Effects of tree species diversity on insect herbivory

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By

Simon Morath

School of Biological Sciences
Royal Holloway, University of London
Egham, Surrey, TW2O 0EX
United Kingdom

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Declaration of Authorship

This thesis and the work presented herein is my own work, conducted whilst enrolled in the School of Biological Sciences as a candidate for the degree of Doctor of Philosophy. This work has not been submitted for any other degree or award in any other University or educational establishment. Where I have consulted the work of others, this is clearly stated.

Signed: ______________________

SIMON MORATH

Date: ________________________

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Abstract

It is generally believed that tree species growing in mixed forest stands are less susceptible to insect herbivore damage than if grown in monocultures, but previous studies have been largely observational and focussed mainly on tree species richness effects. In this thesis, I examined effects of three components of forest diversity (tree species richness, intraspecific genotypic diversity and functional diversity) on insect herbivores using three long-term forest diversity experiments in Finland and Germany. I have also explored the sources of variation in and the mechanisms behind the effects of tree diversity on insect herbivores. I found that all three components of forest diversity significantly influenced insect herbivore abundance and damage. Tree species richness effects depended on the insect herbivore feeding guild, but also changed within season and between years. As a result, silver birch (*Betula pendula*) experienced both associational resistance (reduced damage in mixed stands) and associational susceptibility (higher damage in mixed stands) to different insect herbivores and in some instances this altered temporally. In contrast, tree species richness effects on insect herbivory were spatially consistent and not mediated by tree size (physical apparency), physical properties of leaves or natural enemies. Interestingly, tree species richness and genotypic diversity had opposite effects on leaf miners; leaf miner abundance and species richness were lower in species-rich stands, but higher in mixtures containing several genotypes of silver birch. To test the effects of tree functional diversity, I created a functional diversity index based on constitutive emissions of monoterpenes and isoprene by different tree species and showed that tree species which emitted low levels of volatiles experienced associational resistance in stands with high diversity of volatile emissions. This suggests that increasing chemical complexity in mixed stands may interfere with host finding ability of herbivores.

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List of abbreviations

AR	Associational resistance
AS	Associational susceptibility
FD	Functional diversity
SDH	Semiochemical diversity hypothesis
GLM	General linear model
DBH	Diameter at breast height
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance

Chapter 1

1.1 General introduction

Forests and woodlands throughout the world are acknowledged to be critically important habitats in terms of the biological diversity they contain, the ecological functions they serve (Hooper *et al*. 2005) and the vital ecosystem services they provide (Peterson *et al*. 1998). Biotic agents such as insect and mammalian herbivores and pathogenic bacteria and fungi have marked effects on the health, productivity and quality of tree species in forests. Within a climate change context, these biotic agents combine with abiotic factors such as increasing temperature, ozone, and CO_2 levels and act in an unknown quantity on forest ecosystems (Folke *et al*. 2004; Steffen *et al*. 2004). Before sensible predictions can be made regarding the extent of these effects on forest ecosystems, a fuller understanding of forest ecosystem functioning is required (Hyvonen *et al*. 2007); this is certainly the case with respect to interactions between insect herbivory and tree species richness (Jactel & Brockerhoff 2007), tree species functional diversity (Scherer-Lorenzen *et al.* 2007), and genotypic diversity (Hughes *et al.* 2008). Moreover, the crucial importance of developing a greater understanding of insect interactions in forests is further emphasised as recent experiments and predictions have indicated that many aspects of insect outbreak behaviour will intensify as the climate warms (Logan *et al.* 2003). This thesis is part of a 4 year EU (FP7) funded project BACCARA (Biodiversity and Climate Change a Risk Analysis) www.baccara-project.eu.

Forests in Europe are among the most intensively managed in the world, with only 0.4% of the European forest area covered by non-managed protected forests (Jactel *et al.* 2009). Indeed, forest management-mediated changes in forest extent, composition and structure in Europe have favoured even-aged disturbance-prone monocultures that have been promoted over natural mixed and deciduous forests (Spiecker *et al.* 2004), influencing susceptibility to disturbances (Fettig *et al.* 2007; Jactel *et al.* 2009). Despite recent initiatives in many European countries to plant more mixed woodlands, forests take several decades to change, and current structures and species composition are often the result of policies that were promulgated several decades earlier (Mason 2007).

Some forest ecosystem services are well known and include water conservation, prevention of soil erosion, climate regulation, carbon sequestration, biodiversity conservation, timber and recreation (Nadrowski *et al.* 2010). The boreal forests (the main focus of research in this thesis) sequester 43% of the 958 Pg (10^{15}g) of carbon in closed canopy forests and their soils (Volney & Fleming 2000) and cover 1.02×10^9 ha (over 30%) of the forested lands on Earth. Biodiversity loss is of particular concern in forests, that are thought to contain over half of the known terrestrial plant and animal species (Hassan *et al.* 2005). In addition to forest biodiversity and conservation concerns, loss of tree species in forests may have dramatic and detrimental effects on the functioning of ecosystems (Hooper *et al.* 2005). Insect herbivore regulation being one of the services provided by forest ecosystems that will diminish as forest diversity diminishes (Jactel & Brockerhoff 2007; Castagneyrol *et al.* 2013).

Researching forest diversity effects on insect herbivory is not only interesting from an ecological perspective, enabling us to better understand the complexity of forest ecosystem function, but is also important from a practical forest management and forest crop protection view point. With agricultural crops for example, during the typical 1 or 2 year rotation, farmers are able to utilise certain protective measures i.e. pesticides during a known flight period for a particular insect pest. In forests, the possibility of attack is repeated throughout the many decades of their rotation (Price 1989). Intervention to control insect herbivore outbreaks and high abundance levels (besides controlling for expected and common problems during establishment e.g. *Hylobius abietis* on some conifers) can be costly and almost always severely impact total revenue from timber harvesting at the end of crop rotation (De Turckheim 1993). This is commonly due to the generally low profit margins that production forestry has in many countries where financial cost of crop protection measures are often not redeemed (Price 1989). If, as is commonly believed (Belyea 1923; Graham 1959; Klimetzek 1990), tree stand diversification reduces the likelihood of forest outbreaks and damage from insect herbivores, mixed planting can offer a cheap and viable alternative to minimise damage from insect herbivory and pest outbreaks. If a focal tree species receives less damage when planted in mixture compared to monoculture, it can be said to experience associational resistance (AR) (Tahvanainen & Root 1972). The reverse can also be true, where a focal tree species experiences associational susceptibility (AS) (White & Whitham 2000) when subject to greater herbivore damage when planted in mixtures, compared with monocultures. In forest ecosystems the investigation of the associational resistance and associational susceptibility phenomena, when they occur and why is a matter still to be fully resolved and forms the back bone of this thesis.

1.1.1 Approaches to study forest diversity effects on insect herbivory

Single-species stands (monocultures) of trees are generally believed to be more vulnerable to herbivore attacks than mixed species stands (Elton 1958; Pimentel 1961; Koricheva *et al.* 2006; Vehvilainen *et al.* 2007). However, most of the research to date concerning insect herbivory in forest ecosystems has been collated from observational studies as indicated by Leuschner *et al*. (2009), for example Futuyma & Wasserman (1980). A lot of the evidence and reasoning concerning the effects of stand diversification on pests and pathogens comes from agriculture, where crop diversification to control insect herbivore pests is common (Andow 1991; Trenbath 1993; Mundt 2002). Forest ecosystems are far more complex than these simpler systems in terms of structure, longevity, diversity of biological interactions, as well as greater variation in management techniques. Observational and correlative studies simply compare severity and frequency of pest outbreaks and disease epidemics between low and high tree species diversity forests within and between forest zones (boreal, temperate and tropical) (Pimentel 1961), or specifically between managed and unmanaged forests/plantations (Watt 1992). The present study uses previously established experiments that manipulate tree species richness, tree genetic diversity or functional diversity. Although experimental studies are not flawless in quantifying effects of tree diversity on herbivory (Leuschner *et al.* 2009), they do offer many advantages over other study types. For example, with most observational and correlative studies it is not possible to separate tree species diversity effects from other factors such as environmental variables and land use history. As a result observational and correlative studies lack a certain strength or integrity compared to experimental studies (Scherer-Lorenzen *et al.* 2005). Experiments used in this thesis manipulate stand diversity by creating synthetic stands using mixed planting techniques (Chapter 2). Establishing single-species and mixed stand plots in this manner is considered preferable to so called removal experiments, where in order to achieve a variety of tree species diversity levels tree species or functional groups are physically removed. This procedure affects stand density and causes disruption that may influence the variable of interest (Koricheva *et al*. 2006).

1.1.2 Components of forest diversity and their role in control of insect herbivory

This thesis examines the role of 3 components of forest diversity: tree species richness and species composition, intraspecific genetic diversity, and functional diversity (FD).

All of these components have to some extent been shown to influence insect herbivory in forest systems except FD, but this has been studied in grasslands (Koricheva *et al.* 2000; Symstad *et al.* 2000). The notion that low tree species diversity forests and monocultures receive greater herbivory damage than mixed stands is largely accepted, with some authors recommending stand diversification as a means of controlling forest pests (Belyea 1923; Klimetzek 1990), although the scientific evidence is equivocal (Gibson & Jones 1977; Koricheva *et al.* 2006). In comparison, the manipulation of genetic and functional diversity of forest stands has only rarely been experimentally tested and the picture is less clear with regard to the effect on insect herbivory.

Species diversity

In 2005, an extensive meta-analysis revealed that mixed stands suffer less pest damage and have smaller pest populations than single species stands (Jactel *et al.* 2005), with the trend being more notable with specialist insect herbivores. Boreal forests (the subject of most of this thesis) were under represented in this meta-analysis, with only 5 of the 54 included studies involving this forest type. A more representative meta-analysis of forest experiments from the temperate and boreal forest zones (Vehvilainen *et al.* 2007) revealed a more complicated picture. Responses of herbivores to tree species diversity were found to be dependent on insect herbivore guild and tree species identity. For example, insect herbivory on silver birch was significantly lower in mixtures compared with monocultures, but on alder (*Alnus glutinosa*) the pattern was reversed and insect damage was greater in mixtures. Plant species composition effects on herbivores have been suggested to be more important than effects of plant species diversity (Koricheva *et al.* 2000; Mikola *et al.* 2002; Riihimaki *et al.* 2005; Jactel & Brockerhoff 2007). In particular, Jactel & Brockerhoff (2007) noted that diversity effects on herbivores were greatest when mixed forests comprised taxonomically more distant tree species and when the proportion of non-host trees was greater than that of host trees; this, in addition to tree species richness effects on insect herbivores, also brings into question the role of genetic diversity and functional diversity of tree stands.

Genetic diversity

Studies investigating the role of tree genotypic diversity in structuring the insect herbivore community on woody plants are rare and none exist for silver birch. Although traditionally plant genotypic diversity was assumed to have relatively small effects on biodiversity (Hughes *et al.* 2008), some studies using herbaceous plants (Crutsinger *et al.* 2006; Hughes *et al.* 2008; Cook-Patton *et al.* 2011) indicated that genotypic diversity, may actually affect ecosystem processes in a quantitatively similar way and with similar ecological consequences as species diversity. It has been shown that genotypic diversity can replace the role of species diversity by enhancing biomass production, plant density and faunal abundance in coastal ecosystems (Reusch *et al.* 2005). Increasing genetic diversity of plant species may therefore provide conservation value in a similar manner to increasing tree species diversity. In 2011, Cook-Patton *et al.* published the first ever direct comparison of the consequences of plant genotypic diversity and species diversity on communities and ecosystem function. Using herbaceous plants, they found that above ground primary production increased with both increasing species and genotypic diversity. Furthermore, they found that arthropod species richness also increased with both types of diversity. On tree species, genotypic diversity of willow reduced insect herbivory (Peacock & Herrick 2000; Peacock *et al.* 2001), whist a recent study on oak saplings showed that at the plot level ectophagous herbivores increased in abundance with increasing genotypic diversity (Castagneyrol *et al.* 2012). The effect of intra-specific diversity of woody plants on insect herbivory appears to be variable and requires further investigation.

Functional diversity

The functional diversity (FD) concept is a measure of the value and range of species and organismal traits that influence ecosystem functioning (Tilman 2001) by quantifying biological diversity in ways that account for functional and phenotypic differences (Cadotte *et al.* 2011). Over the last decade or so, the effects of functional diversity of plant communities on ecosystem functioning have received increasing attention. A trait based, causal view of the diversity of communities may therefore be more meaningful than species richness or composition (McGill *et al.* 2006; Cadotte *et al.* 2011). Indeed, recent experiments as well as meta-analyses indicate that FD is one of the best predictors of ecosystem functioning available (Petchey & Gaston 2006; Hoehn *et al.* 2008; Griffin *et al.* 2009; Cadotte *et al.* 2011). The FD concept allows one to focus more on specific traits thought to be associated with the observed phenomenon, enabling functional richness to be determined *a posteriori* (Scherer-Lorenzen *et al*. 2007). Therefore, specific mechanisms (discussed below) can be directly tested. As with plant species richness, FD studies are more common in grasslands, whilst forest systems are poorly studied in this respect. Functional diversity rather than species diversity explained the majority of ecosystem processes i.e. plant productivity, plant nitrogen content, light penetration (Tilman *et al.* 1997), litter decomposition (Scherer-Lorenzen 2008), and insect herbivory (Scherber *et al.* 2006a). In forest ecosystems, FD studies are lacking, however a long term forest diversity experiment (Bechstedt) has been recently established in Germany to address this deficiency (Scherer-Lorenzen *et al.* 2007). The design of the Bechstedt experiment is based on traits primarily indicative of tree growth, morphology, resource use and nutrient cycling. To my knowledge no prior investigation into the effects of FD on insect herbivory in forest systems has occurred.

1.1.3 Mechanisms of forest diversity effects on herbivores

Both the AR (Jactel & Brockerhoff 2007; Kaitaniemi *et al.* 2007; Sholes 2008; Sobek *et al.* 2009) and AS Vehvilainen *et al*. 2007; Sobek *et al*. 2009; Schuldt *et al*. 2010) phenomena have been found in forest ecosystems. The two main hypotheses put forward to account for AR are the enemies hypothesis (Elton 1958) and the resource concentration hypothesis (Root 1973). The natural enemies hypothesis (Elton 1958; Root 1973; Sheehan 1986; Russell 1989) predicts that predators and parasites of insect herbivores are often more abundant in diverse plant communities (polycultures) compared to monocultures, as the majority of enemies are generalist species and survive more successfully on the greater richness of herbivores found in these systems. The resource concentration hypothesis suggests that specialist insects more easily locate, remain and reproduce in large areas of simple systems containing their host plants such as monocultures (or single species stands), consequently attaining greater species richness (Niemela 1983) and higher loads (density per unit mass of the host-plant species) when their food plants grow in high-density patches in pure stands (Otway *et al.* 2005) compared to in polyculture. In the literature there is considerable debate regarding support for these two hypotheses and it is therefore also important to account for and discuss the mechanisms that drive them (Castagneyrol *et al*. 2013). How the relative roles and mechanisms of plant species diversity determine the extent of insect herbivory in forest ecosystems remains an intriguing and perplexing question within forest ecology (Pimentel 1961; Vehvilainen *et al.* 2007). It is suggested that plant diversity has a significant effect on the abundance and species richness of insect herbivores and their predators (Balvanera *et al.* 2006; Vehvilainen *et al.* 2008). In contrast to AR, the AS hypothesis predicts that plants in diverse stands may suffer more from herbivore attack than plants in single-species stands (Brown & Ewel 1987; Plath *et al.* 2011). Associational susceptibility is suggested to occur with generalist insect herbivores (Jactel & Brockerhoff 2007), as they benefit from the broader diet range available in diverse plant communities (Schoonhoven *et al.* 2005; Unsicker *et al.* 2008). Additionally, AS effects may occur when the focal plant species is a less-preferred host growing in close proximity to a highly preferred host (Atsatt & Dowd 1976), allowing a spill-over effect of generalist herbivores after depletion of the favoured host plant species (White & Whitham 2000). There have been a number of predictive and mechanistic hypotheses describing the interactions between plants and herbivores (mainly originating from studies in agricultural/grassland systems). As yet, none of the hypotheses have been developed into a robust general theory (Finch & Collier 2000).

All plants have their own resistance mechanisms to insect herbivores. Broadly, defence reactions can be separated into constitutive defences − traits produced continuously in the plant, regardless of the presence of herbivores, and induced defences − traits produced or increased after initial herbivore damage that then deter further damage (Boege & Marquis 2005). The AR phenomenon acts as an additional defence mechanism (Hamback *et al.* 2000; Jactel & Brockerhoff 2007; Vehvilainen *et al.* 2007; Schuldt *et al.* 2008; Sholes 2008; Orians & Bjorkman 2009; Castagneyrol *et al.* 2013) and as alluded to above was originally thought to be mediated by the resource concentration hypothesis (Root 1973). The resource concentration hypothesis applies to specialist insects and not so much to generalist insects (Root 1973; Schoonhoven *et al.* 2005). Specialist (or monophagous) insects have narrow host plant ranges and often locate larger expanses of their host plant. For a specialist herbivore, any surrounding non-host vegetation decreases the favoured host's apparency/accessibility, by decreasing the likelihood of encounter (Feeny 1989). In contrast, generalist (polyphagous) herbivores benefit from dietary mixing and can often be found at higher levels in plant mixtures; when this leads to damage on less preferred host plants due to depletion of the primary host, this can be referred to as associational susceptibility (AS) (Brown & Ewel 1987; Mulder *et al.* 1999; White & Whitham 2000; Otway *et al.* 2005; Schuldt *et al.* 2010). Within simpler systems i.e. grasslands, the mechanisms behind associational resistance are considered complex, being rather diverse and numerous and thought to act synergistically (Hamback & Beckerman 2003). Neighbouring plants can reduce herbivore damage in several ways (1) by their effects on the predator community, (2) by reducing the ability of herbivores to find their host plants, and (3) by reducing the time herbivores remain on their host plants (Hamback *et al.* 2000).

The natural enemies hypothesis (see above) is one mechanism leading to AR. Natural enemies may also be more abundant in more diverse plant communities because of better provision of alternative energy sources for them including nectar, pollen and honeydew as well as the presence of a more favourable microclimate, greater abundance of suitable overwintering sites or refuges from environmental disturbances (Landis *et al.* 2000). Consequently, it is thought that generalist natural enemies often suppress herbivore populations more in polycultures than in monocultures (Russell 1989). Stiling *et al.* (2003) emphasise the importance of understanding plant community composition on specialist enemies, particularly parasitoids because of the prominent role parasitoids play in biological control and that this is an understudied area. The differential predation pressure on insect herbivores suggested by the enemies hypothesis can also be mediated by plant density and patch size (Otway *et al.* 2005). Studies testing the enemies hypothesis in forest ecosystems are relatively scarce (Riihimaki *et al*. 2005) but some have shown support for the hypothesis (Kemp & Simmons 1978; Cappuccino *et al.* 1998; Jactel *et al.* 2004). Earlier studies in the Satakunta tree species diversity experiment (one of the experiments used in this thesis) provided mixed support for the enemies hypothesis. Minimal evidence of natural enemy effects was found for the autumnal moth (*Epirrita autumnata*) (Riihimaki *et al*. 2005), whereas survival of the European pine sawfly (*Neodiprion sertifer*) on Scots pine was higher in pine monocultures; this was attributed to greater abundance of ants in higher diversity plots (Kaitaniemi *et al*. 2007). Among ground dwelling predatory arthropods, only staphylinids were more abundant in higher tree diversity plots (Vehvilainen *et al*. 2008). In the present thesis, the focus was on ants, spiders and ladybirds, which were the most abundant predators in the Satakunta experiment and more likely to affect foliar insect herbivores.

Volatile chemicals that emanate from plants can provide a trigger to flying receptive insect herbivores that they are passing over suitable host plants (Finch & Collier 2000). Many thousands of plant volatile compounds have been identified (Teranishi & Kint 1992; Zhang & Schlyter 2004). Differences in the composition and relative concentration of constitutive terpenoid volatiles between tree species may be instrumental in the preference of insects for their hosts and the rejection of their non-hosts (Chararas *et al.*

1982; Edwards *et al.* 1993). For example, the bark beetle, *Ips pini*, adjusts its post-landing behaviour in response to monoterpene content of its host, particularly to the concentrations of the monoterpenes β-pinene and limonene (Wallin & Raffa 2000). Koricheva *et al*. (2006) point to studies (Byers *et al.* 1998; Zhang & Schlyter 2003) that show non-host angiosperm volatile compounds to have inhibitory effects on conifer beetles, similarly, high levels of coniferous monoterpene emission can disrupt angiosperm scolytids.

The greater the diversity of tree species growing in an area, the greater and more complex the biochemical emissions present that can interrupt a specific insects ability to detect and choose suitable host trees. It is suggested that this can reduce the incidence of outbreaks and the theory to explain this is referred to as the semiochemical diversity hypothesis (Zhang *et al.* 2001), also referred to as olfactory masking. Olfactory masking can reduce the likelihood of herbivores locating host plants resulting in AR (Zhang & Schlyter 2004; Koricheva *et al.* 2006), but has rarely been tested experimentally in natural habitats (Jactel *et al.* 2011). Olfactory guided host finding by insect herbivores can be disrupted by greater volatile complexity in more tree species rich/diverse communities as compared to less rich and diverse communities (Jactel *et al.* 2011), therefore functional diversity (FD) of tree stands may be important in predicting insect herbivore damage as different tree species vary in their volatile emissions.

Additional factors affecting insect herbivory that may be mediated by plant diversity effects include physical leaf traits i.e. leaf area, leaf thickness and leaf toughness (Feeny 1970; Ayres & Maclean 1987; Basset 1991; Martel & Kause 2002). Leaf chemistry is of course known to affect insect herbivores (Matsuki & Maclean 1994); yet in some instances, physical leaf traits can influence patterns of insect herbivory to a greater extent than leaf chemistry (Clissold *et al.* 2009). Indeed, hypotheses and mechanisms behind plant species diversity effects on insect herbivory can also change as a result of sampling time (i.e. when in the season sampling is conducted). For example, maturation of leaves as the growing season progresses initiates physical and chemical alterations within them (Schoonhoven *et al.* 2005). On silver birch for example, leaf water, nitrogen and phosphorous content declined with leaf maturation whilst leaf toughness increased; consequently larval growth rate of the specialist insect herbivore *Epirrita autumnata* decreased with leaf maturation (Ayres & Maclean 1987). On willow, leaf traits including toughness were found to significantly influence early season insect herbivores (Matsuki

& Maclean 1994). The distribution of insect herbivores changes throughout the growing season. It is commonly thought, in accordance with optimal defence theory (Feeny 1976), that specialist (monophagous) insects generally prefer young tissue and have a species richness peak in the early season, whilst generalist (polyphagous) insects should fare better on mature leaf tissue. This trend was originally applied to oak (Feeny 1970) where leaves are produced mostly during the early growing season (Niemela & Haukioja 1982). Other tree species such as birch and alder produce new leaves throughout the growing season and consequently lepidopteran insect herbivore species richness was found to be more consistent over the growing season and not peak in the early season as with oak (Niemela & Haukioja 1982; Niemela 1983). The influence of physical leaf traits on insect herbivory are addressed within this study for these reasons and partly also because of the ease of assessment as compared, for example, to leaf secondary metabolites including tannins, lignins as well as alkaloids and phenolic glycosides. By measuring physical leaf characteristics it will be possible to firstly, ascertain if forest diversity affects leaf traits of individual tree species and secondly, if forest diversity increases the diversity of leaf traits within a stand to influence insect herbivore damage.

Tree species diversity effects on herbivory may also be mediated by tree size, with larger trees being more physically apparent. Plant apparency can simply be defined as the likelihood of a plant being found by herbivores (Feeny 1970; Endara & Coley 2011). In one respect, trees may be considered apparent because as long lived organisms they are more likely to be discovered by herbivores in comparison, for example, with annual plants (Castagneyrol *et al.* 2013); however, tree apparency in terms of accessibility to insect herbivores is the focus in this thesis and is measured as tree size. A recent study discusses the importance of plant apparency as an overlooked driver of associational resistance to insect herbivory, finding that damage by leaf miners on oak decreases when oaks are less apparent as a result of taller neighbouring non host trees (Castagneyrol *et al.* 2013). Similarly, processionary caterpillar (*Ochrogaster lunifer*) was lower in abundance on acacia trees when acacia was concealed by non hosts (Floater & Zalucki 2000). In addition to the apparency of focal trees being influenced by the height of their most proximal neighbours and stand level tree diversity (Feeny 1976; Castagneyrol *et al.* 2013); growth of individual trees may be differentially affected by mammalian responses to tree diversity, e.g. moose (Milligan & Koricheva 2013).

Abiotic factors may also influence the likelihood of detection by and potential vulnerability of a focal plant to its herbivores (Barbosa *et al.* 2009). The proximity, size and shape of neighbouring trees can interfere with the light levels received by the focal plant as well as alter humidity and temperature (microclimate). Light, for example, is a crucial factor in forest ecosystems and will influence insect herbivory because it is important for controlling the production of leaf defences (Moore *et al.* 1991). Tree canopy, leaf distribution, time of bud break and the physical structure and biochemical processes occurring in leaves are all controlled by the amount of light entering the forest and can influence herbivore colonisation and herbivory on focal plants (Osisanya 1970). Another example is the availability and quality of soil nutrients (which may be influenced by neighbouring plants) needed for defence and growth of focal plants, which in turn may affect vulnerability to herbivores (Barbosa *et al.* 2009). For example, nitrogen and carbon content in oak leaves was significantly higher when oak was planted in a mixture with alder compared to pure oak plots and oak/spruce plots, as alder fixes nitrogen (Moore & Francis 1991). Elevated leaf nitrogen concentration has been noted to result in increased growth rates of herbivores (Onuf *et al.* 1977) and found to cause higher population numbers and damage levels (Onuf *et al.* 1977; Myers & Post 1981).

Very little is known regarding effects of plant genetic diversity on insect herbivory, consequently the mechanisms behind these effects are also fairly elusive. Plant genotypic diversity effects on arthropods could be additive or non additive (Johnson *et al*. 2006; Tack & Roslin 2011). An additive effect occurs, for example when different plant genotypes support different species or abundances of insect herbivores, which are added up in a mixture of these plant genotypes resulting in higher herbivore abundance and species richness. The majority of the mechanisms discussed above in relation to plant species richness effects on insect herbivores may also apply to genotypic diversity. For example, more genetically diverse plant patches may provide a greater variety of niche environments for insect herbivore predators in accordance with the enemies hypothesis. The birch clones used in the Satakunta genetic diversity experiment are known to vary with regard to leaf traits, tree size and architectural complexity, as well as in resistance to moose (Jia *et al.* 1997), leaf rust (Poteri *et al.* 2001), voles, hares, stem lesions and cankers (Vihera-Aarnio & Velling 2001). It is therefore predicted that insect herbivory will also be influenced by genetic differences between the clones. However, focal and neighbouring plants that are more closely related are more likely to share herbivores (Barbosa *et al.* 2009). To this end, genetic diversity effects on herbivory within a single plant species may have weaker effects on insect herbivores than plant species diversity (Andow 1991; Tonhasca & Byrne 1994).

1.1.4 Thesis aims

The overall aim of this thesis is to research the effects of forest diversity on insect herbivory using three long-term forest diversity experiments to explore when and why associational resistance (AR) and associational susceptibility (AS) phenomena occur. I address three main questions: [1] To what extent do different components of forest diversity (tree species richness, species composition, genetic diversity and FD) affect insect herbivores in forest systems? [2] How variable are these effects temporally, spatially, between tree species and between herbivore types/feeding guilds? [3] What are the mechanisms of these effects (natural enemies, tree apparency – physical and chemical, leaf traits)?

1.1.5 Structure of the thesis

Chapter 2 explains the experimental set up and design of the three long term forest diversity experiments used in this thesis and the method employed to sample insect herbivory within them.

In Chapter 3, I use the Satakunta tree species diversity experiment in Finland to investigate the effect of tree species diversity on insect herbivore guilds of silver birch. I test whether these effects are consistent between guilds, spatially and temporally and whether tree species diversity effects are mediated by natural enemies, tree size and physical leaf characteristics.

In Chapter 4, also using the Satakunta tree species diversity experiment, I focus on the effects of tree species diversity on the abundance and species richness of the leaf mining feeding guild on silver birch and black alder.

In Chapter 5, using the Satakunta silver birch genetic diversity experiment in Finland, I examine the effect of intraspecific/genetic diversity of silver birch on the leaf mining guild.

In Chapter 6, the role of plant functional diversity is explored. Utilising the Bechstedt experimental forest site in Germany, I research the plant functional diversity concept and the influence of the diversity of constitutive tree volatile defence compounds on insect herbivory. My research here is stimulated by (among others) the work of Tilman *et al.* (1997), Scherer Lorenzen *et al*. (2008) and Scherber *et al*. (2006a) who show that plant functional diversity when compared to plant species diversity *per se* explained better the majority of ecosystem processes studied.

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

Chapter 2

Experimental sites and insect monitoring

2.1 Experimental sites and experimental design

The experimental sites used for this study are all long term forest biodiversity experiments, whereby either tree species diversity, tree genetic diversity, or tree functional diversity have been manipulated using experimental designs that are intended to elucidate some of the complexity of forest ecosystem functioning.

2.1.1 Tree species diversity experiment, (Satakunta, Finland)

Located in the south west of Finland (61ºN, 21ºE) in the boreal forest zone, the Satakunta tree species diversity experiment was planted in 1999 on three clear cut areas (20-30 kilometres from each other) which are approximately two hectares each. The three experimental areas are between 20 and 50 metres above sea level, with each area being flat (minimal slope). All three areas consist of podzol soils. Prior to the current use all areas were Norway spruce dominated mature forest. The experiment uses a species pool of five: *Betula pendula* (silver birch), *Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), *Alnus glutinosa* (common or black alder) and *Larix sibirica* (Siberian larch). The first three, birch, Norway spruce and Scots pine are the three most commonly planted tree species in Finland and are of significant economic importance. Alder was included in the experiment because it is a nitrogen fixing species which might affect the nutritional status and growth of other tree species in the mixture. In addition, it belongs to the same family (Betulaceae) as birch and shares a number of insect herbivores. Siberian larch is native in western Russia, but is commonly planted as an exotic conifer in Finland. It is a deciduous conifer, unlike evergreen pine and spruce, therefore providing a transition within the experiment from deciduous broadleaves (birch and alder) to evergreen conifers. Each of the the 3 areas consists of 38 plots randomly allocated to 19 treatments (Table 2.1) providing a gradient from purely deciduous broadleaf stands to mixed broadleaf-conifer stands and purely coniferous evergreen stands (Fig. 2.1). The 19 treatments (Table 2.1) represent monocultures of all 5-tree species, seven 2-species mixtures, six 3-species mixtures and one 5-species mixture, which are replicated on each site to give the 38 plots mentioned above. Each plot is 20 x

20 metres and contain 13 rows with 13 trees in each row (169 trees per plot) planted at 1.5 metre spacing. Mixed species plots are composed of equivalent number of trees per species (i.e. in a two species mixture, species A and B will both consist of 84 trees), the planting design is randomised within the plots. One year after planting, trees that had experienced mortality were re-planted. In May 2010, the experimental plots were cleaned from natural regeneration. At the time of study (2009-2011), trees in the Satakunta forest diversity experiment were between 1.33 and 11.89 meters tall and canopy closure was achieved on most plots.

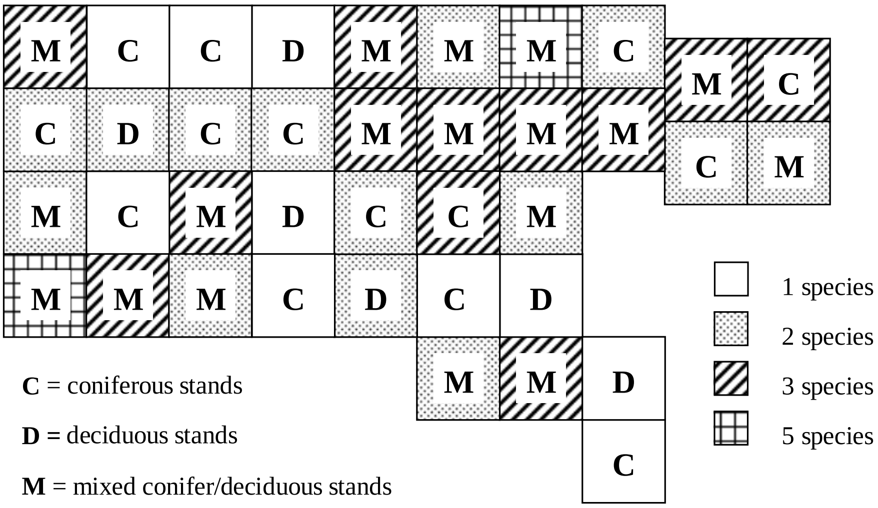


Figure 2.1 Plot layout of one of the three areas at the Satakunta tree species diversity experiment (courtesy of J. Koricheva).

Table 2.1 The 19 treatments planted at the Satakunta forest diversity experiment Finland

No of tree species in mixture	Treatment	Treatment abbreviations
1	pine	P
	spruce	S
	larch	L
	birch	B
	alder	A
2	pine + birch	PB
	spruce + birch	SB
	birch + alder	BA
	pine + larch	PL
	pine + spruce	PS
	spruce + larch	SL
	spruce + alder	SA
3	pine+ birch+ alder	PBA
	pine+ larch+ birch	PLB
	larch+ birch+ alder	LBA
	pine+ spruce+ birch	PSB
	pine+ spruce+ larch	PSL
	spruce+ larch+ alder	SLA
5	pine+ spruce+ larch+ birch+ alder	PSLBA

Plate 2.1 Birch monoculture plot - Satakunta tree diversity experiment, 2009 (photo: J. Koricheva).

Plate 2.2 Birch and spruce plot - Satakunta tree diversity experiment 2009 (photo: J. Koricheva).

2.1.2 Genotypic forest diversity experiment (Satakunta, Finland)

The Satakunta genotypic forest diversity experiment was established in SW Finland (61ºN, 21ºE) in 2000 and represents a 2 ha clear cut area on podzol soil approximately 30 metres above sea level, which consists of 48 (20 x 20m) plots planted with different genotypes of silver birch (*Betula pendula*). The surrounding vegetation is mostly mature *Picea abies* managed forest. Planting distance between trees was 2 metres and each plot contains 100 trees. Plastic vole protectors (Agrame Oy, Finland) were used to reduce early mortality due to vole damage.

Eight genotypes of silver birch were used in the experiment (36, K1659, K5834, K2674, V5952, V5818, O154, JR1/4). The above genotypes are of southern Finnish origin (61-63°N), have been obtained by micro-propagation of vegetative buds of mature birch trees and represent a range of varying responses in their susceptibility to fungal pathogens and mammalian herbivores (Jia *et al.* 1997; Poteri *et al.* 2001; Vihera-Aarnio & Velling 2001).

Plots are randomly assigned to the following genotypic diversity treatments: single-genotype plots, two-genotype mixtures (5 different combinations), four-genotype mixtures (5 different combinations), and an eight-genotype mixture (Fig. 2.2.). Five out of 8 genotypes had 2-3 replicates of single-genotype plots, but for the remaining 3 genotypes (K2674, V5818 and K5834) only a single plot was planted due to problems with the micro-propagation. Plots with multiple genotypes contained the same numbers of plants of each genotype, but the positions of each genotype were randomized. Each particular genotype combination was replicated 2-6 times within the experimental area, permitting separation of the effects of genotype diversity and genotype composition. The only experimental intervention after establishment was some replanting of dead trees between 2001 and 2003 and cleaning (removal of natural regeneration), that took place in 2005 and in 2009. The silver birch genotypic diversity experiment was sampled twice in 2011 (early season and late season) and only for leaf miner abundance and species richness. At the time of study (2011), birch trees in the Satakunta genotypic diversity experiment had DBH measurements between 3 and 105 cm, approximately equating to 0.5 and 10 metres in height; as basal stem diameter correlates with tree height in young silver birch trees (Kaitaniemi & Lintunen 2008). Canopy closure was achieved on most plots.

1 – clone V5818 (violet label, 1 replicate)
2 – clone V5952 (yellow label, 3 replicates)
3 – clone JR ¼ (green label, 2 replicates)
4 – clone 36 (orange label, 3 replicates)
5 – clone K2674 (pink label, 1 replicate)
6 – clone K1659 (white label, 3 replicates)
7 – clone O154 (red label, 3 replicates)
8 – clone K5834 (blue label, 1 replicate)
9 – clone V5818 + clone V5952 (3 replicates)
10 – clone 36 + clone V5952 (3 replicates)
11 – clone V5818 + clone K1659 (3 replicates)
12 – clone O154 + clone K5834 (2 replicates)
13 – clone 36 + clone K1659 (3 replicates)
14 – clone O154+clone K2674+clone V5952+clone V5818 (2 replicates)
15 – clone V5818+clone V5952+clone 36+clone K1659 (3 replicates)
16 – clone JR¼+clone K2674+clone O154+clone K5834 (2 replicates)
17 – clone JR¼+clone K2674+clone V5952+ clone 36 (2 replicates)
18 – clone JR¼+ clone K5834+clone K1659+clone 36 (2 replicates)
19 – all 8 clones (6 replicates)

Figure 2.2 Treatments of the Satakunta silver birch genetic diversity experiment (48 plots in total, over 1 area).

2.1.3 BIOTREE experiment (Thuringia, Germany)

BIOTREE (BIOdivestity and Ecosystem Processes in Experimental TREE Stands) experiment was established in 2003-2004 in close cooperation between the Max Planck Institute for Biogeochemistry and the State Forest Research Institution of Thuringia (Scherer-Lorenzen *et al.* 2005). Prior to establishment of the forest experiment, the sites were used for agricultural crops until 1975 when it was converted to grassland. There are three sites in Thuringia (Bechstedt, Mehrstedt and Kaltenborn) representing the temperate forest zone, they are all within a radius of 70 kilometres of each other. In this thesis I used only the Bechstedt experimental site (11º05'E, 50º54'N) in which tree species richness has been kept constant (4 species per plot); but tree functional diversity (FD) has been manipulated by selecting combinations of tree species with different functional traits out of the total pool of 16 tree species which are common in natural forest communities surrounding the experimental plantation (Table 2.2, Scherer-Lorenzen *et al*. 2007). Nine traits indicative of tree growth, morphology, resource use and nutrient cycling were used to characterize functional attributes of each tree species. FD indices for all possible 4-species mixture combinations were then calculated using the method of Petchey & Gaston (2002) and 24 species combinations were selected to represent the whole range of possible values of functional diversity (Table 2.2, Scherer-Lorenzen *et al*. 2007). The description of the 9 traits used and the calculation of FD index are discussed in Chapter 6.

The plot size is 1,700m^{2}, with each tree species panted in 11 circular groups of 20 individuals, (area of 38.5 m^{2}). The planting design has been selected to avoid early out competition of climax tree species by pioneers, i.e. birch out-competing oak (Fig. 2.3). The Bechstedt forest experiment has an elevation of 400-405m above sea level, consisting of limestone bedrock and stagnic vertisol soil type (Scherer-Lorenzen *et al.* 2007). The site is fenced to exclude large mammal grazing. Mowing, to keep weeds at bay took place during the establishment phase. No further management of the site had taken place prior to insect herbivore monitoring in 2009.

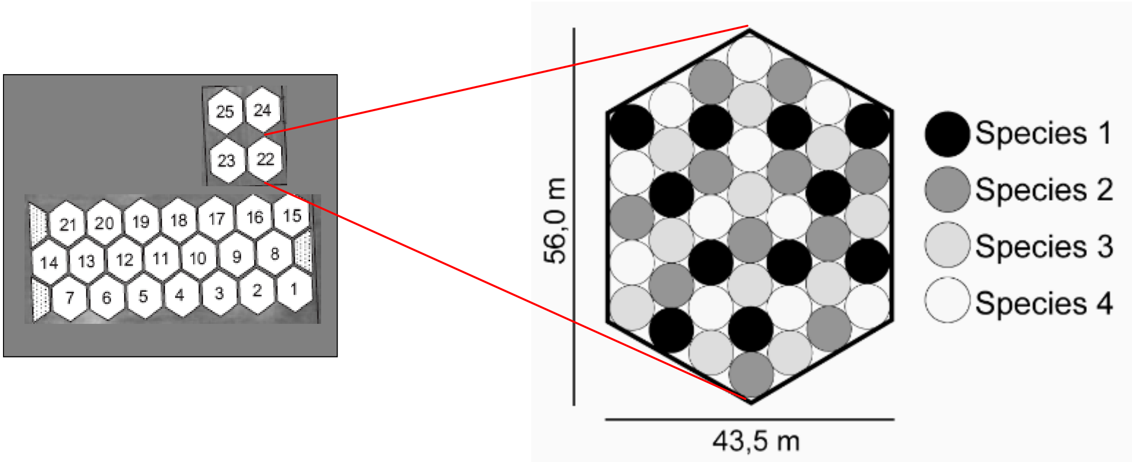
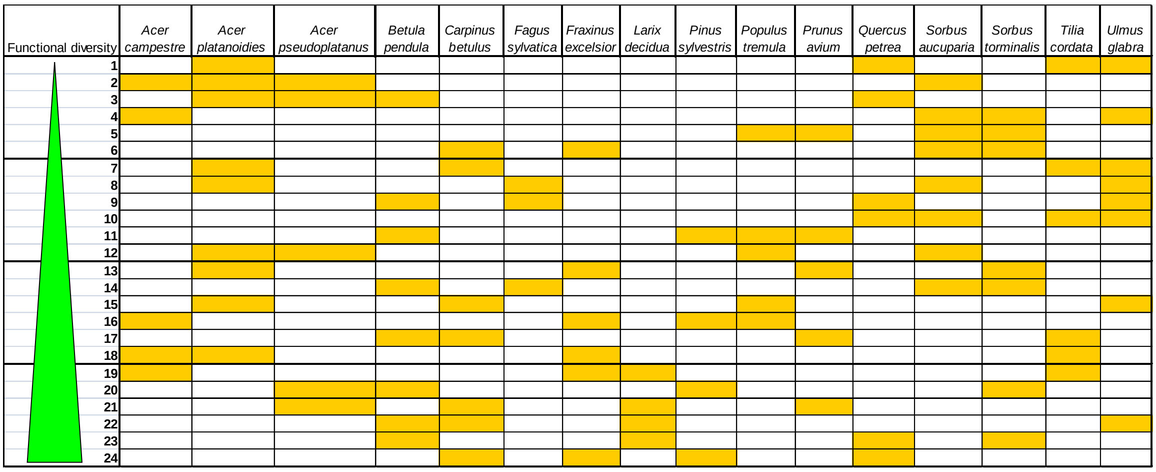


Figure 2.3 Plot layout at the Bechstedt experiment (Source: Scherer-Lorenzen *et al*. 2007)

Table 2.2 Tree species planted at Bechstedt to achieve different functional diversity levels. Yellow bars indicate tree species used at each diversity level from 1 (lowest) to 24 (highest). Adapted from Scherer-Lorenzen *et al.* (2007).



Functional diversity	*Acer campestre*	*Acer platanoidies*	*Acer pseudoplatanus*	*Betula pendula*	*Carpinus betulus*	*Fagus sylvatica*	*Fraxinus excelsior*	*Larix decidua*	*Pinus sylvestris*	*Populus tremula*	*Prunus avium*	*Quercus petrea*	*Sorbus aucuparia*	*Sorbus torminalis*	*Tilia cordata*	*Ulmus glabra*
1		🟨										🟨			🟨	🟨
2	🟨	🟨	🟨										🟨			
3		🟨	🟨	🟨								🟨				
4	🟨												🟨	🟨		🟨
5										🟨	🟨		🟨	🟨		
6					🟨		🟨						🟨	🟨		
7		🟨			🟨										🟨	🟨
8		🟨				🟨							🟨			🟨
9				🟨		🟨						🟨				🟨
10												🟨	🟨		🟨	🟨
11				🟨					🟨	🟨	🟨					
12		🟨	🟨							🟨			🟨			
13		🟨					🟨				🟨			🟨		
14				🟨		🟨							🟨	🟨		
15		🟨			🟨					🟨						🟨
16	🟨						🟨		🟨	🟨						
17				🟨	🟨						🟨				🟨	
18	🟨	🟨					🟨								🟨	
19	🟨						🟨	🟨							🟨	
20			🟨	🟨					🟨					🟨		
21			🟨		🟨			🟨			🟨					
22				🟨	🟨			🟨								🟨
23				🟨				🟨				🟨		🟨		
24					🟨		🟨		🟨			🟨				

The species planted to obtain each diversity level are shown in Table 2.2. At the time of study (2009), trees in the Bechstedt experiment were between 0.6 and 6.6 meters tall and canopy closure was achieved only on a few plots.

2.2 Insect herbivory monitoring protocol

2.2.1 Satakunta tree species diversity experiment

Ten trees per species per plot were randomly selected for *in situ* monitoring during the first visit in 2009. The trees were selected from the centre (core area) of each plot to reduce edge effects. The selected trees were tagged and subsequently used for all other monitoring to allow for the possibility of repeated measures analysis. Four branches per tree, two in the lower canopy facing opposite directions and two in the upper canopy facing opposite directions, were randomly selected for the monitoring. For alder and birch 25 leaves per branch (100 leaves per tree) were monitored. (Note: the same branches on an individual tree were not repeatedly monitored). Step ladders and telescopic pruners were utilised to sample the upper canopy on some of the taller birches (i.e. those between 8 and 12m in height). Alder was not monitored in 2009. In 2010 herbivory on alder was recorded, but only leaf miner data were used.

The tree species diversity experiment at Satakunta was visited in 2009, 2010 and 2011 and sampled both in the early and late season on these years. This helped to gain perspective of insect damage over a complete season and reflects the seasonal emergence of insect herbivores, as different types of herbivores are present at different times of the season (Chapter 1). In all years, the early season (2 to 3 weeks after bud break) sampling period was the last week of May to the beginning of June (i.e. the first two weeks); the late season sampling period was always at the end of July/ beginning of August. At each time point monitoring took approximately 3 weeks to complete. The order of monitoring of areas was the same between all years and time points (first was area 1, then area 3 and then area 2) to help reduce variability when comparing between year data.

In 2009, many of the plots in areas 2 and 3 were excluded from monitoring because they were overgrown with naturally regenerating pine, birch and rowan which altered the tree species composition of plots. Therefore, only area 1 was fully sampled in 2009. In early May 2010, the Satakunta experimental plots were cleaned of naturally regenerating tree species and all 3 areas were sampled in 2010 and 2011 (both early and late season) with the exception of 3 plots in area 2 that had not established due to repeated moose browsing. The omitted plots were numbers 49 (LBA), 58 (BA), and 67 (A); see Table 2.1 for abbreviation definitions.

Insect herbivore damage was measured mostly at guild level and included: chewing, skeletonising, mining, leaf-rolling, leaf-tying, and leaf-galling. Chewing damage and skeletonising damage was classified separately and for each examined leaf as % leaf area missing: (1) less than 5%, (2) 5-25%, (3) 26-50%, (4) 51-75% and (5) 76-100%. Percent leaf area damage was first calculated per branch (for skeletonising and chewing damage separately) by multiplying the midpoint of each category by the number of leaves in this damage category, summing the values and dividing by the number of leaves (25). Averaging the four branches provided overall percent leaf area damage per tree for chewing and skeletonising insects.

As well as recording leaf damage, the relative abundances of sap-feeding and sucking insect herbivores observed (aphids and leafhoppers), chewing insect herbivores (lepidopteran and sawfly larvae, beetles), and natural enemies (spiders, ants and ladybirds), were recorded as the total number of insects per tree (per 100 leaves).

In some instances data were recorded at insect herbivore species level. This occurred for silver birch aphid (in all 3 years), gallers in 2010 and 2011 were recorded separately because they are produced by two different mite species (*Aceria leionotus* and *Acalitus rudis*, Eriophyidae), and leaf miners in 2010 and 2011. The details of leaf miner species richness monitoring are described in Chapter 4.

2.2.2 Satakunta genotypic diversity experiment

This experiment was visited twice in 2011 (early season and late season) to account for different species of leaf miners present at different times during the season; and the same trees monitored on each occasion. Leaf miner abundance was recorded to species level on 5 trees per clone per plot (i.e. in a monoclonal plot 5 trees were monitored, in a four clone mixture 20 trees were monitored). Similar to the Satakunta tree species diversity experiment, four branches, two in the lower canopy and two in the upper canopy were randomly selected from opposite sides of each tree. Fifty leaves per branch were examined (200 leaves per tree). In order to access the upper canopy telescopic pruners were used. Number of leaf miner species present per branch and abundance of each leaf miner species were recorded.

2.2.3 Herbivory monitoring protocol at Bechstedt

The Bechstedt experiment was sampled once during July 2009. Herbivory monitoring protocol was similar to that used in the Satakunta experiment. Within each of the 25 hexagonal plots at Bechstedt, 6 clusters of each of the 4 tree species (as close to the centre of each plot as possible, to avoid edge effects) were selected for the monitoring. Within each circular cluster, 1 tree from the 20 present (again from the centre) was randomly selected. This provided 6 trees per species per plot, and 2 branches from the lower canopy and 2 branches from the upper canopy at opposite sides of each tree were randomly selected for the herbivory assessment. The herbivory monitoring protocol described above (section 2.2.1) used at Satakunta was practically identical to that used at Bechstedt, although an additional damage category (bud galls) was added. Bud galls were recorded on *Carpinus betulus, Fagus sylvatica,* and *Quercus petraea*. Step ladders were sufficient to access the upper canopy of the tallest trees as the experiment was younger and trees smaller than at Satakunta.

2.3 Tree growth and leaf traits

In addition to herbivore damage, surveyed trees at the Satakunta tree species diversity experiment were measured in order to assess tree growth and physical leaf traits. Leaf traits were measured during the late season monitoring of 2010 at area 1 only; whereas tree growth was measured in 2010 and 2011 during the late season and at all areas.

Measures of tree growth characteristics included tree height, diameter at breast height (DBH) S-N and W-E, and crown projection (S-N and W-E) which was assessed as the distance from the trunk to the tips of the longest living branches in each direction.

Leaf traits measured included leaf area, leaf thickness, and leaf toughness. One undamaged leaf per branch (the same branches as used for herbivory monitoring) was sampled for leaf trait measurements, resulting in 4 leaves per tree sampled. Four toughness and thickness 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. Leaf area was calculated by photographing the leaves against a scale under glass (to hold them flat) and using Image J software (Rasband 2005) to determine leaf area in mm^2. Specific leaf area (SLA) was calculated by dividing surface area (mm^2) by dry leaf weight (g); leaves were dried for 24 hours at 60 degrees Celsius.

Chapter 3

Tree species diversity effects on insect herbivory on silver birch in the boreal forest zone

3.1 Introduction

Human activities can influence the composition and therefore functioning of many natural habitats and ecosystems including forest ecosystems which provide habitats for a variety of organisms and serve many important ecological functions (Hooper *et al.* 2005). Insects form the majority of faunal diversity in forests (Weisser 2004). By directly influencing the growth and species composition, insect herbivory can significantly affect ecosystem processes such as plant production and nutrient cycling (Weisser 2004; Thebault & Loreau 2006); thus highlighting the complex interplay between trophic levels and the importance of understanding tree species diversity effects on insect herbivory (Schuldt *et al.* 2010).

The majority of earlier studies investigating relationships between plant diversity and insect herbivory have been conducted in agricultural and grassland ecosystems (Andow 1991; Tonhasca & Byrne 1994; Jactel *et al.* 2005; Scherber *et al.* 2006b; Unsicker *et al.* 2006). Some of these studies (Andow 1991; Tonhasca & Byrne 1994; Jactel *et al.* 2005; Scherber *et al.* 2006b; Unsicker *et al.* 2006) concluded that herbivores decrease in abundance with increasing plant species diversity and hence plants growing in mixed stands experience 'associational resistance' (AR) to herbivores (Tahvanainen & Root 1972). Other studies of plant diversity effects on insect herbivores (Mulder *et al.* 1999; Otway *et al.* 2005; Schuldt *et al.* 2010) have shown the opposite effect to AR, a phenomenon referred to as associational susceptibility (AS), whereby a plant species experiences greater damage when planted in more diverse plots (see Chapter 1). Similar research in forest ecosystems has begun more recently, but also produced conflicting results as illustrated by the titles of two recent publications: 'Tree species diversity reduces insect herbivory by forest insects' (Jactel & Brockerhoff 2007) and secondly 'Tree diversity promotes insect herbivory...' (Schuldt *et al.* 2010).

The observed variation in insect herbivore responses to plant diversity could be partly explained by the diet breadth of herbivores. In general, AR is more commonly exhibited to specialist herbivores whereas AS response is usually displayed to generalist herbivores (Schoonhoven *et al.* 2005). For instance, meta-analysis by Jactel & Brockenhoff (2007) has found that oligophagous species responded strongly and negatively to forest diversity, but the response was not so strong and more variable in polyphagous species. This may be because specialist herbivores target larger patches of their host plant resulting in higher abundances in mono-specific patches as compared to smaller or diluted patches (the resource concentration hypothesis (Root 1973)). Furthermore, resource concentration may also affect distribution of specialist herbivores between individual plants in mono-specific patches. For instance, larger plants provide more resources and may have greater abundance of insect herbivores even when host plant density is low (Marques *et al.* 2000; Barbosa *et al.* 2009). For example, herbivore density in sparse plantings of oak can be many times greater than that on plants in dense stands (Futuyma & Wasserman 1980). In contrast, generalist herbivores can benefit from dietary mixing and have greater resources at their disposal in more diverse plots (Schoonhoven *et al.* 2005; Unsicker *et al.* 2008; Schuldt *et al.* 2010). This Chapter compares responses to forest diversity of a variety of insect herbivores with different degrees of feeding specialisation, thus allowing the detection of general patterns. Mechanisms now thought to drive insect herbivore responses to forest diversity are considered to be numerous and complex and likely to interact synergistically (Hamback & Beckerman 2003). Some of these mechanisms include abundance and distribution of predators, disruption and masking of volatile cues, physical interference, tree apparency, and foliage quality; these are discussed in Chapter 1 and excellently reviewed in Barbosa *et al.* (2009). In this Chapter I focus on the role of natural enemies, tree apparency and physical leaf traits in mediating tree diversity effects on insect herbivores. Studies testing the enemies hypothesis (Chapter 1) in forest ecosystems are relatively scarce (Riihimaki *et al.* 2005). In the present study, the focus was on ants, spiders and ladybirds, which were the most abundant predators in the Satakunta experiment and most likely to affect foliar insect herbivores.

Physical leaf properties such as leaf area, thickness and toughness are known to influence insect herbivore performance (Feeny 1970; Ayres & Maclean 1987; Basset 1991; Martel & Kause 2002) and can sometimes affect patterns of herbivory more than leaf chemistry (Clissold *et al.* 2009). These traits are easily measurable and are included in this study as covariates to investigate if forest diversity effects on herbivores can be mediated by differences in leaf and tree characteristics. Insect survival and larval growth can be influenced by many other leaf quality traits including plant primary and secondary metabolites (Matsuki & Maclean 1994). For instance, secondary metabolites including tannins and lignins as well as alkaloids and phenolic glycosides are acknowledged to be significant factors in herbivore defence but are beyond the scope of this study. In addition to leaf traits, the effects of tree species diversity on insect herbivores may be mediated by tree size or apparency to herbivores (Castagneyrol *et al.* 2013). The plant apparency hypothesis refers to ease of host finding which is also linked to plant size; it predicts that some plant species may not benefit from AR because they are conspicuous or 'apparent' to herbivores (Feeny 1976; Sholes 2008). If tree growth and apparency differ between pure and mixed stands, this could result in higher herbivore abundance in stands where host tree species grow best. From the insect herbivore perspective, plant apparency may also manifest itself via chemical odours or volatile emissions, which is the subject of Chapter 6.

Many of the previous experimental studies of forest diversity effects on insect herbivores used data from a single time point (Coley & Barone 1996; WoonIk *et al.* 1997; Barone 2000; Schuldt *et al.* 2010), a number of studies repeated observations yearly (Berisford & Kulman 1967; Brown *et al.* 1988; Moore *et al.* 1991), but few studies looked at within-season variation and area effects (Vehvilainen *et al.* 2006). Given the complexity, longevity and biodiversity of forest ecosystems, such studies can only provide snapshots of forest diversity effects in space and time. In contrast the present study utilises a long-term experiment, set up in three different areas and reports results from 2 consecutive years as well as 2 dates within each growing season; hence allowing assessment of both temporal and spatial variation in tree species diversity effects on insect herbivore guilds which have been reported to occur previously (Vehvilainen *et al.* 2006). What is also evident from some previous experimental studies on trees is that they have been conducted either on small planted saplings or on young potted plants that clearly differ from a natural forest environment. For example, potted plants are likely to experience higher nutrient, light and water stress whilst being less likely to produce herbivore-induced volatile signal compounds, thus potentially obscuring effects of polyculture (Andow 1991; Holopainen 2008). At the time of sampling, the Satakunta experiment enabled the study of 11-12 year old trees, which had past the establishment phase and most of the plots containing birch had already reached canopy closure.

This study investigates the effects of tree species diversity on insect herbivore abundance on silver birch (*Betula pendula* Roth) in an established, long term forest diversity experiment in the boreal forest zone. Silver birch has been selected as the focal tree species in this study because not only does it support a diverse array of insect herbivore species (Shaw 1984; Atkinson 1992), but is also one of the most commonly planted tree species in Finland and commercially the most important deciduous tree species in Fenno-Scandinavia (Heiramo & Ruutu 1994; Vihervuori *et al.* 2008). Boreal forests, in particular, have been poorly studied in terms of the effects of tree species diversity on insect herbivores (Vehvilainen *et al.* 2007). Specifically, the following questions have been addressed:

1) Does tree species diversity influence insect herbivore abundance and damage on silver birch in accordance with the 'associational resistance' or 'associational susceptibility' phenomenon?
2) Are tree species diversity effects on insect herbivores consistent between guilds?
3) Are tree species diversity effects on insect herbivores stable temporally and spatially?
4) Can insect predators (spiders, ants, and ladybirds) explain variation in herbivore damage in accordance with the enemies hypothesis?
5) Are tree species diversity effects on insect herbivores mediated by changes in tree growth and physical leaf trait characteristics?

3.2 Methods

Insect herbivory monitoring at the Satakunta experiment in Finland took place during the early season and the late season of 2009 and 2010. In both years the early season monitoring was conducted during the first two weeks of June and the late season monitoring took place at the end of July to early August. In 2009, some plots in areas 2 and 3 were not sampled due to poor plot condition (Chapter 2). In May 2010 all plots were cleared of naturally regenerating woody species (Chapter 2), allowing insect herbivory monitoring in all plots.

Ten birch trees from the core (centre) area of each plot were randomly selected for *in situ* monitoring during the first visit in 2009. These trees were tagged and subsequently used for all other monitoring to allow for the possibility of repeated measures analysis. The monitoring procedure is fully described in Chapter 2. Covariate measurements for tree size and physical leaf trait characteristics are also described there.

Insect damage and guilds

In the majority of cases insect damage was recorded as guild-specific, i.e. chewing, skeletonising, gallers, rollers, mining, leaf folders and tiers, etc (see Chapter 2). The only guilds or categories abundant enough for parametric analysis, having appropriate distribution of model residuals, were chewing, skeletonising, galling, rolling, silver birch aphids and insect predators. Leaf miners are the subject of analysis in Chapters 4 & 5 and not included in the present Chapter. The leaf chewing insects observed during monitoring were sawfly or lepidopteran larvae and arguably are the most likely candidates for causing the majority of the leaf chewing damage observed. Birches in Finland support more than 40 species of leaf chewing sawflies that belong to many different genera (Hanhimaki 1989; Nyman 2007). According to Atkinson (1992), silver birch in the UK can support up to 116 leaf eating lepidopteran species, over half of which (64 species) are specialists. Spring and early summer insect herbivore communities consist primarily of free-living larval Lepidoptera of the family Geometridae. Notable amongst these are the genera *Operophtera*, *Epirrita*, *Erranis*, and *Agriopis*, other free-living lepidopteran larvae present in early spring are members of the noctuid and tortricid families (Fisher *et al.* 1999). No insect species were observed during the monitoring periods that cause skeletonising damage, but this was likely to have been caused by early larval instars of late season lepidopteran's, sawflies or beetles (J. Koricheva, personal communication).

The two types of leaf galls studied in the present Chapter are caused by mites, *Acalitus rudis* (Canestrini) and *Aceria leionotus* (Nalepa) (Arcania: Eriophyidae). Although not insects the leaf gall damage they cause is herein referred to generally under 'insect herbivory damage'. Gall mites are small with body length usually 0.14 - 0.40mm, are very host specific and have poor dispersal ability (usually by wind). Female mites overwinter in the buds or axils of short shoots and colonise the expanding leaves in spring (Koricheva *et al.* 1996).

Leaf rolls on silver birch are made by many insect groups including weevils, moths, sawflies and midges. Their shelters can be very similar in appearance and larvae need to be checked for species level identification (Nyman 2007), which was not possible in the present study. A common and distinct species of leaf roller in Finland (and at Satakunta) is *Deporaus betulae* (Coleoptera: Rhynchitidae), which is oligophagous on birches, alder (*Alnus* spp.) and hazel (*Corylus* spp.).

Silver birch aphid, *Euceraphis betulae* (Koch) (Homoptera: Drepanosiphidae), was the most abundant aphid species on birch in Satakunta and was monitored and analysed individually; this species produces several generations each year. In summer, adults are winged females produced parthenogenetically. During autumn, winged males and wingless egg-laying females are produced, sexual reproduction occurs and eggs are laid which survive over winter. *Euceraphis betulae* aphid is known to cause periodic outbreaks during mass migrations (Nieminen *et al.* 2000) and prefers growing leaves to mature ones (Hajek & Dahlsten 1986).

Total herbivore damage

Skeletonising and chewing damage were scored as percent leaf area damage, other damage types were scored as percent of leaves damaged. To gain a perspective on total herbivore damage, all 9 feeding guilds recorded were used and each herbivory category was considered in both early and late season of 2010. For each damage category each tree was assigned a value between 1 and 4 as follows:

1= between the lowest value and 25% of maximum damage recorded for this category.
2= between 26% of damage and 50% of maximum damage recorded for the category.
3= between 51% of damage and 75% of maximum damage recorded for the category.
4= between 76% of damage and maximum damage recorded for the category.

Maximum damage was taken as the highest recorded damage level of a particular guild/ insect, on a per tree basis. For each tree, the new variables for each damage category were summed to provide an index value of total insect herbivore damage per tree.

3.2.1 Data analysis

All statistical analyses were performed using SPSS version 19.0. Herbivore damage at Satakunta for questions 1 and 2 was examined using repeated measures linear mixed models. ‘Season’ (early vs. late season) or where applicable ‘year’ (2009 vs. 2010) were used as a repeated factor to study temporal effects. Fixed factors included in the model were ‘area’, ‘time’ and ‘number of species’ (tree species diversity).

Since in 2009 only area 1 was fully sampled, 2009 data analysis is restricted to area 1 only. Spatial effects are tested in 2010 when all 3 areas were fully sampled. For each herbivore guild, data from 2009 and 2010 were first analysed separately; allowing for analysis of differences between early and late season within each year. Then 2009 and 2010 data for area 1 were combined to allow comparison between years; this was done separately for early and late season damage and for both categories of damage (early and late season) combined. Because skeletonising leaf damage on birch occurred mostly in the late season, no seasonal comparison could be performed for this guild. To analyse the effects of total insect herbivore damage, all 9 feeding guilds recorded were used (see above) and analysis was restricted to 2010 data only; repeated measures linear mixed models were used in the manner just described above.

In 2009, two species of gall mites were recorded on birch as a combined ‘leaf gall’ category, whereas in 2010, the two species were recorded separately. For the analysis, however, they were combined because *Acalitus rudis* galls were insufficiently abundant to analyse separately. Similarly, different types of predators (spiders, ants and ladybirds) were analysed together as the 3 categories were insufficiently abundant to analyse separately. In the linear mixed models the unstructured repeated covariance was used and models utilised the Restricted Maximum Likelihood (REML) estimation method. Where time was not a factor in the model, i.e. analysis of early or late or combined (early + late) herbivory separately, then a univariate GLM was utilised. Square root transformations were used in order to normalise the data distribution of all herbivory categories.

ANCOVA

To test whether variation in insect herbivory could be explained by abundance of natural enemies, leaf traits and tree growth, these variables were used as covariates in general linear models. In the GLM, the dependent variable was the level of damage by each individual feeding guild, the fixed factor was always tree species diversity and area and the covariate were included as described below. Results of analyses with and without covariates were compared to establish whether adjusting for variation in natural enemy abundance, leaf traits and tree growth modify the effects of forest diversity on insect herbivory. When running each ANCOVA compliance with the assumptions of the analysis was assured, namely independence of the covariate and treatment effect and homogeneity of regression slopes (Field 2005).

Predators as a covariate

Each of the five damage categories were analysed separately using ANCOVA in the following ways:

1) Early season damage levels with early season predator abundance as a covariate.

2) Late season damage levels with late season predator abundance as a covariate.

3) Early and late season damage combined with early and late season predator abundance combined as a covariate. The only exception was for skeletonising damage which only occurred in the late season and was therefore only analysed as late season damage with late season predator abundance.

Leaf traits and tree size as covariates

Leaf traits (thickness, toughness and area) were measured in the late season of 2010 at area 1 only (Chapter 2) and therefore used as covariates only for late season damage and herbivory in 2010 at area 1. Note that leaf traits were measured on the same trees and branches as insect herbivory, but on different (undamaged) leaves. One undamaged leaf from each branch on which herbivory was assessed was used (*cf.* Matsuki & MacLean 1994). Undamaged leaves were selected to eliminate the possibility that herbivory directly affects the leaf trait measured.

Tree growth traits (height, stem diameter at breast height (DBH) and crown projection) were measured in late season 2010 in all 3 areas and used as covariates for both early and late season 2010 herbivory data; because these traits, unlike leaf traits, exhibit small seasonal variation. The same approach was used when predators were considered as a covariate for herbivore damage.

3.3 Results

A summary of the statistical results of this Chapter is presented in Table 3.1. The focus of this study is on tree species diversity effects; main effects of season, year and area are reported in Table 3.1, but are not discussed in the text unless diversity effects on herbivory differed over time or among areas.

Table 3.1 Chapter 3 results overview: tree species diversity effects on insect herbivory and influence of covariates. **S**= significant effects, **ns**= non-significant effects, **n/a**= not applicable. Covariates: **+** = positively correlated with herbivory, **-** = negatively correlated with herbivory. T1= early season T2= late season.

Factors	**Chewing**	**Skeletonising**	**Gallers**	**Rollers**	**S. birch aphid**	**Predators**
TEMPORAL						
Season 2009	**S**	n/a	**S**	**S**	**S**	ns
Season 2010	**S**	n/a	**S**	**S**	**S**	**S**
Year						
T1-T1	**S**	n/a	**S**	**S**	**S**	**S**
T2-T2	ns	**S**	**S**	ns	**S**	ns
T1T2-T1T2	**S**	n/a	**S**	**S**	**S**	ns
SPATIAL (area)	ns	**S**	**S**	ns	**S**	**S**
(area × season)	**S**	n/a	**S**	**S**	**S**	ns
DIVERSITY						
2009						
Diversity	**S**	ns	**S**	ns	ns	ns
Diversity × season	**S**	n/a	ns	**S**	ns	ns
Diversity × area	n/a	n/a	n/a	n/a	n/a	n/a
2010						
Diversity	ns	**S**	**S**	ns	**S**	ns
Diversity × season	ns	n/a	ns	ns	**S**	ns
Diversity × area	ns	ns	ns	ns	ns	ns
Diversity × year						
T1-T1	**S**	n/a	ns	ns	ns	ns
T2-T2	ns	ns	ns	ns	**S**	ns
T1&T2-T1&T2	ns	n/a	ns	ns	ns	ns
COVARIATES 2010						
Predators						
T1	**S+**	ns	**S+**	**S+**	ns	**-**
T2	ns	ns	ns	**S+**	ns	**-**
T1.T2	ns	ns	**S+**	**S+**	ns	**-**
						-
Leaf traits						
Leaf area	ns	ns	ns	ns	ns	**-**
Thickness	ns	**S+**	ns	ns	ns	**-**
Toughness	**S+**	**S-**	ns	ns	ns	**-**
Tree growth						
Height						
T1	**S+**	n/a	**S+**	**S+**	**S+**	**-**
T2	**S+**	**S-**	**S+**	**S-**	**S+**	**-**
T1.T2	**S+**	n/a	**S+**	n/a	**S+**	**-**
Crown projection						
T1	n/a	n/a	**S+**	n/a	**S+**	**-**
T2	n/a	**S-**	**S+**	n/a	**S+**	**-**
T1.T2	n/a	n/a	**S+**	n/a	**S+**	**-**
D.B.H.						
T1	**S+**	n/a	**S+**	**S+**	**S+**	**-**
T2	n/a	**S-**	**S+**	**S-**	**S+**	**-**
T1.T2	n/a	n/a	**S+**	n/a	**S+**	**-**

3.3.1 Chewing damage

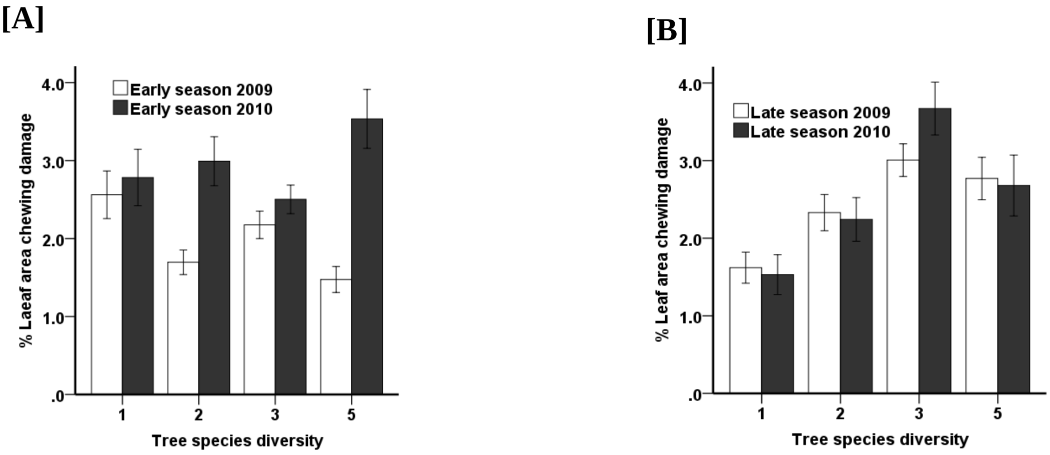


Figure 3.1 Chewing damage on silver birch over the tree species diversity gradient in area 1 2009- 2010 for early **[A]** and late **[B]** season. Error bars are ± 1 SE of mean.

In 2009, tree species diversity had significantly different effects on chewing damage in early and late season ($F_{3, 350.388} = 3.65$, P= 0.013; season × diversity interaction: $F_{1, 350.388} = 5.09$, P= 0.002): chewing damage decreased with tree diversity in early season (Fig. 3.1A), but increased with tree diversity in the late season (Fig. 3.1B).

In 2010, effects of tree species diversity on chewing damage were non-significant ($F_{3, 966.888} = 2.41$, P= 0.066) and consistent across season (season × diversity interaction: $F_{3, 966.888} = 1.20$, P= 0.30) and over the three areas (area × diversity interaction: $F_{3, 966.888} = 2.41$, P= 0.05) with more damage observed in 5- (early season, Fig. 3.1A) and 3-species mixtures (late season, Fig. 3.1B).

When 2009 and 2010 data were combined in the analysis, tree species diversity effects were significant comparing early season 2009 with early season 2010 as the direction of the effect differed between the years (Fig 3.1A) ($F_{3, 318.298} = 4.19$, P= 0.06). For late season and both seasons combined tree species diversity effects for chewing damage were not significant ($F_{3, 313.342} = 0.92$, P= 0.43 & $F_{3, 340.867} = 0.65$, P= 0.58 respectively).

3.3.2 Skeletonisers

Skeletonising damage was unaffected by tree species diversity in 2009 ($F_{3,\,177} = 0.22$, P= 0.881), but significantly increased with tree species diversity in 2010 in all three areas ($F_{3,\,514} = 5.19$, P= 0.002, area × diversity interaction: $F_{6,\,514} = 0.26$, P= 0.96, Fig. 3.2). When 2009 and 2010 data were combined, tree diversity effects were significant ($F_{3,\,193.862} = 2.87$, P= 0.040) and consistent between years (year × diversity interaction: $F_{3,\,193.862} = 1.49$, P= 0.71).

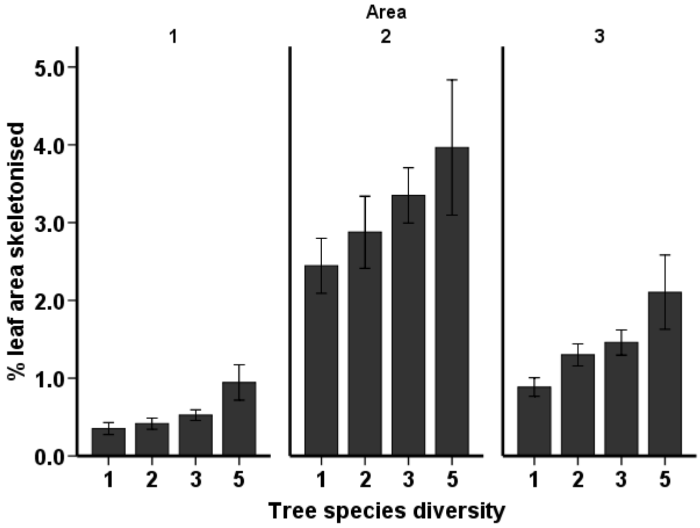


Figure 3.2 Skeletonising damage on silver birch in 2010 for the 3 areas. Error bars are ± 1 SE of mean.

3.3.3 Leaf galls

In 2009, gall abundance was significantly affected by tree species diversity; galls were less abundant in 5-species mixtures than in other treatments ($F_{3,\,348.307} = 7.52$, P= <0.001, Fig. 3.3) and this effect was observed both in the early and late season (season × diversity interaction: $F_{3,\,348.307} = 0.66$, P= 0.57).

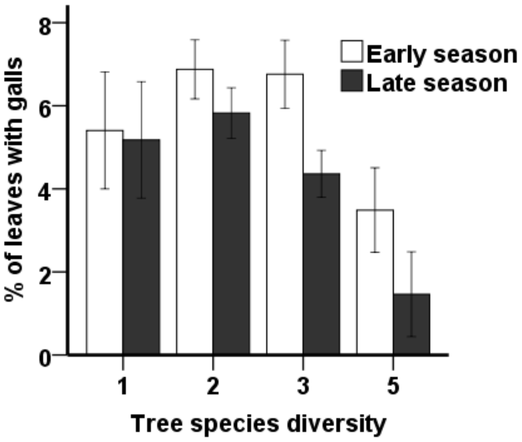


Figure 3.3 Percentage of leaves with galls on silver birch during early and late season of 2009, area 1. Error bars are ± 1 SE of mean.

In 2010, gall abundance was also significantly affected by tree species diversity ($F_{3, 953.131} = 4.86$, P= 0.002) and showed very similar patterns in all 3 areas (area × diversity interaction: $F_{6, 953.131} = 2.17$, P= 0.05, Fig. 3.4) with the effect being observed both in early and late season (diversity × season interaction: $F_{3, 953.131} = 0.38$, P= 0.77, Fig. 3.4). Lowest gall abundance was again observed in 5 species mixtures (Fig. 3.4).

Tree species diversity effects on gall abundance were also consistent between years when comparing early season damage (tree diversity × year interaction: $F_{3, 269.812} = 0.86$, P= 0.46), late season damage ($F_{3, 265.610}$= 0.36, P= 0.78) and both seasons combined ($F_{3, 277.951}$= 0.560, P= 0.64).

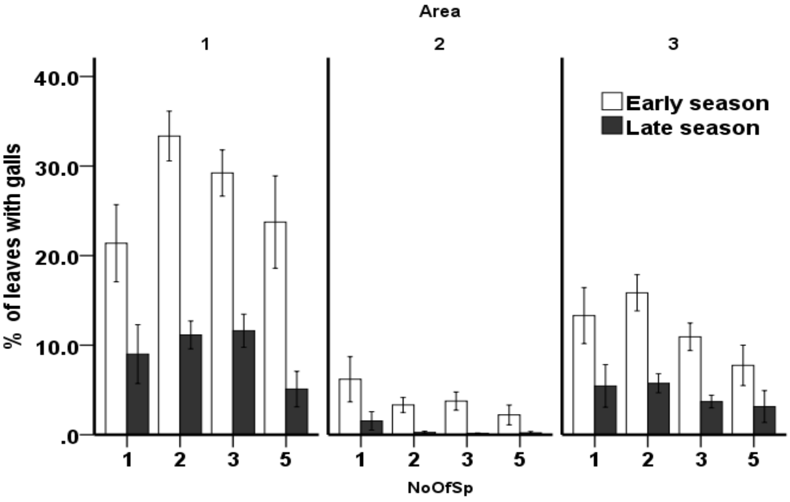


Figure 3.4 Percentage of leaves with galls on silver birch in 2010 at all 3 areas (over early and late season). Error bars are ± 1 SE of mean.

3.3.4 Leaf rollers

In 2009, tree species diversity had a non-significant effect on abundance of leaf rollers ($F_{3,\ 241.854}$ = 2.41, P= 0.06, Fig. 3.5), but direction of the effect differed between seasons (season × diversity interaction: $F_{3,\ 241.854}$ = 4.35, P= 0.005) with higher roller abundance in 5-species mixture in the early season, and lowest abundance in this treatment in the late season (Fig. 3.5).

In 2010, roller abundance was unaffected by tree species diversity ($F_{3,\ 692.517}$ = 0.99, P= 0.39) and no temporal or spatial variation in diversity effects occurred (season × diversity interaction: $F_{3,\ 692.517}$ = 0.43, P= 0.72, area × diversity interaction: $F_{6,\ 692.517}$ = 0.79, P= 0.58).

When 2009 and 2010 data were analysed together, the effect of tree species diversity and all interactions were non-significant (diversity effect: $F_{3,\ 229.668}$ = 1.22, P= 0.30, diversity × year interaction $F_{3,\ 229.668}$ = 1.40, P= 0.24).

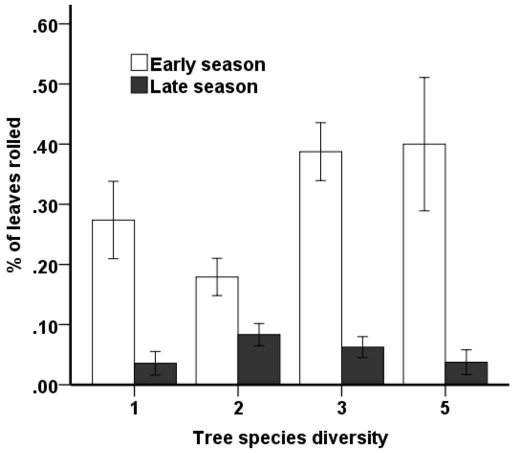


Figure 3.5 Abundance of leaves rolled on silver birch during early season of 2009, area 1. Error bars are ± 1 SE of mean.

3.3.5 Silver birch aphid (*Euceraphis betulae*)

In 2009, aphid abundance was low (Fig. 3.6) and unaffected by tree species diversity ($F_{3,\ 235.748}$ = 1.758, P= 0.16; season × diversity interaction: $F_{3,\ 235.748}$ = 0.89, P= 0.44).

In early summer 2010, aphid densities reached outbreak levels and were significantly affected by tree species diversity ($F_{3,\ 755.072} = 6.43$, P= <0.001) with higher aphid numbers in birch monocultures, particularly in areas 2 and 3 (Fig. 3.7). By late summer 2010, aphid densities fell dramatically resulting in significant season × diversity interaction ($F_{3,\ 755.072} = 3.52$, P= <0.015), but aphid numbers remained higher in birch monocultures than in mixed stands, especially in area 1 (Fig. 3.7).

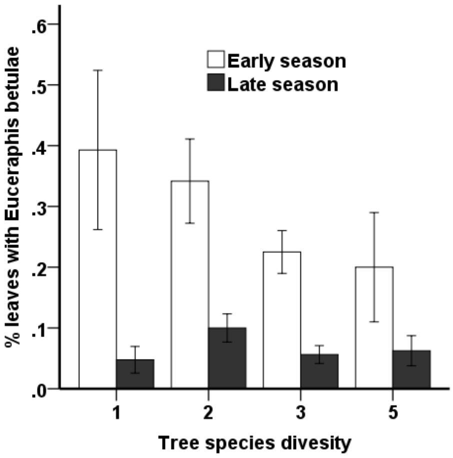


Figure 3.6 Percentage of leaves with silver birch aphid (*Euceraphis betulae*) in 2009. Error bars are ± 1 SE of mean.

Aphid abundance was similarly affected by tree species diversity in all areas (area × diversity interaction: $F_{3,\ 755.072} = 1.46$, P= 0.19). Between years, tree species diversity effects were significant only when comparing aphid abundance in late season ($F_{3,\ 177.020} = 3.09$, P= 0.029), but not when comparing early season data (P= 0.10) or both seasons combined (P= 0.25).

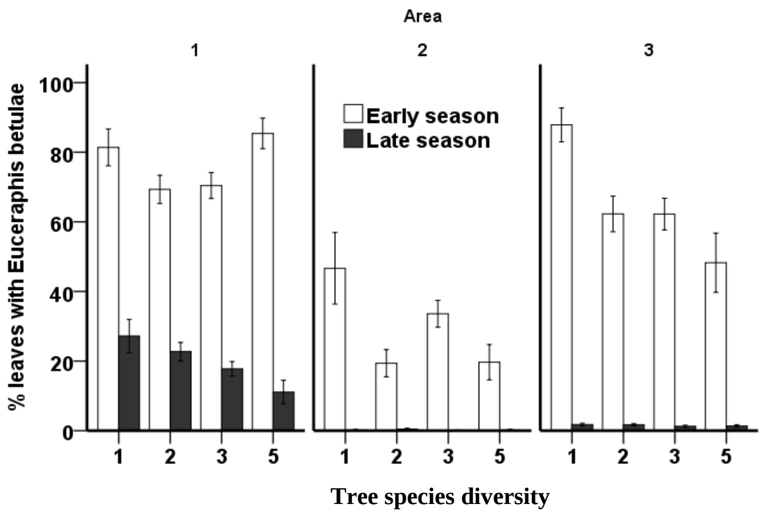


Figure 3.7 Percentage of leaves with silver birch aphid (*Euceraphis betulae*) in 2010 at all 3 areas. Error bars are ± 1 SE of mean.

3.3.6 Total insect herbivore damage

Total insect herbivore damage in 2010 was greater in the early season compared to the late season ($F_{1,\ 850.274} = 523.94$, P= <0.001, Fig. 3.8) but unaffected by tree species diversity ($F_{3,\ 850.274} = 0.74$, P= 0.53) with the same trends observed in both early and late season (season × diversity interaction: $F_{3,\ 850.274} = 0.78$, P= 0.50), and no discernible spatial effects (area × diversity interaction $F_{3,\ 850.274} = 0.91$, P= 0.48).

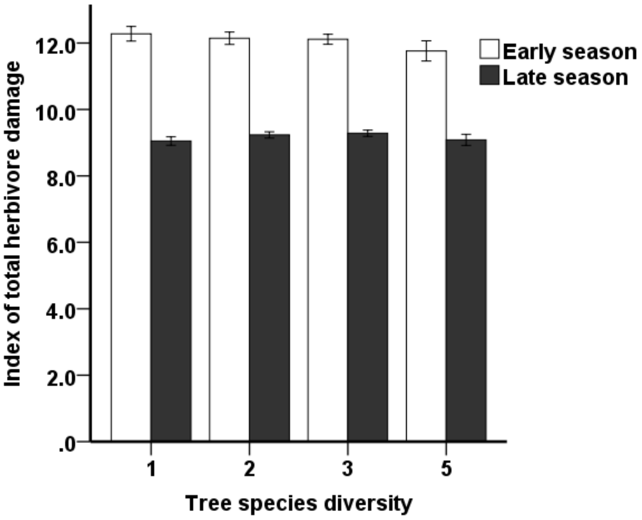


Figure 3.8 Standardised total herbivore damage on birch during early and late season of 2010 Error Bars are +/- 1 SE.

3.3.7 Predators

Tree species diversity had no significant effect on the abundance of predators (2009: $F_{3, 347.005} = 0.90$, P= 0.43; 2010: $F_{3, 664.225} = 1.71$, P= 0.16) and higher tree diversity plots did not contain higher numbers of predators; this finding was consistent across seasons for both years (season × diversity interaction 2009: $F_{3, 347.005} = 0.22$, P= 0.88; 2010: $F_{3, 664.225} = 1.12$, P= 0.34) and across areas in 2010 (area × diversity interaction: $F_{3, 664.225} = 1.72$, P= 0.16).

The effects of tree species diversity on predator abundance were non-significant and consistent between years when comparing early season data, late season data, and both seasons combined (year × diversity interaction: $F_{3, 255.833} = 0.40$, P= 0.75, $F_{3, 332.225} = 0.41$, P= 0.84 and $F_{3, 277.923} = 0.38$, P= 0.77 respectively).

When predator abundance was included in the analysis as a covariate it was positively associated with chewing damage and galler abundance in the early season and with roller abundance throughout the season. However, abundances of skeletonisers and silver birch aphid were not affected by predator abundance (Table 3.2). Importantly, accounting for variation in predator abundance in the analysis did not change the significance of the tree species diversity effects on herbivores for any of the feeding guilds (Table 3.2).

Table 3.2 ANCOVA: Birch herbivory damage with predator abundance as a covariate (2010). Tree diversity effects (without covariate) are reported on the left. Tree diversity effects with predator covariate included (separated by dotted line) are reported on the right.
T1= early season damage with early season predators. **T2**= late season damage with late season predators
T1.T2= early and late season damage combined with early and late season combined predators.
(Except skeletonisers which are late season damage with late season predator abundance only)
ETA= Partial ETA squared, proportion of variance explained by a particular variable (0-1)
Direc= direction of the relationship between predator abundance covariate (when significant) with damage type. **n/a** = not applicable. Significant *P* values are emboldened.

	Tree Diversity			**Tree Diversity + Predator Covariate**						
Damage type	***F***	***P***	**ETA**	***F***	***P***	**ETA**	***F***	***P***	**Direc**	**ETA**
Chewing T1	2.330	0.074	0.014	2.068	0.103	0.012	4.131	**0.043**	**+ve**	0.008
Chewing T2	1.467	0.223	0.009	1.457	0.225	0.009	0.013	0.911	n/a	0.000
Chewing T1.T2	2.311	0.075	0.013	2.126	0.096	0.012	1.416	0.235	n/a	0.003
Skeleton' T2	3.999	**0.018**	0.020	3.327	**0.019**	0.019	0.421	0.517	n/a	0.001
Galls T1	2.814	**0.030**	0.015	3.172	**0.024**	0.018	10.859	**0.001**	**+ve**	0.021
Galls T2	2.030	0.109	0.012	1.998	0.113	0.012	1.840	0.176	n/a	0.004
Galls T1.T2	0.012	0.110	0.012	2.385	0.068	0.014	11.924	**0.001**	**+ve**	0.023
Rollers T1	0.993	0.396	0.006	0.968	0.411	0.006	5.905	**0.015**	**+ve**	0.011
Rollers T2	0.068	0.977	0.000	0.068	0.991	0.000	7.124	**0.008**	**+ve**	0.014
Rollers T1.T2	1.044	0.373	0.006	0.788	0.501	0.005	7.602	**0.006**	**+ve**	0.015
Aphid T1	4.086	**0.007**	0.023	4.348	**0.005**	0.025	3.027	0.083	n/a	0.006
Aphid T2	2.274	0.079	0.017	2.427	0.065	0.014	2.808	0.094	n/a	0.005
Aphid T1.T2	3.932	**0.009**	0.023	4.023	**0.008**	0.023	0.641	0.424	n/a	0.001

3.3.8 Leaf characteristics

Tree diversity did not influence any of the tested leaf traits (Appendix 3.1). The effect of tree species diversity on each of the damage categories did not change significance as a result of adding any of the leaf trait characteristics into the analysis as a covariate (Appendix 3.1). Therefore, independently of tree diversity, chewing and skeletonising damage were the only herbivory categories significantly influenced by the measured leaf trait characteristics. Chewing damage was more abundant on trees with tougher leaves ($F_{1,\ 180}$ = 18.88, P <0.001), with leaf toughness explaining 9.7% of variation in chewing damage. Skeletonising damage was more abundant on thicker leaves ($F_{1,\ 180}$ = 5.04, P= 0.026) with leaf thickness explaining 2.8% of the variation in damage, and on less tough leaves ($F_{1,\ 180}$ = 14.16, P <0.001) with leaf toughness explaining 7.4% of the variation in damage. Abundance of gallers, rollers and silver birch aphid were unaffected by leaf traits (area P≥ 0.472, thickness P≥ 0.102, toughness P≥ 0.089). It is noteworthy that leaf thickness and leaf toughness were not significantly correlated (r = −0.141, P= 0.06).

3.3.9 Tree growth characteristics

Tree growth traits were not influenced by tree species diversity (Appendix 3.2B). Inclusion of tree growth traits as covariates in the analysis did not affect the significance of tree diversity effects for any of the herbivore categories (Appendix 3.2A). Similarly to leaf traits, some tree growth characteristics did influence insect herbivory but independently of tree species diversity.

Tree height had a significant effect on all five insect herbivore categories. Chewing and galling damage and abundance of silver birch aphid were higher on taller trees, a trend that occurred in both early and late season. In contrast, skeletonising damage was significantly more abundant on smaller trees. Finally, leaf rollers were more abundant on taller trees during the early season and on smaller trees during the late season (Appendix 3.2A).

Crown projection (a measure of canopy width) had no effect on chewing damage and abundance of rollers, but higher abundance of leaf galls and silver birch aphid occurred on trees with larger crown projections (Appendix 3.2A). Similarly, skeletonising damage was significantly less abundant on trees with smaller crowns. All measured tree growth traits were significantly and positively correlated (height and crown projection: $r = 0.632$, P= <0.001, n=181; height & DBH: $r = 0.844$, P= <0.001, n=181; crown projection & DBH: $r = 0.699$, P= <0.001, n=181).

3.4. Discussion

3.4.1 Tree species diversity effects on herbivores

Tree species diversity significantly affected abundance of all insect herbivore guilds except rollers (Table 3.1). Interestingly, birches growing in mixed stands exhibited both AR and AS responses depending on the herbivore in question. Chewing damage in early 2009, silver birch aphids and leaf galls were less abundant on birch in mixed stands in accordance with the associational resistance (AR) phenomenon and the results from other studies in forest ecosystems (Jactel & Brockerhoff 2007; Kaitaniemi *et al.* 2007; Sholes 2008; Sobek *et al.* 2009) and herbaceous ecosystems (Andow 1991; Unsicker *et al.*

2006); although it is noted that some studies have found evidence to the contrary (Vehvilainen *et al*. 2007; Sobek *et al*. 2009). Intriguingly, birch showed a strong AR response to silver birch aphid when at outbreak levels in 2010. This is an important finding in two respects; firstly, birch is less damaged by an outbreak species when planted in polyculture; secondly, sap feeding insect damage is reported to be far more detrimental to woody plants than defoliating insects (Zvereva *et al.* 2010) indicating that reducing damage caused by silver birch aphid is important. The mechanisms leading to this phenomenon are unclear from this study. As a specialist insect the aphid may be able to locate larger patches of its host (birch) in monoculture, therefore the resource concentration hypothesis (Chapter 1) is a likely explanation, although, see section 3.4.2 for discussion of importance of tree height (tree apparency) for silver birch aphid.

Predominantly, chewing damage is higher on birch when it is planted in mixtures (AS), as observed during late season 2009 and throughout 2010, although damage on birch was lowest when planted in 5 species mixture in early 2009 (AR). Birch also clearly showed AS effects to skeletonising damage and leaf rollers during the early season. Feeding specialisation of insect herbivores may provide one reason why AR trends occur with some guilds and AS with others. Gall mites and silver birch aphid are specialist herbivores, with lowest abundance occurring on birches growing in mixtures (AR) agreeing with the resource concentration hypothesis (Root 1973); additionally these two categories are also more abundant in the early season. Specialists are predicted to be more abundant in the early season compared with late because insect herbivores with narrow host plant ranges can show preference for the more nutritious young growing leaves, having adapted to avoid greater toxins therein (Niemela 1983; Schoonhoven *et al.* 2005; Sholes 2008).

Conversely to chewing and skeletonising damage, silver birch displayed AS effects that is likely to have been caused by more generalist insects; this is supported as generalists usually have greater abundance during late season (Schoonhoven *et al.* 2005). To some extent this logic fails with leaf rolls in this study because although they are considered to be caused by more specialist insects and had greater abundance during early season, contrary to expectation, birch showed AS and not AR trends to this damage type. Rollers on birch are likely to be oligophagous species (Kozlov *et al.* 2011) which may not respond as strongly to tree species diversity as strictly monophagous insects (i.e. silver birch aphid), although, see Niemela (1983) who suggest that constant new leaf production on birch throughout the growing season may reduce early season peaks in specialist insect herbivore species richness, this may also be the case with species abundance. In addition, a recent study has indicated that specialist insects can cause more damage in higher diversity plots (Plath *et al.* 2012), with differences in search mode suggested as the most likely explanation why specialist insects would behave in this manner.

Specialist insects are predicted to respond to plant diversity in accordance with the resource concentration hypothesis (Root 1973), by locating larger expanses of their host plant and therefore be less prevalent in diverse mixtures than in the monocultures, agreeing with the findings of this study. Data herein on chewers, aphids and galls support the findings from an extensive meta-analysis in forest systems (Jactel *et al.* 2005) and analysis of crop plant systems and algal communities (Tonhasca & Byrne 1994; Hillebrand & Cardinale 2004; Balvanera *et al.* 2006). The general AR effect/trend observed on birch to the silver birch aphid was also found when comparing pine in monocultures to pine in mixtures; where pine demonstrated AR effects to sucking insects, mites and aphids (WoonIk *et al.* 1997).

This study indicates that both AS and AR effects occurred on birch in response to the 5 insect herbivore guilds analysed. No such directional trends are observed with total insect herbivore load in 2010 (Fig. 3.8) and tree species diversity effects were non-significant. As different guilds show opposite responses to diversity, it is unsurprising that total herbivore damage that includes damage from all guilds shows no pattern because individual AS and AR effects cancel each other out. The long term effects of each damage type on birch need to be realised to ascertain the detrimental effects. For instance, is 5% leaf area chewing damage more detrimental than 5% leaf damage caused by aphids, in terms of tree growth, cost of induced defence and vulnerability to further or secondary attack and duration of tree responses? For example, a recent meta-analysis has shown that sap feeders impose a more severe overall negative impact on plant performance than defoliators (Zvereva *et al.* 2010); largely due to lower abilities of woody plants to compensate for sap feeding damage in terms of growth and photosynthesis. Furthermore, the impact of endemic herbivory has recently been shown to exceed impacts from herbivore outbreaks (Wolf *et al.* 2008). It is acknowledged however, that the method used in the present study is not ideal to assess total insect herbivore damage. It is suggested that future studies measure and record individual damages using the same criteria, i.e. percent leaf area damage or percent of leaves damaged (not both). Further investigation into long term effects of each type of damage on the health, productivity etc. of tree species will need to be evaluated.

Spatial and temporal effects

Abundance of most insect herbivore categories varied between areas (Table 3.1), although importantly, the effects of tree species diversity were spatially consistent for all analysed herbivore categories. Studies of forest ecosystems in relation to spatial effects of diversity on insect herbivores are scarce. Tree diversity effects on insect herbivore damage and abundance on silver birch in boreal forests have been shown to vary (at a much larger spatial scale) between Finland and Sweden, with experimental design and differing densities of the tree compositions between sites indicated as the cause (Vehvilainen *et al.* 2006).

Regarding temporal variation, tree species diversity effects tended to be stronger in 2010. This may be because: 1) the analysis of 2009 data was restricted to area 1 only, therefore less statistical power in 2009 to detect diversity effects, 2) most herbivores had higher densities in 2010, hence greater scope for detecting variation in density in 2010 and higher chance to detect trends and direction of effects. Tree species diversity effects were consistent between seasons for all guilds analysed except silver birch aphid in 2010. This result was caused by the dramatic decline in aphid abundance between early season and late season 2010 (Fig. 3.7), with no observable pattern of aphid abundance over tree diversity in areas 2 and 3 during the late season. A detectable AR trend was observed on birch in area 1 during the late season of 2010; the reason may be that area 1 was sampled approximately one week before area 2 and three weeks before area 3, aphid numbers had not declined to the same extent in area 1 when it was sampled.

3.4.2 Mechanisms of forest diversity effects on insect herbivores: the influence of predators, physical leaf traits and tree apparency.

Predators

Support for the enemies hypothesis is common in studies dealing with agricultural systems (Russell 1989; Andow 1991) where predators and parasites are more abundant and effective at suppressing herbivores in polycultures compared with monocultures. In contrast, support for the enemies hypothesis is scarce in studies from forest ecosystems (Riihimaki *et al.* 2005; Kaitaniemi *et al.* 2007; Schuldt *et al.* 2011). Similarly to previous studies in forests, the current study showed no effects of tree species diversity on predator abundance and therefore no support for the enemies hypothesis (Table 3.2). Some authors have postulated that in forest ecosystems predator abundance may be linked more with the presence of certain tree species rather than tree species diversity *per se* (Schuldt *et al.* 2008; Vehvilainen *et al.* 2008; Sobek *et al.* 2009). This Chapter did not directly test tree species composition effects, but did find that the abundance of chewing and galling damage in early season and rolling damage in both early and late season 2010 was positively correlated with predator abundance. This is still contrary to what would be expected according to the enemies hypothesis and indicates that in this study spiders ants and ladybirds do not lower the abundance of the insect herbivore categories analysed; the abundances of the remaining insect herbivore categories (skeletonisers and birch aphids) were not correlated with predator abundance.

There were no changes in tree species diversity effects on herbivores when predator abundance was included in the analysis as a covariate (Table 3.2), indicating that predators do not mediate tree species diversity effects on herbivores. It is noteworthy that the current study did not assess direct effects of predation on herbivores and only recorded predator densities, which were fairly low during the monitoring periods. Studies that have found support for the enemies hypothesis also considered parasitoids (Sobek *et al.* 2009) which were not included in the present study. In addition, insectivorous birds have also been shown to significantly reduce leaf damage on silver birch (Giffard *et al.* 2012) and on oak (Marquis & Whelan 1994); these effects were stronger in higher diversity plots thus supporting the enemies hypothesis.

In the present study, fluctuating weather conditions (i.e. wind and rain from more clement conditions) could have had the effect of reducing predator presence, making them harder to spot during monitoring and result in under-recording. Sampling methods often differ between studies; beating tree branches into collection nets (Ozanne 2008; Sobek *et al.* 2009) would, for example reduce this issue and is recommended in future studies of this nature. It is also acknowledged that monitoring twice during the season is not sufficient to fully test the enemies hypothesis and that the full effects of predation need to be studied, not just predator abundance (Riihimaki *et al.* 2005).

Leaf traits

An important finding regarding leaf traits was that although they did in some instances influence insect herbivory, these effects were not mediated by tree species diversity. It was then discovered that none of the leaf traits changed or altered the effect of tree species diversity on the insect herbivores studied and therefore forest diversity does not influence insect herbivory via physical leaf traits. Therefore, independently of forest diversity effects, higher abundance of chewing damage was found on trees that have tougher leaves, whereas skeletonising damage was higher on trees with less tough leaves but also on trees with thicker leaves. The higher numbers of skeletonisers on less tough and thicker leaves would be logical as these insects would expend less energy to penetrate and process leaf material thereby accessing a greater available food source in the thicker leaves. The damage is likely to have been caused by the early larval instars of late season lepidopterans, sawflies or beetles (Koricheva 2012), although insects causing the damage during the monitoring were not observed, only the damage itself. Insects that skeletonise rather than eat/chew through the leaf lamina are unable to deal with tough parts of the leaf such as veins, which may explain the negative correlation between skeletonising damage and leaf toughness.

There is limited literature on the effects of leaf toughness, thickness and area on the insect guilds within this study. Ayres & MacLean (1987) found *Epirrita autumnata* performance decreased on tougher birch leaves (as they are less digestible), but also found *E. autumnata* to be affected by a covarying suite of characteristics that change throughout leaf maturation. It is important to note that the leaf trait ANCOVA in the present study was conducted on late season herbivore damage (because leaf traits were measured in late season). Therefore, as discussed above, the chewing insects on which the analysis was conducted are more likely to be generalist insects (supported by the AS trend shown in Fig. 3.1). This may explain why chewing damage is significantly positively correlated with leaf toughness, as generalists are also thought to target older, tougher and therefore chemically less well defended leaves that are available later in the growing season (Matsuki & Maclean 1994; Schoonhoven *et al.* 2005). It would be interesting to study physical leaf traits and herbivore abundance in the early season, for example, Matsuki & Maclean (1994) found leaf toughness to be significant only during the early season for insect herbivores on willow, speculating that early season herbivores maybe more sensitive to traits of leaf quality such as toughness, nitrogen and water content than late season herbivores.

Tree apparency

Plant apparency can be simply defined as the likelihood of a plant being found by herbivores (Feeny 1970). It is not that surprising that tree height is significantly and positively correlated with the abundance of most insect herbivore categories. However, tree height and other growth characteristics were clearly not mediated by tree species diversity effects. In this Chapter (independently of tree diversity influences), tree height positively correlated with insect herbivore guilds in the early season and again in the late season, except for leaf rollers that are positively correlated in early season but negatively correlated in the late season (which is difficult to explain) and skeletonisers that are significantly negatively correlated. Height, crown projection and DBH are to some degree correlated as all clearly increase with tree growth. It is, however, tree height that appears to be a more important characteristic influencing insect herbivore guilds, as demonstrated with the chewers that are not significantly correlated with crown projection or DBH.

Tree height was not very important for chewing and rolling herbivores as it only explained up to 2.5 % of the variation in the above types of damage. However, for the skeletonisers, gallers and silver birch aphid, tree height was a far more important determinant of their abundance because it explained between 18 to 35% of the variation in abundance of these herbivores. Again, this could be linked to feeding specialisation and search mode of these insect herbivores as gallers and silver birch aphid are certainly monophagous and are likely to be more abundant in monocultures of their host plant; in line with the prediction of the resource concentration hypothesis and are more strongly affected by tree apparency than generalists (Matsuki & Maclean 1994). Silver birch aphid and gallers are also at their lowest abundance in the high diversity plots indicating that both patch level and tree level selection of birch takes place.

For skeletonisers however, highest abundance was in the highest tree diversity plots, at odds with the prediction of the resource concentration hypothesis. Moreover, as skeletonisers were significantly more abundant on smaller birches they would appear to be selecting the most hidden and discrete trees in terms of density (patch) and in tree size. This may be advantageous in terms of predator avoidance but also suggests skeletonising insects employ a different mechanism for host finding to aphids and gallers. It is possible that mechanistically the search mode of insects, particularly the detection of volatile compounds may explain this further (Chapter 6). Another possibility is that smaller, more shaded trees will likely produce less well chemically defended leaves because the cost of production would be traded against the amount of solar energy received.

Importantly, it was found that tree growth characteristics were not influenced by tree richness. Additionally, when these traits are included as covariates the effect of tree species diversity remained unchanged. An interesting study has recently demonstrated that tree apparency of oak saplings is affected by tree species diversity and this has a significant negative effect on the abundance of leaf miner herbivores (Castagneyrol *et al.* 2013). However, tree apparency in their study was measured as a function of neighbouring tree height, whilst I studied growth traits on trees that herbivory had been measured. Additionally, silver birch is a fast growing and rather apparent pioneer tree species and likely to be less affected in terms of apparency by neighbouring non-host trees as compared to oak, which is a slow growing climax species and likely to vary significantly more in height as a result of tree diversity. Indeed, faster growing trees are predicted to have weaker defences as a consequence (to grow or defend dilemma); this is predicted by the growth-differentiation hypothesis (Herms & Mattson 1992). Therefore, as well as being more apparent to insect herbivores the taller birches in the present study may have less secondary metabolites, resulting in higher levels of herbivory.

3.5 Conclusions

This study shows that silver birch experiences significant AR effects to the damage caused by gallers, silver birch aphid, and in some instances early season chewing damage; in these instances planting birch in high tree diversity mixtures will provide a measure of resistance. These insects also select larger more apparent trees, supporting the resource concentration and tree apparency hypotheses. However, birch also experiences AS to skeletonising insects, late season chewing and early season rolling damage and in some instances early season chewing damage. Skeletonisers were shown to select the least physically apparent trees, i.e. in plots with their host at lowest density as well as selecting smaller birches. An important finding was that tree growth characteristics are not mediated by tree species diversity. Leaf traits were only significant for chewing (more abundant on tougher leaves) and skeletonising (more abundant on less tough leaves and thicker leaves) but these leaf trait effects were also not mediated by tree species diversity.

An additional finding of this study was in 2010, silver birch demonstrated a significant tree species diversity response to silver birch aphid in line with the AR phenomenon. AR effects like this can be used to reduce damage from important outbreaking insect herbivores, especially insects with feeding modes impacting on their hosts to a greater extent than insects with less detrimental feeding modes. Despite significant responses of silver birch to tree species diversity being demonstrated with the 5 guilds rigorously tested, the measure of total insect herbivore damage indicated that tree species diversity had no significant affect on birch when total insect herbivore damage was considered. This emphasises the importance of knowing what damage type or guild are more detrimental to birch, and is suggested as an important avenue for further research.

Tree species diversity effects were shown to be consistent spatially, with greater damage in the early season; likely due to the presence of more specialist insects that are known to be more prevalent in the early season, with many having narrow phenological windows. Finally, with the predators and insect herbivores studied, no support for the natural enemies hypothesis, or predators influencing insect herbivore abundance was found. It is suggested that additional insect herbivore predators, parasites and insectivorous birds be included in future studies of this nature and that measuring predation directly on all insect herbivore guilds is required to fully elucidate the natural enemies hypothesis in forest systems.

Appendix 3.1 ANCOVA - Birch herbivory with leaf trait covariates. All leaf traits were measured at area 1 only and in the late season and therefore modelled with late season herbivory at area 1 only. **ETA =** Partial ETA squared, the proportion of variance that a variable explains that is not explained by other variables in the analysis (scaled between 0-1). Direc= relationship/ direction between covariate (when significant) with damage type. Significant P values are emboldened.

Dependent variable	Model	Factor	F	P	Direc	ETA
Chewing	1	Tree Div	6.068	0.01	n/a	0.093
	2	Tree Div	6.068	**0.01**	n/a	0.094
		Lf Area	0.333	0.565	n/a	0.002
	3	Tree Div	6.399	**<0.001**	n/a	0.098
		Thickness	2.131	0.146	n/a	0.012
	4	Tree Div	7.031	**<0.001**	n/a	0.107
		Toughness	18.877	**<0.001**	**-ve**	0.097
Skeletonising	1	Tree Div	3.876	**0.010**	n/a	0.062
	2	Tree Div	3.876	**0.010**	n/a	0.062
		Lf Area	0.082	0.775	n/a	0.000
	3	Tree Div	3.739	**0.012**	n/a	0.060
		Thickness	5.036	**0.026**	**-ve**	0.028
	4	Tree Div	4.137	**0.007**	n/a	0.066
		Toughness	14.158	**<0.001**	**+ve**	0.074
Gallers	1	Tree Div	1.372	0.253	n/a	0.023
	2	Tree Div	1.357	0.257	n/a	0.023
		Lf Area	0.401	0.472	n/a	0.000
	3	Tree Div	1.493	0.218	n/a	0.025
		Thickness	1.125	0.264	n/a	0.007
	4	Tree Div	1.388	0.248	n/a	0.023
		Toughness	0.089	0.766	n/a	0.001
Rollers	1	Tree Div	1.368	0.254	n/a	0.023
	2	Tree Div	1.401	0.244	n/a	0.023
		Lf Area	0.144	0.705	n/a	0.001
	3	Tree Div	1.201	0.311	n/a	0.020
		Thickness	2.706	0.102	n/a	0.020
	4	Tree Div	1.429	0.236	n/a	0.024
		Toughness	0.422	0.517	n/a	0.002
Aphid	1	Tree Div	3.171	**0.026**	n/a	0.051
	2	Tree Div	3.002	**0.032**	n/a	0.049
		Lf Area	0.212	0.646	n/a	0.001
	3	Tree Div	2.854	**0.039**	n/a	0.046
		Thickness	2.613	0.108	n/a	0.015
	4	Tree Div	3.294	**0.022**	n/a	0.053
		Toughness	2.933	0.089	n/a	0.016

Assumptions of independence: The effect of tree species diversity on each covariate was non-significant:

Lf Thickness: F$_{3, 180}$ = 1.89, P= 0.142

Lf Toughness: F$_{3, 180}$ = 1.20, P= 0.311

Lf Area: F$_{3, 180}$ = 0.932, P= 0.524

Appendix 3.2A ANCOVA: Tree growth traits as covariates (2010).

Tree diversity effect without covariate reported on far left. Next 3 columns report tree diversity effect with covariate added and influence of covariate (separated by dashed line).

ETA= Partial ETA squared, proportion of variance explained by a particular variable (0-1)

Direc= relationship/ direction between covariate (when significant) with damage type. **n/a** = not applicable. Significant *P* values are emboldened

NOTE: SK damage for T2 reported only as damage from guild only occurred in late season

Damage & Time		Tree diversity			Tree diversity + Height							Tree diversity + C. Projection							Tree diversity + DBH						
		F	***P***	**ETA**	***F***	***P***	**ETA**	***F***	***P***	**Dir**	**ETA**	***F***	***P***	**ETA**	***F***	***P***	**Dir**	**ETA**	***F***	***P***	**ETA**	***F***	***P***	**Dir**	**ETA**
Chewing	**T1**	2.243	0.082	0.013	2.290	0.078	0.013	3.704	0.055	+ve	0.007	1.866	0.131	0.011	2.677	0.102	n/a	0.005	1.915	0.126	0.011	53.70	0.054	+ve	0.012
	T2	1.536	0.204	0.009	1.832	0.140	0.011	12.765	**<0.001**	**+ve**	0.025	1.724	0.161	0.010	0.509	0.476	n/a	0.476	1.775	0.151	0.011	2.035	0.154	n/a	0.004
	T1.T2	1.954	0.120	0.011	2.157	0.092	0.013	2.734	0.099	n/a	0.005	1.972	0.117	0.012	0.196	0.658	n/a	0.000	1.957	0.119	0.012	0.570	0.411	n/a	0.000
SK	**T2**	3.804	**0.010**	0.022	3.702	**0.011**	0.023	272.38	**<0.000**	**-ve**	0.350	5.053	**0.002**	0.029	106.41	**<0.001**	**-ve**	0.174	3.993	**0.008**	0.023	151.935	**<0.001**	**-ve**	0.233
Gallers	**T1**	3.026	**0.029**	0.017	2.997	**0.031**	0.015	188.63	**<0.001**	**+ve**	0.272	2.946	**0.032**	0.015	95.193	**<0.001**	**+ve**	0.159	2.832	**0.035**	0.014	143.824	**<0.001**	**+ve**	0.223
	T2	2.030	0.109	0.012	1.822	0.170	0.009	72.334	**<0.001**	**+ve**	0.125	1.617	0.184	0.010	37.832	**<0.001**	**+ve**	0.070	1.527	0.199	0.010	64.382	**<0.001**	**+ve**	0.114
	T1.T2	3.249	**0.022**	0.019	2.876	**0.044**	0.016	186.86	**<0.001**	**+ve**	0.270	2.683	**0.050**	0.015	94.041	**<0.001**	**+ve**	0.157	2.712	**0.047**	0.017	146.989	**<0.001**	**+ve**	0.227
Rollers	**T1**	0.885	0.339	0.005	0.684	0.562	0.004	5.633	**0.018**	**+ve**	0.011	0.668	0.572	0.004	2.448	0.118	n/a	0.005	0.534	0.659	0.003	5.231	**0.023**	**+ve**	0.010
	T2	0.146	0.932	0.001	0.304	0.822	0.002	12.429	**<0.001**	**-ve**	0.024	0.174	0.914	0.001	0.840	0.323	n/a	0.001	0.335	0.800	0.002	6.886	**0.009**	**-ve**	0.009
	T1.T2	0.927	0.428	0.005	0.845	0.469	0.005	0.686	0.408	n/a	0.001	0.755	0.520	0.004	1.463	0.227	n/a	0.003	0.711	0.546	0.004	1.208	0.272	n/a	0.002
Aphid	**T1**	3.090	**0.027**	0.018	5.564	**0.001**	0.032	172.32	**<0.001**	**+ve**	0.254	7.932	**0.000**	0.045	111.06	**<0.001**	**+ve**	0.180	8.093	**0.000**	0.046	175.01	**<0.001**	**+ve**	0.259
	T2	2.014	0.111	0.012	0.811	0.488	0.005	111.68	**<0.001**	**+ve**	0.181	3.635	**0.013**	0.021	131.83	**<0.001**	**+ve**	0.207	2.064	0.104	0.012	97.583	**<0.001**	**+ve**	0.163
	T1.T2	3.932	**0.009**	0.023	5.186	**0.002**	0.030	168.99	**<0.001**	**+ve**	0.251	9.963	**0.000**	0.056	151.17	**<0.001**	**+ve**	0.230	8.479	**0.000**	0.048	179.25	**<0.001**	**+ve**	0.264

Appendix 3.2B

Assumptions of independence: The effect of tree species diversity on each covariate was non-significant, (over all 3 areas individually and over all areas combined).

Covariate	Area	Result
Tree Height	A1	$F_{3,\,180} = 3.11$, $P = 0.055$
	A2	$F_{3,\,152} = 0.66$, $P = 0.578$
	A3	$F_{3,\,178} = 2.72$, $P = 0.077$
	A-all	$F_{3,\,510} = 1.05$, $P = 0.350$
Crown Projection	A1	$F_{3,\,180} = 1.80$, $P = 0.098$
	A2	$F_{3,\,152} = 1.37$, $P = 0.241$
	A3	$F_{3,\,178} = 1.44$, $P = 0.376$
	A-all	$F_{3,\,510} = 0.93$, $P = 0.494$
D.B.H.	A1	$F_{3,\,180} = 2.44$, $P = 0.098$
	A2	$F_{3,\,152} = 1.37$, $P = 0.241$
	A3	$F_{3,\,178} = 1.05$, $P = 0.393$
	A-all	$F_{3,\,510} = 1.06$, $P = 0.320$

Chapter 4

Effects of tree species diversity and tree species composition on leaf miner abundance and species richness

4.1 Introduction

The focus of Chapter 3 was on tree species diversity effects on herbivory by different insect herbivore feeding guilds on one tree species, silver birch. The research described in the present Chapter probes deeper into the effects of tree species diversity and also tree species composition on insect herbivores within the same feeding guild, the leaf miners, on two broadleaf tree species, silver birch and black alder. While several previous studies examined forest diversity effects on leaf miner abundance (Vehvilainen *et al.* 2007; Orians & Bjorkman 2009; Castagneyrol *et al.* 2013), to my knowledge this is the first study exploring effects of tree species diversity on species richness of leaf miners.

Leaf miners are endophytic herbivores, the larvae feed between the upper and lower epidermis of leaves creating leaf mines. Adults are free living, emerging from pupae, often within the mine, to mate and lay eggs (Stiling *et al.* 1999). Leaf mines are distinctive enough for species level identification and persist on trees for some time after a mine is abandoned by its creator, thus enabling assessment of both abundance and species richness of leaf miners throughout the season and providing a convenient system for ecological research (Hespenheide 1991). The four insect orders containing the majority of leaf mining species are Lepidoptera, Coleoptera, Hymenoptera and Diptera (Hespenheide 1991), all four of these orders are represented in the boreal forests.

Assigning leaf damage to the causal insect herbivore allows one to apply information regarding that specific insect's behaviour and ecology to the patterns and trends of damage caused to trees throughout gradients of different forest mixtures. For example, a general and important distinction would be: is the insect in question monophagous, oligophagous or polyphagous? Indeed, meta-analysis by Jactel & Brockerhoff (2007) showed that forest diversity reduces herbivory by oligophagous species while the responses of polyphagous species are variable. Leaf miners are mostly monophagous and oligophagous and I predicted that birch and alder would show a decrease in leaf miner abundance and species richness when planted in mixtures compared with monocultures, i.e. associational resistance (Tahvanainen & Root 1972) possibly driven by the resource concentration hypothesis (Root, 1973, Chapter 1). Indeed, Vehvilainen *et al.* (2007) found this trend and that leaf miners were the only insect herbivore guild they studied that showed consistent responses to forest diversity. AR effects to leaf miners were also observed in a tropical forest system (Orians & Bjorkman 2009). However, Sobek *et al.* (2009) using a tree diversity gradient in Germany found no evidence of associational resistance effects towards leaf miners on beech and maple. Research investigating tree diversity effects on abundance of leaf miners is scarce.

This Chapter also investigates the effect of tree species composition (in addition to tree species richness) because plant species composition effects on herbivores have been suggested to be more important than that of plant species diversity (Koricheva *et al.* 2000; Mikola *et al.* 2002; Riihimaki *et al.* 2005; Jactel & Brockerhoff 2007). In particular, Jactel & Brockerhoff (2007) noted that diversity effects on herbivores were greatest when mixed forests comprised taxonomically more distant tree species and when the proportion of non-host trees was greater than that of host trees. In the Satakunta experiment, previous studies have shown that ants, as predators of many insect herbivores are more abundant on birches and pines due to the presence of ant-tended aphid colonies on these trees; this resulted in lower autumnal moth larvae survival in birch-pine stands compared with birch growing with spruce or alder (Riihimäki *et al.* 2005).

The present study compares patterns of abundance and species richness of leaf miners between two tree species, silver birch and black alder. Focus is on the broadleaved trees at Satakunta because leaf mining on the conifers (spruce, larch and pine) in both 2009 and 2010 was very sparse except for one *Coleophora* species on larch. Previous work (Vehvilainen *et al.* 2007) showed that in many cases insect herbivore responses to tree species were tree species specific and that birch generally experienced associational resistance in mixtures, whilst alder showed associational susceptibility.

Leaf miners are sedentary during the larval stage and are confined to the leaf on which the egg was deposited by the female. This results in intimate interactions between leaf mining larvae and host plant tissues (Thompson & Pellmyr 1991) and makes oviposition site choice by leaf miner females extremely important. Natural selection should favour females with an ability to discriminate between hosts of different suitability for larval development (Levins & Macarthur 1969; Jaenike 1978; Mayhew 1997). This is an aspect of the ‘preference–performance hypothesis’ (PPH) (Refsnider & Janzen 2010), also referred to in the literature as the naive adaptionist hypothesis or the mother-knows-best hypothesis (Gripenberg *et al.* 2010). Choice of the oviposition site by leaf miners has been shown to be affected by many different variables, i.e. leaf thickness (Cunningham *et al.* 1999; Schoonhoven *et al.* 2005; Bairstow *et al.* 2010), leaf toughness (Sanson 2006; Clissold *et al.* 2009) and leaf area (Faeth 1991). In addition, larger trees may be more easily discovered by leaf miners (i.e. plant apparency); consequently supporting a greater abundance and diversity of these specialists (Andow 1991). Tree growth might be affected by tree species diversity and composition (Piotto 2008). In addition, birch and alder have been damaged by moose since establishment of the Satakunta experiment and this might also affect tree height. Therefore, this study also investigates if differences in miner abundance and species richness between stands are due to variation in leaf and tree characteristics and whether forest diversity effects on leaf miners are mediated by changes in the above characteristics.

The following questions are addressed in the present study:

i. Does abundance of leaf miners decrease in accordance with the associational resistance phenomenon if their hosts are planted in combination with other tree species?
ii. Are these tree species diversity effects similar for leaf miner species richness?
iii. How does tree species composition influence the leaf mining community?
iv. Are tree species diversity effects on leaf mining community similar between black alder and silver birch?
v. Do physical leaf traits and tree growth characteristics influence leaf miners and mediate effects of forest diversity on the leaf mining community.

4.2 Methods

Leaf miner abundance and species richness monitoring took place in the Satakunta forest diversity experiment between 11th-24th June (early season) and 26th July-12th August (late season) in 2010 and 2011. Black alder and silver birch were the two tree species studied for leaf miners. Birch was present in 9 and alder in 7 out of 19 treatments used in the Satakunta experiment (Table 4.1). See Chapter 2 for detailed site and design description.

Table 4.1: Treatments in which birch and alder are present (**B**=birch, **A**=alder, **P**=pine, **S**=spruce, **L**=larch)

Tree Species	**1 sp mix**	**2 sp mix**	**3 sp mix**	**5 sp mix**	**N^{o} of treatments per area**
Birch	**B**	P**B**, S**B**, **B**A	PS**B**, PL**B**, P**B**A, L**B**A	PSL**B**A	**9**
Alder	**A**	S**A**, B**A**,	SL**A**, PB**A**, LB**A**	PSLB**A**	**7**

Ten trees per species per plot were monitored; the same ten trees on which all other insect monitoring was conducted (see Chapter 2 for tree sampling methodology). Leaf mines were identified and recorded *in situ* using detailed and extensive images and keys (Spencer 1972; Heath *et al.* 1976; Robbins 1990; Csóka 2003) and from the 'Leaf Miners of Europe' website (http://www.bladmineerders.nl/index.htm), and the 'British Leaf Miners' website (http://www.leafmines.co.uk/index.htm).

4.2.1 Analysis

For both alder and birch separately, leaf miner abundance and species richness were analysed at the tree level (per 100 leaves). Data were collected at the branch level, this is explained in Chapter 2. Leaf miner species richness was also assessed at the plot level. Species richness at the plot level was calculated by summing together the number of different leaf mining species present per plot on the 10 trees sampled (and is expressed as richness per 1000 leaves). This differs from leaf miner species richness at the tree level which is an average of the number of leaf miners per tree of the 10 trees sampled. Assessment of leaf miner species richness at plot level was conducted to find out whether different trees within the plot are colonized by different species of leaf miners, while leaf miner species richness per tree may be the same.

Tree species diversity and species composition effects (at 2 and 3 tree species levels) were assessed using linear mixed effect models in SPSS version 19 with 'time' as a repeated factor. Where required, data were square root transformed to normalise model residuals. Effects of tree species diversity and tree species composition were considered in separate models and abundance or species richness of miners was the dependent variable. In addition, time (either season or year), area and all two way interactions with either tree species richness or tree species composition were included in the model in order to test for all biologically relevant interactions. The significance fixed effects was determined using an *F* statistic where the degrees of freedom were approximated with the Satterthwaite method (Norusis 2005).

Tree species diversity effects on individual leaf miner species

Species specific abundances of leaf miners were analysed at the tree level using univariate ANOVA in the manner described above. Within season effects were not possible to test because leaf miners abundant enough to analyse were specific to either early season or late season (not both). Where significant tree species diversity effects were encountered LSD post-hoc tests were conducted to elucidate significant differences between tree species diversity levels. When running each ANCOVA, compliance with the assumptions of the analysis was assured, namely independence of the covariate and treatment effect and homogeneity of regression slopes (Field 2005).

Influence of physical leaf traits on leaf miners

Leaf traits on alder and birch were measured in 2010 during the late season in area 1 only (see Chapter 2). Results reported here use these measured leaf traits tested against leaf miner abundance and richness from the same time and location (i.e. leaf miner abundance and richness from area 1 from the late season in 2010). Measured leaf traits (leaf thickness, leaf toughness, leaf area (LA) and specific leaf area (SLA)) were tested as covariates in a univariate ANCOVA in SPSS version 19. Models were run with and without each covariate to test whether tree species diversity effects on leaf miners change when variation due to leaf traits is adjusted for. See Chapter 2 for details of leaf trait measurements.

Tree growth as a measure of apparency for leaf miners

Tree growth characteristics were tested as covariates in a univariate ANCOVA. Models were run with and without each covariate. See Chapter 2 for details of tree growth measurements. On alder, in order to study effects of tree growth on the leaf mining community, each area was considered individually. This is because in 2010 tree species diversity effects were inconsistent spatially for abundance and species richness of leaf miners. On birch, in order to study effects of tree growth on the leaf mining community areas were combined because tree species diversity effects were consistent over area, see results section 4.3.5.

4.3 Results

In total, 5364 leaf mines which belonged to 45 different miner species were recorded on alder and birch, over the course of the study. Miner species richness was higher on birch (29 species) than on alder (16 species). More species of leaf miners on both birch and alder were present in the late season compared to the early season; this is consistent between 2010 and 2011 (Table 4.2). On alder, miner abundance followed the same patterns as miner species richness with more mines observed in the late season in both years. In contrast, miner abundance on birch was higher in the early season both in 2010 and 2011 (Table 4.3). Miner abundance and species richness also varied between years with more mines and more species recorded on both birch and alder in 2011 than in 2010 (Table 4.2).

Table 4.2 Summary of leaf miner abundance and species richness on birch and alder in 2010 and 2011, during early (T1) and late (T2) season at the Satakunta tree species diversity experiment. Sampling effort (No. of trees monitored) was the same at both time points.

	Birch T1	**Birch T2**	**Total Per yr**	**Alder T1**	**Alder T2**	**Total Per yr**
Miner abundance						
2010 -	509	348	**857**	610	636	**1246**
2011 -	653	644	**1297**	711	1253	**1964**
Miner Sp richness						
2010 -	10	16	**22**	4	11	**13**
2011 -	15	22	**28**	4	16	**16**

On alder, abundance of miners was particularly high in late season of 2011 (Table 4.2) due to a much greater abundance of *Phyllonorycter froelichiella* and *Bucculatrix cidarella* in 2011 than in 2010 (Table 4.3).

Table 4.3 Alder & birch leaf miner species and abundances in 2010 and 2011. T1=early season, T2=late season.

Alder miner species	Author	Order/ Family	2010		2011	
			T1	T2	T1	T2
Caloptilia elongella	Linnaeus (1761)	Lepidoptera, Gracillariidae	**358**	**104**	**335**	**189**
Coleophora serratella	Linnaeus (1761)	Lepidoptera, Coleophoridae	**132**	0	**66**	**9**
Incurvaria pectinea	Haworth (1828)	Lepidoptera, Incurvariidae	**42**	**1**	**105**	**11**
Phyllonorycter strigulatella	Lienig & Zeller (1846)	Lepidoptera, Gracillariidae	**78**	0	**205**	0
Bucculatrix cidarella	Zeller (1839)	Lepidoptera, Bucculatricidae	0	**224**	0	**383**
Fenusa dohrnii	Tischbein (1846)	Hymenoptera, Tenthredinidae	0	**118**	0	**109**
Heliozela resplendella	Stainton (1851)	Lepidoptera Heliozelidae	0	**98**	0	**60**
Phyllonrycter Sp	n/a	Lepidoptera, Gracillariidae	0	**6**	0	**5**
Phyllonorycter rajella	Linnaeus (1758)	Lepidoptera, Gracillariidae	0	**47**	0	**31**
Phyllonorycter stettinensis	Nicelli (1852)	Lepidoptera, Gracillariidae	0	**2**	0	**2**
Phyllonorycter froelichiella	Zeller (1839)	Lepidoptera, Gracillariidae	0	**19**	0	**298**
Phyllonorycter kleemannella	Fabricus (1781)	Lepidoptera, Gracillariidae	0	**3**	0	**9**
Phyllonorycter ulmifoliella	Hübner (1817)	Lepidoptera, Gracillariidae	0	0	0	**4**
Stigmella sp (unidentifiable)	n/a	Lepidoptera, Nepticulidae	0	**1**	0	**33**
Agromyza alnivora	Spencer (1969)	Diptera, Agromyzidae	0	**13**	0	**79**
Orchestus testaceus	Müller (1776)	Coleoptera testaceus	0	0	0	**13**
Heterarthrus vagans	Fallén (1808)	Hymenoptera, Tenthredinidae	0	0	0	**18**

Birch miner species	Author	Order/ Family	2010		2011	
			T1	T2	T1	T2
Eriocrania sangii	Wood (1891)	Lepidoptera, Eriocraniidae	**4**	0	**1**	0
Eriocrania salopiella	Stainton (1854)	Lepidoptera, Eriocraniidae	**4**	0	**2**	0
Eriocrania cicatricella	Zetterstedt (1839)	Lepidoptera, Eriocraniidae	**2**	0	**2**	0
Eriocrania semipurpurella	Stephens (1835)	Lepidoptera, Eriocraniidae	**1**	0	**8**	0
Eriocrania sp.	n/a	Lepidoptera, Eriocraniidae	0	0	**77**	0
Eriocrania sparmanella	Bosc (1791)	Lepidoptera, Eriocraniidae	0	**9**	0	**7**
Stigmella lapponica	Wocke (1862)	Lepidoptera, Nepticulidae	**19**	0	**35**	0
Unknown sp. 1	n/a	n/a	0	**12**	**11**	**8**
Coleophora serratella	Linnaeus (1761)	Lepidoptera, Coleophoridae	**344**	**19**	**198**	**56**
Incurvaria pectinea	Haworth (1828)	Lepidoptera, Incurvariidae	**70**	0	**204**	**8**
Phyllonorycter ulmifoliella	Hübner (1817)	Lepidoptera, Gracillariidae	**20**	**59**	**14**	**39**
Bucculatrix demaryella	Duponchel (1840)	Lepidoptera, Bucculatricidae	**3**	**103**	**10**	**236**
Orchestes rusci	Herbst (1795)	Coleophora, Curculionidae	**42**	**4**	**69**	**45**
Ectoedemia minimella	Zetterstedt (1839)	Lepidoptera, Nepticulidae	0	**4**	**3**	**9**
Scolioneura vicina	Konow (1894)	Hymenoptera, Tenthredinidae	0	0	**3**	0
Agromyza alnibetulae	Hendel (1931)	Diptera, Agromyzidae	0	0	**10**	**2**
Parornix betulae	Stainton (1854)	Lepidoptera, Gracillariidae	0	0	**6**	**11**
Stigmella sp.	n/a	Lepidoptera, Nepticulidae	0	**9**	0	**3**
Stigmella luteella	Stainton (1857)	Lepidoptera, Nepticulidae	0	**1**	0	**17**
Stigmella lapponica	Wocke (1862)	Lepidoptera, Nepticulidae	0	**25**	0	**47**
Stigmella sakhalinella	Puplesis (1984)	Lepidoptera, Nepticulidae	0	0	0	**13**
Stigmella continuella	Stainton (1856)	Lepidoptera, Nepticulidae	0	0	0	**4**
Stigmella betulicola	Stainton (1856)	Lepidoptera, Nepticulidae	0	0	0	**9**
Ramphus pulicarius	Herbst (1795)	Coleoptera, Curculionidae	0	**2**	0	**31**
Phylloporia bistrigella	Haworth (1828)	Lepidoptera, Heliozelidae	0	**52**	0	**10**
Phyllonorycter cavella	Zeller (1846)	Lepidoptera, Gracillariidae	0	**15**	0	**17**
Phylloporia corylifoliella	Hubner (1896)	Lepidoptera, Gracillariidae	0	**1**	0	0
Scolioneura betuleti	Klug (1816)	Hymenoptera, Tenthredinidae	0	**22**	0	**39**
Heterarthrus nemoratus	Fallén (1808)	Hymenoptera, Tenthredinidae	0	**2**	0	**6**
Profenusa thomsoni	Konow (1886)	Hymenoptera, Tenthredinidae	0	**9**	0	**20**
Anoplus plantaris	Naezén (1794)	Coleoptera, Curculionidae	0	0	0	**7**

4.3.1 Tree species diversity effects on the leaf mining community

On both birch and alder, leaf miner species richness and leaf miner abundance followed very similar trends along the tree species diversity gradient; when the abundance of leaf miners increased so did the species richness of the leaf miners (Fig. 4.1).

For black alder leaf miners, tree species diversity effects on miner abundance and species richness varied between areas in both 2010 and 2011 (Fig. 4.1 A-B, area × No. of species interactions in Table 4.4 & 4.5). In areas 2 and 3, leaf miner abundance and species richness were highest in mixtures containing 2 or 3 tree species, whereas at area 1, alder monocultures had the highest miner abundance and species richness (Fig. 4.1, A-B). Although in the majority of cases at maximum tree species diversity the lowest species richness and abundance of leaf miners occurred (Fig. 4.1 A-B). The above effects were consistent within season (season × No. of Sp interaction, Tables 4.4 & 4.5).

When 2010 and 2011 data were combined, effect of tree species diversity was significant for alder leaf miner abundance ($F_{3,\ 577.039} = 3.22$, $P= 0.022$) and species richness ($F_{3,\ 629.978} = 3.39$, $P= 0.018$). Tree diversity effects were consistent across years (year × diversity interaction: $F_{3,\ 577.039} = 2.02$, $P= 0.10$ & $F_{3,\ 629.978} = 1.44$, $P= 0.23$ respectively for miner abundance and species richness), but varied spatially (diversity × area interaction: $F_{6,\ 577.039} = 6.713$, $P< 0.001$ & $F_{6,\ 629.978} = 4.96$, $P< 0.001$ respectively).

For silver birch leaf miners, tree species diversity effects on miner abundance and species richness were significant in 2010, but not in 2011 (Tables 4.4 & 4.5). In 2010, miner abundance and species richness on birch increased with number of tree species per plot (Fig. 4.1C), in 2011 the same trend was observed for miner species richness but not for abundance (Fig. 4.1D). Tree species diversity effects on birch miner abundance and species richness were consistent spatially and temporally (no significant area × No. of species or season × No. of species interactions, Tables 4.4 & 4.5).

When 2010 and 2011 data were combined, effect of tree species diversity on birch leaf miner abundance was non-significant ($F_{3,\ 900.618} = 1.68$, $P= 0.17$) whereas for leaf miner species richness it was significant ($F_{3,\ 953.013} = 2.73$, $P= 0.043$). For leaf miner abundance and species richness tree diversity effects were consistent across years (year × diversity interaction: $F_{3,\ 900.618} = 0.64$, $P= 0.60$ & $F_{3,\ 953.013} = 0.28$, $P= 0.83$ respectively) and consistent spatially (diversity × area interaction: $F_{6, 900.618} = 1.01$, $P = 0.40$ & $F_{6, 953.013} = 1.63$, $P = 0.14$ respectively). Leaf miner response to tree species diversity was more variable on alder than on birch (comparing Fig. 4.1 A&B to C&D).

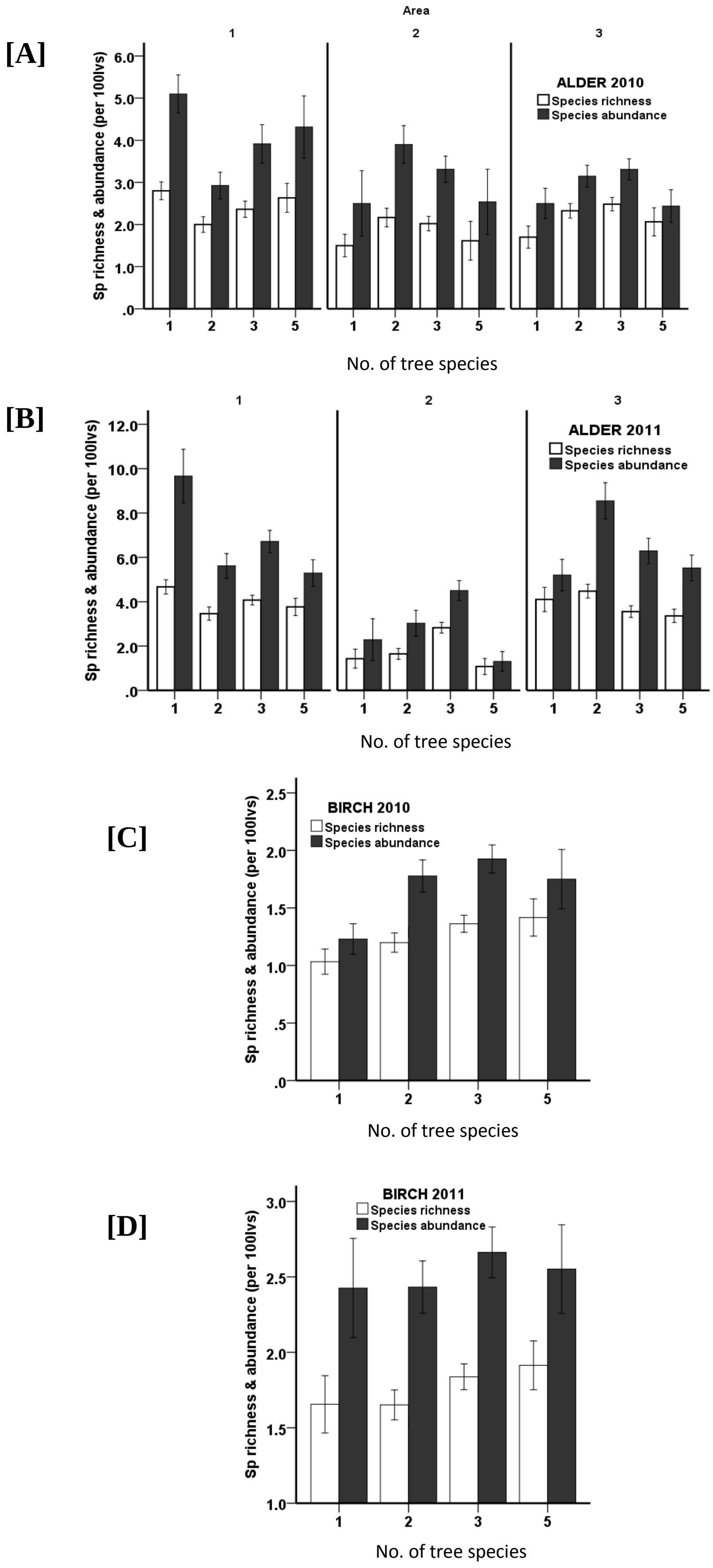


Figure 4.1 Mean leaf miner species richness and abundance per 100 leaves (early and late season combined) for **[A]** alder 2010 **[B]** alder 2011 **[C]** birch 2010 **[D]** birch 2011 over the tree species diversity gradient. Error Bars are +/- 1 SE.

Table 4.4 Linear mixed model results for tree species diversity effects on leaf miner species richness per tree. All factors were treated as fixed. Significance was determined using an *F*-statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05).

		2010		**2011**	
FACTORS	**Numerator df**	**Alder**	**Birch**	**Alder**	**Birch**
Season	1	3.689	5.825*	39.521***	5.388*
Area	2	5.300*	8.461***	46.551***	2.672*
No. of sp	3	1.179	2.362*	3.935*	1.064
Season × Area	2	5.767*	0.844	7.585**	4.004*
Season × No. of Sp	3	2.878	0.631	1.936	2.168
Area × No. of Sp	6	3.122*	1.506	5.364***	1.365
Season × Area × No. of sp	6	0.696	2.102	1.361	1.063
	Denominator df	*687.573*	*1007.166*	*681.397*	*958.718*

Table 4.5 Linear mixed model results for tree species diversity effects on leaf miner abundance. All factors were treated as fixed. Significance was determined using an *F*- statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05).

		2010		**2011**	
FACTORS	**Numerator df**	**Alder**	**Birch**	**Alder**	**Birch**
Season	1	0.995	23.162***	28.062***	0.969
Area	2	7.069**	7.428**	38.245***	2.519
No. of sp	3	0.555	2.606*	4.510*	0.453
Season × Area	2	3.840*	0.023	4.933*	5.058*
Season × No. of Sp	3	2.370	1.459	1.829	1.297
Area × No. of Sp	6	3.516*	1.411	5.822***	0.846
Season × Area × No. of sp	6	0.934	1.681	2.204*	1.192
	Denominator df	*715.977*	*881.104*	*684.877*	*961.439*

Species richness of leaf miners at the plot level

On alder, tree species diversity effects on miner species richness per plot were significant neither in 2010 nor in 2011 and were consistent over area (Appendix 4.1 A-B); species richness of leaf miners at the plot level showed very similar patterns to those described above for species richness of leaf miners at the tree level. When 2010 and 2011 data were combined for alder, tree diversity effects on species richness of miners at plot level were non-significant ($F,_{3,\ 48.406} = 1.84$, P= 0.15).

On birch, tree species diversity effects on leaf miner species richness per plot were significant in 2010, but not in 2011 (Appendix 4.2 A-B); In 2010, plot level leaf miner species richness increased with number of tree species per plot (as it did at the tree level, see above). This trend was not present in 2011 where birches in monoculture, 3 and 5 species mixtures contained equal species richness of leaf miners (Appendix 4.2). When 2010 and 2011 data were combined for birch, tree diversity effects on species richness of miners were non-significant ($F_{3, 75.035} = 0.87$, $P = 0.46$).

4.3.2 Tree species composition effects on the leaf mining community

Only tree species composition effects on abundance of leaf miners are presented graphically (Fig. 4.2 & 4.3) because the patterns of tree species composition effects on leaf miner abundance and leaf miner richness were very similar (as it was for tree species diversity effects in Fig. 4.1).

Alder in two species mixtures

In 2010, leaf miners on alder were not significantly influenced by tree species composition (Table 4.6). In 2011, tree species composition effects on the abundance of leaf miners on alder varied spatially and temporally (area × Sp mix and season × Sp mix interactions: Table 4.6). In area 1, miner abundance was higher in alder spruce-mixtures than in birch-alder mixtures, in area 3 the pattern was opposite to area 1 and in area 2 no differences in abundance of leaf miners between 2-species mixtures was observed (Fig. 4.2B). When 2010 and 2011 data were combined, tree species composition effects on leaf miner abundance and species richness were consistent between years (year × composition $F_{2, 152.004} = 0.28$, $P = 0.75$ & $F_{2, 175.986} = 0.19$, $P = 0.83$ for miner abundance and species richness respectively).

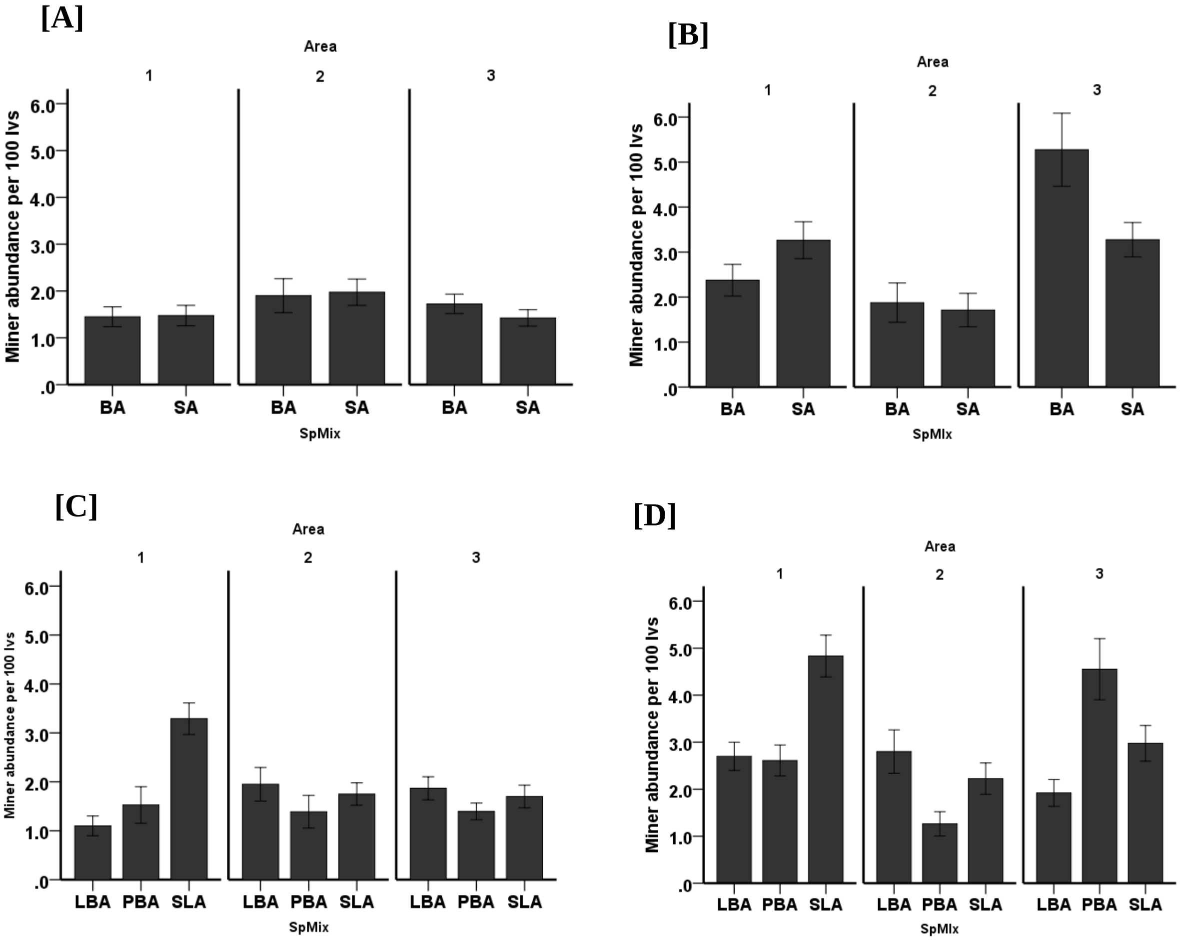


Figure 4.2 Effects of stand (tree) species composition on the abundance of leaf miners on alder (early and late season combined) over all 3 areas on alder in **[A]** 2 species mixtures 2010, **[B]** 2 species mixtures 2011 **[C]** 3 species mixtures 2010, **[D]** 3 species mixtures 2011. (**BA**= birch & alder, **SA**= spruce and alder, **LBA**= larch, birch & alder, **SLA**=spruce, larch & alder. Error Bars are +/- 1 SE.

Alder in three species mixtures

In 3 species mixtures, tree species composition effects on alder leaf miners were significant and varied spatially and temporally in both 2010 and in 2011 (area × Sp mix and season × Sp mix interactions: Table 4.6). In both 2010 and 2011, alder growing in SLA mixtures had greatest abundance in area 1 (Fig. 4.2C-D), whereas patterns in areas 2 and 3 varied between years (Fig. 4.2C-D).

When 2010 and 2011 data were combined, tree species composition effects on leaf miner abundance and species richness were consistent between years (year × Sp mix: $F_{2, 261.540} = 0.52$, $P= 0.60$ & $F_{2, 262.065} = 1.40$, $P= 0.25$ respectively).

Table 4.6 Linear mixed model results for tree species diversity effects on leaf miner abundance on alder. All factors were treated as fixed. Significance was determined using an *F*- statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05).

	2 SPECIES MIX's			**3 SPECIES MIX's**		
FACTORS	Numerator df	**2010**	**2011**	Numerator df	**2010**	**2011**
Season	1	0.033	26.862***	1	2.378	22.516***
Area	2	1.479	14.383***	2	0.586	10.009***
Sp Mix	1	0.078	0.352	2	9.617***	4.412*
Season × Area	2	0.442	8.008***	2	7.731**	7.875*
Season × Sp Mix	1	2.863	4.062*	2	5.457**	1.908
Area × Sp Mix	2	0.355	4.180*	4	7.130***	9.875***
Season × Area × Sp Mix	2	3.089*	9.795**	4	1.045	2.061
	Denominator df	*206.478*	*199.903*	*Denominator df*	*305.375*	*298.860*

Birch in two and three species mixtures

In 2010, leaf miner abundance on birch growing in 2 species mixtures was significantly influenced by tree species composition (Table 4.7). Effects of tree species composition were consistent spatially (area × Sp mix interaction: Table 4.7) with birch having lowest abundance of miners in all 3 areas when grown in mixtures with pine (Fig. 4.3A). Tree species composition effects on abundance of leaf miners however, did vary between season (season × sp mix interaction), but not spatially (area × sp mix interaction), Table 4.7. In 2011, leaf miner abundance on birch growing in 2 species mixtures were not significantly affected by tree species composition (with these effects being consistent spatially and temporally, Table 4.7, Fig. 4.3B). On birch in 2 species mixtures, tree species composition effects on leaf miner abundance and species richness was also consistent between years ($F_{2,\ 284.252}$ = 2.97, P= 0.06 & $F_{2,\ 287.722}$ = 2.93, P= 0.05 for leaf miner abundance and species richness respectively).

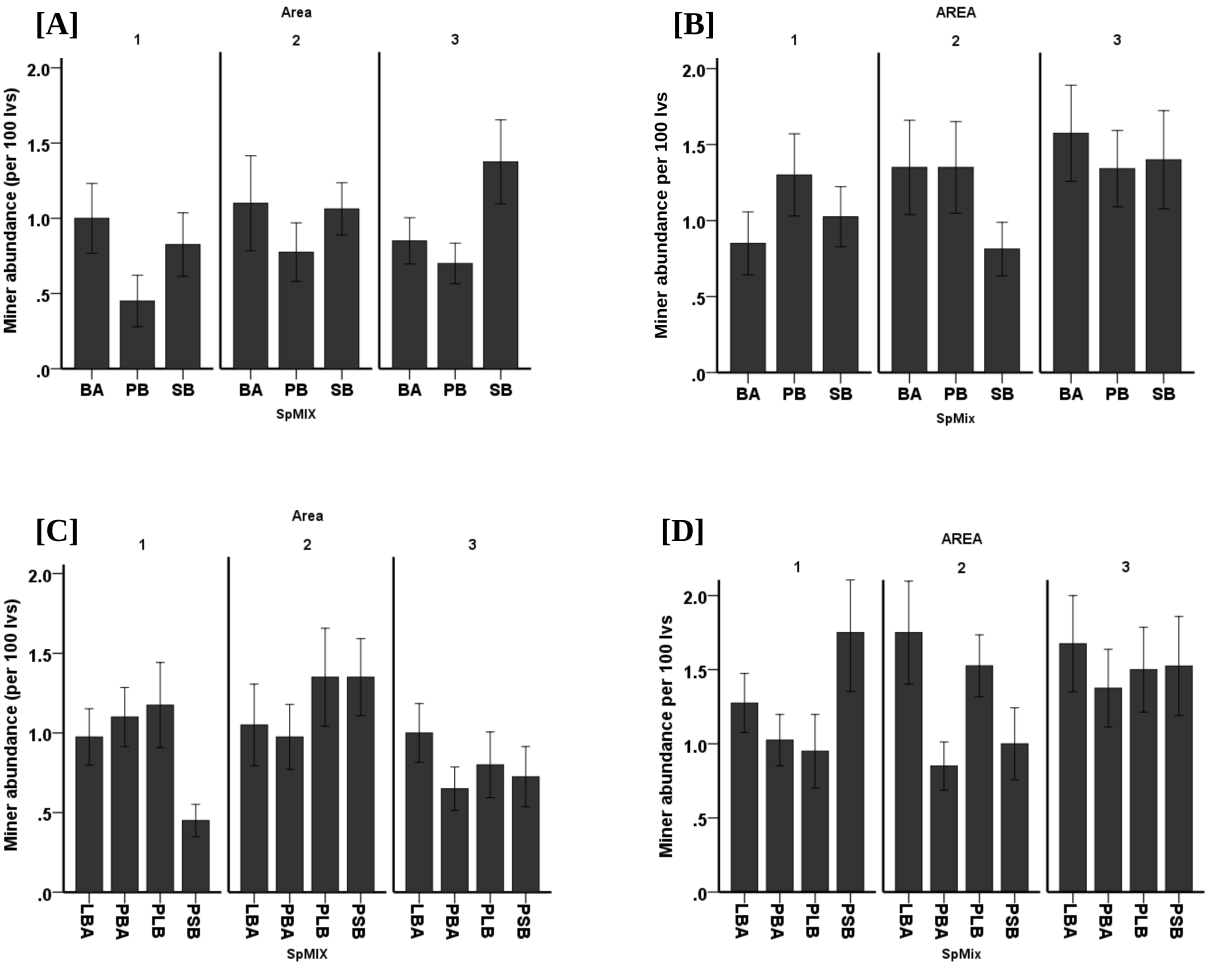


Figure 4.3 Effects of stand (tree) species composition on the abundance of leaf miners on birch (early and late season combined) over all 3 areas on birch in **[A]** 2 species mixtures 2010, **[B]** 2 species mixtures 2011 **[C]** 3 species mixtures 2010, **[D]** 3 species mixtures 2011. (**BA**= birch & alder, **SA**= spruce and alder, **LBA**= larch, birch & alder, **SLA**=spruce, larch & alder. Error Bars are +/- 1 SE.

Table 4.7 Linear mixed model results for tree species composition effects on birch leaf miner abundance. All factors were treated as fixed. Significance was determined using an *F*- statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05).

	2 SPECIES MIX's			**3 SPECIES MIX's**		
FACTORS	Numerator df	**2010**	**2011**	Numerator df	**2010**	**2011**
Season	1	23.253***	0.796	1	19.674***	3.108
Area	2	2.187	1.946	2	3.693*	1.109
Sp Mix	2	4.689*	1.047	3	0.795	1.805
Season × Area	2	0.920	4.321*	2	2.630	3.259*
Season × Sp Mix	2	3.616*	2.305	3	1.511	0.511
Area × Sp Mix	4	0.702	0.872	6	1.747	1.620
Season × Area × Sp Mix	4	3.007*	1.821	6	1.254	2.713
	Denominator df	*270.076*	*286.409*	*Denominator df*	*380.085*	*426.955*

In 3 species mixtures, tree species composition effects on the abundance of leaf miners were not significant in 2010 or in 2011; with tree composition effects on birch being consistent spatially and temporally (Fig. 4.3 C-D, Table 4.7). On birch in 3 species mixtures, tree species composition effects on leaf miner abundance and species richness was also consistent between years (year × species mixture: $F_{3,\ 391.069}$ = 1.14, P= 0.33 & $F_{3,\ 422.335}$ = 0.69, P= 0.56 for leaf miner abundance and species richness, respectively).

4.3.3 Tree species diversity effects on individual leaf miner species

On alder in 2010, four species of leaf miners were abundant enough to be analysed individually: *C. elongella*, *B. cidarella*, *F. dohrnii* and *C. serratella*. In 2011 there were six miner species abundant enough for analysis: *C. elongella, B. cidarella, F. dohrnii, I. pectinea, P. strigulatella and P. froelichiella*. Of the above species, only *C. serratella* and *I. pectinea* are polyphagous and can feed on both birch and alder in the Satakunta experiment whereas the rest of the species are monophagous on alder.

Abundance of the majority of alder leaf miner species tended to decrease with tree species diversity (Fig. 4.4 A&B) although tree species diversity effects were significant only for *P. froelichiella* ($F_{3,\ 358}$ = 3.70, P= 0.012) which showed a non linear response with highest miner abundance observed in 2 and 3 species mixtures (Fig. 4.4B). LSD post-hoc pair wise tests revealed significant differences in *P. froelichiella* abundance between 1 and 2 species mixtures (P= 0.040), between 1 and 3 species mixtures (P=0.021), between 2 and 5 species mixtures (P= 0.06) and between 3 and 5 species mixtures (P= 0.031).

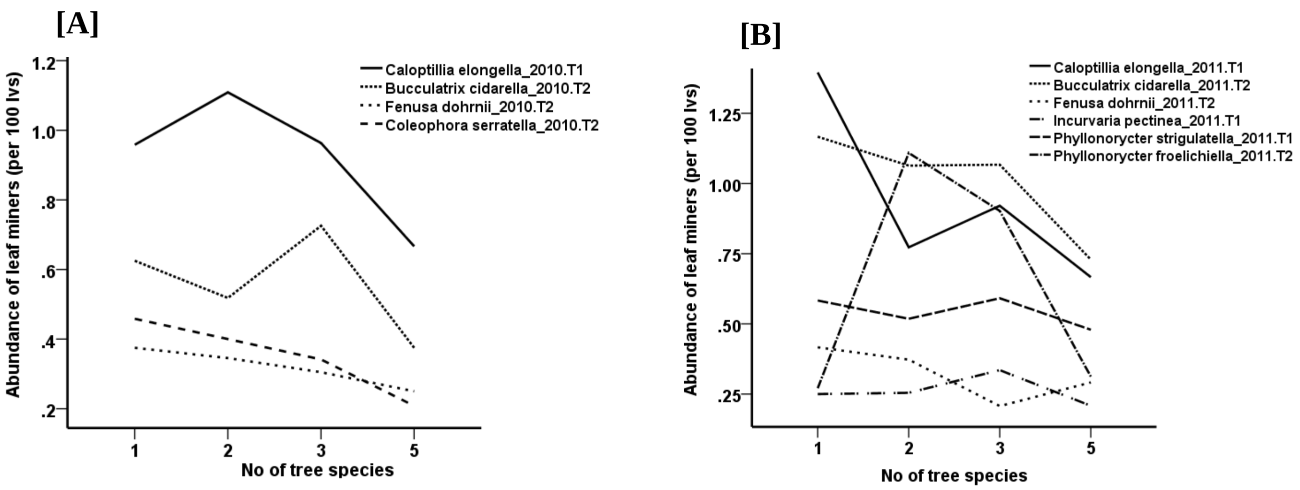


Figure 4.4 Species specific leaf miner abundance on alder over tree species diversity gradient in **[A]** 2010 and **[B]** 2011.

When data for *B. cidarella*, *C. elongella* and *F. dohrnii* were combined across both years, tree species diversity effects remained non-significant but varied between areas (area × diversity interaction: Appendix 4.3).

Out of the four leaf miner species abundant enough for analysis on birch in 2010 (Table 4.3, Fig. 4.5A), only abundance of *C. serratella* was significantly affected by tree diversity ($F_{3, 494}$ = 3.09, P= 0.027) with highest abundance observed in 2- and 3-species mixtures Fig. 4.5A). LSD post-hoc pair wise tests revealed significant differences between 1 and 2 species mixtures (P= 0.011), between 1 and 3 species mixtures (P=0.036) and between 2 and 5 species mixtures (P= 0.037). The response was not consistent over the tree species diversity gradient with the lowest leaf miner abundance occurring in monoculture, the highest in two and three species mixture, with a linear decline in *C. serratella* abundance over 3 and 5 tree species diversity levels Fig. 4.5A).

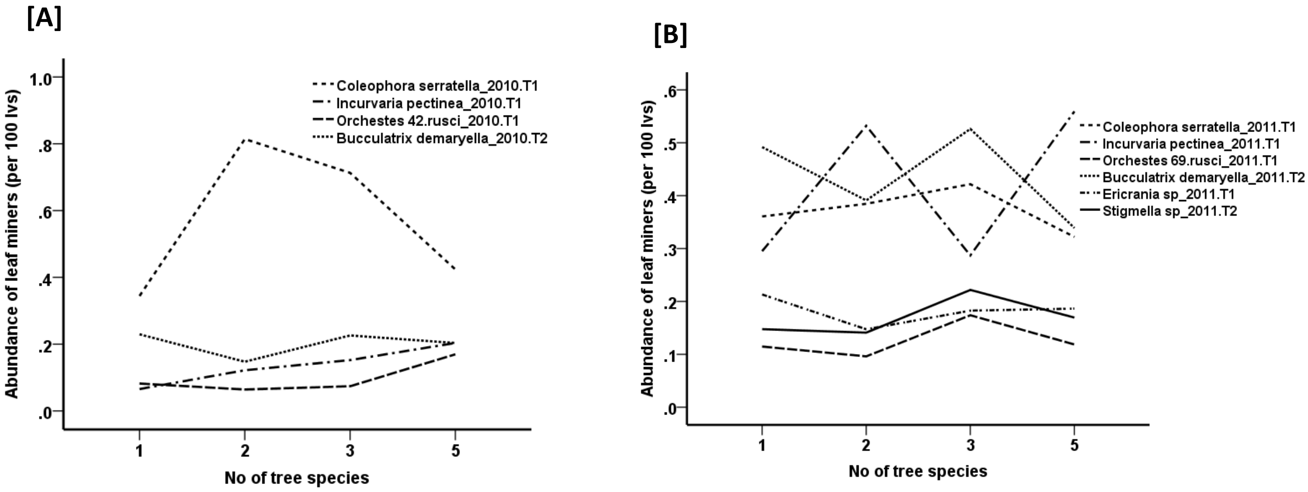


Figure 4.5 Species specific leaf miner abundance on birch over tree species diversity gradient in **[A]** 2010 and **[B]** 2011.

None of the 5 leaf miner species analysed on birch in 2011 (Table 4.3, Fig. 4.5B; *O. rusci* was too low in abundance for analysis but included on the graph to compare with Chapter 5 data in the discussion Chapter) were significantly affected by tree species diversity (P≥ 0.10; season × diversity interaction P≥ 0.12).

When abundances of leaf miner species (that were present in both 2010 and 2011) on birch were combined across 2 years, the effects of tree species diversity remained non significant (Appendix 4.4).

4.3.4 Effects of leaf traits on leaf miner abundance and richness

Tree species richness did not significantly influence any of the leaf traits (Appendix 3.1), confirming independence of the covariate and treatment effect.

For alder, incorporating leaf traits as covariates did not alter the effect of tree species diversity. Leaf thickness, toughness, and area did not explain any of the error variance (P≥ 0.32). Leaf miner abundance and richness did however increase with decreasing specific leaf area (SLA) the effect was significant ($F_{1,\ 131}$ = 3.84, P= 0.05) for the abundance of leaf miners and significant for species richness of leaf miners ($F_{1,\ 131}$ = 5.02, P= 0.027) but only explained 2.8 and 3.7% of the model variance.

On birch, leaf traits did not alter the effect of tree species diversity. Leaf traits did not explain any of the error variance on the abundance and species richness of leaf miners:

Leaf thickness (P≥ 0.86), toughness (P≥ 0.15), leaf area (P≥ 0.51) and specific leaf area (P≥ 0.86).

4.3.5 Tree growth as a measure of apparency for leaf miners

For analysing effects of tree growth on the leaf mining community of alder in both 2010 and in 2011 each area was considered individually. This is because in 2010 tree species diversity effects on abundance and species richness of leaf miners were inconsistent spatially (area × diversity interaction: $F_{6, 369} = 3.39$, P= 0.003 & $F_{6, 369} = 2.63$, P= 0.017 for miner abundance and species richness respectively). Similarly, in 2011, tree species diversity × area interactions were also significant ($F_{6, 356} = 5.31$, P <0.001 for miner abundance and $F_{6, 356} = 4.94$, P <0.001 for miner richness).

In area 2 in 2010, leaf miner abundance and species richness were significantly higher on taller trees with larger crown projections (Table 4.8). This trend did not occur in the other areas, although in area 1, species richness of leaf miners was marginally higher on taller trees with larger crown projections (Table 4.8). In no instances did tree growth traits influence the effect of tree species diversity.

Table 4.8 Effect of tree growth (height and crown projection) on leaf miner abundance and species richness (using *ANCOVA*) for alder in 2010
ETA = proportion of variance that a variable explains that is not explained by other variables in the analysis (values between 0 and 1). Significance was determined using an *F*- statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05). Direction: + = positively correlated with herbivory.

Model	**Factor**	**DF**	**F**	**Direction**	**ETA**	**F**	**Direction**	**ETA**	**F**	**Direction**	**ETA**
Leaf miner abundance			*Area 1*			*Area 2*			*Area 3*		
1	Tree Div	3	3.431*	n/a	0.072	1.680	n/a	0.050	1.767	n/a	0.039
2	Tree Div	3	4.097*	n/a	0.086	1.474	n/a	0.045	1.010	n/a	0.023
	Height	1	2.439	n/a	0.018	20.829***	**+ve**	0.183	3.173	n/a	0.024
3	Tree Div	3	3.936*	n/a	0.083	1.684	n/a	0.052	1.011	n/a	0.023
	C. projection	1	2.459	n/a	0.018	8.866**	**+ve**	0.088	1.941	n/a	0.015
Leaf miner richness			*Area 1*			*Area 2*			*Area 3*		
1	Tree Div	3	2.147	n/a	0.046	1.227	n/a	0.037	2.394	n/a	0.052
2	Tree Div	3	2.170	n/a	0.048	1.342	n/a	0.041	1.837	n/a	0.042
	Height	1	5.962*	**+ve**	0.054	35.068***	**+ve**	0.274	3.533	n/a	0.027
3	Tree Div	3	2.791	n/a	0.050	1.359	n/a	0.042	1.758	n/a	0.040
	C. projection	1	5.267*	**+ve**	0.049	19.374***	**+ve**	0.174	2.227	n/a	0.019
Denominator DF			**135**			**96**			**131**		

Table 4.9 Effect of tree growth (height and crown projection) on leaf miner abundance and species richness (using *ANCOVA*) for alder in 2011
ETA = proportion of variance that a variable explains that is not explained by other variables in the analysis (values between 0 and 1). Significance was determined using an *F*- statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05). Direction: + = positively correlated with herbivory.

Model	**Factor**	**DF**	**F**	**Direction**	**ETA**	**F**	**Direction**	**ETA**	**F**	**Direction**	**ETA**
Leaf miner abundance			*Area 1*			*Area 2*			*Area 3*		
1	Tree Div	3	5.043*	n/a	0.107	5.881*	n/a	0.164	3.594*	n/a	0.077
2	Tree Div	3	6.547**	n/a	0.136	4.984*	n/a	0/144	2.974*	n/a	0.044
	Height	1	8.361*	**+ve**	0.063	28.026***	**+ve**	0.239	31.49***	**+ve**	0.197
3	Tree Div	3	2.623*	n/a	0.059	4.939*	n/a	0.144	3.273*	n/a	0.071
	C. projection	1	0.917	n/a	0.007	22.414***	**+ve**	0.203	38.12***	**+ve**	0.229
Leaf miner richness			*Area 1*			*Area 2*			*Area 3*		
1	Tree Div	3	2.884*	n/a	0.056	7.183***	n/a	0.193	2.723	n/a	0.055
2	Tree Div	3	3.707*	n/a	0.082	6.148**	n/a	0.172	2.500	n/a	0.043
	Height	1	13.05**	**+ve**	0.095	28.794***	**+ve**	0.244	11.62***	**+ve**	0.083
3	Tree Div	3	2.403*	n/a	0.043	6.196**	n/a	0.174	2.534	n/a	0.056
	C. projection	1	0.248	n/a	0.010	24.407***	**+ve**	0.217	19.38***	**+ve**	0.131
Denominator DF			**129**			**93**			**132**		

During 2011, in all 3 areas the taller alder trees with wider canopies supported greater species richness and abundance of leaf miners, (Table 4.9), with exception of area 1 where tree canopy size effect was non-significant, yet tree height effect still was. Again, in no instances did tree growth traits influence the effect of tree species diversity.

For analysing effects of tree growth on the leaf mining community of birch in both 2010 and in 2011, areas 1, 2 and 3 were combined because area × diversity interaction was not significant: $F_{6, 515}$ = 1.19, P= 0.31, for leaf miner abundance & $F_{6, 515}$ = 1.44, P= 0.20 for leaf miner species richness); the same situation in 2011 ($F_{6, 500}$ = 0.80, P= 0.57 for abundance & $F_{6, 500}$ = 1.42, P= 0.21 for species richness of leaf miners).

In 2010, the abundance and species richness of leaf miners on silver birch were not influenced by tree height or crown projection (abundance: P≥ 0.44, species richness P≥ 0.07). In 2011, a greater abundance and species richness of leaf miners was present on trees with larger canopy width ($F_{1, 500}$ = 3.76, P= 0.05 & $F_{1, 500}$ = 4.50, P= 0.034 respectively) although tree height did not affect abundance and species richness of leaf miners (P≥ 0.37 & 0.35 respectively). Tree diversity effects were not significantly influenced by the measured tree growth characteristics. In no instances (for both 2010 & 2011) did the effect of the covariate change the effect of tree species diversity.

4.4. Discussion

4.4.1 Tree species diversity effects on the leaf mining community of alder and birch

Leaf miner abundance and species patterns

Leaf miner abundance and species richness followed very similar patterns to each other when considered over both tree species diversity and tree species composition. In instances where leaf miner abundance increased so did miner species richness. This means that higher abundance of leaf mines is the consequence of more species of leaf miners, rather than the presence of a few dominant species. This needn’t have been the case as it is possible to have an increasing abundance of leaf miners with constant richness (no species addition or losses) or diminishing richness (species losses, i.e. when one species becomes dominant e.g. in an outbreak situation depleting common food resources to the detriment of other species (Lawton & Hassell 1981; Bylund & Tenow 1994). This in itself was an unexpected finding, however, linear patterns in abundance and species richness of miners over tree species diversity gradient were not present for alder but were for birch.

In 2010, leaf miner abundance and species richness on birch increased with tree species diversity (Fig. 4.1 C) this same (AS) trend for leaf miner richness on birch was also observed at the plot level (Appendix 4.2), similar patterns occurred in 2011 on birch but results did not achieve significance (Fig 4.1D & Appendix 4.2). This finding is at odds with a meta-analysis by (Vehvilainen *et al.* 2007) who showed that leaf miner abundance is consistently and significantly lower in mixed stands (AR); Vehvilainen *et al.* (2007) suggest that the high feeding specialisation, sessile nature and search behaviour of leaf miners may be responsible for the trend on silver birch.

The 5 tree species mixtures comprise birch at a lower density (20%) compared to less diverse plots that contain birch (33% in 3 species, 50% in two species and 100% in monoculture plots); despite this, on birch the abundance and species richness of leaf miners is higher in more species diverse mixtures, a trend in line with the predictions of the associational susceptibility phenomenon (Brown & Ewel 1987; Barbosa *et al.* 2009).

One explanation might be that abiotic conditions are more favourable to leaf miners, (Osisanya 1970) in the higher tree diversity plots, that may more closely resemble a natural forest system by possessing a more varied forest structure and/or detectably different (from leaf miner viewpoint) light and humidity levels as compared with plots with lower tree species diversity. This is an intriguing possible explanation and few studies have investigated whether this mechanism might lead to AR or AS (Barbosa *et al.* 2009). The microclimate within forest stands composed of different tree species can be expected to be fairly different (Vehvilainen *et al.* 2008) and can be more stable in more diverse systems (Ulrich 1992). Furthermore, humidity has been shown to commonly influence distribution, fecundity and growth rate of many insects (Bach 1993). Insect distribution and flight activity of adults in the forest environment is also affected by wind speed which will be lower through the higher tree diversity plots (Cloudsley-Thompson 1962), possibly favouring leaf miners. The more closed canopy conditions in the 5 tree species mixtures might also provide more shade leaves shown to be more favourable to miners (Faeth 1991). The subject of microclimates is further complicated as fluctuations influence the emission and dispersion of plant volatiles (Randlkofer *et al.* 2010).

Volatile mixing has been suggested to deter and confuse specialist insects resulting in AR effects, this arises because of the greater complexity of non host volatiles (NHV) interfering with host cues. This mechanism is referred to as the ‘semiochemical diversity’ hypothesis (SDH) (Zhang & Schlyter 2003). However, results of this study suggest leaf miners are not affected by the SDH because alder and birch (considered separately) in the 5 species mixtures virtually always contain equal or greater leaf miner abundance and species richness compared to the monoculture plots of the same tree species. If the mechanisms of the SDH were influencing the leaf miners, one would expect fewer miners in the highest tree species diversity plots.

Tree species diversity and spatial variation in the leaf mining community

When considering the whole leaf mining community, tree species diversity effects appear more variable on alder than on birch. The effect of tree species diversity on alder leaf miner abundance and species richness between areas is inconsistent giving rise to significant tree diversity × area interaction in both 2010 and 2011 (Table 4.4 & 4.5). For birch leaf miners, the tree diversity × area interaction was not significant. A possible explanation for this is variation between areas of certain environmental variables that affect alder more than birch, such as soil macronutrients or water availability. These variations could influence the amount of nitrogen alder can fix and in turn impact on the insect herbivore community. For example, fluctuation in pH, nutrient availability, temperature, and water status, greatly influences the growth, survival, and metabolic activity of nitrogen fixation bacteria (Mohammadi *et al.* 2012). Leaf miner abundance was considerably higher on alder than on birch, although leaf miner species richness was higher on birch than on alder. Higher abundance of leaf miners on alder might be due to the generally higher nitrogen levels in alder foliage (effects of nitrogen on insect herbivory are further discussed in section 4.4.4).

4.4.2 Tree species composition effects on leaf mining community on alder and birch

For tree species composition, spatial effects are similar to that of tree species diversity in as much as the effects of tree species composition differ between areas for alder whilst for birch they do not (Table 4.6 & 4.7). On the whole tree species composition effects on the leaf miner community are rather variable. For example, by looking at responses of birch in two species mixture in 2010 (Fig. 4.3A), it is clear that pine-birch (PB) mixtures contain the least mining damage over all 3 areas. In 2011, however, (Fig. 4.3B) PB mixtures experience the highest or equal highest abundance of leaf mines as other 2-species mixtures. These rather inconsistent responses of leaf miner abundance to tree species composition occur for both tree species at different diversity levels and seasons with no clear trends and patterns being readily observed. It is possible (as alluded to above) that important factors could be missing from the model that may help explain these patterns, i.e. abiotic conditions, (other potential unrecorded explanatory variables are discussed in section 4.4.3).

4.4.3 Responses of individual species of leaf miners to tree species diversity

The only leaf miner species found to be significantly affected by tree species diversity were *Phyllonorycter froelichiella* on alder in 2011 and *Coleophora serratella* on birch in 2010. Both species showed non-linear responses to tree species diversity with highest abundance at 2- and 3-species mixtures. For the remaining miner species analysed, tree species diversity effects remained non-significant when 2010 and 2011 data were combined (Appendix 4.3 and 4.4), although abundance of most species of leaf miners on alder tended to decrease with increasing tree species richness (Fig. 4.4). The finding that leaf miner abundance and species richness are in most cases lower in 5-species mixtures for alder, whilst on birch there is more of an even distribution of mines over tree species diversity supports the finding (from an extensive meta analysis) that the direction of forest diversity effects on insect herbivores is largely dependent on the host tree species (Vehvilainen *et al.* 2007). The responses of individual leaf miners over tree species diversity (for both tree species) are also, in most cases, non-linear and in instances where responses are linear in one year for a particular leaf mining species (i.e. *Bucculatrix cidarella* on alder and *Incurvaria pectinea* on birch), they are not in the next. This suggests that tree species diversity effects on leaf miner abundance are not consistent and rather variable temporally.

In all instances there are a fairly low number of leaf miners present. It is possible that had greater numbers been encountered for a particular species (e.g. in a peak year or an outbreak situation), variation in spatial and temporal responses to tree diversity may have been smaller and clearer trends and more stable patterns observed. This idea is supported in this study because the only two species of leaf miner that were significantly affected by tree species diversity (*Phyllonorycter froelichiella* on alder in 2011 and *Coleophora serratella* on birch in 2010 both show humped shaped responses to tree species richness (highest abundance at intermediate diversity levels); *C. serratella* showing a different response in 2011 when less abundant (than in 2010) and not significantly influenced by tree species diversity. For those leaf miner species present in 2010 and 2011, when data were combined across years tree diversity effects remained non-significant.

It is likely that unrecorded factors may influence the leaf mining community. For example, the different patterns between years for some species of leaf miner (e.g. *Caloptilia elongella* on alder and *C. serratella* and *I. pectinea* on birch) may be due to variation in the numbers of predators and parasites, shown by Faeth *et al.* (1981) to be among the most important factors governing the leaf mining society. Similarly, the highest abundance of leaf mining insects on willow trees were found to be on branches where ants were excluded (Nygard *et al.* 2008). Leaf mining insects rank as having the most species of parasitoids per host of any feeding guild (Connor & Taverner 1997). It would be a worthwhile addition to repeat this study and investigate the direct influence of predators and parasites on structuring the leaf mining community, including how tree species diversity and composition affect natural enemies.

4.4.4 Covariates: leaf traits and tree apparency as predictors of leaf mining

Leaf traits

Leaf miner species richness on alder was shown to significantly increase with decreasing specific leaf area (SLA); whilst the abundance of leaf miners on alder was significant. With the exception of this result, the leaf trait characteristics leaf toughness, leaf thickness, leaf area and specific leaf area did not correlate with species richness and abundance of leaf miners on alder nor on birch. These results suggest that the four leaf traits measured do not affect leaf miner oviposition site choice at the tree level (Refsnider & Janzen 2010). It is possible, however, that measured leaf traits (thickness, toughness, area) may be of importance within the tree level, i.e. at the branch or leaf level (De Sibio & Rossi 2012). At spatial scales smaller than individual plants, very little is known about the chemical and physical traits that influence the choice of suitable ovipositing sites by leaf miners (Ishino *et al.* 2011). Indeed, other leaf traits such as leaf colour and density of trichomes, for example, have been shown to influence leaf miner densities (Dai *et al.* 2011), suggesting that other physical leaf traits affecting miners might also need to be considered. Leaf trichomes can significantly deter feeding damage and oviposition ability of leaf miners (Gross & Price 1988; Hawthorne *et al.* 1992). However, despite the obvious physical barrier that leaf trichomes present, Dai *et al.* (2011) indicate that some leaf miner species will select foliage more densely covered with trichomes. This is thought to occur because a higher leaf trichome density will disturb the searching behaviour of parasitic wasps as well as lower feeding competition from ectophagous insects, bringing into play the role natural enemies. On Acacia (albeit over a large climatic gradient of 950 km), Bairstow *et al.* 2010 showed that leaf miner richness increased with both increasing SLA (opposite to this study on alder) and decreasing leaf thickness. Their results support the idea from Sinclair and Hughes (2008) who show (using 15 leaf miner species and 36 plant species) that ovipositing leaf miners utilise foliage that is less sclerophyllous and consequently more suitable, by selecting longer and thinner leaves. Over larger climatic gradients, as studied in Bairstow *et al*. (2010), variation in leaf traits (i.e. thickness and SLA) will be much greater than those observed in the present study due to the direct influence that climate has on plant traits (Connor *et al.* 1994); that will vary considerably more over 950 km as compared to a few hectares of area 1 in Satakunta. In addition, Bairstow *et al*. (2010) examined variation in leaf traits across many acacia species whilst the present study focused only on two tree species.

Furthermore, within the present study it was only possible to investigate the effects of leaf traits on miners in 2010, at one of the three experimental areas and only during the late season. It is quite possible that extending the data set to include multiple areas and time points (within season and between years) may show different patterns; just as it has been shown in Chapter 3 and other studies (Faeth *et al.* 1981; Plath *et al.* 2011), that the effects of tree species diversity and composition on insect herbivory can be inconsistent over time.

It is perhaps not surprising that the abundance of leaf miners is so much higher on alder (a nitrogen fixer) than on birch (Table 4.2); as larger amounts of N are associated with greater abundance of insect herbivores (Schoonhoven *et al.* 2005). It is surprising, however, that no significant effects of tree species composition on leaf miner abundance have been found (i.e. on birch in mixture with alder compared to birch without alder). It has been shown that growing non N-fixing plants in ployculture with N-fixing plants can significantly increase N content of foliage in the former (Moore & Francis 1991; van Ruijven & Berendse 2005). In this study there are no empirical data on N content from experimental tree foliage during the study period. This makes it difficult to ascertain if, or how much greater the levels of N were in birch growing in mixture with alder, although, seemingly not different enough to impact on leaf miner abundance or species richness. It has been suggested that leaf mining insects may utilise a bet-hedging strategy and oviposit pseudo-randomly ‘here and there’ in the hope that something works out when they encounter variable foliage quality (in terms of physical attributes, defensive compounds and nutrition) within and among host plants (Hopper 1999; Gripenberg *et al.* 2007). This may also explain a lack of significant leaf trait effects on the leaf mining community. One study found female oviposition of the common monophagous holly leaf miner *Phytomyza ilicis* was significantly negatively correlated with foliage nitrogen levels. They found the result surprising as (nitrogen is usually regarded as a critical nutrient for phytophagous insects (Strong *et al.* 1984) admitting the result was likely to be spurious given number of correlations carried out (Valladares & Lawton 1991).

Tree size as a measure of apparency

The apparency of alder, as measured by tree height and crown projection, was shown to significantly and positively influence leaf miner species richness and abundance. In 2010, this effect was only significant in area 2 where tree height explained 18.3% of variation in leaf miner abundance and 27.4% of variation in miner species richness (Table 4.8). Area 2 was initially much more heavily damaged by moose (with many broken stems present) causing greater variation in the height of alder compared with that in the other areas. Between late 2010 and early 2011, areas 1 & 3 had sustained new moose damage affecting alder more than birch (birch being taller and out of moose reach). This might have led to leaf miner abundance and richness in all 3 areas in 2011 being significantly positively affected by tree size. In areas 2 & 3 alder height explained between 19.7% and 23.9% of variation in miner abundance and up to 24.4% of variation in leaf miner richness (Table 4.9). This suggests a connection between moose browsing, alder height and degree of leaf miner infestation; browsing and stem breakage by moose increases variation in tree height and the smaller (moose damaged) trees become less apparent to leaf miners. It was also observed during monitoring that alder was affected by available moisture and the trees in drier parts of the sites were smaller, appearing more stressed than the taller trees growing well in the damper areas. This factor, clearly affecting tree height will also affect tree apparency from the leaf miner perspective in a similar manner to moose browsing. Of course, the effects of mammal browsing on trees and the resultant effects on insect herbivory may also be generated by the causal effects on available food quality (as well as the quantity) (Den Herder *et al.* 2009).

Leaf miners on birch were generally unaffected by tree height and crown projection, although in 2011 leaf miner species richness and abundance were marginally higher on larger trees, which again suggests that moose browsing during winter of 2010-11 might have increased variation in tree heights. However, as birch trees within the experimental plots at Satakunta are considerably taller than alder trees, they are overall more apparent to leaf miners. This provides a likely explanation as to why there are significantly positive interactions between tree size and leaf miner abundance and species richness on alder but not on birch. A recent study on oak saplings found that leaf miner abundance decreased with decreasing oak apparency (Castagneyrol *et al.* 2013). In their study they measured apparency as a function of neighbouring tree height whereas the present study only measured height of focal herbivory trees. Considering the height of neighbouring trees and assessing within plot interactions at this level, may explain better the behaviour of some insect herbivores.

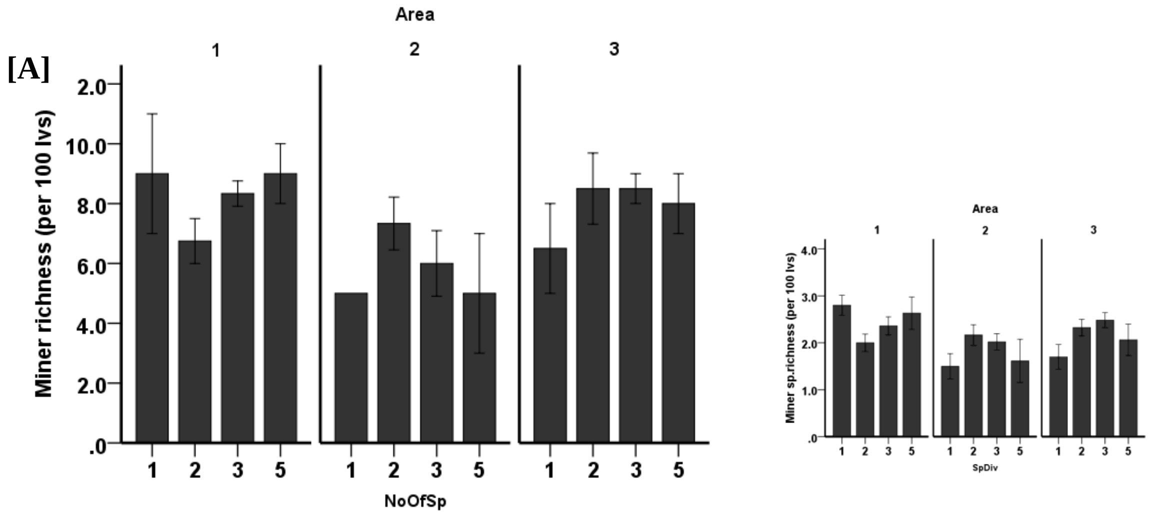
In addition to variation in leaf traits and tree size *per se*, plant architecture (structural complexity), a combination of plant size, growth form and variety of above ground structures (Araujo *et al.* 2006) has been commonly used to explain variation in insect herbivory among plant species (Lawton 1983; Alonso & Herrera 1996; Boege 2005). Although not actually measured in this study the architectural complexity of birch is greater than alder (it being generally taller with many more branches and leaves). This may explain the greater number of species of leaf miner encountered on birch, 30 vs. 17 on alder; agreeing for example with Araujo *et al.* (2006), who show species richness of galling insects is positively correlated with increasing architectural complexity. In all instances the effect of adding covariates to the models did not alter or change the effect of tree species diversity (Section 4.3.4). Therefore, the significant covariate effects discussed above appear to act independently of tree diversity.

4.5 Conclusions

Leaf miner community responses to tree species diversity differed between alder and birch; although associational resistance effects were not present in either species. In alder, tree species diversity effects were harder to elucidate and were more varied than in birch. In birch, associational susceptibility was the main discernible trend resulting in miners becoming more abundant and species rich with increasing tree species richness. The effects of tree species diversity were found to be the same during early and late season and between years. On both alder and birch it is shown that high leaf miner abundance was a result of higher leaf miner species richness.

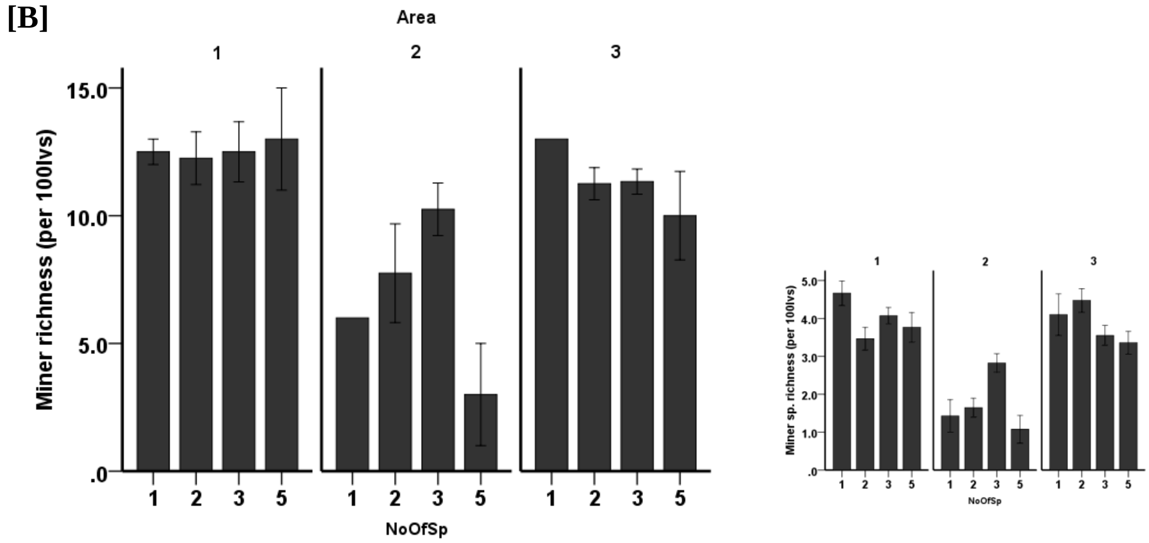
No discernible tree species composition effects were detectable and effects for both tree species were variable and inconsistent spatially and temporally. Analysing leaf traits (alder and birch) demonstrated that ovipositing females do not appear to select or choose their laying sites at the tree level. Alder leaf miners select taller trees with wider canopies probably because the alders varied in size more than birches and larger trees are simply more apparent. It was speculated that leaf miner responses to tree species diversity in alder are area dependent and linked with soil nutrients and available nitrogen in alder foliage, whilst in birch it is down to more favourable conditions in light, temperature and humidity. It is likely that these and other explanatory variables are missing from the models presented; these would be required in future studies to further answer questions about leaf miner host plant choice and interactions with tree species diversity and compositional effects.

Appendix 4.1 Plot level (per 1000 leaves) leaf miner species richness on alder over the tree species diversity gradient (early and late season combined) for **[A]** 2010 and **[B]** 2011. Error Bars are +/- 1 SE. *Small graph to the right shows patterns at the tree level for comparative purposes (this data is also shown in Figure 4.1).* Note: similar patterns in leaf miner species richness at the plot and the tree level. Univariate ANOVA results for plot level analysis also shown.



Univariate ANOVA output:

Sp Div: *$F_{3,38}$ = 0.765, P=0.875*
Area: *$F_{2,38}$ = 4.536,* ***P=0.020***
Sp Div x Area: *$F_{6,38}$ = 1.195, P=0.339*

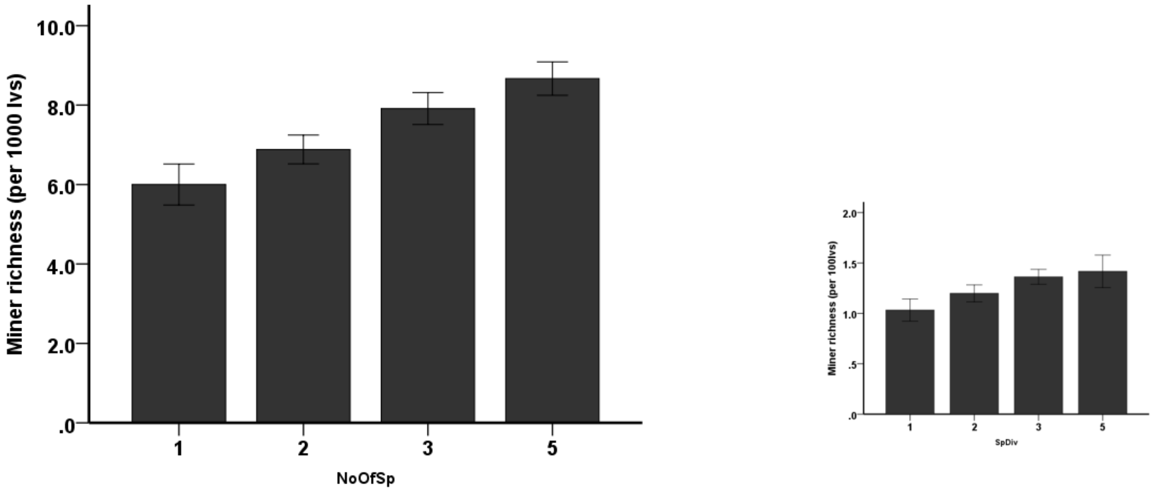


Univariate ANOVA output:

Sp Div: *$F_{3,38}$ = 1.945, P=0.146*
Area: *$F_{2,38}$ = 14.307,* ***P=0.000***
No of Sp x Area: *$F_{6,38}$ = 1.515, P=0.211*

Appendix 4.2 Plot level (per 1000 leaves) leaf miner species richness on birch over the tree species diversity gradient (early and late season combined) for **[A]** 2010 and **[B]** 2011. Error Bars are +/- 1 SE. *Small graph to the right shows patterns at the tree level for comparative purposes (this data is also shown in Figure 4.1).* Note: similar patterns in leaf miner species richness at the plot and the tree level. Univariate ANOVA results for plot level analysis also shown.

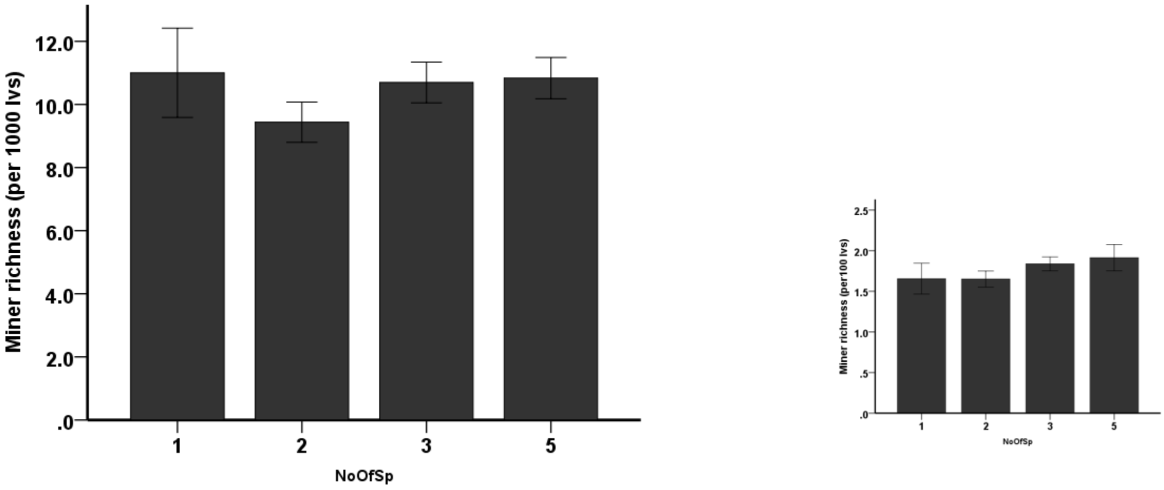
[A]



Univariate ANOVA output:

Sp Div: *$F_{3,51} = 4.531$*, ***$P = 0.008$***
Area: *$F_{3,51} = 4.432$*, ***$P = 0.018$***
Area x Sp Div: *$F_{3,51} = 0.470$, $P = 0.826$*

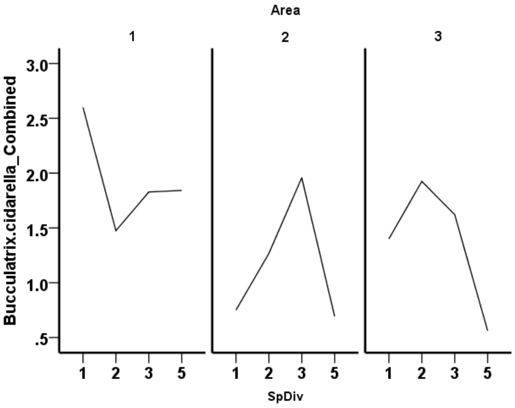
[B]

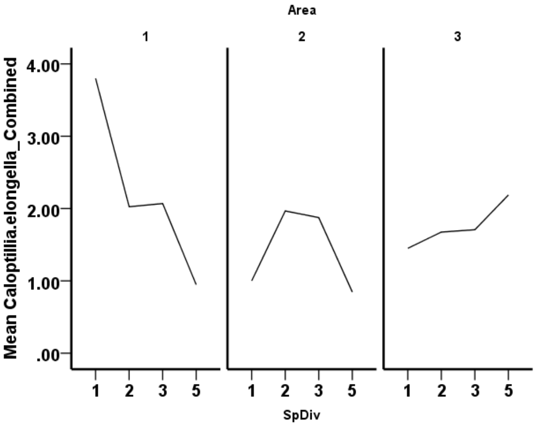


Univariate ANOVA output:

Sp Div: *$F_{3,50} = 0.854$, $P = 0.473$*
Area: *$F_{3,50} = 1.180$, $P = 0.318$*
Area x Sp Div: *$F_{3,50} = 0.833$, $P = 0.552$*

Appendix 4.3 Individual leaf mining species abundance (*Bucculatrix cidarella*, *Caloptilia elongella* and *Fenusa dohrnii)* combined (individually) from 2010 and 2011 on <u>alder</u> Graphs split over area due to significant ‘area x sp’ div interaction term (see table below)





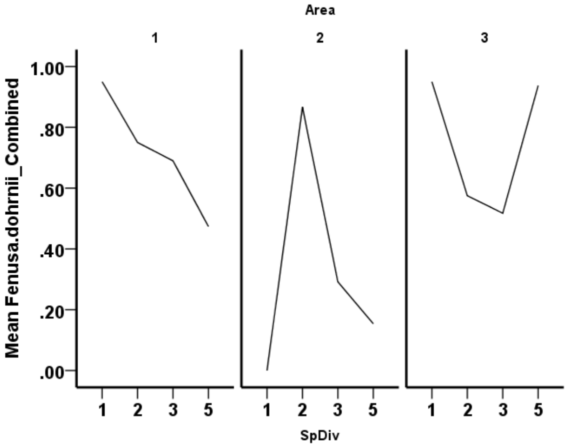


Table of univariate ANOVA showing effect of area, tree diversity and interaction term for *Bucculatrix cidarella*, *Caloptilia elongella* and *Fenusa dohrnii* combined from 2010 and 2011 on <u>alder</u>

FACTORS	**Numerator df**	***Bucculatrix cidarella***	***Caloptilia elongella***	***Fenusa dohrnii***
Area	2	**F** 4.707, **P**= 0.010	**F** 4.323, **P**= 0.014	**F** 4.101, **P**= 0.010
Sp Div	3	**F** 2.400, **P**= 0.068	**F** 1.648, **P**= 0.178	**F** 1.308, **P**= 0.272
Area x Sp Div	6	**F** 2.405, **P**= 0.027	**F** 4.328, **P**= 0.000	**F** 2.033, **P**= 0.061
	Denominator	*369*	*369*	*369*

Appendix 4.4 Individual leaf mining species (*Coleophora serratella*, *Incurvaria pectinea*, *Orchestes rusci*, and *Bucculatrix cidarella)* combined (individually) from 2010 and 2011 on <u>birch.</u> Graphs split over area for *Bucculatrix cidarella* due to significant ‘area x sp div’ interaction term (see table below).

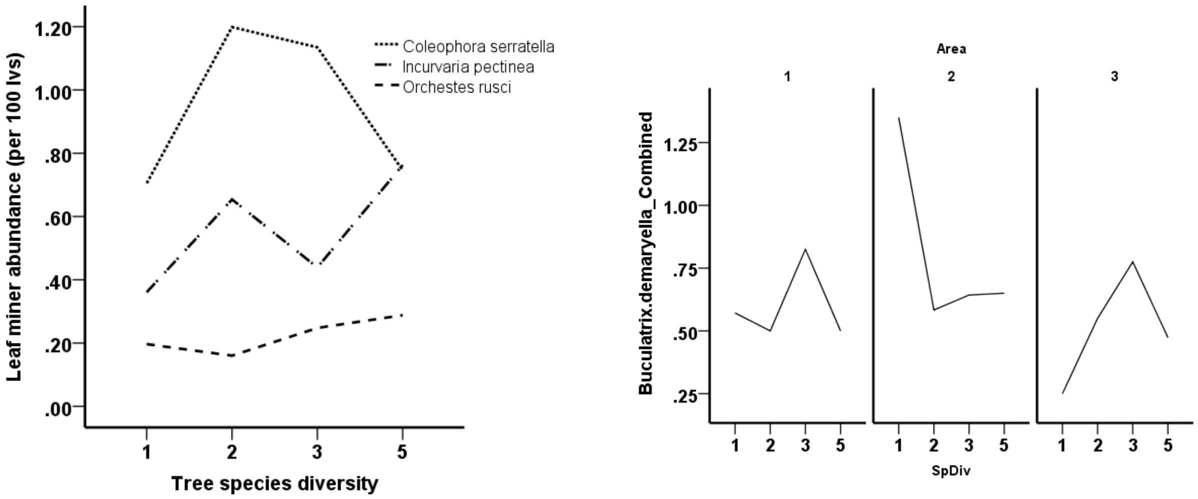


Table of univariate ANOVA showing effect of area, tree diversity and interaction term for *Coleophora serratella, Incurvaria pectinea, Orchestes rusci, and Bucculatrix cidarella* combined from 2010 and 2011 on <u>birch</u>

FACTORS	**Num df**	***Coleophora serratella***	***Incurvaria pectinea***	***Orchestes rusci***	***Bucculatrix cidarella***
Area	2	**F** 1.673, **P**= 0.189	**F** 0.241, **P**= 0.786	**F** 11.30, **P**= 0.000	**F** 2.876, **P**= 0.057
Sp Div	3	**F** 2.134, **P**= 0.095	**F** 1.779, **P**= 0.150	**F** 1.600, **P**= 0.188	**F** 1.736, **P**= 0.159
Area x Sp Div	6	**F** 0.752, **P**= 0.608	**F** 0.689, **P**= 0.659	**F** 0.983, **P**= 0.436	**F** 2.483, **P**= 0.022
Denominator		*505*	*505*	*505*	*505*

Chapter 5

The effect of birch genotypic diversity on the leaf mining community

5.1 Introduction

Chapter 5 investigates how genotypic diversity of silver birch influences the abundance and species richness of leaf miners in the boreal forests of S.W. Finland. The majority of biodiversity research has previously focussed on plant species richness (Reusch *et al.* 2005; Koricheva *et al.* 2006; Cook-Patton *et al.* 2011), though a number of recent studies have shown that plant intra-specific diversity can also influence ecological processes including herbivory (Crutsinger *et al.* 2006; Crutsinger *et al.* 2008; Cook-Patton *et al.* 2011; Tack & Roslin 2011) and that these effects can be pronounced and wide ranging (Bailey *et al.* 2009). Indeed, some studies have suggested that genotypic diversity, (traditionally assumed to have relatively small effects on biodiversity) may actually influence ecosystem processes in a quantitatively similar way with similar ecological consequences to that of species diversity (Crutsinger *et al.* 2006; Hughes *et al.* 2008; Cook-Patton *et al.* 2011).

There are two main approaches utilised in exploring within-species diversity effects. The first approach, exploits naturally hybridising plants; as hybrid genetic classes differ from each other in known ways, they can be used to explore the role of genetic variation on community structure of insect herbivores (Hochwender & Fritz 2004). Studies using this methodology on eucalyptus (Dungey *et al.* 2000) and willow (Hochwender & Fritz 2004) showed that the community structure of insect herbivores was governed by genetic dissimilarities among parent species and hybrid genetic classes; the increased genetic diversity of F1 hybrids in both studies correlated with increased diversity of arthropods. The second method, employed in this and other studies (Crutsinger *et al.* 2006; Johnson *et al.* 2006; Crutsinger *et al.* 2008; Parker *et al.* 2010; Cook-Patton *et al.* 2011; Tack & Roslin 2011) involves the experimental manipulation of the number of genotypes of a particular plant species within plots in order to provide a gradient from low to high genotypic diversity.

Between existing studies there is large variation in the magnitude of plant genetic diversity effects on the herbivore community. Tack & Roslin (2011) point out that system-specific variation in responses to genetic diversity may reflect differences in the genetic distance between the genotypes involved. This may explain why the studies utilising hybrids between species, which are known to differ substantially, often show significant genetic diversity mediated responses in herbivore communities even at a larger spatial scale (Dungey *et al.* 2000; Hochwender & Fritz 2004). In non-hybridising species, differences between the genotypes may not be large enough to have a strong effect on the insect community. For instance, Tack & Roslin (2011) found significant differences in herbivory among individual oak genotypes (tree level) but at a larger spatial scale (plot level) found no effect of genotypic diversity on insect herbivory. However, both Johnson *et al.* (2006) and Cook Patton *et al.* (2011) separately found a significant increase in arthropod diversity in more genetically diverse patches of the biennial herb *Oenothera biennis.* The genotypes in the Satakunta silver birch genetic diversity experiment (used in this study), were specifically selected for their differential resistance to pathogens and mammalian herbivores (Jia *et al.* 1997; Poteri *et al.* 2001; Vihera-Aarnio & Velling 2001); and it is therefore likely that they differ enough genetically to cause effects on insect herbivory as well. Therefore, in the present study (using the birch clone experiment) it is possible to investigate genotype effects i.e. do the different birch clones differ in the abundance of damage and species richness of leaf miners; a requirement for genotypic diversity effects (Hughes *et al.* 2008). Furthermore, it is possible to investigate genotypic diversity effects on leaf miners, specifically to test the effects of increasing clone diversity; which are tested at the tree, clone, and plot level. As alluded to above, the effects of genetic diversity are expected to be stronger at the tree and the clone level compared to plot level.

Plant genotypic diversity effects on arthropods could be additive or non additive (Johnson *et al*. 2006; Tack & Roslin 2011). An additive effect occurs, for example when different plant genotypes support different species or abundances of insect herbivores, which are added up in a mixture of these plant genotypes resulting in higher herbivore abundance and species richness. For example, if genotype ‘A’ and genotype ‘B’ have two different species of leaf miner each, then the two clone mixture ‘AB’ (assuming equal abundance of clones) will contain four species of leaf miner if the mechanism were additive in nature.

Changes in herbivore species richness with plant genotypic diversity that cannot be explained in this manner may be described as non additive or interactive. Non-additive effects can be a direct or an indirect consequence of genetic diversity. It is thought that direct non-additive effects might occur when the genetic diversity of a host patch affects herbivore movement, colonisation and emigration. For example, Crustsinger *et al.* (2006) demonstrated that increased primary productivity in genetically diverse patches explained a non-additive increase in arthropod species richness in mixed patches. Negative non-additive effects may also exist, Tack & Roslin (2011), and this conforms to the concept of associational resistance (Tahvanainen & Root 1972; Hamback *et al.* 2000). Additive and non-additive effects can jointly contribute to the relationship between plant genetic diversity and herbivore community structure (Tack & Roslin 2011). Only a handful of studies have tested for additive and non-additive mechanisms. Johnson *et al.* (2006) and Reusch *et al.* (2005) could not identify any consistent additive or non-additive effects of plant genotypic diversity on herbivore species richness using the biennial herb *Oenothera biennis* and the sea grass *Zostera marina*, respectively. On the other hand, Crutsinger *et al.* (2006) using the herbaceous perennial *Solidago altissima* found evidence of both additive and non-additive genotypic effects on insect herbivores. Another study using *Oenothera biennis* found that species richness increased by ca 20% in monoculture plots relative to genetically diverse plots in the early growing season and the reverse was found in the late growing season (Johnson & Agrawal 2005); they speculated that non-additive effects may have changed direction during the season, *de facto* countering the positive additive effects during the spring.

According to Hughes *et al.* (2008) and Cook-Patton *et al.* (2011), the effects of genetic diversity can impact ecosystem processes in a quantitatively similar way with similar ecological consequences to that of species diversity. In Chapter 4, I have shown that abundance and species richness of leaf miners on silver birch increase with tree species diversity. This Chapter investigates whether leaf miners show similar response to within-species tree diversity. Previous studies have shown that intraspecific diversity of oak is a poor predictor of specialist insect herbivores (Tack *et al.* 2010; Tack & Roslin 2011; Castagneyrol *et al.* 2012), with the latter two studies also focussing on leaf miners. However, these studies used very young trees (2-4 yrs old) and it is possible that the systems were not mature enough to exhibit genetic diversity responses to leaf miners. On the other hand, specialist leaf beetles have been found to be more abundant in monoclonal plots of willow compared to plots of clonal mixtures (Peacock & Herrick 2000; Peacock *et al.* 2001). It is difficult to make predictions on the effects of genetic diversity on insect herbivory on silver birch, given the contrasting results of the relatively few studies conducted. Moreover, leaf miner responses to intraspecific diversity of silver birch have not been investigated before.

In this study, birch genotypic diversity effects on leaf miners are compared over the growing season because of the noticeable time effects (differences between early and late season) on insect herbivores in the species diversity experiment (see Chapter 3 and 4). It is possible that the mechanisms responsible for observed effects on insect herbivores between the tree species and genetic diversity experiments may well differ. For example, Cook-Patton *et al.* (2011), in the only study to date investigating the effects of plant genotypic diversity vs. species diversity, found that arthropod species richness responded less strongly to plant genotypic diversity than to species diversity. In the same experiment they found that quantitatively similar increases in primary production occurred through both genotypic and species diversity treatments and attributed this effect to niche complementarily (decreased intensity of competition). They concluded that resource specialisation influenced the arthropod response to plant species diversity whereas in the plant genotypic diversity experiment, abundance driven accumulation of species (more individual’s hypothesis) was the mechanism. These patterns suggest insects will specialise on host plant species over host plant genotypes.

Once fully understood in various systems, the mechanisms of plant genotypic diversity will be important when informing conservation related decisions and strengthen our knowledge of the ecological mechanisms that influence ecosystem structure and function. For instance, if genotypic diversity effects are discovered to be mostly additive, then conservation management might focus on retaining or adding those plant species with associated diverse insect communities. Whereas, if genotypic diversity effects are mostly non-additive, conservation decisions may centre around conserving a high diversity of host plant genotypes to help increase insect diversity (Johnson *et al.* 2006).

Unlike a lot of the previous experiments on tree genotypic diversity effects on insects where potted tree saplings have been used (White & Whitham 2000; Blande *et al.* 2009; Plath *et al.* 2011; Tack & Roslin 2011), this study has been conducted on trees planted in the ground. Potted plants, by way of comparison, are likely to experience greater light, nutrient or water stress than naturally growing plants, obscuring effects of ployculture

(Andow 1991) and reducing the ability of a plant to produce herbivore-induced volatile signal compounds (Holopainen 2008). At the time of sampling at Satakunta, birch trees were 11 to 12 years old (well past establishment age) and interacted fully with the environment. Consequently, they are ideally suited for investigating genotypic diversity effects of silver birch on the leaf mining community. The Satakunta genotypic diversity experiment is also very suitable for comparing the effects of genotypic diversity to that of tree species diversity (Chapter 4) because both experiments are so similar in design age and location.

To summarise, the objectives of this study were: (1) To compare the effects of intraspecific genotypic diversity of silver birch on leaf miner abundance and species richness. (2) To test the consistency of genotypic diversity effects over time (early season vs. late season). (3) To compare the magnitude of the genotypic diversity at the tree, clone and plot level. (4) To examine whether the effects of birch genotypic diversity on leaf miners are additive or non-additive in nature.

5.2 Methods

5.2.1 Genotypic forest diversity experiment

The genotypic diversity experiment was planted on a two hectare clear cut forest area in Satakunta in 2000. Eight genotypes of silver birch (*Betula pendula*) were used (36, K1659, K5834, K2674, V5952, V5818, O154, JR1/4) and planted in single-genotype stands and 2-, 4- and 8-genotype mixtures. Experimental design is described in detail in Chapter 2.

5.2.2 Study species

Silver birch sustains a diverse insect community (Southwood 1961; Shaw 1984) and is an important native and forestry tree species in Finland. The focus of this Chapter is on the leaf mining guild which is also diverse on birch with all four of the major leaf mining orders (Lepidoptera, Coleoptera, Hymenoptera and Diptera) represented (Hespenheide 1991). Leaf miners are endophagpous insects and as pointed out by Tack and Roslin (2011) and can respond strongly to host-plant genotype due to their intimate association with host plant tissues, which is one of the reasons why this guild was selected for study.

Leaf miners also make a fairly easy and simple system for ecological research because most miner species can be identified by the mine they produce (Plate 5.1); leaf mines persist on leaves for some time (Hespenheide 1991). Species of leaf miners in this study were identified using images and keys from the 'Leaf Miners of Europe' website (*http://www.bladmineerders.nl/index.htm*), and the 'British Leaf Miners' website (*http://www.leafmines.co.uk/index.htm*).

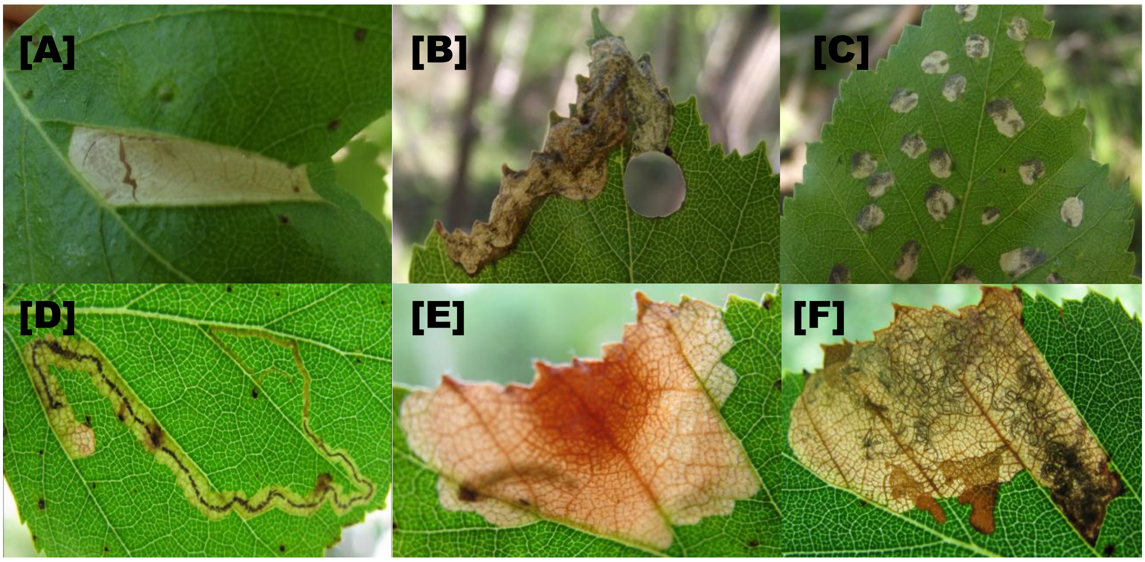


Plate 5.1. Six leaf miner species at the silver birch clone experiment, Satakunta. [**A**] *Coleophora serratella* **[B]** *Orchestes rusci* **[C]** *Incurvaria pectinea* **[D]** *Stigmella lapponica* **[E]** *Heterarthrus nemoratus* **[F]** *Eriocrania* sp. [**Images: A, B, C**, Simon Morath. **Images: D, E, F**, compliments of Julia Koricheva].

5.2.3 Monitoring

Leaf mines on silver birch were identified and recorded *in situ* at two time points during 2011 as it has already been shown that the silver birch herbivore community changes within a growing season (Vehvilainen *et al.* 2006, Chapters 3-4). Chapter 2 describes the general sampling protocol. The causal organisms were identified to species level, in some instances identification was only possible to genus level such as with some *Eriocrania* species (as the larvae needs to be present for species level identification and this was often not the case with this genus). During the late season, *Orchestes rusci* and *Coleophora serratella* mines were identified but not analysed with time as a repeated measure because the mines were old and probably remnants from the early season; there were no signs of fresh second generation mines of these or any other species that occurred in the early season during the late season monitoring. The relative frequencies of mines were calculated on a per (miner) species basis. From these data, species richness per tree, per clone or per plot and species specific and leaf miner abundance per 100 leaves (percentage) was calculated.

5.2.4 Analysis

Analyses of birch genotypic diversity effects on leaf miners were conducted at tree, clone and plot levels using linear mixed effect models in SPSS version 19. This 3-tiered approach is conceptually similar to that employed by Tack & Roslin (2011) and Parker *et al.* (2010) and was used to test for additivity of genotypic diversity effects and to find out at what level birch genotypic diversity affects leaf miners.

For tree level analysis, leaf miner abundance and species richness were averaged per tree and the linear mixed models included clone id, clone diversity and time as fixed factors and all possible two way interactions and the one three way interaction in order to address all the biologically relevant interactions. The same approach was utilised for the analysis at the clone level, except that total number of miner species recorded per clone per plot (based on five trees per clone per plot sampled) was used in the analysis instead of species number per individual tree.

For plot level analysis, the data for leaf miner species richness and abundance were calculated by randomly selecting five trees per plot. This was done to keep the sampling effort per plot constant because more trees were examined in clonal mixture plots compared to monoclonal plots, hence there would be a greater probability to encounter more miner species per plot in mixtures than in monoclonal plots. Again, linear mixed model analysis was utilised and the factors in the model were clone diversity, time and their interaction.

Differences in leaf miner abundance and species richness between birch genotypes were also examined as without an effect at this level, the effect of genotypic diversity and stand composition will likely be negligible (Hughes *et al.* 2008). Although genotype (or ‘clone id’ as referred to herein) is commonly treated as a random factor (Crutsinger *et al.* 2006; Johnson *et al.* 2006; Tack & Roslin 2011), it was treated as a fixed factor in the present study due to the fact that birch genotypes were deliberately selected to represent a broad range of vulnerabilities to herbivory. The normality of residuals was examined and square root, arcsine or log transformed where required in order to improve the normality of data.

5.3 Results

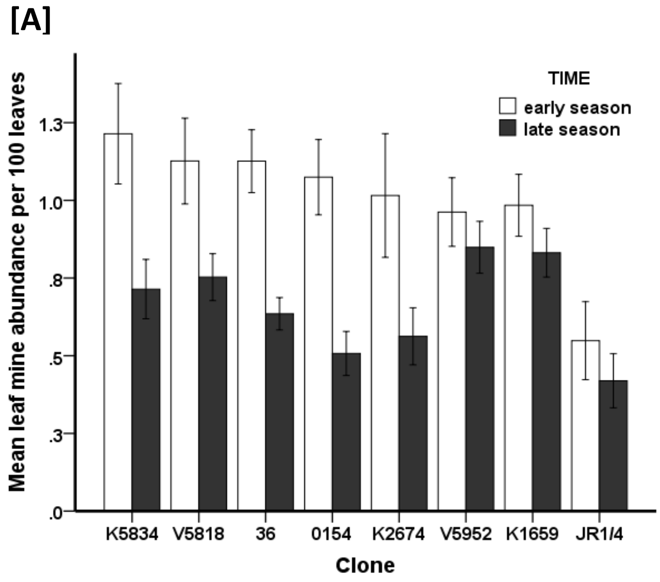
In total, 2009 mines caused by 28 different species of leaf miners were recorded. In the early season, 12 species of leaf miner were identified making up 1201 mines. In the late season, 21 species were indentified making up 808 mines (Table 5.1).

Table 5.1 Birch leaf miner species and abundances in the birch genotypic diversity experiment during 2011, during **T1** (early season) and **T2** (late season).

Leaf miner species	Author	Order / Family	T1	T2
Unknown sp 1	n/a	n/a	**7**	
Unknown sp 2	n/a	n/a	**1**	
Stigmella lapponica	Wocke (1862)	Lepidoptera, Nepticulidae	**14**	
Fenusa pumila	Leach (1817)	Hymenoptera, Tenthredinidae	**5**	
Eriocrania sp	n/a	Lepidoptera, Eriocraniidae	**216**	
Anoplus plantaris	Naezén (1794)	Coleoptera, Curculionidae	**26**	
Orchestes testeceus	Müller (1776)	Coleoptera, Curculionidae	**2**	
Orchestes rusci	Herbst (1795)	Coleophora, Curculionidae	**197**	**88**
Coleophora serratella	Linnaeus (1761)	Lepidoptera, Coleophoridae	**404**	**105**
Incurvaria pectinea	Haworth (1828)	Lepidoptera, Incurvariidae	**271**	**9**
Phyllonorycter ulmifoliella	Hübner (1817)	Lepidoptera, Gracillariidae	**16**	**39**
Bucculatrix demaryella	Duponchel (1840)	Lepidoptera, Bucculatricidae	**36**	**255**
Ectoedemia minimella	Zetterstedt (1839)	Lepidoptera, Nepticulidae	**5**	**26**
Parornix betulae	Stainton (1854)	Lepidoptera, Gracillariidae	**1**	**4**
Stigmella continuella	Stainton (1856)	Lepidoptera, Nepticulidae		**5**
Stigmella luteella	Stainton (1857)	Lepidoptera, Nepticulidae		**18**
Stigmella lapponica	Wocke (1862)	Lepidoptera, Nepticulidae		**63**
Stigmella sakhalinella	Puplesis (1984)	Lepidoptera, Nepticulidae		**5**
Stigmella betulicola	Stainton (1856)	Lepidoptera, Nepticulidae		**3**
Ramphus pulicarius	Herbst (1795)	Coleoptera, Curculionidae		**36**
Phyllonorycter cavella	Zeller (1846)	Lepidoptera, Gracillariidae		**1**
Phylloporia bistrigella	Haworth (1828)	Lepidoptera, Heliozelidae		**11**
Scolioneura betuleti	Klug (1816)	Hymenoptera, Tenthredinidae		**55**
Heterarthrus nemoratus	Fallén (1808)	Hymenoptera, Tenthredinidae		**15**
Profenusa thomsoni	Konow (1886)	Hymenoptera, Tenthredinidae		**28**
Eriocrania sparrmannella	Bosc (1791)	Lepidoptera, Eriocraniidae		**29**
Anoplus roboris	Suffrian (1840)	Coleoptera, Curculionidae		**11**
Agromyza alnibetulae	Hendel (1931)	Diptera, Agromyzidae		**2**
TOTAL			**1201**	**808**

5.3.1 Genotype effects

Clone identity effects on leaf miner abundance were significant and independent of sampling time (Table 5.2) with higher overall miner abundance in early season (Fig. 5.1A). In the early season, lowest miner abundance was on clone JR1/4 and highest miner abundance was on clone K5834. In the late season, lowest miner abundance was also on JR1/4 and highest miner abundance was on clone V5952.



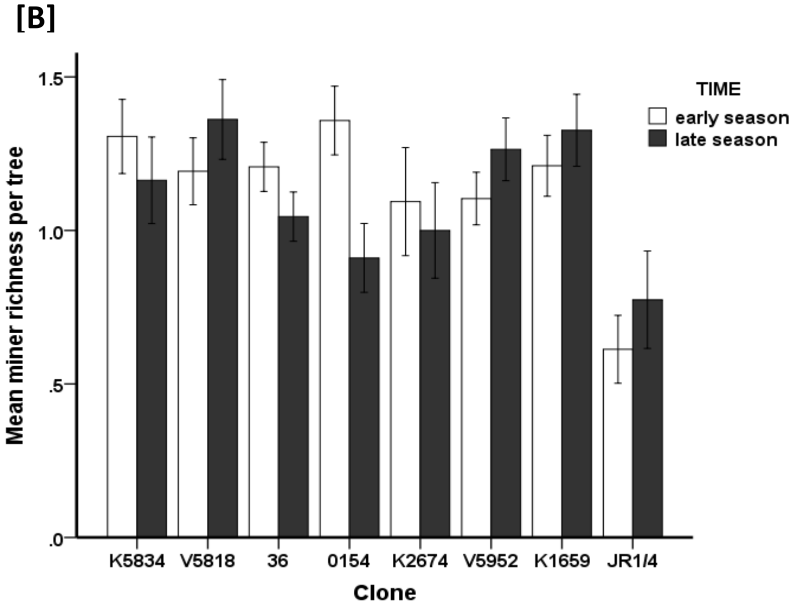


Figure 5.1 Variation between silver birch clones in **[A]** leaf miner abundance [**B]** leaf miner species richness in 2011 per 100 leaves. Error Bars are +/- 1 SE.

In contrast, clone effects on miner species richness differed between early and late season, as indicated by a significant time × clone ID interaction (Table 5.2). Clones K5834, 36, O154, K2674 had greater species richness of leaf miners in the early season, whilst clones V5818, V5912, K1659, JR1/4 have greater species richness in the late season (Fig. 5.1B). Comparing Figures 5.1A with 5.1B, with just a few exceptions, the clones with higher abundance tend to also have greater species richness of leaf miners, the same is true for lower miner abundance and lower species richness.

Clone identity effects were independent of clone diversity effects (Table 5.2) indicating that leaf miner preference for specific clones did not differ depending on the presence of other clones in a plot.

5.3.2 Genotypic diversity effects

Birch genotypic diversity had significant effects on leaf miner species richness at both tree and clone level (Table 5.2); the effect appears to be non-linear with lowest number of species of miners found in 8 clone mixtures (Fig. 5.2A). At tree level, genotypic diversity effect on leaf miner species richness differed between early and late summer (Table 5.2).

Effects of birch genotypic diversity on leaf miner abundance depended on sampling time (as indicated by significant clone diversity × time interactions) at both tree and clone level (Table 5.2). Leaf miner abundance decreased with clone diversity in the early season, but remained constant across the clone diversity gradient in the late season (Fig. 5.2B). In addition, the 3 way interaction time × clone ID × clone diversity was also significant at both clone and tree level, indicating that leaf miner abundance responses to clone diversity depended not only on sampling time, but also on birch clone identity. (Table 5.2)

Table 5.2 Linear mixed model results for clone diversity effects at tree and clone level on leaf miner species richness and leaf miner abundance. All factors were treated as fixed. Significance was determined using an *F*- statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05).

Factors	**Numerator df**	**Miner sp. richness**		**Miner abundance**	
		Clone level	*Tree level*	*Clone level*	*Tree level*
Time	1	2.157	2.265	39.625***	41.072***
Clone ID	7	7.090***	1.792**	3.130**	2.416*
Clone Div	3	2.922*	3.710**	1.703	1.665
Time × Clone ID	7	2.751*	2.147*	1.346	1.463
Time × Clone Div	3	2.037	3.885**	3.386*	3.636*
Clone ID × Clone Div	19	1.021	0.935	1.142	1.385
Time × Clone ID × Clone Div	19	0.701	1.137	1.595*	1.850*
Denominator df		*188.148*	*1069.022*	*187.779*	*929.829*

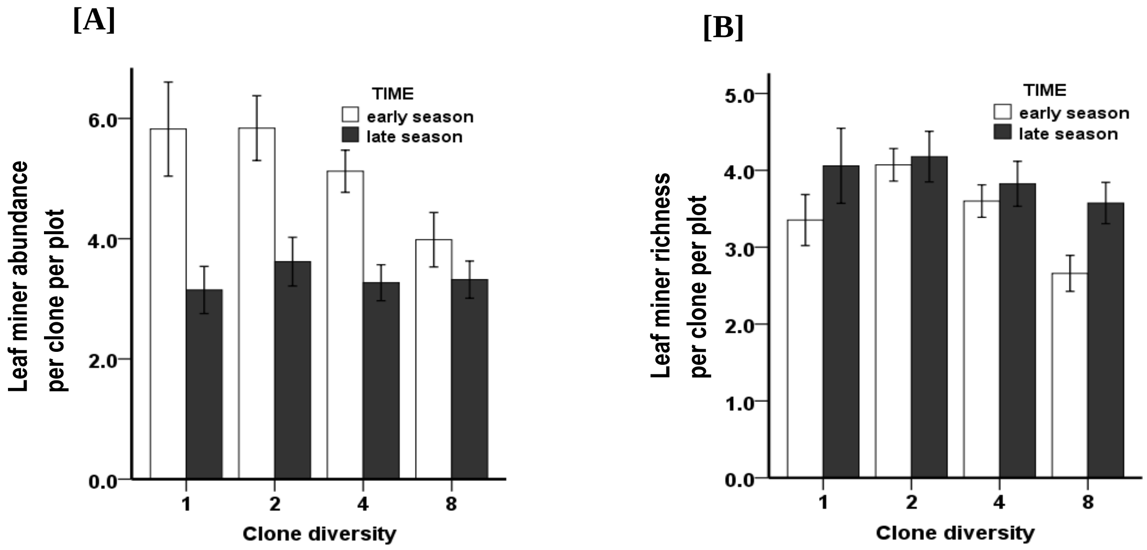


Figure 5.2 Clone diversity effects on **[A]** leaf miner abundance and **[B]** leaf miner species richness. Data are expressed as richness and abundance per clone (same as per 500 leaves). Error Bars are +/- 1 SE.

5.3.3 Plot level effects of genotypic diversity

The structure of the leaf mining community at the plot level did not significantly change as a result of increasing genotypic diversity as there was no significant influence of clone diversity at the plot level on leaf miner abundance ($F_{3,\ 63.954} = 0.218$, P= 0.883) and richness ($F_{3,\ 77.571} = 1.076$, P= 0.365).

Time × clone diversity interaction was significant neither for abundance ($F_{3,\ 63.954}$ = 0.368, P= 0.776) nor for species richness ($F_{3,\ 77.571}$ = 0.509, P= 0.677) of leaf miners at the plot level (Table 5.3). Leaf miner abundance was significantly higher in the early season compared to the late season ($F_{1,\ 63.954}$ = 85.799, P= <0.001, Fig. 5.3A), whereas there were no significant temporal changes in the leaf miner species richness ($F_{1,\ 77.571}$ = 3.231, P= 0.083).

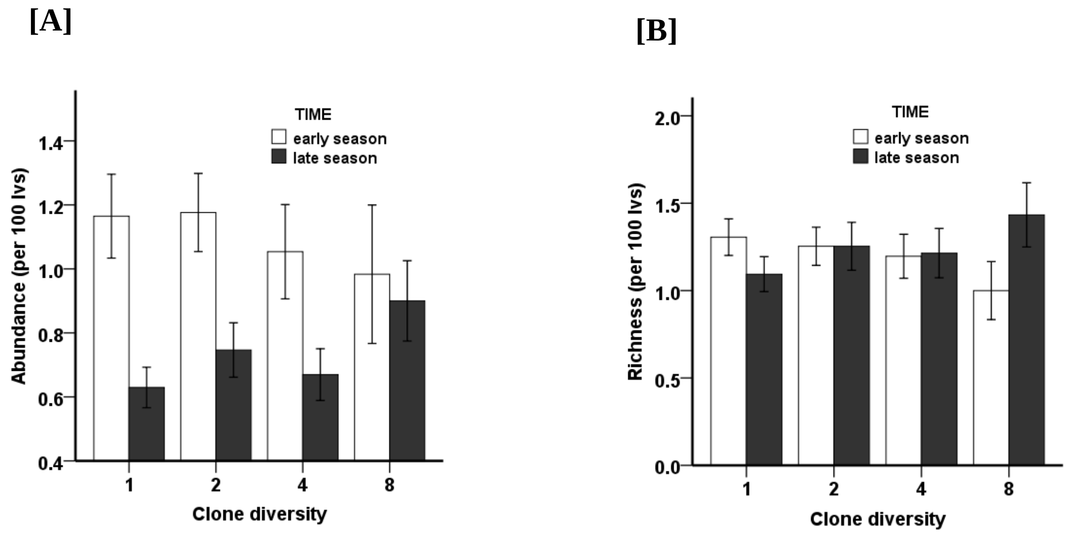


Figure 5.3 Clone diversity effects on mean abundance **[A]** and species richness **[B]** of leaf miners at the plot level. Error Bars are +/- 1 SE.

Table 5.3 Linear mixed model results showing effects of genotypic diversity at plot level on leaf miner species richness and abundance. All factors were treated as fixed. Significance was determined using an *F*- statistic with degrees of freedom assessed using Satterwaithe's method. Significance is given as *** (*P*<0.0001), ** (*P*<0.001), * (*P*<0.05).

Factors	Numerator df	Miner sp richness	Miner abundance
Time	1	3.231	85.799***
Clone Diversity	3	1.076	0.218
Time × Clone Diversity	3	0.509	0.368
	Denominator df	*77.571*	*63.954*

5.3.4 Effects of genotypic diversity on abundance of individual miner species

The leaf miner species that were sufficiently abundant to analyse individually in the early season were *Orchestes rusci* (197 mines), *Incurvaria pectinea* (271 mines), *Coleophora* *serratella*, (404 mines) and *Eriocrania* sp. (216 mines). During the late summer *Bucculatrix demaryella* (255 mines) and *Stigmella* species combined (94 mines) were the only two miner species in sufficient abundance for analysis (Table 5.1).

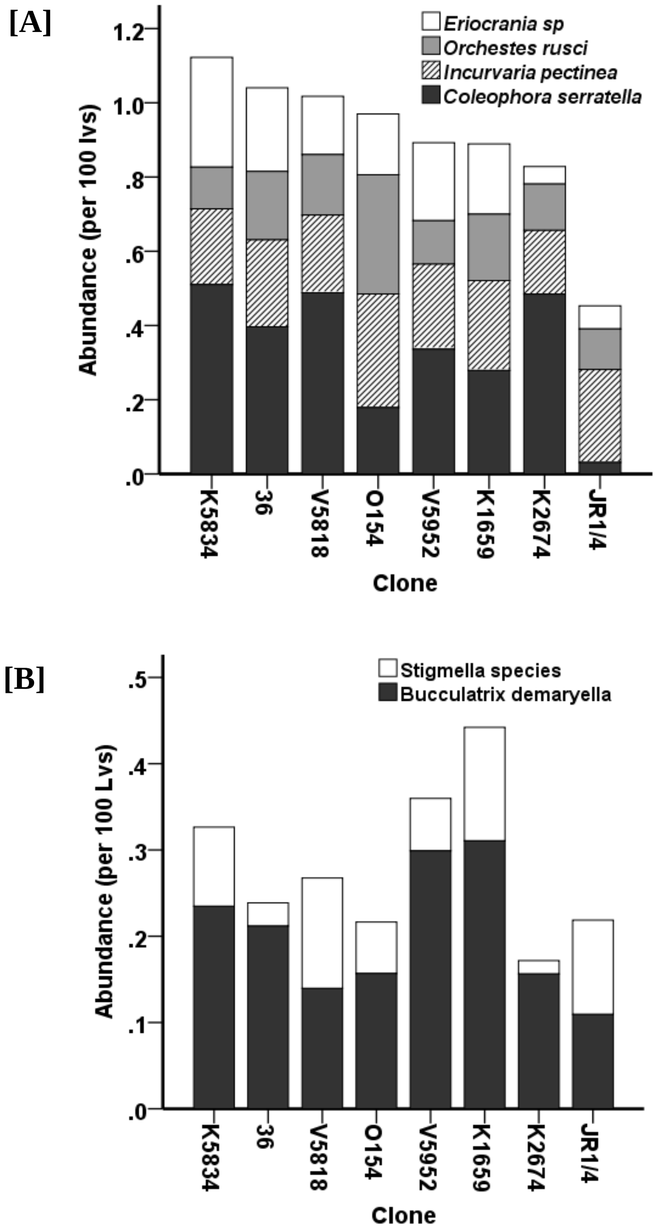


Figure 5.4 Abundance of individual species of leaf miners in early **[A]** and late **[B]** season on 8 different silver birch genotypes.

The effect of clone identity was significant for the early season *Coleophora serratella* miners ($F_{7, 578}$ = 2.191, P = 0.034), and *Eriocrania* sp. ($F_{7, 578}$ = 2.134, P= 0.039) and for the late season leaf miner *Bucculatrix demaryella* ($F_{7, 578}$ = 2.454, P= 0.017). *Incurvaria pectinea* (P= 0.823), *Orchestes rusci* (P= 0.084) and *Coleophora serratella* (P= 0.466) showed no significant genotype preference (Fig. 5.4). Different leaf miner species preferred different clones. For example, early season abundance of *C. serratella* was higher on clone K2674 compared to most other clones, but this same clone had the lowest abundance of Eriocrania species. It appears that no two miner species prefer the same clone (Fig. 5.4).

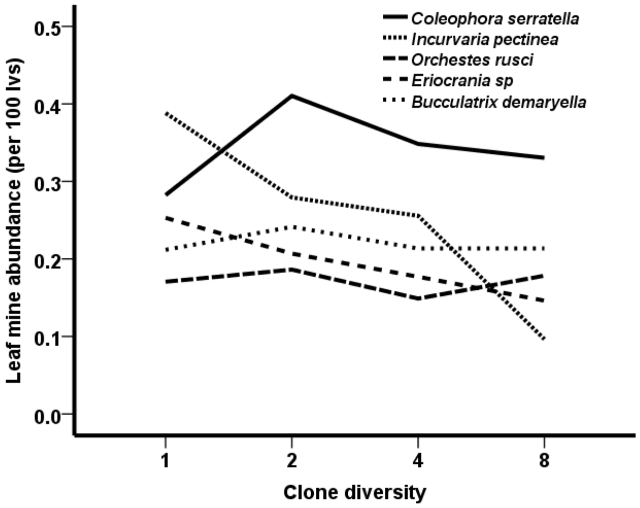


Figure 5.5 Genotypic diversity effects on abundance of individual leaf miner species. (All miner species shown are early season except *Bucculatrix demaryella*).

Eriocrania sp. and *Incurvaria pectinea* showed a significant decline in abundance with increasing genotypic diversity ($F_{3,\ 578}$ = 2.454, P= 0.017 and $F_{3,\ 578}$ = 5.369, P= 0.001 respectively, Fig. 5.5). No significant effects of clone diversity on abundance of other miner species were observed (P≥ 0.101, Fig. 5.5).

5.4 Discussion

5.4.1 Effects of birch genotype identity on leaf miner abundance and species richness

The first important finding of this study was that both leaf miner abundance and species richness varied significantly among birch genotypes. Responses at the level of individual genotype are necessary for genotypic diversity effects to occur at clone and plot level (Hughes *et al.* 2008; Tack & Roslin 2011). Strong effect of birch genotype on leaf miners is not surprising as the birch clones used in the Satakunta experiment are known to differ significantly in their resistance to moose (Jia *et al.* 1997), leaf rust (Poteri *et al.* 2001), and voles, hares, stem lesions and cankers (Vihera-Aarnio & Velling 2001). Tack & Roslin (2011) also showed significant effects of plant genotype on leaf miners on oak. In addition, clones which supported higher leaf miner abundance also tended to have higher leaf miner species richness, suggesting that miner abundance patterns are driven by changes in miner species richness (*cf*. Chapter 4).

At the individual species level, abundance of 3 out of 6 species of leaf miners was significantly affected by identity of birch genotype and different miner species tended to prefer different clones (Fig. 5.4).

5.4.2 Genotypic diversity effects

Overall, both species richness and abundance of leaf miners at tree and clone level tended to be lower in most genotypically diverse plots. In contrast, at the plot level genotypic diversity did not significantly affect the leaf mining community, which confirms the finding of other studies (Tack *et al.* 2010; Tack & Roslin 2011; Castagneyrol *et al.* 2012), that the effect of genotypic diversity varies at different scales and rarely has a strong effect at patch/plot level. The above results suggest that effects of birch genetic diversity on leaf miners are largely non-additive because for additive effects to occur, species richness of leaf miners would be expected to increase with increase in the number of clones in a mixture (Tack & Roslin 2011). In addition, the above results suggest that ovipositing females of leaf miners do not distinguish between the patches composed of one or several birch genotypes. Instead, oviposition decisions appear to be made at the tree and clone level.

At the tree and clone level, the species richness of leaf miners had a non-linear response to increasing clonal diversity, but in both early and late season lowest richness occurred in 8 clone mixture. Leaf miner abundance in early season was also lower in genetically diverse mixtures. Therefore, individual birch trees and genotypes experienced associational resistance (Tahvanainen & Root 1972; Hamback *et al.* 2000; Hughes *et al.* 2008) to leaf miners in mixed stands, possibly due to the fact that it is more difficult for leaf miners to find trees of their preferred genotypes in more diverse mixtures containing fewer trees of each clone. In the late season, however, genetic diversity effects on leaf miner species richness and abundance were much weaker. One potential explanation for this is that in late summer each clone has fully developed foliage and therefore more apparent to leaf miners; whereas in the spring when leaves are just opening, tree apparency may be lower and finding suitable clones is more difficult.

Interestingly, although *I. pectinea* showed no preference for particular birch genotypes, it displayed the strongest response to genetic diversity of all the species of miners examined (Fig. 5.5). The pattern of this response was a clear linear decrease in abundance with increasing genotypic diversity, demonstrating that birch clones are equally susceptible to *I. pectinea* leaf miners, with all clones experiencing strong AR effects when planted in mixture with other clones.

Two common types of non-additive ecological mechanisms are niche partitioning and facilitation/inhibition (Hughes *et al.* 2008), which are often collectively referred to as niche complementarity (Cardinale *et al.* 2007). These mechanisms are often used to explain productivity and resource use of trees in mixed stands. For instance, if birch genotypes differ in resource use in complementary ways, there will be less competition between trees in mixtures of different genotypes and genotypic diversity of the stand will positively influence processes such as total resource utilisation (Tilman 1999). This could indirectly affect leaf miners, for example, by altering chemical or physical leaf traits which are known to affect leaf miners (Sinclair & Hughes 2010), this should be the subject of further investigation. Phenotypic expression of the genotypes may also change as a result of increasing clone diversity. If genotypes are found to express different phenotypes in clonal mixtures than monoclonal stands, the net result would be considered an emergent property of genetic diversity as it will not be predictable from measurements in isolation (Hughes *et al.* 2008). Parker *et al.* (2010) found positive effects of genetic diversity on primrose that included reduced seed loss to specialist insects, increased resistance to voles, as well as increased plant survival and reproductive output; in this instance effects were enhanced by added trophic level complexity (deer and voles). The ‘variance in edibility’ hypothesis (Leibold 1989) was discussed by way of explanation and may also be relevant in the present study; this hypothesis simply argues that more diverse communities are more resistant as they contain less preferred taxa and greater plant phenological diversity.

Another possible mechanism explaining negative non-additive effects of birch genotypic diversity on leaf miners could be increased predation and parasitism of leaf miners in plots of increasing genetic diversity – in accordance with the predictions of the natural enemies hypothesis. Studies testing whether increasing birch intra-specific diversity supports greater numbers of insect predators and parasites could not be found and is worthy of further research.

Other studies (Tack *et al.* 2010; Tack & Roslin 2011) show environmental variation is responsible for as much or more of the variation in the focal insect community and outweighs the importance of genetic diversity. It seems that some studies showing genetic diversity effects (Dungey *et al.* 2000; Wimp *et al.* 2005; Barbour *et al.* 2009) including the present study, have eliminated much of the variation associated with spatial location by selecting genotypes collected from a large geographical area (maximizing genotypic variation) and planting in a single common garden, thereby minimising environmental variation and emphasising genetic diversity effects.

The findings at plot level (which is more relevant to the level that forestry related decisions are made) indicate that increasing genetic diversity of silver birch does not significantly reduce insect herbivory. This study is restricted to leaf miners and as already indicated in Chapter 3, responses of insects to tree diversity can be both positive and negative depending on herbivore guild. Moreover, Castagneyrol *et al.* (2012) have already shown that on oak, genotypic diversity does not affect leaf miners at the plot level, although genotypic diversity can positively affect ectophagous insect herbivores (chewers, rollers and skeletonisers). Similarly, Cook-Patton *et al.* (2011) have shown that ectophagous herbivores increase with genotypic diversity on herbaceous plants. In addition, the consequences of tree genetic diversity on total insect herbivore damage remains largely unknown (Castagneyrol *et al.* 2012).

5.5 Conclusions

Using the leaf mining guild it has been shown that the effects of genetic diversity in silver birch are strong at the tree and genotype level, but peter out at the plot level. The study demonstrates non-additive effects of genetic diversity (similar to associational resistance) because leaf miner species richness was at lowest abundance at maximum clone diversity.

Importantly, on silver birch the effects of interspecific (Chapter 4) and intraspecific diversity (Chapter 5) had opposite effects on leaf miners. Silver birch experienced AS to miners when planted in more tree species diverse plots and AR when planted in more genetically diverse plots. To my knowledge, this is the first time that the effects of species diversity and genetic diversity have been compared in forest systems. The mechanisms behind these results certainly warrant further investigation.

Similarly to the findings of Chapter 4, species abundance of leaf miners appears to be related to leaf miner species richness. It is also important to establish whether phenotypic expression of the eight genotypes throughout genotypic diversity differs and to relate these differences to changes in herbivory. The study of other insect herbivore guilds at species level would also be useful to increase our understanding of the benefits of increasing genetic diversity relative to insect herbivore damage. Moreover, at present the effects of tree genetic diversity on total insect herbivore damage are relatively unknown.

Chapter 6

Effects of tree functional diversity on insect herbivory

6.1 Introduction

Chapters 3-5 of this thesis are concerned with effects of tree species richness on insect herbivory, which has been the main focus of biodiversity–ecosystem functioning research for the last few decades (Mouillot *et al.* 2011). When plant diversity is quantified as a number of plant species or number of genotypes per species (including when calculating diversity indices such as Shannon-Weiner or Simpson) it is assumed that each species or genotype is equally different. Plant species, however, differ from each other in terms of specific traits and the effect of plant diversity is likely to depend on the extent of trait dissimilarity between the species in a particular ecosystem or community (Tilman 2001; Petchey & Gaston 2002; Ricotta 2005; Leps *et al.* 2006). The functional diversity (FD) concept captures the latter aspect of diversity and is a measure of the value and range of species and organismal traits that influence ecosystem functioning (Tilman 2001).

Although by no means a new concept, functional diversity research is currently gaining pace as species' functional characteristics have been shown to strongly influence ecosystem properties (Hooper *et al.* 2005; Liao & Wang 2010). Much of this work is conducted with ecosystem conservation and restoration practices in mind by identifying functional traits in communities required for tolerating environmental disturbances and perturbations (Liao & Wang 2010). Linking certain life-history characteristics of insect herbivores, such as their feeding guild, with behavioural responses to plant volatiles could also reveal some wide-ranging applicability of plant volatiles in pest-management strategies (Szendrei & Rodriguez-Saona 2010). The FD approach has also been used to investigate more specific ecosystem processes. For instance, in grassland ecosystems functional diversity rather than species diversity explained the majority of ecosystem processes, such as plant productivity, plant nitrogen content and light penetration (Tilman *et al.* 1997), litter decomposition (Scherer-Lorenzen 2008), and insect herbivory (Scherber *et al.* 2006a). Species richness of arthropods has been found to be affected by plant functional group composition in grasslands (Koricheva *et al.* 2000; Symstad *et al.* 2000). Plant functional diversity effects on insect herbivory in forest ecosystems have not been previously studied. It has been suggested that mechanisms behind stronger FD effects compared to species richness effects relate to the fact that functional attributes of plant species affect ecological processes more than taxonomic identity (Diaz & Cabido 2001; Hooper *et al.* 2002). Categorical classifications such as number of species or Simpson index have several limitations; for example, information about variation of functional trait diversity within groups is lost (Ricotta 2005; Petchey & Gaston 2006).

The present study investigates possible mechanisms that might explain insect herbivory levels using a community FD approach (the measurement of plot level FD), which a thorough search of the literature has revealed is very novel within forest ecosystems (Scherer-Lorenzen *et al.* 2007). This research has been conducted in the Bechstedt (BIOTREE) experiment in Thuringia, Germany, which was originally designed to investigate FD effects on forest ecosystem functioning (Scherer-Lorenzen *et al.* 2007). Two sets of FD indices were used, each one using a different set of functional traits.

1) FD index calculated by Scherer Lorenzen *et al.* (2007) [referred to as Scherer-Lorenzen's index hereafter] which uses nine traits primarily indicative of tree growth, morphology, resource use and nutrient cycling.

2) FD index calculated using monoterpene and isoprene emissions by tree species (referred to as volatile/ chemical FD index hereafter).

The selection of traits to use when devising FD indices is important (Ricotta 2005), as the traits have to be relevant for the response variable studied. The FD index established by Scherer-Lorenzen was used as the basis of the design for the Bechstedt experiment and intended to account for the importance of functional traits in explaining diversity effects on ecosystem functioning (Scherer-Lorenzen *et al.* 2007). I test whether it is possible to predict insect herbivory with this FD index. Some of the 9 traits included in this index (listed in section 6.2) may not be directly relevant for insect herbivory. I therefore designed a second index based on plant volatile emission diversity.

I expected that FD index based on volatile emissions would predict insect herbivory better than the Scherer-Lorenzen FD index since many insect herbivores are known to respond directly to the presence/absence and relative concentrations of individual volatile compounds during host finding (Huber & Bohlmann 2004; Reddy & Guerrero 2004; Zhang & Schlyter 2004). Terpenoid emissions from plants, particularly the monoterpenes and sesquiterpenes are used by specialist insect herbivores to identify their host plants (Huber & Bohlmann 2004; Zhang & Schlyter 2004). *Ips pini* for example has been shown to alter its post-landing behaviour in response to total monoterpene content, and in particular to the concentrations of the monoterpenes β-pinene and limonene (Huber & Bohlmann 2004). Receptors for commonly occurring terpenoids such as the ocimenes are found in several moth species (Anderson *et al.* 1995). On the other hand, volatile terpenoid emissions may also act as deterrents for generalist herbivores and volatiles of non-host species might deter specialist herbivores (Jactel *et al.* 2011).

In addition to monoterpenes, I included into the volatile FD index emissions of isoprene, which is the most abundant volatile compound emitted by vegetation (Loivamaki *et al.* 2008). Until recently isoprene was assumed to act primarily as plant protection against abiotic stresses, e.g. excessive temperature and ozone (Laothawornkitkul *et al.* 2008a; Unsicker *et al.* 2009). Recent studies (Loivamaki *et al.* 2008; Laothawornkitkul *et al.* 2008b) have, however, shown that isoprene influences insect herbivore feeding decisions. Therefore isoprene was included as a trait in the FD index as it is present in the atmosphere of forest/tree communities, contributes to the diversity of volatiles in this environment and may influence insect herbivores.

The ‘semiochemical diversity hypothesis’ (SDH) (Zhang & Schlyter 2003) predicts that the greater the diversity of tree species growing in an area, the more complex the composition of non-host volatiles (NHV), resulting in a reduced searching efficiency of specialist herbivores (Schiebe *et al.* 2011). This is also referred to in the literature as olfactory masking and is considered an important mechanism for associational resistance. Therefore, I predicted that insect herbivory, particularly by specialised herbivores using olfactorial cues for host plant choice, will be lower in tree stands with higher diversity of emitted volatiles. Furthermore, I predicted that tree species which are low emitters will experience associational resistance in plots of greater volatile diversity as it will be harder for herbivores associated with these tree species to detect them in mixtures with high emitters.

To summarise, Chapter 5 investigates to what extent tree species FD can explain variation in insect herbivory. Specifically, it addresses the following questions: (1) Can insect herbivory levels at the Bechstedt experiment be predicted by the Scherer-Lorenzen FD index? (2) Does diversity of volatile emissions by trees explain insect herbivory levels better than that of the Scherer-Lorenzen FD index? (3) Does tree species FD have similar effects on insect herbivores belonging to different feeding guilds? (4) Do insects associated with low volatile emitting tree species show stronger responses to chemical diversity of stands compared with those associated with high-emitting tree species?

6.2 Methods

Bechstedt (BIOTREE) experiment

There are twenty four plots at the Bechstedt site and each of these plots contains four different tree species from a total pool of sixteen species (see Chapter 2 for detailed experimental description). Each plot therefore has been assigned a different FD value based on trait dissimilarity. Keeping tree species richness in each plot constant (4 species) prevents confounding the effects of FD with those of species richness (Scherer-Lorenzen *et al.* 2007), as co-linearity exists between species richness and functional group richness (Schmid *et al*. 2002). The design of the Bechstedt experiment was based on a FD index (Scherer-Lorenzen's index) using traits indicative of tree growth, morphology, resource use and nutrient cycling. The 9 traits used are listed below; the scales utilised when assigning attributes to the tree species are detailed in Scherer-Lorenzen *et al*. (2007).

1) leaf type
2) light requirements as adults
3) height growth vigour
4) mean annual increment
5) rooting vigour
6) crown architecture
7) root architecture
8) leaf N concentration
9) C/N concentration of the litter

The method used to calculate FD was the Petchey and Gaston method (Petchey & Gaston 2002). The steps involved are: (1) to obtain a trait matrix for all species, (2) to convert the trait matrix into a distance matrix, (3) to cluster the distance matrix in order to produce a dendrogram, and (4) to calculate the total branch length of the dendrogram

(Petchey & Gaston 2002). The calculations of the Petchey-Gaston FD index are incorporated into the F-Diversity software (Di Rienzo *et al.* 2008), in line with the procedure used in Scherer- Lorenzen *et al.* (2007).

Volatile emissions FD index

The traits selected for establishing the volatile FD index were various monoterpenes and isoprene (Table 6.1). Data on monoterpene and isoprene emissions by the tree species used in the Bechstedt experiment were collected from the literature (Table 6.1) and the monoterpenes used in this study were the ones represented most commonly in the literature. While sesquiterpenes also contribute to olfactory host finding (Huber & Bohlmann 2004; Zhang & Schlyter 2004), the data in the literature on the levels emitted from the tree species at Bechstedt are extremely limited. Consequently sesquiterpenes were excluded as a trait from the volatile FD index. F-diversity software (Di Rienzo *et al.* 2008) was used to calculate FD values using the volatile traits. Petchey and Gaston (2002) method was used to calculate volatile FD index as this method was used to calculate Scherer-Lorenzen’s index.

Table 6.1 Volatile emissions by tree species used in the Bechstedt experiment ($\mu g\ g^{-1}\ h^{-1}$). Data on emissions of isoprene and individual monoterpenes by 16 tree species were obtained from literature (Hov *et al.* 1983; Isidorov *et al.* 1985; Petersson 1988; Arey *et al.* 1991; Hewitt & Street 1992; Winer *et al.* 1992; Koenig *et al.* 1995; Harley *et al.* 1996; Kempf *et al.* 1996; Schuh *et al.* 1997; Steinbrecher 1997; Steinbrecher *et al.* 1997; Hakola *et al.* 1998; Janson *et al.* 1999; Rinne *et al.* 1999; Geron *et al.* 2000; Hakola *et al.* 2000; Lindfors *et al.* 2000; Rinne *et al.* 2000; Zimmer *et al.* 2000; Rinne *et al.* 2000b; Janson & de Serves 2001; Kellomaki *et al.* 2001; Hakola *et al.* 2006; Rinne *et al.* 2009). Data from the online resource: Biogenic Volatile Organic Compounds (BVOC) Data Base was also utilised <http://bai.acd.ucar.edu/Data/index.shtml>.

Tree species	Isoprene	Limonene	a-Pinene	b-Pinene	Sabinene	Camphene	D3-Carene	Myrcene	Terpinolene	b-Phellandrene	1_8-Cineole	a-Thujene	Ocimenes	other terpenes
Acer campestre	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Acer platanoides	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Acer pseudoplatanus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Betula pendula	0.025	0.059	0.210	0.249	0.715	0.025	0.166	0.001	0.032	0.039	0.000	0.000	0.980	0.001
Carpinus betulus	0.000	0.005	0.002	0.003	0.003	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000
Fagus sylvatica	0.005	0.012	0.011	0.058	0.040	0.000	0.000	0.000	0.002	0.025	0.000	0.003	0.000	0.028
Fraxinus excelsior	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Larix decidua	0.050	0.002	8.200	0.001	0.000	0.004	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000
Pinus sylvestris	0.025	0.190	0.240	0.249	0.740	0.020	1.135	0.875	0.032	0.039	0.124	0.002	0.980	0.001
Populus tremula	51.000	0.330	0.560	0.162	0.079	0.059	0.856	0.000	0.000	0.000	0.001	0.000	0.368	0.030
Prunus avium	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Quercus petraea	19.265	0.105	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.011
Sorbus aucuparia	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000
Sorbus torminalis	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tilia cordata	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ulmus glabra	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Insect herbivore damage at Bechstedt

Insect herbivory monitoring was conducted at Bechstedt on 5-18 July 2009 using the sampling protocol detailed in Chapter 2. For all tree species at Bechstedt, percent leaf area damage by skeletonising and chewing insects was calculated. For all other damage types and also for insects present, the measure was percentage of leaves damaged or percentage of leaves bearing specific insects.

Each damage type recorded for each tree species was averaged to provide a mean damage level per tree species per plot. Pearson's correlation coefficients between the various herbivory categories and the FD indices were then calculated using SPSS version 19. For example *Acer platanoides* at Bechstedt occurred in 9 of the 24 plots. Each of these plots has a unique FD value (dependent on the other tree species present in each plot), therefore the mean herbivory level on *Acer platanoides* in each plot (for each damage type separately) was correlated with the plot-specific FD values. Individual correlation coefficients were then combined across tree species or types of herbivores by using meta-analysis.

Meta-analysis

Meta-analysis was conducted using Meta-Win 2.0 software (Rosenberg *et al.* 2000). Pearson's correlation coefficients between insect herbivory and FD indices were z-transformed and weighted by their sample size (Rosenberg *et al.* 2000). The transformed coefficients were combined across studies using the mixed-effects model. Bias-corrected 95% bootstrap confidence intervals generated from 4999 iterations (Adams *et al.* 1997) were used to define the significance of the relationship between insect herbivory and FD indices. A relationship was considered significant if the confidence interval did not include 0. Where appropriate at the end of the analysis, the mean z-values were back transformed to the Pearson correlation coefficients and coefficient of determination (r^2) was calculated (Sokal & Rohlf 1995), which indicates the proportion of the variation in herbivory determined by the variation in plot FD. The sign and magnitude of the correlations between herbivory and FD was compared for different types of herbivory and different tree species using a chi-square test statistic, Q_b (Gurevitch & Hedges 1993). Q_b statistics were used to test for variation in the mean effect size between herbivore feeding guilds and between different tree species. *Fagus sylvatica* was excluded from all analysis as it was only present in 3 of the 24 plots (Chapter 2), however its volatile contribution was included into FD index calculation for the plots where it was present. The two coniferous species *Larix decidua* and *Pinus sylvestris,* were excluded from the analysis as they did not receive a great deal of insect herbivory, but their volatile contributions were included in the FD index calculation for the plots where they were present.

To test if insect herbivores associated with low emitting tree species show stronger responses to volatile diversity as compared to herbivores associated with high emitting tree species, Spearman rank correlations were calculated using SPSS version 19. Firstly tree species were ranked in terms of total emissions based on the information in Table 6.1. Tree species emission rank (1= highest, 13= lowest) was then correlated with the correlation between volatile FD and total herbivory for each tree species. The same procedure was again used to test if individual insect herbivore guilds associated with low emitting tree species show stronger responses to volatile diversity (compared to higher emitters) by again correlating tree species emission rank but this time with correlation between volatile FD and each insect herbivore guild for each tree species. Lack of significant correlation between tree species emission rank and correlation between herbivory and volatile FD index indicated that the strength and direction of the relationship between herbivory and volatile FD is independent of whether the host tree species is a low or a high emitter. When a significant correlation between tree species emission rank and FD-herbivory correlation was found, the data were plotted to examine whether, as predicted, low emitters experience associational resistance in plots of greater volatile diversity.

6.3 Results

6.3.1 Can insect herbivory levels at the Bechstedt experiment be predicted utilising Scherer-Lorenzen’s FD index?

When all herbivore types and all tree species were combined in the analysis, Scherer-Lorenzen’s FD index explained overall only 0.48% of variation in herbivory (mean z-transformed correlation = 0.0693, 95% CI -0.0307 to 0.1708, n=138). There were however, marginally significant differences in the strength of FD-herbivory correlations between different herbivore types (Qb= 13.5168, df= 8, P= 0.066). Interestingly, the majority of herbivore types displayed positive rather than negative correlations with Scherer-Lorenzen's FD index, although most of the correlations were weak and non-significant (Table 6.2). The only two groups of herbivores which showed significant correlations with Scherer-Lorenzen's FD index were leaf tiers and sucking insects. Leaf tiers were more abundant in plots with a higher FD index, with FD index explaining nearly 30% of variation in their abundance (Table 6.2). In contrast, sucking insects (aphids and leafhoppers) were less abundant in plots with a high FD index, where FD index explained ca. 11% of variation in their abundance (Table 6.2).

Table 6.2 Relationship between herbivory by different feeding guilds and plot functional diversity. N= # of tree species with damage type. E+ = mean effect size (z-transformed). %var = percentage of variation in herbivory explained by corresponding FD index.

		FD- Scherer-Lorenzen			*FD- using volatile emissions*		
Feeding guild	**N**	**E+**	**95% CI**	**% var**	**E+**	**95% CI**	**% var**
Chewing	13	-0.0516	-0.2713– 0.1534	0.27	-0.0884	-0.4426 – 0.2151	0.78
Skeletonising	13	0.1521	-0.2193– 0.5717	2.31	**0.3239**	**0.0979 – 0.5537**	**10.49**
Leaf miners	13	0.1672	-0.0634– 0.3823	2.80	0.1687	-0.0618 – 0.4140	2.85
Rollers	13	0.1433	-0.0300– 0.3288	2.05	0.0869	-0.1957 – 0.4111	0.76
Leaf tiers	6	**0.5468**	**0.3159– 0.8464**	**29.90**	-0.0984	-0.3543 – 0.2884	0.97
Leaf galls	13	0.1479	-0.0689– 0.3240	2.19	-0.2507	-0.5940 – 0.0530	6.29
Bud galls	2	0.0157	-0.3989– 0.3473	0.02	**-0.4629**	**-0.7447 – -0.2469**	**21.43**
Suckers present	13	**-0.3307**	**-0.5954– -0.0809**	**10.94**	0.1013	-0.1903 – 0.3939	1.03
Chewers present	9	0.0548	-0.2515– 0.2932	0.30	0.2334	-0.1378 – 0.7111	5.45

6.3.2 Does diversity of volatile emissions by trees predict insect herbivory levels?

Overall, the volatile FD index explained 0.18% of variation in herbivory (mean z-transformed correlation = 0.0430, 95% CI -0.0410 to 0.1322, n=138).

When all the deciduous tree species were considered together, there was no significant difference between the damage categories: chewing, skeletonising, mining, rollers, tiers, leaf galls, bud galls, suckers present and chewers present (Qb= 11.5632, df=8, P= 0.195).

The only two herbivore groups that were significantly correlated with volatile FD were the bud gallers which were less abundant in semiochemically diverse stands and skeletonisers which were more abundant in stands of trees producing more diverse volatile mixtures (Table 6.2).

6.3.3 Do insect herbivores associated with low-emitting tree species show stronger responses to volatile diversity as compared to herbivores associated with high-emitting tree species?

The prediction that the tree species which are weaker emitters should have stronger correlations between volatile FD and total herbivory did not hold true as correlation between tree species emission rank and FD-herbivory correlation was not significant (r_s = 0.225, n=13, P= 0.230). Among individual herbivore feeding guilds, significant negative correlations between tree species emission rank and FD-herbivory correlations were found for chewing damage, leaf rollers and leaf galls (Table 6.3). The scatter plots (Fig. 6.1) indicated that these negative correlations were due to lower emitting tree species (high emission ranks) having fewer chewing rolling and galling damage when planted in plots of greater volatile diversity (negative FD-herbivory correlations). On the other hand, tree species which are high emitters (low emission ranks) tended to display positive FD-herbivory correlations for the above 3 feeding guilds, suggesting that chewing, rolling, and galling damage on these species was higher in plots of greater volatile diversity (Fig 6.1)

Table 6.3 Spearman rank correlation between tree species emission rank and herbivory - plot volatile FD relationship. Negative correlations indicate that the lower are tree species volatile emissions, the stronger are correlations between herbivory and volatile diversity.

Herbivory guild	**N**	**Correlation**	***P***	**%Variation**
Chewing	13	**-0.533**	**0.027**	**28.41**
Skeletonising	13	-0.203	0.253	4.12
Leaf miners	13	0.148	0.314	2.19
Rollers	13	**-0.412**	**0.048**	**16.97**
Leaf tiers	6	-0.086	0.436	0.74
Leaf galls	13	**-0.401**	**0.050**	**16.08**
Suckers	13	0.033	0.457	0.11
Chewers	9	-0.267	0.244	7.12

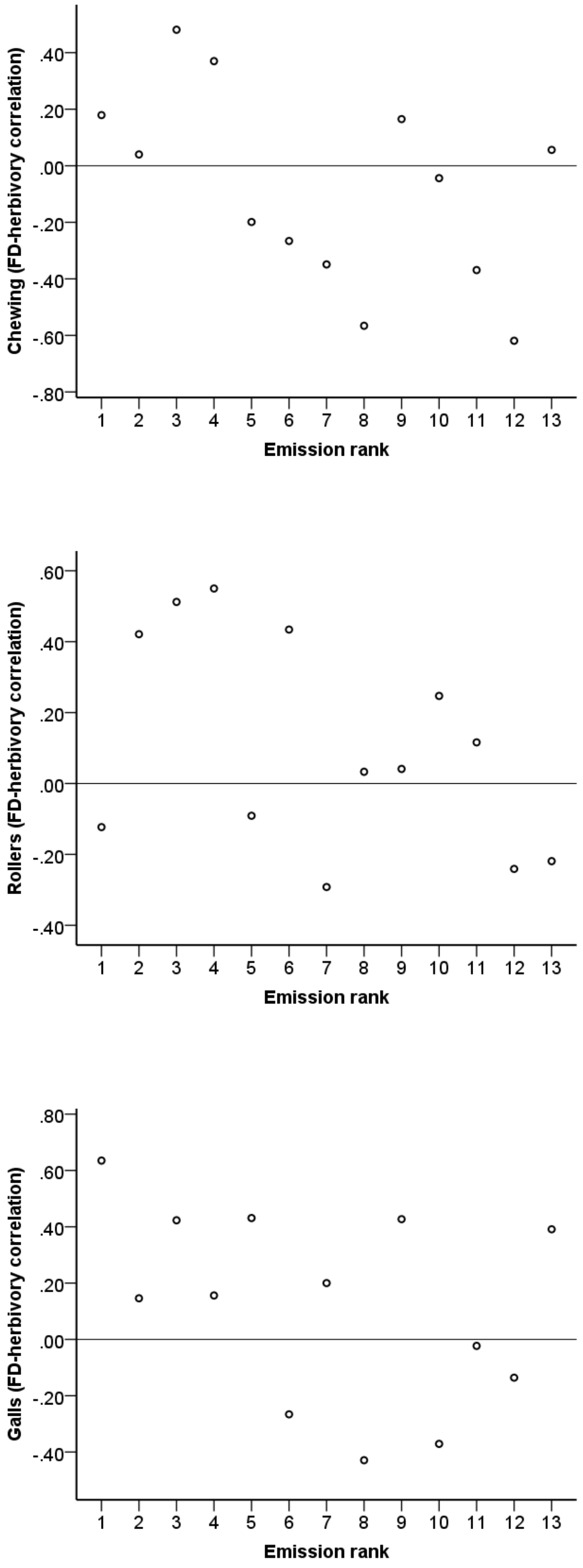


Figure 6.1 Scatter plots between tree species emission rank (1- highest emitter; 13- lowest emitter) and FD-herbivory correlation for chewing, rolling, and galling damage.

6.4 Discussion

6.4.1 Insect herbivory and the Scherer-Lorenzen FD index

As expected, the FD index established by Scherer-Lorenzen did not explain much of the variation in overall herbivory (0.48%), because different feeding guilds responded differently to functional diversity (Table 6.2). Similar lack of response of total herbivory to tree species richness due to variation in responses between different feeding guilds was observed in Chapter 3. However, abundance of 2 out of 9 insect herbivore guilds out of the 9 investigated was significantly associated with the Scherer-Lorenzen index. Leaf tiers increased in abundance with increasing FD whilst sucking insects (aphids, leafhoppers and whiteflies) were less common in higher FD plots. Many leaf tier species are known to be generalists. For example a common leaf tier species, *Byctiscus betulae* (Coleoptera: Attelabidae) can tie leaves of birch (*Betula* sp.), aspen (*Populus tremula*), and also willows (*Salix* sp.) (Nyman 2007). It is possible that more functionally diverse stands can support more species of leaf tiers with different requirements for leaf type, leaf nitrogen concentration etc. A greater species richness of leaf tiers in a stand may lead to higher abundances, as shown in Chapter 4, for leaf miners. Increase in leaf tier abundance in functionally diverse stands may also have a large impact on abundance and species richness of other arthropods (including other leaf tiers) which are known to re-occupy the abandoned shelters (Lill *et al.* 2007; Wang *et al.* 2012). For instance, Wang *et al*. (2012) have shown that the presence of leaf ties on oak increased arthropod species abundance 10–35 times, depending on the plant species.

In contrast to leaf tiers, the vast majority of sucking insects are specialists. For instance, 76% of all British aphids as well as most species of leafhoppers associated with British trees are strictly monophagous (Schoonhoven *et al.* 2005). Plant diversity generally impedes host finding and is a common explanation why specialists are more abundant in less diverse or resource rich patches (Root 1973). Furthermore, aphids and whiteflies are known to use foliage colour to discriminate between host and non-host species (Hamilton & Brown 2001; Archetti & Leather 2005; Archetti 2006) and variation in leaf type and leaf N is likely to create variation in foliage colour which might interfere with host location by these herbivores in plots with higher FD. The lower abundance of sucking insects in functionally diverse plots concurs with the findings in Chapter 3 where silver birch aphid was less abundant in more species rich plots.

Some of the 9 characteristics used to generate the Scherer-Lorenzen index, e.g. root architecture, and rooting vigour are unlikely to have direct effects on above ground insect herbivory. Certainly, further investigative work will be required to ascertain the mechanisms behind the Scherer-Lorenzen index i.e. what aspects of tree growth, morphology, resource use and nutrient cycling are important for insect herbivory. This also emphasises the importance of trait selection to specifically address scientific questions. For instance, it was not really possible to explore specific mechanisms relating to insect herbivore damage with the Scherer-Lorenzen FD index; rather it provides a general indication of what factors may be involved. A number of authors, (Rao 1982; Petchey & Gaston 2002; Ricotta 2005; Leps *et al.* 2006; Diaz *et al.* 2007) have stated that selecting functional traits of species to utilise and which to disregard, as well as deciding on suitable measurements of selected traits, is of paramount importance and needs to be substantiated with sound scientific and ecological reasoning. FD was noted to be inadequately explained and/or trait choice inadequately justified in over 50% of publications (Petchey & Gaston 2006), (see Leps *et al*. 2006 and Petchey & Gaston, 2006 for a good overview of trait selection). The number and type(s) of traits used and how they are measured will ultimately influence the FD value assigned to a community (Scherer-Lorenzen *et al.* 2007). Another consideration affecting the assigned FD value of a community is the mathematical method employed to calculate it, of which there are many (Leps *et al.* 2006; Petchey & Gaston 2006; Casanoves *et al.* 2010).

6.4.2 Herbivory and volatile diversity

Contrary to expectations, the newly calculated volatile FD index explained even less of the variation in overall herbivory (0.18%) than Scherer-Lorenzen's FD index, although % of variance in herbivory explained by the volatile index was higher than that by the Scherer-Lorenzen index for 6 out of 9 feeding guilds (Table 6.2). Only two feeding guilds showed significant associations with the chemical FD index (skeletonisers and bud gallers). Bud galls were less common in plots with the higher volatile diversity. Gallers are specialist herbivores and it is possible that increased volatile complexity prevents galling species from finding their hosts because they are unable to detect the host volatiles/cues or they are deterred or repelled by greater diversity of non-host volatiles, or both. Bud galls, however, only occurred on *Quercus petraea* (sessile oak) and *Carpinus betulus* (hornbeam) and the detected correlation must therefore be viewed with caution as n=2. In contrast to bud gallers, skeletonising damage increased with increasing plot volatile diversity, indicating that skelotinisers were not impeded by volatile diversity. Skeletonising damage is likely to have been caused by the early larval instars of lepidopterans, sawflies or beetles (J. Koricheva personal communication). Many of these insects are generalists (Atkinson 1992; Nyman 2007) and might be less dependent on specific plant volatile cues to find host plants than specialist insects. Increase in skeletonising damage with volatile diversity in the present Chapter concurs with the finding in Chapter 3, where birch demonstrated clear AS trends to skeletonising damage, experiencing greater damage in higher diversity plots.

6.4.3 Differences in effects of volatile diversity on herbivores associated with low and high emitters

It was predicted that lower-emitting tree species would experience associational resistance to insect herbivore damage when planted in plots of higher volatile diversity. The lower emitting tree species did not receive less total insect herbivore damage when planted in higher volatile FD plots compared to lower volatile FD plots, contrary to this prediction. It was revealed, however, that low emitting tree species do receive significantly less chewing, rolling and galling damage when planted in higher volatile FD plots compared to lower volatile FD plots (Table 6.3, Fig. 6.1). A possible explanation as to why low emitting tree species experienced AR effects to chewing, rolling and galling damage when planted in higher volatile diversity plots is via the mechanisms proposed in the semiochemical diversity hypothesis (Zhang & Schlyter 2003), whereby insect olfactory ability to locate host plants is impeded by greater volatile diversity. It is thought that this mechanism affects mainly specialist insects, although it is noted that many rolling and chewing species may also be generalist (Nyman 2007). Data collected for this research from the Bechstedt experiment were insufficient to be able to attribute insect damage to causal insect species. This is necessary to explore and increase our understanding of the mechanisms further. It is also noted that many gall-inducing species are specialists, yet some of these will not even be insect i.e. some galls are induced by small arthropods belonging to the subclass Acari in the class Arachnida, e.g. *Acalitus* sp. on birch.

Interestingly, tree species which are high emitters appeared to experience associational susceptibility to chewing, rolling and galling damage in plots of high volatile diversity (Fig. 6.1). This could be an artefact as most of the tree species used in the Bechstedt experiment are relatively low emitters (Table 6.1) and the presence of high emitter species in the plot was likely to result in high value for the plot FD index, leading to a positive correlation between tree emission rank and FD-herbivory relationship.

Several factors could explain why the FD index based on volatile diversity was a relatively poor predictor of herbivory in the present study. It was expected that the volatile index would be a better predictor of herbivory compared to the Scherer-Lorenzen index. However the Scherer-Lorenzen index did correlate with 2 guilds, as did the volatile FD index. Many insect herbivores are known to respond directly to volatile compound diversity during host finding (Huber & Bohlmann 2004; Reddy & Guerrero 2004; Zhang & Schlyter 2004); however significant correlations with Scherer-Lorenzen index suggests that volatile diversity alone does not predict insect herbivory. Indeed, it is known that structural complexity of trees (incorporated in Scherer-Lorenzen’s index) affects microclimatic conditions, in turn influencing emission and dispersion of plant volatiles (Vickers 2006; Randlkofer *et al.* 2010). It is possible that these more morphological traits need to be included in an FD index with volatile diversity to better predict insect herbivore damage.

In addition, values used herein, for emissions of monoterpenes and isoprene from trees were taken from the literature and may differ from the actual amounts emitted by trees in Bechstedt. For example, the level of emissions from a tree is influenced, among other factors, by maturity of the foliage and amount of foliage damage (Unsicker *et al.* 2009). For instance, the majority of constitutive volatile compounds released from healthy trees become inducible volatiles after foliage damage when these compounds are produced in larger quantities and altered ratios (Holopainen 2004). In these instances, the line between constitutive and inducible volatile compounds becomes somewhat blurred. The present study only included constitutive volatile emissions from undamaged vegetation as traits and could not consider altered compositions or ratios of these compounds which may very well affect insect herbivory. Abiotic factors such as temperature and light also influence emissions (Zhang & Schlyter 2004; Barbosa *et al.* 2009). It is therefore quite likely that the assigned plot volatile FD based on literature searching was very different to that encountered by insect herbivores in the plots, potentially explaining the weak correlations between the volatile FD index and herbivory.

The volatile FD index also did not include green leaf volatiles (GLVs), sesquiterpenes, or homoterpenes, all of which have been suggested or shown to influence insect herbivore host finding (Reddy & Guerrero 2004). In a meta-analysis of studies on insect herbivore behaviour to plant volatiles, aldehyde GLVs (particularly phenyl acetaldehyde) were shown to be the most effective attractants (Szendrei & Rodriguez-Saona 2010), their high volatility thought to make them more likely to be detected by insects than other volatiles. GLVs are produced both constitutively and upon wounding (induced) and detectable by a diverse group of insects including both specialists and generalists (Schoonhoven *et al.* 2005). If insect herbivore damage increases as a result of attraction to induced defence compounds then the FD index based on constitutive defence alone may not be as predictive as previously anticipated.

Furthermore, this study did not account for the volatile emissions from ground vegetation that can also interfere with the olfactory senses of insect herbivores. For example, in Finland, birch leaves have been shown to adsorb and re-release the specific arthropod-repelling C_{15} volatile compounds ledene, ledol and palustrol produced by a rhododendron species that grows in the ground vegetation layer (Himanen *et al.* 2010). Moreover, some terpenoids known to be detectable by moth species i.e. linalool, geraniol, and β-caryophyllene (Anderson *et al.* 1995; Rostelein *et al.* 2005) were not included in the FD index due to limited emission data in the literature.

Additionally, due to limitations and variation in olfactory receptor neurons (ORNs) between insect herbivore species, considerable variation exists in their sensitivities and detectability of plant volatile compounds (Bruyne & Baker 2008). This means that volatiles emitted by a given vegetation is probably not the same as that experienced by a given arthropod when passing through that vegetation. The technique of quantifying the volatile emissions in a community as in this study could be very informative, however, more studies are needed at insect species level to discover what compounds are important for host finding, their lower limits of detection and what specific compounds mask and interfere with host finding ability. It has been suggested that three elements of information are assessed by insects responding to olfactory information, odour identity, odour intensity, and a temporal variation, which is how these two vary in time (Hilderbrand & Shepherd 1997; Bruyne & Baker 2008); indeed, most phytophagous insect species home in on specific ratios among components (Visser 1986). A method to incorporate this level of information in an FD index if devised and utilised may help explain better the variation in herbivory damage observed. The terpenoid linalool for example, has been shown to inhibit some ORNs in some insect species (De Bruyne *et al.* 2001); it is therefore suggested that the positive or negative effect of each volatile compound be considered in a similar FD index in order to account for the affect of the compound on olfactory guidance.

6.5 Conclusions

This study shows that the FD index established by Scherer-Lorenzen, used as the basis of the design for the Bechstedt experiment and originally intended to account for the importance of functional traits in explaining diversity effects on ecosystem functioning, only correlated with 2 out of 9 insect herbivore guilds. This indicated that traits indicative of tree growth, morphology, resource use and nutrient cycling, when combined are not particularly good at predicting insect herbivory. Limited support was, however, found for the ‘semiochemical diversity hypothesis’ (Zhang & Schlyter 2003). Again, only two guilds correlated with the volatile FD index and only one (bud galls) negatively. Insects causing chewing, galling, and rolling damage were shown to respond negatively and were less abundant on lower emitting tree species when these species were planted in higher diversity plots; providing some support for the SDH. Assessing plot FD of volatiles and trying to predict herbivory levels does show promise and it is suggested that this technique be refined in order to account for the complexity of volatile compounds used by insects in host finding and insects variability in detecting them. Ultimately though, more information on specific insect species olfactory responses is required. Compared to pheromone systems (more extensively studied) there are many more compounds in plant produced mixtures, and their ratios of volatiles are far more variable.

Chapter 7

General discussion

In this thesis I researched the effects of forest diversity on insect herbivory using three long-term forest diversity experiments to explore associational resistance (AR) and associational susceptibility (AS) phenomena, when they occur and why. In this Chapter, I discuss key findings of my thesis in relation to my original aims (section 7.1) and the implications of these findings for forest management (section 7.2.), evaluate my experimental approach (section 7.3) and give suggestions for future work (section 7.4).

7.1 Thesis aims

The three main questions addressed in this thesis were: [1] To what extent do different components of forest diversity (tree species richness and composition, genetic diversity and FD) affect insect herbivores in forest systems? [2] How variable are these effects temporally, spatially, between tree species and between herbivore types/feeding guilds? [3] What are the mechanisms of these effects (natural enemies, tree apparency – physical and chemical, leaf traits)? Below I review my key findings in relation to the above questions.

7.1.1. Effects of tree inter-specific, intra-specific and functional diversity on insect herbivory

Summary of main findings

This thesis investigates and quantifies the results of manipulating 3 components of tree diversity on insect herbivory; demonstrating that species diversity, genetic diversity and functional diversity of tree species, all independently influence insect herbivory.

In Chapter 3, silver birch was shown to receive significantly different amounts of insect herbivore damage when planted in monocultures compared to mixed stands. These effects of forest diversity on insect herbivory depended not only on the insect herbivore feeding guild, but also changed within season and between years; with silver birch experiencing both AR and AS effects. In general, silver birch experiences significant AR effects to the damage caused by gallers, silver birch aphid and in some instances early season chewing damage. However, birch also experienced AS to skeletonising insects, late season chewing and early season rolling damage and in some instances early season chewing damage. One finding was that silver birch experienced AR to the damaging effects of the silver birch aphid when at outbreak levels in 2010, this is rather important as damage from sucking insects has been shown to impact more severely on woody plants than defoliation (Zvereva *et al.* 2010). The effects of tree species diversity was consistent spatially and was shown to not be mediated by tree size (physical apparency), physical properties of leaves or natural enemies.

Tree species diversity effects were shown to not only influence abundance, but also species richness of insect herbivores. An unexpected finding of Chapter 4 was that increasing species richness of insect herbivores was responsible for the observed increases in abundance. Chapter 4 focussed on the leaf mining guild and contrary to expectation (as miners are specialist herbivores) demonstrated that silver birch experienced strong AS effects, showing that both abundance and species richness of leaf miners increased with increasing tree species diversity. On alder, these trends were not found and responses were more difficult to interpret. Higher species richness and therefore abundance of leaf miners on birch occurred on taller (more apparent) trees although these effects were not mediated by tree species diversity.

Intra-specific diversity of silver birch (Chapter 5) was also shown to significantly influence insect herbivore abundance and species richness, re-iterating the importance of intra-specific plant diversity effects on predicting insect herbivore damage (Crutsinger *et al.* 2006; Cook-Patton *et al.* 2011; Hughes 2012). Importantly and contrary to the effects of tree species diversity (Chapter 4) increasing genotypic diversity lead to AR effects with more genetically diverse clonal mixtures experiencing fewer mines and containing fewer species of miners. The genetic diversity effects observed in this study were in accordance with similar studies (Tack *et al.* 2010; Tack & Roslin 2011; Castagneyrol *et al.* 2012) and were manifested at the tree and clone level but not at the plot level.

A third, understudied aspect of plant species diversity (Scherer-Lorenzen *et al.* 2005), functional diversity (FD), was also found to influence insect herbivory (Chapter 6). Two separate FD indices were tested. The first FD index (the Scherer-Lorenzen index) was designed to investigate FD effects on forest ecosystem functioning and was used to design the Bechstedt experiment. Traits incorporated in this index included aspects of tree growth, morphology, resource use and nutrient cycling. Abundance of only 2 of the 9 insect herbivore guilds investigated correlated with the Scherer-Lorenzen index. Leaf tying damage was higher in more functionally diverse plots and sucking insects were less abundant in more functionally diverse plots compared to lower functionally diverse plots. The second FD index based on volatile compound emissions was expected to correlate better with insect herbivore damage than Scherer-Lorenzen's index as it used monoterpene emissions of host trees used by insect herbivores in host finding. Skeletonising damage was higher in more functionally diverse plots. Importantly, some support for the semiochemical diversity hypothesis (Zhang & Schlyter 2003) was found because the lower emitting tree species exhibited AR effects and received less chewing damage, leaf rolling and leaf galling damage when planted in higher volatile diversity plots compared to lower diversity plots.

7.1.2 Mechanisms of forest diversity effects on herbivores in relation to main findings.

Cross comparing results between chapters, one interesting finding is that the AR effects observed on silver birch to silver birch aphid, a sap feeding insect (Chapter 3) were also identified when examining the effects of the Scherer-Lorenzen FD index (Chapter 6), where sucking insects were less abundant in plots of higher FD. The 9 traits used to generate the FD index that correlated with sucking damage were indicative of tree growth, morphology, resource use and nutrient cycling. This made it difficult to ascertain exactly what aspect of tree species diversity caused the observed effect. Sucking insects are however, unlikely to be deterred from higher tree diversity plots due to volatile masking or interference from non host species preventing identification of their primary host, as per the assumptions of the semiochemical diversity hypothesis (Zhang & Schlyter 2003). This is because of the lack of correlation between sucking insects and plot volatile FD (Chapter 6). Moreover, many aphid species are thought to use visual cues (Holopainen 2008). With cases where AR effects were observed, including the leaf miners which were more abundant in higher tree species diversity plots in 2010 and 2011 (Chapter 4), abiotic factors may be responsible for AR effects (Barbosa *et al.* 2009; Clissold *et al.* 2009) and suggested as an important avenue for further research. Indeed, microclimate within forest stands composed of different tree species can be expected to be fairly different (Vehvilainen *et al.* 2008) and can be more stable in more diverse systems (Ulrich 1992) that more closely resemble a natural forest compared to monocultures. In particular, humidity has been shown to commonly influence distribution, fecundity, and growth rate of many insect species (Bach 1993).

Another interesting finding from Chapters 3 & 6 relates to skeletonising damage that increased both with tree species richness (Chapter 3) and increasing volatile FD (Chapter 6). It is likely that skeletonisers as a largely generalist feeding guild do not need to rely on specific plant volatile cues to find host plants to the same extent as sucking insects, or if they do use volatile cues then they are able to detect them unhindered by the presence of other non host volatile compounds. The potential mechanisms identified in the present study warrant further investigation at insect species level in order to explore and confirm these suggestions.

It was necessary to move away from insect herbivore guild responses in Chapter 3 in order to focus on leaf miner species specific responses to tree species diversity in Chapter 4; this enabled exploration of tree diversity effects on insect herbivore species richness as well as on insect herbivore abundance. It was found that on both black alder and silver birch, high leaf miner abundance was a result of higher leaf miner species richness and not just greater abundance of a few common species. Birch clearly showed AS to leaf miners whilst responses of alder were less obvious. Plant species composition effects on herbivores have been suggested to be more important than that of plant species diversity *per se* (Koricheva *et al.* 2000; Mikola *et al.* 2002; Riihimaki *et al.* 2005; Jactel & Brockerhoff 2007). The tree species composition effects on leaf miners studied in Chapter 4 on both silver birch and black alder were, however, unclear; effects were inconsistent spatially and temporally. It is possible that leaf miners are not particularly responsive to tree species composition and other guilds need to be investigated. Jactel *et al.* (2007) found composition of mixtures to be more important than species richness in a meta-analysis and noted composition effects on herbivory were greater when mixed forests comprised taxonomically more distant tree species.

Contrary to some studies (Hughes *et al.* 2008; Cook-Patton *et al.* 2011), the effects of interspecific (Chapter 4) and intraspecific diversity (Chapter 5) had opposite effects on leaf miners in this thesis. Silver birch experienced AS to miners when planted in more tree species diverse plots and AR when planted in more genetically diverse plots. To my knowledge, this is the first time that the effects of species diversity and genetic diversity have been compared in forest systems. Genotypic diversity has been shown to be a poor predictor of diversity of specialist herbivores (Tack *et al.* 2010; Castagneyrol *et al.* 2012). Abundance of ectophagous herbivores has been shown to increase with genotypic diversity in most other studies (Peacock & Herrick 2000; Peacock *et al.* 2001; Cook-Patton *et al.* 2011; Castagneyrol *et al.* 2012).

Natural enemies, including predators and parasites have been shown to be more abundant in environments with higher plant diversity compared to lower plant diversity, leading to AR effects (Russell 1989; Andow 1991). In Chapter 3 of this study, predators were not more abundant in higher diversity plots, thus no support for the enemies hypothesis was found; agreeing with other studies in forest systems. For instance spiders were found to decrease in abundance with increasing tree diversity in tropical systems (Schuldt *et al.* 2011), spiders were also not influenced by tree diversity in boreal forests (Riihimaki *et al.* 2005). Rather interestingly in the study of Schuldt *et al.* (2011) the diversity of hunting modes (related to species richness) of spiders increased with tree species diversity. Predator hunting mode may be a key functional trait explaining variation in the nature of top-down control of ecosystems (Schmitz 2008) and was a missing variable in the present study. It is acknowledged that the enemies studied (Chapter 3) may not have been responsible for the majority of predation mediated mortality and indeed a full range of predators (down to species level) including birds as well as parasitoids need to be assessed and their direct effects on insect herbivores need to be studied rather than their abundance alone. However, also in forest systems Riihimäki *et al.* (2005) only found partial support for the enemies hypothesis when measuring direct effects of parasitism on *Epirrita autumnata*. Results from agricultural systems largely support the enemies hypothesis (Pimentel 1961; Russell 1989; Andow 1991; Coll & Bottrell 1994) leading some authors to suggest that the applicability of this hypothesis varies with ecosystem type (Vehvilainen *et al.* 2007). Kaitaniemi *et al.* (2007) questioned the relevance of the enemies hypothesis in forest systems, when on the one hand they found greater ant predator abundance with corresponding lower sawfly abundance in pine-birch mixtures compared to pine monocultures, agreeing with the enemies hypothesis. On the other hand, they noted that the same trees had fewer spider and heteropterans (also enemies of sawflies) as a result of higher ant abundance, indicating interference between enemy groups. Predator responses can also be influenced by scale of the plots, for instance Bommarco & Banks (2003) noticed that predators were affected by plant diversity in intermediate sized plots but not in large scale plots, probably because in smaller plots predators could move about freely and aggregate in plots of higher plant diversity. Without doubt further work in this area should focus on species richness of predators and study direct effects of predation rather than predator abundance.

Physical leaf characteristics, recognised to be important factors influencing insect herbivory (Feeny 1970; Ayres & Maclean 1987; Basset 1991; Coll & Bottrell 1994; Martel & Kause 2002) and tree apparency, the likelihood of a tree being found by herbivores (Feeny 1970; Endara & Coley 2011; Castagneyrol *et al.* 2013) were investigated in Chapters 3 & 4. The observed tree species diversity effects and patterns on insect herbivory were shown not to be mediated by physical leaf traits and tree apparency. Although several of these traits explained some of the variation in insect herbivore damage, these effects were independent of tree species diversity effects. For example, chewing damage was more prevalent on tougher leaves and attributed to generalist insects; because the leaves were measured in the late season, these leaves are likely to be chemically less well defended (Matsuki & Maclean 1994; Schoonhoven *et al.* 2005). Skeletonising damage was found to be higher on less tough leaves, presumably because skeletonisers are unable to deal with tough parts of the leaf such as veins. All other herbivore guilds were not influenced by physical leaf traits. Some studies find that leaf traits explain variation in herbivory only with early season insect herbivores (Matsuki & Maclean 1994). It may be that leaf characteristics undergo more rapid change in the early season and that specialist insects are adapted to a small phenological window of opportunity, where an insect herbivore is predicted to not survive outside a specific leaf age interval of its host plant (Martel & Kause 2002); hence alteration in leaf characteristics will more likely affect specialists in the early season.

Tree height, independently of tree species diversity, was shown to influence insect herbivory. Chapter 3 showed tree height was significantly and positively correlated with the abundance of all insect herbivore categories in the early season and the majority of insect herbivore categories in the late season. In Chapter 4, species richness and abundance of leaf miners on birch were significantly greater on taller trees, suggesting that plants offering a larger resource have greater herbivore species richness and abundance (Bach 1980; Evans 1983; Marques *et al.* 2000). For skeletonisers, gallers and silver birch aphid, tree height explained between 18-35% of variation in herbivory. As specialist insects, gallers and silver birch aphid were more abundant on taller trees and in plots where their host was more abundant. In Chapter 4, the same finding occurred with leaf miners on alder where miner abundance and species richness were both greater on taller trees. Skeletonisers on the other hand (Chapter 3) were significantly more abundant on smaller trees and in plots of higher diversity (where their host was more diluted). An interesting study has recently demonstrated that tree apparency of oak saplings is affected by tree species diversity with a significant negative effect on the abundance of leaf miner herbivores (Castagneyrol *et al.* 2013). Tree apparency in their study was, however, measured as a function of neighbouring tree height, whilst I studied growth traits on trees that herbivory had been measured. Additionally, silver birch is a fast growing and rather apparent pioneer tree species and less likely to be affected in terms of apparency by neighbouring non host trees and therefore plot diversity/composition, as compared to oak which is a slow growing climax species and likely to vary significantly more in height as a result of tree diversity. This highlights that the biology of the tree species may be something that needs to be considered in the study of tree diversity and herbivore resistance.

7.2 Implications for forest management

In this study, when analysing tree species diversity effects in Chapter 3, insect herbivory in most instances (with the exception of the silver birch aphid) was fairly low, e.g. ca. 1.5-3.6% leaf area chewing damage and ca. 0.5-4% skeletonising damage. This is in accordance with observed herbivory levels in other forest experiments, where (with few exceptions) herbivory is generally low (Vehvilainen *et al.* 2006). However, some studies suggest that impacts of endemic herbivory on forest ecosystems over the long term may even exceed impacts from herbivore outbreaks (Crawley 1985; Wolf *et al.* 2008). As described above, tree diversity effects have been demonstrated with most insect herbivore guilds studied (Chapter 3). Birch was shown to experience both AR and AS effects depending on the insect herbivore guild in question and consequently when total insect herbivore damage was considered, no significant tree species diversity effects were detected. Silver birch, however, experienced AR and benefited from being planted in mixtures when silver birch aphid was at outbreak levels. This suggests that in any particular forest area, the identification of insect herbivore species which are more likely to outbreak and/or are more detrimental to silver birch in the long term is important. Forest management strategies could then focus on these identified insects/guilds and manipulate species diversity to minimise damage from them. For example, Zvereva *et al.* (2010) show that damage from sap feeding insects impose a more severe overall negative impact on tree performance than damage from defoliators, due to lower ability of woody plants to compensate from sap feeding damage in terms of both growth and photosynthesis. Furthermore, forest management strategies may very well have to consider temporal aspects of tree species diversity. For instance, during the early season of 2009, chewing damage on birch was lower in mixed stands compared to monocultures but during the late season chewing damage on birch was higher in mixed stands. Similarly, Vehvilainen *et al.* (2006) found that silver birch experienced less early season damage when mixed with pines compared to when birch was grown in monoculture and pointed out that lower early season damage in birches associated with pines may be of great importance to trees. This is due to the fact that young leaves are of higher value for a plant than old leaves and therefore damage caused in the early season may have more detrimental consequences for a tree than at the end of the summer (Harper 1989; Vehvilainen *et al.* 2006). Although, in the case of birch compared with for example, oak, new leaves are produced throughout the growing season (Niemela & Haukioja 1982). Additionally, complicating the matter further, this study indicated that contrary to less early season chewing damage in mixtures compared to monocultures in 2009, in 2010 the trend was reversed and birch growing in mixtures received greater early season chewing damage than monocultures; further confounding the temporal component of utilising tree species diversity as a means of lowering insect herbivore damage. In the majority of instances though, chewing damage was higher on silver birch when it was growing in monoculture and therefore, the most common findings/patterns should form the basis of forest management decisions; the exceptions need to be identified and treated as such.

At stand/plot level, this study suggests that planting mixtures of silver birch genotypes does not significantly reduce damage caused by leaf miners despite variation among genotypes in resistance to damage. When planting different genotypes, it may still be useful to remove genotypes that are particularly susceptible as although not the case in this study, it is likely that including susceptible clones in a plot could cause higher genetic diversity plots to receive more insect herbivore damage. From a forestry perspective, planting fast growing genotypes may also be economically desirable and this is an aspect that warrants further investigation alongside insect herbivore resistance.

As regards manipulating functional diversity of tree stands in order to reduce insect herbivore damage, the research conducted herein is insufficient to suggest worthwhile forest management strategies at the present time. As a tool to further explore the mechanisms that function within tree communities leading to AR or AS, FD can no doubt be exceptionally useful, providing adequate attention is given to selecting traits that specifically address scientific questions (Rao 1982; Petchey & Gaston 2002; Ricotta 2005; Leps *et al.* 2006; Diaz *et al.* 2007). The relationship between insect herbivore damage and FD of volatile compounds in mixtures of differing compositions (as investigated in this study) can only really be expected to be effective for focal and neighbouring plants in close proximity (Barbosa *et al.* 2009). Manipulating volatile FD in this way may be a useful forest management strategy if the results of doing so are better understood. This is because, as one manipulates volatile FD via the planting of differing tree species mixtures, one is also manipulating a myriad of other biological factors and associations.

7.3 Experimental approach

In this thesis, the effects of tree species, genetic and functional diversity on insect herbivory was investigated using an experimental design approach that utilised planted (synthetic) stands/plots. As discussed in Chapter 1, forest diversity experiments are powerful tools as they control factors such as area, plot size, planting density and in particular, environmental variables and by doing so they reduce the effects of confounding factors. It is however, crucial to note that planted synthetic stands utilised throughout this study often deviate from natural forest systems or even production forest stands in several important ways (Huston & McBride 2002) and as such, caution must be applied when scaling results of the studies in this thesis to larger landscape scales. Differences between experimental stands and natural forests occur in tree age distribution, trophic structure and horizontal and vertical canopy structures (Koricheva *et al.* 2006; Leuschner *et al.* 2009). Many natural forests contain trees of several age classes as they are established via natural regeneration (Scherer-Lorenzen *et al.* 2005). Different age classes of trees will influence stand structure, highlighted as an important element of forest diversity (Franklin *et al.* 2002). This need not be considered when scaling results from the present study to most forestry situations as the majority of plantations are still even aged, although there is increasing drive to continuous cover forests that contain a variety of age classes (Mason 2007).

Late successional/climax (shade tolerant) tree species in the natural environment establish and grow in the understory of fast growing/pioneer (shade intolerant) tree species. In commercial forestry, if late successional tree species are desired as a final crop they are established in a similar manner, in the understory of other trees. However, both the Satakunta tree diversity experiment and the Bechstedt FD experiment utilise monoculture patches of late successional species, i.e. spruce, beech and oak that were established on clear cut or open areas in full light conditions. These trees when planted in far from natural conditions never grow well and are permanently stressed, which might affect their relationships with insect herbivores. For example, stress can make these trees smaller (less apparent) and from an insect herbivore view point they may be difficult to spot physically or chemically. Also, leaf quality may be affected in terms of chemical and physical defence, again impacting on insect herbivores.

Stand density is known to be a very important factor affecting pests and pathogens (Burdon *et al.* 1992) and data from this study has been taken from stands at one density. In a production forest system saplings are planted at a closer density to that employed within the experiments and then thinned at several stages throughout a rotation period. Plot size is also a subject of much debate in forest diversity experiments (Scherer-Lorenzen *et al.* 2005). For instance, in agricultural systems, plot size strongly influences the magnitude of crop diversity effects on insect herbivores; effects are enhanced in smaller plots because insects can readily move between the plots aggregating in monocultures (Bommarco & Banks 2003). In addition, with tree stands, smaller plots i.e. less than 0.5ha, edge effects can be substantial resulting in a lack of stable microclimate (Leuschner *et al.* 2009); a homogenous fetch of at least 50-100 metres is required for microclimate and water turnover to stabilise (Jones 2000). Canopy architecture is also sensitive to edge effects (Rothe & Binkley 2001). On the other hand, tree diversity effects were shown to be stronger in larger plots for chewers and rollers, weaker for leaf miners, whilst no effect was detected for gallers and sucking insects (Vehvilainen *et al.* 2007). It may be wise therefore, to utilise data from forest experiments that use synthetic tree communities and data from observational studies from larger production and natural forests when considering the role of forest diversity on ecosystem processes, including insect herbivory (Leuschner *et al.* 2009). Finally, the data in this thesis are from fairly young forests (ca. 10-12 years old at time of sampling), this makes it difficult to extrapolate these results to older stands. For example, resistance to herbivores has been shown to change through the different stages of the life cycle of plants (Boege & Marquis 2005; Barton & Koricheva 2010). Considering the long life span of trees, the work in this thesis on herbivores and their natural enemies has been largely based on observations at a single ontogenetic stage. Using a meta-analysis technique, Barton & Koricheva (2010) indicate that in woody plants, chemical defence increased during the seedling stage, followed by an increase in physical defences during the vegetative juvenile stage; this highlights the importance of long term studies of forest diversity effects through different ontogenetic and successional stages.

7.4 Suggestions for future work

It is important for further work to develop our understanding of the mechanisms that operate within each of the tree species diversity components studied in this thesis. This will ultimately lead us to a better understanding of how these forest diversity components (tree species, genetic, and functional diversity) interact together to influence insect herbivores. We still do not know the effective distance at which neighbouring plants influence focal plants and if the mechanisms that bring about AR and AS at different spatial scales differ (Barbosa *et al.* 2009). Mechanisms that are thought to drive AS and AR observed in this study are discussed herein rather speculatively in some instances and consequently some suggested un-recorded factors are put forward. For example, host finding behaviour may be different with leaf miners compared to other insect herbivores; plot abiotic conditions, particularly humidity and wind speed will vary considerably between plots of different diversity levels and may interact with tree species diversity effects to influence insect herbivores. Light is also a crucial factor in forest ecosystems and will influence insect herbivory as it is important for controlling the production of leaf defences (Moore & Francis 1991). Tree canopy leaf distribution, time of bud break and the physical structure and biochemical processes occurring in leaves are controlled by the amount of light entering the forest, further emphasising that tree density manipulations are important to include in forest experiments.

Importantly, this study has shown that the effect of tree species diversity on insect herbivory when considered as a whole (i.e. all insect herbivore damage combined) is not influenced by tree species diversity (Chapter 3). From a practical point of view this has emphasised the importance of future work to be conducted in a manner better suited to elucidate what damage types or feeding guilds are the most detrimental to a tree species i.e. what insects are more likely to outbreak or what guilds, at endemic levels, are the more damaging in the long term; allowing the manipulation of tree species diversity to reduce what is deemed the most detrimental and risky damage types to forest health and productivity. The effects of tree species richness in this thesis are focussed on birch and alder at the tree level; it will be important to also assess stand level effects and ascertain responses of all trees in a plot.

This study also shows that species diversity, genetic diversity and FD of tree species all independently influence insect herbivory and can lead to AS or AR effects on certain tree species. It is unlikely that these components of diversity are independent of each other and it is suggested that methods are devised so that these effects be considered in unison. For example, potted trees of certain species of interest could be placed within the plots of the birch genotype diversity experiment to explore what effects genotypic diversity of silver birch may have on insect herbivore damage of younger alternative tree species combinations; will increasing the genetic diversity of surrounding vegetation lead to AR or AS effects in understory crops?

Finally, from a forest management point of view, the effects of tree species diversity on insect herbivory alone are insufficient to inform decisions on what mixtures or compositions to plant. This is because above ground insect herbivory poses just one of many serious threats to forest trees. It is suggested that multi-disciplinary approaches are employed to study the effects of tree species diversity on forest health and productivity. For instance, below-ground herbivory, mammalian herbivory and the effects of forest pathogens could also be considered. It is already well known that some tree fungal pathogens i.e. pine twisting rust (caused by *Melampsora pinitorqua* Rostr.) are heteroecious, requiring two unrelated hosts (pine and aspen) to complete their life cycle (Koricheva *et al.* 2006); planting these trees in a mixture increases susceptibility of pine to this damaging pathogen (Mattila *et al.* 2001). Insects can also be vectors, transmitting serious tree killing pathogens, e.g. bark beetles possessing virulent fungal associations (Christiansen & Solheim 1990). The manipulation of tree species diversity may also have a role to play in more complicated tree related disorders. For example, Acute Oak Decline (AOD) is presently thought to be caused by a complex combination of multiple agents, including the buprestid beetle *Agrilus biguttatus,* various species of bacteria and changing abiotic conditions (related to climate change) (Denman & Webber 2009).

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