seedlings may be particularly susceptible to natural enemies such as fungal pathogens due to growth-defence trade-offs (Augspurger & Kelly 1984; Dalling & Hubbell 2002; Wright 2002; Fine et al. 2006). Compensating or accentuating density-dependent mortality could therefore commonly occur at the seedling stage in seed-bank forming species.

1.3.3.2 Summary:

Smaller seeded species are more likely to be incorporated into the soil seed bank where microbial pathogens may be the key mortality agents. Pathogen mediated seed mortality can alter the density and diversity of the seed bank, with potential consequences for the diversity of successfully recruiting individuals within the regeneration niche of both forest and grassland ecosystems.

1.3.4 Seedling mortality

Large vertebrate grazers (e.g. ungulates), smaller grazers (e.g. rodents and lagomorphs), molluscs, insect herbivores of numerous guilds (e.g. leaf chewers, leaf miners, stem borers etc.), and pathogens can all act as the primary seedling mortality agents for individual plant species (Crawley 1997). The effects of natural enemies on seedling growth and survival have been extensively documented (Crawley 1989; Gilbert 2002) and the consequences of enemy mediated seedling mortality for plant community diversity have been commonly documented. For instance large generalist herbivores such as deer can severely reduce seedling regeneration causing long-term declines in species richness and altering the structure of later age classes and canopy composition even at the scale of whole woodlands (Russell et al. 2001; Rooney & Waller 2003; Kirby 2010; Fisher et al. 2010). However, Bakker et al. (2006) demonstrated that although large grazing vertebrates in low productivity grassland systems can directly reduce diversity, grazing in high productivity grasslands can enhance diversity by reducing the negative effects of light limitation on seedling establishment and species richness. Small vertebrate grazers such as rabbits and voles can also influence seedling recruitment (Hulme 1994) by selectively feeding on nutritious/palatable plant species and reducing diversity (Edwards & Crawley 1999). In tropical forests insect herbivores can greatly reduce seedling densities, alter species composition (Bagchi et al. 2014), and may even be a driving factor in determining the geographic distribution of species (Coley & Barone 1996; Gaviria & Engelbrecht 2015). Pathogenic fungi and oomycetes are possibly the most widely studied group of microbial natural enemies in natural systems and can cause especially high mortality in humid conditions at high seedling densities (Augspurger & Kelly 1984; Packer & Clay 2000; Bell 2006; Bagchi et al. 2010).

1.3.4.1 Density- and distance-dependent seedling mortality

As for seed life stages, natural enemy mediated negative density-dependent and positive distance-dependent survival is suggested to have the potential to enhance plant community diversity.

Vertebrates (Yamazaki et al. 2009), insects (Crawley 1989), molluscs (Pigot & Leather 2008) and pathogens (Augspurger 1983; Augspurger & Kelly 1984; Packer & Clay 2000; Bell et al. 2006; Bagchi et al. 2014) have all been demonstrated to generate positive distance- and negative density-dependent seedling survival. These processes have been studied far more extensively in tropical forest ecosystems following the conception of the Janzen-Connell hypothesis (see meta-analyses by Hyatt et al. 2003 and Comita et al. 2014). Numerous studies document an increase in plant diversity due to negatively density-dependent seedling recruitment, but did not test that natural enemies were the causal link (e.g. Webb & Peart 1999; Harms et al. 2000; Lambers et al. 2002; Comita et al. 2010; Metz et al. 2010). Therefore evidence of diversity enhancing Janzen-Connell effects is limited, with few studies testing for negatively density-dependent seedling performance linking enhanced community diversity to identified natural enemies (but see Petermann et al. 2008; Bagchi et al. 2014).

Fungal pathogens are suggested to be particularly likely to enhance community diversity by generating frequency stabilising Janzen-Connell effects relative to insects and vertebrates (Gilbert 2002; Freckleton & Lewis 2006). For instance, Gilbert (2005) stated that abundance limiting 'density-dependent disease development, at least for fungal and oomycete-caused diseases, is probably the rule in both tropical and temperate systems'. Supporting such suggestions are the abundant examples of fungal pathogen-mediated negatively density-dependent (e.g. Bell et al. 2006; Yamazaki et al. 2009; Bagchi et al. 2010; Lin et al. 2012; Liu et al. 2015) and positive distance-dependent seedling survival (e.g. Augspurger 1984; Yamazaki et al. 2009), with demonstrations of their action increasing tropical seedling diversity (Bagchi et al. 2014) and facilitating plant coexistence (Petermann et al. 2008). Fungal pathogens are also suggested to be relatively more likely to generate over-compensating density-dependent mortality relative to insects and vertebrates, the form of density-dependence that would most strongly enhance community diversity through frequency stabilisation (Freckleton & Lewis 2006; Bagchi et al. 2010). However not all plant species within a community may exhibit pathogen-mediated negatively density-dependent or positive distance-dependent seedling survival (Gilbert 2005;

Gripenberg et al. 2014) and the prevalence of these effects at a community level has yet to be quantified.

Negatively density-dependent seedling performance and survival have increasingly been documented in the context of plant-soil feedback (PSF) effects (e.g. reviewed by Klironomos 2002; Kulmatiski et al. 2008; van der Putten et al. 2013; Bever et al. 2015). PSF effects occur when plants alter the chemical, physical and biotic characteristics of soils with consequences for the subsequent performance of both conspecific and heterospecific individuals (Bever et al. 1997). Negative biotic PSF effects have been frequently documented in both tropical and temperate plant species (Klironomos 2002; Kulmatiski et al. 2008; Mangan et al. 2010) and have been demonstrated to enable the coexistence of different plant functional groups in experimental temperate grassland communities (Petermann et al. 2008). However, rare species have been shown to suffer to a greater extent when grown on conspecific soils than abundant species, suggesting that the strength of negative plant-soil feedback effects may *cause* interspecific differences in abundance, rather than the strength of density-dependent feedbacks being a *consequence* of conspecific adult abundance in the community (Klironomos 2002; Mangan et al. 2010). It is therefore possible that negative PSF effects can suppress the abundance of rare species decreasing community evenness and diversity, in addition to generating Janzen-Connell effects which provide a rare species advantage. Investigations linking species-specific PSF effects to community diversity are lacking and greatly needed to assess the importance of these mechanisms in structuring plant communities.

1.3.4.2 Summary

Seedlings are subject to mortality caused by a broad range of vertebrate, invertebrate and microbial natural enemies, each capable of greatly reducing seedling densities and influencing community diversity. Fungi and fungus-like oomycete pathogens have been identified as groups particularly likely to enhance plant diversity by generating negatively density-dependent and positive distance-dependent survival, and have been demonstrated to enhance plant diversity in

both tropical and temperate systems.

1.4 Conclusions

Mortality at early life stages is suggested to be particularly influential in determining plant community diversity. Natural enemies can significantly influence the performance of seeds and seedlings, with consequences for population dynamics, and both local and regional patterns of plant diversity when compensatory mortality at later life stages is limited. Through their differential effects on plant species' performances, natural enemies may enhance plant diversity when generating negatively density-dependent survival, or when creating spatial or temporal refuges for the persistence of weaker competitors by limiting competitive exclusion. Conversely, enemy mediated reductions in diversity can occur when generating mortality that reduces community evenness or species richness. How greatly an enemy may impact plant recruitment and community diversity will be influenced by the intensity of mortality it causes, its host-specificity, and its response to spatially varying resource densities.

1.5 Thesis outline

The chapters within my thesis aim to address gaps in our knowledge within two key contexts identified above where natural enemies are predicted to have prominent influences on plant recruitment and diversity: pre-dispersal insect seed predation in tropical forests, and fungal-pathogen mediated mortality within the seed bank. I conduct observational and manipulative experiments in two contrasting species rich plant communities, tropical forests in Panama and temperate grasslands in the UK, where understanding the processes determining plant community diversity are of great practical and theoretical value.

In **Chapter 2** I use seed rearing and dissection to assess whether pre-dispersal insect seed predators attacking an abundant tropical tree species can generate negatively density-dependent seed survival, and identify the spatial scale at which predation rates are structured. I discuss the potential consequences of pre-dispersal seed predation on recruitment success and community diversity in tropical forests.

In **Chapter 3** I use similar methods to investigate whether the intensity and host-specificity of pre-dispersal seed predation by insects varies across a tropical forest species richness and rainfall gradient. I discuss the potential for increased natural enemy pressure at wetter sites to drive positive associations of precipitation and plant species richness on regional scales.

In **Chapter 4** I propagate seedlings from the soil seed bank of compositionally distinct temperate grassland communities to assess the role of soil fungi and oomycetes in determining seedling community diversity. I aim to identify whether species-specific plant-soil feedback mechanisms can be linked to observed community level effects.

In **Chapter 5** I propagate seedlings from the soil seed bank of eight tropical forest sites across a tropical species richness and rainfall gradient (as in Chapter three). I examine whether soil fungi and oomycetes influence seedling community diversity and whether the intensity of natural enemy pressure increases with humidity contributing to regional scale patterns of diversity.

Finally in **Chapter 6** I provide a general discussion highlighting key themes arising from my work, and present areas where future research will be valuable in identifying the importance of natural enemies for plant diversity in my focal contexts and more widely.

Chapter 2:

Spatial variation in pre-dispersal insect seed predation in a rainforest tree

Christopher T. Jeffs, Owen T. Lewis, S. Joseph Wright & Sofia Gripenberg

Seed predator, *Chelofonyx* sp. Photo credit Indira Simon.

2.1 Abstract

1. Insect seed predators are a significant source of seed mortality in many plant species. Where seed predation generates negatively density-dependent survival (i.e., higher predation at high plant or seed densities), it can limit the dominance of individual plant species and enhance community diversity. However, the intensity and density-dependence of mortality imposed by pre-dispersal seed predators (which attack seeds before they are dispersed from the parent plant) is poorly known, particularly in species-rich tropical forests.

2. We investigated pre-dispersal seed predation in a common rainforest canopy tree (*Quararibea asterolepis*, Malvaceae) within four mapped forest plots on Barro Colorado Island, Panama.

3. Variation in pre-dispersal seed predation by insects was estimated by dissecting mature seeds and immature fruits. Predation rates of mature seeds differed significantly between forest plots, but were independent of tree size (diameter at breast height, DBH) and the density of reproductive-sized conspecifics within 50 m. None of the variables examined significantly explained variation in predation rates of immature fruits.

4. Seed predation was caused largely by a single weevil species (*Conotrachelus* sp., Coleoptera: Curculionidae) with smaller contributions from *Chelofonyx* sp. (Coleoptera: Curculionidae) and *Anastrepha* sp. (Diptera: Tephritidae). None of the seed predator species individually showed tree-size or density-dependent emergence frequencies. However, the abundance of *Conotrachelus* sp. varied significantly between forest plots, consistent with patterns in the dissection-based estimates of seed predation.

5. The number of viable seeds produced by a tree was strongly and positively correlated with tree size measured as DBH, but not with summed neighbourhood DBH or seed predation rate. For *Q. asterolepis*, spatial variation in the production of viable seeds is likely to be driven predominantly by tree size, rather than seed predation.

2.2 Introduction

The processes that generate and maintain the astonishing levels of plant diversity in tropical forests are a longstanding focus of ecological research (Wright 2002). Mechanisms enhancing plant community diversity typically limit opportunities for competitive exclusion to occur, for instance through niche partitioning (Tilman 1997), utilisation of the regeneration niche by weaker competitors (Grubb 1977), or by limiting abundances through population regulating processes (Crawley 1990). Natural enemies - such as pathogens, herbivores and seed predators - directly affect plant performance and therefore competitive ability and the probability of coexistence (Petermann et al. 2008; Wright 2002). In particular, enemy mediated mortality at early life stages such as in seeds and seedlings has been identified as having important consequences for plant population dynamics and community structure (Webb & Peart 1999; Harms et al. 2000; Green et al. 2014).

Most studies investigating enemy-mediated mortality in tropical trees have focused on dispersed seeds and young seedlings. This work has documented a variety of natural enemy groups as important sources of mortality, including microbial pathogens (Augspurger & Kelly 1984; Bell et al. 2006; Mangan et al. 2010; Swinfield et al. 2012), herbivores (Blundell & Peart 1998; Sullivan 2003; Norghauer et al. 2006), and insect (Wright 1983; Herrera 1989; Levey & Byrne 1993) and vertebrate seed predators (Schupp 1992; Wyatt & Silman 2004; Paine & Beck 2007). Mortality occurring earlier in the reproductive cycle, such as in seeds that have not yet been dispersed from the parent plant, is less widely studied. Insects are a prominent source of pre-dispersal seed predation (Janzen 1971; Crawley 2013; Chapter 3) and have the potential to affect plant recruitment and community diversity (Janzen 1970; Kolb et al. 2007; Lewis & Gripenberg 2008). However, there are few studies investigating insect pre-dispersal seed predators in tropical forests.

A leading explanation for the high diversity of plants in tropical forests is the Janzen-Connell

hypothesis, which proposes that specialised natural enemies of plants enhance diversity by causing negatively density-dependent plant survival (Janzen 1970; Connell 1971). This negative feedback mechanism provides a rare species advantage and prevents competitive exclusion, enhancing local plant diversity. Patterns of plant performance consistent with the Janzen-Connell mechanism have been documented widely in tropical forests (Harms et al. 2000; Comita et al. 2010; Bagchi et al. 2014). For enemies to cause Janzen-Connell effects, they need to be relatively host specific (Janzen 1970; Sedio & Ostling 2013). The limited data available suggest that insect pre-dispersal seed predators of tropical rainforest trees have high host-specificity, with polyphagous species typically specializing on congeneric or confamilial host species (Janzen 1980; Nakagawa et al. 2003; Lewis & Gripenberg 2008; S. Gripenberg unpublished data; Chapter 3). Additionally, since local seed densities are greatest within the canopy (i.e. prior to seed dispersal), the potential for density-dependent processes might be particularly high in the canopy environment (Janzen 1971; Jones & Comita 2010). Negatively density-dependent seed survival could result from seed predators being more attracted to individuals or neighbourhoods with greater resources abundances, or by them dispersing more easily between host individuals growing in close proximity (Hammond & Brown 1998). We are aware of only one previous study testing for negatively density-dependent seed survival mediated by pre-dispersal insect seed predators in tropical forests (Jones & Comita 2010). As a result, little data are available to assess whether density-dependent seed mortality caused by pre-dispersal enemies is a common phenomenon, and whether it plays a role in the maintenance of tropical plant diversity.

Density-dependent survival inflicted by seed predators could also be positive or show non-linear patterns. For example, at the highest seed abundances seed predators may become satiated (Ashton 1988; Toy 1991; Bagchi et al. 2011; Mezquida & Olano 2013), potentially reversing patterns of negative density-dependence observed at lower fruit abundances (e.g. Jones & Comita 2010). Satiation reduces the proportion of predated seeds, favouring clumped distributions of conspecific plant individuals and leads to a lowering of diversity at the community level, i.e. the opposite of the Janzen-Connell hypothesis (Janzen 1970; Bagchi et al. 2011).These responses

could occur at different spatial scales, e.g. to resource densities provided by individual plants as well as to the abundance of resources in the wider neighbourhood. Satiation in insect seed predators is suggested to be a common phenomenon in tropical forests. Large interannual variations in tree crop sizes limit the population sizes of host-specific seed predators in low fecundity years, and thus the ability to respond demographically in following high abundance years (see Wright et al. 2005).

Alternatively, seed predation rates may vary spatially, independent of resource densities e.g. due to environmental variation affecting local seed predator population sizes and attack rates (Delerue et al. 2014). In such instances, similarity in predation rates may decline with distance between individuals and neighbourhoods (e.g. Rossi et al. 2011) or even larger regional spatial scales. The scale at which predation rates are structured will depend largely on dispersal ability, a factor that is little known for insect seed predators (Lewis & Gripenberg 2008). Investigations into spatial structuring remain rarely documented in pre-dispersal seed predators.

Seed addition experiments in tropical forests and elsewhere suggest that most plant species are seed-limited (reviewed by Turnbull et al. 2000; Clark et al. 2007): the addition of seeds results in increased population sizes. If seed predators contribute to spatial variation in the production of viable seeds, their actions could have consequences for seedling recruitment and resultant community composition and diversity (Turnbull et al. 2000; Nathan & Muller-Landau 2000; Wright et al. 2005; Svenning & Wright 2005). Even in the absence of density-dependence, intraspecific variation in the production of viable seeds generated by pre-dispersal seed predators could have effects in the plant community, although not in a frequency stabilising fashion.

Pre-dispersal insect seed predators can also drive rates of premature fruit abscission (e.g. Greig 1992; Fernandez et al. 2008; Hosaka et al. 2009; Jones & Comita 2010), where fruits containing damaged seeds are prematurely abscised to allow the plant to allocate resources to viable seeds (Stephenson 1981; Toy & Toy 1992; Ghazoul et al. 1998). Fruit abortion mediated by pre-

dispersal insect seed predators further contributes to the effects of this guild on the input of viable seeds into the community. However, the causes of spatial variation in premature fruit abscission rates have rarely been investigated in the context of density-dependent pre-dispersal seed predation.

To assess the role of insects in generating intraspecific variation in pre-dispersal seed mortality, we collected abscised fruits from a common tree species, *Quararibea asterolepis* (Malvaceae), from four multi-hectare mapped plots within a Panamanian lowland forest. Collected seeds were used to estimate tree-specific levels of insect seed predation through the dissection of immature and mature seeds after rearing internally feeding insect seed predators. Our study aimed to assess whether seed predation rates were dependent on 1) the crop size of the individual tree, 2) the density of conspecifics in the local neighbourhood (within a radius of up to 50 m), and 3) the density of conspecifics at larger spatial scales (between mapped plots within the forest). We also assessed whether fruit abortion rates were driven by pre-dispersal seed predation rates or conspecific density at each studied spatial scale. Our overall aim was to evaluate the relative importance of seed predation and tree fecundity in determining intraspecific variation in the output of viable seeds and its potential implications for spatial patterns of seedling recruitment.

2.3 Methods

2.3.1 Study site and species

Our study was conducted between July and December 2011 on Barro Colorado Island (BCI) (9.1500° N, 79.8500° W), a moist lowland tropical forest in Panama (Leigh 1999). *Quararibea asterolepis*, Pittier (Malvaceae), is a monoecious canopy forming species with trees typically fruiting once per year (Wong et al. 1990). Fruits contain one or two seeds (average 1.58 seeds; S J. Wright unpublished data) and are animal dispersed. It is the second most abundant tree species >20 cm DBH in the 50 ha CTFS-ForestGEO forest dynamics plot on BCI (Wong et al. 1990; Condit et al. 2013).

In July 2011, we visited each *Q. asterolepis* individual with a DBH >20 cm (corresponding closely to the minimum reproductive size of 16 cm observed for this species; Wong et al. 1990) within four mapped plots outside the 50ha forest dynamics plot (one 12 ha, one 7 ha, and two 6 ha plots, pairwise distances between plots were 100 m - 600 m; Figure 2.1). DBH measurements were from 2004, the most recent census in the mapped plots prior to 2011. After dead and non-flowering individuals were excluded (only 2% of those visited), we randomly selected 28 reproductive *Q. asterolepis* individuals for study. To minimise spatial autocorrelation in the data set we excluded individuals growing within 50 m of an individual that had already been selected.

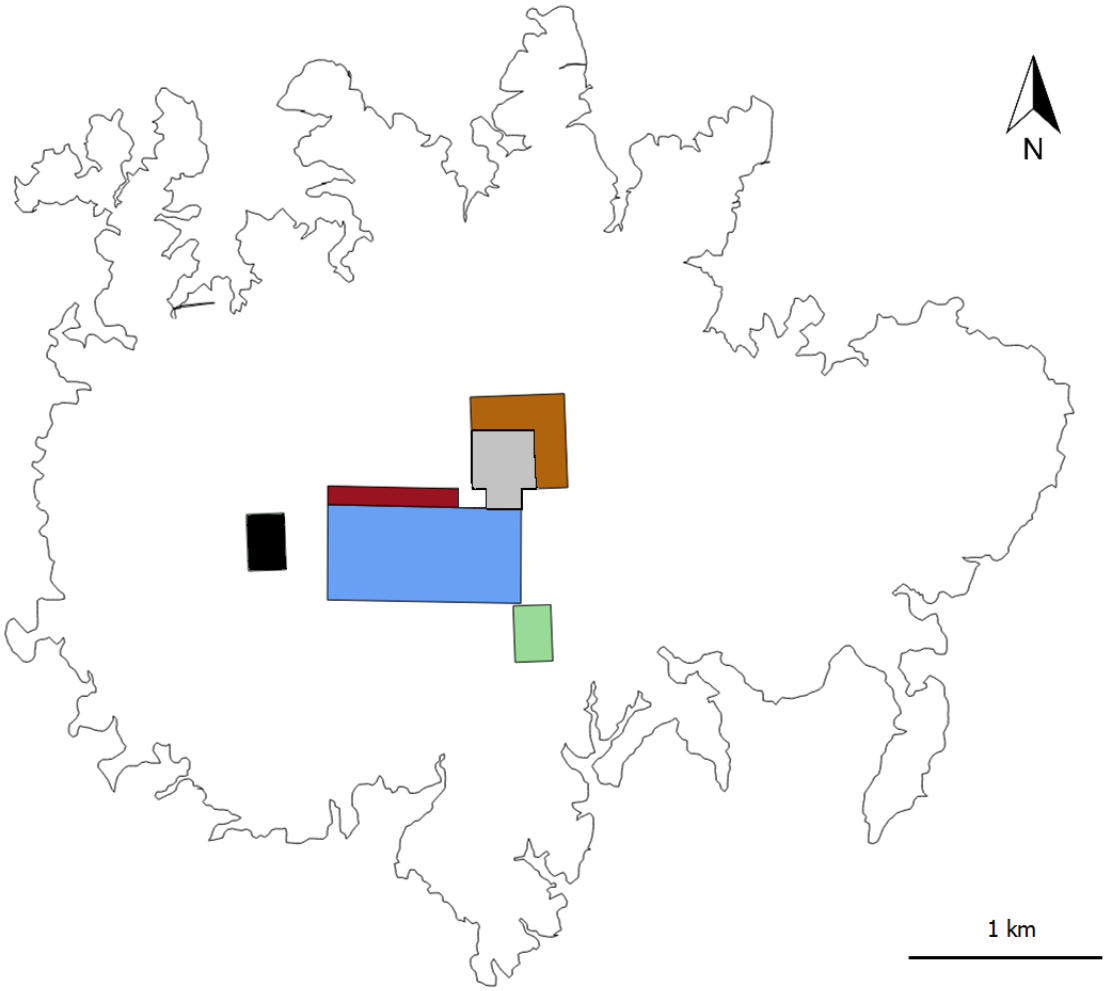

Figure 2.1. Location of mapped plots on Barro Colorado Island. Focal trees were located in four mapped plots: Zetek (black, 6 ha), Drayton (green, 6 ha), 7ha (red), and 12ha (grey). The 12ha plot consists of a smaller portion of a larger 25ha mapped plot (brown), and two hectares of a 10 ha mapped plot parallel to the top of the 50ha forest dynamics plot (blue; Condit et al. 2013). The 7ha plot also lies within the 10 ha mapped plot parallel to the top of the 50ha forest dynamics plot. Coordinate data for images were supplied by Patrick Jansen.

2.3.2 Focal tree size, fecundity and conspecific density

Due to the challenges of measuring DBH for buttress rooted species such as *Q. asterolepis*, updated DBH measures for trees within the mapped plots were taken only in 2014. The far smaller number of remaining trees (non-focal trees outside the mapped plots or 50ha plot measured in 2011 and 2010 respectively) had their DBH values for 2014 estimated using a linear regression model. The model estimated DBH based on observed growth between the 2004 and 2014 censuses for mapped plot trees (supplementary information S2.7.2). We took this decision, rather than estimating 2011 values for all trees, to reduce the number of projections used in analyses. DBH increases per year were <2% of starting DBH and caused few, minor, changes in DBH rank between focal individuals (data not presented) suggesting this decision is likely to have a negligible effect on results presented.

As crop size estimates using quadrats were potentially noisy due to seed loss (from rolling and removal by rodents) and measures were not available for non-focal trees, DBH provided a consistent measure for tree size and conspecific density calculation (see below) for all individuals. Fruit crop size was strongly, positively correlated with DBH in *Q. asterolepis* (supplementary information S2.7.1), and thus DBH provided a suitable proxy for crop size/fecundity in our analyses.

The 'density' of conspecific individuals in the neighbourhood (henceforth 'neighbourhood density') of each of our 28 focal individuals was calculated using two methods. Whilst each method is not a true density measure (e.g. the number of individuals per m^2), each reflects aspects of density seed predators are known to be responsive to i.e. resource abundance and spatial isolation (Hammond & Brown 1998). First we used a summed DBH measure based on all living conspecific individuals of reproductive size (>20 cm DBH) within a given radius every 5 m between 5 m and 50 m from each focal tree. Only trees with signs of reproduction during the fruiting season we studied were included in density calculations.

Second, we calculated a spatial connectivity index for each focal tree using the connectivity kernel (Hanski 1994; supplementary information S2.7.3). The contribution of each neighbouring conspecific individual to the index was dependent on its DBH and proximity to the focal tree i.e. DBH contributions to the index were negatively exponentially weighted by distance from the focal tree, assuming diminishing influences with distance of neighbouring individuals. We refer to these two neighbourhood density calculations as either 'summed DBH' or 'negative exponential connectivity' respectively.

For both density measures the contribution of the focal tree's DBH to the total was subtracted so that separate individual DBH and neighbourhood density explanatory variables could be fitted in statistical analyses. Where selected trees were <50 m from a plot edge, we mapped additional trees >20cm DBH outside the plot to complete the 50 m radius of each study tree. As one of the study plots was immediately adjacent to the CTFS-ForestGEO 50ha forest dynamics plot on BCI (Condit et al. 2013), tree census data from the 50ha plot was incorporated into our calculations of conspecific neighbourhood densities.

To allow us to estimate tree-specific total fruit crop size, we estimated canopy area and average fruit densities per m^2 of canopy for each of our focal trees. The location of the canopy edge was judged using binoculars underneath focal trees and by examining the distribution of abscised flowers on the ground. Canopy area was estimated by marking and measuring the widest and narrowest perpendicular transects across the canopy and calculating an ellipsoid area based on these distances (Area $= \pi ab$, where a = ½ length of the longest transect 'a', and b = ½ length of the shortest transect 'b'). To estimate fruit densities within the canopies, we placed nine 0.5 m^2 quadrats on the ground under each of our focal trees. One quadrat was placed at the centre of the marked canopy area and another eight quadrats were placed along the transects at 1/3 and 2/3 of the distance from the centre quadrat to the margin of the crown. For each tree, we summed the counts of all immature and mature *Q. asterlolepis* fruits (classified based on their size and colour; supplementary information S2.7.4) falling into each of the nine quadrats over the entire fruiting

period and used the data to calculate tree-specific fruit densities per 1 m^2 of canopy. We estimated the total fruit crop size of an individual tree by multiplying fruit density per 1 m^2 of canopy by the canopy area of that tree.

We estimated the production of mature fruits as the total estimated fruit crop size multiplied by the proportion of mature fruits collected within quadrats. Distinguishing between mature and immature fruits also enables us to calculate premature fruit abscission rates, i.e. the proportion of the total fruit crop being shed at an immature stage. The production of viable seeds was calculated as mature fruit production multiplied by 1.58 (the mean number of seeds per fruit), multiplied by the proportion of non-predated mature seeds (estimated by dissection; see below).

2.3.3 Assessing seed predation rates

We visited each focal individual on average every four days during the fruiting period (July to November 2011). At each visit, we collected all fruits within the nine 0.5 m^2 quadrats and classified them as mature or immature.

We reared all collected fruits in transparent plastic pots (round 9 cm diameter pots for small numbers of fruits or rectangular 10 × 15 × 6 cm pots for larger samples) lined with absorbent paper and with netting on the lids. Fruits collected were kept in separate pots for each tree × maturity stage × collection date combination (but fruits collected from different quadrats under a tree were pooled). We split fruits/seeds evenly into two groups reared for one month or three months in separate pots to gauge whether detection of seed predation by dissection or emergence of adult seed predators differed in relation to rearing period. Immature fruits were counted at the fruit level as seeds within each fruit could not be separated and counted. Mature seeds were individually counted after pulp was removed to reduce unnatural putrefaction of fruits reared at high densities which might harm insect development. Frugivorous mammals regularly removed pulp of *Q. asterolepis* fruits before depositing intact seeds (C. Jeffs pers. obs.). All pots were stored in semi-natural conditions on shelves in a shadehouse within the forest close to the BCI

field station. Pots were checked three times per week and all emerging adults were collected and stored in 95% ethanol for later identification. To facilitate identification of larvae that emerged, a proportion (30 - 50% per tree) were transferred to pots of moist, sterilised soil in an attempt to rear them to maturity with the remainder stored in 95% ethanol. Adult insects were sorted according to morphospecies and identified to the lowest taxonomic rank possible by Hector Barrios (Curculionidae) and Marleny Rivera (Diptera).

At the end of the rearing period, all fruits were dissected in the laboratory under a stereo microscope. Seeds with holes in the seed casing and containing frass and/or insect adults or larvae were scored as predated (Nakagawa et al. 2005; supplementary information S2.7.4). Tree-specific predation rates were calculated as the proportion of all reared immature fruits or mature seeds showing signs of predation. Separate predation rates were calculated for immature fruits and mature seeds reared for one month and three month for that tree. The dataset on emerging insect individuals was also used to assess the responses of each seed predator morphospecies to tree DBH and neighbourhood density.

2.3.4 Statistical analyses

We conducted all statistical analyses using the statistical software R, (version 3.1.3, R Development Core Team 2015).

To test for density-dependent mature seed predation rates, we used a generalised linear model with a quasibinomial error distribution (to account for overdispersion). Neighbourhood density, focal individual DBH, and the DBH × neighbourhood density interaction were fitted as explanatory variables. The mapped plot in which each focal individual was located was also added as a 'block' effect to account for potential spatial autocorrelation and to test for differences in predation rates between forest locations. To rectify problems with model assumptions present in a parallel generalised linear model for immature fruits, we fitted a linear model with the same explanatory variables but with the proportion of immature fruits predated arc-sine square-root

transformed and using a normal error structure. Immature fruit predation rates were weighted by sample size (the number of immature fruits dissected per tree).

We also tested whether the emergence frequency of individual seed predators was density-dependent. As multiple seed predators can emerge from a single seed (C. Jeffs pers. obs.) we could not analyse emergence as a proportion. Separate generalised linear models with a quasipoisson error distribution (to account for overdispersion) were therefore fitted to emergence counts for each seed predator morphospecies reared. In addition to focal individual DBH, neighbourhood density, the DBH × neighbourhood density interaction, and plot as explanatory variables, sample size (the number of seeds reared) was also included to account for a positive correlation between sample size and seed predator emergence frequencies (data not presented). For *Chelofonyx* sp. emergence, individuals from one and three month reared mature seeds (which did not differ significantly in emergence frequency; N=26, V=27, P=0.350) were combined to rectify problems with model assumptions. Insect emergence material for immature fruits was insufficient for analysis (42 emerging seed predator individuals across all samples).

To test whether premature fruit abscission rate covaries with pre-dispersal seed predation, we fitted a generalised linear model with a quasibinomial error distribution to the proportion of immature fruits produced per tree. Individual DBH, neighbourhood density, the DBH × neighbourhood density interaction, plot, and immature fruit dissection-based predation rate were fitted as explanatory variables. Additionally, we conducted a paired t-test to compare predation rates between immature fruits and mature seeds. If predation triggers fruit abortion, we predict estimated predation rates to be higher for immature fruits than for mature seeds (Stephenson 1981; Fernandez et al. 2008).

To identify the factors best explaining intraspecific variation in viable seed production, we used a linear model with DBH, neighbourhood density, plot, and mature seed predation rate fitted as explanatory variables. The number of viable seeds produced per tree was log transformed to

improve model criticisms. For all analyses, we selected the neighbourhood density measure and radius explaining most variation in each predation or abortion rate for presentation (supplementary information S2.7.3).

Minimum adequate models were selected using a stepwise model selection procedure following Crawley et al. (2007), with non-significant highest-order interactions removed first, followed by the least significant factor and leaving only significant variables explaining observed variation. The block variable, plot was retained in all models irrespective of significance.

2.4 Results

A total of 5,846 immature fruits and 6,421 mature seeds were collected from 26 trees (two of the focal trees did not produce fruit). From these fruits and seeds, we reared a total of 780 insects representing three seed predator morphospecies; two morphospecies of weevil (Coleoptera: Curculionidae) and one tephritid fly (Diptera: Tephritidae). Of the weevils, morphospecies *Chelofonyx* sp. was less abundant (67 adult individuals) than *Conotrachelus* sp. (41 adults, 561 larvae; identity of larvae verified through soil pot rearing). One *Anastrepha* sp. (Diptera: Tephritidae) which appears to be feeding on *Quararibea asterolepis* seeds rather than on the fruit pulp (I. Simon pers. obs.) was also reared (111 adults). A subset of individuals of all three morphospecies have been DNA barcoded and their Barcode Index Numbers (Ratnasingham & Hebert 2013) are BOLD:ABV0294 (*Chelofonyx* sp.), BOLD:ACC9894 (*Conotrachelus* sp.) and BOLD:AAR3722 (*Anastrepha* sp.).

2.4.1 Effects of tree size, density and location on seed predation rate

Estimated predation rates via dissection were significantly lower for material reared for one month relative to three months for both mature seeds (paired t-test; t_{25}= -10.17, P<0.001) and immature fruits (paired t-test; t_{25}= -6.53, P<0.001), potentially due to decomposition of the seed

interior removing signs of seed predation. We therefore only analysed immature fruits and mature seeds dissected after one month of rearing in our analyses. The mean mature seed predation rate across trees (based on dissection one month after collection) was 26.5% (range 6.7% - 52.5%), and for immature fruits it was 22.9% (range 6% - 47.7%).

The mean mature seed predation rate was unrelated to the DBH of the individual or the density of conspecifics within the neighbourhood (summed DBH within a 35m radius). A single outlying and influential data point was removed which did not qualitatively change results compared to the complete dataset, but also improved normality as assessed by a normal quantile plot. Mature seed predation rates differed significantly among forest plots (Table 2.1; Figure 2.2). No factors significantly explained variation in predation rates of immature fruit (all P>0.05). Parallel analyses using tree-specific crop size instead of DBH found qualitatively unchanged results, with significant differences in predation rate detected between plots only (supplementary information S2.7.5).

Table 2.1. Generalised linear model results for factors influencing the predation rate of mature seeds for reduced and complete datasets. P values calculated via analysis of deviance during stepwise model simplication. Significant variables are in bold.

Model	Variable	Df	Deviance	F	P
Mature seeds	(Global model)	-	88.76	-	-
(N=25)	DBH × Neighbourhood density (35m)	1	95.50	1.366	0.258
	Neighbourhood density (35m)	1	101.17	1.129	0.301
	DBH	1	110.65	1.874	0.186
	Plot	**3**	**258.24**	**9.337**	**<0.001**
Mature seeds	(Global model)	-	146.51	-	-
(N=26)	DBH × Neighbourhood density (35m)	1	147.14	0.0813	0.779
	DBH	1	153.80	0.905	0.353
	Neighbourhood density (35m)	1	170.22	2.241	0.149
	Plot	**3**	**284.46**	**4.922**	**0.004**

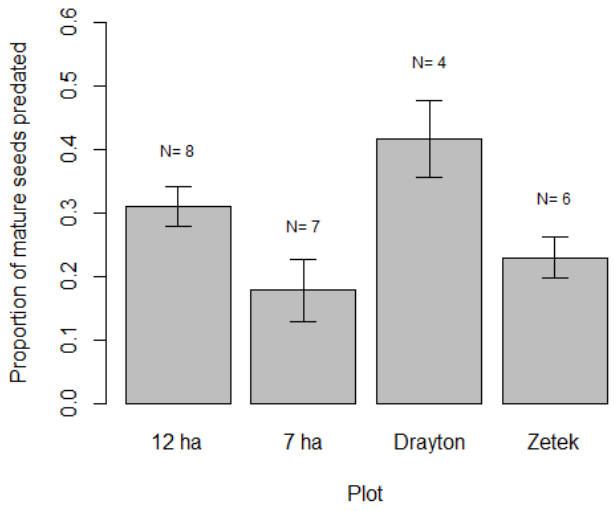

Figure 2.2. Bar chart of the mean (±SE) proportion of mature seeds predated within each mapped forest plot. Plot specific means calculated as the average of focal individual-specific mean predation rates. Data are presented for mature seeds dissected one month after collection. A single outlying and influential data point has been removed.

Differences in seed predation rates between plots could occur if seed predators respond to variation in the abundance of resources at larger, multi-hectare scales. To test for this possibility, we conducted post hoc Spearman's rank correlation analyses. There were no clear associations of predation rate with summed DBH of all trees within plots, or the density of trees within plots (summed DBH divided by area of plot in hectares). However, there were suggestions of a positive correlation between the mean DBH of all conspecific trees within a plot and mean mature seed predation rate (N=4, rho=1, P=0.083; Figure 2.3).

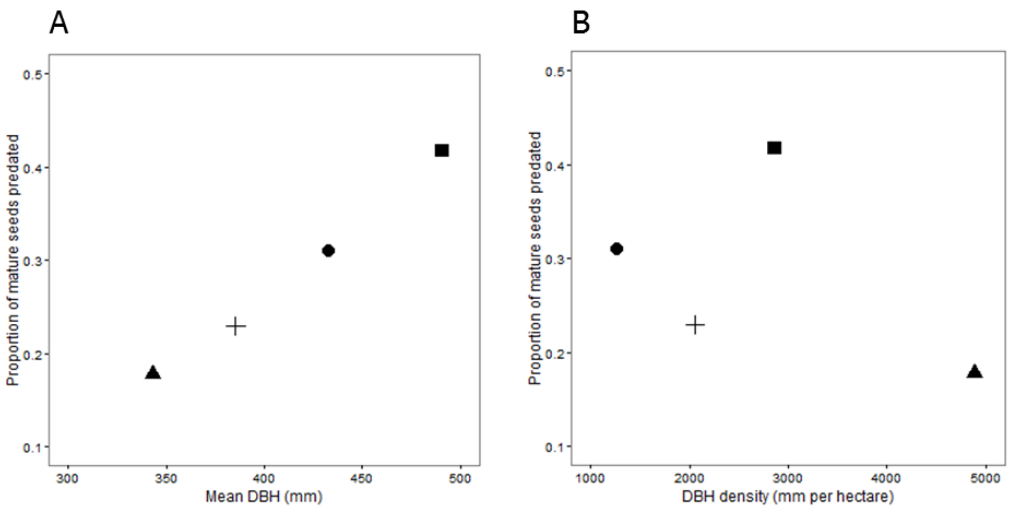

Figure 2.3. Figure 2.3. Scatterplots of the associated between plot level factors and mean seed predation rates. Figures present a) mean DBH of all trees within plots, and b) DBH density (mean summed DBH divided by area of plot) against mean mature seed predation rate of all focal trees per plot. Plots are represented as follows: 7 ha (triangle), Zetek (cross), 12 ha (circle), and Drayton (square)

2.4.2 Incidence and density-dependence of individual seed predator species

For analyses on both *Chelofonyx* sp. and *Conotrachelus* sp. emergence frequencies, the same tree representing a single influential data point was removed (this individual differed to the one removed for dissection analyses).The emergence of *Chelofonyx* sp. did not vary significantly in relation to DBH, neighbourhood density or plot (all P>0.05). It is possible that total *Chelofonyx* sp. emergence (67 individuals) was too low to detect patterns.

Conversely, the frequency of *Conotrachelus* sp. differed significantly between plots, although not in relation to focal tree DBH and neighbourhood density (Table 2.2; Figure 2.4.). *Conotrachelus* sp. frequency was highest in the plot where predation rates based on dissection were highest. Analyses of *Anastrepha* sp. incidence failed to meet model assumptions for any model attempted, perhaps because individuals emerged from only 10 out of 26 trees.

Table 2.2. Generalised linear model results testing for factors influencing the emergence frequency of each weevil seed predator morphospecies. Neighbourhood density values are of summed density measures with radii presented in brackets. P values calculated via analysis of deviance during stepwise model simplication. Significant variables are in bold.

Morphospecies	Variable	Df	Residual Deviance	F	P
Chelofonyx sp.	(Global model)	-	24.678	-	-
	DBH × Neighbourhood density (45 m)	1	24.732	0.0349	0.854
	Neighbourhood density (45 m)	1	25.364	0.4347	0.519
	DBH	1	27.649	1.6217	0.219
	Plot	3	36.367	1.9969	0.149
	Sample size	1	28.117	0.3218	0.577
Conotrachelus sp.	(Global model)	-	105.39	-	-
	DBH × Neighbourhood density (20 m)	1	128.31	3.6975	0.071
	DBH	1	141.34	1.8274	0.193
	Neighbourhood density (20 m)	1	155.20	1.8638	0.188
	Plot	3	529.42	16.055	**<0.001**
	Sample size	1	155.20	0.000	0.999

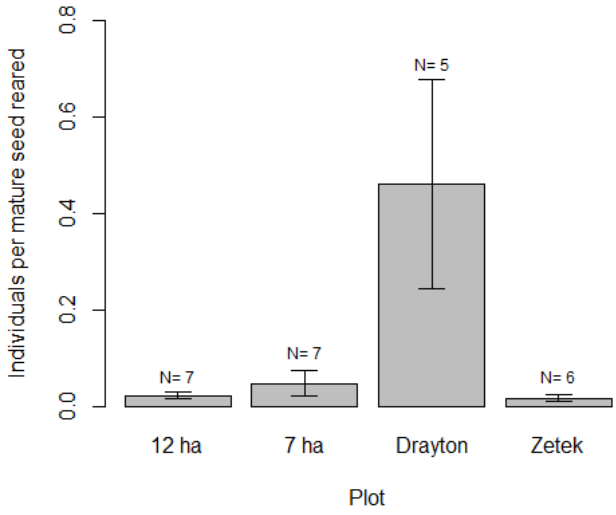

Figure 2.4. Bar chart of the mean (±SE) number of *Conotrachelus* sp. individuals emerging per mature seed reared for each mapped plot. A single outlying point was removed.

2.4.3 Premature fruit abscission rate

Premature fruit abscission rates (the proportion of immature fruits produced per tree) did not differ significantly in relation to focal individual DBH, neighbourhood density or dissection based immature fruit predation rate (all P>0.05). Predation rates estimated based on dissection were not significantly different for immature fruits and mature seeds (paired t-test; t_{24}=-0.819, P=0.421).

2.4.4 Determinants of the production of viable seeds

There was a strong positive association between DBH and total fruit crop size varying over four orders of magnitude (supplementary information S2.7.1; Figure 2.5). Similarly, after two exceptionally low fecundity individuals (with zero and four viable seeds produced respectively) acting as influential outliers were removed, the number of viable seeds produced per tree significantly increased with DBH, but no association with neighbourhood density or predation rate of mature seeds was detected (Table 2.3).

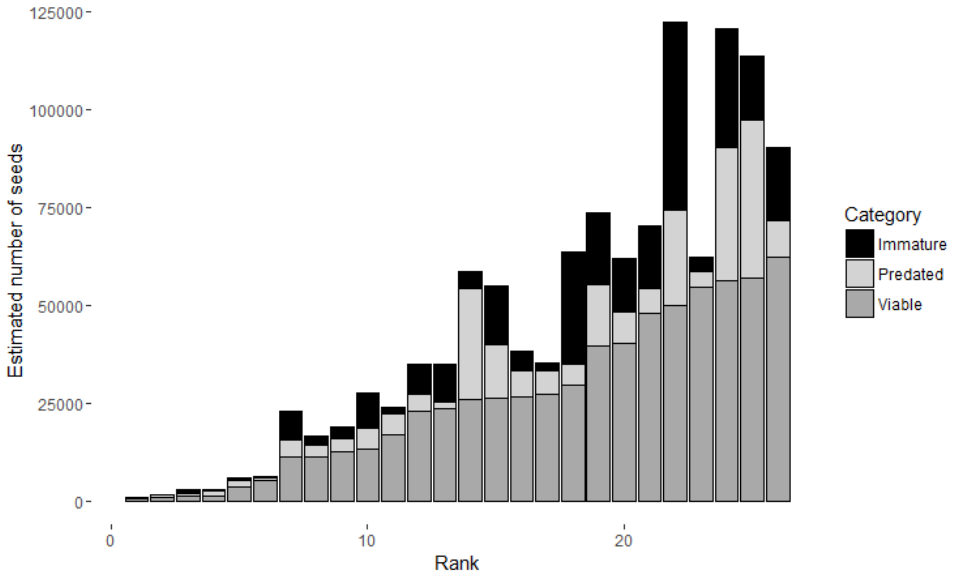

Figure 2.5. Stacked bar plot showing factors influencing focal tree-specific viable seed production. Individual focal trees are ranked by number of viable mature seeds produced (dark grey), with number of predated mature seeds (light grey) and immature inviable seeds (black). The two lowest ranked individuals were removed from fruit production analyses.

Table 2.3. Linear model results assessing the factors influencing the production of viable seeds based on model simplification by analysis of deviance. The neighbourhood density value is a summed density measures with radii presented in brackets. P values calculated by analysis of deviance during model simplication. Significant variables are in bold. N=24.

Variable	Df	Residual Deviance	AIC	F	P
(Global model)	-	4.1370	41.915	-	-
Mature seed predation rate	1	4.3890	41.334	2.0447	0.146
Neighbourhood density (20 m)	1	5.2285	43.535	3.4429	0.080
DBH	1	7.2898	49.511	7.4906	**0.013**
Plot	3	6.4608	42.614	1.4926	0.249

2.5 Discussion

We found no evidence that insects inflict density-dependent pre-dispersal seed predation in the tropical tree species *Quararibea asterolepis.* This was the case both at the individual tree and at the local neighbourhood scale. However, mean predation rates differed significantly between forest locations, potentially due to a positive relationship between plot-level seed predation rates and the mean DBH of all trees within each plot. Overall, strong positive associations between DBH and fruit crop size meant that tree-size effects far outweighed influences of seed predation in determining a tree's production of viable seeds and in creating spatial variation in the output of viable seeds.

2.5.1 Density-dependence and spatial variation in pre-dispersal seed predation rates

Enemy-mediated negatively density-dependent seed survival has the potential to enhance plant community diversity via the Janzen-Connell mechanism (Janzen 1970; Connell 1971). However, frequency stabilising effects within a community are expected to be strongest when density-dependence is overcompensating (Freckleton & Lewis 2006; Bagchi et al. 2010), a mechanism

yet to be demonstrated in seed predators.

Previous work found that saplings of *Q. asterolepis* suffered greater herbivory levels when near to neighbouring conspecific adults during an outbreak year of defoliating moths (Wong et al. 1990). However, we found no evidence of greater seed predation at higher individual or neighbourhood scale resource abundances. As *Q. asterolepis* is the second most abundant tree species >10 cm DBH within the 50 ha forest dynamics plot on BCI (Hubbell 2005; Condit et al. 2013), current evidence suggests that even if enemy mediated negatively density-dependent survival occurs at later life stages it may not prevent numerical dominance of *Q. asterolepis.* Mangan et al. (2010) found that soil fungi mediated negative feedbacks on seedling performance were stronger in rarer woody plant species on BCI. Whether such correlations occur for herbivore and seed predator mediated mortality in tropical forests are unknown (but see Xu et al. 2015).

A study focusing on the tree species *Jacaranda copaia*, also on Barro Colorado Island, demonstrated that the proportion of seeds killed by insect pre-dispersal seed predators increased with both individual fecundity and neighbourhood level fruit densities (Jones & Comita 2010). However, predicted mean increases in seed predation between the most isolated and abundant neighbourhoods were modest, and predator satiation occurred at the highest combinations of individual and neighbourhood fecundity. Although there was no evidence of predator satiation in the current study, patterns of seed survival consistent with predator satiation are commonly seen in tree species including those on BCI (see Wright et al. 2005) and famously in the dominant mast-fruiting Dipterocarpaceae of S.E. Asian tropical forests (Ashton 1988; Bagchi et al. 2011; also see Xu et al. 2015 for examples in subtropical forests). Predator satiation may therefore commonly overcome effects of negative density-dependent seed predation at lower resource densities (e.g. Jones & Comita 2010; Visser et al. 2011) limiting the prevalence of diversity-enhancing overcompensating negative density-dependence. Increased seed survival at the highest densities would favour clumping in tree distributions and lower diversity if compensating negative-density dependent mortality was absent in later life stages (Janzen 1970; Bagchi et al.

2011). Overall, a wider number of species need to be assessed before strong conclusions can be drawn on the prevalence of positive and negative density-dependent survival mediated by pre-dispersal insect seed predators.

Observations in tropical palms have documented that the responses of seed predators to density can differ across the spatial scales assessed, causing post-dispersal seed survival to be positively distance-dependent and negatively density-dependent at smaller spatial scales, but positively density-dependent or density-independent at large spatial scales (e.g. Schupp 1992; Visser et al. 2011). Therefore to ensure that the appropriate scale of density-dependence can be identified, studies should aim to incorporate both local and population level density variation where possible (Lewis & Gripenberg 2008; Gripenberg et al. 2014). Studies should also quantify all causes of mortality independently, as different seed predator species sharing a host can respond differentially to density at the same or different spatial scales (e.g. post-dispersal Visser et al. 2011; pre-dispersal Mezquida & Olano 2013).

2.5.2 Differences in pre-dispersal seed predation between forest locations

Differences in mean predation rates between forest plots in our study were substantial (ranging from 17.8% - 41.7%). The absence of small scale density-dependence but larger differences between spatially separated populations may indicate a mobile suite of seed predators attacking *Q. asterolepis*, but with dispersal limitation able to create distinct seed predation patterns between forest locations. Alternatively seed predation rates are known to vary spatially in response to differences in abiotic conditions (e.g. Delerue et al. 2004), a possibility that was not assessed in our study. Whatever the cause, density-independent seed predation rates varying at the individual level can appear spatially structured at larger spatial scales, as in our study.

Although sample size was small (four plots), our study tentatively reveals a positive relationship between plot-specific mean predation rate and the mean DBH of conspecific trees within a plot. As crop size increases disproportionately with DBH in *Q. asterolepis* (supplementary information

S2.1), seed predator population size may also increase disproportionately more rapidly on larger trees. A plot with fewer but larger trees may thus exceed the predator accumulation potential of plots with a greater number of trees and greater summed DBH if the latter is composed of smaller individuals. Consequently, high predation rates could occur on small individuals within a neighbourhood if large neighbouring trees are a source of abundant and mobile seed predator populations that are not responding to the density of resources at the individual tree scale. Without larger sample sizes, such a hypothesis remains highly speculative. Quantification of seed predator dispersal abilities and population growth rates in relation to resource densities are greatly needed to understand the spatial structuring of seed predation rates (Lewis & Gripenberg 2008).

Our data suggest that the most abundant seed predator species (the weevil *Conotrachelus* sp.) may drive overall seed predation rates in *Q. asterolepis*. The other co-occurring seed predator species, the weevil *Chelofonyx* sp. and tephritid fly *Anastrepha* sp., were far less abundant and showed no apparent spatial structuring. Factors associated with each plot location within the forest (e.g. mean DBH or abiotic conditions) may thus be important for determining spatial variation in predation rates by each seed predator species attacking *Q. asterolepis*, although co-occurring seed predator species can respond differently to the distribution of shared hosts (Dracxler et al. 2011).

2.5.3 Implications of pre-dispersal seed predation for seedling recruitment

Our results demonstrated that total fruit crop size (and the production of viable seeds) increased steeply with DBH for *Q. asterolepis*. There was no evidence for positive density-dependent fruit set (e.g. through enhanced pollination at greater densities; Jones & Comita 2010; Fedriani et al. 2015) which can also result in a net increase in viable seed production even when predation generates negatively density-dependent seed survival (e.g. Jones & Comita 2010). Overall, tree size appears to be of greater importance than neighbourhood density or pre-dispersal seed predation in generating spatial variation in the input of viable seeds.

Our results also suggest that insect seed predators did not trigger the premature abscission of fruits in *Q. asterolepis*, as predation rates of immature fruits (mean 22.9%) and mature seeds (mean 26.5%) did not differ significantly. Nonetheless, seed predator emergence rates were significantly lower in immature fruits relative to mature seeds, suggesting premature fruit abscission is fatal and can still partially limit the local accumulation of seeds predators (Stephenson 1981).

Pre-dispersal seed predators will have their most substantial effects on recruitment in species and communities where seed limitation is most prevalent and strong (Turnbull et al. 2000) such as is suggested for tropical forests (Svenning & Wright 2005; Clark et al. 2007). The predation rates of mature seeds we observed (mean 26.5%, range 6.7% – 52.5%) indicate the potential of insect pre-dispersal seed predators to influence seedling recruitment in *Q. asterolepis* if seed limitation occurs. However, additional and compensating mortality factors post-dispersal may diminish the importance of pre-dispersal seed predation for recruitment (Edwards & Crawley 1999; Crawley 2013). This is especially likely if post-dispersal seed predators are more responsive to resource densities (Wright et al. 2005).

2.5.4 Conclusions

Current evidence for negatively density-dependent seed survival mediated by pre-dispersal insect seed predators within tropical forests is limited, and thus the ability of this guild to enhance community diversity is unknown. Our results reveal a lack of density-dependent seed predation in a common tropical tree, *Q. asterolepis*, at the individual and local neighbourhood scales. However, there was significant spatial structuring of predation rates at larger spatial scales, between forest locations. Assessments of the demographic responses of pre-dispersal seed predators to spatial variation in the density of their resources are greatly needed (Lewis & Gripenberg 2008), with the direction and frequency of such responses needing to be identified. Studies linking tree-specific pre-dispersal seed predation and resultant seedling recruitment rates

will be valuable in assessing the likely importance of this enemy guild to plant population dynamics and community diversity.

2.6 Acknowledgements

We especially thank Catalina Fernandez, Indira Simon and Maritza Moya for their invaluable assistance in the lab and field over the course of the project. Thanks also to Ace North for generating neighbourhood density values. This project was funded by the Academy of Finland (S. Gripenberg) and the Mike Soper Bursary Fund (University of Oxford) (C. Jeffs).

2.7 Supplementary information

S2.7.1 Relationship between DBH and fruit crop size

DBH was significantly positively correlated with total fruit crop size (Spearman's rank correlation, N= 26, S = 1285.44, rho = 0.56, P = 0.003; Figure S2.6). DBH was selected for use in all analyses as a proxy for fecundity ('resource abundance') due to the greater accuracy in measuring DBH relative to crop size itself. The density of fruiting conspecifics in the neighbourhood and DBH were not significantly correlated (Pearson's $r < 0.3$, $P>0.05$ for all measures of neighbourhood density).

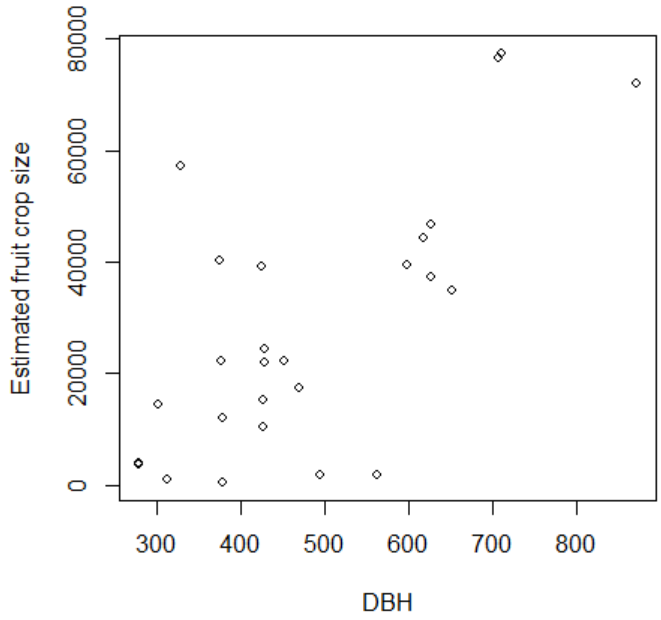

Figure S2.6 Scatterplot of tree specific fruit crop size against DBH. Both immature and mature fruits are counted to calculate total fruit crop size. N=26. Two focal individuals with exceptionally low numbers of collected fruits (zero and four) have been excluded.

S2.7.2: Measurement of DBH

The most recent DBH measurements on trees within our study plots were made in 2014, three years after our field data collection season. Trees in the 50ha plot (within 50m of some of our focal study trees) were measured in 2010, and the DBHs of trees mapped outside the study plots were measured in 2011. To make DBH data collected in different years were comparable, a linear model was used to predict DBH in 2014. We modelled the increase in DBH (in cm) between 2004 and 2014 (the two years for which census data on trees in our study plots were available) as a function of DBH in 2004 (allowing for changes in growth with tree size/age). The model predicted growth over 10 years, and could be used to estimate increases in DBH for trees with diameter measurements taken in 2010 or 2011. As trees in the 50ha plot and those mapped outside the 7ha were not visited in 2011 (the time of our study), we are making the assumption they were reproductive in 2011. Since almost all (98%) of the trees visited in 2011 were reproductive, any inaccuracies should have a minor effect on results.

S2.7.3: Neighbourhood density calculation and selection of type and radius for each model

To calculate the negative exponential connectivity value of each focal tree (*i*), we use the connectivity kernel (equation S1; Hanski 1994) which comes from the dispersal of propagules (insect seed predators) at a rate proportional to patch size (*A*, i.e. DBH) for each neighbouring conspecific tree (*j*), and where dispersal is according to a dispersal kernel relating to the distance in metres (*d*) between the focal tree and each conspecific neighbour (equation S2).

Equation S1: $$connectivity_i = \sum_{j \neq i} e^{-\alpha d_{ij}} A_j$$

Equation S2: $$e^{-\alpha d_{ij}}$$

The mean dispersal distance (α) is calculcated as equation S3:

Equation S3: $$\int_{r=0}^{\infty} 2\,\pi r^2 e^{-\alpha r}\, dr = \frac{4\,\pi}{\alpha^3}$$

so that if mean dispersal distance is 10m, we have (equation S4):

Equation S4: $$10 = \frac{4\,\pi}{\alpha^3}; \alpha = \sqrt[3]{4\,\pi/10} = 1.85664$$

Using mean dispersal distances of 5 m, 10 m, 15 m, 20 m, and 25 m, the corresponding α values used in our calculations are 2.33921, 1.85664, 1.62192, 1.47361, and 1.36798 respectively.

Selection of neighbourhood density measure and radius: All neighbourhood density metrics (summed DBH or negative exponential connectivity) and neighbourhood radii were fitted in separate global models and the model with the lowest residual deviance was chosen for model simplification (Table S2.4). For summed DBH values, a minimum radius of 20 m was used (similar to a minimum of 25 m used by Jones & Comita 2010) as smaller radii showed low variation in density values. For negative exponential connectivity values, a minimum mean dispersal distance of 10 m was selected as smaller values resulted in a low range of density values. If model criticisms indicated poorly specified final models for the density metric and radius initially selected, we selected the density metric and radius with the next lowest residual deviance until model criticism problems were rectified.

Table S2.4. Comparisons of global generalised linear models with different neighbourhood density measures and radii. The density metric and radius presented in final analyses is presented in bold. Residual deviance values are presented to five significant figures.

Density metric	Residual Deviance of global model						
	Mature seed predation rate*	**Immature fruit predation rate+**	**Chelofonyx sp. emergence ***	***Conotrachelus* sp. emergence**	***Anastrepha* sp. emergence**	**Fruit abortion rate**	**Production of viable seeds***
Summed DBH 50m	99.200	37.626	33.410	107.01	120.55	**2393.1**	4.6193
Summed DBH 45m	88.876	37.466	33.432	108.34	133.64	2416.5	4.6482
Summed DBH 40m	90.051	35.363	36.034	117.88	118.25	2452.8	4.3442
Summed DBH 35m	**88.762**	36.419	37.139	114.08	126.37	2529.7	4.6478
Summed DBH 30m	93.761	35.452	38.383	107.63	118.46	2565.4	4.4287
Summed DBH 25m	93.350	37.629	37.530	110.95	111.30	2588.5	4.4173
Summed DBH 20m	93.913	35.769	***31.727***	**104.74**	**106.19**	2572.7	**3.5212**
Connectivity 25m	90.926	**32.435**	29.727	121.25	114.52	2556.2	3.8696
Connectivity 20m	90.754	32.475	29.719	121.13	114.62	2548.5	3.9198
Connectivity 15m	90.567	32.552	29.703	120.98	114.79	2539.0	3.9748
Connectivity 10m	90.567	32.683	**29.672**	120.81	115.16	2528.5	4.0862

+Values for immature fruit predation rate were arcsine square-root transformed to rectify violations of model assumptions in generalized linear models with a quasibinomial error structure

*Values presented are those of models fit to datasets with outlying influential data points removed

S2.7.4: Fruit development stages and evidence of seed predation

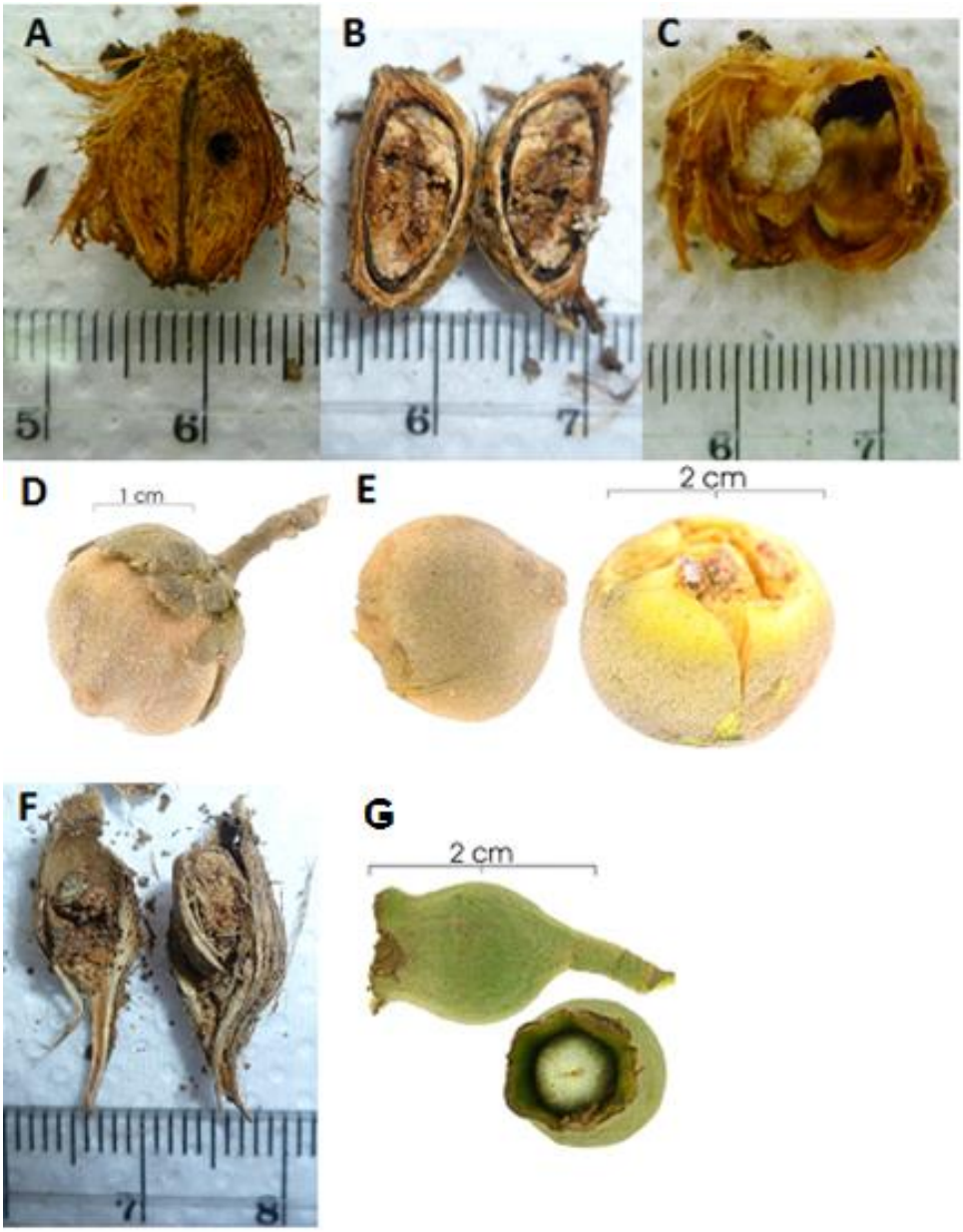

Figure S2.7. Development stage classification and evidence of insect predation for mature seeds and immature fruits. Evidence of insect predation for mature seeds: a) insect emergence hole, b) feeding damage and frass within a dissected seed, c) presence of a seed predator larvae (*Conotrachelus* sp.). Size and colouration of mature fruits (d, e). Evidence of insect predation for immature seeds: f) frass and feeding damage. Size and colouration of immature fruits (g). Photos a, b, c, and f property of C. Jeffs. Photos d, e, and g (credit; Steven Paton) were extracted from the Smithsonian Tropical Research Institute Plant Images Database: http://striweb.si.edu/esp/tesp/plant_images_info.html

S2.7.5: Tree-specific crop size as an alternative explanatory variable

Focal tree specific crop size was used as an alternative explanatory variable to DBH in analyses of factors determining predation rates, but did not qualitatively change results. For mature seeds, neither crop size, neighbourhood density (40m radius) nor their interaction significantly explained variation in mature seed predation rates. Mature seed predation rates different significantly between plots (Table S2.5). Analyses on immature fruit dissection rates failed to meet model assumptions.

Table S2.5. Alternative generalised linear model results for mature seed predation rate using crop size as an alternative to tree-specific DBH. P values calculated via analysis of deviance during stepwise model simplification. Significant variables are in bold.

Model	Variable	Df	Deviance	F	P
Mature seeds	(Global model)	-	158.82	-	-
(N=25)	Crop size × Neighbourhood density (50m)	1	164.30	0.656	0.428
	Crop size	1	164.34	0.005	0.944
	Neighbourhood density (50m)	1	170.22	0.752	0.396
	Plot	**3**	**284.46**	**4.922**	**0.009**

Chapter 3:

Intensity and host-specificity of pre-dispersal seed predation by insects across a tropical precipitation gradient

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Lars Markesteijn & Owen T. Lewis

Rainforest bordering the Panama Canal. Photo credit Chris Jeffs

3.1 Abstract

1. Insects that feed on and kill seeds before they are dispersed from the parent plant (pre-dispersal seed predators) can be a substantial source of mortality for many plants, with consequences for their population dynamics and diversity.

2. Natural enemies such as seed predators may contribute towards the commonly observed positive relationship between plant diversity and precipitation if high-precipitation sites experience a higher intensity of seed predation or a higher degree of seed predator host-specificity.

3. To assess the intensity and specificity of seed predation we collected 31,751freshly-abscised fruits and seeds from 51 tropical forest woody plant species at eight sites spanning a steep precipitation and plant species richness gradient in Panama.

4. Pooled across 31 species with sufficient sample sizes, a mean of 22.5% of seeds were predated per species. Based on 15 species occurring at a minimum of two sites, predation rates were not significantly associated with site humidity (either annual rainfall or dry season water deficit).

5. A total of 46 seed predator morphospecies was reared from seeds representing 17 plant species. Host-specificity was high in our sample, with 91% of seed predator morphospecies associated with just one plant species.

6. Our results confirm that pre-dispersal insect seed predators are highly host-specific and cause significant seed mortality across plant species, suggesting this enemy guild could influence plant population dynamics and diversity in species-rich rainforest plant communities. However, our results do not support that pre-dispersal insect seed predators contribute to the positive relationship between precipitation and plant species richness.

3.2 Introduction

The survival of seeds and the initial establishment of seedlings are key stages determining plant population dynamics and community structure (Webb & Peart 1999; Harms et al. 2000; Green et al. 2014). Most plant species experience a degree of seed recruitment limitation, so factors affecting viable seed production can directly influence seedling recruitment and community diversity (Turnbull et al. 2000; Clark et al. 2007).

One potentially important source of seed mortality is pre-dispersal predation by insects, particularly Coleoptera, Lepidoptera, Diptera and Hymenoptera (Janzen 1971). These insects consume and kill seeds *in situ* on the plant, directly influencing the relative abundances of viable seeds across species (Fenner & Thompson 2005; Lewis & Gripenberg 2008; Crawley 2013). For example, specialised insect seed predators may generate negatively density-dependent seed survival, favouring rare species and promoting species coexistence (Janzen 1970; Hammond & Brown 1998).

Variations in the intensity of density-dependent mortality and the host-specificity of natural enemies could also contribute to gradients in plant diversity in relation to latitude (Janzen 1970) or rainfall (Givnish 1999; Brenes-Arguedas et al. 2009; Comita et al. 2014; Spear et al. 2015). On local and global scales, rainfall is a strong predictor of plant species richness (Pyke et al. 2001; Kreft & Jetz 2007). Greater annual rainfall and less intense dry seasons can also increase the survival of desiccation-intolerant organisms such as insects, resulting in greater population sizes (Coley & Barone 1996; Givnish 1999). Therefore, if rainfall is positively correlated with the intensity of mortality from seed predators, the strength of negatively density-dependent survival, and/or the degree of host-specificity, natural enemies may contribute towards the widely-observed positive relationship between plant diversity and precipitation.

Despite the potential importance of pre-dispersal insect seed predators in structuring tropical forest plant communities (Janzen 1970), assessments of their host-specificity (Janzen 1980; Nakagawa et al. 2003; S. Gripenberg unpublished data), density-dependence (Ashton 1988; Jones & Comita 2010; Bagchi et al. 2011) and effects on plant recruitment are scarce. To address these gaps in knowledge, we investigated patterns in pre-dispersal seed predation by insects for multiple woody plant species at sites spanning a steep rainfall and plant species richness gradient across the Isthmus of Panama, Central America. We test whether pre-dispersal insect seed predation intensity and host-specificity varied across the rainfall and species richness gradient, and use our data to highlight research priorities for understanding the ecological role of pre-dispersal insect seed predators in tropical forests. We reason that our study represents the first to look at within-species trends in mortality across the natural ranges of a diverse group of study species spanning an entire precipitation gradient.

3.3 Methods

3.3.1 Study sites and sample collection

From March to July 2014, freshly abscised fruits, pods, and seeds were collected under all fruiting woody species encountered across eight sites spanning the Isthmus of Panama precipitation and species richness gradient (Figure 3.1; Table 3.1). Each study site was located around a 1 ha permanent forest plot (part of the CTFS-ForestGEO network; Condit et al. 2013). To increase sample sizes, fruits were collected more widely around the plots, but typically within a 1.5 km radius of the plot. Wherever possible, seeds were collected from multiple individuals per species per site to account for inter-individual variation in predation rates. The vast majority of seeds collected were recently abscised (gauged by their fresh condition and location on surface of the leaf litter layer surface in repeatedly sampled locations) and therefore our samples are thought to primarily consist of seeds infested by insects pre-dispersal. Whilst it is possible that post-dispersal

insect seed predators may also infest our samples on the ground prior to collection, we believe this contribution to be minimal.

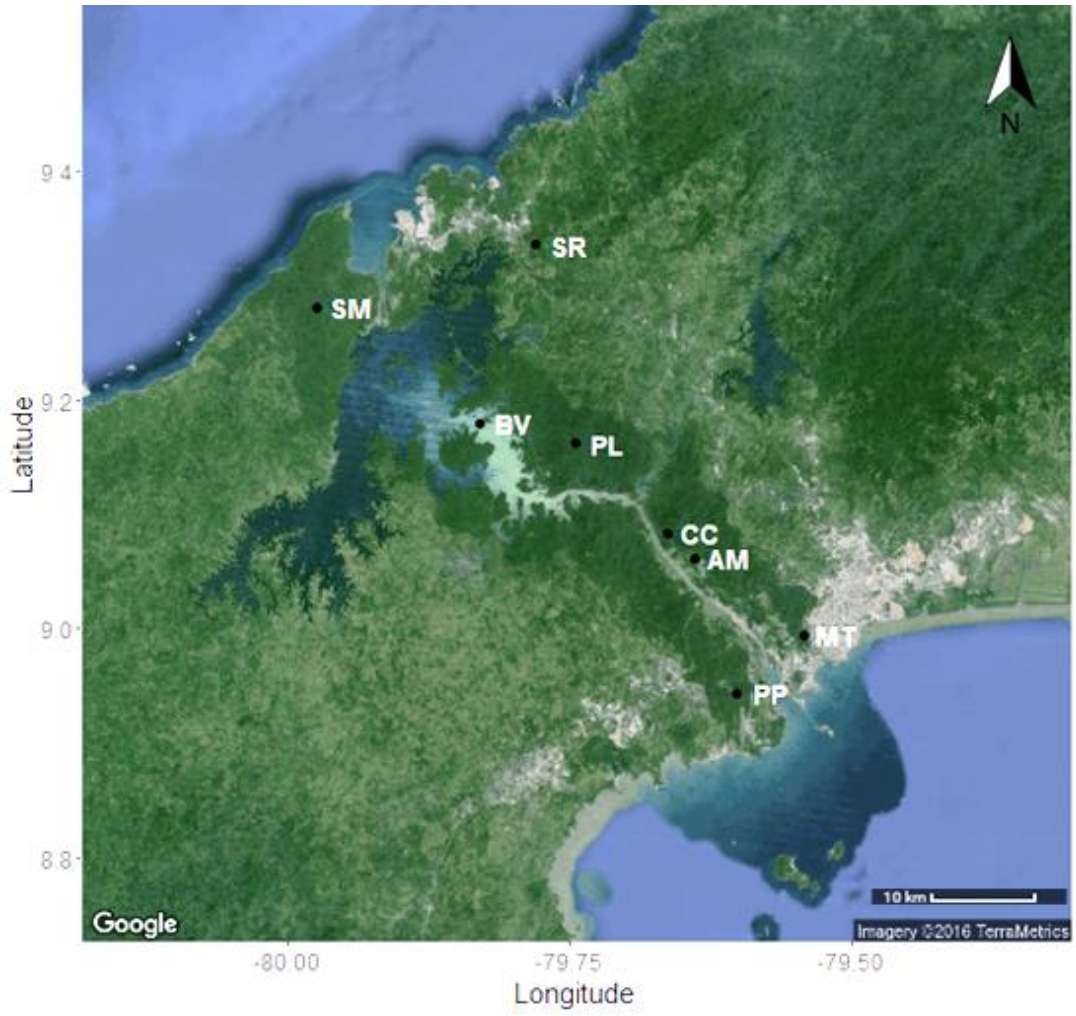

Figure 3.1. Position of forest sites sampled across the Isthmus of Panama rainfall and plant species richness gradient. From lowest to highest annual rainfall, the sites are PP (Panama Pacifico), MT (Metropolitano), AM (Parque Soberania, ANAM office), CC (Parque Soberania, El Charco), PL (Oleoducto 'Pipeline Road'), BV (Buena Vista Peninsula), SM (Fort Sherman), SR (Santa Rita). Image credit Google, ©2016 TerraMetrics. Figure produced using ggmap package (Kahle & Wickham 2013) in R statistical software (version 3.1.3, R Development Core Team 2015).

Table 3.1. Locations and characteristics of study sites. Data for annual rainfall (mm), dry season deficit (the peak volume of rain required to reach field capacity soil water content over the dry season), and species richness (the number of free-standing woody species >1cm diameter at breast height) were collected within a central 1 ha plot at each site (Condit et al. 2013).

Site	Latitude	Longitude	Annual rainfall (mm)	Dry Season deficit (mm)	Species richness
Panama Pacifico	08.9435000	-079.6015333	1756	-571.688	69
Metropolitano	08.9945889	-079.5430000	1874	-574.926	49
ANAM	09.0619500	-079.6394000	2007	-579.902	34
El Charco	09.0840500	-079.6634000	2051	-579.148	84
Oleoducto	09.1617600	-079.7453000	2330	-549.423	132
Buena Vista	09.1793611	-079.8296000	2595	-510.470	85
Fort Sherman	09.2808694	-079.9747000	2848	-491.669	161
Santa Rita	09.3355778	-079.7807417	3054	-487.530	170

3.3.2 Intensity of seed predation

To allow insect seed predators to develop and emerge, samples were reared within plastic pots with fine mesh lids, lined with absorbent paper, in a ventilated, shaded room at ambient environmental conditions. Seeds, pods and fruits were housed separately for each species, parent individual, and collection date. Where samples included different seed and fruit development stages (e.g., abscised immature fruit as well as mature fruit), these were also housed separately to account for succession in seed predator attack (Hosaka et al. 2009). Small volumes of water were added to some containers periodically to maintain humidity. Samples were reared for three months after collection before being dissected, allowing insects to develop and emerge for later identification (see below). Fruits and seeds were then dissected under a stereomicroscope and predation rates were calculated for each sample as the number of predated seeds (indicated by the presence of exit holes, larvae/adults or frass, and feeding damage) divided by the total number of

seeds in the dissected sample. Separate site-specific predation rates were calculated for each species, in addition to an average seed predation rate per species with samples pooled across sites.

Generalised linear mixed effects models with sample size weighted predation rates (using the cbind function; R statistical software v3.1.3, R Development Core Team 2015) as a function of site humidity and species as a random factor did not meet model assumptions of homogeneity of variance, normality of errors, or absence of influential outliers. Instead, we calculated Spearman's *rho* coefficients for the correlation between seed predation and site humidity (separate models for annual rainfall and dry season deficit) for each species. A one-sample Wilcoxon signed rank test was then conducted to assess whether the median effect size based on the correlation between predation rate and either humidity variable was significantly different from zero. Only species present at a minimum of two sites and with ≥20 seeds dissected per site were included.

3.3.3 Host-specificity

Adult insects emerging during rearing and obtained via dissection were stored in separate vials of 95% ethanol or pinned and dried (Lepidoptera) for later morphospecies level identification under a dissection microscope and using S.Gripenberg's reference collection for seed predators reared from woody plant species on Barro Colorado Island. Seed predator host-specificity was assessed using a food web approach (Lewis & Gripenberg 2008) with all identified seed predator morphospecies and the plant species from which they were reared from presented, and the relative frequency of all host-insect interactions plotted. The number of plant species per site yielding reared insects was insufficient to produce site-specific food webs and assess variation in host-specificity across the humidity gradient. A semi-quantitative food web of all insect material pooled across sites was therefore constructed (R bipartite package, Dormann et al. 2015).

3.4 Results

A total of 31,751 seed and fruit units were collected from 51 woody plant species (supplementary information S3.7.1). 311 adult individuals of 46 insect seed predator morphospecies were obtained from 17 of these plant species by rearing or dissection. 13,977 seeds were dissected from 31 woody plant species. Mean seed predation rates varied greatly among species (mean 22.5%, ranging from 0 – 100%, median across species 21%, interquartile range 9 – 29%; Figure 3.2).

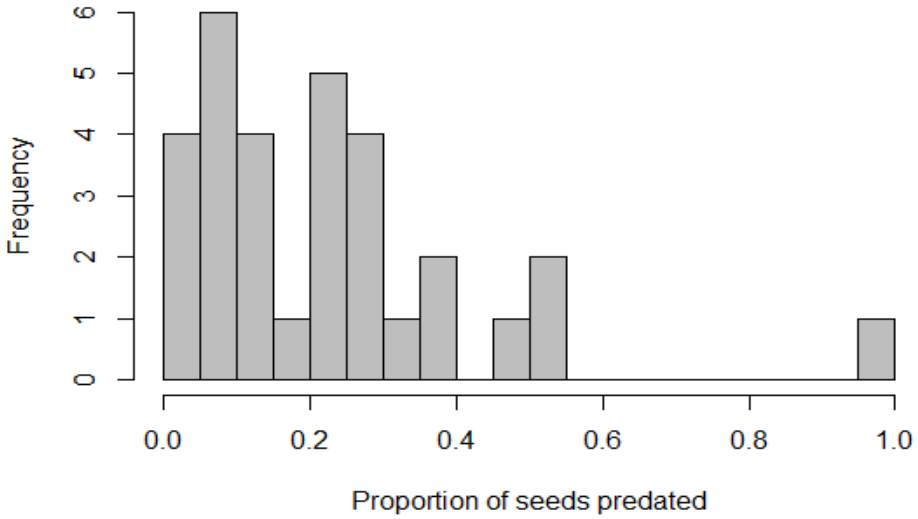

Figure 3.2. Histogram of the proportion of seeds predated per plant species. Seeds were pooled across sites for each species(N=31). Only species with ≥20 seeds dissected are included.

3.4.1 Intensity of predation across the rainfall gradient

There were 15 plant species collected from a minimum of two sites (and with ≥20 seeds per site) available for testing the association between predation rate and humidity. Eleven of 15 species demonstrated a negative association of predation rate and annual rainfall level (Figure 3.3), but the median effect size across species was not significantly different from zero (N=15, V=40, P=0.246). The median effect size across species was also not significantly different from zero for dry season deficit (N=15, V=63, P=0.882).

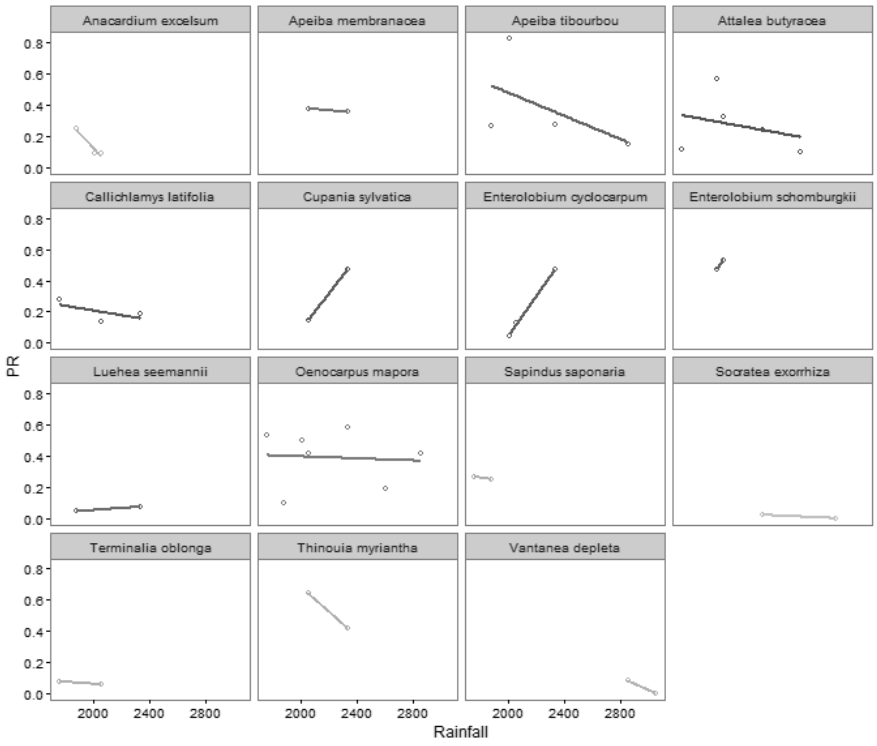

Figure 3.3. Scatterplots of the proportion of seeds predated per species against annual rainfall level (mm). Only species with ≥20 seeds dissected per site from a minimum of two sites are included (*n*=15). Regression lines added to aid interpretation.

3.4.2 Host-specificity of insect seed predators

Of 46 seed predator morphospecies, the majority were Coleoptera (28) and Lepidoptera (15) with fewer being Diptera (3). The food web of pooled seeds across sites demonstrated that host-specificity was high (Figure 3.4). Of the 46 seed predator morphospecies, 43 were monophagous, one was associated with two congeneric host species (*Enterolobium cyclocarpum* and *E. schombergkii*, Fabaceae), two were associated with pairs of host plant species within the same family (one Arecaceae, one Fabaceae), and one was associated with hosts in two different families (Bignoniaceae and Fabaceae). Host plant species varied in the number of seed predator morphospecies reared (Figure 3.5) with evidence that the number of seed predator morphospecies reared per species increased with sample size (generalised linear model, Poisson errors, one outlier removed, N=16, χ^2=19.512, P<0.001).

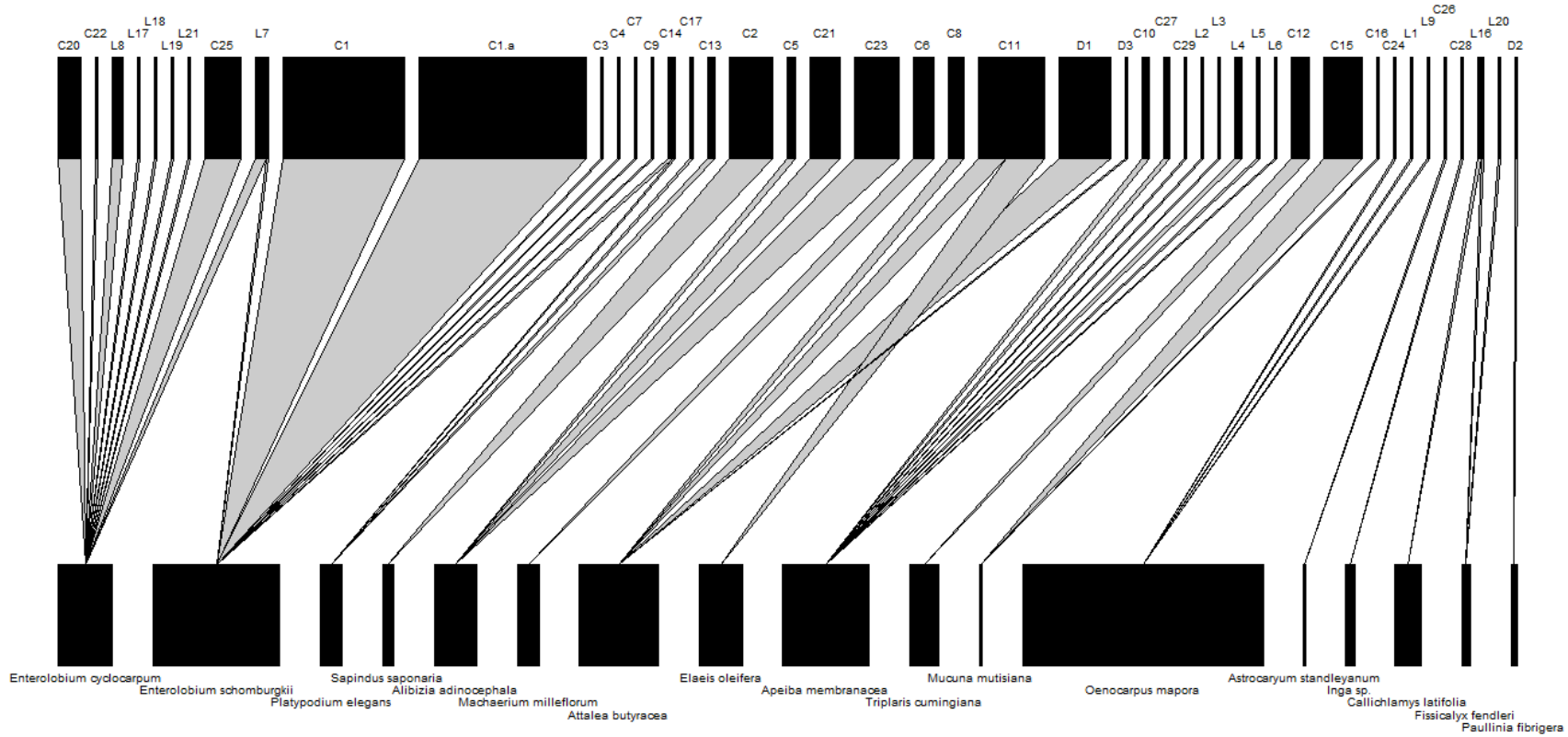

Figure 3.4. Food web of seed predator species and host plants. Bar widths of seed predators reflect their abundance across all samples; bar widths for hosts reflect the summed abundances of all seeds reared per plant species (i.e. 'sampling effort'). For plant species with multi-seeded fruits, the number of seeds dissected was used as an estimation of sampling effort as true seed number was unknown. Insect seed predator morphospecies are as follows. Coleoptera; unknown family C1, C1a, C3, C4, C5, C7, C8, C9, C10, C11, C12, C13, C14, C24, C25, C26, C27, C28, C29; Curculionidae:Apioninae C6, C21; Chrysomelidae:Bruchinae C2; C15, C16, C23; Cerambycidae C17, C20, C22. Diptera: unknown family D2; Richardiidae D1; Calliphridae D3. Lepidoptera: unknown family L1, L16, L18, L19, L20, L21; Pyralidae L16; Pyralidae:Phycitinae L3, L6, L17; Gelechiidae L4, L7, L8; Blastobasidae L5, L9. Plant species are as follows. Arecaceae: *Astrocaryum standleyanum, Attalea butyracea, Elaeis oleifera, Oenocarpus mapora*. Bignoniaceae: *Callichlamys latifolia*. Fabaceae: *Albizia adinocephala, Enterolobium cyclocarpum, Enterolobium schomburgkii, Fissicalyx fendleri, Inga* sp., *Machaerium milleflorum*, *Mucuna mutisiana*, *Platypodium elegans*. Malvaceae: *Apeiba membranacea*. Polygonaceae: *Triplaris cumingiana*. Sapindaceae: *Paullinea fibrigera, Sapindus saponaria*.

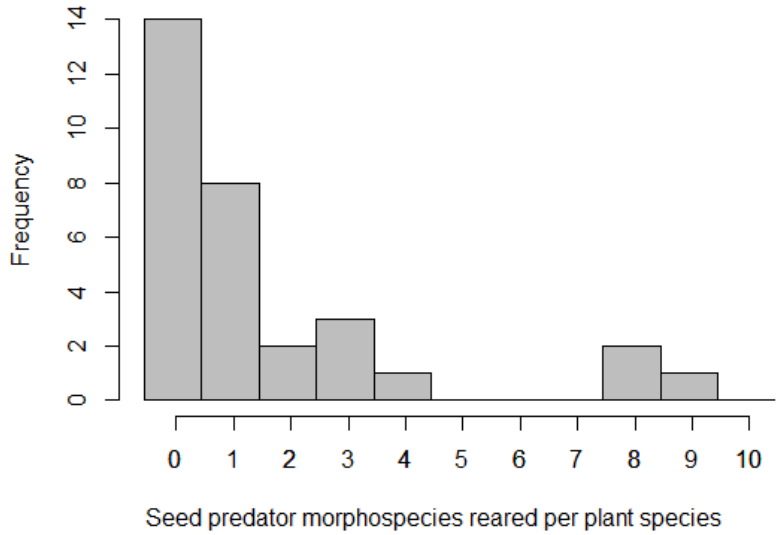

Figure 3.5. Histogram of the number of seed predator morphospecies reared per plant species. Only plant species with a total of ≥50 seeds reared are included (*n*=31).

3.5 Discussion

3.5.1 Trends in seed predation rates in relation to rainfall

Previous studies assessing variation in enemy pressure across a rainfall gradient have compared a single wet and dry site only (Brenes-Arguedas et al. 2009; Spear et al. 2015; Gaviria & Engelbrecht 2015, but see Weißflog et al. 2015), with some transplanting species to a site outside of their natural range (Spear et al. 2015; Gaviria & Engelbrecht 2015). Such designs have limitations in a) true replication, making differences attributable to rainfall uncertain, and b) field relevance, exposing most study species to novel communities of enemies and potentially excluding specialist mortality agents. This means our study arguably represents the first to sample locations spanning the entire gradient enabling true replication, and looking at within-species trends in mortality across their natural ranges.

Contrary to prediction, we found no tendency for the intensity of pre-dispersal seed predation by insects to increase with rainfall. Previous studies have investigated other categories of plant enemy in relation to rainfall and humidity, with a variety of results. Pathogen-related mortality of understory seedlings of multiple plant species was higher at wetter relative to drier sites across the Panama rainfall gradient (Brenes-Arguedas et al. 2009; Spear et al. 2015). However, insect herbivores showed no such trend or caused more damage in dry sites (Baltzer & Davies 2012; Gaviria & Engelbrecht 2015; A. Weißflog 2015). Current evidence therefore suggests that natural enemy groups may differ in their response to precipitation. Humidity and soil moisture can directly affect fungal activity and infection (Griffin 1963; Martin & Loper 1999; Swinfield et al. 2012; Hersh et al. 2012) whilst similar direct effects of natural variation in humidity on the demography and activity of insects are less obvious. More intense and more extended dry seasons may not be sufficient to decrease insect population sizes (Coley & Barone 1996). In addition, wet forest plant species may be better protected against enemies, as they need to invest less in drought tolerance (Coley & Barone 1996; Gaviria & Elgelbrecht 2015), resulting in lower mortality at

wetter sites. Similar trends in seed predation rates could occur if plants invest more heavily in the chemical defences within seeds at wetter sites.

3.5.2 Intensity of seed predation and variation across species

We detected great variation in the intensity of seed predation among species. Whilst predation rates could be very high on particular individuals (frequently >70%; C. Jeffs unpublished data), the majority of species and individuals had modest or low mean predation rates. This supports evidence that pre-dispersal insect seed predators can sometimes generate high mortality levels >90% (Janzen 1971; Fenner & Thompson 2005; Crawley 2013), although low and modest predation rates appear to be more typical (Jones & Comita 2010; Crawley 2013; Ctvrtecka et al. 2014; Chapter 2). It is possible that post-dispersal seed predation may contribute to our estimated predation rates, although our collection of freshly abscised seeds minimises this contribution. Many tropical plant species in central Panama are suggested to be seed-limited (Svenning & Wright 2005) meaning even modest seed predation rates may reduce seedling recruitment. For instance, substantial predation rates were observed for both *Cupania sylvatica* (29.6% predation rate) and *Oenocarpus mapora* (46.3%), species which demonstrate significant seed limitation (Svenning & Wright 2005).

3.5.3 Host-specificity of seed predators

Host-specificity in our food web was high, in agreement with other studies documenting high host-specificity in pre-dispersal insect seed predators (Janzen 1980; Novotny & Basset 2005; Pinzon-Navarro et al. 2010; Ctvrtecka et al. 2014; S. Gripenberg unpublished data). High host-specificity is consistent with a role for pre-dispersal insect seed predators in enhancing community diversity if generating negatively density-dependent survival. In a review of insect herbivore and granivore guilds by Novotny & Basset (2005), seed predators were found to be the most host-specific guild. Our results also demonstrate that host-specificity of pre-dispersal insect seed predators is comparable to the most host family-specialised guilds of tropical herbivores (Novotny et al. 2010). Our results support observations that monophagy is common in insect seed

predators (Janzen 1980; Pinzon-Navarro et al. 2010; Ctvrtecka et al. 2014). Cases of polyphagy typically involve congeneric or confamilial hosts in our study and in previous work (Hopkins et al. 1983; Novotny et al. 2005; Pinzon-Navarro et al. 2010; Ctvrtecka et al. 2014), suggesting taxonomic specialisation. However, without sequencing samples reared from different hosts it is not possible to rule out the possibility that cryptic, host-associated seed predator species may be present (Pinzon-Navarro et al. 2010). Where congeneric host species occur sympatrically, seed predators may still generate frequency stabilising negative density-dependence when they attack hosts differentially (e.g. Hosaka et al. 2009). Alternatively, polyphagous seed predators may link host population dynamics through apparent competition, as observed in vertebrate post-dispersal seed predators (Garzon-Lopez et al. 2015), with potential consequences for community diversity (Lewis & Gripenberg 2008). Despite high host-specificity, low and even modest seed predation intensities may not be sufficient to generate frequency-stabilising Janzen-Connell effects (Ctvrtecka *et al* 2014).

3.5.4 Conclusion

Our study provides the first assessment of variation in seed predation rates along a precipitation gradient. The results do not support the prediction of an increase in pest pressure at wetter sites (Givnish 1999). Longer-term studies with increased host plant coverage, as well as assessments across additional rainfall gradients, are required to test the robustness and generality of this result.

Our results add to existing evidence that the host-specificity of pre-dispersal seed predators is high, supporting a potential diversity enhancing role of these seed predators if they do also generate negatively density-dependent survival. However in most species, the intensity of pre-dispersal seed predation may not be sufficient to cause frequency-stabilising mortality. Efforts are now needed to test for the prevalence of density-dependent pre-dispersal seed predation within a community and its variation in strength with precipitation. Such assessments are challenging as they will require data collection at multiple spatial scales to identify the scale at which seed

predators respond to the abundance of resources (e.g. Schupp 1992; Visser et al. 2011). The complementary or compensatory action of pre- and post-dispersal mortality agents also needs greater attention (Crawley 2013).

Even when acting in a density-independent manner, pre-dispersal seed predators may still have important consequences for seed-limited species and potential consequences for community diversity even when few species are affected. Experiments linking species-specific predation rates and seed limitation assessments will be of particular value in assessing the impact of seed predators on recruitment and community diversity. Investigations relating how seed predation intensity relates to seed and fruit traits are also of great value in predicting the susceptibility of unassessed species (e.g. Pinzon-Navarro et al. 2010; Beckman & Muller-Landau 2011; Ctvrtecka et al. 2014).

3.6 Acknowledgements

We thank Joe Wright for valuable discussions, Marleny Rivera for assistance in insect identification, and Tom Lewis, Joe Lewis and Lily Lewis for assistance in seed dissections. This work was supported by a Varley-Gradwell Travelling Fellowship to C. Jeffs and NERC Standard Grant NE/J011169/1 to O. Lewis.

3.7 Supplementary information

S3.7.1: Plant species list, sampling sizes, and predation rates (Table S3.2).

Table S3.2. Plant species list with species-specific seed predation rates and locations of sample collections. Species-specific predation rates (PR) calculated as the percentage of seeds predated based on total number of seeds dissected (Seeds) pooled across sites. Sites where seeds from each species were collected are shown. Sites are arranged left to right from lowest to highest annual rainfall: PP (Panama Pacifico), MT (Metropolitano), AM (Parque Soberania, ANAM office), CC (Parque Soberania, El Charco), PL (Oleoducto 'Pipeline Road'), BV (Buena Vista Peninsula), SM (Fort Sherman), SR (Santa Rita).

Species	**Family**	**Seeds**	**PR**	PP	MT	AM	CC	PL	BV	SM	SR
Albizia adinocephala	Fabaceae	778	13.8		X			X			
Anacardium excelsum	Anacardiaceae	249	11.2		X	X	X	X		X	
Apeiba membranacea	Malvaceae	1852	35.8				X	X			
Apeiba tibourbou	Malvaceae	940	36.4		X	X		X		X	
Astrocaryum standleyanum	Arecaceae	41	29.3				X	X	X	X	
Attalea butyracea	Arecaceae	431	29.2	X		X	X	X	X		
Bactris sp.	Arecaceae	-	-	X							
Bixa orellana	Bixaceae	-	-							X	
Bursera simaruba	Burseraceae	-	-				X				
Callichlamys latifolia	Bignoniaceae	419	21	X			X	X			
Cavanillesia platanifolia	Malvaceae	34	8.8	X	X					X	
Chamaedorea tepejilote	Arecaceae	-	-							X	
Combretum decandrum	Combretaceae	-	-	X	X	X	X	X			
Cupania rufescens	Sapindaceae	-	-					X			
Cupania scrobiculata	Sapindaceae	-	-								
Cupania sylvatica	Sapindaceae	226	29.6			X	X	X			
Doliocarpus multiflorus	Dilleniaceae	-	-								X
Elaeis oleifera	Arecaceae	76	100	X		X		X			
Enterolobium cyclocarpum	Fabaceae	1165	9.1			X	X	X			
Enterolobium schomburgkii	Fabaceae	2722	50.5		X	X	X	X		X	
Fissicalyx fendleri	Fabaceae	38	21.1		X	X					
Geonoma congesta	Arecaceae	-	-							X	

Table S3.2 ***Continued***

Species	**Family**	**Seeds**	**PR**	PP	MT	AM	CC	PL	BV	SM	SR
Geonoma cuneata	Arecaceae	-	-							X	
Hippocratea volubilis	Hippocrataceae	-	-					X			
Inga sp.	Fabaceae	95	14.7	X				X		X	
Iriartea deltoidea	Arecaceae	-	-								X
Jacaranda copaia	Bignoniaceae	-	-			X					
Luehea seemannii	Tiliaceae	582	5.2		X			X	X		
Machaerium arboreum	Fabaceae	-	-					X			
Machaerium milleflorum	Fabaceae	262	10.3		X	X		X			
Machaerium riparium	Fabaceae	22	22.7					X			
Mucuna mutisiana	Fabaceae	-	-				X			X	
Oenocarpus mapora	Arecaceae	1589	46.3	X	X	X	X	X	X	X	X
Ormosia macrocalyx	Fabaceae	30	0							X	
Arecaceae sp.	Arecaceae	-	-							X	
Paullinia fibrigera	Sapindaceae	-	-				X				
Platypodium elegans	Fabaceae	66	15.2					X			
Pourouma bicolor	Cecropiaceae	176	9.1					X		X	
Prionostemma aspera	Celastraceae	-	-					X			
Protium sp.	Burseraceae	-	-						X		
Sapindus saponaria	Sapindaceae	227	26.4	X	X						
Schizolobium parahyba	Fabaceae	131	3.1			X		X			
Serjania atrolineata	Sapindaceae	-	-					X			
Serjania cornigera	Sapindaceae	155	31.6							X	
Serjania decapleuria	Sapindaceae	33	21.2				X	X			
Serjania mexicana	Sapindaceae	19	21.1					X			
Socratea exorrhiza	Arecaceae	218	1.8					X		X	X
Terminalia oblonga	Combretaceae	721	7.6	X			X				
Thinouia myriantha	Sapindaceae	59	52.5				X	X			
Triplaris cumingiana	Polygonaceae	394	4.6	X	X						
Vantanea depleta	Humiriaceae	145	6.9								X

Chapter 4:

Soil fungi increase the diversity of perennial plants recruiting from grassland seed banks

Christopher T. Jeffs, Clive Hambler, Stephen Harris, Simon Mortimer & Owen T. Lewis

Flowers recruiting from grasslands within Wytham Woods. Photo credit Chris Jeffs.

4.1 Abstract

1. Biotic plant-soil feedbacks (PSF), where plants alter the characteristics of soils with consequences for plant performance, are a prominent mechanism by which soil biota such as fungi can alter plant diversity. Conspecific PSF are widely documented for individual plant species, but their consequences for community diversity in the field are poorly known. Furthermore, studies of field systems where soils have known long-term histories of preconditioning are lacking.

2. We used manipulative experiments to test the effects of soil fungi and fungus-like oomycetes on the diversity of plants germinating and establishing from calcareous grassland seed banks, and whether community level effects were driven by density-independent or density-dependent effects of fungi on species' abundances.

3. We propagated seed banks, under fungicide and water control treatments, from compositionally distinct calcareous grasslands with associated data on the history of soil preconditioning (fine-scale data on plant abundance) spanning 12 years. Fungicide treatment significantly reduced the diversity of perennial plants recruiting relative to controls, but this effect was marginally non-significant when annuals, biennials and woody species were included.

4. Across species (n=40), fungicide did not affect abundance in a consistent direction. Of 13 species with sufficient data, only one (*Prunella vulgaris*) showed evidence suggesting a negative PSF effect: fungicide increased abundance more in samples from areas of greater conspecific preconditioning over 12 years. Low abundances for individual species may have restricted our ability to identify species-specific fungicide effects.

5. Summed subtle effects of fungi on the abundances of multiple species are likely to drive our observed changes in plant diversity. To our knowledge, this is the first study to investigate spatial variation in long-term soil preconditioning levels on the diversity of grassland communities.

4.2 Introduction

Understanding the biotic and abiotic factors affecting plant community diversity is of great practical and theoretical interest (Crawley 1997; Wright 2002). Mortality during early plant life stages is suggested to have the greatest influence on community diversity and composition (Webb & Peart 1999; Harms et al. 2000; Green et al. 2014) and has therefore been a particular focus in investigating the processes structuring plant communities.

A prominent context in which plant performance and community diversity can be affected is through plant-soil feedbacks (PSF). PSF occur when plants alter the chemical, physical or biotic characteristics of soils with consequences for the performance of conspecifics or heterospecifics (Bever et al. 1997). Direct and indirect interactions with fauna and microorganisms within the soil (e.g. invertebrates, nematodes, protozoa, bacteria and fungi) can have great consequences for plant recruitment and diversity (De Deyn et al. 2003; Bonkowski 2004; van der Heijden et al. 2008; Bever et al. 2015) and thus biotic PSF effects have important community structuring implications. For instance, biotic plant-soil feedback effects on seedling performance driven by pathogenic and mutualistic soil microorganisms have been suggested to influence the relative abundances of species, with rarer species experiencing stronger negative feedbacks (in both tropical forests and temperate grasslands; Klironomos 2002; Mangan et al. 2010), and more abundant species experiencing stronger positive feedbacks (in a Canadian grassland, Klironomos 2002).

Fungal and fungi-like oomycete (henceforth 'fungal') pathogens and mycorrhizal fungi are ubiquitous in soils and have been demonstrated to profoundly affect seed and seedling survival (Gilbert 2002; Hodge & Fitter 2013). As a consequence, fungi can affect plant community composition and diversity through a variety of mechanisms (e.g. Grime et al. 1987; Dalling et al. 1997; van der Heijden et al. 1998; Bagchi et al. 2014; Bever et al. 2015). Fungal pathogens can

increase plant evenness by imposing negative density-dependent survival on locally abundant species (Bagchi et al. 2014), or decrease evenness and richness by differentially affecting weaker competitors (Hodge & Fitter 2013; Bever et al. 2015). Fungal pathogens can generate negatively density-dependent (Bell et al. 2006; Yamazaki et al. 2009; Bagchi et al. 2010; Lin et al. 2012; Liu et al. 2015) and positive distance-dependent survival (Augspurger 1984; Packer & Clay 2000; Yamazaki et al. 2009; Liang et al. 2015), enhancing the diversity of seedlings (e.g. Bagchi et al. 2014). Enemy-mediated conspecific feedbacks are a prominent explanation for high tropical plant diversity (the Janzen-Connell hypothesis: Janzen 1970; Connell 1971). However, strong negative PSF are common in temperate grasslands (Klironomos 2002; Kulmatiski et al. 2008) and may have important effects on plant diversity and composition in these ecosystems (Petermann et al. 2008; Bever et al. 2015).

Similarly, beneficial mycorrhizae and endophytic fungi can increase species richness and diversity by enhancing the establishment and survival of rare species (Gange et al. 1990; Gange et al. 1993), for example by conferring protection against pathogens (Arnold et al. 2003; Dalling et al. 2011; Daguerre et al. 2015). Mycorrhizae may also increase community evenness through enhancing the performance of rare species relative to numerically dominant, stronger competitors (Grime et al. 1987; Bever et al. 2001; van der Heijden 2003). However, positive density-dependent associations with mycorrhizae can allow some species to become locally dominant, reducing diversity (Hartnett & Wilson 1999; Bever 2002).

PSFs are commonly studied using soils preconditioned in greenhouses (often as monocultures) over a limited number of generations (Klironomos 2002; Kulmatiski et al. 2008; Petermann et al. 2008). However, microbial communities develop and persist over long periods (Wallenhammar 1996; Hwang et al. 2012; Kardol et al. 2013), can interact with multiple host species (Husband et al. 2002; Gallery et al. 2010), and microbe-microbe interactions can alter disease incidence (Daguerre et al. 2015). Thus, PSFs measured using short-term and monoculture preconditioned soils are likely to differ from those for field soils sampled from established, multi-species

communities (e.g. Kulmatiski & Kardol 2008; Brandt et al. 2013). The smaller number of studies relating PSFs to soil-preconditioning in the field typically collect data on current plant densities only (e.g. Kos et al. 2013). Studies relating PSF strengths to long-term soil preconditioning history, particularly in herbaceous communities, are lacking despite having been highlighted as a key area for focus in PSF studies (Kulmatiski & Kardol 2008). Additionally, whilst positive and negative PSF have been demonstrated for an increasing number of species in grasslands (Klironomos 2002; Kulmatiski et al. 2008; Harrison & Bardgett 2010; van der Putten 2013; Bever et al. 2015), simultaneous experimental demonstrations of how species level PSF translate into effects on community structure are rare (Kulmatiski et al. 2008; but see Petermann et al. 2008) meaning that the consequences of PSF for community diversity are unknown. The diversity structuring role of PSF effects may be of particular importance in low-nutrient calcareous grassland communities in Europe which exhibit relatively high flowering plant species-richness, the maintenance of which is of conservation interest.

To address this research gap we propagated seed banks from compositionally distinct calcareous grasslands with species-specific soil preconditioning data spanning 12 years. We compared the plant assemblages establishing under fungicide and water control treatments to assess whether a) fungi affect the diversity of plants propagating from the soil, b) plant diversity alterations can be related to the effects of fungi on species-specific abundances, and c) intraspecific variation in plant performance is driven by spatial variation in long term field soil preconditioning by conspecifics. To our knowledge, this is the first study to investigate the effects of spatial variation in long-term soil preconditioning levels on PSF effects within grassland communities.

4.3 Methods

4.3.1 Study site and soil core collection

We collected soil samples from a 10 ha calcareous grassland site, Upper Seeds (51°46'10.5"N 1°19'55.3"W), and neighbouring grasslands located within Wytham Woods, Oxfordshire, UK (Figure 4.1). Formerly pasture land and cultivated for crops until 1981, the Upper Seeds long-term restoration experiment was initiated in 1985 to investigate the effects of sheep grazing (during the spring or autumn versus a control) and scrub removal management within two 90 m x 90 m plots each consisting of nine 30 m x 30 m paddocks (see Gibson et al. 1987 for full details). Within each paddock are four 1m x 1m permanent community plant community quadrats. Management treatments were not of focal interest in our study, but created distinct plant communities (Gibson 2010) increasing the ability to generalise the results of our experiment and thus were included in analyses (see below).

The 1m x 1m quadrats have presence/absence data at a resolution of twenty-five 20x20cm sub cells per quadrat from censuses conducted twice annually between 2000 – 2011, except for 2008 and 2010. Twelve additional plant community quadrats are located within each adjacent ancient sheep and rabbit-grazed grassland areas (Sundays Hill and Bowling Alley) and a heavily sheep grazed Spring + Autumn 'Heavy Grazed' area. Seed bank regeneration allowing re-establishment of species absent from the above-ground flora (Grubb 1977; Wellstein et al. 2007) is important in grasslands where vertebrate grazing creates disturbed areas and bare ground (Fenner & Thompson 2005). The seed bank can therefore be integral to grassland diversity, especially those undergoing restoration, despite the low proportion of area where propagation is possible (Kalamees & Zobel 2002).

Between April 1st – 8th 2013, we took soil samples from 108 quadrats across all Upper Seeds and adjacent grassland sites. For each quadrat each soil sample was comprised of 20 soil cores (2.5

cm diameter, 6 cm deep), with five cores taken from each side of the quadrat. We stored samples separately in Ziploc bags in a cool, dark location for between two and nine days before sowing. A cold spring in 2013 meant April collection pre-empted field germination.

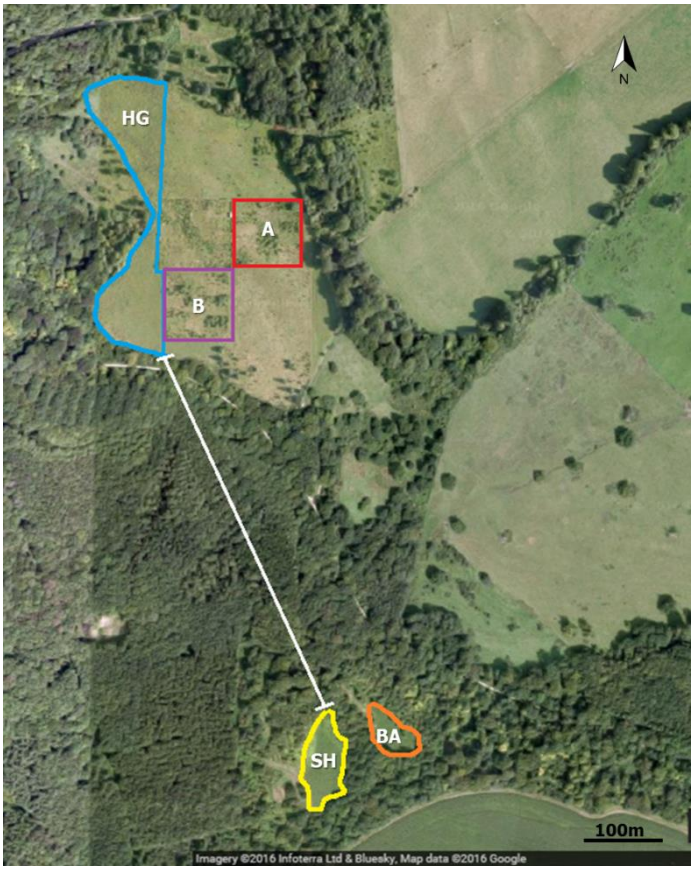

Figure 4.1. Map of sampled grassland sites at Upper Seeds and Wytham Woods. Community quadrats and soil sampling was conducted within the Upper Seeds grassland containing a Heavy Grazed (HG) area and two plots (A + B) of paddocks with Spring, Autumn and Control grazing treatments. Soil sampled were also collected from the Sunday's Hill (SH) and Bowling Alley (BA) ancient, species-rich grassland areas.

4.3.2 Experimental set up

We crumbled and homogenised the soil sample from each quadrat (removing stones, turf and

large roots) and divided it equally. Each half was sown in a 0.5 cm thick layer, to optimise the proportion of germinating seeds (Dalling et al. 1995), on top of a 4 cm thick sterilised substrate soil medium within two 23 cm × 18 cm × 5 cm seedling trays. Within each pair, we assigned one tray to a 'fungicide' and one to a water 'control' treatment.

We collected substrate soil from a disused grassland site at the Sawmill, Wytham Woods (51°46'26.5"N 1°19'29.7"W) and sterilised it before use (supplementary information S4.7.1). As the sterilised substrate soil outweighs the soil core layer 8:1, variation in nutrient levels among quadrats should be minimal whilst still introducing ample microbial inoculum for infection (e.g. Liu et al. 2015).

We established seed trays on April 9th – 10th 2013 and the experiment ran until 8th August. Tray pairs for each quadrat were randomly distributed across nine bench locations within a greenhouse. Trays were watered as necessary. The greenhouse was air conditioned, holding temperatures at 20^{0}C between April and July, and between 18 - 31^{0}C in August. Aphid pests were detected in multiple trays, so all experimental trays were treated on the 10th July using a contact insecticide/detergent solution to minimise non-target effects on plant performance.

We selected two broad spectrum fungicides to exclude both oomycete and fungal pathogens ('SL567A' [active ingredient metalaxyl-M 465.2 g/l] and 'Amistar' [active ingredient azoxystrobin 250 g/l] respectively, Syngenta, UK) which can generate substantial seed and seedling mortality in many plant species (Packer & Clay 2000; Bell et al. 2006; Petermann et al. 2008; Bagchi et al. 2014). Amistar can also reduce mycorrhizal colonisation in numerous plant families particularly when used as a soil drench (Diedhiou et al. 2004; Tbaileh 2012). Fungicide application was conducted two days, two weeks (both 0.17 ml SL567A and 0.4 ml Amistar per litre of water) and six weeks (0.17 ml SL567A and 0.13 Amistar per litre) after sowing. Fungicide was applied using a manual spray bottle, using volumes sufficient to wet the upper soil layers and

leaf surfaces without run off. Control trays received the same volume of water.

4.3.3 Data collection

Germinating seedlings were individually marked daily. Due to their difficulty in identification, all germinating grasses and sedges were removed by hand and not included in analyses. These species comprise under 18% of the total angiosperm richness in the quadrat plant community data (data not presented). Identification censuses were conducted weekly using seedling (Chancellor 1966; Hanf 1973; Muller 1978) and adult (Rose 2006) identification keys and verified by S. Harris. Seedlings that died before species-level identification were identified to as low a taxonomic level as possible or referenced to persisting, morphologically similar seedlings in the same trays.

Based on the individuals surviving at the end of the experiment, for each tray we recorded the abundance of each species, total abundance, species richness, and diversity as the effective number of species (exponential Shannon's diversity index; Jost 2006).

As starting seed bank densities were homogenised and fungicide exposure at the dormant seed stage was limited, any community level effects of fungicide in our study are likely to be due to effects on the mortality of seedlings during the germination phase pre- and post-emergence. Fungal effects on mortality are thus likely to occur shortly after germination, with propagules on seeds and in the soil infecting plants after extension of the radicle and shoot, or post-emergence via root contact or splash transmission onto leaf tissue (Gilbert 2002; Gallery et al. 2010; Swinfield et al. 2012).

To assess the extent to which conspecifics had preconditioned the soil in each quadrat, we used data from the long-term field censuses. These data score plant species as present or absent in each of 25 sub cells per $1m^2$ quadrat. A cell occupancy score of 0 - 25 was used as a proxy for 'density' for each species in each quadrat for a given year. Data were available for 2000 - 2011

(except 2010 and 2008, where data were estimated as the average of the two adjacent years). Data are also missing for 2012, the year immediately preceding the experiment. The potential for each species to precondition soils in each quadrat was estimated in three ways, using either 1) total number of cells occupied by conspecifics in 2011 (reflecting a low residence time of host-associated soil biota), 2) sum of cells occupied by conspecifics between 2000 - 2011, and 3) a linearly weighted version of (2) where the contribution of a cell to the total preconditioning score is weighted by time since occupation to reflect a situation where conspecifics contribute less to preconditioning as time progresses. This was calculated for each species by multiplying the cell occupancy score from each year by 1 to 12 for years from 2000 to 2011, and summing these values across years. Vegetation data were not available for Heavy Grazed, Bowling Alley and Sunday's Hill sites. Congeneric preconditioning values were also calculated by summing independent preconditioning values of congeneric species.

4.3.4 Statistical analyses

4.3.4.1 Community level metrics: We calculated community variables using PAST software (version 2.17c; Hammer et al. 2001) and the 'Vegan' package (Oksanen et al. 2015) in R statistical software (version 3.1.3, R Development Core Team 2015). Linear mixed effects models (lme4 package; Bates et al. 2015) were conducted for analyses on the effective number of species (log transformed to improve model fit). Species richness and total abundance were analysed using generalised linear mixed effects models with Poisson errors and a glmerControl 'bobyqa' optimiser (lme4 package) to rectify convergence problems. We fitted fungicide as a fixed effect, and quadrat and greenhouse position as random effects. To account for variation introduced by sites or quadrat management types, we added either a 'site' or 'management type' fixed effect to each analysis. Only one of these two variables could be added at a time due to perfect nesting of Heavy Grazed levels.

The minimum adequate model with the lowest AIC value after stepwise model simplification (Crawley 2007) determined whether the site or management model was selected for presentation.

As Management and Site are part of the experiment design, these factors were not removed during simplification of the selected model. Diagnostic plots of all analyses demonstrated that model assumptions were met.

Perennials are more likely to modify soil conditions than short-lived annuals, so a second set of analyses was conducted on the subset of perennial species. Annuals, shrubs and trees were not abundant enough to allow community analyses. *Medicago lupulina* and *Senecio jacobea* were also included in the perennial community due to their commonly perennial growth habit in our sites.

4.3.4.2 Mortality effects: We used Paired Wilcoxon signed-rank tests to compare the proportion of individuals dying post-emergence between fungicide and control trays.

4.3.4.3 Species-specific effects of fungicide on abundance: Paired Wilcoxon signed-rank tests were used to compare the final abundance of each species between fungicide and control trays. Only species present in at least five tray pairs were analysed, and Benjamini-Hochberg adjusted P values were calculated to correct for multiple testing. Mixed effects models failed to meet model assumptions.

We also used one sample Wilcoxon signed rank tests to assess whether a) the direction of the effect of fungicide on abundance was significantly biased in a positive or negative direction across all species, and b) the proportion of individuals was greater within fungicide relative to control trays across all species (tray pair specific proportions averaged per species). We calculated species-specific mean direction of change values as the average direction value across tray pairs where a decrease, no change and increase in abundance due to fungicide addition are assigned values of -1, 0 or 1 respectively.

4.3.4.4 Density-dependent effects of fungicide on species-specific abundances: To test for density-dependent fungicide effects, we used only species with a minimum of fifteen tray pairs for logistic regression analyses. Conspecific soil preconditioning level (using summed, linear or most recent preconditioning values in separate analyses) was plotted against a binary 'direction of change' score of 0 for a decrease or 1 for an increase in abundance due to fungicide addition. Tray pairs where there was no difference in abundance between treatments (mean 16% of tray pairs per species) were excluded. For both analyses, tray pairs were only included if at least one individual of the focal species occurred in one tray of the pair.

4.4 Results

A total of 6260 seedlings germinated, with 5283 individuals surviving to the end of the experiment (many reaching adult and flowering stages). Final abundances averaged 24.5 plants per tray (SE ± 1.7) and ranged from 0 – 136. A single, outlying tray pair where no seeds germinated was removed from all analyses.

4.4.1 Do fungi affect the abundance, richness and diversity of plants recruiting from the soil?

4.4.1.1 Diversity: For the full set of species, there was a marginally non-significant tendency for the effective number of species to be lower in fungicide addition trays (Table 4.1). For perennial species only, the effective number of species was significantly lower in the fungicide treatment. In both cases there was a significant site effect (Figure 4.2; Table 4.1). (See supplementary information S4.7.2 for analyses on alternative measures of diversity).

4.4.1.2 Species richness: No factors significantly explained variation in species richness for either the full set of species, or perennial species considered separately (Figure 4.2; Table 4.1).

4.4.1.3 Total abundance: For the complete community of all species, site was marginally non-significant. For the perennial only community, site was strongly significant (Figure 4.2; Table 4.1). No other factors significantly explained variation in total abundance for either community type.

4.4.1.4 Total post-emergence mortality: Mortality post-emergence was moderate (mean 15.7%, 985/6272 of all individuals, with 60% of the individuals dying not identifiable to any informative taxonomic rank). The proportion of individuals dying within tray pairs did not differ significantly between fungicide and control trays (N = 61, V 754, P = 0.237). This was also true when analysing only perennial species (N = 54, V 627.5, P = 0.324).

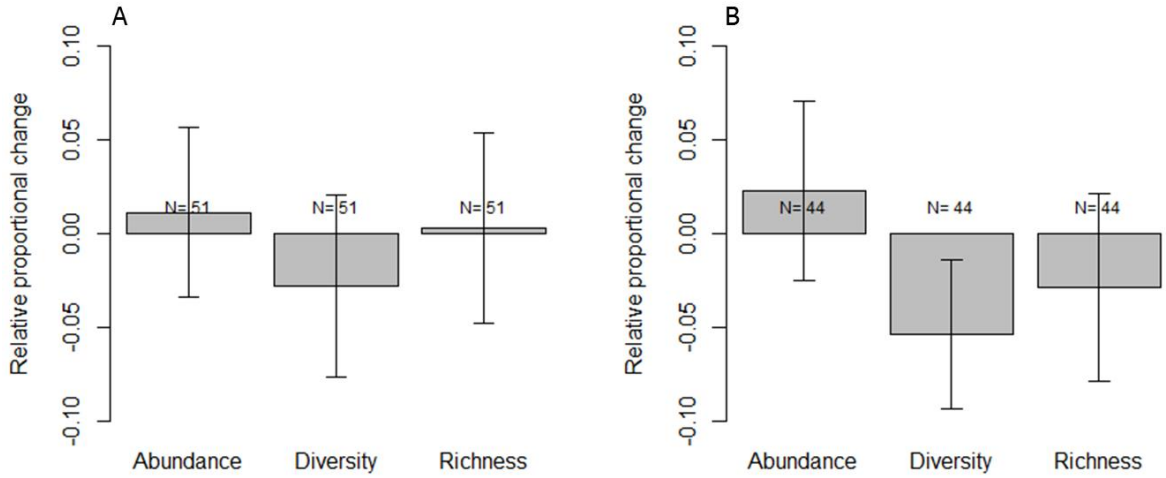

Figure 4.2. Bar chart of fungicide effect sizes for community structure variables. Mean proportional change in total abundance, species diversity (the effective number of species) and species richness between fungicide and water control trays for a) all species, and b) perennial species only. Relative proportional change due to fungicide addition calculated for each tray pair as the absolute difference (fungicide tray minus control tray value) divided by the value of control tray. Error bars show standard errors of the mean.

Table 4.1. Results of mixed-effects model analyses testing for the effect of fungicide on community structure variables. Analyses tested for the difference in mean diversity (the effective number of species; exponential Shannon's index), species richness and total abundance between control and fungicide addition trays. Separate results are presented for analyses on each variable including either all or only herbaceous perennial species (N = number of trays). P values were calculated via analysis of deviance tests, comparing the difference in the fit of two models including and removing each variable. For each response variable, explanatory variables are ordered in the sequence of removal from the maximal model. Variables in bold are statistically significant.

Response	Variable	d.f.	AIC	χ^2	P value
Log(Exponential Shannon's Index)	(Maximal model)		-111.49		
All species	Site:Fungicide	4	-116.23	3.267	0.514
N=102	Fungicide	1	-114.93	3.297	0.069
	Site	**4**	**-107.82**	**15.115**	**0.004**
Log(Exponential Shannon's Index)	(Maximal model)		-91.099		
Perennials species only	Site:Fungicide	4	-91.506	7.594	0.108
N=88	**Fungicide**	**1**	**-88.866**	**4.640**	**0.031**
	Site	**4**	**-85.716**	**13.790**	**0.008**
Species Richness	(Maximal model)		461.03		
All species	Site:Fungicide	4	453.63	0.601	0.963
N=102	Fungicide	1	452.23	0.592	0.442
	Site	4	452.10	6.471	0.167
Species Richness	(Maximal model)		392.96		
Perennials species only	Management:Fungicide	7	379.51	0.554	0.999
N=88	Management	7	373.94	8.426	0.297
	Fungicide	1	378.13	0.618	0.432
Total Abundance	(Maximal model)		852.33		
All species	Site:Fungicide	4	847.06	2.732	0.604
N=102	Fungicide	1	845.41	0.354	0.552
	Site	4	845.94	8.526	0.074
Total Abundance	(Maximal model)		727.62		
Perennials species only	Site:Fungicide	4	723.71	4.086	0.395
N=88	Fungicide	1	721.78	0.069	0.793
	Site	**4**	**727.58**	**13.798**	**0.008**

4.4.2 Do fungi affect the abundance of individual species?

Of 40 species analysed, significant negative effects of fungicide addition on final abundance were found only for *Aethusa cynapium* and *Clinopodium vulgare*, whilst no significant positive effects were detected. No significant fungicide effects remained after accounting for multiple testing using Benjamini-Hochberg adjusted P-values (all P>0.05; Table 4.2). Across all species, there was no significant tendency for fungicide to affect seedling abundance in either a positive or negative direction (analyses for both mean direction of change and proportion of individuals in fungicide trays P>0.05; Figure 4.3).

Repeat analyses on species-specific germination frequencies (prior to post-emergence mortality) were qualitatively the same as species-specific final abundance analyses, except that *Viola hirta* now had sufficient tray pairs for analysis but was non-significantly affected by fungicide addition (supplementary information S4.7.3).

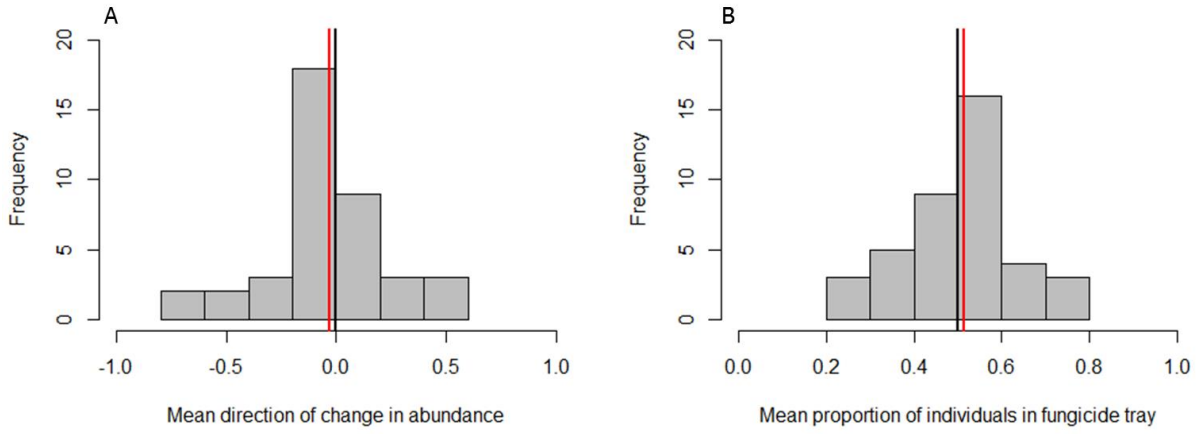

Figure 4.3. Histograms of the effect of fungicide on species-specific abundances. A) Histogram showing the mean direction of change in abundance due to fungicide addition across species relative to control across species. B) Histogram showing the mean proportion of abundance in fungicide tray relative to control across species (average per species across tray pairs). The red lines show the mean fungicide effect sizes, and the null hypotheses in each case are indicated by black vertical lines.

Table 4.2. Results of paired Wilcoxon signed rank tests of the effect of fungicide on the final abundance of each species. Mean and SD values are presented to aid interpretations of the effect size and direction for each species. The replication (N; number of tray pairs), critical statistical (V; sum of ranks showing positive shift), and Benjamini-Hochberg adjusted P values (P.adj) for each species are also presented. Species in bold are statistically significant.

Species	Growth form	Mean Control (±SD)		Mean Fungicide (±SD)		N	V	P	P. adj
Aethusa cynapium	Annual	3.11	(6.72)	1.44	(3.61)	9	32	**0.042**	1
Anagallis arvensis	Annual	0.72	(0.65)	0.62	(0.68)	29	207	0.649	1
Aphanes arvensis	Annual	0.33	(0.52)	0.83	(0.41)	6	3	0.233	1
Betula sp.	Woody	0.60	(0.55)	0.8	(0.45)	5	2	0.773	1
Cerastium fontanum	Perennial	0.71	(0.91)	0.64	(0.50)	14	45.5	1	1
Cirsium eriophorum	Perennial	0.81	(0.98)	0.56	(0.51)	16	52	0.644	1
Cirsium vulgare	Perennial	1.00	(0.71)	0.4	(0.55)	5	8	0.345	1
Clinopodium vulgare	Perennial	1.79	(1.67)	0.83	(0.87)	24	203	**0.045**	1
Crepis capillaris	Annual	3.89	(6.03)	4.06	(8.29)	18	83	0.767	1
Epilobium 'hairy'	Perennial	0.50	(0.52)	0.5	(0.52)	14	52.5	1	1
Epilobium 'smooth'	Perennial	0.67	(0.71)	1.33	(1.0)	9	7	0.124	1
Galium mollugo	Perennial	1.27	(1.01)	0.46	(0.69)	11	35.5	0.131	1
Galium verum	Perennial	1.20	(1.01)	1.33	(1.68)	15	38.5	1	1
Geum urbanum	Perennial	0.95	(1.16)	0.71	(0.69)	38	339	0.455	1
Glechoma hederacea	Perennial	1.05	(1.00)	0.83	(1.02)	41	378	0.283	1
Hypericum 'A'	Perennial	7.69	(11.57)	7.62	(11.52)	55	661.5	0.623	1
Hypericum 'B'	Perennial	15.74	(23.66)	15.49	(20.35)	49	447	0.577	1
Kickxia elatine	Annual	0.8	(0.45)	0.2	(0.45)	5	12	0.233	1
Kickxia spuria	Annual	0.6	(0.70)	0.6	(0.52)	10	22.5	1	1
Linaria vulgaris	Perennial	0.8	(0.79)	0.9	(0.99)	10	20	0.790	1
Lotus corniculatus	Perennial	1.67	(1.72)	1.33	(1.30)	12	32.5	0.627	1
Medicago lupulina	Perennial	2.35	(2.59)	2.27	(2.51)	52	408.5	0.798	1
MS 'Quad1'	Unknown	0.4	(0.55)	0.6	(0.55)	5	6	0.766	1
Oxalis acetosella	Perennial	1.43	(1.28)	1.43	(1.84)	40	311	0.584	1

Table 4.2. ***Continued***

Species	Growth form	Mean Control (±SD)		Mean Fungicide (±SD)		N	V	P	P. adj
Pastinaca sativa	Biennial	0.6	(0.55)	0.6	(0.89)	5	7.5	1	1
Plantago sp.	Perennial	4.05	(5.95)	2.93	(5.79)	29	215.5	0.059	1
Potentilla reptans	Perennial	5.83	(11.41)	5.97	(11.56)	63	745	0.986	1
Prunella vulgaris	Perennial	1.28	(1.37)	1.59	(2.00)	32	138	0.324	1
Ranunculus repens	Perennial	1.03	(1.08)	1.15	(1.04)	33	189	0.519	1
Reseda luteola	Biennial	0.5	(0.65)	1.43	(1.28)	14	16	0.071	1
Rubus sp.	Woody	1.89	(1.67)	1.53	(1.58)	36	287	0.121	1
Rumex obtusifolius	Perennial	0.4	(0.55)	1.2	(0.84)	5	1.5	0.265	1
Sonchus asper	Annual	0.57	(0.54)	0.43	(0.54)	7	16	0.777	1
Thlaspi arvense	Annual	0.33	(0.52)	0.83	(0.41)	6	3	0.233	1
Trifolium repens	Perennial	0.72	(0.75)	0.72	(1.02)	18	76.5	1	1
Urtica dioica	Perennial	2.68	(4.79)	3.39	(7.48)	31	199.5	0.698	1
Verbascum sp.	Biennial	0.92	(1.02)	0.83	(0.76)	24	114	0.742	1
Veronica chamaedrys	Perennial	2.28	(2.47)	2.06	(2.83)	47	352	0.347	1
Veronica serpyllifolia	Perennial	3.0	(5.17)	2.44	(4.04)	9	20	0.831	1
Vicia sp.	Annual	0.77	(0.97)	0.82	(0.64)	17	32	0.592	1

4.4.3 Do the effects of fungi on species-specific abundances differ in relation to conspecific soil preconditioning level?

Of the thirteen species analysed, only *Prunella vulgaris* demonstrated a significant response to historic conspecific density (N = 25, $\chi^2 = 7.57$, P = 0.006; Figure 4.4), with fungicide addition increasing abundance at higher levels of long-term soil preconditioning (summed densities between 2000-2011). For *P. vulgaris*, soil preconditioning estimated by linear weighed density between 2000-2011 was also significant, but preconditioning level in 2011 alone was not. The Benjamini-Hochberg adjusted P-value was marginally non-significant (P = 0.077).

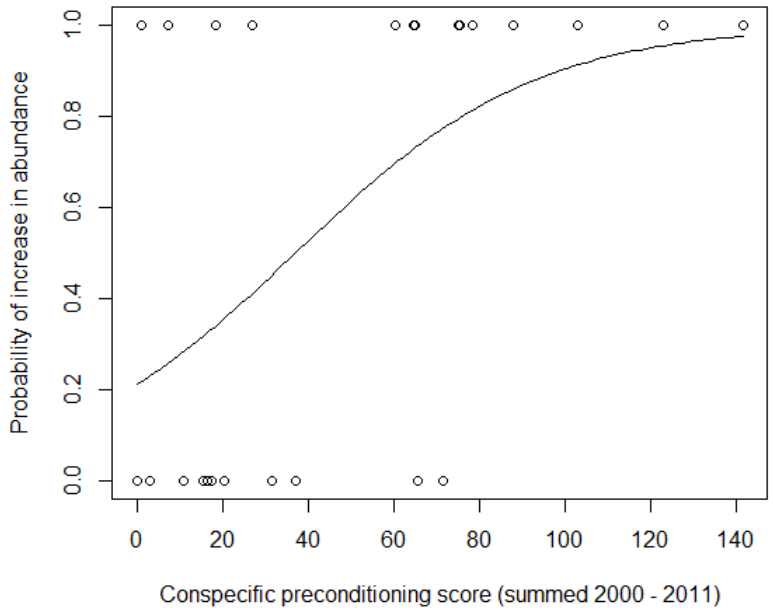

Figure 4.4. Effect of fungicide on the abundance of *Prunella vulgaris* in relation to conspecific soil preconditioning level. Binary logistic regression curve fitted. Data points refer to the direction of change in abundance due to fungicide addition (1 = increase, 0 = decrease) of each tray pair in relation to summed density of *P. vulgaris* adults in quadrats in 2000 - 2011.

4.5 Discussion

To our knowledge, this study is the first to use field soils with long-term plant community data to quantify density-dependent plant-soil feedback effects and their consequences for the diversity of herbaceous grassland communities. Our results demonstrate that fungicide treatment can decrease the diversity of individuals recruiting from grassland seed-banks, consistent with a role for soil fungi in elevating community diversity. However, significant diversity effects occurred only for perennial species, suggesting that different plant growth forms and life-history strategies may have different net responses to fungi (Kulmatiski et al. 2008). Significant effects of fungicide on the abundance of individual species were not observed, and density-dependent PSF effects were rare suggesting that the community-level effects on diversity may be the sum of subtle effects on multiple species.

4.5.1 Do fungi affect the diversity of seedlings propagating from the soil?

In our experiment, plant diversity was reduced when excluding fungi. This effect was significant for the perennial species, but not for all growth forms analysed together. Previous studies have also documented contrasting strengths of PSFs for different plant growth forms. For example in a meta-analysis Kulmatiski et al. (2008) found that temperate grasses and forbs showed large negative PSF, whereas PSF for trees and shrubs were not significantly different from zero. Grasses were excluded from our study but are known to demonstrate the most negative PSF effect sizes of grassland growth forms (Kulmatiski et al. 2008) and can drive effects of fungi on grassland diversity (Grime et al. 1987; Peters & Shaw 1996; Allan et al. 2010; Bonanomi et al. 2012). For instance, mycorrhizal fungi increased the abundance and biomass of subordinate species at the expense of a dominant grass in a mesocosm grassland experiment, with evenness and diversity increasing as a result (Grime et al. 1987). Similarly, Allan et al. (2010) found that foliar fungal pathogens decreased the relative biomass of dominant grasses resulting in increased grassland community evenness and species richness. If grasses had been included in our study the

effects of fungicide treatment might have been larger than the relatively modest effect sizes we observed (~5%). However, greater effects of disease on herbaceous species relative to grasses have also been observed to determine community diversity (Peters & Shaw 1996), making predictions of community level responses to fungal exclusion difficult.

Post-emergence mortality, although appreciable, did not differ significantly between fungicide treatments. Our observed effects of fungicide on plant diversity may therefore be due to effects on mortality at the pre-emergence seed and seedling stages, which can have distinct effects on seedling recruitment rates even when mortality post-emergence is low (Gallery et al. 2010; Chapter 5).

The mechanisms causing our observed diversity alterations are uncertain. As fungicide addition significantly reduced the effective number of species based on exponential Shannon's diversity index but not when calculated as inverse Simpson's index (supplementary information S4.7.2; Jost 2006), reduced species richness may drive our observed diversity reductions rather than decreases in evenness (Magurran 2004). Given the large number of species represented by singletons in our experimental trays, one mechanism by which fungicide addition could reduce diversity is by inhibiting mycorrhizal colonisation and resultant seedling establishment and species richness. Such a mechanism was observed in compositionally similar UK temperate grassland communities (Gange et al. 1993).

4.5.2 Do fungi affect the abundance of individual species?

No species tested showed a significant difference in mean abundance between fungicide addition and water control trays. The sum of subtle absolute or net effects of fungi on the survival of multiple plant species therefore appears to determine our detectable changes in community diversity, rather than being driven by large alterations in the abundance of a small number of species (as in Grime et al. 1987; Dalling et al. 1997).

Relative to studies planting known, higher starting densities of seeds or seedlings within experimental soils (e.g. Leishman et al. 2000; Blaney & Kotanen 2001; McCarthy-Neumann & Kobe 2010a, 2010b; Gallery et al. 2010; Liu et al. 2012), our ability to detect the effects of fungicide on the abundance of individual species may have been limited due to the unknown, low mean abundances and incidences of many species across trays. Additionally, the use of broad spectrum fungicides that exclude both antagonistic and beneficial fungi (Hodge & Fitter 2013) may generate neutral net effects on abundance (e.g. Liang et al. 2015) or effects smaller than if AMF and pathogens were excluded separately. Spatial differences in beneficial and pathogenic fungal communities could also generate intraspecific variation in the net effect of fungi on abundance (Husband et al. 2002; Liu et al. 2015; Wubs & Bezemer 2016) masking overall fungicide effects.

4.5.3 Do the effects of fungi on species-specific abundances differ in relation to conspecific soil preconditioning level?

Of 13 species tested, only the perennial herb, *Prunella vulgaris*, showed evidence suggesting a negative density-dependent feedback effect on abundance consistent with long-term accumulation of host-specific pathogens. Again the low abundances and incidences of our propagated species may reduce our ability to detect PSF effects that may be observable at higher replication. Kulmatiski & Kardol (2008) suggested that non-significant PSF effects were more commonly reported in multi-species versus single-species studies (81% vs 31% respectively) indicating a publication bias. A 92% non-significant PSF rate in our study is thus comparable to the wider literature. In addition, multi-species and field conditioned soils as used in our experiment typically find weaker PSF strengths than monoculture soils, potentially due to the effects of competition mediating PSF effects or due to non-additive microbial interactions (Kulmatiski et al. 2008; Kulmatiski & Kardol 2008; Brandt et al. 2013), thus further reducing detection of significant PSF effects.

Species with low or invariant field abundances, as occurred for many of our focal species, may

not exhibit detectable density-dependence (e.g. Kos et al. 2013). Our presence/absence quadrat data may have also been too coarse to accurately reflect density variation, or conspecifics outside quadrats may have added error.

Alternatively, antagonists and beneficial fungi may balance to yield no net density effect. For example, distance-dependent seedling survival in a temperate tree was only observable when grown on soil inoculated with pathogens or arbuscular-mycorrhizal fungi (AMF) independently, but not when both were added (Liang et al. 2015). Such experiments utilising soils inoculated with washes of AMF, pathogenic and combined soil biota fractions to identify PSF effects are becoming more common (e.g. Klironomos 2002; McCarthy-Neumann & Kobe 2010a; 2010b; Liang et al. 2015). Applying soil washes to artificial seed banks of known starting composition are a potentially powerful tool for linking the species-specific effects of beneficial and antagonistic fungi to resultant changes in community structure.

Our experiment is not able to assess the relative importance of specialist and generalist fungi in generating our observed community diversity alterations. However, our results are not consistent with host-specific enemies generating strong negative density-dependence and increasing community evenness as predicted by the Janzen-Connell hypothesis (e.g. Petermann et al. 2008; Bagchi et al. 2014).

4.5.4 The spatial scale of PSF effects and their implications for community diversity

The strength and direction of PSF effects may determine site level abundance in seedlings of tropical tree (Mangan et al. 2010) and herbaceous grassland species (Klironomos 2002), with rarer species experiencing stronger negative feedbacks. On smaller 'within-site' scales, heterogeneity in conspecific soil preconditioning can generate spatial variation in recruitment success (Brandt et al. 2013; Kos et al. 2013; Wubs & Bezemer 2016) with potential consequences for community structure. In order to identify the ecological relevance of PSF effects, we suggest experiments growing plants on field soils from separate locations (e.g. Wubs & Bezemer 2016)

are preferable to those pooling field soils (e.g. Liang et al. 2015) which may overlook spatial variation in PSF effects.

The spatial scale of heterogeneity in PSF effects is also likely to differ between plant growth forms (Bever et al. 2015). For examples, the zone of influence (i.e. the area where plant mediated soil modification occurs) is smaller for herbaceous species relative to trees, meaning PSF create finer-scale spatial heterogeneity in herbaceous plant recruitment. Additionally, perennial species culture soils for generations in the same location resulting in the local accumulations of species-specific fungi whilst annuals are more ephemeral in their precise locations of growth. Rarer herbaceous species or those utilising ephemeral bare ground are therefore less likely to colonise exact locations previously occupied by conspecifics and be subject to conspecific PSF over extended time periods. Therefore, whilst the potential strength of conspecific PSF effects may be stronger and more negative for annuals than perennials (Kulmatiski et al. 2008), the probability of growing in conspecific preconditioned soils in the field is greater for perennials. The ecological significance of PSF effects may thus differ between plant growth forms.

4.5.5 Conclusions

Investigations utilising field soils of known long-term preconditioning histories, as in this study, are of great value in assessing the true ecological relevance of PSF effects. However, due to practical constraints such studies remain critically rare in herbaceous communities. Our results demonstrate that even when PSF effects are not common or strong, soil fungi can enhance herbaceous perennial species diversity through subtle effects on the abundance of multiple species. Whilst the susceptibility of grassland species to biotic negative plant-soil feedbacks may be common (Klironomos 2002; Kulmatiski et al. 2008), their prevalence under natural field conditions and abundances may be low (Kulmatiski et al. 2008). Accordingly, simultaneous assessments of PSF and alternative mechanisms affecting plant growth such as competition and soil chemistry (e.g. Ehrenfeld et al. 2005; McCarthy-Neumann & Kobe 2010a; 2010b; Mehrabi et al. 2015) are also needed to assess the ecological relevance of biotic PSF in community

structuring.

4.6 Acknowledgements

We thank Phil Smith for his assistance in maintaining the experiment, Laura West-Wilson for soil preparation and plant care, Richard Comont for assistance in plant identification, and Alan Gange and Valerie Brown for valuable advice when planning this experiment. We also thank Oxford's Wytham Committee for granting permission to sample soil and the late Charlie Gibson for collecting the plant community quadrat data.

4.7 Supplementary information

S4.7.1: Substrate soil sterilisation procedure

To sterilise soil we removed organic matter rich top soil and turf, sieved subsoil (0.25 cm wire mesh) to remove stones and large invertebrates, and then autoclave sterilised soil in foil covered metal pans (121^0C holding temperature for 30 minutes) in two cycles 48 h apart. To test the efficacy of the sterilisation process, we made swabs of field and sterilised soils on three replicated Petri dishes of bacterial and fungal culturable media (potato dextrose agar; Sigma-Aldrich, UK) which were incubated at 37^0C for 48 hours. Sterilisation resulted in the complete removal of culturable microbes and no seeds germinated from control trays of watered sterilised soil.

S4.7.2: Results of mixed effects models using alternative measures of community diversity

To test for the effects of fungicide addition on the diversity of individuals recruiting from the soil seed bank, in addition to the effective number of species calculated as exponential Shannon's diversity index, the inverse Simpson's diversity index was also analysed (Table S4.3). These two diversity indices are affected primarily by species richness and evenness respectively (Magurran 2004; Magurran & McGill 2011). For separate analyses including all species or only herbaceous perennial species, only site significantly explained variation in. For the herbaceous perennial species only community, the fungicide effect on inverse Simpson's diversity was marginally non-significant with mean diversity lower in fungicide addition trays.

Table S4.3. Results of mixed-effects model analysis on the difference in mean diversity measured as the effective number of species; inverse Simpson's index. between control and fungicide addition trays. Separate results are presented for analyses on each variable including either all or only herbaceous perennial species (N = number of trays). P values were calculated via analysis of deviance tests, comparing the difference in the fit of two models including and removing each variable. For each response variable, explanatory variables are ordered in the sequence of removal from the maximal model. Variables in bold are statistically significant.

Response	Variable	d.f.	AIC	χ^2	P value
Log(Inverse Simpson's index)	(Maximal model)		-111.50		
All species	Site:Fungicide	4	-114.29	5.206	0.267
N=102	Fungicide	1	-113.65	2.638	0.104
	Site	**4**	**-105.94**	**15.709**	**0.003**
Log(Inverse Simpson's index)	(Maximal model)		-98.485		
Perennials species only	Site:Fungicide	4	-97.112	9.373	0.052
N=88	Fungicide	1	-96.118	2.994	0.084
	Site	**4**	**-89.661**	**14.456**	**0.006**

S4.7.3: Effect of fungicide on germination frequencies of species

In addition to testing for differences in species-specific final abundances, parallel Wilcoxon signed-rank analyses were conducted on data of species-specific total germination frequencies (i.e. prior to post-emergence mortality). The results were qualitatively the same as the final abundance analyses (Table S4.4), except that *Clinopodium vulgare* was now non-significant prior to adjusting P values for multiple testing. In addition, *Viola hirta* now had sufficient tray pairs for analysis although it was non-significantly affected by fungicide addition.

Table S4.4. Results of paired Wilcoxon signed rank tests of the effect of fungicide on the germination frequency of each species. Mean and SD values are presented to aid interpretations of the effect size and direction for each species. The replication (N; number of tray pairs), critical statistical (V; sum of ranks showing positive shift), and Benjamini-Hochberg adjusted P values (P.adj) for each species are also presented.

Species	**Growth form**	**Mean Control (±SD)**		**Mean Fungicide (±SD)**		**N**	**V**	**P**	**P. adj**
Aethusa cynapium	Annual	3.11	(6.72)	1.44	(3.61)	9	32	**0.042**	1
Anagallis arvensis	Annual	0.72	(0.65)	0.62	(0.68)	29	207	0.649	1
Aphanes arvensis	Annual	0.33	(0.52)	0.83	(0.41)	6	3	0.233	1
Betula sp.	Woody	0.60	(0.55)	0.8	(0.45)	5	2	0.773	1
Cerastium fontanum	Perennial	0.71	(0.91)	0.64	(0.50)	14	45.5	1	1
Cirsium eriophorum	Perennial	0.81	(0.98)	0.56	(0.51)	16	52	0.644	1
Cirsium vulgare	Perennial	1.00	(0.71)	0.4	(0.55)	5	8	0.345	1
Clinopodium vulgare	Perennial	1.88	(1.83)	0.88	(0.90)	24	214.5	0.061	1
Crepis capillaris	Annual	3.84	(6.09)	4.011	(8.10)	19	73	0.883	1
Epilobium 'hairy'	Perennial	0.50	(0.52)	0.5	(0.52)	14	52.5	1	1
Epilobium 'smooth'	Perennial	0.67	(0.71)	1.33	(1.0)	9	7	0.124	1
Galium mollugo	Perennial	1.27	(1.01)	0.46	(0.69)	11	35.5	0.131	1
Galium verum	Perennial	1.47	(1.06)	1.53	(2.0)	15	53	1	1
Geum urbanum	Perennial	0.95	(1.16)	0.71	(0.69)	38	339	0.455	1
Glechoma hederacea	Perennial	1.05	(1.00)	0.83	(1.02)	41	378	0.283	1
Hypericum 'A'	Perennial	8.55	(12.89)	8.64	(13.15)	55	548.5	0.873	1
Hypericum 'B'	Perennial	15.98	(24.36)	16.38	(22.04)	50	470.5	0.323	1
Kickxia elatine	Annual	0.8	(0.45)	0.2	(0.45)	5	12	0.233	1
Kickxia spuria	Annual	0.6	(0.70)	0.6	(0.52)	10	22.5	1	1
Linaria vulgaris	Perennial	0.8	(0.79)	0.9	(0.99)	10	20	0.790	1
Lotus corniculatus	Perennial	2.08	(1.93)	1.67	(1.56)	12	42	0.441	1
Medicago lupulina	Perennial	2.34	(2.56)	2.26	(2.52)	53	429.5	0.795	1
MS 'Quad1'	Unknown	0.4	(0.55)	0.6	(0.55)	5	6	0.766	1
Oxalis acetosella	Perennial	1.5	(1.36)	1.43	(1.84)	40	322.5	0.449	1

Table S4.4. ***Continued***

Species	Growth form	Mean Control (±SD)		Mean Fungicide (±SD)		N	V	P	P. adj
Pastinaca sativa	Biennial	0.6	(0.55)	0.6	(0.89)	5	7.5	1	1
Plantago sp.	Perennial	4.19	(7.05)	3.1	(6.95)	31	248	0.062	1
Potentilla reptans	Perennial	5.98	(11.51)	6.11	(11.65)	63	682	0.952	1
Prunella vulgaris	Perennial	1.46	(1.65)	1.8	(2.30)	35	175.5	0.353	1
Ranunculus repens	Perennial	1.06	(1.17)	1.15	(1.03)	33	209	0.614	1
Reseda luteola	Biennial	0.5	(0.65)	1.43	(1.28)	14	16	0.071	1
Rubus sp.	Woody	1.89	(1.67)	1.53	(1.58)	36	287	0.121	1
Rumex obtusifolius	Perennial	0.4	(0.55)	1.2	(0.84)	5	1.5	0.265	1
Sonchus asper	Annual	0.57	(0.54)	0.43	(0.54)	7	16	0.777	1
Thlaspi arvense	Annual	0.33	(0.52)	0.83	(0.41)	6	3	0.233	1
Trifolium repens	Perennial	0.72	(0.75)	0.72	(1.02)	18	76.5	1	1
Urtica dioica	Perennial	2.68	(4.79)	3.39	(7.48)	31	199.5	0.698	1
Verbascum sp.	Biennial	0.92	(1.02)	0.83	(0.76)	24	114	0.742	1
Veronica chamaedrys	Perennial	2.34	(2.15)	2.54	(2.81)	47	338	0.485	1
Veronica serpyllifolia	Perennial	3.0	(5.17)	2.44	(4.04)	9	20	0.831	1
Vicia sp.	Annual	0.77	(0.97)	0.82	(0.64)	17	32	0.592	1
Viola hirta	Perennial	1.17	(0.17)	0.75	(0.41)	6	18.5	1	1

Chapter 5:

Fungi and soil moisture affect the diversity of seedlings germinating from tropical forest seed banks

Christopher T. Jeffs, Lars Markesteijn & Owen T. Lewis

Humid rainforest in Gamboa, Panama. Photo credit Chris Jeffs

5.1 Abstract

1. Fungal pathogens have been implicated in enhancing seedling diversity in the shaded understory of tropical forests. However, many tropical forest plants recruit from the soil seed bank under high- illumination light gap conditions. Effects of fungal pathogens on plant diversity are poorly studied for these species.

2. The effects of soil moisture levels on plant-fungus interactions in these species is also poorly known. If the diversity-enhancing effects of fungal pathogens are higher at high-rainfall sites, this could contribute to the observed positive correlation between precipitation and plant diversity.

3. Using manipulative experiments, we investigated the independent and interacting effects of fungi and soil moisture on seedlings germinating under light gap conditions. Seedlings were allowed to germinate from soil core samples collected from eight sites across a steep gradient of precipitation and plant diversity across the Isthmus of Panama, under fungicide and watering treatments.

4. Fungicide treatment significantly increased the total abundance of recruiting seedlings. Of 48 species tested, only the abundant herbaceous *Blechum* sp., showed a significant fungicide effect, being more abundant in fungicide trays. The effects of fungicide on diversity differed significantly among sites, with suggestions that soil fungi can mediate both increases and decreases in diversity.

5. The effects of fungicide treatment on community structure were not greater at high soil moisture levels or for wetter sites. High light conditions may have reduced post-emergence mortality, reducing the community structuring influence of pathogenic fungi relative to humid, forest understory environments.

6. Soil moisture level effects on plant abundance and diversity differed significantly among sites. Since soil moisture conditions vary markedly depending on season, the time of gap creation is likely to have a strong influence on the resulting composition and diversity of seedling communities.

5.2 Introduction

The biotic and abiotic processes that maintain and structure plant diversity in species-rich tropical rainforests have received widespread attention (reviewed by Wright 2002). A particular area of focus has been the early stages of plant demography, with mortality at the seed and seedling stages suggested to most strongly influence community diversity and composition (Webb & Peart 1999; Harms et al. 2000; Comita et al. 2014; Green et al. 2014). Many tropical forest plants produce seeds that can persist for years or even decades in the soil, and which propagate when light gaps form, for example following treefalls (Dalling & Brown 2009). Rapid emergence from these seed banks confers a competitive advantage on seedlings (Garwood 1983; Kennedy & Swaine 1992); the processes that influence seed and seedling performance following light gap creation are therefore likely to have an important influence on the diversity and composition of established plants (Grubb 1977; Brokaw 1985; Wright 2002).

Interactions with soil microbes can strongly influence seed and seedling performance. For example, soil fungi and fungus-like oomycete pathogens can greatly reduce plant growth and survival, and infection is typically highest at the seed and seedling stages (Gilbert 2002). Fungal pathogens play an important role in determining tropical seedling survival under shaded, understorey conditions (Augspurger 1983; Bagchi et al. 2010; Alvarez-Loayza & Terborgh 2011; Liu et al. 2015), and can alter seedling community diversity by imposing negatively density-dependent survival (Bagchi et al. 2014). Pathogens are also an important source of mortality in many seed-banking pioneer species within the soil (Dalling, Swaine & Garwood 1998; Leishman et al. 2000; Schafer & Kotenen 2003). Fungal pathogens can contribute to seasonal increases in tropical seed bank species diversity by causing high mortality in abundant, small-seeded species (Dalling et al. 1997). Seed-banks are comprised of mostly small-seeded, *r*-selected species such as pioneers, whose seedlings are also likely to be particularly susceptible to natural enemies such as fungal pathogens because they exhibit trade-offs between growth and defence (Augspurger & Kelly 1984; Dalling & Hubbell 2002; Wright 2002; Fine et al. 2006). However, fungal effects on

recruitment success can operate differentially at the seed stage and in seedlings both pre-emergence (the germination phase) and post-emergence (Gallery et al. 2010). It is unknown whether soil pathogens affecting seed-banking species at the germination and post-emergence phases can structure the diversity of seedling assemblages, as has been shown to occur in the forest understory (Bagchi et al. 2014).

Beneficial fungi can also influence plant diversity through their effects on plant growth and survival (Bever et al. 2001). Mycorrhizae and fungal endophytes can protect against pathogen attack and enhance seedling survival (Arnold et al. 2003; Dalling et al. 2011; Daguerre et al. 2015). They can also reduce evenness and community diversity if dominant species particularly benefit from their action (Hartnett & Wilson 1999; Bever 2002). For example, fungi-mediated positive density-dependent survival can give an advantage in succession to early colonising species prior to pathogen accumulation (Reynolds et al. 2003). Conversely, mycorrhizae may increase species richness (Gange et al. 1993) or increase evenness (Grime et al. 1987; van der Heijden 2003) by enhancing the survival and establishment of rare species with the potential to increase community diversity (Bever et al. 2001). As small-seeded tropical pioneer species are suggested to be more dependent on mycorrhizae for initial survival and growth than shade tolerant species (Kiers et al. 2000), the relative influence of mycorrhizae on diversity may differ between understory and light gap seedling communities.

The activity of both pathogenic and mutualistic fungi is closely linked to humidity and soil moisture (Augspurger & Kelly 1984; Gilbert 2002; Schafer & Kotanen 2003). Mortality caused by desiccation-intolerant natural enemies such as fungi is also expected to increase with annual rainfall by maintaining large population sizes in the absence of a harsh dry season (Givnish 1999; Brenes-Arguedas et al. 2009; Spear et al. 2015, but see Coley & Barone 1996; Gaviria & Engelbrecht 2015). Working on understory seedlings in Panama, both Brenes-Arguedas et al. (2009) and Spear et al. (2015) found that seedlings suffered elevated mortality from pathogens at wetter forest sites. Yet further work is needed to investigate the consequences of such patterns on

community diversity (Comita et al. 2014) and identify whether similar patterns occur under high-light, gap conditions. High irradiance reduces humidity in light gaps (Bazzaz & Pickett 1980; Denslow 1980), which may explain reduced mortality from fungal pathogens under high light conditions (Augspurger & Kelly 1984; see also Augspurger 1984; Hood et al. 2004; Swinfield et al. 2012; McCarthy-Neumann & Ibanez 2013). It is possible that increased irradiance in light gaps may reduce levels of pathogen-mediated mortality for otherwise susceptible species, and limit the community structuring effects of pathogens relative to effects observed in the humid forest understory (e.g. Bagchi et al. 2014). In the case of mutualistic fungi, light levels and soil moisture are also important. Endophytic fungi can switch between negative, neutral and beneficial effects on plant growth under differing light conditions (Alvarez-Loayza et al. 2011). The abundance and richness of the mycorrhizal community can vary in relation to plant species composition (Husband et al. 2002) and environmental conditions such as soil moisture (Rabatin 1979; Anderson et al. 1984). Mycorrhizal root colonisation can be variably affected by soil moisture levels, with observations of high infection in moist soils (Lodge 1989) but frequent observations of reductions in soils experiencing high precipitation levels and poor drainage (Lodge 1989; Boucher & Malajczuk 1990; Hartnett & Wilson 1999). Examples of how mycorrhizal infection levels respond to altered soil moisture are lacking from tropical forest habitats.

Here, we investigate the independent and interacting effects of fungi and soil moisture in structuring seedling communities germinating from tropical forest seed banks under light gap conditions. We applied fungicide and soil moisture treatments to soil cores and investigated the consequences for the abundance, richness, diversity and composition of surviving seedlings. Our soil seed banks were sampled from eight forest sites across the Isthmus of Panama, spanning a steep gradient of precipitation, dry season length and plant species richness (Engelbrecht et al. 2007; Condit et al. 2013). We predicted that the effects of fungi on community structure would differ in response to soil moisture levels, through differing influences on the activity of pathogenic and beneficial fungi. We also predicted that the effect of fungi on community structure would be greater for seed banks sampled from wetter sites, where we expect fungal

pathogens to be more abundant, diverse and active, and mycorrhizal associations to be reduced (Boucher & Malajczuk 1990; Givnish 1999; Hartnett & Wilson 1999). To our knowledge, our study is the first to test for variation in the community structuring effects of soil fungi across a well replicated regional humidity gradient.

5.3 Methods

5.3.1 Soil sampling and experimental setup

The eight soil sampling sites used in the study (Figure 5.1) span a steep positive precipitation and species richness gradient (Engelbrecht et al. 2007; Condit et al. 2013). Soil samples were taken from ten locations within a 1 ha plot per site (80 samples in total), and were separated by at least 35 m.

Five soil cores (17.8 cm diameter, 3 cm) separated by a minimum of 3 m surrounding a 5 x 5m area were taken from each location and stored in plastic bags (for between 9 - 15 days) in a cool, dark basement until sowing. Leaf litter from each soil core area was also stored in separate plastic bags for later use. Soil sampling ran towards the end of the dry season between 7th – 21st April 2014, a period corresponding to near-peak seed bank density and richness in central Panama (Dalling et al. 1997).

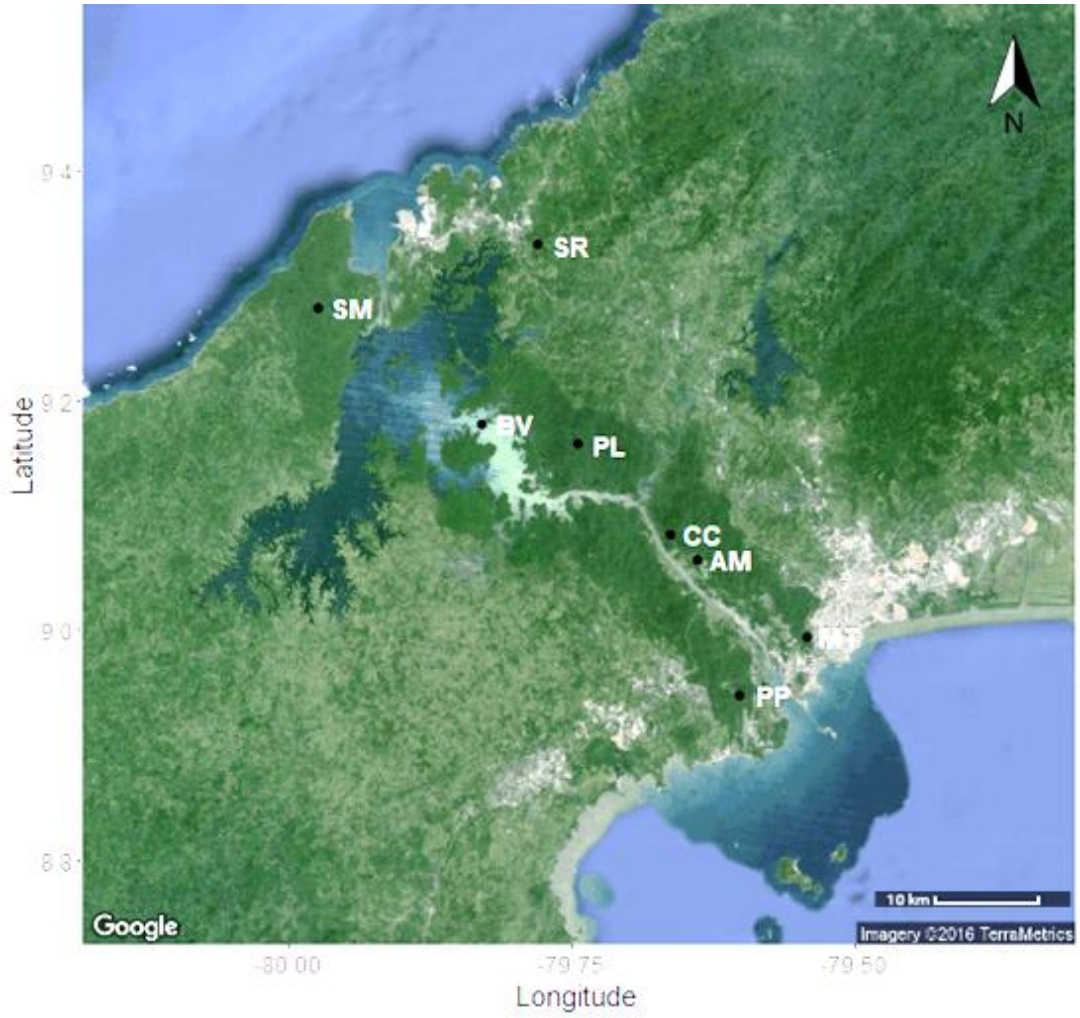

Figure 5.1. Position of forest sites sampled across the Isthmus of Panama rainfall and plant species richness gradient. From lowest to highest annual rainfall, the sites are PP (Panama Pacifico), MT (Metropolitano), AM (Parque Soberania, ANAM office), CC (Parque Soberania, El Charco), PL (Oleoducto 'Pipeline Road'), BV (Buena Vista Peninsula), SM (Fort Sherman), SR (Santa Rita). Image credit Google, ©2016 TerraMetrics. Figure produced using ggmap package (Kahle & Wickham 2013) in R statistical software (version 3.1.3, R Development Core Team 2015).

Table 5.1. Positions and characteristics of the study sites. Data for the minimum gravimetric soil water content (GSWC; percentage of dry mass), annual rainfall (mm), dry season deficit (the peak volume of rain required to reach field capacity soil water content over the dry season), and species richness (the number of tree species >1cm diameter at breast height) were collected within a central 1 ha plot at each site (Condit et al. 2013, GSWC data L. Markesteijn unpublished data).

Site	Latitude	Longitude	Annual rainfall (mm)	Range GSWC	Mean GSWC	Dry season deficit (mm)	Species richness
Panama Pacifico	08.943500	-079.601533	1756	21.22 - 66.20	45.71	-571.688	69
Metropolitano	08.994589	-079.543000	1874	21.07 - 69.58	45.31	-574.926	49
ANAM	09.061950	-079.639400	2007	22.58 - 61.80	43.87	-579.902	34
El Charco	09.084050	-079.663400	2051	16.76 - 69.52	46.25	-579.148	84
Oleoducto	09.161760	-079.745300	2330	19.74 - 75.36	52.65	-549.423	132
Buena Vista	09.179361	-079.829600	2595	27.14 - 79.48	57.18	-510.470	85
Fort Sherman	09.280869	-079.974700	2848	39.70 - 80.92	69.07	-491.669	161
Santa Rita	09.335578	-079.780742	3054	28.22 - 85.93	70.39	-487.530	170

Between 22nd – 26rd April 2014, soil cores from each location were pooled and homogenised, and the soil was divided into two equal volumes, then spread as a 1cm layer in two seedling trays (53 × 25 × 7cm) on the surface of a 3cm layer of common, sterilised forest soil substrate (collected from a single pit in Chilibre; 9.162628 ° N, -79.618027 ° W towards the centre of the gradient). This method aims to minimise between-site variation in soil abiotic conditions, whilst providing sufficient inoculum to infect seedlings (Liu et al. 2015). To remove soil biota, substrate soil was steam sterilised for 12 hours at 121^{0}C. A 25 g sample of the dry leaf litter sample associated with each soil core was macerated in a blender and scattered onto each tray as an additional source of fungal propagules. Trays were lined with perforated foil to standardise drainage, and with a layer of absorbent paper to minimise loss of soil and fungal propagules during watering.

Each tray in the pair was randomly assigned to either a fungicide or no-fungicide control treatment. Trays from each pair were placed adjacent to each other in randomised positions in two shadehouses, with 40 pairs of trays and equal representation of sites in each shadehouse. The light level in both shadehouses was ~25% of the ambient (verified with a photometer) equivalent to light levels in a medium size forest gap in central Panama (Denslow et al. 1998). The walls and roof of each shadehouse were lined with clear plastic to prevent additional water entering trays and altering soil moisture treatment levels (see below).

To check the efficiency of substrate soil sterilisation, and to check for tray contamination from dispersal of seeds into shadehouses, four control trays containing only sterilised substrate soil were also established in each shadehouse. Only two seedlings germinated in these trays, indicating low rates of contamination. These species did not occur in our experimental trays.

5.3.2 Fungicide treatment

Fungicide treatment trays were hand-sprayed until leaf wetting occurred with Amistar® (Syngenta Ltd.; active ingredient; *azoxystrobin*, 0.1 ml Amistar in 1000 ml of water). This is a broad-spectrum fungicide, effective against fungi and fungi-like oomycetes, is known to influence seedling diversity in tropical forests (Bagchi et al. 2014). Equivalent volumes of water were sprayed on control trays. Fungicide application took place away from control trays to avoid contamination. Fungicide was applied two days after sowing, and on a further three occasions, at two-week intervals. All trays were also treated once monthly with the insecticide Engeo® (Syngenta Ltd.; active ingredient, *thiamethoxam*: 0.05 ml in 1000 ml water) to prevent damage by insect herbivores entering the shadehouses.

5.3.3 Water treatment

Five locations per site were assigned randomly to a ‘high’ (90% gravimetric soil water content [GSWC]) or ‘low’ (50% GSWC) soil moisture treatment. Monthly surface GSWC measurements taken between April 2014 and March 2015 at our study sites showed an annual mean range of

20.4 – 66.8% for the four driest sites, and 28.7 – 80.4% for the four wettest sites (Table 5.1). Our selected GSWC levels are therefore likely to reflect natural between-site and seasonal variations in GSWC, particularly at wet sites, with the potential to influence fungal activity within the soil.

Maximum GSWC of the sterilised soil substrate was estimated after repeatedly watering a 4cm layer of substrate soil to field capacity over a 12 hour period. Ten 10 g subsamples were then taken and dried at 90^0C in a drying oven until mass loss ceased. The percentage difference in mass between 100% and 0% water holding soils was then used to set tray mass targets to ensure 50% and 90% maximum GSWC was maintained during the experiment. Each tray was weighed every morning (using a balance accurate to the nearest gram) to calculate how much water should be added to maintain the desired GSWC. Daily water loss varied in relation to weather conditions but never exceeded a 15% decrease from maximum GSWC, still within realistic values for our sites. As only a 1cm layer was used compared to 3cm of substrate soil, variation in GSWC due to site soil differences was likely to be minimal. In all treatments, foliage of seedlings in the trays was also wetted with manual spray bottles after morning watering and in the evening to better mimic sporadic episodes of daily rainfall.

5.3.4 Data collection and seedling identification

Germinating seedlings were marked daily using individually numbered plastic cocktail sticks and germination date recorded. Seedling mortality and disease or desiccation symptoms were assessed during daily censuses. The experiment ran for ten weeks, sufficient time for the majority of seedlings to germinate (Dalling et al. 1995), and a sufficient growing period for substantial mortality to occur (Augspurger & Kelly 1984). Seedlings were identified by experienced botanists during bi-monthly censuses and a final census at the end of the experiment (July 1st - 3rd). Where species-level identifications were not possible, unique morphospecies names in addition to the lowest assignable taxonomic rank possible were recorded. Initial seed densities within each tray are unknown. As soil samples for each location were homogenised before establishing experimental trays, and fungicide exposure at the dormant seed stage was limited,

effects of fungicide on survival are likely to be concentrated at the germination phase, both pre- and post-emergence.

5.3.5 Data Analyses

Seedling census data were used to calculate inverse Simpson's diversity index (henceforth 'effective number of species'; Jost 2006), species richness, total abundance, and composition, as well as the abundance of individual species, for each experimental tray.

5.3.5.1 Community structure analyses: Analyses excluded pairs of trays where one or both of the trays had <15 seedlings, since diversity indices can be sensitive to low abundances (Legendre & Legendre 2012), and species richness is highly correlated with abundance at low sample sizes. Mean seedling abundance in each tray included in the analyses was 50, with only 10% of trays having fewer than 20 individuals. No trays from Panama Pacifico had sufficient abundances for analysis for this or any subsequence analyses.

All community structure analyses were conducted using mixed effects models in R statistical software (lme4 package, Bates et al. 2015; R version 3.1.3, R Development Core Team 2015). A linear mixed effects model with normal errors was fitted for analyses on the effective number of species, and generalised linear mixed effect models with Poisson errors were used for species richness and total abundance. Species richness analyses used a glmerControl 'bobyqa' optimiser to solve convergence problems.

For each analysis, fungicide and water treatments, site-specific humidity (see below), and two-way interactions between all factors were fitted. The location of origin for each soil sample was also included as a random effect to pair fungicide and water control trays. To account for microclimate variation, shadehouse was added as a fixed effect, and bench position within shadehouse as a random effect. Shadehouse was not fitted as a random effect as a factor with two levels cannot have an estimated variance and one with fewer than five levels cannot have a

distribution fit to them. The minimum adequate models were selected using a stepwise model selection procedure following Crawley et al. (2007), with non-significant highest-order interactions removed first, followed by the least significant factor and leaving only significant variables explaining observed variation. As shadehouse was a factor of the experimental design, it was retained in all models irrespective of significance. P values were calculated via analysis of deviance model comparisons, where the difference model fit is tested between models with and without the factor of interest.

Four possible measures of site-specific humidity were available: mean GSWC, minimum GSWC, (both, L. Markesteijn unpublished data), dry season water deficit, and annual rainfall (both, data from Condit et al. 2013). There were strong linear correlations between all variables, so separate models including each humidity variable were compared (with fungicide and water interactions included) and the model with the lowest AIC selected for further analysis and interpretation (supplementary information S5.7.1). For the effective number of species (log transformed to improve model fit) and species richness analyses, models fitting each humidity variable did not meet model assumptions, with all demonstrating an increasing trend in residuals with fitted values indicating a missing linear predictor. Therefore analyses were re-fitted with 'site' as a categorical variable which improved model fit. For total abundance, model fit was similar for mixed effects models fitting each humidity variable and a site categorical variable. The site variable was marginally better behaved and was therefore presented for consistency.

We also conducted regression analyses to directly test whether the fungicide effect size on each community response variable (mean relative change in diversity, richness or abundance per tray pair calculated as the absolute difference between fungicide and control trays divided by the value of the control tray) varied across the humidity gradient. In each model we fitted one of the four humidity variables, water treatment, and the humidity × water interaction as explanatory variables. We also fitted a model with site as a categorical variable. For each community response variable, we presented the global model with the explanatory variable giving the lowest residual

deviance.

5.3.5.2 Species level abundance: We used Wilcoxon signed rank tests to investigate whether the abundance of each species differed significantly between fungicide and water control trays. Only species with a minimum of three tray pairs where at least one seedling is present were used. Generalised linear mixed effects models using species-specific abundances per tray as replicates failed to meet model assumptions of normality and homogeneity of errors.

We also used one sample Wilcoxon signed rank tests to assess whether a) the direction of the effect of fungicide on abundance was significantly biased in a positive or negative direction across all species, and b) the proportion of individuals was greater within fungicide relative to control trays across all species (tray pair specific proportions averaged per species). We calculated species-specific mean direction of change values as the average direction value across tray pairs where a decrease, no change and increase in abundance due to fungicide addition are assigned values of -1, 0 or 1 respectively.

5.3.5.3 Community composition: To assess the factors influencing community composition, for each tray pair we calculated the Sorensen similarity index: $S = 2N_s / (C_a + C_b)$, where N_s equals the number of shared species and C_a and C_b equal the total number of species in each fungicide addition and control tray, respectively (Magurran & McGill 2011). Using generalised linear models with normal errors, we then tested whether Sorensen index (i.e. the effect of fungicide on composition) varied in relation to humidity (either rainfall or dry season deficit), soil water treatment, and their interaction. As for previous analyses, we omitted trays with fewer than 15 individuals.

5.4 Results

A total of 5,370 seedlings representing 112 morphospecies germinated across all 160 experimental trays. Overall, 62.5% of morphospecies were eventually assigned to a known species identification, 18.75% to genus, and 9.82% to family. Only 8.93% of morphospecies had an unknown taxonomic affiliation. Five pairs of trays from four locations were removed from the analyses due to incomplete census data, complete failure of germination, or insect herbivory (prior to insecticide application) and seedling identification.

5.4.1 Do fungi affect the abundance, richness and diversity of seedlings recruiting from the soil seed bank?

5.4.1.1 Effective number of species: Significant site × water and site × fungicide interactions were detected, whilst the fungicide × water interaction was non-significant (Figure 5.2; Table 5.2). Mean diversity was lower in the fungicide treatment in four, and lower in the control treatment for three sites. Mean diversity in high water trays was greater for four sites and lower in two of the six sites relative to low water trays.

5.4.1.2 Species richness: Significant differences in species richness were detected between sites only. All other factors and higher order interactions were non-significant (Table 5.2).

5.4.1.3 Total abundance: For total seedling abundance there was a significant fungicide effect and a significant site × water interaction (Figure 5.2; Table 5.2). Mean total abundance was greater within fungicide trays for all sites analysed. Mean total abundance in high water trays was greater for three and lower in three of the six sites relative to low water trays.

Table 5.2. Results of mixed-effects model analyses testing for the effects of fungicide, soil water level and site on community structure. N equals the number of trays analysed. P values were calculated via analysis of deviance tests, comparing the difference in the fit of two models including and removing each variable. For each response variable, explanatory variables are ordered in the sequence of removal from the maximal model. Statistically significant factors are in bold.

Response	Variable	d.f.	AIC	χ^2	P value
Log(Inverse Simpson's Index)	(Maximal model)		-89.980		
N=84	Fungicide:Water	1	-91.638	0.343	0.558
	Shadehouse	**1**	**-86.187**	**7.451**	**0.006**
	Site:Fungicide	**6**	**-86. 481**	**17.157**	**0.009**
	Site:Water	**5**	**-88.382**	**13.256**	**0.021**
Species Richness	(Maximal model)		395.64		
N=84	Site:Fungicide	6	387.24	3.600	0.731
	Site:Water	5	381.13	3.889	0.566
	Fungicide:Water	1	380.13	0.997	0.318
	Water	1	378.60	0.473	0.492
	Fungicide	1	378.61	0.487	0.485
	Water	1	377.09	0.473	0.492
	Shadehouse	1	377.34	2.256	0.133
	Site	**6**	**391.29**	**24.680**	**<0.001**
Total Abundance	(Maximal model)		681.17		
N=84	Site:Fungicide	6	679.66	0.495	0.482
	Fungicide:Water	1	670.81	0.086	0.769
	Shadehouse	1	669.30	0.495	0.482
	Fungicide	**1**	**675.52**	**6.712**	**0.010**
	Site:Water	**5**	**676.03**	**15.217**	**0.010**

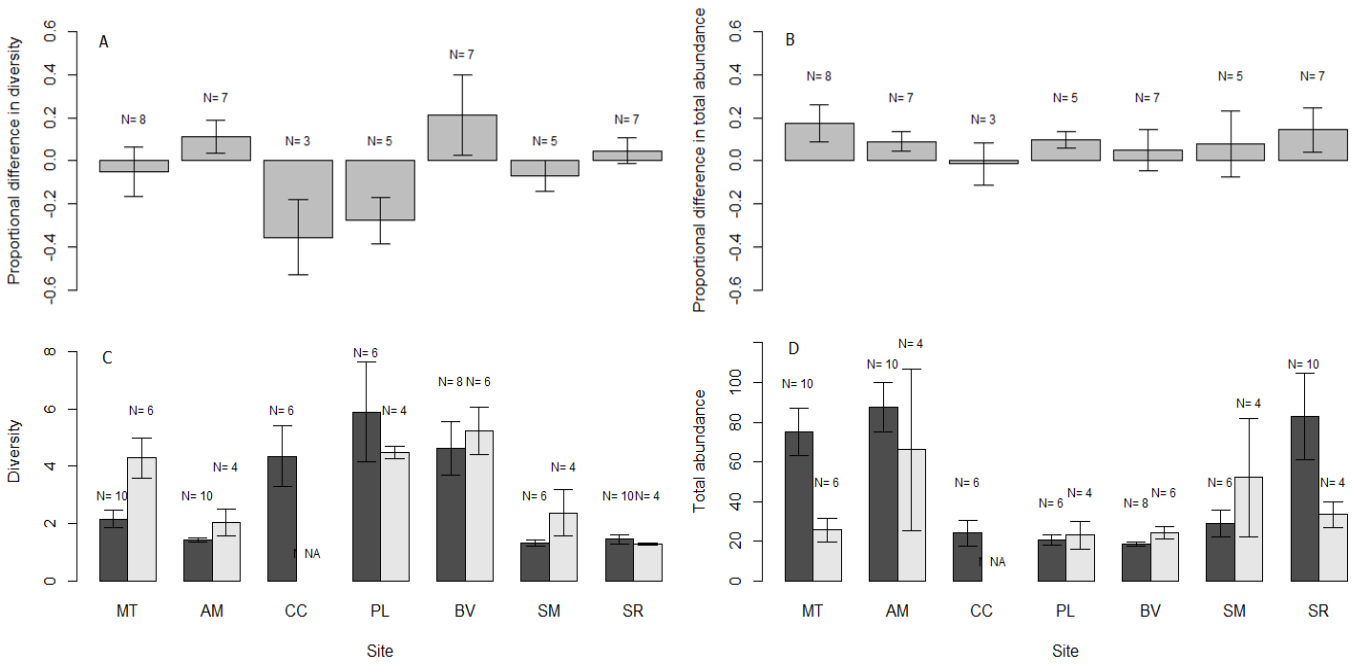

Figure 5.2. Bar plots showing the effects of fungicide and soil moisture treatments on the mean proportional change in community structure. Bar plots are presented for the effect of fungicide on the proportional changes in mean A) effective number of species, and B) total abundance, split by site. N = number of tray pairs. Barplots are also shown for the absolute changes in mean C) effective number of species and D) total abundance, as a function of water level treatment ('high' = black, 'low' = grey) and site. N = number of individual trays in final analysis. Proportional change between trays accounts for the paired nature of the fungicide treatment. Proportional change is calculated as the absolute difference between the control and treatment tray for each pair, divided by value of the control tray. Negative values indicate that fungicide causes a reduction in the effective number of species or species richness relative to controls. Sites are arranged in order from lowest to highest annual rainfall, MT (Metropolitano), AM (Parque Soberania, ANAM office), CC (Parque Soberania, El Charco), PL (Oleoducto 'Pipeline Road'), BV (Buena Vista Peninsula), SM (Fort Sherman), SR (Santa Rita). No low water trays for El Charco possessed sufficient total abundances for analysis.

5.4.2 Do fungicide effects on community structure and composition vary across a humidity gradient?

The effect of fungicide on community diversity, richness, or abundance did not vary significantly with any humidity variable or between sites, and no water treatment effect or interaction with humidity was significant (all analyses P>0.05).

There was a marginally non-significant negative relationship between mean GSWC and Sorensen index of community similarity ($F_{1,40}$=3.946, P=0.054; Figure 5.3). Fungicide appears to have a stronger effect on community composition at wetter sites.

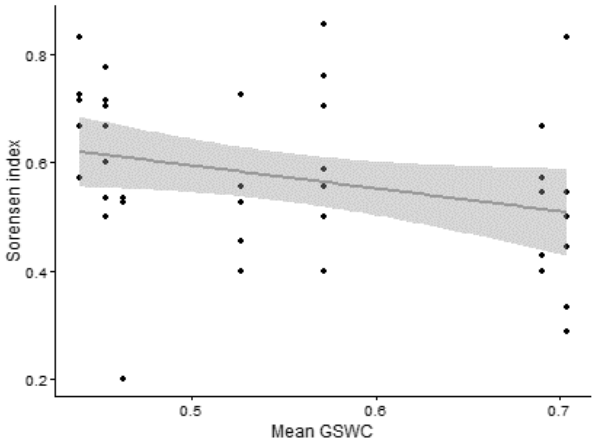

Figure 5.3. Sorensen similarity index between individual tray pairs within sites, as a function of mean GSWC. When there are no unique species in one sample relative to its paired sample, Sorensen index equals 1. Fitted lines show linear model predictions and the shading indicates 95% confidence intervals.

5.4.3 Do fungi affect the abundance of individual species?

Of 48 species tested, only *Blechum* sp. differed significantly in abundance between fungicide treatments. Fungicide addition increased the abundance of this species (Table 5.3). This significance was maintained even after Benjamini-Hochberg adjusted P-values were used to account for multiple testing. There was no significant community level mean direction in fungicide addition effect on abundance (t = -0.884, df = 47, P = 0.381) or the proportion of individuals within the fungicide treatment tray (t = -0.751, df = 47, P = 0.457) (Figure 5.4).

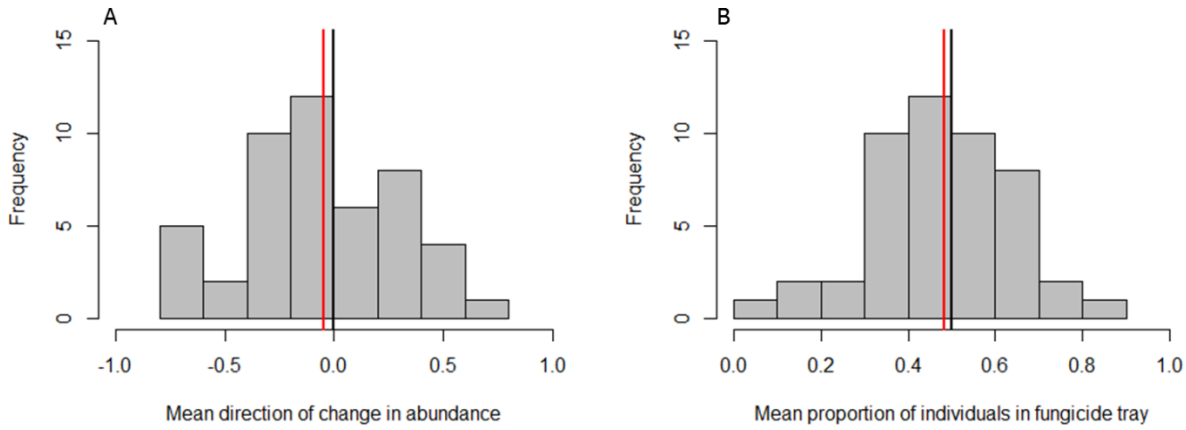

Figure 5.4. Histograms of the mean fungicide-mediated effect on species-specific abundances. A) Histogram showing the mean direction of change in abundance due to fungicide addition across species relative to control across species. B) Histogram showing the mean proportion of abundance in fungicide tray relative to control across species (average per species across tray pairs). The red lines show the mean effect sizes, and the null hypotheses in each case are indicated by black vertical lines.

Table 5.3. Results of paired Wilcoxon signed rank tests of the effect of fungicide on the final abundance of each species. Mean and SD values are presented to aid interpretations of the effect size and direction for each species. The replication (N; number of tray pairs), critical statistical (V; sum of ranks showing positive shift), and Benjamini-Hochberg adjusted P values (P.adj) for each species are also presented. Species in bold are statistically significant.

Species	Family	Mean Control (SD)		Mean Fungicide (SD)		N	V	P value	P. adj
Apeiba sp.	Tiliaceae	1.67	(1.53)	1.33	(0.58)	3	4	0.773	1
Apeiba membranacea	Tiliaceae	1.64	(1.87)	1.50	(1.70)	36	262	0.541	1
Aristolochia maxima	Aristolochiaceae	1.40	(0.89)	0.60	(0.55)	5	12.5	0.203	1
Aristolochia tonduzii	Aristolochiaceae	1.00	(1.22)	0.80	(0.45)	5	6	0.850	1
***Blechum* sp.**	**Acanthaceae**	**18.5**	**(31.5)**	**22.3**	**(33.8)**	**67**	**386**	**<0.001**	**0.02**
Bonamia trichantha	Convolvulaceae	1.00	(0.82)	0.75	(0.96)	4	6	0.850	1
Boraginaceae sp.	Boraginaceae	1.00	(0.82)	1.00	(1.41)	4	5	1.000	1
Calathea sp.	Marantaceae	0.80	(0.45)	0.20	(0.45)	5	12	0.233	1
Cecropia insignis	Urticaceae	0.75	(0.50)	3.25	(5.19)	4	1.5	0.586	1
Cecropia obtusifolia	Urticaceae	2.54	(2.64)	3.58	(3.74)	24	99	0.378	1
Costus sp.	Costaceae	0.25	(0.50)	1.00	(0.82)	4	2	0.345	1
Enterolobium schomburgkii	Fabaceae	1.00	(0.63)	1.00	(1.10)	6	7.5	1.000	1
Erythroxylum sp.	Erythroxylaceae	0.50	(0.58)	1.25	(1.26)	4	2	0.345	1
Ficus insipida	Moraceae	0.83	(0.75)	1.67	(1.37)	6	1.5	0.269	1
Ficus sp.	Moraceae	1.71	(2.06)	2.43	(2.70)	7	9	0.444	1
Hampea sp.	Malvaceae	0.50	(0.58)	0.50	(0.58)	4	5	1.000	1
Heisteria sp.	Erythropalaceae	1.03	(1.05)	1.66	(2.32)	29	144	0.275	1
Jacaranda copaia	Bignoniaceae	0.60	(0.55)	0.40	(0.55)	5	9	0.766	1
Luehea seemannii	Tiliaceae	1.67	(1.15)	1.58	(2.31)	12	47	0.542	1
MALE1*	Unknown	0.14	(0.38)	1.29	(1.25)	7	3.5	0.071	1
Maprounea guianensis	Euphorbiaceae	0.83	(0.41)	0.67	(0.52)	6	4	0.773	1
NEURLO*	Unknown	0.71	(0.49)	0.43	(0.53)	7	14	0.484	1
Ochroma pyramidale	Bombacaceae	0.67	(0.52)	0.33	(0.52)	6	14	0.484	1
Ossaea quinquenervia	Melastomataceae	4.25	(3.10)	3.00	(2.94)	4	5	0.423	1
Passiflora auriculata	Passifloraceae	0.50	(0.58)	0.50	(0.58)	4	5	1.000	1
Pavonia sp. '1'	Malvaceae	3.21	(3.04)	3.14	(2.98)	43	320	0.940	1

* Morphospecies name assigned in raw data.

Table 5.3. ***Continued***

Species	Family	Mean Control (SD)		Mean Fungicide (SD)		N	V	P value	P. adj
Pavonia sp. '2'	Malvaceae	5.33	(7.51)	4.00	(5.20)	3	1	1.000	1
PELU1*	Unknown	0.50	(0.55)	1.17	(0.75)	6	1.5	0.265	1
Perebea xanthochyma	Moraceae	1.80	(2.39)	1.60	(2.07)	5	6	0.850	1
Piper sp.	Piperaceae	2.29	(2.98)	2.71	(7.18)	7	21	0.254	1
Pittoniotis trichantha	Rubiaceae	2.25	(2.06)	2.00	(2.16)	4	2	1.000	1
Psychotria sp. '1'	Rubiaceae	2.17	(7.60)	2.83	(7.54)	41	318	0.426	1
Psychotria sp. '2'	Rubiaceae	5.64	(12.3)	5.09	(12.0)	33	300	0.304	1
Psychotria deflexa	Rubiaceae	1.25	(1.44)	0.88	(1.54)	16	69	0.294	1
Schefflera morototoni	Araliaceae	0.40	(0.55)	1.20	(1.10)	5	3	0.270	1
Solanaceae sp.	Solanaceae	1.00	(1.00)	0.33	(0.58)	3	4.5	0.586	1
Solanum sp.	Solanaceae	0.57	(0.65)	1.43	(1.74)	14	12	0.112	1
Stigmaphyllon ellipticum	Malpighiaceae	0.25	(0.50)	0.75	(0.50)	4	2.5	0.424	1
Stigmaphyllon hypargyreum	Malpighiaceae	1.33	(0.58)	0.67	(0.58)	3	3	0.346	1
Terminalia amazonia	Combretaceae	2.10	(2.51)	2.10	(2.23)	10	29	0.916	1
Tilesia baccata	Asteraceae	0.63	(1.06)	1.25	(1.04)	8	6	0.187	1
Tiliaceae sp.	Tiliaceae	0.60	(0.55)	0.80	(0.84)	5	6	0.766	1
Trema micrantha	Ulmaceae	1.31	(1.58)	1.66	(1.76)	29	141	0.551	1
Trichospermum galeottii	Tiliaceae	0.80	(0.92)	0.90	(0.88)	10	21	0.901	1
Trophis caucana	Moraceae	1.00	(0.00)	0.25	(0.50)	4	6	0.149	1
Urticaceae sp.	Urticaceae	1.75	(1.71)	0.25	(0.50)	4	8.5	0.269	1
Vitis tiliifolia	Vitaceae	1.00	(1.29)	0.69	(1.11)	13	52	0.267	1
Zanthoxylum ekmanii	Rutaceae	0.82	(0.87)	0.64	(0.50)	11	20	0.821	1

* Morphospecies name assigned in raw data.

5.5 Discussion

Our results demonstrate that soil fungi can reduce seedling recruitment, and among sites can differentially affect the diversity of seedlings propagating from tropical forest seed-banks under light gap conditions. As the effects of fungi on community structure were not greater at the higher soil moisture level or for samples from wetter sites across the humidity gradient, our prediction of greater fungal influence with higher humidity was not supported. However soil moisture level did significantly affect seedling diversity and enhance total abundance in a site-specific manner. Post emergence mortality was also low, consistent with evidence of reduced fungal-pathogen virulence under high light conditions (Augspurger & Kelly 1984; Hood et al. 2004). The effects of fungi on recruitment success and community structure may therefore be more concentrated at the pre-emergence stages in light gaps (e.g. Dalling et al. 1997; Gallery et al. 2010) relative to forest understory communities where humid conditions promote greater post-emergence mortality (Bagchi et al. 2014).

5.5.1 Effects of fungi on community structure

We observed that the influence of excluding fungi on the diversity of seedlings recruiting from the soil seed bank differed between sites, with suggestions of both positive and negative effects. These results were consistent when analysing alternative measures of diversity (supplementary information S5.7.2). Increases in diversity could be mediated by a release of low abundance species from pathogen pressure with fungicide addition, increasing species richness and evenness, or by the exclusion of beneficial fungi that increase the numerical dominance of abundant species (Grime et al. 1987; van der Heijden et al. 1998). Conversely, fungicide-mediated decreases in diversity may occur through the exclusion of beneficial fungi essential for the establishment of rare species resulting in lower species richness (Gange et al. 1993), or by abundant species being released from pathogen pressure which in turn decreases evenness. It is possible that multiple mechanisms listed above contribute to our observed differences in diversity responses to

fungicide among sites. Rare species and singletons were common in our trays, meaning the contrasting effects of excluding pathogens and mycorrhizae on the establishment of rare species may have varied among trays. This may result in no net overall effect on species richness, but observable changes in diversity at the site level. Variation in the relative importance of pathogenic and beneficial fungi among sites may reflect spatial heterogeneity in fungal communities perhaps linked to local variations in plant community composition or unmeasured abiotic factors (Husband et al. 2002; Liu et al. 2015). Diversity also differed significantly between shadehouses. Unknown differences in abiotic conditions (e.g. temperature) may have affected species-specific abundances between shadehouses, with no overall effect being detected of shadehouse on species richness or total abundance.

Increases in total abundance with fungicide addition across sites in our study are consistent with seedlings experiencing a release from pathogen pressure, potentially altering community evenness and diversity. The numerically dominant herb *Blechum* sp. (>50% of all individuals in our experiment) significantly increased in abundance with fungicide addition, but did not appear to completely drive fungicide-mediated changes in diversity through influencing evenness (supplementary information S5.7.3). The sum of subtle net effects of fungi on the survival of multiple plant species may therefore also contribute to changes in community diversity in our experiment, rather than effects being driven only by a small number of dominant or greatly affected species (as in Grime et al. 1987; Dalling et al. 1997). Unknown starting seed bank densities combined with low mean abundances and incidences across trays are likely to have reduced our ability to detect other species-specific fungicide effects. Such factors are likely to be a general challenge for studies that utilise natural variation in abundances in species-rich communities, as seed banks and seedling communities are typically composed of many low abundance species with a small number of species with high abundances (Dalling et al. 2002).

Observed differences in species-specific abundances and community structure are also likely to result from differential mortality pre-emergence during the germination phase (see Gallery et al.

2010), as post-emergence seedling mortality was low (4.1%) and was unaffected by fungicide addition. High light conditions (see below), regular watering and average soil moisture levels above the dry season extremes of our sites may also have limited pathogen and desiccation mediated mortality (Dalling et al. 2002; see below). Fungal-pathogen mediated mortality within the seed bank over more extended time periods than tested in this study can also extensively influence seed bank diversity (e.g. Dalling et al. 1997) and seedling recruitment in the field.

Mean site-specific negative effects of fungicide on diversity (5 - 36% reductions) were comparable to field studies applying the same fungicide to forest understory seedlings in Belize. Bagchi et al. (2014) documented a 16% diversity reduction due to the removal of fungal-pathogens generating negatively density-dependent survival. Taken together, these results suggest fungi may thus have important roles in structuring seedling communities in both understory and light gap environments, but likely through differing mechanisms. Effects of fungicide on beneficial fungal-associations may also be of increased relative influence in light gaps due to less intense pathogen-mediated mortality. Experiments utilising field-soil washes of AMF, pathogenic, and combined soil biota fractions (as in Klironomos 2002; McCarthy-Neumann & Kobe 2010a; 2010b; Liang et al. 2015) applied to artificial seed banks are a potentially powerful tool to isolate the relative effects of beneficial and antagonistic fungi on species-specific performances and community structure.

5.5.2 Interactive effects of fungi and soil moisture level on community structure

The effect of fungicide on community structure did not differ between high and low soil moisture levels, suggesting the activity of soil fungi was not altered by water availability. Increases in soil watering volume were also insufficient to increase seedling mortality in a tropical tree in understory shadehouses in Belize, with leaf wetting instead being vital for disease incidence (Swinfield et al. 2012). Factors influencing leaf surface moisture may therefore be integral to disease and explain low seedling mortality in our study. For instance, numerous temperate and tropical forest plant species experience reduced disease mortality in high light relative to shaded

conditions (Augspurger & Kelly 1984; Hood et al. 2004; Ichihara & Yamaji 2009; McCarthy-Neumann & Ibanez 2013). High light can markedly reduce disease mortality even when soil moisture level is constant (e.g. McCarthy-Neumann & Ibanez 2012; 2013). For example Augspurger & Kelly (1984) demonstrated that when planted under low conspecific densities within shadehouses, mean mortality in 18 tree species on BCI was 4.5% in high light versus 31.3% in low light conditions. Overall, evidence suggests that rapid drying of leaf surfaces in high light conditions may reduce the probability of fungal infection relative to low light, high humidity conditions typical of the forest understory (Garwood 1983). Shadehouses generally do not mimic non-uniform periods of shading that occur in true gaps such as when the sun is low in the sky early and late in the day (Dalling et al. 1999). A shorter period of direct illumination may reduce leaf drying and enhance infection, leading to comparable mortality in small light gaps and forest understory conditions (e.g. McCarthy-Newman & Kobe 2010a). Alternatively, high light can reduce disease incidence by enhancing the ability of a plant to compensate for disease damage, by stimulating rapid lignification, or by increasing or switching the direction of the effect of mycorrhizae or endophytes on performance (McCarthy-Neuman & Kobe 2010a and citations within).

Whilst our results did not support a fungicide × soil moisture interaction, higher soil moisture level independently enhanced seedling germination, increasing total abundance. These effects may also drive observed site-specific effects of water level on diversity, demonstrating the importance of both biotic and abiotic factors in structuring seedling communities. Even through short dry spells can result in seedling desiccation and mortality by reducing surface soil moisture levels (Dalling et al. 2002), desiccation is particularly intense in the dry season where dry spells are more frequent and intense (Santiago et al. 2004). The season of light gap formation may therefore alter the relative importance of abiotic and biotic factors on seedling survival and resultant community structure.

5.5.3 Effects of fungi on community structure across the rainfall gradient

The effects of fungicide on community abundance, richness and diversity did not increase with humidity, and thus did not support our prediction of greater influences of fungal pathogens at wetter sites (following Givinish 1999). Whilst there were suggestions of greater differences in community composition under fungicide treatment at wetter sites (higher mean site GSWC) in our study, this effect was marginally non-significant. Previous observations have documented greater fungal-pathogen mediated seedling mortality within the forest understory at wetter forest sites across the Isthmus of Panama precipitation gradient (Brenes-Arguedas et al. 2009; Spear et al. 2015). However, these studies compared a single wet and dry site only making differences attributable to rainfall uncertain (Brenes-Arguedas et al. 2009; Spear et al. 2015; also see Gaviria & Engelbrecht 2015). Our experiments overcome this design limitation, with multiple sites spanning the entire gradient providing true replication and increasing our ability to test the influence of humidity on plant-enemy interactions. Field experiments documenting seedling recruitment in newly formed light gaps under enemy exclusion treatments (e.g. pesticides and mesh exclosures) will be a valuable development of our work. To understand precisely how the effects of fungi on community structure may change with humidity, experiments must now link their effects on species-specific performances to community level consequences under differing humidity conditions.

5.5.4 Conclusion

Expanding on demonstrations that fungal-pathogens enhance seedling diversity in the tropical forest understory (Bagchi et al. 2014), our results highlight that soil fungi can significantly alter the diversity of seedlings propagating from soil seed banks under light gap conditions, with the direction and magnitude of the effect differing among sites at a regional scale. Our results suggest that the influence of fungi on seedling diversity may be determined by effects on pre-emergence mortality within light gaps where pathogen infection of seedlings is reduced, whilst post-emergence mortality is likely to be more influential in shaded understory environments. However even the release of seedlings from fungal-pathogen mediated mortality may not be sufficient to

promote greater establishment to older age classes in light gaps if compensating mortality factors operate. In such instances, interspecific competition, herbivory, and abiotic mortality factors after seedling recruitment may minimise or mask the effects of fungi acting pre-emergence on plant recruitment and diversity.

5.6 Acknowledgements

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5.7 Supplementary information

S5.7.1 Selection of humidity variables for community structure analyses

Site was selected as the explanatory variable for presentation for all community structure variables (Table S5.4). Whilst AIC was lowest with site fitted for both diversity (effective number of species, inverse Simpson's Index) and total abundance, minimum GSWC (gravimetric soil water content) produced the lowest AIC for species richness. However for species richness model criticisms with minimum GSWC showed violations of assumptions, which were rectified by fitting site as the explanatory variable. Site was thus also subsequently presented for species richness in final analyses.

Table S5.4. AIC values from mixed effects models fitting different humidity variables as explanatory factors of tray diversity, richness and total abundance. Fungicide, water level, humidity, and all two-way interactions fitted as explanatory variables. Diversity (effective number of species, inverse Simpson's Index), rainfall, mean and minimum GSWC variables were log transformed to improve model fit. The model selected for initial model simplification is italicised, with the model presented in the final results in bold. AIC values are presented to five significant figures.

	AIC (d.f.)				
Response	**Site**	**Rainfall**	**Mean GSWC**	**Minimum GSWC**	**Dry season deficit**
Diversity	***-89.980 (25)***	-28.133 (11)	-28.735 (11)	-35.500 (11)	-28.009 (11)
Total abundance	***681.17 (24)***	706.02 (11)	706.90 (11)	707.24 (11)	706.62 (11)
Species richness	**395.64 (24)**	390.25 (11)	389.64 (11)	*387.52 (11)*	392.30 (11)

S5.7.2. Effect of fungicide on an alternative measure of diversity

To test for the effects of fungicide addition on the diversity of individuals recruiting from the soil seed bank, in addition to the effective number of species calculated as the inverse of Simpson's diversity index, the exponential of Shannon's diversity index was also analysed (Table S5.5). These two diversity indices are affected primarily by evenness and species richness respectively (Magurran 2004; Magurran & McGill 2011). As for Inverse Simpson's index, a significant fungicide × site interaction was detected as well as a significant shadehouse effect. All other variables and interactions besides shadehouse were non-significant (Table S5.5). Unlike Inverse Simpson's index, diversity measured as the exponential Shannon's index did not detect a significant water x site interaction, although water independently was marginally non-significant.

Table S5.5. Results of mixed-effects model analyses testing for the effects of fungicide, soil moisture, and site on an exponential Shannon's index. N equals the number of trays analysed. P values were calculated via analysis of deviance tests, comparing the difference in the fit of two models including and removing each variable. For each response variable, explanatory variables are ordered in the sequence of removal from the maximal model. Statistically significant factors are in bold.

Response	Variable	d.f.	AIC	χ^2	P value
Log(Exponential Shannon's index)	(Maximal model)		-82.050		
N=84	Fungicide:Water	1	-83.172	0.878	0.349
	Site.rank:Water	5	-85.084	8.088	0.152
	Water	1	-83.440	3.644	0.056
	Site.rank:Fungicide	**6**	**-78.622**	**16.818**	**0.010**
	Shadehouse	**1**	**-76.245**	**9.195**	**0.002**

S5.7.3. Influence of Blechum sp. on the effect size of fungicide and soil moisture on diversity

Post hoc analyses were conducted to determine whether the observed significant fungicide x site and water x site interaction effects on community diversity are driven by the responses of the numerically dominant *Blechum* sp. (which had significantly higher abundance in fungicide addition trays). When *Blechum* sp. individuals were removed, an insufficient number of tray pairs had seedling abundances above the ≥15 individual threshold to conduct community analyses. Therefore the effective number of species mixed effects model was re-fitted with the proportion of individuals within a tray that are *Blechum* sp. added as an additional explanatory variable, with its interactions with water and fungicide treatments also included.

The proportion of *Blechum* sp. was significantly negatively correlated with the effective number of species, likely by causing decreased evenness. A significant fungicide × site interaction was detected, however, the fungicide × site interaction is significantly weaker in the model including the proportion of *Blechum* sp. relative to the original analysis without this factor (P=0.009 [Table 5.2] vs P=0.045 [Table S5.6]). These results therefore suggest that the response of *Blechum* sp. to fungicide addition may contribute greatly to the observed significant fungicide × site interaction, although it is not entirely responsible. Additionally, in the new analysis water had a significant effect on diversity (Table S5.6) contrary to the water × site interaction detected in previous analyses (Table 5.2). Diversity was higher in the low water treatment, suggesting greater *Blechum* sp. germination under high water level treatment may decrease evenness and diversity where it is abundant.

Repeat analyses on total abundance were also conducted, including the proportion of *Blechum* sp. within a tray pair and its two-way interactions with fungicide and water level (Table S5.6). Results were qualitatively unchanged from analyses without the additional variables (Table 5.2), with a significant fungicide effect and water × site interaction. Whilst *Blechum* sp. is significantly more abundant in fungicide addition trays, this effect does not appear to drive fungicide mediated increases in total abundance at the tray level. Additional species may thus also experience

increased abundances as a result of fungicide addition.

Table S5.6. Results of mixed-effects model analyses testing for the effects of fungicide, soil moisture, site, and the proportion of individuals within a tray that are *Blechum* sp. ('Blechum') on mean diversity and total abundance. N equals the number of trays analysed. P values were calculated via analysis of deviance tests, comparing the difference in the fit of two models including and removing each variable. For each response variable, explanatory variables are ordered in the sequence of removal from the maximal model. Statistically significant factors are in bold.

Response	Variable	d.f.	AIC	χ^2	P value
Log(Inverse Simpson's Index)	(Maximal model)		-102.62		
N=84	Blechum:Water	1	-104.38	0.243	0.622
	Blechum:Fungicide	1	-106.02	0.366	0.545
	Fungicide:Water	1	-107.52	0.496	0.481
	Site:Water	5	-107.36	10.162	0.071
	Shadehouse	**1**	**-98.669**	**10.688**	**0.001**
	Blechum	**1**	**-88.382**	**20.975**	**<0.001**
	Water	**1**	**-103.56**	**5.795**	**0.016**
	Site:Fungicide	**6**	**-106.49**	**12.872**	**0.045**
Total abundance	(Maximal model)	1	685.39	0.774	0.379
N=84	Blechum:Water	1	683.41	0.222	0.882
	Site:Fungicide	6	674.78	3.367	0.762
	Fungicide:Water	1	673.02	0.236	0.627
	Blechum:Fungicide	1	672.61	1.595	0.207
	Blechum	1	670.81	0.199	0.656
	Shadehouse	1	669.30	0.495	0.482
	Fungicide	**1**	**675.52**	**6.712**	**0.010**
	Site:Water	**5**	**676.03**	**15.22**	**0.010**

Chapter 6:

General discussion and general conclusions

Christopher T. Jeffs

General Discussion

As detailed discussions have been provided in Chapters 2 – 5, within this general discussion I highlight themes emerging from my thesis and highlight methodological considerations for future studies. First I focus on the need to assess the spatial and temporal consistency of the effects of natural enemies on recruitment and diversity. Second, I evaluate how variations in abiotic conditions may alter the influence of natural enemies on plant performance and community structure, and contribute to spatial patterns of diversity. Third, I highlight the importance of compensatory mortality in determining the lasting influences of enemy-mediated mortality at early life stages on plant community diversity.

6.1 Spatial and temporal variation in natural enemy activity

The effects of natural enemies on plant performance are inherently variable with great differences in effects between individuals, spatial locations, and points in time both within and between species (Crawley 2013; Chapters 2 - 5). The consequences of spatial and temporal variation in the effects of enemies on species-specific performances are essential to understand consequent patterns of plant community diversity.

6.1.1 Within and between site spatial variation in enemy-mediated mortality

When enemy-mediated mortality is spatially structured, areas where mortality of a superior competitor is high can reduce the probability of competitive exclusion of an inferior competitor. Our results in Chapter 2 identified that mean pre-dispersal insect seed predation rates in a common rainforest tree were independent of conspecific density, but differed significantly between locations within a single forest site. Similarly, Chapter 3 identified large intraspecific variation in mean pre-dispersal seed predation rates between forest sites across the Isthmus of

Panama. If spatial differences in mortality are consistent across years for different species (e.g. Kollman et al. 1998), they may enhance community diversity by providing refuges from enemy pressure allowing the persistence of inferior competitors in locations of low mortality (Crawley 2013). Spatially limited experiments risk presenting misleading conclusions on the influence of enemies on species-specific mortality rates. Therefore, well replicated sampling from spatially separated locations is always preferable.

In addition to spatial variation in species-specific performances, the influence of natural enemies on community diversity may also vary spatially. For instance, in Chapter 5 we observed that the effects of soil fungi on the diversity of seedlings propagating from tropical forest seed banks differed between sites, with apparent variation in both the magnitude and direction of the effects. As species differ in their susceptibility to natural enemy attack (Augspurger & Kelly 1984; Coley & Barone 1996; Xu et al. 2015; Chapter 3), dissimilarities in plant species composition, even over small distances, are likely to contribute to spatial variation in the influence of enemies on community diversity. Again, the results of spatially limited experiments may not be representative. To assess the consistency of how natural enemies influence plant recruitment and diversity, wide community coverage through sampling of compositionally distinct species assemblages is necessary (e.g. Chapters 3, 4 and 5).

6.1.2 The scale at which density-dependent mortality occurs

The range of spatial scales at which conspecific density is measured greatly influences conclusions on the importance of natural enemies to plant recruitment. Assessments of density-dependent seedling survival commonly utilise natural variation in seedling densities by monitoring small field plots (e.g. 0.25 m^2 – 1 m^2 Bell et al. 2006; Bagchi et al. 2014). However, Gripenberg et al. (2014) highlighted that as seedling and adult densities are likely to be correlated, observations of negatively density-dependent survival in small seedling plots may actually reflect enemy responses to conspecific abundances at larger spatial scales. The form of density-dependent survival for a given plant species can also differ in relation to the spatial scale at which

mortality is assessed. For instance, Schupp (1992) demonstrated that negatively density-dependent post-dispersal seed survival can occur at small spatial scales due to the attraction of rodent seed predators, but satiation can simultaneously operate where seed densities are high at larger spatial scales. Studies testing for density-dependent mortality should therefore aim to incorporate multiple spatial scales where density variation is assessed (e.g. separate individuals, local neighbourhoods and habitat locations; Chapter 2), and utilise varying distances from conspecifics, to ensure the appropriate scale of density-dependence can be identified if present (Gripenberg et al. 2014).

6.1.3 Temporal variation in enemy-mediated mortality and the form of density-dependence

The timescale during which seed and seedling survival is assessed can also greatly influence results. For instance, the time elapsed since seed burial strongly influences levels of seed-bank mortality (Dalling et al. 1997; Leishman et al. 2000). Insect mediated seed predation rates can vary throughout a fruiting season relating to the speed of demographic responses (Janzen 1972; Wright 1990), and severe enemy-mediated seedling mortality can be missed if not tracked even days after germination (Bell et al. 2006). Extended census periods also increase the probability of enemy-mediated effects being documented, especially if enemy activity is dependent on transient periods where suitable conditions for incidence occur (Gripenberg et al. 2014).

The temporal consistency of observed density-dependent processes is also important, with differences in the strength and form of density-dependence occurring between years or even seasons (Lin et al. 2012). Major interannual differences in crop sizes are common in many plant species (Wright et al. 2005; Crawley 2013), and can cause temporal variation in seedling recruitment rates and observations of enemy-mediated mortality. In low fecundity years, negative density-dependence may predominate, whilst in high fecundity years there is an increased probability of satiation (Janzen 1971). Conclusions on the diversity-enhancing effects of natural enemies may therefore be limited in single-season studies without knowledge of variation in crop sizes and resultant seed and seedling abundances (Xu et al. 2015).

6.2 Variation in abiotic conditions

Variation in abiotic conditions such as temperature and humidity can alter levels of enemy-mediated mortality (Bale et al. 2002; Alexander 2010). This variation can aid understanding of how enemies may contribute to patterns of diversity at local, regional and latitudinal spatial scales. In addition, understanding how abiotic conditions affect enemy-plant interactions aids predictions of the potential consequences of climate change (e.g. altered mean temperate, precipitation, and extreme event frequencies; Meehl et al. 2007).

6.2.1 Within site variation in abiotic conditions

Fine scale spatial variation in abiotic conditions can occur within sites. For example, differences in soil moisture can follow topographic, edaphic and seasonal variation in precipitation (e.g. Becker et al. 1988). Soil moisture level is known to affect the intensity of mortality within the seed bank (Schafer & Kotanen 2003; but see Leishman 2000) which may influence seedling recruitment and diversity after gap creation in both grassland and forest ecosystems. Small scale spatial variation in soil moisture could alter the relative influence of biotic and abiotic factors on plant performance e.g. soil-borne pathogen infection versus desiccation-mediated mortality (Schafer & Kotanen 2003). Such effects contribute to local variation in species-specific recruitment success and resultant spatial distributions. Experimentally altered soil moisture levels (Leishman et al. 2000; Chapter 5) in addition to utilising naturally occurring variation in field locations (Schafer & Kotanen 2003) could be used to assess the consequences of altered abiotic conditions on enemy-mediated mortality. Such assessments will be of great value in predicting the consequences of climate change-mediated alterations in mean precipitation levels (e.g. Leishman et al. 2000) and the frequency of droughts and floods.

Habitat disturbance can also create distinct within-site variation in abiotic conditions. In both temperate and tropical forests, light levels reaching the forest floor can be increased relative to

undisturbed forest by in natural light gaps (Denslow 1980), in forest edge habitat (Foggo et al. 2001), and in logged areas (Nystrand & Ganstrom 2000). As discussed in Chapter 5, reductions in humidity caused by high light can potentially reduce pathogen-mediated seedling mortality (Augspurger & Kelly 1984; Swinfield et al. 2012; but see Benitez-Malvido & Lemus-Albor 2005). Lower forest floor moisture can also reduce the activity of desiccation-sensitive slug herbivores (Nystrand & Ganstrom 1997), leading to severely reduced seedling mortality in logged relative to unlogged temperate forest sites where light penetration is increased (Nystrand & Ganstrom 2000). However, both increased and decreased herbivory and seed predation rates have been documented in edge habitat relative to the forest interior, although greater frequencies of pest and pathogen outbreaks appear to be a common consequence of fragmentation (reviewed by Foggo et al. 2001). Spatial variation in light levels within forest systems therefore appear to have the potential to alter the intensity of enemy-mediated mortality and patterns of recruitment. However, associated changes in plant species composition will affect community level assessments of how altered enemy activity levels influence community diversity. This is supported by documentations of initial increases in species richness in newly formed edge environments (Foggo et al. 2001). In addition, local levels of herbivory and disease-mediated mortality could also increase in disturbed environments as pioneer and ruderal species with lower investment in defence relative to growth than shade-tolerant species become more common (Coley & Barone 1996). Such observations make it difficult to predict how altered enemy activity will affect diversity in disturbed areas. Controlled growth chamber or greenhouse experiments separating the effects of species identity (and associated traits), humidity, temperature, and light level will enable the identification of the causal factors determining the influence of forest disturbances on plant-enemy interactions and resultant recruitment and diversity.

6.2.2 Large scale variation in precipitation and soil moisture

Based on proposals by Janzen (1970) and Givnish (1999), enemy-mediated plant mortality and the strength of negative density-dependence are predicted to increase with site rainfall. Natural enemies can therefore contribute to large scale patterns of diversity, with greater plant diversity in

moist tropical relative to temperate latitudes and wetter forest relative to drier forest sites. In a recent meta-analysis, Comita et al. (2014) found little support for a latitudinal gradient in the strength of negative density-dependence (but see Johnson et al. 2012) but negatively density-dependent survival was stronger in wetter relative to drier forest sites. The Isthmus of Panama provides an ideal location for assessing how natural enemy pressure varies with humidity on regional scales, with a series of mapped forest plots spanning 60 km covering a doubling in precipitation and species richness (Condit et al. 2013). In Chapters 3 and 5 we utilised eight sites spanning the entire Panama rainfall gradient to investigate the effect of humidity on within-species patterns of pre-dispersal seed predation and pathogen-mediated mortality in light gap conditions. By providing multiple data points across a humidity gradient, our design built on previous studies utilising only a single pair of wet and dry sites to test for humidity × enemy interactions on plant performance (Brenes-Arguedas et al. 2009; Spear et al. 2015; Gaviria & Engelbrecht 2015 but see Weiß*f*log et al. 2015). In Chapter 5 we also expanded on species-specific studies, by simultaneously testing for consequences of altered humidity and enemy activity on seedling diversity. Utilising this design, experiments could test whether the intensity of density-dependent mortality varies across the humidity gradient, linking adult conspecific densities in individual mapped plots to variations in the intensity of seed and seedling mortality in small scale plots where densities are manipulated. The use of pesticides and mesh exclosures (e.g. Fricke et al. 2014; Bagchi et al. 2014) will enable identification of the enemy groups responsible for observed patterns. Efforts to expand to parallel humidity gradients will be valuable in testing the generality of these patterns.

6.3 Compensatory mortality and redundancy

Survival at early life stages is proposed to be of greater influence in determining plant population dynamics and community structure than at later life stages (Webb & Peart 1999; Harms et al.

2000; Bagchi et al. 2014; Green et al. 2014). Studies focusing on seed and seedling survival are thus essential to identifying the mechanisms by which natural enemies influence recruitment and diversity. However, the influence of mortality at early life stages can be limited due to interspecific competition (Edwards & Crawley 1999) or enemies (Crawley 1997; Clark et al. 2007) limiting establishment at later life stages e.g. through generating negatively density-dependent compensatory survival. The consequences of early-stage mortality for adult community structure should therefore be followed across intermediate life stages to establish causality. Such processes can generate effective functional redundancy of natural enemies at early life stage, with later acting enemies being of greater influence on recruitment success. How common compensatory mortality is in plant communities, is unknown. Density-independent processes such as stochastic extreme climatic events (e.g. frosts and droughts) and outbreaks of pests and diseases may cause intense mortality even at large community scales, masking effects at earlier life stages (Crawley 1997). Quantifying the relative importance of enemies, interspecific competition, and abiotic mortality factors acting at different life stages are challenging, but highly valuable to identify the key factors determining plant population dynamics and community diversity.

Communities of short lived herbaceous species provide ideal model systems for simultaneously assessing the mechanism, causal agent, and consequence of mortality at a particular life stage on plant diversity. In Chapter 4 our results documented a fungus-mediated increase in the diversity of seedlings emerging from the soil seed bank. Whilst not quantified, a large proportion of individuals were of reproductive stages when the experiment ended, suggesting the effects of natural enemies on community diversity may persist to maturation at least over a single generation. It is also feasible to maintain experimental exclusions of natural enemies and track community diversity across life stages, and even generations, for herbaceous communities (e.g. Allan & Crawley 2011; Gibson et al. 2010). Short generation times also allow direct quantification of the effects of natural enemies acting at distinct life stages on lifetime fitness (e.g. through assessments of the impacts of herbivory on viable seed production; e.g. Maron 1998)

with the consequences for population dynamics being assessed.

In tropical forest communities, as assessed in Chapters 2, 3 and 5, a far greater time period separates the adult stage from processes at the seed and seedling stages, increasing the time over which compensatory mortality can occur. For forest communities with long generation times it is therefore typically necessary to investigate the influence of enemies on diversity in two stages. First, through experimental investigations of the mechanisms by which natural enemies affect plant performance and diversity at particular life stages (e.g. Augspurger & Kelly 1984; Packer & Clay 2000; Bagchi et al. 2014; Chapter 5). Second, through observational studies using mapped plot data to demonstrate diversity altering effects and differences between different age classes within the field (e.g. Connell et al. 1984; Webb & Peart 1999; Harms et al. 2000; Hubbell et al. 2001; Comita et al. 2010; Metz et al. 2010). In particular, evidence linking the action of enemy-mediated negative-dependent survival on early life stages to resultant adult community structure remains elusive.

General conclusions

In my thesis I have investigated the role of natural enemies in influencing both local and regional patterns of diversity in two contrasting species-rich plant communities: tropical forests in Panama, and temperate grasslands in the UK.

Chapters 2 and 3 validated that pre-dispersal insect seed predators can cause significant reductions in the number of viable seeds produced by tropical tree species, with implications for seedling recruitment in seed-limited species. In addition, Chapter 3 reinforced previous evidence of high host-specificity in insect seed predators, supporting proposals that this enemy guild can enhance diversity if generating negatively density-dependent survival (Janzen 1970). Linking the consequences of pre-dispersal seed predation on recruitment rates to community diversity has yet to be demonstrated in tropical forest communities. Combinations of seed addition experiments and insecticide application in tree canopies and will be valuable in quantifying seed limitation and testing for the consequence of pre-dispersal insect seed predators on seedling recruitment rates.

Chapters 4 and 5 demonstrated that soil fungi can influence the diversity of seedlings propagating from the soil seed bank for both temperate grassland and tropical forest ecosystems. Observations from Chapter 4 using temperate grassland seed banks suggested that the diversity enhancing effects of soil fungi may persist to the adult stage in herbaceous communities. In Chapter 5, our experiment propagating tropical forest seed banks under high light conditions provided support that high light conditions may limit pathogen-mediated seedling mortality, with experimentally altered soil moisture levels having no effect on how fungal-pathogens influence survival post-emergence. Overall, I believe Chapters 4 and 5 demonstrate that the propagation of field collected seed banks under experimental enemy exclusion treatments and varying abiotic conditions provide a valuable system for investigating the processes that structure plant communities in gap environments. However, to overcome the limitations of this method in testing the factors

influencing species-specific performances (i.e. low abundances of many species and unknown starting seed densities, Chapters 4; Chapter 5) I suggest that future experiments should utilise two methods where possible, in addition to using enemy exclusion and abiotic treatments. First, propagating field soils from spatially separated locations to assess a) natural variation in seed bank community structure, and b) the factors structuring seedling communities. Second, planting commercial or collected seed on sterilised soils inoculated with field soil microbial washes (e.g. Klironomos 2002; McCarthy-Neumann & Kobe 2010a; 2010b; Liang et al. 2015) to evaluate the biotic factors influencing species-specific performances. Sowing artificial seed banks (of commercial or collected seed) with known composition and starting densities are also a valuable tool for linking species-specific effects to community level consequences. Collecting soils from locations with known preconditioning history will allow the testing of distance and density-dependent mechanisms (as in Chapter 4).

In contrast to predictions (see Givnish 1999; Chapter 3; Chapter 5), our results in Chapters 3 and 5 did not demonstrate that pre-dispersal seed predation rate and the influence of fungal-pathogens on seedling diversity were stronger in wetter forest sites across steep precipitation-diversity gradient. We therefore found no evidence supporting the role of these enemy guilds in contributing to regional scale patterns of diversity. To our knowledge, these experiments provided the first truly replicated tests for the effects of humidity on natural enemy pressure.

In combination, Chapters 2 - 5 within my thesis highlight the great variability in the influence of natural enemies on plant performance and diversity. Specifically our results demonstrate great among-site spatial variation in a) the intensity of pre-dispersal seed predation within and among species, and b) the influence of soil fungi on the diversity of individuals recruiting from soil seed banks in tropical forests. Studies utilising multiple spatially separated locations over repeated seasons and generations are greatly needed to evaluate the consistency of the manner in which natural enemies influence plant recruitment and diversity.

Overall the results within my thesis suggest that pre-dispersal insect seed predators and soil fungi by affecting plant performance at early life stages have the potential to significantly influence the diversity of species-rich tropical forest and temperate grassland plant communities. With greater understanding of the biotic and abiotic processes determining plant community structure, we improve our ability to conserve plant diversity and maintain the essential ecosystem services they provide. Such understanding will be highly valuable in predicting how the influence of natural enemies on plant diversity may be altered when facing anthropogenic climate change and habitat degradation.

References

Allan, E., Van Ruijven, J. & Crawley, M.J., 2010. Foliar fungal pathogens and grassland biodiversity. *Ecology*, 91(9), 2572–2582.

Allan, E. & Crawley, M.J., 2011. Contrasting effects of insect and molluscan herbivores on plant diversity in a long-term field experiment. *Ecology letters*, 14(12), 1246–53.

Alvarez-Buylla, E.R. & Martinez-Ramos, M., 1990. Seed bank versus seed rain in the regeneration of a tropical pioneer tree. *Oecologia*, *84*(3), 314-325.

Alvarez-Loayza, P., White Jr, J.F., Torres, M.S., Balslev, H., Kristiansen, T., Svenning, J.C. & Gil, N., 2011. Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. *PloS one*, *6*(1), p.e16386.

Alvarez-Loayza, P. & Terborgh, J., 2011. Fates of seedling carpets in an Amazonian floodplain forest: intra-cohort competition or attack by enemies? *Journal of Ecology*, *99*(4), 1045-1054.

Andersen, A.N., 1987. Effects of seed predation by ants on seedling densities at a woodland site in SE Australia. *Oikos*, 171-174.

Andersen, A.N, 1988. Insect seed predators may cause far greater losses than they appear to. *Oikos*, 52(3), 337–340.

Anderson, R.C., Liberta, A.E. & Dickman, L.A., 1984. Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. *Oecologia*, *64*(1), 111-117.

Arnold, A.E., Mejía, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N. & Herre, E.A., 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences*, 100(26), 15649-15654.

Ashton, P.S., 1988. Dipterocarp biology as a window to the understanding of tropical forest structure. *Annual Review of Ecology and Systematics*, 347-370.

Augspurger, C.K., 1983. Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of its seedlings from fungal pathogens. *The Journal of Ecology*, 759-771.

Augspurger, C.K., 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology*, *65*(6), 1705-1712.

Augspurger, C.K. & Kelly, C.K., 1984. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling denisty, and light conditions. *Oecologia.*, 61(2), 211–217.

Babweteera, F., Savill, P. & Brown, N., 2007. *Balanites wilsoniana*: Regeneration with and without elephants. *Biological Conservation*, 134(1), 40–47.

Bagchi, R., Philipson, C.D., Slade, E.M., Hector, A., Phillips, S., Villanueva, J.F., Lewis, O.T., Lyal, C.H., Nilus, R., Madran, A. & Scholes, J.D., 2011. Impacts of logging on density-dependent predation of dipterocarp seeds in a South East Asian rainforest. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *366*(1582), 3246-3255.

Bagchi, R., Gallery, R.E., Gripenberg, S., Gurr, S.J., Narayan, L., Addis, C.E., Freckleton, R.P. and Lewis, O.T., 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*,*506*(7486), 85-88.

Bagchi, R., Swinfield, T., Gallery, R.E., Lewis, O.T., Gripenberg, S., Narayan, L. & Freckleton, R.P., 2010. Testing the Janzen-Connell mechanism: pathogens cause overcompensating density dependence in a tropical tree. *Ecology letters*, *13*(10), 1262-1269.

Bakker, E.S., Ritchie, M.E., Olff, H., Milchunas, D.G. &Knops, J.M., 2006. Herbivore impact on grassland plant diversity depends on habitat productivity and herbivore size. *Ecology letters*, *9*(7), 780-788.

Baltzer, J.L. & Davies, S.J., 2012. Rainfall seasonality and pest pressure as determinants of tropical tree species' distributions. *Ecology and evolution*,*2*(11), 2682-2694.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., Eigen, C. & Rcpp, L., 2015. Package 'lme4'. https://cran.r-project.org/web/packages/lme4/ lme4.pdf.

Bazzaz, F.A. & Pickett, S.T.A., 1980. Physiological ecology of tropical succession : a comparative review. *Annual Review of Ecology and Systematics*, 11(1980), 287–310.

Beck, H., Forget, P.M., Lambert, J.E., Hulme, P.E. & Vander Wall, S.B., 2005. Seed predation and dispersal by peccaries throughout the Neotropics and its consequences: a review and synthesis. In P.-M. Forget, J. E. Lambert, P. E. Hulme, and S. B. Vander Wall (Eds.). Seed fate: Predation, dispersal and seedling establishment, pp. 77–116. CABI Publishing,

Wallingford, UK.

Beckman, N.G. & Muller-Landau, H.C., 2011. Linking fruit traits to variation in predispersal vertebrate seed predation, insect seed predation, and pathogen attack. *Ecology*, 92(11), 2131–2140.

Bell, T., Freckleton, R.P. & Lewis, O.T., 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters*, 9(5), 569–574.

Benítez-Malvido, J. & Lemus-Albor, A., 2005. The Seedling Community of Tropical Rain Forest Edges and Its Interaction with Herbivores and Pathogens. *Biotropica*, *37*(2), 301-313.

Bever, J.D., Westover, K.M. & Antonovics, J., 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*, 561-573.

Bever, J.D., 2002. Negative feedback within a mutualism: host–specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London B: Biological Sciences*, *269*(1509), 2595-2601.

Bever, J.D., Mangan, S.A. & Alexander, H.M., 2015. Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics*, *46*, 305-325.

Blaney, C.S. & Kotanen, P.M., 2001. Post-dispersal losses to seed predators: an experimental comparison of native and exotic old field plants. *Canadian Journal of Botany*, *79*(3), 284-292.

Blundell, A.G. & Peart, D.R., 1998. Distance-dependence in herbivory and foliar condition for juvenile *Shorea* trees in Bornean dipterocarp rain forest. *Oecologia*, 117, 151–160.

Bonanomi, G., Mingo, A., Incerti, G., Mazzoleni, S. & Allegrezza, M., 2012. Fairy rings caused by a killer fungus foster plant diversity in species-rich grassland. *Journal of Vegetation Science*, *23*(2), 236-248.

Bonkowski, M., 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist*, *162*(3), 617-631.

Boucher, N. & Malajczuk, N., 1990. Effects of high soil moisture on formation of ectomycorrhizas and growth of karri (*Eucalyptus diversicolor*) seedlings inoculated with *Descolea maculata*, *Pisolithus tinctorius* and *Laccaria laccata*. *New phytologist*, *114*(1), 87-91.

Brandt, A.J., Kroon, H., Reynolds, H.L. & Burns, J.H., 2013. Soil heterogeneity generated by plant–soil feedbacks has implications for species recruitment and coexistence. *Journal of Ecology*, *101*(2), 277-286.

Brenes-Arguedas, T., Coley, P.D. & Kursar, T. A., 2009. Pests vs. drought as determinants of plant distribution along a tropical rainfall gradient. *Ecology*, 90(7), 1751–1761.

Brokaw, N.V., 1985. Gap-phase regeneration in a tropical forest. *Ecology*,*66*(3), 682-687.

Chancellor, R.J., 1966. T*he identification of weed seedlings of farm and garden*. Blackwell Scientific Publications, Oxford.

Chapin III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E. & Mack, M.C., 2000. Consequences of changing biodiversity. *Nature*,*405*(6783), 234-242.

Chesson, P.L., 1985. Coexistence of competitors in spatially and temporally varying environments: a look at the combined effects of different sorts of variability. *Theoretical Population Biology*, 28(3), 263–287.

Clark, C.J., Poulsen, J.R., Levey, D.J. & Osenberg, C.W., 2007. Are plant populations seed limited? a critique and meta-analysis of seed addition experiments. *The American Naturalist*, 170(1), 128-142.

Coley, P.D. & Barone, J.A., 1996. Herbivory and plant defenses in tropical forests. *Annual review of ecology and systematics*, 305-335.

Comita, L.S., Muller-Landau, H.C., Aguilar, S. & Hubbell, S.P., 2010. Asymmetric density dependence shapes species abundances in a tropical tree community. *Science*, 329(5989), 330-332.

Comita, L. S., Queenborough, S. A., Murphy, S. J., Eck, J. L., Xu, K., Krishnadas, M., & Zhu, Y. 2014. Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance-and density-dependent seed and seedling survival. *Journal of Ecology*, 102(4), 845-856.

Condit, R., Engelbrecht, B., Pino, D., Turner, B.,Pérez, R. 2013. Panama Tree Distribution Database. DOI http://dx.doi.org/10.5479/data.bci.20130204.

Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In *Dynamics of Numbers in Populations* (eds P. J. Den Boer and G. R. Gradwell), 298–312. PUDOC, Wageningen.

Connell, J.H., Tracey, J.G. & Webb, L.J., 1984. Compensatory recruitment, growth, and mortality as factors maintaining rain forest tree diversity. *Ecological monographs*, *54*(2), 141-164.

Crawley, M.J., 1989. Insect herbivores and plant population dynamics. *Annual review of entomology*, *34*(1), 531-562.

Crawley M.J. 1997. Plant-herbivore dynamics. In *Plant Ecology*. ed. M.J. Crawley, 2:401-74. Oxford: Blackwell Science. 717

Crawley, M.J., 2013. Seed predators and plant population dynamics. In M. Fenner, ed., *Seeds: the ecology of regeneration in plant communities*. CAB International, Wallingford, UK.

Crawley, M.J., 2012. *The R book*. John Wiley & Sons.

Crawley, M.J. & May, R.M., 1987. Population dynamics and plant community structure: competition between annuals and perrenials. *Journal of theoretical Biology*, *125*(4), 475-489.

Crawley, M. & Long, C.R., 1995. Alternate bearing, predator satiation and seedling recruitment in *Quercus robur* L. *Journal of Ecology*, 683-696.

Ctvrtecka, R., Sam, K., Brus, E., Weiblen, G. D., & Novotny, V. 2014. Frugivorous weevils are too rare to cause Janzen–Connell effects in New Guinea lowland rain forest. *Journal of Tropical Ecology*, 30(06), 521-535.

Daguerre, Y., Siegel, K., Edel-Hermann, V. & Steinberg, C., 2014. Fungal proteins and genes associated with biocontrol mechanisms of soil-borne pathogens: a review. *Fungal Biology Reviews*, *28*(4), 97-125.

Dalling, J.W., 2005. The fate of seed banks: factors influencing seed survival for light-demanding species in moist tropical forests. In: Forget, P.-M., Lambert, J.E., Hulme, P.E., VanderWall, S.B. (Eds.), *Seed Fate: Predation, Dispersal and Seedling Establishment*. CABI Publishing, Wallingford, UK.

Dalling, J.W. & Brown, T.A., 2009. Long-Term Persistence of Pioneer Species in Tropical Rain Forest Soil Seed Banks. *The American Naturalist*,*173*(4), 531-535.

Dalling, J.W., Swaine, M.D. & Garwood, N.C., 1995. Effect of soil depth on seedling emergence in tropical soil seed-bank investigations. *Functional Ecology*, 119-121.

Dalling, J.W., Swaine, M.D. & Garwood, N.C., 1998a. Dispersal patterns and seed bank dynamics of pioneer trees in moist tropical forest. *Ecology*,*79*(2), 564-578.

Dalling, J.W., Hubbell, S.P. & Silvera, K., 1998b. Seed dispersal, seedling establishment and gap partitioning among tropical pioneer trees. *Journal of Ecology*, *86*(4), 674-689.

Dalling, J.W., Muller-Landau, H.C., Wright, S.J. & Hubbell, S.P., 2002. Role of dispersal in the recruitment limitation of neotropical pioneer species. *Journal of Ecology*, *90*(4), 714-727.

Dalling, J.W., Davis, A.S., Schutte, B.J. & Elizabeth Arnold, A., 2011. Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *Journal of Ecology*, *99*(1), 89-95.

Dalling, J.W. & Hubbell, S.P., 2002. Seed size, growth rate and gap microsite conditions as determinants of recruitment success for pioneer species. *Journal of Ecology*, *90*(3), 557-568.

Dalling, J.W., Lovelock, C.E. & Hubbell, S.P., 1999. Growth responses of seedlings of two neotropical pioneer species to simulated forest gap environments. *Journal of Tropical Ecology*, 15(6), 827–839.

Dalling, J.W., Swaine, M.D. & Garwood, N.C., 1997. Soil seed bank community dynamics in seasonally moist lowland tropical forest, Panama.*Journal of tropical ecology*, *13*(05), 659-680.

De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., de Ruiter, P.C., Verhoef, H.A., Bezemer, T.M. & van der Putten, W.H., 2003. Soil invertebrate fauna enhances grassland succession and diversity. *Nature*,*422*(6933), 711-713.

Delerue, F., Gonzalez, M., Atlan, A., Pellerin, S. & Augusto, L., 2014. Pre-dispersal seed predation of gorse (Ulex europaeus) along gradients of light and plant density. *New Zealand Journal of Ecology*, 268-278.

Dennis, A., Lipsett-Moore, G.J., Harrington, G.N., Collins, E.A., Westcott, D.A., Forget, P.M., Lambert, J.E., Hulme, P.E. & Vander Wall, S.B., 2005. Seed predation, seed dispersal and habitat fragmentation: does context make a difference in tropical Australia. In: Forget, P.-M., Lambert, J.E., Hulme, P.E., VanderWall, S.B. (Eds.), *Seed Fate: Predation, Dispersal and Seedling Establishment*. CABI Publishing, Wallingford, UK.

Denslow, J.S., 1980. Gap partitioning among tropical rainforest trees. *Biotropica*, 47-55.

Denslow, J.S., Ellison, A.M. & Sanford, R.E., 1998. Treefall gap size effects on above- and below-ground processes in a tropical wet forest. *Journal of Ecology*, 86(4), 597–609.

Díaz, S. & Cabido, M., 1997. Plant functional types and ecosystem function in relation to global change. *Journal of vegetation Science*, 463-474.

Diedhiou, P.M., Oerke, E.C. & Dehne, H.W., 2004. Effects of the strobilurin fungicides azoxystrobin and kresoxim-methyl on arbuscular mycorrhiza. *Journal of Plant Diseases and Protection*, 545-556.

Dracxler, C.M., Pires, A.S. & Fernandez, F. A., 2011. Invertebrate Seed Predators are not all the Same: Seed Predation by Bruchine and Scolytine Beetles Affects Palm Recruitment in Different Ways. *Biotropica*, 43(1), 8–11.

Edwards, G.R. & Crawley, M.J., 1999. Rodent seed predation and seedling recruitment in mesic grassland. *Oecologia*, *118*(3), 288-296.

Ehrenfeld, J.G., Ravit, B. & Elgersma, K., 2005. Feedback in the plant-soil system. *Annu. Rev. Environ. Resour.*, *30*, 75-115.

Engelbrecht, B.M., Comita, L.S., Condit, R., Kursar, T.A., Tyree, M.T., Turner, B.L. & Hubbell, S.P., 2007. Drought sensitivity shapes species distribution patterns in tropical forests. *Nature*, *447*(7140), 80-82.

Fedriani, J.M., Wiegand, T., Calvo, G., Suárez-Esteban, A., Jácome, M., Żywiec, M. & Delibes, M., 2015. Unravelling conflicting density-and distance-dependent effects on plant reproduction using a spatially explicit approach. *Journal of Ecology*, *103*(5), 1344-1353.

Fenner, M. & Thompson, K., 2005. *The ecology of seeds*. Cambridge University Press.

Fernández, M., Lobo, J., Chacón, E. & Quesada, M., 2008. Curculionid beetles in aborted flower buds and immature fruits of *Ceiba pentandra* (Bombacaceae). *Plant Ecology*, *194*(1), 1-4.

Fine, P.V., Miller, Z.J., Mesones, I., Irazuzta, S., Appel, H.M., Stevens, M.H.H., Sääksjärvi, I., Schultz, J.C. & Coley, P.D., 2006. The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology*, *87*(sp7), S150-S162.

Fisher, N., Brown, N.D., & Savill, P.S. 2010. Conservation management of Wytham Woods. In Savill P, Perrins C, Kirby K, Fisher N eds., *Wytham Woods: Oxford's Ecological Laboratory*. Oxford University Press, Oxford.

Foggo, A., Ozanne, C.M., Speight, M.R. & Hambler, C., 2001. Edge effects and tropical forest canopy invertebrates. *Plant Ecology*, *153*(1-2), 347-359.

Freckleton, R.P. & Lewis, O.T., 2006. Pathogens, density dependence and the coexistence of tropical trees. *Proceedings of the Royal Society B: Biological Sciences*, 273(1604), 2909–2916.

Fricke, E.C., Tewksbury, J.J. & Rogers, H.S., 2014. Multiple natural enemies cause distance-dependent mortality at the seed-to-seedling transition. *Ecology Letters*, 17(5), 593–598.

Gallery, R.E., Moore, D.J.P. & Dalling, J.W., 2010. Interspecific variation in susceptibility to fungal pathogens in seeds of 10 tree species in the neotropical genus *Cecropia*. *Journal of Ecology*, 98, 147–155.

Gange, A.C., Brown, V.K. & Farmer, L.M., 1990. A test of mycorrhizal benefit in an early successional plant community. *New Phytologist*, *115*(1), 85-91.

Gange, A.C., Brown, V.K. & Sinclair, G.S., 1993. Vesicular-arbuscular mycorrhizal fungi: a determinant of plant community structure in early succession. *Functional Ecology*, 616-622.

Garzon-Lopez, C.X., Ballesteros-Mejia, L., Ordoñez, A., Bohlman, S.A., Olff, H. & Jansen, P.A., 2015. Indirect interactions among tropical tree species through shared rodent seed predators: a novel mechanism of tree species coexistence. *Ecology letters*, *18*(8), 752-760.

Garwood, N.C., 1983. Seed germination in a seasonal tropical forest in Panama: a community study. *Ecological Monographs*, 159-181.

Gaviria, J. & Engelbrecht, B.M.J., 2015. Effects of Drought, Pest Pressure and Light Availability on Seedling Establishment and Growth: Their Role for Distribution of Tree Species across a Tropical Rainfall Gradient. *Plos One*, 10(11), p.e0143955.

Ghazoul, J., Liston, K. A & Boyle, T.J.B., 1998. Disturbance-induced density-dependent seed set in *Shorea siamensis* (Dipterocarpaceae), a tropical forest tree. *Journal of Ecology*, 86, 462–473.

Gibson, C.W.D., Watt, T.A. & Brown, V.K., 1987. The use of sheep grazing to recreate species-rich grassland from abandoned arable land. *Biological Conservation*, *42*(3), 165-183.

Gibson, C.W.D. 2010. The ecology of Upper Seeds - an old-field succession experiment. In Savill P, Perrins C, Kirby K, Fisher N eds., *Wytham Woods: Oxford's Ecological Laboratory*. Oxford University Press, Oxford.

Gilbert, G.S., 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology*, *40*(1), 13-43.

Gilbert, G.S., Burslem, D.F.R.P., Pinard, M.A. & Hartley, S.E., 2005. Dimensions of plant disease in tropical forests. *Biotic interactions in the tropics: their role in the maintenance of species diversity*, 141-164.

Givnish, T.J., 1999. On the causes of gradients in tropical tree diversity. *Journal of Ecology*, 87(2), 193–210.

Green, P.T., Harms, K.E. & Connell, J.H., 2014. Nonrandom, diversifying processes are disproportionately strong in the smallest size classes of a tropical forest. *Proceedings of the National Academy of Sciences*, 111(52), 18649–18654.

Green, T.W. & Palmbald, I.G., 1975. Effects of insect seed predators on *Astragalus cibarius* and *Astragalus utahensis* (Leguminosae). *Ecology*, 1435-1440.

Greig, N., 1992. Predispersal seed predation on five *Piper* species in tropical rainforest. *Oecologia*, 93(3), 412–420.

Griffin, D.M., 1963. Soil moisture and the ecology of soil fungi. *Biological Reviews*, *38*(2), 141-166.

Grime, J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American naturalist*, 1169-1194.

Grime J.P., Mackey J.M.L., Hillier S.H., & Read D.J. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* **328**: 420–422.

Gripenberg, S., Bagchi, R., Gallery, R.E., Freckleton, R.P., Narayan, L. & Lewis, O.T., 2014. Testing for enemy-mediated density-dependence in the mortality of seedlings: field experiments with five Neotropical tree species. *Oikos*, *123*(2), 185-193.

Grubb, P.J., 1977. The maintenance of Species-Richness in Plant Communities: the Importance of the Regeneration Niche. *Biological Reviews*, 52, 107–145.

Hammer, O., Harper D.A.T., & Ryan P.D. 2001. PAST: Palaeontological Statistics Software package for education and data anlysis. *Palaeontologia Electronica* 4(1):9

Hammond, D.S., Brown, V.K., Newbery, D.M., Prins, H.H.T. & Brown, N.D., 1998. Disturbance, phenology and life-history characteristics: factors influencing distance/density-dependent attack on tropical seeds and seedlings. In *Dynamics of tropical communities: the 37th symposium of the British Ecological Society, Cambridge University, 1996.* (51-78). Blackwell Science Ltd.

Hanf, H. 1973 *Weeds and their seedlings*. Ipswich, BASF United Kingdom Limited.

Hanski, I., 1994. A practical model of metapopulation dynamics. *Journal of Animal Ecology*, 151-162.

Hartnett, D.C. &Wilson, G.W., 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, *80*(4), 1187-1195.

Hardin, G., 1960. The competitive exclusion principle. *Science*, *131*(3409), 1292-1297.

Harms, K.E., Wright, S.J., Calderón, O., Hernández, A. & Herre, E.A., 2000. Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, *404*(6777), 493-495.

Harrison, K.A. & Bardgett, R.D., 2010. Influence of plant species and soil conditions on plant–soil feedback in mixed grassland communities. *Journal of Ecology*, *98*(2), 384-395.

Hautier, Y., Saner, P., Philipson, C., Bagchi, R., Ong, R.C. & Hector, A., 2010. Effects of seed predators of different body size on seed mortality in Bornean logged forest. *PloS one*, *5*(7), p.e11651.

Hector, A. &Bagchi, R., 2007. Biodiversity and ecosystem multifunctionality. *Nature*, *448*(7150), 188-190.

Hersh, M.H., Vilgalys, R. & Clark, J.S., 2012. Evaluating the impacts of multiple generalist fungal pathogens on temperate tree seedling survival. *Ecology*, 93(3), 511–520.

Herrera, C.M., 1989. Vertebrate frugivores and their interaction with invertebrate fruit predators: supporting evidence from a Costa Rican dry forest. *Oikos*, 185-188.

Hodge, A. & Fitter, A.H., 2013. Microbial mediation of plant competition and community structure. *Functional Ecology*, *27*(4), 865-875.

Hood, L.A., Swaine, M.D. & Mason, P.A., 2004. The influence of spatial patterns of damping - off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal coloniza. *Journal of Ecology*, 92, 816–823.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L. & O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, *486*(7401), 105-108.

Hosaka, T., Yumoto, T., Kojima, H., Komai, F. & Noor, N.S.M., 2009. Community structure of

pre-dispersal seed predatory insects on eleven *Shorea* (Dipterocarpaceae) species. *Journal of Tropical Ecology*, *25*(06), 625-636.

Hubbell, S.P., Ahumada, J.A., Condit, R. & Foster, R.B., 2001. Local neighborhood effects on long-term survival of individual trees in a Neotropical forest. *Ecological Research*, *16*(5), 859-875.

Hulme, P.E., 1994. Seedling herbivory in grassland: relative impact of vertebrate and invertebrate herbivores. *Journal of Ecology*, 873-880.

Hulme, P. & Benkman, C.W. 2002. Granivory. In Herrera, C. & Pellmyr, O. (eds) *Plant–Animal Interactions: An Evolutionary Approach*: 185–208. New York: Blackwell Scientific Publications.

Hulme, P.E. and Kollmann, J., 2004. Seed predator guilds, spatial variation in post-dispersal seed predation and potential effects on plant demography: a temperate perspective. pp. 9 - 30. In: Forget, P.-M., Lambert, J.E., Hulme, P.E., VanderWall, S.B. (Eds.), *Seed Fate: Predation, Dispersal and Seedling Establishment*. CABI Publishing, Wallingford, UK.

Husband, R., Herre, E.A., Turner, S.L., Gallery, R. & Young, J.P.W., 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology*,*11*(12), 2669-2678.

Hutchings, M.J. 1997. The structure of plant populations. Plant Ecology(ed. M. J.Crawley), 475–531. Blackwell Science, Oxford.

Hutchinson, G.E., 1957. The multivariate niche. In *Cold Spring Harbour Symposium of Quantitative Biology.* 22. 415-421.

Hwang, S.F., Ahmed, H.U., Zhou, Q., Rashid, A., Strelkov, S.E., Gossen, B.D., Peng, G. & Turnbull, G.D., 2013. Effect of susceptible and resistant canola plants on *Plasmodiophora brassicae* resting spore populations in the soil. *Plant pathology*, *62*(2), 404-412.

Hyatt, L.A., Rosenberg, M.S., Howard, T.G., Bole, G., Fang, W., Anastasia, J., Brown, K., Grella, R., Hinman, K., Kurdziel, J.P. & Gurevitch, J., 2003. The distance dependence prediction of the Janzen-Connell hypothesis: a meta-analysis. *Oikos*, *103*(3), 590-602.

Ichihara, Y. & Yamaji, K., 2009. Effect of light conditions on the resistance of current-year *Fagus crenata* seedlings against fungal pathogens causing damping-off in a natural beech forest: fungus isolation and histological and chemical resistance. *Journal of chemical ecology*, 35(9), 1077–85.

Janzen, D.H., 1970. Herbivores and the number of tree species in tropical forests. *American naturalist*, 501-528.

Janzen, D.H., 1971. Seed Predation by Animals. *Annual Review of Ecology and Systematics*, 2(1), 465–492.

Janzen, D.H., 1980. Specificity of seed-attacking beetles in a Costa Rican deciduous forest. *Journal of Ecology*, 68(3), 929–952.

Janzen, D. H. 1972. Association of a Rainforest Palm and Seed-Eating Beetles in Puerto Rico. Ecology, 53(2), 258-261.

Jones, F.A. & Comita, L.S., 2010. Density-dependent pre-dispersal seed predation and fruit set in a tropical tree. *Oikos*, 119(11), 1841–1847.

Jost, L., 2006. Entropy and diversity. *Oikos*, 113, 363–375.

Kalamees, R. & Zobel, M., 2002. The role of the seed bank in gap regeneration in a calcareous grassland community. *Ecology*, *83*(4), 1017-1025.

Kardol, P., Martijn Bezemer, T. & van der Putten, W.H., 2006. Temporal variation in plant–soil feedback controls succession. *Ecology Letters*, *9*(9), 1080-1088.

Kardol, P., Deyn, G.B., Laliberte, E., Mariotte, P. & Hawkes, C.V., 2013. Biotic plant–soil feedbacks across temporal scales. *Journal of Ecology*,*101*(2), 309-315.

Kennedy, D.N. &Swaine, M.D., 1992. Germination and growth of colonizing species in artificial gaps of different sizes in dipterocarp rain forest. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *335*(1275), 357-367.

Kiers, E.T., Lovelock, C.E., Krueger, E.L. & Herre, E.A., 2000. Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecology Letters*, *3*(2), 106-113.

Kirby, K.J. 2010. The Trees in the Woods 1945-2007. In Savill P, Perrins C, Kirby K, Fisher N eds., *Wytham Woods: Oxford's Ecological Laboratory*. Oxford University Press, Oxford.

Klironomos, J.N., 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, *417*(6884), 67-70.

Kolb, A., Ehrlen, J. & Eriksson, O., 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspectives in Plant Ecology, Evolution and Systematics*, 9(2), 79–100.

Kollmann, J., Coomes, D.A. & White, S.M., 1998. Consistencies in post-dispersal seed predation of temperate fleshy-fruited species among seasons, years and sites. *Functional Ecology*, *12*(4), 683-690.

Kos, M., Veendrick, J. & Bezemer, T.M., 2013. Local variation in conspecific plant density influences plant–soil feedback in a natural grassland. *Basic and applied ecology*, *14*(6), 506-514.

Kreft, H. & Jetz, W., 2007. Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences*, *104*(14), 5925-5930

Kulmatiski, A. & Kardol, P., 2008. Getting plant—soil feedbacks out of the greenhouse: experimental and conceptual approaches. In *Progress in botany*(449-472). Springer, Berlin Heidelberg.

Kulmatiski, A., Beard, K.H., Stevens, J.R. & Cobbold, S.M., 2008. Plant–soil feedbacks: a meta-analytical review. *Ecology Letters*, *11*(9), 980-992.

Lambers, J.H.R. & Clark, J.S., 2002. Effects of dispersal, shrubs, and density-dependent mortality on seed and seedling distributions in temperate forests. *Canadian Journal of Forest Research*, 33(5), 783–795.

Lambert, J.E. & Chapman, C.A., 2005. The fate of primate-dispersed seeds: Deposition pattern, dispersal distance and implications for conservation. 137-150. In: Forget, P.-M., Lambert, J.E., Hulme, P.E., VanderWall, S.B. (Eds.), *Seed Fate: Predation, Dispersal and Seedling Establishment*. CABI Publishing, Wallingford, UK.

Legendre, P. & Legendre, L.F., 2012. *Numerical ecology* (Vol. 24). Elsevier.

Leigh Jr, E.G., 1999. *Tropical Forest Ecology: A View from Barro Colorado Island: A View from Barro Colorado Island*. Oxford University Press, USA.

Levin, D.A. & Kerster, H.W., 1974. Gene flow in seed plants. In*Evolutionary biology* (139-220). Springer US.

Levins, R. & Culver, D., 1971. Regional coexistence of species and competition between rare species. *Proceedings of the National Academy of Sciences*, *68*(6), 1246-1248.

Louda, S.M., 1982. Limitation of the recruitment of the shrub *Haplopappus squarrosus* (Asteraceae) by flower-and seed-feeding insects. *The Journal of Ecology*, 43-53.

Louda, S.M., Potvin, M.A. & Collinge, S.K., 1990. Predispersal seed predation, postdispersal

seed predation and competition in the recruitment of seedlings of a native thistle in sandhills prairie. *American Midland Naturalist*, 105-113.

Louda, S.M. & Potvin, M.A., 1995. Effect of inflorescence-feeding insects on the demography and lifetime of a native plant. *Ecology*, *76*(1), 229-245.

Liang, M., Liu, X., Etienne, R.S., Huang, F., Wang, Y. & Yu, S., 2015. Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. *Ecology*, *96*(2), 562-574.

Leishman, M.R., Masters, G.J., Clarke, I.P. & Brown, V.K., 2000. Seed bank dynamics: the role of fungal pathogens and climate change. *Functional Ecology*, *14*(3), 293-299.

Levey, D.J. & Byrne, M.M., 1993. Complex ant-plant interactions: rain-forest ants as secondary dispersers and post-dispersal seed predators.*Ecology*, 1802-1812.

Lewis, O.T. & Gripenberg, S., 2008. Insect seed predators and environmental change. *Journal of Applied Ecology*, 45(6), 1593–1599.

Lin, L., Comita, L.S., Zheng, Z. & Cao, M., 2012. Seasonal differentiation in density-dependent seedling survival in a tropical rain forest. *Journal of Ecology*, *100*(4), 905-914.

Liu, Y., Yu, S., Xie, Z.P. & Staehelin, C., 2012. Analysis of a negative plant–soil feedback in a subtropical monsoon forest. *Journal of Ecology*,*100*(4), 1019-1028.

Liu, Y., Fang, S., Chesson, P. & He, F., 2015. The effect of soil-borne pathogens depends on the abundance of host tree species. *Nature communications*, *6*.

Lodge, D.J., 1989. The influence of soil moisture and flooding on formation of VA-endo-and ectomycorrhizae in *Populus* and *Salix*. *Plant and Soil*,*117*(2), 243-253.

Lonsdale, W.M., 1993. Losses from the seed bank of mimosa pigra - soil micro-organisms vs temperature fluctuations. *Journal of Applied Ecology*, 30(4), 654–660.

Magurran, A.E. & McGill, B.J., 2011. Challenges and opportunities in the measurement and assessment of biological diversity. *Biological Diversity: Frontiers in measurement and assessment.* Oxford University Press. Oxford.

Magurran, A.E., 2013. *Measuring biological diversity*. John Wiley & Sons. Oxford

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M., Valencia, M.C., Sanchez, E.I. & Bever, J.D., 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, *466*(7307), 752-755.

Manson, R.H. & Stiles, E.W., 1998. Links between microhabitat preferences and seed predation by small mammals in old fields. *Oikos*, 37-50.

Martin, F.N. & Loper, J.E., 1999. Soilborne plant diseases caused by *Pythium* spp: ecology, epidemiology, and prospects for biological control. *Critical reviews in plant sciences*, *18*(2), 111-181.

McCarthy-Neumann, S. & Ibáñez, I., 2012. Tree range expansion may be enhanced by escape from negative plant-soil feedbacks. *Ecology*, *93*(12), 2637-2649.

McCarthy-Neumann, S. & Ibanez, I., 2013. Plant–soil feedback links negative distance dependence and light gradient partitioning during seedling establishment. *Ecology*, 94(4), 780–786.

McCarthy-Neumann, S. & Kobe, R.K., 2010a. Conspecific plant–soil feedbacks reduce survivorship and growth of tropical tree seedlings. *Journal of Ecology*, *98*(2), 396-407.

McCarthy-Neumann, S.& Kobe, R.K., 2010b. Conspecific and heterospecific plant–soil feedbacks influence survivorship and growth of temperate tree seedlings. *Journal of Ecology*, *98*(2), 408-418.

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, et al. (2007) Global climate projections. In *Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. (Eds S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor, HL Miller) 747–845. (Cambridge University Press: Cambridge, UK)

Mehrabi, Z., Bell, T. & Lewis, O.T., 2015. Plant-soil feedbacks from 30-year family-specific soil cultures: phylogeny, soil chemistry and plant life stage. *Ecology and evolution*, *5*(12), 2333-2339.

Metz, M.R., Sousa, W.P. & Valencia, R., 2010. Widespread density-dependent seedling mortality promotes species coexistence in a highly diverse Amazonian rain forest. *Ecology*, 91(12), 3675–3685.

Mezquida, E.T. & Olano, J.M., 2013. What makes a good neighborhood? Interaction of spatial scale and fruit density in the predator satiation dynamics of a masting juniper tree. *Oecologia*, 173(2), 483–492.

Müller, F.M., 1978. *Seedlings of the North-Western European Lowlands: A Flora of Seedlings*.

Junk Publishers, The Hague.

Nakagawa, M., Takeuchi, Y., Kenta, T. & Nakashizuka, T., 2005. Predispersal Seed Predation by Insects vs. Vertebrates in Six Dipterocarp Species in Sarawak, Malaysia. *Biotropica*, *37*(3), 389-396.

Nakagawa, M., Itioka, T., Momose, K., Yumoto, T., Komai, F., Morimoto, K., Jordal, B.H., Kato, M., Kaliang, H., Hamid, A.A. & Inoue, T., 2003. Resource use of insect seed predators during general flowering and seeding events in a Bornean dipterocarp rain forest. *Bulletin of entomological research*, *93*(05), 455-466.

Nathan, R. & Muller-Landau, H., 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution*, 15(7), p.278.

Norghauer, J.M., Malcolm, J.R. & Zimmerman, B.L., 2006. Juvenile mortality and attacks by a specialist herbivore increase with conspecific adult basal area of Amazonian Swietenia macrophylla (Meliaceae). *Journal of Tropical Ecology*, 22(04), p.451.

Notman, E.M., Villegas, A.C., Forget, P.M., Lambert, J.E., Hulme, P.E. & Vander Wall, S.B., 2005. Patterns of seed predation by vertebrate versus invertebrate seed predators among different plant species, seasons and spatial distributions. pp. 55-75. In: Forget, P.-M., Lambert, J.E., Hulme, P.E., VanderWall, S.B. (Eds.), *Seed Fate: Predation, Dispersal and Seedling Establishment*. CABI Publishing, Wallingford, UK.

Novotny, V., Miller, S.E., Baje, L., Balagawi, S., Basset, Y., Cizek, L., Craft, K.J., Dem, F., Drew, R.A., Hulcr, J. & Leps, J., 2010. Guild-specific patterns of species richness and host specialization in plant–herbivore food webs from a tropical forest. *Journal of Animal Ecology*, *79*(6), 1193-1203.

Novotny, V. & Basset, Y., 2005. Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society B: Biological Sciences*, 272(1568), 1083–1090.

Nystrand, O. & Granström, A., 1997. Forest floor moisture controls predator activity on juvenile seedlings of Pinus sylvestris. *Canadian Journal of Forest Research*, *27*(11), 1746-1752.

Nystrand, O. & Granström, A., 2000. Predation on Pinus sylvestris seeds and juvenile seedlings in Swedish boreal forest in relation to stand disturbance by logging. *Journal of Applied Ecology*, *37*(3), 449-463.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H., 2015. vegan: Community Ecology Package. R

package version 2.0-10. 2013. http://CRANR-projectorg/package=vegan.

Packer, A. & Clay, K., 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, *404*(6775), 278-281.

Packer, A. & Clay, K., 2003. Soil pathogens and *Prunus serotina* seedling and sapling growth near conspecific trees. *Ecology*, *84*(1), 108-119.

Paine, C.T. & Beck, H., 2007. Seed predation by neotropical rain forest mammals increases diversity in seedling recruitment. *Ecology*, *88*(12), 3076-3087.

Pautasso, M., Aas, G., Queloz, V. & Holdenrieder, O., 2013. European ash (*Fraxinus excelsior*) dieback–A conservation biology challenge. *Biological Conservation*, *158*, 37-49.

Petermann, J.S., Fergus, A.J., Turnbull, L.A. & Schmid, B., 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology*, *89*(9), 2399-2406.

Peters, J.C. & Shaw, M.W., 1996. Effect of artificial exclusion and augmentation of fungal plant pathogens on a regenerating grassland. *New Phytologist*, 134(2), 295–307.

Pigot, A.L. & Leather, S.R., 2008. Invertebrate predators drive distance-dependent patterns of seedling mortality in a temperate tree *Acer pseudoplatanus*. *Oikos*, *117*(4), 521-530.

Pinzón-Navarro, S., Barrios, H., Múrria, C., Lyal, C.H. & Vogler, A.P., 2010. DNA-based taxonomy of larval stages reveals huge unknown species diversity in neotropical seed weevils (genus *Conotrachelus*): relevance to evolutionary ecology. *Molecular phylogenetics and evolution*, *56*(1), 281-293.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rabatin, S.C., 1979. Seasonal and edaphic variation in vesicular-arbuscular mycorrhizal infect of grasses by *Glomus tenuis*. *New Phytologist*, *83*(1), 95-102.

Reynolds, H.L., Packer, A., Bever, J.D. & Clay, K., 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology*, *84*(9), 2281-2291.

Ricklefs, R.E., 1977. Environmental heterogeneity and plant species diversity: a hypothesis. *The American Naturalist*, *111*(978), 376-381.

Rooney, T.P. & Waller, D.M., 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Ecology and Management*, *181*(1), 165-176.

Rose F, & O'Reilly C. 2006. T*he Wild Flower Key: How to Identify Wild Flowers, Trees and Shrubs in Britain and Ireland*. The Penguin Group, London.

Roslin, T., Laine, A.L. & Gripenberg, S., 2007. Spatial population structure in an obligate plant pathogen colonizing oak *Quercus robur*. *Functional Ecology*, *21*(6), 1168-1177.

Rossi, M.N., da Silva Rodrigues, L.M., Ishino, M.N. & Kestring, D., 2011. Oviposition pattern and within-season spatial and temporal variation of pre-dispersal seed predation in a population of *Mimosa bimucronata* trees.*Arthropod-Plant Interactions*, *5*(3), 209-217.

Russell, F.L., Zippin, D.B. & Fowler, N.L., 2001. Effects of white-tailed deer (*Odocoileus virginianus*) on plants, plant populations and communities: a review. *The American Midland Naturalist*, *146*(1), 1-26.

Santiago, L.S., Kitajima, K., Wright, S.J. & Mulkey, S.S., 2004. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. *Oecologia*, *139*(4), 495-502.

Schafer, M. & Kotanen, P.M., 2004. Impacts of naturally-occurring soil fungi on seeds of meadow plants. *Plant Ecology*, 175(1), 19–35.

Schafer, M. & Kotanen, P.M., 2003. The influence of soil moisture on losses of buried seeds to fungi. *Acta Oecologica*, 24(5-6), 255–263.

Schupp, E.W., 1992. The Janzen-Connell model for tropical tree diversity: population implications and the importance of spatial scale. *The American Naturalist*, *140*(3), 526-530.

Sedio, B.E. & Ostling, A.M., 2013. How specialised must natural enemies be to facilitate coexistence among plants?. *Ecology letters*, *16*(8), 995-1003.

Silman, M.R., Terborgh, J.W. & Kiltie, R.A., 2003. Population regulation of a dominant rain forest tree by a major seed predator. *Ecology*, *84*(2), 431-438.

Silvius, K.M., Forget, P.M., Lambert, J.E., Hulme, P.E. &Vander Wall, S.B., 2005. Frugivore-mediated interactions among bruchid beetles and palm fruits at Barro Colorado Island, Panama: implications for seed fate. *Seed Fate: Predation, Dispersal and Seedling Establishment*, 45-54.

Slatkin, M., 1974. Competition and regional coexistence. *Ecology*, 128-134.

Smith, C.C. & Follmer, D., 1972. Food preferences of squirrels. *Ecology*, 82-91.

Snyman, H.A. 2005. The effect of fire on the soil seed bank of a semi-arid grassland in South Africa. *South African Journal of Botany*, *71*(1), 53-60.

Spear, E.R., Coley, P.D. & Kursar, T.A., 2015. Do pathogens limit the distributions of tropical trees across a rainfall gradient?. *Journal of Ecology*,*103*(1), 165-174.

Sullivan, J.J., 2003. Density-dependent shoot-borer herbivory increases the age of first reproduction and mortality of neotropical tree saplings. *Oecologia*, 136(1), 96–106.

Svenning, J.C. & Wright, S.J., 2005. Seed limitation in a Panamanian forest. *Journal of Ecology*, 93(5), 853–862.

Swinfield, T., Lewis, O.T., Bagchi, R. & Freckleton, R.P., 2012. Consequences of changing rainfall for fungal pathogen-induced mortality in tropical tree seedlings. *Ecology and evolution*, *2*(7), 1408-1413.

Tbaileh, T., 2012. *Effect of Azoxystrobin and Arbuscular Mycorrhizal Fungal Colonization on Four Non-Target Plant Species* (Doctoral dissertation, University of Ottawa).

Tilman D. 1997.Mechanisms of plant competition. In Crawley M.J., ed. *Plant ecology , 2nd edn*. Oxford: Blackwell Science,239–261.

Thompson, K., 1987. Seeds and seed banks. *New Phytologist*, *106*(s1), 23-34.

Thompson, K.B.S.R., Band, S.R. & Hodgson, J.G., 1993. Seed size and shape predict persistence in soil. *Functional Ecology*, 236-241.

Tomita, M., Hirabuki, Y. &Seiwa, K., 2002. Post-dispersal changes in the spatial distribution of Fagus crenata seeds. *Ecology*, *83*(6), 1560-1565.

Toy, R.J.,1991. Interspecific flowering patterns in the Dipterocarpaceae in West Malaysia: implications for predator satiation. *Journal of Tropical Ecology*, 7(01), p.49.

Toy, R.J. & Toy, S.J., 1992. Oviposition preferences and egg survival in *Nanophyes shoreae* (Coleoptera, Apionidae), a weevil fruit-predator in South-east Asian rain forest. *Journal of Tropical Ecology*, 8(02), p.195.

Turnbull, L.A., Crawley, M.J. & Rees, M., 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos*, *88*(2), 225-238.

Uriarte, M., Condit, R., Canham, C.D. & Hubbell, S.P., 2004. A spatially explicit model of

sapling growth in a tropical forest: does the identity of neighbours matter?. *Journal of Ecology*, *92*(2), 348-360.

Van Der Heijden, M.G., Wiemken, A. & Sanders, I.R., 2003. Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. *New Phytologist*, *157*(3), 569-578.

Van der Heijden, M.G., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, *396*(6706), 69-72.

van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, 11(3), 296–310.

van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A. & Suding, K.N., 2013. Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, *101*(2), 265-276.

Vander Wall, S.B., Kuhn, K.M. & Beck, M.J., 2005. Seed removal, seed predation, and secondary dispersal. *Ecology*, *86*(3), 801-806.

Velho, N., Isvaran, K. & Datta, A., 2012. Rodent seed predation: effects on seed survival, recruitment, abundance, and dispersion of bird-dispersed tropical trees. *Oecologia*, *169*(4), 995-1004.

Visser, M. D., Muller-Landau, H. C., Wright, S. J., Rutten, G., & Jansen, P. A. 2011. Tri-trophic interactions affect density dependence of seed fate in a tropical forest palm. *Ecology letters*, 14(11), 1093-1100.

Wagner, M. & Mitschunas, N., 2008. Fungal effects on seed bank persistence and potential applications in weed biocontrol: a review. *Basic and Applied Ecology*, *9*(3), 191-203.

Wallenhammar, A.C., 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. *Plant Pathology*, *45*(4), 710-719.

Webb, C.O. & Peart, D.R., 1999. Seedling density dependence promotes coexistence of Bornean rain forest trees. Ecology, 80(6), 2006-2017.

Weißflog, A., Markesteijn, L., Lewis, O.T. &Engelbrecht, B.M.J. 2015. Contrasting patterns of

insect herbivory and predation pressure across a tropical rainfall and tree species richness gradient. Masters thesis. Bayreuth University. Germany.

Wellstein, C., Otte, A. & Waldhardt, R., 2007. Seed bank diversity in mesic grasslands in relation to vegetation type, management and site conditions. *Journal of Vegetation Science*, *18*(2), 153-162.

Westerman, P. R., Borza, J. K., Andjelkovic, J., Liebman, M., & Danielson, B. 2008. Density-dependent predation of weed seeds in maize fields. *Journal of Applied Ecology*, 45(6), 1612-1620.

Wong, M., Wright, S.J., Hubbell, S.P. & Foster, R.B., 1990. The spatial pattern and reproductive consequences of outbreak defoliation in *Quararibea asterolepis*, a tropical tree. *The Journal of Ecology*, 579-588.

Wright, S.J., 1983. The dispersion of eggs by a bruchid beetle among *Scheelea* palm seeds and the effect of distance to the parent palm. *Ecology*, 1016-1021.

Wright, J.S., 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia*, 130(1), 1–14.

Wright, S.J., Muller-Landau, H.C., Calderón, O. & Hernandéz, A., 2005. Annual and spatial variation in seedfall and seedling recruitment in a neotropical forest. *Ecology*, *86*(4), 848-860.

Wright, S.J., 1990. Cumulative satiation of a seed predator over the fruiting season of its host. *Oikos*, 58(3), 272–276.

Wubs, E.R. & Bezemer, T.M., 2016. Effects of spatial plant–soil feedback heterogeneity on plant performance in monocultures. *Journal of Ecology*.

Wyatt, J.L. & Silman, M.R., 2004. Distance-dependence in two Amazonian palms: effects of spatial and temporal variation in seed predator communities. *Oecologia*, 140(1), 26–35.

Xu, Y., Shen, Z., Li, D. & Guo, Q., 2015. Pre-Dispersal Seed Predation in a Species-Rich Forest Community: Patterns and the Interplay with Determinants. *PloS one*, *10*(11), p.e0143040.

Yamazaki, M., Iwamoto, S. & Seiwa, K., 2009. Distance- and density-dependent seedling mortality caused by several diseases in eight tree species co-occurring in a temperate forest. *Plant Ecology*, 201(1), 181–196.