

The mechanisms of forest diversity effects on insect herbivores

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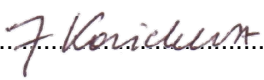
Chapter 3 - JK and I designed the experiment and JK performed the field work. I performed all statistical analyses and prepared the manuscript (and revisions) for publication.

Chapter 4 - JK designed the study and, together with SB, collected leaf samples and assessed insect herbivory in the field. Laboratory analyses of leaf traits were performed by GI, J-PS and EP-F. I assisted with the early stages of leaf trait measurements in the lab, did all statistical analyses and prepared the manuscript for publication.

Chapter 5 - JK designed the study, HM and JK performed moose browsing assessments in 2011. SM and JK performed moose browsing assessment in 2010 and insect herbivory assessments in 2010 and 2011. I did all statistical analyses and prepared the manuscript (and revisions) for publication.

Chapter 6 - JK and I designed the study, KR did the bird surveys and JK conducted assessment of predation rates. I did all statistical analyses and prepared the manuscript (and revisions) for publication.

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Abstract

Insect herbivory is often reduced in plant species mixtures compared to monocultures. Although several hypotheses have been proposed to explain this pattern of associational resistance, empirical tests of these mechanisms are rare in forests and seldom account for the structural or trophic complexity intrinsic to natural ecosystems. In this thesis, I addressed this knowledge gap by testing both traditional and novel mechanisms underlying forest diversity effects on insect herbivores.

Using a long-term forest diversity experiment in Finland, I explored whether forest diversity effects are driven by concurrent changes in environment or host tree traits, and by interactions with other taxonomic groups (insectivorous birds and mammalian browsers). Reduction in insect herbivory in mixed stands was frequently linked to changes in canopy cover around a focal tree. For instance, associational resistance of Norway spruce to a galling adelgid was driven by increased shading by taller heterospecific neighbours in mixed stands. For silver birch, canopy cover decreased in mixed stands resulting in reduced foliar quality and, thus, associational resistance.

I also showed that interactions between and within trophic levels can influence tree diversity effects on insect herbivores. Winter browsing by moose altered both the magnitude and direction of forest diversity effects on birch insect herbivores the following summer whereas avian insectivory increased with tree species diversity at the neighbourhood scale demonstrating that birds might facilitate associational resistance. Both of the above patterns were linked to differences in canopy cover between pure and mixed stands.

Taken together, this work identifies novel pathways linking plant diversity and insect herbivory and highlights the role of structural and trophic complexity as mediators of forest diversity effects on insect herbivores. My findings also suggest that manipulations of tree diversity and canopy cover may offer an effective management strategy against insect pests in planted forests.

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Chapter 1: INTRODUCTION

1.1. Biodiversity and ecosystem functioning

Human activities continue to cause widespread degradation of habitats, influencing both the structure and composition of ecosystems worldwide (MEA 2005). Loss of producer diversity in particular has been shown to negatively affect productivity (Tilman et al. 1996, Hector et al. 1999, Cardinale et al. 2011), nutrient cycling (Scherer-Lorenzen et al. 2007), soil carbon storage (Gamfeldt et al. 2013) and pest resistance (Jactel and Brockerhoff 2007). However, despite an abundance of literature exploring links between biodiversity and ecosystem functions (BEF), empirical studies on functions other than productivity have often focussed on the patterns of BEF relationships without explicit reference to the underlying mechanisms governing them. As biodiversity loss continues unabated, ecological research must advance from correlative approaches towards a more nuanced understanding of BEF relationships so we are better equipped to predict and mitigate the consequences of biodiversity change (Duncan et al. 2015).

In this thesis, I explore the mechanisms underpinning plant diversity effects on one key ecosystem function and service: insect pest resistance. Insects are among the most diverse and important taxonomic groups present in almost all habitats (Mayhew 2007). In terrestrial ecosystems, thousands of insect species can play numerous important roles as herbivores, pollinators, seed dispersers, predators, parasitoids, detritivores and ecosystem engineers (Weisser and Siemann 2004). However, how their activity varies with plant diversity is still a matter of debate. This issue has come to light for phytophagous insects especially as they can both modify vegetation composition (Bagchi et al. 2014) and respond to changes in habitat diversity (Jactel and Brockerhoff 2007, Barbosa et al. 2009). Herbivorous insects may have important positive effects on some ecosystem functions (e.g. nutrient cycling, Metcalfe et al. 2014) but, more often, they are shown to negatively impact the quality, health and productivity of plants (Hartley and Lawton 1987, Ayres and Lombardero 2000, Nykänen and Koricheva 2004, Zvereva et al. 2010, 2012). In particular, mass outbreaks of insect pests and their devastating impacts on plants are well documented (Pimentel 1961, Mattson and Haack 1987, Jepsen et al. 2008, Kollberg et al. 2015). However, even low-level or background herbivory may significantly influence host plants (Zvereva et al. 2010, 2012). Such deleterious effects of insect herbivores on plants may be amplified with climate change (Ayres and Lombardero 2000, Wolf et al.

2007, Kozlov 2008) and thus, an improved understanding of the strength and nature of insect herbivore responses to diversity is urgently required.

1.2 Plant diversity effects on insect herbivores

Plant susceptibility to insect pests and pathogens has frequently been observed to decrease with plant diversity (Jactel and Brockerhoff 2007). This pattern, coined “associational resistance” by Root (1973), has been demonstrated in multiple ecosystem types. For instance, Root (1973) observed that mixing agricultural crops reduced herbivore damage. Similarly, in a meta-analysis by Jactel and Brockerhoff (2007), it was shown that herbivore damage is on average higher in pure forest stands as opposed to mixed stands. Such positive effects of diversity on pest resistance have, for over a century, prompted ecologists to advocate for the diversification of planted ecosystems. For example, Boppe (1889) explicitly recommended the planting of mixed-species forests over monocultures to limit insect herbivory. However, while agricultural and forestry practices have continued to evolve under the assumption of associational resistance, numerous empirical observations have produced contradictory results. Evidence has shown that plants growing in more diverse plots may be more (rather than less) susceptible to herbivory (White and Whitham 2000, Plath et al. 2012). This phenomenon of “associational susceptibility” was first described by Brown and Ewel (1987) and is reportedly more common for generalist rather than specialist insect herbivores (Jactel and Brockerhoff 2007). Given these contradictions in the literature between associational resistance and susceptibility, it is important to understand the mechanisms driving insect herbivore responses to tree diversity to be able to predict the magnitude and direction of these effects.

1.2.1 Relative abundance and quality of host plants

Mechanisms of associational effects have been linked to variation in the relative quantity of host plants with diversity (Barbosa et al. 2009). One of the hypotheses that is most commonly quoted to account for associational resistance is the “resource concentration hypothesis” (Root 1973). As the majority of insects feed on a narrow range of host plants (Forister et al. 2015), increasing plant diversity reduces the abundance of host plants in favour of non-hosts. The resource concentration hypothesis states that oligophagous insects are more likely to find and remain in patches of high host plant density. For many herbivorous insects, host finding is believed to be based on both visual and olfactory cues, thus as diversity increases, the probability of an insect locating a host is expected to

decline as non-hosts physically obstruct or chemically mask the host from searching herbivores (Jactel et al. 2011, Dulaurent et al. 2012). At the same time, insects with narrow host ranges, particularly those that complete their entire life cycle on a single host, are less likely to emigrate from patches with high host plant density. Therefore, high host plant density not only increases the probability of immigration to but also decreases emigration from monospecific stands (Hambäck et al. 2000).

Since its formulation, the resource concentration hypothesis has largely been supported in the literature (Root 1973, Otway et al. 2005, Heiermann and Schütz 2008, Sholes 2008, Björkman et al. 2010, Plath et al. 2012) but, even in the presence of associational resistance, there is also evidence of negative or neutral effects of resource concentration on herbivore loads (Cromartie Jr. 1975, Bach 1980, Futuyma and Wasserman 1980, Bañuelos and Kollmann 2011, Régolini et al. 2014). Thus, while the density of resource plants is an important driver of insect population dynamics, it is unlikely to be the sole determinant of negative plant diversity effects on insect herbivores. Tests of the resource concentration hypothesis have been limited by the fact that they rarely account for the mechanisms governing host plant selection by insect herbivores. In particular, host plant apparency – the probability of a plant being found by an herbivore – has long been known to influence plant-insect interactions (Endara and Coley 2011) but has only recently come under scrutiny. Castagneyrol et al. (2013) presented some of the first empirical evidence showing that increased density of taller heterospecific neighbours in mixed stands reduced the physical apparency of a host plant and thus drove associational resistance to leaf mining insects. Therefore, both the relative density and relative size of host plants can act together to influence herbivore abundance across diversity gradients (Castagneyrol et al. 2013, Régolini et al. 2014).

In addition to interfering with host-finding processes, neighbour plants might also directly or indirectly influence the probability of host acceptance. As insects are well known to be influenced by variation in host plant physical and chemical properties (Loranger et al. 2012, Agrawal and Weber 2015, Caldwell et al. 2016) and host traits are influenced by neighbouring plants (Baier et al. 2002, Barton and Bowers 2006, Broz et al. 2010), it has been suggested that trait variation may underpin associational effects (Barbosa et al. 2009). However, tests of trait-mediated mechanisms across diversity gradients have provided little evidence for this. For example, three recent studies examined whether associational resistance might be mediated by concurrent declines in anti-herbivore

defences (Mraja et al. 2011, Moreira et al. 2014, Wäschke et al. 2015). Although these studies detected higher concentrations of defensive compounds with increasing plant diversity, none of them was able to link it to the insect herbivores in question. This is perhaps unsurprising as a recent meta-analysis showed that plant secondary metabolites do not significantly predict resistance to insect herbivores (Carmona et al. 2011). Rather herbivore susceptibility is better predicted by physical leaf traits and plant general morphology (Clissold et al. 2009, Carmona et al. 2011, Schuldt et al. 2012) possibly because phytochemical compounds are primarily used as host selection cues so, in an interspecific context, variation in host physical properties may have a stronger bearing on herbivore damage and abundance. Nevertheless, even though larger plants may retain greater herbivore abundance (Bach 1980, Marques et al. 2000, Castagneyrol et al. 2013), associational effects are not necessarily related to changes in plant size with diversity (Moreira et al. 2014, Haase et al. 2015).

Studies so far have found stronger support for associational susceptibility mediated by changes in overall stand quality. For instance, generalist herbivores might accumulate in species-rich stands as insects feeding on a varied diet consisting of multiple plant species have been found to perform better than those feeding on a single species (Unsicker et al. 2008, Karban et al. 2010). However, mixed planting could instead result in herbivore “spill-over” from the preferred host to an alternate neighbouring host of inferior quality that may otherwise have escaped attack (Atsatt and O’Dowd 1976, White and Whitham 2000). Therefore, both the quality and relative proportions of viable hosts can act together to influence herbivory across diversity gradients.

With the possibility for such complex interactions between the availability, accessibility and quality of plants with stand diversity, more unifying approaches may be required that link together these different effects. As pointed out by Nadrowski et al. (2010), most studies fail to account for site-related covariates and are therefore unable to separate effects of diversity from that of environmental heterogeneity. This is important as associations between plant species of different growth rates or morphology often yield more structurally complex habitats with variable microclimates (Chen et al. 1999). For example, the presence of taller heterospecific neighbours may result in less light incident on shorter host plant species. As detailed studies have shown that shaded plants often possess higher quality foliage (Roberts and Paul 2006), changes in the extent of overstorey canopy cover with diversity may predict the growth and nutritional quality of

host plants better than diversity per se and, therefore, drive associational patterns. Greater insights into plant diversity effects on insect herbivores may thus be achieved by explicit considerations of concurrent changes in the abiotic environment with diversity.

A shift in focus towards host plant quality and environmental heterogeneity may also be beneficial as, in comparison to resource concentration, spill-over and diet-mixing hypotheses, the above mechanisms are not restricted to specific herbivore types. Root (1973) introduced the resource concentration hypothesis with the caveat that it would apply only to monophagous or oligophagous insects that would have difficulty locating a host plant when diluted by heterospecific neighbours. At the same time, contagion mechanisms such as the spill-over and diet-mixing hypotheses essentially apply only to insects with generalists feeding behaviours as both necessitate switching host plants with neutral or positive effects on subsequent herbivore populations. As several meta-analyses in forest ecosystems have shown, all herbivores do not respond the same way to producer diversity (Vehviläinen et al. 2007, Jactel and Brockerhoff 2007). Although associational resistance is more commonly observed for specialist insects herbivores as compared to generalists (Jactel and Brockerhoff 2007, Castagneyrol et al. 2014a), the magnitude of associational effects may also depend on the feeding guild of the herbivore and the species identity of the host tree (Vehviläinen et al. 2007). Diversity effects may even change direction within seasons and with forest stand age (Vehviläinen et al. 2007, Morath 2013). Therefore, studies may benefit from a more nuanced understanding of underlying mechanisms that can explain associational effects across herbivore types and context-dependent variation.

1.2.2 Multi-trophic interactions

Long before the surge in interest in BEF relationships, community structure was well known to strongly influence the functioning of ecosystems. For instance, the population dynamics of insect pests are governed by both bottom-up effects of the plant community and top-down effects of predators or parasitoids (Lawton and Strong Jr. 1981, Bernays and Graham 1988). In response to frequent observations of more severe pest damage in monocultures rather than mixtures and a higher abundance of insect predators in more diverse habitats (Pollard 1968), Root (1973) put forward the “enemies” hypothesis. Coined at the same time as the “resource concentration” hypothesis, this theory additionally proposes that associational resistance is driven by increased natural enemy effectiveness in mixed as opposed to pure stands (Root 1973). Specifically, more diverse

habitats provide a variety of microhabitats and niches that contribute to a stable, species-rich prey community and also offer additional resources such as nectar or pollen. As a result higher densities of predators and parasites are maintained and top-down control of herbivores is enhanced as plant diversity increases (Root 1973).

In contrast to the three hypotheses mentioned earlier (resource concentration, spill-over and diet-mixing), the enemies hypothesis is perhaps the most applicable across herbivore communities as insects rarely escape both predation and parasitism at all stages of their life cycle. Even so, tests of the enemies hypothesis have produced conflicting results. While some studies have found support for the hypothesis (Russell 1989, Tonhasca 1993, Sobek et al. 2009), several also find that the prediction that natural enemies are more effective in species mixtures is only partially true with stronger effects of plant species composition than species richness per se (Letourneau 1987, Riihimäki et al. 2005) or negative responses (Björkman et al. 2010, Schuldt et al. 2011, Zou et al. 2013). In addition, relationships between diversity and top-down control appear to vary between ecosystem types (Zhang and Adams 2011) and across spatial scales (Bommarco and Banks 2003). Thus, functional predator effects on insects are difficult to generalise to explain associational effects on a broad herbivore community.

Overall, the mechanisms governing associational effects remain elusive suggesting that additional habitat components may need to be considered. In a review by Cardinale et al. (2012), the authors show that although food web interactions are key mediators of ecosystem functioning, studies are yet to incorporate trophic complexity into BEF research. This is particularly the case for the enemies hypothesis as, even though insect herbivores may be fed upon by both invertebrate and vertebrate predators (Letourneau et al. 2009), the vast majority of studies have focussed on arthropod predators alone. Indeed, the pool of herbivores, predators, parasites and pathogens from which we draw biocontrol agents constitute over 50% of species on Earth (Waage 1991). Insectivorous birds, for example, have been completely overlooked despite strong evidence that they are sensitive to habitat complexity at multiple scales (MacArthur and MacArthur 1961, Poch and Simonetti 2013, Huang et al. 2014). Hence, as highlighted by Moreira et al. (2016), future work should document the linkages between plant diversity and top-down feedbacks mediated by insectivorous birds. Furthermore, with clear evidence for positive effects of habitat structural complexity on the abundance of invertebrate natural enemies (Langellotto and Denno 2004) and rates of bird insectivory (Poch and Simonetti 2013),

future studies should explore how effects of structural and vegetational diversity may interact to impact top-down control.

In addition to predator-prey interactions, the majority of ecosystems include complex networks with dozens to thousands of species that interact directly or indirectly through diverse pathways (Sotomayor and Lortie 2015). Therefore, in addition to exploring interactions across trophic levels (plant-herbivore, plant-predator, herbivore-predator), it may also be important to examine interactions within trophic levels (horizontal diversity, Duffy et al. 2007). Nonetheless, only recently has the role of trophic complexity in driving or modifying associational effects come under scrutiny. For example, Axelsson and Stenberg (2012) suggested that in the presence of multiple herbivore types the effect of herbivory by one species could theoretically influence the magnitude or direction of associational herbivory by a second herbivore. Specifically, if one herbivore is under the influence of associational effects and thereby discriminates between individuals, the effects of herbivory on host plants may impact the probability of host selection and, therefore local densities of other herbivores (Ohgushi 2005).

Although such plant-mediated indirect interactions are likely to be common in most ecosystems, relationships between herbivores are very rarely dealt with in BEF studies (Moreira et al. 2016). In the case of mammalian herbivores especially, only one study is known to have tested whether interactions between insects and mammalian herbivores might moderate associational patterns (Parker et al. 2010). Associational susceptibility is commonly observed for mammals (Vehviläinen and Koricheva 2006, Milligan and Koricheva 2013) so increased browsing with diversity may negatively affect insects and drive associational resistance. However, even in the absence of such foraging preferences, plant regrowth responses to browsing may depend on habitat structure and shading (Danell et al. 1985) and thereby link mammalian and insect herbivores indirectly. Nonetheless, Parker et al. (2010) did not observe any changes in browsing in response to genotypic diversity, nor any effects on seed loss to herbivorous insects. Therefore, much more could be done to develop our understanding of how plant-mediated interactions impact associational patterns and may prove more fruitful across species rather than genotypic diversity gradients and where herbivore species exhibit contrasting phenology.

1.3 Understanding mechanisms in forest ecosystems

Forests cover over 30% of the global land area (Keenan et al. 2015) and as the primary habitat for the majority of species, they are likely to lose biodiversity across multiple trophic levels at much faster rates compared to other ecosystems (Thomas et al. 2004). The loss of tree species may have dramatic and detrimental effects on the functioning of ecosystems (Hooper et al. 2005). However, in spite of increasing interest in BEF relationships, studies in forests are rare compared to other ecosystems (Cardinale et al. 2011) with the vast majority of empirical evidence for associational effects on herbivores documented in herbaceous systems. For instance, tests of Root's resource concentration and enemies hypotheses have largely been conducted in agricultural/grassland ecosystems (Bommarco and Banks 2003, Otway et al. 2005, Björkman et al. 2010, Letourneau et al. 2011). Due to slower growth rates and greater longevity of trees compared to herbaceous plants, similar experimental manipulations of producer diversity in forests have been more difficult to implement.

With the emergence of experimental platforms in forests in the past decade, ecologists can now adopt mechanistic approaches to BEF relationships (Verheyen et al. 2015). However, in contrast to herbaceous systems, the first experimental tests of relationships between tree diversity and pest resistance have produced contradictory results. Effects of tree species richness on herbivores have been found to vary in direction (Jactel and Brockerhoff 2007, Schuldt et al. 2010, Plath et al. 2012) and magnitude (Vehviläinen et al. 2007) depending on insect feeding behaviour or, the age, size and planting density of forest stands. Early hypotheses proposed to explain associational effects are well supported in herbaceous systems but have received only partial support in forest ecosystems. For example, experimental studies in forests have found little support for the enemies hypothesis (Riihimäki et al. 2005, Schuldt et al. 2011, Zou et al. 2013). Furthermore, studies frequently observe stronger effects of tree species composition (Vehviläinen et al. 2006, Castagneyrol et al. 2014b) and identity (Nadrowski et al. 2010) on insect herbivores rather than tree species richness per se. Thus, it is important to investigate the mechanisms of forest diversity effects on herbivores as they might differ from those in herbaceous ecosystems.

Recent years have seen a dramatic increase in the number of manipulative experimental studies that have investigated the effects of tree diversity on various aspects of ecosystem functioning (Verheyen et al. 2015). Although such experimental platforms are not without

their criticisms (Leuschner et al. 2009), in comparison to observational approaches, treatment replication across similar sites means that diversity effects are easier to separate from other confounding variables (e.g. historical land use), facilitating mechanistic explanations of BEF relationships (Scherer-Lorenzen et al. 2005). Furthermore, even though planted experimental sites differ from primary forest, in Europe at least, managed forests dominate with less than 1% of forest area covered by non-managed protected forests (Jactel et al. 2009). In addition, tree plantations may be considered one of the largest forms of terrestrial novel ecosystems, gradually increasing in size since 1990 and now accounting for 6.6% of all forested areas globally (Lindenmayer et al. 2015). Results from the Global Forest Resources Assessment 2015 have also shown that although natural forest areas have declined by 9.4% between 1990 and 2015, plantation areas have increased by 6% in the same period (Keenan et al. 2015). However, despite a wealth of research on the benefits of mixed-species planting (Boppe 1889, Koricheva et al. 2006, Erskine et al. 2006, Gamfeldt et al. 2013), less than 0.1% of commercial plantations are polycultures (Nichols et al. 2006).

The vast majority of plantations in Europe typically comprise of even-aged monocultures that are more susceptible to disturbances from herbivores, pathogenic fungi, wind and fire (Jactel et al. 2009). With respect to insect herbivores especially, most woody species have evolved to tolerate major and minor damage by insect pests (Haukioja and Koricheva 2000). However, even low-level background herbivory impacts tree growth with some evidence of resource allocation towards photosynthetic tissues rather than woody parts (Zvereva et al. 2012). As trees are long-lived and can host hundreds or even thousands of insects species (Atkinson 1992, Southwood et al. 2004), the possibility of herbivore attack may be repeated over extended periods leaving more time for low-level and outbreak herbivory to negatively affect wood production. In addition to these effects of native insects, the introduction of exotic pests presents one of the greatest threats to the world's forests with evidence of significant economic losses due to reduced timber stocks (Mingyang and Haigen 2005, Holmes et al. 2009). Therefore, it is more important now than ever to understand how best to secure forests against insect pests.

The current challenge for forest managers is to manage plantations in ways that are cost-effective and meet production requirements. Diversification may form one important strategy against insect pests, but studies so far have not been able to reveal consistent causal links between diversity and herbivory. As forests are long-lived, interactions

between trees may take longer to manifest and associations between woody species may yield structurally complex environments with more variable microclimates in comparison to herbaceous plant communities. These issues of scale, heterogeneity and longevity present significant constraints on expensive pest control methods and further emphasise the importance of developing long-term and cost-effective strategies. In contrast to short-rotation agricultural crops, forests are managed at decadal scales and therefore, policy changes can take years to manifest. Accordingly, any decisions made now must be supported by a clear mechanistic understanding of the benefits and consequences of stand diversification.

As outlined earlier, studies on mechanistic links between forest diversity and herbivory would benefit from improved considerations of producer and consumer traits, structural and environmental heterogeneity and trophic complexity (Moreira et al. 2016). The importance of these novel mechanisms in relation to more established hypotheses needs to be evaluated. From an applied perspective, these data will shed light on how tree herbivore resistance is likely to be affected by stand diversification. In particular, by adopting a community approach to research on associational effects, my work will facilitate predictions of changes in tree pest resistance depending on the extent of tree species diversity and trophic complexity in a given forest.

1.4 Thesis aims

In this thesis, I use a long-term forest diversity experiment in Satakunta, Finland to test established mechanisms and identify novel pathways linking forest diversity and insect herbivores. Specifically, I address two main questions: I) are effects of forest diversity on insects mediated by concurrent changes in environmental factors or host tree traits? (Chapter 3 and 4) and II) are associational effects regulated or maintained through interactions with other taxonomic groups such as mammalian herbivores or birds? (Chapters 5 and 6)

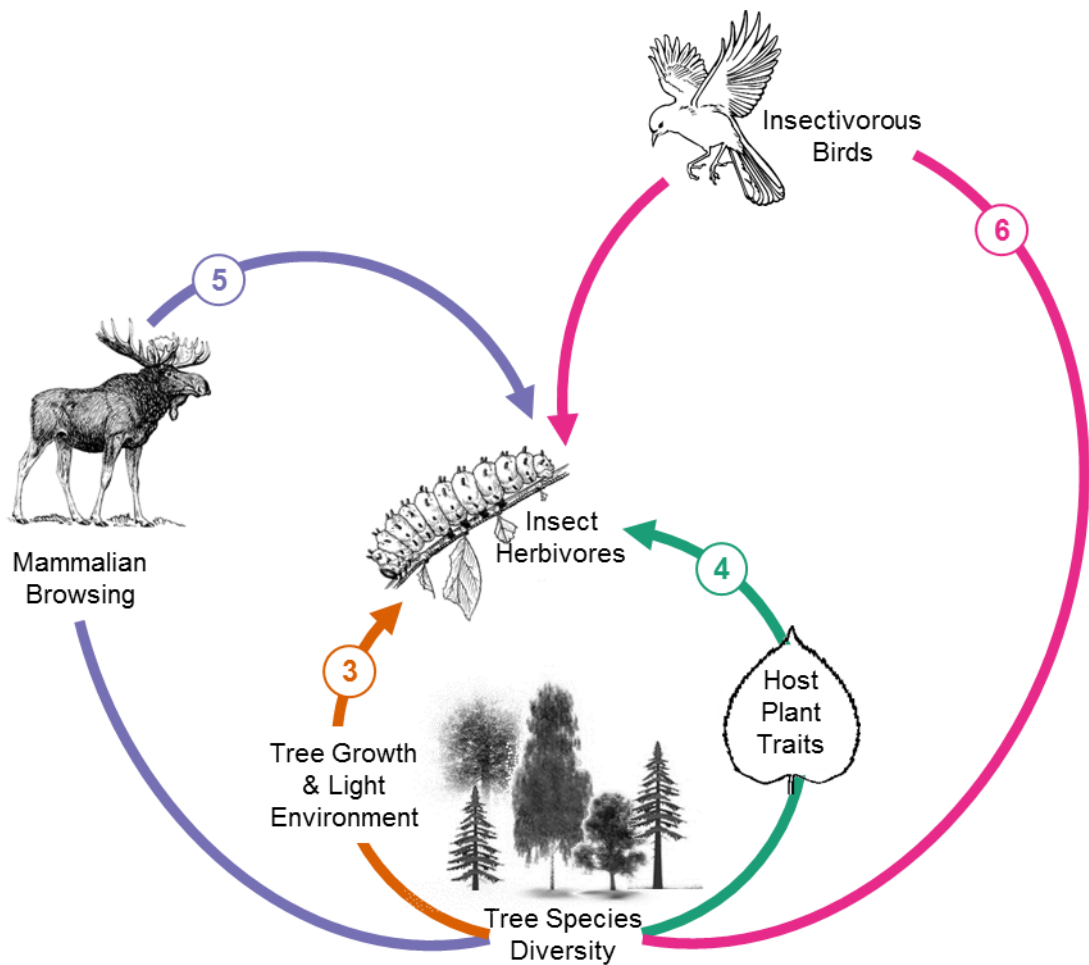


Figure 1.1 Schematic overview of the mechanistic pathways explored in chapter's ③, ④, ⑤ and ⑥

1.5 Structure of the thesis

Chapter 2 describes the Satakunta forest diversity experiment in detail and the methods employed to sample insect herbivory and assess stand structure.

In Chapter 3, I explore to what extent associational resistance of Norway spruce to a specialist galling adelgid is mediated by changes in host tree growth and the light environment.

In Chapter 4, I investigate the effects of tree species diversity on insect herbivore guilds of silver birch, testing whether concurrent changes in leaf traits underpin associational effects.

In Chapter 5, I examine whether positive interactions between mammalian browsers and insect herbivores might modify patterns of birch associational resistance and explain temporal and spatial variation in forest diversity effects on insects.

In Chapter 6, I test the enemies hypothesis in a novel context by examining forest diversity effects on avian predation of insect herbivores.

In Chapter 7, I review and critically evaluate my findings in relation to the aims of the thesis, discuss the implications of these findings for forest management, and provide suggestions for future work.

Chapter 2: METHODS

In this chapter, I provide an overview of the methods used in this thesis. More detailed descriptions of protocols can be found in the subsequent chapters (3-6).

2.1 An experimental approach to BEF studies

In order to examine relationships between biodiversity and the functioning of ecosystems, perhaps one of the simplest approaches would be to identify communities differing in one aspect of biodiversity and compare specific ecosystem responses. However, such comparative approaches are limited by the fact that sampled sites are often spatially-separated so effects of biodiversity may be confounded by variation in environmental factors or land-use history between stands (Scherer-Lorenzen et al. 2005). As a result, such methods cannot be used to establish causality or identify the underlying mechanisms of BEF relationships. Experimental approaches have therefore been developed that allow for effects of biodiversity to be studied in a controlled environment. Biodiversity treatments (most often gradients of plant species richness) are established in planted stands while keeping extrinsic conditions such as climate or land-use history as constant as possible. A variety of ecosystem processes and functions can then be monitored as response variables to changing biodiversity.

For practical reasons, the vast majority of biodiversity experiments have focussed on small, fast-growing, early successional model systems such as grasslands (Tilman et al. 1996, Hector et al. 1999). More recently, however, ecologists have begun to perform manipulative biodiversity experiments where multiple tree species are planted in monocultures and mixed-species stands and replicated in a randomised design. Establishing plots in this manner is considered preferable to removing or adding species to stands as these procedures often modify stand density and can cause disturbance to a site that may influence the variable of interest (Scherer-Lorenzen et al. 2005). Furthermore, new planting also offers more opportunity to homogenise planting sites and establish plots varying in species richness and composition. These manipulations of stand composition help to provide more reliable estimates of ecosystem functioning by permitting the separation of species identity effects from species richness effects while still accounting for potentially confounding factors due to site conditions and local environmental gradients.

Although experimental approaches allow for unambiguous interpretation of cause-effect relationships, they have been criticised for their lack of realism with small species pools, diversity manipulations in only one trophic level and a failure to account for non-random species loss (Lepš 2004, Srivastava and Vellend 2005). However, in the case of forests, plantation areas are growing in size even though the area of natural forest areas is declining globally (FAO 2010). As the vast majority of plantations are planted as monocultures (Nichols et al. 2006), more detailed information is needed on whether species-mixing (rather than species loss) may provide higher yields and other ecosystem services (Verheyen et al. 2015). Tree species diversity experiments therefore remain a promising approach to study BEF relationships with new platforms planted worldwide and integrated within the global network TreeDivNet (www.treedivnet.ugent.be, Verheyen et al. 2015).

2.2 The Satakunta tree species diversity experiment

In this thesis, I explore the mechanisms of forest diversity effects on herbivores using the Satakunta tree species diversity experiment in south-western Finland. Established in 1999, the experiment is the oldest of all 20 experiments in the global network of tree diversity experiments (TreeDivNet) and is the only existing forest diversity experiment in the boreal forest zone established specifically for studying the link between forest diversity and ecosystem functioning. The experiment consists of three separate areas (area 1, 61°42'N, 21°58'E; area 2, 61°39'N, 22°09'E; area 3, 61°40'N, 21°42'E) located 13-17 km from each other. All areas are between 20 and 50 meters above sea level and have minimal slope. Each area was established in similar-sized (approximately 1.5 ha) clear-cuts of formerly conifer-dominated forest that was felled in 1998. All three areas have podzol soils and are surrounded mainly by mature *Picea abies* managed forest.

The tree species pool in the Satakunta experiment consists of five tree species: silver birch (*Betula pendula* Roth, B); black alder (*Alnus glutinosa* L., A); Scots pine (*Pinus sylvestris* L., P); Norway spruce (*Picea abies*, S); non-native Siberian larch (*Larix sibirica*, L). Birch, pine and spruce are the most economically important trees in Finland and commonly occur in both natural and plantation forests. Siberian Larch is native to Russia but frequently cultivated in Finland (Redko and Mälkönen 2005). As a deciduous conifer species, it provides a transition from completely evergreen coniferous (pine, spruce) to deciduous broadleaf stands (birch and alder). Alder was included in the experiment because it is a nitrogen-fixing species and could therefore alter the nutritional status and

growth of other tree species. In addition, as it belongs to the same family (Betulaceae) as birch, both species share a number of insect herbivores.

Within each area, tree seedlings were planted in 38 plots (20m x 20m) which are randomly allocated one of 19 treatments representing a diversity gradient from monocultures of each species ($n = 5$), to two-species ($n = 7$), three-species ($n = 6$) and a 5-species mixture (Fig. 2.1, 2.2, Table 2.1). Each treatment is therefore replicated six times, once in each area. Within each plot, 13 tree seedlings were planted in 13 rows (total 169 trees) at 1.5m intervals (Fig. 2.1). One-year-old seedlings were used for all species except Norway spruce where two-year-old seedlings were used instead. Species mixtures contained equal proportions of each species (Table 2.1) but tree species were randomly allocated a position to mimic natural stands (Fig. 2.1).

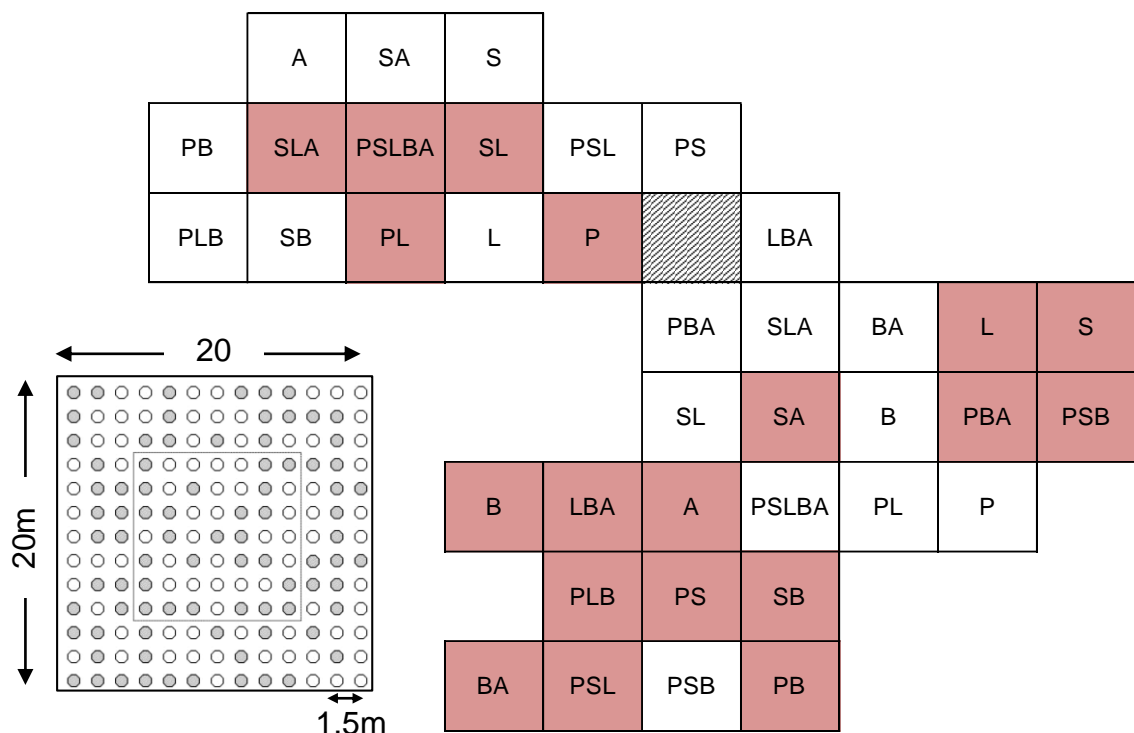


Figure 2.1 The layout of plots in one of the experimental areas in the Satakunta tree species diversity experiment. Treatment combinations of Scots pine (*Pinus sylvestris*, P), Norway spruce (*Picea abies*, S), Siberian larch (*Larix sibirica*, L), silver birch (*Betula pendula*, B) or black alder (*Alnus glutinosa*, A) are randomly allocated a position in each area. Half of all plots in each area were thinned and these are highlighted in red. Trees within each plot 20 x 20 m were randomly planted 1.5m apart and experimental trees were only selected from the plot interior, excluding trees in the outer three rows.

Table 2.1 The 19 tree species mixtures in the Satakunta tree species diversity experiment. Tree species richness ranges from one to five and species mixtures consist of different combinations of Scots pine (*Pinus sylvestris*, P), Norway spruce (*Picea abies*, S), Siberian larch (*Larix sibirica*, L), silver birch (*Betula pendula*, B) or black alder (*Alnus glutinosa*, A) with each species planted in equal proportions in mixed stands. Treatments used in each chapter are also indicated.

Treatment Number	Tree Species Richness	Tree Species Proportions at planting					Chapter			
		P	S	L	B	A	3	4	5	6
1	1	1								✓
2			1				✓			
3				1						
4					1			✓	✓	✓
5						1				✓
6	2	0.5	0.5				✓			
7		0.5		0.5						
8			0.5	0.5			✓			
9		0.5			0.5			✓	✓	✓
10			0.5		0.5		✓	✓	✓	
11			0.5			0.5	✓			
12					0.5	0.5		✓	✓	✓
13	3	0.33	0.33	0.33			✓			
14		0.33	0.33		0.33		✓	✓	✓	
15		0.33		0.33	0.33			✓	✓	
16			0.33	0.33		0.33	✓			
17		0.33			0.33	0.33		✓	✓	✓
18				0.33	0.33	0.33		✓	✓	
19	5	0.2	0.2	0.2	0.2	0.2	✓	✓	✓	✓

Since initial planting in 1999, the Satakunta experiment has received minimal management interventions and no chemical inputs. To ensure treatment establishment, all dead saplings were replaced in 2000 and again in 2001 in plots where mortality exceeded 10%. No replanting has been done since and plots were cleared of naturally regenerating woody vegetation in spring 2010. Within each area, one replicate of each experimental treatment was thinned in 2013 in line with traditional management practices of young forest stands (Fig. 2.1). After accounting for natural mortality, trees were selectively

removed from each plot to reduce the overall density to 50%. In the case of mixed-species plots, trees of each species were removed in such a way as to maintain equivalent species proportions as at planting. Data used in this thesis were collected between spring 2010 and summer 2014 when trees were 11-16 years of age and 2-12 m in height. Canopy closure had also been achieved in almost all plots by the start of the first field season (Fig. 2.2).

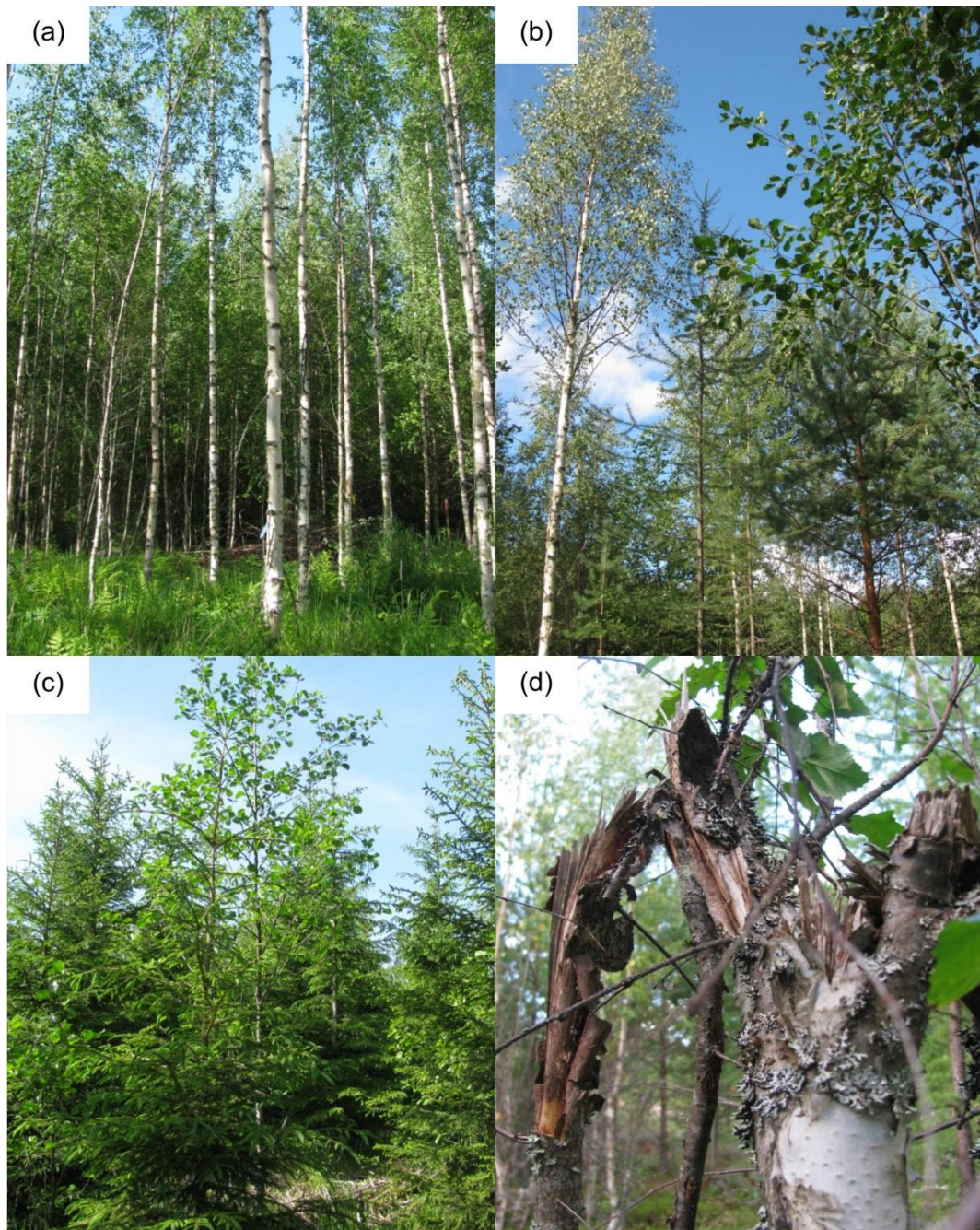


Figure 2.2 Images from the Satakunta tree species diversity experiment taken in summer 2014. A birch monoculture (a), five-species mixture (b) and a spruce and alder plot (c) are pictured along with evidence of moose browsing damage on birch (d). All photos courtesy of J. Koricheva

Plots in the Satakunta experiment are not fenced and hence are accessible to a range of herbivores. Mammalian herbivores, in particular, have caused widespread damage to trees in the Satakunta experiment. For example, damage to seedlings by vole species (e.g. *Microtus agrestis* L.) was considerable during the first winter after planting (Vehviläinen and Koricheva 2006). Among the large mammalian herbivores, moose (*Alces alces* L.) caused the largest amount of damage to trees in the Satakunta experiment. Other cervids such as white-tailed deer (*Odocoileus virginianus* Zimmerman), fallow deer (*Dama dama* L.) and roe deer (*Capreolus capreolus* L.) are also known to occur in the Satakunta experimental site but at much lower densities (Vehviläinen and Koricheva 2006, Milligan and Koricheva 2013). These mammals preferentially browse on short, young trees during the winter (Jalkanen 2001), removing twigs and even breaking main stems to access foliage that would otherwise be out of reach (Fig 2.2). The effects of tree species diversity and the detrimental effects of browsing on tree size are explored in detail in Chapter 5 using data collected in 2010/2011 on browsing, tree growth and herbivory. Six plots in area 2 had to be abandoned from 2011 onwards due to poor establishment and accidental tree harvesting.

In addition to the plot treatments defined in the Satakunta experimental design (Table 2.1), the role of neighbourhood diversity was also considered in each results chapter. The susceptibility of a focal tree to herbivore attack can be altered by the identity, diversity and abundance of its neighbouring species (Underwood et al. 2014). As Satakunta plots are relatively small, and studies have also shown that diversity effects can be traced down to interactions at the individual neighbourhood scale (Potvin and Dutilleul 2009), I posited that diversity in the immediate vicinity of host trees may have stronger effects on insect herbivores than plot diversity. In addition, recent work by Castagneyrol et al. (2013) showed that different components of diversity may also influence herbivory at both plot and neighbourhood scales. Therefore, neighbourhood species richness and composition was assessed based on the eight closest neighbours of a focal tree. However, contrary to expectations, effects of neighbourhood tree species richness and composition on herbivory were not significant in chapters 3-5. Only in Chapter 6 were stronger effects of neighbourhood species richness detected and we expand on this within the chapter and the overall discussion (Chapter 7).

2.2 Insect herbivory monitoring

Trees in the Satakunta experiment host a multitude of insect herbivores (Fig. 2.3). Indeed, one of the main focal species in this study is silver birch which supports over 100 leaf-eating lepidopteran species (Atkinson 1992). While species-level analyses were beyond the scope of this work (except Chapter 3), insect herbivores were grouped into guilds according to their feeding behaviours. Data were collected on the extent of leaf chewing damage (Chapters 4, 5 and 6), the number of leaf galls (Chapters 3 and 4), leaf miners and leaf rollers (Chapter 4). In Chapter 6, I grouped insect herbivores according to their accessibility to foraging birds. Thus, in addition to assessing the extent of leaf chewing damage, I also recorded the presence of exposed chewing insects and the abundance of concealed feeding insects (leaf galls, miners and rollers). Although not all types of herbivore damage were caused by insects (e.g. two mite species commonly caused galls on birch: *Acalitus rudis* and *Aceria leionotus*), for simplicity, I use the term “insect herbivores” throughout to refer to all phytophagous arthropods observed in the Satakunta experiment.

In all cases, visual assessments of insect herbivore abundance and damage were performed on a fixed number of branches sampled from focal trees. Relative abundances of leaf-chewers (lepidopteran and sawfly larvae, beetles), galls (caused by adelgids or mites, Acarina: Eriophyidae), miners (Lepidoptera, Coleoptera, Hymenoptera and Diptera), rollers (weevils, moths and sawflies) were also recorded as the total number of insects per tree (Fig. 2.3). For chewing damage, leaves were classed according to the percentage leaf area missing – <5%, 6-25%, 26-50%, 51-75% and 76-100% – and the number of leaves in each damage category was then multiplied by the midpoint of each category. These values were summed and divided by the total number of sampled leaves per branch then averaged across all branches to provide an estimate of overall percentage leaf area damage per tree. Such visual estimates of herbivory (as opposed to digitizing damaged leaves for example) are often criticised for their lack of accuracy. However, evidence shows that while the precision of these assessments is dependent on surveyor experience, visual estimates provide equally accurate measures in comparison to digital assessments of leaf area loss (Johnson et al. 2015). Furthermore, from a practical perspective they still offer the fastest and most cost-effective approach to assess leaf area damaged by insect herbivores.

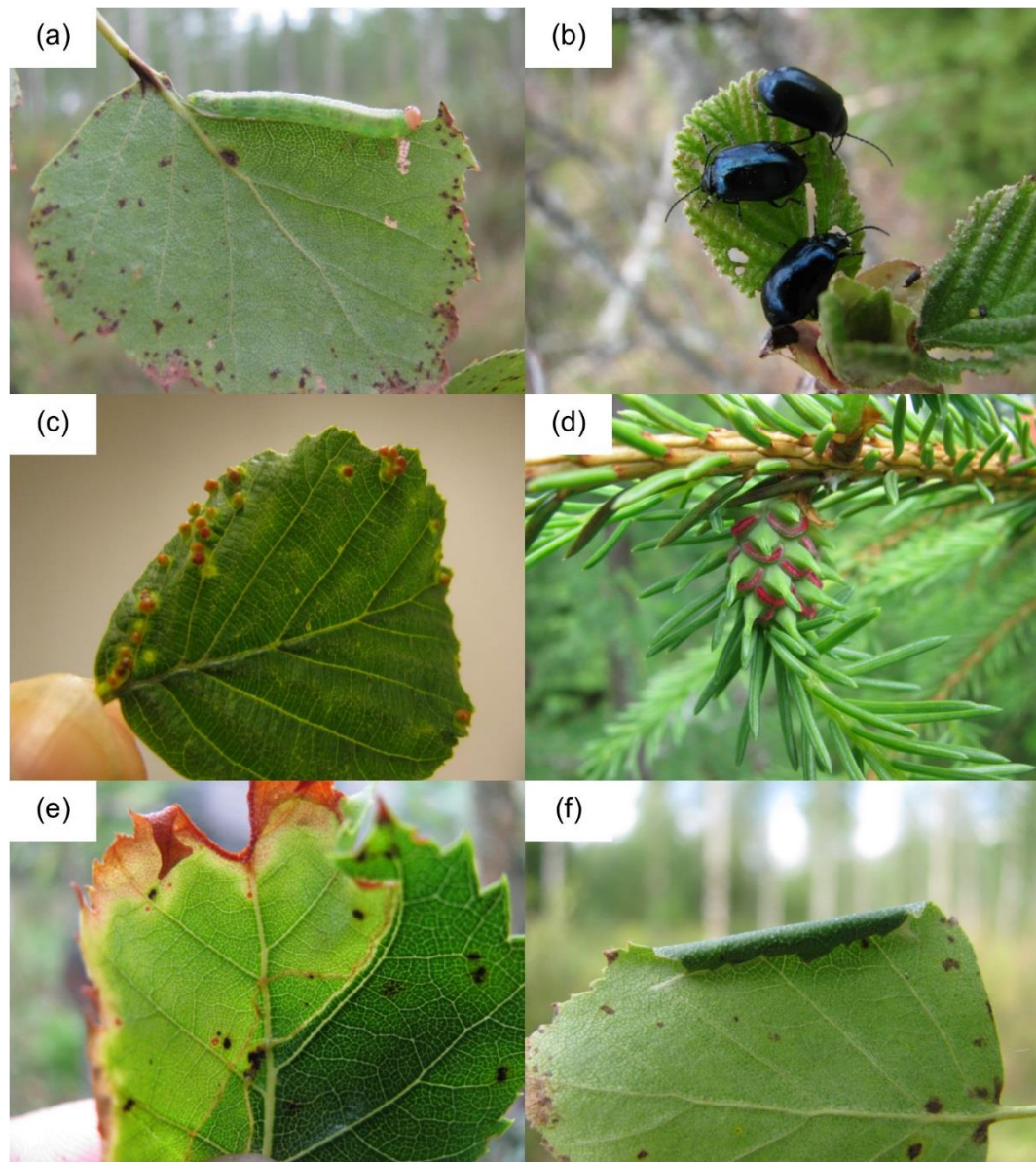


Figure 2.3 Common insect herbivores in the Satakunta tree species diversity experiment: (a) sawfly larva on birch (*Amauronematus* sp.), (b) leaf alder beetle, *Agelastica alni*, (c) *Eriophyes laevis* mite galls on alder, (d) pineapple gall by *Adelges abietis* on spruce, (e) leaf miner *Phylloporia bistrigella* on birch, and leaf rollers (g). All photos courtesy of J. Koricheva

Further criticisms could be levelled at this approach as herbivory was characterised at the whole-tree level based on restricted sampling of a few branches. This is particularly important on tall trees as herbivory can differ markedly between the lower and upper canopy (Fortin and Mauffette 2002) but these intra-canopy differences cannot be incorporated where the upper branches are out of reach to surveyors. In the Satakunta experiment, step ladders and telescopic pruners were used to access the upper canopy of the tallest trees in the Satakunta experiment (birch, >8m). However, while this was sufficient to reach the upper canopy in 2010, further tree growth meant that even the mid

canopy was difficult to reach by the last sampling point in spring 2014 (Chapter 4). Thus, it was not always possible to sample the full canopy on all trees but as the focus is on relative rather than absolute differences in herbivory between plots, I consider that this method is still appropriate to meet the aims of this thesis.

In three of the four results chapters of this thesis, I focus on herbivory on individual tree species and therefore select plots containing these focal tree species (Table 2.1). For example, in Chapter 3, I concentrate on one gall maker, the pineapple gall adelgid (*Adelges abietis*, Fig. 2.3) that specialises on spruce. Therefore, sampling was focussed on spruce trees growing in the nine spruce-containing treatments (Table 2.1). Ten spruce trees were randomly selected from the plot interior and the sampling protocol, described in detail in the chapter, was developed from previous studies on this herbivore species (Fidgen et al. 1994).

In Chapters 4 and 5, I focus on birch insect herbivory and therefore use the nine birch-containing treatments (Table 2.1). Either five (Chapter 4) or ten birch trees (Chapter 5) were randomly selected from the plot interior to minimise edge effects. Sampling effort was reduced to five as opposed to ten trees of birch per plot in Chapter 4 to allow for the detailed assessment of leaf physical traits in the field and the collection of samples for chemical analysis. In both chapters, insect herbivore assessments were performed in the early season (June) and late season (late July-early August). Repeated sampling was performed to gain perspective on the seasonal emergence of insect herbivores and the consequences for leaf area damage. Focal trees selected in the early season were tagged for re-sampling in the late season and herbivory assessed on four branches per tree (low-mid canopy) at each time point. A fixed number of leaves were also sampled per branch and per tree: 100 leaves per tree in 2010 and 2011 (25 leaves per branch, chapter 5) and 200 leaves per tree in 2014 (50 leaves per branch, Chapter 4) when birch trees were larger.

In Chapter 6 insect herbivory was assessed as a predictor of bird predation. As birds may preferentially forage on particular tree species, I used three different tree species (birch, alder and pine) and the stands containing them to capture predation by the community of insectivorous birds in Satakunta (Table 2.1). Pine trees in the experimental site have generally hosted very few insect herbivores so herbivore assessments were focussed on birch and alder only as they are more likely to host insect prey. Natural insect herbivory was monitored on six trees per plot on which artificial caterpillars (modelled from clay)

were installed, recording predation by the attack marks left by birds. Sampling effort was adjusted according to the species richness of the plot: six trees per species per plot were sampled in monocultures, three trees per species per plot sampled in 2-species mixtures and two trees/species/plot sampled in 3- and 5-species mixtures. As in Chapter 5, four branches were selected per tree and 25 leaves per branch (100 leaves per tree) monitored on both birch and alder. However, herbivore assessments only took place during the bird breeding season in May/June 2013 and no surveys were performed in the late season.

With the exception of Chapter 6, all other results chapters focus on a single tree species. This was a deliberate decision in Chapter 3 as the study system was specifically chosen to determine whether species diversity effects on a specialist insect herbivore are mediated by the growth of a host tree or changes in the immediate light environment. Such an analysis would be difficult to do across multiple host species as not only would they host different insect herbivore communities but their growth rates and, therefore, their relative position in the canopy would also vary making interpretation nearly impossible. In Chapters 4 and 5, I also focus on birch alone but the mechanisms explored could potentially have been tested across the entire species pool in the Satakunta experiment. However, herbivore densities have been shown to be very low on two out of the five species in Satakunta (larch and pine, Morath 2013) and, densities on the other Betulaceae species, alder, are significantly lower (Chapter 6). Thus, other than herbivory on spruce, assessments across all species may not have yielded drastically different results.

2.3 Canopy Cover

One of the key aims of this thesis was to determine whether diversity effects on herbivores are mediated by concurrent changes in the abiotic environment. The structure of the forest canopy governs the distribution of light, local precipitation, humidity and temperature (Chen et al. 1999). While direct measurements of light in forest stands are possible, the majority of sensors for direct measurements must be in place for long periods of time to capture light variation at a single point (Jennings et al. 1999). With the additional time and the costs involved, indirect measurements of the light regime are often favoured over direct measures of light intensity. Estimates of canopy cover, in particular, are widely used in forest research and management to classify stand structure (Wisdom et al. 2000) and as effective proxies for light (Lhotka and Loewenstein 2006, Pannek et al. 2013).

Although there are several ground-based methods for assessing overstorey cover (e.g. hemispherical photography, spherical densitometers), the wide angle view means that these measures offer inaccurate depictions of canopy cover characteristics (Stumpf 1993). In particular, by introducing an angle, the resulting estimates are strongly influenced by tree height so cover values increase beneath progressively taller trees (Jennings et al. 1999). Canopy cover estimates are made more accurate and repeatable by ensuring that the observer is looking vertically upwards and that the measure is for a point and not an area (Jennings et al. 1999, Lhotka and Loewenstein 2006).

This is the approach used in Chapters 3-5 where canopy cover was estimated using the GRS densitometerTM (Geographic Resource Solutions, Arcata, CA, USA). The instrument contains a mirror and sighting guides to ensure the observer can project a vertical line-of-sight upwards and record whether the view is obstructed by the canopy or not. To estimate canopy cover around the focal trees, the percentage of views that were obstructed by canopy at 10 evenly spaced positions around the crown edge of each tree was estimated. In addition to being a quick and cost-effective method, the method integrates information gathered in both vertical and horizontal planes and therefore provides a detailed depiction of the light environment around focal trees (Stumpf 1993).

2.4 General statistical approaches

In all chapters, effects of plot species richness and composition on insect herbivores were explored in addition to the specific mechanism under scrutiny. Despite varying levels of herbivory in space and time, tree diversity effects on the damage and abundance of insect herbivores were consistent so data were pooled across years (Chapter 5), seasons (Chapter 4 and 5), thinned and unthinned plots (Chapters 3, 4 and 6) and across all experimental areas (all chapters). However, to account for non-independence of plots within areas, statistical relationships were assessed in mixed effects models where the inclusion of random-effect terms is permitted (Zuur et al. 2009). Further descriptions of data analyses are given in each chapter, tailored to individual study aims.

Chapter 3

Going undercover: increasing canopy cover around a host tree drives associational resistance to an insect pest

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Going undercover: increasing canopy cover around a host tree drives associational resistance to an insect pest

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Abstract

Neighbouring heterospecific plants are often observed to reduce the probability of herbivore attack on a given focal plant. While this pattern of associational resistance is frequently reported, experimental evidence for underlying mechanisms is rare particularly for potential plant species diversity effects on focal host plants and their physical environment. Here, we used an established forest diversity experiment to determine whether tree diversity effects on an important insect pest are driven by concomitant changes in host tree growth or the light environment. We examined the effects of tree species richness, canopy cover and tree growth on the probability of occurrence, the abundance, and the volume of galls caused by the pineapple gall adelgid (*Adelges abietis* L.) on Norway spruce. Although tree diversity had no effect on gall abundance, we observed that both the probability of gall presence and gall volume (an indicator of maternal fecundity) decreased with tree species richness and canopy cover around host spruce trees. Structural equation models revealed that effects of tree species richness on gall presence and volume were mediated by concurrent increases in canopy cover rather than changes in tree growth or host tree density. As canopy cover did not influence tree or shoot growth, patterns of associational resistance appear to be driven by improved host tree quality or more favourable microclimatic conditions in monocultures compared to mixed-stands. Our study therefore demonstrates that changes in forest structure may be critical to understanding the responses of herbivores to plant diversity and may underpin associational effects in forest ecosystems.

Keywords

Biodiversity and ecosystem functioning, forest, galls, plant-herbivore interactions, preference, Satakunta forest diversity experiment

Introduction

Producer diversity has profound ecological consequences on ecosystem function and on the structure of associated communities of consumers (Tilman et al. 1996, Hector et al. 1999, Cardinale et al. 2011). For instance, plant species diversity is frequently observed to decrease the vulnerability of a focal plant to herbivore attack (Jactel and Brockerhoff 2007, Barbosa et al. 2009). This pattern, known as associational resistance, has been well documented and is traditionally explained by two main hypotheses. The enemies hypothesis attributes associational resistance to higher predator recruitment with increasing diversity and thus a stronger suppression of herbivores (Root 1973, Grez and Gonzalez 1995, Hamback and Englund 2005, Muiruri et al. 2016). Secondly, the resource concentration hypothesis states that as herbivores frequently forage in a density-dependent manner, increasing the number of plant species at a constant plant density reduces the probability of finding a preferred host plant species, which in turn lowers herbivore abundance and damage (Root 1973, Letourneau 1987, Tonhasca 1993, Riihimäki et al. 2005). While both hypotheses are well-supported in the literature, associational effects on insect herbivores remain notoriously difficult to predict (Barbosa et al. 2009, Himanen et al. 2010, Axelsson and Stenberg 2012, Muiruri et al. 2015) partly because experimental studies of associational effects often fail to account for factors that co-vary with plant diversity (Huston 1997, Nadrowski et al. 2010). This makes it difficult to establish causal links between producer diversity and insect herbivory and limits mechanistic understanding of associational effects.

While studies reporting diversity effects on herbivores are on the rise in forest ecosystems (Jactel and Brockerhoff 2007, Moreira et al. 2014, Abdala-Roberts et al. 2015, Haase et al. 2015), the underlying biotic and/or abiotic factors driving associational resistance are rarely explored even though the size, complexity and longevity of forests can make for a more spatially heterogeneous environment. For instance, as tree diversity increases, interactions between plant species of differing growth rates inevitably yields structurally complex forest stands with spatially variable microclimates (Chen et al. 1999). In addition, competitive interactions between species can affect relative growth rates of individual host trees (Piotto 2008, Kaitaniemi and Lintunen 2010), which in turn may influence insect herbivory at the stand-level (Haase et al. 2015). These competitive interactions may also yield changes in the light environment around a focal host tree as the extent of shading they experience by neighbours increases (Lang et al. 2011). Thus, as tree diversity increases, concurrent changes in host tree growth and proximate light

conditions may play an important role in insect herbivore distributions and underpin observed patterns of associational resistance.

With growing interest in the mechanistic basis of diversity-ecosystem functioning relationships (Duncan et al. 2015, Moreira et al. 2016), the role of structural and environmental heterogeneity in forests has recently come under scrutiny. For example, Castagneyrol et al. (2013) showed that faster growing and taller trees might be more susceptible to insect herbivory, in accordance with the plant vigour hypothesis (Cornelissen et al. 2008). At the same time, Castagneyrol et al. (2013) demonstrated that associational resistance to leaf-mining insects might be driven by host trees being obscured by taller neighbouring heterospecifics as diversity increases. However, as tree diversity increases, competitive interactions between species may lead to changes in tree crown area, which in turn may affect light availability in the understorey (Lang et al. 2011). Such changes in the light environment are particularly important as they can directly or indirectly influence host plant susceptibility to insect herbivores. For example, Roberts and Paul (2006) demonstrated that shading within and between tree canopies often has a positive effect on insect herbivory due to light modulating foliar quality and increasing anti-herbivore defences. In addition, as shown by Stoepler and Rehill (2012), the effects of light on insect herbivores may also act independently of the host plant in question. Insects may avoid host plants in high-light environments where natural enemies may be more active and effective at locating prey (Perfecto et al. 2004, Stoepler and Lill 2013) or, they may be more prone to desiccation in the warmer and drier microclimate (von Arx et al. 2012). Thus, changes in the light environment may have overarching consequences for both host trees and their insect pests and may therefore explain patterns of herbivory better than tree species richness per se. Nonetheless, the role of natural variation in the light environment in driving associational effects remains untested.

In this paper, we explore the mechanisms by which tree species diversity affects a galling adelgid (*Adelges abietis*). This adelgid forms pineapple-shaped galls on Norway spruce (*Picea abies*) and can negatively impact tree health and vigour (Havill and Footitt 2007). With no known natural enemies, adelgid responses to diversity are likely to reflect interactions between the host plant and the environment rather than changes in predation risk (Björkman 1998). Previous studies have found that pineapple galls are larger in size on faster growing spruce trees (Flaherty and Quiring 2008). In addition, studies on pineapple gall adelgids have also shown that stem mothers may prefer to initiate galls and

oviposit on unshaded branches (Fidgen et al. 1994) indicating that the light environment may be important in the selection and performance of pineapple galls. Therefore, we hypothesised that effects of tree species richness on pineapple gall adelgids are mediated either by changes in tree size or in shading by neighbouring tree species.

Our study was conducted in the Satakunta forest diversity experiment in south-west Finland, where we explored effects of tree species diversity on the (i) presence, (ii) abundance and (iii) volume of pineapple galls on spruce trees growing in 1, 2, 3 and 5-species mixtures. These three variables were chosen to help identify the proximate mechanisms that might influence (i) the selection of trees by stem-mothers, (ii) the accumulation of pineapple gall adelgids on a given tree and, (iii) the performance of mothers and offspring in ensuring gall development. We also measured tree height and diameter as well as canopy cover around each focal spruce tree in pure and mixed-species stands and used structural equation modelling to determine whether gall distributions along richness gradients are driven directly or indirectly by changes in spruce tree size or shading by neighbouring trees. Therefore, by examining herbivore responses to variation in host plant growth and light conditions across the diversity gradient, this study advances our understanding of mechanistic links between plant diversity and insect herbivores.

Methods

Study species

The pineapple gall adelgid, *Adelges abietis* (Hemiptera: Adelgidae), is an aphid-like insect that is monophagous on Norway spruce where it forms pineapple-shaped galls (Havill and Footitt 2007). The entire life cycle is completed on spruce and most individuals stay on the tree on which they were born leading to a highly clustered distribution (Havill and Footitt 2007). The parthenogenetic females, known as fundatrices, overwinter with their stylets inserted in a bud and if attack is successful, the bud develops into a gall (Plumb 1953). Once stem-mothers mature, they oviposit in spring, laying their eggs beneath a wax cover on the swelling bud. The newly-hatched gallicolae crawl into the gall chambers and their feeding enhances further development to form pineapple-shaped galls (Fig. 1a). The resulting multi-chambered pineapple gall grows and eventually dehisces in autumn when the next generation of adelgids emerge, leaving the empty gall behind (Fig. 1b, Havill and Footitt 2007).

We recorded gall presence, gall abundance and gall volume on a given tree. Gall presence reflects not only the probability of stem-mothers selecting a given tree but also the probability of stem-mothers performing well enough to successfully induce gall formation as rates of gall induction success by stem mothers can be quite low (McKinnon et al. 1999). The second variable, gall abundance, was also used as an indicator of stem-mother abundance on a given tree as all galls are initiated by one or more stem-mothers (Plumb 1953). As most adelgids remain on the same host tree on which they were born, abundance is therefore a measure of past success of gall induction on a given tree. Finally, the third variable, gall volume is considered to be a good indicator of gall performance as larger pineapple galls result from stronger galling stimulus from stem mothers (Flaherty et al. 2010). Previous studies on this adelgid species have also shown that bigger galls host a larger number and size of gallicolae that also have a higher fecundity than gallicolae from smaller galls (McKinnon et al. 1999). Therefore, gall volume indicates the performance of both stem-mothers and their daughters as well as the success of these gallicolae in producing the third generation.

Experiment

The study was carried out at the Satakunta forest diversity experiment (www.sataforestdiversity.org) in south-western Finland. Established in 1999, the experiment is located in three separate areas (area 1, 61°42'N, 21°58'E; area 2, 61°39'N, 22°09'E; area 3, 61°40'N, 21°42'E) planted with five tree species: Scots pine (*Pinus sylvestris*); Norway spruce (*Picea abies*); non-native Siberian larch (*Larix sibirica*); silver birch (*Betula pendula*); and black alder (*Alnus glutinosa*). Each area consists of 38 plots (20m x 20m) which are randomly allocated one of 19 treatments representing a richness gradient from monocultures to 2-species, 3-species and a 5-species mixture. Each plot, in turn, consists of 13 rows, with 13 trees planted at 1.5m intervals (total 169 trees) and tree species randomly allocated a position. To ensure establishment of trees in the experiment, all dead seedlings were re-planted in 2000 and, again, in 2001 in plots where mortality exceeded 10%. No chemical inputs have been used in the experiment but plots have been cleared of naturally regenerating vegetation in 2010 to maintain plot treatment and species densities. During this study, plot thinning also took place in June 2013, selecting trees for removal such that species densities remained constant. However, as gall mothers overwinter on their host tree, effects of thinning on spruce trees and gall abundance were not expected to emerge during the experimental period.

Gall measurements

In the present study, two out of the three experimental areas were used (area 1 and area 3). We used all spruce-containing treatments available in the experiment: the spruce monoculture, four 2-species combinations (spruce + alder, spruce + birch, spruce + larch, spruce + pine), three 3-species mixtures (spruce + larch + alder, spruce + pine + birch, spruce + pine + larch) and the five species mixture (spruce + pine + larch + birch + alder). Each treatment was replicated at two plots within each study area (4 replicates in total).

In June 2013, 10 spruce trees were randomly selected from each plot and the presence and abundance of pineapple galls was recorded on eight randomly chosen branches from the mid- and upper-canopy of each tree. Twenty lateral shoots were examined per branch, in keeping with the sampling strategy used for the same adelgid species by Fidgen *et al.* (1994). Trees and shoots found to have pineapple galls were marked for subsequent sampling later in the summer season.

In August 2013, when galls had reached their final size, we assessed gall volume on all experimental spruce trees. Galls occurring on branches with more than 5 galls were excluded from this analysis as both the survival and size of emerging gallicolae has been shown to be negatively affected by gall densities (Sopow and Quiring 2001). The length and two diameters (measured at right angles to each other) of each gall was measured with callipers and gall volume was calculated using the following equation (McKinnon *et al.* 1999):

$$4.1888 \times \frac{\text{length}}{2} \times \left(\frac{\text{average diameter}}{2} \right)^2$$

With these data we observed no significant relationship between gall numbers and gall volume ($\chi^2=0.68$, $df=1$, $p=0.409$), thus our measures of gall volume were independent of gall abundance. To explore the relationship between gall volume and the number of gallicolae, 50 galls of different size were collected from spruce trees in different plots and treatments. The volume of each gall was recorded as above and galls were dissected to count the number of feeding cavities in each gall. A strong positive relationship was observed ($R^2=0.518$, Supporting Information Appendix 1) confirming that gall volume is a good indicator of stem-mother fecundity.

Canopy cover and tree size

To determine changes in canopy cover with tree species richness, we measured the canopy cover around all experimental spruce trees in June 2013. We used the GRS densitometer™ (Geographic Resource Solutions, Arcata, CA, USA) to record the percentage of views obstructed by canopy at 10 evenly-spaced positions around each of the spruce trees. Such visual estimates are commonly used to assess light availability with evidence that canopy cover assessments are well correlated with light intensity (Pannek et al. 2013). Measurements were taken around the edge of the focal tree crowns. As spruce tree crowns are conical in shape, increases in percentage canopy cover reflect increased shading by neighbouring trees and not self-shading.

In August 2013, both the height and diameter at breast height (1.3m, DBH) were measured for all experimental spruce trees as a measure of cumulative growth since the start of the experiment. In addition, on trees harbouring galls, the lengths and diameters of the shoot fostering the gall (“mother shoot”) and another shoot on the same branch (“neighbouring shoot”) were measured. These measurements were used as indicators of the growth potential of the galled shoot which is assumed to be positively related to adelgid performance (Björkman 1998).

Statistical analysis

To aggregate branch-level measurements of galls to the tree-level, we calculated the mean gall volume as well as the total number of galls observed on all sampled branches per tree. Thus, gall presence equates to the presence of at least one gall on one of the eight sampled branches and gall abundance to the total number of galls found on these branches. As only 113 trees of the 353 sampled were infested with galls, we firstly constructed zero-altered and zero-inflated Poisson models to account for the excess zeroes in the data (Zeileis et al. 2008). Results were comparable between zero-altered models, where ungalled trees are excluded from the count part, and zero-inflated models where all trees are included (Supporting Information Appendix 2). However, results differed between the binary and count parts of the models and we therefore performed all analyses on gall presence/absence and gall abundance separately to identify distinct mechanisms driving the presence and density of galls. Additionally, to better separate stem mother preferences (and performance) for a given host tree (indicated by gall presence) from mechanisms governing the accumulation of pineapple galls on the same host tree, we also omitted ungalled trees in all subsequent analyses of gall abundance.

For each of the three response variables (gall presence/absence, gall abundance, and gall volume), we performed analyses to determine their responses to tree species diversity, changes in canopy cover, and the size of the focal tree. Effects of tree species composition on galls were also tested but as these effects were negligible, we focus on tree species richness only. Initial models were fitted to test for differences in gall presence, abundance and volume between the two study areas (area 1 and 3) and between thinned and unthinned plots. While gall densities were higher in area 3, effects of richness, canopy cover or tree size on galls were independent of study area or thinning (Supporting Information Appendix 3 Table A2). Therefore, we present results from models fit to data pooled from both study areas and both thinned and unthinned plots.

All of the analyses were conducted in R software (R Core Team 2015) using the lme4 package (Bates et al. 2012). All models featured “plot” as a random factor and area as a fixed factor as well as either (1) tree species richness, (2) canopy cover, (3) tree height or (4) tree DBH as additional continuous explanatory variables. Additional models were also constructed to explore whether effects of canopy cover were dependent on tree size by including either tree height or DBH in interaction with canopy cover (canopy cover x height/DBH). Generalized linear mixed models (GLMMs) were used for predicting gall presence/absence and gall abundance. Gall presence/absence was modelled with a binomial error structure and gall abundance with a Poisson error structure in GLMMs. Linear mixed models (LMMs) were used for gall volume as data were successfully log transformed to meet assumptions of homogeneity of variance.

To determine the effect of stand species richness on tree size, we used LMMs to assess changes in tree height or DBH with increasing species richness. Although spruce tree heights and DBH were positively correlated ($\chi^2=1081.7$, $df=1$, $p<0.001$), previous studies have observed stronger effects of tree density and diversity on tree diameter rather than height (Lanner 1985, Piotta 2008). Thus, both variables were tested here separately to isolate any differences in their responses to tree species richness and their effects on pineapple galls. For canopy cover, as it was estimated as a percentage of obstructed canopy (in increments of 10), effects of tree species richness were tested in GLMMs where canopy cover was modelled as a binomial response variable (percentage canopy / percentage sky) bounded between 0 and 100. Relationships between canopy cover and tree size were also explored by repeating these binomial GLMMs with either tree height

or tree DBH in place of tree species richness. All models for canopy cover and tree size were finally repeated with gall presence included as an additional explanatory variable. This was done to determine whether infested spruce trees exhibited different properties to ungalled trees across gradients of species richness and canopy cover and, therefore, ascertain whether stem-mother preferences are context-dependent. For these and earlier models, chi-squared and p values are reported from an ANOVA of (G)LMMs using the car package in R (Fox and Weisberg 2011).

Structural equation modelling

Piecewise Structural Equation Models (piecewise SEM) were used to test the hypothesis that tree species richness effects on pineapple galls are mediated by concurrent changes in spruce tree size or canopy cover around spruce trees. In contrast to traditional SEMs, piecewise SEMs permit the inclusion of hierarchical and non-normally distributed data by piecing multiple (G)LMMs into one causal framework (Lefcheck 2015). However, as piecewise SEMs do not permit inclusion of covariance structures, models were fit to separately test whether tree species richness effects on pineapple gall presence are mediated by changes in either canopy cover, tree height or tree DBH.

The “piecewiseSEM” package in R (available at <https://github.com/jslefcche/piecewiseSEM>) was used to generate the causal network with all component models fit with (G)LMMs as described earlier. Overall fit of the models was assessed using Shipley’s test of direct separation which evaluates the probability that none of the paths missing from the hypothesised network contain useful information (Shipley 2009). Models were rejected if a chi-squared test of Fisher’s C statistic fell below the significance level ($p < 0.05$) indicating that models are inconsistent with the data. Accepted models were then compared using the second-order Akaike’s Information Criterion (AICc) which increases as the relative likelihood of the model decreases (Burnham and Anderson 2004). Models with $\Delta AICc \geq 10$ are considered to be unsupported by the data and can therefore be omitted. For all three pineapple gall variables, attempts to incorporate both canopy cover and tree size variables in the SEM led to a large increase in AICc ($\Delta AICc \geq 10$) and therefore, we only present models including either canopy cover or tree size variables individually.

Results

Effects of tree species richness, canopy cover and spruce tree size on galling adelgids

The likelihood of galls being present on spruce trees decreased significantly with the number of tree species per plot (Fig. 2a, Table 1). In contrast, the abundance of pineapple galls on infested spruce trees did not vary with plot species richness (Fig. 2b, Table 1). Gall volume decreased with tree species richness but this effect was only marginally significant (Fig. 2c, Table 1). A similar pattern was observed for canopy cover as the number of galls on spruce did not vary with canopy cover (Fig. 2b, Table 1) but both the probability of gall presence and the volume of galls was reduced as canopy cover increased from 0 (focal tree completely unshaded) to 100 (focal tree completely shaded) (Fig. 2a, c, Table 1). In tests of interactive effects of canopy cover and tree size on pineapple galls, we found that these effects of canopy cover were in fact dependent on spruce tree diameter (Supporting Information Appendix 4). Gall presence significantly increased with tree DBH (Fig. 2a, Table 1) but these positive effects were much stronger where canopy cover exceeded 20% (canopy cover x DBH: $\chi^2=3.93$, $df=1$, $p=0.048$), thus galls were least likely to be present on small trees growing under high canopy cover (Supporting Information Appendix 4 Fig. A2a). In addition, we found that tree size generally had a positive effect on gall abundance but this was only significant for tree height (Fig. 2b, Table 1). Nevertheless, the weak positive effects of tree DBH on the number of galls per tree was reversed when canopy cover was high (canopy cover x DBH: $\chi^2=5.95$, $df=1$, $p=0.015$). Galls were therefore most abundant on large unshaded or, small shaded spruce trees (Supporting Information Appendix 4 Fig. A2b). Finally, we found that effects of canopy cover on gall volume were independent of tree size (Supporting Information Appendix 4) and gall volume also did not significantly vary with either tree height or DBH (Fig. 2c, Table 1).

Within trees infested by galls, the size of shoots had no effect on the number of galls per tree ($p>0.530$), but it did have a positive effect on gall volume. Both the average shoot length and diameter were positively related to mean gall volume on individual trees (shoot length: $\chi^2=5.34$, $df=1$, $p=0.021$; shoot diameter: $\chi^2=3.85$, $df=1$, $p=0.049$). Thus, although tree height had no direct effect on gall volume, differences in shoot size may have impacted gall development. However, as shoot size was unrelated to tree species richness (Supporting Information Appendix 5 Table A5), this cannot explain species richness effects on pineapple galls.

Relationships between tree species richness, canopy cover and spruce tree size

Preliminary analysis revealed that canopy cover around spruce trees was significantly lower in area 3 compared to area 1 (Supporting Information Appendix 3 Table A2). Tree species richness had a strong positive effect on canopy cover with focal spruce trees being more shaded in mixed stands than in spruce monocultures ($\chi^2=11.7$, $df=1$, $p<0.001$, Fig. 3a). This effect was independent of study area (richness x area: $\chi^2=0.0$, $df=1$, $p=0.947$) or thinning (richness x thinning: $\chi^2=0.01$, $df=1$, $p=0.914$). Effects of tree species richness on canopy cover were also consistent between galled and ungalled trees (Fig 3a, richness x gall presence/absence: $\chi^2=0.64$, $df=1$, $p=0.425$) but the vast majority of gall-infested trees had less than 50% canopy cover by neighbouring trees (Fig. 3a).

At the time of the study, spruce trees averaged 548.7 ± 2.3 cm in height and 59.1 ± 0.3 mm in DBH and did not differ in size between study areas or in thinned verses unthinned plots (Supporting Information Appendix 3 Table A2). We observed that tree size generally decreased with tree species richness (Fig. 3b, c), however, this pattern was not significant (height: $\chi^2=1.40$, $df=1$, $p=0.236$ and DBH: $\chi^2=3.09$, $df=1$, $p=0.079$). Examining galled and ungalled trees separately, we observed that effects of tree species richness on spruce size differed between infested and uninfested trees (Fig. 3b, c). Negative effects of tree species richness on tree size were observed for ungalled trees while trees with galls were equivalent in size regardless of plot species richness. Although this was only significant for tree DBH (Fig. 3c, richness x gall presence: $\chi^2=4.1$, $df=1$, $p=0.046$) and not tree height (Fig. 3b, $\chi^2=2.6$, $df=1$, $p=0.108$), the consistent pattern suggests that adelgids counteract the negative effects of species richness by selecting the largest trees in more diverse stands. These preferences are reflected in our previous analysis of interactions between canopy cover and tree size (Supporting Information Appendix 4) and in relationships between canopy cover and tree size as we observe that galled trees are consistently larger than ungalled trees where canopy cover is high (Fig. 3b and c, canopy cover x galls presence/absence: $p<0.001$).

Structural Equation Modelling

For gall presence, only one SEM model was selected demonstrating that effects of tree species richness on the probability of galls being present is almost entirely mediated by changes in canopy cover (Fig. 4a). Comparable models with canopy cover replaced by either tree height or tree DBH were a poor fit to the data ($p<0.05$, Fig 4a) and more

complex models incorporating tree size and canopy cover simultaneously did not improve model fit. In contrast, SEMs for gall abundance did not reveal any direct or indirect effects of tree species richness on gall abundance (Fig. 4b). Although the top model with canopy cover and tree species richness was also selected ($p > 0.05$, $\Delta AICc \geq 0.140$), it was no different to similar putative models with height or DBH ($\Delta AICc < 10$). Even though strong effects of tree species richness on canopy cover were detected, this was uncoupled from any effect of tree size on gall abundance. Finally, SEMs for gall volume revealed a similar pattern to that seen for gall presence in that the top selected model ($p > 0.05$, $\Delta AICc \geq 3.63$) included tree species richness and canopy cover only (Fig. 4c). As $\Delta AICc$ between this top model and other candidate models was low ($\Delta AICc < 10$), we could not omit these models entirely (Fig. 4c). However, since estimates for tree species richness effects on either tree height or DBH were small (< 0.1), we conclude that tree size is not a strong determinant of gall volume across the diversity gradient.

Discussion

While plant associational effects on insect herbivores have been studied extensively in the literature, experimental data on mechanisms driving patterns of associational resistance (or susceptibility) are lacking (Barbosa et al. 2009). In addition, although plant responses to herbivory are well known to depend on both plant growth and environmental conditions (Roberts and Paul 2006, Cornelissen et al. 2008), these factors are scarcely ever included in models of biodiversity and ecosystem functioning. Of the few studies that address this discrepancy, Castagneyrol et al. (2013) is the only known study to demonstrate that associational resistance in forest ecosystems can be mediated by simultaneous changes in stand structure with diversity. Here we not only show that changes in stand structure drive associational resistance, but we go one step further with structural equation models to directly test for causal links between tree species richness, changes in canopy cover or tree size and the presence of an important insect pest. We observed that, despite weak positive effects of tree growth on pineapple gall adelgids, changes in canopy cover with tree species richness underpin associational resistance of focal spruce trees to pineapple gall adelgids. Thus, relationships between producer diversity and insect herbivores may be the result of predictable changes in environmental conditions.

Canopy cover as a mechanism driving associational resistance

According to the resource concentration hypothesis, the density of pineapple gall adelgids would be expected to decrease with tree species richness as a result of reduced host plant density which, in turn, decreases the likelihood of detection of preferred hosts (Root 1973). While we did observe associational resistance in this experiment, this appeared to be independent of resource concentration as tree diversity had no direct effect on any of the three gall responses. Instead, the effects of tree species richness were mediated by the concurrent increases in canopy cover. Strong relationships between tree species richness and canopy cover were, most likely, due to the fact that spruce was one of the shortest tree species planted in the Satakunta experiment (Muiruri et al. 2015). As a result, spruce trees are frequently shaded by the faster growing tree species in mixed stands (silver birch, larch and Scots pine) and are therefore less likely to host galls. To the best of our knowledge, this study is the first demonstration of canopy cover acting as a mediator of associational resistance to insect herbivores.

Given that spruce tree growth was unaffected by changes in canopy cover, we suggest that observed tree species richness effects on pineapple galls were driven by some property relating to canopy cover by neighbouring trees. Although we did not directly measure light, a recent study by Pannek et al. (2013) showed that visual estimates of canopy cover correlate well with measures of light intensity in over 100 deciduous forests. Thus, patterns of associational resistance may reflect stem mother preferences for high-light environments leading to selection of trees with canopy cover below 50% (Fig. 3) and initiation of galls in the mid- and upper-canopy branches which are not shadowed by other branches (Fidgen et al. 1994). Similar light-dependent responses of herbivores are documented with light commonly found to suppress herbivory by leaf-chewing insects (Roberts and Paul 2006). However, several studies also find the opposite that light may promote herbivory as a result of concurrent increases in foliar quality or reduced anti-herbivore defences (Roberts and Paul 2006 and references therein). In our study system, for example, stem mothers may choose to oviposit on upper shoots where nitrogen content may be double that in the lower canopy (Tarvainen et al. 2013). In addition, it could be that unshaded spruce trees in monocultures are nutritionally superior compared to spruce trees in mixed stands. However, recent studies exploring changes in host plant quality with diversity have been unable to link observed changes in host chemistry with species richness effects on primary consumers (Mraja et al. 2011, Moreira et al. 2014, Wäschke

et al. 2015). Thus, the role of canopy cover in driving patterns of associational resistance may not necessarily be mediated by changes in host plant foliar quality.

In the literature on insect responses to light, there are clear examples of direct herbivore responses to the environment outweighing higher host quality (Sipura and Tahvanainen 2000, Stoepler and Rehill 2012). For instance, work by Sipura and Tahvanainen (2000) demonstrated that, despite better quality foliage in shaded environments, leaf beetles performed better in open habitats where higher and more variable daily temperatures accelerate larval growth. A similar mechanism may occur here as reduced canopy cover in monocultures may result in higher temperatures (Morecroft et al. 1998, Chen et al. 1999) but also increase the variability of the understory microclimate (Rambo and North 2009, von Arx et al. 2012). At the same time, higher temperatures in unshaded monocultures may increase evaporative demand (Matejka et al. 2004), ensuring the delivery of water and nutrients to stem mothers and developing galls. Even if adelgid offspring are insensitive to the external environment within the gall, they may benefit from the higher transpiration rates in open, species-poor habitats where spruce trees are better nutritive sinks as compared to spruce trees in mixed, shaded stands. More generally, it seems that associational resistance to pineapple gall adelgids may be mediated by changes in canopy cover and microclimatic differences along the species-richness gradient.

Effects of host tree growth on pineapple galls

Previous work on pineapple gall adelgids has found that adelgids that successfully induce galls often perform better on faster growing modules or trees (McKinnon et al. 1999, Flaherty et al. 2010). Our results support the above conclusions and show that pineapple galls benefit from increased tree growth in accordance with the plant vigour hypothesis (Cornelissen et al. 2008). However, we found that associational resistance of spruce to adelgids was not due to changing spruce vigour as spruce growth was not affected by tree species richness. The only indication that tree species richness and spruce growth may interact was that, even though spruce tree size generally decreased with tree species richness, trees harbouring galls were consistent in size and significantly higher in DBH compared to uninfested trees in mixed plots (Fig. 3c). In contrast to previous studies (Cunningham et al. 2006), we found that spruce tree growth did not generally correlate with canopy cover but galled trees were larger in shaded plots (Fig 3). As galls were also more likely to be found on trees with large DBH, even those growing under high canopy

cover (Supporting Information Appendix 4), it appears that stem-mother preferences for larger trees may act to counter the negative effects of tree species richness. However, our findings also suggest that even if stem-mothers settle on small spruce trees in shaded, mixed stands, adelgids may still persist and accumulate (Supporting Information Appendix 4) possibly to avoid the risks associated with migration (Hopper 1999, Havill and Foottit 2007). Thus, although increased canopy cover in high species mixtures reduces the probability of infestation and resulting gall size, it does not guarantee immunity from or prevent the proliferation of adelgids on a given host tree.

Conclusions

Results from this study show that although tree growth may be an important determinant of adelgid success, changes in the light environment around spruce predict the presence and fecundity of pineapple gall stem-mothers better than tree species richness per se. Furthermore, tree species richness may have no direct effects on pineapple galls but the associated increase in the density of taller heterospecific neighbours may instead drive changes in pineapple gall presence and volume by modifying canopy cover. From an applied perspective, our findings indicate that mixed planting of spruce trees with faster-growing heterospecifics may offer an alternative and important strategy in adelgid pest management. This is important as few management options exist for gall-forming adelgids because insecticide sprays are ineffective on adelgid offspring sheltered within galls and adelgids also have few natural enemies that could offer sufficient biological control (Havill and Foottit 2007). From a theoretical viewpoint, our results suggest that canopy cover may be critical to understanding the mechanisms of associational effects in forest ecosystems. Our findings also indicate that future studies may benefit from considering herbivore presence separately from abundance to improve our understanding of plant-insect interactions with changing producer diversity. More generally, this study highlights the importance of examining both direct and indirect effects of plant diversity on consumers in order to develop a mechanistic understanding of diversity-functioning relationships.

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Table

Table 1. Factors affecting pineapple gall presence, abundance and volume on spruce. Separate models were run for each of the three gall response variables with either tree species richness, canopy cover, tree height or tree diameter at breast height (DBH) as explanatory variables. Area was included in models (not in interaction) with gall presence and abundance but is omitted here for clarity. Significant effects are shown in bold text.

Response variable		χ^2	df	p
Gall Presence	Richness	9.1	1	0.003
	Canopy Cover	21.7	1	<0.001
	Tree Height	4.5	1	0.034
	Tree DBH	8.5	1	0.004
Gall Abundance	Richness	0.40	1	0.527
	Canopy Cover	0.23	1	0.628
	Tree Height	5.17	1	0.023
	Tree DBH	0.67	1	0.413
Gall Volume	Richness	3.8	1	0.052
	Canopy Cover	8.7	1	0.003
	Tree Height	1.3	1	0.263
	Tree DBH	2.0	1	0.156

Figure legends

Figure 1. The pineapple gall (*Adelges abietis*) in spring (a) and, after dehiscence, the empty gall left-behind the following year (b).

Figure 2. Gall responses to tree species richness, canopy cover, spruce tree height and DBH. Changes in the probability of galls occurring on spruce, the abundance of galls on galled trees and, their volume are shown in panels a, b and c respectively. Smoothed means are shown in red for each plot.

Figure 3. Relationships between tree species richness, canopy cover, tree height and tree DBH. Smooth density estimates are drawn for each variable - (a) canopy cover, (b) tree height) and (c) tree DBH - in the first column. Their responses to tree species richness are shown in the second column, and relationships between canopy cover and tree height/DBH are plotted in the third column. In all cases, data are shown from spruce trees where galls were either absent or present. Overall effects (black, dashed line) are also shown in the second and third columns to illustrate the mean relationships across both galled and ungalled trees.

Figure 4. Structural equation models for effects of tree species richness (RICH), canopy cover (CAN COV) and tree size (HEIG=Height, DBH=DBH) on either (a) gall presence (GAL PRE) or (b) gall abundance (GAL ABU) and (c) volume (GAL VOL). Blue arrows indicate positive relationships and red arrows indicate negative relationships. Standardised path coefficients are indicated near the arrows and the thickness of arrows corresponds to the magnitude of these coefficients. Overall fit was evaluated using Shipley's test of d-separation: Fisher's C statistic (if $p > 0.05$, then no paths are missing and the model is a good fit) and the second-order Akaike's Information Criterion (AICc). Models for gall presence were a poor fit with tree height or DBH ($p > 0.05$) therefore these SEMs are illustrated in grey.

Figures

Figure 1

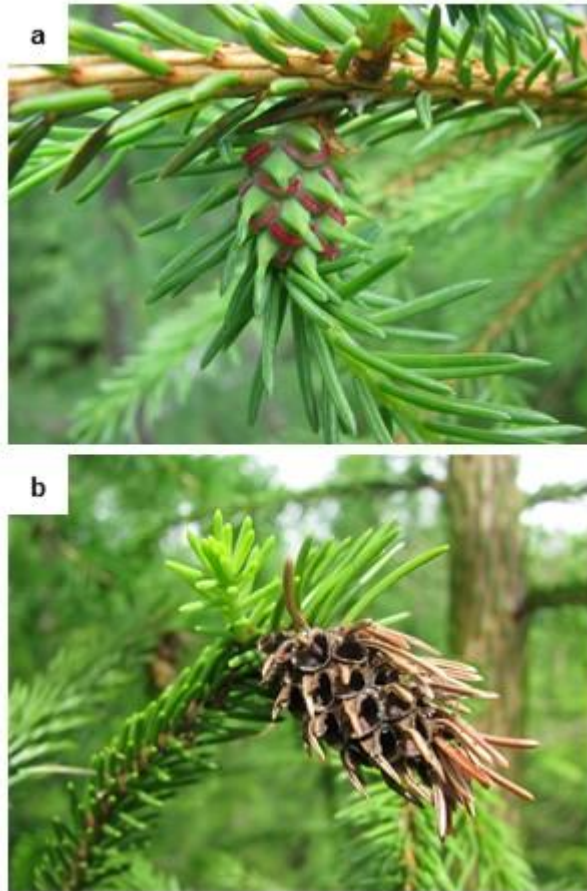


Figure 2

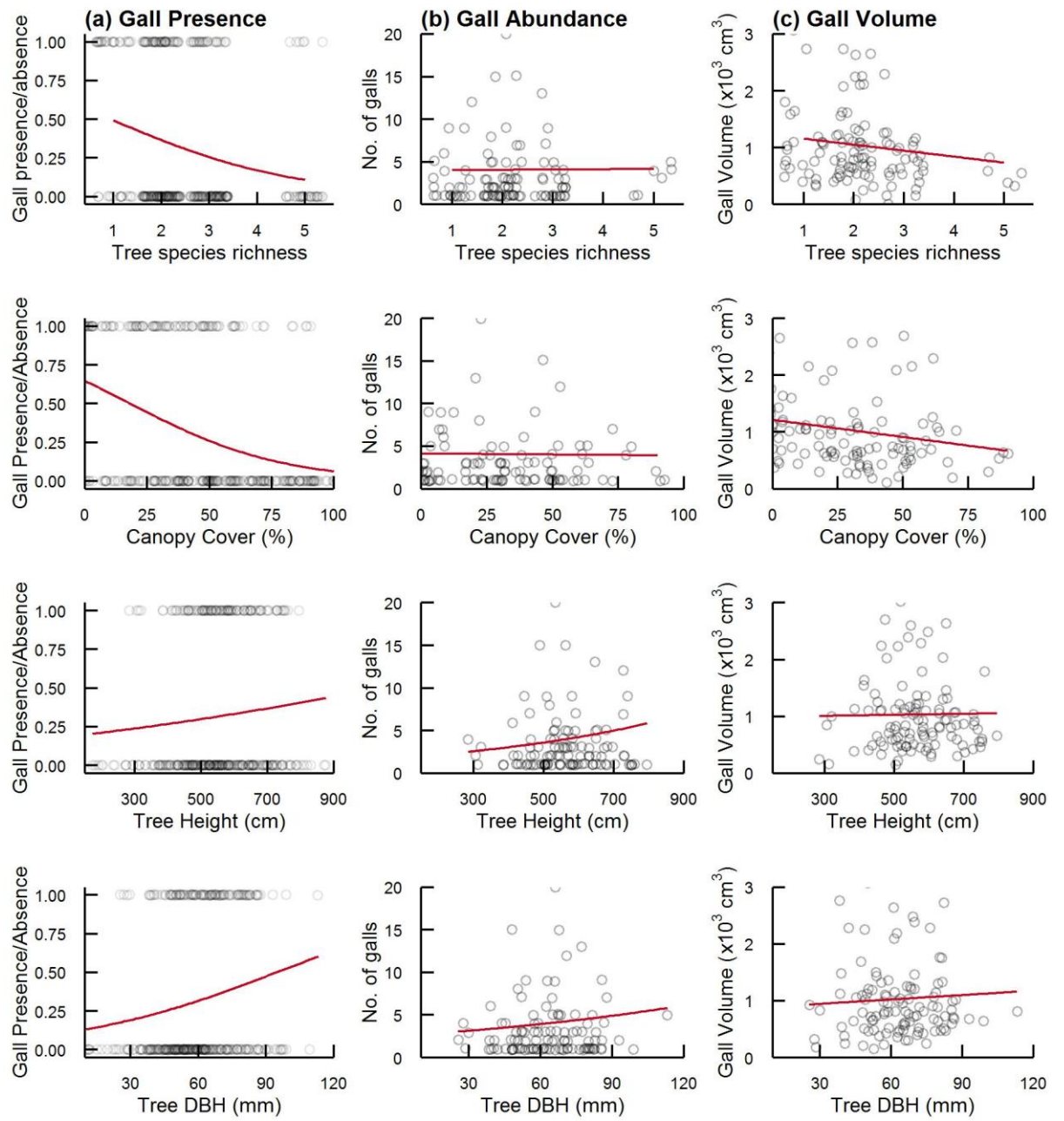


Figure 3

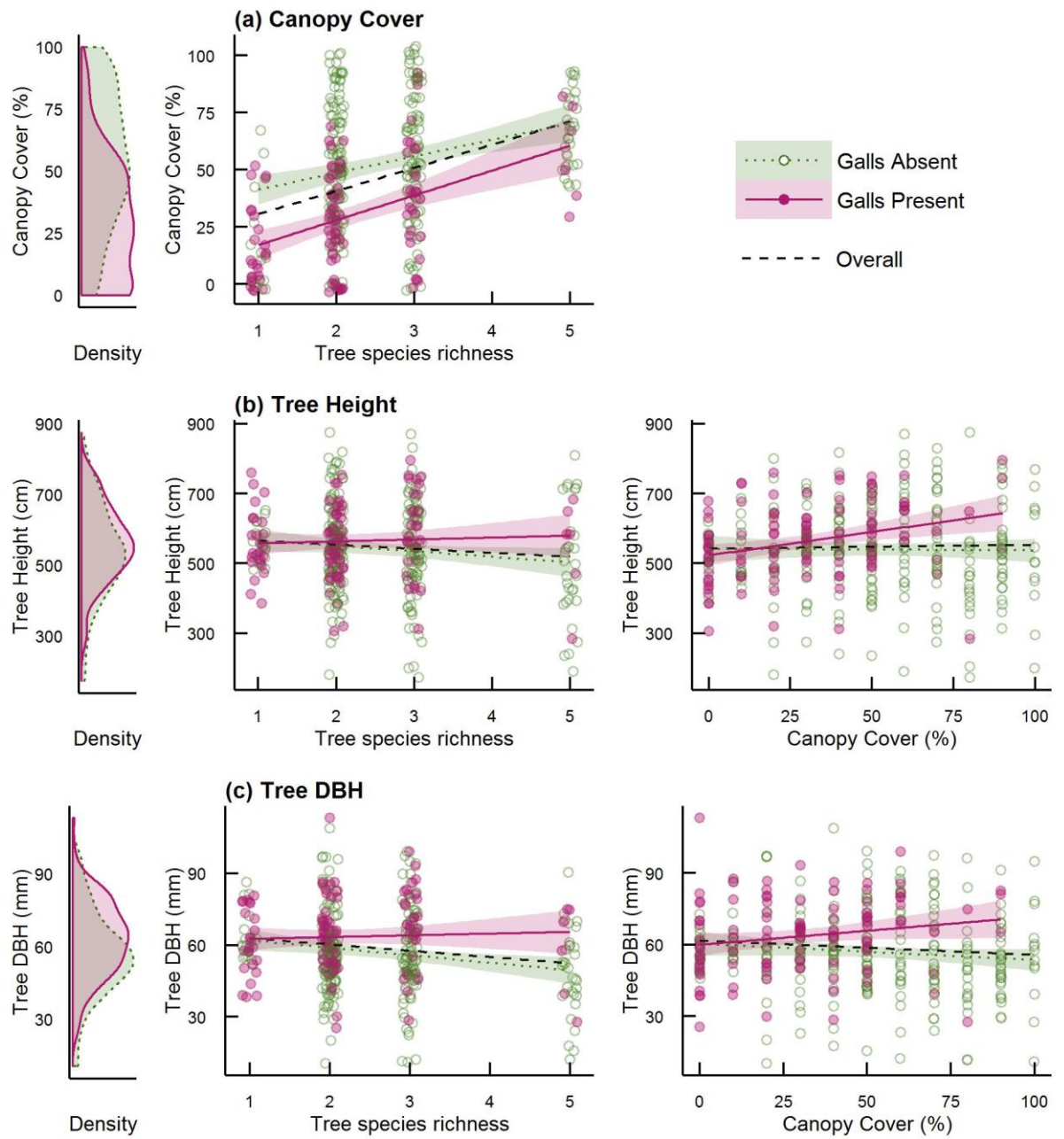
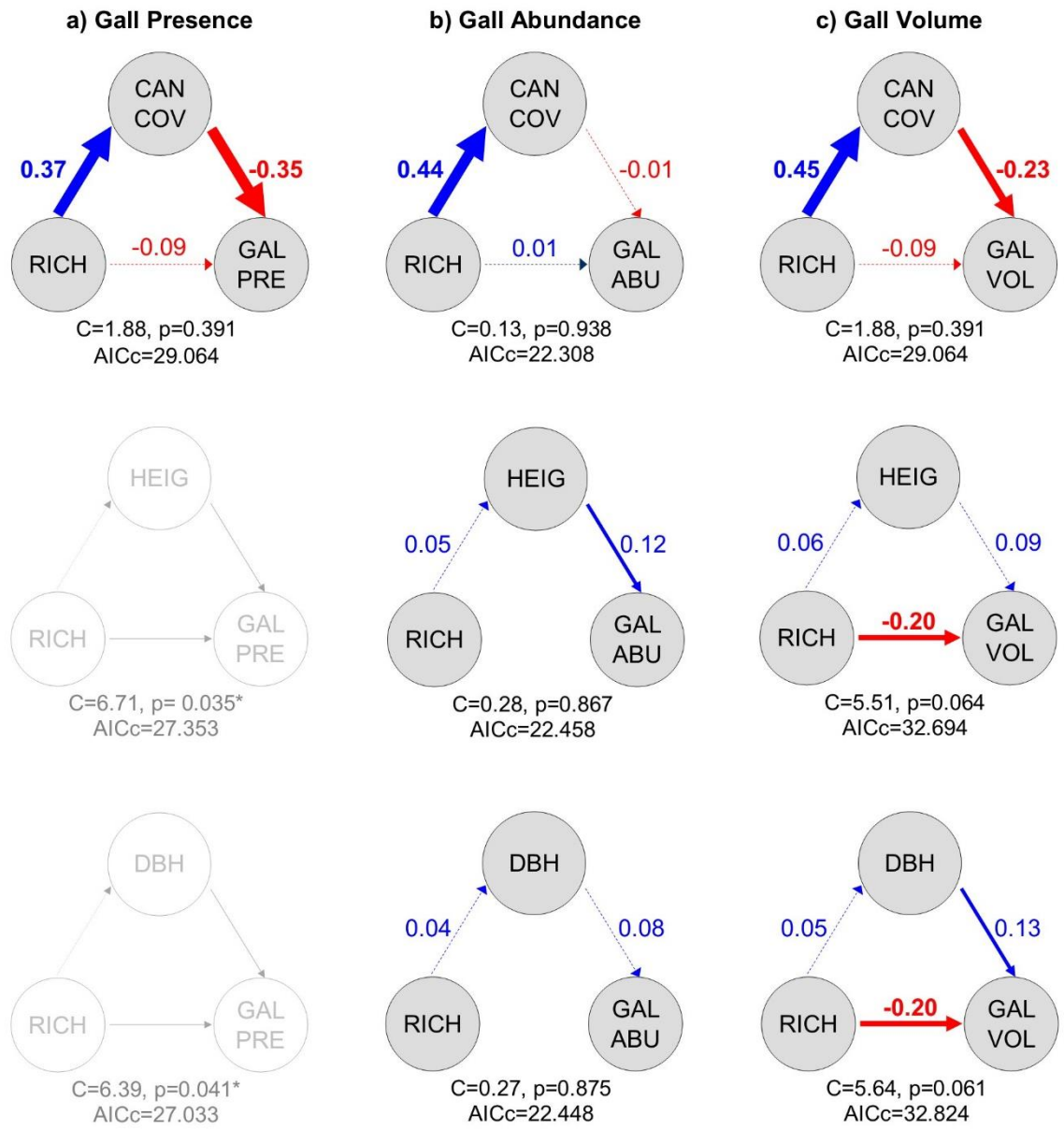


Figure 4



Supporting Information

Going undercover: increasing canopy cover around a host tree drives associational resistance to an insect pest

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Appendix 1 – Volumetric Relationship

The size of a gall positively correlated with the number of cavities within a gall (Fig. 5.3, $F_{(1,53)}=58.7$, $p<0.0001$) therefore, gall volume can be used as an indicator of fecundity and, therefore, high-performing mothers.

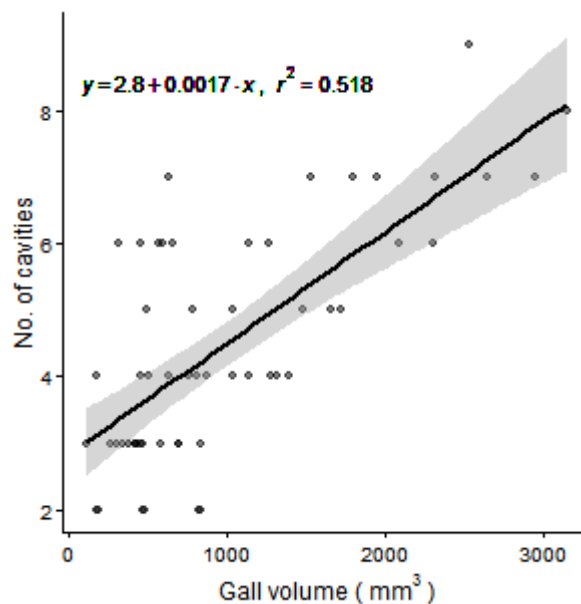


Figure A1. Relationship between gall volume and the number of cavities found in dissected galls. A smoothed mean line ($\pm 95\%$ CI) is shown as well as the fitted equation and r^2 from a linear model.

Appendix 2 – Initial analysis of all gall count data

Using all gall count data, we initially constructed Poisson GLMMs to assess the effects of tree species richness, canopy cover, tree height and DBH. However, all models were a poor fit to the data to an excess of zero values: of the 353 experimental trees, only 113 hosted galls on the sampled branches. We therefore constructed zero-altered Poisson (ZAP) and zero-inflated Poisson (ZIP) regression models using the *pscl* package in R (Zeileis et al. 2008) to account for the large number of zeros in these data. Both ZIP and ZAP models are two part models where the chance of getting a non-zero result is modelled with a binomial distribution, and then, count data are modelled separately. The main difference between them is in a ZIP model zeroes are included in the count model and in the ZAP they are not. Therefore, we are able to test effects of each predictor variable and also investigate whether the increased information in the count data (with ungalled trees included) altered gall responses.

Table A1. Results from zero-altered (ZAP) and zero-inflated poisson (ZIP) models examining effects of tree species richness, canopy cover, tree height and DBH on gall densities. Both model types report results from the binomial and count parts of the model separately. Significant effects are in bold text

	Binomial				Count				
	ZAP	Estimate	SE	z	p	Estimate	SE	z	p
Richness		-0.612	0.148	-4.14	<0.001	-0.064	0.056	-1.14	0.254
Canopy Cover		-0.850	0.146	-5.81	<0.001	0.043	0.064	0.67	0.503
Height		0.299	0.130	2.30	0.022	0.255	0.063	4.07	<0.001
DBH		0.483	0.133	3.64	<0.001	0.125	0.054	2.34	0.020
ZIP									
Richness		0.594	0.151	3.93	<0.001	-0.074	0.055	-1.33	0.184
Canopy Cover		0.891	0.156	5.70	<0.001	0.037	0.063	0.58	0.561
Height		-0.233	0.141	-1.66	0.098	0.244	0.063	3.87	<0.001
DBH		-0.489	0.145	-3.38	<0.001	0.112	0.054	2.08	0.038

NB: The binary part of the models exhibits opposite signs as ZAP models predict the probability of a non-zero response and ZIP models predict the probability of excess zeros.

Comparing ZIP and ZAP models, we found that the inclusion of zeros in the count part of ZIP models yielded similar results for all four predictor variables. In addition, results from ZIP/ZAP models are similar to those reported in the main text with gall abundance influenced by tree size, especially tree height, and gall presence affected by all four predictor variables. However, as these models did not allow for the inclusion of random factors, in the main text, we prefer to report results from separate analyses of gall presence and abundance in (generalized) mixed-effects models where “plot” is specified as a random factor.

Appendix 3 – Effects of study area and plot thinning

To determine whether observed effects might be confounded by area or thinning, we examined how the three gall response variables, canopy cover and spruce growth varied between the two study areas (area 1 and 3) and between thinned and unthinned plots.

Table A2. Effects of study area and plot thinning on the three gall responses, canopy cover and spruce growth.

	Area			Thinning		
	χ^2	df	p	χ^2	df	p
Canopy Cover	4.36	1	0.037	6.61	1	0.010
Tree Height	0.69	1	0.405	0.06	1	0.806
Tree DBH	0.05	1	0.821	0.02	1	0.888
Gall Presence	16.10	1	<0.001	1.42	1	0.234
Gall Abundance	17.29	1	<0.001	0.00	1	0.958
Gall Volume	0.00	1	0.973	0.00	1	0.995

Table A3. Results from models testing whether effects of tree species richness, canopy cover and tree growth on pineapple galls differ between the two study areas. Gall presence/absence was modelled with a binomial error structure, gall abundance with a Poisson error structure in GLMMs and gall volume was log transformed to meet assumptions of homogeneity of variance. (df=1 in all cases)

	<i>Area*</i> Richness		Canopy cover		Tree Height		Tree DBH	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Gall Presence	0.91	0.340	0.02	0.889	1.89	0.169	2.35	0.125
Gall Abundance	0.01	0.913	0.24	0.623	0.81	0.367	0.02	0.877
Gall Volume	0.96	0.328	2.23	0.135	1.95	0.162	1.3	0.258

Canopy cover was reduced by plot thinning but thinning had no effect on any other variable (Table A2). As galls were more likely to occur and were more abundant in area 3 as compared to area 1, we performed further analysis to test whether effects of study area might interact with any of the other variables. However, as none of the interactions with area were found to be significant (Table A3), all subsequent analyses were performed with data pooled across thinned and unthinned plots and, across both study areas.

Appendix 4 – Interactive effects of canopy cover and tree size on pineapple galls

We ran models to determine whether effects of canopy cover were dependent on changes in tree height or DBH finding that negative effects of canopy cover on gall presence and abundance are dependent on spruce tree size.

Table A4. Results from models testing the interactive effects of canopy cover and either tree height or diameter at breast height (DBH) on each gall response. Significant effects are in bold text

<i>Canopy Cover*</i>	Height			DBH		
	χ^2	df	p	χ^2	df	p
Gall Presence	1.66	1	0.198	3.93	1	0.048
Gall Abundance	0.16	1	0.690	5.95	1	0.015
Gall Volume	1.01	1	0.315	2.11	1	0.147

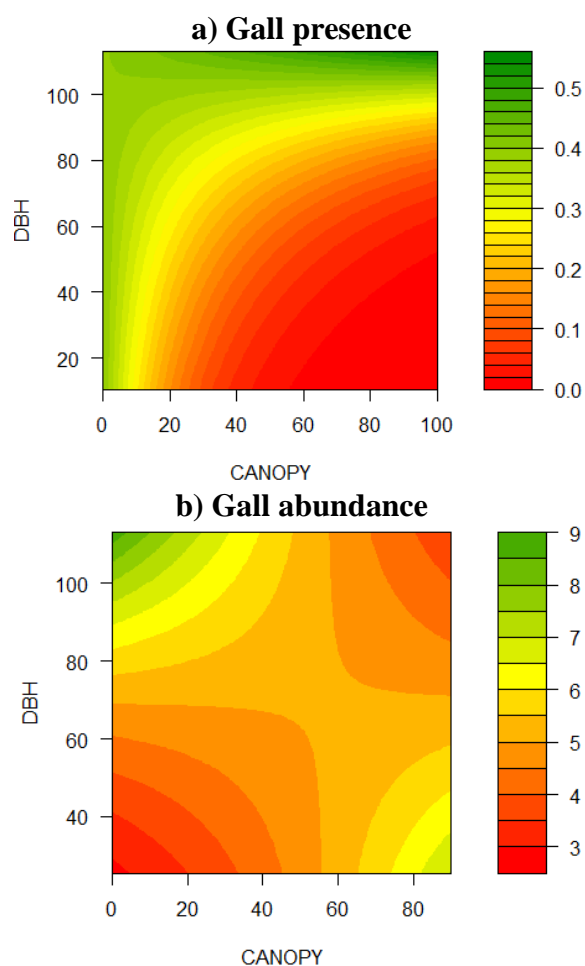


Figure A2. Interactive effects of canopy cover and tree size on a) gall presence and b) gall abundance. Colour scale represents the increased predicted proportion of galled trees (a) or increased number of galls per tree (b) along crossed gradients of canopy cover (CANOPY, %) and diameter at breast height (DBH, mm).

Appendix 5 – The size of galled “mother” shoots and ungalled “neighbour” shoots

Spruce shoots infested with galls were consistently larger than ungalled shoots. Mother shoots averaged 181.8mm (± 13.7) in length and 4.8mm (± 0.3) in diameter while neighbouring ungalled shoots were 127.2mm (± 9.2) long and 3.3mm (± 0.2) in diameter. The size of galled shoots was positively related to that of neighbouring ungalled shoots (shoot length: $\chi^2=40.8$, $df=1$, $p<0.001$; diameter: $\chi^2=46.1$, $df=1$, $p<0.001$). In addition, as the height of trees increased, both galled and ungalled shoots decreased in size but tree DBH had no effect on shoot size (Table A5). Similarly, neither tree species nor canopy cover had any effect on shoot size (Table A5).

Table A5. Results from models examining the factors influencing the length and diameter of galled “mother” and ungalled “neighbour” shoots. Significant effects are in bold text

	Shoot type: Mother			Neighbour		
	χ^2	df	p	χ^2	df	p
<i>Shoot length</i>						
Tree species richness	2.00	1	0.158	0.24	1	0.624
Tree Height	3.30	1	0.069	7.13	1	0.008
Tree DBH	1.29	1	0.257	3.50	1	0.061
Canopy Cover	0.00	1	0.951	0.10	1	0.755
<i>Shoot Diameter</i>						
Tree species richness	1.48	1	0.224	0	1	0.996
Tree Height	5.56	1	0.018	5.66	1	0.017
Tree DBH	2.80	1	0.094	2.58	1	0.108
Canopy Cover	1.19	1	0.276	1.19	1	0.276

Chapter 4

Forest diversity effects on insect herbivores: do leaf traits matter?

Manuscript to be submitted to the *Journal of Ecology*

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Forest diversity effects on insect herbivores: do leaf traits matter?

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Running headline: Leaf traits underpin associational resistance

Summary

1. Increasing plant diversity is frequently observed to reduce insect herbivore damage and abundance (associational resistance). Although there is evidence to suggest that plant-plant interactions can alter foliar quality and anti-herbivore defences within a focal plant, few studies have explored whether changes in producer diversity can influence host plant traits and, in turn, have cascading effects on herbivores.
2. Here, we used a long-term forest diversity experiment in south-west Finland to explore whether tree diversity effects on the physical and chemical leaf traits of silver birch (*Betula pendula*) drive associational resistance. Leaf damage by chewing insect herbivores and the abundance of galls, miners and rollers were measured on birch trees growing in 1-, 2-, 3-, and 5-species mixtures. Sixteen physical and chemical leaf traits were measured on the same focal trees and local canopy cover assessed.
3. Although herbivory consistently decreased with tree species richness and most herbivore types were influenced by leaf traits, none of the traits significantly varied with tree species richness. Associational resistance was instead mediated by changes in individual leaf traits – leaf area and SLA – in response to either host dilution or reduced canopy cover with tree species richness.
4. Structural equation modelling revealed that host dilution with increasing tree species richness resulted in smaller birch leaves which led to reduced chewing damage and roller abundance. Concurrent declines in canopy cover with host dilution also reduced SLA, leading to reduced gall abundance across the diversity gradient. However, while these trait-mediated effects were important, effects of tree species richness could also operate independently of foliar quality for chewing herbivores. In addition, leaf miner abundance was primarily driven by host dilution rather than leaf traits, consistent with the “resource concentration hypothesis”.
5. *Synthesis.* Our results show that leaf trait variation might promote associational resistance but these patterns are determined by host dilution and reduced canopy cover rather than tree species richness per se. Therefore, accounting for concurrent changes in stand structure and key foliar traits is important for the interpretation of diversity effects and predictions of associational patterns.

Keywords

Biodiversity and ecosystem functioning, boreal forest, plant-herbivore interactions, Satakunta forest diversity experiment, trait-mediated effects

Introduction

Neighbouring plants are frequently observed to decrease the vulnerability of a focal plant to herbivore attack (Jactel & Brockerhoff 2007; Barbosa *et al.* 2009). This pattern of associational resistance has largely been attributed either to an increase in the effectiveness of natural enemies or a reduced concentration of resources for specialist herbivores in more diverse stands (Root 1973). In particular, the “resource concentration hypothesis” has received a great deal of interest in the literature as the strength of associational effects have been shown to depend on both the frequency and density of host plants (Root 1973; Letourneau 1987; Tonhasca 1993; Riihimäki *et al.* 2005; Underwood, Inouye & Hambäck 2014). However, while the resource concentration hypothesis is well supported, little work has been done to compare effects of host concentrations to changes in host plants triggered by their neighbours. Thus, a mechanistic understanding of associational effects is limited due to a focus on external influences without accounting for changes intrinsic to the focal plant.

Much of the research into associational effects has instead concentrated on the traits of neighbouring plants and how they influence herbivory on a focal plant. For instance, studies have shown that the presence of well-defended plants may repel herbivores from a stand (Wahl & Hay 1995; Hjältén & Price 1997). Alternatively, the presence of more palatable species nearby may either draw herbivores to focal plants (White & Whitham 2000) or lure them away from less palatable hosts (Tahvanainen & Root 1972). The roles of repellent and attractant neighbour plants have been studied extensively in the literature (Atsatt & O’Dowd 1976; Ruttan & Lortie 2014) but more recent studies have also explored the effects of nearby heterospecifics on focal plant traits. Neighbouring plants have been shown to influence host plant quality by modifying soil nutrient availability (van Ruijven & Berendse 2005; Ayres *et al.* 2007), altering the composition of insect-repelling volatiles (Himanen *et al.* 2010) or the levels of defence-related secondary metabolites in the host plant (Baier *et al.* 2002; Barton & Bowers 2006; Broz *et al.* 2010). In addition to the presence of heterospecific neighbours, plants are also sensitive to the presence and density of conspecifics (Callaway 2002; Biedrzycki & Bais 2010) and can modulate their anti-herbivore defences accordingly (Karban & Shiojiri 2009). As herbivores may be influenced by both plant chemical (Feeny 1970; Forkner, Marquis & Lill 2004) and physical traits (Ayres & Maclean 1987; Pérez-Harguindeguy *et al.* 2003; Pearse 2011; Haase *et al.* 2015), changes in host plant properties across diversity gradients may therefore underpin associational effects.

Several recent studies have shown increases in anti-herbivore defences in mixed stands, but failed to demonstrate the link between these changes and herbivore abundance or damage (Mraja *et al.* 2011; Moreira *et al.* 2014; Wäschke *et al.* 2015). This may be due to the focus on single feeding guilds and defensive chemistry over other indicators of foliar nutritional quality. A meta-analysis by Carmona *et al.* (2011) showed that plant susceptibility to herbivores was largely dictated by gross plant morphology and physical resistance traits rather than host plant chemistry and the effects depended on herbivore specialisms. As studies have shown that associational resistance patterns are more consistent for monophagous or oligophagous insects but not for polyphagous insect species (Andow 1991; Kaitaniemi *et al.* 2007; Jactel & Brockerhoff 2007), similar considerations of dietary breadth are also important in understanding associational effects. Carmona *et al.* (2011) has also demonstrated variable effects of traits on different herbivore feeding guilds. Studies are therefore needed that consider a broader range of host plant properties and encompass a variety of herbivores in order to clarify trait-mediated mechanisms of associational resistance.

Finally, as neighbour effects on host plant traits may be driven by environmental changes, studies would also benefit from examining abiotic factors that vary with habitat complexity. This is particularly important in forest ecosystems where interactions between woody species can yield structurally heterogeneous environments with potentially long-term consequences for host trees and their susceptibility to insect pests. For instance, differential growth rates between tree species or different stand species compositions (Piotto 2008; Muiruri *et al.* 2015) result in variable canopy structure and light availability in the understorey (Lang *et al.* 2011). As reviewed by Roberts and Paul (2006), such changes in canopy structure and light may in turn modify foliar quality and anti-herbivore defences. Thus, measured host plant traits may not only reflect changes in forest structure and the type of neighbouring species but also mirror both abiotic and biotic changes along the diversity gradient. Despite these important effects, environmental factors have not been implicated in trait-mediated mechanisms in forest ecosystems (Moreira *et al.* 2014). Thus, more work is needed that explores the role of host plant traits in driving associational effects in complex forest ecosystems.

In this study, we aimed to test the hypothesis that changes in host plant traits drive insect herbivore abundance and damage on silver birch (*Betula pendula*) trees with increasing

tree species diversity and associated dilution by heterospecific neighbours. We assessed insect chewing damage and the abundance of three different feeding guilds (leaf galls, miners and rollers) on 16-year old birch trees growing in monocultures and, 2-, 3- and 5-species mixtures in the Satakunta forest diversity experiment, south-west Finland. We also measured a comprehensive list of morphological and chemical traits known to influence insect herbivores with the aim of identifying which traits vary with tree species richness and therefore mediate associational resistance to the four insect guilds. While chewing insects and leaf rollers may be both generalists and birch-specialists, gall-makers and the majority of miner species are host-specific organisms with poor dispersal ability. As endophagous herbivores, both gallers and miners have an intimate relationship with their host and may therefore be more sensitive to changes in host density and quality in comparison to leaf chewers and rollers that can relocate to better foliage (Thompson & Pellmyr 1991; Skoracka 2006). Thus, we used structural equation models to determine the relative importance of direct and indirect pathways between tree species richness and herbivory, comparing responses between feeding guilds.

Materials and Methods

Experimental design

This study was conducted in the Satakunta forest diversity experiment in south-western Finland (www.sataforestdiversity.org). The experiment was planted in 1999 and is made up of three separate areas (area 1, 61°42'N, 21°58'E; area 2, 61°39'N, 22°09'E; area 3, 61°40'N, 21°42'E) planted with five tree species: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.), Siberian larch (*Larix sibirica* Ledeb.), silver birch (*Betula pendula* Roth.) and black alder (*Alnus glutinosa* L.). Each of the three areas consists of 38 plots (20 x 20m), randomly allocated to 19 treatments representing a gradient from monocultures to 2-, 3- and 5-species mixtures. Trees were planted in 13 rows at 1.5m intervals and each species was randomly allocated a position. In 2000 and 2001, dead seedlings were re-planted in plots where mortality exceeded 10% to ensure establishment of trees in the experiment. No chemical inputs have been used in the experiment but plots have been cleared of naturally regenerating vegetation in 2010 to maintain plot treatment and species densities. In June 2013, half of the experimental plots in each area were thinned so that species proportions in mixtures remained constant (i.e. 50:50 in 2-species, 33:33:33 in 3-species and 20:20:20:20:20 in 5-species mixtures) but overall tree density was halved.

Five birch trees were randomly selected in 2014 from the plot interior of each birch-containing treatment: the birch monoculture, three different 2-species mixtures (birch + alder, birch + pine, birch + spruce), four different 3-species mixtures (birch + alder + larch, birch + alder + pine, birch + larch + pine, birch + pine + spruce) and the 5-species mixture (birch + alder + larch + pine + spruce). One thinned and one unthinned replicate of each treatment are available per area.

Insect herbivore assessment

Insect herbivore damage and the abundance of leaf galls, miners and rollers (Fig. 3) was assessed on all experimental trees. We sampled insect herbivores during the early (early June) and late summer (late July-early August) to capture changes in insect herbivores at different times during the season. A total of 200 leaves were sampled from four randomly selected branches in the lower- to mid-canopy of each experimental birch tree and four categories of herbivory/herbivore abundance were identified: (1) chewing damage, (2) gall abundance, (3) leaf miner abundance and (4) leaf roller abundance.

For each examined leaf, insect chewing damage was scored in situ as follows: (i) 0.1–5% of leaf area damaged, (ii) 6–25% of leaf area damaged, (iii) 26–50% of leaf area damaged, (iv) 51–75% of leaf area damaged, or (v) more than 75% of leaf area damaged. Percentage leaf area damage was first calculated per branch by multiplying the midpoint of each category by the number of defoliated leaves, summing the values and dividing by 50. Averages across branches were then calculated to obtain an estimate of percentage chewing damage per tree. Leaf chewing insects observed during monitoring were sawfly or lepidopteran larvae and are considered to be likely culprits for observed chewing damage as birch trees have been shown to support a species-rich community of the same herbivores (Hanhimäki 1989; Atkinson 1992).

Abundance estimates for the remaining feeding guilds were conducted by counting the number of leaves with galls, mines or leaf rolls (Fig. b-d) out of the 200 leaves sampled. Leaf galls were caused by two species of gall mites (Acarina: Eriophyidae): *Acalitus rudis* (Canestrini) and *Aceria leionotus* (Nalepa), leaf mines by different species of Lepidoptera, Hymenoptera, Coleopteran or Diptera, and leaf rolls by weevils, moths and sawflies (Nyman 2007). While leaf rolling herbivores are all generalists, gall mites and the majority of leaf miners are birch specialists.

Canopy cover assessment

Canopy cover above the focal trees was assessed for a subset of trees in unthinned plots only, sampling 68 of the 125 experimental trees. Canopy cover was estimated with the GRS densitometer™ (Geographic Resource Solutions, Arcata, CA, USA) by recording the percentage of views that were obstructed by canopy at 10 evenly spaced positions around the crown edge of each birch tree (to exclude self-shading). Similar visual estimates have been used to assess light availability in forest ecosystems with evidence that canopy cover assessments are well correlated with light intensity (Lhotka & Loewenstein 2006; Pannek, Ewald & Diekmann 2013).

Leaf trait measurements

Leaf physical and chemical traits were measured on fully expanded undamaged short shoot leaves in early summer on the same trees used to assess insect herbivory. Leaf collection was stratified according to the height and aspect of each tree, with four branches sampled, two from the lower canopy and two from the mid-canopy and leaves within these strata chosen at random. For the determination of leaf thickness and toughness, one undamaged leaf per branch was sampled and four measurements were made per leaf. Thickness was measured in mm using a digital micrometer accurate to 4 decimal places. Toughness was measured using a Mitutoyo dial tension gauge with a 0.3mm needle. To assess leaf area, specific leaf area (SLA) and leaf dry matter content (LDMC), five additional undamaged leaves were sampled from each experimental birch tree. Leaf area was calculated by photographing the leaves against a scale and using Image J software (Abràmoff, Magalhães & Ram 2004). Sampled leaves were dried for 24 hours at 60°C and SLA was calculated by dividing the surface area (cm²) by the dry leaf weight (g). LDMC was then computed as the ratio of leaf dry weight to fresh weight and water content (%) was calculated from the difference in fresh leaf weight from the dry leaf weight divided by the dry weight.

For the laboratory assessment of leaf traits, 120g of fully expanded, undamaged birch leaves (approximately 100 birch leaves) were collected in June 2014. Petioles were removed at collection and samples transported from the field in cool bags and subsequently freeze dried. All samples of leaf material were divided into two portions one of which was ball-milled to a fine powder (Retsch UK Ltd, Hope Valley) and the other milled to pass a 1mm screen of a Glen Creston mill (Glen Creston, London).

All ball-milled samples were analysed for total carbon and nitrogen concentration using an elemental analyser (Thermo Finnigan, FlashEA 1112 Series) and the carbon-nitrogen ratio (C:N) subsequently calculated. In order to quantify acid detergent fibre (ADF), lignin, condensed tannins and protein precipitating tannins in the leaves and needles, the overall approach adopted was to analyse a subset of samples using standard wet-chemistry methods and produce a predictive calibration using near infrared spectroscopy (Foley *et al.* 1998), which was applied to the remaining samples to obtain estimates for these chemical constituents. For this method, all ball-milled samples of birch leaves were scanned in reflectance mode in the range between 1100-2500 nm, at 2 nm intervals, using a FOSS NIRSystems 5000 monochromator (FOSS, Hoganas, Sweden), with a ring cup sampling cell and a transport module attachment, in a constant laboratory environment (average temperature: 23°C; humidity < 15%).

The resulting near infrared spectra from each sample were reduced to principal component scores and population structuring algorithms were applied to select the most representative samples to use as calibration and validation sets (Shenk and Westerhaus 1991, Supporting Information, Appendix 1). Calibration and validation samples were subsequently analysed for ADF, lignin, condensed tannins and protein precipitation. Analysis of ADF, which incorporates the cellulose, lignin and lignified-nitrogen contents of plant cell-wall material (Van Soest 1982), and sequential digestion of the residue to determine lignin content, were carried out according to the methods of Van Soest (1963), on samples milled to pass a 1mm screen. Phenolic compounds were extracted from the ball-milled samples by three sequential extracts of 30mg in 3ml of 80% methanol, pooling the supernatants following centrifugation. Condensed tannins were analysed by the Butanol-HCl method for proanthocyanidins (Porter, Hrstich & Chan 1986), and their protein precipitating capacity was quantified using the radial diffusion assay with 50% methanol as the assay solvent (Hagerman 1987). Both assays were standardised using condensed tannin extracted from a bulk sample of birch leaves collected at Torphins, Aberdeenshire, UK, and purified using Sephadex LH20 (Hagerman and Butler 1980; modified according to Hagerman 2011).

A suite of calibrations were performed for each trait correlating near infrared absorbance and wet chemistry values. Different types of correction treatments were applied in each to enhance weak signals and remove baseline effects on the spectra (Geladi *et al.* 1985, Barnes *et al.* 1989, Supporting Information, Appendix 1). Once optimised, the best

calibration equation was then applied against validation samples and the predicted near infrared spectra compared to the actual spectra. Further details of this analysis are reported in the Supporting Information, Appendix 1 with results indicating good calibration of all four variables ($R^2 > 0.6$) but more effective estimation of ADF and condensed tannins ($R^2 > 0.6$) compared to lignin ($R^2 = 0.529$) or protein precipitation ($R^2 = 0.297$).

For the assessment of total phenolics and oxidative capacity, freeze-dried fine powder of each sample ($20 \text{ mg} \pm 0.5 \text{ mg}$) was weighed into new 2 ml microcentrifuge tube. 1.4 ml of acetone/water (80:20 V/V) was added to the tube and samples were vortexed for 5 min and macerated at 4°C overnight. Each tube was placed on a planary shaker for 3 hours (280 rotations/min), followed by centrifugation for 10 minutes. The supernatant was transferred to a new microcentrifuge tube and acetone was removed in an Eppendorf concentrator (5301, Eppendorf AG). The plant pellet was then re-extracted with 1.4 ml of acetone/water solution (80:20, V/V), the supernatants were combined and acetone removed once more. Aqueous samples were frozen at -20°C and lyophilized. The freeze-dried phenolic extract was re-suspended in 1 ml of Milli-Q purified water, vortexed for 5 min, and centrifuged for 10 min. The supernatant was pipetted and placed into a new 1.5 ml microcentrifuge tube. Measurements of total phenolics and oxidative capacity were carried out with a 96 well plate reader using the protocol outlined by (Salminen & Karonen 2011). Gallic acid was used as the quantitation standard. The percentage of easily oxidized phenolics (% easily oxidized) was calculated by dividing the amount of easily oxidized phenolics by the total phenolic content and multiplying by 100.

Statistical analysis

Preliminary analyses showed that although insect herbivore responses (chewing damage, gall abundance, miner abundance and roller abundance) differed between seasons, effects of tree species richness and leaf traits were identical for herbivory in both the early and late season. Consequently, further analyses was performed on mean herbivory across both seasons. Mixed effects models were used to determine whether effects of diversity on herbivores differed between the three experimental areas and the two thinning treatments. Although tree species richness effects on galls were stronger in thinned plots, tree species richness effects on all other herbivores guilds were consistent between study areas and thinning treatments (Supporting Information, Appendix 2). Separate analyses of leaf galls

in thinned and unthinned treatments showed very similar results, therefore, we present analysis and results from data pooled across all areas and thinning treatments. Finally, for all herbivore guilds, we also examined effects of tree species composition and host dilution where host dilution was calculated as the inverse of birch tree density after accounting for mortality. However, as we found no significant effects of composition on any of the four herbivore responses, we focus here on the effects of tree species richness and host dilution only.

To identify trait-mediated effects of diversity on birch insect herbivores, we first tried to minimise redundancy between the 16 measured traits (leaf area, SLA, thickness, toughness, LDMC, water content, lignin, ADF, carbon content, nitrogen content, C:N, condensed tannins, PPT, total phenolic content, easily oxidized phenolics and the percentage of easily oxidized phenolics). We conducted principle component analysis to identify traits that scaled closely with one another and used correlation matrices to detect strong relationships between them ($r > 0.8$, Supporting Information, Appendix 3). Collinear pairs of traits included water content and LDMC ($r = -1$), nitrogen and C:N ($r = -0.95$), and easily oxidized phenolics with either total phenolic content ($r = 0.81$) or condensed tannin content ($r = 0.87$). In all cases, we retained the most comprehensive trait that summarises information on multiple leaf properties and discarded the others. Therefore, we retained LDMC instead of water content, C:N rather than nitrogen content, and total phenolic content rather than either condensed tannin content or easily oxidized phenolic content. With the remaining 12 traits, we used linear mixed-effects models (LMMs) to determine their individual responses to either tree species richness or host dilution, where host dilution is the inverse proportion of birch trees in a plot. Plot was specified as the random factor of the linear-mixed effects model. To satisfy assumptions of normality, SLA, LDMC, Lignin, ADF, Carbon, Phenolics and % Easily Oxidized were all log-transformed and Protein Precipitation was square root transformed.

Similar univariate LMMs were also used to determine the effects of tree species richness and host dilution on insect chewing damage and gall, miner or roller abundance. We also used univariate LMMs to determine effects of individual traits and their relative importance compared to tree species richness or host dilution on the basis of model AICc (second-order Akaike's Information Criterion) and Akaike weights. The AICc value is used to compare models with the same response variable but different explanatory variables and Akaike weights indicate the support for a given model compared to all other

candidate models (Anderson *et al.* 2001; Burnham & Anderson 2004). Models with lower AICc values were considered to be better than other candidate models but could only be termed the single best model if the Akaike weight (w_{ic}) exceeded 0.9 (Anderson *et al.* 2001). Where Akaike weights did not exceed this value, differences in the AICc were used as an indicator of the relative likelihood of the model. Candidate models differing least from the best model ($\Delta AICc \leq 2$) are considered to be well supported but those differing most ($\Delta AICc \geq 10$) can be omitted (Burnham & Anderson 2004). Miner abundance was square root transformed and the remaining three herbivore responses log-transformed to meet assumptions of normality. Plot was specified as a random factor in all models to account for variation between plot replicates. In order to improve the interpretability of all models, we centred all continuous variables before inclusion in models by subtracting the sample mean from all observations) and scaled them (by dividing the input variables by their sample standard deviation) before including them in any model (Schielzeth 2010).

To explore the role of canopy cover in modifying traits and driving associational resistance, we repeated the above models with data from the subset of trees for which canopy cover was measured. For each of the 12 traits and 4 herbivore response variables, we tested effects of canopy cover in univariate models. We also tested how canopy cover varied with tree species richness and host dilution in separate generalised mixed-effects models where canopy cover was modelled as a binary response variable (percentage canopy / percentage sky) bounded between 0 and 100 with plot specified as a random factor.

All statistical tests were conducted in R software v.3.2.3 (R Core Team 2015) using the lme4 package (Bates, Maechler & Bolker 2012) for mixed-effects models. Model residuals were examined for homogeneity of variance and we report AICc and Akaike weights (w_{jc}) from the MuMIn package (Barton 2015) as well as chi-squared and corresponding p values from ANOVA using the car package (Fox & Weisberg 2011). For the presentation of results, we computed regression coefficients ($\pm 95\%$ CI) from linear models without the inclusion of the random factor, to examine the effect of each explanatory variable on a given response.

In a final step, we used Piecewise Structural Equation Models (piecewise SEM) to assess the relative importance of tree species richness and host dilution on insect herbivore

responses and determine whether these effects are mediated through changes in the top selected trait (identified from model comparisons). The “piecewiseSEM” package in R (available at <https://github.com/jslefcche/piecewiseSEM>) is unlike traditional SEMs in that it permits the inclusion of hierarchical data by piecing multiple mixed-effects models into one causal framework (Lefcheck 2015). Here, we combined component models into one causal network for each herbivore response and assessed the overall fit of the piecewise SEM using Shipley’s test of direct separation that determines the probability of an informative path missing from the hypothesised network (Shipley 2009). Models were rejected if a chi-squared test of Fisher’s C statistic falls below the significance level ($p < 0.05$) indicating that models are inconsistent with the data. As we were only interested in the primary pathways linking tree species richness to each herbivore response, we present here accepted models which were simplified, where possible, by removing pathways with small standardised coefficients (< 0.1). As canopy cover was also found to influence the same traits that were important for gall and miner abundance, we constructed SEMs using the 68 trees where canopy cover, leaf traits and herbivory were all measured. These models better illustrated trait-mediated effects on galls and miners (all path coefficients > 0.1) so previous models on the full dataset ignoring canopy cover are only reported in the Supporting Information, Appendix 5.

Results

Tree diversity effects on birch insect herbivores and leaf traits

Insect herbivore damage and abundance were generally reduced by stand richness and host dilution (Fig. 1). Chewing damage and leaf miner abundance decreased significantly with tree species richness and host dilution (Table 1, Fig. 1a, e) whereas only marginal effects were detected for gall abundance (richness: $\chi^2 = 3.05$, $df = 1$, $p = 0.081$, dilution: $\chi^2 = 3.37$, $df = 1$, $p = 0.066$, Fig. 1c) and neither tree species richness nor host dilution effects were significant for leaf roller abundance (richness: $\chi^2 = 0.97$, $df = 1$, $p = 0.324$, dilution: $\chi^2 = 0.52$, $df = 1$, $p = 0.469$, Fig. 1g). In contrast to insect herbivore responses, most birch leaf traits were unaffected by tree species richness or host dilution (Supporting Information, Appendix 4). Only ADF marginally decreased with tree species richness ($\chi^2 = 3.31$, $df = 1$, $p = 0.069$) and leaf area significantly decreased with host dilution ($\chi^2 = 4.22$, $df = 1$, $p = 0.040$).

Relative effects of traits and diversity on insects

Of the measured traits in this study, only leaf area, leaf thickness, and C:N had significant effects on insect herbivore damage. Percentage chewing damage was positively related to leaf area and leaf thickness but decreased with C:N (Table 1, Fig. 1a). Model comparison with AICc demonstrated that leaf area was the best predictor of insect chewing damage as this model had the lowest AICc value ($\Delta\text{AICc}>10$) and a high weighting ($w_{ic}>0.9$, Table 1, Fig. 1b).

Gall abundance was also influenced by a number of birch leaf traits with positive effects of SLA and leaf area and negative effects of C:N, LDMC and protein precipitation (Fig 1c). Nonetheless, despite clear effects of several traits, SLA emerged as the best determinant of gall abundance in model selection ($\Delta\text{AICc}>2$, Table 1, Fig. 1d).

In contrast to insect herbivore damage and gall abundance, leaf traits were not important determinants of leaf miner abundance (Table 1, Fig. 1e). Total phenolic content was the top ranked trait in model comparisons with a positive but marginal effect on miner abundance (Fig. 1e, $\chi^2=3.71$, $df=1$, $p=0.054$).

Finally, while tree species richness had no significant effect on leaf roller abundance, the number of rollers on birch increased with leaf area, total phenolic content and protein precipitation by tannins. Leaf area emerged as the top-selected trait explaining roller abundance ($\Delta\text{AICc}>10$, $w_{ic}>0.9$, Table 1).

The role of canopy cover

As birch leaf traits were linked to herbivory but not tree species richness, we explored whether concurrent changes in canopy structure with diversity may instead drive associational resistance. We observed that birch canopy cover decreased with tree species richness (Fig. 2a) and host dilution (Fig. 2b) but this effect was only significant for the latter (richness: $\chi^2=1.97$, $df=1$, $p=0.161$, dilution: $\chi^2=4.95$, $df=1$, $p=0.026$). While canopy cover had no direct effect on any of the herbivore response variables ($p>0.162$), it was found to affect a number of traits important for herbivory (Fig. 2c). With a subset of 68 trees where canopy cover was assessed, we observed a significant positive effect of canopy cover on SLA ($\chi^2=4.48$, $df=1$, $p=0.034$) and negative but marginal effects on leaf toughness ($\chi^2=3.29$, $df=1$, $p=0.070$) and phenolic content ($\chi^2=3.32$, $df=1$, $p=0.068$).

Structural Equation Modelling

For both chewing damage and roller abundance, tree species richness effects were mediated by changes in leaf area with host dilution (Fig. 3a and d). However, tree species richness effects on leaf chewers also operated independently of resource density or foliar traits (Fig. 3a). For gall and miner abundance, tree species richness effects appeared to be mediated by host dilution but not by traits (Supporting Information, Appendix 5). However, on the subset of trees where canopy cover was measured, we observed that concurrent declines in canopy cover with birch dilution modified leaf traits and, in turn, drove distributions of galls and miners (Fig. 3b and c). While this pathway through canopy cover adequately explained gall distributions, for leaf miners we found that they were directly affected by host dilution and this outweighed any effects of phenolic content or tree species richness.

Discussion

Neighbouring plants play an important role in insect herbivore distributions as they can modify host plant properties and thereby mediate associational resistance (Barbosa *et al.* 2009). However, while increasing diversity frequently reduces insect herbivory, changes in host plant traits have rarely been evoked in mechanistic explanations of associational effects (Mraja *et al.* 2011; Moreira *et al.* 2014; Wäschke *et al.* 2015). Therefore, identifying the key properties that capture the effects of plant-herbivore and, plant-plant interactions presents an important challenge as we move towards a more predictive ecology. Specifically, it would improve our understanding of how plant traits interact with the environment to drive insect pest damage and abundance in ecosystems. Here, we show that from a comprehensive list of leaf properties, only a few morphological traits – leaf area and SLA – were important determinants of herbivory on birch. Contrary to predictions however, these traits did not vary with tree species richness but were instead influenced by host dilution and canopy cover. Therefore, we demonstrate for the first time that associational resistance may be triggered by trait responses to stand structure rather than tree diversity per se.

Trait variation with forest structure contributes to associational resistance

In this study, we observed that tree species richness consistently reduced herbivore damage and abundance. However, contrary to previous findings, species richness effects were independent of herbivore feeding specialisation (Jactel & Brockerhoff 2007). Tree

species richness had variable effects on the two specialist feeding guilds, with significant negative effects of tree species richness on miner abundance but not gall abundance (Table 1, Fig. 1). Similarly, negative effects of species richness were significant for chewing herbivores but not rollers (Table 1, Fig. 1). While we also predicted that the specialist and endophagous herbivores – leaf miners and gall formers – would be more sensitive to changes in birch foliar quality, we found little evidence for this as all guilds, except leaf miners, were significantly influenced by several traits. In addition, individual leaf traits were almost always selected as the best determinants of herbivory over any other variables: leaf area was the best predictor of insect chewing damage and roller abundance and gall abundance was best predicted by SLA (Fig. 1, Table 1). These effects of leaf morphology surpassed effects of any other analysed trait as well as effects of plot species richness or host dilution.

Previous studies exploring trait-mediated effects of plant diversity have shown that plant diversity may result in increased investment in anti-herbivore defences (Mraja *et al.* 2011; Moreira *et al.* 2014; Wäschke *et al.* 2015). However, here we found that none of the sixteen measured traits significantly varied with tree species richness even though most herbivores responded to both physical and chemical traits. Only acid detergent fibre (ADF) – a measure of structural carbohydrate and fibre – marginally decreased with species richness but, as ADF did not significantly influence insect herbivore damage or abundance, it cannot explain variation in pest damage. Rather, insect herbivore distributions appear to be more closely related to birch density and canopy cover and effects of these factors on birch leaf traits. Leaf area significantly increased with host dilution and we detected positive effects of increasing canopy cover on SLA and marginally significant negative effects on phenolic content (Fig 2). As birch trees are the tallest species in the Satakunta experiment (Muiruri *et al.* 2015), mixed-species plots have a higher density of shorter tree species within the plot and therefore a lower canopy cover around birch trees. While we did not measure light availability directly on focal birch trees, previous work by Pannek *et al.* (2013) showed that visual estimates of canopy cover correlate well with measures of light intensity in over 100 deciduous forests. Therefore, birch trees surrounded by tall conspecifics in monocultures experience the lowest light intensities and produce leaves with a higher leaf area per unit of dry leaf weight.

Increasing canopy cover is known to trigger the investment of resources to photosynthetic tissue and shaded plants are therefore found to produce larger leaves with a higher SLA

(Chapin, Matson & Mooney 2002). This is also the case for birch as it has previously been shown to form leaves in the lower canopy that have a higher SLA (Niinemets & Kull 1994), larger leaf area (Sack *et al.* 2006) and reduced phenolic concentrations (Henriksson *et al.* 2003), in accordance with our findings. Foliage from shady environments is in general more favourable for herbivore growth and development (Roberts & Paul 2006) and there is evidence that leaves from the lower canopy of birch are also of better quality for common chewing insects (*Epirrita autumnata*, Suomela *et al.* 1995a) and are preferred over leaves in the upper canopy by leaf galls (*Acalitus rudis*, Buchta *et al.* 2004) and rollers (*Deporaus betulae*, Riihimäki *et al.* 2003). Although leaf area may be less sensitive to stand canopy cover than specific leaf area (Niinemets & Kull 1994), even with a subset of data, we found that increases in canopy cover also had a positive effect on leaf area. Therefore, changes in leaf morphology with diversity as a result of reduced canopy cover may govern associational resistance on birch.

Variation in birch leaf structure could also be dictated by competitive processes rather than canopy cover. Similar to previous studies on birch, we observed no effect of tree species richness on leaf area (Pollastrini *et al.* 2014) but a significant negative effect of birch dilution. This suggests that reduced intraspecific competition with host dilution drives changes in leaf area. Indeed, it has been shown that birch trees grow more vigorously in competition with conspecifics than they do when grown with heterospecific neighbours (Lintunen & Kaitaniemi 2010; Kaitaniemi & Lintunen 2010). The resulting increase in tree growth and leaf size is likely to favour insects with evidence of improved performance of chewing insects on tall, large-leaved trees (Senn, Hanhimäki & Haukioja 1992). Leaves with a larger area are also favoured by leaf rollers as these leaves are not only easier to roll due to less torque (Horváth 1988), but they may also provide more internal leaf mass in the coil to feed larvae and better conceal growing larvae from predators (Lind *et al.* 2001).

These positive effects of leaf area on chewing insects could also be mediated by positive effects of leaf area on leaf rollers as it has been shown that leaf shelters are frequently colonised by chewing herbivores and their presence enhances the overall diversity of insect herbivores on trees (Lill & Marquis 2003, 2004). At the same time, studies have observed delayed compensatory growth in birch with larger leaves produced in the year after herbivory (Danell, Huss-Danell & Bergström 1985; Kozlov *et al.* 2012). Birch leaf area may therefore be a positive indicator of both past and present herbivory across tree

diversity gradients. However, it only seems to be important for external feeders as galls and miners feed internally on a small portion of a leaf and may therefore be more sensitive to the density or chemistry of leaves rather than the total leaf area (Koricheva *et al.* 1996).

Associational effects can also operate independently of leaf traits

In addition to the trait-mediated effects of diversity detected, we found that tree species richness effects may operate independently of birch leaf traits or canopy cover. For chewing damage specifically, tree species richness influenced leaf area consumed irrespective of variation in leaf size with host dilution (Fig. 3a). This could be because generalist chewing herbivores are drawn away from focal birch trees in accordance with the attractant-decoy hypothesis (Ruttan & Lortie 2014). However, as insects cannot feed on both conifers and broadleaves, the only other potential host tree in Satakunta would be alder and the absence of any effects of tree species composition or alder density would work against this prediction. One possibility could be that as birch tree height decreases with species richness (Muiruri *et al.* 2015) plots may become less apparent to birch searching herbivores (Endara & Coley 2011; Castagneyrol *et al.* 2013). However, as the density of short neighbours increases at the same time, individual birch trees in more diverse plots may instead be more accessible to herbivores. The direct effects of tree species richness on chewing damage may instead be mediated by some other unmeasured trait or by higher predation risk as diversity increases (Muiruri, Rainio & Koricheva 2016).

We also found that the density of birch trees was consistently an important factor for all herbivore guilds. Since the original formulation of associational resistance by (Root 1973), the resource concentration hypothesis remains one of the long-established theories cited to explain negative effects of diversity (Root 1973; Letourneau 1987; Tonhasca 1993; Riihimäki *et al.* 2005). In accordance with the hypothesis, we found that tree species richness effects were largely mediated by host dilution effects on the abundance of galls, miners and rollers. Similar to Castagneyrol *et al.* (2013), we found that host dilution was the primary factor driving leaf miner abundance but not any other herbivore feeding guild in this study. Most of birch leaf miner species in the Satakunta experiment are specialists and are thus more likely to concentrate where their resource is abundant (Root 1973). In addition, even though host selection for oviposition by leaf miner females has been shown to be influenced by several leaf traits (Clissold *et al.* 2009), the high feeding specialisation and sessile nature of leaf miners at the larval stage may be the

dominant driver of their distributions as miners are less likely to emigrate from a resource-rich stand. As a result, leaf miners are often found to respond most consistently to forest diversity and host dilution in comparison to other herbivore guilds (Vehviläinen, Koricheva & Ruohomäki 2007; Castagneyrol *et al.* 2013). Despite these direct effects of host dilution on mining herbivores, the abundance of miners was also partially influenced by total phenolic content (Fig. 1e, 3c). Previous studies have also detected elevated phenolic content on birch leaves with leaf mines (Valladares & Hartley 1994; Johnson *et al.* 2002) but, as these studies only compared mined and undamaged birch leaves it remains unclear whether leaf miners select trees based on their phenolic content or induce chemical changes in birch. Furthermore, it has been shown that even though mining larvae feed within a leaf, they can bypass host plant defences simply by consuming only those tissues that are of greater quality than the whole leaf (Kimmerer & Potter 1987). As a result, attempts to relate whole leaf traits to the abundance of mining herbivores may yield misleading results. In any event, phenolic content only had a marginally significant effect on leaf miner abundance thus we are unable to put as much confidence into this trait-mediated pathway in comparison to other guilds.

Morphological traits predict herbivore resistance better than chemical traits

With the exception of leaf miners, most insect feeding guilds were significantly influenced by both physical and chemical leaf traits (Fig. 1). For example, C:N was an important variable for chewing damage and gall abundance, and protein precipitation of tannins predicted both gall and roller abundance. Recent studies investigating the role of traits in associational effects have primarily focussed on defensive chemical compounds but have not found any links with insect herbivores (Mraja *et al.* 2011; Moreira *et al.* 2014; Wäschke *et al.* 2015). For instance, Moreira *et al.* (2014) showed that tree species diversity had a positive effects on stem polyphenol and condensed tannin content but this did not explain differences in herbivore damage in polycultures versus monocultures. Although we assessed leaf phenolics and tannins, their protein precipitation capacity and even the less explored oxidative capacity of tannins (Salminen & Karonen 2011), it appears that morphological traits consistently dominate over chemical properties in effects on birch insect herbivory (Fig. 1, Table 1).

Our findings are in agreement with previous work showing that morphological traits may be more important determinants of herbivory on plants than nutritive and chemical defence traits (Clissold *et al.* 2009; Carmona *et al.* 2011; Schuldt *et al.* 2012; Caldwell,

Read & Sanson 2016). In the case of birch trees especially, foliar phenolics may not be important for insect performance (Suomela, Ossipov & Haukioja 1995b) and there is evidence that herbivory on birch triggers positive effects on growth and foliar quality but only a weak defensive responses (Hanhimäki & Senn 1992). As we observed consistent effects of leaf traits on both early and late season herbivory on birch, it seems that trait variation occurs in the longer-term possibly as a result of delayed induced resistance to herbivory. We therefore consider that our findings are relevant throughout the growing season and suggest that morphological traits may be a better indicator of past damage and a good predictor of herbivory on birch across different habitat contexts.

Conclusions

Although the measurement of plant traits has often been suggested as a useful tool to improve our understanding of herbivory across diversity gradients, studies on associational effects have rarely implicated leaf traits (Andrew, Roberts & Hill 2012). Until now, studies of plant diversity have been limited in their focus on single herbivore types and have rarely explored the role of leaf traits even though they often yield predictable changes in insect herbivore distributions and may have wider consequences for ecosystems (Wright *et al.* 2004). Furthermore, with damage of northern birch forests by leaf-chewing and leaf-mining insects set to double with expected climate warming (Kozlov 2008), it is even more important to understand how the structure and diversity of forest plantations can be managed to limit birch foliar losses and consequences for productivity.

Here, we not only explored the effects of tree species richness and stand structure on multiple herbivore types but also determined the role of trait variation in driving these relationships. Our results demonstrate that leaf traits are important to study in the context of associational effects as they reflect both abiotic (light) and biotic (conspecific interactions) changes along the diversity gradient. Morphological leaf traits appear to be especially important determinants of herbivory across most insect guilds used in this study, predicting both insect damage and abundance depending on the diversity or canopy cover around a focal tree. More research on leaf traits accounting for environmental and structural differences between forest stands may therefore improve our understanding of biodiversity-resistance relationships and enhance our ability to predict associational patterns across spatial and temporal scales.

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Table

Table 1. Results from the comparison of univariate mixed-effects modelling for herbivore responses to tree species richness, host dilution and birch leaf traits. Only statistics from significant models are reported and the direction of each significant effect given. For each herbivore response, differences in Akaike's Information Criterion relative to the top model are reported (ΔAICc) and the Akaike weights (w_{ic}) are also given and indicate the weight of evidence for a model relative to all other candidate models.

	Model	χ^2	p		ΔAICc	w_{ic}
Chewing	Leaf Area	21.3	<0.001	+	0.00	0.99
Damage	Thickness	9.51	0.002	-	10.4	0.01
	Tree Species Richness	9.51	0.002	+	11.0	0.01
	Host Dilution	7.38	0.007	-	12.8	0.00
	C:N	5.76	0.016	-	14.3	0.00
Gall	SLA	15.6	<0.001	+	0.00	0.71
Abundance	C:N	11.8	<0.001	-	2.59	0.19
	LDMC	9.06	0.003	-	5.23	0.05
	Protein Precipitation	5.34	0.021	-	8.32	0.01
	Leaf Area	4.61	0.032	+	9.00	0.01
Miner	Host Dilution	6.14	0.013	-	0.00	0.36
Abundance	Tree Species Richness	4.64	0.031	-	1.55	0.18
Roller	Leaf Area	13.3	<0.001	+	0.00	0.98
Abundance	Phenolic Content	10.6	0.001	+	10.2	0.01
	Protein Precipitation	6.15	0.013	+	11.1	0.00

Figure legends

Figure 1. Responses of insect chewing damage (a, b), gall abundance (c, d), miner abundance (e, f) and roller abundance (g, h) to tree species richness, host dilution and leaf traits. Coefficients of regression ($\pm 95\%$ CI) estimated from linear models are shown in the left panel where the shaded area relates to the best univariate model with the lowest Akaike's information criterion (AICc). The top selected variable highlighted in the left panel also corresponds to the x-axis in the right panel. A smoothed mean response (\pm SE) is shown in the right panel to illustrate the effects of the selected variable on each insect herbivore response. Significance codes: $p < 0.001$, '***', $p < 0.01$, '**', $p < 0.05$, '*', $p < 0.1$, '.'

Figure 2. Canopy cover responses to tree species richness and host dilution and its effect on birch leaf traits. Smoothed means are drawn for effects of tree species richness (a, dashed line) and host dilution (b, solid line \pm SE) on canopy cover around birch trees but effects were only statistically significant for the latter. Estimated coefficients of regression and their 95% CI are also shown in (c) for responses of leaf traits to changes in canopy cover. Significance codes: $p < 0.001$, '***', $p < 0.01$, '**', $p < 0.05$, '*', $p < 0.1$, '.'

Figure 3. Structural equation models to illustrate direct and indirect effects of tree species richness on chewing damage (a) and the abundance of galls (b), miner (c) and rollers (d). Standardised path coefficients are indicated near the arrows and the thickness of arrows corresponds to the magnitude of these coefficients. Positive relationships are shown in blue and negative relationships in red. Overall model fit was determined with Shipley's test of d-separation (Fisher's C statistic) where models were considered a good fit if $p > 0.05$. SEMs for gall and miner abundance were based on a subset of data to determine the role of canopy cover.

Figures

Figure 1

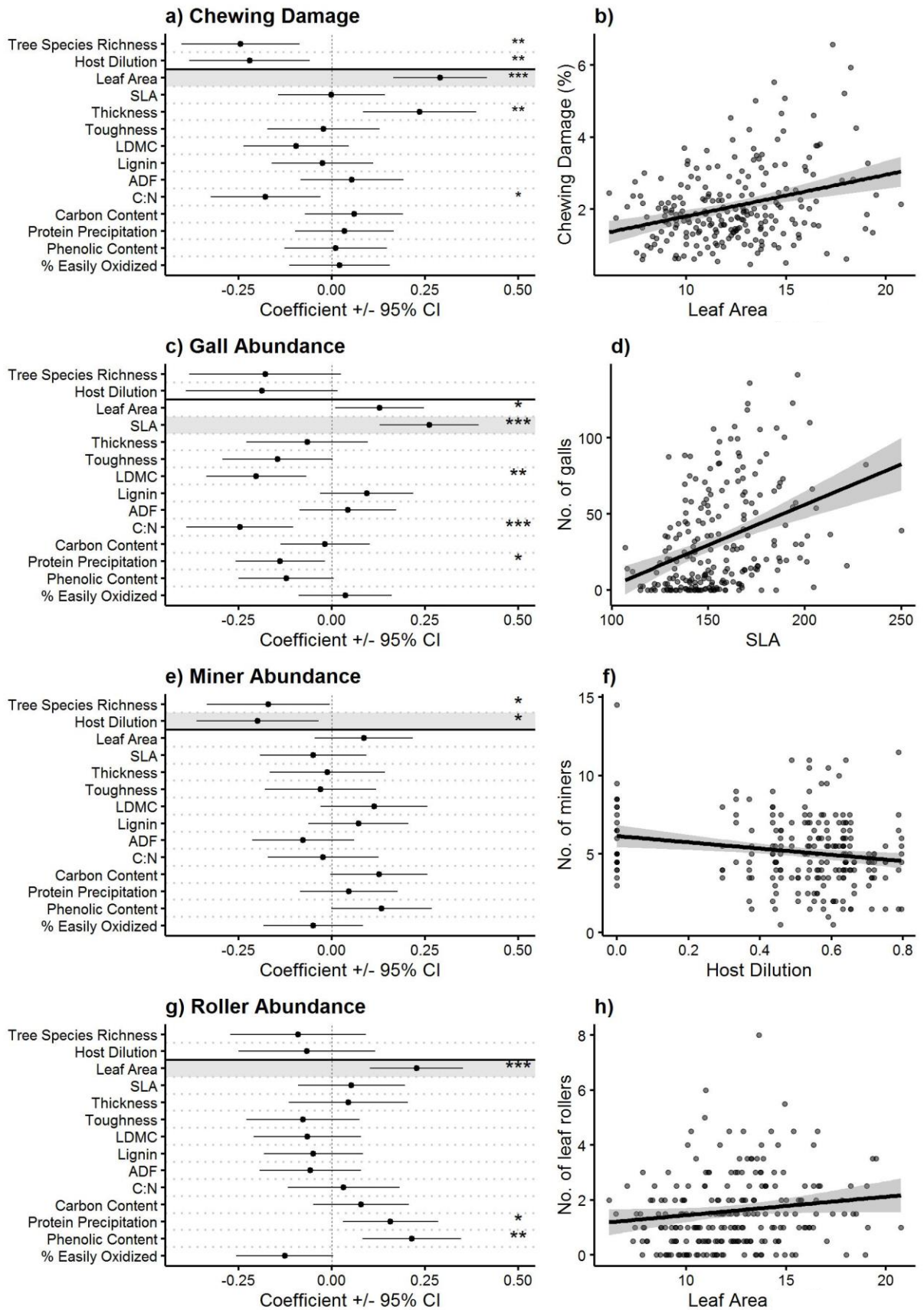


Figure 2

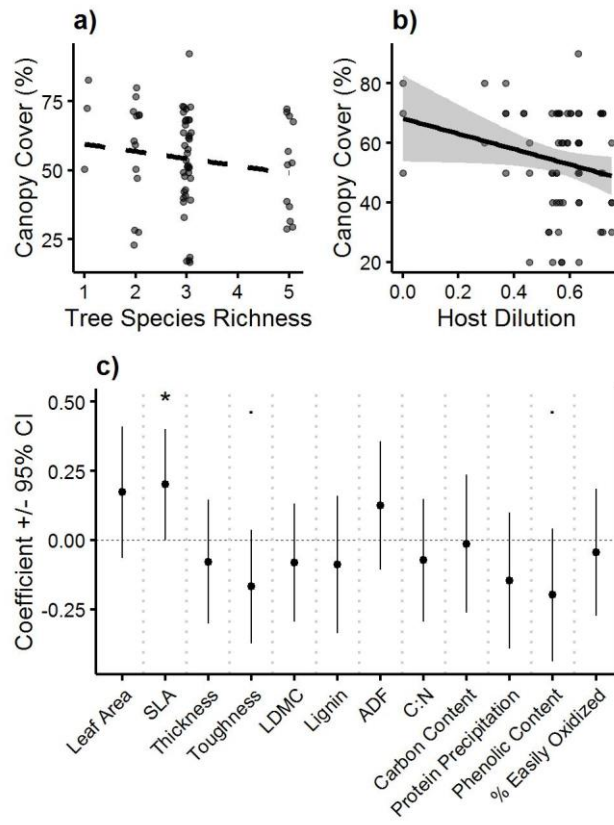
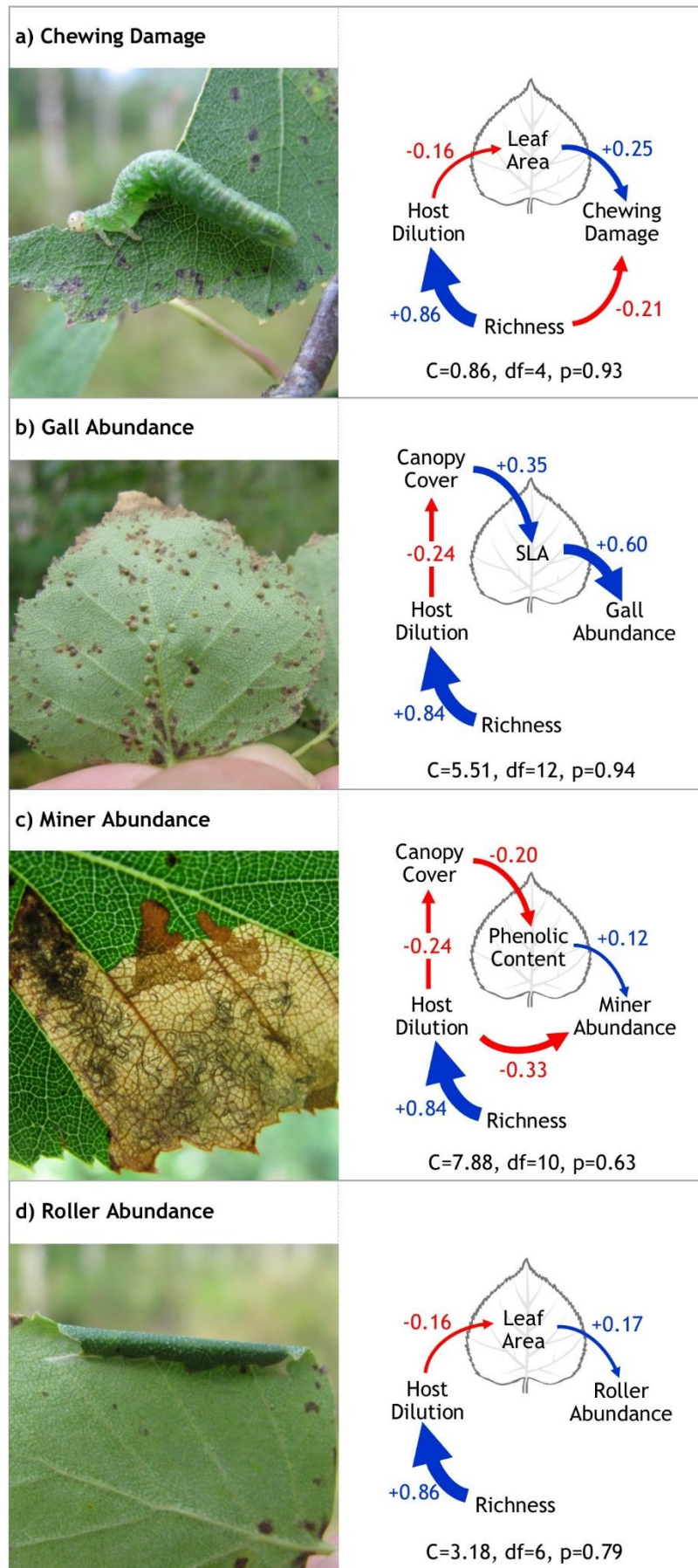


Figure 3



Supporting information

Forest diversity effects on insect herbivory: do leaf traits matter?

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Appendix 1 – Near infrared reflectance spectroscopy (NIR) for the determination of ADF, lignin, condensed tannins and protein precipitating tannins

Near infrared spectra of all samples were reduced to principal component scores, Mahalanobis distance to the mean spectra of the population was calculated and the distribution of the sample population along the first three components was assessed. Subsequently, CENTER and SELECT population structuring algorithms (Shenk & Westerhaus 1991) were applied, using WINISI III v.1.63 software, to select the most representative samples to use as calibration and validation sets. These algorithms structure the population on the basis of the standardized Mahalanobis distance between each spectrum and select those samples with the highest number of neighbours, within a given distance. The neighbours are then dismissed and the procedure is repeated until all spectra have been considered.

A suite of 12 calibrations, correlating NIR absorbance and wet chemistry values for each parameter, were carried out by applying modified partial least squares regression in combination with a number of spectral pre-treatments, including four types of derivative (up to 4th order derivative) and three scatter correction options: multiplicative scatter correction (MSC) (Geladi, MacDougall & Martens 1985), standard normal variate and de-trend SNVD (Barnes, Dhanoa & Lister 1989) or no scatter correction treatment. These pre-treatments are commonly used to enhance weak signals and remove baseline effects

on the spectra, but are dependent on the dataset; hence different combinations need to be applied to find the most appropriate one.

Amongst the statistics produced from this regression, r^2_{cal} and standard error of cross-validation (*SECV*) were used to select the best equation for each parameter (that with highest r^2_{cal} and lowest *SECV*). The selected equations were applied on the validation samples and subsequently NIR predicted vs. actual values were compared. The statistics derived from this comparison were r^2_{val} , standard error of prediction (*SEP*), slope and bias, and were used to select equation that would produce the most accurate predictions, i.e. that with r^2_{val} and slope closest to 1, and with *SEP* and bias closest to zero.

Table S1. Calibration and validation results of the selected best NIRS equations developed to predict acid detergent fibre (ADF), lignin, condensed tannins and protein precipitating tannins. All equations were developed using modified partial least squares regression (MPLS) and a combination of derivation and scatter correction treatments (Equation details). Derivation procedures are expressed in the form of (a, b, c, d) where a: order of derivative, b: the gap or number of data points over which the derivative is calculated, c: number of data points over which first smoothing is applied and d: number of data points over which the second smoothing is applied.

Constituent	Equation details	Calibration results						Validation results				
		N_{cal}	Mean	<i>SD</i>	r^2_{cal}	<i>SECV</i>	<i>I-VR</i>	N_{val}	r^2_{val}	<i>SEP</i>	Slope	Bias
ADF	1,4,4,1 + MSC	136	18.01	3.62	0.96	0.76	0.96	15	0.634	0.912	0.767	-0.342
Lignin	4,10,10,1	138	6.54	1.25	0.82	0.57	0.79	15	0.529	0.735	0.975	-0.576
CT	4,10,10,1 + SNVD	139	7.11	2.75	0.96	0.70	0.93	15	0.730	1.095	0.84	-0.390
PPT	1,4,4,1 + SNVD	113	38.81	13.38	0.62	9.02	0.55	36	0.297	9.25	0.75	4.02

N_{cal} : Number of samples used for calibration, *SD*: Standard deviation, R^2_{cal} : coefficient of determination in calibration, *SECV*: standard error of cross validation, *I-VR*: coefficient of determination in cross-validation, N_{val} : number of samples in the validation set, r^2_{val} : coefficient of determination in validation, *SEP*: standard error of prediction.

Barnes, R.J., Dhanoa, M.S. & Lister, S.J. (1989) Standard Normal Variate

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Appendix 2 – Effects of area and thinning treatment on insect herbivore responses

Table S2. Results from linear mixed-effects models to determine variation in tree species richness effects on each herbivore guild between the three different areas and the thinning treatments.

	Chewing			Galls			Miners			Roller		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
Richness	8.14	1	0.004	8.17	1	0.004	3.81	1	0.051	0.07	1	0.797
Area	7.99	2	0.018	74.2	2	<0.001	0.48	2	0.787	1.96	2	0.376
Thinning	0.91	1	0.339	2.04	1	0.153	0.01	1	0.928	0.67	1	0.414
Richness*Area	0.05	2	0.978	1.15	2	0.563	0.78	2	0.678	4.82	2	0.090
Richness* Thinning	1.73	1	0.189	6.15	1	0.013	2.56	1	0.110	0.00	1	0.995
Area*Thinning	1.65	2	0.438	13.1	2	0.001	1.51	2	0.471	1.17	2	0.558
Richness*Area* Thinning	3.03	2	0.220	3.92	2	0.141	0.02	2	0.989	0.11	2	0.947

Appendix 3 – Selection of birch leaf traits for analysis

Figure S1. Trait separation and correlations. Principal component analysis was used to illustrate separation of all 16 measured traits. Correlations between all traits are also shown where the colour code corresponds to values in the correlation matrix in Table S2.

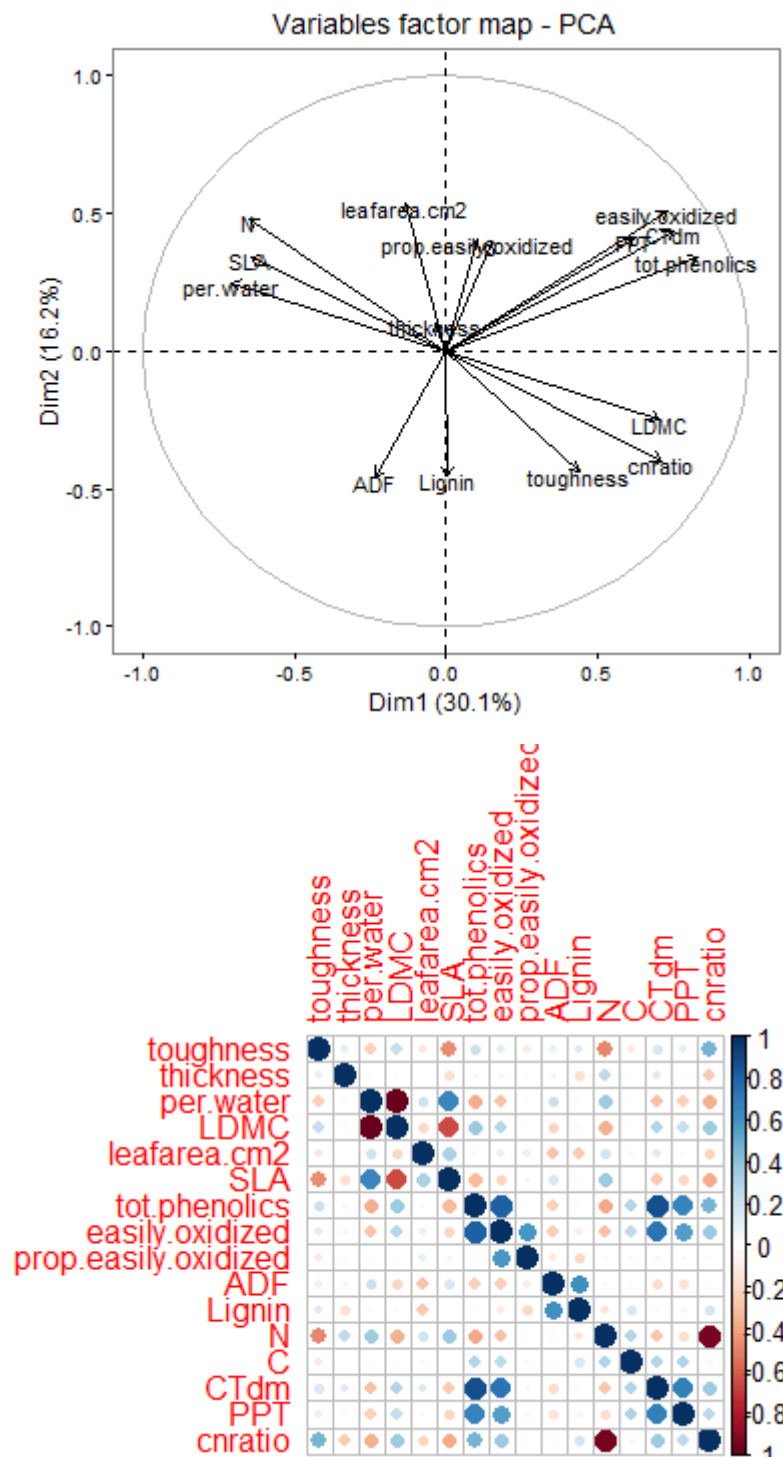


Table S3. Correlation matrix of all 16 measured traits. Values > 0.8 are in bold text and one of these correlated variables was removed as explained in the main text.

	Toughness	Thickness	Water	LDMC	Leaf Area	SLA	Phenolics	Easily Oxidized	% Easily Oxidized	ADF	Lignin	N	C	CTannins	PPT	C:N
Toughness																
Thickness	0.1															
Water	-0.22	-0.01														
LDMC	0.22	0.01	-1													
Leaf Area	-0.13	-0.01	0.21	-0.21												
SLA	-0.46	-0.16	0.65	-0.65	0.31											
Phenolics	0.22	-0.06	-0.37	0.37	0.04	-0.32										
Easily Oxidized	0.12	-0.05	-0.29	0.29	0.1	-0.22	0.81									
%Easily Oxidized	-0.11	-0.02	0.01	-0.01	0.09	0.07	0.01	0.58								
ADF	0.09	0.07	0.21	-0.21	-0.28	0.16	-0.24	-0.25	-0.1							
Lignin	0.15	-0.14	0.03	-0.03	-0.26	-0.01	0.02	-0.11	-0.2	0.61						
N	-0.49	0.25	0.37	-0.37	0.19	0.37	-0.39	-0.3	0.02	-0.04	-0.14					
Carbon Content	-0.12	0.01	0	0	-0.04	-0.01	0.29	0.26	0.03	-0.02	0.16	0.3				
Condensed Tannins	0.17	0.15	-0.3	0.3	0.14	-0.27	0.87	0.74	0.06	-0.18	-0.03	-0.27	0.3			
PPT	0.1	0.03	-0.23	0.23	0.04	-0.21	0.66	0.56	0.05	-0.15	-0.07	-0.17	0.3	0.69		
C:N	0.47	-0.25	-0.37	0.37	-0.21	-0.39	0.46	0.36	-0.02	0.03	0.18	-0.95	-0.06	0.35	0.27	

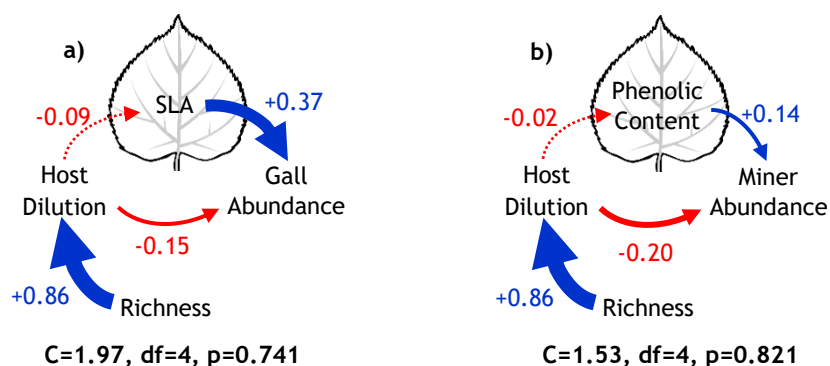
Appendix 4 – Effects of tree species richness, host dilution and canopy cover on birch leaf traits and herbivory

Table S4. Results from univariate linear mixed-effects models testing effects of tree species richness, host dilution and canopy cover on the final 12 traits selected. Canopy cover was only measured on some of the experimental trees so these models are based on a subset of the full dataset. Significant and marginally significant effects are in bold text and the direction of these effects is given.

Trait	Richness		Dilution		Canopy Cover		
	χ^2	p	χ^2	p	χ^2	p	
Leaf area	2.40	0.122	4.22	0.040	* -	2.11	0.146
SLA	1.34	0.246	0.72	0.396		4.48	0.034 * +
Thickness	0.08	0.778	0.10	0.749		0.60	0.440
Toughness	0.00	1.000	0.47	0.494		3.29	0.070 . -
LDMC	1.90	0.169	2.26	0.133		0.75	0.388
Lignin	0.70	0.404	0.46	0.499		0.67	0.414
ADF	3.31	0.069	1.83	0.177		0.95	0.330
C:N	0.01	0.938	0.06	0.813		0.74	0.390
Carbon	0.90	0.342	0.06	0.808		0.02	0.876
Protein Precipitation	0.51	0.477	0.18	0.671		1.84	0.176
Phenolic Content	0.01	0.911	0.10	0.757		3.32	0.068 . -
% Easily Oxidized	0.01	0.939	0.00	0.977		0.21	0.651

Appendix 5 – Results of piecewise structural equation modelling excluding canopy cover effects on SLA and phenolic content.

Figure S2. Structural equation models for (a) gall abundance and (b) miner abundance using the full dataset. Blue arrows indicate positive relationships and red arrows indicate negative relationships. Standardised path coefficients are given and the thickness of arrows corresponds to the magnitude of these coefficients. Overall fit was evaluated using Shipley's test of d-separation (Fisher's C statistic) where models were a good fit to the data if $p > 0.05$.



Chapter 5

Moose browsing alters tree diversity effects on birch growth and insect herbivory

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Moose browsing alters tree diversity effects on birch growth and insect herbivory

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Running headline: Moose alter tree diversity effects on birch

Summary

1. Producer diversity is known to affect a wide range of ecosystem processes including plant growth and insect pest resistance. Consumers such as mammalian herbivores too have been shown to modify plant growth and insect herbivory by triggering changes in host plants. However, few studies have investigated whether consumer effects interact with plant species diversity effects on a focal plant.
2. To unravel consumer-diversity interactions, we recorded both the presence and intensity of winter browsing by moose (*Alces alces*) on silver birch (*Betula pendula*) in a long-term forest diversity experiment in Finland and measured birch tree growth as well as insect chewing damage during the following growing season.
3. Although browsing on birch by moose was not affected by tree species richness, the intensity of moose damage altered tree diversity effects on birch tree growth. At minor browsing intensity, tree height, trunk diameter and canopy projections showed positively-humped relationships with tree diversity, peaking at 3-species mixtures. Growth of moderately browsed trees increased with tree species richness, but growth of severely browsed birch trees was unaffected.
4. Moose browsing also altered the direction of tree diversity effects on insect herbivory on birch. Unbrowsed trees experienced lower insect chewing damage in mixed stands (associational resistance) whilst browsed trees suffered more insect chewing damage in diverse stands (associational susceptibility). Increasing browsing intensity also reversed the relationship between tree species richness and insect chewing damage from negative to positive.
5. The observed interactions between moose browsing and tree species richness effects could be explained by lower canopy cover of more diverse stands compared to birch monocultures, leading to increased re-growth capacity and more high-quality foliage of browsed birch trees in more open diverse stands.
6. Our findings demonstrate that both the presence and intensity of mammalian browsing may modify the magnitude and even the direction of tree diversity effects on tree growth and susceptibility to insect herbivory. Differences in consumer impact among studies may thus potentially explain much of the observed variability in plant diversity effects on ecosystem functioning and must therefore be taken into account in future studies.

Keywords

Biodiversity and ecosystem functioning, boreal forest, plant-herbivore interactions, Satakunta forest diversity experiment

Introduction

Human activities have resulted in dramatic losses of biodiversity around the globe with associated detrimental effects on ecosystem functioning (Balvanera *et al.* 2006; Cardinale *et al.* 2011). Forests, in particular, have suffered significant biodiversity losses due to deforestation, creation of single-species plantations, habitat fragmentation and climate change (Saunders, Hobbs & Margules 1991; Brook, Sodhi & Ng 2003; Thomas *et al.* 2004). However, despite the ecological importance of forests, the vast majority of research on the relationship between biodiversity and ecosystem functioning has been based on grassland or aquatic systems (Balvanera *et al.* 2006; Cardinale *et al.* 2011). Only recently has the attention switched to forest ecosystems with evidence emerging for positive relationships between tree species richness and multiple ecosystem functions (Jactel & Brockerhoff 2007; Piotta 2008; Gamfeldt *et al.* 2013; Vilà *et al.* 2013). However, the relationship between forest diversity and function may also take other forms with some studies finding negative (Schuldt *et al.* 2010; Hynynen, Repola & Mielikäinen 2011; Plath *et al.* 2012; Milligan & Koricheva 2013), non-significant (Vehviläinen, Koricheva & Ruohomäki 2008; Lang *et al.* 2012) or hump-shaped relationships with increasing tree species richness (Scherer-Lorenzen, Luis Bonilla & Potvin 2007; Gamfeldt *et al.* 2013).

The mechanisms underlying variability in diversity-function relationships are still poorly understood. Although an increasing number of studies have shown that tree species composition effects on ecosystem processes such as primary productivity and herbivore resistance can be stronger than effects of tree species richness (Koricheva *et al.* 2006; Nadrowski, Wirth & Scherer-Lorenzen 2010), this does not appear to explain the differences in responses to tree species richness. For plant-herbivore interactions, we still lack predictive frameworks for when tree diversity will reduce (associational resistance) versus enhance herbivory (associational susceptibility) (Barbosa *et al.* 2009). Similarly, the effects of tree diversity on growth appear to be context-specific, depending on the identity of the focal species, its neighbours or other stand properties (Vilà *et al.* 2003; Zhang, Chen & Reich 2012). A more community-based approach may therefore be

required to elucidate which factors might modify the direction or magnitude of diversity-function relationships.

In addition to the bottom-up effects of producer diversity, primary consumers are well known to strongly influence functional processes in ecosystems (Duffy 2002), impacting nutrient cycling (Pastor *et al.* 1993; Forkner & Hunter 2000), productivity (Weisser & Siemann 2004; Persson, Bergström & Danell 2007) and producer diversity (Heikkilä & Tuominen 2009; Speed, Austrheim & Hester 2013; Bagchi *et al.* 2014). Mammalian herbivores, in particular, can have widespread effects in forests, influencing stand development (Edenius *et al.* 2002), biomass production (Persson *et al.* 2007), tree growth (Bergstrom & Danell 1987) and stand species composition (Pastor *et al.* 1993). Such top-down effects are well documented in many systems but few have considered how consumer activity and diversity effects may interact. To our knowledge, only two studies have explored the role of mammalian herbivores in diversity-function relationships in herbaceous plant communities (Parker, Salminen & Agrawal 2010) and in an experiment with tree seedlings (Cook-Patton, Laforgia & Parker 2014). However, the effects of mammalian herbivores on diversity-function relationships for trees in established forest stands have not yet been explored.

Here, we use a long-term forest diversity experiment in a Finnish boreal forest to investigate consumer impact on diversity-function relationships. Specifically, we focus on the interactive effects of mammalian browsing by moose (*Alces alces* L.) and tree species diversity on the growth and susceptibility of silver birch (*Betula pendula* Roth.) to insect herbivores. Silver birch is one of the tree species preferred by moose (Milligan & Koricheva 2013), responding to winter browsing with reduced growth and increased foliar quality for insect herbivores (den Herder *et al.* 2009). Tree species richness and composition have been shown to affect silver birch growth (Kaitaniemi & Lintunen 2010; Hynynen *et al.* 2011) and insect herbivory (Vehviläinen, Koricheva & Ruohomäki 2007), although the magnitude and direction of these effects varies among studies (Vehviläinen *et al.* 2006; Morath 2013). Given that moose browsing can also be affected by tree species richness and composition (Vehviläinen & Koricheva 2006; Milligan & Koricheva 2013), we may therefore expect moose browsing preferences to interact with tree diversity effects on tree growth and susceptibility to herbivory.

In boreal systems, moose are known to cause the most extensive damage to trees in winter (Jalkanen 2001) and the damage they cause has been shown to not only reduce tree growth (Bergström & Danell 1995; den Herder *et al.* 2009) but also trigger changes in host tree traits that increase insect herbivore damage in the following growing season (Danell & Huss-Danell 1985; den Herder *et al.* 2009). Although considerable research has gone into the effects of mammalian browsing on tree susceptibility to insect pests, very few studies have explored diversity effects in a multi-herbivore context (Axelsson & Stenberg 2012). The vast majority of previous work on plant species diversity effects on herbivores has focussed on interactions between a focal plant species and a single herbivore (Barbosa *et al.* 2009). However, most plants interact with a diverse suite of herbivores, each of which can modify plant traits and hence alter host plant susceptibility to subsequent attack by another herbivore (Ohgushi 2005). As such, it has been suggested that in a multi-herbivore system including both mammalian and insect herbivores, the effect of herbivory by one species could theoretically influence the direction and/or strength of plant diversity effects on the second herbivore (Axelsson & Stenberg 2012). As moose browsing and insect herbivory on birch are temporally separated in this system, our study system provides a unique opportunity to establish cause and effect in plant-mediated interactions between both herbivore types across the diversity gradient.

In this study, we monitored moose browsing during two consecutive years and tested two main hypotheses: that the presence and intensity of winter browsing by moose would alter tree species richness and composition effects on (1) birch tree growth and (2) insect herbivore damage. Tree growth and herbivory were assessed each summer and we also explored changes in canopy cover and neighbouring tree heights with tree diversity to elucidate the mechanisms underpinning these interactive effects.

Materials and methods

(a) Experimental site

All data were collected from a long-term forest diversity experiment in Satakunta, SW Finland. Established in 1999, the experiment is made up of three separate areas (area 1, 61°42'N, 21°58'E; area 2, 61°39'N, 22°09'E; area 3, 61°40'N, 21°42'E) planted with five tree species: Scots pine (*Pinus sylvestris* L., P), Norway spruce (*Picea abies* L., S), Siberian larch (*Larix sibirica* Ledeb.), silver birch (*Betula pendula* Roth., B), and black alder (*Alnus glutinosa* L., A). Each area consists of 38 plots (20m x 20m) which are

randomly allocated one of 19 treatments representing a species richness gradient from monocultures to 2-, 3- and 5-species mixtures; each treatment is replicated twice per area. Each plot, in turn, consists of 13 rows with 13 trees planted 1.5m apart (total 169 trees per plot) in a substitutive design with tree density equal across plots and tree species positions randomised within each plot. Ten birch trees were randomly selected from each of the birch-containing treatments in all experimental areas giving six plot replicates of birch monoculture (B), each 2-species (B+A, B+P, B+S) and 3-species (B+A+L, B+A+P, B+L+P, B+P+S) mixture, and the 5-species mixture (B+A+L+P+S). Plots are not fenced and hence all experimental trees are exposed to natural moose browsing. Moose browsing and insect herbivory were assessed on the same individuals in 2010 and 2011, with a total of 516 trees surveyed.

The height, trunk diameter at breast height (DBH) and crown projection (average of N-S and W-E projections) of each experimental tree were measured at the end of the growing season each year (2010 and 2011). Tree height was also measured for the other four tree species in the area on 10 randomly chosen individuals per species per plot. All trees within each species were comparable in size at planting therefore any differences in growth might be attributed to browsing history and neighbouring tree species richness or composition. In August 2014, we also recorded canopy cover around experimental birch trees as an additional measure to help identify changes in the light environment with tree diversity. As half of the plots in Satakunta were thinned in 2013, we measured canopy cover for trees in unthinned plots only, sampling 250 of the 516 experimental birch trees. Canopy cover was estimated with the GRS densitometerTM by recording the percentage of views that were obstructed by canopy at 10 evenly-spaced positions around the crown edge of each tree.

(b) Moose browsing monitoring

Data on winter browsing by moose were collected in May-June 2010 and 2011. Moose browsing was recorded for each birch tree by scoring presence or absence of browsing and the intensity of browsing damage in the lower part of the canopy accessible to moose (up to 300cm from the ground). The intensity of browsing was scored as: none, minor (<25% of available branches browsed), moderate (26-75% of available branches browsed) or severe (>75% of available branches browsed). Using these measurements, we also calculated two indices of browsing damage for plot level analysis. The proportion

of birch trees browsed per plot was assessed by calculating the percentage of live birch trees in a plot that had suffered browsing damage regardless of the intensity of browsing. The intensity of browsing was assessed by multiplying the number of trees in each of the browsing categories (minor, moderate and severe) by the category midpoint. These values were each divided by the total number of birch trees and were then summed together to give the percentage moose browsing damage on birch (Vehviläinen & Koricheva 2006; Milligan & Koricheva 2013). No signs of recent moose browsing were observed in August 2014 while doing canopy cover measurements.

At the time of the study, birch trees were 11 to 12 years old and averaged 790 ± 7.8 cm in height. Although foliage is considered to be beyond the reach of moose above 300 cm, all experimental birch trees had accessible branches during the browsing assessment in spring 2010 and 2011. Measurements of the lowest live branches at the end of the study (August 2011) revealed that the mean height of lowest live branches on birch trees was 212.2 ± 5.0 cm (J Koricheva, unpublished data). As evidence of moose damage can remain visible for several years, our measurements were cumulative and reflect both current and past browsing. Moose populations were higher in the Satakunta region in the winter of 2010/2011 with 3353 individuals compared to 3095 in the winter of 2009/2010 (Riistaweb 2014), equating to a density of 2.75 and 2.54 individuals per 10 km², respectively. In addition, the experimental areas experienced a greater than average snowfall during the winter of 2010/11 with snow cover averaging 49 cm in February–March 2011 period compared to 38 cm during the same period in 2009/2010 (Finnish Meteorological Institute, personal communication). As a result, moose browsing also increased in winter 2010/2011 with 24% more trees damaged in the winter of 2010/2011 compared to the previous winter of 2009/2010. Whilst other smaller deer species can cause similar browsing damage (Jalkanen 2001), faecal pellet counting revealed that moose densities were much higher in the study area relative to the densities of white-tailed (*Odocoileus virginianus* Zimmerman), fallow (*Dama dama* L.) or roe deer (*Capreolus capreolus* L.) with 97% of faecal pellet groups from moose (Milligan & Koricheva 2013). Thus, we consider moose to be the primary agents of browsing damage to birch trees in the study area.

(c) Insect herbivory monitoring

Insect herbivory data were collected on the same ten birch trees per plot which were used for moose browsing monitoring during the summers of 2010 and 2011 at two sampling

periods: early summer (early June) and late summer (late July-early August) to reflect different species of insect herbivores feeding at different times through the season. A total of 100 leaves were sampled per tree from four randomly selected branches in the lower to mid canopy which were reached from a ladder. For each leaf, insect chewing damage was scored *in situ* as follows: (1) 0.1-5% leaf area damaged, (2) 6-25% leaf area damaged, (3) 26-50% leaf area damaged, (4) 51-75% leaf area damaged and (5) more than 75% of leaf area damaged. The number of leaves in each class was subsequently multiplied by the mid-point of the category and the values were summed to obtain an estimate of chewing damage per branch. These values, in turn, were averaged for all branches separately to obtain an estimate of percentage leaf area chewing damage per tree.

(d) Statistical analysis

Preliminary analyses have shown that the effects of tree species richness and browsing on tree growth and insect herbivory were similar across both years and seasons of sampling (results not shown). Therefore, for the sake of clarity, we report results from analyses on data pooled across all sampling periods. A linear mixed-effects modelling approach was used because it allows us to account for both temporal (repeated measures on the same trees in early and late summer of both 2010 and 2011) and spatial autocorrelation (multiple plots in each area) as well as permitting the inclusion of nested random-effect terms (Zuur *et al.* 2009). Prior to testing for interactive effects of moose browsing and tree species richness on birch, we tested whether moose browsing on birch was independent of tree species richness. Linear mixed models were used to check whether the two indices of browsing damage - the average intensity of moose browsing per plot and the proportion of birch trees browsed per plot - were affected by tree species richness. Both variables were log transformed and tested against tree species richness as a fixed factor; area and plot (nested within area) were used as random effects.

We initially ran separate linear mixed-effects models for each growth variable (tree height, DBH, crown projection) and insect chewing damage (log-transformed) with tree species richness as the only fixed effect, specifying area, plot and tree as nested random effects (i.e. Area/Plot/Tree). To test our first hypothesis that moose browsing influences tree species richness effects on tree growth (height, DBH or crown projection), we ran models that included as the fixed factors tree species richness, browsing (either presence/absence or intensity) and their interaction terms (i.e. richness x browsing) and used the same random effect structure. To test our second hypothesis that the responses

of insect herbivory to tree species richness are influenced by browsing, we repeated the same model with the log-transformed insect herbivory data as the response variable and the same fixed and random effects specified. Effects of tree species composition were tested in the 2- or the 3-species mixtures only as species composition of monocultures (B) and 5-species mixtures (BAPSL) did not vary. To test for species composition effects, the same models as for species richness effects were used, but tree species richness was replaced with tree species composition (i.e. composition x browsing). Finally, to examine whether insect chewing damage is affected by tree growth, we ran models with the same random effects structure but with either tree height, DBH or crown projection as the only fixed factor to avoid covariation with tree species richness, composition or browsing.

To assess differences in the structural or light environment for birch trees with increasing tree species richness, we compared the height of birch to other tree species in the study area across all levels of diversity. After excluding data from plots with no birch trees, we ran a similar model to determine the effect of tree species richness on the mean stature of all trees in a plot. In a separate analysis, we calculated mean birch height and mean neighbour height for each plot (excluding birch monocultures) to test whether heterospecific neighbour height varied with tree species richness and if birch height was predicted by neighbour tree height. As we detected a significant relationship between neighbour height and tree species richness, we excluded richness from the latter model, testing only whether the effect of neighbour height on birch height was dependent on the browsing status of birch. Finally, to test our hypothesis that tree diversity affects light availability, we examined the effects of tree species richness and composition on canopy cover around birch in 2014. As we did not discover any recent damage by moose to the experimental trees, we did not include the interaction with browsing in this model. All models specified area and plot as nested random effects, except those for canopy cover where only area was included as a random factor as there were no plot replicates available in 2014.

All data were analysed in R software version 2.15.2 (R Core Team 2012) using the *lmer* function in the *lme4* package to fit mixed-effects models. Tree species richness was treated as a continuous variable in all models, running both linear and second order polynomials that were ranked on the basis of their second-order Akaike's Information Criterion (AICc) obtained using the *AICcmodavg* package (Appendix 1, Supporting Information). Most models were a better fit with tree species richness as a linear variable

but models with birch growth were improved with richness as a polynomial (Table S1, Supporting Information). We report chi-squared and corresponding p-values from ANOVA of the best models using the car package (Fox & Weisberg 2011). Pairwise comparisons of means and of slopes for interactive effects of tree species richness and browsing intensity were calculated with the Bonferroni correction using R's *testInteractions* function in the phia package (de Rosario-Martinez 2013) but the pairwise comparisons between slopes were only possible for models on insect herbivory where tree species richness was included as a linear variable.

Results

During the course of the study in 2010 and 2011, 516 trees were monitored, 53% of which were browsed by moose. Tree species richness had no significant effect on the proportion of browsed birch trees per plot ($\chi^2=2.3$, $df=1$, $p=0.130$) or the average intensity of moose browsing on birch ($\chi^2=2.3$, $df=2$, $p=0.128$). Tree species composition also had no effect on browsing on birch in 2-species mixtures for both indices of browsing (proportion-browsed: $\chi^2=3.6$, $df=2$, $p=0.164$, average intensity: $\chi^2=3.5$, $df=2$, $p=0.175$) and in 3-species mixtures (proportion-browsed: $\chi^2=0.7$, $df=3$, $p=0.865$, average intensity: $\chi^2=2.6$, $df=3$, $p=0.459$).

Birch tree height, DBH, crown projection

When browsed and unbrowsed trees were analysed together, tree species richness had no significant effect on birch height ($\chi^2=4.22$, $df=2$, $p=0.121$). Moose browsing reduced birch tree height from 888 ± 7.6 cm on unbrowsed trees to 646 ± 12.8 cm irrespective of tree species richness (Table 1, Fig. 1a) and increasing browsing intensity also significantly reduced birch tree height (Table 1, Fig. 1b). The effect of tree species richness on tree height depended on browsing intensity (significant browsing intensity x species richness interaction, Table 1, Fig. 1b). Whilst the height of unbrowsed trees declined linearly with tree species richness, the height of trees with minor damage showed a hump-shaped relationship with plot richness. These trees with minor browsing were notably shorter than unbrowsed trees in monocultures and 2-species mixtures but were similar in height to unbrowsed trees in 3-species and 5-species (Fig. 1b). The height of moderately damaged trees also displayed a curvilinear relationship with tree species richness, increasing from monocultures to 3-species mixtures and levelling off in 5-species mixtures (Fig. 1b). In contrast, severely browsed trees were of similar heights across the species richness gradient (Fig. 1b).

Considering both browsed and unbrowsed trees together, we observed that tree species richness had no overall effect on birch DBH ($\chi^2=1.23$, $df=2$, $p=0.540$) or canopy projection ($\chi^2=1.41$, $df=2$, $p=0.495$). However, moose browsing had a negative effect, reducing DBH from $73\pm 0.8\text{mm}$ to $54\pm 1.2\text{mm}$ and crown projection from $259\pm 2.6\text{cm}$ to $222\pm 3.1\text{cm}$ (Table 1). Browsing intensity also modified the effect of tree species richness on both horizontal growth variables (Table 1). Trees with none or minor browsing damage displayed a positively-humped relationship with tree species richness, peaking at the 3-species level (Appendix 2, Figs S1-2, Supporting Information). DBH and canopy projection of moderately browsed trees increased with tree species richness but severely browsed trees were not affected by tree species richness.

In analysis of the effect of tree species composition, we observed significant differences between all 2-species mixtures for each tree growth variable (Table 1). Birch growth was highest for birch trees in B+A mixtures, intermediate in B+P and lowest in B+S mixtures (Fig. 2 and Appendix 2, Supporting Information). However, post-hoc comparisons were only significant for mean DBH between B+A and B+S plots ($\chi^2=6.6$, $df=1$, $p=0.030$). These changes in tree size between treatments at the 2-species level were independent of the presence and the intensity of browsing (Table 1, Fig. 2 and Figs S3-4, Supporting Information). Similarly, we observed no significant interaction between browsing and tree species composition in the 3-species mixtures and no significant differences were detected in birch height, DBH or crown projection in the 3-species mixtures (Table 1, Fig. 2 and Appendix 2, Supporting Information).

Neighbouring trees & canopy cover

Birch trees were the tallest of the five tree species in the experimental area regardless of plot species richness ($\chi^2=4279.9$, $df=4$, $p<0.0001$). The effect of plot species richness was significantly different between birch and other tree species ($\chi^2=33.3$, $df=8$, $p<0.0001$). Whilst the height of unbrowsed birch trees declined linearly with tree species richness, heights of alder and larch trees exhibited hump-shaped rather than linear relationships with tree species richness (growing tallest in 3-species mixtures); heights of pine and spruce trees were similar in all plots (Appendix 3, Fig. S5a, Supporting Information). Using data from birch-containing treatments only, we found that the mean stature of trees (irrespective of species identity) decreased with tree species richness ($\chi^2=28.4$, $df=2$,

$p < 0.0001$, Appendix 3, Fig S5b, Supporting Information) but did not vary with tree species composition ($p > 0.118$).

The average height of heterospecific neighbours in birch stands showed a hump-shaped relationship with tree species richness, with neighbour heights peaking in 3-species mixtures ($\chi^2 = 6.35$, $df = 2$, $p = 0.042$, Fig. 1a). In addition, we detected a significant positive linear relationship between the mean height of birch trees and their neighbours within a plot ($\chi^2 = 10.2$, $df = 1$, $p = 0.0014$) but found that this effect was independent of browsing presence/absence ($\chi^2 = 0.19$, $df = 1$, $p = 0.665$). However, the effect of neighbour height of birch growth became less apparent with increasing browsing intensity ($\chi^2 = 13.3$, $df = 3$, $p = 0.004$, Appendix 3, Fig S6 Supplementary Information). Although the heights of minor and moderately browsed birch trees increased with neighbour height, no relationship was observed for severely browsed trees (pairwise comparison of slopes of severely browsed and unbrowsed trees: $\chi^2 = 11.3$, $df = 1$, $p = 0.005$). Of all the birch trees monitored in this study, only severely browsed trees were similar in height to heterospecific neighbours ($\chi^2 = 50.6$, $df = 1$, $p = 0.635$). All other birch trees grew taller than heterospecifics ($p < 0.0001$).

In 2014, canopy cover around birch trees decreased linearly with tree species richness ($\chi^2 = 6.10$, $df = 1$, $p = 0.0135$, Appendix 3, Fig. S7 Supplementary Information) and varied with tree species composition in 3-species mixtures ($\chi^2 = 8.84$, $df = 3$, $p = 0.031$) but not 2-species mixtures ($\chi^2 = 1.72$, $df = 2$, $p = 0.423$). Canopy cover in 2-species mixtures was highest in B+A plots followed by B+P then B+S. In 3-species mixtures, pairwise comparisons revealed that canopy cover around focal birch trees was significantly higher in B+P+S compared to B+A+L mixtures ($\chi^2 = 8.55$, $df = 1$, $p = 0.021$, Appendix 3, Fig S7, Supporting Information).

Insect herbivory

When browsed and unbrowsed trees were analysed together, insect herbivory on birch was not affected by tree species richness ($\chi^2 = 0.158$, $df = 1$, $p = 0.691$). However, both the presence of browsing and increasing intensity of browsing significantly increased insect herbivore damage on birch (Table 2, Fig. 3). Presence of moose browsing reversed the direction of tree species richness effects on insect herbivory (Table 2, Fig. 3a). Unbrowsed trees experienced less chewing damage as tree species richness increased whilst insect chewing damage on browsed trees increased with tree species richness (Fig. 3a). When the intensity of browsing was taken into account, we observed that whilst

severely browsed trees experienced the highest insect chewing damage overall compared to unbrowsed trees (post-hoc pairwise comparison of means: $\chi^2=8.2$, $p=0.025$), the slope of tree species richness effects on herbivory was only significantly different between unbrowsed trees and trees with minor browsing damage (Fig. 3b, post-hoc pairwise comparison of slopes: $\chi^2=7.6$, $p=0.035$). The lack of significant differences between the slope of tree species richness effects on herbivory for unbrowsed trees and trees with moderate and severe browsing could be due to large variation in chewing damage on severely and moderately browsed trees within each tree species richness category. Herbivore damage on birch trees with minor and severe browsing increased with tree species richness but did not vary on moderately browsed trees (Fig. 3b). Interactive effects of tree species richness and browsing presence/absence on insect herbivory were still significant after the exclusion of severely browsed trees ($\chi^2=5.80$, $df=1$, $p=0.016$) and the exclusion of both severely and moderately browsed trees ($\chi^2=6.83$, $df=1$, $p=0.009$) thus, differential responses of browsed and unbrowsed trees were still evident in the presence of minimal browsing damage.

Tree species composition had no significant effect on insect chewing damage and did not significantly interact with browsing in either 2- or 3-species mixtures (Table 2, Fig. 4). Insect chewing damage was independent of birch tree height ($\chi^2=0.2$, $df=1$, $p=0.672$) and DBH ($\chi^2=1.7$, $df=1$, $p=0.192$) but significantly increased with crown projection ($\chi^2=6.64$, $df=1$, $p=0.010$).

Discussion

Although consumers are well known to affect ecosystem functioning and manipulate producer diversity, few studies have yet demonstrated the effects of consumer impact on diversity-function relationships. Most of these studies have been conducted in grasslands and aquatic systems with insects or microbes as primary consumers (Mulder *et al.* 1999; Naeem, Hahn & Schuurman 2000; Fox 2004; Schnitzer *et al.* 2011). To our knowledge, our study is the first to explore the interactive effects of mammalian herbivores and plant species richness and composition in established young forest as well as to assess effects of both presence/absence and the intensity of mammalian browsing. The results of this study support our hypothesis that winter browsing by moose may influence tree diversity effects on birch tree growth and resistance to insect herbivores. We observed that intensity of moose browsing not only altered the shape of the relationship between tree growth and tree species richness but also reversed the effects of tree species richness on insect

herbivores. Interestingly, the only two previous studies exploring interactions between effects of mammalian herbivores and plant diversity have shown that browsing effects enhance rather than counter the positive effects of plant species (Cook-Patton *et al.* 2014) and genetic diversity (Parker *et al.* 2010) on the performance of tree seedlings or herbaceous plants, respectively. Both Parker *et al.* (2010) and Cook-Patton *et al.* (2014) cite browsing selectivity and associational protection as the main mechanisms through which browsing shifted polyculture output to less palatable but high-performing species or genotypes. However, our study focussed on one tree species that was browsed with the same probability and intensity across all levels of diversity. Therefore, interactive effects of moose browsing and tree species richness on birch growth and insect herbivory cannot be explained by moose selective browsing or tree diversity effects on moose browsing. Rather we suggest that changes in the canopy cover and neighbouring tree heights along the species richness gradient influence birch responses to browsing and explain the patterns observed. Below we discuss these mechanisms in detail, explaining our results and the implications of our study for future biodiversity research.

Tree growth

We observed that browsing intensity modified the effects of tree species richness on all three growth variables: height, DBH and crown projection. Whilst the height of unbrowsed birch trees linearly declined with tree species richness, the DBH and crown projection of unbrowsed trees did not vary with tree species richness. However, for all growth variables, trees with minor or moderate browsing showed humped or positive curvilinear relationships to tree species richness, whilst severely browsed trees were of similar size regardless of plot species richness.

The observed interactions between effects of tree species richness and moose browsing cannot be attributed to an increase in the number of browsed birch trees or a higher intensity of moose browsing on birch in more species rich stands because, in accordance with previous studies on moose winter browsing in the Satakunta experiment (Vehviläinen & Koricheva 2006; Milligan & Koricheva 2013), we found that the proportion of browsed birch trees and the intensity of browsing on birch was independent of tree species richness or composition. In addition, as all birch trees in the experimental area were of equivalent size at planting, differences in birch growth cannot simply be attributed to moose preferentially targeting smaller trees. Although we have not experimentally manipulated moose browsing in our study, the cumulative character of

moose browsing and known tendency for moose to browse the same trees year after year (Bergqvist, Bergström & Edenius 2003) suggest that the observed differences in birch growth between browsed and unbrowsed trees are more likely to reflect the effects of repeated browsing over several years than moose preference for shorter, more accessible trees. While taller trees may eventually escape moose browsing, over 76% of the examined birch trees had foliage accessible to moose and the upper parts of the canopy might also have been reached by moose breaking main stems to feed on twigs that would otherwise be out of reach (Telfer & Cairns 1978).

We therefore suggest that the interaction between moose browsing and tree species richness effects could be explained, not by moose preferences, but by differential responses of birch trees to browsing at different levels of tree species richness. Birch is a shade-intolerant pioneer species and the tallest tree species in the Satakunta experiment. As tree species richness increases, so too does the abundance of shorter tree species, leading to the observed decline in the mean height of trees from birch monoculture to more species rich plots. The resulting increase in canopy openness with species richness detected in this study reduces the pressure on individual birch trees to overtop each other leading to shorter unbrowsed birch trees as tree species richness increases (Fig. 1a). Milligan & Koricheva (2013) found increasing moose browsing intensity with tree species richness at plot level and this might have also contributed to observed differences in canopy cover. In birch monoculture, stronger competition for light may also explain why even birch trees with minor browsing damage exhibit a significant reduction in growth relative to unbrowsed trees (Fig. 1b). Previous work by Danell, Huss-Danell & Bergström (1985) showed that birch trees demonstrate better compensatory growth following browsing by moose in open rather than in shaded habitats. As we observed a decline in canopy cover around birch trees with increasing tree species richness, we suggest that improved compensatory growth in more species rich stands may therefore explain why unbrowsed trees and trees with minor or moderate browsing exhibit increasingly similar growth at higher levels of species richness (Figs 1b).

Curvilinear relationships between the height of browsed birch trees and tree species richness (Fig. 1) appear to result from a combination of intra- and interspecific competition. As explained above, strong intraspecific competition for light and high canopy cover in birch monocultures limit re-growth of browsed birch trees in these stands due to shading from unbrowsed trees. However, we have also found that birch trees tend

to grow taller in the presence of taller heterospecifics (Fig. S6, Supporting Information), and the height of other tree species peaked in 3-species stands (Fig 1a), largely due to presence of larch (the second tallest tree species after birch) in 3-species mixtures, but not in 2-species mixtures. As a result, the hump-shaped relationship between neighbour heights and tree species richness (Fig. 1a) is mirrored by birch trees with minor browsing (Fig. 1b, S1-2, Supporting Information); their height peaks in 3-species mixtures due to competition with taller heterospecifics and lower canopy cover in these stands allowing better re-growth as compared to birch monocultures. For moderately browsed trees, increased re-growth in more open, species rich stands outweighs competitive interactions with shorter neighbours as they are not only shaded by unbrowsed trees but also by taller birch trees with minor browsing. The higher relative density of tall neighbours results in maximal re-growth occurring in the most species rich plots producing a saturating rather than a hump-shaped curve. As browsing intensity increases, more leading and lateral shoots are likely to be removed impeding both vertical and horizontal growth (Bergstrom & Danell 1987; den Herder *et al.* 2009; Speed *et al.* 2013) and thereby reducing the competitive ability of birch trees. Severely browsed trees, in particular, are more likely to suffer from multiple stem breakage by moose, which directly reduces tree height and suppresses growth (Rea 2011). Thus, the higher the degree of damage caused by moose, the higher the plot species richness required to mitigate the negative effects of browsing. However, for severely browsed trees, growth is affected so dramatically that trees might be overtopped by neighbouring heterospecifics and left unable to compensate for the damage even in 5-species mixtures.

Insect herbivore damage

The only study which has examined interactive effects of mammalian browsing and plant diversity on insects is by Parker *et al.* (2010), who found no interactions between effects of deer browsing and plant genetic diversity on vole and insect herbivory. Thus, our study is the first empirical demonstration of interactive effects of mammalian herbivory and plant species richness on insect herbivores.

Winter browsing by moose reversed the effects of tree species richness on insect herbivory from a negative relationship on unbrowsed trees (associational resistance) to a positive relationship (associational susceptibility, Fig. 3a). Despite large variations in chewing damage on moderately and severely browsed trees, contrasting responses to tree species richness were still apparent when only trees with minor browsing damage were

considered. The observed patterns cannot be attributed to changes in tree size as a result of browsing because insect herbivory was independent of birch tree height and DBH. Although insect herbivory was positively correlated with crown projection, this relationship cannot explain insect herbivore preference for browsed trees and interactive effects of browsing and species richness on insect herbivory because tree species richness had no effect on crown projection, and browsing reduced rather than increased crown projection. Instead, the above interaction is likely to be explained by differential regrowth of browsed trees at different species richness levels.

Moose browsing has been shown to stimulate changes in birch physiology and morphology, inducing compensatory growth of dormant buds to produce long shoots with more nutritious leaves than unbrowsed shoots (Danell & Huss-Danell 1985; den Herder *et al.* 2009). Previous studies have shown that the development of birch buds into long-shoots in response to browsing is more frequent in open rather than shaded habitats (Danell *et al.* 1985). As we observed a decrease in canopy cover with tree species richness, we propose that re-growth in the more open diverse stands is likely to be more vigorous than in the monocultures where browsed trees are shaded by their taller counterparts. In addition, as regrowth of birch may also require a higher intensity of browsing damage (Bergström & Danell 1995), stronger associational susceptibility may therefore occur with higher browsing intensity (Fig. 3b). The improved regrowth would therefore make browsed trees in species rich stands more attractive to chewing insects and reverse the effects of resource dilution on insect herbivore damage.

Species composition effects

Moose browsing was not found to interact with species composition effects on tree size or insect herbivory. However, birch height, trunk diameter and crown projection were all significantly higher in 2-species mixtures with nitrogen-fixing black alder than with Scots pine or Norway spruce (Table 1). Presence of nitrogen-fixing species in a mixture is known to have a positive effect on growth of non-fixing tree species (Piotto 2008). In contrast, no effect of tree species composition was observed for insect herbivory on birch. Given that three out of five tree species used in the Satakunta experiment are conifers, it is unlikely that any chewing herbivores would be able to feed on both birch and conifers, so lack of differences in insect herbivory on birch between mixtures with spruce or pine is not surprising. Although many chewing herbivores are able to feed on both birch and alder, alders were 4-5m shorter than birches in our experiment (Appendix 3, Fig. S5,

Supporting Information) and may therefore have been less apparent to chewers than birches, resulting in similar herbivory levels on birch in mixtures with alder and conifers.

Weak compositional effects suggest that the observed interactive effects of browsing and tree species richness were unlikely to be caused by moose modifying interactions among tree species. But rather, as the experiment used a substitutive design, the interactions may reflect differences in how browsed trees respond to decreasing birch density as tree species richness increased. In particular, reduced birch density likely explains the decline in canopy cover with tree species richness and concurrent changes in re-growth capacity. However, canopy cover also varied with tree species composition in 3-species mixtures and we observed that moose browsing had a negative rather than positive effect on insect herbivore damage in 2010/2011 in the same plots where canopy cover was highest (B+A in 2-species and B+P+S in 3-species mixtures). This observation further supports our hypothesis that interactive effects of browsing and tree diversity (both species richness and composition) result from differential re-growth responses driven by differences in the light environment.

Conclusions

With the majority of forests in the temperate and boreal region under intensive management, the increased provision of early successional stage forest and suitable winter forage has benefited populations of moose and other cervids (Lavsund, Nygrén & Solberg 2003; Côté, Rooney & Tremblay 2004). Thus, the role of mammalian herbivores as disturbance factors in forests is likely to become more important. In this study, the assessment of browsing by moose was conducted such that measurements reflected both old and recent damage. As moose are known to return to the same winter ranges (Sweanor & Sandegren 1989) and browse the same trees year after year (Bergqvist *et al.* 2003), our results reflect the cumulative impact of browsing damage over several years. Birch trees mediated the interaction between temporally separated herbivores such that browsing by moose in winter reversed the effect of tree species richness on insect herbivory the following summer. Although the observed change in magnitude of insect damage between browsed and unbrowsed trees was small, low levels of persistent insect damage (<2%) have been shown to reduce birch growth and fitness (Zvereva, Zverev & Kozlov 2012). Thus the effects of browsing on tree growth may be further compounded by increases in background insect herbivore damage.

Our study provides the first evidence that not only presence/absence but also intensity of mammalian browsing may modify the effects of tree species richness on tree growth and insect herbivory. This reinforces the conclusion by Parker *et al.* (2010) and Cook-Patton *et al.* (2014) that understanding the interactions between mammalian herbivores and plant diversity effects is very important for developing realistic predictions for the consequences of biodiversity loss. If we had ignored the browsing status of birch trees, we would have erroneously concluded that tree species richness overall has no effect on insect herbivory on birch and would have missed an important nonlinear responses of growth in browsed trees to species richness. We also suggest that variation in presence and intensity of mammalian browsing between different forest diversity experiments may at least partly explain conflicting results of existing studies. For instance, experimental studies of diversity-function relationships that exclude mammalian herbivores by fencing may be more likely to observe associational resistance to insect herbivores whereas unfenced studies may be more likely to show associational susceptibility. Moreover, fenced diversity experiments may also under- or overestimate (depending on the variable measured) the effects of forest diversity on tree growth. Differences in consumer impact among studies may thus potentially explain much of the observed variability in plant diversity effects on ecosystem functioning. Our results suggest that mammalian herbivores are key modifiers of ecosystem properties along the diversity gradient and should be included in future work on the impacts of biodiversity loss.

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Data Accessibility

All data for this paper are deposited in the Dryad Digital Repository: doi:[10.5061/dryad.fv090](https://doi.org/10.5061/dryad.fv090) (Muiruri *et al.* [2015](#)).

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Tables

Table 1. Factors affecting birch tree height, DBH and crown projection. Results from the best linear mixed-effects models with tree species richness as a second-order polynomial are shown. Separate models were run for the presence/absence and intensity of browsing. Significant effects are in bold text.

		Browsing: Presence/absence						Intensity							
		Height			DBH			Crown Projection		Height			DBH		Crown Projection
		df	χ^2	p	χ^2	P	χ^2	p	df	χ^2	p	χ^2	p	χ^2	p
All plots	(richness) ²	2	4.8	0.089	1.4	0.504	1.2	0.542	2	3.9	0.140	1.4	0.502	1.6	0.440
	browsing	1	52.3	<0.001	57.3	<0.001	34.0	<0.001	3	211.2	<0.001	96.4	<0.001	61.0	<0.001
	(richness) ² :browsing	2	2.5	0.281	3.3	0.195	1.4	0.508	6	28.5	<0.001	14.1	0.028	12.7	0.047
2-species mixtures	composition	2	6.2	0.045	7.8	0.020	7.6	0.022	2	4.9	0.087	6.2	0.044	6.1	0.048
	browsing	1	19.0	<0.001	6.5	0.011	6.0	0.014	3	69.5	<0.001	22.6	<0.001	12.4	0.006
	composition:browsing	2	0.3	0.848	0.9	0.627	1.1	0.577	6	5.8	0.451	9.5	0.147	11.4	0.077
3-species mixtures	composition	3	4.2	0.245	3.9	0.269	3.0	0.384	3	2.6	0.461	3.4	0.334	2.1	0.544
	browsing	1	25.2	<0.001	40.0	<0.001	19.6	<0.001	3	121.9	<0.001	67.3	<0.001	48.1	<0.001
	composition:browsing	3	1.6	0.660	1.3	0.731	2.66	0.447	9	6.5	0.690	3.8	0.924	10.3	0.330

Table 2. Factors affecting insect chewing damage on birch. Results from the best linear mixed-effects models with tree species richness as a linear variable are shown. Separate models were run for the presence/absence and intensity of browsing. Significant effects are in bold text.

		Browsing: Presence/absence			Intensity		
		df	χ^2	p	df	χ^2	p
All plots	richness	1	0.3	0.610	1	0.4	0.545
	browsing	1	5.7	0.017	3	11.0	0.012
	richness:browsing	1	7.4	0.007	3	7.9	0.048
2-species	composition	2	1.1	0.570	2	1.2	0.537
Mixtures	browsing	1	1.4	0.244	3	6.0	0.110
	composition:browsing	2	2.0	0.368	6	4.2	0.649
3-species	composition	3	2.7	0.444	3	2.7	0.444
Mixtures	browsing	1	0.9	0.337	3	3.1	0.378
	composition:browsing	3	1.5	0.683	9	8.8	0.452

Figure Legends

Figure 1. Relationships between birch tree height (cm) and tree species richness for (a) unbrowsed and browsed trees, and (b) different levels of browsing intensity. The relationships between tree species richness and overall birch tree height and mean height of heterospecific neighbours are also shown in (a). Lines represent the best fit with a polynomial function and mean heights (\pm SE) are plotted for each mixture.

Figure 2. Effect of tree species composition in 2-species (left side) and 3-species mixtures (right side) on birch tree height. Means (\pm SE) are given for (a) unbrowsed and browsed trees, and (b) different levels of browsing intensity. The monoculture (B) and 5-species mixture (BAPSL) are not shown as their compositions did not vary. B=silver birch, A=black alder, P=Scots pine, S=Norway spruce, L=Siberian larch.

Figure 3. Relationships between insect chewing damage (%) on birch and tree species richness for (a) unbrowsed and browsed trees, and (b) different levels of browsing intensity. The overall relationship between tree height and tree species richness is also shown in (a). Lines represent the best fit with a linear function and mean insect chewing damage (\pm SE) are plotted for each mixture.

Figure 4. Effect of tree species composition in each 2-species (left side) and 3-species mixture (right side) on insect chewing damage (%) on birch. Means (\pm SE) are given for (a) unbrowsed and browsed trees, and (b) different levels of browsing intensity. The monoculture (B) and 5-species mixture (BAPSL) are not shown as their compositions did not vary. B=silver birch, A=black alder, P=Scots pine, S=Norway spruce, L=Siberian larch.

Figures

Figure 1

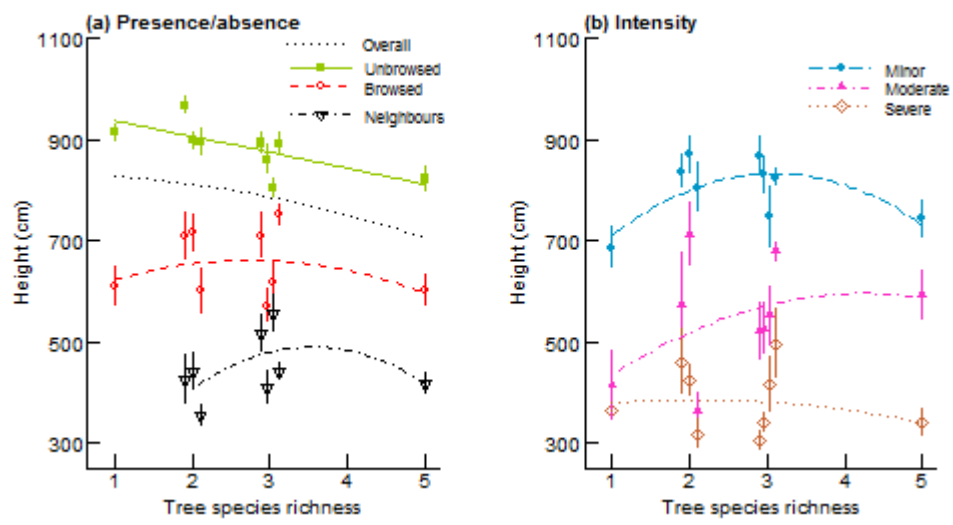


Figure 2

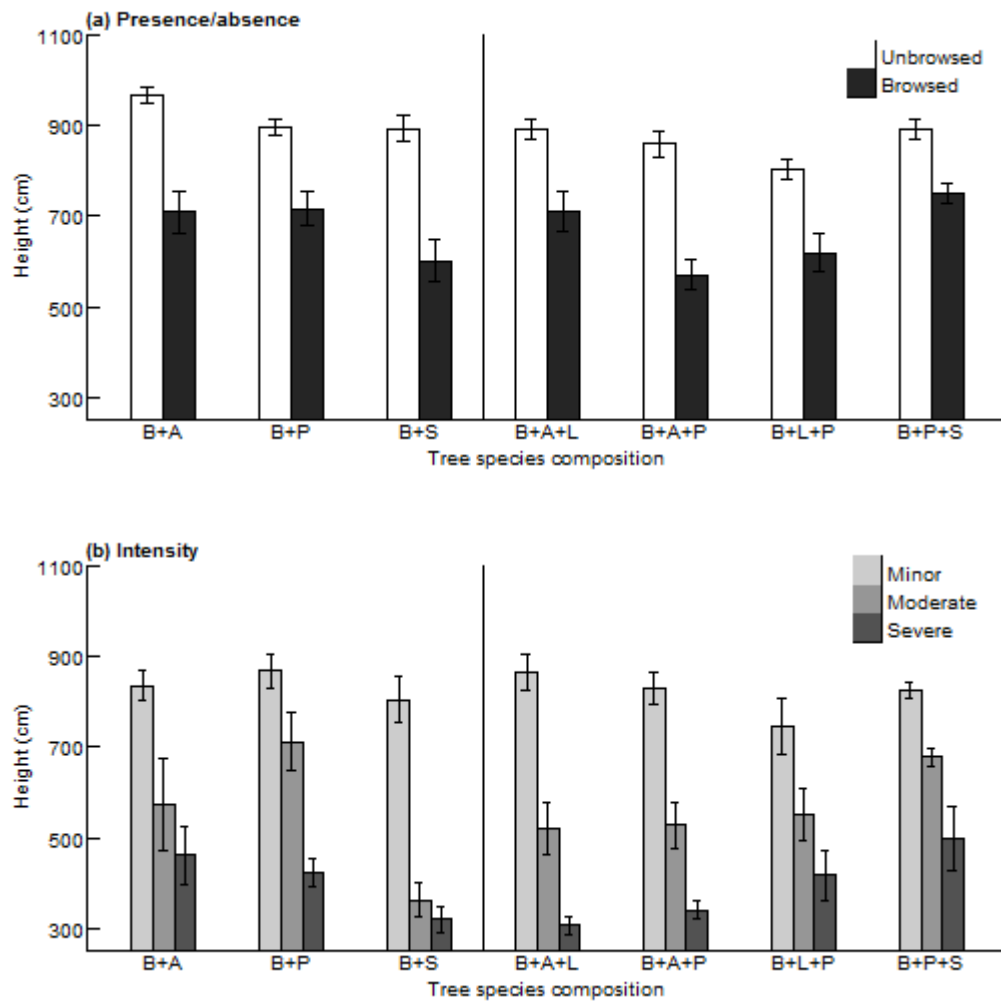


Figure 3

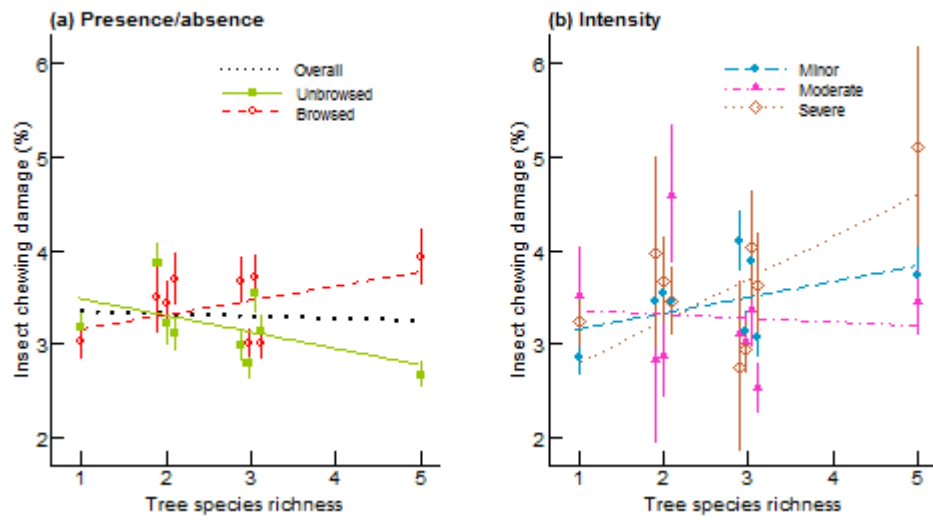
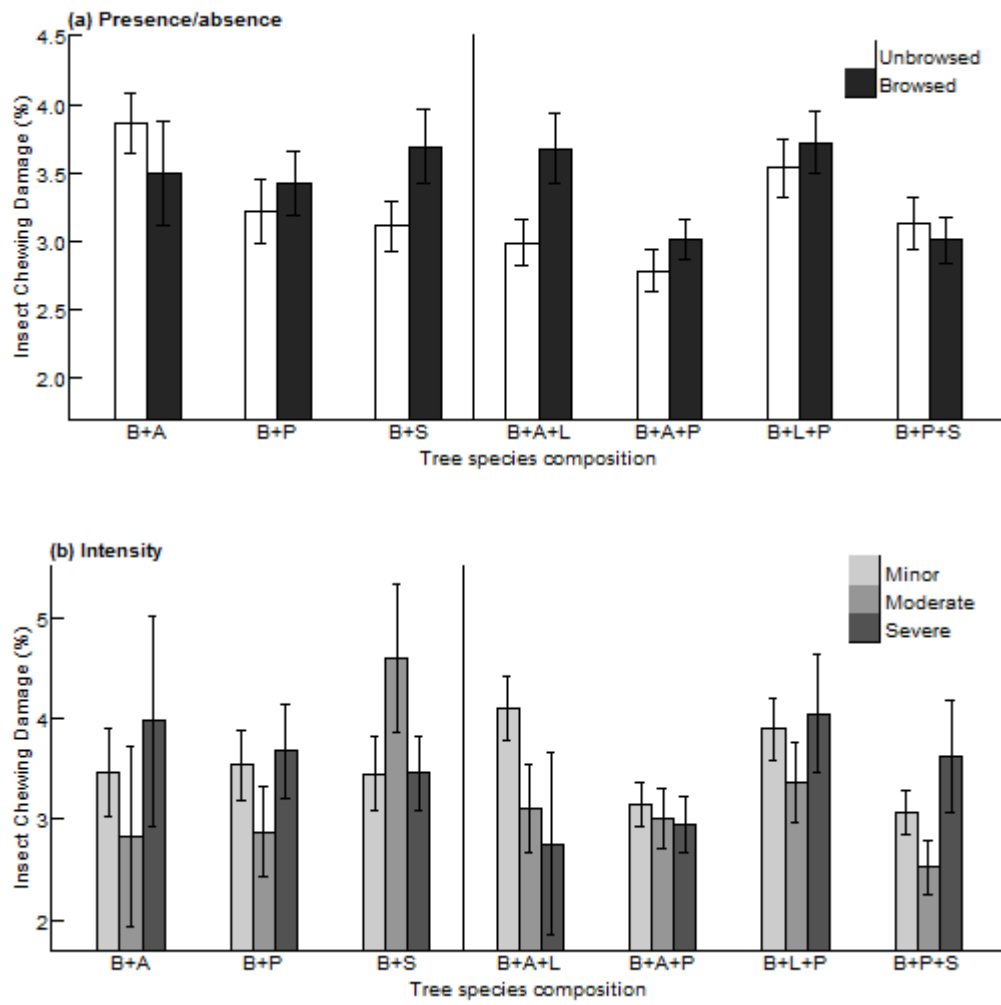


Figure 4



Moose browsing alters tree diversity effects on birch growth and insect herbivory

Evalyne W. Muiruri, Harriet T. Milligan, Simon Morath, Julia Koricheva

Online Supporting information for article in *Functional Ecology*

APPENDIX 1 – Model comparisons

All models were fit using restricted maximum likelihood estimations and residuals were checked for assumptions of normality and homoscedasticity. In the models that included tree species richness as a fixed effect, we ran the models twice with richness treated as either a linear or second-order polynomial variable. We then compared these models on the basis of their AICc (second-order Akaike's Information Criterion). This is a value used to compare models using the same response variable but different explanatory variables (Burnham & Anderson 2004). All models on browsing per plot, insect herbivory and canopy cover showed a considerably better fit with tree species richness as a linear variable (Table S1, $\Delta\text{AICc} > 5.9$). However, in models with birch growth variables, AICc values were lower when tree species richness was treated as a polynomial (Table S1, $\Delta\text{AICc} > 4$) indicating considerably less support for linear effects of tree species richness on birch growth (Burnham and Anderson 2004). The only exceptions were in models for tree species richness and browsing (presence/absence) effects on DBH and for tree species richness effects on canopy cover where the linear and non-linear models were equally supported (Table S1, $\Delta\text{AICc} < 2$) and we report statistics in the main text from the models with the lowest AICc value (non-linear for DBH model, linear for canopy cover) factor.

Table S1. Model comparisons where tree species richness was treated as either a linear or quadratic term. AICc values and corresponding Δ AICc are reported with the lowest AICc value, corresponding to the best model, shown in bold text. Where browsing is included as a fixed effect, models were run for presence/absence of browsing and repeated for the intensity of browsing.

	Browsing: Presence/absence		Intensity	
	AICc	Δ AICc	AICc	Δ AICc
Proportion browsed per plot ~ richness	-60.6	9.5		
Proportion browsed per plot ~ (richness) ²	-51.1			
Percentage damage per plot ~ richness	277.5	5.9		
Percentage damage per plot ~ (richness) ²	283.4			
Height ~ richness	13024.2	4.3		
Height ~ (richness) ²	13019.9			
Height ~ richness*browsing	12966.3	8.5	12768.4	30.5
Height ~ (richness) ² *browsing	12957.8		12738.4	
Diameter ~ richness	8097.3	0.7		
Diameter ~ (richness) ²	8096.6			
Diameter ~ richness*browsing	8045.0	1.1	8003.6	8.2
Diameter ~ (richness) ² *browsing	8043.9		7995.4	
Crown projection ~ richness	10923.9	3.1		
Crown projection ~ (richness) ²	10920.8			
Crown projection ~ richness*browsing	10885.5	4.7	10844.4	20.6
Crown projection ~ (richness) ² *browsing	10880.8		10823.8	
Insect chewing damage~ richness	2738.5	8.6		
Insect chewing damage~(richness) ²	2747.1			
Insect chewing damage~ richness*browsing	2740.2	15.5	2760.5	26.2
Insect chewing damage~(richness) ² *browsing	2755.7		2786.7	
Height of all species ~ richness*species	58620.1	1.6		
Height of all species ~ (richness) ² *species	58618.5			
Mean height in birch plots ~ richness	3753.3	6.1		
Mean height in birch plots ~ (richness) ²	3747.2			
Heterospecific neighbour height in birch plots ~ richness	1058.6	10.5		
Heterospecific neighbour height in birch plots ~ (richness) ²	1048.1			
Canopy cover ~ richness	2134.6	0.7		
Canopy cover ~ (richness) ²	2135.3			

APPENDIX 2 – DBH and Canopy Projection

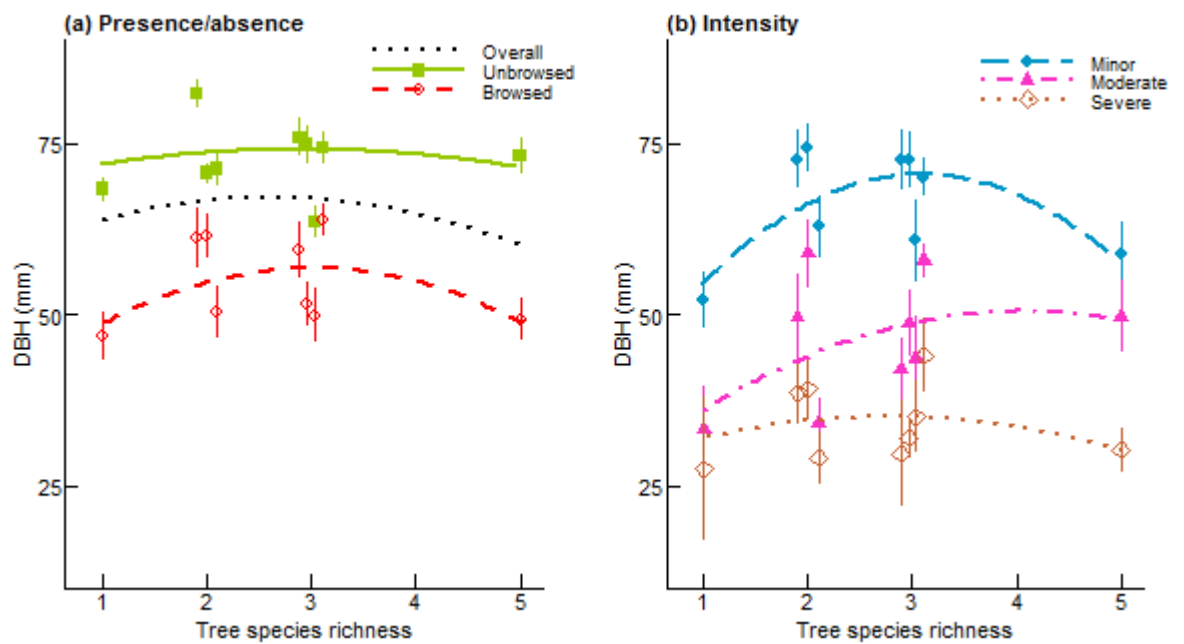


Figure S1. Relationships between birch trunk DBH (mm) and tree species richness for (a) unbrowsed and browsed trees and (b) different levels of browsing intensity. The overall relationship between tree height and tree species richness is also shown in (a). Lines represent the best fit with a polynomial function and mean DBH (\pm SE) are plotted for each mixture.

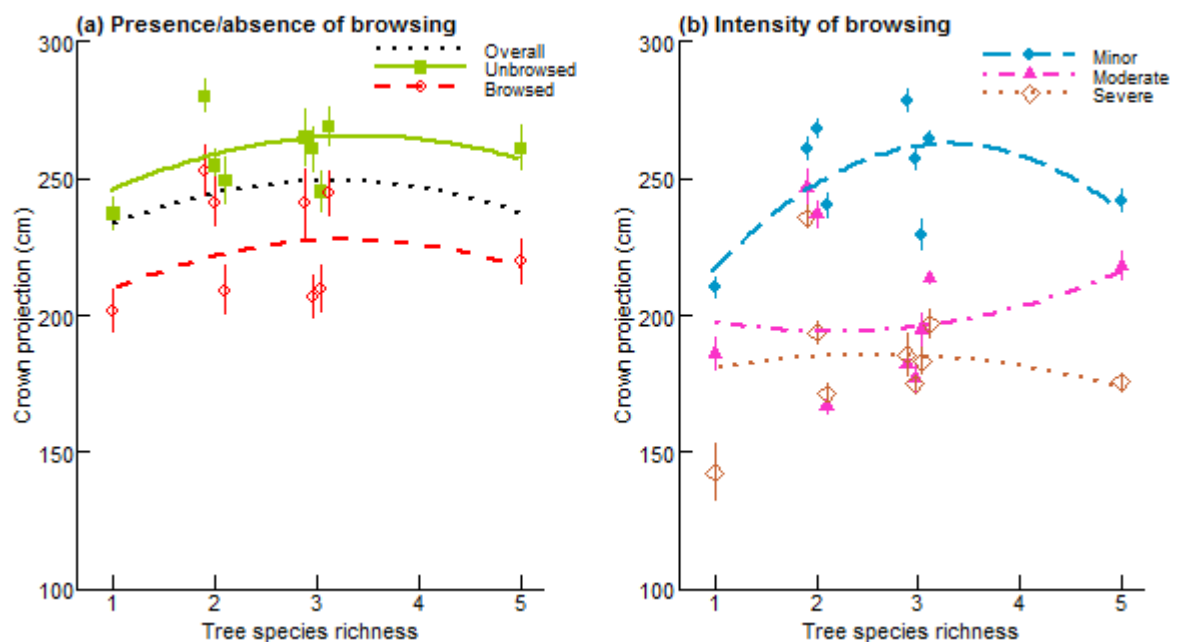


Figure S2. Relationships between birch crown projection (cm) and tree species richness for (a) unbrowsed and browsed trees and (b) different levels of browsing intensity. The overall relationship between tree height and tree species richness is also shown in (a). Lines represent the best fit with a polynomial function and mean crown projections (\pm SE) are plotted for each mixture.

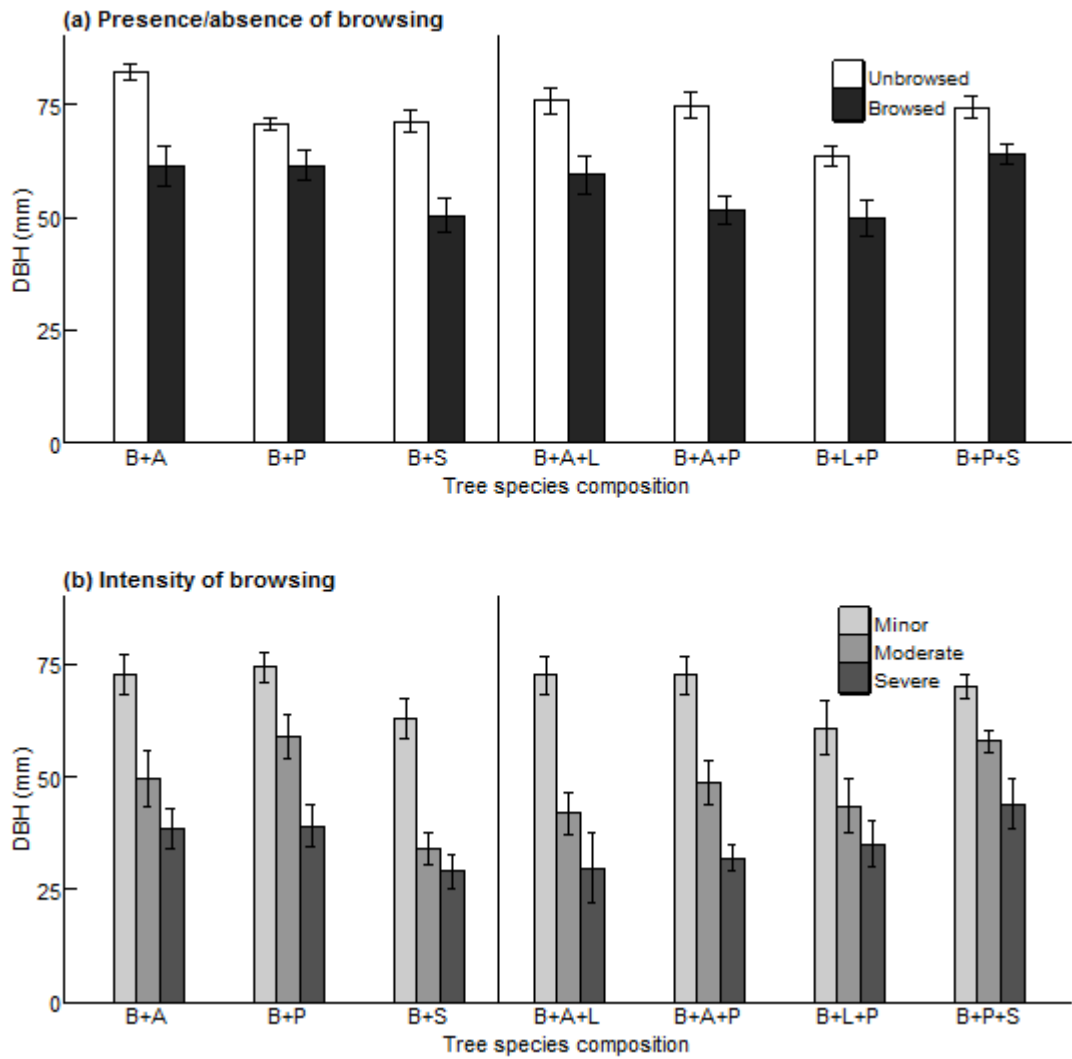


Figure S3. Effect of tree species composition in each 2-species (left side) and 3-species mixture (right side) on birch DBH. Mean (\pm SE) is given for (a) unbrowsed or browsed trees or, (b) increasing browsing intensity. The monoculture (B) and 5-species mixture (BAPSL) are not shown as their compositions did not vary. B=silver birch, A=black alder, P=Scots pine, S=Norway spruce, L=Siberian larch.

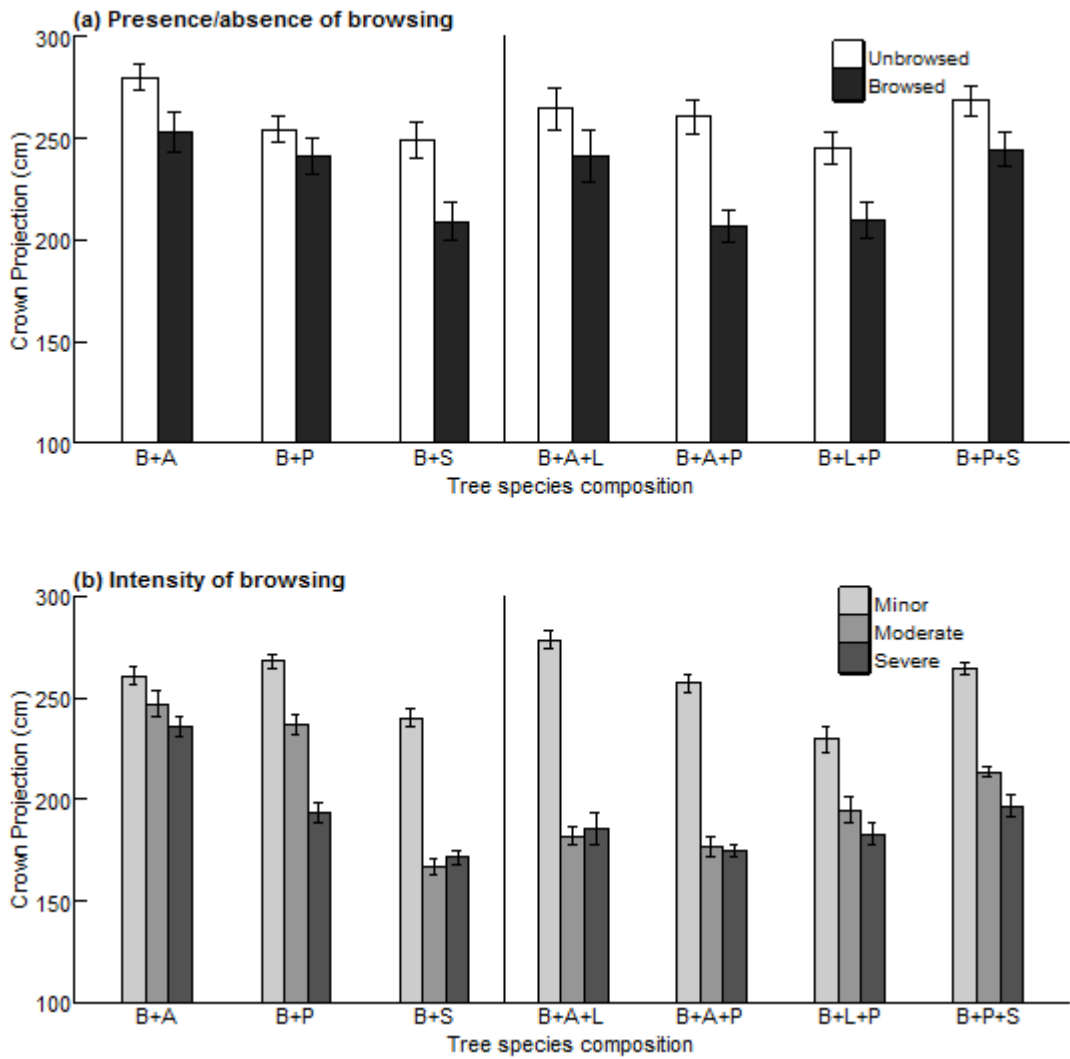


Figure S4. Effect of tree species composition in each 2-species (left side) and 3-species mixture (right side) on birch crown projection. Mean (\pm SE) is given for (a) unbrowsed or browsed trees or, (b) increasing browsing intensity. The monoculture (B) and 5-species mixture (BAPSL) are not shown as their compositions did not vary. B=silver birch, A=black alder, P=Scots pine, S=Norway spruce, L=Siberian larch.

APPENDIX 3 – Neighbouring trees & canopy cover

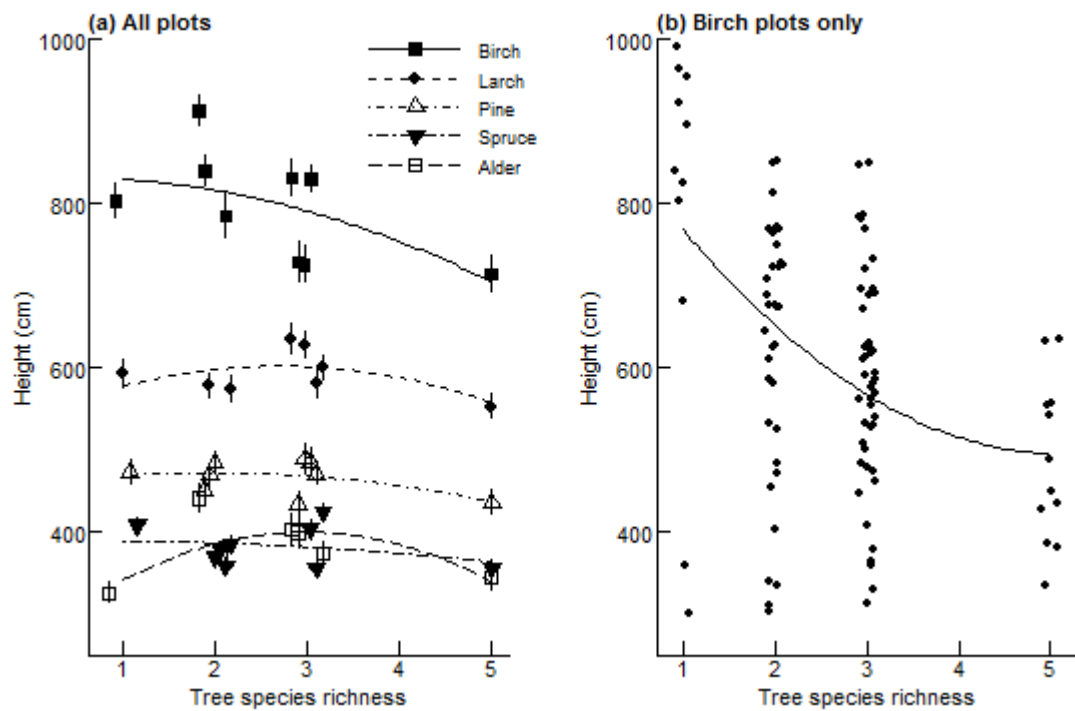


Figure S5. Effect of tree species richness on tree height in (a) all plots and (b) in birch plots only. Mean (\pm SE) tree heights are plotted in (a) for all trees including larch, pine, spruce and alder in each treatment. All pairwise comparisons are significant ($p < 0.0001$) except spruce and alder ($\chi^2 = 5.02$, $df = 1$, $p = 0.375$). Plot means are shown in (b) for all treatments in both years and a smoothed mean is drawn for the average height of trees in birch-containing stands.

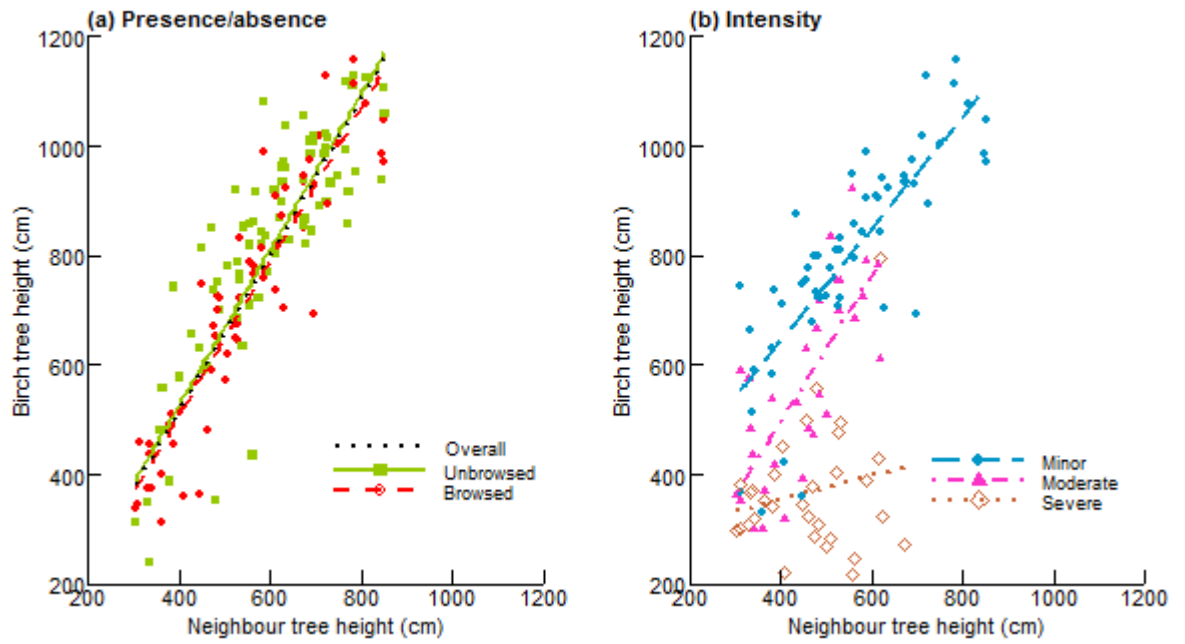


Figure S6. Effect of mean neighbour tree height on the height (cm) of (a) unbrowsed and browsed birch trees and (b) birch trees with different levels of browsing intensity. The overall relationship between neighbour and birch tree height is also shown in (a). Lines represent the best fit from a linear model.

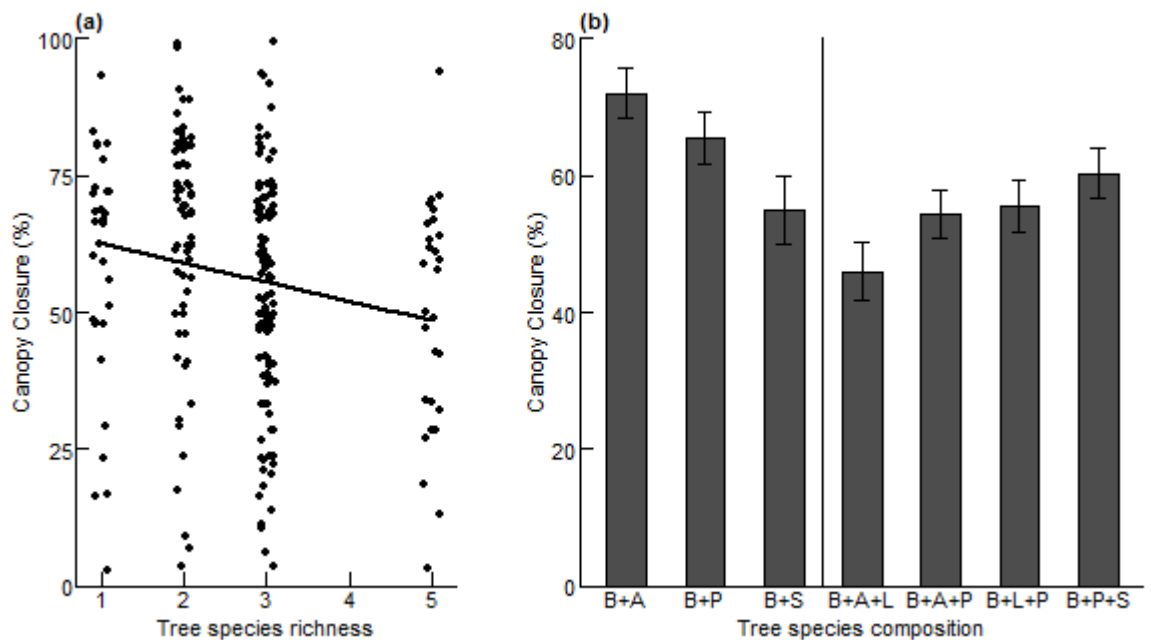


Figure S7. Effects of tree species richness (a) and composition (b) on canopy cover (%) around focal birch trees. A smoothed mean is drawn from a linear model in (a) and means (\pm SE) are plotted in (b) for each 2-species and 3-species mixture. The monoculture (B) and 5-species mixture (BAPSL) are not shown as their compositions did not vary. B=silver birch, A=black alder, P=Scots pine, S=Norway spruce, L=Siberian larch.

Chapter 6

Do birds see the forest for the trees? Scale-dependent effects of tree diversity on avian predation of artificial larvae.

Manuscript published in *Oecologia* with Electronic Supplementary Materials

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Do birds see the forest for the trees? Scale-dependent effects of tree diversity on avian predation of artificial larvae

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As part of her PhD thesis, Evalyne Muiruri proposed the idea to analyse effects of diversity on bird predation at two spatial scales and to test effects of structural heterogeneity. Here, we present the first empirical demonstration of scale-dependent effects of tree diversity on avian insectivory. Predation rates were increased with tree species richness at small but not large spatial scales and were independent of structural complexity in forest stands. Findings from this study present a timely contribution to the rapidly developing fields of biodiversity-ecosystem functioning and multi-trophic interactions.

Declaration of authorship: JK designed the study, KR and JK conducted fieldwork, EWM performed statistical analyses and wrote the manuscript. All authors have been involved in editing the manuscript drafts.

Abstract

The enemies hypothesis states that reduced insect herbivory in mixed-species stands can be attributed to more effective top-down control by predators with increasing plant diversity. Although evidence for this mechanism exists for invertebrate predators, studies on avian predation are comparatively rare and have not explicitly tested effects of diversity at different spatial scales, even though heterogeneity at macro- and micro-scales can influence bird foraging selection. We studied bird predation in an established forest diversity experiment in SW Finland, using artificial larvae installed on birch, alder and pine trees. Effects of tree species diversity and densities on bird predation were tested at two different scales: between plots and within the neighbourhood around focal trees. At the neighbourhood scale, birds preferentially foraged on focal trees surrounded by a higher diversity of neighbours. However, predation rates did not increase with tree species richness at the plot level and were instead negatively affected by tree height variation within the plot. The highest probability of predation was observed on pine, and rates of predation increased with the density of pine regardless of scale. Strong tree-species preferences observed may be due to a combination of innate bird species preferences and opportunistic foraging on profitable-looking artificial prey. This study therefore finds partial support for the enemies hypothesis and highlights the importance of spatial scale and focal tree species in modifying trophic interactions between avian predators and insect herbivores in forest ecosystems.

Keywords

Biodiversity and ecosystem functioning, insectivorous birds, insect pests, Satakunta forest diversity experiment, tri-trophic interactions

Introduction

Insect herbivores can have significant impacts on key ecosystem functions such as nutrient cycling, productivity and carbon sequestration (Metcalf et al 2014). These effects may be further compounded by losses in plant diversity, and many studies have shown that insect herbivore damage and abundance is higher in less diverse plant communities (associational resistance, Kaitaniemi et al. 2007; Jactel and Brockerhoff 2007; Barbosa et al. 2009). Root (1973) was the first to suggest that natural enemies of insect herbivores may drive the observed patterns of associational resistance by being more effective as predators in diverse plant communities compared to monocultures. This

prediction, termed the enemies hypothesis, was based on the observation that more species-rich habitats often support a higher diversity of prey species, provide refuges and offer additional resources such as pollen and nectar for invertebrate predators (Root 1973; Russell 1989). The enemies hypothesis has received much experimental scrutiny and support from studies in agricultural ecosystems and grasslands (Tonhasca 1993; Siemann et al 1998; Sobek et al 2009; Letourneau et al 2011; Straub et al 2014), however, fewer tests of this hypothesis have been conducted in forest ecosystems. These studies have produced mixed results with some reporting negative effects of tree diversity on predator effectiveness (Schuldt et al 2011; Zou et al 2013) and others showing stronger effects of tree species composition (Riihimäki et al 2005; Kaitaniemi et al 2007; Vehviläinen et al 2008), density (Sperber et al 2004; Schuldt et al 2008) or tree species identity (Sobek et al 2009) rather than tree species richness per se (Zhang and Adams 2011). Thus, more studies are required to better understand relationships between diversity and top-down control of insect pests in forest ecosystems.

An additional limitation of previous tests of the enemies hypothesis is that they have almost exclusively been performed for arthropod predators (Russell 1989; Andow 1991) even though insect herbivores are fed upon by both invertebrate and vertebrate predators (Letourneau et al 2009). Birds, in particular, have received little attention even though they are widely considered to be important control agents of insect pests in forest stands (Mäntylä et al 2011; Bereczki et al 2012) and can deliver a key ecosystem service (Whelan et al 2015). In addition, the diversity and abundance of avian predators has not only been shown to respond to increased structural and floristic diversity (MacArthur and MacArthur 1961; Bereczki et al 2014; Huang et al 2014), but also vary with densities of individual tree species (Wiens and Rotenberry 1981; Mason 1997). Nevertheless, very few studies have examined effects of tree diversity on avian predation in forest ecosystems (Giffard et al 2012; Poch and Simonetti 2013; Giffard et al 2013; Bereczki et al 2014) and of these studies, none have directly tested the effects of increasing tree species richness or explored the effects of tree species composition and individual tree species densities on bird predator effectiveness. Recent work by Poch and Simonetti (2013) has shown that higher bird predation occurs in structurally complex forest plantations with more developed and diverse understorey. Therefore, just as top-down control by arthropod predators was hypothesised to increase with plant diversity and associated structural complexity (Root 1973), positive effects of diversity on bird predation may be driven by increased structural complexity rather than diversity per se.

Finally, the vast majority of studies testing the enemies hypothesis have done so at a single spatial scale and thus, we still know little about the scale at which the enemies hypothesis applies (Zhang and Adams 2011). Spatial scale is believed to be an important determinant of the strength of prey-predator interactions (Langellotto and Denno 2004; Gripenberg and Roslin 2007) and effects of plant diversity on these relationships may vary with spatial scale (Bommarco and Banks 2003). The review by Bommarco and Banks (2003) found that effects of plant diversity on the effectiveness of arthropod predators was strongest in small ($<16\text{m}^2$) plots, intermediate in intermediate-sized ($28\text{-}196\text{m}^2$) but absent in large ($>256\text{m}^2$) plots; these patterns could be due to easier redistribution of predators to the more favoured mixed stands in experiments of smaller plot size. For birds, a similar pattern may arise as, even though they can travel further than arthropods in search of prey, their capacity for direct assessment of insect abundance is greater within a microhabitat compared to larger spatial scales (Strode 2009). Optimal foraging theory predicts that natural selection favours behaviours that maximise energy intake per unit time spent foraging (Stephens and Krebs 1986). Thus, even in the absence of detectable prey, birds may have evolved to use alternative indicators such as the signs of leaf damage (Heinrich and Collins 1983) or chemical cues from insect-damaged plants (Mäntylä et al 2008; Amo et al 2013) to locate insect-rich trees within small spatial scales. At larger scales, the patchy distribution of many insect herbivores may drive forage selection towards patches where the host plants of their favoured prey dominate as a strategy to minimise search time (Arvidsson and Klaesson 1986; Mason 1997). Therefore, different factors might act as drivers of bird predation depending on the spatial scale of observation. As habitat selection by birds is understood to occur in a hierarchical manner (Johnson 1980), a combination of different drivers at each spatial scale may act to maximise overall foraging efficiency, in accordance with optimal foraging theory.

The primary goal of this study was to experimentally test whether bird predation increases with tree species richness, as predicted by the enemies hypothesis. We used an established forest diversity experiment in SW Finland to examine the effects of tree species diversity, prey availability and habitat structural heterogeneity on bird foraging preferences. To assess bird predation, artificial larvae (modelled from plasticine) were installed on alder, birch and pine trees in stands of varying tree species diversity. This technique of presenting artificial prey has risen in popularity in prey-predator studies as it facilitates field assessment of relative predation rates (Howe et al 2009) and the marks left by

predators in plasticine are identifiable to a coarse taxonomic level (Low et al 2014). In keeping with most tests of the enemies hypothesis, we explored how bird predation rates vary with diversity at plot level and test whether these effects are mediated by changing structural complexity. Secondly, we examine avian predation responses to tree diversity at finer spatial scales, focussing on the local neighbourhood of a focal tree. Finally, we compare the importance of natural herbivore abundance and damage on experimental trees relative to the importance of neighbourhood diversity in predicting bird predation rates.

Methods

Study site and design

The study was carried out at the Satakunta forest diversity experiment established in 1999 in south-western Finland. The experiment consists of three separate areas with 38 plots (20 x 20 m) in each area. Diversity treatments represent monocultures and 2-, 3-, and 5-species combinations of the following five tree species: Scots pine (*Pinus sylvestris*); Norway spruce (*Picea abies*); Siberian larch (*Larix sibirica*); silver birch (*Betula pendula*); and black alder (*Alnus glutinosa*). Each plot consists of 13 rows with 13 trees planted at 1.5m intervals (total 169 trees) and the position of different tree species in mixed stands was randomised. Replanting of species was carried out in 2000 for all plots and, in 2001 for plots where mortality exceeded 10%. Other than the manual removal of naturally regenerating woody vegetation in spring 2010, no management interventions have been used in the Satakunta experiment since planting.

In the present study, we used two out of the three experimental areas (area 1, 61°42'N, 21°58'E and area 3, 61°40'N, 21°42'E) and focussed on three focal tree species: pine, birch and alder. These species were chosen as they host caterpillar larvae (e.g. *Epirrita autumnata* Borkhausen. on birch and alder and, *Neodiprion sertifer* Geoff. on pine) that could easily be modelled from plasticine. The other species present in the study areas are attacked mostly by small sucking insects (aphids or adelgids) which might be considered less profitable prey (Naef-Daenzer et al 2000) and therefore receive less bird predation compared to caterpillars. We therefore selected trees for this experiment from the seven treatments containing pine, birch or alder: three monocultures (pine, birch and alder), two 2-species mixtures (pine + birch, birch + alder), one 3-species mixture (pine + birch + alder) and the 5-species mixture (pine + birch + alder + spruce + larch). There were two replicates of each treatment per area but no pine-alder combination was present in the

original experimental design so only two out of the three possible 2-species mixtures were available for this study. For each plot, six trees were selected within the interior, avoiding selection of adjacent trees and substituting tree species in mixtures such that six trees were sampled in monocultures, three trees per species were sampled in 2-species mixtures, and two trees per species were sampled in 3- and 5-species mixtures. Insect herbivore abundance and damage were assessed on experimental trees in early June 2013 prior to the start of the bird predation experiment. Pine trees in the study area have been observed to have very low herbivore densities (J. Koricheva, unpublished data) and hence assessment of insect herbivores was only performed on birch and alder trees. We assessed the presence/absence of exposed chewing insects, the abundance of concealed-feeder insects (e.g. leaf miners or rollers) and the extent of leaf area damaged (%) by defoliating insects on the same focal trees used in the predation experiment (Online Resource 1).

Bird predation assessment and surveys

The experiment was timed to coincide with the peak bird nesting period when insects compose the majority of the diet fed to nestlings (Naef-Daenzer et al 2000). On 8th and 9th June 2013, five artificial larvae were installed on each experimental tree (30 larvae per plot). The larvae were modelled from odourless, light green plasticine to an approximate size of 2-3cm in length and 3-4 mm in diameter (Fig. 1a). The size of the larvae was chosen based on previous studies using artificial caterpillars on the same tree species (Mäntylä et al 2008) and to represent the average size of larvae of the autumnal moth (*E. autumnata*) and the European pine sawfly (*N. sertifer*), both of which are common defoliators on alder, birch and pine trees in Finland. Artificial larvae were installed on branches which were 1.5-3m above ground, corresponding to the mid canopy for alder and pine and to the lower canopy for birch. Five larvae per tree were distributed between different branches from all sides of the canopy to avoid systematic differences in sun/shade exposure, and secured to a branch using metal wire (diameter 0.35mm). Following installation, the condition of the artificial larvae was checked five times: 3, 6, 9, 11 or 12 and 15 or 16 days after installation. Predation attempts by birds were recorded on larvae if they exhibited marks that were consistent with bird pecking damage and could not otherwise be explained (e.g. not a scratch by a nearby branch, Fig 1b, c). Although wood ants are highly abundant in the study area, we found no evidence of ants preying on the artificial larvae in this experiment or when the artificial larvae were offered to wood ants near their nests. After each larva was checked, those that were damaged were either remoulded where possible or replaced.

To identify possible culprits for predation on artificial larvae, surveys of bird territories were conducted shortly after dawn on 22nd May, 7th June and 12th June 2013. The surveyor (KR) walked a path which ensured good coverage of the experimental areas and recorded breeding bird species on the basis of sightings, singing or other acoustic encounters. As the home range size of birds in the experiment exceeded a single plot, only the overall diversity and abundance of individual bird species was assessed in each study area. To determine which species were preying on artificial larvae, we installed camera traps around three pine trees in one pine monoculture in June 2014 as this was the plot where the highest predation rates were observed the previous summer. About 30 artificial caterpillars per tree were installed and camera traps were in operation for one month.

Tree height variation

In order to examine the role of structural complexity on bird predation, we used tree height measurements from 2011 where ten randomly chosen trees of each species were assessed in each experimental plot (Muiruri et al 2015). For each plot, we calculated a mean and standard deviation of tree heights, using data for all species combined in mixtures. The coefficient of variation (referred to as Tree Height Variation from here on) was then calculated by dividing the standard deviation by the mean tree height per plot. Plots with higher tree height variation are considered to be more structurally complex with greater heterogeneity in vertical canopy structure.

Statistical analysis

To investigate the effects of tree diversity on bird predation, we used four continuous variables as predictors of bird damage to artificial larvae in each plot: (1) tree species richness, (2) proportion of pine, (3) birch and (4) alder trees out of the total number of live trees in a plot (hereafter referred to as pine, birch or alder density, respectively). In addition, for plot level analysis only, we used a fifth variable – tree height variation – as a predictor of bird predation. Although tree species compositions were similar at plot and neighbourhood scales, randomised species arrangements at planting and tree mortality resulted in some focal trees with different proportions or fewer heterospecific neighbours than expected in the 2-, 3- or 5-species mixtures or, no neighbours at all. Thus, as damage to larvae was recorded on individual trees, we also gathered information on the neighbourhood of each experimental tree, recording variables 1-4 from the eight trees surrounding the focal tree.

We used generalised linear mixed-effects models (GLMM) to account for the nested design and to allow specification of an error family. In order to assess bird predation, we modelled the probability of predation of artificial larvae as a bounded binary response variable (larvae damaged/not damaged) with a binomial error structure, specifying a random error structure with individual trees nested within plot (plot/tree). Due to the regularity of the experimental design at Satakunta the variables (1-4) describing tree diversity at the plot and neighbourhood scales were not independent, therefore, no more than one of the four diversity variables could be included in models at any one time. However, initial models were run to determine whether effects of all diversity variables (from either plot or neighbourhood level) on bird predation were dependent on the study area used or the time of observation (area x time x variable [1-4]). As neither area nor time significantly interacted with any diversity variable at either spatial scale, we performed all subsequent analysis on predation across all sampling points, retaining area as a fixed factor in subsequent models (not in interaction with other variables) to account for natural variation in bird activity between the two study areas.

For analysis at plot level, we first calculated the mean number of larvae damaged per plot and ran generalized linear-models (GLM) with the binomial response variable (mean number of larvae damaged per plot, number of larvae installed in each plot) against area and each variable (1-4) or tree height variation separately (ie. area + variable[1-4] or area + tree height variation). A similar approach was used at the neighbourhood scale, this time running models for neighbourhood-level predictor variables (1-4) or tree species identity. Binomial GLMMs were run for the response variable (number of damaged larvae per tree, number of larvae installed per tree) against each individual predictor variable using plot as a random factor. Tree species composition effects were assessed for mixtures at each species richness level separately but as no significant differences were detected at either plot or neighbourhood level, we focus our discussion on variables 1-4.

In order to determine which variables (at plot or neighbourhood level) best predicted bird predation, we ranked univariate models on the basis of their AICc values (second-order Akaike's Information Criterion) and used Akaike weights as an indicator of the weight of evidence in support of a given model, compared to other candidate models (Anderson et al 2001; Burnham and Anderson 2004). Models with lower AICc values were therefore considered to be better than other candidate models but could only be termed the single

best model if the Akaike weight exceeded 0.9 (Anderson et al 2001). Where Akaike weights did not exceed this value, differences in the AICc were used as an indicator of the relative likelihood of the model. Candidate models differing least from the best model ($\Delta\text{AICc} \leq 2$) are considered to be well supported but those differing most ($\Delta\text{AICc} \geq 10$) can be omitted (Burnham and Anderson 2004). In addition to model comparison, we also calculated R^2 values to estimate the variance explained by fixed factors only (R^2_m) or, both fixed factors and random factors together (R^2_c) (Nakagawa and Schielzeth 2013).

Different bird species might exhibit foraging preferences for individual tree species or the insect prey they host (Holmes and Robinson 1981; Gabbe et al 2002). Therefore, we ran similar analysis for each tree species separately to test the relative importance of components of neighbourhood diversity in determining bird predation rates. In particular, for birch and alder trees, we use AICc weighing to determine whether predation rates are driven more by changes in neighbourhood diversity (variables 1-4), natural insect abundance (both exposed and concealed insects) or insect herbivore damage (understood to enhance bird predation rates). Further GLM and GLMM models were used to determine the effect of plot and neighbourhood diversity variables (1-4) on tree height variation and insect herbivore damage (log transformed) respectively. Effects of diversity on the presence/absence of exposed chewing and the abundance of concealed-feeding insects on birch and alder were also examined using GLMMs with a poisson error distribution specified for count data. All statistical tests were conducted in R software version 2.15.2 (R Core Team 2012) using the lme4 package (Bates et al 2012). Model residuals were examined for homogeneity of variance and we report AICc and Akaike weights from the MuMIn package as well as Chi-squared and corresponding p-values from ANOVA using the car package (Fox and Weisberg 2011).

Results

Bird species present in the study area

A total of 19 different bird species and 140 bird territories were recorded during all three bird surveys (Online Resource 2). Of all the bird species present, willow warblers (*Phylloscopus trochilus* L.) were the most abundant in both experimental areas and across all censuses, occupying 40% of all observed territories (Online Resource 2). Other common bird species in the study areas included chaffinches (*Fringilla coelebs* L., 10% of observed territories), robins (*Erithacus rubecula* L., 6% of territories), garden warblers (*Sylvia borin* Bodd. 6% of territories), and lesser whitethroats (*Sylvia curruca* L., 5% of

territories). At the start of the predation experiment, both the diversity and abundance of birds were similar in the two experimental areas. Nine bird species were observed in 29 territories in area 1 and eleven species in 27 bird territories were observed in area 3.

Patterns of bird predation

The number of attacks on artificial larvae increased linearly over time ($\chi^2=48.0$, $df=1$, $p<0.001$). This pattern was more pronounced in area 1 than in area 3 (time x area: $\chi^2=34.6$, $df=1$, $p<0.001$) with overall number of attacks being higher in area 1 ($\chi^2=11.3$, $df=1$, $p<0.001$). However, despite these patterns, no significant two-way or three-way interactions were detected between time, area and each of the four main diversity variables at either spatial scale (Online Resource 3). Similarly, effects of tree height variation at the plot level and tree species identity on bird predation were independent of area or time (Online Resource 3). Therefore, we conducted all subsequent analysis on the total number of larvae damaged per tree across all sampling points and excluding interaction terms with 'area' in further models.

Throughout the experiment, we observed that while artificial larvae on birch or alder usually received single beak marks (Fig. 1b), larvae on pine frequently exhibited multiple beak marks (Fig. 1c) and were occasionally detached or missing entirely from the wire installation. Video footage from trap cameras from June 2014 showed a great tit (*Parus major*) pecking repeatedly at an artificial larva on pine, suggesting that great tits, possibly together with other Parid species, may have been responsible for the heavy damage on the artificial larvae on pine.

Plot-level analysis

Bird predation was not significantly affected by plot tree species richness (Fig. 2a, Table 1) but decreased with tree height variation within a plot (Fig. 2a inset, Table 1). The densities of pine, birch and alder had opposite effects on bird predation (Fig. 3a, Table 1). The number of larvae damaged significantly increased with the density of pine but decreased with increasing proportions of birch or alder (Fig 3a, Table 1). Although tree height variation increased with plot species richness ($F=12.6$, $df=1$, $p=0.001$), it did not depend on densities of alder ($F=0.9$, $df=1$, $p=0.362$), birch ($F=1.5$, $df=1$, $p=0.234$) or pine ($F=0.2$, $df=1$, $p=0.667$). Model comparisons based on AICc identified the density of pine as the variable best accounting for bird predation at the plot level compared to other predictor variables and explained the most variance (Table 1). The second-ranked

predictor was birch density but as $\Delta AICc > 10$, this model had essentially no support compared to the top model with pine density.

Neighbourhood-level analysis

Bird predation on artificial larvae significantly increased with species richness of the neighbouring trees (Fig 2b, Table 1). Some experimental trees in alder monocultures had no neighbours as a result of tree mortality. However, even after exclusion of these trees from the analysis, tree species richness still had a significant positive effect on the total number of larvae damaged per tree ($\chi^2=4.8$, $df=1$, $p=0.028$). Similar to the plot-level analysis, the probability of predation decreased with a higher proportion of alder and birch among the neighbouring trees but increased with pine density (Fig. 3b, Table 1).

Regardless of tree species diversity, tree species identity had a significant effect on the number of larvae damaged per tree (Table 1). Of the 551 damaged larvae, 358 (65%) were on pine trees (222 from pine monocultures), 129 (23%) on birch and 64 (12%) on alder (all *post hoc* pairwise comparisons significant, $p < 0.001$). In model comparisons, the single best explanatory variable for the number of artificial larvae damaged per tree was the species identity of the focal tree, explaining the most variance (highest R^2_m value) compared to any other model (Table 1). However, responses to diversity did not differ between the three species (tree species identity x richness: $\chi^2=0.5$, $df=2$, $p=0.769$, tree species identity x pine density: $\chi^2=2.1$, $df=2$, $p=0.356$). Only the effects of birch and alder density varied between the three focal tree species. Increasing birch density in the neighbourhood had a strong negative effect on predation rates on pine trees but only weak negative effects on predation on birch and alder (tree species identity x birch density; $\chi^2=6.3$, $df=2$, $p=0.042$, Fig 3b). At the same time, predation of artificial larvae on birch trees decreased with alder density but no relationship was observed for predation on pine or alder focal trees (tree species identity x alder density; $\chi^2=11.0$, $df=2$, $p=0.004$, Fig 3b). However, this pattern might be partially attributed to the fact that we did not have any plots with a pine/alder two-species combination so proportions of alder around pine trees rarely exceeded 33% (Fig. 3b).

Tree species-specific analysis

Bird predation on pine trees did not vary significantly with tree species richness or the density of alder in the neighbourhood (Fig. 2b, 3b, Table 1). However, the number of damaged larvae increased with the density of pine in the neighbourhood and declined

with the density of birch (Fig 3, Table 1). In model comparisons, the neighbourhood density of pine emerged as the best predictor of bird predation on larvae installed on pine, closely followed by the density of birch in the neighbourhood ($\Delta\text{AICc} < 2$, Table 1). For artificial larvae on either birch or alder trees, bird predation appeared to increase with both neighbourhood species richness and pine density and decrease with birch or alder density (Fig 2b and 3b). However, neither the diversity variables nor insect herbivore damage or the abundance of concealed feeding insects significantly predicted predation of artificial larvae on birch and alder (Table 1). Predation of artificial larvae on alder was independent of the presence/absence of exposed chewing insects but, on birch trees, predation was higher when exposed chewing insects were present (Table 1, Online Resource 4). Model comparison ranked the presence of exposed chewing insects as the most important determinant of predation on birch, followed by alder density ($\Delta\text{AICc} < 2$, Table 1). In contrast, for alder trees, even the abundance of concealed-feeding insects, which was identified as the best explanatory variable had a weak but non-significant (negative) effect on the number of larvae damaged on alder trees (Table 1, Online Resource 4).

Natural insect herbivory on birch and alder

In a comparison of natural herbivory between the two broadleaved species, the percentage leaf area damage was significantly higher on birch compared to alder trees ($\chi^2=24.8$, $df=1$, $p < 0.001$), but the presence of exposed insects or the abundance of concealed insect herbivores did not differ between the two species ($p \geq 0.531$). Tree species richness surrounding focal trees also had no significant effect on initial insect herbivore damage ($p \geq 0.180$) or the presence of exposed chewing insects ($p \geq 0.918$) on either tree species. However, the abundance of concealed insects was reduced with increasing neighbourhood tree species richness on birch ($\chi^2=4.5$, $df=1$, $p=0.033$) but not on alder ($\chi^2=2.5$, $df=1$, $p=0.111$). Neighbourhood densities of alder, birch or pine had no effect on insect herbivore damage, the abundance of concealed insects or the presence of exposed insect herbivores ($p \geq 0.295$).

Discussion

The results of our study provide partial support for the enemies hypothesis as we found that bird predation increased with tree species richness at the neighbourhood scale. However, effects of tree species richness were scale-dependent and absent at the plot level. To our knowledge, this is the first demonstration of differential responses of avian

predators to forest diversity at two different spatial scales. Use of model prey in this experiment permitted a standardised, rapid assessment of relative predation rates across the diversity gradient and between different tree species (Howe et al 2009). Although natural prey offer more complex sensory cues compared to artificial larvae, the same number of identical green and odourless artificial larvae were installed in each plot and, as such, we consider that their use could not have modified natural bird behaviour in a way that would affect conclusions with respect to effects of tree diversity, structural heterogeneity or spatial scale.

Effects of tree species richness at different spatial scales

Variable effects of diversity on predation at different spatial scales have previously been observed for arthropod predators. Plant-insect-predator interactions have been found to be stronger at small spatial scales (Langellotto and Denno 2004; Gripenberg and Roslin 2007) and the positive effects of plant diversity on top-down control by arthropod predators might even disappear at larger spatial scales (Bommarco and Banks 2003). Bommarco and Banks (2003) attributed the disappearance of plant diversity effects on arthropod predators at larger spatial scale to more effective re-distribution of arthropod predators in smaller experimental plots. However, birds are far less limited by dispersal distances than arthropod predators and can easily seek out preferred forage habitats further afield. Even during the breeding season when bird foraging occurs largely near the nest site, home range sizes of birds still exceed the area of a single plot (Online Resource 2).

When the enemies hypothesis was first proposed, Root (1973) suggested that stronger top-down control in diverse habitats is mediated by increased structural complexity where more niches were available for predators to exploit. This mechanism was supported by Poch and Simonetti (2013) who showed that bird predation rates were higher in more structurally complex forest plantations that had a higher abundance and diversity of woody species in the understorey. However, we found that, despite increased structural complexity with tree species richness, bird predation decreased with increases in tree height variation (Fig. 2a, inset). Although greater structural complexity may enhance the number of niches a predator can exploit, prey might be better concealed, increasing search time. As a result, structurally complex habitats may be considered less suitable foraging locations. For example, willow warblers, the most common bird species in the study area, have been shown to establish territories more frequently in stands where trees are of a

similar size (Stostad and Menéndez 2014). Therefore, structural heterogeneity may reduce rather than enhance bird predation independently of plot species richness. As predator responses to structural complexity have been shown to change in magnitude but not direction across spatial scales (Langellotto and Denno 2004), bird predation at the neighbourhood level is unlikely to increase with structural heterogeneity within the microhabitat. Thus, structural complexity can explain neither tree species richness effects at plot and neighbourhood levels nor differential responses to tree species richness between the two scales.

Instead, scale-dependence of tree species richness effects on bird predation may result from differences in prey visibility that manifest themselves only at fine spatial scales. Bird predation rates on insects have been shown to increase where plant cover is reduced (Groner and Ayal 2001). As tree species richness increases, presence of tree species differing in growth rates and foliage structure may result in less horizontal canopy space used and thus, a more open canopy (Lang et al 2011). This has previously been shown in the Satakunta experiment, with canopy cover around birch trees decreasing with tree species richness (Muiruri et al. 2015). As a result, artificial larvae may be more visible to birds when the focal trees are surrounded by heterospecifics but these effects are likely to be restricted to small spatial scale and unlikely to manifest at plot level. Improved visibility of insect prey on trees in more open forest stands may present a key advantage as palatable caterpillars may be visually more cryptic or have more cryptic behaviour compared to unpalatable insect prey, hiding amongst foliage and feeding in such a way as to minimise their apparent damage (Heinrich and Collins 1983). Thus, neighbourhood species richness effects on avian predation may be driven by differences in the light environment minimising search time and the energetic costs of foraging.

At the plot level, the capacity for direct visual assessment of prey is hampered and birds may instead rely on other indicators of a suitable foraging patch such as the presence or absence of host tree species of their favoured prey (Wiens and Rotenberry 1981; Mason 1997). As insect prey abundance can vary significantly over space and time, insectivorous birds often have to visit different parts of the environment continually to assess prey availability to the detriment of immediate foraging efficiency (Smith and Dawkins 1971). However, with the use of different cues within each spatial scale, insectivorous birds might be able to efficiently explore the landscape, concentrating their searches on selected patches for visible and easily accessible prey. This strategy would enable birds to exploit

new resources as soon as they become available, minimising the time spent locating insect prey while maximising food intake for adults and nestlings in accordance with the optimal foraging theory (Stephens and Krebs 1986).

Effects of tree species density and identity

Strong foraging preference of insectivorous birds for certain tree species have been well documented in forests (Holmes and Robinson 1981; Gabbe et al 2002; Strode 2009). In this experiment, we observed that predation was consistently higher on artificial larvae installed on pine than on birch and alder. Moreover, different beak marks on damaged artificial caterpillars indicated that different bird species were responsible for predation on pine and the broadleaf tree species. Individual pecks on caterpillars installed on birch and alder (Fig. 1b) were likely to be caused by small passerines such as the willow warblers, the most abundant bird species in the study area. In contrast, the multiple large beak marks found on artificial larvae on pine trees (Fig. 1c) were likely caused by the great tits, as confirmed by the camera trapping. This generalist insectivorous bird has been shown to preferentially forage on pine trees (Eeva et al 1997) and is known to be a highly innovative, opportunistic forager capable of social learning (Aplin et al 2015). Artificial larvae used in this experiment may have presented a new and attractive resource for breeding birds which often try to find the largest, most profitable prey for their nestlings (Diaz et al 1998; Naef-Daenzer et al 2000; Hino et al 2002) regardless of nutritional quality (Brodmann and Reyer 1999). Opportunistic pecking by seed-eating birds would also be consistent with damage seen on artificial larvae (Fig. 1c) as they may have stronger beaks to pry seeds out of cones (van der Meij and Bout 2004).

Tree species-specific differences in bird predation rates may also be driven by different properties of pine compared to birch or alder. For example, the low complexity of pine canopy relative to broadleaved trees may increase the accessibility and visibility of artificial prey enhancing predation of artificial larvae on pine (Šipoš and Kindlmann 2013). At the same time, a higher colour contrast between the light green of the artificial larvae and foliage may make artificial prey more conspicuous to birds on the darker pine foliage compared to birch and alder. However, as larvae were placed on branches rather than on leaves, contrasts between model prey and bark in both colour and texture might be just as important as foliage colour, if not more so. Thus, differences in predation on artificial larvae between the three focal tree species would be difficult to predict based on background matching alone.

Regardless of scale, increases in pine density (and reduced birch and alder density) consistently increased the probability of predation on artificial larvae (Fig. 2). Passerine birds often conduct concentrated searches for prey within microhabitats (Naef-Daenzer and Keller 1999) so any trees neighbouring pine may also be more susceptible to attack by virtue of their proximity and those neighbouring birch or alder, less so. However, insectivorous birds may also return repeatedly to profitable patches (Naef-Daenzer and Keller 1999) and this might explain why predation of artificial larvae increased during the experimental period. Experiments using the same technique of model prey over the same duration usually find that predation increases initially then decreases as birds learn that the artificial prey offer no nutritional reward (Mäntylä et al 2008). We hypothesise that the continuous increase in predation in this experiment was due to increased recruitment of 'naïve' birds from outside the study area. In particular, as birds might develop a search image for a given prey item during feeding (Tinbergen 1960), the newly-fledged birds of early broods observed outside experimental plots may be responsible for the continued increase in predation rates.

Effects of insect damage and natural prey abundance

We hypothesised that focal trees with more insect herbivore damage or a higher abundance of insect prey might experience higher predation rates. However, contrary to previous work showing that birds prefer to forage on insect-damaged trees (Mäntylä et al 2008; Amo et al 2013), leaf area damage by insect herbivores had no effect on predation rates on either birch or alder. Similarly, despite evidence suggesting concealed insects are under intense bird predation (Xiong et al 2010), we also observed no effect of concealed insect herbivore damage on the probability of larval attack on birch or alder trees. This is perhaps not surprising as, although concealed-feeding insects are sedentary and therefore potentially easy targets for avian predators, the concealed insects measured in this experiment (leaf rollers, folders and miners) are quite small (<10mm) and the difficulty of localising prey within shelters also increases search and handling time for birds for little reward in return. The only indication that density-dependent predator-prey interactions occurred in this experiment was found on birch where predation was higher on trees initially infested with exposed chewing insects (Online Resource 4). However, this could not explain the effects of tree species richness on bird predation because there was no significant difference between natural herbivory on birch trees surrounded by birches or by other tree species.

Conclusions

In this study we have shown that, in accordance with the enemies hypothesis, bird predation rates increase with tree species richness but only at the small spatial scale. However, contrary to Root's predictions, our findings suggest that positive relationships between tree diversity and bird predation are not due to increased structural complexity of a forest stand but rather due to improved ability for prey assessment. With the economic benefits of birds coming under scrutiny (Whelan et al 2015), our findings not only show that birds contribute a key ecosystem service but their regulation of insect pests might be dependent on species richness at fine spatial scales only. Together with the strong tree-species foraging preferences apparent in this experiment, this suggests that greater control of insect pests by insectivorous birds may be achieved by introduction of preferred tree-species and planting a mix of species together rather than patches of individual species in production forests.

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Table

Table 1. Models describing the probability of bird attack to artificial larvae. Response variables were either at plot or neighbourhood level and variables were introduced separately into models with study area as the only other fixed factor (omitted here for clarity). Models were ranked on the basis of their AICc, where $\Delta AICc \leq 2$ indicate almost equivalent models, and the Akaike weights indicate the weight of evidence for a model relative to all candidate models. R^2 values are given for GLM models at plot level and both marginal (R^2_m , i.e. for fixed effects) and conditional (R^2_c i.e. for both fixed and random effects) R^2 values are reported for GLMM models at neighbourhood-level. To explore species-specific responses, we ran all neighbourhood models of predation on each tree species separately.

Spatial Scale	Variable	χ^2	df	p	AICc	$\Delta AICc$	Weight	R^2_m (R^2_c)
Plot	Pine density	40.0	1	<0.001	116.1	0.00	1	0.19
	Birch density	21.0	1	<0.001	133.6	17.51	0	0.17
	Alder density	10.5	1	<0.001	142.2	26.18	0	0.14
	Tree height variation	4.3	1	0.038	147.6	31.53	0	0.11
	Tree species richness	0.0	1	0.964	152.0	35.91	0	0.09
Neighbourhood (All)	Tree species identity	50.4	2	<0.001	660.5	0.00	1.00	0.19 (0.37)
	Pine density	25.2	1	<0.001	691.5	31.00	0.00	0.20 (0.34)
	Tree species richness	6.2	1	0.013	707.3	46.81	0.00	0.14 (0.38)
	Birch density	4.5	1	0.034	709.3	48.84	0.00	0.14 (0.35)
	Alder density	4.0	1	0.044	709.6	49.16	0.00	0.14 (0.36)
(Pine only)	Pine density	9.8	1	0.002	237.2	0.00	0.68	0.29 (0.51)
	Birch density	9.4	1	0.002	238.7	1.58	0.31	0.25 (0.44)
	Alder density	0.0	1	0.828	248.0	10.79	0.00	0.18 (0.46)
	Tree species richness	0.0	1	0.983	248.0	10.84	0.00	0.18 (0.46)
(Birch only)	Exposed chewing insects	5.2	1	0.022	255.2	0.00	0.46	0.14 (0.31)
	Alder density	3.6	1	0.056	256.3	0.56	0.27	0.14 (0.31)
	Birch density	1.9	1	0.167	257.9	2.20	0.12	0.14 (0.39)
	Pine density	0.1	1	0.742	260.1	4.34	0.04	0.12 (0.30)
	Tree species richness	0.0	1	0.845	260.2	4.40	0.04	0.12 (0.29)
	Concealed insects	0.0	1	0.898	260.2	4.41	0.04	0.12 (0.30)
	Insect herbivore damage	0.0	1	0.825	260.2	4.43	0.04	0.12 (0.23)
(Alder only)	Concealed insects	3.1	1	0.076	153.3	0.00	0.33	0.29 (0.35)
	Insect herbivore damage	2.4	1	0.124	154.0	0.67	0.24	0.29 (0.35)
	Tree species richness	1.1	1	0.304	155.2	1.85	0.13	0.27 (0.37)
	Birch density	1.0	1	0.326	155.8	2.50	0.10	0.27 (0.31)
	Alder density	0.3	1	0.578	156.4	3.07	0.07	0.28 (0.33)
	Pine density	0.0	1	0.825	156.6	3.26	0.07	0.27 (0.32)
	Exposed chewing insects	0.0	1	0.825	156.5	3.16	0.06	0.26 (0.31)

Figure Legends

Fig. 1 Artificial larvae secured to tree branches showing (a) no damage, (b) single beak mark and (c) multiple pecks by birds.

Fig. 2 Bird predation responses to tree species richness (a) within a plot and (b) in the neighbourhood around a focal tree. Lines represent the best fit with a linear function and the number of larvae damaged by birds (mean \pm SE) are plotted for each tree species composition in (a) and for individual tree species in (b). The effect of tree height variation on the number of larvae damaged at the plot level is shown inset. Trees with no immediate neighbours were assigned a tree species richness level of zero.

Fig. 3 Bird predation responses to densities of pine, birch and alder either (a) within a plot or (b) in the neighbourhood around a focal tree. Solid lines represent the best fit with a linear function across all plots in (a) and for all focal trees in (b). Separate lines are also drawn in (b) for each of the three focal tree species: pine, birch and alder.

Figures

Figure 1

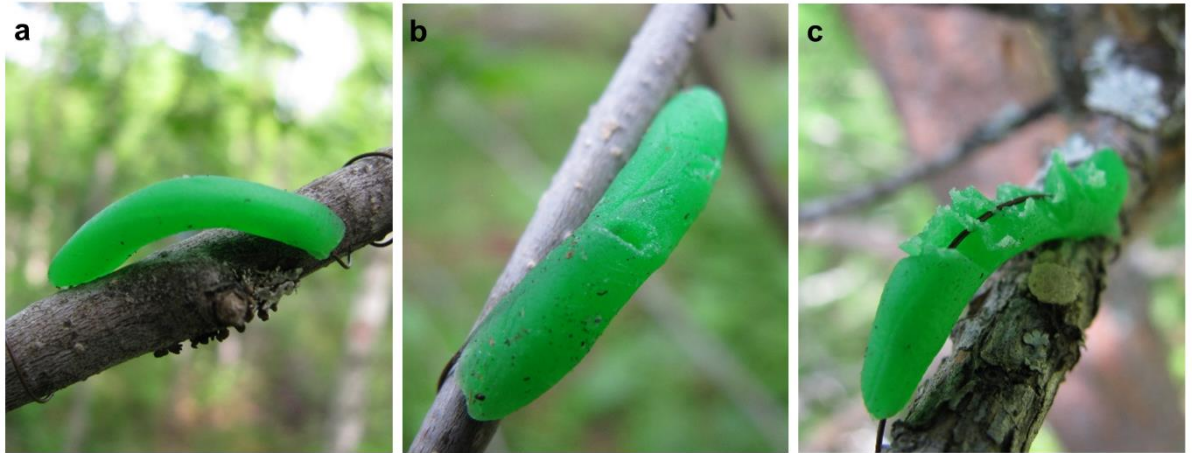


Figure 2

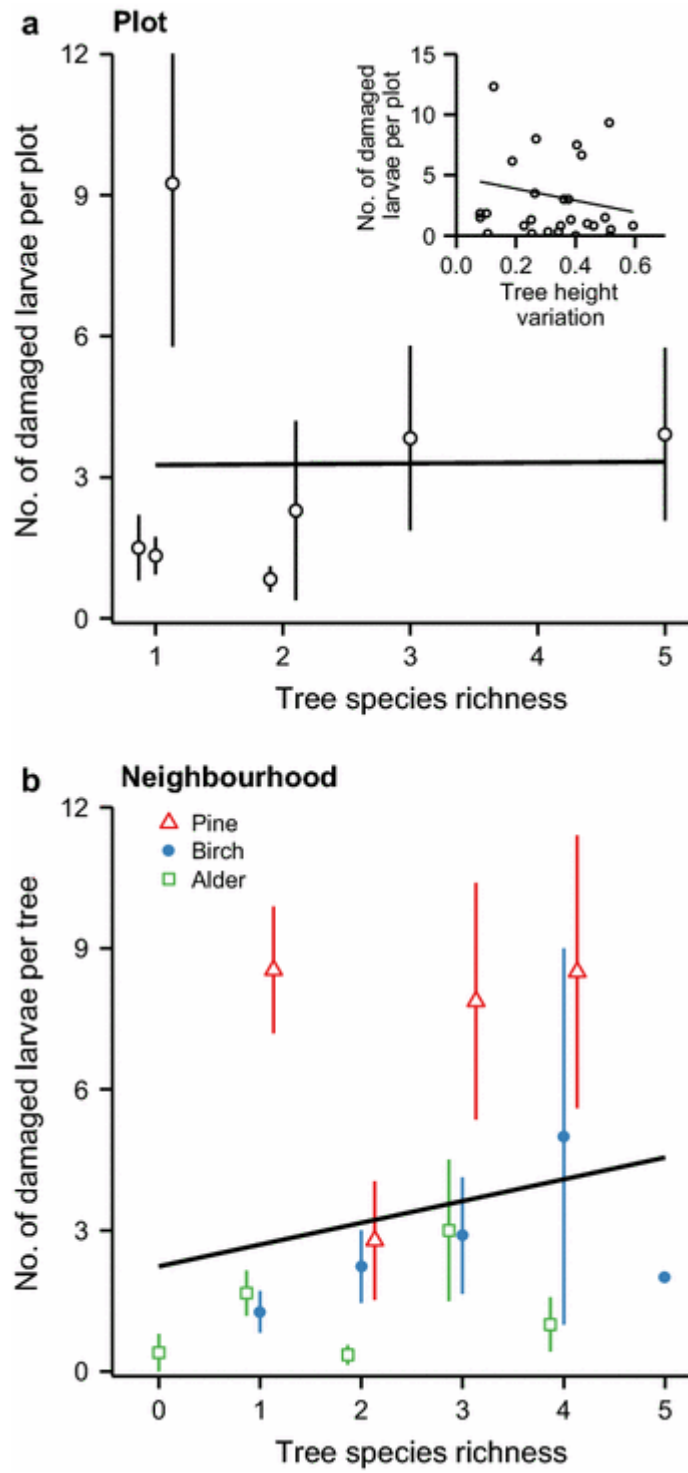
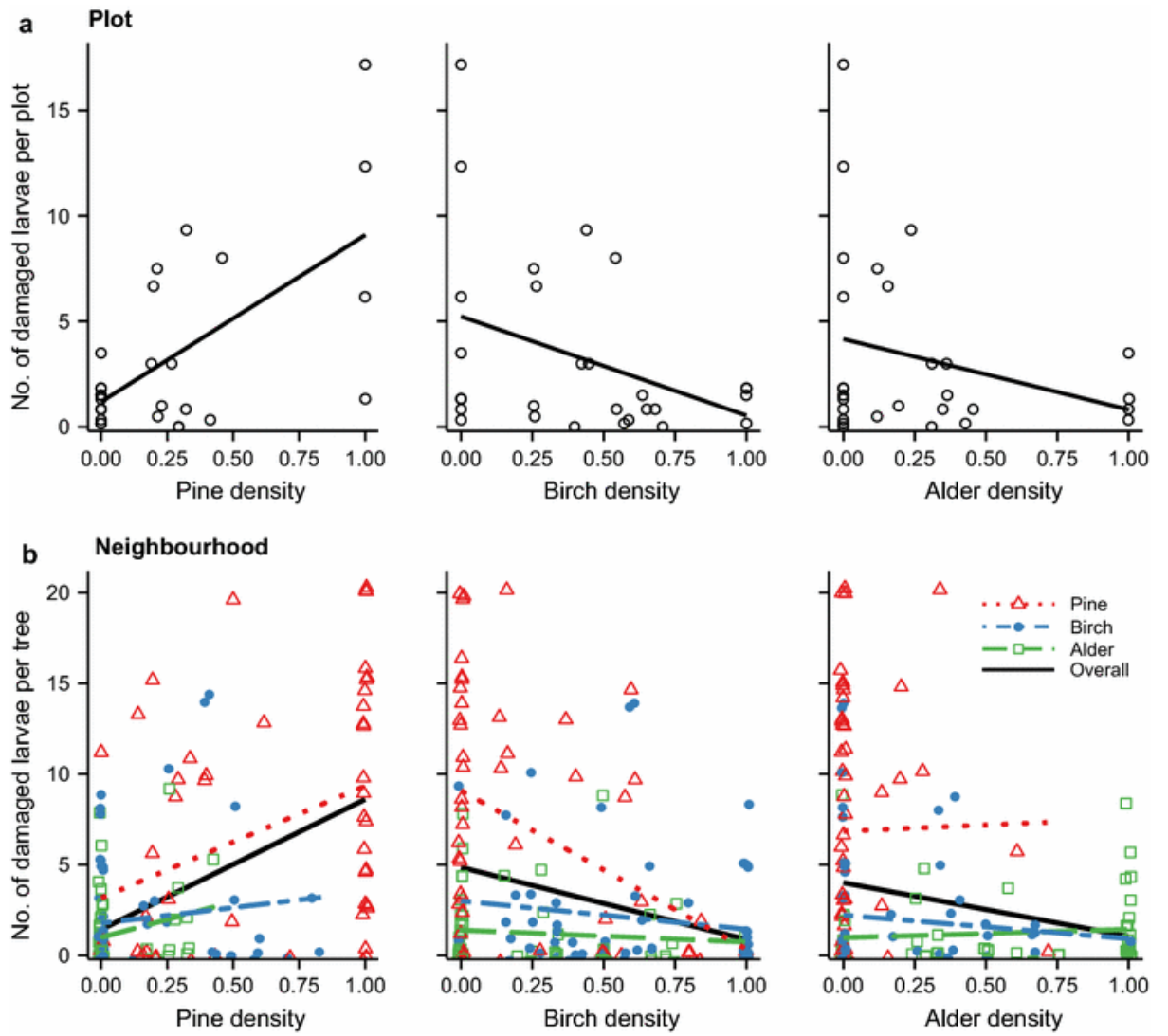


Figure 3



Electronic Supplementary Materials

Do birds see the forest for the trees? Scale-dependent effects of tree diversity on avian predation of artificial larvae

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Online Resource 1 - Insect herbivory monitoring

Fifty leaves were sampled from two branches facing opposite directions from the lower canopy, recording the number of exposed chewing insects (*Agelastica alni* L., weevils, Lepidoptera and sawfly larvae) as well as the number of leaves exhibiting rolling, folding or mining damage by concealed insect herbivores. As the number of exposed chewing insects was low and patchily distributed, we used their presence or absence as a variable instead. For each leaf, chewing and skeletonising damage was also scored in situ as follows: (1) 0.1-5% leaf area damaged, (2) 6-25% leaf area damaged, (3) 26-50% leaf area damaged, (4) 51-75% leaf area damaged and (5) more than 75% of leaf area damaged. The number of leaves in each class was subsequently multiplied by the mid-point of the category and the values summed to obtain an estimate of herbivore damage per branch. These values, in turn, were averaged for each tree, generating percentage values of leaf area damage per tree.

Online Resource 2 - Bird species, their life-history traits and number of territories observed during bird surveys at the Satakunta experiment. The first survey (22nd May 2013) was conducted prior to data collection on insect herbivores or bird predation. The second survey (7th June 2013) was conducted the day before the installation of artificial larvae and the third survey (12th June 2013) was completed during the predation experiment on the 5th day after installation. Life trait data taken from (Barbaro and van Halder 2009) and references therein.

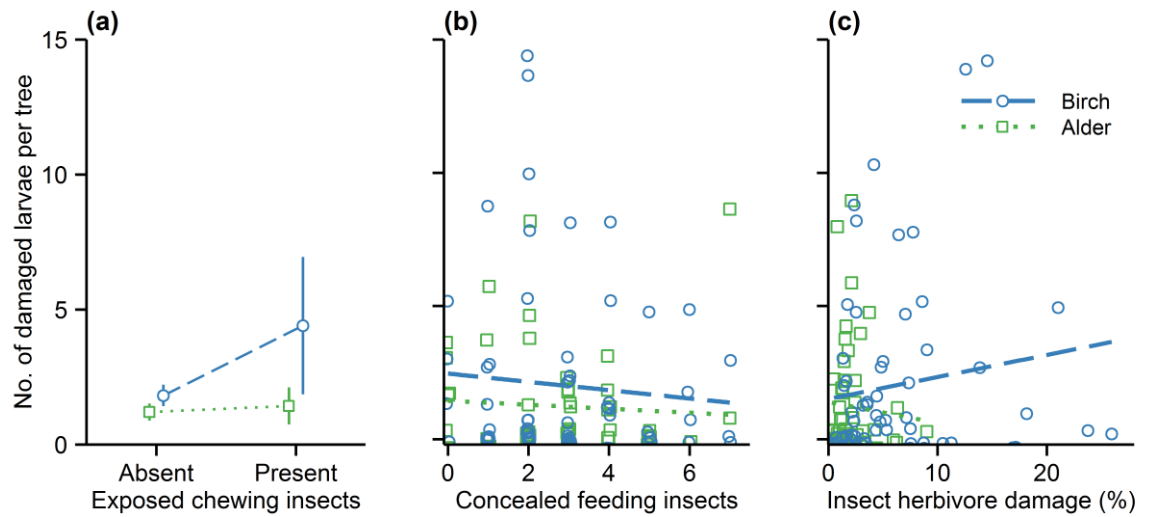
Bird species		No of territories/ Survey						
Latin name	Common name	Foraging strategy	Breeding diet	Home range size	1 st survey	2 nd survey	3 rd survey	Total
<i>Anthus trivialis</i>	Tree pipit	ground gleaner	insects	medium (1–4 ha)	1	2	2	5
<i>Carpodacus erythrinus</i>	Scarlet rosefinch	ground gleaner	seeds/ insects	small (<1 ha)	1	0	0	1
<i>Carduelis spinus</i>	Siskin	canopy gleaner	seeds/ insects	large (>5 ha)	2	0	0	2
<i>Emberiza citrinella</i>	Yellowhammer	ground gleaner	insects/ seeds	small (<1 ha)	2	2	0	4
<i>Erithacus rubecula</i>	Robin	understorey gleaner	insects/ seeds	medium (1–4 ha)	1	2	5	8
<i>Fringilla coelebs</i>	Chaffinch	canopy gleaner	insects/ seeds	small (<1 ha)	4	5	5	14
<i>Phylloscopus collybita</i>	Chiffchaff	canopy gleaner	insects	small (<1 ha)	1	1	1	3
<i>Parus cristatus</i>	Crested tit	canopy gleaner	insects/ seeds	medium (1–4 ha)	1	0	1	2
<i>Phylloscopus trochilus</i>	Willow warbler	canopy gleaner	insects	small (<1 ha)	9	27	20	56
<i>Parus major</i>	Great tit	canopy gleaner	insects	small (<1 ha)	1	2	2	5
<i>Prunella modularis</i>	Dunnock	ground gleaner	insects	small (<1 ha)	4	0	2	6
<i>Parus montanus</i>	Willow tit	canopy gleaner	insects/ seeds	medium (1–4 ha)	0	1	0	1
<i>Regulus regulus</i>	Goldcrest	canopy gleaner	insects	medium (1–4 ha)	1	1	0	2
<i>Sylvia atricapilla</i>	Blackcap	understorey gleaner	insects/ seeds	small (<1 ha)	0	4	0	4
<i>Sylvia borin</i>	Garden warbler	canopy gleaner	insects/ seeds	small (<1 ha)	2	3	3	8
<i>Sylvia curruca</i>	Lesser whitethroat	canopy gleaner	insects	small (<1 ha)	2	3	2	7
<i>Scolopax rusticola</i>	Woodcock	ground gleaner	worms/ insects	medium (1–4 ha)	1	0	0	1
<i>Turdus iliacus</i>	Redwing	ground gleaner	worms/ insects	small (<1 ha)	2	2	1	5
<i>Turdus philomelos</i>	Song thrush	ground prober	insects/ seeds	medium (1–4 ha)	1	1	4	6
Total No of territories					36	56	48	140
Total No of species					17	14	12	19

Online Resource 3 – Interactions between time, study area and selected variables. A summary of results from initial binomial GLMMs including time (T) from installation of the artificial larvae, study area (A) and the predictor variables of interest in this experiment is presented below. The four diversity variables; richness, pine density, alder density and birch density were tested at both plot and neighbourhood level separately. In addition, we report statistics from models substituting diversity variables with either tree height variation within a plot or, tree species identity of the focal tree.

Diversity variables												Additional Variables			
Plot	Richness			Pine Density			Alder Density			Birch Density			Tree Height Variation		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
T	46.3	1	<0.001	45.4	1	<0.001	45.1	1	<0.001	45.0	1	<0.001	45.0	1	<0.001
A	11.3	1	<0.001	18.0	1	<0.001	12.3	1	<0.001	15.2	1	<0.001	12.1	1	<0.001
Variable	0.11	1	0.730	19.1	1	<0.001	1.09	1	0.296	8.21	1	0.004	0.12	1	0.727
T x A	31.2	1	<0.001	32.6	1	<0.001	32.8	1	<0.001	34.1	1	<0.001	30.7	1	<0.001
T x Variable	0.11	1	0.735	0.02	1	0.882	1.30	1	0.255	1.48	1	0.223	0.26	1	0.611
A x Variable	0.91	1	0.340	0.02	1	0.896	0.13	1	0.718	0.07	1	0.795	1.03	1	0.310
T x A x Variable	2.93	1	0.087	0.09	1	0.770	0.28	1	0.598	0.01	1	0.919	0.68	1	0.410

Diversity variables												Additional Variables			
Neighbourhood	Richness			Pine Density			Alder Density			Birch Density			Tree Species Identity		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
T	45.5	1	<0.001	45.4	1	<0.001	45.0	1	<0.001	44.8	1	<0.001	45.7	2	<0.001
A	10.8	1	0.001	18.4	1	<0.001	12.8	1	<0.001	14.9	1	<0.001	13.2	2	<0.001
Variable	2.08	1	0.150	22.0	1	<0.001	1.73	1	0.189	5.97	1	0.015	21.5	2	<0.001
T x A	32.1	1	<0.001	32.9	1	<0.001	32.3	1	<0.001	34.5	1	<0.001	33.2	2	<0.001
T x Variable	0.01	1	0.910	0.20	1	0.651	1.57	1	0.210	2.19	1	0.139	1.58	2	0.453
A x Variable	0.03	1	0.855	0.04	1	0.836	1.02	1	0.311	0.32	1	0.573	2.10	2	0.350
T x A x Variable	0.56	1	0.455	0.04	1	0.847	0.26	1	0.610	0.05	1	0.830	3.14	2	0.208

Online Resource 4 – Effects of insect herbivore abundance and damage on bird predation rates. The effects of (a) the presence of exposed chewing insects, b) the abundance of concealed feeding insects or c) percentage of leaf area damaged by insects on the number of damaged artificial larvae is shown for either birch or alder trees. The mean (\pm SE) number of damaged larvae is shown in (a) and smoothed means drawn in panels (b) and (c) for each species separately.



Chapter 7: GENERAL DISCUSSION

The role of biodiversity in supporting ecosystem functioning is now well established by numerous experimental and meta-analytical studies (Tilman et al. 1996, Hector et al. 1999, Balvanera et al. 2006, Cardinale et al. 2011, Zhang et al. 2012). A strong imbalance in research within different ecosystems can nevertheless be noted, with a preponderance of work in herbaceous rather than woody plant communities (Cardinale et al. 2011). Due to the slower growth rate and greater longevity of woody plants, experimental manipulations of forest diversity have been slow to materialise. While the number of experimental studies in forests has steadily increased during the last two decades (Verheyen et al. 2015), they delivered conflicting results on the magnitude and mechanisms of associational effects on insect herbivores (Riihimäki et al. 2005, Vehviläinen et al. 2006, Schuldt et al. 2010, 2011, Plath et al. 2012, Moreira et al. 2014, 2015). As a result, causal linkages between tree species diversity and insect herbivores have recently come under scrutiny (Moreira et al. 2016) and the main aim of my thesis has been to explore the importance of different mechanisms of associational effects in boreal forests. In this chapter, I discuss the main findings of my thesis in relation to the original questions (section 7.1), evaluate the generality of my findings (section 7.2), consider their implications in theoretical and applied contexts (section 7.3) and offer suggestions for future work (section 7.4).

7.1 Main findings

This thesis aimed to address two main questions I) are effects of forest diversity on insects mediated by concurrent changes in environment or host tree traits? and (II) are associational effects regulated or maintained through interactions with other taxonomic groups such as mammals or birds? Below I review the central findings of this work in relation to the two questions above.

7.1.1 Canopy cover and host tree properties

In Chapters 3 and 4, I presented the first empirical evidence of forest diversity effects on insect herbivores triggered by stand structure and the properties of a host tree. Specifically, I showed that associational responses of two dissimilar tree species – an evergreen conifer and a deciduous broadleaf – are primarily driven by canopy cover rather than tree diversity per se.

Results from Chapter 3 demonstrated that spruce trees acquired resistance to a gall-forming adelgid through increased canopy cover in species-rich stands (Fig. 7.1). As diversity increases so does the density of species taller than spruce in the Satakunta experiment. Spruce trees therefore experienced more shading in mixed-species plots and pineapple galls caused by *Adelges abietis* were both smaller in size and less likely to occur on shaded spruce trees in mixed plots. It therefore appears that associational effects may be the result of insect herbivores actively discriminating between trees (and plots) by their canopy structure rather than tree diversity per se.

While pineapple gall adelgids preferences for unshaded environments have previously been documented (Fidgen et al. 1994), it was unclear whether these patterns were purely driven by changes in the abiotic environment or whether they could be driven by concurrent changes in foliar properties. In Chapter 4, therefore, I extended the work in Chapter 3 by differentiating between direct and trait-mediated effects of canopy cover on insects. I found that, although associational resistance on birch trees was partially triggered by changes in canopy cover (Fig. 7.1), these effects were purely trait-mediated. Unlike spruce, birch trees experienced less canopy cover in mixed-species stands where host dilution was matched by increasing density of shorter heterospecifics (Fig. 7.1). Plants growing in shady environments typically invest more in photosynthetic tissues (Chapin et al. 2002) and in birch reduced canopy cover in mixed stands resulted in smaller specific leaf area, making these leaves less favourable to gall-formers. Additional weak positive effects of canopy cover on leaf area may also have influenced leaf roller abundance and the extent of chewing damage across the diversity gradients. Thus, it appears that, even in the absence of herbivore preferences for specific light conditions (as in Chapter 3), canopy cover may still play an important role in associational resistance by modifying leaf traits that are key determinants of insect herbivory. Taken together, findings from Chapters 3 and 4 clearly demonstrate that canopy configuration may be an important and previously overlooked driver of associational resistance in forest ecosystems. Moreover, I showed the role of canopy cover in two different tree species and a variety of insect herbivores, which suggests that this mechanism of associational resistance is likely to be widespread across different host species and herbivore types.

While much of the early work on associational effects focussed on host plant density as a driver of tree diversity effects on insects (Root 1973, Otway et al. 2005, Heiermann and Schütz 2008, Sholes 2008, Björkman et al. 2010, Plath et al. 2012), I found that these

effects are not quite so straightforward. By exploring a range of causal linkages between tree diversity and herbivory, I showed in Chapter 4 that associational effects may not just be canopy or trait-mediated but may also act via multiple pathways simultaneously. In accordance with the resource concentration hypothesis (Root 1973), the dilution of birch trees in mixed stands consistently reduced herbivore damage and abundance on birch. However, tree species richness effects on insects could also operate independently of resource density, or elicit host dilution that may directly or through changes in canopy cover alter leaf properties and, in turn, influence herbivory. While these interactions are quite complex, they reflect the fact that in both the Satakunta experiment and many natural ecosystems changes in tree species richness, resource density and canopy cover are difficult to extricate from each other. Yet, by exploring variation in host plant traits and both horizontal stand and vertical canopy structure, I was able to build a much clearer picture of how and why tree diversity influences herbivory.

Studies exploring leaf trait changes in response to plant diversity have emerged only recently and most of them focussed on diversity effects on plant growth and anti-herbivore defences (Mraja et al. 2011, Moreira et al. 2014, Wäschke et al. 2015). In line with my findings, these studies have not been able to attribute associational resistance to variation in host plant growth (Chapters 3 & 5, Moreira et al. 2014) or the levels of anti-herbivore defences (Chapter 4, Mraja et al. 2011, Moreira et al. 2014, Wäschke et al. 2015). Instead, my research suggests that only variation in leaf morphology (SLA and leaf area) underpins herbivory on birch. In Chapter 4 I present the first empirical evidence of tree diversity effects mediated by stand structure and variation of physical traits (Fig. 7.1). The finding that physical leaf traits were more important in mediating plant diversity effects on herbivores than chemical traits is in agreement with meta-analysis by Carmona et al. (2011) which has shown that effects of morphological leaf traits outweigh those of chemical or nutritive compounds on insect herbivores (Carmona et al. 2011). To summarize, in answer to the first question of this thesis, I showed that effects of forest diversity on insects are indeed mediated by concurrent changes in the environment and host tree traits. More specifically, forest diversity effects on insects were mediated by host dilution and changes in canopy cover as well as the resulting variation in physical leaf traits.

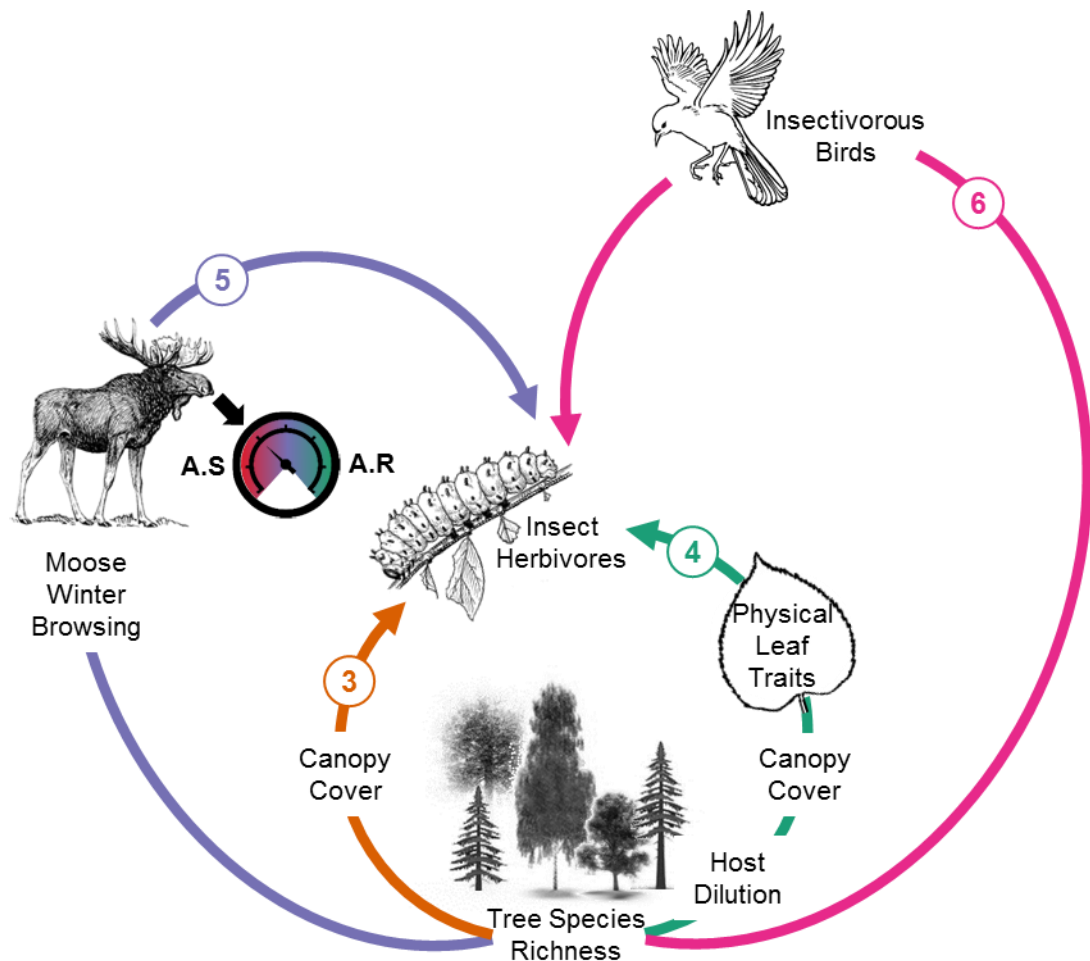


Figure 7.1 Schematic diagram of results based on the conceptual diagram from Chapter 1. Associational resistance was found to be triggered by canopy cover and changes in physical leaf traits with host dilution (Chapters ③ & ④) and potentially driven by bird predation in mixed stands over monocultures (Chapter ⑥). Moose browsing in winter, however, modified the magnitude and direction of associational effects, ultimately reversing associational resistance (A.R) to associational susceptibility (A.S, Chapter ⑤).

7.1.2. Multi-trophic interactions

While identifying the abiotic or host plant properties underlying associational effects is essential, it is also important to understand the effects of plant diversity on other taxonomic groups that might interact with phytophagous insects. One mechanism that has largely been ignored is the potential for one herbivore type to influence diversity effects on a second herbivore as a result of plant-mediated interactions among herbivores (Ohgushi 2005). In Chapter 5, I explored one such interaction by examining how the presence and intensity of moose browsing on birch in the winter months influenced subsequent insect herbivory in the summer. Although moose browsing at the plot level had been shown to increase with tree species richness, at tree species level the probability and degree of browsing on birch was consistent across the diversity gradient (Milligan and Koricheva 2013). Thus, the observed effects of browsing on birch could not simply

be due to moose causing more severe damage in mixed stands. Rather, effects of browsing on insects may have been the result of improved compensatory regrowth in mixed stands.

The main conclusion of Chapter 5 was that browsing on birch trees reversed associational resistance observed on unbrowsed trees to associational susceptibility on more heavily browsed trees (Fig. 7.1). While I did not specifically assess browsing effects on birch, previous studies have found that birch trees respond by producing producing larger shoots with larger leaves (Danell and Huss-Danell 1985, Danell et al. 1997) that, in turn, are favoured by chewing insects (Chapter 4, Senn et al. 1992). This compensatory regrowth has also been shown to increase with browsing intensity and to be stronger in open as opposed to shaded stands (Danell et al. 1985). Thus, heavily browsed trees in mixed plots with low canopy cover may be more attractive to defoliating herbivores than either slightly browsed trees in mixtures or even unbrowsed trees in monocultures. The trait-mediated pathway described in Chapter 4 for insect chewing damage is therefore overturned by increasing moose browsing intensity.

This novel finding provides a possible explanation for the variation in herbivore responses to plant diversity and the dichotomy between associational resistance and associational susceptibility observed in the literature (Jactel and Brockerhoff 2007, Barbosa et al. 2009, Schuldt et al. 2010). Tree species diversity may have a more consistent negative effect on birch insect herbivory in the absence of browsing (Chapter 4) but, where mammalian herbivores occur at a high density, their manipulation of host leaf traits reverses this pattern (Chapter 5). Moose and other cervids also favour early successional stage forest (Bergqvist et al. 2003), so diversity-herbivore interactions may be less variable in older forests (as in Chapter 4 - data collection in 2014) compared to younger stands with more accessible foliage (as in Chapter 5 - data collection in 2010/2011). The detrimental effects of browsing on tree morphology and productivity (Chapter 5, Edenius et al. 2002) mean that young forest diversity experiments are commonly fenced to exclude mammalian herbivores and are therefore more likely to report associational resistance rather than susceptibility. However, patterns of associational resistance seem to be stronger in older forest stands (Vehviläinen et al. 2007). This could be because browsing mammals generally prefer early successional stage forest (Bergqvist et al. 2003). Alternatively, tree diversity effects may simply have had longer to manifest in older stands. Therefore, it is possible that future work will yield more uniformity and consistent mechanisms in associational effects regardless of whether or not mammalian herbivores are excluded.

Results from Chapter 5 also have wider implications for BEF research. By exploring multiple ecosystem properties, I was able to show that mammalian browsers play a functional role in forest diversity effects, the likes of which have only been reported in grasslands and aquatic systems with insects or microbes as the focal consumers (Mulder et al. 1999, Naeem et al. 2000, Schnitzer et al. 2011). Although browsing reduced both tree size and herbivore resistance, tree growth and defoliation were not strongly related. Nevertheless, these effects were important to test as tree growth and herbivory often correlate (Senn et al. 1992, Zvereva et al. 2012) but their responses to tree diversity are rarely explored together (Castagneyrol et al. 2013, Haase et al. 2015). In other systems, more distinct trade-offs between resource allocation to tree growth and anti-herbivore defences may be revealed (Herms and Mattson 1992) and may depend on the identity or diversity of neighbouring species (Moreira et al. 2014). Therefore, current approaches to testing tree diversity effects on insects need to be revised to explore shifts in resource allocation triggered by biotic stressors as well as account for trophic complexity and trait-mediated interactions between herbivores.

In addition to these regulatory effects of mammalian herbivores, effects of tree species richness on the top-down control of insect pests must also be considered. Predation is an important and ubiquitous force that can determine ecosystem structure and function. In particular, for avian predators, their interactions with phytophagous insects may not only shape insect habitat preferences (Thompson and Pellmyr 1991) but also cascade down to improve plant productivity (Mäntylä et al. 2011). However, even though birds are widely known to provide an important and economically valuable pest control service (Whelan et al. 2015), the habitat features that influence their predation rates have rarely been considered in forests (Whelan 2001, Poch and Simonetti 2013). In Chapter 6, therefore, I present one of the very first studies examining avian predation rates across gradients of tree species diversity and structural complexity.

In line with the “enemies hypothesis” put forward by Root (1973), predation on artificial caterpillar larvae increased with tree species richness. However, this effect depended on the scale of analysis and was independent of structural complexity. Rather than diversity increasing niche availability for predators, results from this chapter suggest that some other factor operating only at small spatial scales may encourage stronger top-down control in mixed stands. I therefore suggest that increased predation with neighbourhood diversity could be due to reduced canopy cover in mixed stands (as seen in Chapters 4

and 5). As bird predation rates have been shown to increase where plant cover is reduced (Groner and Ayal 2001), lower canopy cover in mixed stands may have improved the visibility of prey to foraging birds and driven increased top-down control in more species-rich plots. Thus, on top of regulating insect herbivore preference and performance (Chapters 3-5), changes in canopy structure with stand diversity may encourage associational resistance by reducing the survival of insect herbivores. However, these effects may depend on the foraging behaviours of the bird community and, specifically, their preferences for individual tree species.

Taken together, results of Chapters 5 and 6 show that associational effects may indeed be regulated and maintained through interactions with other taxonomic groups (Question II). Insectivorous birds can contribute to associational resistance but these effects may be thwarted by the action of mammalian browsers. However, studies in Scandinavian boreal forest have also shown that moose browsing on birch reduces the abundance of insectivorous birds, possibly due to loss of nesting sites through browsing or trampling (Mathisen and Skarpe 2011). Top-down control could therefore be more relaxed in the presence of moose, further amplifying the reversal of associational resistance to associational susceptibility. To my knowledge, such complex interactions between mammals, invertebrates and insectivorous birds are rarely explored (Mathisen and Skarpe 2011, Mathisen et al. 2012) and have yet to be incorporated into BEF studies.

Overall, my findings across all research chapters indicate that forest canopy structure plays a critical role in effects of diversity on insects. Changes in canopy cover, and light intensity as a consequence, not only drive the preference and performance of insect herbivores (Chapter 3) and foliar traits (Chapter 4) but also modify tree responses to biotic stresses (Chapter 5) and the foraging behaviour of predators (Chapter 6). In contrast to previous findings from this and other forest diversity experiments (Riihimäki et al. 2005, Vehviläinen et al. 2006, Schuldt et al. 2011, Castagneyrol et al. 2014b), tree species composition did not emerge as a predictor of herbivory and therefore could not be implicated in mechanisms of associational resistance. The only indication that species composition may be important was observed in Chapter 6 where I found that the pine monocultures and mixtures with a high proportion of pine received more predation. Variation between the three separate areas in the Satakunta experiment may have obscured any composition effects but with data from only these sites, it is difficult to offer any alternative explanation for why herbivory was independent of stand composition.

Nevertheless, the fact that canopy structure was implicated in separate studies conducted at different times and on different tree species suggests that idiosyncratic patterns in other forest plantations may be easily resolved if only stand structure is accounted for. Hence, results from this thesis demonstrate that both the abiotic environment and food web interactions are key determinants of plant diversity effects on insect herbivores.

7.2 Critical evaluation of experimental approach

The conclusions of this thesis were derived from the analysis of herbivory in planted stands where forest diversity was manipulated. As discussed in Chapter 2, such experimental approaches were implemented early on in agricultural and grassland ecosystems (Tahvanainen and Root 1972, Root 1973, Hector et al. 1999, Loreau and Hector 2001) but have only recently been adopted in research to understand effects of tree diversity on forest insects (Verheyen et al. 2015). Previously, our understanding of the patterns and mechanisms of associational effects in forests came from observational or comparative studies (Futuyma and Wasserman 1980, Scherer-Lorenzen et al. 2005). However, as many of the forests in the northern temperate zone have been managed for some time, plot history could alter stand structure and function and thus confound effects of diversity with historical influences (Mund and Schulze 2005). Furthermore, unless site conditions are very similar, environmental differences between them may overshadow any effects of forest biodiversity on an ecosystem process. Experimental studies are therefore required that can complement observational studies by providing a framework in which to analyse putative causal relationships between tree species identity or diversity and ecosystem functioning (Leuschner et al. 2009).

As outlined in Chapter 2, the relevance and generality of experimental tests of BEF relationships has been heavily debated. Although experimental platforms offer controlled environments to unambiguously test for links between diversity and function, among the main criticisms of this approach is the applicability of these experimental tests to natural systems (Lepš 2004, Srivastava and Vellend 2005). For instance, experimental studies that rely solely on random manipulations of species communities to determine BEF relationships are liable to reach erroneous conclusions about cause and effect by ignoring other confounding variables (Huston 1997). These so-called “hidden treatments” include abiotic and biotic factors, the non-random selection of species and sampling effects (Huston 1997). However, these limitations may be overcome by carefully assessing

confounding variables in each plot (e.g. canopy cover, predation, species densities etc.) and including them in statistical analyses to isolate causal pathways.

Thus, forest diversity experiments continue to be powerful tools to assess BEF relationships (Schmid and Hector 2004) and new experimental platforms have emerged in the past decade that are used widely in scientific research. For example, for plant-herbivore interactions, the global network of tree diversity experiments (TreeDivNet, Verheyen et al. 2015) has already provided evidence of associational resistance (Castagneyrol et al. 2013, 2014b, Setiawan et al. 2014) and susceptibility (Vehviläinen et al. 2007, Schuldt et al. 2010, Haase et al. 2015) and has also begun to contribute to our mechanistic understanding of diversity effects on insect herbivores (Riihimäki et al. 2005, Castagneyrol et al. 2013, Moreira et al. 2014, Haase et al. 2015). Nevertheless, it is clear that the planted synthetic stands used throughout this study differ from most natural and production forests by their age, small scale, homogenous planting densities and tree species assemblages.

Of the forest diversity experiments included in TreeDivNet, Satakunta is the only experiment in the boreal zone and the oldest of all experimental sites with trees up to 16 years of age. Situated in south-west Finland and planted with common and economically important tree species, results from Satakunta are most likely to apply to planted forest in the boreal zone. As tree species diversity in boreal forests is relatively low compared to temperate and tropical forests, a maximum species pool of five is not only a realistic representation of the local community but the addition or loss of a single tree species may be expected to have larger effects on ecosystem functioning as compared to other forest biomes. Indeed, this appears to be the case as experiments in temperate regions have more commonly reported weak associational effects (Haase et al. 2015, Verheyen et al. 2015) in contrast to consistent patterns of associational resistance observed in this study. However, as Vehviläinen et al. (2007) also observed that tree species diversity effects increase with tree age, effects seen here may contradict those from other experiments due to differences in age rather than location.

Although BEF relationships have been shown to strengthen over time (Eisenhauer et al. 2012), results from this thesis were obtained only from young forest stands (11-16-year-old trees) so it is difficult to extrapolate results to older plantation or natural forest. Not only might herbivore resistance increase with tree age (Barton and Koricheva 2010) but,

as forests mature, they also become more structurally complex. In particular, tree species differences in growth form and morphology may amplify as forests age and regeneration in natural stands lead to a more vertically complex canopy structure (Brokaw and Lent 1999, Franklin et al. 2002). This vertical complexity in the forest contributes to faunal diversity (MacArthur and MacArthur 1961) with instances of increased herbivore richness even where plant species diversity declined over time (Southwood et al. 1979). Thus, findings from this thesis are unlikely to apply to naturally established forests as the even-aged stands in Satakunta bear little resemblance to the heterogeneous age, canopy and trophic structures typical of naturally regenerating forest (Scherer-Lorenzen et al. 2005).

Rather, as discussed in Chapter 2, the identified mechanisms are most likely to apply to plantation forests. Time continues to be a limiting factor for the conclusions that can be drawn from this experimental work. This is because, considering the long life span of trees, data collected in this thesis was largely obtained at a single ontogenetic stage. Trees in older plantations may interact with a different community of herbivores (Jeffries et al. 2006) and exhibit different functional traits to those in younger plantations (Barton and Koricheva 2010). Additionally, plantations are managed at the scale of decades with different methods employed to control the regeneration, composition, health, quality, growth and harvest of trees. More work is therefore required to understand how forest diversity effects respond to silvicultural practices and vary through different ontogenetic stages. For now, Satakunta is the oldest forest diversity experiment and we can begin to tentatively link mechanisms observed here to more mature plantations or stands with minimal management. However, even if my findings are not relevant to all points in a forest's life span, the work presented here still addresses a crucial stage in stand development that is of great relevance to forest establishment and management (Greenberg et al. 2011).

Due to the high costs of establishing and managing large forest stands, experimental plots are often restricted to small sizes (rarely more than 0.25 hectares). However, plot size can not only restrict the type of processes that can be assessed (Scherer-Lorenzen et al. 2005) but it can also dictate diversity effects on herbivores. For example, Bommarco and Banks (2003) suggested that insects in agricultural systems are better able to redistribute between plots and aggregate in monocultures when plots are small. However, studies in forest diversity experiments find stronger effects of diversity in large plots instead

(Vehviläinen et al. 2007) possibly because they are more likely to include the more sparsely distributed specialist insects that are more sensitive to diversity and resource density. The latter aligns with my own observations that only plot but not neighbour diversity affected herbivore response variables in Chapters 3-5. Nevertheless, edge effects could be substantial in small plots in the Satakunta experiment introducing considerable variation in microclimate between plots (Leuschner et al. 2009, Rothe and Binkley 2011). With environmental conditions and canopy structure shown to be influenced by the proximity to the forest boundary (<15m, Williams-Linera 1990), even the selection of trees in the interior of the plot may not be enough to overcome effects of plot size on canopy architecture and, in turn, the consequences for herbivore distributions. Thus, care must be taken in scaling results of the studies in this thesis to larger stands or landscape scales.

In addition to considerations of plot size, stand density is known to be an important factor governing tree growth, canopy structure, foliar quality and herbivory (Burdon et al. 1992, Underwood and Halpern 2012). With thinning in the Satakunta experiment taking place in 2013, data for most chapters were collected from treatments replicated at two densities (169 trees in 20x20m plots halved to 84 trees after thinning). The only exception was Chapter 5 where data collection was performed prior to thinning (2010/2011). While I observed effects of thinning on herbivory and canopy cover (Chapters 3 and 4), these density effects did not interact with diversity. My findings instead suggest that it is the dilution of host species rather than actual planting density that influences insect herbivore distributions. Nevertheless, spatial arrangement remains an important variable to account for in comparisons with other forest types as more even planting schemes maximise competitive interactions with neighbours, impacting canopy structure and host tree properties (Lang et al. 2010, 2011).

Despite the limitations of an experimental approach, by focussing on only one long-term tree species diversity experiment, I am able to draw more detailed mechanistic insights of associational effects that could not necessarily be gained from meta-analysis of different experiments. I am not only able to demonstrate the important role of canopy cover in driving diversity effects but also show that other foraging taxa can directly or indirectly impact herbivory. Such detailed assessments of canopy structure and multi-trophic interactions would be difficult to accomplish in more than one experiment. However, by doing so, I am able to highlight the importance of accounting for the

presence and density of mammalian browsers and the avian community. As both mammalian browsers and insectivorous birds are globally present in forests, it is likely that the mechanisms described in Chapters 5 and 6 operate across experimental and other even-aged forest stands.

In the majority of research chapters in this thesis (Chapters 3-5), I focussed on the herbivores of an individual tree species rather than herbivory at the plot level. This approach is appropriate in Satakunta as only birch and alder that belong to the same family (Betulaceae) may share herbivores whereas the remaining tree species have essentially non-overlapping communities of insects. However, as the primary focus of BEF research is on ecosystem-level consequences of biodiversity change, it has been argued that the state of a particular species cannot constitute an ecosystem-level function as the particular species only occurs in certain ecosystems (Weisser and Siemann 2004). In the case of herbivorous insects, a focus on individual host-insect interactions is limited as the effect of species diversity on herbivore damage does not necessarily reflect the totality of damage caused by the entire community of herbivores at the plot scale (Southwood et al. 2004, Wielgoss et al. 2012). At the same time, however, a focus on individual tree species avoids the problems of sampling effect, where highly-preferred tree species may drive up herbivory at stand-level (Mulder et al. 1999). In addition, as BEF studies in forests frequently report stronger effects of species identity than diversity (Vehviläinen et al. 2007, Haase et al. 2015), it has been suggested that these studies would profit more from information at the scale of individual tree species for an improved understanding of mechanisms (Nadrowski et al. 2010).

As with the focus on an individual experiment, concentrated efforts on individual tree species also facilitate more detailed analysis of plant-insect interactions particularly in terms of tree properties (Chapters 3 and 4) and a more complete assessment of the herbivore community (Chapters 4 and 5). By focussing on Norway spruce and silver birch – two of the most common and economically important tree species in Finnish boreal forests – I am also able to reveal detailed mechanisms and provide suggestions for the management of these species in forest plantations. Nevertheless, recent work by Haase et al. (2015) has also shown that tree species-specific responses to diversity may be highly variable and differ from responses at plot-level. In Chapter 6, where I explored plant diversity effects on top-down control, I therefore tested for and demonstrated contrasting effects at forest plot versus neighbourhood and individual species scales. Future studies

would therefore benefit from examining herbivore responses to diversity on multiple tree species in a stand as well as assessing community-level responses to diversity.

In this thesis, most of the herbivore measures were restricted to specific guilds rather than species for practical reasons. This approach may mask important interactions between insect species and their different feeding behaviours. For instance, some but not all leaf mining species on birch may render leaves unsuitable for other insect herbivores (Fisher et al. 1999, Johnson et al. 2002). Although coarse analyses of interactions between guilds may have been possible in this study, they are unlikely to yield clear results as such interactions are often only detected at very high herbivore densities (Fisher et al. 1999) and may depend on the scale of analysis (e.g. branch vs. tree-level, Valladares and Hartley 1994). Nonetheless, such important interactions between insect species and guilds may determine the net effect of herbivory on focal trees and should be explored in future work. The focus on foliar herbivory in this study also ignores the important role of belowground herbivores, microbial organisms and any feedbacks between them and the aboveground herbivory measured (Johnson et al. 2012). For example, mycorrhizal interactions are known to favour herbivory on leaves aboveground (Koricheva et al. 2009). These biotic interactions and feedbacks are also sensitive to plant neighbour effects but the relative importance of neighbour effects on above and belowground herbivory are rarely explored (Kos et al. 2015). Thus, although guild-specific herbivory was sufficient for our purposes here, a more detailed analysis of above- and belowground fauna in the future may shed light on more dynamic interactions that ultimately affect ecosystem functioning.

7.3 Implications

7.3.1. Theoretical implications

The overriding theme of this work is the importance of canopy structure in driving herbivore distributions across gradients of diversity. Despite calls for more detailed analysis of factors co-varying with diversity (Nadrowski et al. 2010), very few studies have attempted to explore what role such “hidden treatments” could have in determining the magnitude and direction of plant diversity effects (Riihimäki et al. 2005, Castagneyrol et al. 2013, Moreira et al. 2014). Results from this thesis indicate that measures of canopy cover or light intensity could provide a unifying path across all mechanisms and help to explain the observed context-dependency of tree diversity effects on insects (Vehviläinen et al. 2007). With the light responses of many plant and herbivore species already recorded, predictions of associational effects could already be made based on the relative

position of a tree within a canopy and the natural preferences of the insect herbivore in question. However, much more work is needed on canopy structural dynamics across age classes to be able to develop generalised approaches to pest management through canopy manipulations.

The findings of this thesis also highlight some of the shortfalls of mechanistic research so far. For instance, host plant growth and anti-herbivore defences (and the trade-offs between them: Herms and Mattson 1992) have both been implicated in theories of associational effects (Moreira et al. 2016). However, physical leaf traits appear to have almost entirely escaped attention in mechanistic studies even though they are consistently found to be the best predictors of herbivory by different guilds (Clissold et al. 2009, Carmona et al. 2011, Schuldt et al. 2012, Caldwell et al. 2016). Furthermore, with mammalian herbivores able to trigger changes in leaf morphology (Danell and Huss-Danell 1985), and physical defences shown to increase with age in woody plants (Barton and Koricheva 2010), it is clear that physical traits deserve more attention in BEF studies as they synthesise information about environmental and biotic stress that may underpin forest diversity effects.

The important role of both mammalian browsers and insectivorous birds observed in this study further suggests that a better understanding of the community as a whole is needed to identify when and how forest diversity affects insect herbivores. Multiple studies have already recommended the integration of diversity within trophic levels (horizontal diversity) and across trophic levels (vertical diversity) (Duffy et al. 2007, Axelsson and Stenberg 2012). However, few studies have clearly documented the role of multi-trophic interactions in mediating diversity effects on arthropods even though these interactions are likely to produce a wider variety of diversity-functioning relationships (Duffy et al. 2007). If anything, research has almost gone the opposite way. Many forest diversity experiments in TreeDivNet deliberately exclude mammalian herbivores. While this is often necessary to secure the establishment of the experiment, it brings to question what effect is really being tested. If browsing can reverse associational effects but mammals are barred from experimental sites, then the patterns reported can hardly be extrapolated to nearby plantations where mammals are free to roam. This is particularly problematic as the continuing rise of planted forest areas (Keenan et al. 2015) has benefitted large mammalian herbivores that forage in young forest stands (Lavsund et al. 2003, Côté et al. 2004). In addition, as large herbivores are known to have detrimental effects on birds and

other animal species (Mathisen and Skarpe 2011, Foster et al. 2014), mammalian browsing may have much wider consequences for forest diversity effects. Potential consequences for productivity, pest resistance and biodiversity may be better explored in BEF studies by manipulating both presence/absence of mammalian herbivores and forest diversity and exploring potential interactions between their effects as done in Cook-Patton et al. (2014).

In this thesis, I present a clear starting point for future work on this subject: expanding the focus from diversity effects on insects to the dynamic influences of different taxonomic or functional groups that are ubiquitous across different habitats and can modify foliar quality for insects (Chapter 5) or enhance top-down control (Chapter 6). With a better understanding of these interactions, a more detailed assessment could be achieved within habitats to better mimic the trophic structures of natural ecosystems and improve predictions of biodiversity loss at multiple trophic levels (Cardinale et al. 2012).

7.3.2. Importance for forestry

My findings offer a variety of options for the management of tree health in production forest and commercial plantations. For instance, the highlighted role of canopy cover in directing changes in tree growth (Chapter 5), foliar properties (Chapter 4) and herbivory (Chapter 3) suggests that tree pest resistance could be effectively managed by accounting for the structure and light environment around focal tree species. The management of light in forests has already come under scrutiny; for instance, Lieffers et al. (1999) proposed that the control of light in northern or boreal forests could be achieved by managing the growth of the lower strata of trees in un-even-aged and mixed-species stands or reducing overstorey density in plantation forests through thinning. In the case of Norway spruce, management of canopy cover is rather more straightforward as traditional forestry practices include the planting of slow-growing, shade-tolerant species (spruce) with, so-called, nurse trees that are larger, faster-growing pioneer species (e.g. silver birch). Total growth is not only higher in these birch-spruce mixtures compared to single-species plots (Lundqvist et al. 2014) but, as spruce appears to acquire resistance to adelgids through shading by taller neighbours, birch nurse trees may not only provide the appropriate light environment but also inadvertently contribute to herbivore resistance in spruce.

Where canopy cover has a negative effect on herbivory (Chapter 4), more appropriate light regimes could be developed by decreasing initial planting density, pruning to reduce overstorey density or commercial thinning in plantation forests. These approaches are problematic as, while they may permit higher light transmission to the lower parts of the canopy, they do so temporarily and would therefore need to be repeated to sustain maximal herbivore resistance. They may also be impractical solutions to foresters as low planting densities may be unfeasible where land is at a premium. In addition, high-quality timber can still be grown without artificial pruning of birch (Hynynen et al. 2009). Nonetheless, where both artificial pruning and thinning are applied, they may contribute to herbivore resistance and may be part of the reason why both strategies are applied to ensure a high yield of top quality birch timber (Hynynen et al. 2009).

In addition to the issues of focal tree health are the concerns about the benefits and the costs of planting species-rich rather than species-poor plantations. In this work, I show that forest diversification may have a consistent positive impact on spruce and birch resistance to insect herbivory and pest accumulation. However, my finding that these effects are primarily driven by stand structure suggests that herbivore resistance can just as well be achieved by silvicultural practices outlined above rather than forest diversification. While species-mixing has been recommended for a long time in commercial forestry (Boppe 1889), plantations are still overwhelmingly species-poor (Nichols et al. 2006). Thus it appears, that much more evidence is needed to convince foresters of the benefits of mixed forests. Here, I showed that tree species richness had either neutral (tree growth) or positive (herbivore resistance) effects on focal trees. This agrees with recent work demonstrating equivalent yields between mono- and mixed-species forests and improved resistance to biotic and abiotic disturbances with increasing diversity (Knoke et al. 2008). For example, in Chapter 5, I have shown that moose browsing effects on birch growth are mitigated by tree species richness even though insect herbivory increased. In addition, I have shown in Chapter 6 that top-down control of insect pests may increase with species richness at fine spatial scales. This thesis therefore finds some support for mixed-species planting as it can benefit tree health and ultimately contribute to multiple ecosystem functions (Gamfeldt et al. 2013). However, much more work is needed at operational scales to determine whether the costs and practicalities of managing mixed-species forests are at least matched by similar profits in the long term.

7.4 Future Work

Conclusions from this thesis suggest that future studies could benefit from a more integrative approach to diversity effects on herbivores. Specifically, community ecologists could work more closely with ecophysiologicals to better understand how abiotic factors influence direct biotic interactions and feedbacks on forest ecosystems. For instance, high resolution remote sensing techniques such as LiDAR (Light Detection and Ranging) have recently emerged as powerful tools for the assessment of tree crown and canopy structures (Omasa et al. 2007, Lintunen 2013). This technique provides precise data at a high grain resolution that can cover large spatial extents. It has already been used to relate habitat heterogeneity to arthropod (Müller et al. 2014) and bird diversity (Huang et al. 2014) at local to landscape scales. However, to my knowledge, no studies have yet used this approach to predict insect herbivore damage and abundance. At the small spatial scale, the approach could be applied to existing forest diversity experiments to determine whether canopy structure can really explain associational patterns and why they differ between sites. At larger scales, the approach may be used initially in correlative studies of forest diversity effects on herbivory. In particular, where the composition of tree species is known, the generality of findings from existing literature could already be explored, incorporating variation in previous land-use, stand age and current management strategies.

As useful as 3D information may be on individual plants, stands and larger spatial scales, the long-lived nature of forest ecosystems means these patterns need to be understood over temporal scales as well. In particular, with global mean surface temperatures expected to increase by up to 4.8°C by the end of the 21st century (IPCC 2014), temporal analyses are particularly important to understand how diversity-herbivore interactions may vary as forests age and the climate changes. The strength of relationships between biodiversity and ecosystem functioning may be expected to increase with forest age (Eisenhauer et al. 2012) but will these patterns hold in a changing climate? With respect to herbivorous insects, climate change will likely modify the magnitude and direction of plant-herbivore interactions (Kozlov 2008, Bauerfeind and Fischer 2013) and the strength of their top-down control (Rodríguez-Castañeda 2013). Thus, understanding how plant-herbivore interactions vary across climatic and species-richness gradients presents one of the most important challenges in this field. One of the main reasons for this is that experimental manipulations of many of the components of climate change (e.g. manipulating temperatures or carbon emissions) in forests are likely to be expensive and

unrealistic. However, at least three forest diversity experiments in France, Italy and Canada have begun to explore effects of drought stress on diversity-herbivore interactions (B. Castagneyrol, S. Mereu, W. Parker, personal communication). I suggest that future work should continue in this vein and contribute to existing models (Harfoot et al. 2014) or build new ones that can illuminate mechanisms, facilitate predictions at multiple scales and ultimately inform policy (Mace 2013).

From a trophic perspective, my work here clearly shows that accounting for food web complexity is important to improve our understanding of how and why diversity-function relationships may arise. With the advent of forest diversity experiments (Verheyen et al. 2015), ecologists now have more options than ever to explore the functional roles of different taxonomic groups. For instance, large herbivore impacts on BEF relationships may be experimentally assessed in protected and unprotected stands. Exclusion treatments may be set up from the start, or initiated later on to simulate more dynamic changes in mammal populations (Côté et al. 2004). Subsequent measures of above and belowground processes may shed light on the large scale impacts of browsing and feedbacks to aboveground productivity and herbivory (Bardgett and Wardle 2003).

Similar exclusion studies on birds may be possible on small tree seedlings but are not feasible for larger trees. Although survival studies could also be performed, I suggest that future work on avian insectivory across forest diversity gradients should continue to use model prey. As bird predation often leaves no trace of prey, artificial larvae made from clay offer the best option to unambiguously assess the frequency of attacks and the identity of predators (Low et al. 2014). While I only explored effects of diversity on predation in Chapter 6, more could be done to determine whether the observed foraging patterns are determined by fine scale changes in foliage structure or host plant traits (Whelan 2001, Mäntylä et al. 2004). At the same time, both the species diversity and abundance of birds can vary as a result of local land-use or global environmental or anthropogenic factors (Kissling et al. 2012, Huang et al. 2014). Since bird predation is often positively related to avian abundance and diversity (Barbaro et al. 2012, 2014), the strength of avian pest control may also vary across geographic scales as well as with increasing forest diversity. This topic forms the basis of a new project I am coordinating in forest diversity experiments located in Europe, North and Central America with a view to determine the effects of forest diversity on bird insectivory at the global scale. In my humble opinion, mechanistic studies linking species diversity, trophic interactions and levels of ecological function across spatial scales are the next frontier in ecology.

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